

GMP-conform production of [^{68}Ga]Ga-NeoB for positron emission tomography imaging of patients with gastrointestinal stromal tumor

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Abstract

Background: [^{68}Ga]Ga-NeoB is a novel DOTA-coupled Gastrin Releasing Peptide Receptor (GRPR) antagonist with high affinity for GRPR and good in vivo stability. This study aimed at (1) the translation of preclinical results to the clinics and establish the preparation of [^{68}Ga]Ga-NeoB using a GMP conform kit approach and a licensed $^{68}\text{Ge}/^{68}\text{Ga}$ generator and (2) to explore the application of [^{68}Ga]Ga-NeoB in patients with gastrointestinal stromal tumors (GIST) before and/or after interventional treatment (selective internal radiotherapy, irreversible electroporation, microwave ablation).

Results: Validation of the production and quality control of [^{68}Ga]Ga-NeoB for patient use had to be performed before starting the GMP production. Six independent batches of [^{68}Ga]Ga-NeoB were produced, all met the quality and sterility criteria and yielded 712 ± 73 MBq of the radiotracer in a radiochemical yield of $>50\%$, a radiochemical purity of $>95\%$ and a molar activity of 14.2 ± 1.5 GBq/ μmol within 20 min synthesis time and additional 20 min quality control. Three patients (2 females, 1 male, 51–77 yrs of age) with progressive gastrointestinal stromal tumor metastases in the liver or peritoneum not responsive to standard tyrosine kinase inhibitor therapy underwent both [^{68}Ga]Ga-NeoB scans prior and after interventional therapy. Radiosynthesis of ^{68}Ga -NeoB was performed using a kit approach under GMP conditions. No specific patient preparation such as fasting or hydration was required for [^{68}Ga]Ga-NeoB PET/CT imaging. Contrast-enhanced PET/CT studies were performed. A delayed, second abdominal image was acquired at 120 minutes p.i. of administration of the first dose of [^{68}Ga]Ga-NeoB.

Conclusions: A fully GMP compliant kit preparation of [^{68}Ga]Ga-NeoB enabling the routine production of the tracer under GMP conditions was established for clinical routine PET/CT imaging of patients with metastatic GIST and proved to adequately visualize tumor deposits in the abdomen expressing GRPR. Patients could benefit from additional information derived by [^{68}Ga]Ga-NeoB diagnosis to assess the presence of GRPR in the tumor tissue and monitor antitumor treatment.

Introduction

Gastrointestinal stromal tumors (GIST) are rare soft tissue mesenchymal tumors occurring in the gastrointestinal tract and are thought to be derived from the cells of Cajal, which drive peristalsis in the intestine [1, 2]. In GIST with high risk of developing metastases, early detection of metastases detection of GIST with high sensitivity and by non-invasive methods would be an important improvement to allow for immediate treatment and to monitor or predict of the efficacy of therapy which currently is mainly influenced by assessing the type of mutations in *KIT* or *PDGFRA* gene [3].

In this regard, morphological and functional imaging methods may be important for detection, staging and to follow-up of GIST patients undergoing therapy. Computed tomography (CT) is the most frequently used morphological imaging procedure, although it lacks sensitivity and/or specificity. Functional imaging with positron emission tomography (PET) using 2-deoxy-2- ^{18}F fluoro-d-glucose combined with

computed tomography ($[^{18}\text{F}]\text{FDG}$ PET/CT) is the most commonly used nuclear medicine functional imaging modality in clinic routine and has shown to be advantageous over morphological imaging procedures alone when assessing therapy response. However, earlier studies using $[^{18}\text{F}]\text{FDG}$ PET/CT for GIST detection reported only the low/moderate sensitivity [4]. Hence, more accurate, specific and sensitive non-invasive diagnostic tools visualizing GIST are needed.

The gastrin-releasing peptide receptor (GRPR), also called bombesin receptor 2 (BB2) [5], is a target for noninvasive PET imaging of various types of cancer [6, 7]. Since overexpression of the GRPR has been reported in various cancer types, e.g. prostate cancer [8], breast cancer [9], gastrointestinal stromal tumors (GISTs) [10] and other tumors [11]. Targeting the GRP receptor with radioligands has a significant impact on the specific and sensitive detection and treatment of GRPR-expressing tumors [12]. Bombesin (BBN) is a peptide with high affinity to the GRPR [13, 14]. Therefore, various radiolabeled bombesin-based peptide ligands have been extensively used to target GRPR-expressing tumors such as GISTs [10, 15, 16]. Derived from BBN, the truncated BBN_{7-14} sequence was developed, showing nearly the same affinity to GRPRs but with higher stability than bombesin. Hence, BBN_{7-14} has been used for the development of various radiopharmaceuticals with positron emitters such as ^{18}F [17], ^{64}Cu [18] and ^{68}Ga [19] for PET, with gamma emitters such as $^{99\text{m}}\text{Tc}$ [20] for single photon emission computed tomography (SPECT), and with radionuclides such as ^{90}Y [21], ^{111}In [22] and ^{177}Lu [23] for endoradiotherapy. Additionally, optimization of the stability and affinity of the bombesin analogs has been explored by changing l- to d-amino acids [24], utilizing triazole backbones in the peptide [25] and applying multimerization [26, 27]

Recently, $[^{68}\text{Ga}]\text{Ga-NeoB}$ (formerly known as NeoBOMB1) was developed, a novel 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetra acetate (DOTA)-coupled bombesin-based GRPR antagonist, which showed a high affinity for GRPR ($\text{IC}_{50} = 1.17 \pm 0.06 \text{ nM}$ [28]) and high tumor uptake in preclinical studies in a xenograft mouse model ($30.7 \pm 3.9 \text{ \%ID/g}$ 4h post injection (p.i.) in PC3 tumor-bearing mice) accompanied by an good *in vivo* stability (5 min p.i. >95% intact, 30 min p.i. >90% intact) [28]. $[^{68}\text{Ga}]\text{Ga-NeoB}$ might have significant impact on the detection and treatment of GRPR expressing tumors such as like GIST [29]. Labelled with therapeutic radionuclides the peptide NeoB could also be useful for the treatment of imatinib-resistant GIST [7].

The purpose of our study was to explore the applicability of $[^{68}\text{Ga}]\text{Ga-NeoB}$ for the determination of the status of GIST in patients with different GRPR expression levels confirmed by previous biopsies of those lesions. Furthermore, the GMP compliant production of $[^{68}\text{Ga}]\text{Ga-NeoB}$ was established in our good manufacturing practice (GMP) environment including risk management, installation qualification (IQ), operation qualification (OQ) and validation of the process in six independent productions of $[^{68}\text{Ga}]\text{Ga-NeoB}$ following most recent guidelines [30].

In addition, the applicability of $[^{68}\text{Ga}]\text{Ga-NeoB}$ for visualization of GIST metastases before and/or after selective and patient-oriented specific interventional therapy (selective internal radiotherapy (SIRT), irreversible electroporation (IRE), microwave ablation (MWA)) was assessed.

Methods

Radiochemistry

The $^{68}\text{Ge}/^{68}\text{Ga}$ -generator used holds a license for patient application according to the European pharmacopeia and was purchased from Eckert&Ziegler (1.85 GBq, GalliaPharm, Eckert & Ziegler, Berlin, Germany). The NeoB radiolabeling kit was received from Advanced Accelerator Applications S.A. (AAA). The kit consisted of a vial containing the lyophilized NeoB precursor and additives and a second vial containing the reaction buffer. For automated generator elution, we used an automated synthesis module (Scintomics GRP, Fürstfeldbruck, Germany) together with a cartridge kit for ^{68}Ga -radiolabeling using this module (SC-103, ABX, Radeberg, Germany). Analytical (radio-)HPLC was performed on a Shimadzu HPLC system (Nakagyo-ku, Kyōto, Japan), equipped with a reverse phase column (Merck LiChrospher 100 RP-18; 125x3 mm), a UV-diode array detector (254 nm) and a scintillation radiodetector (Pomo, Elysia-Raytest, Straubenhardt, Germany). The solvent system used was a gradient of acetonitrile:water (containing 0.1% TFA) (0–20 min: 10–90% acetonitrile). [^{18}F]FDG was commercially obtained (Life Radiopharma f-con GmbH, Germany).

^{68}Ga -Radiolabeling

The whole radiotracer production was performed inside a hot cell isolator (cleanroom class A, ITD, Dresden-Rossendorf, Germany) under GMP conditions 4 mL 0.1 M suprapur HCl were automatically eluted through the $^{68}\text{Ge}/^{68}\text{Ga}$ -generator and through a sterile filter (Millex-GS 0.22 μm , SLGSV255F, Millipore) directly into the reaction vial containing the NeoB precursor ($50 \pm 5 \mu\text{g}$, $31.7 \pm 0.6 \text{ nmol}$), resulting in a total reaction mixture volume of 3 mL containing $1100 \pm 100 \text{ MBq } ^{68}\text{Ga}$. Subsequently, the kit labeling buffer ($0.57 \pm 0.3 \text{ mL } 1 \text{ M formic acid, pH } 5$) and the antioxidant (gentisic acid) was added. Silicon-coated cannulas ($0.6 \times 60 \text{ mm}$, Sterican, B. Braun) were used throughout the whole synthesis process. Radiolabeling was performed at pH 3.6–4.0 for 7–10 minutes at 89°C inside the reaction vial in a heating block (95°C). The resulting solution contained $712 \pm 73 \text{ MBq } [^{68}\text{Ga}]\text{Ga-NeoB}$ in a radiochemical purity (RCP) of 96–99%, as confirmed by radio-thin-layer chromatography (radio-TLC) and radio-HPLC ($t_{\text{R}} = 9.7 \text{ min}$).

Quality control under GMP conditions for the patient use

The quality control (QC) of the injectable radiotracer solution was performed on an Elysia-Raytest QC-Cubicle compact unit within a cleanroom class D environment. The in-house production of radiopharmaceuticals is regulated in the German Pharmaceuticals Act and the European Pharmacopeia. There are monographs for example for [^{68}Ga]Ga-Octreotide [31] on which the quality control (QC) was based on. The following criteria were tested: the pH value has to be between 3.0–4.0. A bubble-point test of the sterile filter was performed. The radiochemical purity as determined by radio-TLC and radio-HPLC have to exceed $> 97\%$ and $> 95\%$, respectively. The half-life of the product has to be $1.133 \pm 0.1 \text{ h}$ and was determined using an activimeter (Elysia Raytest). The nuclide purity has to exceed 99.999% and was

determined by gamma spectroscopy at an energy of the γ -line to be 511 ± 70 keV. The product was also tested for endotoxins using an Endosafe unit (Charles River, Wilmington, MA, USA), and the endotoxin level had to be below 35 EU/mL in a maximum application volume of 5 mL. Finally, the product was externally tested for sterility.

Validation of production under GMP conditions

The validation of the production process was performed on six independent $[^{68}\text{Ga}]\text{Ga-NeoB}$ syntheses which met the above mentioned criteria.

The generator was eluted automatically using the mentioned GRP module equipped with an 20 mL syringe by pushing 3 mL of 0.1 M HCl through the generator (2 mL/min) and a sterile filter into the reaction vial containing the precursor for $[^{68}\text{Ga}]\text{Ga-NeoB}$ [32].

$^{68}\text{Ga-NeoB}$ PET/CT Imaging Protocol

A lowdose-CT (Siemens Biograph mCT, Biograph 40 VA44A, 32 + 8-line-CT, PETSyngo software VG51C) and then early whole-body PET/CT images were acquired from vertex to mid thighs with 8 bed positions and 3-min emission scans per bed position at 60 min after intravenous administration of the $[^{68}\text{Ga}]\text{Ga-NeoB}$ of 1.5–2 MBq/kg (135–229 MBq) into the antecubital vein. Contrast-enhanced PET/CT studies were performed on a 40-slice PET/CT scanner with 80 ml arterial contrast (Imeron). A delayed, second abdominal PET image was acquired at 120 minutes p.i. of the $[^{68}\text{Ga}]\text{Ga-NeoB}$. Two experienced nuclear medicine physicians manually drew regions of interest on the liver lesions for each image using 3-dimensional ellipsoid isocontouring with the assistance of the corresponding CT images. The results were expressed as SUV_{mean} and SUV_{max} .

Results

Validation runs for $[^{68}\text{Ga}]\text{Ga-NeoB}$

The results of the validation runs are summarized in Table 1. Runs with a different buffer amount were performed to evaluate and validate the possible effect of inaccuracies during the addition by different operators. The elution of the generator was performed with 5.5 mL 0.1 M HCl (EZAG), resulting in 5.0 mL ^{68}Ga -solution in the reaction vial and not less than 0.5 mL buffer solution (maximum 0.55 mL). In comparison to productions A (0.50 mL buffer solution) the insignificant higher amount (0.55 mL buffer solution) in productions B resulted in the same RCP using the TLC-method, but the RCP determined by HPLC demonstrated a slightly higher RCP. The production-SOP for $[^{68}\text{Ga}]\text{Ga-NeoB}$ has to instruct to use at minimum an amount of buffer of 0.50 mL. In conclusion, all six consecutive runs were inside of all the specifications yielding 712 ± 73 MBq of $[^{68}\text{Ga}]\text{Ga-NeoB}$ in a radiochemical yield of > 95%. The room conditions were proper and the devices are qualified for the production; thus the production process as carried out was demonstrated to be valid for patient production of $[^{68}\text{Ga}]\text{Ga-NeoB}$.

Table 1
Results of the validation runs of [⁶⁸Ga]Ga-NeoB.

Run #	batch #		Reaction			Quality control				
	Buffer	Charge	buffer [ml]	HCl* [ml]	temp. time	yield** [MBq]	pH	radionuclide purity	⁶⁸ Ga-content	RCP: HPLC / TLC
A-1	CT00516004		0.50	5.0	95.1°C	765.2	3.5	1.13 h	96.1%	yes
	F03517002				10 min			> 99.999%	99.0%	
A-2	CT00516004		0.50	5.0	94.9°C	716.0	3.5	1.12 h	96.4%	yes
	F03517002				9 min			> 99.999%	98.7%	
A-3	CT00516004		0.50	5.0	95.1°C	568.0	3.4	1.12 h	97.0%	yes
	F03517002				8 min	***		> 99.999%	99.9%	
B-1	CT00516004		0.55	5.0	95.0°C	745.6	3.8	1.13 h	98.5%	yes
	F03517002				8 min			> 99.999%	99.2%	
B-2	CT00516004		0.55	5.0	95.0°C	756.2	3.8	1.12 h	97.0%	yes
	F03517002				10 min			> 99.999%	98.5%	
B-3	CT00516004		0.55	5.0	95.2°C	722.0	3.7	1.13 h	98.7%	yes
	F03517002				7 min			> 99.999%	99.4%	

*: Elution volume minus 0.5 mL dead volume of the tubing

** : measured 2–5 minutes after cool down

***: second generator elution of the day (first elution 2.5 h before)

[⁶⁸Ga]Ga-NeoB: Clinical PET/CT investigations

For an implementation in clinical routine, three patients (2♂, 1♀, 51–77 a) with biopsy proven, metastatic GIST were examined with the GMP-produced [⁶⁸Ga]Ga-NeoB via PET/CT for staging purposes after they had been treated by antiproliferative drug therapy (imatinib, sunitinib, regorafenib) followed by SIRT, IRE or MWA. Image acquisition, attenuation correction, fusion, reconstruction and post-processing was performed on a dedicated workstation. SUV_{max} was determined both on the initial whole-body and on the later focused imaging.

Patient #1 (male, 57 years old, small bowel GIST with peritoneal and progressive liver metastases after 3rd line therapy carrying an exon 11 and a 2ndary exon 17 mutation) received [^{18}F]FDG PET/CT for staging and two of the progressive liver metastases in segment VII and segment II/III were depicted. The patient underwent subsequent SIRT therapy. Four months after therapy the patient received [^{68}Ga]Ga-NeoB (229 MBq) for follow-up staging (Fig. 2) with low accumulation in the lesion in liver segment VII (SUV_{max} early of 1.4, SUV_{max} late of 3.3), but with persistence of the radiotracer in liver segment II/III (SUV_{max} early of 6.3, SUV_{max} late of 16.1), indicating still vital tumor tissue. Thus, the patient again underwent IRE on the left lobe (segment II/III). In comparison to the preliminary examination with [^{18}F]FDG, two newly occurring demarcated peritoneal metastases in the right hemiabdomen with increased nuclide uptake (SUV_{max} early of 3.3 and 5.7, SUV_{max} late 6.4 and 17.9, respectively) were detected with [^{68}Ga]Ga-NeoB. In addition, physiological distribution of [^{68}Ga]Ga-NeoB was found in the study area. At a three-month [^{18}F]FDG PET/CT follow-up the patient showed further progressive disease.

Patient #2 (female, 77 years old, small bowel GIST with progressive peritoneal and soft tissue metastases under third line therapy carrying an exon 11 and two different secondary mutations in exon 13) received [^{68}Ga]Ga-NeoB (202 MBq) PET/CT for staging. A previously unknown isolated hypodense liver lesion in segment VII (SUV_{max} early of 11.2, SUV_{max} late of 16.6) was found (Fig. 3). Additionally, an abdominal wall metastasis in the left lower abdomen was found, which was not previously known from a three-month preliminary [^{18}F]FDG PET/CT and was later proven histologically by surgical resection. In addition, physiological distribution of [^{68}Ga]Ga-NeoB was visible in the study area. The single metastasis in liver segment VII was treated by IRE and no lesions were found by magnetic resonance imaging (MRI) follow-up 11 months later. Therefore, the patient was considered to have experienced a complete response.

Patient #3 (female, 51 years old, multiple progressive hepatic metastases of GIST of the stomach carrying a D842V mutation in PDGFR α and being pretreated as treated by SIRT and microwave ablation at previously known hepatic metastases. Three months later the patient received [^{68}Ga]Ga-NeoB (135 MBq) PET/CT for staging (Fig. 4). An inhomogeneous, flat tracer accumulation within the uterine cavity with decrease of uptake in the temporal course (SUV_{max} 60 min p.i. of 26.5, SUV_{max} 120 min p.i. of 7.1, SUV_{max} 180 min p.i. of 6) was found, most likely a physiological enrichment. In addition, physiological radiopharmaceutical distribution in the study area was observed. However, there was no pathologically increased uptake of [^{68}Ga]Ga-NeoB in the known hepatic metastatic lesions which had an unchanged morphology in comparison to the three-month previously performed [^{18}F]FDG PET/CT. No tumor uptake and no new metastases were found in the region of interest also at later time-points indicating a stable disease. In the 120 min p.i. images, an increased nuclide uptake was found in the region of the gall bladder neck (Fig. 4b). However, after fatty eating, there was no correlation in 180 min p.i. images (Fig. 4c). An additional MRI follow-up examination six months after therapy was performed, which confirmed a stable disease.

Discussion

[⁶⁸Ga]Ga-NeoB was produced routinely under GMP compliant conditions and was introduced in the clinical routine in three patients with different diagnostic scenarios. In the [⁶⁸Ga]Ga-NeoB studies two new lesions in patient 1 and one more lesion in patient 2 were detected, which were not known previously. These results suggest that [⁶⁸Ga]Ga-NeoB imaging may be an additional useful tool for detection of new lesions or metastases compared to conventional [¹⁸F]FDG PET/CT or MRI with unclear outcome. The uptake of the radiotracer in the newly detected metastases is mainly related to the overexpression of GRPR, which was histologically documented in our cases. However, patient #3 was stable between therapy and [⁶⁸Ga]Ga-NeoB PET/CT follow-up and the patient had a complete response.

Additionally, patient #1 was assessed with a progressive disease because of increased high uptake in segment II/III and newly detected lesions, whereas the lesion in segment VII was confirmed stable showing no [⁶⁸Ga]Ga-NeoB uptake after SIRT therapy. The reason for the higher uptake in segment II/III could be a possible increased GRPR expression in growing GIST lesions or in metabolic active phases [29]. Although patient #1 underwent SIRT and IRE, he showed a progressive disease in the follow-up examination after four months. It could be suggested, that endoradiotherapy with [¹⁷⁷Lu]Lu/[²²⁵Ac]Ac-NeoB would be an additional, more specific therapeutic option to SIRT, IRE or MWA in patients with high GRPR-expressing lesions in multiple organs. However, it should be taken into account that if tumorous lesions known from morphologic imaging do not show a [⁶⁸Ga]Ga-NeoB uptake, this might also be related to low GRPR expression [29].

It is well known that GIST have a wide spectrum of mutations, some of it with very low incidence [33]. Although 70–75% of GIST harbour imatinib-sensitive mutations of KIT [34], secondary resistances are often acquired within 2 years [22, 29, 35, 36]. The mechanisms of primary and secondary therapy resistance in GIST are not completely understood. It could be speculated that patients showing no uptake in [⁶⁸Ga]Ga-NeoB PET/CT in lesions known from conventional [¹⁸F]FDG PET/CT or MRI imaging might have progressive disease and therapy resistance due to mutations affecting GRPRs. [⁶⁸Ga]Ga-NeoB imaging could be a useful additional modality regarding monitoring of the effectiveness of a therapy of GIST patients and assisting to choose the type of therapy.

Conclusion

[⁶⁸Ga]Ga-NeoB was successfully integrated in the clinical diagnostic procedures by routine production under GMP conditions. The application of the tracer could be introduced to the care of patients with GIST metastases in the liver and abdominal cavity. With the combination of PET/CT it was possible to evaluate therapy response in GIST patients with liver metastases. Patients who underwent [¹⁸F]FDG PET/CT with an inconclusive result on therapeutic response could benefit of an additional diagnostic approach with [⁶⁸Ga]Ga-NeoB for characterization of GRPR-expressing tumors. In future, ¹⁷⁷Lu/²²⁵Ac-

labeled NeoB may also be used for endoradiotherapy of high GRPR-expressing tumors. However, further clinical diagnostic studies are warranted prior to a therapeutic approach.

Declarations

ETHICS APPROVAL

The application of [⁶⁸Ga]Ga-NeoB in patients with GIST was approved by the ethical vote 2014-912W-MA from 13.01.2015.

CONSENT FOR PUBLICATION

All data is consent for publication.

AVAILABILITY OF DATA AND MATERIAL

The data used and analysed during the current study are available from the corresponding author on reasonable request.

COMPETING INTERESTS

The authors declare that they have no competing interests

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AUTHORS' CONTRIBUTIONS

MP, LR and BW were responsible for the radiopharmaceutical translation to clinical routine production und GMP conditions. MP, CW and PH analyzed and interpreted the patient data. MP, CW and PH were major contributor in writing the manuscript. SD, SOS and BW were responsible for funding of the project. SD, SOS and PH were responsible for selection of patients and study design. All authors read and approved the final manuscript. Study concept and design:

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Figures

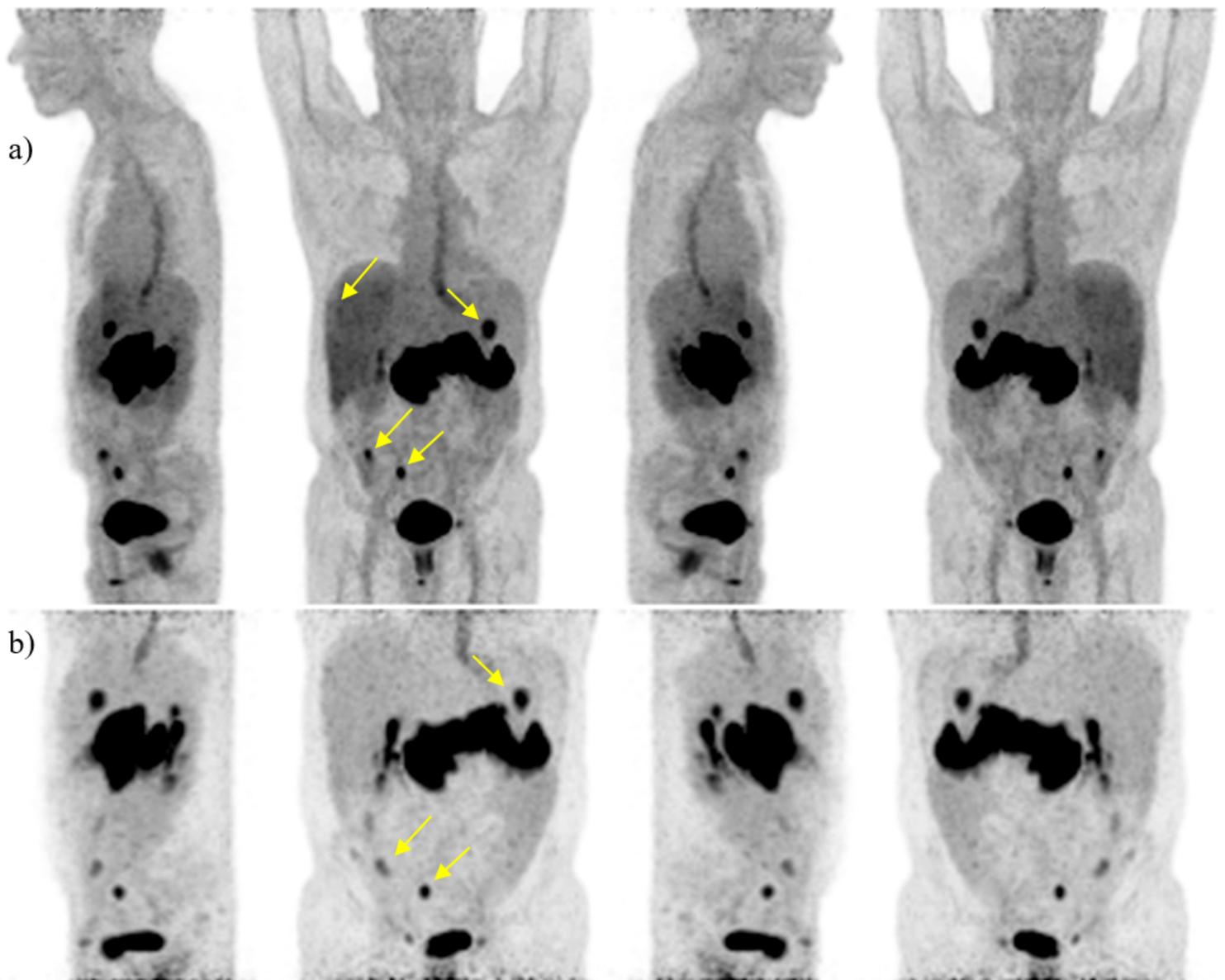


Figure 1

a) Maximum-intensity-projections (MIP) of (^{67}Ga , 57) 1 h p.i. b) 2 h p.i. left, front, right, behind.

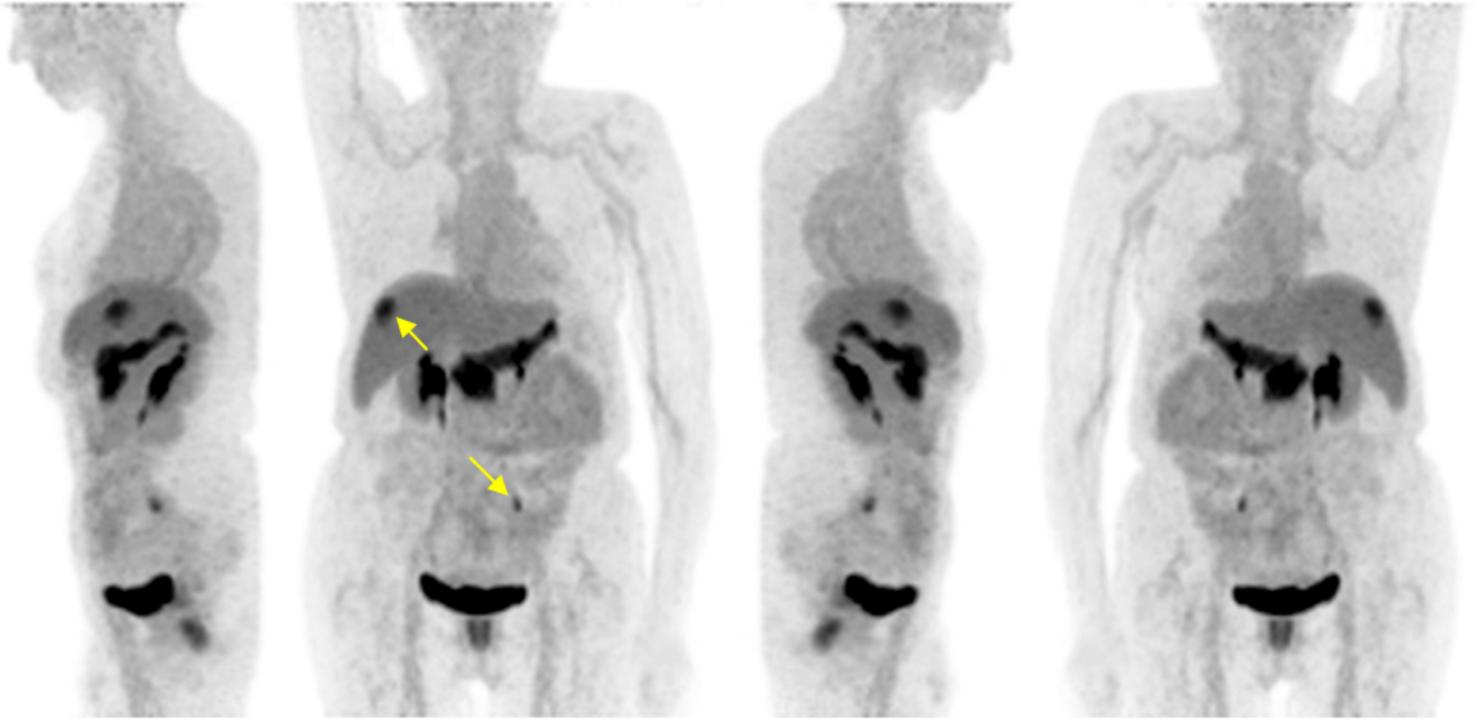


Figure 2

MIP of (a, 77) 1 h p.i. b) 2 h p.i. left, front, right, behind.

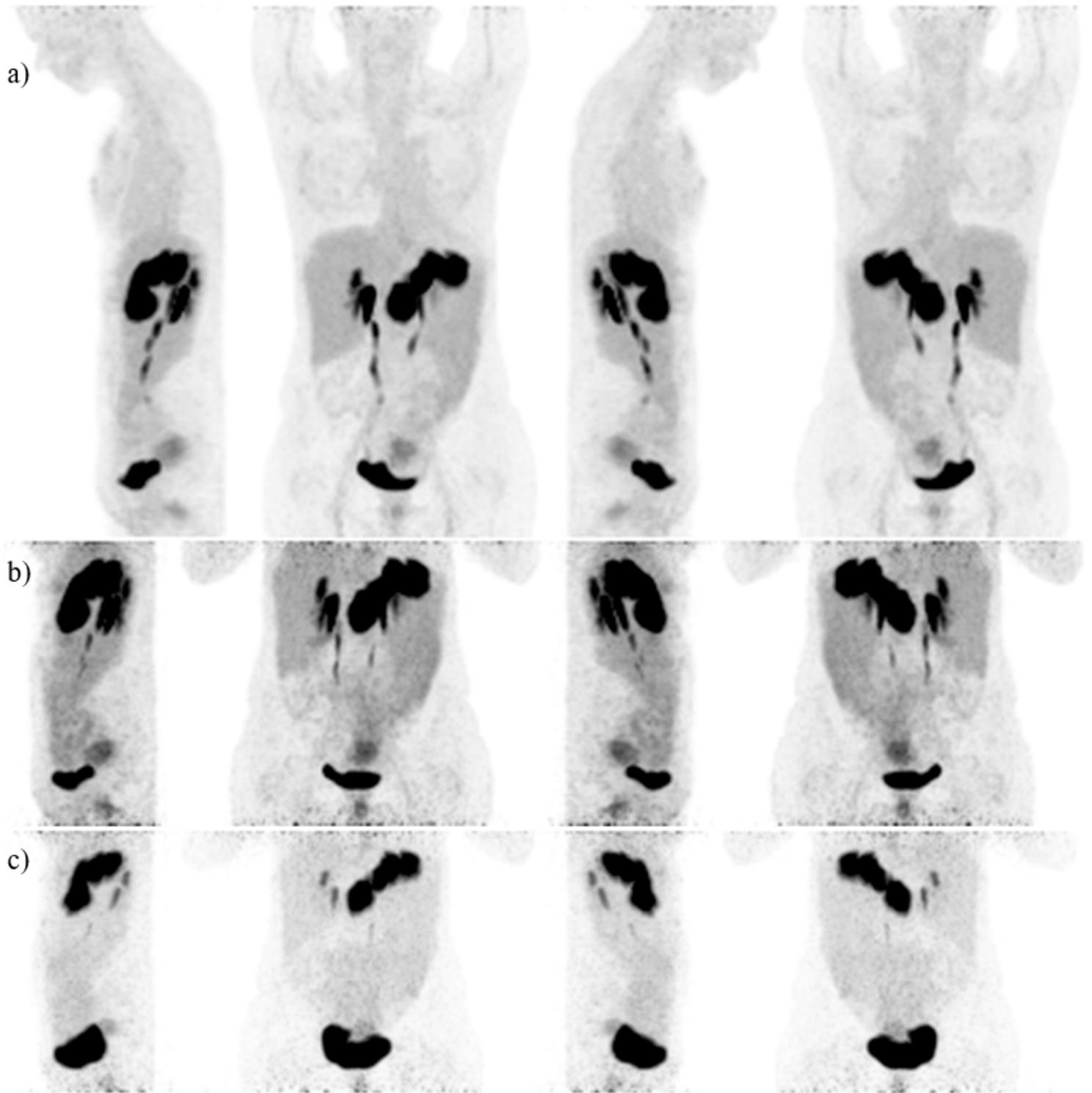


Figure 3

a) MIP of (\square , 57) 1 h p.i. b) 2 h p.i., and c) 3 h p.i. left, front, right, behind.