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Mini-Review

Nuclear Receptor Imaging In Vivo—Clinical and Research Advances

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Abstract

Nuclear receptors are transcription factors that function in normal physiology and play important roles in diseases such as cancer, inflammation, and diabetes. Noninvasive imaging of nuclear receptors can be achieved using radiolabeled ligands and positron emission tomography (PET). This quantitative imaging approach can be viewed as an in vivo equivalent of the classic radioligand binding assay. A main clinical application of nuclear receptor imaging in oncology is to identify metastatic sites expressing nuclear receptors that are targets for approved drug therapies and are capable of binding ligands to improve treatment decision-making. Research applications of nuclear receptor imaging include novel synthetic ligand and drug development by quantifying target drug engagement with the receptor for optimal therapeutic drug dosing and for fundamental research into nuclear receptor function in cells and animal models. This mini-review provides an overview of PET imaging of nuclear receptors with a focus on radioligands for estrogen receptor, progesterone receptor, and androgen receptor and their use in breast and prostate cancer.

Key Words: estrogen receptor, progesterone receptor, androgen receptor, cancer, positron emission tomography

Abbreviations: AR, androgen receptor; CT, computed tomography; ER, estrogen receptor; FDA, US Food and Drug Administration; FDG, 2-deoxy-2-[^{18}F]fluoro-D-glucose; FDHT, 16β-^{18}F-fluoro-5α-dihydrotestosterone; FES, 16α-[^{18}F]fluoro-17β-estradiol; FFNP, 21-^{18}F-fluorofuranylnorprogesterone; FXR, farnesoid X receptor; GR, glucocorticoid receptor; mCRPC, metastatic castration-resistant prostate cancer; MRI, magnetic resonance imaging; PET, positron emission tomography; PPARγ, peroxisome proliferator-activated receptor γ; PR, progesterone receptor; PSMA, prostate-specific membrane antigen; RXR, retinoid X receptor; SUV_{max}, maximum standardized uptake value; VDR, vitamin D receptor.

Nuclear receptors are transcription factors that regulate gene expression in normal development, reproduction, metabolism, and homeostasis. These proteins are also key regulators in cancer, inflammation, diabetes, and metabolic disease [1]. The nuclear receptor superfamily consists of 48 proteins in humans, which share a conserved functional domain organization including a ligand-binding domain, DNA-binding domain, amino-terminal region, and hinge region [2].

Nuclear receptors are important targets for therapy, with approximately 13% of US Food and Drug Administration (FDA)-approved drugs targeting nuclear receptors [3, 4]. In oncology, ligands targeting the estrogen receptor (ER) and androgen receptor (AR) are routinely used in the clinical treatment of breast cancer and prostate cancer, respectively. Additionally, molecular imaging of nuclear receptors can be a noninvasive way to identify whether the target for therapy is present to help guide optimal use of targeted therapies.

Positron emission tomography (PET) radiopharmaceuticals used for imaging nuclear receptors are small molecules labeled with a positron emitting radioisotope such as ^{18}F. Due to the intracellular localization of nuclear receptors, radiolabeled antibodies are not effective. After intravenous injection, the PET radiopharmaceutical distributes throughout the body and accumulates in organs and tumors expressing the targeted nuclear receptor. PET scanners coupled with computed tomography (PET/CT) or magnetic resonance imaging (PET/MRI) provide visual localization and quantitative information regarding receptor binding and occupancy. Steroidal PET radiopharmaceuticals typically demonstrate nanomolar to subnanomolar binding affinity for their respective steroid receptor, produce high-quality images using subpharmacologic mass doses, and thus do not functionally activate or inhibit the targeted nuclear receptor with minimal adverse events compared to conventional intravenous contrast agents used for CT and MRI [5].

Several targeted and selective PET imaging radiopharmaceuticals for nuclear receptors have been developed with various preclinical and clinical applications (Fig. 1). Potential uses span from basic and translational research to clinical use with recent approval by the FDA for an ER PET radiopharmaceutical, 16α-[^{18}F]fluoro-17β-estradiol (FES). This article reviews PET imaging of nuclear receptors with a focus on recent ER, progesterone receptor (PR), and AR imaging clinical trials and a brief review of other less well-studied nuclear receptor

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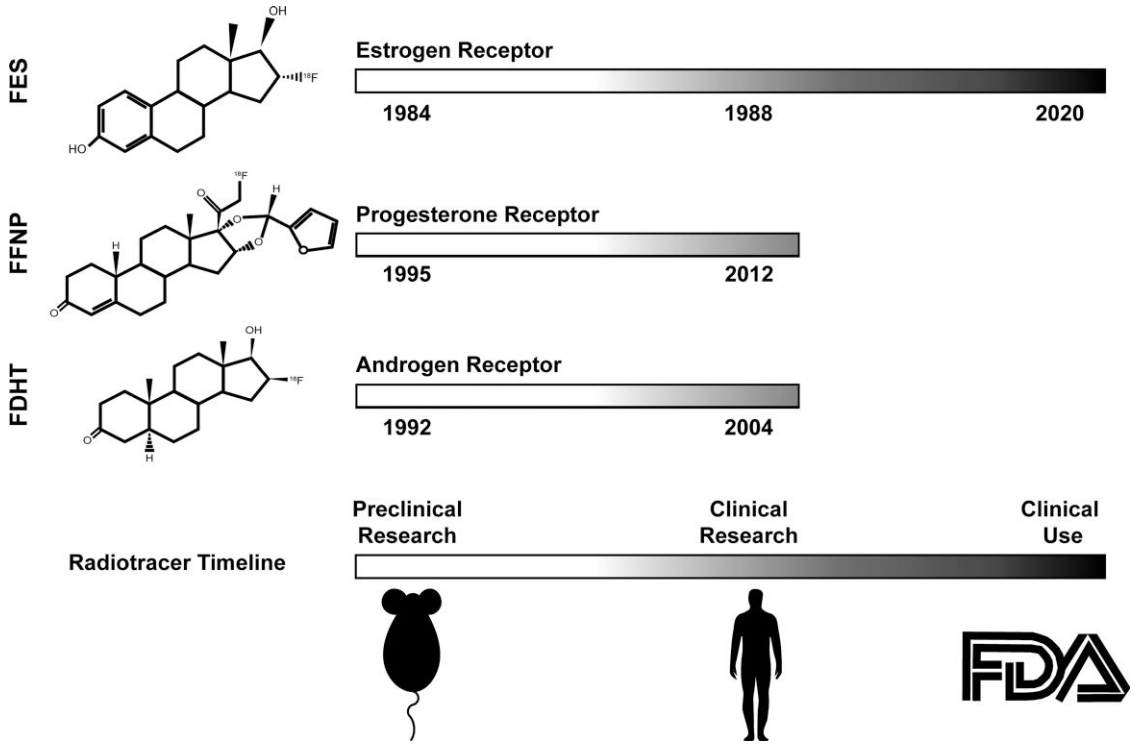

Figure 1. Development of positron emission tomography (PET) radiopharmaceuticals targeting nuclear receptors from basic preclinical research culminating in US Food and Drug Administration (FDA) approval. 16α-[^{18}F]fluoro-17β-estradiol (FES) has been approved for clinical use in patients with estrogen receptor–positive breast cancer. 21-^{18}F-Fluorofuranylnorprogesterone (FFNP) and 16β-^{18}F-fluoro-5α-dihydrotestosterone (FDHT) have both reached clinical trials for patients with breast cancer and prostate cancer, respectively. ^{11}C-YJH08 is a new radioligand specific to glucocorticoid receptor (not shown) with an ongoing first-in-human study in patients with prostate cancer. Various PET radiopharmaceuticals (not shown) targeting other nuclear receptors (PPARα, PPARγ, VDR, RXR, FXR) have been developed and tested in preclinical research.

targets. The rationale for development and significance for clinical use and for quantitative tools in fundamental research into nuclear receptor function will be discussed. For additional details regarding the development of PET imaging agents for steroid hormone receptors in breast and prostate cancer, readers are referred to this comprehensive review [6]. It should be noted that while FES has received FDA approval for clinical use, all other steroid ligand radiopharmaceuticals mentioned in this mini-review are for investigational purposes only.

Estrogen Receptor

Breast cancer is the most common malignancy in women, excluding nonmelanoma skin cancer. The incidence of breast cancer has risen over the past several decades, primarily due to increasing local-stage and hormone receptor–positive disease with 80% of invasive breast cancers being ER positive (ER+) [7]. Breast cancer is the second leading cause of death for women after lung cancer and is the leading cause of death in Black and Hispanic women [8].

2-Deoxy-2-[^{18}F]fluoro-D-glucose (FDG) is clinically the most commonly employed molecular imaging agent to identify local and metastatic breast cancer, and to evaluate response to therapy of patients with metastatic breast cancer [9]. FDG is a glucose analogue that undergoes transport across the cell membrane by glucose transporters (GLUT) and becomes phosphorylated by hexokinase as the first step in the glycolytic pathway, effectively trapping it within the cell. Many breast cancers have high reliance on glucose metabolism (Warburg effect) resulting in high FDG uptake [10-13]. However, there are limitations for FDG PET in the evaluation of breast cancer, namely that 1) some breast malignancies, such as invasive lobular breast carcinoma, have notoriously low FDG uptake [14] and 2) FDG is unable to identify functional hormone receptor status [15]. FDG uptake is positively associated with tumor grade and Ki-67 proliferation index, and is inversely proportional to ER status [16], affording an opportunity for improved lesion detection and signal-to-noise with ER imaging [17]. A meta-analysis of 7 studies including 171 patients compared the diagnostic accuracy of FES and FDG PET/CT for ER+ breast cancer and found that FES was more sensitive than FDG for lesion-level detection at the time of disease relapse with a trend toward statistical significance [18].

FES was the first nuclear receptor imaging agent approved by the FDA for clinical use in 2020 "with PET imaging for the detection of ER+ lesions as an adjunct to biopsy in patients with recurrent or metastatic breast cancer" [19]. FES was developed in the 1980s at the University of Illinois [20] and was the first receptor-based radiopharmaceutical to be successfully evaluated in humans [6, 21]. There are 2 subtypes of ER, ERα and ERβ, both of which function as ligand-dependent transcription factors. While ERβ is expressed in physiologic breast, ovarian, and prostate tissues, ERα is more commonly overexpressed in ER+ malignancies, with ERβ levels decreasing as the cancer progresses [22]. Although FES has high, nanomolar-range binding affinity both for ERα and ERβ, FES has a 6.3-fold preferential affinity for ERα [23]. ERβ-specific radiopharmaceuticals have been developed but have not yet been evaluated in human studies [23-26]. To the best of our knowledge, there have been no publications investigating whether FES recognizes membrane-bound ER.

FES is an ER PET radiopharmaceutical, and ERα expression has been shown to correlate with clinical response to hormonal treatment in several malignancies including breast cancer and endometrial cancer [27, 28]. FES uptake requires an intact ER ligand-binding pocket and thus detects ER that is available and capable of binding ligand [29]. However, FES uptake is not affected by common clinically important activating mutations in *ESR1*, the gene coding for the ERα protein [30, 31]. While FES PET can be performed regardless of diet or allergies to iodinated contrast, it is important to note that patients should be imaged before starting therapies with selective ER modulators (tamoxifen) and selective ER downregulators (fulvestrant), which bind to the estrogen-binding pocket of the receptor. Tamoxifen and fulvestrant can block ER for up to 8 weeks and 28 weeks, respectively, and can cause false-negative results.

Following FDA approval, FES has become generally available for integration into clinical practice [17, 32]. According to the recently released Appropriate Use Criteria from the Society of Nuclear Medicine and Molecular Imaging, clinical scenarios for breast cancer for which FES PET imaging is appropriate include (1) detecting ER status when other imaging tests are equivocal or suspicious, (2) assessing ER status in lesions that are difficult to biopsy or when biopsy results are nondiagnostic, (3) after progression of ER+ metastatic disease for considering second-line endocrine therapy, and (4) at the initial diagnosis of ER+ metastatic disease for considering first-line endocrine therapy [33]. FES can be useful as a diagnostic problem-solving tool, for instance when there is clinical suspicion of disease recurrence or progression despite negative conventional imaging to identify occult ER+ metastatic breast cancer (Fig. 2) or to determine the distribution of ER+ disease in patients with a combination of metastatic breast cancer and a separate non–ER-expressing malignancy [34-37]. Although FDG PET can be used to monitor response to therapy, FES PET is not recommended for this clinical scenario [33]. Given the extensive molecular imaging trials of FES dating back to the 1980s, a complete description of the clinical studies using FES in the evaluation of breast cancer is beyond the scope of this mini-review but can be further explored in other recent reviews [38]. Selected points involving recent studies in the past few years are highlighted next.

Currently, ER expression in primary and metastatic breast cancer is determined by immunohistochemistry [39]. However, biopsy may lead to sampling errors and may not be feasible because of its invasive nature or the location of the metastatic lesion. Also, heterogeneity in ER expression between the primary and metastatic lesions result in discrepancies in ER expression in 16% to 40% of patients [39, 40], and poor diagnostic and therapeutic outcomes can be seen when treatment-management decisions are based solely on primary lesion immunohistochemistry [41]. A high correlation has been found between FES uptake and immunohistochemistry findings for determination of ER status for metastatic disease [42]. To demonstrate the utility that FES has to provide whole-body, real-time interrogation of ER+ metastatic disease, a recent study combined primary data and a meta-analysis of 556 patients who underwent FES PET to identify ER+ status in metastatic disease and found an excellent positive predictive value and negative predictive value of 93% and 85%, respectively [43].

Additionally, tumor ER expression may change over time, especially in the setting of ER-targeted therapies leading to clinical dilemmas regarding both the correct diagnosis and the best choice of therapy. FES has been shown to accurately estimate ER expression of all tumor lesions and can be reliably used for individualized therapy decision-making [44]. A retrospective study of 56 patients with known ER+ metastatic breast cancer who underwent FES PET/CT before combined endocrine and palbociclib (cyclin-dependent kinase 4/6-inhibitor) therapy found that patients with only FES-positive lesions had a substantially longer progression-free survival compared to those ER+ breast cancer patients who had at least one FES-negative lesion (23.6 months compared to 2.4 months) [45]. Similarly, a retrospective study of 75 patients with ER+ metastatic breast cancer found that patients with ER heterogeneity (both FES-positive and FES-negative lesions) responded better to chemotherapy than endocrine therapy, and did not improve with combined chemotherapy and endocrine therapy [46]. The utility of FES to guide clinical management can be especially useful in patients who have both ER+ breast cancer and at least one other primary malignancy. A retrospective analysis of 83 patients with conventional imaging findings indeterminate for ER+ metastatic disease found that 87% of patients had their clinical dilemma solved by FES PET [34].

In addition to breast cancer, the role of FES PET in other known ER-dependent malignancies, such as endometrial and ovarian cancers, has been evaluated in clinical trials. Endometrial cancers are classified into type I and type II tumors. Type I, including endometrioid adenocarcinomas, are estrogen dependent, and are often preceded by endometrial hyperplasia. Type II tumors, including serous or clear cell carcinomas, are commonly estrogen independent, are less well differentiated, and have poorer prognoses. Several studies have used FES PET in combination with FDG to characterize endometrial carcinomas [47, 48]. A prospective study of 67 patients with endometrial carcinoma demonstrated that low FES uptake in the primary tumor is strongly associated with adverse prognostic factors, and that FES uptake is an independent prognostic factor for progression-free survival [49]. Among other factors, ERα loss is associated with lymphovascular space involvement [28] and metastatic spread in part because angiogenesis and other steps required for metastatic progression are modulated by sex-steroid hormones [50]. There are few studies reporting the efficacy of FES PET in the evaluation of ovarian carcinoma [51]. One interesting study found that, in patients with metastatic disease, FES PET correlated with histology at the time of debulking, but not at primary diagnosis, suggesting some degree of transformation in metastatic disease [52]. As with other molecular imaging techniques, such as with FDG PET, ovarian metastatic disease evaluation with FES PET is limited in cystic lesions and requires a large solid component for accurate quantitative measurement [53].

Progesterone Receptor

Transcription of the gene encoding progesterone receptor (*PGR*) is controlled by ER with increased expression in response to estrogen stimulation. As with ER, PR protein expression is determined by tissue immunohistochemistry and is routinely used clinically as a tumor biomarker in breast cancer [54-58]. Measuring changes in PR protein expression in metastatic breast cancer can serve as an indicator of ER functional activity and hormonal responsiveness [57, 59, 60].

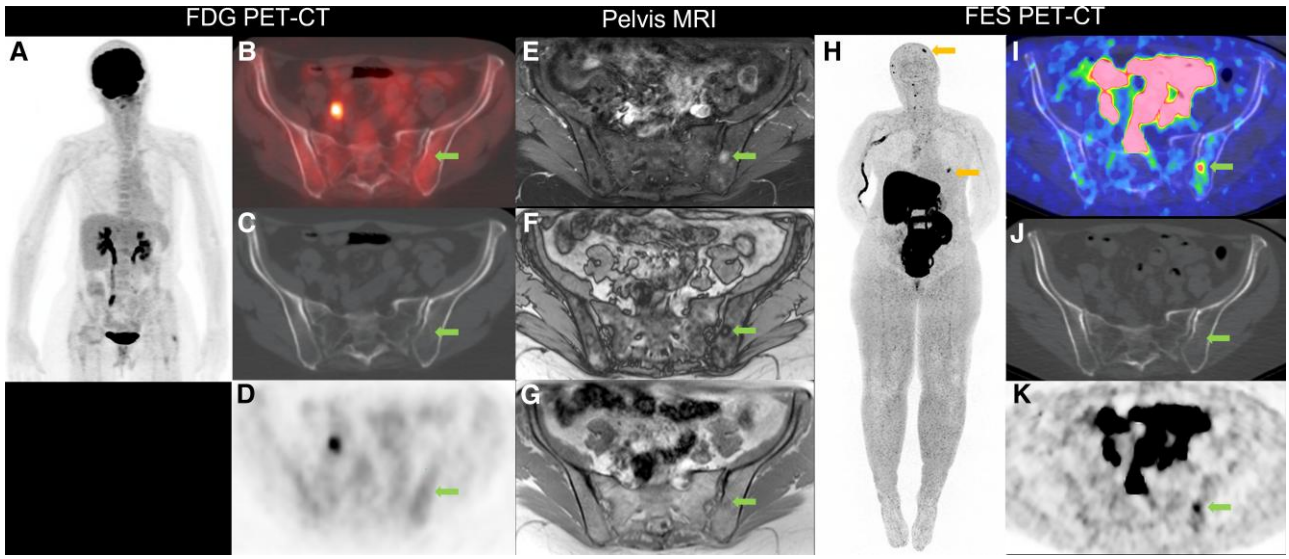

Figure 2. Restaging 16α-[^{18}F]fluoro-17β-estradiol (FES) positron emission tomography/computed tomography (PET/CT) imaging of a woman with estrogen receptor–positive (ER+) breast cancer. A woman with a history of strongly ER+ right breast adenocarcinoma treated with bilateral mastectomy and adjuvant endocrine therapy presented clinically with continually increasing tumor markers and CA 15-3 of 121 U/mL. Images shown from restaging FDG (2-deoxy-2-[^{18}F]fluoro-D-glucose) PET/CT including A, maximum intensity projection (MIP) and selected transaxial fused FDG B, PET/CT; C, CT; and D, FDG PET was negative for local or metastatic disease (arrows), as was bone scintigraphy (not shown). Abdominal and pelvis magnetic resonance imaging (MRI) scan was also interpreted as negative for metastatic disease with an enhancing lesion in the left iliac bone as seen on E, T1+ contrast; F, out of phase; and G, in phase being interpreted as red marrow. FES PET/CT was conducted for definitive staging with H, FES MIP demonstrating several FES-avid osseous lesions (arrows) and transaxial fused FES I, PET/CT; J, CT; and K, FES PET identifying ER+ metastatic disease in the left iliac bone and no CT correlate (arrows). Subsequent biopsy of the left iliac FES-positive lesion was consistent with ER+ breast metastatic disease.

Additionally, there is growing evidence indicating a more direct role of PR in breast cancer biology and crosstalk with ER [61-63], which has fueled clinical trials testing new antiprogestin therapeutics selectively targeting PR [64-67]. Thus, PR imaging may be useful as an early-response biomarker for ER-targeted endocrine therapy response and as a potential predictive biomarker for PR-targeted therapies.

21-[^{18}F]Fluorofuranylnorprogesterone (FFNP) is the most studied PR-targeted radioligand in preclinical [68-74] and clinical research [75, 76] (Fig. 3). Also developed at the University of Illinois, FFNP has high binding affinity for PR [68, 69]. Furthermore, FFNP has comparable binding both to PR-A and PR-B isoforms, the 2 main isoforms expressed in breast cancer [72, 73, 77]. To the best of our knowledge, there have been no publications investigating whether FFNP recognizes membrane-bound PR.

Preclinical studies have shown how FFNP imaging can measure real-time changes in PR expression as an indicator of ER functionality and endocrine therapy sensitivity in hormone receptor–positive breast cancer [70, 71, 73, 74]. The stimulatory effect of estradiol on PR expression in T47D human breast cancer cells and tumor xenografts was evident by increased FFNP uptake within 1 to 2 days of treatment [73]. Conversely, the inhibitory effect of estrogen deprivation via ovariectomy or ER inhibition via fulvestrant treatment on PR expression in STAT1-deficient mouse mammary tumors was also demonstrated by decreased FFNP uptake within 3 to 4 days [70, 71]. This imaging phenotype occurred only for endocrine-sensitive tumors, whereas endocrine-resistant tumors showed no significant changes in FFNP uptake [70, 71]. Furthermore, early assessment of PR expression dynamics using FFNP PET predicted inadequate tumor growth inhibition with endocrine therapy in xenografts expressing activating mutations of the *ESR1* gene, another mechanism of endocrine resistance [74]. Together these studies demonstrated that imaging the molecular changes in the expression of a downstream estrogen-regulated target gene can serve as a surrogate measure of endocrine sensitivity within a few days of treatment before anatomic changes in tumor size can be measured.

Results from clinical studies of FFNP PET imaging for patients with breast cancer align with the conclusions drawn from preclinical research and provide important human safety data for potential clinical use. In 2012, the "first-in-human" study of FFNP PET/CT involving 20 patients with breast cancer reported no adverse events or abnormal vital signs with a resulting radiation exposure for the patient comparable to standard clinical PET imaging agents (eg, FDG) [75]. Tumor uptake of FFNP peaked a few minutes after injection and remained stable through 60 minutes with no significant washout [75]. When corrected for normal breast tissue background, tumor FFNP uptake measured with PET/CT correlated with PR expression scores based on immunohistochemistry and was greater in PR+ cancers compared to PR-negative cancers [75]. Subsequent work by this group demonstrated that an increase in tumor FFNP uptake by at least 6.7% after a 1-day stimulation with estradiol predicted endocrine therapy response with 100% specificity and sensitivity in their prospective, single-center phase 2 study of 43 postmenopausal women with advanced and metastatic ER+ breast cancer [76]. Longer overall survival was observed in the responding participants. Thus, FFNP PET has been shown to be a safe method for measuring tumor PR expression, and changes in tumor FFNP uptake after estradiol stimulation are highly predictive of endocrine therapy response and survival in patients with advanced ER+ breast cancer.

PR also plays an important role in benign and malignant gynecologic processes. No studies have been published yet using FFNP PET imaging for gynecologic disease; however, there

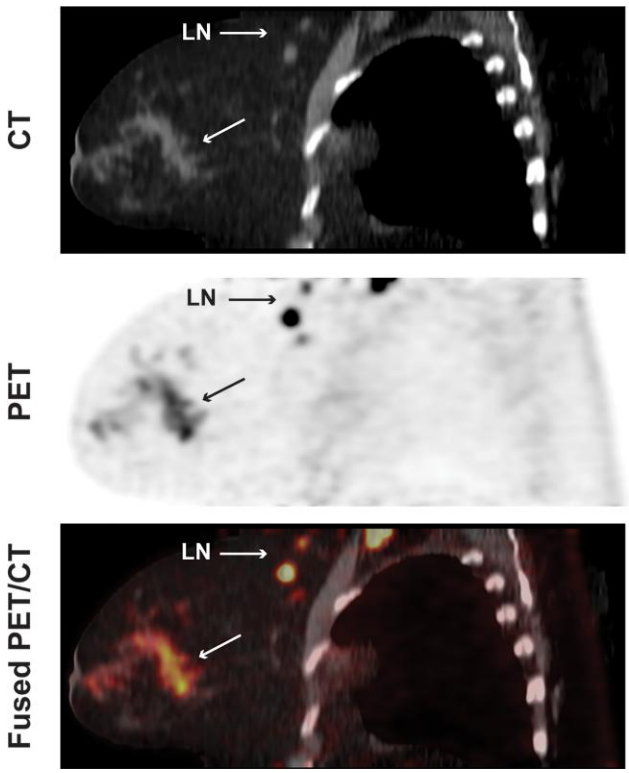

Figure 3. 21-^{18}F-Fluorofuranylnorprogesterone (FFNP) positron emission tomography/computed tomography (PET/CT) imaging of a woman with biopsy-proven progesterone receptor–positive (PR+) breast cancer and axillary lymph node metastasis. FFNP PET/CT images (sagittal view) of a postmenopausal woman with estrogen receptor–positive (ER+)/PR+/HER2– invasive lobular carcinoma show radioligand uptake in the biopsy-proven malignancy involving the upper left breast (arrow) and multiple left level 1 axillary lymph nodes (LN arrow). The patient was imaged under a research protocol.

are 2 clinical trials on ClinicalsTrials.gov. The purpose of one study (NCT05483023) is to evaluate the utility of FFNP PET/MRI to predict response to progestin hormonal therapy for patients with complex atypical hyperplasia and endometrial carcinoma. Another study (NCT05480995) aims to assess the sensitivity and specificity of FFNP PET/MRI for the diagnosis of endometriosis. Thus, the potential clinical utility of PR-targeted imaging could extend beyond breast cancer to also include gynecologic disease.

There are some limitations to FFNP as a PET imaging agent for PR. A current practical challenge that may improve in the future is that FFNP is not yet approved by the FDA or commercially available and thus must be obtained close to the site of use typically by a local cyclotron and radiopharmacy [78, 79]. Based on the timeline and number of clinical trials performed before FDA approval of FES, more studies are needed before FFNP will likely gain approval for use in clinical practice. An inherent limitation of FFNP is that the hepatic metabolism results in high background activity and inhibit detection and quantification of uptake in liver lesions, similar to FES. Nonsteroidal PR-targeted radioligands and delayed time point imaging may improve this limitation [80]. Lastly, FFNP has been shown to also bind glucocorticoid receptor (GR) in vitro, which may be a confounding factor when both PR and GR are highly expressed [80]. However, FFNP uptake in one mouse mammary tumor model system was shown to be blocked by coadministration of the progestin R5020, which binds specifically to PR, but was not blocked with dexamethasone, which is specific to GR, supporting the conclusion that FFNP uptake appropriately reflects PR binding in vivo [71]. Other radiolabeled progestins, including those with very low GR-binding affinity, have been developed and tested in preclinical models, but not yet in humans [80-85].

Androgen Receptor

Prostate cancer is the second most common cancer among men in the United States, with 1 out of 8 men diagnosed during their lifetime [86]. When identified early, patients with prostate cancer can undergo definitive radical prostatectomy or radiotherapy. However, up to 30% of patients with prostate cancer will eventually develop metastatic castration-resistant prostate cancer (mCRPC) [87, 88]. Androgen deprivation therapy remains a mainstay of treatment in mCRPC despite androgen insensitivity and is used in conjunction with bone-modifying agents and chemotherapy [89]. Advances in the field of targeted molecular imaging and radionuclide therapy for mCRPC has led to the widespread adoption of PET amino acids (^{18}F Fluciclovine; Axumin) and prostate-specific membrane antigen (PSMA) ligands (^{68}Ga PSMA-11; Locometz, Illucix) and radionuclide therapy including bone-specific radionuclide agents (^{223}Ra dichloride; Xofigo) and PSMA targeted radiotherapy (^{177}Lu PSMA-617; Pluvicto) [90]. PSMA imaging in particular has eclipsed the ability of androgen receptor (AR) imaging to identify mCRPC lesions Similar to ER and PR expression in breast cancer, AR expression is heterogeneous and expression can change over time, particularly when patients are placed on hormonal therapy. Regardless, noninvasive PET imaging of AR expression has shown some utility in documenting the extent of the disease and can identify patients who may benefit from AR-targeting therapy. 16β-^{18}F-Fluoro-5α-dihydrotestosterone (FDHT) is an AR-specific radiopharmaceutical and analogue of 5α-dihydrotestosterone (DHT) [91-93]. FDHT is rapidly metabolized, and the downstream radiolabeled metabolites have persistent affinity for blood proteins resulting in a relatively high background uptake [94]. Despite this limitation, FDHT demonstrates high repeatability and interobserver reproductivity in men with mCRPC [95].

FHDT was first evaluated in clinical studies in 2004 [96, 97], and while early trials with FDHT concentrated on evaluating metastatic staging and response to antiandrogen therapy, FDHT has limited sensitivity for mCRPC compared to other PET radiopharmaceuticals such as ^{68}Ga- and ^{18}F-labeled prostate-specific membrane antigen (PSMA)-based tracers, ^{18}F-fluciclovine, and ^{11}C-choline. Early studies of FDHT PET identified only 63% to 78% of radiographically proven metastases [96, 97]. As with FES and FFNP, FDHT has greater utility in interrogating the AR bioavailability and likelihood of success in patients being considered for continued AR therapy. A prospective study of 133 men with mCRPC who underwent both FDG PET and FDHT PET found that AR expression (as determined by FDHT PET) and glycolytic activity–Warburg effect (as determined by FDG PET) are independent factors of mCRPC progression and that patients with the most FDHT-negative lesions had the worst outcomes when treated with antiandrogen therapy [98]. Conversely, a retrospective analysis of 38 patients with mCRPC also undergoing evaluation both with FDG PET and FDHT PET found that patients with the most FDHT uptake had the shortest survival [99].

However, it should be noted that this particular study accrued patients between 2008 and 2009, before approval of modern antiandrogen pharmaceuticals such as enzalutamide. Several smaller phase 1 and 2 trials have used FDHT to identify AR-rich disease before and during enzalutamide and apalutamide therapy, but the authors did not correlate FDHT uptake or change in FDHT uptake to outcomes [100, 101].

While AR expression is most commonly associated with prostate cancer, AR is also abundantly expressed in breast cancer with up to 75% to 95% of ER+ and 10% to 35% of triple-negative (ER–, PR–, and HER2–) breast cancers expressing AR [102-104]. Several studies have shown that FDHT can be used to identify AR expression in breast cancers [105], and AR stimulation has been used in clinical trials to inhibit breast cancer tumor growth [106-108]. A prospective study was performed in 10 patients with ER+ breast cancer who underwent steroid hormone receptor interrogation both with FES and FDHT PET to evaluate the interreader visual and quantitative agreement [109]. For the 120 identifiable lesions, the study found a high visual positive and negative interobserver agreement with FES PET (84% and 83%, respectively) but low agreement with FDHT PET (49% and 74%). Conversely, both FES and FDHT PET had good quantification agreement of 0.98 and 0.78, respectively. The authors felt that this was due to the relatively low FDHT uptake thus requiring quantitative analysis of FDHT for complete evaluation in patients with breast cancer. A feasibility study using FDHT PET to predict response to antiandrogen therapy with bicalutamide in 21 patients with AR+ breast cancer patients found a baseline sensitivity of 66% in radiographically identifiable lesions, and a total of 21 new lesions were identified with FDHT PET [110]. However, while there was a decrease in radiotracer uptake after treatment with bicalutamide, FDHT PET could not predict which patients would have a response to antiandrogen therapy. Similarly, a phase 2 clinical trial with a novel oral nonsteroid androgen agonist, GTx-024, used FDHT PET to noninvasively interrogate whole-body AR expression in patients with metastatic ER+ breast cancer during therapy [111]. Nine women underwent FDHT PET, and patients who were found to have the most clinical benefit from AR stimulation had the largest decline in radiotracer uptake on the post FDHT PET. Of note, there was no correlation between baseline FDHT PET and circulating estradiol or testosterone levels. These trials in breast cancer targeting AR with androgen agonists or antagonists are likely constrained by the same limitations in studies with FDHT in patients with prostate cancer, in that AR ligand therapy (agonist or antagonist) will block the bioavailable AR and therapy does not necessarily correspond to a decrease (and thus response) in the AR protein expression itself [97].

Glioblastoma is the most common and aggressive form of primary brain malignancy, and AR is found overexpressed in a majority of glioblastomas [112]. AR antagonists have been shown to induce dose-dependent death of several glioblastoma cell lines and in vivo reduction in tumors. A feasibility study of 12 patients (6 men and 6 women) with high-grade glioma underwent FDHT PET to noninvasively evaluate AR expression [113]. Five of 12 glioblastoma lesions demonstrated significantly higher FDHT accumulation compared to normal brain with a maximum standardized uptake value (SUV_{max}) tumor/control of 1.6 to 3.4, and lesions with greater FDHT demonstrated a linear correlation with AR protein expression on histopathological analysis. While the SUV of the lesions was relatively low (SUV_{max} 0.45-2.3), the negligible FDHT uptake in normal brain parenchyma provided a good contrast to the area of AR-expressing glioma. This trial provides initial data for the potential use of FDHT to identify patients who may benefit from AR antagonist therapy.

To date, FDHT is the only positron-labeled AR-targeted radiopharmaceutical to enter clinical trials; however, efforts have been made to improve binding affinity and metabolic stability [114]. For example, enzalutamide is a pure AR antagonist routinely used in hormonal therapy in prostate cancer, and the radiolabeled ^{18}F-enzalutamide has been found to have improved binding affinity and metabolic stability compared to FDHT [115]. Future clinical trials are planned to determine if ^{18}F-enzalutamide will be able to improve on the low signal-to-noise that has hampered FDHT trials.

Other Nuclear Receptors

Compared to ER, PR, and AR, there are fewer published data for PET imaging agents targeting other nuclear receptors. GR and mineralocorticoid receptor are included in the nuclear receptor 3C subfamily along with PR and AR [116]. GR regulates many cellular processes, including catabolism and apoptosis. The transcriptional activity of GR in peripheral tissues is activated by binding to corticosteroids, which are synthesized and secreted by the adrenal cortex [117]. Corticosteroid production is controlled by a negative-feedback endocrine loop termed the hypothalamus-pituitary-adrenocortical axis. In healthy organisms, a pulse of high corticosteroid production and secretion typically occurs transiently after periods of stress, whereupon homeostasis is restored by corticosteroid metabolism in peripheral tissues. Dysregulation of GR signaling is known to stimulate several diseases including endocrine disorders, pulmonary diseases, mood disorders, and even cancers [118, 119]. For instance, hyperactivation of GR in tumor cells overrides the effects of cytotoxic chemotherapy in breast and ovarian cancer [120]. Moreover, high expression of GR in newly diagnosed triple-negative breast cancer appears to result in an especially fatal form of this already aggressive subtype [121]. However, accurate evaluation of the role that GR plays in human biology has been limited by the lack of an in vivo means to measure GR expression [122].

Several studies have focused on developing radioligands for GR with a particular interest in brain imaging [123-132]. Further development of these early GR-targeted radioligands was limited by a variety of technical issues including metabolic instability, defluorination, inability to cross the blood-brain barrier, insufficient brain uptake, and lack of specific binding. Investigators concluded that ligands with improved metabolic stability and higher receptor-binding affinity and selectivity were needed. Promising nonsteroidal radioligands with specific binding to GR have recently emerged from researchers at the University of California San Francisco that appear to overcome the limitations of previous agents [133-136]. Preliminary results from their ongoing first-in-human study (NCT04927663) evaluating ^{11}C-YJH08 PET imaging for detecting GR expression in mCRPC were recently presented [137]. To date, there are no reported positron-labeled mineralocorticoids that have been evaluated in humans.

Radioligands targeting peroxisome proliferator-activated receptor γ (PPARγ) [138-144] and peroxisome proliferator-activated receptor α (PPARα) [145] have been synthesized and

studied as potential PET imaging agents using various rodent models. PPARγ has several functions including regulating fat metabolism and is also seen in several pathologies including cancer, neurodegenerative diseases, and inflammation [146, 147]. PPARγ agonists have shown promising results by inducing cell cycle arrest and apoptosis in various malignancies in addition to preventing local invasion and metastasis [148]. Targeted PET radiopharmaceuticals to measure not only the level of PPARγ but also ligand-binding ability would provide a more accurate prediction of the outcome of PPARγ agonist treatment. PPARγ-targeted PET radiopharmaceutical development has been hindered by poor specificity and metabolic instability [139]. No human clinical trials have yet been reported for PPARγ positron-containing ligands.

The vitamin D receptor (VDR) binds the hormone calcitriol, also known as 1,25-dihydroxyvitamin D_3, and regulates calcium homeostasis [149]. In addition to the classic role in bone health, the actions of VDR modulates the immune system and can inhibit proliferation, stimulate differentiation, and induce apoptosis in various normal and malignant cells [150]. Thus, VDR-targeted pharmaceuticals and imaging agents may have broad applications in the fields of oncology, endocrinology, immunology, and bone disease. Bonasera et al [151] developed ^{11}C-labeled 1,25$(OH)_2$ D_3 with the goal of in vivo measurement of VDR expression and ligand occupancy. Their study showed high affinity in vitro binding to purified VDR. Preclinical PET imaging studies have not yet been published using this radioligand.

The retinoid X receptor (RXR) is an important DNA binding partner for several nuclear receptors, including VDR, PPARs, thyroid receptor, and retinoic acid receptor [152]. RXR is activated by 9-cis retinoic acid and plays a role in many biological processes including development, cellular differentiation, metabolism, and cell death [153, 154]. ^{18}F-Labeled and ^{11}C-labeled radioligands for RXR have been developed and studied in rodents and nonhuman primates as potential imaging biomarkers for treatment of central nervous system diseases [155-160]. However, translation of these RXR radioligands has not yet progressed to first-in-human studies.

The farnesoid X receptor (FXR) is activated by bile acids, controls bile acid synthesis, and modulates lipid and glucose metabolism [161]. FXR also plays a role in cancer and cardiovascular disease [162]. Jia et al [163] developed and evaluated an ^{18}F-labeled bile acid compound as a potential PET imaging agent. The authors demonstrated high in vitro and in vivo metabolic stability of this agent and feasibility through PET/CT imaging of athymic nude mice. They propose potential future application as a PET imaging agent for early detection of FXR-related diseases and that further research is needed.

Conclusion

Considerable work has been achieved in the synthesis and initial preclinical testing of radioligands targeting nuclear receptors. Like therapeutic drug development, many agents do not proceed as far as first-in-human studies. However, the recent FDA approval of FES is a success story and clinical implementation of FES PET imaging for patients with recurrent and metastatic breast cancer is increasing at many institutions. Additional research is needed for FFNP and FDHT to be ready for clinical use.

Search Strategy and Selection Criteria

An electronic literature search was performed using PubMed to identify potential studies published in English until August 2022. The search terms "*positron emission tomography*" and "*estrogen receptor*," "*progesterone receptor*," "*androgen receptor*," "*glucocorticoid receptor*," "*vitamin D receptor*," and "*retinoid X receptor*" were used. Relevant studies were retrieved, and their references were reviewed to identify any additional studies. Articles relevant to the scope of this mini-review were included.

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Data Availability

Data sharing is not applicable to this article as no data sets were generated or analyzed during the present study.

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