PET Imaging of Steroid Receptor Expression in Breast and Prostate Cancer

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Abstract: The vast majority of breast and prostate cancers express specific receptors for steroid hormones, which play a pivotal role in tumor progression. Because of the efficacy of endocrine therapy combined with its relatively mild side-effects, this intervention has nowadays become the treatment of choice for patients with advanced breast and prostate cancer, provided that their tumors express hormone receptors. However, in case of breast cancer it is well known that part of the patients have hormone receptor-negative tumors at diagnosis, whereas other patients have discordant receptor expression across lesions. In addition, receptor expression can change during therapy and result in resistance to this therapy. Besides several lines of hormonal treatments, also other strategies to affect the hormone receptors are currently under investigation, namely histone deacetylases (HDAC) and heat shock protein (HSP) inhibitors. Knowledge of the actual receptor status can support optimal treatment decision-making and the evaluation of new drugs. Positron emission tomography (PET) is a non-invasive nuclear imaging technique that allows monitoring and quantification of hormone receptor expression across lesions throughout the body. Several PET tracers have been developed for imaging of the most relevant hormone receptors in breast and prostate cancer: i.e. the estrogen, progesterone and androgen receptors. Some of these PET tracers have been successfully applied in early clinical studies. This review will give an overview of the current status of PET imaging of hormone receptors in breast and prostate cancer.

Key Words: Breast cancer, prostate cancer, estrogen receptor, progesterone receptor, androgen receptor, endocrine therapy, positron emission tomography, imaging.

1. INTRODUCTION

Worldwide, breast and prostate cancer are common causes of death among women and men, respectively. Breast and prostate cancer are the best known examples of hormone dependent tumors, although other tumors like ovarian tumors and endometrial cancer are also frequently characterized by hormone dependency. In hormone dependent tumors, the hormone receptors play a key role in tumor proliferation and disease progression. The primary signal for the activation of steroid hormone receptors (SR) is binding of the hormone (Fig. **1**). In the absence of hormone, steroid receptor monomers are associated with heat shock protein (HSP) complexes and as a rule are only phosphorylated to a small extent. Upon binding of the hormone, receptors dissociate from the HSPs and form dimers. These hormone receptor dimers, translocate from the cytoplasm to the nucleus, bind to target gene-specific sites containing hormone response elements (HRE) and recruit a series of co-activator complexes to regulate target gene transcription [1]. Since they can activate oncogenes and inhibit the expression of tumor-suppressor genes, the steroid hormone receptors are important intermediates in the progression of breast and prostate cancer and therefore are key targets for treatment in patients with breast and prostate cancer. At diagnosis, 70% of the breast cancer patients are positive for estrogen receptor (ER) and/or progesterone receptor (PR) expression, whereas the androgen receptor (AR) is expressed in 80-90% of the patients with prostate cancer. The vast majority of hormone receptor expressing tumors are sensitive for endocrine treatment, which aims to inhibit the hormone receptor-mediated pathway for tumor proliferation. Endocrine therapy has become the essential part of treatment for hormone receptor positive patients with primary and metastatic breast cancer and patients with advanced prostate cancer, with a favorable benefit-to-toxicity ratio. Despite significant advances in primary cancer treatment, many patients will develop a systemic relapse. At the time of systemic relapse and during treatment, receptor expression can change [2-5]. To achieve an effective hormone receptor-mediated treatment, knowledge of the actual receptor status of the primary tumor and metastases would be benificial (Cancer Information Summaries: Adult treatment; http://www.cancer.gov/cancertopics/pdq/adulttreatment). It is however often hard to obtain fresh tumor tissue due to the location of systemic metastasis. Non-invasive molecular imaging techniques to monitor the actual status or occupancy of the steroid receptors could therefore be of additional value for therapy management of patients with breast and prostate cancer. This review will give an overview of the current status of the application of the mo-

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lecular imaging technique, PET, for visualization of steroid hormone receptor expression in breast and prostate cancer.

2. STEROID HORMONE RECEPTORS IN BREAST CANCER

Endocrine therapy has become the treatment of choice for many patients with metastatic breast cancer. The most frequently applied endocrine drugs are tamoxifen (antagonist of the ER, but with agonistic properties at low concentrations), fluvestrant (a pure ER antagonist; induces degradation of the receptor) and aromatase inhibitors (inhibit the production of estrogens). The importance of steroid hormone receptors in breast cancer was already recognized over 40 years ago, when radiolabeled estrogens were found to concentrate preferentially in the estrogen-influenced target organs of animals and in human breast cancers. At diagnosis, 70% of the breast cancer patients have tumors with positive ER or PR expression. Out of these patients, 50% to 60% will respond to anti-hormonal treatment, whereas the remain fraction will not (probably due to intrinsic resistance). For breast tumors that do not express these hormone receptors, endocrine therapy is not effective. Among the patients with steroid hormone receptor expressing tumors, the ER–/PR+ phenotype represents only 3% to 5% of patients, which would suggest that determination of the ER status is of primary importance for therapy management. However, determination of the PR status could still be relevant because it is a reflection of an intact ER signaling pathway. For example, expression of the PR distinguishes two subsets of ER-positive tumors that may require different treatment strategies. ER-positive tumors without PR expression were found to respond less likely to the selective ER modulator tamoxifen than the ER-positive tumors that also expressed PR [6, 7]. Because of their relative resistance to tamoxifen, the ER+/PR- tumors should therefore preferably be treated initially with an aromatase inhibitor.

After an initial response to tamoxifen or aromatase inhibitor treatment, all patients will eventually become resistant to anti-hormonal treatment (acquired resistance) [8-10]. Remarkably, loss of ER expression (ER-) has only been demonstrated in 17-28% of patients with acquired resistance [11] and mutations of the ER are rare and have mainly been found in tumors that were immunohistochemically classified as ER- [12]. Thus, the majority of the tumors with acquired anti-hormonal resistance still expresses the ER. A major mechanism of acquired resistance to endocrine therapy is the development of increased sensitivity of breast cancer cells for estrogen or (partial) ER agonists like tamoxifen [8]. This first phase of acquired resistance to tamoxifen is characterized by tamoxifen or estrogen-stimulated tumor growth. Removal of estrogen with an aromatase inhibitor or blocking the ER with the pure antagonist fulvestrant prevents tumor growth and provides an alternative therapy for patients that have become resistant to tamoxifen. Laboratory studies have demonstrated that a second phase of acquired resistance can occur after prolonged estrogen deprivation. In this phase of acquired resistance, tumor cells have become hypersensitive to estrogens and endocrine treatment is ineffective. In fact, at this stage tumor cells have become so hypersensitive to estrogens that they are killed by physiological concentrations of the hormone. This mechanism was first demonstrated in in-vitro experiments, in which ER expressing tumor cells that were maintained estrogen-free for years showed a switch in response to estradiol from stimulation of proliferation into induction of apoptosis [13, 14]. Similarly, estradiol caused a rapid regression of ER expressing tumors in athymic mice that had been treated with tamoxifen for a long period of time [15]. Interestingly, estrogen-hypersensitivity of tamoxifen-resistant tumor cells is accompanied by a 4 to 10-fold increase in ER expression. A clinical parallel to the aforementioned laboratory observations of estrogen-hypersensitivity is shown in a number of studies in respectively 523, [16], 143 [17] and 32 patients [18], which demonstrated that treatment with estrogen showed objective responses in 30 to 42% of the breast cancer patients that were previously treated with one or more lines of hormonal treatment. Another mechanism of acquired tamoxifen-resistance in breast tumors is cross-talk of the ER pathway with other signal transduction pathways, such as growth factor receptor signaling pathways (e.g. EGFR, HER-2). In this situation, tumor growth is stimulated by growth factor receptor signaling, whereas the classic ER genomic function is repressed. [19].

Since the steroid receptor pathway can be affected in several manners during treatment, different alternative treatments would be applicable in therapy resistant patients. Selection of the most suitable treatment of therapy-resistant tumors could be based on the actual ER expression levels in the tumor. If steroid receptor expression is lost, the treatment of choice would be chemotherapy. When the ER density is increased, estrogen treatment may be a good alternative. For resistant tumors with intermediate ER density, treatment with an aromatase inhibitor or fulvestrant would be the best option. Moreover, exiting new avenues may be openend by the development of new drug as HDAC and HSP inhibitors, which affect by their action by modulation of the expression of the ER [20-22]. Molecular imaging techniques, such as PET, to monitor the presence and to quantify expression levels of the ER, PR and AR could be of additional value for therapy management**.**

3. PROSTATE CANCER: THE ANDROGEN RECEPTOR.

Since approximately 80-90% of prostate cancers is androgen-dependent at initial diagnosis [23], endocrine therapy of prostate cancer is always directed toward the reduction of serum androgens and inhibition of the AR signaling. However, the initial response to anti-androgen therapy is almost always followed by a relapse to an unresponsive, hormone-refractory stage. In prostate cancer, the same mechanism of anti-hormonal therapy resistance have been found as in breast cancer [24]. In contrast to ER expression in breast cancer, the hormone-refractory stage in prostate cancer is rarely associated with a loss of AR expression [25]. On the contrary, the AR gene is over-expressed in approximately 20 to 30% of the hormone refractory tumors [23, 26]. Edwards *et al.* investigated AR protein expression in hormone-sensitive and hormone-refractory tumors from the same patient [26]. They found that AR expression levels were higher in hormone-resistant tumors than in matched hormone-sensitive tumors. Others found that 30-40% of men whose disease progresses during anti-androgen therapy experienced

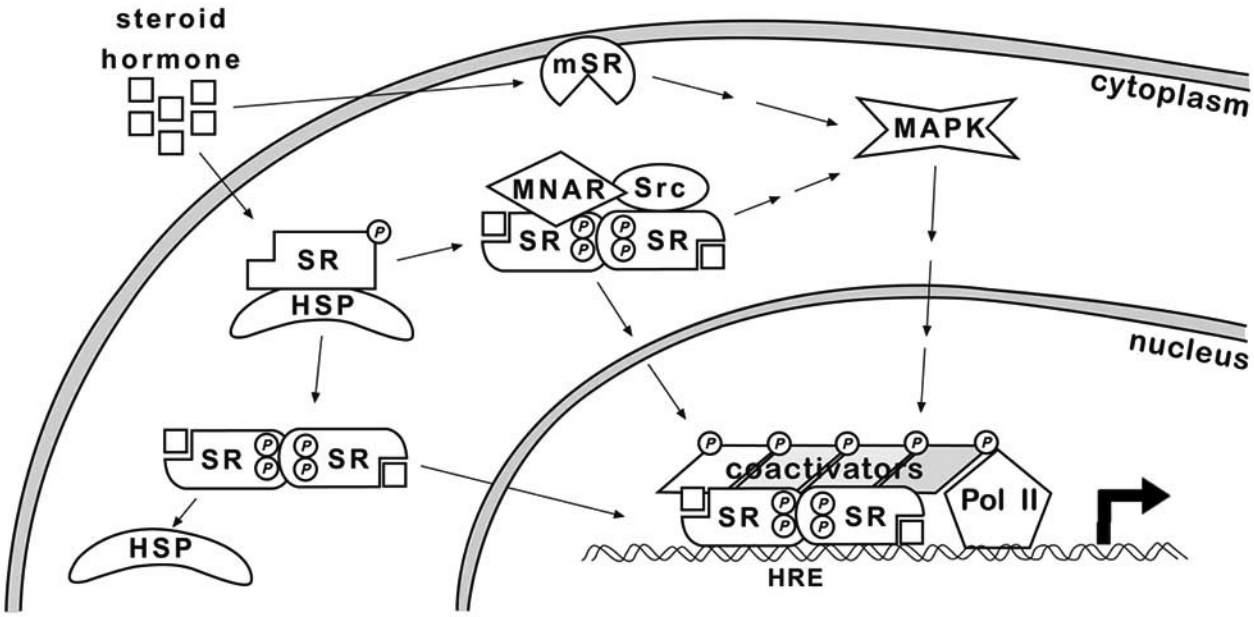


Fig. (1). Mechanism of steroid hormone action as published by Weigel and Moore [1] (reproduction is allowed). In the absence of hormone, steroid receptor monomers (SR) are associated with heat shock protein complexes (HSP) and are typically basally phosphorylated. Upon binding hormone (1), receptors dissociate from HSPs and dimerize (2). The dimer binds to target gene-specific sites containing hormone response elements (HRE) (3), and recruits a series of coactivator complexes to regulate target gene transcription (4). Site-specific phosphorylation of receptors increases subsequent to hormone binding, with some increases occurring rapidly, and others with delayed kinetics. Upon steroid binding, some receptors also interact with Src (steroid receptor coactivator) and MNAR (modulator of nongenomic action of estrogen receptor) (5), activating Src and downstream MAPK (mitogen-activated protein kinases) (6). Membrane-associated steroid receptors (mSR) also bind hormone and initiate signaling cascades (7). While some of these are classical steroid receptors, others bear no homology to the steroid receptor superfamily.

a fall in serum prostate-specific antigen (PSA) after discontinuation of therapy [27]. These results suggest the development of hypersensitivity to androgens, in analogy to the phase II type of resistance to anti-estrogen therapy in breast cancer. In animal model hormone refractory prostate cancer cells became highly sensitive to androgens. Besides androgen hypersensitivity that is accompanied by enhanced AR expression, there are several other mechanisms that lead to resistance to androgen ablation [28]. For example, increased 5-alpha reductase activity causes conversion of the less potent AR agonist testosterone to its metabolite dihydrotestosterone, which is 10 times more potent and therefore can more efficiently induce AR signaling [29, 30]. Other mechanisms could involve enhanced levels of AR transcriptional co-activators and cross-talk between the AR signaling pathway and other signal transduction pathways that activate the Src/MAPK kinase cascade (Fig. **1**).

As follows from the above, the AR density can change during treatment. Depending on the kind of changes in AR expression different follow-up treatments may be required. In rare cases where AR expression is lost, the treatment of choice will be chemotherapy. In case of increased AR expression (hypersensitivity), discontinuation of anti-androgen therapy or even supplementation of androgens has the preference. If normal AR levels are maintained when resistance develops, alternative treatment could be aimed at further decreasing the AR agonist concentrations in blood, blocking the receptor with full AR antagonists or preventing cross talk between the AR pathway and other signal transduction pathways that activate the Src/MAPK kinase cascade. Thus, for optimal treatment decision-making, molecular imaging techniques to monitor the actual density of the AR and to visualize the effect of the intervention could be of additional value**.**

In addition to clinical decision-making, imaging may also play a role in the development of new drugs. Currently, new drugs are being developed that can modulate hormone receptor expression. Promising candidates among these drugs are histone deacetylases (HDAC) and heat shock protein (HSP) inhibitors. HDACs are enzymes that deacetylate the aminoteminal tails of histones, causing structural changes in chromatin that regulated transcription. HDAC inhibitors can also destabilize the AR by interfering with the binding of HSP90 to the AR. HSP90 inhibitors cause AR degradation by competing with ATP for binding. Thus, HDAC and HSP90 inhibitors induce the breakdown of the hormone receptors and thus interrupt the hormone receptor signaling pathway that is responsible for tumor growth. The HSP90 inhibitors and HDCAs are currently in early clinical phase I/II trials [31]. Imaging of AR density by quantitative PET imaging could help the development of these drugs.

4. DEVELOPMENT OF PET TRACERS FOR STEROID RECEPTORS

PET is a nuclear imaging technique that could be an attractive alternative for repeated biopsies of tumor tissue as a tool for guiding of treatment. PET can visualize and quantify physiological and biochemical parameters in-vivo by administering a radioactive tracer to a patient. The distribution of the tracer is monitored over time using a dedicated PET

camera. The data that are acquired by the PET camera are subsequently converted in quantitative 3D-images of the tracer distribution as a function of time. With pharmacokinetic modeling paradigms, the dynamic PET data can provide quantitative in-vivo measures of biochemical and physiological parameters. This technique is basically non-invasive and allows monitoring of the whole body in a single session. Nuclear imaging techniques like PET could therefore offer the unique opportunity to detect and quantify the steroid receptor expression levels in patients with hormone responsive tumors, provided that a suitable tracer for the receptor of interest is available. Thus, imaging-based tumor characterization may provide the required input for guiding systemic therapy in patients and may assist drug development. In the following sections, we will describe the current status of PET imaging methods for the most relevant steroid receptors in breast and prostate cancer: ER, PR and AR.

4.1. PET Tracers for Imaging of Estrogen Receptors

In the 1980's, the estrogen derivative 16α-[^{18}F]fluoro-17β-estradiol ([^{18}F]FES) was developed for imaging of the ER (Fig. **2**). To date, [^{18}F]FES is still the most frequently applied PET tracer for ER imaging. A simplified, two-step procedure for labeling [^{18}F]FES in high radiochemical yields was described by Römer *et al.* [32] and subsequently adapted for automated clinical productions [33-35]. [^{18}F]FES proved stable upon storage in aqueous ethanol solution for up to 24 h. In-vivo, [^{18}F]FES showed favorable characteristics as an imaging agent for the ER in tumor-bearing rat and mouse models [36-40]. Highest tracer uptake was found in the uterus and ovaries, both organs with high ER expressions. The ER consists of 2 subtypes, called ERα and ERβ. Studies in ERα and in ERβ knock-out mice demonstrated that [^{18}F]FES preferentially binds to the ERα-subtype [36]. In rats, co-injection of estradiol resulted in a dose-dependent reduction in tracer uptake in these organs at an injected dose of 1 μg and higher [41]. In addition, receptor occupancy by tamoxifen after pretreatment of the rats with the drug could be measured by titration of [^{18}F]FES uptake in relevant organs. In rodents, [^{18}F]FES is also able to detect ER expression in positive breast tumors, either by ex-vivo biodistribution or microPET imaging [38, 40].

In rat plasma, [^{18}F]FES is converted into a hydrophilic metabolite with a metabolic half-life of approximately 30 min [42]. [^{18}F]FES is also fairly rapidly metabolized into glucuronides and sulfates in human plasma [43]. The unmetabolized [^{18}F]FES is mainly reversibly bound to albumin and sex hormone binding globulin (SHBG) in plasma, where it is largely protected from metabolism.

The feasibility of in-vivo quantification of [^{18}F]FES binding parameters, using either equilibrium analysis or graphical analysis was demonstrated in rat brain [42]. Both analysis approaches allowed quantitative measurement of ER in receptor-rich brain regions, such as pituitary and hypothalamus, but not in brain areas with low receptor levels like hippocampus. In contrast, [^{18}F]FES uptake was found to be flow-dependent in tissues with very high ER concentrations, such as uterus and ovaries and therefore tracer uptake may underestimate ER levels in these organs [41]. Since receptor density in breast tumors is substantially lower than in uterus, quantification of ER density in tumors by [^{18}F]FES PET should be feasible. In fact, FES uptake was found to correlate with the ER concentration (B_{max}) in the tumor from in-vitro assays, although the correlation coefficient was relatively low ($r = 0.45$, $p<0.05$) [38]. [^{18}F]FES has already been used to monitor ER expression in several patient studies (see section 4.1).

In order to develop an improved tracer, moxestrol (17α-ethynyl-11β-methoxy-estradiol), which is one of the most potent estrogens, has been labeled with fluoro-18 for PET imaging of ER. 16β-[^{18}F]fluoromoxestrol ([^{18}F]βFMOX) was

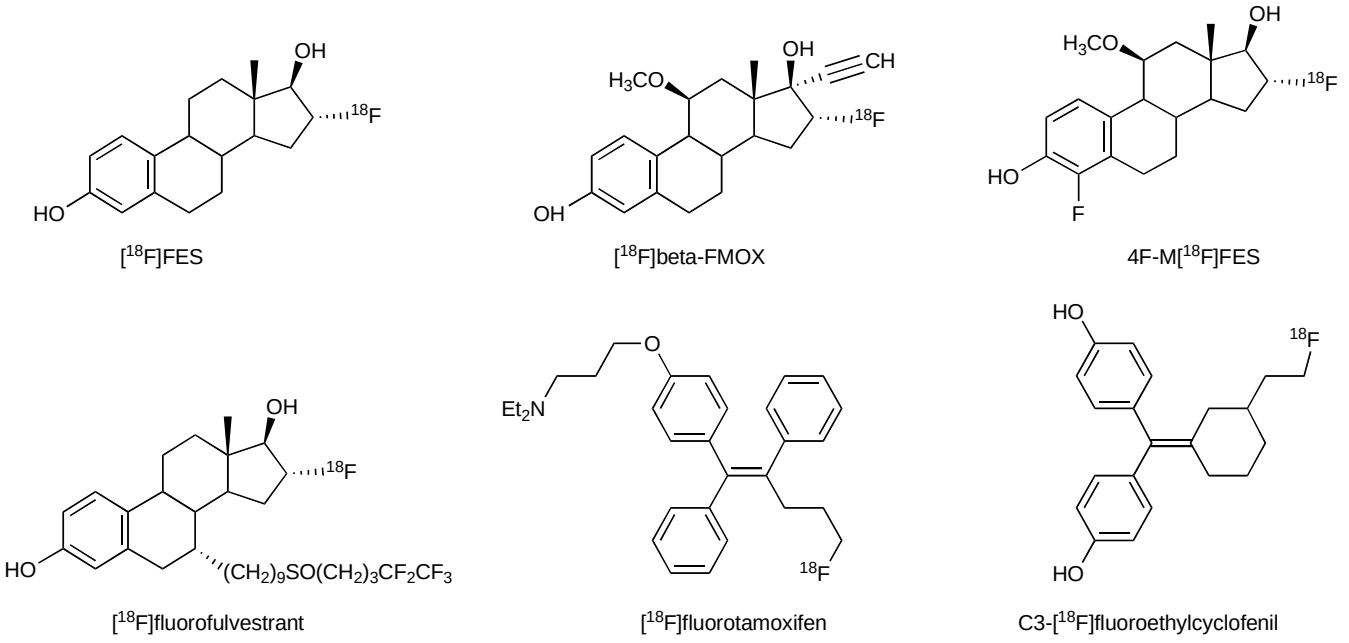


Fig. (2). Structures of PET tracers for imaging of the estrogen receptor.

evaluated in animal experiments and displayed the most promising characteristics for a PET tracer among a series of other 17-ethynylestradiols [44-46]. Uterine uptake of [^{18}F]βFMOX in immature rats was approximately twofold higher than that of [^{18}F]FES. In contrast to [^{18}F]FES, [^{18}F]βFMOX also exhibited specific binding in organs with low levels of ER expression, such as kidney, muscle and thymus. This suggests that [^{18}F]βFMOX could be a more sensitive tracer of ER than [^{18}F]FES. The improved tracer uptake of [^{18}F]βFMOX in target organs is most likely due to its higher metabolic stability, which results in an extended bioavailability [46]. Dosimetry in immature female rats indicated that the radiation burden of [^{18}F]βFMOX was within acceptable limits for clinical application [46]. Despite the encouraging results in rat studies, [^{18}F]βFMOX proved unable to detect ER-positive lesions in breast cancer patients [47]. This lack of specific uptake was ascribed to fast metabolism of the tracer in humans. In contrast to [^{18}F]FES, [^{18}F]βFMOX had low affinity for SHBG and consequently is not protected against metabolic degradation.

Besides [^{18}F]βFMOX, several other modifications in the structure of [^{18}F]FES have been investigated in order to improve the binding affinity and/or stability of the tracer, such as the introduction of an 11β-methoxy or a 7α-methyl substituent [39, 48]. Among these tracers, 11β-methoxy-4,16α-[16α-^{18}F]difluoroestradiol (4F-M[^{18}F]FES) showed the highest uterine uptake and uterus-to-background ratios. This compound has low binding affinity for SHBG. Its properties remain to be further investigated in humans.

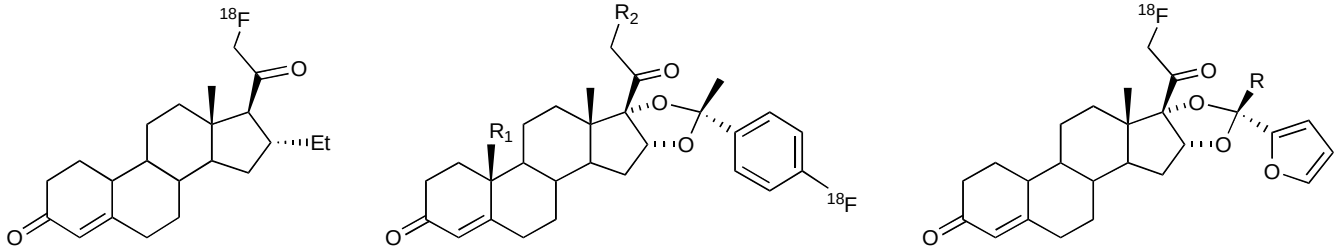
In addition to tracers that were derived from estradiol, a few tracers have been based on drugs that are used in hormonal therapy. For example, the ER antagonist fulvestrant was labeled at the 16α-position with fluorine-18 [49]. However, introduction of the [^{18}F]fluorine atom strongly reduced the binding affinity of the compound and uterine uptake in immature rats, making the tracer unsuitable for PET imaging. Also the selective ER modulator tamoxifen was labeled with fluoro-18 [50]. In rats, [^{18}F]fluorotamoxifen exhibited specific uptake in uterus and mammary tumors that could be blocked by co-administration of estradiol. However, uterine uptake and the percentage of displaceable binding of [^{18}F]fluorotamoxifen was much lower than that of fluoro-18 labeled steroids like [^{18}F]FES. Still, [^{18}F]Fluorotamoxifen was evaluated in a pilot PET study in 10 women with ER-positive breast tumors [51]. Twenty-three lesions were evaluated, of which 2 out of 3 lesions were scored as true negative and 16 out of 20 lesions as true positive. Tracer uptake did not correlate with ER concentration in the primary lesion. It was suggested that [^{18}F]fluorotamoxifen might have some use in predicting response to treatment, as tracer uptake in tumors that responded well to tamoxifen treatment was higher than in poorly responding tumors, but this was only the case when bone lesions were excluded. However, the statistical power of this study was limited, because only a small number of patients were included in the study.

Cyclofenil derivatives form another class of non-steroidal ER ligands that have been labeled with ^{18}F, ^{11}C and ^{94}Tc for PET imaging [52-55]. Despite high in-vitro binding affinities of these compounds, specific uptake in rats was disappointingly low.

Thus, [^{18}F]FES remains the only validated PET tracer for ER that is currently used in clinical studies, although its characteristics are not ideal. So far, attempts to develop a tracer with better properties for ER imaging have yielded disappointing results. Therefore, the efforts to develop better alternatives for [^{18}F]FES have increased.

4.2. PET Tracers for Imaging of Progesterone Receptors

So far, only a few tracers for PET imaging of the PR have been investigated (Fig. **3**). Two decades ago, encouraging preclinical results have already been obtained with the high affinity PR ligand 21-[^{18}F]fluoro-16α-ethyl-19-norprogesterone ([^{18}F]FENP) [56, 57]. In estrogen-primed rats, [^{18}F]FENP showed high levels of specific uterine uptake, with uterus-to-blood ratios of 14 [57] to 26 [56] at 60 min after tracer injection. Uterine uptake could be blocked by pretreatment with unlabeled FENP (≥ 83% reduction in uptake), indicating that uterine uptake was receptor-mediated. However, considerable [^{18}F]FENP uptake was also observed in fat and bone, reflecting the high lipophilicity and metabolic defluorination rate of the compound, respectively [56]. High tracer uptake in fat could hamper imaging of breast lesions, since the breast contains a high proportion of adipose tissue. In addition to the specific tracer uptake in the uterus, receptor-mediated uptake of [^{18}F]FENP was also observed in PR-positive mammary carcinoma in mice, although the uptake in the tumor was substantially lower than in the uterus [57]. In a pilot study in 8 patients with PR-positive primary breast carcinoma, however, tumor-background ratios of [^{18}F]FENP were low and consequently the tumors could only be detected in 50% of the patients [58]. Moreover, [^{18}F]FENP uptake did not correlate with PR expression lev-



[^{18}F]FENP

4-fluorophenyldioxolane derivatives
(R_1 = H, CH_3, R_2 = OH, H)

furanyldioxolane derivatives
(R = H, CH_3)

Fig. (3). Structures of PET tracers for imaging of the progesterone receptor.

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