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18F-4FMFES and 18F-FDG PET/CT in ER+ endometrial carcinomas: preliminary report

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ABSTRACT

In this study, the preliminary results of a phase II clinical trial investigating the use of the Estrogen Receptor (ER) targeting PET tracer 4-fluoro-11β-methoxy-16α-[^{18}F]fluoroestradiol (18F-4FMFES) and [^{18}F]-fluorodeoxyglucose (18F-FDG)-PET in endometrial cancers will be accounted. In parallel, non-invasive interventions will be attempted to slow down progression of 18F-4FMFES metabolites in the intestines to reduce abdominal background.

Methods: In an ongoing study, 25 patients that received prior pathological confirmation of an ER+ endometrial cancer or endometrial intraepithelial neoplasia agreed to participate to the ongoing clinical trial. Patients were scheduled for 18F-FDG and 18F-4FMFES PET/CT imaging in random order and within 2 weeks. Patients were administered either 4 mg loperamide *per os* before 18F-4FMFES tracer injection or repeated intravenous injection of 20 mg hyoscine N-butylbromide during 18F-4FMFES-PET/CT. Regions-of-interest (ROIs) covering the whole abdomen and excluding the liver, bladder and uterus were drawn for the 18F-4FMFES-PET images, and a threshold of SUV > 4 was applied. The volume of the resulting region was compared between the different interventions to estimate the extent of the intestinal background.

Results: Repeated injection of hyoscine N-butylbromide substantially reduced the intestinal background volume, whereas loperamide had a significant but moderate effect. 18F-4FMFES tumor uptake ranged between SUV_{Max} 3.0 and 14.4 (9.4 ± 3.2), whereas 18F-FDG uptake spreaded between SUV_{Max} 0 and 22.0 (7.5 ± 5.1). Tumor-to-background ratio were significantly higher for 18F-4FMFES (16.4 ± 5.4) than for 18F-FDG (7.4 ± 4.6). Significant differences were observed between grade 1 and higher-grade tumors concerning 18F-4FMFES uptake and contrast. 18F-FDG uptake, and the 18F-FDG/18F-4FMFES uptake ratio.

Conclusions: It is possible to improve 18F-4FMFES abdominal background using hyoscine N-butylbromide. Both 18F-FDG and 18F-4FMFES-PET are suitable for detection of ER+ endometrial cancers, although 18F-4FMFES yielded a better tumor contrast than 18F-FDG.

Keywords

Endometrial carcinoma

18F-4FMFES

Abdominal background

INTRODUCTION

Endometrial cancers affected 382,069 women worldwide in 2018, and 89,929 died from the disease *(1)*. About two-thirds of endometrial cancers are diagnosed at an early, localized stage, for which prognosis is very favorable. The Estrogen Receptor (ER) is expressed in nearly 80% of uterine tumors *(2)*, a subset of patients which has an improved 5-year disease-free survival compared to ER- disease *(3,4)*. Moreover, adjuvant hormone therapies success rate was shown to be dependent upon ER status for endometrial cancers *(5,6)*. As such, knowledge of ER status is increasingly evidenced to be crucial for this disease, both for prognosis and therapy management.

Current diagnostic tools for endometrial cancers include transvaginal echography, computed tomography (CT) and magnetic resonance imaging (MRI) *(7)*. More recently, the use of [^{18}F]-fluorodeoxyglucose (18F-FDG) PET imaging is spreading and contributes to the detection and staging of those cancers *(8,9)*. However, 18F-FDG only indicates the relative avidity of tissues and tumors for glucose and as such is prone to false-negatives (hypometabolic tumors) and false-positives such as inflammation and physiological uptake *(10,11)*. As such, even if it supplements anatomical imaging such as CT and MRI, 18F-FDG have a sensitivity and specificity ranging from poor to moderate for endometrial cancers *(8)*.

In order to improve imaging of endometrial cancers and at the same time allow non-invasive assessment of ER status, a few groups explored the use of the estrogen-like [^{18}F]-16α-fluoroestradiol (18F-FES) PET tracer in the clinical setting. FES tumor uptake was shown to correlate well with the biopsy-determined ER status in endometrial cancers *(12,13)*. The successive use of 18F-FDG-PET and FES-PET enabled to discriminate between low-and high-grade endometrial carcinomas *(14)*. 18F-FDG-to-18F-FES tumor uptake ratio also correlated well with the progression-free and the overall survival in uterine sarcomas *(15,16)*.

More recently, FES-PET was shown in a prospective study to better evaluate endometrial cancer patient outcome than 18F-FDG-PET, further displaying the potential of ER imaging for this disease *(17)*. Despite those successes, FES-PET has some shortcomings, including slow blood clearance and rapid metabolization *(18,19)*, both factors increasing non-specific signal and hence reducing tumor detectability.

To palliate the main weaknesses of FES, our group developed an alternative ER-targeting molecule, 4-fluoro-11β-methoxy-16α-[^{18}F]fluoroestradiol (18F-4FMFES) *(20,21)* that was shown to resist hepatic metabolism in humans. Its very low binding to plasma globulins resulted in a 5-fold reduction of the tracer blood pool in the clinical setting *(22,-23)*. Combined, those two factors substantially reduced 18F-4FMFES accumulation in non-specific organs over FES, resulting in a much lower background signal *(23)*. Consequently, it was observed that 18F-4FMFES generated a significantly better tumor contrast than FES in a phase II clinical study on a breast cancer cohort, which allowed detection of more ER+ lesions than previously possible *(23)*. Preliminary reports indicated that 18F-4FMFES complements standard 18F-FDG-PET imaging in breast cancer patients *(24)*.

The recent success of 18F-4FMFES-PET in ER+ breast cancers in the clinical setting foretell its usability for ER+ endometrial cancers as well. Given the high prevalence of ER *(2)* and the importance of ER status *(3,4)* in endometrial cancers, this novel ER-targeting PET imaging modality might improve the diagnostic and the non-invasive ER status determination of those cancers. 18F-FDG tumor uptake was shown to follow an inverse relationship with ER expression in breast cancers *(25,26)*, and that combined 18F-FDG and FES-PET was shown as superior to each tracer alone in breast *(27)* and endometrial cancers *(14,15)*. As such, the 18F-4FMFES-PET procedure was paired and compared with 18F-FDG-PET within a two-week interval to evaluate their complementarity for this new indication.

Hence, this report shows the preliminary trends and observations of a phase II clinical trial evaluating 18F-4FMFES and 18F-FDG-PET in an endometrial cancer cohort. In parallel, we investigated the impact of using drugs to slow down intestinal transit in combination with diuretics, as the hepatobiliary and urinary metabolites of 18F-4FMFES generate intense low-abdomen background which could impair endometrial cancer assessment.

MATERIALS AND METHODS

The study was approved by the Sherbrooke University Hospital clinical research ethics committee and institutional board, performed under the authority of Health Canada and registered on ClinicalTrials with the ID# NCT04823065. All patients signed a written informed consent form, and the procedure was explained in lay terms by the investigators. Eligible patients were recruited after biopsy and as recommended by the gynecologic oncologists. Eligibility criteria included patients with newly diagnosed endometrial cancer, with positive ERα status histologically confirmed. Exclusion criteria included pregnancy and concomitant endocrine therapy. In this ongoing study aiming to recruit 72 patients with ovarian and uterine cancers of various origins, the first 25 endometrial cancer patients recruited were examined using both 18F-FDG and 18F-4FMFES-PET, as planned. Among them 23 patients were burdened with ER+ endometrial carcinoma (including 16 that had the endometroid endometrial adenocarcinoma subtype) and 2 were diagnosed with endometrial intraepithelial neoplasia. Four of those patients were pre-menopausal, whereas 21 patients were post-menopausal. The gynaecologic oncology team staged the patients according to the post-surgery pathology report. Table 1 summarizes the patient characteristics in more detail.

Radiochemistry

^{18}F was prepared by the ^{18}O(p,n)^{18}F reaction on ^{18}O-enriched water as target material using the TR-19 or TR-24 cyclotrons (Advanced Cyclotron Systems, Inc.) of the Sherbrooke Molecular Imaging Center. 18F-4FMFES precursor synthesis *(20)*, its labeling *(21)* using an optimized automated procedure *(28)* and its preparation, formulation, and quality control procedures *(23)* were as described previously. Apparent molar activity for 18F-4FMFES ranged between 20 and 123 GBq/µmol and were similar to those reported in the literature *(23,24)*.

Pharmacological Interventions to Slow Down Intestinal Transit

Patients were not allowed to drink after injection of 18F-4FMFES until the end of the imaging procedure. In addition, for 18F-4FMFES examinations patients received either 4 mg loperamide *per os* 15 minutes before injection (n = 12), or 20 mg of the anticholinergic drug hyoscine N-butylbromide intravenously at 0, 20 and 40 minutes after tracer administration (n = 11). Two patients received no additional intervention and were pooled with the 18F-4FMFES-PET scans previously performed in breast cancer patients (n = 31) for the intestinal transit assessment analysis *(23)*.

PET Imaging

Patients were injected intravenously in the arm using a catheter with 210.6 ± 20.5 MBq of 18F-4FMFES in a total volume of 10 mL of physiologic saline (0.9% NaCl). Thereafter, the line was flushed with 20 mL saline. Within an interval of less than 2 weeks, the same patients were injected with 320.3 ± 102.7 MBq of 18F-FDG. Both scans occurred in a random order. Patients were injected with 40 mg of the diuretic furosemide shortly after tracer injection to void the bladder for both imaging procedures.

All acquisitions were performed using a Discovery MI PET scanner (GE Healthcare) from midthigh to vertex, including the upper limbs. One hour after injection, a low-dose CT acquisition was initiated, followed immediately by a PET acquisition (3-5 overlapping bed positions, 2 min each). All PET images were reconstructed using a 3-dimensional time-of-flight weighted line-of-response row-action maximum-likelihood algorithm, with attenuation correction derived from the CT attenuation map. The accuracy of the absolute count calibration of the scanner was validated against a uniform phantom containing ^{18}F at a known concentration. The measured activity was expressed as Standard Uptake Value (SUV) for each voxel.

Image Analysis

Images were visualized and analysed using MIM software, version 6.0 (MIM Software Inc.). Images were qualitatively evaluated with a focus on the apparent extent of the low-abdomen background emanating from the intestinal radio-content by a nuclear medicine specialist. A region-of-interest (ROI) covering the whole abdomen and excluding the liver, bladder and uterus was drawn. An arbitrary SUV > 4 threshold, corresponding to a background value for which 80% of primary tumors observed during this study would be undetected or equivocal with 4FMFES-PET, was applied on the ROI and the volume of the resulting contour was extracted.

A volumetric region-of-interest (ROI) was drawn on each detectable tumor foci, and ROIs were also drawn in the area surrounding tumors (tumor background). The maximum-intensity voxel (SUV_{max}) was taken for tumor and uterine ROI quantification, whereas the averaged value of the voxels included in the ROIs (SUV_{mean}) was used for background regions. Tumor contrast was evaluated by the ratio of tumor uptake to its proximal background (T/B). Lesions with a T/B ratio of less than 3.0 were considered equivocal.

Statistical Analysis

Data were reported as mean ± SD for n ≥ 3 and as mean only for n < 3. Statistical analyses were performed using the Prism software, version 7.0.4 (GraphPad Software Inc.). One-way ANOVA using the Tukey method for multiple comparisons was applied to compare 18F-4FMFES and 18F-FDG uptake, 18F-FDG/18F-4FMFES uptake ratio and T/B ratios in tumors. The threshold for significance was *a priori* set to a p-value of less than 0.05 for each compared group.

Results

Drug-Induced Intestinal 18F-4FMFES Slow-Down

As it was observed in the past with breast cancer patients *(23,24)*, the natural elimination pathway of 18F-4FMFES generated extensive abdominal contamination without any additional intervention (Fig. 1a). Both the use of 4 mg loperamide 5 minutes prior 18F-4FMFES injection and repeated injection of 20 mg hyoscine N-butylbromide at 0, 20 and 40 minutes after 18F-4FMFES injection appeared to be successful to slow down progression of the radioactive intestinal bolus. The use of the diuretic furosemide along with 18F-4FMFES injection reduced the bladder volume and uptake in most patient. Together, the combination of furosemide and hyoscine N-butylbromide improved the diagnostic quality of 18F-4FMFES-PET for endometrial cancers (Fig. 1a).

Application of a SUV > 4 threshold on an abdominal ROI allowed standardized estimation of the intestinal volume containing significant contamination with 18F-4FMFES radiometabolites (Fig. 1b). In the absence of intervention, the measured volume reached 1117.8

± 413.4 ml, which was significantly reduced by the administration of either loperamide (677.9 ± 471.2 ml; $p < 0.01$) or hyoscine N-butylbromide (495.7 ± 341.9 ml; $p < 0.001$). However, the background 18F-4FMFES uptake in the immediate vicinity of the primary endometrial tumor was not significantly different between the control (SUV_{Mean} = 0.66 ± 0.12), loperamide (SUV_{Mean} = 0.58 ± 0.13) and hyoscine N-butylbromide (SUV_{Mean} = 0.63 ± 0.15) groups.

PET Image Qualitative Assessment

Firstly, both 18F-FDG and 18F-4FMFES-PET were able to produce high-contrast visualization of endometrial carcinoma (Fig. 2). Two patients had sentinel node involvement with size ranging between 2 and 5 mm in diameter according to pathology and lymphoscintigraphy. Those lesions could not be detected by PET imaging with either tracer and were considered within normal range by CT. One patient had an endometroid endometrial adenocarcinoma that was only detectable using 18F-4FMFES-PET; the 18F-FDG-PET examination returned negative (Fig. 3). Pathology of the surgical specimen confirmed the presence of a 2 cm grade 1 endometroid tumor. Two patients yielded a ubiquitous 18F-FDG uptake (T/B = 1.9 and 2.2, respectively) that were clearly detected using 18F-4FMFES-PET (SUV_{Max} = 11.1 and 8.5; T/B = 19.9 and 18.9, respectively). Two other patients harbored sub-centimeter endometrial intraepithelial neoplasia lesions, which were both better visualized using 18F-4FMFES (average SUV_{Max} = 5.7; T/B = 11.7) than with 18F-FDG (average SUV_{Max} = 3,1; T/B = 4.8).

In one case, 18F-FDG-PET spotted an inguinal node focus (SUV_{Max} = 5.2; T/B = 7.2) that was 18F-4FMFES-negative, but control 18F-FDG-PET/CT at later time showed reduced uptake and stable size reminiscent of a benign node (Fig. 4). 18F-FDG-PET was thus considered false-positive for this node assessment. Another patient had a 18F-4FMFES-positive (SUV_{Max} = 3.0; T/B = 5.0), 18F-FDG-negative right iliac sentinel node (Fig. 5). Dissection of 10 nodes at

surgery (including the suspected one) all returned negative in the pathology report, as well as a control 18F-FDG-PET at 9-month post-initial assessment showing no abnormal uptake at this site, meaning a false-positive result for 18F-4FMFES for this case.

Semi-Quantitative Assessment

Endometrial tumor uptake values obtained with 18F-4FMFES-PET (SUV_{Max} = 9.4 ± 3.2) were in average slightly higher than those obtained with 18F-FDG-PET (SUV_{Max} = 7.5 ± 5.1), but the difference was not significant. Uptake were not significantly different between endometroid lesions and endometrial carcinomas with either tracer (Fig. 6a). 18F-FDG uptake followed a continuous increase according to grade, with a significant difference between grade 1 (SUV_{Max} = 4.0 ± 2.0) and grade 2 lesions (SUV_{Max} = 8.0 ± 4.9; $p < 0.05$), as well as between grade 1 and grade 3 tumors (SUV_{Max} = 9.7 ± 3.0; $p < 0.01$). 18F-4FMFES uptake peaked in grade 2 tumors at SUV_{Max} = 11.4 ± 2.3, which was significantly higher from grade 1 (SUV_{Max} = 6.9 ± 2.6; $p < 0.05$) but not from grade 3 (SUV_{Max} = 9.2 ± 3.1; $p = 0.53$) tumors (Fig. 6a).

Contrast values, as defined by the tumor uptake on the surrounding background uptake ratio (T/B), were 2.3-fold higher ($p < 0.0001$) using 18F-4FMFES over 18F-FDG (16.9 ± 6.3 and 7.4 ± 4.6, respectively). T/B ratios were significantly different between grade 1 lesions (10.5 ± 3.8) and grade 2 (18.0 ± 4.4; $p < 0.01$) and grade 3 tumors (17.5 ± 5.6; $p < 0.05$) using 18F-4FMFES-PET (Fig. 6b). Such T/B relationship according to grade were not found for 18F-FDG-PET (Fig. 6b), as the slight differences observed were not significantly different.

18F-FDG-to-18F-4FMFES uptake ratio were also measured according to grade (Fig. 6c), similarly to previous publications *(13-15,17)*. While the 18F-FDG/18F-4FMFES ratio was similar between grade 1 and 2 tumors (0.65 ± 0.35 and 0.77 ± 0.40, respectively), a significant increase ($p < 0.05$) over grade 1 was observed for grade 3 tumors with a value of 1.25 ± 0.64.

DISCUSSION

In this preliminary assessment, the use of combined 18F-FDG and 18F-4FMFES-PET imaging was investigated in recently diagnosed ER+ endometrial cancer patients. Firstly, the application of interventions aiming to slow down the progression of the intestinal bolus after 18F-4FMFES injection in order to improve image quality in the abdomen produced variable results. Baseline 18F-4FMFES image quality in the abdominal region was relatively poor because of the abundant presence of radioactive intestinal content. Pre-dosing with loperamide, a peripheral opioid mainly used for control of diarrhea, moderately reduced the distribution of the abdominal contamination. Increasing dosage of loperamide might yield better results, at the cost of the associated discomfort of prolonged constipation for the patient. In contrast, repeated injection of hyoscine N-butylbromide during tracer administration, a routine procedure for radiological assessment of the intestines, substantially slowed down the intestinal content and improved overall abdominal 18F-4FMFES image quality in assessed patients. Even if the PET/CT assessment of anatomical planes usually allow distinction between uterus and intestines and even if the pharmacological interventions did not impact the uterine region background, such an intervention might be useful for non-ambiguous diagnostic of locoregional metastases using 18F-4FMFES-PET in advanced stage patients.

While both tracers yielded similar uptake overall in endometrial tumors, detectability was noticeably improved using 18F-4FMFES over 18F-FDG, as measured by the increased tumor-to-background obtained. As a result, all primary tumor assessed were clearly visualized using 18F-4FMFES-PET, whereas 2 patients obtained a ubiquitous signal (T/B lower than 3) at the tumor site using 18F-FDG-PET. Moreover, one patient was 18F-FDG-/18F-4FMFES+, with CT and surgery sample examination instead showing presence of a 20 mm lesion and confirming a false negative for 18F-FDG. One patient presented with a suspect sentinel node uptake with 18F-FDG-PET that was 18F-4FMFES-negative, but its subsequent biopsy infirmed the

presence of cancer in the assessed tissue, meaning a false-positive for 18F-FDG. Only one confirmed false positive case was found for 18F-4FMFES-PET, where a node with substantial 18F-4FMFES uptake (and 18F-FDG-negative) was exempt from cancer cell in the pathology examination. While anecdotal, those few examples in our relatively modest sample size might suggest a better overall sensitivity and specificity for 18F-4FMFES over 18F-FDG in ER+ endometrial cancers, as well as a good complementarity between the two tracers.

The 2 cases of endometrial interepithelial neoplasia observed so far in our study showed a slightly higher uptake for 18F-4FMFES than with 18F-FDG along with a 2.4-fold higher T/B, and as such it could be interesting to investigate further the use of 18F-4FMFES-PET for this hard-to-detect small-sized subclass of endometrial lesion. Of equal interest would be other less frequent uterine cancers that were previously investigated with FES-PET, including ER+ mesenchymal *(13)* and sarcoma *(15)* lesions, and our group will actively seek to recruit patients harboring those subtypes during the ongoing trial.

A significantly higher tumor uptake could be observed for 18F-4FMFES-PET for grade 1 lesions over grade 2 tumor, whereas 18F-FDG-PET uptake was significantly different between grade 1 and grade 2 and 3 tumors. This trend is contradicting a previously published result where grade 1 cancers yielded significantly higher FES uptake than higher grade tumors *(12)*, which will need to be further investigated. Tumor-to-background ratios for 18F-FDG were unable to discern between grade. In contrast, 18F-4FMFES T/B ratios were able to properly differentiate low-grade lesions from grade 2 and 3 tumors. As such, 18F-4FMFES uptake and T/B values can both be useful to distinguish between low- and high-grade endometrial tumors.

18F-FDG-to-18F-4FMFES uptake ratio were also measured. A significantly higher 18F-FDG/18F-4FMFES ratio was measured for grade 3 compared to grade 1 lesions, similar to what was previously observed for the 18F-FDG/FES ratio in endometrial cancers *(12,14,15,17)*. Higher 18F-FDG/FES was also correlated with worse progression-free and overall survival *(17)*.

As such, the 18F-FDG/18F-4FMFES ratio could be equally useful to differentiate tumors of different grades or patient outcome than the 18F-FDG/FES ratio previously evaluated.

So far, all recruited patients were newly diagnosed and early stage, which disabled the comparison of 18F-FDG and 18F-4FMFES according to stage. Relying on a previous study *(12)*, where a non-significant trend of lower FES and higher 18F-FDG uptake was observed for advanced endometrial cancers, the same tendency is expected to be observed using the similar 18F-4FMFES tracer. A related drawback of this low-stage patient sample is the lack of metastatic disease in this study. While the assessment of primary lesions with 18F-4FMFES-PET was an essential first step to evaluate the endometrial tumor targeting properties of the tracer, PET imaging procedures are expected to reach their full usefulness on patients with disseminated diseases that are more challenging to adequately assess using standard procedures. Further studies will be needed to evaluate 18F-4FMFES-PET in advanced endometrial cancer.

Disclosure

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KEY POINTS

Question: Will 18F-4FMFES-PET, along with pharmaceutical interventions to reduce abdominal background, improve the detection of ER+ endometrial cancers and allow grade segmentation in combination with 18F-FDG-PET?

Pertinent findings: The use of hyoscine N-butylbromide in repeated intravenous injection significantly reduced the extent of the abdominal background resulting from the natural elimination of 18F-4FMFES. 18F-4FMFES-PET yielded better tumor contrast than 18F-FDG-PET in ER+ endometrial cancers. Both tracers succeeded to distinguish between low- and high-grade cancers.

Implications for patient care: Because of the high tumor contrast it displayed, 18F-4FMFES-PET in combination with repeated injection of hyoscine N-butylbromide could improve the locoregional and whole-body assessment of advanced ER+ endometrial cancers compared to 18F-FDG-PET.

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TABLE 1: Summary of patient's characteristics

Parameter	Data
Patients (n)	25
Age (years)	63.4 ± 10.5 (median 66, range 41-79)
Pre-menopausal (n)	4
Post-menopausal (n)	21
Histology	n
Endometrial carcinoma	23
Endometrial intraepithelial neoplasia	2
Grade	n
1	5
2	12
3	8
Treatment	n
Loperamide (4 mg)	12
Hyoscine N-butylbromide (3 × 20 mg)	11
No treatment	2 (plus 31 breast cancer patients *(23)*)

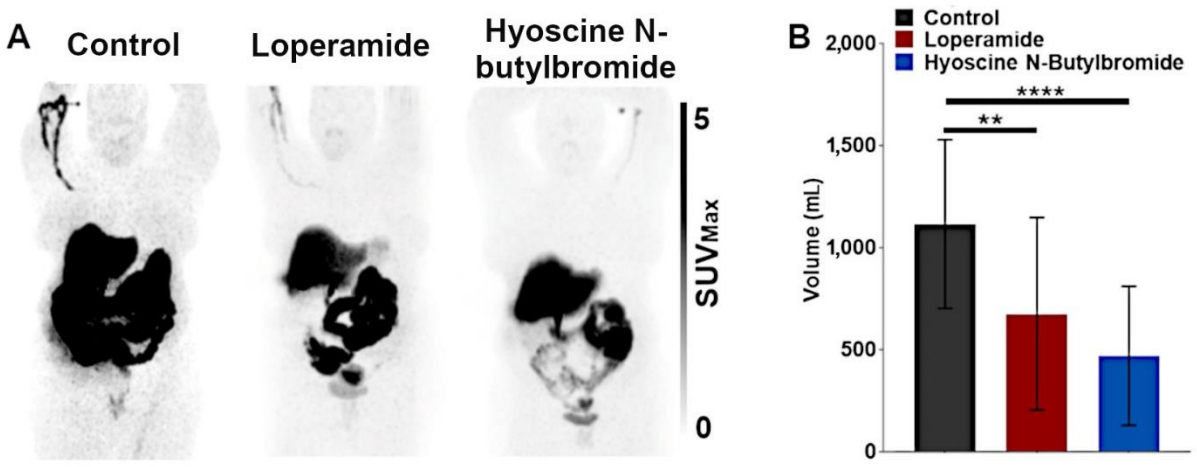

FIGURE 1: A) Qualitative maximum intensity projection (MIP) whole-body assessment of the effect of pharmacological interventions to slow down progression of the intestinal radio-bolus. Without the use of any intervention (Control; left panel), 18F-4FMFES-PET typically produces an intense abdominal uptake dependent of the progression of the radiometabolites excreted by the gallbladder in the intestines. The ingestion of 4 mg loperamide 15 minutes prior injection of the radiotracer yielded mitigated results (center panel). Repeated intravenous injection of 20 mg hyoscine N-butylbromide at 0, 20 and 40 minutes after 18F-4FMFES injection apparently reduced lower abdomen background and slowed down the intestinal radio-transit (right panel). B) Measured volume extracted from the application of a SUV > 4 threshold on an abdominal ROI. Both the use of loperamide and hyoscine N-butylbromide significantly reduced the intestinal background volume. **: $p < 0.01$; ****: $p < 0.001$.

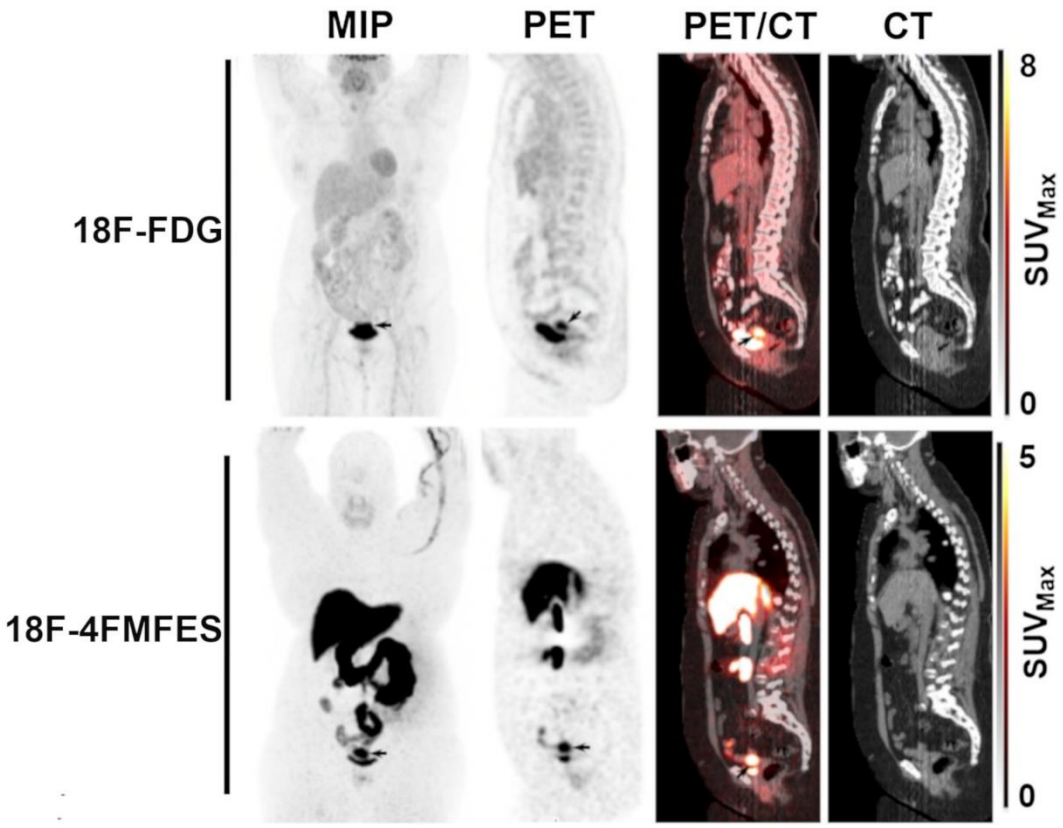

FIGURE 2: Representative case of an endometrial carcinoma (black arrows) imaged with 18F-FDG (top row) and 18F-4FMFES (bottom row) PET/CT, displayed in frontal maximum intensity projection (MIP) and in sagittal views.

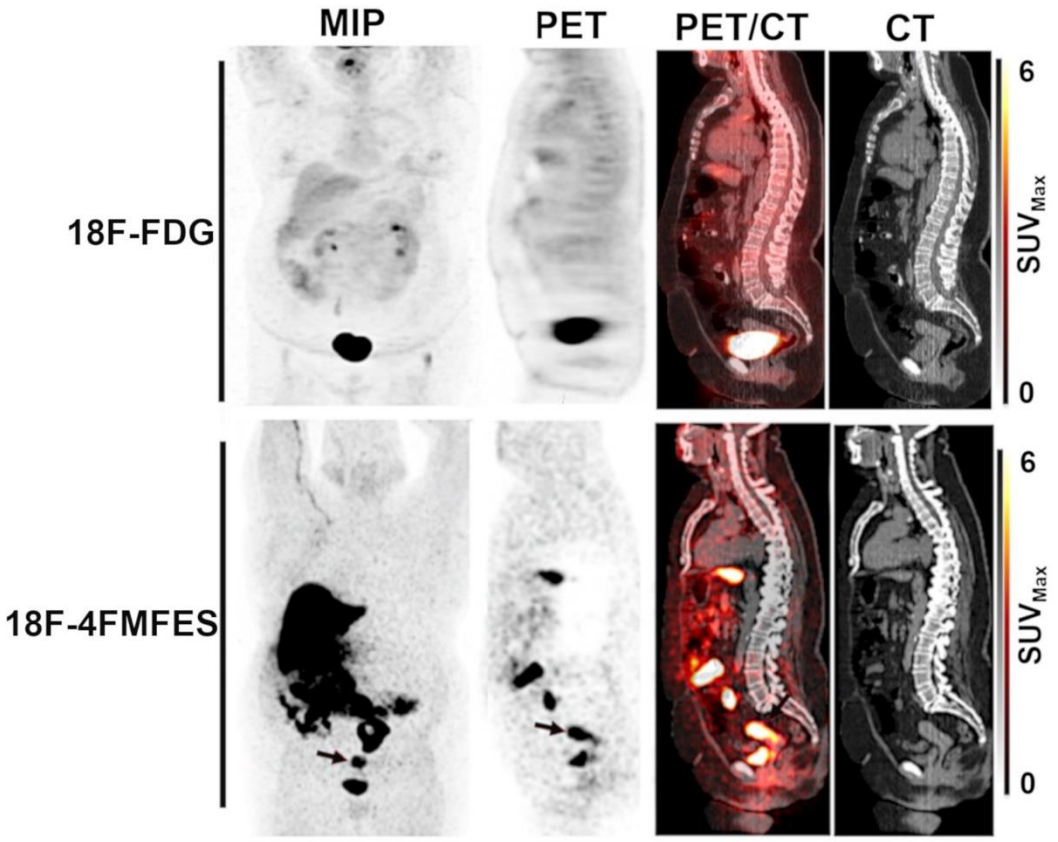

FIGURE 3: A 69 years-old endometroid adenocarcinoma patient with an 18F-FDG-negative, 18F-4FMFES-positive primary lesion. 18F-FDG-PET did not yield any abnormal uptake in the uterus, whereas 18F-4FMFES-PET revealed an intense signal (SUV_{Max} = 9.6; black arrows) over a 44 × 32 × 25 mm region. Post-surgery pathology report measured the size of the lesion to be 20 mm in its long axis, meaning 18F-4FMFES overestimated the size of the tumor in this case.

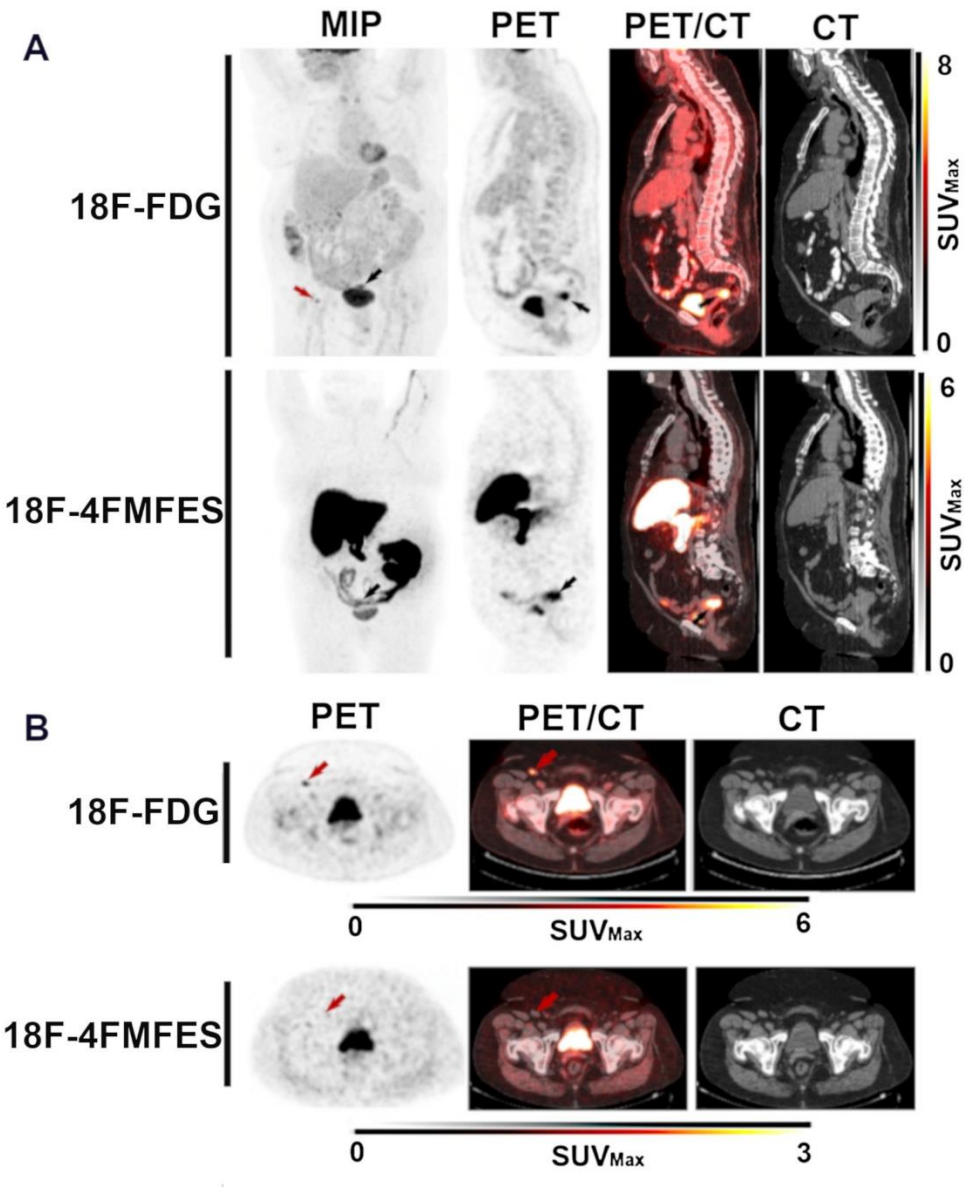

FIGURE 4: A 75 years-old endometroid adenocarcinoma patient with an 18F-FDG false-positive inguinal node. A) Endometroid adenocarcinoma primary lesion, with SUV_{Max} uptake of 12.3 for 18F-FDG and 8.9 for 18F-4FMFES (black arrows). B) Suspicion of a right inguinal node metastasis with 18F-FDG (red arrows), which yielded an SUV_{Max} of 5.2 (T/B = 7.2). The node was 18F-4FMFES-negative and of normal appearance in the CT image. Pathology examination considered the inguinal node as normal, meaning the 18F-FDG signal was a false-positive.

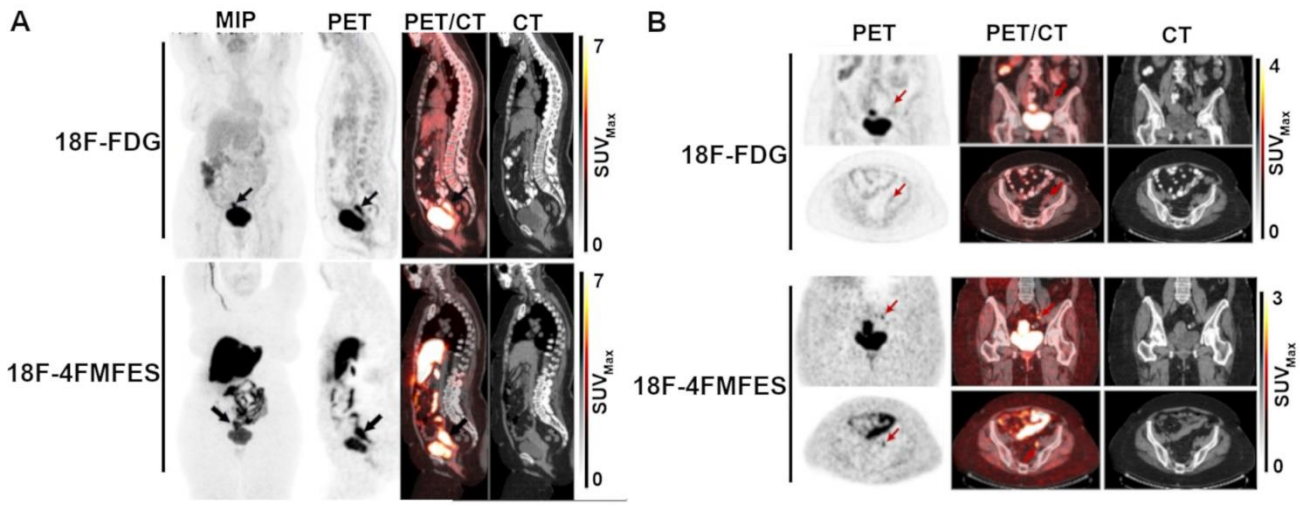

FIGURE 5: A 67 years-old endometrial carcinoma patient with a 18F-4FMFES false-positive iliac node. A) Endometrial carcinoma primary lesion, with SUV_{Max} uptake of 12.9 for 18F-FDG and 12.7 for 18F-4FMFES (black arrows). B) Coronal (top) and transaxial (bottom) views centered on a suspected left iliac sentinel node metastasis with 18F-4FMFES (red arrows), which yielded an SUV_{Max} of 3.0 (T/B = 5.0). The node was 18F-FDG-negative and of normal aspect in the CT image. Pathology examination after surgery considered the iliac node as normal, confirming a false-positive for 18F-4FMFES.

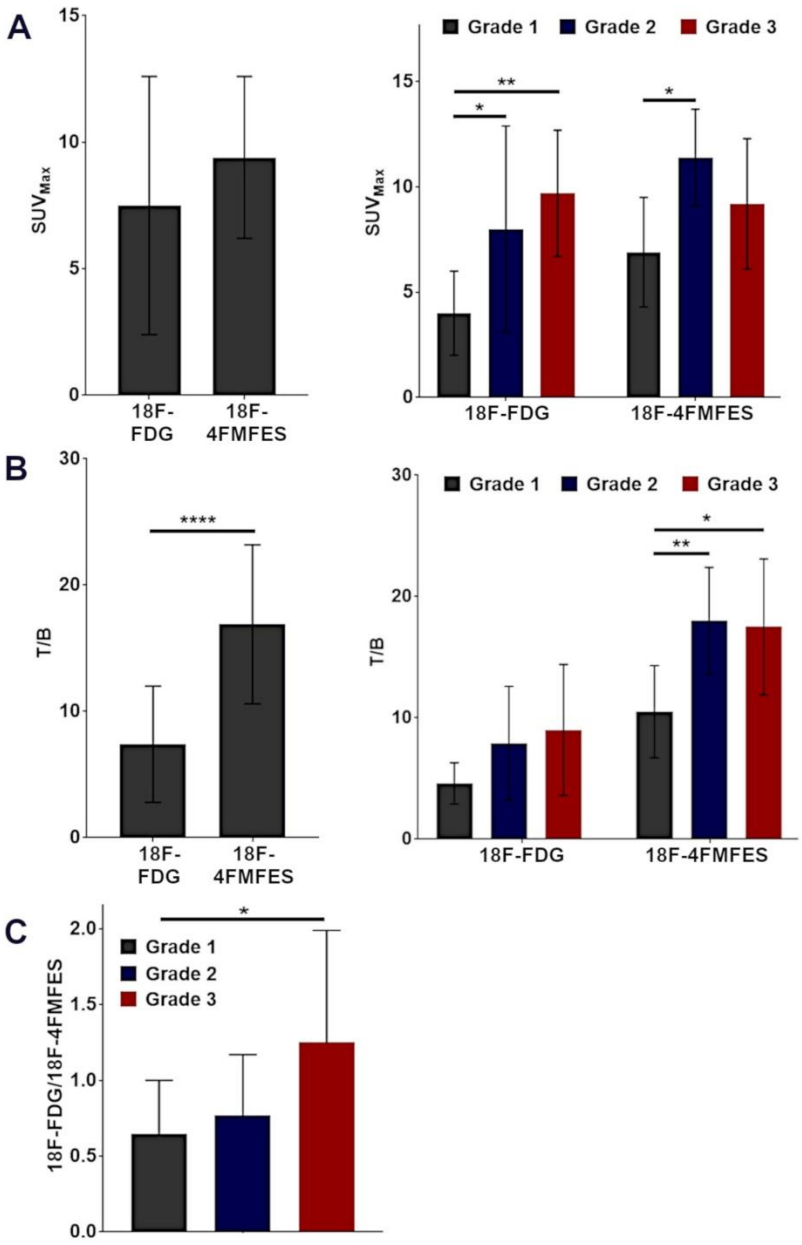

FIGURE 6: Semi-quantitative 18F-FDG and 18F-4FMFES uptake and tumor-to-background (T/B) values. A) 18F-FDG and 18F-4FMFES uptake (SUV_{Max}) for the whole sample (left panel) and according to grade (right panel) B) 18F-FDG and 18F-4FMFES T/B values for the whole studied sample (left panel) and according to grade (right panel) **C)** 18F-FDG and 18F-4FMFES T/B values according to grade.

:** $p < 0.05$; *:** $p < 0.01$; ******:** $p < 0.001$

Graphical Abstract