Supplemental Appendix 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*).

References

1. Lemmens HJM, Bernstein DP, Brodsky JB. Estimating Blood Volume in obese and morbidly obese Patients. *Obes Surg*. 2006;16:773-776.
2. Stabin MG, Sparks RB, Crowe E. OLINDA/EXM: the second-generation personal Computer Software for internal Dose Assessment in Nuclear Medicine. *J Nucl Med*. 2005;46:1023-7.
3. Glatting G, Kletting P, Reske SN, Hohl K, Ring C. Choosing the optimal Fit Function: Comparison of the Akaike Information Criterion and the F-Test. *Med Phys*. 2007;34:4285-4292.
4. Hardiansyah D, Begum NJ, Kletting P, Mottaghy FM, Glatting G. Sensitivity Analysis of a physiologically based pharmacokinetic Model used for Treatment planning in Peptide Receptor Radionuclide Therapy. *Cancer Biother Radiopharm*. 2016;31:217-224.

5. Walker RC, Stabin M, Smith GT, Clanton J, Moore B, Liu E. Measured human Dosimetry of 68Ga-DOTATATE. *J Nucl Med*. 2013;54:855-860.

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).

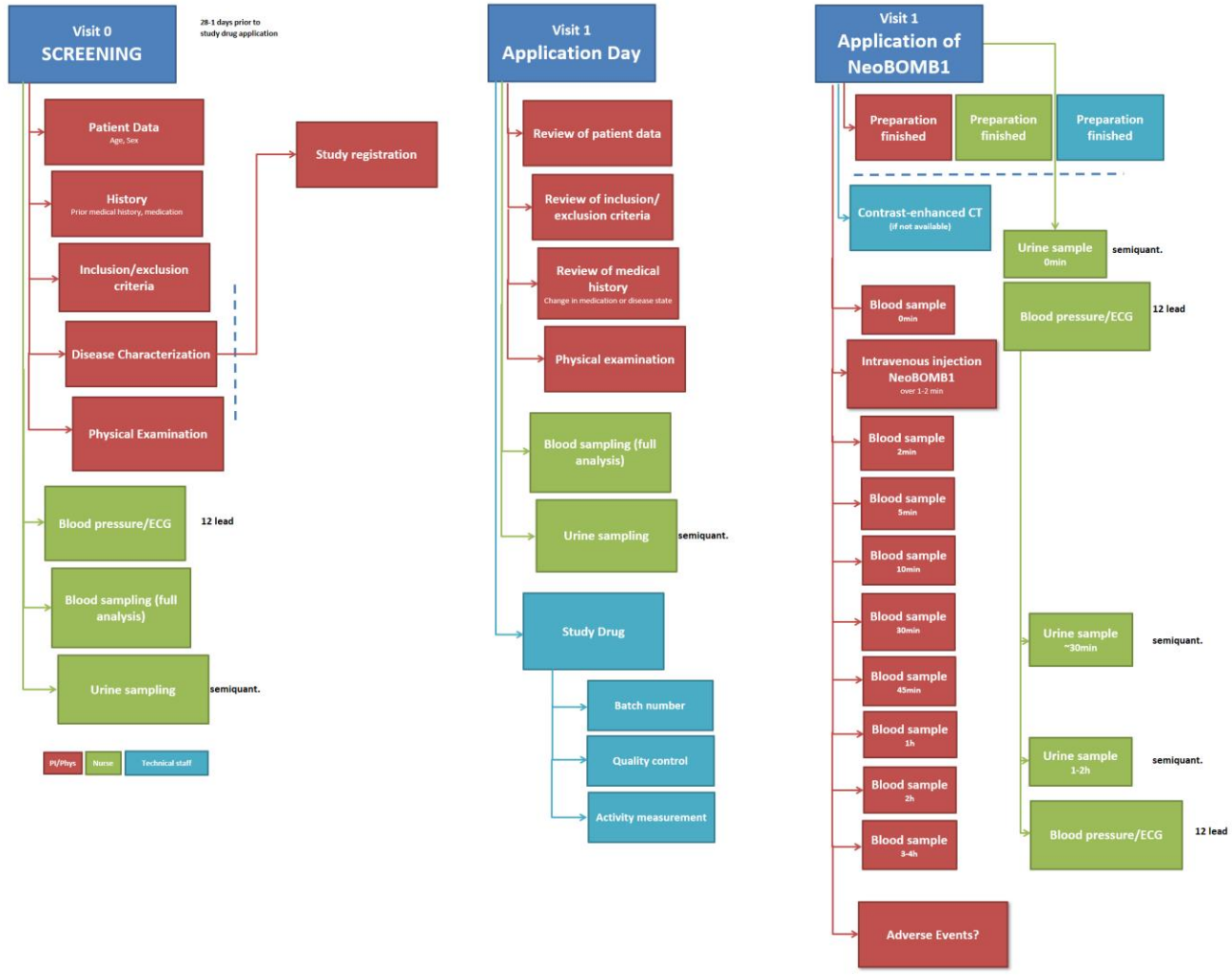
Participant	Adverse events	Max. CTCAE	Resolved?	Serious adverse events
#1	None	n/a	n/a	none
#2	**mild neutrophilia** (visit 2, resolved), **mild hypophosphatemia** (visit 3, resolved)	1	yes	none
#3	None	n/a	n/a	none
#4	None	n/a	n/a	none
#5	None	n/a	n/a	none
#6	None	n/a	n/a	none

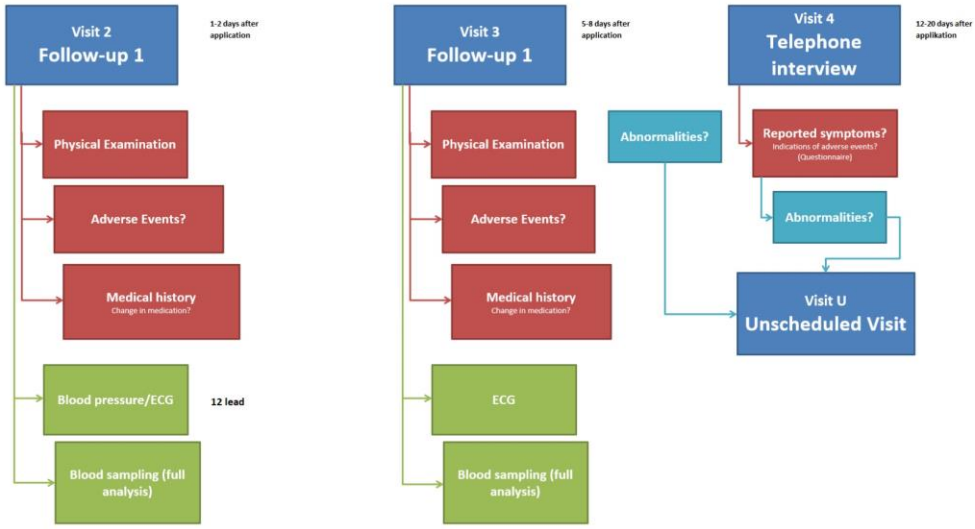
Supplemental Table 4: Dose estimates after administration of ^{68}Ga-NeoBOMB1 in six patients (patient 1 to 6), mean ^{68}Ga-NeoBOMB1 doses, standard deviations (Std.) of the ^{68}Ga-NeoBOMB1 doses and doses for ^{68}Ga-DOTATATE.

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**

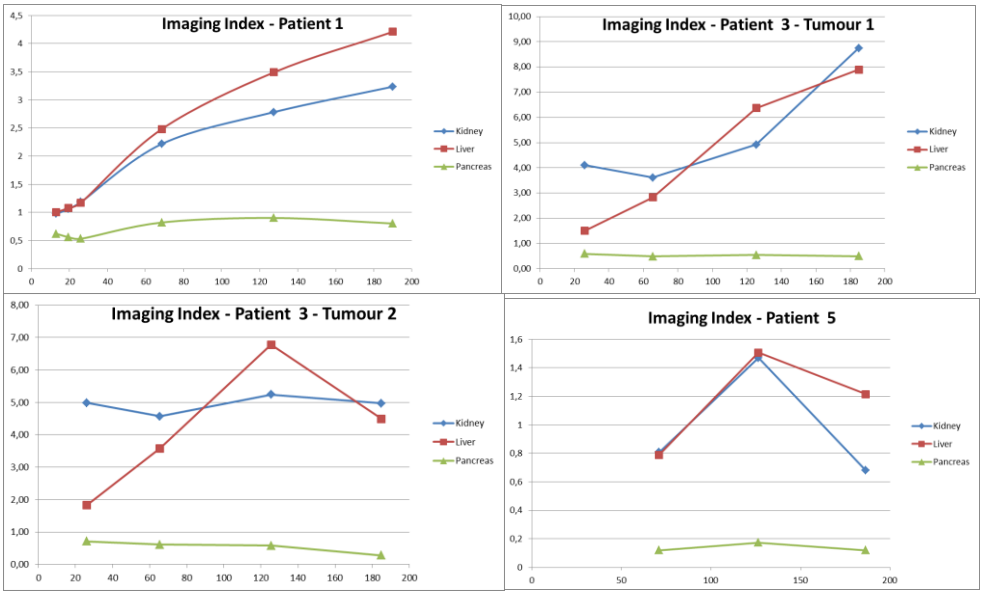
[$] Dose calculations for ^{68}Ga-DOTATATE (no bladder voiding considered) (*5*).

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

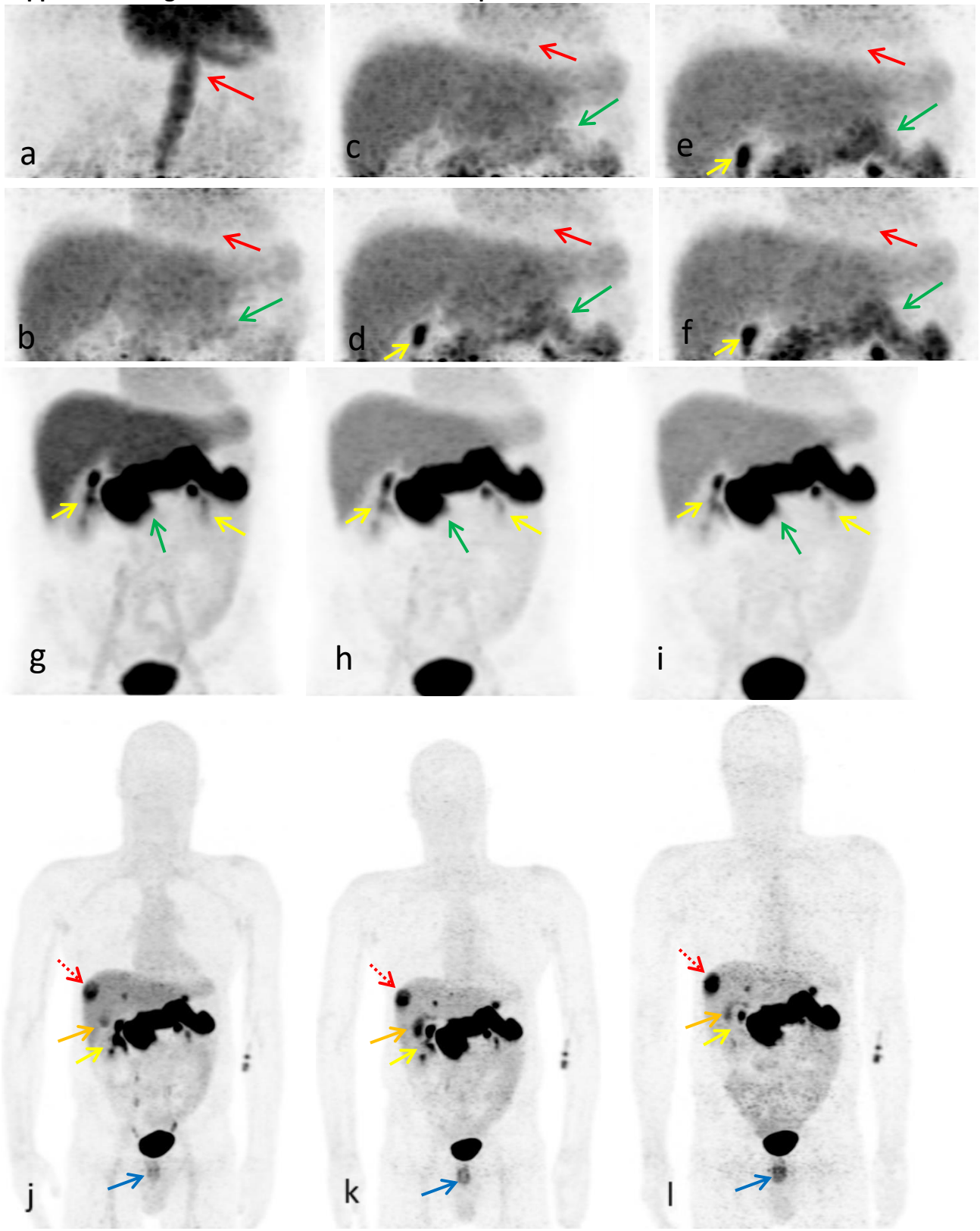


Figure 3: ^{68}Ga-Neobomb PET/CT of participant 6 with gastrointestinal stromal tumour of the ileum and histologically verified liver metastases. Maximum intensity projections (MIP) of dynamic imaging within the first five minutes post

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).