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First-in-Human PET Imaging of Tissue Factor in Patients with Primary and Metastatic Cancers Using ^{18}F-labeled Active-Site Inhibited Factor VII (^{18}F-ASIS): Potential as Companion Diagnostic

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ABSTRACT

Tissue factor (TF) expression in cancers correlates with poor prognosis. Recently, the first TF-targeted therapy was approved by the US Food and Drug Administration for cervical cancer. To unfold the potential of TF-targeted therapies, correct stratification and selection of patients eligible for treatments may become important for optimization of patient outcomes. TF-targeted PET imaging based on ^{18}F-radiolabeled active-site inhibited versions of the TF natural ligand coagulation factor VII (^{18}F-ASIS) has in preclinical models convincingly demonstrated its use for non-invasive quantitative measurements of TF expression in tumor tissue. ^{18}F-ASIS PET imaging thus has the potential to act as a diagnostic companion for TF-targeted therapies in the clinical setting.

Methods In this first-in-human trial we included 10 cancer patients (4 pancreatic, 3 breast, 2 lung, and 1 cervical cancer patient) for ^{18}F-ASIS PET imaging. The mean and standard deviation of administered ^{18}F-ASIS activity was 157 ± 35 MBq (range, 93–198 MBq). PET/CT acquisition was performed after 1, 2, and 4 hours. The primary objectives were to establish the safety, biodistribution, pharmacokinetics, and dosimetry of ^{18}F-ASIS. Secondary objectives included quantitative measurements of standardized uptake values (SUV) in tumor tissue with PET and evaluation of the correlation (Pearson correlation) between tumor SUV_{max} and *ex vivo* TF expression in tumor tissue.

Results Administration of ^{18}F-ASIS was safe, and no adverse events were observed. No clinically significant changes in vital signs, electrocardiograms, or blood parameters were observed following injection of ^{18}F-ASIS. Mean ^{18}F-ASIS plasma half-life was 3.2 hours, and the radiotracer was predominantly excreted in the urine. For an administered dose of 200 MBq of ^{18}F-ASIS, effective whole-body dose was 4 mSv and no prohibitive organ-specific absorbed doses were found. Heterogeneous radiotracer uptake was observed across patients and within tumors. We found a trend of a positive correlation between tumor SUV_{max} and *ex vivo* TF expression (p=0.08, r=0.84, n=5).

Conclusion ^{18}F-ASIS can safely be administered to cancer patients for PET imaging of TF expression in tumors. The trial marks the first test of a TF-targeted PET radiotracer in humans (first-in-class). The

findings represent important first steps towards clinical implementation of ^{18}F-ASIS PET imaging of TF expression.

Keywords

Active site inhibited factor VII (ASIS); Tissue factor; PET/CT; First-in-human; Phase I clinical trial

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DISCLOSURE

AK and CHN are inventors/hold intellectual property rights on a patent covering tissue factor Imaging. No other potential conflicts of interest relevant to this article exist.

INTRODUCTION

Personalized medicine based on targeted therapies is predicted to shape the future of oncology in the coming decades. An emerging oncological target is the transmembrane glycoprotein tissue factor (TF) that functions as the main initiator of the extrinsic coagulation cascade (*1*). In addition to its role in coagulation, TF expression is also linked to several cancer hallmarks including tumor growth, angiogenesis, and metastatic potential (*2,3*). Abundant TF expression has been reported in most solid tumors, and TF expression levels are associated with disease stage and poor overall survival in pancreatic cancer (*4*), cervical cancer (*5*), non-small cell lung cancer (*6-8*), and breast cancer (*9*).

TF-targeted therapies are currently under translation into the clinical treatment of cancer patients. In 2019, reports from the first phase 1-2 clinical trial of the TF-targeted antibody-drug conjugate *tisotumab-vedotin* in patients with recurrent, advanced, or metastatic solid tumors showed an objective tumor response in 16% of the patients (*10*). Recently, a 24% response rate was demonstrated in a phase 2 trial in previously treated recurrent or metastatic cervical cancer patients (*11*), and the United States Food and Drug Administration approved the therapy in September 2021 for this indication (*12*).

With the emergence of TF-targeted therapies, robust methods for quantifying TF expression in primary tumors and metastases are needed for efficient patient selection and stratification. Whole-body PET imaging can reduce the risk of sampling error from within tumor and between tumor heterogeneity seen in *ex vivo* analyses of tumor biopsies (*13*). Hence, PET imaging of TF expression is attractive as a companion imaging diagnostic agent for identifying patients eligible for TF-targeted therapies and may have the potential to increase response rates.

We have developed a TF-targeted PET radiotracer based on the natural ligand, factor VII (FVII). When vascular injury occurs, FVII is activated to FVIIa by the exposed TF on the endothelial cells and sets off the coagulation cascade (*1*). Through inhibition of the active site in FVIIa, the resulting active-site inhibited FVIIai (ASIS) binds to TF with an affinity approximately 5-fold higher than FVIIa

without activating the coagulation system (*14*). For TF-targeted PET imaging, ASIS is radiolabeled with N-succinimidyl 4-[^{18}F]fluorobenzoate (^{18}F-SFB) to form ^{18}F-ASIS (*15*). Preclinical studies with xenograft tumor-bearing mice have demonstrated high and specific ^{18}F-ASIS uptake in tumor tissue that reflects the level of TF expression determined *ex vivo* (*16*). Spurred on by the promising preclinical results, we moved forward with the clinical translation of ^{18}F-ASIS PET imaging in cancer patients.

Here we report our first-in-human trial on ^{18}F-ASIS PET in cancer patients. The primary objectives were to demonstrate the safety, biodistribution, pharmacokinetics, and dosimetry of ^{18}F-ASIS. As a secondary objective, we investigated radiotracer accumulation in tumors with PET and its correlation with TF expression in *ex vivo* analyses of matched tumor samples.

MATERIALS AND METHODS

Study Design

We performed the study as an open-label, phase 1 clinical trial approved by the Danish Medicines Agency (EudraCT no. 2015-005583-42) and the Ethical Committee of the Capital Region of Denmark (protocol H-18015477). Patients signed written informed consent prior to inclusion. The study was conducted in accordance with the requirements for Good Clinical Practice including independent monitoring by the Good Clinical Practice unit of Copenhagen University Hospital and the trial was registered at ClinicalTrials.gov (NCT03790423). Eligible patients were ≥ 18 years, diagnosed with breast, lung, pancreatic, cervical, or ovarian cancer, and capable of understanding the patient information in Danish and giving full informed consent. Exclusion criteria were pregnancy/breast-feeding, weight above 140 kg or history of allergic reaction attributable to compounds of similar chemical or biologic composition to ^{18}F-ASIS.

From January to November 2019, after giving informed consent, 10 patients with pancreatic cancer (n=4), breast cancer (n=3), lung cancer (n=2), and cervical cancer (n=1) were included in

the study and referred to a ^{18}F-ASIS PET/CT imaging series. The mean and standard deviation of the administered mass of ^{18}F-ASIS was 0.67 ± 0.12 mg (range, 0.41–0.84 mg). The mean administered activity was 157 ± 35 MBq (range, 93–198 MBq) yielding a mean specific activity of 245 ± 84 MBq/mg (range, 126-412 MBq/mg) at the time of injection. Sequential whole-body PET/CT imaging was performed 1 hour (h), 2h, and 4h after injection of ^{18}F-ASIS. Patients were monitored for changes in vital signs, electrocardiograms, and blood parameters before and after radiotracer administration. Adverse events were registered up to 48 hours after administration of ^{18}F-ASIS and coded according to the Common Terminology Criteria for Adverse Events (CTCAE) v5.0. Blood sampling and urine collection was performed for pharmacokinetic analyses. The study design is summarized in Figure 1. A detailed study description is provided in the supplemental information. When available, tumor biopsies or surgically excised primary tumor tissue and local lymph nodes were collected, and TF expression was analyzed with immunohistochemistry (IHC) and enzyme-linked immunosorbent assay (ELISA).

Inhibition of FVIIa

FVIIa (Novo Nordisk A/S, Bagsværd, Denmark) was dissolved in water and 5 equivalents of D-Phe-Phe-Arg-chloromethyl ketone (fFR-cmk, Bachem, Bubendorf, Switzerland) were added for inhibition of FVIIa to produce ASIS. After inhibition (1h, 4°C), excess of inhibitor was removed by dialysis (Slide-a-lyzer, MWCO 10, Thermo Fisher Scientific, Copenhagen, Denmark) in 50 mM N-2-Hydroxyethylpiperazine-N'-2-Ethanesulfonic Acid (HEPES,150 mM NaCl, 10 mM $CaCl_2$, pH 7.4, Sigma-Aldrich, Brøndby, Denmark) overnight. The content of fFR-cmk and the concentration of ASIS was analyzed by high-pressure liquid chromatograph (HPLC) using an Aeris C4 column (3.6 µm, 150 x 4.6 mm, Phenomenex, Værløse, Denmark) and 1.5 ml/min solvent flow with a gradient method: 0-2 min 17% B; 2-5 min 60% B, 6 min 60% B, 6-7 min 17% B, 7-8 min 17% B with solvent phases 0.1% trifluoroacetic acid (TFA) in H_2O (A) and 0.1% TFA in acetonitrile (MeCN) (B). Aliquots (500 µl) were stored at -80°C prior to labeling.

Synthesis of ^{18}F-ASIS

ASIS was labeled with the ^{18}F-containing prosthetic group ^{18}F-SFB. ^{18}F-SFB was produced in a three-step, one pot synthesis on a qualified TracerLab$_{MX}$ module (GE Healthcare, Brøndby Denmark) with a final solid-phase extraction purification in 80% MeCN. ^{18}F-SFB was subsequently evaporated to dryness in a single vial. ASIS (500 µl) was added to the vial for labeling at room temperature for 30 min followed by purification with a PD10 column (Sigma-Aldrich, Brøndby, Denmark) into formulation buffer (10 mM GlyGly, 150 mM NaCl, and 10 mM $CaCl_2$, pH 7.5). The final product was sterile filtered in a laminar airflow bench and a sample was drawn for quality control. The shelf-life of ^{18}F-ASIS was evaluated up to 4h after end-of-synthesis.

Quality Control of ^{18}F-ASIS

All analytical methods were validated according to the International Council of Harmonization guidelines (*17*). The radiochemical purity, unspecified ^{18}F-labeled impurities, and ^{18}F-fluoride were determined with radio-HPLC while the content of ASIS was determined by ultraviolet-detector HPLC (UV-HPLC), both using the same gradients as described above. Residual MeCN from the ^{18}F-SFB synthesis was determined by gas chromatography. Color-spot tests were used to determine the content of tetrabutylammonium hydrogen carbonate and HEPES in the final product. The immunoreactivity of ^{18}F-ASIS was determined by Lindmo assay using a high TF expressing cell-line (BxPC-3, CRL-1687™, ATCC, Virginia, United States) according to previously described procedures (*18*). Quality control parameters are summarized in Supplemental Table 1.

Plasma and Urine Pharmacokinetics

The activity of urine, whole blood, and plasma samples was measured on a Cobra II TM Gamma Counter (PACKARD, Meriden, CT, USA). The plasma samples were prepared from whole blood samples by centrifugation (3,500 rpm, 4 min) and filtering of the supernatant plasma through a

0.45 µM syringe filter. The radiotracer half-life was determined from the activity concentrations in plasma decay-corrected to the blood sampling time points (approximately 1h, 2h, and 4h after injection). The accumulated percentages of excreted radiotracer in urine were determined from the ratio between the accumulated activity in urine and the injected radiotracer activity dose decay-corrected to the urine sampling time points (approximately 1h, 2h, and 5h after injection). Metabolites in plasma and urine samples were analyzed by radio-HPLC with a Posi-RAM Module 4 using the same gradients as described above.

Image Acquisition

Image acquisition was performed on a Siemens Biograph 128 mCT PET/CT (Siemens Healthineers, Erlangen, Germany) with PET acquisition commenced 1h, 2h, and 4h after injection of ^{18}F-ASIS. Unless otherwise contraindicated, patients were injected with intravenous iodine-based contrast (Optiray 300 mg I/ml, 70-100 ml, injection rate 1.5-2.5 ml/s) using an automated injection system. Detailed descriptions of the PET and CT imaging parameters (including acquisition times and reconstruction parameters) are provided in the supplemental information.

Biodistribution and Dosimetry

Dosimetry was based on the PET images (n=10) supplemented with sampled urine-data (n=8). For each patient, organ, and time-point, tissue activity concentration was calculated as the average of the mean values from 3 volumes of interests (VOIs) drawn in the following organs/regions: adrenal, bone, brain, blood pool, ascending and descending colon, heart wall, kidney, liver, lung, red marrow (L3–L5 vertebrae), small intestines, spleen, stomach contents, and thyroid using MIRADA DBx version 1.2.0 (Mirada Medical, Denver CO, USA). OLINDA/EXM 2.0 software (Vanderbilt University, Nashville, TN, USA and HERMES Medical Solutions, Stockholm, Sweden) was used for calculation of do-

simetry parameters using the organ masses of the OLINDA male adult phantom (*19,20*) and the absorbed doses for organs and effective dose with tissue weighting factors according to International Commission on Radiological Protection (ICRP) 103 (*21*). A detailed description of the dosimetry calculation and biodistribution data processing is provided in the supplemental information.

Image Analysis

The PET/CT images were evaluated by a highly experienced team consisting of a nuclear medicine specialist and a radiologist. Size measurements of the primary tumor and metastases (if any) were performed on the diagnostic CT. In tumor lesions identified on the CT, radiotracer accumulation was measured on the PET images and reported as standardized uptake values (SUV). Spherical VOIs maximizing a volume encompassed by the tumor lesion perimeter based on the CT images were used for uptake quantification, and the tumor lesion maximal SUV (SUV_{max}) and mean SUV (SUV_{mean}) were recorded on the PET scan. Tumor-to-blood ratios were calculated as tumor lesion SUV_{max} divided by the blood pool SUV_{mean}. Any additional foci identified only on the PET, judged indicative of a primary tumor or metastases by the readers, were recorded. SyngoVIA Version VB30A-HF04 (Siemens Healthineers, Erlangen, Germany) was used for the image analysis.

***Ex Vivo* Tumor Tissue Samples**

Tumor tissue samples were obtained from surgically resected tissue or from tumor biopsies performed in relation to routine clinical procedures. Samples were processed for measurement of TF expression with ELISA and IHC. Details on tissue preparation, ELISA measurements, and IHC preparation is provided in the supplemental information. TF expression on IHC was stratified as low, intermediate, or high based on visual assessment.

Statistical Methods

The radiotracer plasma half-life was determined from mono-exponential linear regression models (one-compartment models) fitted to the decay-corrected time-activity curves in plasma (n=8). The relationship between the PET 4h tumor SUV_{max} and *ex vivo* measurements of TF expression by ELISA was analyzed with Pearson correlation (n=5). Two-sided P-values <0.05 were considered statistically significant. Data is presented as mean ± standard deviation unless otherwise noted. All statistical analyses were performed using R version 3.6.1 (R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

Radiochemistry

^{18}F-SFB was prepared in 29.4 ± 25.9 % non-decay corrected radiochemical yield (n=10 batches). ^{18}F-ASIS was achieved in 221 ± 58 MBq non-decay corrected activity yield (n=10 batches). ^{18}F-ASIS was produced with a radiochemical purity ≥95%, and unspecified ^{18}F-labeled impurities and ^{18}F-fluoride were both determined to ≤2%. The concentration of ASIS was 0.08 ± 0.01 mg/ml. Tetrabutylammonium hydrogen carbonate and HEPES content were <0.1 mg/ml and <20 µg/ml, respectively. An immunoreactivity of ≥75% was found for all 10 batches. Summary results of all quality control parameters are provided in Supplemental Table 1.

Patient Characteristics and Safety

The characteristics of the patients are shown in Table 1. There were no adverse events and no clinically significant changes in vital signs (Supplemental Table 2), blood parameters (Supplemental Table 3), nor electrocardiograms observed in any of the 10 patients.

Biodistribution, Pharmacokinetics, and Dosimetry

Biodistribution. A representative imaging series demonstrating the radiotracer distribution on the 1h, 2h, and 4h PET on the maximum intensity projection (MIP) is shown in Figure 2 for patient 5. The MIPs for the additional 9 patients are shown in Supplemental Figure 1. Organ-specific radiotracer uptake expressed as SUV_{mean} is shown in Figure 3. The highest uptakes were observed in the urinary bladder followed by the kidneys and the liver. The brain, bone, muscle, red bone marrow, and lung had low and decreasing uptakes suggesting no radiotracer accumulation.

Pharmacokinetics and dosimetry. Time-activity curves measured in plasma (n=8) are shown in Figure 4A. The plasma half-life was 3.2 ± 0.6 h. Urinary excretion accounted for the majority of ^{18}F-ASIS elimination and more than 40% of the injected dose was accumulated in the urine within 5h after injection (Figure 4B). Radio-HPLC run on plasma samples showed no major metabolites. Radio-HPLC run on urine samples showed urinary excretion of a smaller ^{18}F-radiolabeled fragment suggesting renal metabolism of ^{18}F-ASIS. Representative chromatograms of plasma samples collected 1h, 2h, and 4h after injection and urine samples collected 1h, 2h and 5h after injection are shown in Figure 4C. The dosimetry output from the OLINDA/EXM dosimetry software is shown in Table 2. The highest dose was received by the urinary bladder wall (118 µGy/MBq) followed by the kidneys (76 µGy /MBq), liver (67 µGy/MBq), and spleen (60 µGy/MBq). The effective dose was 20 µSv/MBq corresponding to 4 mSv for a target 200 MBq dose.

Radiotracer Accumulation in Tumor and Correlation with *Ex Vivo* Tumor Tissue

^{18}F-ASIS accumulation in tumor lesions quantified as SUV_{max} and tumor-to-blood ratios are shown in Figure 5. Heterogeneous SUV_{max} patterns between patients were observed: For patients 3 and 4 (both primary pancreatic tumors) and 5 (lung metastasis) SUV_{max} increased on the 2-4h PET

compared with the 1h PET. Contrary, in patients 7 and 8 (both primary breast tumors), low uptakes were observed at all three time points. The remaining patients showed relatively intermediate SUV_{max} that remained stable or slightly increased with time. Compared with the other patients, the 4h SUV_{max} for patients 3 and 4 were relatively high. The tumor-to-blood ratios showed a similar pattern.

Within tumor and within patient heterogeneity in radiotracer accumulation was also observed. Patient 10 (breast cancer) had heterogeneous radiotracer accumulation in the primary tumor (Figure 6A) with 4h SUV_{max} in the intermediate range (2.86). A corresponding small tissue sample taken immediately from the surgically resected tumor showed low *ex vivo* TF expression measured with both ELISA and IHC (Figure 6B). However, TF IHC staining of the full mastectomy specimen, performed following the pathology examination, showed areas with intermediate TF expression (Figure 6C). The pathology examination demonstrated two separate primary tumors. This patient also had an axially sentinel node metastasis that was not enlarged on CT, showed no apparent focal accumulation on PET, and had low TF expression on IHC (Figure 6D).

There was a trend of a positive correlation between 4h PET SUV_{max} and TF expression measured *ex vivo* on matched tumor tissue samples although not statistically significant (p=0.08, r=0.84, n=5). TF IHC-stained images in matched tumor tissue samples were available for 7 patients. Representative examples of low, intermediate, and high TF expression on IHC with corresponding 4h PET/CT images are shown in Figure 7. A summary of the PET/CT findings, quantitative plasma and *ex vivo* tumor TF expression, and TF IHC staining patterns is shown in Table 3.

DISCUSSION

We report here the first-in-human experience of the tissue factor (TF)-targeted radiotracer ^{18}F-ASIS in cancer patients. The trial marks the first test in humans of a PET radiotracer targeting TF

(first-in-class). Our main finding was that injection of ^{18}F-ASIS was safe, and no adverse events were observed. The effective radiation dose of 4 mSv from administration of 200 MBq of ^{18}F-ASIS is lower than that received after a standard ^{18}F-FDG injection (*22*). None of the calculated organ-specific absorbed doses were prohibitive for administration of 200 MBq of ^{18}F-ASIS. As an indication of the specific tumor-targeting ability of ^{18}F-ASIS, we observed a trend of a positive correlation between tumor SUV_{max} and quantitative TF expression determined *ex vivo* (p=0.08, r=*0.84*). These initial findings represent important first steps towards the clinical implementation of ^{18}F-ASIS PET imaging as a companion diagnostic tool for TF-targeted therapies.

The biodistribution and pharmacokinetic data indicated that the primary elimination route of ^{18}F-ASIS was through the kidneys. The low bone uptake is supportive of high metabolic stability, as freely circulating ^{18}F-fluoride would expectedly result in high bone accumulation (*23*). The 3.2h ^{18}F-ASIS plasma half-life was comparable to the 3.8h half-life observed for an unlabeled version of ASIS at similar dose (*24*) suggesting that the radiolabeling does not fundamentally alter the elimination of the radiotracer from plasma. Compared to antibody- and antibody fragment based TF-targeted radiotracers with long circulation time resulting in optimal tumor to background contrast after several days in preclinical models, e.g. ^{64}Cu and ^{89}Zr-labeled NOTA-ALT-836 (*25,26*), the relatively fast elimination of ^{18}F-ASIS makes this radiotracer better suited for same day imaging.

The between patient and cancer type heterogeneity in radiotracer tumor accumulation and *ex vivo* TF expression observed in the study is in line with the varying degree of TF expression across cancer types reported in the literature (*2,16,27*). Pancreatic tumors have particularly high TF expression in agreement with our findings. The within tumor heterogeneity seen in both radiotracer accumulation on PET and on *ex vivo* TF IHC staining of full surgical specimens serves as an example of the potential of PET imaging for evaluation of TF expression. As PET imaging captures the whole-body tumor burden, identification of hotspots that could be otherwise missed on a biopsy is possible with PET. Importantly,

the sentinel node metastasis without enlargement on CT, and with no apparent focal PET accumulation, had low TF expression on IHC, which suggests that PET was not false negative. Conclusions should of course not be inferred from single observations, but the results encourage further investigation.

The trend of a positive correlation between tumor SUV_{max} and quantitative TF expression measured *ex vivo* (r=0.84, p=0.08) suggests that ^{18}F-ASIS accumulation depends on the levels of TF in tumors. It may be argued that the radiotracer accumulation in tumors were modest. Importantly, this does not pose a limitation to the use of ^{18}F-ASIS PET as a whole-body non-invasive companion diagnostic or prognostic tool based on tumor TF expression if robust correlations between PET-derived tumor radiotracer accumulation and actual TF expression can be established. The relationship between SUV_{max} and *ex vivo* TF expression presented in this study suggests such a correlation. The observed trend is in line with our preclinical results in xenografted tumor mouse models that showed a strong and statistically significant positive correlation between tumor SUV_{max} on 4h ^{18}F-ASIS PET and TF expression measured in excised tumor tissue (*16*). The specificity of ^{18}F-ASIS for targeting TF was supported by the qualitative relationship between the tumor SUV_{max} and TF IHC staining patterns of surgical specimens that generally were in agreement. These preliminary results suggest that ^{18}F-ASIS PET imaging can be used for non-invasive measurement of TF expression in tumor tissues which may ultimately assist in identifying patients eligible for TF-targeted therapies. However, future later-phase clinical studies are needed to validate these findings in larger populations.

CONCLUSION

^{18}F-ASIS can safely be administered to cancer patients for TF-targeted PET imaging. The trial marks the first test of a TF-targeted PET radiotracer in humans (first-in-class). The effective whole-body dose for a 200 MBq activity dose was 4 mSv and no prohibitive organ-specific absorbed doses were observed. Plasma half-life was 3.2h and renal elimination accounted for most of the radiotracer excretion. The findings represent important first steps towards the clinical implementation of ^{18}F-ASIS for PET imaging of TF expression, which could assist in patient prognostication and selection of eligible patients for TF-targeted therapies. Future later-phase studies are needed to validate these initial findings.

DISCLOSURE

AK and CHN are inventors/hold intellectual property rights on a patent covering tissue factor imaging. No other potential conflicts of interest relevant to this article exist.

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KEYPOINTS

QUESTIONS: Can ^{18}F-ASIS safely be administered to cancer patients for PET imaging of tissue-factor in tumors?

PERTINENT FINDINGS: In this first-in-human clinical trial of 10 cancer patients, administration of 200 MBq ^{18}F-ASIS was safe, and no adverse events were reported. The effective whole-body dose was 4 mSv and no prohibitive organ-specific absorbed doses were observed.

IMPLICATIONS FOR PATIENT CARE: The trial marks the first test in humans of a PET radiotracer targeting tissue factor (first-in-class). The findings represent important first steps towards implementation of ^{18}F-ASIS PET imaging of tissue factor in cancer patients for prognostication and selection of patients for tissue factor-targeted therapies.

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FIGURES

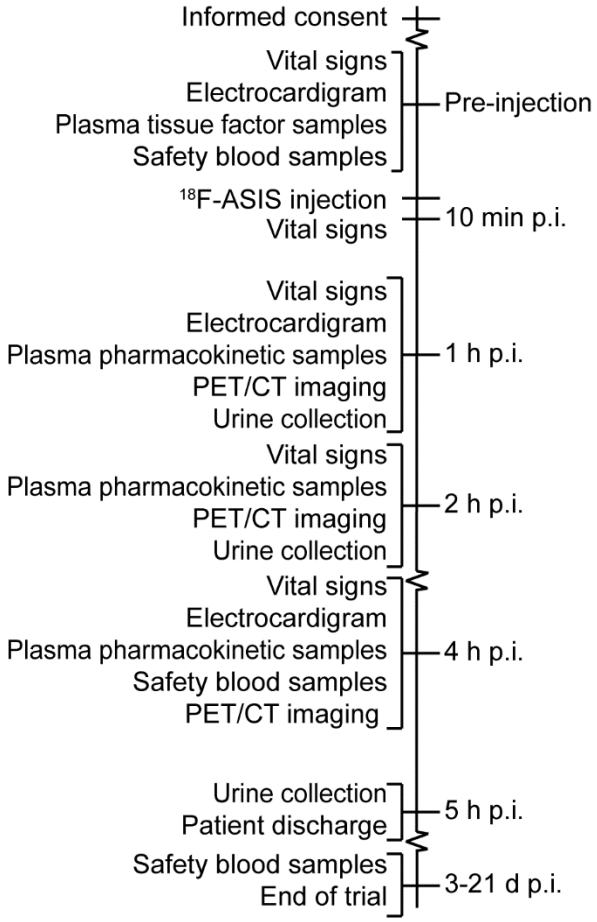


FIGURE 1 Schematic overview of the study design. d: days. h: hours. p.i.: post-injection.

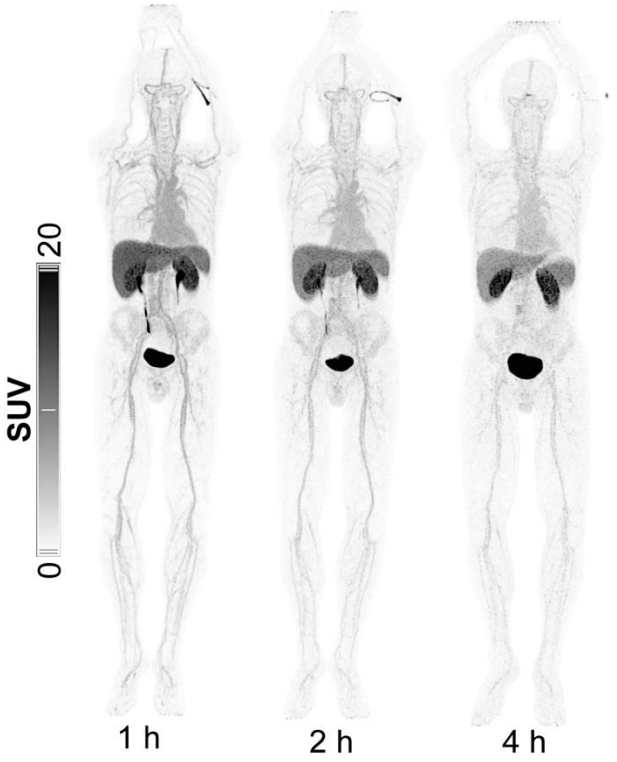


FIGURE 2 Representative maximum intensity projection (MIP) showing the distribution of ^{18}F-ASIS for patient 5. h: hours.

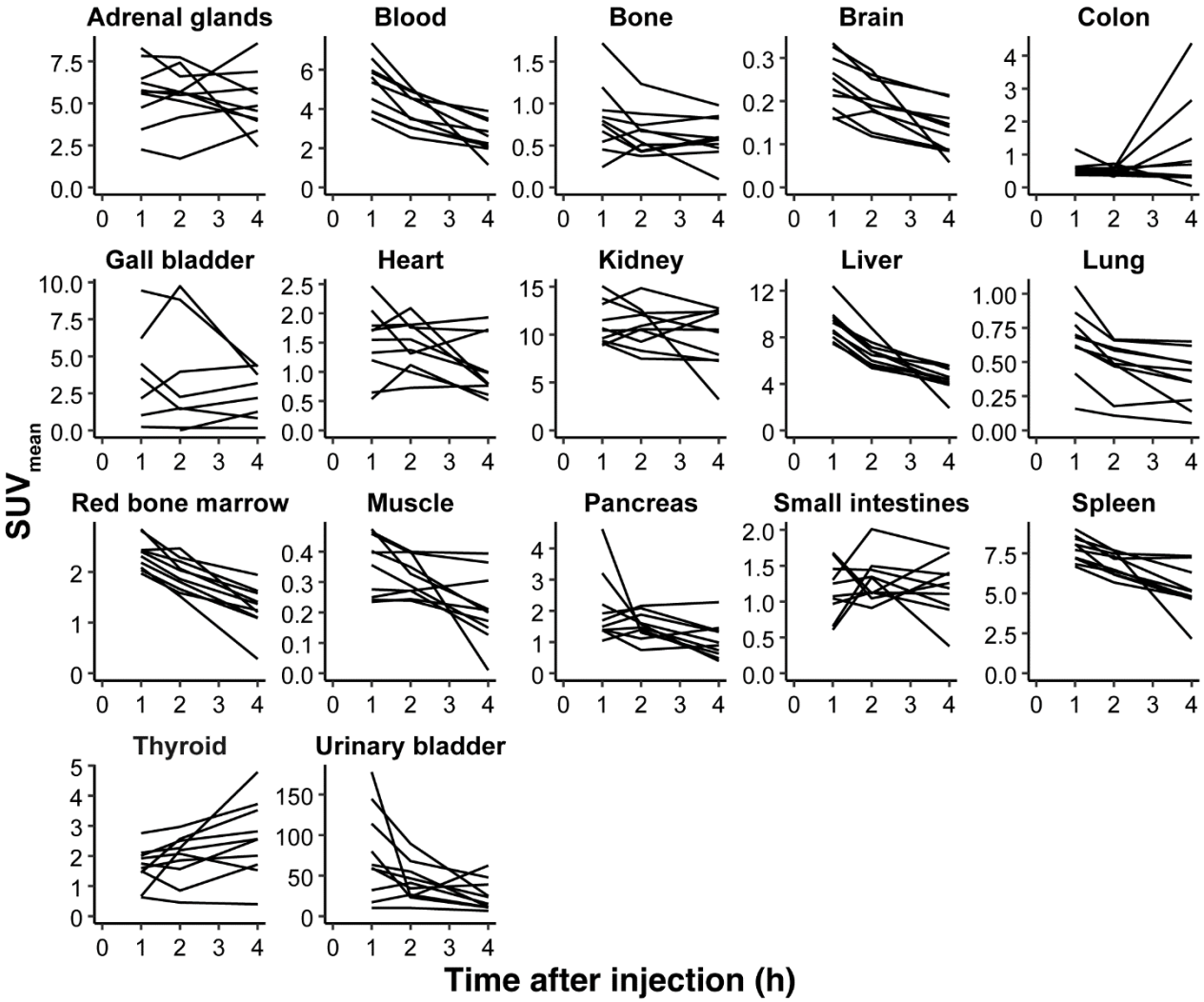


FIGURE 3 Distribution of ^{18}F-ASIS in organs (n=10). h: hours. SUV_{mean}: mean standardized uptake value

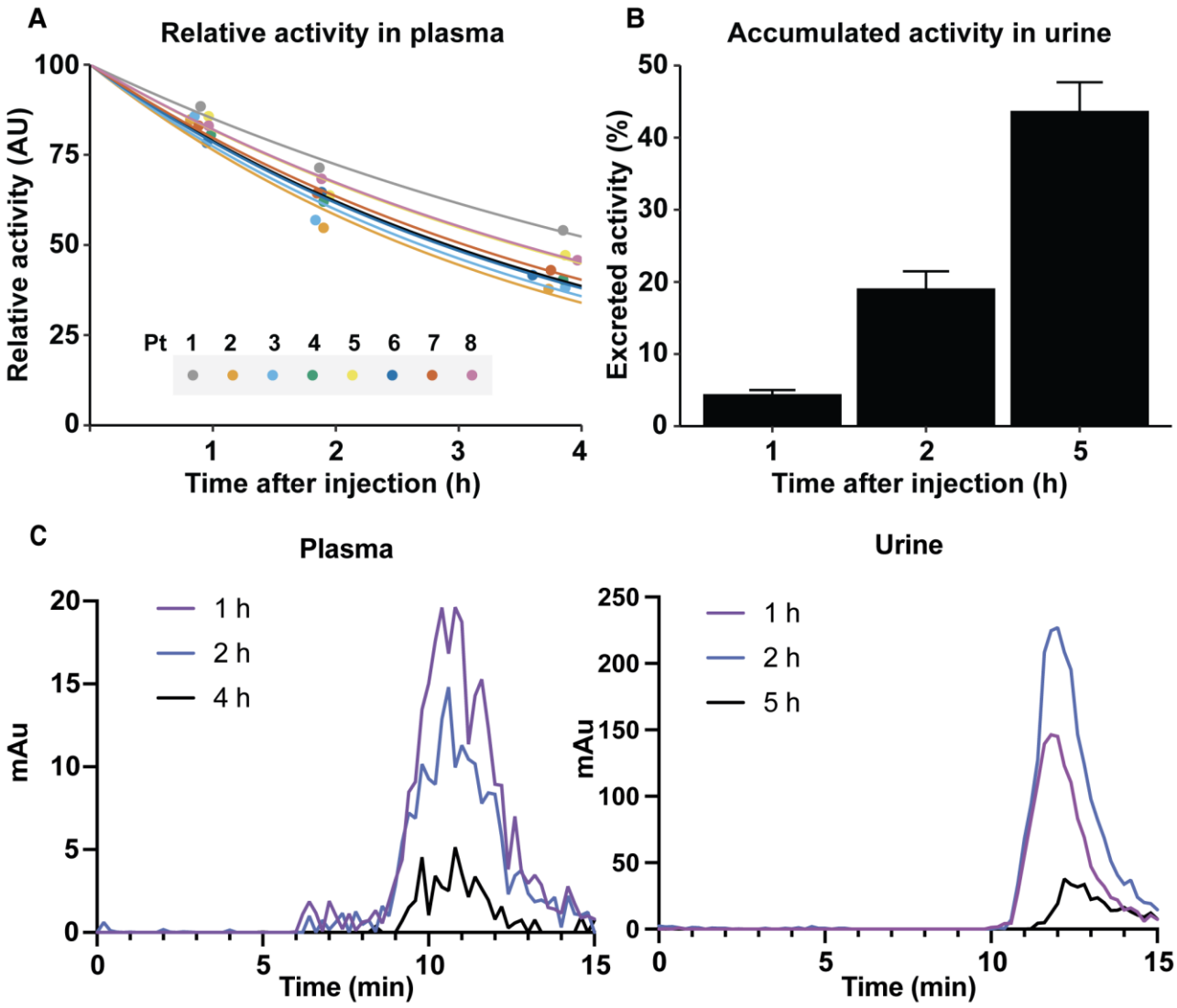


FIGURE 4 A Normalized time-activity curves of plasma samples with mono-exponential fits (n=8). **B** Accumulated percentages of activity excreted in urine (n=8). **C** Representative radio-high-pressure liquid chromatograph (radio-HPLC) chromatograms from plasma showing no major metabolites (left) and representative radio-HPLC from urine showing urinary excretion of a smaller ^{18}F-radiolabeled fragment (right). AU: arbitrary units. h: hours. mAu: absorbance units.

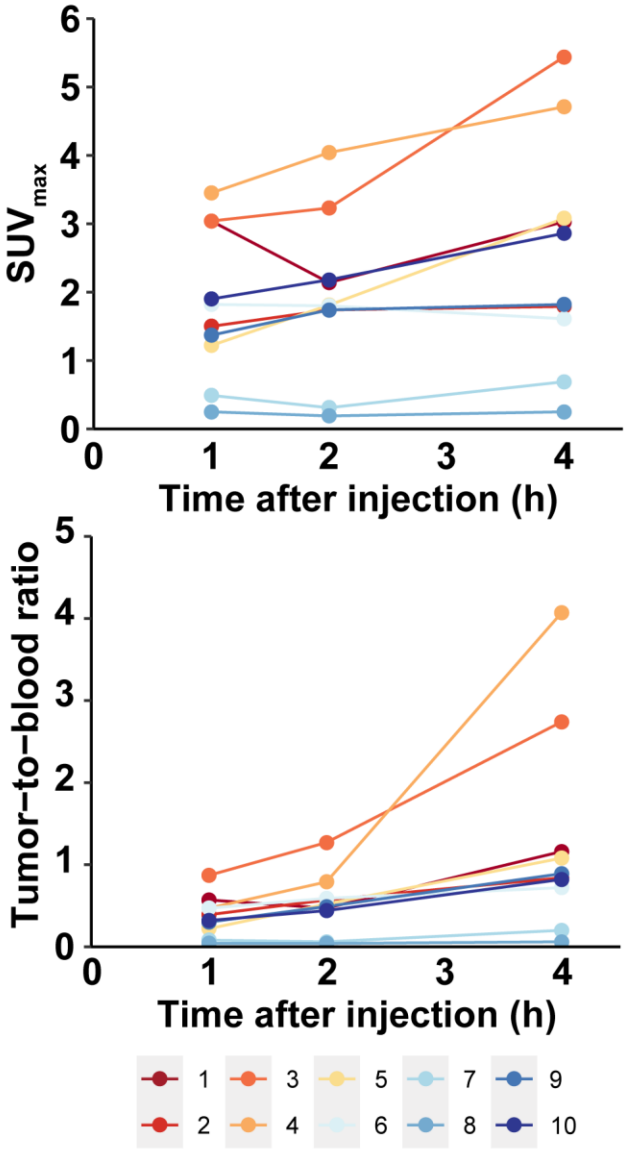


FIGURE 5 Tumor maximum standardized uptake values (SUV_{max}) (top) and tumor-to-blood ratios (Tumor SUV_{max} divided by blood pool SUV_{mean}) on the 1h, 2h, and 4h PET (bottom). Colors refer to the patient numbers shown below.

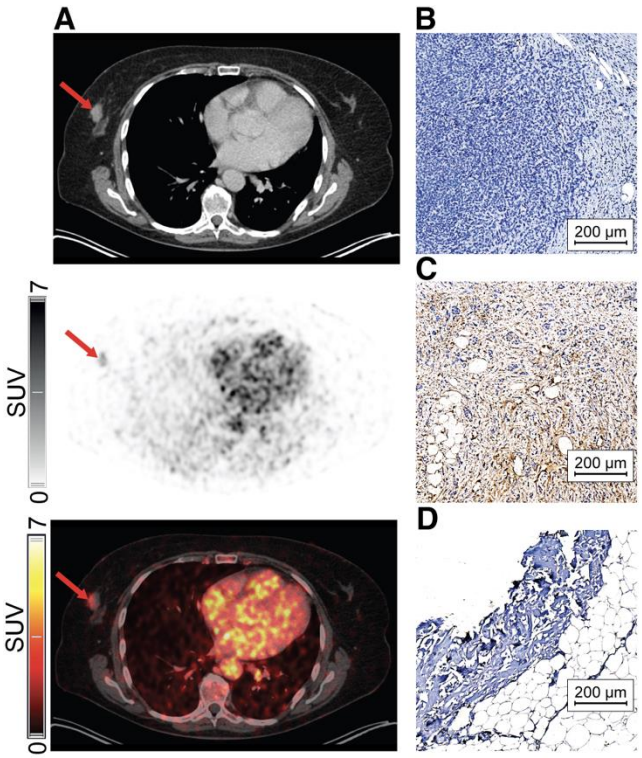


FIGURE 6 Patient 10 with breast cancer. **A** Primary breast tumor with relatively intermediate 4h PET SUV_{max} (2.86) shown on (top to bottom) CT, PET and fused PET/CT, respectively. Arrows mark tumor location. **B** Small sample taken from the tumor lesion immediately following surgery with low TF expression on immunohistochemistry (IHC). **C** Portion of the mastectomy specimen showing intermediate TF expression on IHC performed after the pathology examination. **D** Axillary sentinel node metastasis with low TF expression on IHC without apparent focal accumulation in the corresponding axillary area on PET or lymph node enlargement on CT (not shown).

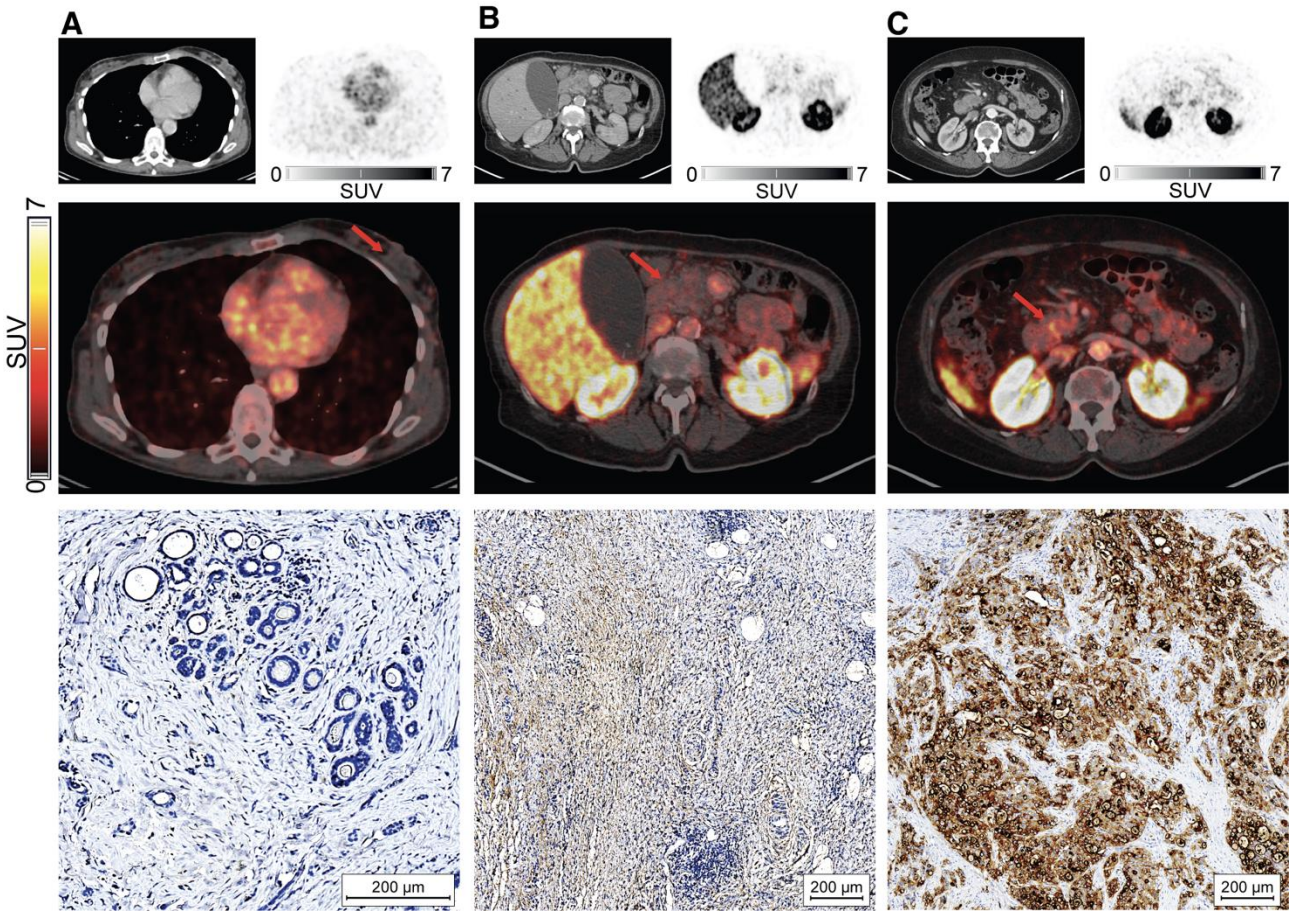


FIGURE 7 A Patient 7: Breast tumor with low SUV_{max} (0.69) and low tissue factor TF expression on immunohistochemistry (IHC) *ex vivo.* **B** Patient 2: Pancreatic tumor with relatively intermediate SUV_{max} (1.79) and intermediate TF expression on IHC. **C** Patient 4: Pancreatic tumor with relatively high 4h PET SUV_{max} (4.71) and high TF expression on IHC. **Top-bottom:** 4h CT, PET and fused PET/CT, and IHC. Arrows mark tumor location on the PET/CT. SUV: standardized uptake values.

TABLE 1 Patient Characteristics

Pt	1	2	3	4	5	6	7	8	9	10
Gender	Female	Female	Female	Female	Male	Female	Female	Female	Female	Female
Age (y)	88	67	69	79	65	58	54	59	43	73
Primary tumor	Pancreas	Pancreas	Pancreas	Pancreas	Lung†	Lung	Breast	Breast	Cervix	Breast
Type [stage]*	PT: DAC	PT: PAC [pT2pN2M0]	PT†: DAC	PT: DAC [pT2N0M0]	PT†: AC MET: AC	PT: AC [pT2bN0M0]	PT: IDC (HER2+1/ ER100%). SN without MET (0/1 LN). [M0]	PT: ILC (HER2+1/ ER100%). SN without MET (0/2 LN). [M0]	PT: SCC	PT‡: ISPC SN with micro MET (1/2 LN). [M0]
Prior cancer treatment	None	None	CTX	None	Surgery and CTX	CTX	None	None	None	None
Concomitant cancer treatment	None	None	None	None	CTX	RDX	None	None	None	None

*Pathology TNM staging is reported in square brackets when available. †Primary tumor removed. ‡Two separate tumors without connection: Tumor 1: HER2+1/ER100%; Tumor 2: HER2-/ER100%. AC: adenocarcinoma. CTX: chemotherapy. DAC: ductal adenocarcinoma. ER: estrogen receptor. HER2: human epidermal growth factor receptor 2. IDC: invasive ductal carcinoma. ILC: invasive lobular carcinoma. ISPC: invasive solid papillary carcinoma. LN: lymph nodes. MET: metastases. PAC: pancreaticobiliary adenocarcinoma. PT: primary tumor. RDX: radiation therapy. SN: sentinel nodes. SCC: squamous cell carcinoma.

TABLE 2 Organ-based Dosimetry

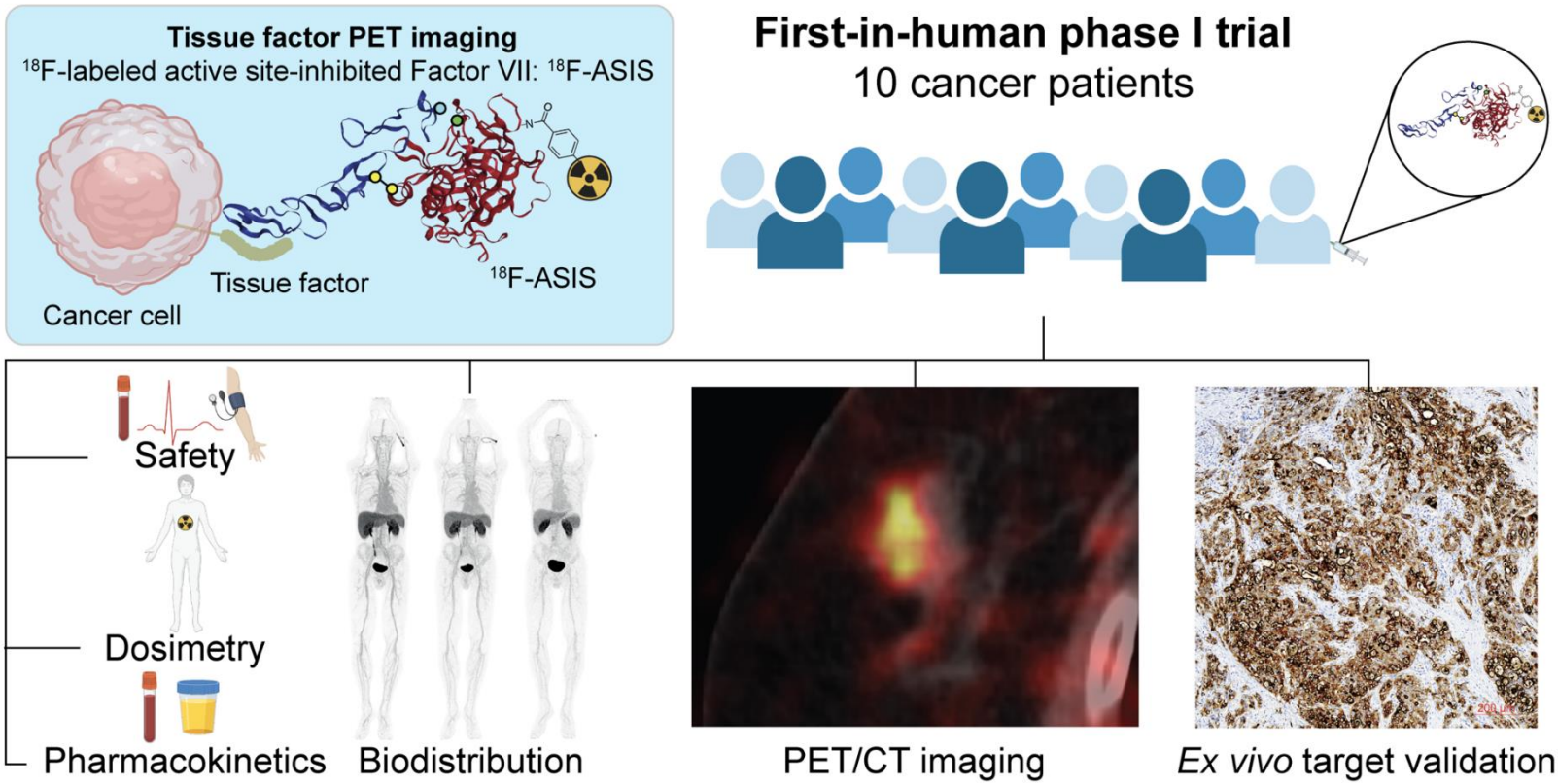
Organ	Total mean absorbed dose (µGy/MBq)
Adrenals	56
Brain	4
Breasts	8
Esophagus	12
Eyes	6
Gallbladder Wall	22
Left colon	21
Small Intestine	25
Stomach Wall	15
Right colon	13
Rectum	17
Heart Wall	17
Kidneys	76
Liver	67
Lungs	10
Ovaries	15
Pancreas	17
Prostate	15
Salivary Glands	7
Red Marrow	15
Osteogenic Cells	16
Spleen	60
Testes	8
Thymus	9
Thyroid	17
Urinary Bladder Wall	118
Uterus	22
Total Body	12
Effective Dose (µSv/MBq)	**20**

TABLE 3 PET/CT Image Findings and *Ex Vivo* Tissue Factor Measurements

Pt	1	2	3	4	5	6	7	8	9	10
Primary tumor	Pancreas	Pancreas	Pancreas	Pancreas	Lung‖	Lung	Breast	Breast	Cervix	Breast
Radiotracer mass (mg)	0.84	0.69	0.71	0.71	0.74	0.41	0.76	0.56	0.74	0.58
Activity dose (MBq)	135	187	198	189	93	169	145	187	117	145
Specific activity* (MBq/mg)	161	271	279	266	126	412	191	334	158	250
Metastases (Pathology/PET/CT)†	÷/÷/÷	÷/÷/÷	÷/÷/÷	÷/÷/÷	+/+/+	÷/÷/÷	÷/÷/÷	÷/÷/÷	÷/÷/÷	+/÷/÷¶
Tumor size (cm)	3.6x3.3	3.5x3.1	4.9x3.8	2.6x2.2	1.2x0.9‖	3.6x3.4	2.8x1.4	0.7x0.8	3.2x2.9	2.4x1.4
SUV_{max} (1h)	3.04	1.50	3.04	3.45‖	1.22	1.82	0.49	0.25	1.37	1.90#
SUV_{max} (2h)	2.14	1.74	3.23	4.04‖	1.81	1.80	0.31	0.19	1.74	2.18#
SUV_{max} (4h)	3.03	1.79	5.44	4.71‖	3.08	1.61	0.69	0.25	1.82	2.86#
SUV_{mean} (1h)	1.41	0.85	1.67	1.93‖	0.83	0.70	0.30	0.20	0.75	1.19#
SUV_{mean} (2h)	1.38	0.96	1.73	2.24‖	1.19	1.26	0.21	0.15	0.92	1.21#
SUV_{mean} (4h)	1.68	0.98	2.94	2.62‖	1.97	1.18	0.40	0.15	1.01	1.73#
ΔT (days)‡	42	4	NA	6	NA	NA	12	5	6	4
TF_{tumor} (µg/mg)	NA	5.93	NA	25.75	NA	NA	1.14	NA	1.27**	PT: 0.67 MET: NA
TF_{tumor} IHC§	Low**	Intermediate	NA	High	NA	NA	Low	Low	Low**	PT: Low / intermediate†† MET: Low
TF_{plasma} (µg/l)	61	54	56	72	73	43	82	66	21	73

*At time of injection. †Presence of metastases based on pathology, PET, and CT, respectively. (+): metastases present, (÷): no metastases. ‡Time between imaging and tissue collection. §Tissue factor (TF) expression on immunohistochemistry (IHC) rated low, intermediate, or high based on visual assessment. ‖Primary tumor removed. SUV and size measured on metastasis. ¶No lymph node enlargement on CT and no apparent focal accumulation on PET. #Heterogeneous radiotracer accumulation observed. **Samples from biopsies. ††Low TF staining on IHC on tissue sample also showing low (0.67 µg/mg) TF expression. Tissue from full mastectomy, obtained from post-pathology evaluation, with intermediate TF expression on IHC. MET: metastases. NA: Not available. PT: Primary tumor.

GRAPHICAL ABSTRACT



SUPPLEMENTAL INFORMATION

Full Methods Section

Study Design

We performed the study as an open-label, phase 1 clinical trial approved by the Danish Medicines Agency (EudraCT no. 2015-005583-42) and the Ethical Committee of the Capital Region of Denmark (protocol H-18015477). Patients signed written informed consent prior to inclusion. The study was conducted in accordance with the requirements for Good Clinical Practice including independent monitoring by the Good Clinical Practice unit of Copenhagen University Hospital and the trial was registered at ClinicalTrials.gov (NCT03790423). Eligible patients were ≥ 18 years, diagnosed with breast, lung, pancreatic, cervical, or ovarian cancer, and capable of understanding the patient information in Danish and giving full informed consent. Exclusion criteria were pregnancy/breast-feeding, weight above 140 kg or history of allergic reaction attributable to compounds of similar chemical or biologic composition to ^{18}F-ASIS.

From January to November 2019, after giving informed consent, 10 patients with pancreatic cancer (n=4), breast cancer (n=3), lung cancer (n=2), and cervical cancer (n=1) were included in the study and referred to a ^{18}F-ASIS PET/CT imaging series. The mean and standard deviation of the administered mass of ^{18}F-ASIS was 0.67 ± 0.12 mg (range, 0.41–0.84 mg). The mean administered activity was 157 ± 35 MBq (range, 93–198 MBq) yielding a mean specific activity of 245 ± 84 MBq/mg (range, 126-412 MBq/mg) at the time of injection. Sequential whole-body PET/CT imaging was performed 1 hour (h), 2h, and 4h after injection of ^{18}F-ASIS.

Blood samples were collected prior to administration of ^{18}F-ASIS for plasma tissue factor (TF) measurements in all patients. In a subset of eight patients (patients 1-8), blood samples were

collected for pharmacokinetic analysis, including radiotracer metabolism and plasma half-life, approximately 1h, 2h, and 4h after injection. In the same eight patients, urine collection was performed throughout the study period and sampled immediately prior to the 1h and 2h PET/CT and following the 4h PET/CT scans (at approximately 1h, 2h, and 5h after injection) for determination of urinary metabolism, excretion, and dosimetry calculations.

Safety measures included observation of the patients by a medical doctor up to 5h after injection of ^{18}F-ASIS and monitoring of heart rate, blood pressure, and pulse oximetry with regular intervals before, during, and after the last PET/CT scan (pre-injection, 10 min, and approximately 1h, 2h, and 4h after injection). Electrocardiograms were performed pre-injection, and approximately 1h, and 4h after injection. Hematologic (hemoglobin, white blood cells, platelets), liver (alanine amino transferase, aspartate transaminase, alkaline phosphatase), and renal function (creatinine, glomerular filtration rate, sodium, potassium), and c-reactive protein (CRP) were measured before radiotracer administration, 4h after injection, and as follow-up on the patient's routine return to the hospital 3–21 days after the study day. Adverse events were registered up to 48 hours after administration of ^{18}F-ASIS and coded according to the Common Terminology Criteria for Adverse Events (CTCAE) v5.0.

When available, tumor biopsies or surgically excised primary tumor tissue and local lymph nodes were collected for target validation by analysis of expression of TF with immunohistochemistry (IHC) and enzyme-linked immunosorbent assay (ELISA).

PET/CT Image Acquisition and PET Image Reconstruction

Image acquisition was performed on a Siemens Biograph 128 mCT PET/CT with an axial-field-of-view (FOV) of 221 mm (Siemens Healthineers, Erlangen, Germany) and PET acquisition commenced 1h, 2h and 4h after injection of ^{18}F-ASIS. Patients were preferably placed with their arms above the head. Prior

to the 1h and 2h PET, whole body (from fingertips to toes) low-dose CT (2 mm slice thickness, 40 mAs exposure, 120 kV kilovoltage peak) were performed. Following the 4h PET, a diagnostic whole-body 2 mm slice thickness CT was performed: Quality reference mAs = 225, kV = 120. Dose and kV modulation were activated meaning that the output from the X-ray tube was modulated according to patient size using the build-in CARE software (Siemens Healthineers, Erlangen, Germany). Unless otherwise contraindicated, patients were injected with intravenous iodine-based contrast (Optiray 300 mg I/ml, 70-100 ml, injection rate 1.5-2.5 ml/s) using an automated injection system. The PET acquisitions covered the whole body (from fingertips to toes). To allow for sufficient count statistics, while keeping the acquisition times at an acceptable level, differential acquisition times were employed. For the 1h and 2h PET, the acquisition time was 3 minutes per bed from head to midthighs and 1 minute per bed from midthighs and downwards. For the 4h PET, the corresponding acquisitions times were 4 and 2 minutes per bed, respectively. PET data were reconstructed iteratively using 3-dimensional ordinary Poisson ordered subsets expectation maximization (3D-OP-OSEM) with point-spread-function using the vendor supplied TrueX algorithm (Siemens Healthineers, Erlangen, Germany). 2 iterations and 21 subsets were used including time-of-flight information (540 ps) and smoothed by a Gaussian filter (2 mm full-width-half-maximum) with a slice thickness of 2 mm. For the 1h and 2h PETs, the corresponding low-dose CTs were used for localization and attenuation correction. For the 4h PET, the diagnostic CT was used.

Dosimetry and Biodistribution

Dosimetry was based on the non-decay corrected PET image sets from the 3 time-points (n=10) supplemented with sampled urine-data (n=8). The following organs were considered: adrenal, bone, brain, blood pool, heart wall, kidney, liver, lung, red marrow (L3–L5 vertebrae), ascending and descending colon, small intestine, spleen, stomach contents, and thyroid. For each patient, organ, and time-point, tissue activity concentration (kBq/mL) was calculated as the average of the mean values from 3 volumes

of interest (VOIs) drawn on the PET images using MIRADA DBx version 1.2.0 (Mirada Medical, Denver CO, USA). For presentation of the organ-specific radiotracer distribution, the average decay-corrected tissue activity concentration was calculated as body-weight adjusted mean standardized uptake values (SUV_{mean}). For the dosimetry calculations, total activity (per patient, organ, and time) was estimated by multiplying these average values by organ masses of the OLINDA male adult phantom (*1,2*). Activity values were normalized to 1 MBq by dividing with injected activity and scaled with the ratio of actual patient weight to the weight of the standard male model (73 kg). Time integrated activity coefficients (TIAC; unit *h*) for each patient and organ were determined by numerical integration up to the third (last) data point and analytical extrapolation to infinity assuming only physical decay. Piecewise linearity was assumed from time zero up to the second data point and a mono-exponential was used between the second and third data points. The resulting organ TIACs were averaged over patients. All data were entered into OLINDA/EXM 2.0 software (Vanderbilt University, Nashville, TN, USA and HERMES Medical Solutions, Stockholm, Sweden). The cumulated decay corrected activity (in MBq) of the excreted urine, normalized to 1 MBq of injection, was plotted over time for all 8 subjects and data fitted to a one phase exponential association (exponential growing towards a limit) using the Excel Solver. The resulting limit and half-life were used as input to the bladder voiding model of OLINDA, yielding the TIAC for bladder contents. A bladder voiding interval of 2 hours was selected for the calculation. The value for “remainder tissue” was determined as the total area (in h) for 1 MBq minus the sum (except bladder) of the organ-specific values minus the value passed to urine (based on the fitted model parameters). The output from OLINDA consists of absorbed doses for organs and effective dose with tissue weighting factors according to International Commission on Radiological Protection (ICRP) 103 (*3*).

Ex vivo Tumor Tissue Samples

Tissue preparation

Tumor tissue samples were obtained from resected surgical specimens or from tumor biopsies performed in relation to routine clinical investigation. Samples intended for quantification of TF by ELISA were immediately frozen in liquid nitrogen and subsequently stored at -80°C until use. Samples intended for histological preparation were fixated in 4% paraformaldehyde for 48h followed by storage in 96% alcohol until embedding into paraffin.

Measurement of Tissue Factor Expression in *Ex Vivo* Tumor Tissue Samples and Plasma

Tissue homogenization

Following thawing, tumor samples were weighed and 1 ml of RIPA buffer (89900, Thermo Fischer Scientific, Waltham, MA, USA) per g of tissue were added in a CKmix tissue homogenizing tube (Bertin Instruments, Rockville, MD, USA). Samples were homogenized on a Precellys Evolution Homogenizer (Bertin Instruments, Rockville, MD, USA) with the following program settings: 2 cycles of 35 sec, 9500 RPM, 4°C.

ELISA measurements

The TF protein concentration in tumor samples and plasma were measured with ELISA using the manufacturer's protocol (Human Coagulation Factor III/Tissue Factor Quantikine ELISA, DCF300, R&D systems, Minneapolis, MN, USA). Standards were applied in duplicates in the range 7.8–500 pg/mL, and a standard curve was fitted to a 3-parameter dose–response curve (Microsoft Excel 2016, Microsoft, Redmond, WN, USA). Samples were diluted to 1:3 (plasma) and 1:300 (tumor samples)

and measured in duplicates, and the TF concentration interpolated from the standard curve. Finally, the TF concentration in the tumor samples was normalized to total protein concentration measured with the Micro BCA™ Protein Assay Kit (23235, Thermo Scientific, Pierce Biotechnology, IL, USA) according to the manufacturer's protocol.

Immunohistochemistry of Tissue Factor in *Ex Vivo* Tumor Tissue

Biopsies where fixated in buffered 4% paraformaldehyde (pH 7.2) followed by preparation in Shandon Excelsior AS Tissue Processor O/N (Thermo Fisher Scientific, Waltham, MA, USA) and embedded in paraffin. Biopsies were cut in sections of 4 µm and dewaxed through xylene to tap water. For antigen retrieval, the sections were heat treated for 15 min in citrate buffer (pH 6). This was followed by a blocking step with Peroxidase-Blocking Solution (S2023, Agilent, Santa Clara, CA, USA) and pre-incubation in 2 % bovine serum albumin for 10 min. Sections were incubated with primary anti-tissue factor antibody (ADG4508, ImmBioMed, Pfugstadt, Germany) in a 1:500 dilution in 2% bovine serum albumin 1 hour at RT. For visualization, the sections were incubated with Envision+ system Anti-Mouse (K4001, Agilent, Santa Clara, CA, USA) for 45 min followed by incubation with DAB+ system (K3468, Agilent, Santa Clara, CA, USA) for 10 min. Counterstaining was performed with Mayer's Hematoxylin. Imaging was performed on Carl Zeiss Axio Lab.A1 (Carl Zeiss Microscopy GmbH, Jena, Germany) and analyzed with ZEN 3.2 Blue Edition (Carl Zeiss Microscopy GmbH, Jena, Germany). Immunohistochemistry TF expression was stratified as low, intermediate and high based on visual assessment.

SUPPLEMENTAL REFERENCES

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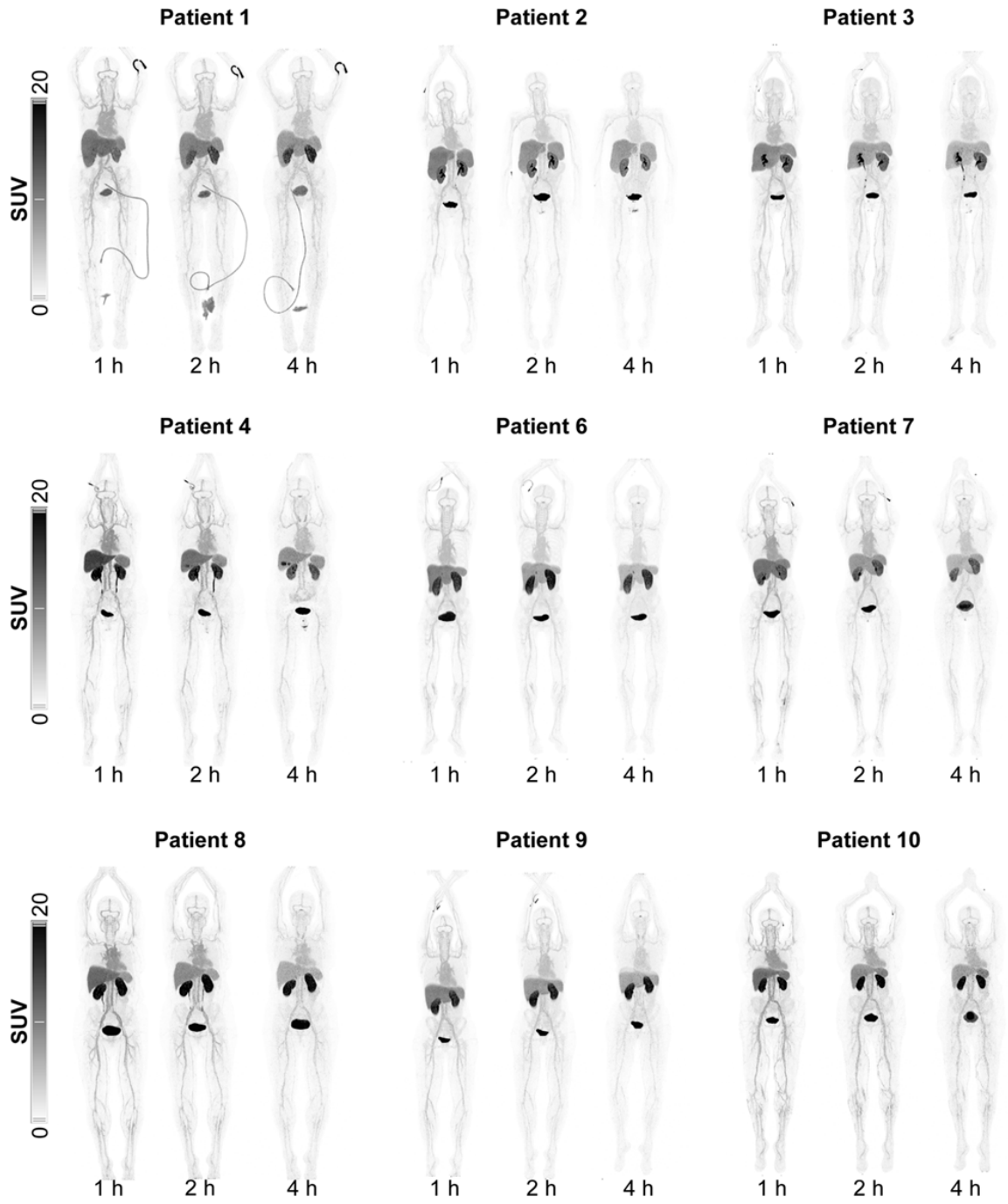
SUPPLEMENTAL FIGURES AND TABLES



SUPPLEMENTAL FIGURE 1 Maximum intensity projections (MIPs) showing the distribution of ^{18}F-ASIS for patients 1-4 and patients 6-10. h: hours.

SUPPLEMENTAL TABLE 1 Quality control parameters for ^{18}F-ASIS

Test	Method	Specification	Results (n=10)
Physical Tests			
Radioactivity	Dose Calibrator	200 MBq – 330 MBq at end of synthesis	220.5 ± 58.1 MBq
Physical appearance	Visual inspection	Clear and colorless solution, free from visible particulates or cloudiness	Complies for all batches
pH	Calibrated pH meter	6.0-8.0	7.2 ± 0.1
Radioactivity and chemical Tests			
^{18}F-Fluoride	HPLC	≤ 2%	0.0 ± 0.0 %
Unspecified ^{18}F-impurities	HPLC	≤ 2%	0.2 ± 0.2 %
Overall radio-chemical purity	HPLC	≥ 95%	99.9 ± 0.2 %
Identification of ^{18}F-ASIS	HPLC	The labeled product corresponds in retention time to an authentic reference standard of ASIS	Complies for all batches
ASIS content	HPLC	0.05 mg/ml - 0.15 mg/ml	0.08 ± 0.01 mg/ml
Acetonitrile	GC	≤ 410 ppm	30.9 ± 82.5 ppm
Tetrabutylammonium ions	Color-spot test	< 0.1 mg/ml	< 0.1 mg/ml for all batches
HEPES	Color-spot test	< 20 µg/ml	< 20 µg/ml for all batches
Protein and cell assays			
Immunoreactive fraction*	Lindmo assay	≥ 75 %	≥ 75 % for all batches

Microbiology tests			
Sterility*	Ph. Eur. test for sterility	Must comply	Complies for all batches
Endotoxins test	Ph. Eur. test for endotoxins	≤ 1EU/ml	≤ 1EU/ml for all batches

Data is presented as mean ± standard deviation unless otherwise indicated. *Not release test. Performed retrospectively following release of every batch. EU: Endotoxin units. HEPES: N-2-Hydroxyethylpiperazine-N'-2-Ethanesulfonic Acid. HPLC: high-pressure liquid chromatograph, GC: gas chromatography.

SUPPLEMENTAL TABLE 2 Vital signs for patients pre-injection, 10 min, 1 hour, 2 hours, 4 hours following injection of ^{18}F-ASIS

	Before injection				10 min post injection				1 hour post injection				2 hours post injection				4 hours post injection			
Pt	Sys	Dia	HR	PO_2	Sys	Dia	HR	PO_2	Sys	Dia	HR	PO_2	Sys	Dia	HR	PO_2	Sys	Dia	HR	PO_2
1	122	60	52	99	132	62	51	98	146	71	52	99	133	61	54	96	136	60	54	100
2	128	80	89	97	131	69	87	98	144	84	91	99	127	77	88	98	136	80	85	100
3	145	92	72	93	153	89	68	100	144	79	81	97	142	73	79	93	154	92	71	100
4	153	76	69	96	161	71	71	96	162	76	81	97	166	78	83	97	170	69	81	95
5	125	75	72	98	119	74	67	98	123	71	63	97	115	80	80	98	128	79	81	96
6	100	59	67	99	100	57	68	99	109	52	52	99	112	62	59	98	98	55	73	99
7	150	94	79	99	146	93	75	100	139	82	77	99	139	82	77	99	143	79	78	98
8	121	78	70	95	116	71	63	93	129	78	63	97	141	85	52	96	134	74	79	93
9	127	66	44	97	112	66	47	100	133	72	44	100	125	73	47	100	131	70	59	99
10	150	72	68	99	136	80	74	98	162	84	66	98	174	83	67	100	146	102	76	98

Sys: Systolic blood pressure, Dia: Diastolic blood pressure HR: Heart rate, PO_2: pulse oximetry.

SUPPLEMENTAL TABLE 3 Safety blood parameters measured before and after injection of ^{18}F-ASIS

Pt	Parameter (normal range)	Pre-injection	4 hours p.i.	Follow-up (*21* days p.i.)
1	Hemoglobin (7.3-9.5 mM)	7.3	6.2*,a	5.5*,b
	Leucocytes (3.5-8.8 10^7/l)	11.2*,b	12.6*,b	14.4*,b
	Platelets (145-390 10^7/l)	429*,b	356	497*,b
	CRP (<10 mg/l)	27*,b	28*,b	14*,b
	eGFR (> 60 ml/min/1.73 m^2)	21*,c	22*,c	34*,c
	Creatinine (50-90 μM)	186*,c	175*,c	123*,c
	Sodium (137-144 mM)	134*,b	132*,b	138
	Potassium (3.5-4.4 mM)	4.1	3.8	4.8*,a
	ALAT (10-45 U/l)	67*,b	62*,b	97*,b
	ASAT (15-35 U/l)	85*,b	80*,b	NA
	ALP (35-105 U/l)	407*,b	353*,b	868*,b
	Comments *Outside normal ranges. [a]Not considered clinically significant. [b]Related to patient's cancer disease (pancreatic cancer). [c]Related to patient's chronic kidney disease. NA: Not available.			
Pt	**Parameter (normal range)**	**Pre-injection**	**4 h. p.i.**	**Value (*18* days p.i.)**
2	Hemoglobin (7.3-9.5 mM)	7.8	6.8*[a]	7.2*[a,c]
	Leucocytes (3.5-8.8 10^7/l)	8.0	7.7	12.8*[a,c]
	Platelets (145-390 10^7/l)	371	NA	958*[a,c]
	CRP (<10 mg/l)	2	2	2
	eGFR (> 60 ml/min/1.73 m^2)	>90	>90	>90
	Creatinine (50-90 μM)	48*[a]	47*[a]	48*[a]
	Sodium (137-144 mM)	136*,a	138	136*,a
	Potassium (3.5-4.4 mM)	3.9	NA	4.0
	ALAT (10-45 U/l)	105*,b	80*,b	37
	ASAT (15-35 U/l)	124*,b	65*,b	69*,b
	ALP (35-105 U/l)	343*,b	287*,b	83
	Comments *Outside normal ranges. [a]Not considered clinically significant. [b]Related to patient's cancer disease (pancreatic cancer). [c]Related to sequelae from post-surgical infection. NA: Not available.			
Pt	**Parameter (normal range)**	**Pre-injection**	**4 hours p.i.**	**Follow-up (*4* days p.i.)**
3	Hemoglobin (7.3-9.5 mM)	6.0*,b	5.7*,b	6.8*,b
	Leucocytes (3.5-8.8 10^7/l)	7.4	6.2	6.7
	Platelets (145-390 10^7/l)	377	343	308
	CRP (<10 mg/l)	1	1	1
	eGFR (> 60 ml/min/1.73 m^2)	90	90	90

	Creatinine (50-90 μM)	55	55	53
	Sodium (137-144 mM)	143	142	143
	Potassium (3.5-4.4 mM)	3.8	4.2	4.1
	ALAT (10-45 U/l)	28	28	31
	ASAT (15-35 U/l)	37[*,a]	47[*,a]	37[*,a]
	ALP (35-105 U/l)	92	89	100
	Comments [*]Outside normal ranges. [a]Not considered clinically significant. [b]Patient has anemia.			
Pt	**Parameter (normal range)**	**Pre-injection**	**4 h. p.i.**	**Value (5 days p.i.)**
4	Hemoglobin (7.3-9.5 mM)	7.6	7.6	7.3
	Leucocytes (3.5-8.8 10^7/l)	11.0[*,a]	11.8[*,a]	12.3[*,a]
	Platelets (145-390 10^7/l)	374	343	309
	CRP (<10 mg/l)	7	6	66[*,a]
	eGFR (> 60 ml/min/1.73 m^2)	83	83	86
	Creatinine (50-90 μM)	61	60	55
	Sodium (137-144 mM)	137	138	135[*,a]
	Potassium (3.5-4.4 mM)	4.7[*,a]	4.4	4.1
	ALAT (10-45 U/l)	24	24	20
	ASAT (15-35 U/l)	23	24	20
	ALP (35-105 U/l)	59	59	64
	Comments [*]Outside normal ranges. [a]Not considered clinically significant.			
Pt	**Parameter (normal range)**	**Pre-injection**	**4 hours p.i.**	**Follow-up (15 days p.i.)**
5	Hemoglobin (8.3-10.5 mM)[#]	7.3[*,a]	7.4[*,a]	8.0[*,a]
	Leucocytes (3.5-8.8 10^7/l)[#]	8.9[*,a]	7.6	9.8[*,a]
	Platelets (145-390 10^7/l)	327	332	370
	CRP (<10 mg/l)	13[*,a]	15[*,a]	36[*,a]
	eGFR (> 60 ml/min/1.73 m^2)	67	69	79
	Creatinine (60-105 μM)[#]	101	99	88
	Sodium (137-144 mM)	140	140	139
	Potassium (3.5-4.4 mM)	4.2	4.4	4.3
	ALAT (10-70 U/l)[#]	25	25	20
	ASAT (15-45 U/l)[#]	28	28	NA
	ALP (35-105 U/l)	123[*,a]	126[*,a]	150[*,a]
	Comments [*]Outside normal ranges. [a]Not considered clinically significant. [#]Different normal ranges due to male gender. NA: Not available.			
Pt	**Parameter (normal range)**	**Pre-injection**	**4 hours p.i.**	**Follow-up (6 days p.i.)**
6	Hemoglobin (7.3-9.5 mM)	6.7[*,a]	7.3	7.4
	Leucocytes (3.5-8.8 10^7/l)	1.9[*,a]	2.0[*,a]	4.3
	Platelets (145-390 10^7/l)	220	222	267
	CRP (<10 mg/l)	1	2	3

	eGFR (> 60 ml/min/1.73 m^2)	74	87	>90
	Creatinine (50-90 μM)	77	67	54
	Sodium (137-144 mM)	135[*,a]	140	138
	Potassium (3.5-4.4 mM)	4.6[*,a]	4.2	4.4
	ALAT (10-45 U/l)	16	18	26
	ASAT (15-35 U/l)	25	26	32
	ALP (35-105 U/l)	54	59	60
	Comments [*]Outside normal ranges. [a]Not considered clinically significant.			
Pt	**Parameter (normal range)**	**Pre-injection**	**4 hours p.i.**	**Follow-up (11 days p.i.)**
7	Hemoglobin (7.3-9.5 mM)	8.5	8.2	8.1
	Leucocytes (3.5-8.8 10^7/l)	7.6	6.3	6.5
	Platelets (145-390 10^7/l)	354	303	311
	CRP (<10 mg/l)	2	2	3
	eGFR (> 60 ml/min/1.73 m^2)	70	87	71
	Creatinine (50-90 μM)	82	74	81
	Sodium (137-144 mM)	136[*,a]	134[*,a]	131[*,a]
	Potassium (3.5-4.4 mM)	4.0	3.7	3.9
	ALAT (10-45 U/l)	22	18	22
	ASAT (15-35 U/l)	32	29	33
	ALP (35-105 U/l)	51	49	47
	Comments [*]Outside normal ranges. [a]Not considered clinically significant.			
Pt	**Parameter (normal range)**	**Pre-injection**	**4 hours p.i.**	**Follow-up (4 days p.i.)**
8	Hemoglobin (7.3-9.5 mM)	8.1	8.2	8.2
	Leucocytes (3.5-8.8 10^7/l)	4.8	5.7	5.7
	Platelets (145-390 10^7/l)	190	189	200
	CRP (<10 mg/l)	1	1	1
	eGFR (> 60 ml/min/1.73 m^2)	87	87	82
	Creatinine (50-90 μM)	67	67	70
	Sodium (137-144 mM)	137	140	142
	Potassium (3.5-4.4 mM)	3.5	3.7	4.1
	ALAT (10-45 U/l)	26	26	23
	ASAT (15-35 U/l)	25	21	23
	ALP (35-105 U/l)	71	62	68
Pt	**Parameter (normal range)**	**Pre-injection**	**4 hours p.i.**	**Follow-up (13 days p.i.)**
9	Hemoglobin (7.3-9.5 mM)	6.0[*,a]	6.2[*,a]	7.3
	Leucocytes (3.5-8.8 10^7/l)	4.5	5.5	5.1
	Platelets (145-390 10^7/l)	174	195	259
	CRP (<10 mg/l)	1	1	NA

	eGFR (> 60 ml/min/1.73 m^2)	72	75	77
	Creatinine (50-90 μM)	86	83	81
	Sodium (137-144 mM)	141	143	136[*,a]
	Potassium (3.5-4.4 mM)	3.8	3.4[*,a]	4.1
	ALAT (10-45 U/l)	13	15	NA
	ASAT (15-35 U/l)	19	18	NA
	ALP (35-105 U/l)	39	42	NA
	Comments [*]Outside normal ranges. [a]Not considered clinically significant. NA: Not available.			
Pt	**Parameter (normal range)**	**Pre-injection**	**4 hours p.i.**	**Follow-up (4 days p.i.)**
10	Hemoglobin (7.3-9.5 mM)	9.0	9.0	9.3
	Leucocytes (3.5-8.8 10^7/l)	7.6	7.3	6.3
	Platelets (145-390 10^7/l)	246	251	239
	CRP (<10 mg/l)	1	1	1
	eGFR (> 60 ml/min/1.73 m^2)	57[*,a]	49[*,a]	58[*,a]
	Creatinine (50-90 μM)	87	98[*,a]	86
	Sodium (137-144 mM)	140	144	141
	Potassium (3.5-4.4 mM)	4.3	3.9	4.2
	ALAT (10-45 U/l)	22	21	24
	ASAT (15-35 U/l)	24	21	23
	ALP (35-105 U/l)	49	49	48
	Comments [*]Outside normal ranges. [a]Not considered clinically significant.			

Safety blood samples measuring hematologic parameters (hemoglobin, leucocytes, platelets), liver parameters (ALAT: alanine amino transferase, ASAT: aspartate transaminase, ALP: alkaline phosphatase), and renal function (creatinine, GFR: glomerular filtration rate, sodium, potassium), and c-reactive protein (CRP) prior to injection, 1 hour after injection and 3-21 days post injection (p.i.)