Supplementary Material

In situ Generated ^{212}Pb-PSMA Ligand in a ^{224}Ra-Solution for Dual Targeting of Prostate Cancer Sclerotic Stroma and PSMA-positive Cells

Vilde Yuli Stenberg[1,2,3], Asta Juzeniene[1,*], Øyvind Sverre Bruland[3,4] and Roy Hartvig Larsen[2]

[1]*Department of Radiation Biology, Institute for Cancer Research, Norwegian Radium Hospital, Oslo University Hospital, Oslo, Norway;* [2]*Nucligen AS, Oslo, Norway;* [3]*Institute for Clinical Medicine, University of Oslo, Oslo, Norway;* [d]*Department of Oncology, Norwegian Radium Hospital, Oslo University Hospital, Oslo, Norway*

METHODS

Supplementary Table S1. Energy of alphas, betas, X rays and gammas of radionuclides in the ^{224}Ra-series.[1,2]

Radionuclide (Half-Life)	Alphas and Betas, Mean Energy	X Rays and Gammas, Energy and % Abundance
^{228}Th (1.91 years)	α, 5.4 MeV	84 keV, 1.2%
^{224}Ra (3.66 d)	α, 5.6 MeV	241 keV, 4.1%
^{220}Rn (55.60 s)	α, 6.3 MeV	
^{216}Po (0.15 s)	α, 6.8 MeV	
^{212}Pb (10.64 h)	β, 0.1 MeV	75 keV, 10.3% 77 keV, 17.1% 87 keV, 6.0% 90 keV, 1.5% 239 keV, 43.6% 300 keV, 3.3%
^{212}Bi (60.55 min)	α, 6.1 MeV (2.2 MeV effective) β, 0.7 MeV (0.4 MeV effective)	727 keV, 6.7% (4.3% effective)
^{212}Po (0.30 μs)[1]	α, 8.8 MeV (5.6 MeV effective)	
^{208}Tl (3.05 min)[2]	β, 0.6 MeV (0.2 MeV effective)	75 keV, 3.4% (1.2% effective) 511 keV, 22.6% (8.1% effective) 583 keV, 85.0% (30.6 % effective) 860 keV, 12.5% (4.5% effective) 2615 keV, 99.8% (35.9% effective)

[1]64% branching.
[2]36% branching.

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SUPPLEMENTARY RESULTS

Supplementary Table S2. Radiochemical purity (RCP) values of ^{212}Pb and ^{212}Bi after radiolabelling of different concentrations of NG001 or PSMA-617 in the solution of ^{224}Ra in equilibrium with progeny. N, the number of independent experiments.

Ligand Concentration (µg/ml)	Specific Activity (MBq/mg)	RCP (%)			
		^{212}Pb	N	^{212}Bi	N
0	0	1.33±0.06	3		
NG001					
2.5	720-907	16.32±4.24	2		
5	350-533	51.85±12.02	3		
10	10-207	90.75±4.66	4	45.69±0.78	2
12.5	500-1130	91.31±1.61	2		
15	127-178	94.50±0.42	2		
33	99-180	94.04±0.87	7		
45	43-59	95.04±1.45	2		
50	16-338	95.59±0.68	5	66.01±12.27	2
60	79-84	94.36±2.21	2		
80	94-104	94.20±0.21	2		
150	6-14	96.69±0.45	2	79.44±8.96	2
500	2-4	96.22±0.27	2	75.25±9.09	2
PSMA-617					
20-35	105-200	93.96±2.73	4		
45	145-200	96.25±2.98	2		
50-60	90-105	96.53±1.88	2		
80-90	30-45	95.20±1.00	3		

Supplementary Table S3. Radiochemical purity (RCP) values of ^{212}Bi-labelled NG001 in the ^{224}Ra-solution after different time points up to 48 h. N, the number of independent experiments.

Incubation Time	RCP (%)	
	^{212}Bi	N
1 h	58.20±3.50	3
24 h	64.99±1.98	2
48 h	68.85±7.18	2

Supplementary Table S4. Radiochemical purity (RCP) (top) and cell binding fraction (bottom) of ^{212}Pb-labelled NG001 in a solution of radium-224 added no scavenger, 2% L-ascorbic acid, 2% HSA or the two scavengers in combination, after different time points up to 48h, n=2-3 independent experiments.

Incubation Time	RCP (%)			
	No Scavenger	2% L-Ascorbic acid	2% HSA	2% L-Ascorbic Acid and 2% HSA
0.5 h	93.53±1.61	92.70±0.75	92.79±1.55	92.65±1.17
1 h	94.04±1.23	92.16±1.23	92.56±0.89	93.06±0.61
24 h	93.46±0.67	92.67±0.85	91.63±1.16	94.16±0.04
48 h	92.52±1.39	92.84±1.09	89.75±2.12	92.44±1.83
	Cell binding fraction (%)			
Incubation time	No scavenger	2% L-Ascorbic acid	2% HSA	2% L-Ascorbic acid and 2% HSA
0.5 h	61.50±4.50	58.84±0.59	61.54±4.99	62.40±4.62
1 h	57.62±4.59	58.03±2.91	59.51±4.19	61.82±3.78
24 h	45.92±5.46	48.01±1.28	47.31±0.28	61.14±1.77
48 h	40.12±5.31	46.69±1.05	40.44±6.08	56.00±10.24

Supplementary Table S5. Percentage of injected activity per gram of tissue (%ID/g) ± SD of [^{212}Pb]PbCl$_2$, [^{212}Pb]Pb-NG001 and [^{212}Pb]Pb-PSMA-617 at 2 h after intravenously injection of ^{224}Ra&^{212}Pb (A), ^{224}Ra&^{212}Pb-NG001 (B) and ^{224}Ra&^{212}Pb-PSMA-617 (C) in athymic mice bearing human prostate C4-2 cancer xenografts, n=3-5 mice. The ingrowth of the carrier-free ^{212}Pb from ^{224}Ra was calculated and subtracted from the measured counts per minute (CPM) values in tissues and standards before %ID/g values were calculated (upper values). %ID/g values for ingrowth of the carrier-free ^{212}Pb from ^{224}Ra were calculated (lower values). BLOQ, below level of quantification; NA, not available.

Organ	%ID/g of ^{212}Pb without ^{212}Pb Ingrowth from ^{224}Ra (^{212}Pb Ingrowth from ^{224}Ra)			P-value		
	A	B	C	A vs B	A vs C	B vs C
Blood	4.47±0.89 (0.03±0.00)	1.34±0.96 (0.04±0.01)	1.37±0.40 (0.05±0.00)	0.002 (>0.05)	0.005 (>0.05)	>0.05 (>0.05)
Urine	21.42±14.08 (3.15±2.99)	117.08±71.55 (3.97±1.66)	247.30±106.95 (5.86±1.90)	>0.05 (>0.05)	0.014 (>0.05)	>0.05 (>0.05)
Testes	0.41±0.07 (0.02±0.01)	0.73±0.65 (0.04±0.02)	0.72±0.24 (0.04±0.01)	>0.05 (>0.05)	>0.05 (>0.05)	>0.05 (>0.05)
Prostate	1.04±0.36 (0.09±0.03)	1.64±1.66 (0.10±0.03)	2.49±1.39 (0.12±0.01)	>0.05 (>0.05)	>0.05 (>0.05)	>0.05 (>0.05)
Salivary gland	BLOQ (1.61±0.47)	BLOQ (1.67±0.50)	BLOQ (2.15±0.48)	NA (>0.05)	NA (>0.05)	NA (>0.05)
Tumour	1.71±1.29 (0.07±0.03)	15.01±4.73 (0.09±0.02)	18.71±4.78 (0.19±0.11)	0.006 (>0.05)	0.003 (>0.05)	>0.05 (>0.05)
Skin	0.62±0.00 (0.08±0.01)	0.54±0.19 (0.10±0.01)	0.49±0.20 (0.14±0.02)	>0.05 (>0.05)	>0.05 (>0.05)	>0.05 (>0.05)

Kidneys	48.47±7.57 (1.20±0.06)	21.39±9.55 (1.77±0.28)	74.17±25.59 (2.34±0.10)	>0.05 (0.014)	>0.05 (<0.001)	0.002 (0.014)
Liver	8.54±1.54 (0.04±0.01)	3.03±1.66 (0.05±0.01)	1.80±0.29 (0.06±0.01)	0.002 (>0.05)	0.001 (>0.05)	>0.05 (>0.05)
Spleen	0.99±0.48 (1.29±0.46)	BLOQ (1.62±0.33)	BLOQ (2.12±0.60)	NA (>0.05)	NA (>0.05)	NA (>0.05)
Small intestine	2.99±0.47 (0.08±0.02)	0.63±0.46 (0.18±0.14)	0.56±0.26 (0.15±0.10)	<0.001 (>0.05)	<0.001 (>0.05)	>0.05 (>0.05)
Large intestine	2.05±0.30 (0.62±0.30)	0.33±0.47 (0.73±0.38)	0.30±0.18 (0.65±0.09)	<0.001 (>0.05)	0.001 (>0.05)	>0.05 (>0.05)
Stomach	0.39±0.11 (0.09±0.02)	0.18±0.12 (0.11±0.01)	0.19±0.19 (0.14±0.02)	>0.05 (>0.05)	>0.05 (>0.05)	>0.05 (>0.05)
Lungs	3.28±0.20 (0.12±0.01)	1.36±0.59 (0.15±0.03)	1.03±0.64 (0.18±0.09)	0.003 (>0.05)	0.002 (>0.05)	>0.05 (>0.05)
Heart	0.98±0.30 (0.04±0.00)	0.38±0.24 (0.04±0.01)	0.77±0.30 (0.09±0.08)	0.037 (>0.05)	>0.05 (>0.05)	>0.05 (>0.05)
Bladder	1.73±0.10 (0.07±0.02)	4.62±2.65 (0.19±0.12)	5.45±2.52 (0.15±0.10)	>0.05 (>0.05)	>0.05 (>0.05)	>0.05 (>0.05)
Femur	7.66±0.27 (2.24±0.23)	1.32±1.27 (2.71±0.47)	BLOQ (3.14±0.12)	>0.05 (>0.05)	NA (>0.05)	NA (>0.05)
Muscle	0.17±0.02 (0.03±0.00)	0.14±0.02 (0.04±0.01)	0.12±0.02 (0.06±0.01)	>0.05 (>0.05)	>0.05 (>0.05)	>0.05 (>0.05)
Brain	0.20±0.03 (0.01±0.01)	0.13±0.05 (0.02±0.01)	0.13±0.07 (0.10±0.14)	>0.05 (>0.05)	>0.05 (>0.05)	>0.05 (>0.05)
Skull	7.30±0.96 (2.26±0.15)	0.81±0.78 (2.52±0.61)	BLOQ (2.70±0.33)	<0.001 (>0.05)	NA (>0.05)	NA (>0.05)

Supplementary Table S6. Percentage of injected activity per gram of tissue (%ID/g) ± SD of ^{224}Ra at 2 h after intravenously injection of ^{224}Ra&^{212}Pb (A), ^{224}Ra&^{212}Pb-NG001 (B) and ^{224}Ra&^{212}Pb-PSMA-617 (C) in athymic mice bearing human prostate C4-2 cancer xenografts, n=3-5 mice.

Organ	**^{224}Ra %ID/g**			**P-value**		
	A	**B**	**C**	**A vs B**	**A vs C**	**B vs C**
Blood	0.30±0.03	0.31±0.08	0.43±0.01	>0.05	0.043	0.036
Urine	40.97±15.08	27.30±11.05	47.74±15.47	>0.05	>0.05	>0.05
Testes	0.21±0.05	0.31±0.14	0.30±0.05	>0.05	>0.05	>0.05
Prostate	0.81±0.26	0.74±0.28	0.98±0.11	>0.05	>0.05	>0.05
Salivary gland	14.00±4.08	11.60±3.34	17.54±4.03	>0.05	0.152	>0.05
Tumour	0.72±0.13	0.69±0.26	1.17±0.19	>0.05	>0.05	0.043
Skin	0.66±0.09	0.67±0.11	1.11±0.19	>0.05	0.009	0.005

Kidneys	10.41±0.47	12.42±2.94	19.10±0.85	>0.05	>0.05	0.007
Liver	0.33±0.06	0.32±0.06	0.53±0.06	>0.05	0.007	0.003
Spleen	11.23±4.01	11.15±1.46	17.30±5.02	>0.05	0.077	>0.05
Small intestine	0.68±0.15	1.27±1.08	1.23±0.80	>0.05	>0.05	>0.05
Large intestine	5.42±2.61	5.24±2.94	5.34±0.78	>0.05	>0.05	>0.05
Stomach	0.80±0.20	0.76±0.12	1.16±0.18	>0.05	>0.05	0.023
Lungs	1.07±0.08	1.09±0.28	1.43±0.76	>0.05	>0.05	>0.05
Heart	0.32±0.01	0.31±0.10	0.74±0.66	>0.05	>0.05	>0.05
Bladder	0.65±0.13	1.28±0.76	1.26±0.79	>0.05	>0.05	>0.05
Femur	19.40±1.97	18.90±3.74	25.57±0.81	>0.05	>0.05	>0.05
Muscle	0.23±0.01	0.27±0.09	0.45±0.07	>0.05	>0.05	>0.05
Brain	0.11±0.05	0.12±0.07	0.81±1.14	>0.05	>0.05	0.437
Skull	19.62±1.28	17.85±5.45	21.98±2.73	>0.05	>0.05	>0.05

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