SUPPLEMENTAL MATERIAL AND METHODS

Two different methods (a ROI placed next to the lumbar spine vs. a ROI in the thigh) were used to correct the red bone marrow dose. ROI for red bone marrow doses were drawn on the lumbar vertebrae L2-4. ROI on the whole body; kidneys; liver; parotid, submandibular, and lacrimal glands; and up to 5 tumor lesions per patient were delineated manually by two experienced nuclear medicine physicians on the anterior and posterior whole-body images performed at approximately 1h, 4h, 24h, 48h and 7d after injection using the open-source DICOM software OsiriX (version 5.1, 64-bit, Pixmeo, Geneva, Switzerland). The geometric mean for each ROI was calculated.

Background ROIs were drawn outside the body. For estimation of the background activity from soft tissue using ROI on the thigh was used. The content of this (tissue) background ROI was appropriately scaled and subtracted from the counts in the kidney ROI only. It was not necessary to use it for the liver due to its size and the fact that there is no accumulating tissue in front or behind it. Neither was the ROI subtracted from the different glands since there is only minimal uptake in overlapping tissue. Calibration factors were calculated based on the whole-body ROI in the first scan and the measurements performed with the probe counter in order to normalize the number of counts to the administered activity. If the uptake in the normal organs overlapped with physiological uptake in healthy tissue or lesions, this uptake was included in the ROI.

For the kidneys, the first whole-body scan, with no visible uptake in the intestine, was used to correct the following scans showing overlap of uptake in the intestine. The self-attenuation correction was neglected, because it had no relevant influence on the tumor, kidneys and glands due to their size. The liver turned out to be no organ at risk and therefore self-attenuation was not considered. No scatter correction was used.

The mean organ masses for normal organs were 1869 g (range, 1265–2144 g) for the liver, 211 g (range, 171–288 g) for the kidneys, 34 g (range, 13–45 g) for the parotid, 13 g (range, 9–22 g) for the submandibular, and 1.1 g (range, 0.5–1.7 g) for the lacrimal glands. For paired organs, masses from both sides were summed and divided by 2. Lesion sizes of the metastases are given in Supplemental table 2.

Finally, absorbed organ and tumor doses for each cycle were calculated using OLINDA/EXM (*1*), the time-integrated activity coefficients were calculated using the

EXM module using a mono- or bi-exponential function depending on the individual data set. In all cases, data measured at least 6 days after injection were included in the fitting procedure, ensuring an adequate description of the exponential tail of the time-activity curve. The absorbed doses for tumor lesions and salivary glands were calculated using the density sphere model and for tumor lesions irradiation from surrounding tissues was not considered.

Pre-therapeutic Dosimetry, Image Analysis and Dosimetric Calculations

Each scan was obtained at a speed of 12cm/min on a dual-headed SYMBIA T6 (Siemens Medical Solutions, Erlangen, Germany) equipped with 9.5mm NaI(Tl) crystals and medium-energy low-penetration collimators. A 20% and a 12% energy window were placed around the 208 keV and 113 keV peak of ^{177}Lu, respectively. The image matrix contained 1024×256 pixels, with pixel size of 2.4×2.4mm^2.

The volumes of normal organs and tumor lesions were calculated using the CT dataset of the corresponding pre-therapeutic ^{18}F-rhPSMA-7.3 PET/CT. To estimate the volume of a lesion, a volume-of-interest with a 20–50% of SUV_{max} isocontour adjusting the volume-of-interest optimal to the anatomical configuration of the lesion was drawn using a dedicated workstation (Syngo.Via, Siemens Healthcare, Erlangen, Germany). Volumes of normal organs were segmented using their contours on CT.

RESULTS

Pre-therapeutic Dosimetry of Bone Marrow

Two of six patients had lesions in the respective areas of the lumbar spine that were used for dosimetry. Using ROIs next to the lumbar spine for correction, two patients were not evaluable due to methodical reasons (negative values). Red bone marrow doses for the four evaluable patients were 0.56±0.57 Gy/GBq vs. 0.22±0.21 Gy/GBq for ^{177}Lu-rhPSMA-7.3 vs. ^{177}Lu-PSMA-I&T, respectively. Excluding the two patients with bone lesions in the lumbar spine pre-therapeutic absorbed doses were 0.12±0.02 Gy/GBq vs. 0.06±0.004 Gy/GBq for ^{177}Lu-rhPSMA-7.3 vs. ^{177}Lu-PSMA-I&T, respectively (see supplemental table 3).

Radioligand treatment (RLT) and Post-treatment Scintigraphy

For subsequent RLT, ^{177}Lu-rhPSMA-7.3 was only considered in cases when it provided a clearly higher TI(kidney) compared with ^{177}Lu-PSMA-I&T. With 7.4 GBq as the established standard activity for ^{177}Lu-PSMA-I&T RLT at our department, activity level for a potential treatment with ^{177}Lu-rhPSMA-7.3 was adjusted to not exceed comparable kidney radiation dose for standard dosing with ^{177}Lu-PSMA-I&T. Given the potential benefit from a higher TI(kidney) patient 2 and patient 4 subsequent underwent PSMA-RLT with ^{177}Lu-rhPSMA-7.3. The other patients received ^{177}Lu-PSMA-I&T.

The mean applied activity for ^{177}Lu-PSMA-I&T in the four patients treated was 7307 ± 145 MBq (range, 7116–7517 MBq). Two patients received a mean of 3639 ± 299 MBq of ^{177}Lu-rhPSMA-7.3 (range 3340-3937).

Post treatment tumor lesions received a mean absorbed dose of 6.64 ± 8.71 Gy/GBq (range, 1.29 –27.65 Gy/GBq) for ^{177}Lu-rhPSMA-7.3 and 1.44 ± 0.76 Gy/GBq for ^{177}Lu-PSMA-I&T (range, 0.23– 2.72 Gy/GBq, see supplemental table 4). Graphs displaying the respective %Injected dose (%ID) of post-therapeutic tumor lesions using a semilogarithmic scale are presented in supplemental figure 3.

Post-treatment

In total 5 patients were evaluated using post-treatment scintigraphy (^{177}Lu-rhPSMA-7.3 n = 2, and for ^{177}Lu-PSMA-I&T n = 3). The mean whole-body post-treatment effective dose for ^{177}Lu-rhPSMA-7.3 was 0.34 Gy (0.09 Sv/GBq, n = 2) and for ^{177}Lu-PSMA-I&T 0.32 Gy (0.04 Sv/GBq, n = 3). The mean absorbed organ doses for ^{177}Lu-rhPSMA-7.3 vs. ^{177}Lu-PSMA-I&T were for the kidneys 5.79 Gy (1.59 Gy/GBq) vs. 4.60 Gy (0.63 Gy/GBq), for the liver 0.74 Gy (0.20 Gy/GBq) vs. 0.33 Gy (0.05 Gy/GBq), for the parotid 5.85 Gy (1.59 Gy/GBq) vs. 3.32 Gy (0.46 Gy/GBq), for the submandibular 7.22 Gy (1.97 Gy/GBq) vs. 0.71 Gy (0.67 ± 0.31 Gy/GBq) and for the lacrimal glands 13.88 (3.82 Gy/GBq) vs. 5.95 Gy (0.82 Gy/GBq, see supplemental table 4). Graphs displaying the respective %Injected dose (%ID) using a semilogarithmic scale are presented in supplemental figures 2 and 3.

Red bone marrow dose lumbar spine ROI

For ^{177}Lu-rhPSMA-7.3 post-treatment the dose was 0.47 Gy (0.14 Gy/GBq; n = 1). For ^{177}Lu-PSMA-I&T post-treatment the absorbed dose in the patient without metastases in the lumbar spine was not evaluable because this patient did not receive

a post therapeutic dosimetry. In the two patients with metastases in the lumbar spine, the post treatment absorbed dose was 1.73 Gy (0.24 ± 0.15 Gy/GBq, n = 2).

Red bone marrow ROIs thigh

For ^{177}Lu-rhPSMA-7.3 vs. ^{177}Lu-PSMA-I&T post-treatment the absorbed dose was 1.05 Gy (0.29 ± 0.06Gy/GBq; n = 2) vs. 2.04 Gy (0.28 ± 0.20 Gy/GBq, n = 3).

DISCUSSION

When investigating differences in radiation doses to normal organs the kidney is usually regarded as the dose limiting organ at risk. Pre-therapeutic kidney doses in our six mCRPC patients were 0.7±0.2 Gy/GBq for ^{177}Lu-PSMA-I&T and 1.7±0.3 Gy/GBq for ^{177}Lu-rhPSMA-7.3, which is ca. 2.3 times higher compared with ^{177}Lu-PSMA-I&T. However, for pre-therapeutic measurements the approximately equivalent amount of radioactivity (1 GBq ^{177}Lu) was administered. The higher absorbed kidney dose of ^{177}Lu-rhPSMA-7.3 is consistent with preclinical data (*2*). Compared to previous dosimetry results, the range of absorbed kidney dose for ^{177}Lu-PSMA-617 is between 0.4±0.2 Gy/GBq and 0.8±0.3 Gy/GBq (*3-7*). For ^{177}Lu-PSMA-I&T the absorbed kidney dose was reported at 0.7±0.2 Gy/GBq (*8*). Taking together, our pre-therapeutic kidney doses for ^{177}Lu-PSMA-I&T are within the range of previous results.

Comparison of absorbed doses to the salivary and lacrimal glands exhibited the highest ratios (2.8-3.2) between ^{177}Lu-rhPSMA-7.3 and ^{177}Lu-PSMA-I&T. However, dosimetry of these organs is known to be highly variable which is very likely based on the difficult assessment of size. E.g. our data for ^{177}Lu-PSMA-I&T are 50% lower (1.92 vs. 3.8 Gy/GBq) compared with a previous report from our group using exactly the same methodology (*8*). Despite numerical high absorbed doses, salivary and parotid glands clinically relevant toxicity is only anecdotical reported and mainly transient (*9*).

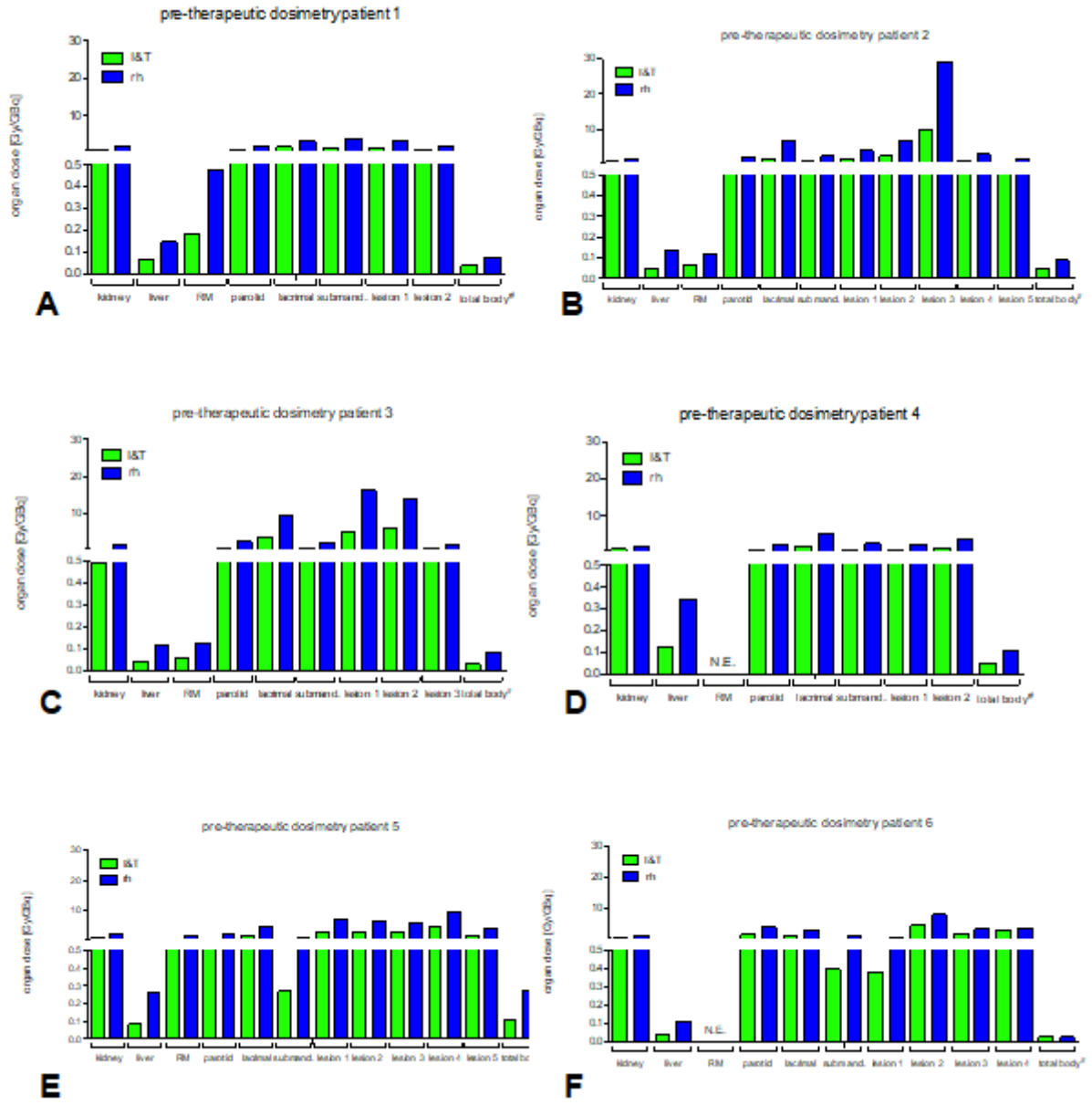
In tumor lesions, a high variability of absorbed doses was observed similar to data reported for ^{177}Lu-PSMA-I&T (*8*) and ^{177}Lu-PSMA-617 (*3,4,6,7*). In total, an effective dose of 6.44±6.66 mGy/MBq for ^{177}Lu-rhPSMA-7.3 and 2.64±2.24 mGy/MBq for ^{177}Lu-PSMA-I&T was delivered to tumor lesions. In detail, effective doses of 4.09±2.57 mGy/MBq vs. 1.70±1.13 mGy/MBq and 11.14±8.83 mGy/MBq vs. 4.51 ±

2.69 mGy/MBq were delivered to bone and lymph node metastases for ^{177}Lu-rhPSMA-7.3 vs. ^{177}Lu-PSMA-I&T, respectively.

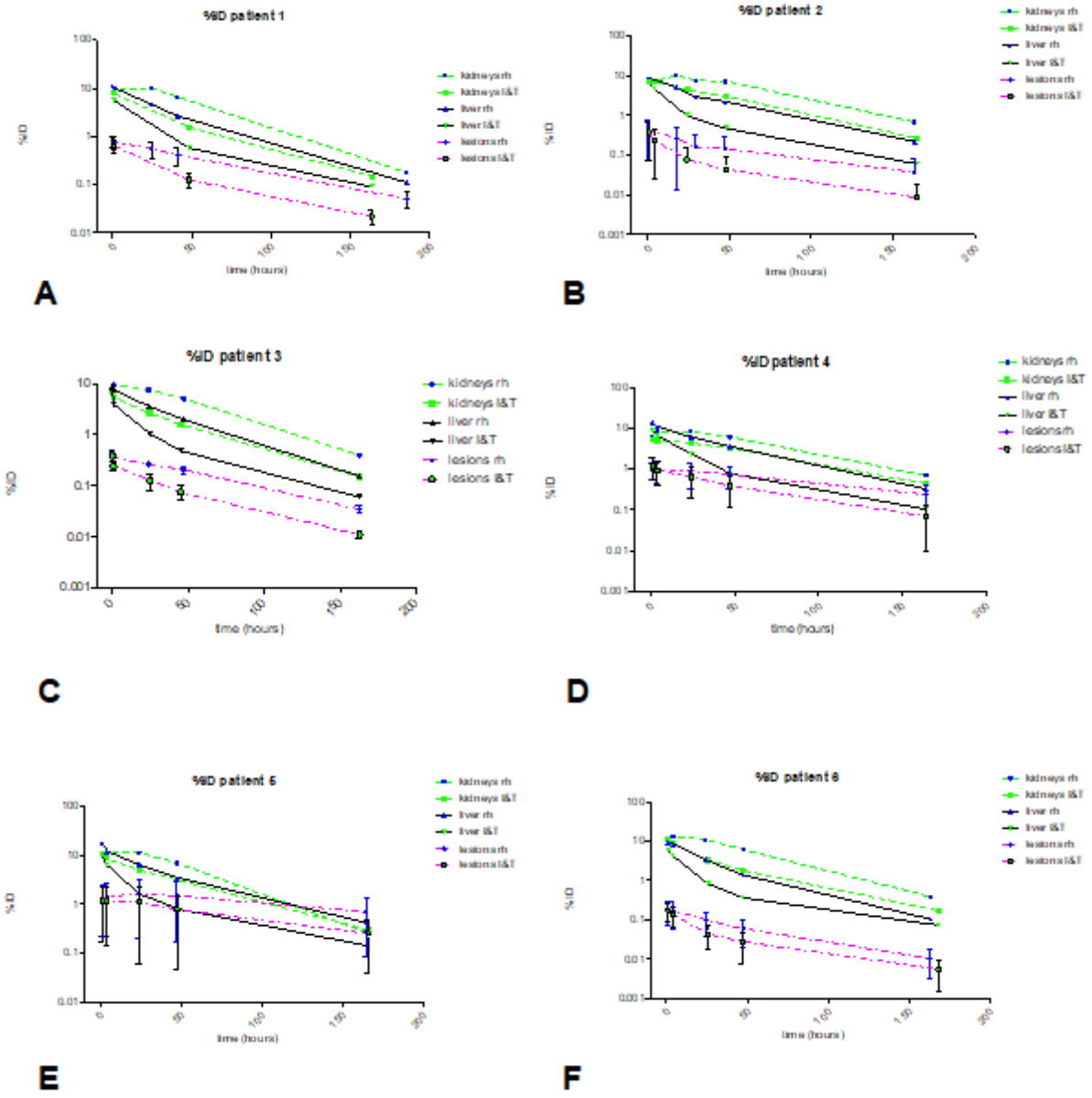
Notably, in comparison to literature our data for ^{177}Lu-PSMA-I&T show slightly lower numbers. However, for dosimetry analyses, tumor lesions with high uptake are usually analyzed as they show better delineation from the surrounding healthy tissue and thus a relatively high absorbed dose. Our retrospective study evaluated patients with mild progression after the previous mCRPC line and limited number of tumor lesions. Hence, we could not select specifically hot lesions as the number of lesions was limited.

At present two different routes of production of ^{177}Lu are commonly used- a direct and an indirect process. The direct route comprises irradiation of an ^{176}Lu enriched target with thermal neutrons in a nuclear reactor and dissolution, whereby a low amount of carrier added, metastable ^{177m}Lu is produced (*10*). It has a long half-life of approximately 160.4d, making the waste disposal and management thereof expensive. Following the indirect way with irradiation of a ^{176}Yb target with thermal neutrons in a reactor, separation and dissolution, non-carrier added ^{177}Lu can be produced, which has a half-life of ca. 6.7d and is less challenging in terms of radioactive waste management (*10*).

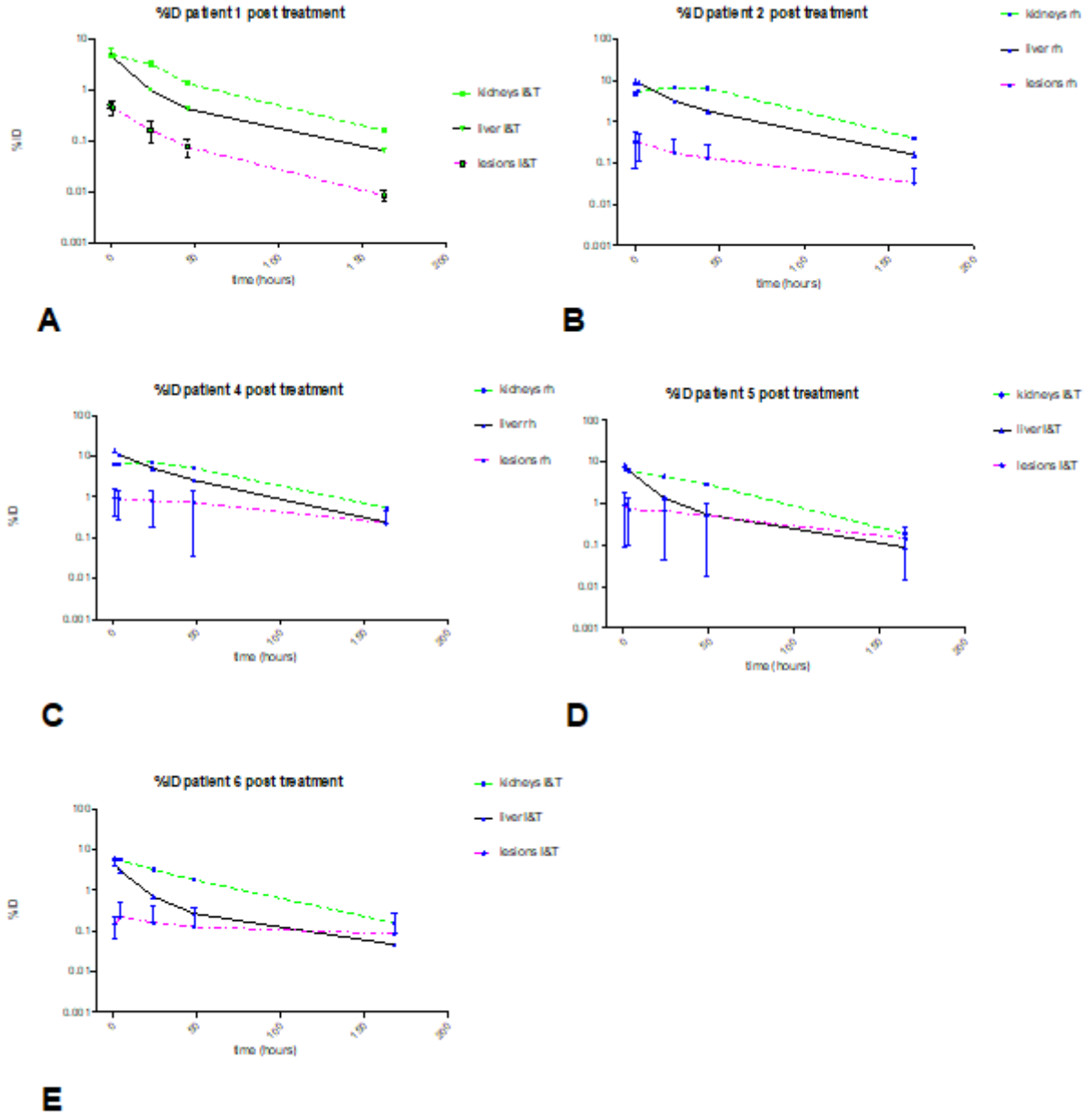
Supplemental Figure 1: Pre-therapeutic organ doses [Gy/GBq] in kidneys, liver, parotid, lacrimal and submandibular glands, tumor lesions and effective dose of the total body [Sv/GBq] determined with ^{177}Lu-rhPSMA-I&T (I&T) and ^{177}Lu-rhPSMA-7.3 (rh) for patients (A-F)**.** # = Sv/GBq



Supplemental Figure 2: Pre-therapeutic %injected dose (%ID) displayed on a semilogarithmic scale for kidneys, liver and tumor lesions pre-treatment for all patients with ^{177}Lu-PSMA-I&T (I&T) and ^{177}Lu-rhPSMA7.3 (rh) (A-F)



Supplemental Figure 3: Post-therapeutic %injected dose (%ID) displayed on a semilogarithmic scale for kidneys, liver and tumor lesions pre-treatment for five patients (no post-therapy scintigraphies are available for patient 3) with ^{177}Lu-PSMA-I&T (I&T) and ^{177}Lu-rhPSMA7.3 (rh) (A-E)



	Age [years]	iPSA [ng/mL]	LDH [U/l]	AP [U/l]	PSA baseline [ng/mL]	Gleason score	Site of metastases	Previous treatments
1	67	16.7	337	67	10.0	9	B	E, A, D + Nivolumab
2	67	6.2	188	49	11.8	10	B, LN	A, E, D
3	67	360	265	68	31.3	n.a.	B, LN	D, A
4	73	8.9	202	46	15.7	9	B, LN	A, E, D
5	69	630	181	46	120	n.a.	B	D, A, E + Nivolumab
6	65	255.4	209	59	1.5	8	B, LN	D, A, Pembrolizumab + Olaparib

Supplemental Table 1: Patient characteristics, LN = Lymph nodes, B = Bones, n.a. = not available, D = Docetaxel, A = Abiraterone, E = Enzalutamide
iPSA = initial PSA, PSA = Prostate-Specific Antigen, LDH = Lactate Dehydrogenase, AP = Alkaline Phosphatase

		Lesion size (ml)
Patient 1		
1	B	21.3
2	B	18.2
Patient 2		
1	B	1.6
2	B	0.9
3	LN	0.2
4	B	7.6
5	B	39.3
Patient 3		
1	LN	1.3
2	LN	2.1
3	B	13.7
Patient 4		
1	B	75
2	LN	10
Patient 5		
1	B	82
2	B	73
3	B	35
4	B	3
5	B	2.8
Patient 6		
1	B	1.14
2	LN	4.3
3	LN	2
4	LN	1

Supplemental table 2: Lesion sizes of tumor lesions based on PSMA-ligand positive tumor volume derived from the pretherapeutic ^{18}F-rhPSMA-7.3-PET. B = Bones, LN = Lymph nodes.

Organ	^{177}Lu-rhPSMA-7.3 (rh)	^{177}Lu-PSMA-I&T (I&T)	ratio rh/I&T
Total body (mSv/MBq)			
mean	0.12	0.05	2.24
SD	0.07	0.03	
upper range	0.28	0.11	
lower range	0.07	0.03	
Kidneys (mGy/MBq)			
mean	1.65	0.73	2.25
SD	0.28	0.18	
upper range	2.13	0.95	
lower range	1.33	0.49	
Liver (mGy/MBq)			
mean	0.19	0.07	2.74
SD	0.09	0.03	
upper range	0.34	0.12	
lower range	0.12	0.04	
Parotid glands (mGy/MBq)			
mean	2.35	0.80	2.93
SD	0.78	0.41	
upper range	4.01	1.71	
lower range	1.70	0.56	
Lacrimal glands (mGy/MBq)			
mean	5.29	1.92	2.75
SD	2.16	0.80	
upper range	9.24	3.70	
lower range	2.93	1.45	
Subman. glands (mGy/MBq)			
mean	2.10	0.67	3.15
SD	0.86	0.31	
upper range	3.54	1.24	
lower range	1.00	0.27	
Red bone marrow (thigh ROI) * (mGy/MBq)			
mean	0.67	0.30	2.23
SD	0.62	0.27	
upper range	1.97	0.87	
lower range	0.20	0.10	
Red bone marrow (four patients) ** (mGy/MBq)			
mean	0.55	0.22	2.49
SD	0.56	0.21	
upper range	1.49	0.58	
lower range	0.11	0.06	
Red bone marrow (only patients 2 and 3) ** (mGy/MBq)			
mean	0.12	0.06	1.98
SD	0.007	0.004	

upper range	0.13	0.07
lower range	0.11	0.06

Supplemental Table 3: Pre-therapeutic effective dose for Whole Body in mGy/MBq and mSv/MBq respectively and Absorbed doses for Normal Organs in mGy/MBq and its ratios for all six patients. SD = standard deviation.

* Calculation using a ROI in the thigh to obtain values from all patients. Values are displayed separately **Please note that in patients 1 and 5, bone metastases were present in the area used for bone marrow dosimetry leading to a clear overestimation of these doses. Evaluation of patients 4 and 6 using a ROI next to the lumbar spine resulted in negative values, therefore resulting in wrong values.

Organ	^{177}Lu-rhPSMA7.3 (rh) n = 2	^{177}Lu-PSMA-I&T (I&T) n = 3
Total Body (mSv/MBq)		
mean	0.09	0.04
SD	0.002	0.03
upper range	0.10	0.08
lower range	0.09	0.02
Kidneys (mGy/MBq)		
mean	1.59	0.63
SD	0.02	0.19
upper range	1.61	0.90
lower range	1.57	0.47
Liver (mGy/MBq)		
mean	0.20	0.05
SD	0.08	0.01
upper range	0.27	0.06
lower range	0.12	0.03
Parotid Glands (mGy/MBq)		
mean	1.59	0.46
SD	0.27	1.47
upper range	1.86	5.40
lower range	1.32	2.25
Lacrimal Glands (mGy/MBq)		
mean	3.82	0.82
SD	0.05	0.20
upper range	3.87	1.07
lower range	3.77	0.57
Submandibular Glands (mGy/MBq)		
mean	1.97	0.30
SD	0.16	0.43
upper range	2.13	1.24
lower range	1.81	0.27
RM (5 pts)* **(mGy/MBq)**	n = 2	n= 3
mean	0.29	0.28
SD	0.06	0.20
upper range	0.35	0.55
lower range	0.24	0.07
RM (3 pts)** n= 1 **(mGy/MBq)**		n=2
mean	0.14	0.24
SD	0	0.15
upper range	0.14	0.38
lower range	0.14	0.09
Tumor Lesions (mGy/MBq)		
mean	6.64	1.44

SD	8.71	0.76
upper range	27.65	2.72
lower range	1.29	0.23

Pts=patients; RM = red bone marrow
*Calculation using a ROI in the thigh for bone marrow correction.
**Calculation using a ROI next to the lumbar spine for bone marrow correction. Please note that in patients 1 and 5 bone metastases were present in the area used for bone marrow dosimetry leading to a clear overestimation of these doses. Patients 4 and 6 were not evaluable.

Supplemental Table 4: Post-treatment effective dose for Whole Body in mSv/MBq in 2 patients treated with ^{177}Lu-rhPSMA7.3 (rh) and 3 patients treated with ^{177}Lu-PSMA-I&T (I&T) respectively and absorbed doses for normal organs in mGy/MBq and its ratios for five patients. Patient 3 did not receive a posttreatment dosimetry.

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