Measuring Residual Estrogen Receptor Availability during Fulvestrant Therapy in Patients with Metastatic Breast Cancer

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ABSTRACT

It is unknown whether the current dose of fulvestrant, an estrogen receptor (ER) antagonist, is sufficient for maximal ER downregulation in patients with metastatic breast cancer. We performed a feasibility study to assess ER availability before and during fulvestrant. Sixteen patients with ER-positive metastatic breast cancer underwent positron emission tomography/computed tomography (PET/CT) at baseline (scan 1), day 28 (scan 2), and day 84 (scan 3) to monitor tumor [18F]fluoroestradiol (FES) uptake. Incomplete reduction in ER availability was predefined as <75% decrease in median tumor FES uptake and a residual standardized uptake value (SUVRmax) of ≥1.5. In total, 131 FES-positive lesions were identified (median SUVRmax of 2.9; range, 1.7–6.5). The median change in patients during fulvestrant treatment was −85% at scan 2, but varied widely (−99% to +60%). Fulvestrant reduced tumor FES uptake incompletely at scan 2 in 6 (38%) of the 16 patients, which was associated with early progression.

SIGNIFICANCE: Serial imaging of tumor estrogen uptake by FES-PET can give insight into the dose needed for ER antagonists to completely abolish ER. FES-PET showed significant residual ER availability in tumors during fulvestrant therapy in 38% of patients, which was associated with early progression. Cancer Discov; 5(1); 72–81. © 2014 AACR.

INTRODUCTION

Fulvestrant, a pure estrogen receptor (ER) antagonist, is used as treatment for advanced and metastatic ER-positive breast cancer. Its method of action is unique in that it not only competitively binds to the ER but also reduces its expression (1). In preclinical studies, fulvestrant was shown to completely downregulate tumor ER expression and inhibit estrogen-mediated tumor growth (2). In early clinical studies, no dose-limiting toxicities were detected, and 250 mg administered intramuscularly every 4 weeks was approved as a standard dose, given that higher doses cannot be administered as a single injection due to the solubility of the drug (3, 4). However, serial biopsy studies showed that ER expression is downregulated incompletely at the 250-mg dose (4). A loading-dose regimen was therefore explored more recently: 500 mg at day 1 and 250 mg at days 14 and 28 and every 4 weeks thereafter, as well as a high-dose regimen consisting of 500 mg at days 1, 14, and 28 and every 4 weeks thereafter. Because of a progression-free survival (PFS) gain in the high-dose regimen from 5.5 to 6.5 months in a phase III trial, the high-dose regimen ultimately became the current standard dose regimen from 5.5 to 6.5 months in a phase III trial, the current standard dose regimen from 5.5 to 6.5 months in a phase III trial, the

Fulvestrant reduced tumor FES uptake incompletely at scan 2 in 6 (38%) of the 16 patients, which was associated with early progression. Fulvestrant reduced tumor FES uptake incompletely at scan 2 in 6 (38%) of the 16 patients, which was associated with early progression. Fulvestrant reduced tumor FES uptake incompletely at scan 2 in 6 (38%) of the 16 patients, which was associated with early progression.
were identified on FES-PET/CT (107 bone lesions, 17 lymph positive lesion at scan 1. A total of 131 FES-positive lesions before and during treatment. All patients had at least one FES-positive response on the CT scan.

The patient requested treatment withdrawal despite partial response could not be determined in 1 patient who withdrew consent after 1 month of therapy.

According to RECIST 1.1 criteria, fulvestrant induced one partial response. Stable disease (SD) was observed in 8 patients ≥ 24 weeks; 3 of these had measurable disease at baseline. In the remaining 5 patients with SD, no evidence of radiologic or clinical progressive disease (PD) was detected, and all 5 had a decrease in tumor marker CA15.3 (range, −5% to −66%). Finally, 4 patients had radiologic PD and 2 had clinical PD. Clinical PD was evident from deterioration of symptoms and a 3- to 12-fold increase in CA15.3. Treatment response could not be determined in 1 patient who withdrew consent after 1 month of therapy.

The median follow-up was 6.5 months (range, 1.9–15.9 months). Of the 16 patients, 15 discontinued fulvestrant, with a median PFS of 6.2 months. The remaining patient had received fulvestrant for more than 16 months. Fulvestrant was well tolerated by nearly all patients. One grade 3 adverse event was observed, a urinary tract infection, after which the patient requested treatment withdrawal despite partial response on the CT scan.

FES-PET/CT Analysis

FES-PET was performed to evaluate tumor ER availability before and during treatment. All patients had at least one FES-positive lesion at scan 1. A total of 131 FES-positive lesions were identified on FES-PET/CT (107 bone lesions, 17 lymph nodes, four lung metastases, one ovarian metastasis, and one perineal metastasis). In addition, the CT scan revealed FES-negative metastases (n = 11 bone lesions and one lymph node) in 5 (31%) of 16 patients. Finally, seven liver metastases were detected. FES uptake could not be reliably measured in liver metastases due to high background FES uptake in healthy liver tissue. Baseline maximum standardized uptake value ($SUV_{max}$) of FES varied greatly among lesions (median, 3.4; range, 1.4–17.4) and patients (median, 2.9; range, 1.7–6.5).

A second scan was available for all patients; 12 patients received the second scan at day 28, 2 patients at day 56 (before the fourth fulvestrant administration), and 2 patients at day 84 (before the fifth fulvestrant administration). A third scan was available for 9 patients, but 7 patients did not receive the third scan due to early progression ($n = 3$), treatment withdrawal ($n = 2$), and logistic reasons ($n = 2$).

At the second scan, the median change in FES uptake for the 16 patients was −85% (−99% to +60%). Median residual tumor $SUV_{max}$ was 1.7 (1.1–3.8). A median reduction in FES tumor uptake of more than 75% was observed in 9 of the 16 patients, whereas incomplete reduction (−58% to +60%) with a residual $SUV_{max} ≥ 1.5$ was observed in 6 patients. One patient had a relative decrease in FES uptake of less than 75%, but residual $SUV_{max}$ was below the threshold of 1.5.

Interestingly, even in the 9 patients with $≥ 75%$ decrease in FES uptake, 4 patients had residual FES uptake ($SUV_{max}$ above 1.5. In the 9 patients for whom three scans were available (in 8 of 9 patients at the predefined time points), residual tumor FES uptake did not decrease between scans 2 and 3 (median $SUV_{max}$, 1.6 vs. 1.7; $P = 0.23$), which suggests that the maximum effect of fulvestrant 500 mg can be measured after only two administrations. Larson–Fox–Gonen plots are provided for representative patients with complete (Fig. 1A) and incomplete (Fig. 1B) reduction in FES uptake at scan 2. FES uptake characteristics before and during therapy are provided in Table 2.

Heterogeneity in the reduction in FES uptake was seen among lesions within individual patients. For example, one individual had six lesions with $≥ 90%$ reduction in FES uptake with a residual $SUV_{max} ≤ 1.5$, whereas in seven other lesions, a modest reduction of 50% to 70% was observed, with residual $SUV_{max}$ as high as 4.9 (Fig. 2). On the basis of our predefined cutoff point, at the second scan, 8 of 16 patients had lesions with complete reduction in FES uptake, but they also had lesions with incomplete reduction. Of all lesions quantified, 58 (44%) of 131 had incomplete reduction in FES uptake on the second FES-PET scan. The incomplete reduction in individual lesions shown at scan 2 persisted during treatment. Paired analysis of the 9 patients with three available scans showed incomplete reduction in FES uptake in 18 (26%) of 70 lesions at scan 2 and 20 lesions (29%) at scan 3.

**Correlation between Treatment Outcome and FES-PET Results**

Baseline tumor FES uptake in metastases of patients having clinical benefit from fulvestrant was similar to that in patients with PD (median $SUV_{max}$, 3.1 vs. 2.5; $P = 0.6$). In our study, a previously published (12, 13) threshold of baseline $SUV_{max} ≥ 2.0$ did not help to identify responding patients. Furthermore, the presence of FES-negative lesions at baseline did not predict therapy failure. PD developed in 2 of 5

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**Table 1. Patient characteristics**

<table>
<thead>
<tr>
<th>Characteristic</th>
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<tr>
<td>Number of patients</td>
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<tr>
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<tr>
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</tr>
<tr>
<td>Range</td>
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<tr>
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<tr>
<td>Median (±SD)</td>
<td>24 (±4)</td>
</tr>
<tr>
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</tr>
<tr>
<td>Female</td>
<td>16</td>
</tr>
<tr>
<td>Male</td>
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</tr>
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<td>Primary tumor receptor status</td>
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</tr>
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<td>PR-positive</td>
<td>11</td>
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<tr>
<td>HER2-positive</td>
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</tr>
<tr>
<td>Site of disease</td>
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<tr>
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<td>3</td>
</tr>
<tr>
<td>Bone + visceral (nodes)</td>
<td>8</td>
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<td>Bone + nodes</td>
<td>3</td>
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<td>2</td>
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<tr>
<td>Prior lines of palliative endocrine therapies</td>
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</tr>
<tr>
<td>Tamoxifen, aromatase inhibitor</td>
<td>14</td>
</tr>
<tr>
<td>Tamoxifen, aromatase inhibitor, megestrol</td>
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</tr>
<tr>
<td>Prior lines of chemotherapy for metastatic disease</td>
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<td>0</td>
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<td>1</td>
<td>4</td>
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</table>

Abbreviations: BMI, body mass index; PR, progesterone receptor; HER2, human epidermal growth factor receptor 2.
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**RESEARCH ARTICLE**

**Figure 1.** Change in FES uptake in the tumor during fulvestrant treatment. **A,** a representative patient with incomplete reduction in FES uptake. **B,** a patient with extensive reduction in FES uptake. Blue dots indicate individual lesions; the red square represents the median of all lesions used for patient-based analysis. At the right side of the plot the corresponding PET images are shown. The line indicates the 75% reduction threshold.

**Table 2.** FES-PET results in individual patients

<table>
<thead>
<tr>
<th>Patient</th>
<th>Tumor sites</th>
<th>FES+lesions (n)</th>
<th>Baseline SUV max</th>
<th>Second SUV max</th>
<th>%Change SUV cor</th>
<th>Progression, mo</th>
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<td>1</td>
<td>Bone, lung</td>
<td>20</td>
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<td>Bone, nodal, lung</td>
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<td>1.4</td>
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<td>1.9</td>
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<td>Nodal, lung</td>
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<td>3.1</td>
<td>−88</td>
<td>11.7</td>
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<td>1.5</td>
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<td>13.3</td>
</tr>
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<td>5a</td>
<td>Bone, nodal, lung, liver</td>
<td>7</td>
<td>2.3</td>
<td>1.0</td>
<td>−100</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Perineal, intestinal</td>
<td>1</td>
<td>1.7</td>
<td>1.1</td>
<td>−68</td>
<td>2.8</td>
</tr>
<tr>
<td>7</td>
<td>Bone, lung, liver</td>
<td>11</td>
<td>2.4</td>
<td>2.4</td>
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<td>1.9</td>
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<td>1.9</td>
<td>1.1</td>
<td>−91</td>
<td>16+ (ongoing)</td>
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<tr>
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<td>Bone-only</td>
<td>11</td>
<td>1.8</td>
<td>2.5</td>
<td>+60</td>
<td>4.7</td>
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<tr>
<td>9b</td>
<td>Bone-only</td>
<td>13</td>
<td>2.8</td>
<td>2.4</td>
<td>−26</td>
<td></td>
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<td>−92</td>
<td>Partial response (W)</td>
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<td>11</td>
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<td>6.8</td>
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<td>12b</td>
<td>Bone, liver</td>
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<td>1.9</td>
<td>−58</td>
<td>1.9</td>
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<tr>
<td>13</td>
<td>Bone, nodal, liver</td>
<td>7</td>
<td>4.1</td>
<td>2.1</td>
<td>−58</td>
<td>3.7</td>
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<tr>
<td>14</td>
<td>Bone, nodal, ovarian</td>
<td>9</td>
<td>6.0</td>
<td>1.1</td>
<td>−89</td>
<td>2.8</td>
</tr>
<tr>
<td>15</td>
<td>Bone, nodal</td>
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<td>5.5</td>
<td>3.8</td>
<td>−45</td>
<td>NE (W)</td>
</tr>
<tr>
<td>16</td>
<td>Bone, skin, intestinal</td>
<td>3</td>
<td>2.7</td>
<td>1.2</td>
<td>−99</td>
<td>6.2</td>
</tr>
</tbody>
</table>

**NOTE:** Data presented are median values of all quantified lesions. Abbreviations: NE, not evaluable; W, withdrew.

+a Two patients had recent tamoxifen treatment and very low baseline uptake. An earlier FES-PET scan while using an aromatase inhibitor provided additional insights (see “Tamoxifen Effects on Baseline PET Measures” in Results).

+b Patients with clinical PD.
patients with heterogeneous disease with at least one FES-negative site and 4 of 10 patients with only FES-positive sites. Consequently, baseline FES-PET was unable to differentiate between patients who would subsequently derive clinical benefit from fulvestrant treatment and those who would not.

However, the magnitude of changes in tumor FES uptake corrected for physiologic background (SUV cor) was significantly larger in patients having clinical benefit from fulvestrant compared with patients with PD (median change SUV cor, −88% vs. −58%; \( P = 0.025 \)). Of 9 patients with ≥75% change in median FES uptake, 8 (89%) had clinical benefit from fulvestrant therapy, compared with only 1 of 6 with <75% decrease. In addition, median PFS was 3.3 months for patients with <75% decrease in FES uptake versus 11.7 months for patients with ≥75% decrease (\( P < 0.05 \)). FES uptake at baseline and during treatment for all individual patients and its relation with response is depicted in Fig. 3. The relative decrease in FES uptake and its relationship with response are shown in Fig. 4. Receiver operating characteristic (ROC) analysis was performed to evaluate whether a threshold other than the predefined 75% would increase the predictive value. ROC analysis showed that the optimal cutoff point (−76%) was close to our predefined threshold of −75%. In a lesion-based analysis, no association between changes in FES uptake in the tumor and size changes on CT scan was detected for the small number of measurable lesions (\( n = 13 \)).

**Correlation between Tumor FES Uptake and Plasma Fulvestrant Levels**

To evaluate whether changes in tumor FES uptake correspond to individual patient plasma fulvestrant levels, blood was drawn on the same day as the PET scan. Plasma fulvestrant levels were determined by liquid chromatography/tandem mass spectrometry (LC/MS-MS). Median patient fulvestrant plasma levels were 33 nmol/L at day 28 and 27 nmol/L at day 84 of treatment (Supplementary Fig. S1A). Plasma fulvestrant levels varied between patients (16–53 nmol/L). Although median fulvestrant levels were slightly higher in patients who had clinical benefit from fulvestrant compared with patients with PD (39.6 vs. 29.4 nmol/L), plasma fulvestrant levels did not correlate with absolute or relative changes in either SUV max or SUV cor at day 28 or at day 84. Apparent serum estradiol levels increased by 0.19 ± 0.05 nmol/L due to cross-reactivity with fulvestrant. The actual serum estradiol levels, corrected for effects of fulvestrant, were 0.06 ± 0.02 nmol/L at day 28 and 0.06 ± 0.03 nmol/L at day 84, and hence remained in the postmenopausal range (Supplementary Fig. S1B).

**Tamoxifen Effect on Baseline PET Measures**

Although it was not prespecified as a study endpoint, we did observe an impact of recent therapies on baseline scan results. This information could allow further optimization of FES-PET/CT study protocols (9). All 4 patients who withdrew from tamoxifen treatment shortly (5–6 weeks) before baseline FES-PET/CT had lower FES uptake when compared with patients who did not recently use tamoxifen (median SUV max, 1.7 vs 4.1; \( P = 0.004 \)). Coincidentally, an earlier FES-PET that was performed during treatment with an aromatase inhibitor was available for 2 of these 4 patients (14).

One patient had clinical benefit despite a reduction in FES uptake of only 32%. In this patient, baseline SUV max was
Figure 3. Pretreatment (squares) and posttreatment (diamonds) background-corrected tumor FES uptake (SUV_{cor}) for all individual patients. Patients are grouped according to their response, in blue patients with partial response and SD, and in red patients with PD. In two patients with recent tamoxifen therapy, a previous baseline PET was available while on aromatase inhibitor therapy. The green squares and diamonds represent the values if the previous PET would have been used as baseline measure.

Figure 4. Waterfall plot showing the relative change in median tumor FES uptake (SUV_{cor}) in individual patients at the second scan compared with baseline. The predefined 75% reduction threshold is indicated. Patients who used tamoxifen until 5 weeks before baseline PET are indicated (*). In two patients with recent tamoxifen therapy, a previous baseline PET was available while on aromatase inhibitor therapy. The shaded bars represent the values if the previous PET would have been used as baseline measure.
In the current study, whereas previous FES uptake (during aromatase inhibitor therapy) was much higher (SUV\text{max}, 3.1) and more tumor lesions were visible. If the scan while on aromatase inhibitor was used as the baseline measure, a median 100% decrease in FES uptake would have been observed during fulvestrant therapy. This would have correctly identified this patient as a responder. The second patient who used tamoxifen until 5 weeks before baseline PET had similarly low uptake at baseline (SUV\text{max}, 1.8). Paradoxically, fulvestrant induced a median 60% increase in FES uptake in this patient. FES uptake at the earlier scan during aromatase inhibitor therapy was higher (median SUV\text{max}, 2.8). If this scan was used as the baseline scan, FES uptake would have decreased by 26% during fulvestrant treatment, hence correctly identifying this patient as nonresponder. Together, these results suggest that tamoxifen and its metabolites partly block FES uptake in the tumor, even after a 5-week drug-free period.

If patients with residual tamoxifen effects were excluded from our analysis, fulvestrant would have decreased median FES uptake in all patients by 86%, by 91% in patients with clinical benefit (n = 7 patients), and ~58% in nonresponding patients (n = 4 patients). Four of 12 patients (33%) would have had incomplete reduction in ER availability. Seven of 8 patients (88%) with >75% decrease in FES uptake would had clinical benefit from fulvestrant therapy, compared with none of 4 patients with incomplete reduction in FES uptake. When data adjusted for previous tamoxifen use were included, the positive predictive value increased to 89% and negative predictive value remained at 100%.

**DISCUSSION**

This is the first serial PET imaging study evaluating the effects of fulvestrant 500 mg on FES uptake in patients with metastatic breast cancer. The fulvestrant-induced reduction in FES uptake in the tumor varied widely and was inadequate in some patients: incomplete reduction in FES uptake after 4 weeks was observed in 38% of the patients.

In contrast to tamoxifen, fulvestrant is a pure ER antagonist that can downregulate ER expression in a dose-dependent fashion without any agonistic effects. Therefore, FES-PET is a very suitable technique to study the ability of fulvestrant to reduce ER availability and to correlate this parameter with treatment response.

Previous studies provided FES-PET data for patients treated with tamoxifen (10, 12). For example, in a study in 40 patients, after 7 to 10 days of tamoxifen therapy, FES uptake decreased by 55% in patients having clinical benefit compared with 19% in nonresponding patients (12). However, this incomplete reduction in FES uptake should not lead to the conclusion that tamoxifen dosing is suboptimal, as it can take several weeks for tamoxifen to reach steady-state levels.

A retrospective study in 11 patients who underwent fulvestrant therapy showed a mean decrease of 49% in FES uptake in tumors (10). This study differed from our current study in several respects. Patients had received lower fulvestrant doses, that is, 250 mg or 500 mg at day 1, followed by 250 mg at days 14 and 28 and every 4 weeks thereafter. Moreover, only one follow-up scan was performed, between 1 and 18 weeks after therapy initiation, and the FES-PET scans were not synchronized with fulvestrant injections. This could have affected the results, because plasma fulvestrant levels can vary 10-fold between two doses (15). We therefore performed FES-PET at stringent time points in all patients, concurrent with determination of plasma fulvestrant levels.

In our study, as in other FES-PET studies, patients were required to discontinue tamoxifen at least 5 weeks before baseline FES-PET to prevent competitive binding (14). However, the lowest median FES uptake was recorded in the 4 patients who used tamoxifen until 5 weeks before FES-PET. In two of these patients, an earlier FES-PET scan was available while they were still on aromatase inhibitor treatment. These PET scans showed more tumor lesions and a higher median SUV\text{max}. It is likely that a generally used 5-week stopping period for tamoxifen is too short to reliably measure baseline ER expression by FES-PET. The reported half-life for tamoxifen is 4 days, but this can be longer for its metabolites with a half-life of even 400 days for N-desmethyltamoxifen (16). Therefore, after discontinuation of tamoxifen there may still be residual effects of tamoxifen metabolites in the tumor for over 5 weeks. Interestingly, we did not observe a correlation between plasma drug levels in individual patients and effects on tumor FES uptake. Thus, measuring plasma fulvestrant levels does not provide information on whether the dose is sufficient for optimal effect at the tumor site. Fulvestrant effects at the tumor site could possibly depend not only on plasma levels, but also on various other factors (e.g., tumor size, vascularization, ER levels, and presence of mutations in the ligand-binding domain of the ER), which could explain the lack of correlation.

The effect of fulvestrant on tumor ER availability could be visualized after only two doses of fulvestrant. In our feasibility study with a small number of patients, a reduction in FES uptake larger than 75% was significantly associated with clinical benefit. Because it takes several months before therapy effects can be reliably measured by anatomic imaging techniques, such as CT scan (17), earlier prediction of treatment response would be valuable. Moreover, ER-positive breast cancer is characterized by bone-dominant disease (18), as was also evident in this study. It is notoriously difficult to evaluate treatment response in bone lesions, and therefore they are considered nonmeasurable by RECIST v1.1 criteria. Therefore, future studies should address whether serial FES-PET can offer an early response measurement in patients with bone-dominant disease.

Our study has some limitations. First, given the character of the study, the sample size was relatively modest. Because of PD and treatment withdrawal, a third scan was available in only 9 patients. In 4 patients, the second scan was delayed for logistic reasons. However, the scans were still performed on the same pharmacokinetic trough, that is, just before dosing of fulvestrant. Given the fact that tumor FES uptake remained stable between scan 2 and scan 3 in the 8 patients with three serial scans available on the exact predetermined scan times, this delay likely did not affect our results. Second, from our data it is difficult to address the effects of FES-negative lesions on fulvestrant efficacy. Given the heavy pretreatment of the patients in our study, CT scan may show bone lesions that are no longer active. This might potentially lead to an overestimation of FES-negative sites (SUV\text{max} <1.5).
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at baseline. On the other hand, CT is relatively insensitive for bone metastases, and therefore some FES-negative sites may have been missed. Others have used FDG-PET together with FES-PET for the identification of FES-negative lesions (19). However, FDG-PET can fail to visualize osteoblastic bone metastases (20). Third, correction of FES uptake for recent tamoxifen treatment using an earlier FES-PET should be regarded as exploratory, as temporal changes in ER expression cannot be excluded. Finally, we did not have access to serial tumor biopsies, which could have enabled us to discriminate between decrease in FES uptake due to downregulation of ER expression and occupancy of ERs with preserved expression. However, ER might also be heterogeneously expressed across tumor lesions within a patient.

We observed that patients with incomplete reduction in FES uptake were more likely to develop PD within 24 weeks of therapy initiation. A recent phase I study on the antiandrogen ARN-509 showed that increasing doses resulted in a plateau in which >90% of tumor androgen uptake was blocked as measured by [18F]fluorodihydrotestosterone (FDHT) PET (21). If we had applied the −90% threshold, only 4 (25%) of 16 patients would have obtained complete reduction in tumor ER uptake. Given the resemblance between fulvestrant and ARN-509—both are nuclear hormone receptor antagonists—and between FES-PET and FDHT-PET, it may be possible that >90% reduction in FES uptake can be obtained with higher doses of fulvestrant. This may also be clinically feasible. In a randomized phase III study comparing fulvestrant 500 mg to 250 mg, toxicity was equally mild in both groups (5, 22). In addition, in a neoadjuvant study in premenopausal patients, a single injection with fulvestrant 750 mg was well tolerated (23). As an alternative to an increased dose, a higher frequency of administration (e.g., every 2 or 3 weeks) might also be an option. However, our results suggest that not all patients need a higher dose, because fulvestrant reduced ER availability sufficiently in 62% of the patients. Conversely, not all patients with complete reduction in ER availability will experience clinical benefit, because other mechanisms aside from inadequate dosing can be responsible for therapy failure. Among these potential mechanisms are ESR1 mutation and upregulation of growth factor receptor pathways (24).

The optimal drug dose that leads to complete reduction in ER availability could be verified with FES-PET. This approach would clearly differ from the current approach for endocrine drugs, where the principle “one-dose-fits-all” is applied, despite the fact that serum and tissue drug levels can vary considerably between patients and at different doses (25). Some authors have therefore suggested therapeutic drug monitoring and dose escalation based on serum or plasma drug levels (26). In addition, FES-PET could prove useful during drug development to evaluate the dose required for optimal ER downregulation for new compounds that block or degrade ER.

METHODS

The study was conducted at the University Medical Center Groningen (UMCG; Groningen, the Netherlands) in accordance with the principles of Good Clinical Practice and the Declaration of Helsinki. The UMCG Institutional Review Board approved the protocol, and patients provided written informed consent before participation. The study was registered in the clinicaltrials.gov database (NCT01377324).

Study Population

Medical oncologists in our center and our referring nonacademic hospitals identified patients for the imaging study. They get regular updates about ongoing trials in our center and have Web-based access to summaries of ongoing trials. Postmenopausal patients with ER-positive metastatic breast cancer were eligible for the study when they had PD after two or three lines of palliative hormonal therapy. Other eligibility criteria were ECOG performance ≤52, life expectancy >3 months, and a creatinine clearance >30 mL/min. Exclusion criteria were previous fulvestrant therapy, the presence of life-threatening visceral metastases, central nervous system metastases, and more than two lines of palliative chemotherapy. To prevent competition between FES and drugs from previous therapies, the patients were required to discontinue drugs known to bind ER for at least 5 weeks before baseline PET imaging (14).

Study Design

We performed an imaging trial in patients on salvage endocrine therapy with standard fulvestrant dosing. The patients were imaged just before each fulvestrant administration. The aim of this feasibility study was to evaluate the effects of fulvestrant on ER availability. The primary endpoint was to evaluate the number of patients and lesions in which fulvestrant incompletely abolished tumor ER availability. Secondary endpoints were the following: (i) the correlation between FES-PET results, plasma fulvestrant levels, and treatment outcome; (ii) the heterogeneity of FES uptake among lesions and between individuals; and (iii) the feasibility of quantifying liver lesions by FES-PET.

Study Measurements

All patients were treated uniformly with fulvestrant 500 mg intramuscularly on days 1, 14, 28, and every 4 weeks thereafter. Baseline measurements included recording of symptoms, performance status, physical examination (including size and weight), laboratory tests (including hormonal status and tumor markers), and a diagnostic CT scan. Clinical follow-up included clinical history, performance status, physical examination, and laboratory tests, and was performed every 4 weeks. Follow-up of serum tumor marker (CA15.3) and diagnostic CT scans was performed after 84 days (concurrently with FES-PET), after 6 months, and thereafter when progression was suspected on the basis of clinical assessment or biochemistry. Response assessment was performed by radiologic imaging using RECIST v1.1 criteria, as well as clinical and biochemical parameters. Patients were considered to have radiologic PD when they had >20% increase in measurable lesions, when new lesions were detected during follow-up, or when there was unequivocal progression of existing lesions (11). Clinical PD could also develop before radiologic response assessment or in the setting of radiographic SD. In these instances, PD was defined as an overall level of substantial worsening such that the overall tumor burden, biochemistry (e.g., tumor markers and liver function), and/or complaints increased sufficiently to merit discontinuation of therapy (27). Final response classification was done by the treating medical oncologist while blinded for FES-PET results. All patients without PD for at least 24 weeks were classified as having clinical benefit derived from fulvestrant (5). Treatment was continued until PD, withdrawal of consent, or severe toxicity. Adverse events were documented and graded during the first 6 months of therapy, according to the Common Terminology Criteria of Adverse Events (version 3.0).

Pharmacodynamic Biomarker

In vivo ER availability was assessed by FES-PET/CT on a hybrid PET/CT camera with a 64-slice CT and high-definition time-of-flight PET (Siemens Medical Systems). FES was produced and administered...
to the patient as described earlier (14, 28). The mean radiochemical yield FES was 30% ± 17%, with a radiochemical purity of 99.9% ± 0.2%, and a specific activity of 209 ± 112 GBq/μmol. The mean injected dose of 18F-FES was 204 ± 23 MBq. A few hours before the next fulvestrant dose, FES-PET/CT was performed at baseline (scan 1), day 28 ± 2 (scan 2), and 84 ± 2 (scan 3). The first and third PET scans were combined with a diagnostic CT scan as part of the staging. FES-PET scan 2 was combined with a low-dose CT for attenuation correction.

FES uptake was quantified at the three time points for tumor lesions detected by PET/CT at baseline, according to the guidelines of the European Association of Nuclear Medicine (EANM; ref. 29). FES uptake for individual lesions was expressed as SUV\text{max}. We used the previously published threshold of SUV\text{max} ≥ 1.5 to define FES-positive lesions (9, 27). When calculating the relative change in FES uptake, only FES-positive lesions were included. FES uptake was also expressed as SUV\text{max}corrected for physiologic background (SUV\text{cor}; ref. 30). Background correction was applied by using the unaffected contralateral site whenever available, or surrounding tissue of the same origin. Liver lesions were excluded from quantitative analysis given the high physiologic background of FES uptake in healthy liver tissue.

We observed that patients who had used tamoxifen until 5 weeks before FES-PET had a very low tumor FES uptake. Therefore, for an exploratory analysis, we used an earlier FES-PET scan while the patients were on aromatase inhibitor therapy as a baseline measure to correct for tamoxifen effects in two patients. Because this resulted in not only higher FES uptake, but also the identification of more FES-avid lesions, we calculated the decline in FES uptake for all lesions.

For patient-based analysis, the median SUV\text{max} and median SUV\text{cor} were calculated up to an arbitrary maximum of 20 lesions. Incomplete reduction in ER availability in a patient was defined as a relative decrease in FES uptake of less than 75% in median tumor lesion SUV\text{cor} and an absolute median tumor lesion SUV\text{max} > 1.5. The 75% cutoff point was chosen on the basis of an earlier approximately 50% decrease with the lower (250 mg) dose of fulvestrant in a retrospective study (10).

PK Assessments

Plasma fulvestrant levels were determined by LC/MS-MS on the same days as scan 2 and scan 3. Serum estradiol levels were determined by fluorescent immunoassay concurrently with scan 1, scan 2, and scan 3. Because fulvestrant is known to cause cross-reactivity with the estradiol assay, calibration curves were obtained to assess the increase in apparent serum estradiol levels for increasing doses of fulvestrant (Supplementary Fig. S2). This calibration curve was used to correct the measured estradiol levels in patient serum for the measured fulvestrant plasma levels on LC/MS-MS. Plasma fulvestrant levels and corrected serum estradiol levels were correlated with findings on FES-PET.

Statistical Analysis

We aimed to include 15 patients to provide an estimate of the proportion of patients with incomplete reduction in FES tumor uptake, defined as described previously (relative decrease <75% in median lesion SUV\text{cor}, and absolute median tumor SUV\text{max} > 1.5). Statistical analysis was performed in IBM SPSS Statistics version 20.0. Tumor FES uptake was tested for normality. The Wilcoxon signed-rank test was used to evaluate changes in FES uptake between scans 2 and 3, and the Mann-Whitney \text{U} test for changes between patients having clinical benefit from fulvestrant versus patients with PD. Receiver operating characteristics analysis was performed to evaluate the optimal threshold to predict therapy outcome by FES-PET.

Disclosure of Potential Conflicts of Interest

M. Brown reports receiving a commercial research grant from Novartis and is a consultant/advisory board for the same. No potential conflicts of interest were disclosed by the other authors.

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Measuring Residual Estrogen Receptor Availability during Fulvestrant Therapy in Patients with Metastatic Breast Cancer

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