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ORIGINAL ARTICLE

^{18}F-FES and ^{18}F-FDG PET/CT imaging as a predictive biomarkers for metastatic breast cancer patients undergoing cyclin-dependent 4/6 kinase inhibitors with endocrine treatment

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Abstract

Objective The aim of this study was to investigate the potential value of dual tracers ^{18}F-FDG and ^{18}F-FES PET/CT in predicting response to Cyclin-Dependent 4/6 Kinase (CDK4/6) inhibitors combined with endocrine therapy for metastatic estrogen receptor (ER)-positive breast cancer patients.

Methods This retrospective study enrolled 38 ER-positive metastatic breast cancer patients from our center who underwent both ^{18}F-FDG and ^{18}F-FES PET/CT scans within 1 month before CDK4/6 inhibitors combined with endocrine therapy. The extracted parameters comprised the maximum standardized uptake value (SUVmax) for both FDG and FES PET, as well as the ratio between FES and FDG SUVmax. Each parameter was dichotomized based on its median threshold. The primary endpoint was progression-free survival (PFS), which was estimated using the Kaplan–Meier method and compared by the log-rank test.

Results After a median follow-up of 15.6 months, progressive disease was observed in 23 out of 38 patients, and the median PFS for the whole cohort was 21.0 months [95% confidence interval (CI) 12.7–29.3]. FES and FDG PET identified 6 patients (15.8%) with FES-negative lesions, suggesting ER heterogeneity in metastatic lesions. The median PFS of these patients was only 5.3 months (95% CI 1.7–8.9), which was substantially shorter than that of patients with 100% FES-positive lesions (median PFS 22.9 months, 95% CI 17.1–28.7, $P < 0.001$). Patients with 100% FES-positive lesions who had high FES/FDG showed significantly shorter PFS compared to those with low FES/FDG (14.9 vs. 30.5 months, $P = 0.003$).

Conclusions This study shows that FDG and FES PET imaging may serve as valuable tools for patient selection in the context of CDK4/6 inhibitor therapy combined with endocrine treatment, and have the potential to function as prognostic biomarkers.

Keywords ^{18}F-FDG · ^{18}F-FES · CDK4/6 inhibitor · Metastatic breast cancer · PFS

Introduction

Breast cancer is the most prevalent malignancy among women, with the highest fatality rate of all female malignant tumors [1]. Hormone receptor positive (HR+) breast

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cancer is the most common subtype, and endocrine therapy is the mainstay of treatment modality [2]. Cyclin-dependent 4/6 kinase (CDK4/6) inhibitors impede the G_1 to S phase transition of the cell cycle and exhibit potential in overcoming endocrine resistance [3, 4]. The combination of CDK4/6 inhibitor and endocrine therapy has become a standard of care for patients with metastatic breast cancer (MBC) who are estrogen receptor (ER)-positive/human epidermal growth factor receptor 2 (HER2)-negative (ER+/HER2−) [5–7]. At present, the combination of CDK4/6 inhibitors palbociclib or abemaciclib with endocrine therapy for patients with ER+/HER2− advanced breast cancer has been approved in China [8, 9]. While the combination of CDK4/6 inhibitors and endocrine therapy has improved patient survival, some patients who do not respond to this, or drug resistance inevitably develops over time [9–11]. Due to the high incidence of adverse effects, exorbitant cost of this combination therapy, and the urgency to initiate alternative treatment promptly, early prediction of response to CDK4/6 inhibitor combined with endocrine therapy is imperative.

The primary biomarker for selecting patients that are candidates for CDK4/6 inhibitors combined with endocrine therapy is the ER status, as determined by the primary lesion is ER-positive or conducting an IHC biopsy of the lesion [12, 13]. Nearly 40% of ER-positive primary breast cancer patients may develop ER-negative metastatic lesions during the course of their disease treatment, and these patients are unlikely to benefit from ER-oriented treatment [14–16]. This highlights the importance of conducting repeated investigations into the ER status of the disease. However, performing representative or repetitive biopsy may not always be feasible due to the location of metastatic lesions and associated risks [17]. In addition, a single biopsy cannot represent multiple lesions throughout the whole body, nor does it provide information on the heterogeneity of ER expression in different lesions, which may have implications for treatment decisions and evaluation of treatment responses [18, 19].

In vivo PET imaging utilizing 16a-[18F]-fluoro-17b-estradiol (^{18}F-FES) is a non-invasive technique that has been developed for visualizing and quantifying the ER status of tumor lesions throughout the patient's body [20–22]. [18F]-Fluorodeoxyglucose (^{18}F-FDG) imaging is commonly performed in conjunction with ^{18}F-FES imaging to assist tumor localization and indicate tumor aggressiveness [23–26]. Other scholars and our own research have investigated the predictive value of FDG or FES PET/CT as a singular imaging biomarker in the combination of CDK4/6 inhibitors and endocrine therapy for breast cancer [27–31]. However, these studies had many limitations, such as the small sample size of FDG PET data, the potential underestimation of metastasis diagnosis by FES PET alone, and the inability to integrate information on tumor ER status and metabolic activity. Therefore, the purpose of the present study was to investigate whether FDG and FES PET/CT imaging can be used for response prediction in patients with ER+/HER2− metastatic breast cancer undergoing treatment with CDK4/6 inhibitors combined with endocrine therapy.

Methods

Patients

We conducted a retrospective study of patients with metastatic breast cancer who underwent an imaging protocol consisting of ^{18}F-FDG and ^{18}F-FES PET/CT prior to initiation of CDK4/6 inhibitor therapy. The image data was collected from December 2016 to August 2022, with a temporal offset of less than 1 month between the two scans. All data were retrospectively collected from the medical records of the Fudan University Shanghai Cancer Center. This clinical research was granted approval by the Ethics Committee and Institutional Review Board of Shanghai Cancer Center at Fudan University, and was in accordance with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. As a retrospective study, informed consent was waived.

Each patient underwent ^{18}F-FES scanning for the following purposes: (1) to assess ER status of metastatic lesions in patients scheduled for endocrine therapy, (2) to evaluate heterogeneity of FES uptake in advanced breast cancer patients, and (3) to determine ER status of metastatic lesions post-endocrine therapy. Enrolled patients received one of the following standard-of-care treatments: (1) palbociclib/abemcaciclib + AI, (2) palbociclib/abemcaciclib + fulvestrant. Premenopausal women with combined ovarian suppression.

PET/CT imaging

FDG-PET: Patients fasted for at least 6 h and had serum glucose levels less than 10 mmol/L before the intravenous injection of FDG at a dose of 3.7 MBq/kg. PET/CT scanning and image reconstruction according to the guidelines of the European Association of Nuclear Medicine (EANM) [32]. Whole-body (head to mid-thigh) PET/CT was performed 60 min after tracer injection using a Siemens Biograph 16 HR PET/CT scanner or Biograph 64 mCT Flow PET/CT scanner (Knoxville, Tennessee, USA). A CT scan (120 kVp, 0.33 s per rotation, with CareDOSE 4D) was performed approximately 60 min after injection of the tracer. The scan used a slice thickness of 3.0 mm and was reconstructed to a 512×512 matrix (voxel size: $0.98 \times 0.98 \times 3.0$ mm^3). Then, PET scans were conducted with a bed speed of 2 mm/s, and PET images were reconstructed using a TrueX + TOF algorithm (2 iterations, 21 subsets, and 5 mm full width at half-maximum) with Gaussian filtering at 5 mm full

width at half-maximum. For all PET reconstructions, the matrix size was 200 × 200, resulting in anisotropic voxels of 4.07 × 4.07 × 3.0 mm^3. The PET images were converted into standardized uptake value (SUV) units by normalizing the activity concentration to the administered dosage of tracers and the patient's body weight.

FES-PET: Patients who received ER antagonists require a 6–8 weeks washout period before FES PET imaging, as these drugs prevent the tracer from binding to the ER and result in false negative ^{18}F-FES. No washout period was required for Aromatase inhibitor (AI). Patients received approximately 222 MBq of ^{18}F-FES intravenous injection within 1–2 min. The PET/CT scan was performed approximately 60 min post-injection of the tracer, with acquisition parameters and image reconstruction methodology consistent with that of ^{18}F-FDG PET/CT.

Image interpretation

The multimodality computer platform (Syngo, Siemens, Knoxville, TN, USA) was used to analyze the FDG and FES PET/CT imaging. The PET images were converted into standardized uptake value (SUV) units by normalizing the activity concentration to the injected dose (ID) of tracers and the patient's body weight (BW). SUV was calculated as (A)/(ID/BW), where A is the tissue tracer uptake in microcuries per gram for the hottest pixel, ID is the injected dose in millicuries, and BW is the body weight in kilograms. A lesion that exhibits obvious tracer accumulation outside the normal distribution or higher than the surrounding physiological uptake during visual analysis is considered positive. Volumes of interest (VOIs) were drawn around the area of positive lesion visible on PET for semi-quantitative analysis to calculate SUVmax. PET/CT images were analyzed qualitatively and semi-quantitatively by two board-certified nuclear medicine doctors (with more than 5 years of work experience) independently, and blinded to the standard evaluation and follow-up data. In the event of a discrepancy between the two physicians, a consensus was established to determine the final reading for statistical analysis. To quantitatively assess the uptake of FES, FDG scans were utilized in conjunction with FES scans to precisely identify and locate lesions. In line with our previous studies, a critical threshold of SUVmax ≥ 1.8 was utilized to define positivity for FES and quantify the expression of ER [22]. FDG PET positive lesions were defined as focal uptake levels exceeding the corresponding background activity, rather than being attributed to physiological or inflammatory processes. In patients with extensive metastatic lesions, following the guidelines of the European Association of Nuclear Medicine (EANM), 20 lesions exhibiting the highest uptake on FDG PET were meticulously selected and identified as FES lesions [32]. The FES/FDG ratio for each lesion was also calculated. Liver metastasis was excluded from the analysis of FES due to physiological uptake in hepatic tissue [33]. Another optimized PET parameter is the FES to FDG ratio, which can serve as a differentiation index and reflect both tumor ER expression and invasiveness [23, 34]. Moreover, this ratio provides a practical first-order compensation for the partial volume effect, making it particularly suitable for breast cancer with high incidence of bone metastasis. A patient's SUVmax or FES/FDG ratio was defined as the median SUVmax or FES/FDG ratio among all lesions detected in that patient.

Assessment of treatment response

Treatment outcome was assessed as progression-free survival (PFS), which was measured from the date of CDK4/6 inhibitor therapy initiation until the first documented disease progression or death. Clinical follow-up and response assessment was according to Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1. Clinical benefit rate (CBR) was defined as the percentage sum of complete responses (CR), partial responses (PR), and stable disease (SD) for at least 24 weeks. Patients with only unmeasurable lesions will be considered to have disease progression if there is clear progression of existing lesions or discovery of new lesions during follow-up.

Statistical analyses

Data were presented as medians (ranges) or patient counts (percentages). To assess the predictive value of PET biomarkers for treatment response at an individual patient level, the median of quantified PET parameters was utilized as a classification threshold.

Survival rates were estimated using the Kaplan–Meier method and compared utilizing the log-rank test. Prognostic factors were investigated through a Cox regression model with a 95% confidence interval in both univariate and multivariate models. A P value less than 0.05 was considered statistically significant. Statistical analyses were conducted using IBM SPSS version 23.0.

Results

Patient characteristics

A total of 50 consecutive eligible patients with HR+/HER2− metastatic breast cancer underwent FDG and FES PET/CT scans prior to initiation of CDK4/6 inhibitor therapy. Twelve patients were excluded from the study: five were lost to follow-up, two discontinued CDK4/6 inhibitor due to side effects, one was synergized with the PIK3CA inhibitor (Alpelisib) as part of clinical trials, and four had undergone

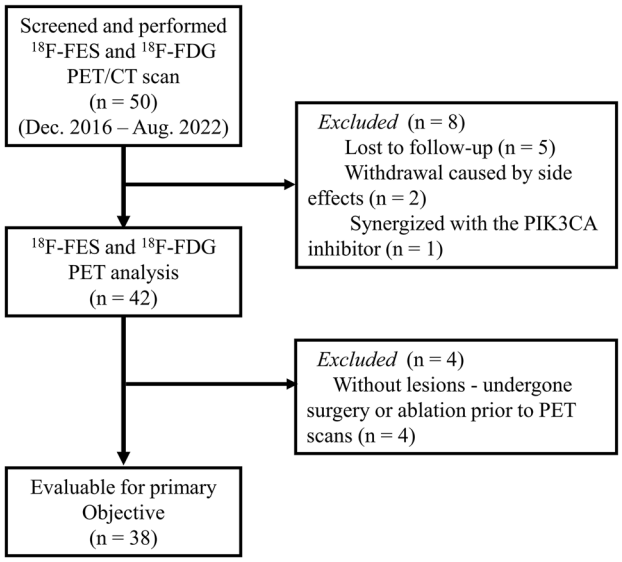

Fig. 1 Patient flowchart for inclusion and exclusion

surgery or ablation prior to PET scans without definitive target lesions (Fig. 1). Therefore, 38 female patients (median age = 56 years, range 29–71 years) were enrolled in our study. Time from breast cancer diagnosis to FES/FDG PET scans ranged from 0 months to more than 20 years, with a median of 6.5 years. Thirty-six patients underwent surgery and had metastatic disease, while 2 patients were diagnosed with de novo stage IV disease. Nearly two-thirds of the patients (65.8%) received a combination of palbociclib and fulvestrant or AI, 13 patients received abemaciclib in combination of fulvestrant or AI. Seven patients underwent premenopausal to postmenopausal transition through the administration of luteinizing hormone releasing hormone (LHRH) agonists. The majority of patients (86.8%) were administered CDK4/6 inhibitors as their first-line therapy, 5 individuals received it as a second-line treatment. Patient and main disease characteristics are summarized in Table 1.

PET/CT analysis

A total of 384 lesions were available for FDG and FES-PET evaluation and analysis in a cohort of 38 patients, 379 lesions showed FDG uptake and 361 lesions were avid in FES PET. The number of lesions analyzed per patient ranges from 1 to 20, with a median of 7 lesions. Lesions were detected in bones ($n = 240$; 62.5%), lymph nodes ($n = 95$; 24.8%), liver ($n = 7$; 1.8%), lung ($n = 23$; 6.0%), pleura ($n = 7$; 1.8%), ovary ($n = 2$; 0.5%), breast ($n = 2$; 0.5%), and soft tissue ($n = 8$; 2.1%). Based on visual analysis, FDG uptake was negative in only five lesions observed in two patients, but FES uptake was positive and located in the lung ($n = 1$) and bones ($n = 4$), respectively. According to qualitative (visual) and

Table 1 Patient demographics and disease characteristics at time of PET scan

Characteristics	$n = 38$	%
Age, years		
Median	56	
Range	29–71	
< 55 years	15	39.5
≥ 55 years	23	60.5
Menopausal status		
Premenopausal[a]	7	18.4
Postmenopausal	31	81.6
Disease-free interval[b]		
> 5 y	21	55.3
≤ 5 y	15	39.5
Histology of primary breast cancer		
IDC	30	78.9
ILC	5	13.2
Unknown	3	7.9
Hormone receptor status		
ER-positive and PR-positive	29	76.3
ER-positive and PR-negative	9	23.7
Metastatic sites		
Nonvisceral	24	63.2
Bone	30	78.9
Bone-only	8	21.1
Visceral disease	14	36.8
Lung	7	18.4
Pleural	4	10.5
Ovarian	2	5.3
Liver	5	13.2
No. of disease sites		
1	14	36.8
2	8	21.1
≥ 3	16	42.1
De novo metastatic disease	2	5.3
Lines of therapy prior to CDK4/6i		
0	33	86.8
1	5	13.2
Prior ET for metastatic disease		
None	38	100
Yes	0	0.0
Prior chemotherapy for metastatic disease		
None	33	86.8
Yes	5	13.2
Endocrine therapy following FES PET		
Palbociclib + AI	10	26.3
Palbociclib + Ful	15	39.5
Abemaciclib + AI	3	7.9
Abemaciclib + Ful	10	26.3
Outcome		
CR	0	0.0
PR	6	15.8

Table 1 (continued)

Characteristics	$n=38$	%
SD	28	73.7
PD	4	10.5
Clinical benefit		
None	4	10.5
Yes	34	89.5
PFS		
Events	23	60.5
Censored	15	39.5
With negative ^{18}F-FES lesions		
None	32	84.2
Yes	6	15.8

IDC, invasive ductal carcinoma; ILC, invasive lobular carcinoma; ER, estrogen receptor; PR, progesterone receptor; ET, endocrine therapy; CR, complete responses; PR, partial responses; SD, stable disease; PD, progressive disease; PFS, progression-free survival; AI, Aromatase inhibitor; Ful, fulvestrant

[a]For premenopausal women, palbociclib combination with endocrine therapy was given upon the administration of gonadotropin-releasing hormone agonist

[b]Patients with stage IV breast cancer at initial diagnosis were excluded ($n=2$)

quantitative analyses (SUVmax < 1.8), it was determined that 16 lesions (8 bones, 6 lymph nodes, and 2 soft tissue) in 6 patients were FES-negative but FDG-positive. Additionally, high FDG PET uptake was observed in 7 liver metastases from 5 patients. However, qualitative and quantitative analyses of FES PET were unattainable due to the liver's substantial physiological FES uptake. The median SUVmax for FDG-positive lesions (excluding 5 FDG-negative lesions, including 1 lung and 4 bones) was determined to be 6.6 (range 2.0–31.9), Similarly, the median SUVmax for FES-positive lesions (excluding 7 liver metastases and 16 FES-negative lesions, including 8 bones, 6 lymph nodes, and 2 soft tissues) was found to be 6.9 (range: 1.8–38.9). At the patient level, the median SUVmax of FDG and FES were 6.3 (range 3.4–12.5) and 7.4 (range 2.9–35.5), respectively. The median FES/FDG ratio was 1.05 (range 0.05–7.20) in both positive lesions and 1.08 (range 0.33–5.95) in patients with 100% FES-positive lesions.

Tumor response

Sixteen patients had measurable lesions at baseline according to RECIST 1.1, 22 patients had unmeasurable lesions, and 8 of them had only bone metastases. Thirty-four of 38 (89.5%) patients derived clinical benefit from CDK4/6 inhibitor combined with endocrine therapy, with 6 PR (15.8%) and 28 SD (73.7%), and only 4 patients experienced PD within 24 weeks. Interestingly, 4/6 patients with FES-negative lesions experienced PD during treatment, while all 32 patients with 100% FES-positive lesions achieved clinical benefit. The comparison between patients with clinical benefit and non-clinical benefit patients is presented in the Supplementary Table.

Prediction of response to combination therapy

At the time of analysis (May 2023), fifteen patients (39.5%) were still on combination therapy, 23 patients discontinued CDK4/6 inhibitor combined with endocrine therapy because of progressive disease (PD). The median PFS was 21.0 months (95% CI 12.7–29.3) in the whole cohort. Before evaluating the predictive response of PET parameters, we analyzed the factors of patients and diseases. It is disappointing that age, disease-free interval (DFI), number of disease sites, presence of visceral disease, number of prior treatment lines for metastatic disease, and types of CDK4/6 inhibitors regimens were not identified as predictors of PFS in the entire cohort (Table 2).

In this study, a total of 6 patients with FES-negative lesions all experienced disease progression, with a median PFS of only 5.3 months (95% CI 1.7–8.9), dramatically shorter than that of patients with 100% FES-positive lesions, and the median PFS was 22.9 months (95% CI 17.1–28.7) (log-rank $P<0.001$, Fig. 2a). Exemplary imaging of patients with FES-negative lesions is depicted in Fig. 3. Patients with 100% FES-positive lesions exhibit a hazard ratio (HR) of 0.01 for PFS (95% CI 0.02–0.11, $P<0.001$) in comparison to those with FES-negative lesions. Of patients with 100% FES-positive tumors who may benefit from CDK4/6 inhibitors combined with endocrine therapy, we conducted exploratory analysis using the identified PET parameters to predict PFS of combination therapy. In the subgroup analysis of patients with 100% FES-positive lesions, the median value of PET parameters was selected as the cut-off point, FDG SUVmax was 6.3, FES SUVmax was 7.4 and FES/FDG ratio was 1.08, respectively (Table 3). Patients with high FDG metabolism suggesting highly invasive tumors showed a HR of 1.73 for PFS (95% CI 0.63–4.78, $P=0.287$) compared to those with low FDG metabolism. In general, high FES uptake suggests better endocrine therapy efficacy; however, our study indicates that low FES uptake is associated with improved PFS. Compared to those with low FES uptake, high FES uptake showed a HR of 2.73 for PFS (95% CI 0.96–7.74; $P=0.059$). Unfortunately, neither FDG nor FES SUVmax has predictive value for PFS ($P>0.05$). FES/FDG is an alternative parameter for weighting FES uptake through metabolic aggression. The median PFS of patients with low FES/FDG (≤ 1.08) was significantly longer

Table 2 Univariate and multivariate Cox regression analyses for prediction of PFS for the entire patients

Parameters	No.	Event	Median PFS (95% CI)	Log-rank	Univariate analysis		Multivariate analysis	
				P value	HR (95% CI)	*P* value	HR (95% CI)	*P* value
Age								
≤56	20	11	26.9 (11.4–42.4)	0.356	1.48 (0.63–3.45)	0.358	NA	
>56	18	12	15.3 (13.5–17.1)					
Disease-free interval (DFI)								
≤5y	15	8	21.0 (NE)	0.385	1.45 (0.61–3.51)	0.387	NA	
>5y	21	14	16.5 (7.1–25.8)					
No. of disease sites								
1–2	22	13	16.5 (6.7–26.3)	0.946	0.97 (0.42–2.24)	0.946	NA	
≥3	16	10	21.6 (12.8–30.4)					
Visceral disease								
No	24	14	21.0 (11.0–31.0)	0.703	0.85 (0.37–1.97)	0.703	NA	
Yes	14	9	21.6 (6.5–36.7)					
Lines of therapy prior to CDK4/6i								
0	33	19	21.6 (13.6–29.6)	0.369	1.71 (0.58–5.08)	0.374	NA	
1	5	4	13.8 (7.8–39.4)					
Types of CDK4/6i								
Palbociclib + AI/Ful	25	17	21.6 (13.6–29.7)	0.705	1.2 (0.45–3.20)	0.705	NA	
Abemaciclib + AI/Ful	13	6	NE					
Presence of FES-negative lesions								
Yes	6	6	5.3 (1.7–8.9)	<0.001*	0.01 (0.02–0.11)	<0.001*	NA	
No	32	17	22.9 (17.1–28.7)					

PFS, progression-free survival; CI, confidence interval; HR, hazard ratio; CDK4/6i, CDK4/6 inhibitors; AI, Aromatase inhibitor; Ful, fulvestrant
*Indicates statistically significant differences ($P < 0.05$); N/A: Analysis not performed; NE: not evaluable

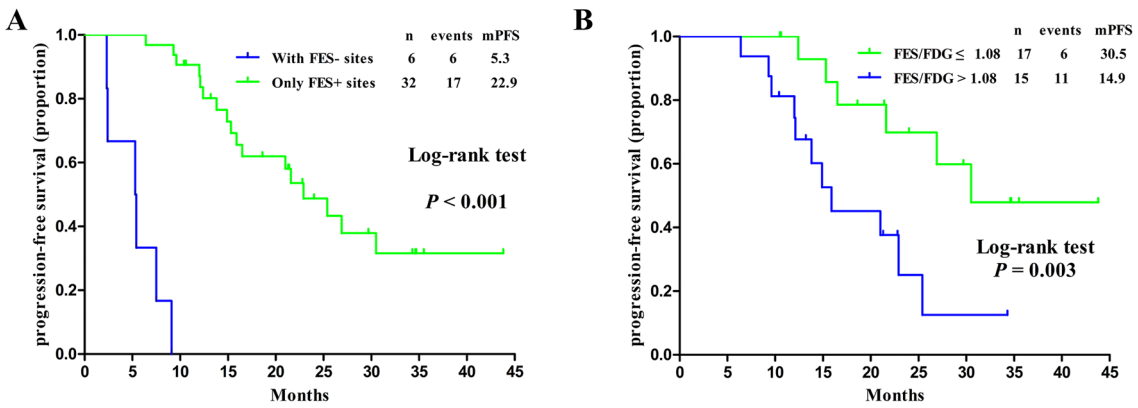

Fig. 2 Kaplan–Meier curve of progression-free survival (PFS) according to PET parameters. **A** Patients were categorized based on the presence or absence of FES-negative lesions to predict PFS in the whole cohort. **B** The PFS predicted by patients in the subgroup cohort with only FES-positive lesions was stratified based on the median FES/FDG. Abbreviations: *FES*−, FES-negative; *FES*+, FES-positive. FES/FDG = FES SUVmax/FDG SUVmax

than that of patients with high FES/FDG (30.5 months vs. 14.9 months, $P = 0.003$, Figs. 2b and 4). Patients with high FES/FDG showed a HR of 4.12 for PFS compared to those with low FES/FDG (95% CI 1.49–11.55; $P = 0.006$).

Discussion

We analyzed PET imaging parameters and tumor response in patients with ER+/HER2− (primary) metastatic breast cancer who received CDK4/6 inhibitors combined with

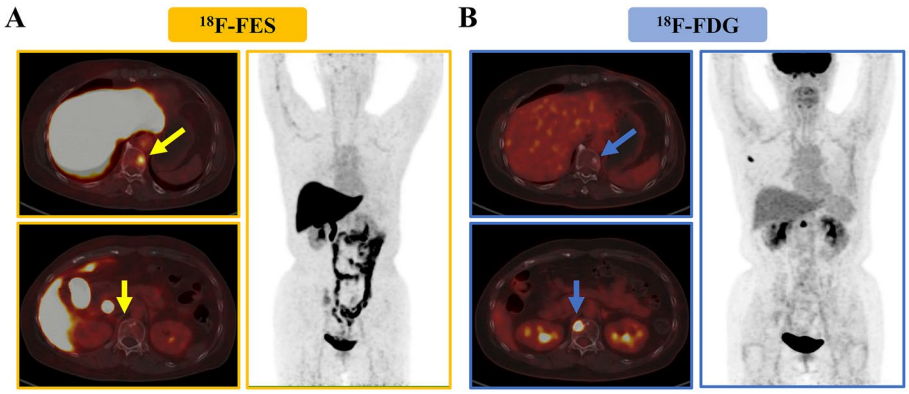

Fig. 3 Representative imaging of patients with FES-negative lesions. This 61-year-old woman showed positive on FES PET for the 10th thoracic vertebrae (**A** yellow arrow, above), but negative FDG (**B** blue arrow, above). On the contrary, FES is negative on the 12th thoracic spine (**A** yellow arrow, below), and FDG is positive (**B** blue arrow, below). The MIP image shows that FDG in the right axillary lymph node is positive, while FES is negative. She was on abemaciclib combined with fulvestrant as first-line of treatment for 9.1 months until progression

Table 3 Univariate and multivariate Cox regression analyses for prediction of PFS in the subgroup of patients with only FES-positive sites

Parameters	No.	Event	Median PFS (95% CI)	Log-rank	Univariate analysis		Multivariate analysis	
				P value	HR (95% CI)	*P* value	HR (95% CI)	*P* value
FDG SUVmax								
≤6.3	16	6	NE	0.282	1.73 (0.63–4.78)	0.287	NA	
>6.3	16	11	21.6 (18.9–24.3)					
FES SUVmax								
≤7.4	16	6	30.5 (NE)	0.051	2.73 (0.96–7.74)	0.059	NA	
>7.4	16	11	21.0 (13.7–28.3)					
FES/FDG[a]								
≤1.08	17	6	30.5 (NE)	0.003*	4.12 (1.49–11.55)	0.006*	NA	
>1.08	15	11	14.9 (11.4–18.3)					

PFS, progression-free survival; CI, confidence interval; HR, hazard ratio; SUVmax, maximum standard uptake value

[a]FES/FDG = FES SUVmax/FDG SUVmax

*Indicates statistically significant differences ($P < 0.05$); N/A: Analysis not performed; NE: not evaluable

endocrine therapy. Despite the greater heterogeneity in pathological type, disease stage, and previous treatment plans among patients in our study, we have identified that the FES/FDG ratio can serve as a prognostic indicator for ER-positive metastatic breast cancer patients undergoing CDK4/6 inhibitor combined with endocrine therapy. Yamamoto et al. proposed that the ratio of FES and FDG-SUV, serving as a link between ER expression and glucose metabolism, could serve as a valuable indicator for assessing the association between neutral hormone receptor status and cell proliferation in uterine tumors [35]. Therefore, based on our findings, we hypothesize that the ratio of FES to FDG-SUV in breast cancer may possess similar parameterized indicators.

To our knowledge, this study is the first to investigate predictive value of non-invasive molecular image biomarkers FDG and FES in ER-positive metastatic breast cancer patients receiving CDK4/6 inhibitor combined with endocrine therapy. Boers et al. [30] and our previous studies [31] have demonstrated that the heterogeneity parameters based on FES PET can be used as an imaging biomarker to predict the response of CDK4/6 inhibitors combined with endocrine therapy. However, FES PET is limited to monitoring ER expression in tumor lesions, which may not accurately evaluate the extent of tumor invasion or detect ER-negative lesions [36, 37]. In addition, the predictive value of FES PET primarily pertains to endocrine therapy mode, whereas CDK4/6 inhibitors are cell cycle-targeted agents that may be more closely associated with tumor proliferation [38] and tumor glycolytic metabolic activity [39]. Some studies [27–29] have also elucidated the value of FDG PET in predicting the response to combination therapy. On the

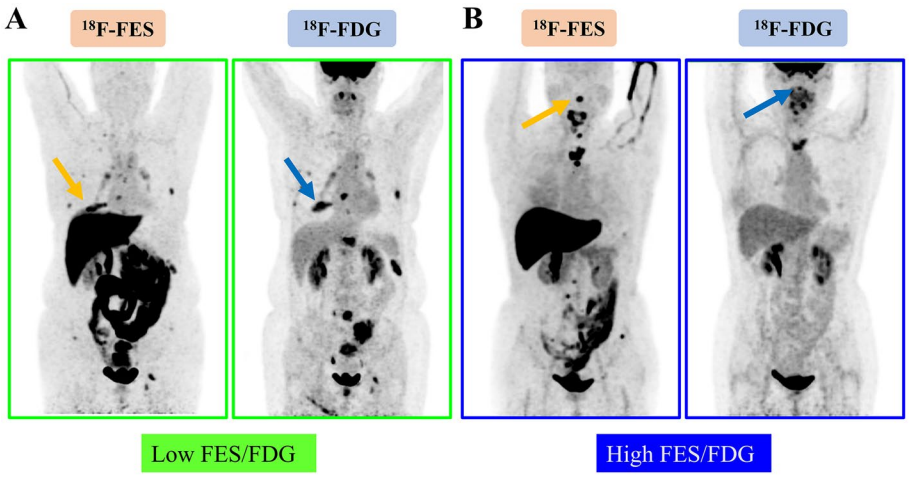

Fig. 4 Representative imaging of patients with 100% FES-positive lesions. **A** Low FES/FDG (≤ 1.08). This 57-year-old woman has three lymph nodes and twelve bone metastases, all of which show positive results on FES and FDG PET scans. The representative lesion was located on the right side of the 9th rib, exhibiting SUVmax values of 7.8 and 8.7 for FES (yellow arrow) and FDG (blue arrow), respectively, with a FES/FDG ratio of 0.89. She was administered palbociclib in combination with letrozole as first-line therapy for a duration of 34.6 months, and no evidence of disease progression was observed at the time of analysis. **B** High FES/FDG (> 1.08). This 63-year-old woman had 5 bone metastases, all of which show positive results on FES and FDG PET scans. The representative lesion was located on the upper cervical spine, exhibiting SUVmax values of 10.7 and 6.1 for FES (yellow arrow) and FDG (blue arrow), respectively, with a FES/FDG ratio of 1.75. She was on palbociclib combined with fulvestrant as first-line treatment for 14.9 months until progression

contrary, FDG PET serves as an indicator of tumor metabolic aggressiveness; however, it lacks the capability to identify functional ER distribution and screen patients who are suitable for endocrine therapy [40–42]. Therefore, a single PET probe molecular image may exhibit bias, whereas the utilization of dual probes could potentially enhance its predictive value.

In line with our previous study, our results suggest that patients with no or partial lesion uptake of FES are unlikely to benefit from CDK4/6 inhibitors combined with endocrine therapy. On the contrary, all patients with 100% FES-positive lesions achieved clinical benefits and had a superior PFS (22.9monts vs. 5.3 months log-rank, $P < 0.001$). For the study of FES PET in endocrine therapy of ER + breast cancer, most reports showed that higher FES uptake had better prognosis compared with patients with low/no FES uptake [23, 43]. Interestingly, we found that patients with low FES uptake have a longer PFS patients compared with patients with high FES uptake in the cohort of patients with 100% FES-positive lesions, and with borderline significance (low FES 30.5 months vs. high FES 21.0 months, $P = 0.051$, HR 2.73). Additionally, other scholars and our previous research have corroborated that the uptake of FES at high or low levels does not serve as an indicator for response to endocrine therapy [31, 44–46]. Indeed, the uptake of FES corresponds positively with ER expression, which may serve as a prerequisite for response to endocrine therapy. For FDG PET, most studies suggest that a higher uptake indicates more aggressive tumor growth and a worse prognosis [23, 47, 48]. Our findings also support this, as patients with low FDG uptake tend to have a more favorable prognosis compared to those with high FDG uptake (HR 1.73). However, the difference between the two groups was not statistically significant ($P = 0.282$). Although individual FES or FDG SUVmax does not have the ability to predict PFS, exploratory research based on the ratio of the two tracers reveals a novel predictive factor. In the cohort of patients with 100% FES-positive lesions, those with low FES/FDG exhibited significantly longer PFS compared to those with high FES/FDG (median PFS, 30.5 months vs. 14.9 months, $P = 0.003$). This is in line with our previous study, which demonstrated that patients with low FES/FDG have a lower risk of progressive disease and longer PFS during fulvestrant treatment [45]. However, our findings appear to contradict previous studies [23], which suggest that a lower FES uptake or lower FES/FDG ratio is indicative of resistance to endocrine therapy and associated with a poor prognosis. The potential reasons are postulated as follows: (1) The population in other studies exhibiting low FES uptake encompasses patients with FES-negative lesions, which may constitute the predominant cohort of individuals displaying endocrine resistance. In our study, even patients with low FES uptake exhibited 100% FES-positivity lesions, thereby maintaining sensitivity to endocrine therapy. (2) In the PALOMA 3 trial [11], the efficacy of fulvestrant plus palbociclib was not affected by the level of expression of ER and PR. Indeed, the presence of ER expression served as a prerequisite for the combination of CDK4/6 inhibitors and endocrine therapy; however, no

direct linear correlation was observed between their expression levels and clinical outcomes [49]. (3) Preclinical studies have found an increase in glucose metabolism observed in CDK4/6 inhibitor resistant cells [50], while high glucose metabolism also reflects an improvement in ER signaling pathway activation, which may enhance the inhibitory effect of endocrine therapy [51]. Therefore, the underlying mechanism of FDG PET metabolism in CDK4/6 inhibitor combined endocrine therapy remains elusive. In our study, among patients with 100% FES-positive lesions, those showing low FES uptake demonstrated a slightly higher PFS compared to those with high FES uptake (borderline significance, $P = 0.051$). This observation may partially account for the improved prognosis associated with a lower FES/FDG ratio.

There are some limitations to this study. First, the sample size is relatively small, and there is significant heterogeneity in the patients' disease characteristics, including pathological types and prior treatment regimens. Furthermore, while the FES to FDG ratio has been systematically investigated in uterine tumors, there is a lack of clear biological characteristics reported for breast cancer, necessitating further research to confirm. Finally, liver metastasis is a crucial prognostic factor in breast cancer, but its quantification on FES PET is challenging due to the liver's physiological high uptake. Therefore, FES/FDG does not include liver metastasis, which may result in partial deviation of the results, and this imaging biomarkers may not be applicable for some patients with liver metastasis.

Conclusion

Our research has demonstrated that the lack of ER expression, as measured by FES PET, can serve as a predictive factor for unlikely to response to CDK4/6 inhibitors combined with endocrine therapy. Synergizing with FDG PET can further stratify and predict survival in patients with 100% FES-positive lesions, thereby enhancing the precision of prognostication.

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Declarations

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2020. CA Cancer J Clin. 2020;70(1):7–30.
2. Johnston SJ, Cheung KL. Endocrine therapy for breast cancer: a model of hormonal manipulation. Oncol Ther. 2018;6(2):141–56.
3. Polk A, Kolmos IL, Kümler I, et al. Specific CDK4/6 inhibition in breast cancer: a systematic review of current clinical evidence. ESMO Open. 2016;1(6):e000093.
4. Goel S, Bergholz JS, Zhao JJ. Targeting CDK4 and CDK6 in cancer. Nat Rev Cancer. 2022;22(6):356–72.
5. Spring LM, Wander SA, Andre F, et al. Cyclin-dependent kinase 4 and 6 inhibitors for hormone receptor-positive breast cancer: past, present, and future. Lancet. 2020;395(10226):817–27.
6. O'sullivan CC, Clarke R, Goetz MP, et al. Cyclin-dependent kinase 4/6 inhibitors for treatment of hormone receptor-positive, ERBB2-negative breast cancer: a review. JAMA Oncol. 2023;2023:1.
7. Huppert LA, Gumusay O, Idossa D, et al. Systemic therapy for hormone receptor-positive/human epidermal growth factor receptor 2-negative early stage and metastatic breast cancer. CA Cancer J Clin. 2023;2023:1.
8. Toi M, Boyle F, Im YH, et al. Adjuvant abemaciclib combined with endocrine therapy: efficacy results in monarche cohort 1. Oncologist. 2023;28(1):e77–81.
9. Li Y, Li W, Gong C, et al. A multicenter analysis of treatment patterns and clinical outcomes of subsequent therapies after progression on palbociclib in HR+/HER2− metastatic breast cancer. Ther Adv Med Oncol. 2021;13:17588359211022890.
10. Wang B, Li R, Wu S, et al. Breast cancer resistance to cyclin-dependent kinases 4/6 inhibitors: intricacy of the molecular mechanisms. Front Oncol. 2021;11:651541.
11. Cristofanilli M, Turner NC, Bondarenko I, et al. Fulvestrant plus palbociclib versus fulvestrant plus placebo for treatment of hormone-receptor-positive, HER2-negative metastatic breast cancer that progressed on previous endocrine therapy (PALOMA-3): final analysis of the multicentre, double-blind, phase 3 randomised controlled trial. Lancet Oncol. 2016;17(4):425–39.
12. Demichele A, Clark AS, Tan KS, et al. CDK 4/6 inhibitor palbociclib (PD0332991) in Rb+ advanced breast cancer: phase II activity, safety, and predictive biomarker assessment. Clin Cancer Res. 2015;21(5):995–1001.
13. Fang H, Huang D, Yang F, et al. Potential biomarkers of CDK4/6 inhibitors in hormone receptor-positive advanced breast cancer. Breast Cancer Res Treat. 2018;168(2):287–97.
14. Currin E, Peterson LM, Schubert EK, et al. Temporal heterogeneity of estrogen receptor expression in bone-dominant breast cancer: 18F-fluoroestradiol PET imaging shows return of ER expression. J Natl Compr Canc Netw. 2016;14(2):144–7.
15. Schrijver W, Suijkerbuijk KPM, Van Gils CH, et al. Receptor conversion in distant breast cancer metastases: a systematic review and meta-analysis. J Natl Cancer Inst. 2018;110(6):568–80.
16. 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(2):277–89.
17. Van Es SC, Van Der Vegt B, Bensch F, et al. Decalcification of breast cancer bone metastases with EDTA does not affect ER, PR, and HER2 results. Am J Surg Pathol. 2019;43(10):1355–60.
18. Liao GJ, Clark AS, Schubert EK, et al. 18F-fluoroestradiol PET: current status and potential future clinical applications. J Nucl Med. 2016;57(8):1269–75.
19. Hu X, Chen W, Li F, et al. Expression changes of ER, PR, HER2, and Ki-67 in primary and metastatic breast cancer and its clinical significance. Front Oncol. 2023;13:1053125.

20. Liu C, Gong C, Liu S, et al. (18)F-FES PET/CT influences the staging and management of patients with newly diagnosed estrogen receptor-positive breast cancer: a retrospective comparative study with (18)F-FDG PET/CT. Oncologist. 2019;24(12):e1277–85.
21. Van Kruchten M, De Vries EGE, Brown M, et al. PET imaging of oestrogen receptors in patients with breast cancer. Lancet Oncol. 2013;14(11):e465–75.
22. 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 18F-fluoroestradiol PET/CT. Clin Nucl Med. 2017;42(6):421–7.
23. Kurland BF, Peterson LM, Lee JH, et al. Estrogen receptor binding (18F-FES PET) and glycolytic activity (18F-FDG PET) predict progression-free survival on endocrine therapy in patients with ER+ breast cancer. Clin Cancer Res. 2017;23(2):407–15.
24. Iqbal R, Yaqub M, Bektas HO, et al. [18F]FDG and [18F]FES PET/CT imaging as a biomarker for therapy effect in patients with metastatic ER+ breast cancer undergoing treatment with rintodestrant. Clin Cancer Res. 2023;29(11):2075–84.
25. Bottoni G, Piccardo A, Fiz F, et al. Heterogeneity of bone metastases as an important prognostic factor in patients affected by oestrogen receptor-positive breast cancer. The role of combined [18F] Fluoroestradiol PET/CT and [18F]Fluorodeoxyglucose PET/CT. Eur J Radiol. 2021;141:10821.
26. Yamada S, Tsuyoshi H, Yamamoto M, et al. Prognostic value of 16α-(18)F-fluoro-17β-Estradiol PET as a predictor of disease outcome in endometrial cancer: a prospective study. J Nucl Med. 2021;62(5):636–42.
27. Taralli S, Lorusso M, Scolozzi V, et al. Response evaluation with (18)F-FDG PET/CT in metastatic breast cancer patients treated with Palbociclib: first experience in clinical practice. Ann Nucl Med. 2019;33(3):193–200.
28. Ozawa H, Higuchi T, Fujimoto Y, et al. Total lesion glycolysis levels as predictive indicators in patients with metastatic and recurrent breast cancer undergoing endocrine therapy with or without CDK4/6 inhibitor. Anticancer Res. 2022;42(10):4813–24.
29. Seifert R, Küper A, Tewes M, et al. [18F]-fluorodeoxyglucose positron emission tomography/CT to assess the early metabolic response in patients with hormone receptor-positive HER2-negative metastasized breast cancer treated with cyclin-dependent 4/6 kinase inhibitors. Oncol Res Treat. 2021;44(7–8):400–7.
30. Boers J, Venema CM, De Vries EFJ, et al. Molecular imaging to identify patients with metastatic breast cancer who benefit from endocrine treatment combined with cyclin-dependent kinase inhibition. Eur J Cancer. 2020;126:11–20.
31. Liu C, Hu S, Xu X, et al. Evaluation of tumour heterogeneity by (18)F-fluoroestradiol PET as a predictive measure in breast cancer patients receiving palbociclib combined with endocrine treatment. Breast Cancer Res. 2022;24(1):57.
32. Boellaard R, Delgado-Bolton R, Oyen WJ, et al. FDG PET/CT: EANM procedure guidelines for tumour imaging: version 2.0. Eur J Nucl Med Mol Imaging. 2015;42(2):328–54.
33. 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(8):1212–8.
34. Zhao Z, Yoshida Y, Kurokawa T, et al. 18F-FES and 18F-FDG PET for differential diagnosis and quantitative evaluation of mesenchymal uterine tumors: correlation with immunohistochemical analysis. J Nucl Med. 2013;54(4):499–506.
35. Yamamoto M, Tsujikawa T, Yamada S, et al. 18F-FDG/18F-FES standardized uptake value ratio determined using PET predicts prognosis in uterine sarcoma. Oncotarget. 2017;8(14):22581–9.
36. Bottoni G, Fiz F, Puntoni M, et al. Diagnostic effectiveness of [(18)F]Fluoroestradiol PET/CT in oestrogen receptor-positive breast cancer: the key role of histopathology. Evidence from an international multicentre prospective study. Eur J Nucl Med Mol Imaging. 2023;50(8):2477–85.
37. Ulaner GA, Mankoff DA, Clark AS, et al. Summary: appropriate use criteria for estrogen receptor-targeted PET imaging with 16α-(18)F-fluoro-17β-fluoroestradiol. J Nucl Med. 2023;64(3):351–4.
38. Elmi A, Makvandi M, Weng CC, et al. Cell-proliferation imaging for monitoring response to CDK4/6 inhibition combined with endocrine-therapy in breast cancer: comparison of [(18)F]FLT and [(18)F]ISO-1 PET/CT. Clin Cancer Res. 2019;25(10):3063–73.
39. Franco J, Balaji U, Freinkman E, et al. Metabolic reprogramming of pancreatic cancer mediated by CDK4/6 inhibition elicits unique vulnerabilities. Cell Rep. 2016;14(5):979–90.
40. Van Geel JJL, De Vries EFJ, Van Kruchten M, et al. Molecular imaging as biomarker for treatment response and outcome in breast cancer. Ther Adv Med Oncol. 2023;15:17588359231170738.
41. Boers J, De Vries EFJ, Glaudemans A, et al. Application of PET tracers in molecular imaging for breast cancer. Curr Oncol Rep. 2020;22(8):85.
42. 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(2):182–90.
43. Van Kruchten M, Glaudemans A, De Vries EFJ, et al. 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(11):1674–81.
44. Van Kruchten M, De Vries EG, Glaudemans AW, et al. Measuring residual estrogen receptor availability during fulvestrant therapy in patients with metastatic breast cancer. Cancer Discov. 2015;5(1):72–81.
45. Liu C, Xu X, Yuan H, et al. Dual tracers of 16α-[18F]fluoro-17β-Estradiol and [18F]fluorodeoxyglucose for prediction of progression-free survival after fulvestrant therapy in patients with HR+/HER2− metastatic breast cancer. Front Oncol. 2020;10:580277.
46. He M, Liu C, Shi Q, et al. The predictive value of early changes in (18) F-fluoroestradiol positron emission tomography/computed tomography during fulvestrant 500 mg therapy in patients with estrogen receptor-positive metastatic breast cancer. Oncologist. 2020;25(11):927–36.
47. Li Y, Liu C, Wang B, et al. Prediction of pretreatment 18F-FDG-PET/CT parameters on the outcome of first-line therapy in patients with metastatic breast cancer. Int J Gen Med. 2021;14:1797–809.
48. Diao W, Tian F, Jia Z. The prognostic value of SUV(max) measuring on primary lesion and ALN by (18)F-FDG PET or PET/CT in patients with breast cancer. Eur J Radiol. 2018;105:1–7.
49. Anurag M, Haricharan S, Ellis MJ. CDK4/6 inhibitor biomarker research: Are we barking up the wrong tree? Clin Cancer Res. 2020;26(1):3–5.
50. Lorito N, Bacci M, Smiriglia A, et al. Glucose metabolic reprogramming of ER breast cancer in acquired resistance to the CDK4/6 inhibitor palbociclib. Cells. 2020;9(3):1.
51. O'mahony F, Razandi M, Pedram A, et al. Estrogen modulates metabolic pathway adaptation to available glucose in breast cancer cells. Mol Endocrinol. 2012;26(12):2058–70.

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