

Supplementary Materials for

First-in-human uPAR PET: imaging of cancer aggressiveness

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Supplementary Materials

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Figure S1

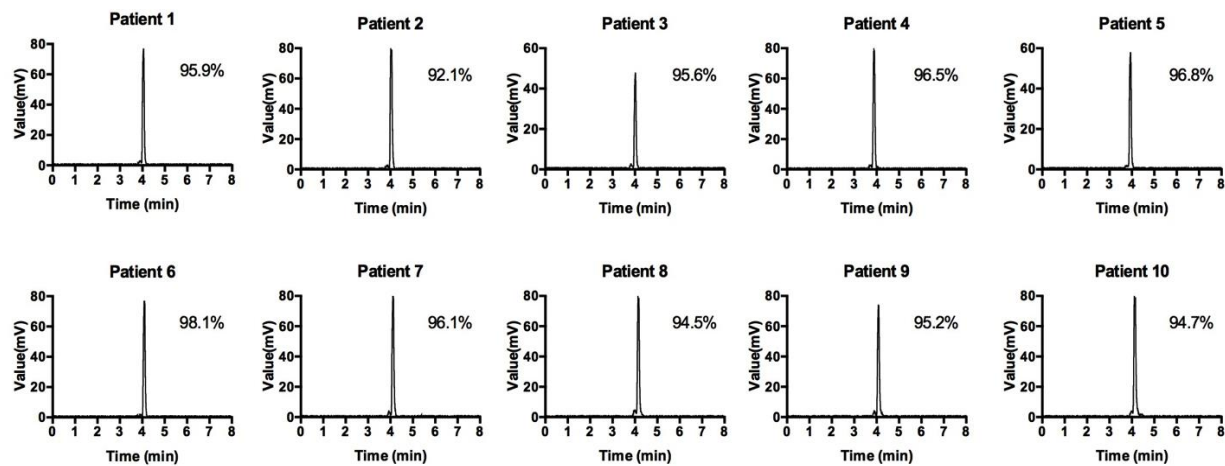


Fig. S1. Batch chromatograms and purity. RP-HPLC Chromatograms, including purity, for all 10 batches of ^{64}Cu -DOTA-AE105 used for injection in the study.

Figure S2

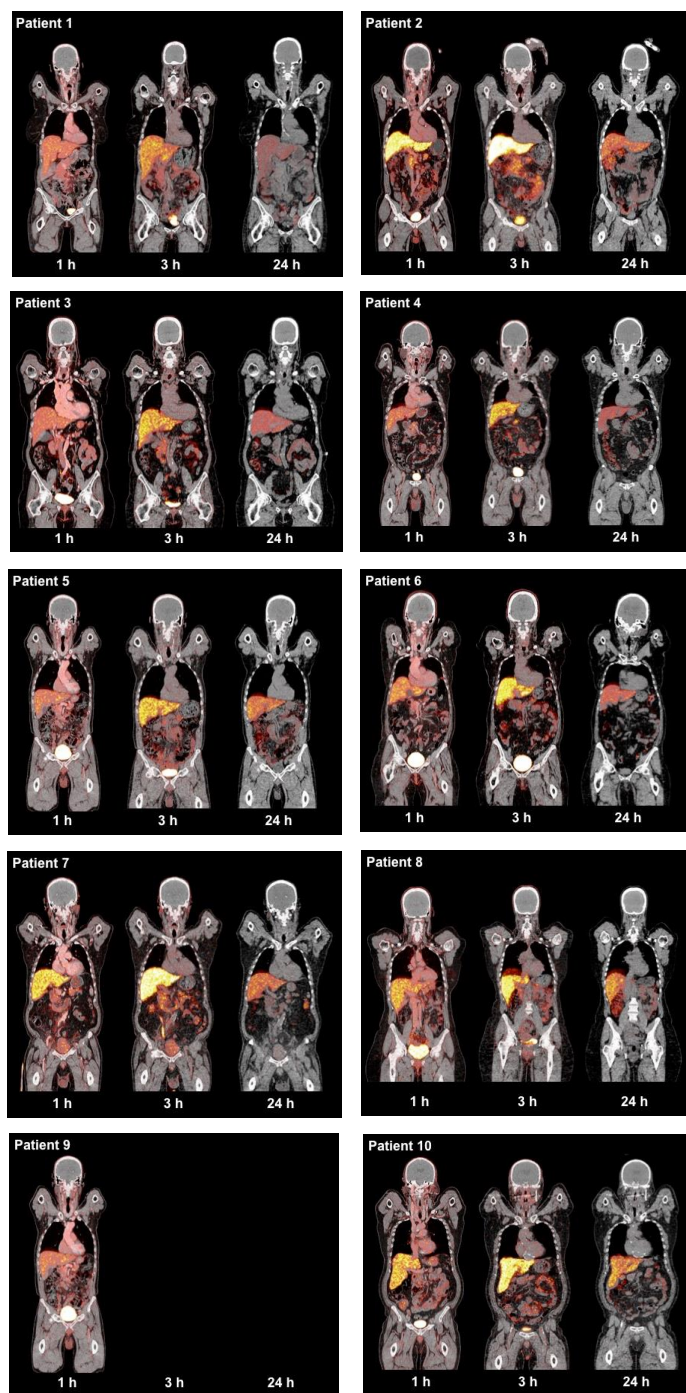


Fig. S2. Serial Whole-body PET/CT images of the biodistributions of ^{64}Cu -DOTA-AE105. PET/CT fusion images for all 10 patients acquired at 1, 3 and 24 hours following i.v. injection of ^{64}Cu -DOTA-AE105.

Figure S3

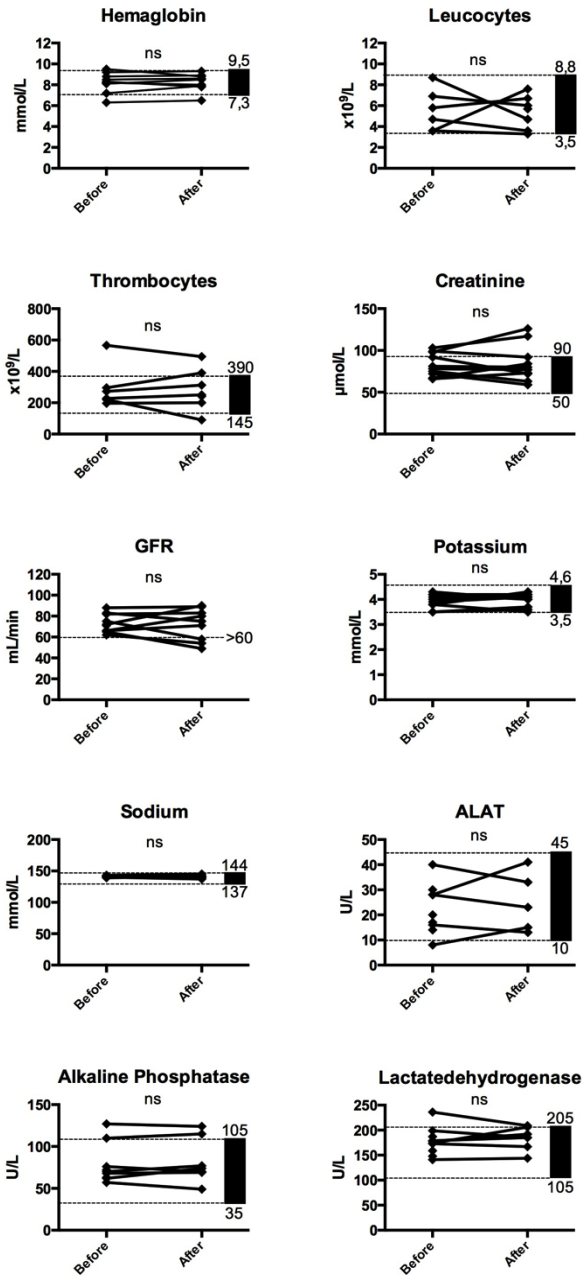
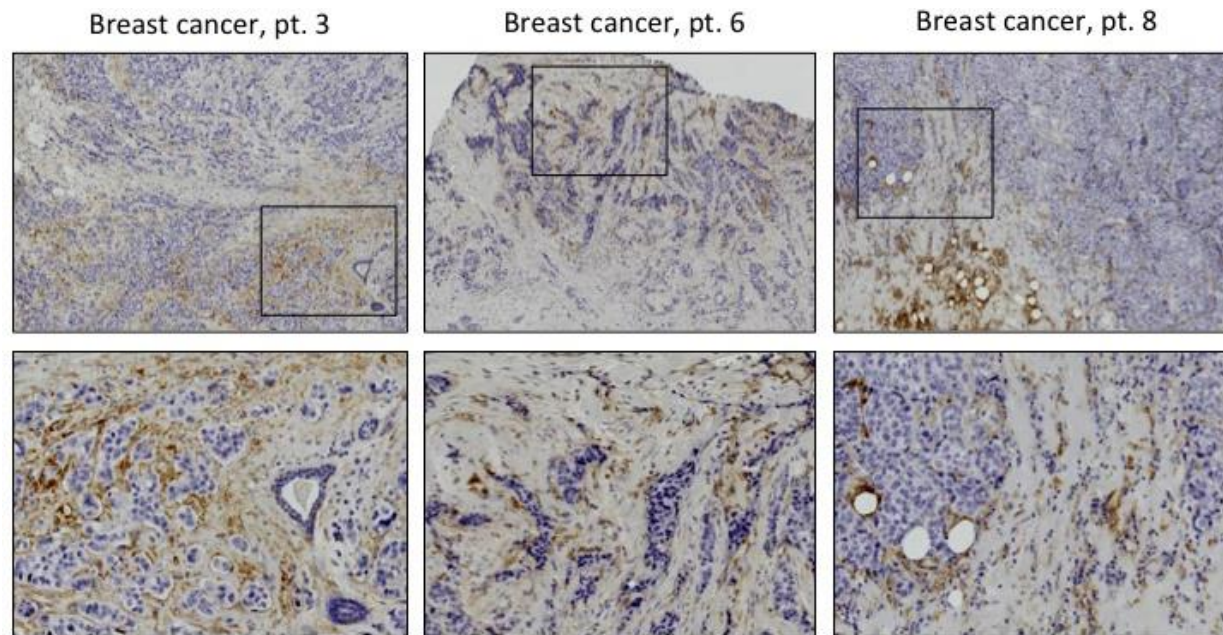


Fig. S3. Blood biochemistry parameters before and after the study in each patient. Individual plot of hemoglobin, leucocytes, thrombocytes, creatinine, estimated glomerular filtration rate (eGFR), potassium, sodium, alanine aminotransferase (ALAT), alkaline phosphatase and lactate-dehydrogenase before and after PET/CT scan with ^{64}Cu -DOTA-AE105. No significant differences in any parameter were found. Black bars in the right side of the figure with top-bottom values indicate reference interval.

Figure S4

A



B

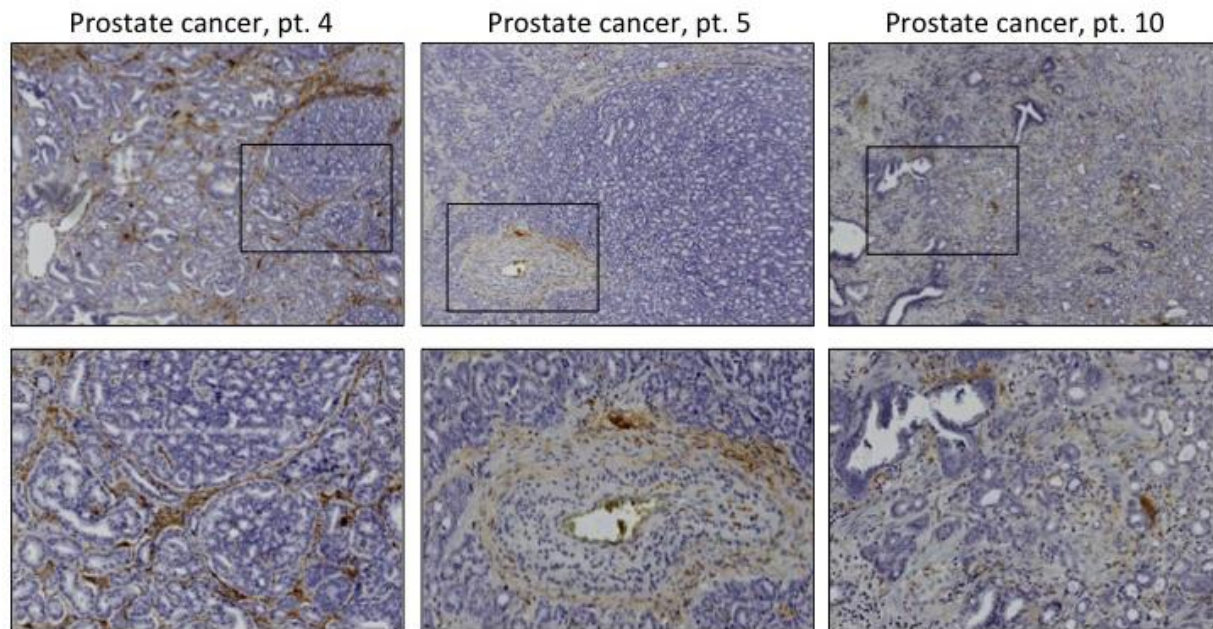


Fig. S4. uPAR immunohistochemistry staining of tumor tissue. (A) Representative uPAR stained slides for each breast cancer patient. uPAR expression was confirmed in all three patients. (B) Representative uPAR stained slides for each prostate cancer patients. uPAR expression was confirmed in all three tumors.

Figure 5S

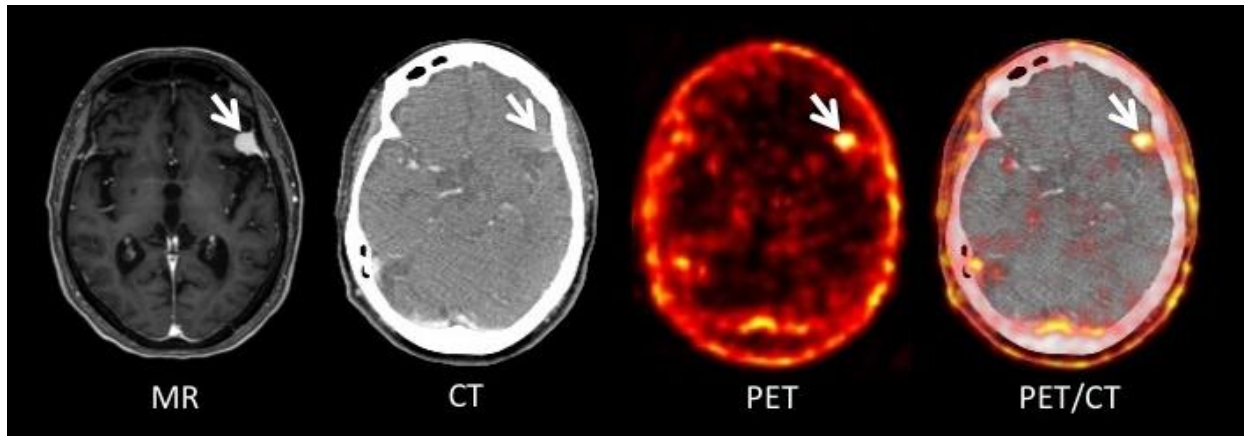


Fig. S5. MR and uPAR PET/CT of meningioma in patient 6. Transversal contrast-enhanced T1 weighted MRI followed by CT, uPAR PET and co-registered PET/CT image of a brain lesion (meningioma, white arrow). High uptake of activity was found in patient 6 with an SUVmean and SUVmax of 2.83 and 4.29, respectively.

Figure S6

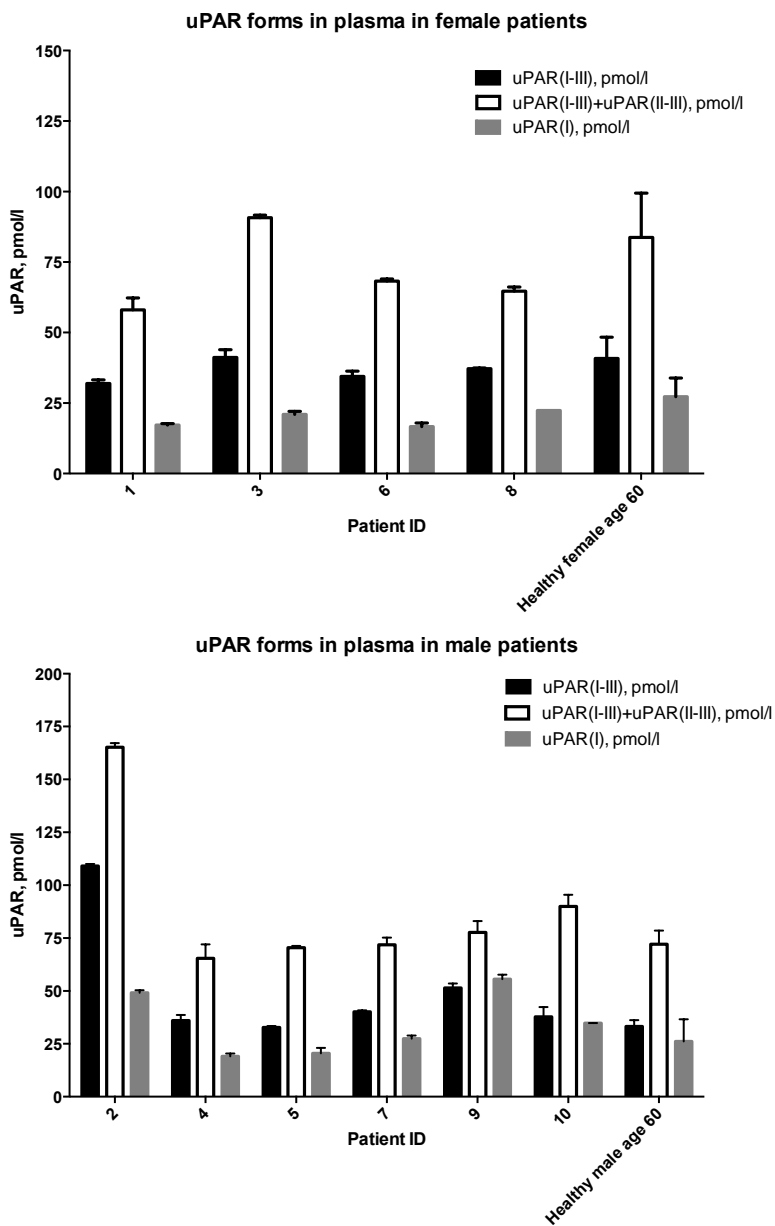
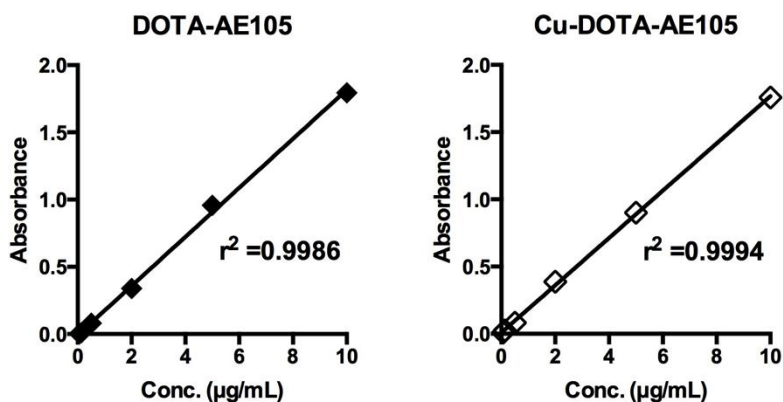


Fig. S6. Soluble uPAR forms in plasma. Circulating uPAR forms were measured in EDTA plasma inform blood samples taken from each patient at the time of injection of ^{64}Cu -DOTA-AE105. Data are presented in female (top) and male (bottom) patients as mean level with %CV values. The geometric mean levels for healthy males and females at age 60 are presented for comparison. uPAR(I-III): full length uPAR, uPAR(II-III): uPAR domain II + domain III, uPAR(I): uPAR domain I.

Figure S7

A Linearity



B Accuracy

DOTA-AE105

Expected concentration (µg/ml)	Found concentration (µg/ml)	Recovery (%)
1.0	0.99	99.3
2.0	1.90	95.0
3.0	3.05	101.5
5.0	5.02	100.5
10.0	10.4	103.6

Specification: recovery: 90-110%

Cu-DOTA-AE105

Expected concentration (µg/ml)	Found concentration (µg/ml)	Recovery (%)
1.0	0.99	99.4
2.0	1.99	99.7
3.0	2.96	98.7
5.0	4.59	91.9
10.0	10.3	102.8

Specification: recovery: 90-110%

C Sensitivity

DOTA-AE105: LOD (S/N=3) = 0.06 µg/ml LOQ (S/N = 10) = 0.125 µg/ml

Cu-DOTA-AE105: LOD (S/N=3) = 0.03 µg/ml LOQ (S/N = 10) = 0.06 µg/ml

Fig. S7. Chemical analysis validation (A) Linearity after analysis using HPLC. (B) Accuracy and (C) sensitivity of the HPLC analysis. All tests were within specification limits.

Table S1: Injected dose, mass, specific activity and purity for each batch

Pt. #	Dose (MBq)	Batch activity (MBq)	SA (GBq/ μ mol)	Purity (%)	Mass (μ g)
1	205	1376	341	95.9	1.01
2	208	649	285	92.1	1.22
3	200	421	232	95.6	1.44
4	201	1141	206	96.5	1.63
5	197.5	552	190	96.8	1.74
6	201.1	697.1	323	98.1	1.04
7	197.3	674.8	266	96.1	1.24
8	211.6	803.9	340	94.5	1.04
9	209	700.1	262	95.2	1.34
10	212.8	819.5	362	94.7	0.98
MEAN	204.3	783.4	280.7	95.6	1.27
SD	5,7	280.9	59.9	1.6	0.27

Table S2: Patient Hematology/biochemistry data																				
	Patient 1		Patient 2		Patient 3		Patient 4		Patient 5		Patient 6		Patient 7		Patient 8		Patient 9		Patient 10	
Parameter	Before	After	Before	After	Before	After	Before	After	Before	After	Before	After	Before	After	Before	After	Before	After	Before	After
Hemaglobin (mmol/L)	6.3	6.5	7.2	7.9	8.3	7.8	8.5	8.6	9.2	9.3	8.1	8.5	9.5	8.8	8.2	8	8.8	8.9	8.3	-
Leucocytes (x10 ⁹ /L)	3.6	7.6	3.6	3.3	5.8	6.7	-	5.7	-	-	4.7	3.6	8.7	4.7	6.9	6	-	-	-	-
Thrombocytes (x10 ⁹ /L)	566	494	295	390	227	250	-	241	-	-	196	200	220	90	272	312	-	-	-	-
Creatinine (μmol/L)	72	59	103	117	67	84	92	72	81	80	75	63	98	126	66	73	78	77	99	92
GFR (ml/min per 1.73 m ²)	72	90	62	54	75	58	71	-	82	83	66	80	65	49	83	75	88	89	66	71
Potassium (mmol/L)	4.3	4	4.2	4	3.8	4.3	3.9	4.1	4.2	4.2	3.5	3.7	3.8	3.5	4	4.1	3.5	3.6	4.1	4.2
Sodium (mmol/L)	139	141	140	137	143	142	141	139	140	142	143	145	141	139	140	139	140	141	142	141
ALAT (U/L)	-	-	28	23	40	33	17	-	20	-	8	15	16	13	28	41	30	-	14	-
Alkaline Phosphatase (U/L)	68	69	110	115	127	124	57	49	72	-	76	70	70	77	62	74	63	-	27	-
Lactatedehydrogenase (U/L)	179	185	178	192	199	185	173	167	187	-	174	206	236	209	141	144	159	-	148	-

- Value is missing

Table S3: Blood pressure and pulse during PET/CT scans

Time	Before			1 min post injection			10 min post injection			1 hour post injection			3 hours post injection			24 hours post injection		
Patient #	Systolic	Diastolic	Pulse	Systolic	Diastolic	Pulse	Systolic	Diastolic	Pulse	Systolic	Diastolic	Pulse	Systolic	Diastolic	Pulse	Systolic	Diastolic	Pulse
1	120	86	69	132	88	72	122	81	72	124	85	73	129	77	74	73*	38*	99*
2	179	89	65	-	-	-	-	-	-	-	-	-	-	-	-	181	90	67
3	137	86	67	143	92	65	156	94	70	156	94	70	162	84	50	144	81	55
4	130	80	76	102	98	86	137	90	75	144	88	76	141	93	87	132	82	72
5	164	94	54	152	92	52	162	96	54	160	91	53	153	86	56	149	96	58
6	170	74	86	170	74	86	138	95	80	143	84	84	126	80	86	146	84	78
7	161	84	70	163	90	68	156	87	67	-	-	-	161	84	70	156	85	72
8	141	80	84	-	-	-	124	77	74	-	-	-	-	-	-	-	-	-
9	191	97	87	190	103	88	181	89	82	208	105	96	Scan not performed			Scan not performed		
10	161	70	68	165	75	67	153	69	64	175	77	69	165	75	67	126	65	63

*Patient 1 was diagnose with a urinary tract injection on the same morning the 24 hour scan was performed and had high fever.

- Value is missing.

Table S4: Observed and predicted dosimetry for uPAR PET

Organ/Tissue	Mean absorbed dose (mGy/MBq)	
	<i>Observed</i>	<i>Predicted*</i>
Adrenals	3,10E-02	2,59E-02
Brain	2,57E-03	2,27E-02
Breast	9,05E-03	2,11E-02
Gallbladder wall	5,19E-02	2,83E-02
Lower large intestine wall	3,27E-02	2,50E-02
Small intestine	4,87E-02	4,92E-02
Stomach wall	2,32E-02	2,52E-02
Upper large intestine wall	4,82E-02	2,69E-02
Heart wall	5,02E-02	7,86E-02
Kidneys	5,62E-02	2,97E-02
Liver	1,75E-01	6,38E-02
Lungs	1,95E-02	1,81E-02
Muscle	1,10E-02	1,28E-02
Ovaries	1,33E-02	2,56E-02
Pancreas	5,55E-02	2,68E-02
Red marrow	1,43E-02	2,41E-02
Osteogenic cells	2,60E-02	5,48E-02
Skin	7,75E-03	1,89E-02
Spleen	2,04E-02	2,88E-02
Testes	8,64E-03	2,14E-02
Thymus	1,01E-02	2,41E-02
Thyroid	1,66E-02	2,21E-02
Urinary bladder wall	4,60E-02	2,93E-02
Uterus	1,31E-02	-
Total body	1,78E-02	2,58E-02
Effective dose (mSv/MBq)	2,76E-02	2,51E-02

*Based on data from mice (*Persson et al. 2013, ref. 24*)

- Value is missing.

Table S5: Raw data for dosimetry estimation

	Bq/mL																	
Patient ID	#3			#4			#5			#6			#8			#10		
Time (h)	1	3	24	1	3	24	1	3	24	1	3	24	1	3	24	1	3	24
Brain	309.7	90.7	28.6	156.6	69.4	69.7	234.7	108.5	42.3	366.2	150.8	31.2	170.8	72.8	36.8	145.8	131.8	37.6
Thyroid	4567.8	2647.3	382.1	1979.6	1736.5	694.8	2571.1	1789.5	351.1	3229.7	2337.3	486.7	3039.6	1422.5	403.3	3194.5	1479.8	287.3
Lung	1483.4	559.6	126.5	979.6	449.7	290.2	705.6	977.0	81.7	1449.9	588.9	144.5	1367.5	507.9	161.6	588.1	274.4	89.6
Heart	5728.1	1722.3	458.7	4074.7	1704.3	505.6	3576.3	1228.7	360.8	6719.7	2481.2	535.3	5268.6	1526.2	297.4	4013.5	1391.5	366.2
Liver	8017.7	12095.6	6001.1	8289.5	11776.3	5594.5	6461.5	10562.9	7484.8	13563.3	20432.9	9971.3	11956.3	17309.5	7720.9	10866.1	17076.5	9285.2
Gall Bladder	531.4	1442.3	653.9	-	-	-	3040.6	3875.9	4066.5	-	-	-	-	-	-	5403.8	5632.2	3645.1
Spleen	3358.7	1597.9	649.7	2678.4	1578.3	573.3	2530.2	1186.7	473.8	3792.7	1957.6	703.3	2978.2	1686.8	492.3	2493.5	1524.7	410.1
Adrenal	3958.8	2508.6	766.3	2560.9	2797.2	936.6	2947.3	2691.4	737.0	3886.9	3554.9	1080.5	1798.3	1999.1	836.7	1690.9	1557.1	618.2
Pancreas	4566.9	4285.0	1548.7	7553.6	9686.7	3196.2	5628.2	4949.3	1340.3	7230.5	7238.8	1296.4	7380.4	4042.2	1586.1	6180.3	7371.7	741.7
Stomach cont.	6585.2	558.8	942.5	3620.4	138.5	943.6	3175.0	395.1	1241.9	5076.6	1089.4	399.5	4273.1	441.8	166.4	4958.7	938.8	683.1
ULI*	1935.5	1649.1	2734.5	1307.8	804.1	369.2	1624.8	1750.7	2433.6	2160.1	4522.7	2464.6	1996.8	1906.6	1784.4	1261.4	1785.1	3571.4
SI	3086.8	2430.8	1986.7	2206.3	1285.8	569.1	2128.8	2378.0	2305.7	2008.3	4277.4	1205.2	2428.7	3408.3	1495.1	3041.1	2445.1	1070.6
LLI"	1828.4	1472.3	740.6	887.5	907.1	1604.2	2001.7	1148.2	957.1	1848.2	1970.5	1545.4	1663.9	1688.2	600.3	2155.2	1448.4	1873.1
Red marrow	1040.5	737.6	278.6	926.1	878.1	557.9	2049.0	1114.2	507.7	1656.2	1612.9	490.0	1517.7	1444.0	327.2	1156.7	960.3	494.1
Kidney	9643.0	6843.7	1290.3	7285.8	5628.2	1220.8	7486.2	5306.0	2945.0	10443.4	6999.2	1650.0	9967.6	8590.9	1133.1	8611.3	4951.6	1741.4
Muscle	929.1	727.2	166.4	1317.5	771.0	208.7	1112.4	721.2	164.9	1292.2	579.5	235.0	1095.6	673.7	229.8	1558.0	1480.6	185.6
Bone	574.5	405.9	107.9	613.5	454.7	124.9	453.8	327.5	60.3	1099.7	625.6	139.2	763.8	516.5	95.7	560.5	260.7	107.3
Urine Bladder	74650.7	87039.5	230.7	124119.7	92533.9	198.5	41123.9	40333.9	45.1	143337.2	56450.5	316.1	29442.1	67343.0	55.5	74438.1	28639.0	34.5

* Upper Large Intestine

^ Small Intestine

" Lower Large Intestine

- Missing value

Table S6 - Process validation batches result

Batch no.	Volume (ml)	Activity (MBq)	Appearance	Radiochemical purity (%)		pH		DOTA-AE105 (mg/ml)	Cu-DOTA-AE105 (mg/ml)	Total DOTA-AE105 content (incl. Cu and other metals) (mg/ml)	Radionuclidic purity			Ethanol (%)	Specific radioactivity (GBq/mmol)	Sterility	Endotoxins (EU/ml)
				EOS	EOS 20 hours	EOS	EOS 20 hours				t _{1/2} (min)	Gamma spectrum	Gamma spektrum after >2 weeks				
1	9.5	1115	Clear and colourless solution, free from visible particulates or cloudiness	93.3	93.7	6.3	5.64	0.07	0.07	0.51	12.6	Pass	Not different to background spectrum	6.2	385	Complies	< 0.0250
2	9.3	840	Clear and colourless solution, free from visible particulates or cloudiness	93.4	92.1	6.29	5.41	0.26	0.12	0.61	12.5	Pass	Not different to background spectrum	6.2	248	Complies	< 0.0500
3	9.4	1300	Clear and colourless solution, free from visible particulates or cloudiness	98	94.3	5.34	5.13	0.17	0.073	0.49	12.6	Pass	Not different to background spectrum	6.67	429	Complies	0.1029

Table S7: Product release criteria	
ID	Specifications
Active Substance	[⁶⁴ Cu]DOTA-AE105 Radioactive half-life: 12.4 – 13.0 hours Gamma Spectrum shows only 511 and 1022 keV peaks
Radioactivity	200 MBq to 1.3 GBq
Appearance	Clear and colourless solution, free from visible particulates or cloudiness
Volume	10 ± 0.5 ml
Half-life (T _{1/2})	T _{1/2} = 12.4-13.0 hours
Radionuclidic purity	>99.9% (<0.1% radionuclidic impurities)
Radiochemical purity	>91 %
pH	Between 4.5 and 8.5
Microbiology	Passes Test for Sterility Ph.Eur.
Microbiology	Endotoxins < 0.125 IU/mL
Microbiology	Bioburden: <10 c.f.u./100 ml
Shelf Life	20 hours
Ethanol	3-10 % (w/v)
DOTA-AE105 content	≤ 1 µg/mL (maximal injected dose of 1.5 µg)
Cu-DOTA-AE105 content	≤ 1 µg/mL (maximal injected dose of 1.5 µg)
Total DOTA-AE105 content (incl. Cu and other metals)	≤ 1 µg/mL (maximal injected dose of 1.5 µg)
Specific Activity at end of synthesis:	> 20 GBq/µmol