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MITIGATE-NeoBOMB1, a Phase I/IIa Study to Evaluate Safety, Pharmacokinetics and Preliminary Imaging of ^{68}Ga-NeoBOMB1, a Gastrin-releasing Peptide Receptor Antagonist, in GIST Patients

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ABSTRACT

Introduction: Gastrin Releasing peptide receptors (GRPRs) are potential molecular imaging targets in a variety of tumors. Recently, a ^{68}Ga-labelled antagonist to GRPRs, NeoBOMB1, was developed for PET. We report on the outcome of a Phase I/IIa clinical trial (EudraCT 2016-002053-38) within the EU-FP7 project Closed-loop Molecular Environment for Minimally Invasive Treatment of Patients with Metastatic Gastrointestinal Stromal Tumours ('MITIGATE') (grant agreement number 602306) in patients with oligometastatic gastrointestinal stromal tumors (GIST).

Materials and methods: The main objectives were evaluation of safety, biodistribution, dosimetry and preliminary tumor targeting of ^{68}Ga-NeoBOMB1 in patients with advanced TKI-treated GIST using PET/CT. Six patients with histologically confirmed GIST and unresectable primary or metastases undergoing an extended protocol for detailed pharmacokinetic analysis were included. ^{68}Ga-NeoBOMB1 was prepared using a kit procedure with a licensed ^{68}Ge/^{68}Ga generator. 3 MBq/kg body-weight were injected intravenously and safety parameters were assessed. PET/CT included dynamic imaging at 5 min, 11 min and 19 min as well as static imaging at 1, 2 and 3-4 h p.i. for dosimetry calculations. Venous blood samples and urine were collected for pharmacokinetics. Tumor targeting was assessed on a per-lesion and per-patient basis.

Results: ^{68}Ga-NeoBOMB1 (50 µg) was prepared with high radiochemical purity (yield >97%). Patients received 174 ± 28 MBq of the radiotracer, which was well tolerated in all patients over a follow-up period of 4 weeks. Dosimetry calculations revealed a mean adsorbed effective dose of 0.029 ± 0.06 mSv/MBq with highest organ dose to the pancreas (0.274 ± 0.099 mSv/MBq). Mean plasma half-life was 27.3 min with primarily renal clearance (mean 25.7 ± 5.4% of injected dose 4h p.i.). Plasma metabolite analyses revealed high stability, metabolites were only detected in the urine. In three patients a significant uptake with increasing maximum standard uptake values (SUVmax at 2h p.i.: 4.3 to 25.9) over time was found in tumor lesions.

Conclusion: This Phase I/IIa study provides safety data for ^{68}Ga-NeoBOMB1, a promising radiopharmaceutical for targeting GRPR-expressing tumors. Safety profiles and pharmacokinetics are suitable for PET imaging and absorbed dose estimates are comparable to other ^{68}Ga-labelled radiopharmaceuticals used in clinical routine.

INTRODUCTION

Radiolabeled peptides for diagnosis and therapy of various malignancies have been a cornerstone of radiopharmaceutical development over the past decades with ^{68}Gallium as the mainstay of modern PET-based tumor imaging. The clinical utility of the "thera(g)nostic" approach has peaked in the products recently obtaining marketing authorization for diagnosis (^{68}Ga-DOTATOC as SOMAKit TOC® in Europe and ^{68}Ga-DOTATATE as NETSPOT® in the US) and therapy (^{177}Lu-DOTATATE as Lutathera®), stimulating research on regulatory peptides binding to other receptors (*1*). One of main targets in this area are gastrin releasing peptide receptors (GRPRs), which are overexpressed in a variety of cancers including prostate (*2*–*6*) and breast cancer (*7*) as well as glioma (*8*,*9*).

A variety of radiolabeled peptides binding to GRPRs, derived from the natural ligand bombesin, have been developed (*10*). However, early bombesin analogues with agonistic properties led to a high rate of unwanted side effects (*11*), thus shifting focus towards antagonistic bombesin analogues, avoiding side effects and possibly leading to higher targeting efficiency and metabolic stability.

NeoBOMB1 is a novel DOTA-coupled GRPR-antagonist generated by modification of the C-terminal Leu13-Met14-NH2 of native bombesin. It can be labelled with ^{68}Gallium resulting in high GRPR affinity, metabolic stability and excellent tumor targeting in various animal models (*12*,*13*).

Gastrointestinal stromal tumors (GIST), a rare sarcoma subtype with an incidence of 4.3 to 22 per million (*14*,*15*), express high levels of GRPR (*16*). 80-85% of patients with GISTs present localized disease at first diagnosis, but metastases is a frequent phenomenon during the course of the disease in up to 85% of patients (*17*). Tyrosine-kinase inhibitors (TKI) such as imatinib, sunitinib or regorafenib have dramatically improved overall survival (*18*), yet up to 80% of patients develop resistance during therapy (*19*). A study within the EU-FP7 project Closed-loop Molecular Environment for Minimally Invasive Treatment of Patients with Metastatic Gastrointestinal Stromal Tumours ('MITIGATE', grant agreement number 602306) investigating several radiolabeled peptides on tumor cell lines revealed ^{68}Ga-NeoBOMB1 to be a very

promising candidate for targeting GIST (*16*). Recently, the development of a new kit formulation for the preparation of ^{68}Ga-NeoBOMB1 (Advanced Accelerator Applications; Colleretto Giacosa TO, Italy) has allowed radiolabeling without complex synthesis and purification procedures.

Here we report on safety, tolerability, pharmacokinetics and preliminary targeting properties of ^{68}Ga-NeoBOMB1. These data originated from the first part of a prospective clinical Phase I/IIa in patients with advanced GIST under current or previous TKI treatment ("A Phase I/IIa study to evaluate safety, biodistribution, dosimetry and preliminary diagnostic performance of ^{68}Ga-NeoBOMB1 in patients with advanced TKI-treated GIST using positron-emission tomography/computer tomography (PET/CT)", EudraCT No. 2016-002053-38).

MATERIALS AND METHODS

Study approval and Registration

Study approval was granted on the 25th of July 2016 by the local Ethical Review Board (Ethics Committee Medical University Innsbruck) and the Austrian Competent Authority (Bundesamt für Sicherheit im Gesundheitswesen) on the 28th of November 2016. All participants signed a written informed consent. Monitoring was provided by the local Clinical Trial Centre (KKS, MUI). The study was registered both within EudraCT (2016-002053-38) and ClinicalTrials.gov (NCT02931929) and was officially initiated in December 2016.

Subjects, Study Plan and Safety assessment

Overall, 6 patients with advanced GIST – defined as metastatic disease without a curative surgical option – were enlisted for full pharmacokinetic analysis, representing a subset of a total of nine patients enrolled in the study. At least 50% of patients were required to have a 1^{st}-, 2^{nd}- or 3^{rd}-line TKI resistance defined by disease progress under treatment. Disease progress was classified by imaging (usually by Choi criteria (*20*)). To minimize a bias of patients with a greater disease burden, two patients with a stable disease were also included. Details on disease status and treatment are provided in Table 1.

For a detailed overview of inclusion and exclusion criteria, please refer to the Supplemental Table 1. Oral and written informed consent was obtained from all participants.

The occurrence of adverse events (AE) and severe adverse events (SAE) were documented and graded in regard to severity and causal correlation to the administration of ^{68}Ga-NeoBOMB1 following the Common Terminology Criteria for Adverse Events v5.0 (National Institute of Health; Bethesda, Maryland, USA).

Participants were screened and enrolled at least 24 hours prior to the tracer administration (visit 0) after review of inclusion and exclusion criteria, medical history and physical status. A pregnancy test was performed in female participants via a urine dipstick test.

Prior to the tracer administration (visit 1), inclusion and exclusion criteria, medical history and physical status were reviewed again. Serial venous blood samples were taken at 0, 2, 5, 10, 30, 45 min and 1, 2 and 3-4h after tracer administration. Furthermore, one urine sample was taken prior and at least one urine sample after administration. Patients were hospitalized for observation until the next morning, re-examined (visit 2) and discharged if no adverse events were observed.

The follow-up examinations (visit 3) took place 5-8 days after tracer administration and included physical examination, history and blood analysis. A final telephone interview (visit 4) was conducted 12-20 days after the tracer administration. Patients were advised to readily report any abnormalities to the

investigators at any time for the study duration and beyond. For a flow-chart of the study plan, please refer to Supplemental Figure 1.

Preparation, Quality Control, Pharmacokinetics and Metabolite Analysis of ^{68}Ga-NeoBOMB1

A kit-based preparation was used for ^{68}Ga-NeoBOMB1, for technical details on preparation, quality control, pharmacokinetic and metabolite analysis please refer to Supplemental Information 1 ("Preparation and Quality Control of ^{68}Ga-NeoBOMB1") (*21*).

PET Imaging

Patients were intravenously injected 127 - 214 MBq ^{68}Ga-NeoBOMB1 (on average 174 ± 28 MBq) corresponding to the total content of the kit. A 5 x 60 sec dynamic PET/CT scan of the upper abdomen (one bed position with an axial field of view of 15.6 cm) was started immediately after tracer injection, followed by three sequential static PET/CT scans covering the whole abdomen and pelvis (three bed positions with two min per bed position) at five, 12 and 19 min p.i. and three late PET/CT scans from the skull vertex to the mid-thigh (7 bed positions, 2 min per bed position) at one, two and three hour p.i.. In total, five low-dose CT (LDCT) scans were performed for attenuation correction of the PET emission data (one for the dynamic PET/CT, one for the time points from 5 to 19 min, and one for each of later time points; low-dose CT scan parameters using BGE smart mA dose modulation: 100 kVp, 15–150 mA, noise index 60, 0.8 s tube rotation, slice thickness 3.75 mm and pitch 1.375).

All scans were performed using a Discovery PET/CT 690 VCT scanner (GE Healthcare, Milwaukee, WI, USA). PET images were reconstructed using an ordered-subset expectation maximization (OSEM) algorithm with 2 iterations and 24 subsets for static images and 32 subsets for dynamic images. The PET images were normalized to units of Bq/mL by applying corrections for sensitivity, attenuation, scatter, dead-time, random coincidences and for decay to image acquisition onset.

Positron emission data were reconstructed using an ordered-subset expectation maximization (OSEM) algorithm (2 iterations, 24 subsets). The images were corrected for attenuation using CT data collected over the same regions as for emission imaging.

CT Imaging

If no recent CT of the thorax and abdomen was available, a diagnostic whole-body CT was acquired after the third whole body PET procedure 3h p.i. after body-weight adjusted intravenous administration (1.5ml/kgBW) of Iopromid (Ultravist 370; Bayer AG, Leverkusen, Germany). BGE smart mA dose modulation was used (100–120 kVp, 80– 450 mA, noise index 24, 0.8 s tube rotation, slice thickness 3.75 mm, pitch 0.984).

Image Analysis

For image analysis, Hermes software (Version P5 gold 4.4-B; Hermes Medical Solutions AB, Stockholm, Sweden) was used. To determine the temporal enhancement, representative organ regions of interest (ROIs) were used to measure standardized uptake values (SUV), then converted into percentage of injected dose per volume [%ID/L]. To determine ideal imaging time windows, tumor-to-organ ratios for liver, kidney and pancreas were calculated in patients with discernible tumor uptake (presented as imaging indices). Lesion tracer uptake was visually assessed using Hermes software on a per-patient and per lesion-basis, qualitative results and percentage of positive lesions grouped by primary/local recurrence and metastases are presented in tabular format.

Dosimetry Calculations

Dosimetry calculations were performed using OLINDA/EXM covering organ and tumor ROIs to determine time-activity curves, details are described in Supplemental Information 1 (“Dosimetry Calculations”) (*22*–*25*).

Statistics and Visual Presentation

All data were collected and stored in Microsoft Excel (Microsoft Corporation; Redmond, USA). Statistical analysis was carried out in GraphPad Prism 8.1.1 (GraphPad Software Inc.; La Jolla, USA).

Continuous data are presented as dot-plots including mean and standard deviation (SD). Alternatively, data are presented as bars with whiskers denoting the SD.

PET-CT images are presented according to the AQARA principle (*26*).

RESULTS

Preparation of ^{68}Ga-NeoBOMB1

^{68}Ga-NeoBOMB1 was prepared with a radiochemical purity of >97%. Radiolabeling timing was performed so that all patients received the total kit content, corresponding to an amount of approximately 50 µg of NeoBOMB1 peptide. Injected activity was between 127 and 214 MBq, dependent on body weight.

Participants, Tolerability, and Adverse Effects

The average age was 68.7 ± 11.5 years and four out of six participants were female (66.7%). Details on demographics and disease state are summarized in Table 1.

Intravenous administration of ^{68}Ga-NeoBOMB1 was well tolerated in all participants. No SAE were observed, minor adverse events after the administration of ^{68}Ga-NeoBOMB1 were observed in one participant (16.7%), yet without a causal relation to the tracer administration (details on pre-existing conditions can be found in the Supplemental Table 2). All AE had resolved upon the last study visit. Details are summarized in Supplemental Table 3.

Blood, Urine and Metabolite Analysis

Results from analysis of ^{68}Ga-NeoBOMB1 in venous blood, plasma and urine samples of the 6 patients are summarized in Figure 1. ^{68}Ga-NeoBOMB1 was rapidly eliminated from blood with <10% of the total injected activity measured after 3h. Mean half-life in blood was 35min with plasma activity levels practically identical, indicating no cellular bound radioactivity. 10.6% and 17.2% of the injected activity was excreted in early and late urine sample, totally 25.7% after 3h (mean of n=6). HPLC analysis revealed mostly intact peptide in plasma samples with some hydrophilic metabolites detectable at 30min and 1h, whereas in urine only hydrophilic metabolites could be found in samples taken both at early and late time points. Retention times of metabolites in urine matched those of metabolites detected in blood, indicating high metabolic stability of ^{68}Ga-NeoBOMB1 and rapid renal excretion.

Pharmacokinetics and Tumor Accumulation

Liver uptake was immediate at 13.4 % ID/L on average with a continuous decline over time (Fig. 2a). Highest ^{68}Ga-NeoBOMB1 uptake was found in the pancreas with a time-dependent increase in activity especially in the head and corpus and highest dose values at the end of the examination up to 45.6 %ID/L, suggesting specific binding (Fig. 2b). Excretion was mainly renal with immediate significant renal uptake plateauing around 20% ID/L and concomitant activity accumulation along the ureters and bladder (Fig. 2f). Nonetheless, a continuous increase in gallbladder activity was found over time up to 12.2 %ID/L on average at 3h (Fig. 2a), hinting at a partial hepatobiliary clearance of ^{68}Ga-NeoBOMB1. Esophagus, colon and rectum all displayed visible accumulation with low uptake ranging from 3 to 7 %ID/L with a time-dependent increase in activity in all participants. Spleen and blood pool activity decreased rapidly correlating well with *in vitro* measurements (Fig. 2f, please also refer to Fig. 1) and simultaneous increase in bladder activity.

In 3 of 6 patients a strong tumor uptake was found with a significant uptake starting 1h p.i. increasing over time, leading to an improved tumor-to-tissue contrast ratio. Two participant showed mixed lesion uptake

with a subset of lesions showing virtually no uptake, one patient exhibited no lesion uptake at all (for further details, please refer to Table 1). Depending on the individual lesions, a ratio of up to 6.9 could be achieved at 2h p.i. (Supplemental Figure 2). Noise levels were highest at 3h p.i. due to decay of ^{68}Ga, indicating an optimal PET-imaging time window between 1.5 and 2.5 h p.i.. SUV_{max} reached 32.1 in one case. For an exemplary illustration of tumor uptake over time, refer to Fig. 3 (another case is presented in Supplemental Figure 3).

Dosimetry Calculations

The dose estimates (i.e. doses) after administration of ^{68}Ga-NeoBOMB1 as well as the mean ^{68}Ga-NeoBOMB1 doses and the standard deviations of the ^{68}Ga-NeoBOMB1 doses are presented in Supplemental Table 4. For these dose calculations, a bladder model with voiding every 2 h was considered. In this same table, doses for a well-established diagnostic radiopharmaceutical ^{68}Ga-DOTATATE are also presented for comparison (no bladder voiding considered) (*25*).

As shown in Supplemental Table 3, the pancreas is by a large margin the organ receiving the highest dose after administration of ^{68}Ga-NeoBOMB1 (mean 0.274 ± 0.099 mSv/MBq). Effective doses for ^{68}Ga-NeoBOMB1 ranged from 0.022 mSv/MBq to 0.040 mSv/MBq (mean 0.029 ± 0.06 mSv/MBq), which is in line with the reported effective dose for ^{68}Ga-DOTATATE (0.026 mSv/MBq) (*25*). When compared to ^{68}Ga-DOTATATE, ^{68}Ga-NeoBOMB1 presented a much higher mean dose for the pancreas (by a factor of approximately 15) and lower mean doses for the spleen and the kidneys (by factors of approximately 0.05 and 0.5, respectively). The rest of the organs presented similar dose values between these two tracers. Higher doses for ^{68}Ga-NeoBOMB1 in the pancreas are due to the high expression of GRP receptors in this organ, which is the targeted receptor for NeoBOMB1 (*27*,*28*).

DISCUSSION

In this study, safety, tolerability and dosimetry of ^{68}Ga-NeoBOMB1, a novel PET radiopharmaceutical targeting GRP receptors were evaluated in 6 patients with metastasized GIST, representing the Phase I of the MITIGATE-NeoBOMB1 trial (EudraCT No. 2016-002053-38). Details from Phase IIa regarding GIST targeting properties in all patients will be reported separately.

In contrast to other bombesin derivates, which mainly have agonistic effects, NeoBOMB1 constitutes a GRP-receptor antagonist, potentially reducing the incidence of side effects. Bombesin antagonists have been demonstrated to possess superior properties compared to their agonist counterparts, leading to improved tumor uptake and lower accumulation in physiologic GRPR-expressing nontarget tissues (*29*,*30*). Furthermore, side effects are lower compared to agonistic bombesin derivates due to lower internalization rates and consequent physiologic activity (*11*). NeoBOMB1 is a part of a family of recently described GRPR antagonists for clinical application, mainly labelled with ^{68}Ga (*5*) or ^{18}F (*3*).

Metabolic stability was very high with no detectable peptide fractions in full blood samples, in contrast to other agents such as SB3, probably due to C-terminus stabilization (*28*). Human pharmacokinetics have only been reported for BAY 86-7548, which appeared less stable with only 19% of peptide intact in plasma 1 hour post administration(*5*).

No causally related adverse events were observed, as only transient mild neutrophilia and hypophosphatemia with a CTCAE grade of 1 were encountered in one participant, most likely caused by the participant's advanced disease stage or intake of sunitinib. No serious adverse events were observed.

After intravenous administration, rapid renal clearance was observed with an increase in gallbladder activity over time, hinting at a relatively minor hepatic clearance, in line with the small and hydrophilic nature of the peptide. Highest organ accumulation was observed in the pancreas – increasing over time, suggesting specific binding to pancreatic GRP receptors as described in rats (*31*). Further apparent

physiological accumulation was found in the gastrointestinal system, notably esophagus, colon and rectum.

Radiation doses were found comparable to other ^{68}Ga-radiopharmaceuticals supporting the suitable safety profile of ^{68}Ga-NeoBOMB1 (*5*,*25*,*32*,*33*). As expected, pancreas with its high physiological expression of GRPR represents the organ receiving the highest radiation dose. Even though not within the primary scope of the study, tumor uptake was also examined. Reubi et al. have already demonstrated a high rate of bombesin-subtype 2 receptor expression in GIST lesions (*34*), regardless of primary or metastatic lesion nature. In our study population, tumor accumulation was observed with the highest tumor-to-organ ratio at 2 to 3 hours post administration. Even if negative in one participant and mixed in two participants (exemplary Fig. 4), tumor uptake was intense in the rest of participants (n = 3), suggesting viability for diagnosis and potentially treatment in a subset of patients with high levels of GRP-receptor expression. This heterogeneity in tumor uptake is unclear, yet may represent changes in tumor biology after prolonged disease duration and under treatment pressure from tyrosine-kinase inhibitors, as most patients examined in this study had been diagnosed several years prior to the study participation. Probably owing to the restricted number of participants, no clear correlation between disease duration, current treatment, mutational status, patient gender or age and ^{68}Ga-NeoBOMB1 uptake could be observed. Interestingly, in another study by Reubi et al. no correlation between receptor status and size, mitotic index or presence of tumor necrosis could be found by even after therapy with Imatinib or chemoembolization (*34*).

Noteworthy, a major challenge in GIST-related studies is the low incidence rate. Further details on ^{68}Ga-NeoBOMB1's diagnostic properties in more patients will be presented separately. GRPR targeting, however, offers diagnosis of other tumor entities, which were also in the focus of studies with other Bombesin analogs (2-12). PET imaging of other tumor entities expressing GRPR, such as breast, prostate or lung cancers with ^{68}Ga-NeoBOMB1 is currently under investigation in a continuing Phase II study possibly also leading to a theragnostic pathway with the corresponding Lu-177 counterpart.

Conclusion

^{68}Ga-NeoBOMB1 is a novel, kit-based GRPR targeting radiopharmaceuticals. Our results show an excellent safety profile, low radiation dose and apparent suitability for diagnostics of GRPR expression. ^{68}Ga-NeoBOMB1 therefore is a promising radiotracer suitable for PET imaging of GRPR expression in oncological patients and may open a pathway for therapeutic applications.

Ethical approval

This article does not contain any studies with animals performed by any of the authors. All procedures performed in this study involving human participants were approved by the ethics committee of the Medical University of Innsbruck and the Austrian Competent Authority (BASG, EudraCT No. 2016-002053-38) and were in accordance with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. All participants signed a written informed consent.

Disclosure

This study was supported by the EU FP7 project: Closed-loop Molecular Environment for Minimally Invasive Treatment of Patients with metastatic Gastrointestinal Stromal Tumours 'MITIGATE', grant agreement number 602306. Advanced Accelerator Applications was one of the industrial partners in the 'MITIGATE' project. Francesca Orlandi and Maurizio Mariani were employees of Advanced Accelerator Applications at the time of the study. GiPharma is fully owned by Advanced Accelerator Applications. Irene Virgolini was principle investigator of the study EudraCT (2016-002053-38). No other potential conflicts of interest relevant to this article exist.

Key points:

- Is the application of ^{68}Ga-NeoBOMB1 safe for PET imaging applications, how are pharmacokinetics, radiation dose and imaging properties of this novel radiopharmaceuticals?
- This study was designed as a Phase I/IIa clinical trial and the outcome of the first 6 patients is reported. ^{68}Ga-NeoBOMB1 showed an excellent safety profile, suitable pharmacokinetics, low radiation dose and promising targeting properties in GIST tumors.
- ^{68}Ga-NeoBOMB1 is a promising radiotracer suitable for PET imaging of GRPR expression in oncological patients and opens a pathway for translation into a therapeutic approach

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TABLE 1: Patient characteristics, injected doses and ^{68}Ga-NeoBOMB1 lesion uptake

Participant	Age [years]	Sex	Primary	Year of first diagnosis	Current status	Current treatment	Body weight [kg]	Injected activity [MBq]	^{68}Ga-NeoBOMB1 uptake	
									Primary/local recurrence	Metastases
#1	76	f	Duodenum	2003	Omental and liver metastasis	Sunitinib	68	179	0/0 (n/a)	2/2 (100%)
#2	73	f	Ileum	2012	Liver metastases	Sunitinib	48	127	0/0 (n/a)	0/1 (0%)
#3	75	m	Duodenum	2016	Liver and lung metastases	Sunitinib	68	214	0/0 (n/a)	2/2 (100%)
#4	83	f	Stomach	2014	Liver metastases, local recurrence	Imatinib	59	158	1/1 (100%)	0/3 (0%)
#5	50	f	Stomach	2013	Local recurrence, liver and peritoneal metastases	none	52	169	2/2 (100%)	1/6 (16.6%)
#6	55	m	Ileum	2014	Liver metastasis	Sunitinib	66	199	0/0 (n/a)	4/4 (100%)

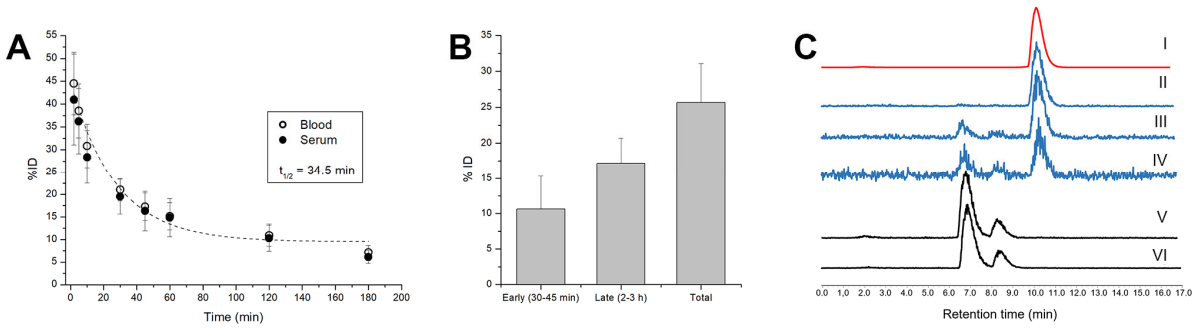

FIGURE 1: Summary of pharmacokinetic analyses of ^{68}Ga-NeoBOMB1 in blood and urine. A: Time activity curve in plasma and serum, mean values of 6 patients, B: Mean urinary excretion (n=6) at early and late time points as well as total after 3h. C: Typical radiochromatograms of ^{68}Ga-NeoBOMB1: A: Reference, B: Plasma 5min, C: Plasma 30min, D: Plasma 60min, E and F: Urine 40min and 3h respectively.

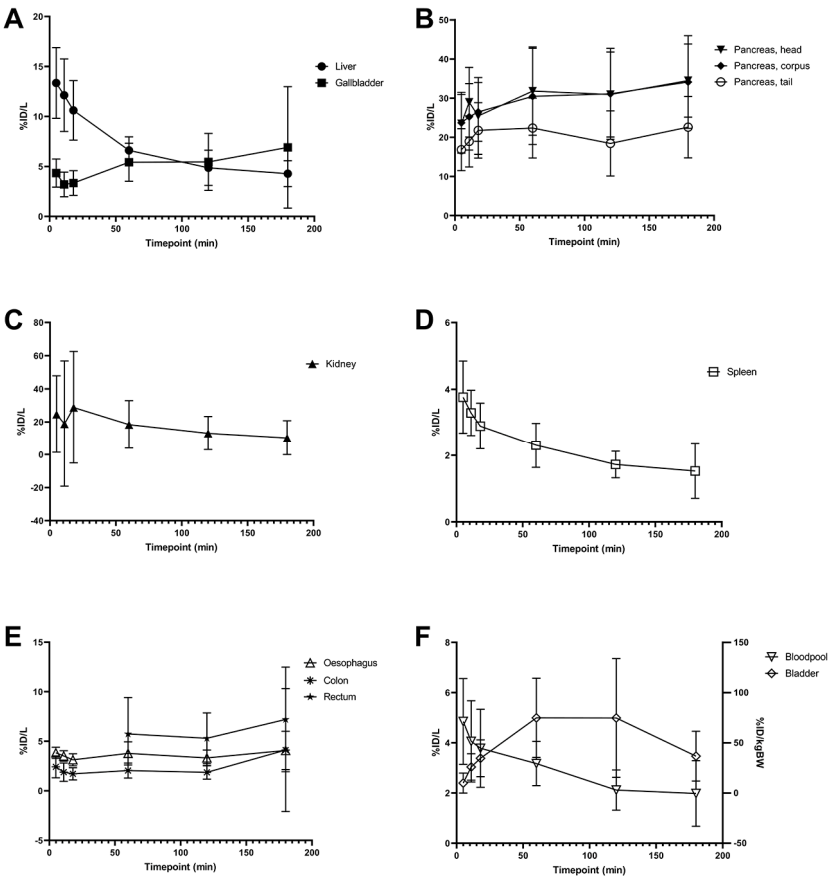

FIGURE 2: Organ-based pharmacokinetic data for liver and gallbladder (A), pancreatic head, corpus and tail (B), kidneys (C), spleen (D), esophagus, colon and rectum (E) as well as blood-pool (left y-axis) and bladder (right y-axis) (F). Note missing rectum values due to small field of view for early timepoints.

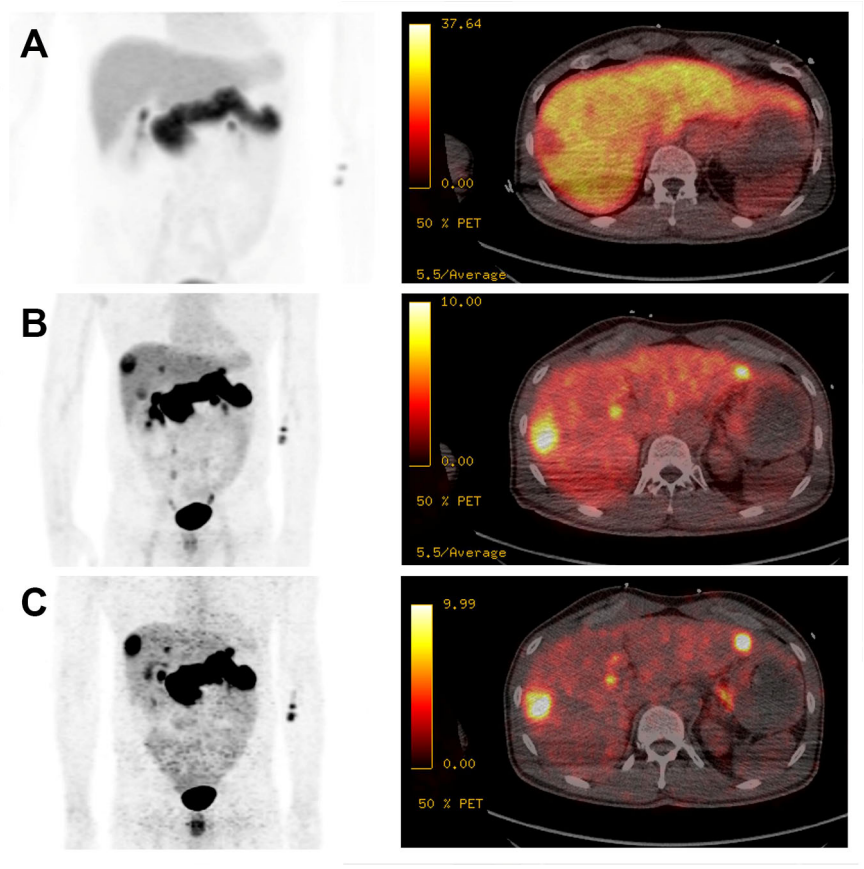

FIGURE 3: ^{68}Ga-NeoBOMB1 PET/CT of participant 6 with gastrointestinal stromal tumor of the ileum and histologically verified liver metastases. Maximum intensity projections (MIP, left) and fused PET-CT images (right) of at 5 min (A), 60 min (B) and 180 min (C) post injection (p.i.).

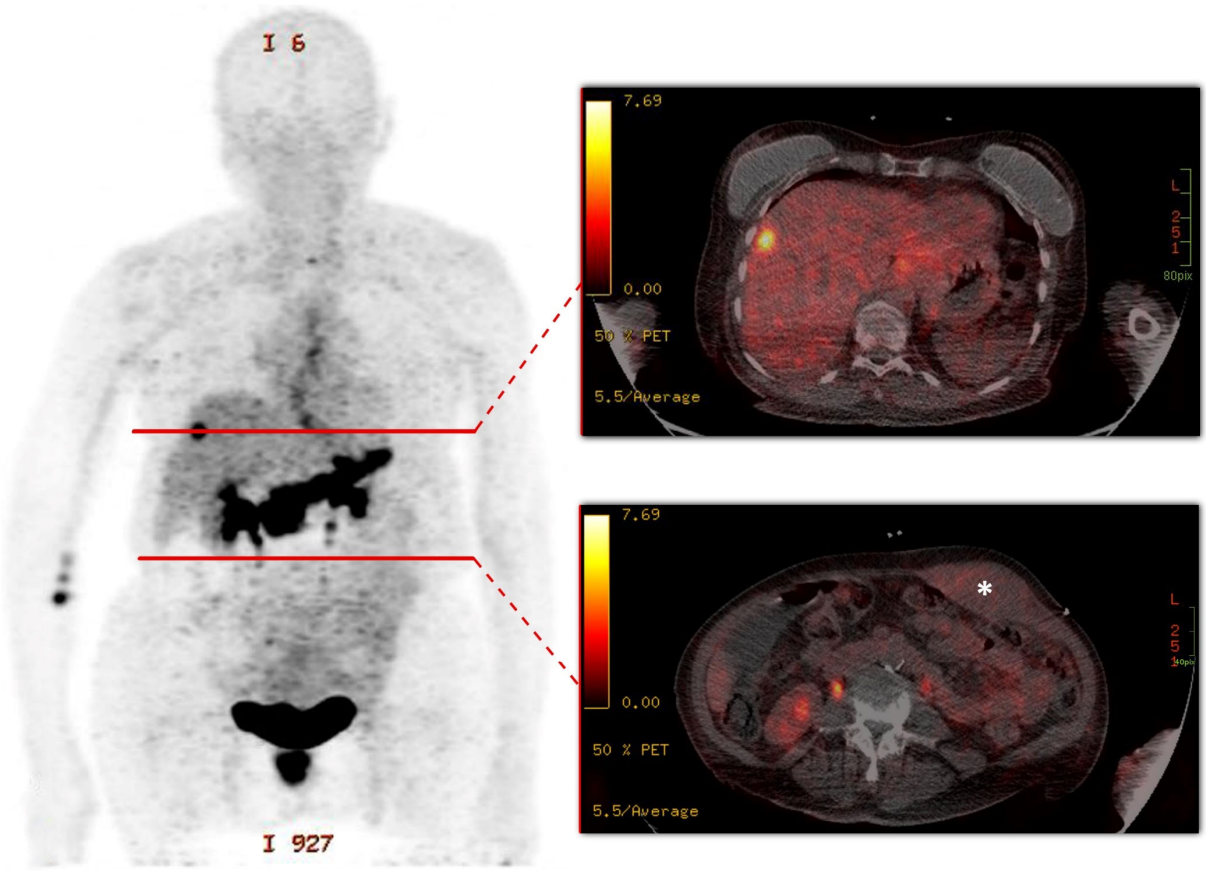

FIGURE 4: ^{68}Ga-NeoBOMB1 PET-CT of participant 1 with duodenal gastrointestinal stromal tumor and mixed tracer uptake illustrated by a maximum intensity projection (MIP, left) and fused PET-CT images (right) 1 h after administration. Strong tracer uptake was observed in a hepatic metastasis and absent uptake was seen in a tumor formation invading the abdominal wall (asterisk). Red lines (left) denote the according section planes for the axial PET-CT slices.

Supplemental Material

Supplemental Information 1

Preparation and Quality Control of ^{68}Ga-NeoBOMB1

The NeoBOMB1 kit was supplied by GiPharma (Saluggia, Italy), consisting of 2 vials and an accessory cartridge containing 660 mg porous silica. Vial 1 (reaction vial) contains 50 µg of NeoBOMB1 in a lyophilized formulation, Vial 2 a buffer solution for adjusting pH.

^{68}Ga solution for radiolabelling was obtained from a ^{68}Ge/^{68}Ga-generator (1850 MBq reference activity, GalliaPharm, Eckert & Ziegler Radiopharma, Berlin, Germany), eluted according to manufacturer`s instructions.

Radiolabelling was performed by adding 5.0 ml of ^{68}Ga solution for radiolabelling directly to Vial 1 of the ^{68}Ga-NeoBOMB1 kit via a sterile filter (Cathivex Filter SLGV0250S, Merck-Millipore, Burlington, MA) and the accessory cartridge. Immediately afterwards, 0.5 ml of buffer from kit vial 2 were added and the vial was incubated at 95 °C for 7 min. After cooling to ambient temperature, a sample of 0.1 ml for quality control was taken and the solution used for injection without further processing.

For the release of ^{68}Ga-NeoBOMB1 radiochemical purity was determined using ITLC-SA strips (Agilent Technologists, Santa Clara, CA), developed in ammonium acetate (5M)/ methanol/water 1:7:2, Rf ^{68}Ga-NeoBOMB1 0.6-0.9, Rf non-complexed ^{68}Ga species 0-0.1. Not more than 3% of ^{68}Ga species were defined as acceptance criteria. The pH was determined using paper strips with an acceptance criterion of 3.2-3.8 and visual inspection was performed to ensure clear solution and absence of particle.

Additionally, reversed-phase high performance liquid chromatography (RP-HPLC) was performed with an UltiMate 3000 UHPLC pump, an UltiMate 3000 autosampler, an UltiMate 3000 column compartment, an UltiMate 3000 variable wavelength detector (Thermo Fisher Scientific, Vienna, Austria) and a GabiStar radiometric detector (Raytest GmbH, Straubenhardt, Germany). An ACE 3 C18, 3 µm 100 Å, 150 x 3.0 mm column (ACE, Aberdeen, UK) with a flow rate of 0.6 mL/min and UV detection at 220 nm were employed.

Acetonitrile (ACN)/H2O/0.1% trifluoroacetic acid (TFA) was used as mobile phase with the following multistep gradient: 0–2.0min 15% ACN, 2- 9.0min 15–60% ACN, 9.0–11.0 min 60% ACN, 11.0–13.0 min 60-80% ACN.

Before administration, three subsequent batches of ^{68}Ga-NeoBOMB1 were prepared and analysed as described above. Additionally, endotoxins and sterility were tested in these samples.

Pharmacokinetics and metabolite analysis

Heparinized venous blood samples (3-4 mL) were obtained from the participants at 2, 5, 10, 30 and 45 min and at 1, 2 and 3 h p.i.. Whole blood and plasma activity concentrations for each time point were determined by measuring the activity in two 0.2 mL whole blood samples and in two 0.2 mL plasma samples (after centrifugation of the heparinized samples) using a Gamma Counter (2480 Automatic Gamma Counter Wizard2 3"; PerkinElmer, Waltham, MA, USA). The percentage of injected dose (% ID) in whole blood and plasma were calculated based on total blood/plasma volumes as described in (*1*). For analysis of metabolites, RP-HPLC was applied as described above. Samples were prepared by mixing 0.2 mL of plasma with 0.2 mL of methanol, followed by rapid centrifugation (2,000 rcf for 2 min) and injection of 50 µL samples in the HPLC.

Urine was collected at 30-50 min and at 2-3 h p.i., the voided volumes were measured, 10 mL-samples were used to determine the urine activity concentration and for metabolite analysis. % ID (injected dose) in the urine was calculated and summed up for determination of the total excreted activity within the first 3 h p.i. 1 mL urine samples were centrifuged (2.000 rcf for 2 min) and 50 µl samples of the supernatant directly injected on HPLC.

Dosimetry Calculations

An automated kinetic-based segmentation method (using the PSEG module in PMOD v3.8 software (21), PMOD Technologies LLC, Zurich, Switzerland) was used to retrieve the activity concentration from

the PET images considering the totality or a large portion of the tumour lesions and organs of interest in order to decrease the possibility of under- or overestimation in the retrieved activity concentration due to heterogeneous drug uptake within an organ. Time activity curves (TACs) for the organs of interest and tumour lesions were obtained by multiplying the retrieved activity concentration at each time point by the organ and tumour volumes. Reference organ masses for the average man and average woman were extracted from OLINDA/EXM software (*2*). Organ masses were linearly scaled according to the patient body weight (BW) and sex. Tumour lesion volumes were obtained from the PET segmentation as previously detailed. A density of 1 g/mL was assumed for organs of interest as well as tumour lesions. The TAC for the bladder content was corrected by the activity in the collected voided urine.

TACs were fitted to a sum of exponential functions to subsequently calculate the values of the areas under the curves (AUCs) by analytically integrating the fitted sum of exponentials from time 0 min to infinity (*3*). Subsequently, the obtained AUCs were divided by the total injected activity to obtain the time-integrated activity coefficients (TIACs) (formerly called "residence times") (*4*). The calculated TIACs for every organ of interest were subsequently entered as an input to the OLINDA/EXM software to perform calculations of the radiation dose estimates (i.e. doses). The sex of the patient and individually scaled organ masses (based on patient BW) were considered in OLINDA/EXM for the dose calculations. A 2h bladder voiding model was used in the OLINDA/EXM software.

The obtained organ doses for ^{68}Ga-NeoBOMB1 were compared with published dose data for ^{68}Ga-DOTATATE, a well-established diagnostic tracer (*5*).

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Supplemental Table 1: Inclusion and exclusion criteria

Main inclusion/ exclusion criteria:	**Inclusion Criteria** ● Understanding and provision of signed and dated written informed consent by the patient or legally acceptable representative prior to any study-specific procedures ● Patients with histologically confirmed advanced GIST ● Previous or current TKI treatment ● A minimum of 50% of patients showing either 1st-, 2nd- or 3rd-line TKI-resistance documented either through RECIST 1.1 criteria, Choi-criteria or FDG-CT/PET and showing presence of at least one surgically untreatable primary or metastasis confirmed with either 18F-FDG PET/CT or structural imaging (CT, MRI) and a minimum of 25% non-resistant patients. ● Karnofsky performance status > 70% ● Age > 21 years. ● Participating men must use a single barrier method for contraception for 1 month after completion of the trial starting at the day of application of ^{68}Ga-NeoBOMB1. ● Women of childbearing age must use two highly effective methods of contraception during the trial and 6 months after its completion if not in menopause (defined as onset of menopause without menstruation for over 1 year) or after hysterectomy. The following contraceptive methods with a Pearl Index lower than 1% are regarded as highly-effective: o Oral hormonal contraception ('pill') (as far as its efficacy is not expected to be impaired during the trial, e.g. with IMPs that cause vomiting and diarrhoea, adequate safety cannot be assumed) o Dermal hormonal contraception o Vaginal hormonal contraception (NuvaRing®) o Contraceptive plaster o Long-acting injectable contraceptives o Implants that release progesterone (Implanon®) o Tubal ligation (female sterilisation) o Intrauterine devices that release hormones (hormone spiral) o Double barrier methods o This means that the following are not regarded as safe: condom plus spermicide, simple barrier methods (vaginal pessaries, condom, female condoms), copper spirals, the rhythm method, basal temperature method, and the withdrawal method (coitus interruptus).

	○ The regulations for contraception are derived from Guideline ICH E8 Chapter 3.2.2.1 Selection of subjects together with ICH M3 Note 4 ● Confirmed GRPR expression (phase II only) **Exclusion Criteria** ● Renal insufficiency with an eGFR < 45 ml/min/1.72m^2 or intolerance to any constituents of intravenous CT-contrast agents, preventing their administration (in cases without an available recent and sufficient contrast-enhanced CT examination) ● Higher than grade 2 hematotoxicity (CTC > 2) ● Other known co-existing malignancies except non-melanoma skin cancer and carcinoma in situ of the uterine cervix, unless definitively treated and without evidence of recurrence for 5 years ● Participation in any other investigational trial within 30 days of study entry with potential interactions regarding the study drugs or the underlying disease ● Pregnancy, breast-feeding ● Patients with concurrent illnesses that might preclude study completion or interfere with study results ● Patients with bladder outflow obstruction or unmanageable urinary incontinence ● Known or expected hypersensitivity to ^{68}Gallium, Bombesin or to any of the excipients of NeoBOMB1. ● Any condition that precludes raised arms position for prolonged imaging purposes. ● Prior administration of a radiopharmaceutical within a period corresponding to 8 half-lives of the radionuclide used on such radiopharmaceutical. ● History of somatic or psychiatric disease/condition that may interfere with the objectives and assessments of the study. ● Clinically significant illness or clinically relevant trauma within 2 weeks before the administration of the investigational product. ● Subjects with any kind of dependency on the investigator or is employed by the sponsor or investigator ● Subjects held in an institution by legal or official order

Supplemental Table 2: Pre-existing conditions

Adverse events observed after the administration of ^{68}Ga-NeoBOMB1 are **bold**. ^{p} … pre-existing

Participant	Pre-existing conditions (^{p}) and Adverse events	Max. CTCAE	Resolved?	Serious adverse events
#1	Fatigue^{p}, headache^{p}, weight loss^{p}, GPT/GOT elevation^{p}, latent hyperthyreosis^{p}, anaemia^{p}, leukocyturia^{p}	-	n/a	none
#2	Leukopenia^{p}, anaemia^{p}, CRP elevation^{p}, proteinuria^{p}, urinary leukocytosis^{p}, **mild neutrophilia** (visit 2, resolved), hypokalaemia^{p}, **mild hypophosphatemia** (visit 3, resolved), hyperthyreosis^{p}	1	yes	none
#3	Anaemia^{p}, lymphocytosis^{p}	-	n/a	none
#4	Anaemia^{p}, fatigue^{p}, chronic kidney disease^{p}, urinary tract infection^{p}	-	n/a	none
#5	Microhaematuria^{p} (chronic IgA-nephritis), liver enzyme elevation^{p}, leukocyturia/haematuria^{p}	-	n/a	none
#6	microhaematuria^{p}, anaemia^{p}	-	n/a	none

Supplemental Table 3: Overview of adverse events (AE) and severe adverse events (SAE) in participants graded by the Common Terminology Criteria for Adverse Events (CTCAE).

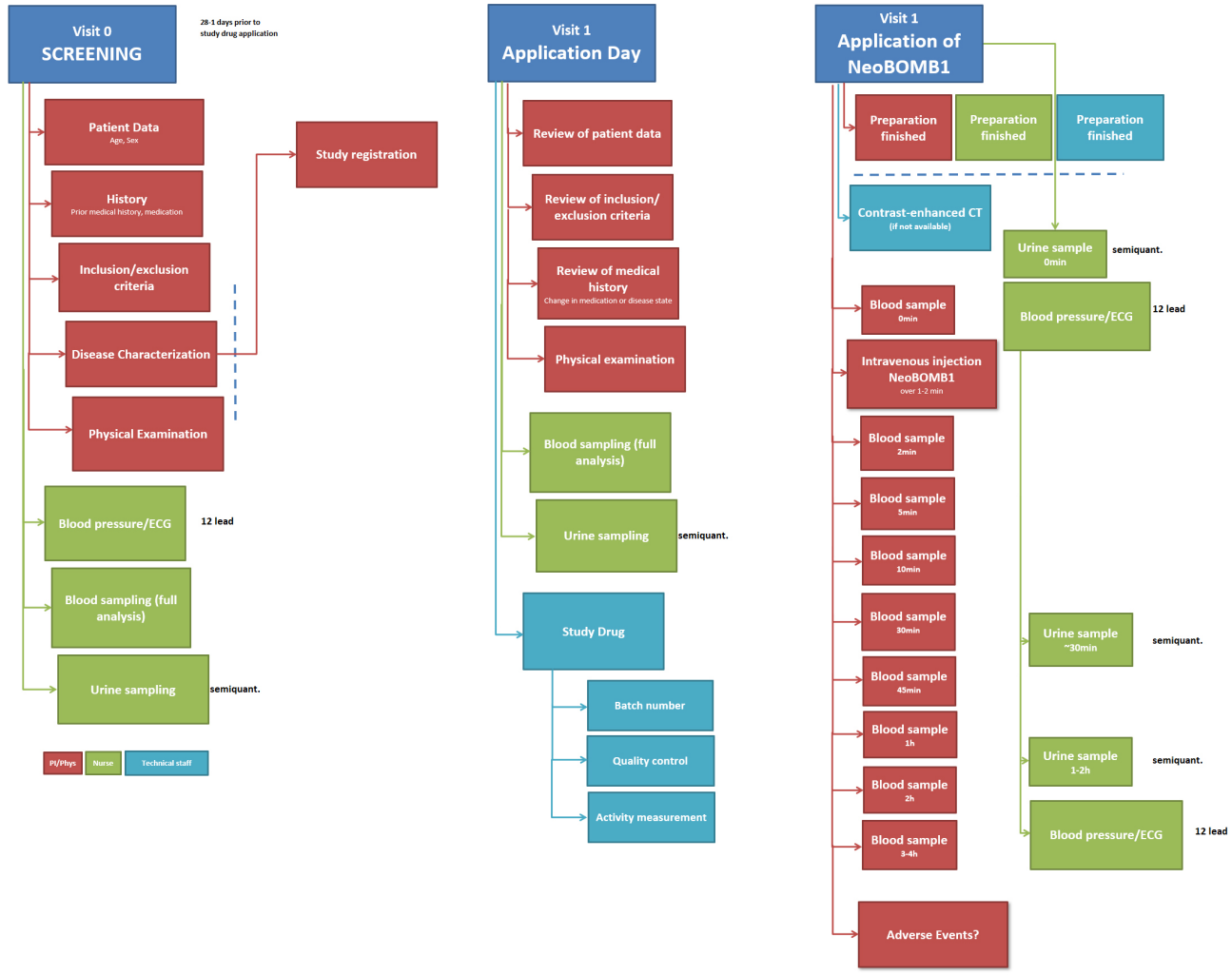
Participant	Adverse events	Max. CTCAE	Resolved?	Serious adverse events
#1	None	n/a	n/a	none
#2	**mild neutrophilia** (visit 2, resolved), **mild hypophosphatemia** (visit 3, resolved)	1	yes	none
#3	None	n/a	n/a	none
#4	None	n/a	n/a	none
#5	None	n/a	n/a	none
#6	None	n/a	n/a	none

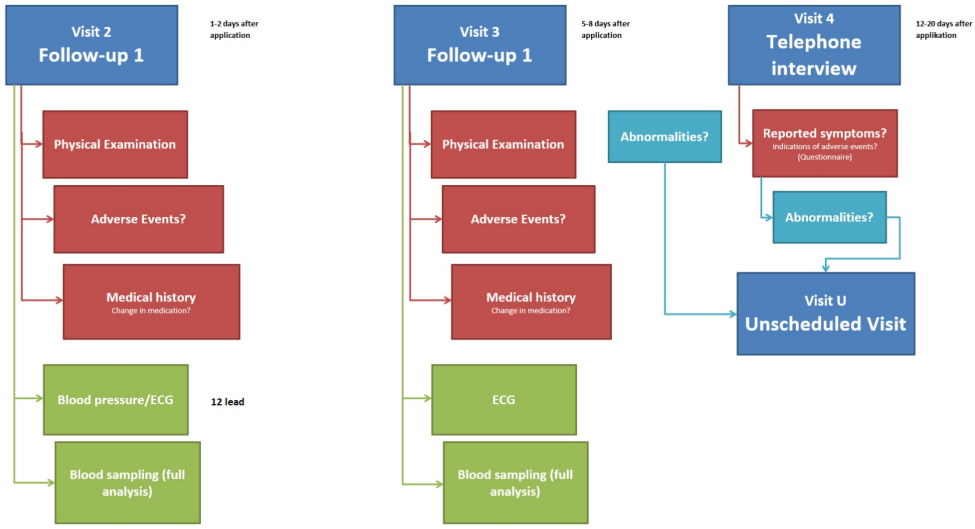
Supplemental Table 4: Dose estimates after administration of ^{68}Ga-NeoBOMB1 in six patients (patient 1 to 6), mean ^{68}Ga-NeoBOMB1 doses, standard deviations (Std.) of the ^{68}Ga-NeoBOMB1 doses and doses for ^{68}Ga-DOTATATE.

Target Organ	Organ dose [mSv/MBq]								
	P1	P2	P3	P4	P5	P6	Mean	Std.	^{68}Ga-DOTATATE[$]
Adrenals	0.0135	0.0165	0.0142	0.0179	0.0184	0.0135	0.0157	0.0022	0.0146
Brain	0.0103	0.0127	0.0088	0.0127	0.0142	0.0084	0.0112	0.0024	0.0099
Breasts	0.0102	0.0127	0.0091	0.0128	0.0141	0.0086	0.0113	0.0023	0.0100
Gallbladder Wall	0.0145	0.0175	0.016	0.0193	0.0193	0.0220	0.0181	0.0027	0.0149
LLI Wall	0.0136	0.0163	0.012	0.0149	0.0164	0.0138	0.0145	0.0017	0.0129
Small Intestine	0.013	0.0156	0.0119	0.0155	0.0168	0.0121	0.0142	0.0021	0.0138
Stomach Wall	0.0127	0.0155	0.0129	0.0168	0.0177	0.0125	0.0147	0.0023	0.0138
ULI Wall	0.0129	0.0155	0.0119	0.0156	0.0168	0.0119	0.0141	0.0021	0.0129
Heart Wall	0.0122	0.0148	0.0116	0.0155	0.0166	0.0109	0.0136	0.0023	0.0123
Kidneys	0.0406	0.0704	0.0466	0.051	0.0524	0.0510	0.0520	0.0100	0.0921
Liver	0.0403	0.0652	0.0744	0.0779	0.0588	0.0605	0.0629	0.0134	0.045
Lungs	0.0114	0.0139	0.0106	0.0144	0.0155	0.0100	0.0126	0.0023	0.0115
Muscle	0.0115	0.014	0.0103	0.0139	0.0152	0.0104	0.0126	0.0021	0.0113
Ovaries	0.0137	0.0164	0.0122	0.0152	0.0167	0.0137	0.0147	0.0018	0.0131
Pancreas	0.106	0.215	0.316	0.304	0.315	0.3890	0.2742	0.0993	0.0167
Red Marrow	0.0095	0.0113	0.0088	0.0115	0.0124	0.0087	0.0104	0.0016	0.0096
Osteogenic Cells	0.016	0.0199	0.014	0.0198	0.022	0.0136	0.0176	0.0035	0.0155
Skin	0.01	0.0125	0.0088	0.0124	0.0137	0.0085	0.0110	0.0022	0.0097
Spleen	0.0126	0.0154	0.0124	0.0146	0.0176	0.0123	0.0142	0.0021	0.282
Testes	0.0119	0.0145	0.0104	0.0135	0.015	0.0115	0.0128	0.0018	0.0112
Thymus	0.0112	0.0137	0.0099	0.0139	0.0153	0.0094	0.0122	0.0024	0.0109
Thyroid	0.0111	0.0135	0.0095	0.0136	0.0151	0.0091	0.0120	0.0024	0.0187
Bladder Wall	0.165	0.195	0.161	0.0515	0.053	0.3620	0.1646	0.1141	0.125
Uterus	0.0159	0.0189	0.0143	0.0157	0.0172	0.0187	0.0168	0.0018	0.0147
Total Body	0.0129	0.0176	0.0131	0.0174	0.0183	0.0129	0.0154	0.0026	0.0134
Effective Dose	**0.0223**	**0.0306**	**0.0263**	**0.0262**	**0.0266**	**0.0404**	**0.0287**	**0.0063**	**0.0257**

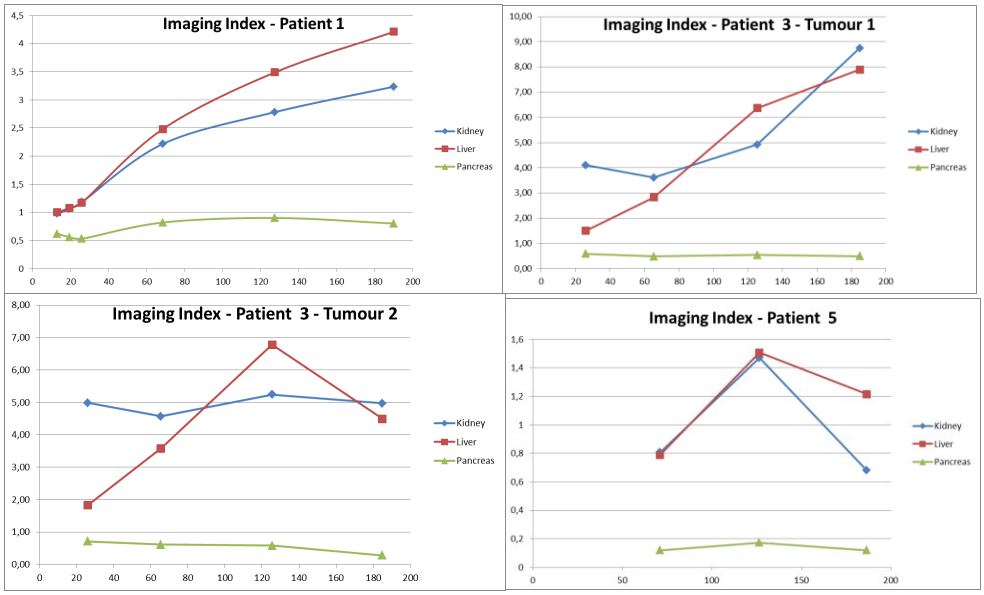
[$] Dose calculations for ^{68}Ga-DOTATATE (no bladder voiding considered) (*5*).

Supplemental Figure 1: Study plan




Visit 2
Follow-up 1
1-2 days after application
Physical Examination
Adverse Events?
Medical history
Change in medication?
Blood pressure/ECG
12 lead
Blood sampling (full analysis)
Visit 3
Follow-up 1
5-8 days after application
Physical Examination
Adverse Events?
Medical history
Change in medication?
ECG
Blood sampling (full analysis)
Abnormalities?
Visit 4
Telephone interview
12-20 days after applikation
Reported symptoms?
Indications of adverse events? (Questionnaire)
Abnormalities?
Visit U
Unscheduled Visit

Supplemental Figure 2: Imaging indices



Imaging indices for major organs for Patient 1, 3 and 5.

Supplemental Figure 3: Pharmacokinetics example

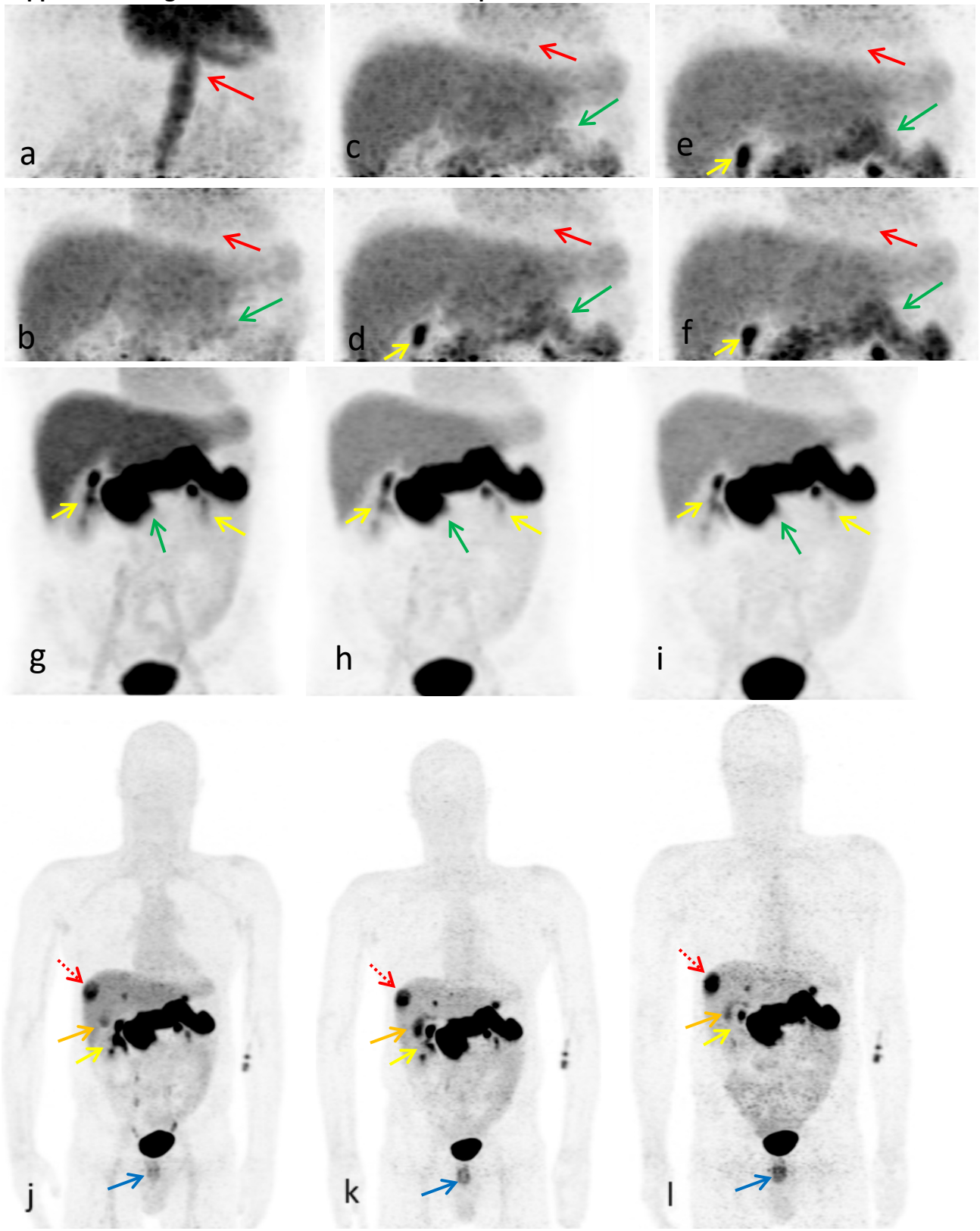


Figure 3: ^{68}Ga-Neobomb PET/CT of participant 6 with gastrointestinal stromal tumour of the ileum and histologically verified liver metastases. Maximum intensity projections (MIP) of dynamic imaging within the first five minutes post

injection (p.i.) are displayed (images: a-f), followed by MIPs of static images at 5, 12 and 19 min p.i. (images: g-i) and MIPs of whole body scans at 60, 120 and 180 min p.i. (images: j-l). As described in figure 1 pharmacodynamics of structures with physiologic tracer uptake are shown (red arrow: vascular activity, green arrow: pancreas, yellow arrow: renal pelvis, orange arrow: gall bladder, blue arrow: anal activity). In addition, lesions with pathologic tracer accumulation are clearly visualised on the scan 60 min p.i., but also at 120 min and 180 min p.i. (j-l: dotted red arrow pointing at one of the lesions in the right lobe of the liver), corresponding to the known metastases on diagnostic CT. The pathologic liver lesions cannot be discriminated on the early dynamic images and the static images 5 min, 12 min and 19 min p.i. (a-f and g-i).