Fully automated, robust and reproducible synthesis of 16α-18F-fluoroestradiol (18F-FES), a PET biomarker for the evaluation of an ER-targeting drug.

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1-Introduction:
Breast cancer is the most common cancer in women, with 1,658,370 new cancer cases diagnosed in 2015. Almost 70% of breast cancers are estrogen receptor positive. The ER concentration in tumor cells is a significant indicator for the degree of disease. The monitoring of estrogen receptors (ER) engagement is important for both patient prognosis and evaluation of the effectiveness of therapy.1 Postion emission tomography (PET) is a powerful non-invasive tool for in vivo imaging and can detect disease biomarker expression in malignant tissues and monitor therapeutic response. 18α-18F-fluoro-17β-estradiol (18F-FES) is a steroid-based PET tracer with high affinity for ER, validated in clinical studies and used as a dependable tracer for evaluating and managing patients with estrogen receptor-positive (ER+) breast cancer (fig.1).2

2-Background:
In order to evaluate the in vivo effect of different dosing regimens of a selective drug inducing ER degradation, currently in development, we have developed an "in-house" automated synthesis of 18F-FES. The automated production was performed with the TRASIS AllInOne® radiosynthesizer (AIO) shielded in a lead-shielded hot-cell and remotely controlled by a workstation. This module is equipped with a complete built-in HPLC system with an injection & column selection valve, a radiation & UV detectors. The pump and the eluent selection box are separate from the production was performed with the TRASIS AllInOne® radiosynthesizer (AIO) shielded in a lead-shielded hot-cell and remotely controlled by a workstation. This module is equipped with a complete built-in HPLC system with an injection & column selection valve, a radiation & UV detectors. The pump and the eluent selection box are separate from the built-in HPLC system: PET imaging, upon 18F-FES uptake in MCF7Y537S tumor bearing mice4. The trapped 18F-fluoride was eluted into sealed reactor (R1) using a solution of kryptofix K222 / K2CO3 (S1) in acidic media, c) semi-preparative HPLC purification. (scheme 1).

3-Synthetic route:
The radio-synthesis of 18F-FES 3 consists of three steps starting from 17β-epiestriol-O-cyclic sulfone (MMSE) 1 as follows: a) Incorporation of activated 18F fluoride ion , from K18F into 1 by a nucleophilic substitution, b) hydrolysis of the protected intermediate 2 in acidic media, c) semi-preparative HPLC purification. (scheme 1).

3-PET imaging studies:
CT and µPET fused images for evaluation of the selective drug upon 18F-FES uptake in MCF7Y537S tumor bearing mice are shown below (figure 4). Reduced tumor uptake of 18F-FES has been observed in mice after injection of the selective drug. Data showed that 18F-FES uptake correlated well with immunohistochemical scoring for ER and indicated that 18F-FES PET might have potential use in the clinic to assist dosage decision in breast cancer patients eligible for the selective drug therapy.4

4-Results:
As shown in the sequence layout (scheme 2), no-carrier-added 18F-fluoride in [18F]water (100GBq-270 mCi) was transferred to the module and passed through a QMA cartridge. The trapped [18F]fluoride was eluted into sealed reactor (R1) using a solution of kryptofix K222 / K2CO3 (S1) in acidic media, water (10%), after complete azeotropic drying (8min.), the precursor 1 (0.5mg) in 1.2 ml of acetonitrile was added to the dry [18F]F and the mixture heated at 105°C for 6 min. 1M H2SO4aq (1 ml) was then added to R1 and the mixture heated at 110°C for 5 min for hydrolysis. After cooling, water (3 ml) was added for dilution and the solution was transferred into the built-in HPLC system for purification conducted with a C18 Kromasil® column (250 x 10 mm, 100-5, Akzo Nobel), using water/EtOH (40/60) solution as mobile phase at 3ml/min@254nm. [18F]- FES eluted at 8min (fig.3). The [18F]-FES was isolated in uncorrected RCY of 11% to 25% (16 runs) with good radiochemical purity (>98%) within a short synthesis time, less than 45 min.

5-Conclusions:
We have described an “in-house” automated, reliable and reproducible radiosynthesis using the TRASIS AllInOne®, AIO synthesizer for the routine production of 18F-FES for preclinical studies. The easy to use AIO module allowed us to develop efficient automated synthetic and purification procedures to obtain [18F]-FES in good radiochemical yields (up to 25% ncp) after just 45 min. Isolated radioactivities were in the range of 0.45 GBq to 3.9 GBq and were sufficient to support PET imaging studies. Note that the specific activity 5A is not a product releasing criterion since 5A has no significant effect on tumor uptake of [18F]-FES (expressed as standardized uptake value, SUV).5