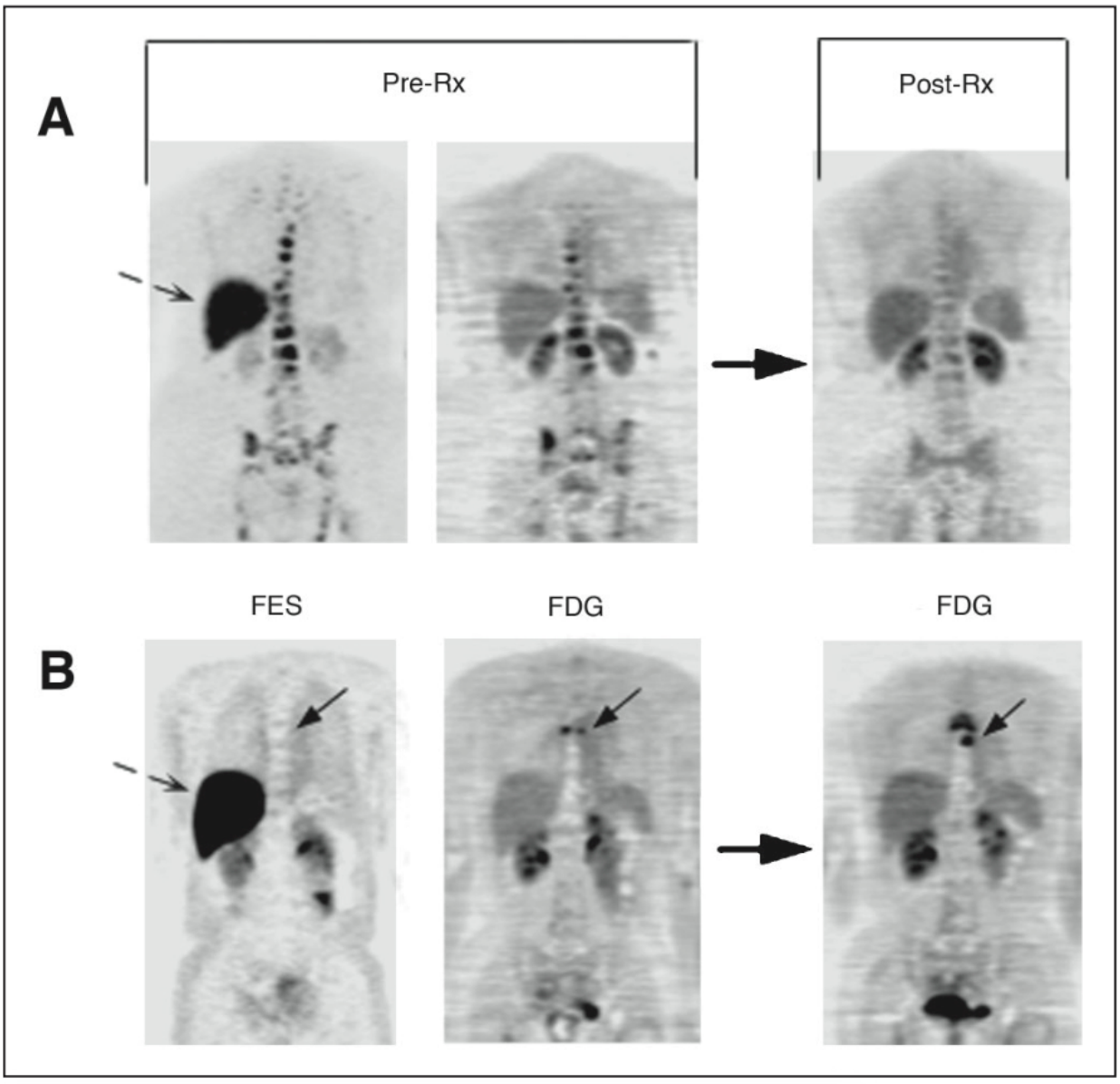

FIGURE 1. Imaging examples from two patients of baseline ^{18}F-FES PET and ^{18}F-FDG PET and follow-up posttherapy ^{18}F-FDG PET.
Dashed arrows point out normal liver ^{18}F-FES uptake. **(A)** Pretreatment ^{18}F-FES PET and ^{18}F-FDG PET scans both demonstrate uptake in numerous osseous metastases, with response seen in posttherapy ^{18}F-FDG PET scan. **(B)** Pretreatment ^{18}F-FES PET scan does not demonstrate ^{18}F-FES uptake in osseous metastases seen on pretreatment ^{18}F-FDG PET scan (solid arrow). Progressive disease is seen on posttherapy ^{18}F-FDG PET scan. (Reprinted with permission of (*25*).)

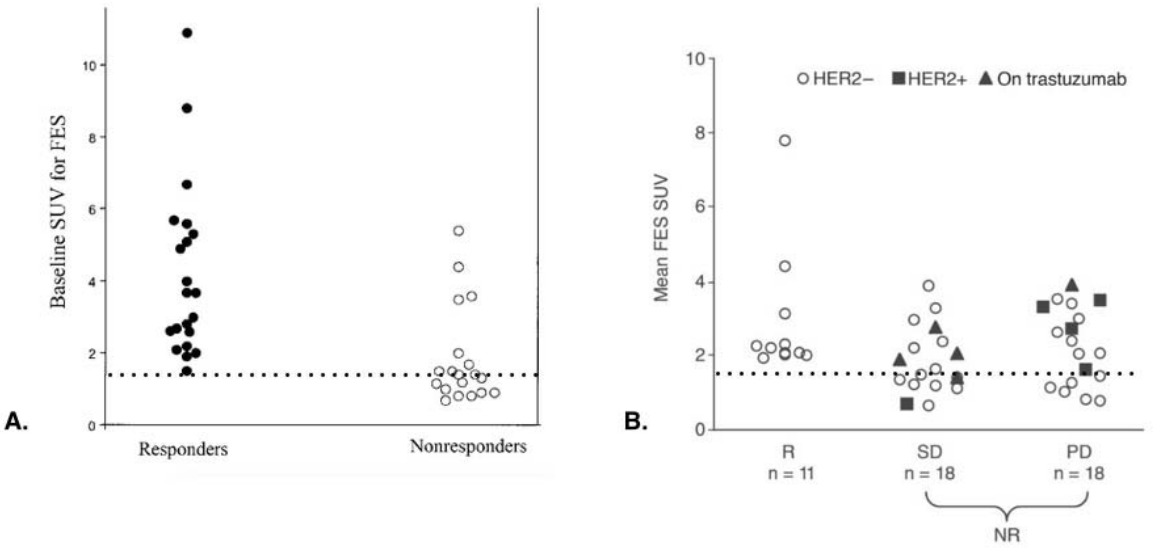

FIGURE 2. Baseline tumor ^{18}F-FES uptake for treatment responders and nonresponders.
(A) Baseline ^{18}F-FES SUV shown for responders vs. nonresponders to tamoxifen. No responders demonstrated SUV < 1.5. (Reprinted with permission of (*24*).) **(B)** Baseline mean ^{18}F-FES SUV is shown for responders (R) and nonresponders (NR) to salvage endocrine treatment. No responders demonstrated SUV < 1.5. SD = stable disease, PD = progressive disease. (Reprinted with permission of (*25*).)

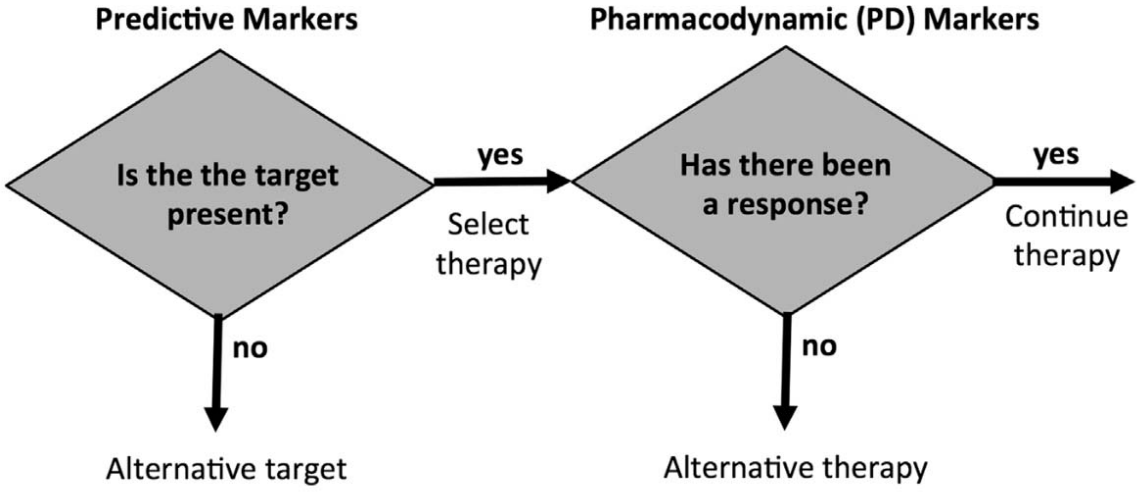

FIGURE 3. Flowchart diagram illustrating potential roles for molecular imaging companion diagnostics.
Baseline ^{18}F-FES PET would first establish presence of ER at the tumor site, followed by repeat ^{18}F-FDG PET after initiation of therapy to assess pharmacodynamic response. (Reprinted with permission of (*32*).)

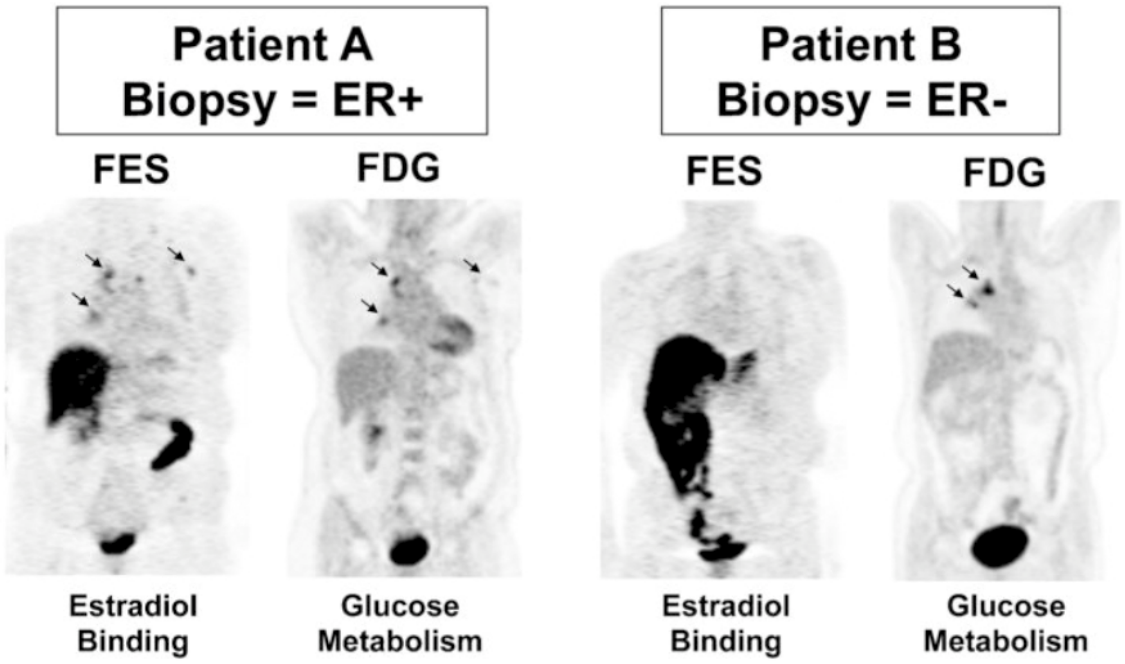

FIGURE 4. Imaging examples of two patients who underwent pretreatment ^{18}F-FES PET and ^{18}F-FDG PET imaging.
Left panel: Patient A had axillary and mediastinal lesions (arrows) seen on both ^{18}F-FES PET and ^{18}F-FDG PET scans. Core biopsy of the axillary lesion was ER-positive by immunohistochemistry. *Right panel*: Patient B had mediastinal lesions (arrows) seen only on the ^{18}F-FDG PET scan. Needle biopsy of a vertebral lesion was ER-negative by immunohistochemistry. (Reprinted with permission of (*16*).)

TABLE 1. Clinical ^{18}F-FES PET studies related to breast cancer

	# of pts	Study Purpose	Summary of Findings
Mintun et al., 1988 (*17*)	13	First attempt at imaging breast masses with ^{18}F-FES PET	^{18}F-FES uptake seen at site of primary tumor, axillary nodal metastases, and one distant metastatic site; 0.96 correlation between ^{18}F-FES uptake and ER concentration measured by in vitro radioligand binding assay
McGuire et al., 1991 (*41*)	16	Evaluate potential of ^{18}F-FES PET for imaging metastatic breast cancer; assess changes in ^{18}F-FES uptake before and after initiation of endocrine therapy	Sensitivity 93% for ^{18}F-FES PET in detecting metastatic lesions; ^{18}F-FES uptake decreased in lesions after initiation of hormonal therapy, suggesting receptor-mediated uptake of ^{18}F-FES
Dehdashti et al., 1995 (*21*)	53	Compare ^{18}F-FES PET and ^{18}F-FDG PET to in vitro radioligand and immunohistochemical ER expression, compare ^{18}F-FDG PET with tumor ER status	88% correlation between ^{18}F-FES uptake and in vitro ER expression; no correlation between ^{18}F-FDG uptake and ER status or ^{18}F-FDG and ^{18}F-FES uptake
Mortimer et al., 1996 (*33*)	43	Evaluate ability of ^{18}F-FDG PET and ^{18}F-FES PET to predict response to hormonal therapy; correlate ^{18}F-FES PET to in vitro ER assay	Using threshold ^{18}F-FES SUV of 1.0, PPV 70%, NPV 66% for treatment response; sensitivity 76%, specificity 100% for in vitro ER status
Dehdashti et al., 1999 (*23*)	11	Correlate pre- and posttamoxifen initiation ^{18}F-FDG PET and ^{18}F-FES PET imaging to treatment response	^{18}F-FDG PET demonstrated subclinical metabolic flare in treatment responders; responders had higher pretreatment ^{18}F-FES uptake and greater decrease in ^{18}F-FES uptake after treatment initiation
Mortimer et al., 2001 (*24*)	40	Determine ability of ^{18}F-FDG PET and ^{18}F-FES PET to detect metabolic flare and changes in tumor ER availability after initiation of tamoxifen	^{18}F-FDG PET demonstrated subclinical metabolic flare in treatment responders; responders had higher pretreatment ^{18}F-FES uptake and greater decrease in ^{18}F-FES uptake after treatment initiation
Linden et al., 2006 (*25*)	47	Determine ability of pretreatment ^{18}F-FES PET to predict response to salvage hormonal therapy in heavily pretreated metastatic breast cancer patients	Significant association between quantitative ^{18}F-FES PET and treatment response; sensitivity of 100%, specificity of 42% with threshold SUV of 1.5
Peterson et al., 2008 (*18*)	17	Compare ^{18}F-FES PET to qualitative and semiquantitative immunohistochemical assay ER expression	SUV threshold of 1.1 demonstrated 94% agreement with immunohistochemistry results
Dehdashti et al., 2009 (*26*)	51	Determine if ^{18}F-FDG PET metabolic flare induced by estradiol challenge and baseline ^{18}F-FES PET correlate with treatment response to AI or fulvestrant	Responders had higher baseline ^{18}F-FES uptake; threshold ^{18}F-FES SUV of 2.0 had PPV 50% and NPV 81% for response; only responders demonstrated metabolic flare
Tonkin et al., 2010 (*34*)	38	Assess utility of pretreatment ^{18}F-FES PET in predicting response to hormone therapy and correlate ^{18}F-FES PET with ^{18}F-FDG PET results in metastatic breast cancer	53% of patients had lesions with discordant ^{18}F-FES and ^{18}F-FDG uptake; discordant lesions demonstrated only stable disease at best
Kurland et al., 2011 (*35*)	91	Describe within-patient and between-patient heterogeneity of ^{18}F-FES PET uptake	Within-patient ^{18}F-FES uptake and ratio of ^{18}F-FES to ^{18}F-FDG uptake clustered around patient's average value; wide variance of average ^{18}F-FES uptake between patients (intraclass correlation coefficient 0.6); 37% had low or absent ^{18}F-FES uptake
Linden et al., 2011 (*37*)	30	Utilize ^{18}F-FES PET to evaluate in vivo pharmacodynamics of ER binding of various endocrine therapies	Treatment with tamoxifen or fulvestrant demonstrated greater degree of blockade than with AIs; rate of complete blockade greater with tamoxifen than with fulvestrant
Peterson et al., 2011 (*22*)	239	Assess factors that affect quantitative ^{18}F-FES uptake	^{18}F-FES uptake had inverse relationship with SHBG; no relationship with plasma estradiol, patient age, or rate of ^{18}F-FES metabolism; direct relationship with body mass index but not lean body mass

van Kruchten et al., 2012 (*65*)	33	Evaluate utility of ^{18}F-FES PET in ER-positive breast cancer patients presenting with a clinical dilemma	^{18}F-FES PET utilized to evaluate equivocal lesions on standard workup, ER status of patients with metastases, and origin of metastatic lesions. ^{18}F-FES PET improved diagnostic understanding in 88% of patients and resulted in change of therapy in 48%
Gemignani et al., 2013 (*20*)	48	Compare ^{18}F-FES PET to in vitro ER expression in patients with operable primary breast cancer	Sensitivity 85%, specificity 75% with threshold ^{18}F-FES SUV of 1.5; ^{18}F-FES SUV did not correlate with ER and PR gene expression
Yang et al., 2013 (*36*)	32	Assess heterogeneity of ER expression with ^{18}F-FES PET and ^{18}F-FDG PET	33.4-fold difference in ^{18}F-FES uptake between patients, 8.2-fold difference in ^{18}F-FES uptake among lesions in the same patient; 28.1% of patients had discordant ^{18}F-FES and ^{18}F-FDG uptake
Peterson et al., 2014 (*16*)	19	Evaluate pretreatment ^{18}F-FES PET association with treatment response and ER expression in newly metastatic breast cancer	No patient with baseline ^{18}F-FES SUV < 1.5 had response; all patients with ER-negative biopsy had low average ^{18}F-FES uptake and at least one ^{18}F-FES-negative site
Wang et al., 2015 (*39*)	30	Utilized ^{18}F-FES PET to validate ER engagement by novel ERα antagonist and degrader	Posttherapy ^{18}F-FES PET demonstrated > 90 % reduction in ^{18}F-FES uptake in 90% of patients
van Kruchten et al., 2015 (*28*)	19	Evaluate ^{18}F-FES PET in predicting response to additive low-dose estradiol therapy in patients with endocrine-refractory metastatic breast cancer	^{18}F-FES SUV threshold of 1.5 had PPV 60% and NPV 80% for response to treatment
van Kruchten et al., 2015 (*38*)	16	Assess change in ^{18}F-FES uptake during fulvestrant therapy in patients with ER-positive metastatic breast cancer	38% demonstrated incomplete reduction in ER availability (< 75% reduction in ^{18}F-FES uptake and residual SUV $\geq$1.5), which was associated with early progression

^{18}F-FES = ^{18}F-fluoroestradiol. ER = estrogen receptor. PET = positron emission tomography. ^{18}F-FDG =^{18}F-fluorodeoxyglucose. SUV = standardized uptake value. PPV = positive predictive value. NPV = negative predictive value. SHBG = sex hormone binding globulin.

TABLE 2. Studies correlating ^{18}F-FES PET with in vitro assay

	Patient Population	Key Outcomes
Mintun et al., 1988 (*17*)	13 postmenopausal women with primary breast masses suspected to be primary breast cancers	High correlation (r = 0.96) between tumor ^{18}F-FES uptake and ER concentration measured by in vitro radioligand binding; poor correlation with progestin-receptor concentration
Dehdashti et al., 1995 (*21*)	32 patients with primary breast masses (Group 1), 21 patients with recurrent or metastatic breast cancer (Group 2)	SUV $\geq$ 1.0 considered positive; 82% agreement between ^{18}F-FES PET and in vitro assay (combination of radioligand binding and immunohistochemistry) in Group 1; 94% agreement in Group 2; overall 88% agreement between ^{18}F-FES PET and in vitro assays
Mortimer et al., 1996 (*33*)	43 patients with locally advanced or metastatic breast cancer	SUV $\geq$ 1.0 considered positive; sensitivity of 76% and specificity of 100% compared to in vitro assay (combination of radioligand binding and immunohistochemistry)
Dehdashti et al., 1999 (*23*)	11 patients with ER-positive breast cancer with newly diagnosed metastatic disease	All 11 patients had SUV > 1.0
Peterson et al., 2008 (*18*)	17 patients with newly diagnosed or recurrent breast cancer	SUV vs. qualitative immunohistochemistry had Spearman correlation coefficient (ρ) of 0.62; SUV vs. semiquantitative Allred had ρ = 0.72; SUV vs. semiquantitative immunohistochemical index had ρ = 0.73; 94% agreement between SUV and immunohistochemistry with SUV threshold of 1.1
Gemginani et al., 2013 (*20*)	48 patients with primary breast cancer at least 1 cm in size without prior treatment	SUV > 1.5 considered positive; sensitivity of 85%, specificity of 75%, PPV 94%, NPV 50%, area under ROC curve of 0.85

^{18}F-FES = ^{18}F-fluoroestradiol. ER = estrogen receptor. SUV = standardized uptake value. PET = positron emission tomography. PPV = positive predictive value. NPV = negative predictive value. ROC = receiver operating characteristic.

TABLE 3. Studies evaluating ^{18}F-FES PET as predictor of response to endocrine therapy

	Patient Population	Key Outcomes
Mortimer et al., 1996 (*33*)	43 patients with locally advanced or metastatic breast cancer	Baseline SUV ≥ 1.0 considered positive; PPV 70%, NPV 66% for treatment response
Dehdashti et al., 1999 (*23*)	11 patients with ER-positive breast cancer with newly diagnosed metastatic disease, for whom tamoxifen treatment was planned	7 responders, 4 nonresponders; responders trended toward higher baseline SUV (4.6 ± 2.2 vs. 2.5 ± 1.9; P = 0.09); all responders had baseline SUV > 2.2
Mortimer et al., 2001 (*24*)	40 endocrine therapy naïve postmenopausal women with ER-positive locally advanced, recurrent, or metastatic breast cancer, for whom tamoxifen treatment was planned	21 responders, 19 nonresponders; responders had higher mean baseline SUV (4.3 ± 2.4 vs. 1.8 ± 1.4; P = 0.0007)
Linden et al., 2006 (*25*)	47 pretreated patients with an ER-positive primary tumor, presenting with recurrent or metastatic breast cancer	No patient with baseline SUV < 1.5 had objective response to salvage endocrine therapy
Dehdashti et al., 2009 (*26*)	51 postmenopausal women with locally advanced or metastatic ER-positive breast cancer, for whom treatment with an AI or fulvestrant was planned	17 responders, 34 nonresponders; responders had higher mean baseline SUV (3.5 ± 2.5 vs. 2.1 ± 1.8; P = 0.0049); with threshold SUV ≥ 2.0, PPV of 50% and NPV of 81% for response
Peterson et al., 2014 (*16*)	19 patients with newly diagnosed metastatic cancer with ER-positive primary tumor	2 of 5 patients with baseline SUV ≤ 1.5 were available for response assessment and both had progression of disease
van Kruchten et al., 2015 (*28*)	19 patients with ER-positive metastatic breast cancer that progressed after ≥ 2 lines of endocrine therapy, with previous response to endocrine therapy	Median SUV_{max} > 1.5 considered positive; PPV 60% and NPV 80% for treatment response
van Kruchten et al., 2015 (*38*)	16 postmenopausal women with ER-positive metastatic breast cancer with progression of disease after ≥ 2 lines of endocrine therapy	No significant difference in baseline median SUV_{max} between responders and nonresponders (3.1 vs. 2.5; P = 0.6)

SUV = standardized uptake value. PPV = positive predictive value. NPV = negative predictive value. ER = estrogen receptor. AI = aromatase inhibitor.

TABLE 4. Studies utilizing ^{18}F-FES PET to demonstrate heterogeneity of disease

	Patient Population	Key Outcomes
Dehdashti et al., 1995 (*21*)	53 patients total, 21 of whom had recurrent or metastatic breast cancer, 13 of whom had multiple sites evaluated on ^{18}F-FES PET	2 of 13 patients (15%) had discordant ^{18}F-FES uptake in at least one site
Mortimer et al., 1996 (*33*)	43 patients with locally advanced or metastatic breast cancer; 17 of whom had multiple sites evaluated on ^{18}F-FES PET	4 of 17 patients (24%) had discordant ^{18}F-FES uptake in one site
Linden et al., 2006 (*25*)	47 pretreated patients with an ER-positive primary tumor, presenting with recurrent or metastatic breast cancer	6 of 47 (13%) patients had at least one qualitatively ^{18}F-FES-negative site
Dehdashti et al., 2009 (*26*)	51 postmenopausal women with locally advanced or metastatic ER-positive breast cancer, for whom treatment with an AI or fulvestrant was planned	16 of 51 (31%) patients had mean SUV $\leq$ 1.0
Tonkin et al., 2010 (*34*)	38 patients with ER-positive metastatic breast cancer undergoing 1st-, 2nd-, or 3rd-line hormone therapy	20 of 38 (53%) had discordant ^{18}F-FES uptake in at least one site (^{18}F-FES-negative, ^{18}F-FDG-positive)
Kurland et al., 2011 (*35*)	91 patients with prior ER-positive biopsy scheduled to initiate, resume, or change endocrine therapy	SUV for individual lesions ranged from 0.0 to 10.2; average SUV ranged from 0.2 to 6.7 between patients, intraclass correlation coefficient was 0.60; 34 of 91 (37%) patients had mean SUV < 1.0; small number of patients with discordant ^{18}F-FES uptake
van Kruchten et al., 2012 (*65*)	33 patients with history of ER-positive breast cancer presenting with equivocal lesions on conventional workup, metastatic disease, or lesions of unknown origin	Wide variance in SUV seen in metastatic lesions (range, 1.2–18.81); 45% of patients had both ^{18}F-FES-positive and ^{18}F-FES-negative lesions
Yang et al., 2013 (*36*)	32 patients with new, recurrent, or metastatic breast cancer	SUV_{max} ranged from 0.5 to 16.7 between patients (33.4-fold difference); SUV_{max} ranged from 1.0 to 8.2 (8.2-fold difference) within a single individual; 9 of 32 (28%) patients had both ^{18}F-FES-positive and ^{18}F-FES-negative lesions
Peterson et al., 2014 (*16*)	19 patients with newly diagnosed metastatic cancer with ER-positive primary tumor	5 of 19 (26%) patients had average SUV $\leq$ 1.5; 6 of 19 (32%) had at least one qualitatively ^{18}F-FES-negative site
van Kruchten et al., 2015 (*28*)	19 patients with ER-positive metastatic breast cancer that progressed after $\geq$ 2 lines of endocrine therapy	6 of 19 (32%) patients had both ^{18}F-FES-positive and ^{18}F-FES-negative lesions; wide variance of SUV_{max} between lesions (range, 0.6–24.3) and patients (1.1–15.5)
van Kruchten et al., 2015 (*38*)	16 postmenopausal women with ER-positive metastatic breast cancer with progression of disease after $\geq$ 2 lines of endocrine therapy	5 of 16 (31%) patients had at least one ^{18}F-FES-negative metastatic lesion

^{18}F-FES = ^{18}F-fluoroestradiol. PET = positron emission tomography. ER = estrogen receptor. AI = aromatase inhibitor. SUV = standardized uptake value. ^{18}F-FDG =^{18}F-fluorodeoxyglucose.

TABLE 5. Studies evaluating impact of endocrine therapy on ^{18}F-FES uptake

	Patient Population	Key Outcomes
McGuire et al., 1991 (*41*)	16 patients with recurrent or metastatic breast cancer; 7 of whom had evaluable ^{18}F-FES PET imaging before and after initiation of endocrine therapy	Mean lesion SUV decreased from 2.2 (±1.23) to 0.80 (±0.42) after initiation of endocrine therapy
Dehdashti et al., 1999 (*23*)	11 patients with ER-positive breast cancer with newly diagnosed metastatic disease, for whom tamoxifen treatment was planned	7 responders, 4 nonresponders; responders had greater posttreatment mean decrease in SUV (2.7 ± 1.7 vs. 0.8 ± 0.5; $P = 0.04$)
Mortimer et al., 2001 (*24*)	40 endocrine therapy-naïve postmenopausal women with ER-positive locally advanced, recurrent, or metastatic breast cancer, for whom tamoxifen treatment was planned	21 responders, 19 nonresponders; responders had higher percentage decrease in SUV from baseline (−54.8% ± 14.2% vs. −19.4% ± 17.3%; $P = 0.0003$) and larger mean change in SUV (−2.5 ± 1.8 vs. −0.5 ± 0.6; $P = 0.0003$)
Linden et al., 2011 (*37*)	30 patients with metastatic breast cancer who had serial ^{18}F-FES PET imaging while undergoing endocrine therapy	Patients on ER blockers (tamoxifen and fulvestrant) had lower average SUV during treatment vs. patients on nonblockers (AIs) (1.5 vs. 2.2; $P = 0.04$); patients on blockers had higher average percentage decline in SUV (54% vs. 14%; $P < 0.001$); 100% of patients treated with tamoxifen vs. 36% of patients treated with fulvestrant demonstrated complete ER blockade (SUV ≤ 1.5)
Wang et al., 2015 (*39*)	30 patients with advanced or metastatic ER-positive breast cancer treated with ARN-810 (novel ERα antagonist and degrader)	27 of 30 (90%) patients demonstrated > 90% decrease of SUV_{max} on posttherapy scan
van Kruchten et al., 2015 (*38*)	16 postmenopausal women with ER-positive metastatic breast cancer with progression of disease after ≥ 2 lines of endocrine therapy	Patients with clinical benefit from fulvestrant (no radiologic or clinical progression of disease for at least 24 weeks) had greater median change in SUV (−88% vs. −58%; $P = 0.025$); patients with incomplete ER blockade (<75% decrease in ^{18}F-FES uptake and residual SUV ≥ 1.5) had shorter median progression free survival (3.3 months vs. 11.7 months; $P < 0.05$)

^{18}F-FES = ^{18}F-fluoroestradiol. PET = positron emission tomography. SUV = standardized uptake value. ER = estrogen receptor. AI = aromatase inhibitor.