Supplemental Figures:

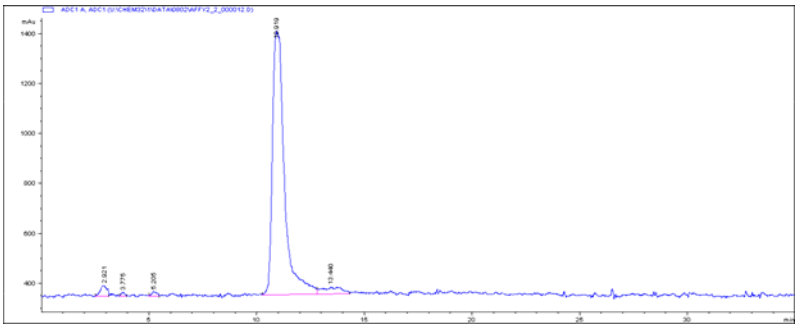

FIGURE 1. HPLC chromatogram of ^{99m}Tc-His6-$Z_{HER2:342}$-Cys. Column: Zorbax Bonus-RP, 4.6 x 250 mm, 5 µm. Solvent A = 0.1% TFA in water; Solvent B = 0.1% TFA in 90/10 acetonitrile/water. Gradient: 20-50% B over 20 minutes.

Animal model comparison using ^{111}In-DOTA-$Z_{HER2:342}$

The biodistribution results of ^{99m}Tc-H_6-$Z_{HER2:342}$-C in SKOV-3-bearing SCID mice (performed at Schering AG) studied at 1, 2 and 4 h p.i., particularly the high liver uptake, deviated from previous data on $Z_{HER2:342}$ biodistribution in BALB/c nu/nu mice. To verify if the deviation was caused by a different animal strain or by the properties of the conjugate, biodistribution results of ^{111}In-DOTA-$Z_{HER2:342}$ in SCID mice were collected at 1, 4 and 24 h p.i. and compared with the published data on biodistribution of ^{111}In-DOTA-$Z_{HER2:342}$ in BALB/c nu/nu mice (Table 2) (*17*).

For preparation of ^{111}In-DOTA-$Z_{HER2:342}$, 37MBq (2.8 µL) of [^{111}In]$InCl_3$ was added to 100 µg DOTA-$Z_{HER2:342}$ acetate salt dissolved in 100 µL 0.2 M ammonium acetate buffer (pH 5.5) and the volume made up to 250 µL with (147.2 µL) 30 mM ammonium acetate buffer / 15 mM ascorbic acid buffer pH 5.5. The reaction solution was heated for 60 minutes at 60°C and purified by RP-HPLC using a water/ethanol gradient. The water/ethanol fraction containing 16 MBq in 3.1 mL was analyzed by TLC (ITLC strips with mobile phase 1% DTPA in water at pH 5.5; ^{111}In product remains at the origin) and RP HPLC using a water (TFA)/acetonitrile gradient (Figure S2, supplemental data). The final sample was used to prepare the solution which was subsequently injected into the animals for biodistribution analysis.

For ^{111}In-DOTA-$Z_{HER2:342}$, the final sample had TLC RCP = 91% and HPLC RCP = 100%.

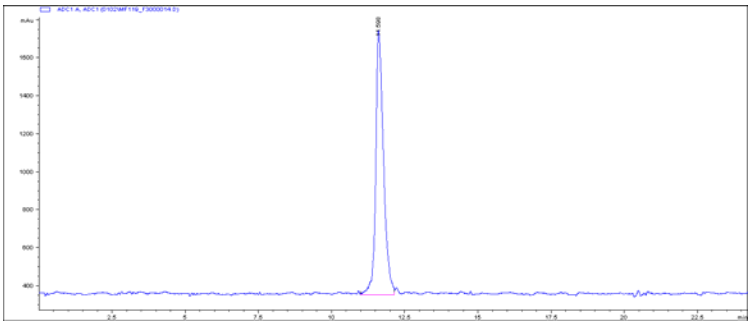

FIGURE 2. HPLC chromatogram of ^{111}In-DOTA $Z_{HER2:342}$. Column: Zorbax Bonus-RP, 4.6 x 250 mm, 5 µm. Solvent A = 0.1% TFA in water; Solvent B = 0.1% TFA in 90/10 acetonitrile/water. Gradient: 10-65% B over 20 minutes. (*note free In-111 does not elute from the HPLC column under the conditions employed, but is detected by the TLC method).

For biodistribution experiments in SKOV-3 xenograft SCID mice, animals were randomized into groups of 3 and injected intravenously with 600 ng ^{111}In-DOTA-$Z_{HER2:342}$ conjugate (94 kBq) in 100 µL PBS.

The general pattern in both strains was characterized by high tumor uptake and rapid clearance of radioactivity from the blood and majority of organs and tissues except kidneys (Table S1). The liver uptake of ^{111}In-DOTA-$Z_{HER2:342}$ in the SCID mice was somewhat higher than in BALB/c nu/nu but much lower than in the case of ^{99m}Tc-H_6-$Z_{HER2:342}$.

Table 1. Biodistribution of ^{111}In-DOTA-$Z_{HER2:342}$ in BALB/c nu/nu* and SCID mice xenografted with SKOV-3 tumors[†]

	1 h p.i.		4 h p.i.		24 h p.i.	
	BALB/c nu/nu*	SCID	BALB/c nu/nu*	SCID	BALB/c nu/nu*	SCID
blood	3.0 ± 0.3	2.5 ± 0.1[#]	1.1 ± 0.1	1.1 ± 0.3	0.32 ± 0.06	0.2 ± 0.1
heart	1.4 ± 0.1	1.2 ± 0.1	0.55 ± 0.07	0.6 ± 0.1	0.40 ± 0.08	0.3 ± 0.1
lung	3.2 ± 0.3	2.6 ± 0.6	0.82 ± 0.07	0.8 ± 0.1	0.61 ± 0.08	0.5 ± 0.1
liver	2.0 ± 0.1	2.8 ± 0.5[#]	1.7 ± 0.1	4.8 ± 1.4[#]	1.7 ± 0.3	3.4 ± 0.9[#]
spleen	1.4 ± 0.2	1.4 ± 0.2	0.61 ± 0.05	1.5 ± 0.7[#]	1.0 ± 0.2	1.5 ± 0.5
pancreas	0.8 ± 0.1	0.6 ± 0.2	0.27 ± 0.06	0.25 ± 0.04	0.37 ± 0.04	0.3 ± 0.1
kidney	243 ± 22	229 ± 34	256 ± 21	232 ± 28	231 ± 34	211 ± 30
stomach	1.6 ± 0.2	1.0 ± 0.1[#]	0.41 ± 0.04	0.4 ± 0.1	0.4 ± 0.2	0.26 ± 0.04
tumor	23± 4	29 ± 3[#]	13 ± 2	19 ± 3[#]	15 ± 2	16 ± 5
skin	2.2 ± 0.3	1.6 ± 0.3[#]	0.76 ± 0.09	1.0 ± 0.4	1.0 ± 0.1	1.0 ± 0.2
muscle	0.6 ± 0.1	0.3 ± 0.1[#]	0.3 ± 0.3	0.1 ± 0.1	0.25 ± 0.08	0.08 ± 0.01[#]
bone	0.9 ± 0.1	0.7± 0.1	0.7 ± 0.3	0.36 ± 0.05	0.8 ± 0.4	0.6 ± 0.1

*Biodistribution published by Orlova et al. (*17*).

[†]Data are expressed as % IA/g and presented as an average from four animals ± SD in the BALB/c nu/nu mice and from three animals ± SD in the SCID mice.

[#]Significant difference between BALB/c nu/nu and SCID in Student's t-test ($p<0.05$).