Diagnostic accuracy and safety of 16α-[^{18}F]fluoro-17β-oestradiol PET-CT for the assessment of oestrogen receptor status in recurrent or metastatic lesions in patients with breast cancer: a prospective cohort study

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Summary

Background A biopsy of first recurrence or metastatic disease is recommended to re-evaluate oestrogen receptor status in patients with breast cancer and to select appropriate treatment. However, retesting for oestrogen receptor status with rebiopsy is not always feasible, depending on lesion location and the risk associated with biopsy, and in these cases clinicians continue to treat patients according to the oestrogen receptor status of the primary tumour. Consequently suboptimal therapy might be offered to these patients. We assessed the diagnostic accuracy and safety of 16α-[^{18}F]fluoro-17β-oestradiol (^{18}F-FES) PET-CT to assess oestrogen receptor status in patients with recurrent or metastatic breast cancer.

Methods We did a prospective cohort study at the Asan Medical Center, Seoul, South Korea. Eligible patients had breast cancer, with first recurrence or metastatic disease at presentation, were 19 years or older, and had an Eastern Cooperative Oncology Group performance status of 0–2. The primary objective was to show the agreement between qualitative ^{18}F-FES PET-CT interpretation and the results of oestrogen receptor expression by immunohistochemical assay, a non-reference standard test. Whole-body ^{18}F-FES PET-CT imaging was done after intravenous injection of 111–222 MBq of ^{18}F-FES, with dosing primarily determined by radiation dosimetry analysis. ^{18}F-FES uptake above background intensity was interpreted as positive. Efficacy was assessed in all patients with histologically confirmed recurrent or metastatic breast cancer who received ^{18}F-FES and had PET-CT images available (intention-to-diagnose analysis), and safety was assessed in all patients who received ^{18}F-FES. This study is registered with ClinicalTrials.gov, number NCT01986569.

Findings Between Nov 27, 2013, and Nov 10, 2016, 93 patients were enrolled. Of the 85 patients included in the efficacy analysis, 47 (55%) were oestrogen receptor-positive and 38 (45%) were oestrogen receptor-negative. Positive status percent agreement between the ^{18}F-FES PET-CT results and oestrogen receptor status by immunohistochemical assay was 76·6% (95% CI 62·0–87·7) and the negative status percent agreement was 100·0% (90·8–100·0). Patients who were oestrogen receptor-positive and had a positive ^{18}F-FES PET-CT result had a significantly higher progesterone receptor expression than those who were oestrogen receptor-positive and had a negative ^{18}F-FES PET-CT result (23 [68%] of 34 patients *vs* 0 of 11 patients; p<0·0001). The most common adverse event was procedural pain in nine (10%) of 90 patients injected with ^{18}F-FES. No adverse events were related to the study drug except injection site pain in one (1%) patient. No serious adverse events were recorded.

Interpretation The high negative percent agreement between ^{18}F-FES PET-CT and oestrogen receptor status by immunohistochemical assay in this cohort suggests that positive ^{18}F-FES uptake by recurrent or metastatic oestrogen receptor-positive breast cancer lesions could be an alternative to oestrogen receptor assays in this setting. Staging assessment should include ^{18}F-FES PET-CT when retesting oestrogen receptor status is not feasible.

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Introduction

Breast cancer is one of the leading causes of cancer-related death in women. For metastatic breast cancer, 5-year cancer-specific survival is no more than 40%.[1] The systemic treatment of breast cancer recurrence or metastasis with available therapies is not curative but improves quality of life and overall survival. A biopsy is recommended for patients with first recurrence or metastatic disease at presentation to obtain histopathological confirmation and to re-evaluate oestrogen receptor status; the results direct the selection of appropriate treatment because of the potential discordance in oestrogen receptor status between

Research in context

Evidence before this study

Oestrogen receptor status of breast cancer can be non-invasively assessed by PET with 16α-[^{18}F]fluoro-17β-oestradiol (^{18}F-FES). We did a literature review of PubMed and Embase to identify reports published in English from Jan 1, 1985, to May 8, 2018. For search terms used, please see the appendix (pp 2–3). We identified five studies that investigated the accuracy of ^{18}F-FES PET in patients with recurrent or metastatic breast cancer. Previous prospective studies were not designed to formally test a specific hypothesis and the results were exploratory. In only two studies, the accuracy of ^{18}F-FES PET was separately assessed in a small number of patients with recurrent or metastatic breast cancer with the use of a prespecified threshold of ^{18}F-FES uptake. The clinical utility of ^{18}F-FES PET in the diagnosis of recurrent or metastatic disease has not been established.

Added value of this study

To our knowledge, this is the first study to investigate diagnostic accuracy and safety of ^{18}F-FES PET-CT for the assessment of oestrogen receptor status in recurrent or metastatic lesions in patients with breast cancer. In this study, we provide an estimate for positive and negative percent agreements between ^{18}F-FES PET-CT and the immunohistochemical assay of oestrogen receptor expression in a consecutive sample of patients. We show a high negative percent agreement, but a moderate positive percent agreement. Patients who were oestrogen receptor-positive and had a positive ^{18}F-FES PET-CT result had a significantly higher progesterone receptor expression than those who were oestrogen receptor-positive and had a negative ^{18}F-FES PET-CT result. ^{18}F-FES administration and PET-CT procedures were well tolerated in all patients.

Implications of all the available evidence

The staging assessment of patients who present with first recurrence or metastatic breast cancer should include ^{18}F-FES PET-CT when retesting oestrogen receptor status is not feasible. Positive ^{18}F-FES PET-CT could be a non-invasive alternative in the diagnosis of oestrogen receptor-positive recurrent or metastatic breast cancer. Future research should focus on whether ^{18}F-FES PET-CT can be done as an initial standard imaging test or at the same time as other standard tests in the setting of recurrent or metastatic disease. Also, patients with oestrogen receptor-positive breast cancer and ^{18}F-FES PET-CT negative results might represent a distinct subset of oestrogen receptor-positive breast cancer. Larger studies are needed to precisely characterise this patient subset.

primary and metastatic breast cancer lesions.[2] However, collecting a biopsy sample from metastatic tissue is not considered mandatory in daily practice and is sometimes not feasible depending on lesion location and the risk associated with biopsy. In these cases, clinicians keep treating patients according to the oestrogen receptor status of the primary breast cancer.[3] Consequently, suboptimal therapy might be offered to these patients.

Oestrogen receptor status of breast tumours can be assessed by PET with 16α-[^{18}F]fluoro-17β-oestradiol (^{18}F-FES).[4,5] ^{18}F-FES binds to oestrogen receptors in vivo, similar to oestradiol, and enables in-vivo imaging of oestrogen receptor expression at recurrent or metastatic tumour sites in the body, without requiring a biopsy sample.[6–10] Previous studies of ^{18}F-FES were not designed to formally test a specific hypothesis, and the results were exploratory. In only two studies,[6,9] the accuracy of ^{18}F-FES PET was prospectively assessed in a small number of patients with recurrent or metastatic breast cancer with the use of a prespecified threshold of ^{18}F-FES uptake (appendix pp 4–6).

In the current study, our primary objective was to show the agreement between qualitative ^{18}F-FES PET-CT interpretation and immunohistochemical evaluation of oestrogen receptor expression, a non-reference standard test, in patients with recurrent or metastatic breast cancer. Secondary objectives were to assess the association between quantitative ^{18}F-FES uptake within a tumour normalised to the whole-body concentration of the injected ^{18}F-FES activity (standardised uptake value; SUV) and the semiquantitative immunohistochemistry index (Allred score) and to assess the safety of ^{18}F-FES PET-CT. Additionally, we explored the association between SUV of ^{18}F-FES PET-CT and serum oestradiol concentration and the concordance of oestrogen receptor status between primary and recurrent or metastatic cancer lesions.

Methods

Study design and participants

We did a prospective, non-randomised, single-centre cohort study. We identified participants on the basis of presenting symptoms and imaging findings at the outpatient and inpatient clinics of the Departments of Surgery and Oncology at the Asan Medical Center, Seoul, South Korea. Eligible patients had breast cancer, with first recurrence or metastatic disease at presentation, were aged 19 years or older, and had an Eastern Cooperative Oncology Group performance status of 0–2. Patients were scheduled to have core needle biopsy or surgery for histological confirmation and determination of oestrogen receptor status of recurrent or distant metastatic cancer lesions within 15 days after ^{18}F-FES PET, or they had already had core needle biopsy of recurrent or distant metastatic cancer within 30 days and discontinued selective oestrogen receptor modulators or fulvestrant for at least 60 days before ^{18}F-FES PET-CT. Other hormonal treatments, including aromatase inhibitors, were allowed. No laboratory tests were required to assess eligibility. Patients were excluded if the recurrent or metastatic lesion scheduled to have a biopsy sample taken from was located in the breast, liver, ovary, uterus, or bone; if they

See Online for appendix

had been given chemotherapy within the previous 3 weeks before the planned study with ^{18}F-FES administration; if chemotherapy, radiotherapy, or immunological or biological therapy was scheduled before the histological confirmation or ^{18}F-FES PET; or if they had a concurrent severe, uncontrolled, or unstable medical disease other than cancer (eg, congestive heart failure, acute myocardial infarction, pulmonary disease, or chronic renal or hepatic disease). The complete selection criteria are listed in the appendix (p 7).

The institutional review board of the Asan Medical Center and the Ministry of Food and Drug Safety of South Korea approved this study protocol. This trial was done in accordance with the Declaration of Helsinki and institutional guidelines. All patients provided written informed consent before participating in the study. A data monitoring committee reviewed safety data and the progress of the study. A clinical monitoring group reviewed oestrogen receptor status results to assess whether the planned number of oestrogen receptor-positive and oestrogen receptor-negative patient populations were achieved.

Procedures

^{18}F-FES was administered as described previously.[11] Oral hydration with water was encouraged before ^{18}F-FES PET-CT. A radioactive dose of 111–222 MBq of ^{18}F-FES was administered as a slow intravenous bolus injection over 1–2 min. The radioactive dose was chosen to provide a high image quality while keeping within acceptable limits to be delivered to patients.[12] PET-CT imaging was done from the skull base to the upper thigh for 2 min acquisition time per bed for each patient, 80–100 min after intravenous injection of ^{18}F-FES with the use of a PET-CT scanner (Discovery PET/CT 690 or Discovery PET/CT 710, GE Healthcare, Milwaukee, WI, USA). Imaging time was determined from previous ^{18}F-FES biodistribution studies to minimise non-specific blood uptake and to maximise tumour contrast.[6,7,13] For attenuation correction and lesion localisation, a low-dose CT scan (100 KVp, 35 mA) was done. Images were reconstructed with the use of the manufacturer-provided iterative algorithm with four iterations and 18 subsets. Blood samples were taken for serum oestradiol radioimmunoassay (Beckman Coulter, Brea, CA, USA) before ^{18}F-FES injection.

The safety of ^{18}F-FES was monitored via vital signs and a physical examination before and 2 h after ^{18}F-FES injection. Adverse events were continuously recorded, beginning with patient enrolment until the last patient contact between 1 and 10 days after ^{18}F-FES administration. We used the Common Terminology Criteria for Adverse Events (version 4.0).

All archived slides of recent or metastatic breast cancer lesions were immunostained for oestrogen receptors with a 6F11 mouse monoclonal antibody (NCL-ER-6F11, Novocastra Laboratories, Newcastle, UK) as previously described.[14] Semiquantitative oestrogen receptor expression was assessed according to the Allred score by breast pathologists who were masked to the results of ^{18}F-FES PET-CT.[15] Proportion of positive cells (0–5) and staining intensity scores (0–3) were summed for a total score of 0–8. Oestrogen receptor status was considered positive if Allred scores were 3 or higher.

The plan for ^{18}F-FES PET-CT image assessment was specified in the protocol. Case report forms to be used by the readers were prepared before enrolment. Image assessment was performed by readers who had not had contact with patients and who were not affiliated to the study site. Readers were three board-certified, nuclear medicine physicians with more than 5 years of experience. The readers reviewed images of all enrolled patients in a random sequence, regardless of the image quality, outside of the study site, and they decided whether or not the ^{18}F-FES PET-CT image quality was adequate for interpretation. The images were defined as technically inadequate when a misregistration of more than 10 mm, or other sources of errors, was present that could affect the accuracy of the qualitative or quantitative assessment of images. Readers were masked to the clinical data and independently assessed ^{18}F-FES PET-CT images at sites that were not otherwise involved in the conduct of the study, but they knew the eligibility criteria and the location of biopsy or surgery sites for ^{18}F-FES PET-CT image assessment and the ^{18}F-FES injection site. The recurrent or metastatic lesion, from which at least one biopsy sample was taken for pathological diagnosis (decision of the responsible physician), was selected as a reference lesion for the comparative ^{18}F-FES PET-CT analysis. If a biopsy sample was taken from more than one lesion, the largest one was selected by imaging studies as a reference lesion for the precise localisation and accurate quantitation, as prespecified in the protocol. Objective image features assessed by the readers included the location, size, and intensity of the reference lesion. Based on the knowledge of normal ^{18}F-FES biodistribution, intensity was assessed on a three-point scale (0, decreased uptake; 1, equivocal uptake; 2, increased uptake) in relation to the background activity, as in previous studies.[6,16] When a focus of increased ^{18}F-FES uptake was present in the reference lesion, it was interpreted as positive. If the intensity was equivocal or decreased, the lesion was interpreted as negative. The final results were reported based on majority interpretation of the three masked readers.

For quantitative analysis, a volume of interest was drawn on reference lesions (Advantage Workstation 4.6 software, GE Healthcare). Maximal SUV (SUVmax) of the lesion of interest was obtained. All SUVs were normalised to the injected dose and bodyweight and were calculated as SUV=activity (Bq/g)/[injected activity (Bq)/bodyweight (g)].

A literature review of PubMed and Embase was done to identify reports of accuracy of ^{18}F-FES PET and oestrogen

receptor status of primary breast cancer to assess oestrogen receptor status of recurrent or metastatic breast cancer (appendix pp 2–6, 9–13).

Outcomes

The primary outcome measure of this study was lesion-level positive and negative percent agreement between qualitative ^{18}F-FES PET-CT interpretation and the immunohistochemical assay. Secondary outcome measures were association between the SUVmax of ^{18}F-FES PET-CT and semiquantitative immunohistochemical assay index (Allred score) and safety variables, including adverse events, vital signs, and physical examination.

Statistical analysis

The statistical analysis plan was prespecified in the protocol and a summary in English is included in the appendix (p 8). We calculated that a sample size of 85 patients (47 oestrogen receptor-positive patients and 38 oestrogen receptor-negative patients) would be sufficient to achieve a statistical power of 80% in detecting differences between the null hypothesis (a positive percent agreement of 55% and negative percent agreement of 82%) and the alternative hypothesis (positive percent agreement of 75% and negative percent agreement of 97%) using a one-sided exact binomial test at a 2·5% significance level (appendix pp 9–14). The alternative hypothesis was based on a systematic review of the literature published between 1985 and 2012; the results are in the appendix (pp 2–6). Acceptance of the null hypothesis would result in a positive predictive value of 79% by ^{18}F-FES PET assuming 55% prevalence of oestrogen receptor positivity in patients with recurrent or metastatic breast cancer. We planned to enrol 94 patients assuming a 10% dropout. We prespeficied in the protocol that patients who were enrolled after reaching the planned target number of patients (47 in the oesotrogen receptor-positive group; or 38 in the oestrogen receptor-negative group) would not be analysed for efficacy of ^{18}F-FES PET-CT.

We described all the data in this study as median values (IQR) for continuous variables or numbers (%) for categorical variables unless otherwise specified. We considered a two-sided p value of less than 0·05 as significant. We did comparisons of quantitative parameters using unpaired *t* tests. We compared categorical data using χ^2, Fisher's exact, or McNemar tests. We assessed inter-reader variability of ^{18}F-FES PET-CT evaluation using the Fleiss's κ statistic. We assessed the association between SUVmax of ^{18}F-FES PET-CT and the semiquantitative immunohistochemical assay index (Allred score) using the Spearman rank correlation coefficient.

We estimated lesion-level positive and negative percent agreements between the ^{18}F-FES PET-CT interpretation and immunohistochemical assay as (1) positive percent agreement=(the number of ^{18}F-FES-positive patients divided by the number of immunohistochemistry-positive patients)×100 and (2) negative percent agreement=(the number of ^{18}F-FES-negative patients divided by the number of immunohistochemistry-negative patients)×100. We calculated positive and negative predictive values accordingly. We presented estimates with 95% Clopper-Pearson's exact CIs. We assessed the primary and secondary outcomes in all patients with histologically confirmed recurrent or metastatic breast cancer who received any amount of ^{18}F-FES and had PET-CT images available (intention-to-diagnose analysis), and we assessed safety in all patients who received ^{18}F-FES.

All statistical tests were done using IBM SPSS Statistics for Windows, version 21, and SAS, version 9.4. This study is registered with ClinicalTrials.gov, number NCT01986569.

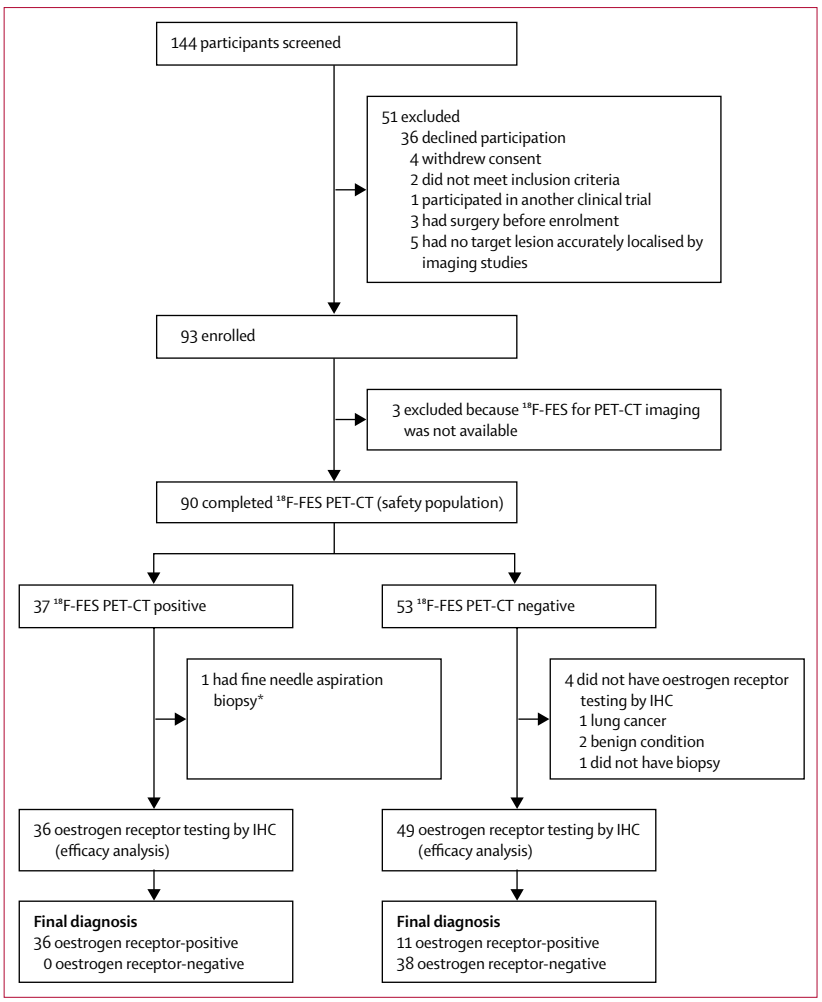

Figure 1: **Study profile**
IHC=immunohistochemical assay. ^{18}F-FES=16α-[^{18}F]fluoro-17β-oestradiol. *Fine needle aspiration specimens do not have enough cells for immuhohistochemical stains; patients who had only core needle biopsy or surgery were included (specified in the eligibility criteria).

	Participants (n=90)
Age, years	55 (46–60)
Menopausal status	
Premenopausal	35 (39%)
Postmenopausal	55 (61%)
Body-mass index, kg/m²	23·0 (21·2–25·5)
ECOG performance status	
0	84 (93%)
1	6 (7%)
Pathology of the primary carcinoma	
Invasive ductal carcinoma	88 (98%)
Invasive lobular carcinoma	2 (2%)
Previous treatment for breast cancer	
Surgery	86 (96%)
Chemotherapy	73 (81%)
Hormonal therapy	50 (56%)
Radiotherapy	47 (52%)
Reason for inclusion in the study	
Locoregional and distant recurrence	86 (96%)
Stage IV metastatic breast cancer	4 (4%)
Time interval from surgery to recurrence, months*	52 (19–108)
Pathological diagnosis of recurrence or metastasis†	
Regional lymph node‡	40 (47%)
Distant lymph node	14 (16%)
Lung	18 (21%)
Chest wall	13 (15%)
Pleura	1 (1%)

Data are median (IQR) or n (%). ECOG=Eastern Cooperative Oncology Group. *Patients with pathologically confirmed recurrence (n=83). †Patients with pathologically confirmed recurrence or metastasis (n=86). ‡Metastases in ipsilateral axillary, internal mammary, supraclavicular, or infraclavicular lymph node(s).

Table 1: **Baseline characteristics**

Role of the funding source

The funders had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data and had the final responsibility for the decision to for publication.

Results

Between Nov 27, 2013, and Nov 10, 2016, 93 (65%) of 144 screened patients were enrolled (figure 1). We consecutively enrolled 47 patients who were oestrogen receptor-positive, 33 patients who were oestrogen receptor-negative (according to immunohistochemical assay results), and eight patients who were finally excluded due to unavailability of oestrogen receptor assay or ^{18}F-FES PET-CT imaging until Aug 23, 2016, when the target number of patients who were oestrogen receptor-positive was reached. For the remaining five additional oestrogen receptor-negative patients needed, we enrolled only patients with oestrogen receptor-negative primary breast cancer after obtaining the approval of the institutional review board. A total of 90 patients were injected with ^{18}F-FES. The median follow-up after ^{18}F-FES injection was 2 days (IQR 1–3). The median specific activity of ^{18}F-FES was 1688 GBq/μmol (IQR 1106–1731). The median administered activity was 204 MBq (IQR 192–211). In total, 85 (94%) of 90 patients (oestrogen receptor-positive, n=47; oestrogen receptor-negative, n=38) who completed ^{18}F-FES PET-CT had PET-CT scans available for efficacy analyses.

The median age of 90 patients injected with ^{18}F-FES was 55 years (IQR 46–60) and all patients were Korean women (table 1). The median time between ^{18}F-FES PET-CT and biopsy or surgery was 5 days (1–12). Readers deemed the ^{18}F-FES PET-CT image quality as adequate for interpretation in all patients. The inter-rater agreement was 0·90 (95% CI 0·78–1·0).

Clinical characteristics of oestrogen receptor-positive and oestrogen receptor-negative patients and corresponding qualitative ^{18}F-FES PET-CT results are listed in table 2. Significant differences were found between the two groups in the time interval from surgery to recurrence, size, progesterone receptor status, HER2 status of reference lesions, and oestrogen receptor status of primary breast cancer (table 2). Of the 47 patients who were oestrogen receptor-positive, ^{18}F-FES PET-CT was positive in 36 and the positive percent agreement of ^{18}F-FES PET-CT for diagnosing oestrogen receptor positivity by immunohistochemistry was 76·6% (95% CI 62·0–87·7; p=0·0018). The positive agreement was not different between patients who received aromatase inhibitors within 30 days of ^{18}F-FES PET-CT and those who did not (5 [56%] of 9 patients *vs* 31 [82%] of 38 patients; p=0·11). Patients who were oestrogen receptor-positive with ^{18}F-FES-positive breast cancer had a significantly higher expression of progesterone receptors than patients who were oestrogen receptor-positive but had a negative ^{18}F-FES PET-CT scan (23 [68%] of 34 patients with progesterone receptor staining available *vs* 0 [0%] of 11 patients p<0·0001; table 2). The time interval from surgery to recurrence was also significantly longer (p=0·0056; table 2). All 38 patients who were oestrogen receptor-negative showed negative ^{18}F-FES PET-CT; the negative percent agreement was 100·0% (95% CI 90·8–100·0; p=0·00053). The positive predictive value was 100% (36 of 36 patients; 95% CI 90·3–100·0) and the negative predictive value was 77·6% (38 of 49 patients; 63·4–88·2).

In the quantitative analysis, the median values of the SUVmax were 4·6 (IQR 2·1–7·9) for oestrogen receptor-positive and 1·2 (1·0–1·5) for oestrogen receptor-negative reference lesions (p<0·0001). When we used a cutoff of 1·5 or higher,[17] the positive percent agreement was 85·1% (40 of 47 oestrogen receptor-positive patients; 95% CI 71·7–93·8) and the negative percent agreements was 78·9% (30 of 38 oestrogen receptor-negative patients; 62·7–90·4). A strong positive association was found between the SUVmax of ^{18}F-FES

	Oestrogen receptor-positive group (n=47)			Oestrogen receptor-negative group (n=38)	p value†
	^{18}F-FES-positive (n=36)	^{18}F-FES-negative (n=11)	p value*		
Age, years	55 (47–59)	60 (51–67)	0·12	54 (42–59)	0·072
Time interval‡, months	..	..	0·0056	..	<0·0001
Median	101 (78–122)	49 (13–81)	..	20 (12–28)	..
Mean (95% CI)	98 (84–111)	55 (23–87)	..	30 (18–42)	..
Biopsy sample site	..	..	0·80	..	0·99
Lymph node	21 (58%)	8 (73%)	..	24 (63%)	..
Lung	8 (22%)	2 (18%)	..	8 (21%)	..
Others	7 (19%)	1 (9%)	..	6 (16%)	..
Tumour size, mm	19 (13–27)	20 (11–32)	0·53	17 (10–22)	0·028
Oestrogen receptor Allred score	..	..	<0·0001	..	<0·0001
0–2	0	0	..	38 (100%)	..
3–6	1 (3%)	10 (91%)	..	0	..
7–8	35 (97%)	1 (9%)	..	0	..
Progesterone receptor status	..	..	<0·0001	..	<0·0001
Positive	23 (64%)	0	..	0	..
Negative	11 (31%)	11 (100%)	..	37 (97%)	..
Not assessed	2 (6%)	0	..	1 (3%)	..
HER2	..	..	0·096	..	0·0029
Positive	2 (6%)	3 (27%)	..	15 (39%)	..
Negative	30 (83%)	8 (73%)	..	22 (58%)	..
Not assessed	4 (11%)	0	..	1 (3%)	..
Maximum SUV	6·5 (4·1–9·6)	1·4 (1·40–1·8)	<0·0001	1·2 (1·0–1·5)	<0·0001
Oestradiol, pg/mL	23·1 (13·3–50·0)	32·9 (10·0–101·0)	0·23	29·3 (18·3–49·2)	0·38

Data are median (IQR) or n (%), unless otherwise stated. All 85 patients in the efficacy analysis group are shown. ^{18}F-FES=16α-[^{18}F]fluoro-17β-oestradiol. SUV=standardised uptake value. *Statistical difference between ^{18}F-FES-positive and negative patients in the oestrogen receptor-positive group. †Statistical difference between oestrogen receptor-positive and oestrogen receptor-negative patients. ‡Interval from surgery to recurrence in 82 patients with recurrence (oestrogen receptor-positive with ^{18}F-FES-positive, n=35; oestrogen receptor-positive with ^{18}F-FES-negative, n=11; and ^{18}F-FES-negative, n=36).

Table 2: **Clinical characteristics by oestrogen receptor status and ^{18}F-FES PET-CT results**

and the oestrogen receptor immunohistochemical assay Allred scores (ρ=0·83; p<0·0001; figure 2). A weak positive association was found between SUVmax of ^{18}F-FES and tumour size (ρ=0·33; p=0·0020). Serum oestradiol concentration was not significantly associated with the SUVmax (ρ=–0·21; p=0·058). The positive percent agreement of patients who were oestrogen receptor-positive with oestradiol concentrations of 30 pg/mL or higher was not different from that of patients with concentrations less than 30 pg/mL (22 [81%] of 27 patients *vs* 14 [70%] of 20 patients; p=0·49). Age, menopausal status, and body-mass index were not associated with the SUVmax of ^{18}F-FES (p>0·05).

The oestrogen receptor status of primary tumours assessed at the Asan Medical Center was available from medical records in 51 patients (36 had oestrogen receptor-negative primary tumours and 15 oestrogen receptor-positive). For patients with oestrogen receptor-positive primary breast cancer, two (13%) of 15 had oestrogen receptor-negative recurrent breast cancer, whereas for those with oestrogen receptor-negative primary breast cancer, two (6%) of 36 had oestrogen receptor-positive

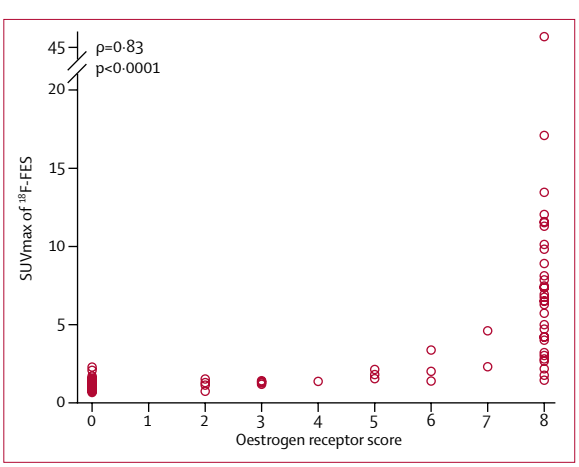

Figure 2: **Association between oestrogen receptor histological Allred score and SUVmax of ^{18}F-FES PET-CT**

SUVmax=maximum standardised uptake value. All patients with ^{18}F-FES PET-CT and immunohistochemical assay available are shown (n=85). ^{18}F-FES=16α-[^{18}F]fluoro-17β-oestradiol.

	Grade 1	Grade 2
Patients with ≥1 treatment-emergent adverse event	10 (11%)	5 (6%)
Nausea	3 (3%)	0
Injection site pain*	1 (1%)	0
Viral upper respiratory tract infection	0	1 (1%)
Procedural pain	5 (6%)	4 (4%)
Dizziness	1 (1%)	0
Headache	0	1 (1%)
Skin irritation	1 (1%)	0

Data are n (%) in the safety population (n=90). Adverse events are listed by preferred terms (Medical Dictionary for Regulatory Activities version 20.0) and graded by the National Cancer Institute Common Terminology Criteria for Adverse Events (version 4.0). All events are presented irrespective of association with study drug. There were no grade 3, 4, or 5 events. *One adverse event was considered to be possibly treatment-related.

Table 3: **Adverse events**

recurrent breast cancer. The positive percent agreement of oestrogen receptor status between primary breast cancer and recurrent or metastatic breast cancer lesions was 86·7% (13 of 15 patients; 95% CI 59·5–98·3) and the negative percent agreement was 94·4% (34 of 36 patients; 81·3–99·3), which were not significantly different to those of ^{18}F-FES PET-CT (p>0·05).

^{18}F-FES administration and PET-CT procedures were well tolerated in all patients. No clinically relevant change in safety parameters was observed. The most common adverse event was procedural pain in nine (10%) of 90 patients injected with ^{18}F-FES (table 3). No adverse events occurred that were related to the study drug except injection site pain in one (1%) patient. No serious adverse events occurred during the study and there were no treatment-related deaths.

The literature review showed that the positive percent agreement of oestrogen receptor status between primary breast cancer and recurrent or metastatic breast cancer lesions was 90% (95% CI 87–93) and the negative percent agreement was 64% (60–68); the results are in the appendix (pp 12–13).

Discussion

In this prospective cohort study, we showed a high negative percent agreement, but a moderate positive percent agreement between ^{18}F-FES PET-CT and oestrogen receptor status assessed histologically. Obtained agreement values of ^{18}F-FES PET-CT were significantly higher than those of the prespecified null hypothesis, which proposed a higher positive predictive value than the oestrogen receptor status of primary breast cancer. ^{18}F-FES PE-CT was well tolerated and no serious adverse events were recorded. To the best of our knowledge, this study is the first registered confirmatory study to assess the diagnostic accuracy of ^{18}F-FES PET-CT against an immunohistochemical assay as the non-reference standard to assess oestrogen receptor status.

The high negative percent agreement of ^{18}F-FES PET-CT in this study indicates that positive ^{18}F-FES uptake represents a reliable, non-invasive alternative in the diagnosis of oestrogen receptor-positive recurrent or metastatic breast cancer to immunohistochemical assessment of oestrogen receptor status. In preclinical studies, it has been previously shown[18–20] that binding of ^{18}F-FES to oestrogen receptor in vivo in mouse models is highly specific. Previous studies in humans[6–10,21] have also shown very high specificity of ^{18}F-FES PET. Only three false positives of ^{18}F-FES uptake have been reported so far.[8,10] One false positive was likely due to a sampling error caused by heterogeneous oestrogen receptor expression.[8,22] The other two false positives were found in a quantitative ^{18}F-FES study[10] in which the quantitative threshold value was chosen post hoc for optimal discrimination between oestrogen receptor-positive and oestrogen receptor-negative lesions. Post-hoc characterisation of ^{18}F-FES uptake based on clinical data can be misleading unless it is prespecified.[23] Of note, no false positives were recorded in studies in which ^{18}F-FES PET was interpreted qualitatively based on knowledge of the normal biodistribution of ^{18}F-FES.[6,7,9] Our results are promising for the diagnosis and staging of patients with recurrent or metastatic breast cancer. The staging assessment of women who present with recurrent or metastatic breast cancer should include ^{18}F-FES PET-CT if retesting hormone receptor status is not feasible. Hormonal therapy can be recommended for patients with positive ^{18}F-FES PET-CT. However, because of the need for retesting of other biomarkers, we might not conclude that positive ^{18}F-FES uptake would eventually replace taking a biopsy sample of recurrent or metastatic lesions. Additional studies are needed to assess whether ^{18}F-FES PET-CT can be performed as an initial standard imaging test or at the same time as other standard tests in the setting of recurrent or metastatic disease.

This study further validates the previously observed correlation between quantitative ^{18}F-FES uptake and oestrogen receptor expression by immunohistochemistry.[8–10,21] The association was not perfect. Positive percent agreement of qualitative interpretation was not high, with 11 patients displaying oestrogen receptor-positive with ^{18}F-FES-negative. We did the oestrogen receptor assessment using standardised techniques and interpretation methods.[15] The discordant results cannot be explained by inaccuracy of the immunohistochemical assay alone.[4,5] Several explanations might account for this discrepancy. First, PET imaging has a limited ability to detect small-sized lesions due to the fundamental limits of PET instrumentation. However, no difference in the size of the recurrent or metastatic lesion was observed between patients with true-positive and false-negative ^{18}F-FES PET-CT (table 2). Second, a tissue sample obtained for immunohistochemical testing might not be representative of the whole tumour lesion. The

association between quantitative ^{18}F-FES uptake and oestrogen receptor expression can be affected by heterogeneous oestrogen receptor expression. Finally, discordant results might simply reflect inherent differences between the immunohistochemical assay and ^{18}F-FES PET-CT as shown from studies comparing in-vitro radioligand binding and immunohistochemical assays.[24] Importantly, patients who were oestrogen receptor-positive with ^{18}F-FES-positive breast cancer had a significantly higher expression of progesterone receptor than those who were oestrogen receptor-positive with ^{18}F-FES-negative breast cancer. No patients with false-negative ^{18}F-FES uptake showed positive progesterone receptor expression. The time interval from surgery to recurrence was also significantly different between the two groups. Evidence suggests that substantial crosstalk occurs between the oestrogen receptor and progesterone receptor pathways.[25,26] Progesterone receptors play a part in modulating oestrogen receptor action. Although the value of progesterone receptor expression in the metastatic setting remains controversial, oestrogen receptor-positive with progesterone receptor-negative breast cancer is considered a distinct subtype of breast cancer characterised by aggressive behaviour and a poorer response to endocrine therapy than tumours that are oestrogen receptor-positive and progesterone receptor-positive.[27] Previous studies[7,9,16] looking at the association between ^{18}F-FES uptake and response to endocrine therapy have also shown that patients who were progesterone receptor-positive but ^{18}F-FES-negative tended to have a decreased response to endocrine therapy. Taken together, an oestrogen receptor-positive but ^{18}F-FES-negative status might not necessarily be considered a false negative. Rather, it might represent a distinct subset of oestrogen receptor-positive but functionally endocrine therapy-resistant breast cancer, such as cancers characterised by oestrogen receptor mutations[28] and splice variants.[29] Larger studies are needed to precisely characterise this subset. One major advantage of ^{18}F-FES PET-CT is that it can be used to non-invasively assess the oestrogen receptor status of several tumour lesions across the whole body.[5] Site-to-site variability in ^{18}F-FES uptake and oestrogen receptor expression in relation to clinical benefit with endocrine therapy should also be assessed further.[4,9]

Factors that are not related to tumour-specific oestrogen receptor status might influence ^{18}F-FES uptake, and thereby introduce bias in accuracy estimates of ^{18}F-FES PET-CT. Competition with circulating oestrogens should theoretically lead to decreased ^{18}F-FES uptake, especially in premenopausal patients.[4] However, we found no significant correlation between serum oestradiol concentrations and ^{18}F-FES uptake. When we stratified the oestrogen receptor-positive patients using the oestradiol concentration threshold of 30 pg/mL, we found no significant difference in positive percent agreement between groups. Contrary to theoretical expectation, serum oestradiol concentration did not significantly affect ^{18}F-FES uptake in previous studies.[9,30,31] After adjusting for the effect of tumour oestrogen receptor expression levels on ^{18}F-FES uptake, oestrogen concentration was not a significant factor influencing ^{18}F-FES uptake.[31] Although whether or not oestrogen concentrations are sufficiently high to have a notable effect on tumour ^{18}F-FES uptake still remains unclear,[32] it does not appear to be a major determinant of the accuracy of ^{18}F-FES PET-CT. Another non-tumour specific factor that influenced the SUVmax of ^{18}F-FES in our patients was tumour size, as previously reported.[31] Partial volume correction can account for the loss of activity attributable to finite resolution and might be needed for improved quantitation of oestrogen receptor status by ^{18}F-FES PET-CT.[8] Nevertheless, quantitative assessment of ^{18}F-FES uptake as a diagnostic method raises concerns about limited applicability due to variances in technical factors affecting the quantitative measurement of SUV.[33] Additionally, the cutoff value for SUV was not determined in previous studies, and was not preplanned in this assessment. In this study, qualitative ^{18}F-FES PET-CT interpretation was robust, with high interobserver concordance, similar to previous results,[9] and it was not affected by tumour size. Qualitative ^{18}F-FES PET-CT interpretation does not seem to raise concerns of bias and applicability in assessing oestrogen receptor status. Quantitative measurement of ^{18}F-FES uptake might be done to improve prediction of endocrine treatment response.

This study has some limitations. First, after reaching the target number of oestrogen receptor-positive patients in August, 2016, we enrolled only patients with oestrogen receptor-negative primary breast cancer. The five patients subsequently enrolled were confirmed to have oestrogen receptor-negative recurrent or metastatic breast cancer. Our study population might therefore not represent a consecutive sample of eligible patients. However, we did not make any inappropriate exclusions that might result in overestimation of negative percent agreement. Second, we did not include patients when the recurrent or metastatic lesion scheduled for biopsy was located in the breast, liver, or bone. More patients had locoregional recurrence than distant metastatic disease. Patients with recurrent or a metastatic lesion located in the breast, liver, ovary, and uterus were excluded because of organ-specific uptake of ^{18}F-FES and its metabolites. Biopsy samples of bone lesions can be associated with substantial morbidity and sampling error. Decalcification might result in loss of oestrogen receptor epitopes and give rise to false-negative immunohistochemistry results.[34] In previous studies[6,9,21] including metastatic bone lesions, oestrogen receptor characteristics in relation to ^{18}F-FES did not differ among recurrent and metastatic sites. In one paper,[35] the ^{18}F-FES uptake in bone metastasis was higher than that of other organs. Thus, we believe that our results might be applicable to metastatic bone lesions. Third, we did not measure the

concentration of sex hormone-binding globulin to examine the association with ^{18}F-FES uptake. A previous study[30] found an inverse association between SUV of ^{18}F-FES and sex hormone-binding globulin; however, it is not certain whether correction for sex hormone-binding globulin would improve quantitation of ^{18}F-FES uptake.[32,36] Fourth, we did not show a higher agreement of ^{18}F-FES PET-CT than indicated by oestrogen receptor status of primary breast cancer obtained from medical records. However, our data showed a higher negative agreement of ^{18}F-FES PET-CT than the previously reported results of oestrogen receptor status of primary breast cancer. Finally, this study is single-centred, which limits the generalisability of the results, and the absence of central review is also a limitation. However, the primary outcome measure of this study is unlikely to be influenced by factors that might limit external validity, and to minimise potential bias ^{18}F-FES PET-CT images were interpreted by masked readers at sites that had not otherwise been involved in the conduct of the study.

In conclusion, ^{18}F-FES PET-CT can non-invasively assess oestrogen receptor status in patients with recurrent or metastatic breast cancer. With high negative percent agreement between ^{18}F-FES PET-CT and oestrogen receptor status, positive ^{18}F-FES uptake can adequately diagnose recurrent or metastatic oestrogen receptor-positive breast cancer and represents an alternative to histological oestrogen receptor assays. Staging assessment should include ^{18}F-FES PET-CT if retesting of oestrogen receptor status is not feasible.

Contributors

SYC, SHA, S-BK, JBL, and DHM conceived and designed the study. DHM supervised the study. SYC, SHA, S-BK, SH, SHL, HJK, BSK, JWL, BHS, JK, J-HA, KHJ, JEK, and HSL contributed to patient recruitment. SYC, SH, SHL, SJO, SJL, S-YK, WJC, HJS, GG, and HSL were involved in data acquisition. SYC and SJL collected the data. SYC, SH, JBL, and DHM were involved in data analysis and interpretation. SYC and DHM wrote the manuscript. All authors contributed to the review of the manuscript.

Declaration of interests

We declare no competing interests.

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References

1 DeSantis CE, Ma J, Goding Sauer A, Newman LA, Jemal A. Breast cancer statistics, 2017, racial disparity in mortality by state. *CA Cancer J Clin* 2017; **67:** 439–48.

2 Aurilio G, Disalvatore D, Pruneri G, et al. A meta-analysis of oestrogen receptor, progesterone receptor and human epidermal growth factor receptor 2 discordance between primary breast cancer and metastases. *Eur J Cancer* 2014; **50:** 277–89.

3 Arslan C, Sari E, Aksoy S, Altundag K. Variation in hormone receptor and HER-2 status between primary and metastatic breast cancer: review of the literature. *Expert Opin Ther Targets* 2011; **15:** 21–30.

4 van Kruchten M, de Vries EG, Brown M, et al. PET imaging of oestrogen receptors in patients with breast cancer. *Lancet Oncol* 2013; **14:** e465–75.

5 Liao GJ, Clark AS, Schubert EK, Mankoff DA. ^{18}F-Fluoroestradiol PET: current status and potential future clinical applications. *J Nucl Med* 2016; **57:** 1269–75.

6 Dehdashti F, Mortimer JE, Siegel BA, et al. Positron tomographic assessment of estrogen receptors in breast cancer: comparison with FDG-PET and in vitro receptor assays. *J Nucl Med* 1995; **36:** 1766–74.

7 Mortimer JE, Dehdashti F, Siegel BA, Katzenellenbogen JA, Fracasso P, Welch MJ. Positron emission tomography with 2-[^{18}F] fluoro-2-deoxy-D-glucose and 16^{alpha}-[^{18}F]fluoro-17^{beta}-estradiol in breast cancer: correlation with estrogen receptor status and response to systemic therapy. *Clin Cancer Res* 1996; **2:** 933–39.

8 Peterson LM, Mankoff DA, Lawton T, et al. Quantitative imaging of estrogen receptor expression in breast cancer with PET and ^{18}F-fluoroestradiol. *J Nucl Med* 2008; **49:** 367–74.

9 Peterson LM, Kurland BF, Schubert EK, et al. A phase 2 study of 16^{alpha}-[^{18}F]-fluoro-17^{beta}-estradiol positron emission tomography (FES-PET) as a marker of hormone sensitivity in metastatic breast cancer (MBC). *Mol Imaging Biol* 2014; **16:** 431–40.

10 Yang Z, Sun Y, Xu X, et al. The assessment of estrogen receptor status and its intratumoral heterogeneity in patients with breast cancer by using ^{18}F-fluoroestradiol PET/CT. *Clin Nucl Med* 2017; **42:** 421–27.

11 Oh SJ, Chi DY, Mosdzianowski C, Kil HS, Ryu JS, Moon DH. The automatic production of 16^{alpha}-[^{18}F]fluoroestradiol using a conventional [^{18}F]FDG module with a disposable cassette system. *Appl Radiat Isot* 2007; **65:** 676–81.

12 Mankoff DA, Peterson LM, Tewson TJ, et al. [^{18}F]fluoroestradiol radiation dosimetry in human PET studies. *J Nucl Med* 2001; **42:** 679–84.

13 Mankoff DA, Tewson TJ, Eary JF. Analysis of blood clearance and labeled metabolites for the estrogen receptor tracer [F-^{18}]-16^{alpha}-fluoroestradiol (FES). *Nucl Med Biol* 1997; **24:** 341–48.

14 Bae YK, Gong G, Kang J, et al. Hormone receptor expression in invasive breast cancer among Korean women and comparison of 3 antiestrogen receptor antibodies: a multi-institutional retrospective study using tissue microarrays. *Am J Surg Pathol* 2012; **36:** 1817–25.

15 Hammond ME, Hayes DF, Dowsett M, et al. American Society of Clinical Oncology/College of American Pathologists guideline recommendations for immunohistochemical testing of estrogen and progesterone receptors in breast cancer. *Arch Pathol Lab Med* 2010; **134:** 907–22.

16 Chae SY, Kim SB, Ahn SH, et al. A randomized feasibility study of ^{18}F-fluoroestradiol PET to predict pathologic response to neoadjuvant therapy in estrogen receptor-rich postmenopausal breast cancer. *J Nucl Med* 2017; **58:** 563–68.

17 van Kruchten M, Glaudemans AW, de Vries EF, Schroder CP, de Vries EG, Hospers GA. Positron emission tomography of tumour [(^{18})F]fluoroestradiol uptake in patients with acquired hormone-resistant metastatic breast cancer prior to oestradiol therapy. *Eur J Nucl Med Mol Imaging* 2015; **42:** 1674–81.

18 Kiesewetter DO, Kilbourn MR, Landvatter SW, Heiman DF, Katzenellenbogen JA, Welch MJ. Preparation of four fluorine-18-labeled estrogens and their selective uptakes in target tissues of immature rats. *J Nucl Med* 1984; **25:** 1212–21.

19 Yoo J, Dence CS, Sharp TL, Katzenellenbogen JA, Welch MJ. Synthesis of an estrogen receptor ^{beta}-selective radioligand: 5-[^{18}F]fluoro-(2R,3S)-2,3-bis(4-hydroxyphenyl)pentanenitrile and comparison of in vivo distribution with 16^{alpha}-[18F]fluoro-17^{beta}-estradiol. *J Med Chem* 2005; **48:** 6366–78.

20 Paquette M, Ouellet R, Archambault M, Croteau E, Lecomte R, Benard F. [^{18}F]-fluoroestradiol quantitative PET imaging to differentiate ER+ and ER^{alpha}-knockdown breast tumors in mice. *Nucl Med Biol* 2012; **39:** 57–64.

21 Venema CM, Mammatas LH, Schroder CP, et al. Androgen and estrogen receptor imaging in metastatic breast cancer patients as a surrogate for tissue biopsies. *J Nucl Med* 2017; **58:** 1906–12.

22 Chung GG, Zerkowski MP, Ghosh S, Camp RL, Rimm DL. Quantitative analysis of estrogen receptor heterogeneity in breast cancer. *Lab Invest* 2007; **87:** 662–69.

23 Whiting PF, Rutjes AW, Westwood ME, et al. QUADAS-2: a revised tool for the quality assessment of diagnostic accuracy studies. *Ann Intern Med* 2011; **155:** 529–36.

24 Pasic R, Djulbegovic B, Wittliff JL. Comparison of sex steroid receptor determinations in human breast cancer by enzyme immunoassay and radioligand binding. *J Clin Lab Anal* 1990; **4:** 430–36.

25 Mohammed H, Russell IA, Stark R, et al. Progesterone receptor modulates ER^{alpha} action in breast cancer. *Nature* 2015; **523:** 313–17.

26 Lim E, Palmieri C, Tilley WD. Renewed interest in the progesterone receptor in breast cancer. *Br J Cancer* 2016; **115:** 909–11.

27 Arpino G, Weiss H, Lee AV, et al. Estrogen receptor-positive, progesterone receptor-negative breast cancer: association with growth factor receptor expression and tamoxifen resistance. *J Natl Cancer Inst* 2005; **97:** 1254–61.

28 Jeselsohn R, Buchwalter G, De Angelis C, Brown M, Schiff R. ESR1 mutations—a mechanism for acquired endocrine resistance in breast cancer. *Nat Rev Clin Oncol* 2015; **12:** 573–83.

29 Groenendijk FH, Zwart W, Floore A, Akbari S, Bernards R. Estrogen receptor splice variants as a potential source of false-positive estrogen receptor status in breast cancer diagnostics. *Breast Cancer Res Treat* 2013; **140:** 475–84.

30 Peterson LM, Kurland BF, Link JM, et al. Factors influencing the uptake of ^{18}F-fluoroestradiol in patients with estrogen receptor positive breast cancer. *Nucl Med Biol* 2011; **38:** 969–78.

31 Gemignani ML, Patil S, Seshan VE, et al. Feasibility and predictability of perioperative PET and estrogen receptor ligand in patients with invasive breast cancer. *J Nucl Med* 2013; **54:** 1697–702.

32 van Kruchten M, Glaudemans AW, de Vries EF, et al. PET imaging of estrogen receptors as a diagnostic tool for breast cancer patients presenting with a clinical dilemma. *J Nucl Med* 2012; **53:** 182–90.

33 Boellaard R. Standards for PET image acquisition and quantitative data analysis. *J Nucl Med* 2009; **50** (suppl 1)**:** 11–20S.

34 Hoefnagel LD, van de Vijver MJ, van Slooten HJ, et al. Receptor conversion in distant breast cancer metastases. *Breast Cancer Res* 2010; **12:** R75.

35 Nienhuis HH, van Kruchten M, Elias SG, et al. (^{18})F-fluoroestradiol tumor uptake is heterogeneous and influenced by site of metastasis in breast cancer patients. *J Nucl Med* 2018; **59:** 1212–18.

36 Wang Y, Ayres KL, Goldman DA, et al. (^{18})F-fluoroestradiol PET/CT measurement of estrogen receptor suppression during a phase I trial of the novel estrogen receptor-targeted therapeutic GDC-0810: using an imaging biomarker to guide drug dosage in subsequent trials. *Clin Cancer Res* 2017; **23:** 3053–60.