Molecular imaging of estrogen receptors

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Chapter 8

Summary and future perspectives
SUMMARY

For patients with estrogen receptor (ER) positive breast cancer, endocrine therapy plays a major role in both the adjuvant and palliative setting. Although endocrine therapies with or without chemotherapy have significantly reduced the recurrence risk as adjuvant therapy, 20-30% of all patients with primary breast cancer will develop distant metastases. Also when metastases have developed, long-term disease stabilization can be achieved by endocrine therapies. For adequate treatment decision-making it is crucial to obtain up-to-date information on the ER status of the tumor(s), since ER expression is the sole predictor for response to endocrine therapy. Moreover, ER status can change during the course of disease in up to 30% of the patients, and therefore treatments based on the ER status of the primary tumor may be inadequate. Therefore, guidelines now advise to determine the ER status at the time of first relapse by obtaining a biopsy of a metastatic lesion. Obtaining a biopsy can however be difficult due to the location of a lesion; and heterogeneous ER expression can occur within one metastasis and among different metastases within an individual, which can lead to sampling errors.

Positron emission tomography (PET) imaging of ER expression by use of the tracer 16α-[18F] fluoro-17β-estradiol (FES) can give functional information about the ER status of all lesions within the body. The aim of this thesis was to address the clinical potential of the 18F-FES-PET technique in breast and ovarian cancer patients.

In chapter 1 we provided a short introduction and outline of the thesis. In chapter 2, we have reviewed the possible applications of 18F-FES-PET imaging for metastatic breast cancer patients. A literature search was performed to acquire preclinical data on the development of ER-targeted PET tracers, and all clinical studies performed until May 2013 were included. The possible applications of 18F-FES-PET in breast cancer patients that were identified by the literature search were: 1) as a diagnostic tool, 2) as a predictive biomarker for endocrine therapy, 3) to evaluate heterogeneity of ER expression among metastases, and 4) to evaluate change in ER binding capacity during treatment with ER antagonists. In a pooled analysis of studies using 18F-FES-PET and concurrent biopsies, 18F-FES-PET had a good sensitivity for ER positive lesions of 84% and an excellent specificity of 98%. Analysis of a limited number of small studies in 138 patients allowed us to evaluate the predictive value of 18F-FES-PET. A maximum standardized uptake value (SUV\text{max}) \geq 1.5 appeared to be the best predictor of response with a positive predictive value of 65% and a negative predictive value of 88%. Finally, we summarized important factors that should be taken into account when performing clinical 18F-FES-PET studies, such as recent therapies affecting tumor 18F-FES uptake and patients’ menopausal status.
In chapter 3 we evaluated whether $^{18}$F-FES-PET could be used as an add-on diagnostic tool in breast cancer patients that presented with a clinical dilemma. Thirty-three breast cancer patients were referred for a $^{18}$F-FES-PET after standard work-up was unable to solve the diagnostic dilemma. Physicians were required to fill in questionnaires prior to $^{18}$F-FES-PET, shortly after and three months after $^{18}$F-FES-PET imaging to evaluate the impact of $^{18}$F-FES-PET on diagnostic understanding and therapy management. The clinical dilemmas for which patients were referred to undergo $^{18}$F-FES-PET imaging could be grouped into three categories: 1) patients with equivocal or conflicting findings on conventional imaging procedures, 2) patients with metastatic breast cancer in which ER status could not be determined by tissue biopsies, or 3) patients with metastases of unknown origin detected by conventional imaging. Although, our patients clearly represented a very selected group of patients in which prior work-up was inconclusive, $^{18}$F-FES-PET improved the diagnostic understanding of the referring physician in 88% of the cases and contributed to a change in therapy in 48% of the patients. This study therefore suggests that $^{18}$F-FES-PET may well be of value as an add-on diagnostic tool. Therefore, future studies are warranted to determine the exact indications in which $^{18}$F-FES-PET can be of additive value.

In chapter 3A $^{18}$F-FES-PET was used in addition to standard work-up in a patient with a rare tumor type: endometrial stromal sarcoma (ESS). In this chapter we provided a review of the literature on the use of endocrine therapies and present a case report. ESS is a rare uterine tumor that frequently expresses the ER. It is commonly treated by progestins and there is limited data for efficacy of ER-targeted therapies. The patient that we presented had no further evidence-based therapies available, and in addition the most recent tumor biopsy had shown ER negative disease. $^{18}$F-FES-PET was performed to evaluate whether ER-targeted therapy could be an option in this patient. Based on high $^{18}$F-FES uptake in all known lesions, treatment with fulvestrant was initiated. Fulvestrant is a pure anti-estrogen that competitively binds to the ER and down regulates ER expression. Treatment with fulvestrant in ESS was not earlier described in literature. Follow-up after 3 and six months revealed a clear reduction in tumor estrogen binding on $^{18}$F-FES-PET as well as a reduction in tumor volume on CT-scan. Based on this case report fulvestrant can be considered in the treatment of ESS after failure of megestrol and aromatase inhibitors. $^{18}$F-FES-PET may contribute to select the right patients for treatment with fulvestrant.

The reduction in $^{18}$F-FES uptake during fulvestrant can potentially give insights in the optimum dose required to inhibit the ER. In breast cancer, 500 mg fulvestrant per intramuscular injection on day 1, 14, 28 and every 4 weeks thereafter, is the approved dose since 2011. While preclinical studies have shown that fulvestrant can completely down-regulate ER expression and block estrogen-induced proliferation, it is unknown whether the dose administered to
patients is sufficient to completely abrogate ER expression. We therefore evaluated tumor $^{18}$F-FES uptake before and during fulvestrant therapy by means of serial $^{18}$F-FES-PET imaging (in chapter 4) in 16 patients with metastatic breast cancer. A relative reduction of $<75\%$ with an absolute uptake value of $\geq 1.5$ (SUV$_{max}$) was predefined as an incomplete reduction in tumor $^{18}$F-FES uptake. Although fulvestrant decreased tumor $^{18}$F-FES uptake in the majority of patients, in $\sim 38\%$ of the patients there was significant residual tumor $^{18}$F-FES uptake. Interestingly, only one of six patients with incomplete reduction in tumor $^{18}$F-FES uptake had clinical benefit from fulvestrant therapy, while eight of nine patients with $\geq 75\%$ relative reduction had clinical benefit. It therefore deserves further attention to evaluate whether $^{18}$F-FES-PET could serve as an early marker of response to fulvestrant.

Whereas the most commonly used endocrine therapies are aimed at the inhibition of the ER by either depleting circulating estrogens or by competitive antagonism, also stimulation by estradiol can generate an anti-tumor effect. Estradiol-induced apoptosis can be observed in breast cancer cells that are cultured in estrogen-deprived conditions for a longer period. It is hypothesized that estrogen-deprivation makes the tumor cells adapt to the low estradiol levels by increasing ER expression. Due to the up-regulation of ER expression the cells can still grow in response to very low estradiol levels, but at the cost of going into apoptosis when exposed to higher concentrations of estradiol. In chapter 5 we described a study in nineteen patients with metastatic breast cancer that were extensively pretreated with anti-estrogen therapies. A $^{18}$F-FES-PET was performed prior to the initiation of high-dose estrogen therapy. Based on the preclinical observations it was hypothesized that high tumor $^{18}$F-FES uptake, as a measure of high ER expression, would predict efficacy of high-dose estrogen therapy. In this study we showed that absent $^{18}$F-FES uptake was predictor of failure of high dose estrogen 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.

Also in ovarian cancer hormone receptors are thought to play a role in carcinogenesis and disease progression. Endocrine therapy is however not advocated since current phase II studies have only shown modest effects. Surprisingly, however, most studies were performed in patients that were not selected for ER expression. In chapter 6 we have evaluated the presence of androgen receptor (AR), ER$\alpha$ and ER$\beta$, and progesterone receptor (PR) in tissue micro-arrays from 121 patients with epithelial ovarian cancer that were uniformly treated in a multicenter phase II study. ER$\beta$ was the most abundant hormone receptor isoform and was present in 73% of all patients, followed by ER$\alpha$ (31%), PR (19%) and AR (10%). Hormone receptor expression was compared with prospectively collected progression-free and overall survival data, which showed a favorable prognosis for patients with hormone receptor negative tumors. Interestingly, similar to reports in breast cancer, we observed...
a relatively high degree of heterogeneity in hormone receptor expression between the primary ovarian tumor and omental metastases within individual patients. This would point towards the possible value of $^{18}$F-FES-PET for determination of ER status and subsequent treatment decision-making.

It was however unknown whether it was feasible to determine tumor ER expression by $^{18}$F-FES-PET in ovarian cancer patients. This was especially of relevance since ERα expression is generally lower in ovarian cancer than in breast cancer; and metastases usually occur within the abdomen where quantification of $^{18}$F-FES uptake may be impaired by high physiological background uptake of $^{18}$F-FES in liver, intestines, uterus and bladder. In a feasibility study in fifteen patients with ovarian cancer $^{18}$F-FES-PET was performed shortly before debulking surgery. This allowed direct comparison of tumor $^{18}$F-FES uptake and tumor hormone receptor status determined via the golden standard, i.e. immunohistochemistry, for multiple lesions. This pilot study showed it is feasible to visualize and quantify $^{18}$F-FES uptake in ovarian cancer lesions. Quantitative $^{18}$F-FES uptake correlated well with semi-quantitative ERα-scores by immunohistochemistry. The sensitivity and specificity of $^{18}$F-FES-PET for ERα positive ovarian cancer lesions were 79% and 100%. Other important observations in this study were that 1) a diagnostic CT for the allocation of ovarian cancer lesions is recommendable, 2) quantification of $^{18}$F-FES uptake in cystic lesions is hampered, and 3) that neo-adjuvant chemotherapy may affect tumor ER expression and $^{18}$F-FES uptake. Future studies are warranted to evaluate whether $^{18}$F-FES-PET can contribute to select ovarian cancer patients for treatment with endocrine drugs.

**FUTURE PERSPECTIVES**

$^{18}$F-FES-PET in breast cancer

To further optimize the use of $^{18}$F-FES-PET, it will be of relevance to dissect the effects of recent therapies on tumor $^{18}$F-FES uptake. In our study with fulvestrant we observed low tumor $^{18}$F-FES uptake in the four patients that withdrew tamoxifen therapy shortly before baseline $^{18}$F-FES-PET. Similarly, in the study with high dose estrogens $^{18}$F-FES uptake appeared to be hampered in patients that were recently treated with tamoxifen and fulvestrant, despite a 5-week drug-free interval. Thus, recent use of ER antagonists can potentially lead to a false-negative $^{18}$F-FES-PET scan. Ideally, a study should be performed in which individual patients undergo a $^{18}$F-FES-PET scan at two time points following withdrawal of tamoxifen and fulvestrant. This could allow evaluation of the period of withdrawal in which tumor $^{18}$F-FES uptake still increases, and the period in which tumor $^{18}$F-FES uptake reaches a plateau.

The studies presented in this thesis were primarily designed as feasibility studies to obtain
new insights in the possible value of $^{18}$F-FES-PET as diagnostic tool, to predict treatment outcome, and to evaluate therapy effects. However, eventually one would like to know whether $^{18}$F-FES-PET can be implemented into clinical practice. Current guidelines advise re-evaluation of tumor ER- and HER2 expression at first relapse by a tumor biopsy, since these receptors can discordantly be expressed between primary tumor and metastases. However, limitations of tumor biopsies include the accessibility of tumor sites, sampling errors due to heterogeneity within a single tumor lesions, and unrepresentative results due to heterogeneity among different metastases. Potentially, some patients are subsequently falsely withheld from endocrine or HER2-targeted therapy, and others receive these therapies while the majority of the tumor burden lacks expression of these targets. While $^{18}$F-FES-PET can be used to evaluate tumor ER expression in multiple tumor lesions within a patient, $^{89}$Zr-trastuzumab-PET can be used to evaluate tumor HER2 expression. In a multi center study (in collaboration with VUMc and Radboud University Medical Center) 200 patients with newly diagnosed metastatic breast cancer will undergo imaging with $^{18}$F-FES-PET, $^{89}$Zr-trastuzumab-PET and FDG-PET, tumor biopsy, and assessment of and circulating tumor DNA (NCT01957332). It will be evaluated whether these novel imaging techniques and liquid biopsies can give clinically relevant information to guide personalized therapy more precisely. If successful, this may result in implementation of these techniques in future clinical practice.

Several novel endocrine treatment strategies are currently explored, among which combination of endocrine drugs with drugs that inhibit growth factor signaling, cyclin-dependent kinase 4/6 inhibitors, epigenetic drugs, novel ER down-regulators, and androgen receptor (AR)-targeted drugs. The mammalian target of rapamycin (mTOR) acts downstream of growth factor receptors and can phosphorylate and activate ERα ligand-independently. For this reason, combination therapy with the aromatase inhibitor exemestane and the mTOR-inhibitor everolimus has been explored in clinical trials. The phase III trial (BOLERO-2) showed that the addition of everolimus to exemestane extended median progression-free survival from 3.2 to 7.8 months. Another interesting new combination is that of cyclin-dependent kinase 4/6 (CDK4/6) inhibitors with endocrine therapies. Activation of the ER by estrogens results in activation of CDK4/6. CDK4/6 on its turn, induces cell cycle progression via activation of E2F (figure 1). Thus, CDK4/6 is a rational target in ER positive breast cancer. Indeed, in preclinical studies CDK4/6 inhibitors prevented cellular proliferation especially in ER positive (luminal) breast cancer cells. In addition, combination of CDK4/6 inhibition with endocrine drugs synergistically blocked tumor growth. In a phase I study, CDK4/6 inhibition was well-tolerated, and in a recent phase II study it showed promising results when combined with
an aromatase inhibitor.\textsuperscript{223} In this study, progression-free survival increased from 7.5 months for letrozole alone to 26.2 months for the combination of letrozole plus the CDK4/6 inhibitor PD991 (palbociclib).

Despite the advances in terms of progression-free survival it will remain of importance to select the right patients for these combination strategies. For example, the combination of everolimus plus exemestane results in progressive disease within 24 weeks of treatment initiation in \textasciitilde50\% of the patients. Moreover, side-effects are associated with the use of everolimus.\textsuperscript{85,220} Finally, the costs of one month of exemestane therapy equals \textasciitilde€10,\- euro, while combination therapy with everolimus equals \textasciitilde€3600,\-.\textsuperscript{224} Likely, also CDK4/6 inhibitors will be associated with an increase in both costs and side-effects and preclinical studies suggest that CDK4/6 inhibition in ER negative tumors may be counter-effective.

These facts underscore that it remains of relevance to identify those patients that are most likely to benefit from combination therapy and patients that won’t. Lost ER expression and heterogeneous ER expression are among possible reasons for therapy failure. Loss of ER expression in metastases compared to the primary tumor is reported in 16-40\% of breast cancer patients with an ER positive primary tumor.\textsuperscript{8,9,18} \textsuperscript{18}F-\textsuperscript{FES}-PET may aid to identify patients that are most likely to respond to these compounds.

In ER negative tumors the \textit{ESR1} (estrogen receptor) gene is usually not mutated, but transcription is repressed by epigenetic modifications which results in an ER negative phenotype. The two most important epigenetic modifications are DNA methylation and histone tail modifications. Histone deacetylase inhibitors and DNA methyltransferase inhibitors can be used to alter gene expression (figure 2).

We and others have previously shown that HDAC-inhibition can re-express the ER in ER negative breast cancer cells. Also, HDAC-inhibition can re-sensitize ER negative breast cancer xenografts to endocrine therapies in mouse studies.\textsuperscript{82} Finally, in a randomized phase
Il study in 130 metastatic breast cancer patients that progressed on a nonsteroidal aromatase inhibitor, exemestane plus the HDAC inhibitor entinostat was compared to exemestane plus placebo. The combination of exemestane plus entinostat improved PFS (4.3 v 2.3 months) and OS (28.1 v 19.8 months).

Serial \(^{18}\text{F}-\text{FES-PET}\) imaging could shed light on the changes of ER expression upon treatment with HDAC inhibitors, the time frame in which changes occur, and the duration of changes, which would provide critical information for the timing of combination therapies. In an ongoing study (NCT01153672) in ER positive breast cancer patients receiving 2 weeks of vorinostat treatment followed by 6 weeks of aromatase inhibitor therapy, changes in ER expression are measured by \(^{18}\text{F}-\text{FES-PET}\) after 2 and 8 weeks of treatment.

Finally, the efficacy of endocrine therapies may be enhanced by drugs with increased ER binding affinity. We have shown that the estrogen receptor downregulator fulvestrant blocks the ER incompletely in ~38% of metastatic breast cancer patients, and that incomplete blockage was associated with early progression. Novel, orally available ER downregulators with high affinity for the ER are currently in preclinical development. \(^{226}\) \(^{18}\text{F}-\text{FES-PET}\) may be used to evaluate the binding characteristics of novel ER-targeted drugs in vivo animal and clinical studies. Ideally,

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**Figure 2.** The figure shows the interactions between epigenetic enzymes (writers, erasers, readers) and nucleosomes. The nucleosome core consists of a histone octamer (mainly two copies each of H2A, H2B, H3 and H4) that is wrapped by a nuclear DNA strand of 147 bp. DNA methylation and hydroxymethylation are depicted as black and grey circles, respectively. DNA methylation is induced by DNA methyltransferases (DNMTs). To inhibit DNA methylation, DNMT inhibitors (DNMTIs) are used to target and suppress DNMTs. Histone tales can be post-transcriptionally modified using enzymes such as histone acetyltransferases (HATs). Histone acetylation can be inhibited by histone deacetylases (HDACs), and HDAC inhibitors (HDACIs) can be used as HDAC suppressors.
18F-FES-PET should be performed in dose-escalation studies which would allow assessment of the most optimal dose to completely block tumor estrogen binding capacity.

Not only ER-targeted drugs, but also treatments targeting the androgen receptor (AR) may be valuable in breast cancer. The AR is expressed in ~75% of all breast tumors, and moreover in 12-30% of the triple-negative tumors.227,228 This offers a potential new treatment option for this subgroup that can nowadays only be treated with chemotherapy.229,230 AR-targeted therapies (flutamide, bicalutamide, nilutamide) are already widely available for the treatment of prostate cancer, and several novel AR-antagonists, such as enzalutamide, abiraterone acetate, and ARN-509 have shown promising results in this patient group.231,232

Tumor AR expression can be evaluated by 16β[18F]fluoro-5α-dihydrotestosterone (18F-FDHT) PET.233 18F-FDHT uptake is observed in metastases from prostate cancer patients.234, and AR-targeted drugs such as enzalutamide and flutamide have shown to block tumor 18F-FDHT uptake.235,236 In a multi-center (in collaboration with VUMc) feasibility study in 20 metastatic breast cancer patients we will evaluate whether tumor 18F-FDHT uptake can be visualized in breast cancer patients (NCT01988324). Biopsies will be obtained to evaluate whether quantitative tumor 18F-FDHT uptake correlates with tumor AR expression. When 18F-FDHT-PET proves feasible in metastatic breast cancer patients, this technique will deserve further exploration to select patients for AR-targeted therapies, and to evaluate AR binding characteristics of these drugs in vivo.

18F-FES-PET in ovarian cancer
We have shown that it is possible to visualize and quantify tumor ERα expression not only in breast cancer, but also in ovarian cancer in chapter 8. Compared to breast cancer, endocrine therapy has moderate to poor efficacy in unselected ovarian cancer patients. It seems rational to select patients for these therapies based on tumor ER expression, however whether this indeed predicts response and which thresholds should be applied is unknown. In breast cancer patients tumor 18F-FES uptake >1.5 (SUV_{max}) was predictive of achieving clinical benefit from endocrine therapy, and moreover patients with tumor 18F-FES uptake below 1.5 were very unlikely to respond. It may however well be possible that other thresholds would apply to ovarian cancer. In our study, three (21%) of 16 patients stood out with visually increased tumor 18F-FES uptake compared to background. In these three patients 18F-FES uptake was high (median SUV_{max} >3.5) when compared to the other patients in which 18F-FES uptake was comparable or only slightly higher than physiological background (median SUV_{max} < 2.3). It deserves further exploration in a prospective clinical study whether those patients with clearly increased 18F-FES uptake are indeed the patients that could benefit from endocrine therapy.