Molecular imaging of estrogen receptors
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Chapter 5

Imaging ER density to guide high dose estrogen therapy in hormone refractory breast cancer patients

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(Submitted)
ABSTRACT

Whereas anti-estrogen therapy is widely applied to treat estrogen receptor (ER) positive breast cancer, paradoxically, also estrogens can induce tumor regression. Up-regulation of ER expression is a marker for estrogen hypersensitivity. We therefore performed an exploratory study to evaluate positron emission tomography (PET) with the tracer 16α-[18F]fluoro-17β-estradiol (18F-FES) as potential marker to select breast cancer patients for estradiol therapy.

Eligible were patients with acquired endocrine-resistant metastatic breast cancer, who progressed after ≥2 lines of endocrine therapy. All patients had prior ER positive histology. Treatment consisted of estradiol 2 mg, 3 times daily orally. Patients underwent 18F-FES-PET/CT imaging at baseline. Tumor 18F-FES uptake was quantified for a maximum of 20 lesions and expressed as maximum standardized uptake value (SUV_{max}). CT-scan was repeated every 3 months to evaluate treatment response. Clinical benefit was defined as time to radiologic or clinical progression ≥24 weeks. Serum tumor markers (CA15.3 and CEA) and serum bone turnover markers were serially assessed.

18F-FES uptake, quantified for 255 lesions in 19 patients, varied greatly between lesions (median 2.8; range 0.6–24.3) and between patients (median 2.5; range 1.1–15.5). Seven (37%) patients experienced clinical benefit of estrogen therapy, eight progressed (PD), and four were non-evaluable due to side effects. The positive and negative predictive value (PPV/NPV) of 18F-FES-PET using SUV_{max} >1.5 were 60% (95% CI: 31–83%) and 80% (95% CI: 38–96%) respectively. Combining 18F-FES-PET/CT with tumor marker response and bone turnover markers resulted in a PPV of 100%.

The high NPV of 18F-FES-PET for response to estradiol therapy deserves further exploration. Taking into account tumor markers and bone turnover markers alongside with 18F-FES-PET may aid to obtain also a high PPV.
INTRODUCTION

Until the introduction of tamoxifen, additive estrogens such as the synthetic diethylstilbestrol (DES) were considered the hormonal treatment of choice in postmenopausal women. In a randomized study in 143 postmenopausal patients with metastatic breast cancer, first line endocrine therapy with DES was equally effective as tamoxifen with a response rate of 41% vs. 33%. Yet, tamoxifen became the preferred agent because it showed fewer side effects. An emerging number of anti-estrogen therapies have become available since. Recently, however, additive estrogen therapy has regained interest by showing efficacy in ~35% of patients that are extensively pre-treated with anti-estrogens. Interestingly an update of the randomized study showed a superior 5-year survival for DES compared to tamoxifen (35% vs. 16%) after 14 years of follow-up. Moreover, in a recent study a lower dose of only 6 mg estradiol rendered similar clinical benefit rates as 30 mg estradiol with fewer side effects. Finally, clinical results suggest that estrogens can restore the sensitivity to anti-estrogens. As the majority of patients will not benefit from additive estrogen therapy a biomarker for patient selection would be helpful.

In preclinical studies, long-term estrogen deprivation triggered hypersensitivity to estrogens, which is accompanied by a 5 to 10-fold increase in ER expression. Thus, patients that have been treated with anti-estrogens for a long time may likewise have become hypersensitive to estrogens. If so, patients that are most likely to benefit from estradiol therapy could potentially be identified by high tumor ER expression. Positron emission tomography (PET) with 16α-[18F]fluoro-17β-estradiol ([18F]-FES) can visualize and quantify ER expression in breast cancer lesions. The aim of this exploratory study was therefore to evaluate [18F]-FES-PET as a potential marker to select breast cancer patients for estradiol therapy.

In the setting of ER positive metastatic breast cancer, response assessment is notoriously difficult due to the high incidence of bone metastases. Bone is the most common site affected in breast cancer. However, bone metastases are regarded non-measurable by the response evaluation criteria in solid tumors (RECIST). This underlines the need for objective measures to predict and evaluate response, for example by molecular imaging techniques. Recent studies have shown that [18F]-FES-PET can predict response to various forms of anti-estrogen therapy. Its value as a biomarker for additive estrogen therapy is however unknown.

In addition to molecular imaging techniques, also serum markers may be valuable to assess response in patients with bone-dominant disease in which response assessment is difficult. The ASCO recommendations for the use of tumor markers in breast cancer indicate that
CA 15.3 and CEA can be considered to monitor treatment effects. Also, bone turnover markers such as procollagen type I amino-terminal propeptide (PINP), carboxyl-terminal telopeptide of type I collagen (CTx), and bone alkaline phosphatase (BALP) are reported to correlate with the number and size of bone metastases in breast and prostate cancer. Therefore, in addition to $^{18}$F-FES-PET, we also evaluated whether tumor markers, and bone turnover markers can aid response prediction.

**METHODS**

**Patients**

The trial was conducted in accordance with the principles of Good Clinical Practice and the Declaration of Helsinki. The protocol was approved by the local medical ethical committee and registered in the ClinicalTrials.gov database (NCT01088477). All patients provided written informed consent.

Eligible patients had acquired endocrine-resistant advanced breast cancer showing progression ≥2 lines of endocrine therapy. All patients had earlier ER positive immunohistochemical tumor staining, and were required to have responded to at least one prior line of anti-hormonal therapy (objective response, or stable disease ≥6 months). Other eligibility criteria were ECOG performance ≤2 and life expectancy ≥3 months. Exclusion criteria were the presence of symptomatic central nervous system lesions, a history of thrombosis, diabetes mellitus, uncontrolled hypercalcemia, treatment with investigational drugs within 30 days before the start of study, dyspnoea at rest due to any cause, and class III or IV congestive heart failure according to the New York Heart Association. Patients were required to withdraw drugs known to bind ER for at least 5 weeks prior to baseline imaging.

**Estradiol Treatment**

Patients were treated with estradiol three times daily 2 mg orally. Therapy was initiated within 4 days after $^{18}$F-FES-PET/CT. In case of toxicity estradiol dosing was reduced to twice daily 2 mg, or shortly interrupted with re-introduction at a lower dose when the symptoms had resolved. Therapy was continued until progressive disease (PD) by radiologic or clinical assessment, withdrawal of consent, or severe toxicity. Toxicity was documented according to the Common Terminology Criteria of Adverse Events v3.0.

**Assessment of Treatment Response**

Baseline measurements included documentation of all symptoms, performance status, physical examination, laboratory tests (including blood counts, kidney function, and liver enzymes), and a diagnostic CT-scan. Clinical follow-up with documentation of symptoms,
performance status, physical examination and laboratory tests were done monthly. A diagnostic CT-scan was performed every 3 months until progression. For patients with measurable disease, response was defined according to RECIST v1.1. Patients with only non-measurable lesions were considered to have PD when there was unequivocal progression of existing lesions or when new lesions were detected at follow-up. In the absence of radiological PD, patients could develop clinical PD, defined as an overall level of substantial worsening such that the overall tumor burden or complaints increased sufficiently to merit discontinuation of therapy. In reference to other studies, patients with time-to-progression ≥24 weeks were considered to have obtained clinical benefit from estradiol therapy.

**Study Measurements**

$^{18}$F-FES was produced and administered to the patient as described earlier. On average $3.4 \pm 1.5$ GBq $^{18}$F-FES was obtained with 100% radiochemical purity and a $325 \pm 274$ GBq/µmol specific activity. Patients received approximately 200 MBq $^{18}$F-FES intravenously. $^{18}$F-FES-PET/CT to evaluate tumor ER expression was performed at baseline on a hybrid PET/CT camera with a 64-slice CT and high definition and time-of-flight PET (Siemens Medical Systems). Low dose CT scan was used for attenuation correction in all patients. Patients were scanned from skull to mid-thigh, 3 minutes per bed position (usually 7-8 bed positions per patient). In all patients, baseline $^{18}$F-FES-PET was combined with a contrast-enhanced diagnostic CT scan. For representative $^{18}$F-FES-PET, CT and $^{18}$F-FES-PET/CT images see figure 1.

Tumor $^{18}$F-FES uptake was quantified according to the guidelines of the European Association of Nuclear Medicine (EANM). Whole-body CT-scan was used to allocate tumor lesions and identify possible $^{18}$F-FES negative lesions. Lesion $^{18}$F-FES uptake was expressed as maximum standardized uptake value ($SUV_{max}$). For patient-based analysis, the median $^{18}$F-FES uptake of an arbitrary maximum of 20 lesions was calculated. Quantification of tumor $^{18}$F-FES uptake was performed while blinded for treatment outcome. Patients and treating physician were held blinded for $^{18}$F-FES-PET results.

Tumor markers (CA-15.3, CEA) and bone turnover markers (PINP, CTx and BALP) were also determined at baseline, and repeated every 3 months or at the time of progression. Patients were considered evaluable for tumor marker response if one of both tumor markers were increased at baseline (CA15.3 >33 kU/L, CEA >5 µg/L). A 10% decrease in tumor marker was scored as -1, an increase of 10% as +1 and between -10 and +10% was scored 0. A sum of scores of <1 was defined as biochemical tumor marker response, and a sum of scores ≥1 as non-response. Patients were considered evaluable for bone turnover markers when they
had evidence of bone metastases on imaging. Serum PINP >95 ng/mL was considered the threshold for increased bone turnover based on literature. For CTx and BALP the optimum thresholds were determined by receiver operating characteristic (ROC) analysis.

**Statistical Analysis**

The expected study time frame was 3 years for inclusion of 50 patients, to evaluate the positive predictive value (PPV) and negative predictive value (NPV) of $^{18}$F-FES-PET/CT by ROC analysis. After 3 years and 21 patients included, the study was terminated. We here report the PPV and NPV for $^{18}$F-FES-PET/CT, which was the predefined primary end point of the study. PPV and NPV were calculated using a ROC analysis for the median tumor SUV$_{\text{max}}$ in patients. The secondary end point was the association between biochemical tumor markers.
Table 1: Patient characteristics

<table>
<thead>
<tr>
<th>Baseline characteristics</th>
<th>All patients (n=19)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male : female</td>
<td>1 : 18</td>
</tr>
<tr>
<td>Age, mean years (range)</td>
<td>57 (36-76)</td>
</tr>
<tr>
<td>Site of metastases, n</td>
<td></td>
</tr>
<tr>
<td>Bone + visceral</td>
<td>14</td>
</tr>
<tr>
<td>Bone-only</td>
<td>3</td>
</tr>
<tr>
<td>Visceral-only</td>
<td>2</td>
</tr>
<tr>
<td>Measurable lesions, n</td>
<td></td>
</tr>
<tr>
<td>Measurable visceral</td>
<td>12</td>
</tr>
<tr>
<td>Non-measurable visceral</td>
<td>4</td>
</tr>
<tr>
<td>Bone-only</td>
<td>3</td>
</tr>
<tr>
<td>Prior systemic therapies, n</td>
<td></td>
</tr>
<tr>
<td>&lt;3 lines</td>
<td>1</td>
</tr>
<tr>
<td>3 or 4 lines</td>
<td>11</td>
</tr>
<tr>
<td>≥5 lines</td>
<td>7</td>
</tr>
<tr>
<td>Menopausal status, n</td>
<td></td>
</tr>
<tr>
<td>Postmenopausal</td>
<td>13</td>
</tr>
<tr>
<td>Following ovariectomy</td>
<td>3</td>
</tr>
<tr>
<td>Goserelin treatment</td>
<td>2</td>
</tr>
</tbody>
</table>

and bone turnover markers and benefit from estradiol, calculated by a Mann-Whitney U test. PPV and NPV were determined for these markers alone and in combination with \(^{18}\)F-FES-PET/CT findings. Analyses were performed in SPSS Statistics version 20.0.

RESULTS

Patient Characteristics
Between May 2010 and May 2013, 30 patients were screened for participation in the trial, out of whom 21 were included and 19 started estradiol therapy, one male and 18 females. Mean age was 57 years (range 36–76). Seventeen patients had bone metastases, in 14 patients accompanied by also visceral or nodal metastases. Two patients had only visceral lesions. All patients had postmenopausal status, which was in two patients achieved by the use of LHRH agonists, while others were truly postmenopausal. Tumor histology was positive for ER in all patients, 12 (63%) were also PR positive, and none were HER2 positive. All patients were heavily pre-treated; 11 patients had already received 3-4 lines of systemic therapy, and 7 patients ≥5 lines. Patient characteristics are summarized in table 1. For an overview of screening, inclusion and exclusion see the CONSORT diagram (figure 2).

Tumor Response
Twelve patients had measurable lesions on baseline CT according to RECIST, four patients had non-measurable visceral lesions and three patients had only bone metastases. Four of
19 (21%) discontinued estradiol because of side effects and were therefore not evaluable for treatment response.

Seven of the remaining 15 patients experienced clinical benefit from estradiol therapy as indicated by stable disease ≥24 weeks. Four had radiological measurable stable disease, and three patients had no new lesions detected on radiological examination, no progression of non-measurable lesions, improvement or stabilization of symptoms, and no evidence of biochemical progression ≥24 weeks. They eventually experienced PD according to RECIST criteria at 26, 28 and 48 weeks respectively.

Finally, eight patients had PD; in five of them there was radiologic PD and in three patients there was substantial clinical deterioration, meriting discontinuation of therapy. One of them had laboratory signs of bone marrow invasion, confirmed with a biopsy, one had rising liver function tests, a 3-fold increase in tumor marker CA15.3 and clinical deterioration, and one
patient had deterioration of pain symptoms from bone lesions, rising alkaline phosphatase and worsening of performance score. Overall clinical benefit rate was 37% in all treated patients (intention-to-treat; n=19 patients). Mean progression-free-survival was 4.7 months (range 0.4–15.3 months). Estradiol therapy induced an increase in serum estradiol levels from 89 ± 15 pmol/L at baseline to 1241 ± 225 pmol/L after 1 month of therapy. The increase in estradiol levels was equal among responders and non-responders.

Toxicity
In four patients (21%) estradiol therapy was terminated prematurely due to adverse events. These side effects were progressive thrombocytopenia (n=1), transient ischemic accident with atrial fibrillation (n=1), mood disorders (n=1) and signs of congestive heart failure (n=1). Other grade 3 serious adverse events requiring hospital admission were tumor flare (n=1), hypercalcemia (n=2), pneumonia (n=1) and atrial fibrillation (n=1). The patient who experienced a tumor flare had rapid increase of pain symptoms at known metastatic sites, starting already the day after initiation of estrogen therapy. Laboratory results were suggestive of tumor flare with increased lactate dehydrogenase and other liver enzymes. Symptoms and laboratory findings resolved after estradiol discontinuation. Interestingly, this patient with grade 3 clinical flare reaction showed highest tumor uptake of 18F-FES (median SUVmax 15.5, maximum SUVmax 24.3). Common but manageable grade 1-2 adverse events were tumor flare, fatigue, nausea, and vaginal bleeding.

Predictive Value of 18F-FES-PET for Response to Estradiol Therapy
18F-FES uptake in tumor lesions was quantified for a total of 255 lesions (214 bone; 24 lung; 12 lymph nodes; 1 breast; 1 soft-tissue; and 1 brain lesion) out of which 42 (16%) were 18F-FES negative (SUVmax <1.5). Twelve out of 19 patients (63%) had only 18F-FES positive lesions, six (32%) had both 18F-FES positive and 18F-FES negative lesions, and one had only 18F-FES negative lesions. Absolute 18F-FES uptake (SUVmax) varied widely between lesions (median 2.8; range 0.6–24.3) and patients (median 2.5; range 1.1–15.5), as is depicted in figure 3. ROC analysis indicated that the most optimum threshold to differentiate between patients with clinical benefit and patients with PD was a median SUVmax of >1.5. This threshold produced a PPV of 60% (95% CI: 31–83%) and a NPV of 80% (95% CI: 38–96%) (figure 4A), with an area under curve of 0.62.

Nine patients terminated treatment with ER antagonists 5 weeks before initiating estrogen therapy. Three patients of them had an earlier 18F-FES-PET obtained in another study (NCT01377324). These patients had much lower 18F-FES uptake than on the earlier scans and several lesions could no longer be observed. For example, one patient had on earlier 18F-FES-PET a median tumor 18F-FES uptake of 6.5, while in the current study SUVmax was only
1.1. This patient benefited from estradiol despite the relatively low tumor $^{18}$F-FES uptake. The remaining six patients had no earlier scans available, but also had relatively low tumor $^{18}$F-FES uptake compared to patients without recent use of drugs that can bind ER. Thus, ER antagonists may reduce tumor $^{18}$F-FES uptake beyond the currently used 5-week drug withdrawal period. In an explorative analysis, using the results of the previous $^{18}$F-FES-PET scans instead of the current PET scans, the PPV and NPV increased to 64% and 100%, respectively (figure 4B).

**Additive Value of Serum Markers**

Two patients had normal tumor markers at baseline, leaving 17 patients evaluable for tumor markers. A response was seen in seven patients. The PPV and NPV for tumor marker response were 67% and 71%, respectively. Tumor marker response added to $^{18}$F-FES-PET results provided a PPV of 100% for $^{18}$F-FES positive patients with a tumor marker response and a NPV of 66% for $^{18}$F-FES negative patients with a non-response in tumor markers (table 2).
Table 2: Association between 18F-FES-PET, tumor markers, bone turnover markers, and treatment outcome

<table>
<thead>
<tr>
<th>Marker</th>
<th>Result</th>
<th>n</th>
<th>Response Classification</th>
<th>Predictive value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>CB</td>
<td>PD</td>
</tr>
<tr>
<td>18F-FES-PET*</td>
<td>Positive</td>
<td>10</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>5</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>Tumor marker</td>
<td>Response</td>
<td>6</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Non response</td>
<td>7</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Not evaluable</td>
<td>2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Combined PET + tumor marker</td>
<td>PET+/ Response</td>
<td>4</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>PET-/ No response</td>
<td>3</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Bone marker</td>
<td>Normal</td>
<td>5</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Increased</td>
<td>8</td>
<td>1</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>Not evaluable</td>
<td>2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Combined PET + Bone marker</td>
<td>PET+/ normal PINP</td>
<td>5</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>PET+/ increased PINP</td>
<td>4</td>
<td>0</td>
<td>4</td>
</tr>
</tbody>
</table>

**Abbreviations:** CB = clinical benefit, PD = progressive disease, NE = non-evaluable, PPV = positive predictive value, NPV = negative predictive value,
* The optimum threshold determined by receiver operating characteristic (ROC) analysis was used to define 18F-FES-PET positivity/negativity (SUV$_{max} >$1.5).

Bone turnover markers were explored as potential effect sensors in patients with bone metastases. Mean levels were 175 ng/mL for PINP (range 17–613 ng/mL), 90 U/L for BALP (range 27–298 U/L), and 467 pg/mL for sCTx (range 26–1369 pg/mL). Change in bone turnover markers was not associated with treatment response. However, the baseline
values were strongly associated with time-to-progression. Baseline PINP levels were 85 vs 234 ng/mL ($P = 0.032$), and sCTX levels 195 pg/mL vs. 623 pg/mL ($P = 0.032$) in patients with clinical benefit and PD, respectively. The PPV and NPV for PINP were 100% (5/5 patients) and 88% (7/8 patients) for PINP ≤95 ng/mL. PPV and NPV were 100% (4/4 patients) and 78% (7/9 patients) for sCTX levels >200 pg/mL. BALP levels were non-informative in this exploratory study. The addition of PINP to $^{18}$F-FES-PET/CT results increased both PPV and NPV values to 100%.

**DISCUSSION**

This is the first exploratory study evaluating $^{18}$F-FES-PET/CT as predictive marker for estradiol therapy in patients with metastatic endocrine resistant breast cancer. While the mechanism of anti-estrogen therapy is well known, this is not the case for the addition of estrogens. Based on preclinical data, we hypothesized that very high $^{18}$F-FES uptake would predict response to estradiol therapy. The value of $^{18}$F-FES-PET, however, turned out to be especially its ability to identify patients that are unlikely to benefit from estradiol therapy as a result of low or absent $^{18}$F-FES uptake in metastases.

There are currently no good upfront predictive biomarkers to select patients for estradiol therapy. Assessing ER status by a biopsy is the current golden standard, but is sometimes unreliable due to heterogeneous ER expression within and among lesions, and detection of non-functional ER. $2'-[^{18}$F$]fluoro-2'-deoxyglucose (^{18}$F-FDG) PET imaging has been tested to predict response to estradiol therapy. A study randomized 66 patients to 6 or 30 mg estradiol daily, 43 patients underwent $^{18}$F-FDG-PET imaging before and 24 h after the initiation of estrogen therapy. A metabolic flare reaction upon estradiol therapy, predefined as a ≥12% increase in tumor $^{18}$F-FDG uptake, had a PPV of 80% (12 of 15 patients) and a NPV of 87% (27/31 patients) for response to estradiol therapy. Metabolic flare on $^{18}$F-FDG-PET in 51 patients subsequently treated with an aromatase inhibitor or fulvestrant had an even higher PPV and NPV of 100% and 94%. $^{18}$F-FES-PET was evaluated earlier in three studies as predictive biomarker before the initiation of aromatase inhibitors, tamoxifen or fulvestrant. In these studies, the PPV of $^{18}$F-FES-PET ranged between 32% and 79% and the NPV between 82% and 100%. which is comparable with our findings. It is hypothesized that a negative $^{18}$F-FES-PET can identify tumors that have lost ER expression during the course of disease. Recently, ESR1 (ER) gene mutations have been described, some of which strongly reduce ligand binding affinity and induce endocrine resistance. These phenomena might explain the good NPV of low tumor uptake of $^{18}$F-FES.

Our study has some limitations. First, the number of patients included was lower than expected. A possible explanation is that, despite the fact that previous studies have shown
the safety and benefits of estradiol therapy, physicians may be reluctant to refer patients for estrogen therapy given that anti-estrogen therapy is the key method to treat patients with ER positive disease. Secondly, we used CT to identify $^{18}$F-FES negative lesions. It is possible that $^{18}$F-FDG-PET together with $^{18}$F-FES-PET increases the sensitivity for $^{18}$F-FES negative lesions, since especially bone lesions are difficult to characterize on CT. Finally, when evaluating the predictive value of $^{18}$F-FES-PET, it is important to take concomitant and recent therapies into account. We observed low $^{18}$F-FES uptake in several previously $^{18}$F-FES-avid lesions in patients that had used ER antagonists up to 5 weeks before $^{18}$F-FES-PET. Possibly, the long half-lives ($t\frac{1}{2}$) of fulvestrant ($t\frac{1}{2} = 40$ days) and of tamoxifen and metabolites ($t\frac{1}{2} = 9$ and 13 days, respectively) could be responsible for the $^{18}$F-FES negative results.\textsuperscript{170–172} In the absence of biopsies, we are however unable to fully dissect whether the observed effects can be attributed to altered ER expression or to spill over effects of recent therapies. In preclinical studies long-term estrogen deprived ER positive breast cancer cells are used to study estrogen-induced apoptosis. After several months of culturing in estrogen-deprived conditions, breast cancer cells adapt to the low levels of estrogens by increasing ER expression.\textsuperscript{173,174} Paradoxically, therapeutic doses of estrogens now no longer induce growth proliferation, but induce apoptosis. More recently, $ESR1$ gene amplification was described in patient-derived mouse xenografts as a possible marker for hypersensitivity to estradiol.\textsuperscript{175} It would therefore be of interest in future studies to combine $^{18}$F-FES-PET/CT with analysis of $ESR1$ gene amplification and mutation in tumor biopsies, in order to potentially improve the selection of patients for estrogen therapy.

Although ER expression is required for response to endocrine agents, ER positive tumors may still fail to respond, e.g. due to cross talk with other pathways. We therefore evaluated whether the addition of tumor and bone turnover markers could improve response prediction. The association of tumor marker response alone with the patient response classification was modest, but when tumor marker response was combined with $^{18}$F-FES-PET the PPV increased to 100%. Clearly, the number of patients in this study was only limited and therefore this observation should be further evaluated in larger studies. The same effects were observed for bone turnover markers, which in this limited number of patients performed better than $^{18}$F-FES-PET alone. Surprisingly, not the changes in bone markers during treatment, but the pre-treatment values were associated with time-to-progression. These markers are known to correlate with the number and size of bone metastases in breast and prostate cancer.\textsuperscript{166–168} Therefore, high serum bone markers may be useful to identify patients that have a poor prognosis independent of the therapy given. Whether bone markers are of prognostic or predictive value needs to be addressed in larger studies, adhering to REMARK criteria.\textsuperscript{176}
We observed a clinical benefit rate of 37% for estradiol 6 mg orally daily, which is comparable to the study by Ellis et al.\textsuperscript{160} Our study is the second evaluating this low-dose regimen, as compared to the previously standard of 30 mg orally daily. The clinical benefit rate observed in our study in patients that were extensively pre-treated (median of 4 prior regimens) provides further evidence for the efficacy of estradiol 6 mg daily. The 21% of patients that terminated treatment prematurely due to toxicity was relatively high; grade 3 adverse events were noted in 42% of the patients. The high incidence of toxicity, however, underlines the value of upfront predictive markers for this treatment.

CONCLUSION

Patients with acquired endocrine resistant metastatic breast cancer may paradoxically benefit from estradiol therapy. The relatively low response rate and toxicity accompanying estradiol therapy warrants exploration of potential biomarkers to predict response. \textsuperscript{18}F-FES-PET may aid to identify patients that are unlikely to respond to estradiol therapy. The addition of other markers, such as bone turn over markers and tumor markers may aid to obtain also a good positive predictive value.

CONFLICT OF INTEREST

The authors declare no potential conflicts of interest.

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Chapter 5

18F-FES-PET to guide estrogen therapy