[18F]-Fluoroestradiol PET: Current Status and Potential Future Clinical Applications

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Word Count: 6488

Financial Support: Susan G. Komen Grant SAC140060, Department of Energy Grant DE-SC0012476, University of Pennsylvania Health System Breast Cancer Translational Center of Excellence, and Educational grant from Blue Earth Diagnostics.

Running Title: 18F-FES-PET Clinical Applications
ABSTRACT

Estrogen receptor (ER) expression in breast cancer is associated with a more favorable prognosis and necessary for response to endocrine therapies. Traditionally, ER expression is assessed by *in vitro* assays on biopsied tumor tissue. However, recent advances have allowed *in vivo* evaluation of ER expression with $^{18}$F-fluoroestradiol (F-FES) positron emission tomography (PET). Clinical studies have demonstrated the use of $^{18}$F-FES-PET as a method for quantifying *in vivo* ER expression and explored its potential as a predictive assay and method of assessing *in vivo* pharmacodynamic response to endocrine therapy. This review outlines the biology and pharmacokinetics of $^{18}$F-FES, highlights the current experience with $^{18}$F-FES in patient studies in breast cancers and other diseases, and discusses potential clinical applications and possible future clinical use of $^{18}$F-FES-PET.

**Keywords (4):**

1. Estrogen receptor
2. Positron emission tomography
3. Breast cancer
4. $^{18}$F-Fluoroestradiol
INTRODUCTION

Breast cancer is the most common cancer in women and the 2nd most common cause of cancer death in women in the United States, with an estimated 231,840 new diagnoses in 2015 (1). Approximately 75% of newly diagnosed patients have estrogen receptor (ER) expressing breast tumors, which are associated with a more favorable prognosis (2). ER expression in breast cancer is traditionally assessed by in vitro assays on biopsied tissue using qualitative and/or semi-quantitative immunohistochemical staining (3). A tumor's ER status predicts likelihood of response to ER-targeted therapy, also known as endocrine or hormone therapy (4). While absence of ER by in vitro assay indicates low likelihood of response and is associated with a worse prognosis, the presence of ER by immunohistochemistry does not necessarily guarantee response to endocrine therapy (5). Nevertheless, it is important to identify ER-positive patients with recurrent and metastatic disease, whom may respond to hormone therapy and potentially avoid toxic side effects of chemotherapy (6).

For patients with advanced or metastatic ER-positive disease, the advent of positron emission tomography (PET) and PET combined with computed tomography (PET/CT) has made it possible to evaluate ER expression in all metastatic lesions without multiple biopsies. Used with 18F-fluorodeoxyglucose (18F-FDG) to detect increased glucose metabolism, PET imaging possesses widespread oncologic applications (7). In breast cancer, 18F-FDG-PET/CT is recommended primarily for locally advanced or metastatic disease when standard staging studies are equivocal or suspicious (8).

Other radiotracers have subsequently been developed to better characterize tumor biology, including 18F-fluoroestradiol (18F-FES). 18F-FES targets ER, enabling in vivo imaging of ER-expressing tissues. In conjunction with 18F-FDG-PET or other standard imaging, 18F-FES-PET has the potential to assess heterogeneity in ER expression and identify sites that have lost ER expression or functionality. 18F-FES-PET has been evaluated in numerous breast cancer clinical studies as a promising method for quantifying in vivo ER expression, predicting response to hormone therapy, and evaluating effective ER blockade (Supplemental Table 1). This review
providers background for practitioners by highlighting the biology and pharmacology of \(^{18}\text{F}\)-FES, reviewing current clinical experience with \(^{18}\text{F}\)-FES, and summarizing its potential applications.

\textbf{\(^{18}\text{F}\)-FES STRUCTURE, SYNTHESIS, PHARMACOKINETICS, AND SAFETY}

Early efforts to develop an ER targeting radiotracer involved labeling steroid and non-steroid compounds with iodine and bromine \((9)\). However, the subsequent advent of PET imaging and \(^{18}\text{F}\) – a small halogen that displayed uptake in target tissues, elimination in non-target tissue, substitution at several positions in various estrogen analogs, and a half-life long enough to allow for multistep synthesis \((10,11)\) – encouraged the development of \(^{18}\text{F}\)-labeled compounds.

In 1984, Kiesewetter et al. found that \(^{18}\text{F}\)-FES exhibited the highest uptake selectivity and target to background ratio among several \(^{18}\text{F}\)-labeled estrogens \((10)\). Newer compounds such \(^{18}\text{F}\)-moxestrol \((^{18}\text{F}\text{-betaFMOX})\) and 4-fluoro-11\(\beta\)-methoxy-16\(\alpha\).\(^{18}\text{F}\)-fluoroestradiol (4F-M-\(^{18}\text{F}\)-FES) demonstrated increased ER binding, with \(^{18}\text{F}\)-betaFMOX also displaying decreased metabolism \((12,13)\). However, \(^{18}\text{F}\)-betaFMOX displays suboptimal uptake in humans, which likely arises from modest binding to sex-hormone binding globulin (SHBG), the main plasma protein estradiol transporter, compared to \(^{18}\text{F}\)-FES \((12)\). Though 4F-M-\(^{18}\text{F}\)-FES demonstrates adequate uptake in humans, tumor uptake comparison studies and further testing are needed. To date, \(^{18}\text{F}\)-FES remains the most widely studied ER PET imaging compound.

\(^{18}\text{F}\)-FES is highly extracted and metabolized by the liver, resulting in rapid early blood clearance and constant total blood activity 10 to 15 minutes after injection \((11)\). By 20 minutes post-injection, only 20\% of the total activity is attributable to unmetabolized \(^{18}\text{F}\)-FES; by 120 minutes, only 10\%. Like estradiol, unmetabolized \(^{18}\text{F}\)-FES is heavily protein bound in blood. Although \(^{18}\text{F}\)-FES has much higher affinity for SHBG than albumin, the higher concentration of albumin in blood results in an approximately 1:1 distribution of \(^{18}\text{F}\)-FES between SHBG and albumin \((14)\). Its non-SHBG-bound metabolites, comprised of glucuronide and sulfate conjugation products \((11)\), are secreted in bile, resorbed via enterohepatic circulation, and renally excreted. The rate of decline in total liver activity is similar to the rate of increase in total bladder activity,
suggesting that $^{18}\text{F-FES}$ metabolites are cleared by the kidneys at nearly the same rate as they are released into circulation by the liver \((11)\). At the highest recommended dose of $2.22 \times 10^8$ becquerel (Bq), the effective dose equivalent is 0.002 millisievert (mSv) per MBq, with the critical organ being the liver at 0.13 mSv per MBq \((15)\). Cumulative experience in published human studies have yet to demonstrate any associated toxicities or adverse events. Collectively, these characteristics make $^{18}\text{F-FES}$ a favorable PET ER imaging tracer.

$^{18}\text{F-FES}$ has been studied as an investigational diagnostic agent in Canada, Europe, and Asia. While it is currently considered an investigational drug in the United States, several American academic centers hold Investigational New Drug (IND) approvals that support studies involving $^{18}\text{F-FES}$-PET and $^{18}\text{F-FES}$-PET/CT. The National Cancer Institute (NCI) also holds an IND (IND 79,005) enabled by a University of Washington study \((16)\) that can support multi-center trials in NCI-supported clinical trials networks. There has been discussion in Europe and the United States seeking regulatory approval for $^{18}\text{F-FES}$ based upon published studies and accruing data from prospective multi-center trials.

**CORRELATION OF $^{18}\text{F-FES}$ UPTAKE AND TUMOR ER EXPRESSION**

Multiple studies have demonstrated correlation between $^{18}\text{F-FES}$ uptake and tumor ER expression measured by conventional \textit{in vitro} assays (Supplemental Table 2). In 1988, Mintun et al. verified the association between $^{18}\text{F-FES}$ uptake and \textit{in vitro} tumor ER concentration measured by radioligand binding among patients with primary breast masses \((17)\). Subsequent studies established the correlation between $^{18}\text{F-FES}$ uptake and results from immunohistochemical assays. Peterson et al. used a standardized uptake value (SUV) threshold of 1.1 to characterize tumors as ER-positive or ER-negative, reporting a correlation of 0.73 between $^{18}\text{F-FES}$ uptake and immunohistochemical index results \((18)\) consistent with those from studies comparing \textit{in vitro} radioligand binding assays to immunohistochemical assays \((19)\).

Peterson et al. also studied the correlation between immunohistochemical assays and other $^{18}\text{F-FES}$ uptake quantification methods, finding that those that accounted for variable blood
clearance and the presence of labeled $^{18}$F-FES metabolites provided no definite advantages over simpler SUV measurements (18). More recently, $^{18}$F-FES uptake and immunohistochemical ER expression have been demonstrated in early stage breast cancers, though with a lower sensitivity than prior studies (20).

Other factors that can affect tumor $^{18}$F-FES uptake have also been evaluated. Prior analyses posited that competition with higher circulating estrogen levels in pre-menopausal women may contribute to false-negative $^{18}$F-FES-PET results (21). However, Peterson et al. subsequently demonstrated no significant difference in average $^{18}$F-FES uptake based on a plasma estradiol threshold of 30 pg/mL, a level typically used to indicate menopausal status (22). In this same study, F-FES uptake was inversely associated with plasma SHBG levels but not with testosterone levels, patient age or disease stage at time of imaging – discrepancies suggesting that although a certain amount of binding to SHBG may be necessary to protect F-FES from metabolism, protein-bound $^{18}$F-FES may be less available to tissue ER receptors and result in decreased $^{18}$F-FES uptake. Thus, measurement of SHBG levels in patients could be considered, especially in clinical scenarios such as the post-partum period when SHBG levels might be expected to be outside the typical range.

This study also revealed only a modest effect of lower injected specific activities on $^{18}$F-FES uptake, suggesting that "cold" $^{18}$F-FES would not significantly saturate tissue ERs at specific activities of greater than approximately 11.1 GBq/mole. However, since a small (approximately 10%) negative effect on $^{18}$F-FES uptake was noted for injected $^{18}$F-FES masses greater than 0.2 nmole/kg, injected mass should aim to be below this value (22).

**BASELINE $^{18}$F-FES UPTAKE AS A PREDICTOR OF RESPONSE TO ENDOCRINE THERAPY**

For patients with ER-positive breast cancer, endocrine therapy can potentially provide effective treatment with fewer side effects and lower morbidity than chemotherapy (6). However, ER positivity only correctly predicts response in 50-60% of treatment-naïve patients (5) as measured by in vitro immunohistochemical assays, which require biopsies and are thus limited by
sampling error and disease heterogeneity. In contrast, $^{18}$F-FES-PET can evaluate ER expression across all tumor sites and present a more complete picture of a patient’s overall ER status.

Studies have demonstrated a correlation between response to endocrine therapy and baseline pre-treatment $^{18}$F-FES uptake (Supplemental Table 3). Both Dehdashti et al. and Mortimer et al. investigated baseline $^{18}$F-FES uptake in ER-positive patients beginning tamoxifen therapy (23,24). Using a threshold SUV of 2.0 for baseline $^{18}$F-FES uptake, they reported a positive predictive value (PPV) of 79-87% and negative predictive value (NPV) of 88-100% for response.

The utility of baseline $^{18}$F-FES-PET imaging has also been demonstrated in patients undergoing salvage therapy with aromatase inhibitors (AIs) and fulvestrant. In a study of patients with heavily pre-treated metastatic breast cancer, Linden et al. established a threshold $^{18}$F-FES SUV of 1.5 for baseline $^{18}$F-FES uptake, below which no patient responded (Supplemental Fig. 1) (25). Using a higher threshold SUV of 2.0, Dehdashti et al. demonstrated an NPV of 81% for response (26). Both studies demonstrated poor PPV of 34-50%, a finding consistent with known decreased objective response rates to endocrine therapy among those with recurrent and previously treated disease (27).

Taken together, these studies demonstrate the value of $^{18}$F-FES-PET in predicting endocrine responsiveness or unresponsiveness. Data from four studies evaluating response to tamoxifen, AIs, and fulvestrant (23-26) reveal that of the 159 patients who underwent pre-treatment $^{18}$F-FES-PET imaging, only 1 with baseline $^{18}$F-FES SUV < 1.5 responded to endocrine therapy by demonstrating disease stabilization (Supplemental Fig. 2) (26).

Applying a SUV threshold of 1.5 to these data, van Kruchten et al. studied the relationship between baseline $^{18}$F-FES-PET and response to low-dose oral estradiol as salvage therapy (28). It is thought that long-term anti-estrogen therapy may induce hypersensitivity to estrogens, whereby estrogen exposure activates apoptosis rather than growth pathways (29). In this scenario, the presence of ER, which could be measured by $^{18}$F-FES-PET, is necessary to
induce these apoptotic pathways. The threshold SUV of 1.5 demonstrated a PPV of 60% and NPV of 80% for response to low-dose estradiol (28).

An alternative approach to predicting response utilizes $^{18}$F-FDG-PET imaging, which has been established as a predictive biomarker in cancers such as lymphoma (30). In breast cancer, a clinical “flare” can occasionally be seen with therapeutic agents possessing ER agonist properties, where initial symptom exacerbation upon therapy initiation predicts subsequent response (31). Studies have shown that $^{18}$F-FDG-PET can also detect subclinical “metabolic flares” in patients who subsequently respond to therapy (23,24). Comparison of $^{18}$F-FDG-PET before and 7-10 days after initiation of tamoxifen, which can manifest transient agonist activity early after initiation of therapy, showed increased $^{18}$F-FDG uptake in patients who subsequently responded, but no significant change in uptake in non-responders. Metabolic flare induced by an estradiol challenge was also predictive of response to AIs and fulvestrant as well as improved survival (26).

Studies supporting both pre-therapy $^{18}$F-FES-PET and early serial $^{18}$F-FDG-PET to predict endocrine responsiveness have generated debate about which approach is more clinically applicable. Both radiotracers show high NPV for endocrine responsiveness, but serial $^{18}$F-FDG-PET possesses higher PPV for response compared to pre-therapy $^{18}$F-FES-PET (23,24). Some also argue that $^{18}$F-FDG-PET is more widely available and used in the setting of metastatic breast cancer. However, serial $^{18}$F-FDG-PET requires two PET scans and exposure to a therapy with ER agonist properties. On the contrary, a single baseline $^{18}$F-FES-PET is able to predict response for various endocrine therapies, prior to any exposure to therapy, directing patients without ER expression away from likely unbenefficial endocrine treatments. In addition, the increasing use of therapeutic strategies combining endocrine and other targeted therapy increases the need to determine ER expression and suitability for combined treatment. One potential framework for combining both approaches would be to first select patients whose tumors express ER by using $^{18}$F-FES to confirm target expression, followed by serial $^{18}$F-FDG-PET or other standard imaging
to predict responsiveness by assessing the pharmacodynamic response to a specific types of therapy (Supplemental Fig. 3) (32).

**ABILITY OF ¹⁸F-FES-PET TO ASSESS WHOLE-BODY TUMOR BURDEN AND HETEROGENEITY OF DISEASE**

One major advantage of ¹⁸F-FES-PET is its ability to non-invasively assess the *in vivo* ER status of several tumor lesions across the whole body simultaneously. Evaluating for lesions with discordant ¹⁸F-FDG and ¹⁸F-FES uptake can determine the heterogeneity of a patient’s disease (Supplemental Fig. 4) (16,25,33,34).

Studies correlating ¹⁸F-FES uptake with *in vitro* ER expression and response to hormone therapies have demonstrated the ability of ¹⁸F-FES-PET to image metastatic disease *in vivo* (Supplemental Table 4) (17,21,33). Among multiple metastatic sites in individual patients, ¹⁸F-FES uptake was concordant with *in vitro* ER expression (33). Patients with discordant *in vitro* ER expression and ¹⁸F-FES uptake (i.e. ER-positive but ¹⁸F-FES negative) tended to have a decreased response to hormone therapy, suggesting that ¹⁸F-FES-PET may identify tumor sites that are ER-positive by *in vitro* assay but functionally hormone therapy resistant (16,33,34).

Kurland et al. specifically studied within-patient and between-patient concordance of ¹⁸F-FES uptake and a previously documented ER-positive biopsy (35). While ¹⁸F-FES uptake and the ratio of ¹⁸F-FES to ¹⁸F-FDG uptake were generally consistent across a single patient, these values varied greatly between patients despite the fact that all but one originally had ER-positive primary tumors. Thirty-four of the 91 patients, many of whom had already undergone treatment with one or more anti-estrogen therapies, had an average ¹⁸F-FES SUV below 1.0, suggesting that exposure to endocrine therapy may impose selective pressure for tumor phenotypes with low or non-functional ER expression.

There was also a small number of patients that demonstrated highly discordant ¹⁸F-FES uptake across sites (i.e., ¹⁸F-FES positive and ¹⁸F-FES negative lesions), a finding that possibly reflects emerging loss of ER expression in only some lesions. In another study evaluating within-
patient concordance of ¹⁸F-FES uptake, discordant ¹⁸F-FES uptake was only seen in patients pre-
treated with endocrine therapy (36).

Potential discrepancies in tumor ER status and ¹⁸F-FES uptake are particularly important
for women with recurrent or metastatic disease. Several studies have demonstrated that although
a primary tumor may have been ER-positive, its metastatic lesions may no longer express ER or
express only non-functional ERs (16,33,34). Because it is clinically infeasible to biopsy all sites of
disease to determine overall ER expression, or undergo repeated biopsies to evaluate tumor
phenotype evolution, ¹⁸F-FES-PET imaging could represent an important adjunct for monitoring
ER expression at the time of disease progression or throughout a treatment course.

**UTILITY OF ¹⁸F-FES-PET IN ASSESSING IN VIVO PHARMACODYNAMICS**

Several investigators have utilized ¹⁸F-FES-PET to study the in vivo pharmacodynamics
of standard endocrine therapies (23,24,37,38) and validate new investigational ER antagonists
(39,40) (Supplemental Table 5). McGuire et al. utilized repeat ¹⁸F-FES-PET imaging to
demonstrate a change in ¹⁸F-FES uptake after initiation of endocrine therapy. Compared to the
baseline ¹⁸F-FES-PET scan, decreased ¹⁸F-FES uptake at known metastatic lesions 7-10 days
after initiation of tamoxifen provided evidence of receptor-mediated tumor uptake of ¹⁸F-FES (41).
Mortimer et al. demonstrated similar decreases in ¹⁸F-FES uptake in patients receiving tamoxifen
(24) and showed that responders had a significantly higher mean percentage decrease in ¹⁸F-
FES uptake after initiation of therapy (54.8% ± 14.2%) compared to non-responders (19.4% ±
17.3%; P=.0003).

Linden et al. evaluated changes in ¹⁸F-FES uptake in patients receiving tamoxifen, AIs, or
fulvestrant. As expected, treatment with tamoxifen, a selective ER modulator, and fulvestrant, a
selective ER downregulator, was associated with a greater decrease in ¹⁸F-FES uptake than
treatment with AIs, which decrease the amount of circulating estrogen and do not act directly on
ER. Van Kruchten et al. also utilized ¹⁸F-FES-PET to study the effects of fulvestrant on ¹⁸F-FES
uptake (38). Thirty-eight percent of patients demonstrated incomplete reduction of ¹⁸F-FES
uptake (defined as less than 75% decrease in median tumor SUV), which was significantly associated with shorter progression-free survival. There was also wide variance in the median change in $^{18}$F-FES SUV before and after initiation of therapy (-99% to +60%), with significantly larger decreases in patients with clinical response compared to those with disease progression (median change in SUV of -88% vs -58%). Neither clinical response nor degree of change in $^{18}$F-FES uptake correlated with plasma drug levels of fulvestrant, pointing to the unique potential of $^{18}$F-FES-PET in evaluating the effects of fulvestrant at the receptor level.

In a related pre-clinical study, Heidari et al. demonstrated that increasing fulvestrant doses in murine xenografts led to parallel decreases in $^{18}$F-FES uptake and ER expression by immunohistochemical assay that did not correlate with $^{18}$F-FDG uptake (42). These findings suggest that changes in ER availability occur before detectable changes in tumor metabolism and growth. Since higher doses (750 mg vs 500 mg) of fulvestrant have been studied with minimal increase in side effects (43), serial $^{18}$F-FES-PET imaging could conceivably be used to measure early blockade of ER to guide individualized ER-antagonist dosing. However, this approach would require further testing to determine its accuracy and impact.

These concepts could also be applied to new investigational endocrine therapies, both to demonstrate effective ER blockade and identify optimal dosing for complete ER downregulation. Wang et al. described a new ER$\alpha$ antagonist, ARN-810, and utilized $^{18}$F-FES-PET to validate ER target engagement (39). Dickler et al. then evaluated ARN-810, aka GDC-0810, in a phase I study, and utilized $^{18}$F-FES PET to assess pharmacodynamic activity and demonstrate greater than 90% suppression of estradiol binding to ER in 90% of patients (40).

**NON-BREAST CANCER USES OF $^{18}$F-FES**

**Uterine Endometrium and Myometrium**

Tsuchida et al. first verified the correlation between $^{18}$F-FES uptake and *in vitro* immunohistochemical measurement of ER concentration in endometrial tissue (44). Subsequent studies demonstrated a significant difference in both $^{18}$F-FES uptake and the ratio of $^{18}$F-FDG to
\(^{18}\)F-FES uptake between endometrial hyperplasia and endometrial cancer, as well as between low-grade and high-grade endometrial cancer (45,46). Compared to endometrial hyperplasia, low-grade endometrial carcinoma displayed significantly lower \(^{18}\)F-FES uptake and higher \(^{18}\)F-FDG uptake, and thus higher \(^{18}\)F-FDG to \(^{18}\)F-FES uptake ratios. In turn, high-grade carcinomas displayed higher \(^{18}\)F-FDG to \(^{18}\)F-FES uptake ratios than low-grade endometrial carcinomas.

\(^{18}\)F-FES-PET also has potential to differentiate benign uterine leiomyomas from malignant uterine sarcomas based on \(^{18}\)F-FES uptake and \(^{18}\)F-FDG to \(^{18}\)F-FES uptake ratio (47,48). Differentiation of sarcoma from leiomyoma can often be difficult with MRI (49) and \(^{18}\)F-FDG-PET findings can be equivocal (50). Similar to endometrial pathologies, lower \(^{18}\)F-FES uptake and higher \(^{18}\)F-FDG to \(^{18}\)F-FES uptake ratio have been associated with malignant sarcomas (47,48). Given the substantial management and prognostic differences between the two entities, \(^{18}\)F-FES-PET can potentially play a role in risk stratification of indeterminate uterine masses.

**Epithelial Ovarian Cancer**

Up to 70% of epithelial ovarian cancers express ER at baseline (51), and \(^{18}\)F-FES-PET has the ability to localize primary and metastatic lesions in such cancers (52,53). Van Kruchten et al. studied the utility of \(^{18}\)F-FES-PET in 15 patients with suspected ovarian cancer, demonstrating a correlation between lesion \(^{18}\)F-FES uptake and immunohistochemical ER expression, as well as 79% sensitivity and 100% specificity using a SUV threshold of 1.8 (53).

As in breast cancer, \(^{18}\)F-FES-PET has potential utility to evaluate and monitor heterogeneity of disease in ovarian cancer. Given results from phase II trials of endocrine therapy for epithelial ovarian cancer (54-56), \(^{18}\)F-FES-PET could play a role in identifying patients who would most likely benefit from endocrine therapy. While promising, however, these results have been modest and more studies are needed to validate preliminary findings.

**Other Uses**
\(^{18}\)F-FES-PET has demonstrated ER expression in normal brain tissues and in meningiomas \((57,58)\). There is limited evidence evaluating the relationship between tamoxifen and development of meningiomas, as well as the utility of tamoxifen in treating refractory meningiomas \((59,60)\). Investigators have also begun studying the ligand-binding domain of human ER as a potential reporter gene and \(^{18}\)F-FES-PET as a probe for confirming successful transfection in gene and cell therapies \((61,62)\). Promising results were demonstrated for various transfection techniques, suggesting additional applications for \(^{18}\)F-FES-PET in basic and translational research studies.

**POTENTIAL CLINICAL USES**

As described in this review, \(^{18}\)F-FES-PET has the ability to quantify regional ER expression in breast cancer and preliminarily in other cancers. As with ER assays of sampled tissue, the key value of \(^{18}\)F-FES-PET is identifying patients whose tumors do not express ER, indicating a lack of endocrine responsiveness. Studies have also demonstrated the utility of \(^{18}\)F-FES as a pharmacodynamic marker for endocrine therapy, especially to assess the degree of blockade by ER antagonists. Below, we review possible clinical applications where \(^{18}\)F-FES-PET might be applicable to current and future practice.

**Breast Cancer Detection and Staging**

\(^{18}\)F-FES-PET is unlikely to supersede \(^{18}\)F-FDG-PET as the primary PET tool for breast cancer staging given limitations such as hepatic metabolism, which precludes visualization of liver metastases, and considerable enterohepatic circulation, which complicates abdominal imaging using \(^{18}\)F-FES-PET \((11)\).

 Nonetheless, because it is highly specific for ER-expressing breast cancers, \(^{18}\)F-FES could be a beneficial adjunct that expands the focus in radionuclide breast cancer beyond \(^{18}\)F-FDG for metastatic staging and possibly beyond \(^{18}\)F-FDG and \(^{99m}\)Tc-sestamibi for primary breast cancer diagnosis \((63,64)\). \(^{18}\)F-FES-PET can clarify and/or detect otherwise poorly visualized sites
seen with $^{18}$F-FDG-PET, particularly in less differentiated cancers (e.g., invasive lobular carcinoma) that tend to be less $^{18}$F-FDG-avid, and also help with false-positive uptake due to inflammation, healing, and other know non-cancer causes of FDG uptake (7,21,34,65). Knowledge of $^{18}$F-FES uptake might also obviate the need for invasive biopsy, particularly in the metastatic setting, potentially improving the cost-effectiveness of metastatic disease workup (66). Finally, our advancing knowledge about predisposing factors for specific types of breast cancer could one day lead to a scenario where patients prospectively identified as high-risk for ER-expressing cancers might benefit from adjunct screening with $^{18}$F-FES-PET.

**Predicting Response to Endocrine Therapy**

Consistent with growing emphasis on precision medicine and individualized care, as well as evidence that $^{18}$F-FES-PET can improve diagnostic understanding and inform therapeutic approaches (65), $^{18}$F-FES-PET could provide a tool for individualized therapy. A particular advantage of $^{18}$F-FES-PET is its ability to evaluate receptor status over the entire tumor volume.

In the clinic, PET ER imaging would have its greatest impact in patients with metastatic breast cancer, where it is not practical to biopsy all sites of disease. As such, clinicians often base their choice of endocrine therapy on ER status in the primary tumor and not in the metastatic sites. However, studies suggest that up to 30% of patients may lose ER expression at one or more sites of disease after undergoing several lines of endocrine therapy (16,28,36,38). Based upon these considerations, the most immediately compelling clinical use of $^{18}$F-FES-PET appears to be as a tool for measuring regional ER-expression and is a logical extension of the current practice of assessing ER expression by tumor biopsy.

Recent trends in therapeutic strategies for ER-expressing breast cancer may increase the utility of $^{18}$F-FES-PET for guiding therapy selection. One such trend is to target multiple breast cancer pathways by combining agents such as everolimus or palbociclib with endocrine therapy (8). Additional combined therapies targeting other pathways (e.g., epidermal growth factor receptor) are likely in the future (67).
The use of combined therapy, where it is difficult to discern the contribution of each agent to therapeutic response, creates an increased need for biomarkers for each target of the combined therapy. An imaging-based biomarker for ER expression to predict and/or assess response could be especially valuable and cost-effective in the setting of patients being considered for combinations of endocrine therapy and other targeted agents.

**Barriers to widespread clinical use of $^{18}$F-FES-PET**

While $^{18}$F-FES-PET represents a promising advancement, barriers to more widespread use also exist. First, additional work is required to prospectively validate its role in different clinical contexts, similar to the process undertaken with $^{18}$F-FDG-PET. Moreover, its utility as one component in a multimarker approach to prognostication and management must be further understood. Finally, data from these efforts will be needed to support regulatory approvals, which could support the use of $^{18}$F-FES-PET in a clinical setting beyond its current investigational approvals and role. All efforts are important given that support for $^{18}$F-FES-PET has arisen from smaller retrospective or single center, prospective studies. Larger, additional studies can clarify the generalizability of the modality’s reported benefits, particularly given the high associated costs and limited availability in most institutions and settings.

**CONCLUSION**

$^{18}$F-FES-PET is a safe and potentially clinically valuable tool for *in vivo* evaluation of ER expression in breast cancer. It correlates well with traditional *in vitro* immunohistochemical methods and has shown potential for predicting endocrine therapy response. Limited studies have also shown the potential utility of $^{18}$F-FES-PET in assessing other ER expressing tumor types, such as those of uterine and ovarian epithelial origin. Advantages of $^{18}$F-FES-PET over *in vitro* methods include its ability to assess whole-body tumor burden and heterogeneity of disease, as well as provide serial information about *in vivo* pharmacodynamics of various endocrine therapies. The studies reviewed in this paper have demonstrated promising potential clinical uses
of $^{18}$F-FES-PET, perhaps most importantly as a tool for individualizing treatment by predicting response to endocrine therapies. Although barriers to widespread use exist, at the time of writing, there are 10 open clinical trials utilizing $^{18}$F-FES-PET, 8 of which are studying its use in breast cancer (68). These and future studies will shed further light on the uses of $^{18}$F-FES-PET in guiding drug development, assessing disease burden, and informing therapeutic decision making.

**DISCLOSURE**

Consultant to GE Healthcare, funding from Siemens Medical, and honoraria from Philips Healthcare (D.A. Mankoff).

**ACKNOWLEDGEMENTS**

This work was supported in part by Susan G. Komen Grant SAC140060, Department of Energy Grant DE-SC0012476, the University of Pennsylvania Health System Breast Cancer Translational Center of Excellence, and an educational grant from Blue Earth Diagnostics. The authors also wish to thank Jonathan Allis for helpful comments.
REFERENCES


29. Song RX, Mor G, Naftolin F, et al. Effect of long-term estrogen deprivation on apoptotic
outcome in diffuse large cell lymphoma and classic hodgkin disease. Cancer. 2006;107:2678-
2687.
33. Mortimer JE, Dehdashti F, Siegel BA, Katzenellenbogen JA, Fracasso P, Welch MJ. Positron
emission tomography with 2-[18F]fluoro-2-deoxy-D-glucose and 16alpha-[18F]fluoro-17beta-
estradiol in breast cancer: correlation with estrogen receptor status and response to systemic
estrogen PET (FES-PET) and glucose PET (FDG-PET) scans and pathologic characteristics
including HER2 and Ki67 to predict for hormone insensitivity in women with metastatic breast
variability in estrogen receptor binding, measured in vivo by 18F-fluoroestradiol PET. J Nucl Med.
2011;52:1541-1549.
computed tomography demonstrate the heterogeneity of breast cancer in vivo? Clin Breast
reveals differences in pharmacodynamics of aromatase inhibitors, tamoxifen, and fulvestrant in


