University of Groningen

Molecular imaging to identify patients with metastatic breast cancer who benefit from endocrine treatment combined with cyclin-dependent kinase inhibition

Boers, Jorianne; Venema, Clasina M; de Vries, Erik F J; Glaudemans, Andor W J M; Kwee, Thomas C; Schuurin, Ed; Martens, John W M; Elias, Sjoerd G; Hospers, Geke A P; Schröder, Carolina P

Published in:
European Journal of Cancer

DOI:
[10.1016/j.ejca.2019.10.024](https://doi.org/10.1016/j.ejca.2019.10.024)

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Final author's version (accepted by publisher, after peer review)

Publication date:
2020

[Link to publication in University of Groningen/UMCG research database](#)

Citation for published version (APA):

Boers, J., Venema, C. M., de Vries, E. F. J., Glaudemans, A. W. J. M., Kwee, T. C., Schuurin, E., Martens, J. W. M., Elias, S. G., Hospers, G. A. P., & Schröder, C. P. (2020). Molecular imaging to identify patients with metastatic breast cancer who benefit from endocrine treatment combined with cyclin-dependent kinase inhibition. *European Journal of Cancer*, 126, 11-20.
<https://doi.org/10.1016/j.ejca.2019.10.024>

Copyright

Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

The publication may also be distributed here under the terms of Article 25fa of the Dutch Copyright Act, indicated by the "Taverne" license. More information can be found on the University of Groningen website: <https://www.rug.nl/library/open-access/self-archiving-pure/taverne-amendment>.

Take-down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Article type: Original research article

Molecular imaging to identify patients with metastatic breast cancer who benefit from endocrine treatment combined with cyclin dependent kinase inhibition

Author list: J. Boers¹, C.M. Venema¹, E.F.J. de Vries², A.W.J.M. Glaudemans², T.C. Kwee³, E. Schuuring⁴, J.W.M. Martens⁵, S.G. Elias⁶, G.A.P. Hospers¹, C.P. Schröder¹

Affiliation list:

1: Department of Medical Oncology, University Medical Center Groningen, University of Groningen, Groningen, the Netherlands

2: Department of Nuclear Medicine and Molecular Imaging, University Medical Center Groningen, University of Groningen, Groningen, the Netherlands

3: Department of Radiology, University Medical Center Groningen, University of Groningen, Groningen, the Netherlands

4: Department of Pathology and Medical Biology, University Medical Center Groningen, University of Groningen, Groningen, the Netherlands

5: Department of Medical Oncology, Erasmus MC, Erasmus University Medical Center, Rotterdam, the Netherlands

6: Department of Epidemiology, Julius Center for Health Sciences and Primary Care, University Medical Center Utrecht, Utrecht University, Utrecht, the Netherlands

Corresponding author

Correspondence to: Dr. Carolina P. Schröder, Department of Medical Oncology, University Medical Center Groningen, Hanzeplein 1, 9700 RB Groningen, the Netherlands. Phone: 31 50 36 12821. E-mail: c.p.schroder@umcg.nl.

List of where and when the study has been presented in part elsewhere: Part of the data was presented at the San Antonio Breast Cancer Symposium 2017.

Clinical trial information: NCT02806050.

ABSTRACT

BACKGROUND: Adding cyclin dependent kinase (CDK) inhibitor to endocrine treatment improves outcome in estrogen receptor positive (ER+) metastatic breast cancer (MBC), but identifying patients who benefit is challenging. Response is potentially associated with ER expression heterogeneity. Positron emission tomography (PET) with 16α -[^{18}F]fluoro- 17β -estradiol (FES) allows whole body ER assessment. We explored whether FES-PET heterogeneity and FES uptake were related to letrozole and palbociclib outcome, in patients with ER+ MBC.

PATIENTS AND METHODS: Patients underwent a baseline FES-PET and ^{18}F -fluorodeoxyglucose (FDG) PET with contrast enhanced computed tomography (CT). FES-PET heterogeneity score (% FES+ lesions divided by all lesions on FDG-PET and/or CT) and FES uptake were related to outcome and 8 week FDG-PET response. Circulating tumor DNA samples for *ESR1* mutation analysis were collected at baseline.

RESULTS: In thirty patients with 864 metastatic lesions, baseline FES-PET heterogeneity was assessed. In 27 patients with 688 lesions, response was evaluated. Median time to progression (TTP) was 73 weeks (95%CI 21 to ∞) in 7 patients with 100% FES+ disease, 27 weeks (14 to 49) in heterogeneous FES+ disease (20 patients), and 15 weeks (9 to ∞) without FES positivity (3 patients; log-rank $P=0.30$). Geometric mean FES uptake was 2.3 for metabolic progressive patients, 2.5 ($P_{\text{vs progression}}=0.82$) for metabolic stable disease, and 3.3 ($P_{\text{vs progression}}=0.40$) for metabolic response ($P_{\text{trend}}=0.21$). *ESR1* mutations, found in 13/23 patients, were unrelated to FES uptake.

CONCLUSION: This exploratory study suggests that FES-PET heterogeneity may potentially identify ER+ MBC patients who benefit from letrozole combined with CDK inhibition. (*NCT02806050*)

Key words: *CDK inhibition; endocrine therapy; biomarker; FES-PET; heterogeneity; breast cancer.*

INTRODUCTION

Cyclin dependent kinase (CDK) inhibitors improve outcome in estrogen receptor positive (ER+) metastatic breast cancer (MBC) patients when combined with endocrine treatment [1–10]. The downside of this combined treatment is that CDK inhibition adds considerable costs, manageable toxicity and increased hospital visits although quality of life is not affected [1, 2, 6, 8, 9]. Response prediction by ER expression is most commonly used in clinical practice [11, 12]. Recently, other potentially promising biomarkers are found for CDK inhibitor benefit, for example cyclin E1 (CCNE1) mRNA expression [13]. However, these biomarkers are not yet used in daily practice. Therefore, we focus on the ER marker. To establish ER expression, a metastasis biopsy is preferred. However, this is not always safely feasible. Furthermore, a biopsy cannot rule out ER heterogeneity. Also standard imaging cannot dichotomize between ER+ and ER- lesions. Whole body positron emission tomography (PET) with 16α -[^{18}F]fluoro- 17β -estradiol (FES) can, by assessment of ER expression of all tumor lesions [14, 15]. Chae et al. showed high agreement between FES-PET results and immunohistochemical ER status [16]. Another study concluded that FES-PET heterogeneity was related to (lack of) response to endocrine treatment [17]. Whether this might also be the case for the combination with CDK inhibition, is presently unknown.

At present, *ESR1* gene mutation analysis in circulating tumor DNA (ctDNA) is a rapidly evolving field. Mutations of the *ESR1* gene, which encodes ER, are found within the ligand-binding domain of ER [18]. These mutations lead to estrogen-independent activation [19].

This feasibility study aimed to explore whether baseline FES-PET heterogeneity and FES uptake were related to outcome and (non) response to treatment with letrozole combined with CDK inhibitor palbociclib. In addition, we explored whether *ESR1* mutations are related to FES uptake.

METHODS

Study design

This is a prospective, single center study performed at the University Medical Center Groningen, the Netherlands (*NCT02806050*). The protocol was approved by the institutional review board. All patients provided written informed consent. At baseline, ¹⁸F-fluorodeoxyglucose (FDG) PET with contrast enhanced computed tomography (CT), and FES-PET were performed, ctDNA samples were also collected (supplementary Figure A.1). If feasible, a metastasis biopsy was obtained at baseline, unless a recent ($\pm$ 6 months previously) biopsy was available. Early response was evaluated with FDG-PET after treatment for 2 weeks. After 8 weeks of treatment, response was evaluated with FDG-PET/CT. Endpoints were: correlation of FES-PET heterogeneity score with time to progression (TTP), FES uptake with (non) response to treatment at 8 weeks, and relation of *ESR1* mutations and FES uptake. We also investigated the relation of FDG uptake at 2 and 8 weeks (supplementary Table B.1).

Patients

Post- and premenopausal (with ovarian function suppression) women with ER+ MBC (>1% staining on biopsy), were eligible. Inclusion criteria included adequate organ function and performance score of ≤ 2 [20]. Patients with symptomatic brain metastases, visceral crisis, previous CDK inhibitors, active cardiac disease or concurrent malignancy were excluded. Treatment consisted of 2.5 mg letrozole daily and 125 mg palbociclib for 21 consecutive days, followed by 7 days off treatment, until unacceptable toxicity or progression.

PET/CT imaging

FES-PET: Patients received ~200 MBq of ^{18}F -FES intravenously. Patients did not have to fast, and discontinued ER antagonists for at least 6 weeks to avoid false negative scans [21]. Whole body (head to mid-thigh) PET/CT was performed 60 min after tracer injection 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. Low dose CT was acquired for attenuation and scatter correction. Reconstructions of scan and quantification were performed according to European Association of Nuclear Medicine Research Limited (EARL) criteria [22].

FDG-PET: Whole body FDG-PET/CT was performed in the same manner, but patients had to fast for at least 6 hours and blood glucose levels had to be < 120 mg/dl. The injected FDG dose was 3 MBq/kg according to EANM guidelines [22].

PET/CT imaging analysis

FDG/FES-PET: We used *syngo.via* imaging software for quantification of tracer uptake. Volumes of interest (VOIs) were drawn around the area of enhanced tumor uptake visible on PET (higher than background) to calculate maximum standardized uptake value (SUV_{max}). Due to high physiological FES uptake, liver lesions are excluded [23]. PET scans were evaluated for metastases by an experienced nuclear medicine physician (AG). Tracer uptake was quantified by two trained observers (CV and JB). SUV_{max} calculations were performed according to EANM guidelines for ^{18}F imaging [22]. In line with previous studies, $\text{SUV}_{\text{max}} \geq 2.0$ was defined as FES+ [14, 15, 17, 24].

Contrast enhanced CT: Chest-abdomen CT scans, evaluated by an experienced radiologist (TK) for metastases, were used to allocate FDG-PET positive lesions to an anatomic substrate to identify FES-PET negative lesions, or vice versa. Lesions only present on CT, were considered metastases if they had a minimum axial diameter of 10 mm.

FES-PET heterogeneity score related to outcome measure TTP

FES-PET heterogeneity score per patient, according to Gennari et al., was defined as percentage of FES+ lesions divided by all lesions visible on FDG-PET or CT at baseline [17]. This score was categorized into three groups (1) 100% of the lesions FES+, (2) 1-99% of the lesions FES+, or (3) 0% of the lesions FES+, and related to TTP.

FES uptake related to response

Per patient: Geometric mean SUV_{max} on FES-PET was calculated in all lesions per patient. For metabolic response per patient, the percent change in SUV_{max} of all lesions was calculated from FDG-PET at baseline and after 8 weeks. Metabolic response per patient was defined as $\geq 30\%$ decrease of SUV_{max} on 8 week FDG-PET, and progression was defined as $\geq 30\%$ increase. Disease without response or progression was considered stable. This response score was partly based on PERCIST [25]. A less laborious method is shown in Appendix B.

Per lesion: The same definition for metabolic response as described above was used. In a second analysis, we excluded lesions with SUV_{max} on FDG-PET less than two times the SUV_{max} in the center of the descending thoracic aorta at baseline FDG-PET, to avoid bias due to lesions with tracer uptake close to background being unable to show a decrease in uptake.

Relation of *ESR1* mutations in ctDNA to FES-PET

From patients with additional consent, blood was sampled for plasma isolation at baseline. CtDNA was analyzed for *ESR1* mutations by next generation deep sequencing using commercial Oncomine Breast cell free nucleic acid panel with a detection limit of 0.1%, according to the manufacturer's instructions. Mutation status was compared to FES uptake, and heterogeneity score.

Statistical Analysis

We used Kaplan-Meier analysis with log-rank testing to relate heterogeneity score to TTP (interval between start of therapy and progression or death), and Firth corrected Cox regression analysis to obtain hazard ratios (HRs) with 95% confidence interval (95%CI). *ESR1* mutation versus heterogeneity score was tested with Fisher Exact test. Per patient metabolic response category was related to per lesion FES uptake accounting for within-patient clustering by a random intercept (using Satterthwaite approximations to degrees of freedom, restricted maximum likelihood for parameter estimation, and likelihood ratio testing under maximum likelihood for statistical inference). We natural-log-transformed FES uptake to obtain approximate normal

distributions, yielding estimates of geometric means following back-transformation of the results. Mixed-effects ordinal logistic regression was used to relate FES uptake to metabolic response category per lesion. We estimated the Pearson correlation for clustered data between natural-log-transformed FES uptake and Box-Cox transformed change in FDG uptake on a lesion-level [26]. A nominal P -value ≤ 0.05 was considered statistically significant. We used R software version 3.2.1 for Macintosh (lme4 1.1-11, lmerTest 2.0-20, survival 2.38-3, coxphf 1.11) and IBM SPSS software version 23 for data analysis.

RESULTS

Patients

Thirty ER+ MBC patients, mean age 55 years, were included between September 2016 and March 2018 (Figure 1 and Table 1). Baseline metastases biopsies, performed in 9 patients, showed ER expression in 8/9 cases. In 3 additional patients, a metastasis biopsy performed within ± 6 months showed ER positivity. In the other patients, a biopsy was considered not feasible. 87% of patients had received at least one previous line of endocrine treatment in the metastatic setting. At the time of analysis (February 2019), 5 patients (17%) were still on combination therapy. Reasons for treatment discontinuation were progressive disease ($n = 21$), and adverse events ($n = 4$). Those who discontinued treatment due to adverse events were monitored until progression or death (2/4 patients). Median follow-up was 34 weeks (5 – 118). Examples of PET images are depicted in Figure 2. The toxicity profile was in line with previous findings (supplementary Table C.1).

FES-PET heterogeneity score related to outcome measure TTP

864 lesions in 30 patients were assessable for FES-PET heterogeneity analysis (Figure 1). The number of lesions per patient varied between 4 and 70. Lesions were present in bone ($n = 733$; 85%), lymph nodes ($n = 100$; 12%), lung ($n = 19$; 2%), breast ($n = 5$; 0.6%), brain ($n = 4$; 0.5%) and adrenal gland ($n = 3$; 0.3%). Most lesions were identified with all three imaging techniques FES- and FDG-PET/CT (supplementary Figure D.1). Seven patients had 100% FES+ disease with a median TTP of 73 weeks (95%CI 21 to ∞); 20 patients had heterogeneous FES uptake, their TTP was 27 weeks (14 to 49); 3 patients had no FES positivity, their TTP was 15 weeks (9 to ∞) (log-rank $P=0.30$, $P_{\text{trend}}=0.19$) (Figure 3). Patients with less than

100% FES positivity (groups 0% and 1-99% are merged) showed a HR of 2.1 for TTP (95%CI 0.81 to 6.91; $P=0.13$) compared to those with 100% positivity.

FES uptake related to response

In 27 patients with 688 lesions, response was evaluated (Figure 1).

Per patient: Geometric mean SUV_{max} on FES-PET varied widely per patient (0.9 – 6.8) (Figure 4). The estimated geometric mean FES uptake was 2.3 (95%CI 1.0 to 5.2) for patients with metabolic progression, 2.5 (1.9 to 3.3; $P_{vs\ progression}=0.82$) for patients with stable disease, and 3.3 (2.2 to 5.1; $P_{vs\ progression}=0.40$) for metabolic responding patients ($P_{trend}=0.21$).

Per lesion: We also analyzed response to treatment on a lesion-level. In total, 454 FES+ and 234 FES- lesions were found (FES SUV_{max} range 0.6 – 13.7; Figure 4, Table 2). FES+ lesions had 1.44 (95%CI 0.88 to 2.36; $P=0.14$) times the odds of belonging to a better metabolic response category than FES- lesions (i.e. stable disease instead of progression, or responsive instead of stable disease). When analyzed continuously, the correlation between FES uptake and change in FDG from baseline to week 8 was -0.20 (95%CI -0.41 to 0.00; $P=0.051$). Using the bias-correction method, no significant differences were observed (Table 2).

Relation of *ESR1* mutations in ctDNA to FES-PET

13/23 patients showed *ESR1* mutations (Table 1, supplementary Figure E.1). No *ESR1* mutation was detected in patients without FES positivity (0/2), while in patients

with 100% FES positivity a mutation was detected in 3/5 patients (E380Q, Y537N, V392I) ($P=0.34$).

DISCUSSION

An issue in ER+ MBC management remains the need to identify patients who benefit from endocrine treatment combined with CDK inhibition. We examined whether ER imaging could potentially support this identification.

This is the first study exploring whole body ER expression heterogeneity in relation to response to endocrine treatment and CDK inhibition. These data suggest that heterogeneity may be a biologically relevant entity in BC, and can potentially support identification of patients who benefit most (or least) from combined treatment.

We found that patients with 100% FES positivity benefitted most from combination therapy compared to those with heterogeneous or no FES uptake (HR 2.1). These results are in line with the preliminary data by Gennari et al., who found that heterogeneity score of 3 largest lesions was related to endocrine treatment response (HR 1.8) [17]. The similar findings in these two studies, despite different patient selection and treatment, underline the possible underlying biology that can be detected with molecular imaging. Although biopsy is the gold standard, it cannot evaluate whole body heterogeneity. Furthermore, molecular imaging is more patient friendly, as patients prefer a scan to a biopsy [27]. Although future general FES-PET availability may appear challenging, due to high costs and limited specialized centers, successful multicenter (inter)national trials including FES-PET, and the common use of ^{18}F for FDG-PET, do support its feasibility in clinical practice. Furthermore, FES-PET could potentially support optimal individualized treatment choice, which in case of expensive medication such as CDK inhibition is likely cost-effective. This was already suggested in a previous computer simulated study, and will be further

evaluated [28]. Therefore, we are currently exploring FES/FDG-PET heterogeneity further in the SONImage study (*NCT04125277*), a side study to the randomized (aromatase inhibitor $\pm$ CDK inhibition) Dutch SONIA trial.

As expected, more FES+ lesions responded to treatment than FES- lesions. However, also a substantial number of FES- lesions showed response. One explanation may be that also other pathways with downstream complex formation between CDK 4/6 and cyclin D might have been inhibited by palbociclib, such as human epidermal growth factor receptor 2 or androgen receptor signaling pathways [29, 30]. Recently, the study by Chae et al. used a FES SUV_{max} cut-off of 1.5 [16]. Based on previous data, in which background measurements exceeded the 1.5 cut-off level, we used a threshold of 2.0, as in previous studies [14, 15, 17, 24]. Also a difference in use of EARL approved parameters and scanning time limit direct comparability. Establishing a generally accepted cut-off value for FES uptake should be priority in studies in this setting, such as the Dutch multicenter IMPACT breast trial (*NCT01957332*). In addition, the most optimal method for quantification is still unknown, and will be performed in the IMPACT trial.

Contrary to expectations, higher FES uptake was observed in patients with *ESR1* mutations compared to those without. An explanation for this could be that the *ESR1* mutation leads to a higher binding affinity of FES to the receptor, or that the receptor can be activated at a lower concentration of estrogens [31]. Currently, the relation between FES uptake and *ESR1* mutation status, as well as circulating tumor cell count, is assessed in the IMPACT breast trial [27, 32]. Other potentially promising

biomarkers for the degree of benefit to CDK inhibitors are: D-cyclin-activating features, tumor *CCNE1* mRNA levels, and FAT1 alterations [13, 33–35].

This study has limitations, including its small sample size, heterogeneous pretreatments and relatively short time to response measurements. In the single arm design, only effect of endocrine treatment combined with CDK inhibition could be related to FES-PET heterogeneity. Another limitation might be that response measurements were only possible according to RECIST in the minority of lesions ($n = 14$). However, this is in line with real world experience in ER+ MBC. Strengths of this study are its comprehensive molecular imaging analysis including (repeated) FDG-PET/CT, as well as the novel FES-PET. The whole body all-lesion analysis of ER expression, in relation to biopsy confirmation and particularly related to response to a highly relevant treatment combination, add to its informative value of ER heterogeneity as biological entity.

Concluding, this exploratory study suggests that FES-PET heterogeneity may potentially identify patients who benefit from combination therapy.

CONFLICT OF INTEREST STATEMENT

No conflicts of interest are to be declared associated with the manuscript.

ACKNOWLEDGEMENTS

This work was supported by an unrestricted research grant by Pfizer Oncology The Netherlands [Grant number WI205548], and Pfizer also provided the palbociclib.

ROLE OF THE FUNDING SOURCE

Pfizer provided palbociclib, but did not participate in data collection or analysis.

REFERENCES

- [1] Finn RS, Martin M, Rugo HS, Jones S, Im SA, Gelmon K, et al. Palbociclib and Letrozole in Advanced Breast Cancer. *N Engl J Med* 2016; 375:1925–36. <https://doi.org/10.1056/NEJMoa1607303>.
- [2] Hortobagyi GN, Stemmer SM, Burris HA, Yap YS, Sonke GS, Paluch-Shimon S, et al. Ribociclib as First-Line Therapy for HR-Positive, Advanced Breast Cancer. *N Engl J Med* 2016; 375:1738–48. <https://doi.org/10.1056/NEJMoa1609709>.
- [3] Cristofanilli M, Turner NC, Bondarenko I, Ro J, Im SA, Masuda N, et al. Fulvestrant plus palbociclib versus fulvestrant plus placebo for treatment of hormone-receptor-positive, HER2-negative metastatic breast cancer that progressed on previous endocrine therapy (PALOMA-3): final analysis of the multicentre, double-blind, phase 3 randomised controlled trial. *Lancet Oncol* 2016; 17:425–39. [https://doi.org/10.1016/S1470-2045\(15\)00613-0](https://doi.org/10.1016/S1470-2045(15)00613-0).
- [4] Sledge GWJ, Toi M, Neven P, Sohn J, Inoue K, Pivot X, et al. MONARCH 2: Abemaciclib in Combination With Fulvestrant in Women With HR+/HER2- Advanced Breast Cancer Who Had Progressed While Receiving Endocrine Therapy. *J Clin Oncol* 2017; 35:2875–84. <https://doi.org/10.1200/JCO.2017.73.7585>.
- [5] Tripathy D, Im SA, Colleoni M, Franke F, Bardia A, Harbeck N, et al. Ribociclib plus endocrine therapy for premenopausal women with hormone-receptor-positive, advanced breast cancer (MONALEESA-7): a randomised phase 3 trial. *Lancet Oncol* 2018; 19:904–15. [https://doi.org/10.1016/S1470-2045\(18\)30292-4](https://doi.org/10.1016/S1470-2045(18)30292-4).
- [6] Finn RS, Crown J, Lang I, Boer K, Bondarenko I, Kulyk SO, et al. Overall

- survival results from the randomized phase II study of palbociclib (P) in combination with letrozole (L) vs letrozole alone for frontline treatment of ER+/HER2– advanced breast cancer (PALOMA-1; TRIO-18). *J Clin Oncol* 2017; 35(suppl 15):[abstract 1001].
- [7] Turner NC, Slamon DJ, Ro J, Bondarenko I, Im SA, Masuda N, et al. Overall Survival with Palbociclib and Fulvestrant in Advanced Breast Cancer. *N Engl J Med* 2018; 379:1926–36. <https://doi.org/10.1056/NEJMoa1810527>.
 - [8] Harbeck N, Iyer S, Turner N, Cristofanilli M, Ro J, André F, et al. Quality of life with palbociclib plus fulvestrant in previously treated hormone receptor-positive, HER2-negative metastatic breast cancer: patient-reported outcomes from the PALOMA-3 trial. *Ann Oncol Off J Eur Soc Med Oncol* 2016; 27:1047–54. <https://doi.org/10.1093/annonc/mdw139>.
 - [9] Rugo HS, Diéras V, Gelmon KA, Finn RS, Slamon DJ, Martin M, et al. Impact of palbociclib plus letrozole on patient-reported health-related quality of life: results from the PALOMA-2 trial. *Ann Oncol Off J Eur Soc Med Oncol* 2018; 29:888–94. <https://doi.org/10.1093/annonc/mdy012>.
 - [10] Im SA, Lu YS, Bardia A, Harbeck N, Colleoni M, Franke F, et al. Overall Survival with Ribociclib plus Endocrine Therapy in Breast Cancer. *N Engl J Med* 2019. <https://doi.org/10.1056/NEJMoa1903765>.
 - [11] DeMichele A, Clark AS, Tan KS, Heitjan DF, Gramlich K, Gallagher M, et al. CDK 4/6 inhibitor palbociclib (PD0332991) in Rb+ advanced breast cancer: phase II activity, safety, and predictive biomarker assessment. *Clin Cancer Res* 2015; 21:995–1001. <https://doi.org/10.1158/1078-0432.CCR-14-2258>.
 - [12] Finn RS, Dering J, Conklin D, Kalous O, Cohen DJ, Desai AJ, et al. PD 0332991, a selective cyclin D kinase 4/6 inhibitor, preferentially inhibits

- proliferation of luminal estrogen receptor-positive human breast cancer cell lines in vitro. *Breast Cancer Res* 2009; 11:R77. <https://doi.org/10.1186/bcr2419>.
- [13] Turner NC, Liu Y, Zhu Z, Loi S, Colleoni M, Loibl S, et al. Cyclin E1 Expression and Palbociclib Efficacy in Previously Treated Hormone Receptor-Positive Metastatic Breast Cancer. *J Clin Oncol* 2019; 37:1169–78. <https://doi.org/10.1200/JCO.18.00925>.
- [14] Nienhuis HH, van Kruchten M, Elias SG, Glaudemans AWJM, de Vries EFJ, Bongaerts AHH, et al. ⁽¹⁸⁾F-fluoroestradiol Tumor Uptake is Heterogeneous and Influenced by Site of Metastasis in Breast Cancer Patients. *J Nucl Med* 2018; 59:1212–18. <https://doi.org/10.2967/jnumed.117.198846>.
- [15] van Kruchten M, de Vries EGE, Brown M, de Vries EFJ, Glaudemans AWJM, Dierckx RAJO, et al. PET imaging of oestrogen receptors in patients with breast cancer. *Lancet Oncol* 2013; 14:e465–e475. [https://doi.org/10.1016/S1470-2045\(13\)70292-4](https://doi.org/10.1016/S1470-2045(13)70292-4).
- [16] Chae SY, Ahn SH, Kim SB, Han S, Lee SH, Oh SJ, et al. Diagnostic accuracy and safety of 16alpha-[(18)F]fluoro-17beta-oestradiol PET-CT for the assessment of oestrogen receptor status in recurrent or metastatic lesions in patients with breast cancer: a prospective cohort study. *Lancet Oncol* 2019; 20:546–55. [https://doi.org/10.1016/S1470-2045\(18\)30936-7](https://doi.org/10.1016/S1470-2045(18)30936-7).
- [17] Gennari A, Brain E, Nanni O, Muñoz Couselo E, Harbeck N, Geiss R, et al. Molecular imaging with 18F-fluoroestradiol (18F-FES) to assess intra-patient heterogeneity in metastatic breast cancer (MBC): a European TRANSCAN program. *Ann Oncol* 2017; 28(suppl 5).
- [18] Jeselsohn R, De Angelis C, Brown M, Schiff R. The Evolving Role of the

- Estrogen Receptor Mutations in Endocrine Therapy-Resistant Breast Cancer. *Curr Oncol Rep* 2017; 19:35. <https://doi.org/10.1007/s11912-017-0591-8>.
- [19] Angus L, Beije N, Jager A, Martens JW, Sleijfer S. ESR1 mutations: Moving towards guiding treatment decision-making in metastatic breast cancer patients. *Cancer Treat Rev* 2017; 52:33–40. <https://doi.org/10.1016/j.ctrv.2016.11.001>.
- [20] Oken MM, Creech RH, Tormey DC, Horton J, Davis TE, McFadden ET, et al. Toxicity and response criteria of the Eastern Cooperative Oncology Group. *Am J Clin Oncol* 1982; 5:649–55.
- [21] Linden HM, Kurland BF, Peterson LM, Schubert EK, Gralow JR, Specht JM, et al. Fluoroestradiol positron emission tomography reveals differences in pharmacodynamics of aromatase inhibitors, tamoxifen, and fulvestrant in patients with metastatic breast cancer. *Clin Cancer Res* 2011; 17:4799–805. <https://doi.org/10.1158/1078-0432.CCR-10-3321>.
- [22] 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* 2015; 42:328–54. <https://doi.org/10.1007/s00259-014-2961-x>.
- [23] van Kruchten M, Glaudemans AWJM, de Vries EFJ, Beets-Tan RGH, Schröder CP, Dierckx RA, et al. PET Imaging of Estrogen Receptors as a Diagnostic Tool for Breast Cancer Patients Presenting with a Clinical Dilemma. *J Nucl Med* 2012; 53:182–90. <https://doi.org/10.2967/jnumed.111.092734>.
- [24] Dehdashti F, Mortimer JE, Trinkaus K, Naughton MJ, Ellis M, Katzenellenbogen JA, et al. PET-based estradiol challenge as a predictive biomarker of response to endocrine therapy in women with estrogen-receptor-

- positive breast cancer. *Breast Cancer Res Treat* 2009; 113:509–17.
<https://doi.org/10.1007/s10549-008-9953-0>.
- [25] O JH, Lodge MA, Wahl RL. Practical PERCIST: A Simplified Guide to PET Response Criteria in Solid Tumors 1.0. *Radiology* 2016; 280:576–84.
<https://doi.org/10.1148/radiol.2016142043>.
- [26] Lorenz DJ, Datta S, Harkema SJ. Marginal association measures for clustered data. *Stat Med* 2011; 30:3181–91. <https://doi.org/10.1002/sim.4368>.
- [27] Eisses B, Angus L, van der Vegt B, Sieuwerts AM, Kraan J, Martens JW, et al. Non-invasive estrogen receptor assessment by [18F]-fluorestradiol (FES)-PET or circulating tumor cells predicts receptor status in patients with metastatic breast cancer. *SABC 2018*; PD4–09.
- [28] Koleva-Kolarova RG, Greuter MJW, Feenstra TL, Vermeulen KM, de Vries EFJ, Parkin D, et al. Molecular imaging with positron emission tomography and computed tomography (PET/CT) for selecting first-line targeted treatment in metastatic breast cancer: a cost-effectiveness study. *Oncotarget* 2018; 9:19836–46. <https://doi.org/10.18632/oncotarget.24869>.
- [29] Reinert T, Goncalves R, Bines J. Implications of ESR1 Mutations in Hormone Receptor-Positive Breast Cancer. *Curr Treat Options Oncol* 2018; 19:24.
<https://doi.org/10.1007/s11864-018-0542-0>.
- [30] Scott SC, Lee SS, Abraham J. Mechanisms of therapeutic CDK4/6 inhibition in breast cancer. *Semin Oncol* 2017; 44:385–94.
<https://doi.org/10.1053/j.seminoncol.2018.01.006>.
- [31] Pakdel F, Reese JC, Katzenellenbogen BS. Identification of charged residues in an N-terminal portion of the hormone-binding domain of the human estrogen receptor important in transcriptional activity of the receptor. *Mol Endocrinol*

- 1993; 7:1408–17. <https://doi.org/10.1210/mend.7.11.8114756>.
- [32] Cristofanilli M, Pierga JY, Reuben J, Rademaker A, Davis AA, Peeters DJ, et al. The clinical use of circulating tumor cells (CTCs) enumeration for staging of metastatic breast cancer (MBC): International expert consensus paper. *Crit Rev Oncol Hematol* 2019; 134:39–45. <https://doi.org/10.1016/j.critrevonc.2018.12.004>.
- [33] Chandarlapaty S, Razavi P. Cyclin E mRNA: Assessing Cyclin-Dependent Kinase (CDK) Activation State to Elucidate Breast Cancer Resistance to CDK4/6 Inhibitors. *J Clin Oncol* 2019; 37:1148–50. <https://doi.org/10.1200/JCO.19.00090>.
- [34] Gong X, Litchfield LM, Webster Y, Chio LC, Wong SS, Stewart TR, et al. Genomic Aberrations that Activate D-type Cyclins Are Associated with Enhanced Sensitivity to the CDK4 and CDK6 Inhibitor Abemaciclib. *Cancer Cell* 2017; 32:761-76. <https://doi.org/10.1016/j.ccell.2017.11.006>.
- [35] Li Z, Razavi P, Li Q, Toy W, Liu B, Ping C, et al. Loss of the FAT1 Tumor Suppressor Promotes Resistance to CDK4/6 Inhibitors via the Hippo Pathway. *Cancer Cell* 2018; 34:893-905. <https://doi.org/10.1016/j.ccell.2018.11.006>.

Table 1. Baseline patient characteristics[†]

Characteristic	Total cohort (n = 30)	100% FES+ [¶] (n = 7)	1-99% FES+ [¶] (n = 20)	0% FES+ [¶] (n = 3)
Age, years	55 ± 10.9	54 ± 10.1	56 ± 11.2	56 ± 14.4
Hormone receptor status of primary tumor*:				
ER+/HER2-	29 (97)	7 (100)	19 (95)	3 (100)
Unknown	1 (3)	-	1 (5)	-
Metastatic tumor characteristics*:				
ER+/HER2-	24 (80)	5 [‡] (71)	18 [‡] (90)	1 [‡] (33)
ER-/HER2- ^{††}	1 (3)	-	-	1 (33)
Unknown	5 (17)	2 (29)	2 (10)	1 (33)
Prior treatment in metastatic setting:				
None	1 (3)	1 (14)	-	-
Hormonal therapy (1-2)	21 (70)	3 (43)	16 (80)	2 (67)
Hormonal therapy (>2)	5 (17)	1 (14)	3 (15)	1 (33)
Chemotherapy (1-2)	10 (33)	2 (29)	8 (40)	-
Chemotherapy (>2)	-	-	-	-
Other ^{¶¶}	1 (3)	-	1 (5)	-
Only hormonal therapy	19 (63)	4 (57)	12 (60)	3 (100)
Only chemotherapy	3 (10)	2 (29)	1 (5)	-
Number of hormonal therapies received	1 [0 – 6]	1 [0 – 6]	1 [0 – 3]	2 [2 – 3]
Number of chemotherapy regimens or hormonal therapies received	2 [0 – 6]	1 [0 – 6]	2 [0 – 5]	2 [2 – 3]
ESR1 mutations**:				
E380Q	5/13	1/3	4/10	0/0
D538G	8/13	0/3	8/10	0/0
Y537N	4/13	1/3	3/10	0/0

S463P	1/13	0/3	1/10	0/0
Y537C	1/13	0/3	1/10	0/0
V392I	1/13	1/3	0/10	0/0
Y537S	2/13	0/3	2/10	0/0

† Values are presented as mean ± standard deviation (SD), median [range] or total number (%).¶

FES-PET heterogeneity score (% FES positive lesions divided by all lesions on FDG-PET and/or CT).

* based on histopathological examination. ‡ 100% FES+: all patients 100% ER positive, except one (3% ER+), 1-99% FES+: all patients ≥70% ER positive, 0% FES+: one patient ER negative and one 100% ER positive. †† This patient had a previous biopsy of a bone metastasis that was ER positive, but a new biopsy of a liver lesion was negative for ER. ¶¶ PD-L1 plus antibody-drug conjugate. ** 13/23 patients showed *ESR1* mutations, and different mutations were observed within one patient.

Abbreviations: ER, estrogen receptor; HER2, human epidermal growth factor receptor 2; FES, 16α-¹⁸F]fluoro-17β-estradiol; *ESR1*, estrogen receptor 1.

Table 2. FES-PET analysis per lesion and response evaluation by 8 week FDG-PET

	Response	Stable	Progression	
FES positive	205 (45%)	186 (41%)	63 (14%)	454
(SUV _{max} ≥ 2.0)	112 (41%)	125 (46%)	34 (13%)	271
FES negative	51 (22%)	132 (56%)	51 (22%)	234
(SUV _{max} < 2.0)	21 (19%)	68 (61%)	22 (20%)	111
	256	318	114	688
	133	193	56	382

Orange: all 688 lesions. Blue: only 382 lesions, corrected for background.

Figure 1. CONSORT diagram

† Bone lesions; * Not evaluable due to high muscle activity on 8 week FDG-PET, physiological FDG uptake, pleural effusion, motion, and 8 week FDG-PET or CT-scan was not performed due to rapid progression during follow-up; ¶ Lymph node, bone, lung.

Figure 2. Examples of PET images of a MBC patient that responded to palbociclib plus letrozole (A, B, C) and one patient that did not respond (D, E, F).

Upper row responder: Baseline FDG-PET (A) shows pathological uptake in axillary lymph nodes (right side) and in nearly all vertebrae and pelvic bones. Image B shows the baseline FES-PET with pathological ER expression in the axial skeleton (including vertebrae, pelvic bones, proximal humeri and femora) and in axillary lymph nodes (right side). After 8 weeks the FDG-PET (C) showing almost complete metabolic response (just some slightly elevated uptake in the axillary lymph nodes). The patient has been on treatment for more than 70 weeks.

Lower row non-responder: Baseline FDG-PET (D) shows pathological uptake in multiple skeletal lesions. Image E shows the baseline FES-PET with only some increased ER expression in thoracic vertebrae. After 8 weeks the FDG-PET (F) showing no metabolic response, even some increase in the pathologic uptake in the multiple skeletal lesions.

Figure 3. Kaplan-Meier curves of the role of FES uptake heterogeneity score on time to progression (TTP).

A **B**

Figure 4. FES uptake per patient and per lesion.

(A) Distribution of metastases per patient and FES uptake of all metastases ($n = 688$) in 27 patients. Overview of FES uptake as SUV_{max} in tumor lesions and illustrating geometric mean SUV_{max} per patient, (B) Waterfall plot showing relative change in tumor FDG uptake in individual lesions ($n = 688$) at 8 week FDG-PET scan compared with baseline. Red bars represent FES+ lesions ($SUV_{max} \geq 2.0$) and blue bars represent FES- lesions.