Clinical Translation of a ‘Click’ Labeled $^{18}$F-Octreotate Radioligand for Imaging Neuroendocrine Tumors

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Clinical Translation of $^{18}$F-FET-βAG-TOCA
ABSTRACT

We conducted the first-in-human study of $^{18}$F-fluoroethyl triazole [Tyr$^3$] octreotide ($^{18}$F-FET-βAG-TOCA) in patients with neuroendocrine tumors (NETs) to evaluate biodistribution, dosimetry, and safety. Despite advances in clinical imaging, detection and quantification of NET activity remains a challenge, with no universally accepted imaging standard. **Methods** Nine patients were enrolled. Eight patients had sporadic NET and one had multiple endocrine neoplasia type 1 (MEN1) syndrome. Patients received 137-163MBq (mean 155.7± 8 MBq) of $^{18}$F-FET-βAG-TOCA. Safety data were obtained during and 24 h after radioligand administration. Patients underwent detailed whole body PET-CT multi-bed scanning over 4 h with sampling of venous bloods for radioactivity and radioactive metabolite quantification. Regions of interest were defined.
to derive individual and mean organ residence times; effective dose (ED) was calculated with OLINDA 1.1. **Results** All patients tolerated $^{18}$F-FET-βAG-TOCA with no adverse events. Over 60% parent radioligand was present in plasma at 60 min. High tumor (primary and metastases)-to-background contrast images were observed. Physiological distribution was seen in pituitary, salivary, thyroid and spleen, with low background distribution in liver, an organ where metastases commonly occur. The organs receiving highest absorbed dose were gallbladder, spleen, stomach, liver, kidneys and bladder. The calculated ED over all subjects (mean ± SD) was $0.029 ± 0.004$ mSv/MBq. **Conclusion** The favorable safety, imaging and dosimetric profile makes $^{18}$F-FET-βAG-TOCA a promising candidate radioligand for staging and management of NETs. Clinical studies in an expanded cohort are ongoing to clinically qualify this agent.

**Key words:** Neuroendocrine, $^{18}$F-fluoroethyl [Tyr$^3$] octreotate analog, PET/CT imaging

**INTRODUCTION**

Neuroendocrine tumors (NETs) are a heterogeneous group. Once thought of as rare tumors, the incidence and prevalence has risen over the last three decades, with figures from the National Cancer Institute’s Surveillance, Epidemiology and End Results Database showing a 520% increase, now surpassing other gastrointestinal tumors and sharing the same incidence rates as tumors of the cervix, and multiple myeloma (1). Gastroenteropancreatic-NETs are the most common subtype with small bowel being the most frequent site (1, 2).
Clinical presentation can vary and depends on the location of the tumor, presence of secretory products, and metastatic potential. Imaging is a pivotal part of clinical workup when staging NET patients, as well as to aid in treatment decisions and predict response to therapy. Current anatomical imaging modalities, namely ultrasound, computed tomography, magnetic resonance imaging and somatostatin receptor scintigraphy (SRS), fall short with low sensitivity (82-93%, 73-83%, 89-93%, and 89%, respectively) and operator dependence (ultrasound), thereby, greatly underestimating the stage of disease (3, 4).

Current anatomical imaging modalities, namely ultrasound, computed tomography, magnetic resonance imaging and somatostatin receptor scintigraphy (SRS), fall short with low sensitivity (82-93%, 73-83%, 89-93%, and 89%, respectively) and operator dependence (ultrasound), thereby, greatly underestimating the stage of disease (3, 4). ^6^Ga-DOTA-radiolabeled somatostatin analogs (^6^Ga-labeled DOTATOC, DOTANOC, or DOTATATE) have been developed for use with PET; more recently ^6^Cu-DOTATATE has also been developed although the isotope has a branching ratio of 0.175 and 12.7 h half-life. ^6^Ga-PET is far superior to SRS in terms of resolution and sensitivity (5-7).

^1^8^F^-somatostatin analogs represent a cyclotron-generated alternative to ^6^Ga analogs. Thus, we developed a novel ^1^8^F^-‘click’-labeled octreotate radioligand, ^1^8^F^-FET-βAG-TOCA, for SSTR-2 receptor imaging (8, 9). ^1^8^F^-FET-βAG-TOCA (Fig.1A), synthesized using a versatile two-step method to label [Tyr3] octreotate via copper catalyzed azide-alkyne cycloaddition reaction (CuAAC; also known as “click chemistry”), was shown to be superior to other ^1^8^F^-octreotate labeled ligands and ^6^Ga-DOTATATE in pre-clinical models, with good tumor uptake and low non-specific liver uptake (8).
We present the first-in-human biodistribution, dosimetry and safety study of this radioligand in patients with NETs.

MATERIALS AND METHODS

Radiopharmaceutical preparation

$^{18}$F-FET-βAG-TOCA was synthesized via the click reaction (9). Briefly, $^{18}$F-fluoride in a solution of oxygen-18 enriched water was transferred with a sweep of argon gas from the cyclotron target to the hotcell containing the automated module for radiochemistry. The $^{18}$F-fluoride was then trapped on an ion exchange cartridge (Sep-Pak QMA-carbonate Light Cartridge), released into the FASTLab reaction vessel, using 1.5 mL of a solution containing Kryptofix® $\text{K}_{222}$ and $\text{K}_2\text{CO}_3$ and evaporated to dryness. A solution of 2-azidoethyl-p-toluenesulfonate (6 µL) in dry acetonitrile (1 mL) was added to the reaction vessel and the solution was heated to 80°C for 15 min. The formed $^{18}$F-fluoroethyl azide was then distilled at 120°C into a vial containing a solution of copper sulphate (3.25 mg) in water (25 µL) and a solution of βAG-TOCA (4 mg; obtained under contract from ABX (ABX Advanced Biochemical Compounds GmbH, Radeberg, Germany) in $N,N$-dimethylformamide (50 µL). Following distillation, 100 µL of a sodium ascorbate solution (56.7 mg in 2 mL of sodium acetate buffer) and 100 µL of a solution of bathophenanthroline disulfonic acid disodium salt trihydrate (192 mg) in 2 mL of water were added. The resulting mixture was mixed and allowed to react at room temperature for five min. The solution was then diluted with water and loaded onto semi-prep HPLC for purification. The semi-prep HPLC column (Phenomenex Jupiter Proteo, 250 x 10 mm, 4 micron, 90 Å) was eluted at a 3 mL/min flow with a pre-mixed solution consisting of water/acetonitrile/ethanol/conc. HCl 740/250/10/1. The product
fraction eluting at the retention time corresponding to $^{18}$F-FET-βAG-TOCA was collected, diluted with 30 mL of ascorbic acid solution and passed through a tC18 light Sep-Pak cartridge (Waters Corp.). Following an initial SepPak cartridge wash with sterile water, $^{18}$F-FET-βAG-TOCA was eluted off the cartridge with ethanol, 0.9% saline for injection and water for injection to produce $^{18}$F-FET-βAG-TOCA formulated in 8 mL of maximum 12 % (v/v) ethanol in 0.1 % saline for injection. In the final step, the resulting formulation solution was filtered through a 0.2µm sterile filter (Millex GV, Sterile, 0.22 µm, Millipore) into its final sterile container. The identity and purity (chemical and radiochemical purity) of the final product were determined by HPLC. Other quality control tests were performed according to European Pharmacopoeia guidelines.

Patients

This was a prospective first-in-human study in nine patients. All patients included were ≥18 yr, with locally advanced or metastatic disease and life expectancy ≥3 mo. Only patients who had a positive $^{68}$Ga-DOTATATE scan within the preceding 6 months were enrolled. Patients who had received chemotherapy within 3 weeks or radiotherapy within 4 weeks, and those with serious underlying medical illness, or unable to tolerate scanning were excluded. Previous imaging was recorded to ascertain disease sites alongside histopathology, gut hormones, chromogranin levels (CgA and CgB) and Ki-67 index. Date and dose of last octreotide/lanreotide injection was recorded where relevant. The Leeds East and Humberside Research Ethics Committee approved this study and all subjects signed written informed consent. The study was conducted according to the Declaration of Helsinki. The administration of radioactivity was
approved by the Administration of Radioactive Substances Advisory Committee, U.K. The Medicines and Healthcare products Regulatory Agency (UK) gave permission to administer the Investigational Medicinal Product (European Clinical Trials number 2013-003152-20).

**Safety**

Safety data was obtained during and 24 h after radioligand administration. Data recorded included vital signs (heart rate, blood pressure, respiratory rate, and body temperature); physical examination; cardiovascular, lung, abdomen, and neurologic examinations; electrocardiogram; and laboratory parameters (serum biochemistry, hematology, coagulation, and urinalysis). Any adverse events were recorded using the common toxicity criteria (version 4.03: http://www.eortc.be/services/doc/ctc/CTCAE_4.03_2010_0614_QuickReference_5x7.pdf).

**Image Acquisition**

Images were acquired on a Siemens Biograph 6 TruePoint PET/CT scanner (with TrueV; extended field of view) with 21.6-cm axial and 60.5-cm transaxial fields of view. An attenuation CT scan of each patient was obtained prior to administration of $^{18}$F-FET-βAG-TOCA, from the vertex to mid-thigh (CT settings: tube potential, 130 kV; exposure, 15 effective mAs; pitch, 1.5; slice thickness, 5 mm; rotation time, 0.6 s; resulting in an effective dose of 2.5 mSv. This was then followed by multi-bed whole body PET scanning protocol on six occasions within a four-hour period. A break after the 4th emission scan allowed voiding to enhance radioligand clearance and was
followed by a second CT and the last two multi-bed whole body PET scans (Supplemental Table.1).

All emission scans were reconstructed using the ordered-subsets expectation maximization algorithm (3 iterations and 21 subsets) with corrections for dead time, scatter, attenuation and radioactive decay. Volumes of interest (VOIs) for as many of the possible ICRP 103 source organs were outlined, using the ANALYZE software package (version 11; Biomedical Imaging Resource, Mayo Clinic). All source organs were manually outlined on screen using a circular paint-brush of fixed diameter and width, by a single investigator to avoid any inter-observer variation.

**Blood activity measurements**

Discrete venous bloods and plasma (at 5, 10, 15, 30, 60, 90 and 150 min post injection) were obtained for radioactivity counting and metabolite analysis, as previously described (8).

**Data analysis, Biodistribution and Dosimetry**

The mean non–decay corrected $^{18}$F activity was obtained for the source organ ROIs at each whole body scan time, resulting in time-activity curves. The curves were decay corrected to the mid-point of each whole-body scan to most closely represent the average activity distribution for the scan. To account for the activity remaining in the body at the end of the scan protocol, the time-activity curves were extrapolated from the last whole-body scan with the simplification that radioactive decay would be the only significant change. The curves were converted to activity per organ using the
volume of organs in ICRP 23 reference man (10) and normalized by the injected activity to give the fractional uptake in each organ as a function of time. These time-activity curves were trapezoidally-integrated to generate organ residence times (τ); the total number of disintegrations in each source organ per unit injected activity.

Bladder radioactivity unlike other organs was calculated taking into account the bladder volume changes over the time course of the scan (11-14). The ED was calculated using firstly the mean residence time over all subjects for each organ and secondly using residence times for individual subjects with OLINDA/EXM v1.1, which uses the organ weighting factors from ICRP 60 (15).

RESULTS

Patients

In this prospective study nine patients (6 female and 3 male subjects) were recruited. Six patients with gastroenteropancreatic NETs and three with lung NETs. One patient had known MEN1 syndrome whilst the other tumors were sporadic. MEN1, an autosomal dominant hereditary syndrome where patients develop pituitary, parathyroid, pancreatic and adrenal tumors, is detected through familial screening. 

$^{68}$Ga-DOTA peptides allow detection of NET lesions in MEN1 syndrome patients and can be used as an additional tool to CT. The mean age and weight ± SD were 56 yr ± 12.8 (range 35-73 yr) and 75.2kg ± 11.8 (66.3-91.2kg), respectively. Study schedule (Fig.1B), patient demographics and clinical characteristics (Table.1) are summarized. Median (range) CgA and CgB levels reflecting in part the burden of disease (16), were 95 pmol/l (24-1,567) and 170 pmol/l (58-1,328), respectively. Tumor staging and
grading were in accordance with ENETS consensus classification systems (17). In our cohort of patients 22% [2/9] were graded as G1 (≤2) and 78% [7/9] were G2.

**Safety**

There were no adverse or clinically detectable pharmacologic events during the study or within 24 h after ¹⁸F-FET-βAG-TOCA injection. No significant changes in vital signs or the results of laboratory studies or electrocardiograms were observed.

**Radiopharmaceutical**

¹⁸F-FET-βAG-TOCA formulated for injection was obtained from fluoride in 6.2 ± 2.9 % non-decay corrected radiochemical yield (range, 2.0-12.1 %) within 100 min. Radiochemical purity was 100 % and specific radioactivity was 374 ± 124 GBq/μmol (range, 224-562 GBq/μmol). The mean ± standard deviation (SD) of the administered mass of ¹⁸F-FET-βAG-TOCA was 0.86 ± 0.26 μg (range, 0.52-1.32 μg). The mean administered activity was 155.7± 8 MBq (range, 137-163 MBq).

**Metabolite analysis**

The metabolism of ¹⁸F-FET-βAG-TOCA was analyzed using radio-HPLC (1200 series system; Agilent). Typical HPLC chromatograms for subject 1 are illustrated in Fig. 2, A-D. Over 60% of parent radioligand was detectable in plasma at 60 min and over 30% of parent radioligand was detectable at 2.5 h (Supplemental Fig.1). There was rapid equilibration of radioactivity in blood with blood/plasma ratio of ~1.5. From the parent plasma data, the elimination half-lives of the distribution and terminal/elimination phase
were calculated as 2.95 ± 0.87 min and 15.87 ± 6.84 min, respectively (fit: $R^2$ range 0.98-1.0) demonstrating rapid radioligand clearance from the blood compartment. The identity of the metabolites are presently unknown; no bone uptake was seen thus precluding significant defluorination.

Image Quality

Images obtained with $^{18}$F-FET-βAG-TOCA PET/CT showed excellent contrast (Fig. 3). The image of patient 1, diagnosed with a small bowel NET and widespread metastases (bone, liver) shows avid uptake of $^{18}$F-FET-βAG-TOCA. Fig.4, shows images of a patient with known MEN 1 syndrome and with lesions localized within the pancreas.

Biodistribution

$^{18}$F-FET-βAG-TOCA derived radioactivity was visually detectable in the vascular compartment, liver, spleen, and kidneys, within the first 6 min of radioligand injection. There were no significant differences in organ biodistribution between male and female patients (Fig.5). Tissue time activity curves generated for the main source organs are shown (Fig.6). Over the next 192 min, increased radioligand localization was seen in gall bladder, spleen and bladder. Physiological uptake was also noted in the pituitary, salivary glands, and thyroid. The radioligand also showed high tumor uptake (Fig. 6C) and tumor/background contrast in all organs including the liver. Three lesions were chosen per patient where available, and in patients with multiple metastases, 3 lesions were chosen from different sites. In patients with multiple liver lesions only, 3 clearly visible lesions were identified and used for measurement of SUV.
The mean residence times ($\tau$) in male and female patients are shown (Table 2). Overall the radioligand was eliminated rapidly from most organs leading to relatively short residence times and low/stable organ radioactivity within 60 min of radioligand injection. Bladder radioactivity was variable; an example of bladder time-activity curve for subject 1 using the 3 parameter fit model is illustrated (Supplemental Fig. 2).

**Dosimetry**

The calculated ED using mean organ residence times over all 9 subjects was 0.029 mSv/MBq. When using time-activity curves for individual subjects, the calculated ED ranged from 0.022 to 0.032 mSv, giving a standard deviation of 0.004 mSv. The estimated mean absorbed dose to all source organs assuming 2-hour bladder voiding scenario in individual patients (1-5) or all patients combined is shown (Fig 7, A-E). The organs that received the highest dose (mSv/MBq) in descending order were: gallbladder wall (0.149 ±0.007), spleen (0.117 ± 0.036), stomach wall (0.076 ± 0.02), liver (0.066 ± 0.009), kidneys (0.065 ± 0.011) and urinary bladder (0.051 ± 0.015).

**DISCUSSION**

$^{18}$F-FET-βAG-TOCA, a SSTR-2 targeting fluorine-18 radioligand has been shown in this first in-man study to be safe and well tolerated. GMP compliant, $^{18}$F-FET-βAG-TOCA was produced on an automated platform; synthesis time of $^{18}$F-FET-βAG TOCA, using the “click reaction” (9) was shown to be shorter than other $^{18}$F-octreotate analogs and results in reasonable radioligand yields (18). $^{18}$F-FET-βAG-TOCA had acceptable metabolic stability with little or no defluorination. Intact parent radioligand was
detectable in plasma by HPLC throughout the study; radioactivity in urine comprised up to 90% of parent radioligand at 90 min after injection (data not shown).

Future use of this radioligand should be cognizant of physiological localization. Low level physiological localization was seen in the pituitary, salivary glands, thyroid and spleen, and elimination via the gall bladder increased over time. Rapid distribution to the liver was noted but over time there was gradual elimination of radioligand in this organ and background activity proved to be less than other previously described $^{18}$F-octreotate based radioligands (18, 19). This is in keeping with pre-clinical studies in mice. $^{18}$F-FET-$\beta$AG-TOCA was selected from a library of compounds mainly due to its low liver uptake - a common site for metastases in neuroendocrine tumors - while retaining reasonably high binding affinity comparable ($^{18}$F-AIF-NOTA-OC) or higher than existing clinically applicable radioligands ($^{68}$Ga-DOTATATE) (8, 9).

The main pharmacokinetic difference between $^{18}$F-FET-$\beta$AG-TOCA and $^{68}$Ga-emitting somatostatin radioligands relates to the highest absorbed dose received by source organs, where in descending order the spleen, bladder, kidneys and liver receive the highest dose with the latter. In comparison, for $^{18}$F-FET-$\beta$AG-TOCA, the gallbladder received the highest absorbed dose. This is to be expected as $^{18}$F-FET-$\beta$AG-TOCA had both renal and biliary elimination, while $^{68}$Ga-based ligands have predominantly renal elimination (20-22). In our cohort of patients with liver metastases the tumor-to-background ratio for $^{18}$F-FET-$\beta$AG-TOCA in liver ($4.23 \pm 2.69$) was broadly similar to that reported for $^{68}$Ga-DOTANOC ($3.4 \pm 2.3$), $^{68}$Ga-DOTATOC ($2.8 \pm 1.6$) and $^{68}$Ga-DOTATATE (2.0, interquartile range 1.4-2.7) (23-25).

The dosimetry of $^{18}$F-FET-$\beta$AG-TOCA was similar to other $^{18}$F-based radioligands. The mean ED was found to be $0.029 \pm 0.004$ mSv/MBq which is comparable to the ED of $^{18}$F-FDG ($0.019$ mSv/MBq) (26). Radiation safety of $^{18}$F-FET-$\beta$AG-TOCA was
inferred from the organ absorbed dose estimates obtained from our study; all values were within the limits suggested by the US Food and Drug Administration Code of Federal regulation Title 21, Part 361.1.

Although a small cohort of patients, we observed no trend between SUV values and blood biomarkers or grade of tumors. Patient 1, for instance, was found to have a high tumor burden reflected by high chromogranin CgA and CgB levels and high SUV of lesions within liver, bone and bowel, however, patient 3 (lung NET), showed low tumor burden with low SUV, but CgA and CgB levels were high. Additionally, patient 7 (pancreatic NET) was found to have very low levels of CgA despite having widespread liver metastases on $^{18}$F-FET-βAG-TOCA PET/CT. This lack of correlation between imaging and biomarkers has also been highlighted in other studies (27, 28) and suggest that additional multianalyte biomarkers such as circulating NET gene transcripts should be considered in the future (29).

The MEN1 syndrome positive patient in our study had previous pituitary surgery and therefore showed no uptake within this region (physiological or pathological). Imaging with $^{18}$F-FET-βAG-TOCA detected multiple NETs within the pancreas, and as expected there were no differences in SUV values of lesions between the MEN1 positive patient and other patients with gastroenteropancreatic-NETs or lung NETs.

$^{18}$F-FET-βAG-TOCA, has shown initial promise with its ease of synthesis, high production yield, and accessibility for large multicenter studies, and clinical studies in an expanded cohort, with a single static whole-body imaging protocol based on this study, are currently ongoing to clinically qualify the ligand in patients with NET, by direct comparison with $^{68}$Ga-DOTATATE PET/CT. The potential of this somatostatin-receptor specific radioligand, with high specificity for SSTR type 2, highlights the possibility for use both in diagnosis and treatment planning, an attractive option in NET.
patients. This concept of “Theranostics” (therapies that combine diagnostic and therapeutic capabilities into a single agent) in nuclear medicine has gained popularity and is one step forward in achieving a “personalized medicine” approach in NET patients. While this concept applies directly to $^{68}$Ga-DOTA radioligands (30) it is envisaged that the $^{18}$F-variant can be used, indirectly, to personalize similar therapies.

**CONCLUSION**

We report a novel ‘click’ $^{18}$F-radiolabeled octreotate PET imaging radiopharmaceutical with appropriate safety, dosimetry and distribution properties, which highlights tumor lesions with high contrast. With the range of treatment modalities available in the management of NETs, the use of an optimal imaging modality, together with blood biomarkers in the clinic, is of great importance in the therapeutic decision making process of NET patients.

**DISCLOSURE**

The costs of publication of this article were defrayed in part by the payment of page charges. Therefore, and solely to indicate this fact, this article is hereby marked “advertisement” in accordance with 18 USC section 1734. This work was supported by the U.K. Medical Research Council grant MR/J007986/1, Experimental Cancer Medicine Centres grant C37/A7283, and National Institute for Health Research (NIHR) Biomedical Research Centre award to Imperial College Healthcare NHS Trust and Imperial College London.

**ACKNOWLEDGMENTS**

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NIHR/Wellcome Trust Imperial Clinical Research Facility for their support of the trial.

AUTHOR CONTRIBUTIONS
All authors have contributed as follows: Protocol development (RS, PM, SD, EA), conducting portions of the study (SRD, RS, KK), data collection (SRD, NK), data analysis (SRD, NK), specimen analysis (RD, SRD), preparing the manuscript (SRD, RS, EA, NK, MH, LC, AN, AF, AS). All authors discussed the results and commented on the manuscript.

COMPETING INTERESTS
GE Healthcare provided in-kind contribution to support radiochemistry on the FASTlab platform. No other potential conflict of interest relevant to this article was reported.

DATA AND MATERIALS AVAILABILITY
Imperial College, London. Inquiries should be directed to the corresponding authors.

REFERENCES


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# TABLE 1
Patient clinical and pathological characteristics

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Primary site</th>
<th>Age (yr)</th>
<th>Sex</th>
<th>Grade</th>
<th>Metastatic</th>
<th>Ki67 (%)</th>
<th>Biomarker (pmol/l)</th>
<th>Previous Treatment</th>
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<tr>
<td>1</td>
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<td>53</td>
<td>F</td>
<td>2</td>
<td>Liver, Bone</td>
<td>&gt;10</td>
<td>1,567</td>
<td>170 Surgery/ RFA/ 177 Lu† Octreotide</td>
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<td>Para-aortic lymph nodes, Left SCF‡</td>
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<td>962 558</td>
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<td>56</td>
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<td>2</td>
<td>Bone</td>
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<td>507 1,328</td>
<td>Octreotide</td>
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<td>1</td>
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<td>143 73</td>
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<td>F</td>
<td>2</td>
<td>Mediastinal lymph nodes, Bone</td>
<td>17</td>
<td>47 58</td>
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<td>70</td>
<td>M</td>
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<td>70 174</td>
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<td>Surgery/ RFA/ 177 Lu† Octreotide</td>
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<td>M</td>
<td>2</td>
<td>None</td>
<td>&lt;5</td>
<td>49 124</td>
<td>Surgery</td>
</tr>
</tbody>
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*Radiofrequency ablation
† ^177^Lutetium-DOTATATE (peptide receptor radionuclide therapy)
‡ SCF Supraclavicular fossa lymph node
§ MEN 1 (multiple endocrine neoplasia type 1)
| Table 2: Mean Residence Times (τ) of $^{18}$F-FET-βAG-TOCA for different organs in Male (n=3) and Female (n=6) subjects |
|---|---|---|---|---|
| Organ | MALE | | FEMALE | |
| | Mean | SD | Mean | SD |
| Adrenals * | 0.002 | 0.0002 | 0.003 | 0.0008 |
| Brain | 0.006 | 0.001 | 0.007 | 0.001 |
| Breasts |  |  | 0.004 | 0.001 |
| Cortical bone | 0.049 | 0.002 | 0.040 | 0.016 |
| Gallbladder † | 0.110 | 0.072 | 0.019 | 0.019 |
| Heart contents | 0.029 | 0.014 | 0.019 | 0.005 |
| Heart wall | 0.020 | 0.010 | 0.013 | 0.004 |
| Kidneys | 0.088 | 0.007 | 0.089 | 0.026 |
| Liver | 0.478 | 0.073 | 0.089 | 0.084 |
| Lungs | 0.063 | 0.009 | 0.067 | 0.025 |
| Lower large intestine | 0.012 | 0.002 | 0.019 | 0.011 |
| Muscle | 0.709 | 0.154 | 0.600 | 0.154 |
| Ovaries ‡ |  |  | 0.0005 | 0.00004 |
| Pancreas | 0.017 | 0.006 | 0.010 | 0.0035 |
| Red marrow | 0.028 | 0.004 | 0.050 | 0.032 |
| Small intestine | 0.094 | 0.065 | 0.121 | 0.055 |
| Stomach | 0.005 | 0.029 | 0.066 | 0.222 |
| Spleen | 0.122 | 0.019 | 0.097 | 0.026 |
| Testes | 0.001 | 0.0002 |  |  |
| Thyroid | 0.001 | 0.0003 | 0.0008 | 0.0003 |
| Upper large intestine | 0.026 | 0.004 | 0.028 | 0.014 |
| Urinary bladder § | 0.075 | 0.019 | 0.101 | 0.028 |
| Uterus |  |  | 0.006 | 0.001 |
| Remainder | 0.523 | 0.250 | 0.628 | 0.250 |

* Adrenal glands could not be visualized in 3 subjects.
† Gallbladder surgically removed in 5 subjects (1 male subject had gallbladder in-situ).
‡ Ovaries could not be visualized in 1 subject due to post-menopausal atrophy and 2 subjects had previous hysterectomy and bilateral salpingo-oophorectomy.
§ Urinary bladder τ is for 2-h voiding model. SD, standard deviation.
Figure Legends

**FIGURE 1.** Chemical structure of $^{18}$F-FET-βAG-TOCA and study design. (A) Schematic diagram of chemical structure of $^{18}$F-FET-βAG-TOCA; CuSO$_4$, Copper (II) sulphate; Na-ascorbate, Sodium ascorbate; BPDS, Bathophenanthroline disulfonate; pH 5 NaOAc H$_2$O, Sodium acetate buffer (pH5), water; DMF dimethyl formamide; MeCN (8:3:10). (B) PET/CT study timeline. FBC, full blood count; U&Es, urea and electrolytes; SUV, standardized uptake values; AUC area under the curve; TAC, time activity curves.
FIGURE 2. Metabolite analysis of $^{18}$F-FET-βAG-TOCA in patient 1. Typical HPLC chromatogram of $^{18}$F-FET-βAG-TOCA in plasma at 5, 30, 60 and 90 min time-points (A, B, C and D respectively), red arrows indicate parent / unmetabolized $^{18}$F-FET-βAG-TOCA. Scaling of B, C and D adjusted to allow for visualization of metabolite peaks I, II and III (blue arrow).
FIGURE 3. $^{18}$F-FET-$\beta$AG-TOCA PET/CT images and corresponding maximum intensity projection images in patient 1 (small bowel NET with widespread metastases in liver and bone). (A and B) Sagittal images, and (C and D) axial slices showing widespread liver and bone metastases.
FIGURE 4. $^{18}$F-FET-βAG-TOCA PET/CT images and corresponding maximum intensity projection images in patient with MEN1 syndrome, with pancreatic NETs. (A and B) axial slices showing multiple lesions within the pancreas (red arrows).
FIGURE 5. Time course biodistribution of $^{18}$F-FET-βAG-TOCA in male and female patients. Maximum intensity-projection images of $^{18}$F-FET-βAG-TOCA in (A) a female patient with liver metastases and (B) a male patient with lung NET.
FIGURE 6. Mean decay corrected time activity curves for source organs and tumors. 
(A and B) SUV mean for bladder, kidneys, gallbladder, pancreas, spleen and liver. (C) 
SUV mean and max for tumors (maximum of 3 lesions chosen per patient).
FIGURE 7. Biodistribution and dosimetry of $^{18}$F-FET-βAG-TOCA. Multibed whole-body PET scanning over 192 min was used to determine the absorbed doses per unit administered activity (mGy/MBq) of major organs and tissues for each patient (the first 5 patients are shown). The organ absorbed doses in all patients is shown with mean effective dose ± SD (standard deviation).
**Supplementary Materials**

**TABLE S1.** Image acquisition protocol.

**FIGURE S1.** (A) Percentage (%) of total radioactivity in the form of parent radioligand remaining in plasma at 5, 60 and 150 min and (B) table of percentage (%) of total radioactivity and metabolite quantification.

**FIGURE S2.** Bladder time-activity curve for subject 1 using the 3 parameter fit model.

### TABLE S1

Image Acquisition Protocol

<table>
<thead>
<tr>
<th>Scan Acquisitions</th>
<th>Min./bed position</th>
<th>No. of bed positions</th>
<th>Vertex to mid thigh</th>
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<tbody>
<tr>
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</tr>
<tr>
<td>1</td>
<td>1</td>
<td>6-7</td>
<td>Vertex to mid thigh</td>
</tr>
<tr>
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<td>2</td>
<td>6-7</td>
<td>Vertex to mid thigh</td>
</tr>
<tr>
<td>3</td>
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<tr>
<td>4</td>
<td>5</td>
<td>6-7</td>
<td>Vertex to mid thigh</td>
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<tr>
<td>Gap</td>
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<tr>
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<td></td>
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<td>Vertex to mid thigh</td>
</tr>
<tr>
<td>6</td>
<td>7</td>
<td>6-7</td>
<td>Vertex to mid thigh</td>
</tr>
</tbody>
</table>

Each scan was 6-7 bed positions, with the inferior border set to mid-thigh and the superior border set at the vertex. The acquisition for each bed position was 1, 2, 5, 5, 7 and 7 min for the six time points after radioligand injection.
<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Percentage (%) Total Radioactivity</th>
<th>Percentage (%) of Total Radioactivity of Individual metabolites I II III</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Parent Radioligand Total Metabolite(s)</td>
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<tr>
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<td>94 5.5</td>
<td>5.5 0 0</td>
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<tr>
<td>10</td>
<td>92 7.6</td>
<td>7.6 0 0</td>
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<tr>
<td>15</td>
<td>90 9.7</td>
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<tr>
<td>30</td>
<td>84 15.6</td>
<td>15.6 0 0</td>
</tr>
<tr>
<td>60</td>
<td>68 32</td>
<td>22.5 3.1 6</td>
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<tr>
<td>90</td>
<td>55 45</td>
<td>35.1 4 6.7</td>
</tr>
<tr>
<td>150</td>
<td>34 66</td>
<td>45 6.1 15</td>
</tr>
</tbody>
</table>

**Figure S1.** (A) Percentage (%) of total radioactivity in the form of parent radioligand remaining in plasma at 5, 60 and 150 min. (B) Table summarizing proportions of parent radioligand and metabolite(s) expressed as percentage (%) of total radioactivity. The unidentified metabolites I, II and III respectively eluted at the following retention times (min) 7, 5.33 and 4.

**FIGURE S2.** Bladder time-activity curves for patient 1 using the 3 parameter fit model. Total bladder activity was estimated as previously (14). Graph (A) illustrates the bladder model in patient 1, with measured and fitted curves, and resulting modelled bladder net activity using a 2 h void interval. Graph (B) shows the modelled bladder net activity compared to the measured data extrapolated for decay beyond the last measured point.