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Original Research

Serial [^{18}F]-FDHT-PET to predict bicalutamide efficacy in patients with androgen receptor positive metastatic breast cancer☆

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KEYWORDS
Bicalutamide;
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[^{18}F]-FDHT-PET

Abstract ***Background:*** The androgen receptor (AR) is a potential target in metastatic breast cancer (MBC), and 16β-[^{18}F]-fluoro-5α-dihydrotestosterone positron emission tomography ([^{18}F]-FDHT-PET) can be used for noninvasive visualisation of AR. [^{18}F]-FDHT uptake reduction during AR-targeting therapy reflects AR occupancy and might be predictive for treatment response. We assessed the feasibility of [^{18}F]-FDHT-PET to detect changes in AR availability during bicalutamide treatment and correlated these changes with treatment response.

☆ **List of where and when the study has been presented in part elsewhere:** Part of the data was presented at the AACR virtual annual meeting 2020.

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Patients and methods: Patients with AR + MBC, regardless of oestrogen receptor status, received an [^{18}F]-FDHT-PET at baseline and after 4–6 weeks bicalutamide treatment. Baseline [^{18}F]-FDHT uptake was expressed as maximum standardised uptake value. Percentage change in tracer uptake, corrected for background activity (SUV_{cor}), between baseline and follow-up PET scan (% reduction), was assessed per-patient and lesion. Clinical benefit was determined in accordance with Response Evaluation Criteria in Solid Tumours (RECIST) 1.1 or clinical evaluation (absence of disease progression for ≥24 weeks).

Results: Baseline [^{18}F]-FDHT-PET in 21 patients detected 341 of 515 lesions found with standard imaging and 21 new lesions. Follow-up [^{18}F]-FDHT-PET was evaluable in 17 patients with 349 lesions, showing a decrease in median SUV_{cor} from 1.3 to 0.7 per-patient and lesion ($P < 0.001$). Median % reduction per-patient was −45% and per-lesion −39%. In patients with progressive disease ($n = 11$), median % reduction was −30% versus −53% for patients who showed clinical benefit (in accordance with RECIST ($n = 3$) or clinical evaluation ($n = 3$); $P = 0.338$).

Conclusion: In this feasibility study, a bicalutamide-induced reduction in [^{18}F]-FDHT uptake could be detected by follow-up [^{18}F]-FDHT-PET in patients with AR + MBC. However, this change could not predict bicalutamide response.

Clinical trial information: NCT02697032.

1. Introduction

Hormone receptors play a vital role in the development of several malignancies [1]. Although oestrogen receptor (ER) expression is routinely determined in breast cancer (BC), this is not the case for androgen receptor (AR) expression [3]. The AR is expressed in 60–85% of all BCs, with a high prevalence among ER + tumours (85–95%), and lower rates in triple-negative BC (10–40%) [2–11]. The AR may be a possible target for therapeutic strategies in BC patients. Studies with AR-targeting drugs, such as bicalutamide, enzalutamide, abiraterone acetate, and orteronel have been conducted in patients with metastatic BC (MBC), including triple-negative [12–14] and ER + tumours [15–19].

Patients with ER −/AR + tumours show a modest clinical benefit rate from AR-targeting drugs [12–14]. Controversies in (pre)clinical studies exist regarding the effectiveness of this strategy in ER +(/AR +) tumours [15–20]. Therefore, the degree of AR blockade will possibly lead to more insight into the prediction of treatment response between the patient subgroups. Monitoring AR expression during treatment can be performed by serial biopsies; however a (metastasis) biopsy is not always feasible and not necessarily representative for the AR status throughout the body, owing to heterogeneity. Another approach to obtain (serial) assessment of whole-body AR expression is the imaging technique positron emission tomography (PET) with the tracer 16β-[^{18}F]-fluoro-5α-dihydrotestosterone ([^{18}F]-FDHT). In previous work, we have shown that AR expression in a metastasis biopsy of BC patients correlates with tracer uptake on the 16β-[([^{18}F]-FDHT-PET) scan [21]. Studies in patients with prostate cancer showed that [^{18}F]-FDHT uptake reduction during AR-targeting therapy reflects AR occupancy and might be predictive for treatment response [22–25]. This makes the follow-up of patients using [^{18}F]-FDHT-PET during AR-targeting treatment a potentially interesting tool for BC patients. In addition, the AR/ER ratio and hormone levels may influence treatment response in patients with BC [26,27].

Therefore, in this study, we assessed the feasibility of [^{18}F]-FDHT-PET to detect changes in [^{18}F]-FDHT uptake during treatment with the AR antagonist bicalutamide in patients with AR + MBC. In addition, we evaluated whether a reduction in [^{18}F]-FDHT uptake, and other exploratory markers including AR/ER ratio, were related to bicalutamide response.

2. Methods

2.1. Study design and patients

This prospective, single-center study (NCT02697032) was conducted at the University Medical Center Groningen, the Netherlands. The institutional review board approved the protocol, and patients provided written informed consent. Postmenopausal patients with AR +, human epidermal growth factor receptor 2–negative MBC were eligible, independent of their ER status. Additional inclusion criteria were measurable disease in accordance with the Response Evaluation Criteria in Solid Tumours (RECIST v1.1) or evaluable disease [28], performance status <2, and adequate organ function. Exclusion criteria included symptomatic brain metastases, or a cardiovascular history <6 months before

screening. In addition, concomitant use of CYP3A4 inhibitors was not allowed.

2.2. Study procedures

Contrast-enhanced computed tomography (CT) of the thorax/abdomen and conventional bone scintigraphy (with single-photon emission CT if necessary) were performed for baseline staging. At baseline, an [^{18}F]-FDHT-PET scan, twelve-lead electrocardiogram, and peripheral blood to measure testosterone and dihydrotestosterone levels were obtained. Wherever feasible, a metastasis biopsy was obtained, except for patients of which a biopsy was taken in the past 6 months. All patients received oral bicalutamide 150 mg alone once daily, until progression or toxicity. The safety assessments are described in the Supplemental. A follow-up [^{18}F]-FDHT-PET scan was performed after 4–6 weeks (day 28–42 ± 3) of bicalutamide treatment based on the steady-state concentration [29] and previous prostate cancer studies [22,24]. Response evaluation was performed after 6 weeks and every 3 months thereafter for measurable disease in accordance with RECIST 1.1 or clinical/imaging assessment for non-measurable disease according to physician's opinion. Clinical benefit as best response was defined as stable disease, partial or complete response (in patients with measurable disease in accordance with RECIST 1.1) or absence of disease progression for ≥24 weeks (in patients with non-measurable disease in accordance with clinical evaluation).

2.3. Pathology assessments

Immunohistochemistry for AR was performed on stored paraffin-embedded tumour samples on primary tumour or metastasis biopsy and was stained for ER (ER + if ≥ 1% of the tumour cells stained positive, according to guidelines [30]) and AR (AR + if >10% of the tumour cells stained positive, in line with a previous study [21]).

2.4. [^{18}F]-FDHT-PET

[^{18}F]-FDHT was produced as previously described [31]. Patients were not required to fast. A whole-body (head to mid-thigh) [^{18}F]-FDHT-PET scan was performed 60 min after a fixed dose of ~200 MBq [^{18}F]-FDHT was injected intravenously. Patients were scanned using a Siemens Biograph 40 or 64-slice mCT with 2-mm reconstructed spatial resolution and emission acquisition time of 3 min per bed position in accordance with the European Association of Nuclear Medicine (EANM) guidelines for ^{18}F imaging [32]. All quantifications were performed on EANM Research Limited reconstructed images. Low-dose CT was acquired for attenuation and scatter correction.

2.5. Imaging analysis

Metastatic lesions were identified on CT and bone scintigraphy. Lesions only present on CT, were considered metastases if they had a minimum diameter of 10 mm. An experienced nuclear medicine physician (A.G.) visually identified [^{18}F]-FDHT-PET lesions with tracer uptake above the background signal, which could not be attributed to physiological uptake or an artifact. Liver lesions were excluded due to high physiological [^{18}F]-FDHT uptake. We used syngo.via VB20 imaging software for quantifying tracer uptake. Guided by CT and/or bone scintigraphy (including lesions not visible on [^{18}F]-FDHT-PET), a volume of interest was drawn around each metastatic lesion to determine tracer uptake (C.V., J.B.). Baseline [^{18}F]-FDHT uptake was expressed as the maximum standardised uptake value (SUV_{max}), including body weight correction. For per-patient analysis, we used the median SUV_{max} and SUV_{max} corrected for physiological background activity (SUV_{cor}) of all lesions within one patient. The following calculation was applied in case of background correction: SUV_{cor} = tumour SUV_{max} − background SUV_{max} of the unaffected contralateral site of the organ, or the surrounding bone structure of the same origin. The percentage change in [^{18}F]-FDHT uptake between baseline and follow-up PET scan was expressed as the percentage difference in SUV_{cor} (% reduction), per-patient and per-lesion, using the following calculation: % reduction = $\{(SUV_{cor2} - SUV_{cor1})/SUV_{cor1}\}$*100%.

2.6. Outcomes

The primary outcome measure was the difference in [^{18}F]-FDHT uptake between baseline and follow-up [^{18}F]-FDHT-PET scan, per-patient and per-lesion. Secondary end-points were the association between changes in [^{18}F]-FDHT uptake and bicalutamide response, the difference in [^{18}F]-FDHT uptake change between BC subgroups, the correlation between baseline [^{18}F]-FDHT uptake and biopsy-based AR expression (Supplemental), and the relation of plasma levels of testosterone or dihydrotestosterone with [^{18}F]-FDHT uptake (Supplemental).

2.7. Statistical analysis

[^{18}F]-FDHT uptake reduction was evaluated as a continuous variable per subgroup, and median values (min − max) are presented. Statistical analyses of the differences in tracer uptake between baseline and follow-up [^{18}F]-FDHT-PET were performed using a non-parametric Wilcoxon signed-rank test. Based on an earlier prostate cancer study [22], to show a minimum decrease of 20% in [^{18}F]-FDHT uptake after 4–6 weeks compared with baseline uptake and a maximum decrease of 50% (delta 30%), with an α of 0.05 and a

power of 80%, 17 evaluable patients were needed. The Mann-Whitney *U* test was used to evaluate differences between patients having benefit from bicalutamide versus progressive patients, or between ER + versus ER − tumours. Progression-free survival (PFS) was defined as interval from start of therapy to disease progression or death. A p-value ≤0.05 was considered statistically significant. Statistical analysis was performed in SPSS software, version 23.

3. Results

3.1. Patients

Twenty-one patients with AR + MBC were included (refer Figure 1). Detailed patient characteristics are provided in Table 1. The last patient discontinued treatment in November 2019. Reasons for treatment discontinuation were progressive disease (n = 20) and adverse events (n = 1).

3.2. Pathology assessments

Metastasis biopsies were available from 15 patients (10 obtained at baseline, and 5 previously). Primary tumour was used in the remaining 6 patients. Median percentage of AR expression in these 21 samples was 100% (range: 50%–100%).

3.3. Treatment response

Median PFS was 8 weeks (range: 2–42). In total, 6 of 21 patients (29%) showed clinical benefit, including 5 of 15 (33%) ER + patients and 1 of 6 (17%) ER − patients (3 stable disease in accordance with RECIST, and 3 in accordance with clinical evaluation; refer Table 1).

3.4. Baseline [^{18}F]-FDHT-PET

At baseline, 545 metastatic lesions in 21 patients were visible on CT scan, bone scintigraphy or [^{18}F]-FDHT-PET scan (refer Table 1 for metastases location). Finally, 536 evaluable lesions were included for baseline [^{18}F]-FDHT-PET analysis (refer Figure 1). Baseline [^{18}F]-FDHT-PET detected 341 of 515 lesions found with standard imaging and 21 new lesions. Consequently, 174 lesions were not visible on [^{18}F]-FDHT-PET but found with standard imaging and were also included in the analysis. The number of lesions per patient varied from 2 to 78. Baseline [^{18}F]-FDHT uptake varied widely between patients (median SUV_{max} 2.6; range: 1.6–5.6) and lesions (3.1; 0.6–20.2). Baseline [^{18}F]-FDHT uptake of patients with progressive disease (n = 15; median SUV_{max} 3.2 [1.6–4.6]) was similar to patients having clinical benefit (n = 6; 2.2 [2.0–5.6]; P = 0.664). Baseline [^{18}F]-FDHT uptake heterogeneity was also observed within patients with up to 7-fold difference in tracer uptake between tumour lesions within one patient. However, this type of heterogeneity was not associated with bicalutamide response (progressive disease 4-fold difference [range: 1–7] vs. clinical benefit 3-fold [range: 2–7]; P = 0.850). Figure 2 shows the differences in [^{18}F]-FDHT uptake between and within patients.

3.5. Follow-up [^{18}F]-FDHT-PET

Four patients did not receive the follow-up [^{18}F]-FDHT-PET scan due to early progression. Therefore, scans from 17 patients (of which 13 ER + and 4 ER −) with a total of 349 lesions were available for analysis (Figure 1). The follow-up [^{18}F]-FDHT-PET scan

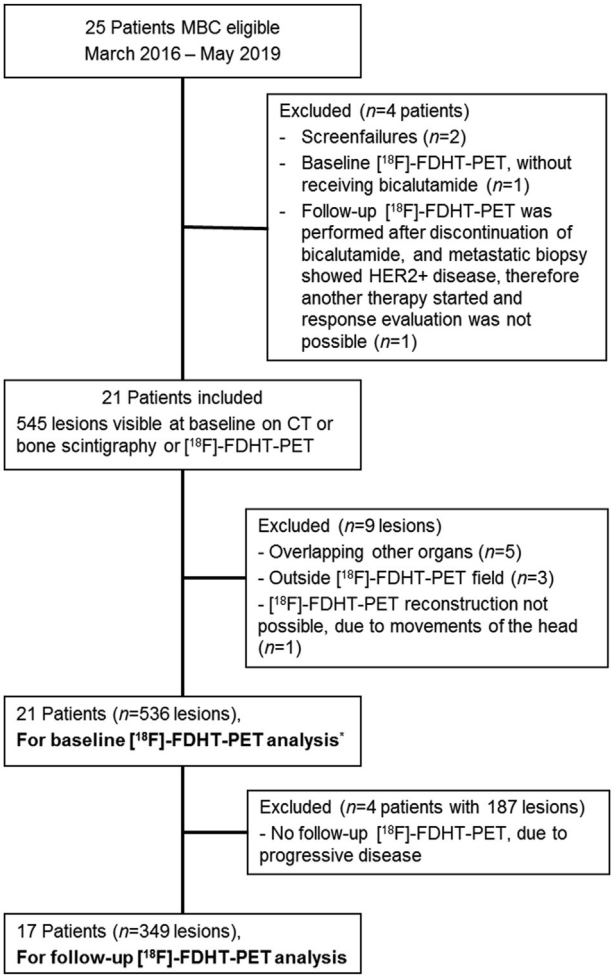

Fig. 1. CONSORT diagram. * Some remarks regarding imaging: in one patient with bone and cutaneous metastases no baseline diagnostic CT scan was performed, and lesions were based on bone scintigraphy; in a second patient, no baseline bone scintigraphy was performed, and lesions were based on diagnostic CT scan; in another patient, bone scintigraphy was performed after starting bicalutamide. If a CT scan was not possible due to allergic reactions to the contrast agent, a magnetic resonance imaging (MRI) or CT scan without contrast was performed. CT, computed tomography.

Table 1
Baseline patient characteristics.[a]

Characteristic	Total cohort (n = 21)
Age, years	65 ± 11
Type:	
Lobular	6 (29)
Ductal	15 (71)
Hormone receptor status primary tumour[b]:	
ER +/AR +	14 (67)
ER −/AR +	5 (24)
ER unknown	2 (10)
Metastatic tumour characteristics[b]:	
ER +/AR +	10 (48)
ER −/AR +	5 (24)
ER/AR unknown	6 (29)
AR expression[c]:	
>50%	20 (95)
10–50%	1 (5)
ER expression[c]:	
>50%	12 (57)
1–50%	2 (10)
<1%	6 (29)
Unknown	1 (5)
Prior systemic treatment in metastatic setting:	18 (86)
ER +	14/15 (93)
Chemotherapy	9/15 (60)
Endocrine therapy ± palbociclib or everolimus	14/15 (93)
ER −	4/6 (67)
Chemotherapy	3/6 (50)
Endocrine therapy[d]	2/6 (33)
Immunotherapy in the context of research	1/6 (17)
Number of prior lines of systemic therapy for advanced BC:	3 [0–8]
ER +	3 [0–5]
ER −	1 [0–8]
Measurable disease:	
Yes	10 (48)
Showed stable disease as best response	3/10 (30)
Progressed at first CT	7/10 (70)
No	11 (52)
Clinical benefit at 24 weeks	3/11 (27)
Clinically progressive disease at 24 weeks	8/11 (73)
Site of tumour lesion:	n = 545 lesions
Bone	503 (92)
Lymph node	18 (3)
Cutaneous	10 (2)
Lung	6 (1)
Primary breast tumour	8 (1)
Interval between start therapy and follow-up [^{18}F]-FDHT-PET[e]:	4 weeks [day 24–76]

CT, computed tomography; [^{18}F]-FDHT-PET, 16β-[^{18}F]-fluoro-5α-dihydrotestosterone positron emission tomography; AR, androgen receptor; ER, oestrogen receptor.

[a] Values are presented as mean ± standard deviation (SD), median (range: min-max) or the percentage of the total is provided between brackets.

[b] Based on histopathological examination.

[c] Based on primary tumour or metastasis biopsy samples.

[d] Primary tumour ER +, and the most recent metastasis biopsy showed ER − disease.

[e] In three cases, the follow-up [^{18}F]-FDHT-PET was outside the intended scan interval, ranging from −4 to +34 days: one early examination and two delayed evaluations (due to surgery, technical or logistic reasons).

showed a median reduction in SUV_{cor} ranging from 1.3 to 0.7 per-patient (Figure 3) and from 1.3 to 0.7 per-lesion ($P < 0.001$). Median % reduction per-patient was −45% (range: −72% to −7%) and per-lesion −39% (−95% to +100%). Examples of a baseline and follow-up [^{18}F]-FDHT-PET scan are depicted in Figure 4. In patients with progressive disease ($n = 11$) median % reduction was −30% (−72% to −7%) versus −53% (−62% to −16%) for patients who showed clinical benefit ($n = 6$, $P = 0.338$). When the ER status of the patient was taken into consideration, there was a trend towards a statistically significant difference in ER + patients between bicalutamide response (progressive disease [$n = 8$] median % reduction: −27% [−53% to −7%] vs. clinical benefit [$n = 5$]: −56% [−62% to −16%; $P = 0.059$; refer Figure 3]). The sample size for ER − patients ($n = 4$) was too small for statistical testing. In ER − patients with progressive disease ($n = 3$), median % reduction was −69% (−72% to −25%) versus −33% for 1 patient who showed clinical benefit (Figure 3). For RECIST evaluable patients, median % reduction was −24% (−53% to −7%) in patients with progressive disease ($n = 7$) versus −56% (−60% to −33%) for patients who showed stable disease ($n = 3$, $P = 0.067$). Owing to the highly limited number of measurable lesions (11 of 349), response evaluation per lesion was not performed.

When the ER status of the patient was taken into consideration, we found a median % reduction of −24% (−95% to +100%) for lesions ($n = 268$) of ER + patients versus −69% (−94% to +59%) for lesions ($n = 81$) of ER − patients ($P < 0.001$; Figure 5).

4. Discussion

In this study, we assessed the feasibility of [^{18}F]-FDHT-PET to detect changes in [^{18}F]-FDHT uptake during bicalutamide treatment in patients with AR + MBC and correlated these changes with treatment response.

This is the first exploratory study with patients with BC showing a decline in [^{18}F]-FDHT uptake during bicalutamide treatment, which was most pronounced in ER −/AR + patients. This imaging approach may potentially support optimal patient identification for AR-targeting in BC subgroups. As the AR is increasingly used as target of interest in both ER −/AR + and ER +/AR + BC, [^{18}F]-FDHT-PET could clearly be of relevance, for example, in future dose-finding trials.

To date, follow-up [^{18}F]-FDHT-PET during treatment has been used only in prostate cancer studies. In 12 patients with metastatic prostate cancer, who underwent a follow-up [^{18}F]-FDHT-PET after receiving flutamide for 1 day, a decline of SUV_{max} was shown ranging from −9% to −70% [23]. These results are comparable with our findings, but a direct comparison between the trials

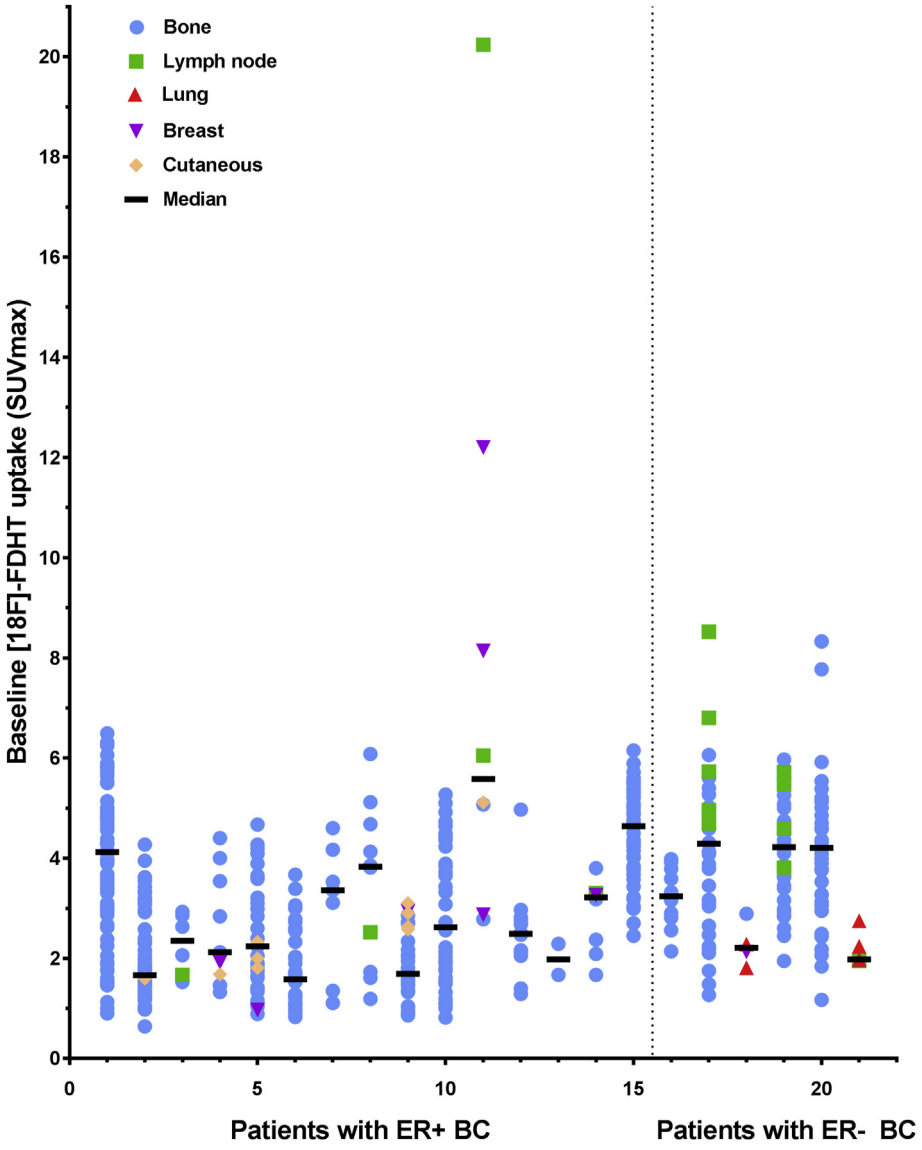

Fig. 2. Baseline [^{18}F]-FDHT uptake of all metastases (n = 536), expressed as the maximum standardised uptake value (SUV_{max}), in 15 patients with ER + BC and 6 patients with ER − BC. BC, breast cancer; ER, oestrogen receptor.

is difficult because different methods for quantification of [^{18}F]-FDHT uptake and background correction are used. In our opinion, serial imaging provides the clearest assessment of tumour tracer uptake (change) in individual patients if background corrected values are used [33]. The fact that we did not observe complete AR blockade may have had several reasons. Low binding affinity of bicalutamide compared with second-generation AR blockers, such as enzalutamide, may be of influence [34]. A relatively large [^{18}F]-FDHT uptake reduction, ranging from −20% to −100%, was previously shown after 4 weeks of enzalutamide in 22 patients with metastatic prostate cancer [22]. In addition, a higher bicalutamide dosage, for instance, with ≥200 mg daily as tested in prostate cancer trials, would likely have led to a larger reduction in tracer uptake [35–37]. However, a nonlinear relationship between doses of >200 mg and plasma level of bicalutamide was seen, and therefore clinical benefit is not expected at higher doses [35].

In our small study, no clear association was observed between changes in [^{18}F]-FDHT uptake and bicalutamide response per-patient. Nonetheless, patients with clinical benefit showed a non-significant trend toward larger reduction in tracer uptake compared with patients with progressive disease. The response measurement in this study may have affected the findings. Clinical benefit, but not [^{18}F]-FDG-PET, was included for response evaluation in addition to RECIST measurement on CT. [^{18}F]-FDG-PET is not included in RECIST due to insufficient clinical validation data [38], and the optimal response evaluation of bone metastases in BC remains a matter of debate. Clinical benefit is clearly a weaker end-point than RECIST. If we had assessed

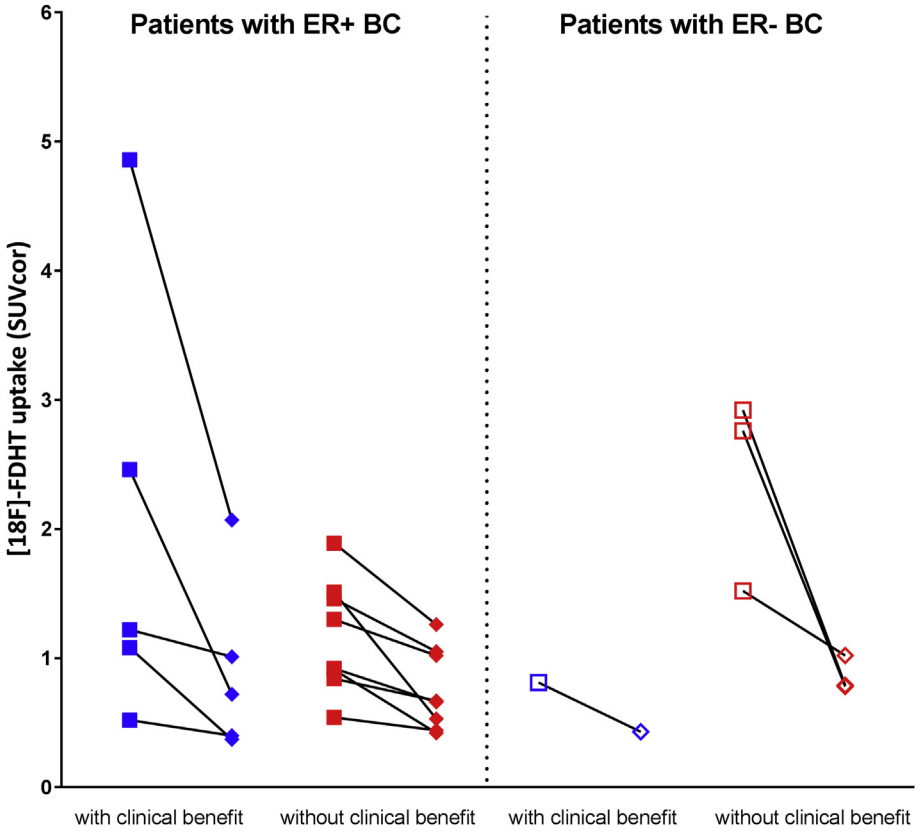

Fig. 3. Only those patients who were evaluable on follow-up [^{18}F]-FDHT-PET were reported. Individual changes in [^{18}F]-FDHT uptake after 4–6 weeks treatment with bicalutamide in patients with ER + disease (n = 13) and ER − disease (n = 4) are shown. The squares represent the background-corrected tumour [^{18}F]-FDHT uptake (SUV_{cor}) at baseline, and the diamonds the tracer uptake (SUV_{cor}) during treatment. Patients are grouped based on their treatment response, in blue patients who had clinical benefit, and in red patients without clinical benefit. [^{18}F]-FDHT-PET, 16β-[^{18}F]-fluoro-5α-dihydrotestosterone positron emission tomography; ER, oestrogen receptor.

bicalutamide response only in RECIST evaluable patients (n = 10), the association almost reached statistical significance. However, this target group was very small to draw firm conclusions, and only stable disease was observed as best response. This could possibly explain why SUV changes were not significantly related to treatment response. Our results are comparable with the study by Scher *et al.* [22], showing that [^{18}F]-FDHT reduction was not related to early metabolic response to enzalutamide, as measured by [^{18}F]-FDG-PET. It is also likely that other factors besides receptor occupation by AR blockers are involved in therapy response, for example heterogeneity in receptor status. Although a remarkable heterogeneity of [^{18}F]-FDHT uptake at baseline within patients was seen, heterogeneity did not predict bicalutamide response.

BC subtype is considered to affect response to AR targeting. Preclinical data have shown that the AR inhibits the growth in ER + tumours but stimulates the growth of ER − tumours [39]. This is supported by preclinical data showing that bicalutamide inhibits proliferation of ER − BC cells [40]. In addition in the present study, the largest reduction in [^{18}F]-FDHT uptake after bicalutamide treatment was seen in ER − metastases. However, we did see a larger proportion of patients with ER + disease deriving clinical benefit from bicalutamide than patients with ER − disease. These apparently conflicting findings are also shown in other (pre)clinical studies [15–20]. This could be related to the degree of AR blockade and balance between ER and AR expression, which warrants further assessment in future studies. Interestingly, in ER + BC AR agonists rather than antagonists may be more effective [41]. Historic studies using androgen in ER + MBC, were ended prematurely due to the severe side effects of virilisation [42]. But selective AR modulators, such as RAD140 and enobosarm, show acceptable toxicity profiles in ongoing trials in patients with ER +/AR + MBC [43,44]. Therefore, the AR remains a target of clear interest in both ER + and ER − BC.

Our study has limitations. First, given the low number of measurable lesions in this population, measurement of treatment response in individual lesions was not

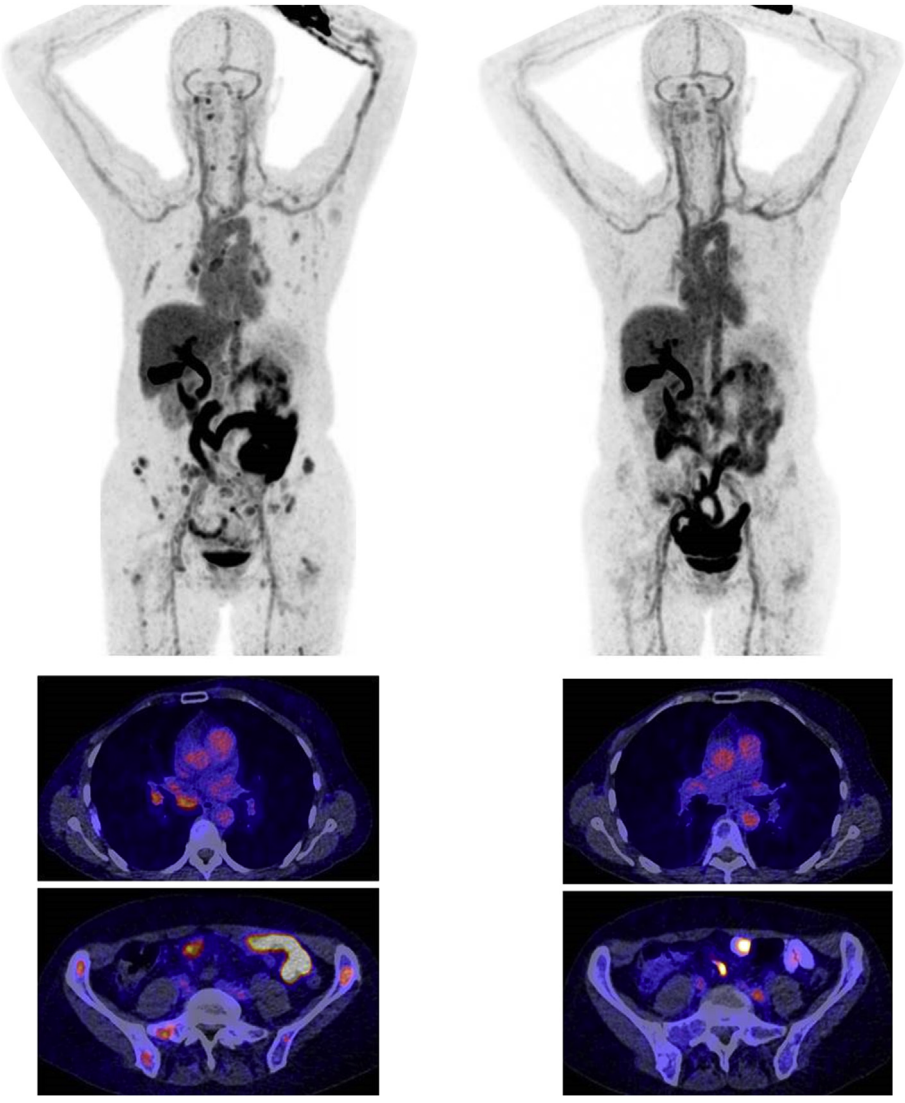

Fig. 4. Example of the baseline (left image) and follow-up [^{18}F]-FDHT-PET scan (right image) in a patient with ER − disease. High AR expression in the tumour lesions (hilar and subcarinal lymph nodes, as well as multiple skeletal lesions including vertebrae, ribs, pelvic bones) are visible and decreased during treatment. The tracer uptake as noticed at baseline was no longer visible at the follow-up [^{18}F]-FDHT-PET scan. Despite the large decrease in tracer uptake, this patient did not show clinical benefit. [^{18}F]-FDHT-PET, 16β-[^{18}F]-fluoro-5α-dihydrotestosterone positron emission tomography; ER, oestrogen receptor; AR, androgen receptor.

possible. Secondly, in this feasibility setting, the number of patients evaluable for primary end-point was limited. Power calculations were based on detecting a minimum decrease of 20% in [^{18}F]-FDHT uptake, but with present insights about repeatability of [^{18}F]-FDHT-PET, a decrease of 30% should be considered in further studies [33,45]. Third, as outlined previously, the inclusion of both patients with ER − and ER + BC adds complexity to the interpretation. Finally, two patients had a delayed [^{18}F]-FDHT-PET evaluation during treatment. We did include these patients in the follow-up analysis because a steady-state of AR blockade is reached after 4–6 weeks with bicalutamide treatment, and these patients were still on treatment during the follow-up analysis. Therefore, we did not expect this delay to have affected the [^{18}F]-FDHT uptake change from baseline. The strengths of this study include the follow-up [^{18}F]-FDHT-PET imaging related to response, all-lesion analysis, and AR expression in relation to pathology confirmation.

Concluding, in this exploratory study, bicalutamide-induced changes in AR availability in patients with AR + MBC could be detected by [^{18}F]-FDHT-PET. In this small study, however, the change in [^{18}F]-FDHT uptake was not significantly related to the response to bicalutamide treatment.

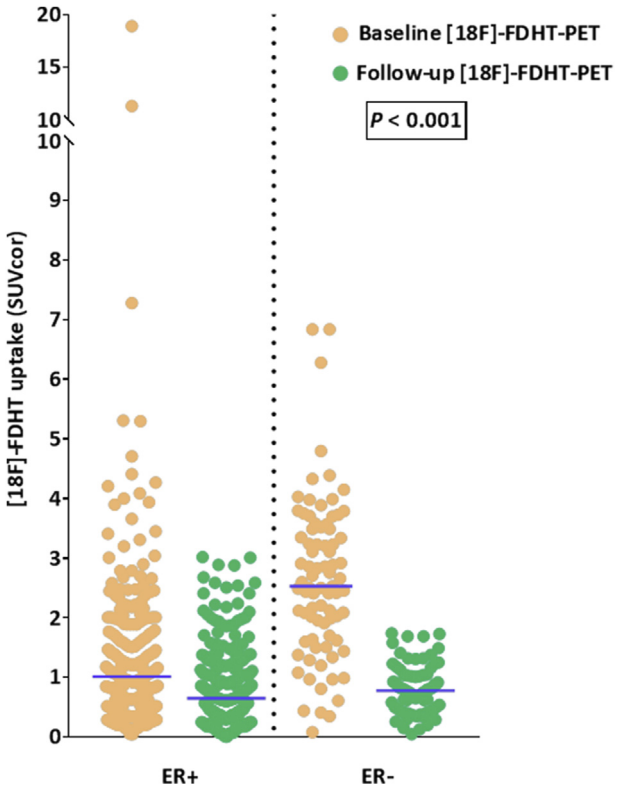

Fig. 5. Baseline (yellow) and follow-up (green) background-corrected tumour [^{18}F]-FDHT uptake (SUV_{cor}) for all individual lesions (n = 349) of patients with ER + and ER − BC. Patients are grouped based on their ER status. In 81 lesions of 10 patients, increased [^{18}F]-FDHT uptake was observed during treatment. BC, breast cancer; ER, oestrogen receptor; [^{18}F]-FDHT-PET, 16β-[^{18}F]-fluoro-5α-dihydrotestosterone positron emission tomography.

Conflict of interest statement

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejca.2020.11.008.

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Author contribution

C.P.S., C.M.V., B.R. and H.H.B. contributed to study concepts. C.P.S., C.M.V., A.W.J.M.G., G.A.P.H., B.R. and E.F.J.d.V. contributed to study design. C.M.V.., J.B. and C.P.S. contributed to data acquisition. A.W.J.M.G. and C.D. contributed to quality control of data and algorithms. All authors contributed to data analysis and interpretation. J.B. and C.M.V. contributed to statistical analysis. J.B., C.M.V., C.P.S., A.W.J.M.G., G.A.P.H. and E.F.J.d.V. contributed to manuscript preparation. C.P.S. contributed to manuscript editing. H.H.B., B.R. and C.D. contributed to manuscript review.

References

[1] Tessel MA, Krett NL, Rosen ST. Steroid receptor and microRNA regulation in cancer. Curr Opin Oncol 2010;22:592–7. https://doi.org/10.1097/CCO.0b013e32833ea80c.

[2] Cimino-Mathews A, Hicks JL, Illei PB, Halushka MK, Fetting JH, De Marzo AM, et al. Androgen receptor expression is usually maintained in initial surgically resected breast cancer metastases but is often lost in end-stage metastases found at autopsy. Hum Pathol 2012;43:1003–11. https://doi.org/10.1016/j.humpath.2011.08.007.

[3] Kraby MR, Valla M, Opdahl S, Haugen OA, Sawicka JE, Engstrøm MJ, et al. The prognostic value of androgen receptors in breast cancer subtypes. Breast Canc Res Treat 2018;172:283–96. https://doi.org/10.1007/s10549-018-4904-x.

[4] Niemeier LA, Dabbs DJ, Beriwal S, Striebel JM, Bhargava R. Androgen receptor in breast cancer: expression in estrogen receptor-positive tumors and in estrogen receptor-negative tumors with apocrine differentiation. Mod Pathol 2010;23:205–12. https://doi.org/10.1038/modpathol.2009.159.

[5] Ogawa Y, Hai E, Matsumoto K, Ikeda K, Tokunaga S, Nagahara H, et al. Androgen receptor expression in breast cancer: relationship with clinicopathological factors and biomarkers. Int J Clin Oncol 2008;13:431–5. https://doi.org/10.1007/s10147-008-0770-6.

[6] Grogg A, Trippel M, Pfaltz K, Ladrach C, Droeser RA, Cihoric N, et al. Androgen receptor status is highly conserved during tumor progression of breast cancer. BMC Canc 2015;15:872. https://doi.org/10.1186/s12885-015-1897-2.

[7] Gonzalez LO, Corte MD, Vazquez J, Junquera S, Sanchez R, Alvarez AC, et al. Androgen receptor expresion in breast cancer: relationship with clinicopathological characteristics of the tumors, prognosis, and expression of metalloproteases and their inhibitors. BMC Canc 2008;8:149. https://doi.org/10.1186/1471-2407-8-149.

[8] Ismael NEHS, Khairy RA, Talaat SM, El-Fattah FAA. Immunohistochemical expression of androgen receptors (AR) in various breast cancer subtypes. Open Access Maced J Med Sci 2019;7:1259–65. https://doi.org/10.3889/oamjms.2019.311.

[9] Kensler KH, Poole EM, Heng YJ, Collins LC, Glass B, Beck AH, et al. Androgen receptor expression and breast cancer survival: results from the nurses' health studies. J Natl Cancer Inst 2018;111:700–8. https://doi.org/10.1093/jnci/djy173.

[10] Kensler KH, Regan MM, Heng YJ, Baker GM, Pyle ME, Schnitt SJ, et al. Prognostic and predictive value of androgen receptor expression in postmenopausal women with estrogen receptor-positive breast cancer: results from the Breast International Group Trial 1-98. Breast Cancer Res 2019;21:30. https://doi.org/10.1186/s13058-019-1118-z.

[11] Collins LC, Cole KS, Marotti JD, Hu R, Schnitt SJ, Tamimi RM. Androgen receptor expression in breast cancer in relation to molecular phenotype: results from the Nurses' Health Study. Mod Pathol 2011;24:924–31. https://doi.org/10.1038/modpathol.2011.54.

[12] Gucalp A, Tolaney S, Isakoff SJ, Ingle JN, Liu MC, Carey LA, et al. Phase II trial of bicalutamide in patients with androgen receptor-positive, estrogen receptor-negative metastatic breast cancer. Clin Cancer Res 2013;19:5505–12. https://doi.org/10.1158/1078-0432.CCR-12-3327.

[13] Traina TA, Miller K, Yardley DA, Eakle J, Schwartzberg LS, O'Shaughnessy J, et al. Enzalutamide for the treatment of androgen receptor-expressing triple-negative breast cancer. J Clin Oncol 2018;36:884–90. https://doi.org/10.1200/JCO.2016.71.3495.

[14] Bonnefoi H, Grellety T, Tredan O, Saghatchian M, Dalenc F, Mailliez A, et al. A phase II trial of abiraterone acetate plus prednisone in patients with triple-negative androgen receptor positive locally advanced or metastatic breast cancer (UCBG 12-1). Ann Oncol 2016;27:812–8. https://doi.org/10.1093/annonc/mdw067.

[15] Schwartzberg LS, Yardley DA, Elias AD, Patel M, LoRusso P, Burris HA, et al. A phase I/Ib study of enzalutamide alone and in combination with endocrine therapies in women with advanced breast cancer. Clin Canc Res 2017;23:4046–54. https://doi.org/10.1158/1078-0432.CCR-16-2339.

[16] Krop I, Abramson V, Colleoni M, Traina T, Holmes F, Estevez L, et al. Results from a randomized placebo-controlled phase 2 trial evaluating exemestane ± enzalutamide in patients with hormone receptor–positive breast cancer. AACR 2018;78(4 Suppl). GS4-07.

[17] O'Shaughnessy J, Campone M, Brain E, Neven P, Hayes D, Bondarenko I, et al. Abiraterone acetate, exemestane or the combination in postmenopausal patients with estrogen receptor-positive metastatic breast cancer. Ann Oncol 2016;27:106–13. https://doi.org/10.1093/annonc/mdv487.

[18] Lu Q, Xia W, Lee K, Zhang J, Yuan H, Yuan Z, et al. Bicalutamide plus aromatase inhibitor in patients with estrogen receptor-positive/androgen receptor-positive advanced breast cancer. Oncol 2020;25. https://doi.org/10.1634/theoncologist.2019-0564. 21-e15.

[19] Rampurwala M, Wisinski KB, Burkard ME, Ehsani S, O'Regan RM, Carmichael L, et al. Phase 1b study of orteronel in postmenopausal women with hormone-receptor positive (HR+) metastatic breast cancer. Invest New Drugs 2017;35:87–94. https://doi.org/10.1007/s10637-016-0403-2.

[20] Cochrane DR, Bernales S, Jacobsen BM, Cittelly DM, Howe EN, D'Amato NC, et al. Role of the androgen receptor in breast cancer and preclinical analysis of enzalutamide. Breast Cancer Res 2014;16:R7. https://doi.org/10.1186/bcr3599.

[21] Venema CM, Mammatas LH, Schröder CP, van Kruchten M, Apollonio G, Glaudemans AWJM, et al. Androgen and estrogen receptor imaging in metastatic breast cancer patients as a surrogate for tissue biopsies. J Nucl Med 2017;58:1906–12. https://doi.org/10.2967/jnumed.117.193649.

[22] Scher HI, Beer TM, Higano CS, Anand A, Taplin M-E, Efstathiou E, et al. Antitumour activity of MDV3100 in castration-resistant prostate cancer: a phase 1-2 study. Lancet 2010;375:1437–46. https://doi.org/10.1016/S0140-6736(10)60172-9.

[23] Dehdashti F, Picus J, Michalski JM, Dence CS, Siegel BA, Katzenellenbogen JA, et al. Positron tomographic assessment of androgen receptors in prostatic carcinoma. Eur J Nucl Med Mol Imag 2005;32:344–50. https://doi.org/10.1007/s00259-005-1764-5.

[24] Rathkopf DE, Morris MJ, Fox JJ, Danila DC, Slovin SF, Hager JH, et al. Phase I study of ARN-509, a novel antiandrogen, in the treatment of castration-resistant prostate cancer. J Clin Oncol 2013;31:3525–30. https://doi.org/10.1200/JCO.2013.50.1684.

[25] Larson SM, Morris M, Gunther I, Beattie B, Humm JL, Akhurst TA, et al. Tumor localization of 16beta-18F-fluoro-5alpha-dihydrotestosterone versus 18F-FDG in patients with progressive, metastatic prostate cancer. J Nucl Med 2004;45:366–73.

[26] Rangel N, Rondon-Lagos M, Annaratone L, Osella-Abate S, Metovic J, Mano MP, et al. The role of the AR/ER ratio in ER-positive breast cancer patients. Endocr Relat Canc 2018;25:163–72. https://doi.org/10.1530/ERC-17-0417.

[27] Bronte G, Rocca A, Ravaioli S, Scarpi E, Bonafe M, Puccetti M, et al. Evaluation of androgen receptor in relation to estrogen receptor (AR/ER) and progesterone receptor (AR/PgR): a new must in breast cancer? J Oncol 2019:393505. https://doi.org/10.1155/2019/1393505.

[28] Eisenhauer EA, Therasse P, Bogaerts J, Schwartz LH, Sargent D, Ford R, et al. New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). Eur J Canc 2009;45:228–47. https://doi.org/10.1016/j.ejca.2008.10.026.

[29] Summary of product characteristics (SmPC) for bicalutamide 150 mg.

[30] Allison KH, Hammond MEH, Dowsett M, McKernin SE, Carey LA, Fitzgibbons PL, et al. Estrogen and progesterone receptor testing in breast cancer: ASCO/CAP guideline update. J Clin Oncol 2020;38:1346–66. https://doi.org/10.1200/JCO.19.02309.

[31] Liu A, Dence CS, Welch MJ, Katzenellenbogen JA. Fluorine-18-labeled androgens: radiochemical synthesis and tissue distribution studies on six fluorine-substituted androgens, potential imaging agents for prostatic cancer. J Nucl Med 1992;33:724–34.

[32] Boellaard R, Delgado-Bolton R, Oyen WJG, Giammarile F, Tatsch K, Eschner W, et al. FDG PET/CT: EANM procedure guidelines for tumour imaging: version 2.0. Eur J Nucl Med Mol Imaging 2014;42:328–54. https://doi.org/10.1007/s00259-014-2961-x.

[33] Vargas HA, Kramer GM, Scott AM, Weickhardt A, Meier AA, Parada N, et al. Reproducibility and repeatability of semiquantitative (18)F-fluorodihydrotestosterone uptake metrics in castration-resistant prostate cancer metastases: a prospective multicenter study. J Nucl Med 2018;59:1516–23. https://doi.org/10.2967/jnumed.117.206490.

[34] Tran C, Ouk S, Clegg NJ, Chen Y, Watson PA, Arora V, et al. Development of a second-generation antiandrogen for treatment of advanced prostate cancer. Science 2009;324:787–90. https://doi.org/10.1126/science.1168175.

[35] Blackledge GR. High-dose bicalutamide monotherapy for the treatment of prostate cancer. Urology 1996;47:44–53. https://doi.org/10.1016/s0090-4295(96)80008-2.

[36] Tyrrell CJ, Iversen P, Tammela T, Anderson J, Björk T, Kaisary AV, et al. Tolerability, efficacy and pharmacokinetics of bicalutamide 300 mg, 450 mg or 600 mg as monotherapy for patients with locally advanced or metastatic prostate cancer, compared with castration. BJU Int 2006;98:563–72. https://doi.org/10.1111/j.1464-410X.2006.06275.x.

[37] Tyrrell C, Denis L, Newling D, Soloway M, Channer K, Cockshott I. Casodex TM 10–200 mg daily, used as monotherapy for the treatment of patients with advanced prostate cancer. Eur Urol 1998;33:39–53. https://doi.org/10.1159/000019526.

[38] Boers J, de Vries EFJ, Glaudemans AWJM, Hospers GAP, Schröder CP. Application of PET tracers in molecular imaging for breast cancer. Curr Oncol Rep 2020;22:85. https://doi.org/10.1007/s11912-020-00940-9.

[39] Hickey TE, Robinson JLL, Carroll JS, Tilley WD. Minireview: the androgen receptor in breast tissues: growth inhibitor, tumor suppressor, oncogene? Mol Endocrinol 2012;26:1252–67. https://doi.org/10.1210/me.2012-1107.

[40] Huang R, Han J, Liang X, Sun S, Jiang Y, Xia B, et al. Androgen receptor expression and bicalutamide antagonize androgen receptor inhibit β-catenin transcription complex in estrogen receptor-negative breast cancer. Cell Physiol Biochem 2017;43:2212–25. https://doi.org/10.1159/000484300.

[41] Lim E, Hickey TE, Selth LA, Chia KM, Milioli HH, Roden D, et al. The androgen receptor is a tumour suppressor in estrogen receptor positive breast cancer. SABCS 2019. Abstract nr GS2-03.

[42] Kono M, Fujii T, Lim B, Sri Karuturi M, Tripathy D, Ueno NT. Androgen receptor function and androgen receptor–targeted therapies in breast cancer: a review. JAMA Oncol 2017;3:1266–73. https://doi.org/10.1001/jamaoncol.2016.4975.

[43] Hamilton E, LoRusso P, Ma C, Vidula N, Bagley RG, Troy S, et al. Phase 1 dose escalation study of a novel selective androgen receptor modulator (SARM), RAD140, in estrogen receptor positive (ER+), human epidermal growth factor receptor 2 negative (HER2-), metastatic breast cancer. SABCS 2019. Abstract nr P5-11-01.

[44] Overmoyer B, Sanz-Altimira P, Partridge AH, Extermann M, Liu J, Winer E, et al. Enobosarm for the treatment of metastatic, estrogen and androgen receptor positive, breast cancer. Final results of the primary endpoint and current progression free survival. SABCS 2014. Abstract nr P1-13-04.

[45] Kramer GM, Yaqub M, Vargas HA, Schuit RC, Windhorst AD, van den Eertwegh AJM, et al. Assessment of Simplified methods for quantification of 18 F-FDHT uptake in patients with metastatic castration-resistant prostate cancer. J Nucl Med 2019;60:1221–7. https://doi.org/10.2967/jnumed.118.220111.