

HER3 PET-imaging: ^{68}Ga -labeled affibody molecules provide superior HER3 contrast to ^{89}Zr -labeled antibody and antibody-fragment based tracers

Results

Characterization

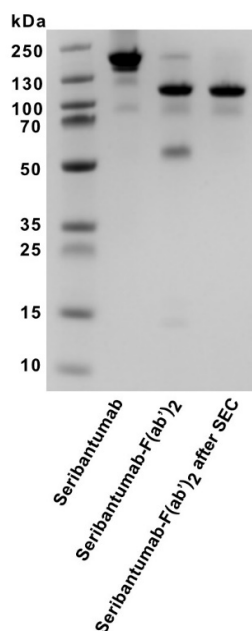


Figure S1: SDS-PAGE showing molecular weight marker (lane 1), intact seribantumab (lane 2), seribantumab-F(ab')₂ after pepsin digestion (lane 3), and successful removal of intact seribantumab and Fc fragments by SEC purification (lane 4).

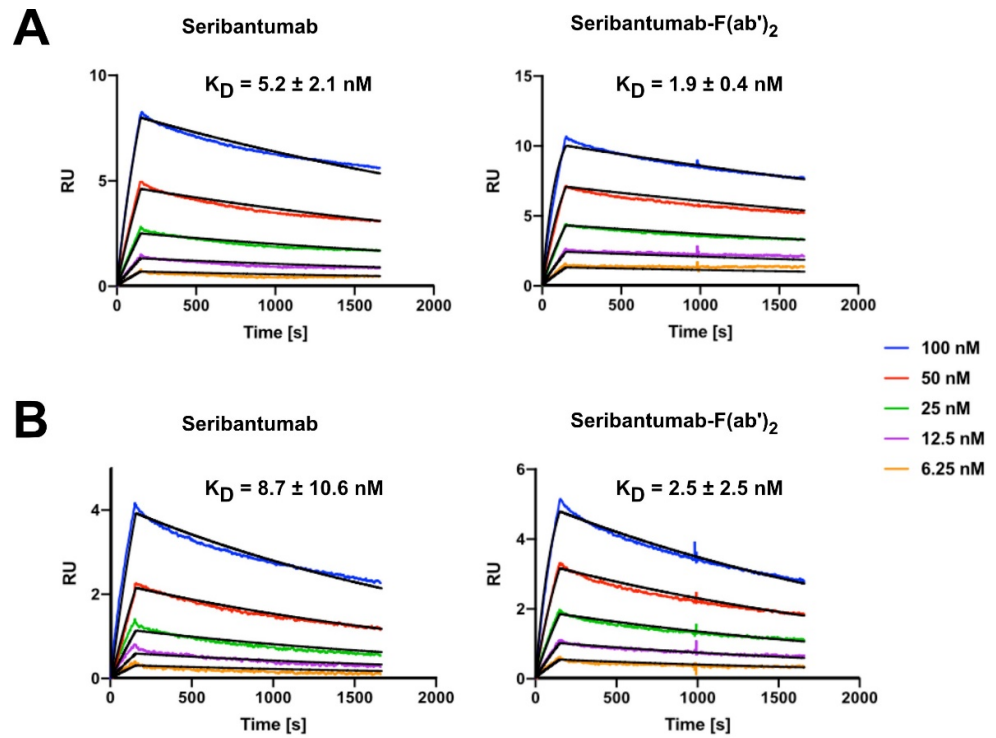


Figure S2: Representative SPR sensorgrams showing binding kinetics against immobilized (A) human HER3 and (B) murine ErbB3 for seribantumab and seribantumab- $F(ab')_2$. The experimental data (colored curves) were fitted using a Langmuir 1:1 model (black curves) to approximate kinetic constants. The K_D -values (\pm SD) are presented as the average obtained from two surfaces with different immobilization levels.

In vivo studies

Table S1: Biodistribution of [⁶⁸Ga]Ga-DFO-seribantumab-F(ab')₂, [⁸⁹Zr]Zr-DFO-seribantumab-F(ab')₂, [⁸⁹Zr]Zr-DFO-seribantumab and [⁶⁸Ga]Ga-Z_{HER3} in balb/c nu/nu mice with HER3-expressing BxPC-3 xenografts presented as %ID/g. Data is presented as average of n=4-6 mice with SD. Data for GI-tract and body is presented as %ID. Significant difference (p<0.05) between groups is indicated by ^a vs [⁶⁸Ga]Ga-DFO-seribantumab-F(ab')₂ ^bvs. [⁸⁹Zr]Zr-DFO-seribantumab-F(ab')₂ 3 h pi, ^c vs. [⁸⁹Zr]Zr-DFO-seribantumab-F(ab')₂ 24 h pi, ^d vs. [⁸⁹Zr]Zr-DFO-seribantumab-F(ab')₂ 48 h pi, ^e vs. [⁸⁹Zr]Zr-DFO-seribantumab 48 h pi, ^fvs. [⁸⁹Zr]Zr-DFO-seribantumab 96 h pi, ^{*}vs. [⁶⁸Ga]Ga-Z_{HER3}. Statistical significance was done by two-tailed unpaired t-test, except for comparison of data from [⁶⁸Ga]Ga-DFO-seribantumab-F(ab')₂ and [⁸⁹Zr]Zr-DFO-seribantumab-F(ab')₂ 3 h pi when a two-tailed paired t-test was used, due to a dual isotope approach.

Organ	[⁶⁸ Ga]Ga-DFO-seribantumab-F(ab') ₂ 3 h pi	[⁸⁹ Zr]Zr-DFO-seribantumab-F(ab') ₂			[⁸⁹ Zr]Zr-DFO-seribantumab		[⁶⁸ Ga]Ga-Z _{HER3} 3 h pi
		3h pi	24 h pi	48 h pi	48 h pi	96 h pi	
Blood	23 ± 4 ^{b,c,d,e,f,*}	14 ± 2 ^{a,c,d,e,f,*}	0.58 ± 0.04 ^{a,b}	0.23 ± 0.04 ^{a,b}	2.1 ± 0.2 ^{a,b}	0.7 ± 0.1 ^{a,b}	0.2 ± 0.07 ^{a,b}
Salivary	4 ± 0.9 ^{b,c,d,f,*}	2.6 ± 0.3 ^{a,c,d,*}	1.9 ± 0.2 ^{a,b,e,f}	1.4 ± 0.2 ^{a,b,e,f}	3.6 ± 0.5 ^{c,*}	3.1 ± 0.3 ^{a,c,*}	1.3 ± 0.1 ^{a,b,e,f}
Lungs	13 ± 2 ^{b,c,d,e,f,*}	7.3 ± 0.6 ^{a,c,d,e,f,*}	1.7 ± 0.2 ^{a,b}	1.3 ± 0.1 ^{a,b}	2.7 ± 0.5 ^{a,b}	1.8 ± 0.2 ^{a,b}	1.05 ± 0.08 ^{a,b}
Liver	32 ± 5 ^{b,c,d,e,f,*}	10.6 ± 0.6 [*]	8.2 ± 0.3 ^{a,*}	8.2 ± 0.9 ^{a,*}	8.6 ± 0.7 ^{a,*}	8.5 ± 0.8 ^{a,*}	3.4 ± 0.4 ^{a,b,c,d,e,f}
Stomach	3.3 ± 0.5 ^{b,c,d,e,f,*}	2.6 ± 0.3 ^{a,c,d}	1.5 ± 0.2 ^{a,b}	1.1 ± 0.2 ^{a,b,f}	2.1 ± 0.4 ^{a,f,*}	1.3 ± 0.1 ^{a,d,e}	1.4 ± 0.2 ^{a,b,e}
Spleen	16 ± 3 ^{b,c,d,e,f,*}	5.9 ± 0.6 ^{a,c,d,*}	3.0 ± 0.4 ^{a,b,*}	3.1 ± 0.3 ^{a,b,*}	3 ± 1 ^{a,b,*}	3.7 ± 0.6 ^{a,*}	0.39 ± 0.0 ^{a,b,c,d,e,f}
Small Intestine	13 ± 2 ^{b,c,d,e,f,*}	6.6 ± 0.7 ^a	3.8 ± 0.2 ^{a,b,d}	2.3 ± 0.4 ^{a,b,c,e}	7 ± 1 ^{a,d,f,*}	2.4 ± 0.2 ^{a,b,e}	4.0 ± 0.9 ^{a,b,e}
Kidney	70 ± 7 ^{e,f,*}	41 ± 3 ^{e,f,*}	48 ± 7 ^{e,f,*}	40 ± 8 ^{e,f,*}	7.0 ± 0.5 ^{a,b,c,d,*}	4.8 ± 0.2 ^{a,b,c,d,*}	230 ± 37 ^{a,b,c,d,e,f}
Tumor	11 ± 3 ^{b,c,d,e,f,*}	7 ± 2 ^{a,d,*}	4.0 ± 0.9 ^a	3.5 ± 0.6 ^{a,b}	5.6 ± 0.4 ^a	4.7 ± 0.8 ^a	2.6 ± 0.3 ^{a,b}
Muscle	1.72 ± 0.10 ^{b,c,d,e,f,*}	1.19 ± 0.10 ^{a,c,d,e,f,*}	0.41 ± 0.06 ^{a,b}	0.32 ± 0.03 ^{a,b}	0.6 ± 0.2 ^{a,b,*}	0.7 ± 0.4 ^{a,b,*}	0.12 ± 0.02 ^{a,b,e,f}
Bone	3.3 ± 1.0 ^{e,*}	2.2 ± 2 ^{e,*}	3.4 ± 0.2 ^{e,*}	3.5 ± 0.7 ^{e,*}	5.9 ± 0.5 ^{a,b,c,d,*}	4 ± 2 [*]	0.24 ± 0.05 ^{a,b,c,d,e,f}
GI	19 ± 2 ^{c,d,e,f,*}	17 ± 7 ^{c,d,e,f,*}	7 ± 1 ^{a,b}	3.3 ± 0.3 ^{a,b,e}	11 ± 0.9 ^{a,b,d,f}	4.7 ± 0.6 ^{a,b,e}	6 ± 1 ^{a,b}
Body	38 ± 6 ^{b,c,d,e,f,*}	19 ± 9 ^{a,*}	16 ± 1 ^{a,*}	14 ± 1 ^{a,e}	25 ± 2 ^{a,d}	21 ± 1 ^{a,*}	6.3 ± 0.7 ^{a,b,c,d,f,*}

Table S2: Tumor-to-organ ratios for [⁶⁸Ga]Ga-DFO-seribantumab-F(ab')₂, [⁸⁹Zr]Zr-DFO-seribantumab-F(ab')₂, [⁸⁹Zr]Zr-DFO-seribantumab and [⁶⁸Ga]Ga-Z_{HER3} in balb/c nu/nu mice with HER3-expressing BxPC-3 xenografts. Data is presented as average of n=4-6 mice with SD. Significant difference (p<0.05) between groups is indicated by ^a vs [⁶⁸Ga]Ga-DFO-seribantumab-F(ab')₂, ^b vs. [⁸⁹Zr]Zr-DFO-seribantumab-F(ab')₂ 3 h pi, ^c vs. [⁸⁹Zr]Zr-DFO-seribantumab-F(ab')₂ 24 h pi, ^d vs. [⁸⁹Zr]Zr-DFO-seribantumab-F(ab')₂ 48 h pi, ^e vs. [⁸⁹Zr]Zr-DFO-seribantumab 48 h pi, ^f vs. [⁸⁹Zr]Zr-DFO-seribantumab 96 h pi, ^{*} vs. [⁶⁸Ga]Ga-Z_{HER3}. Statistical significance was done by two-tailed unpaired t-test, except for comparison of data from [⁶⁸Ga]Ga-DFO-seribantumab-F(ab')₂ and [⁸⁹Zr]Zr-DFO-seribantumab-F(ab')₂ 3 h pi when a two-tailed paired t-test was used, due to a dual isotope approach.

Organ	[⁶⁸ Ga]Ga-DFO-seribantumab-F(ab') ₂ 3 h pi	[⁸⁹ Zr]Zr-DFO-seribantumab-F(ab') ₂			[⁸⁹ Zr]Zr-DFO-seribantumab		[⁶⁸ Ga]Ga-Z _{HER3} 3 h pi
		3h pi	24 h pi	48 h pi	48 h pi	96 h pi	
Blood	0.45 ± 0.09 ^{c,d,f,*}	0.5 ± 0.1 ^{c,d,f,*}	7 ± 2 ^{a,b,d}	15 ± 4 ^{a,b,c,e,f}	2.7 ± 0.4 ^{d,*}	6 ± 2 ^{a,b,d,*}	11 ± 2 ^{a,b,e,f}
Salivary	2.6 ± 0.6 ^{e,f}	2.6 ± 0.5 ^{e,f}	2.1 ± 0.3	2.4 ± 0.7	1.6 ± 0.2 ^{a,b}	1.5 ± 0.3 ^{a,b}	2.0 ± 0.3
Lungs	0.8 ± 0.2 ^{c,e,d,f,*}	0.9 ± 0.2 ^{c,d,e,f,*}	2.3 ± 0.3	2.7 ± 0.6	2.2 ± 0.4	2.7 ± 0.6	2.4 ± 0.3
Liver	0.33 ± 0.05 ^{b,e,*}	0.6 ± 0.2	0.5 ± 0.1 [*]	0.4 ± 0.1 [*]	0.66 ± 0.08	0.6 ± 0.1	0.74 ± 0.05 ^{a,c,d}
Stomach	3 ± 1	2.6 ± 0.6	2.6 ± 0.2	3 ± 1	2.7 ± 0.06	3.6 ± 0.7 [*]	1.9 ± 0.2 ^f
Spleen	0.7 ± 0.1 ^{e,*}	1.2 ± 0.3 [*]	1.3 ± 0.2 [*]	1.1 ± 0.3 [*]	3 ± 1 ^{a,*}	1.3 ± 0.4 [*]	6.4 ± 0.8 ^{a,b,c,d,e,f}
Small Intestine	0.8 ± 0.1 ^{c,f}	1.0 ± 0.2 ^f	1.1 ± 0.2 ^{a,f}	1.5 ± 0.5 [*]	0.9 ± 0.2 ^f	2.0 ± 0.5 ^{a,b,c,e,*}	0.7 ± 0.2 ^d
Kidney	0.1 ± 0.03 ^{e,f}	0.17 ± 0.04 ^{e,f}	0.08 ± 0.02 ^{e,f}	0.09 ± 0.03 ^{e,f}	0.81 ± 0.07 ^{a,b,c,d,f,*}	1.0 ± 0.2 ^{a,b,c,d,e,*}	0.011 ± 0.003 ^{e,f}
Muscle	6 ± 2 [*]	6 ± 1 [*]	10 ± 2 [*]	11 ± 2 [*]	10 ± 4 [*]	8 ± 2 [*]	21 ± 6 ^{a,b,c,d,e,f}
Bone	3.2 ± 0.5 [*]	3.1 ± 0.7 [*]	1.2 ± 0.3 [*]	1.1 ± 0.5 [*]	0.95 ± 0.09 [*]	2 ± 2 [*]	11 ± 2 ^{a,b,c,d,e,f}

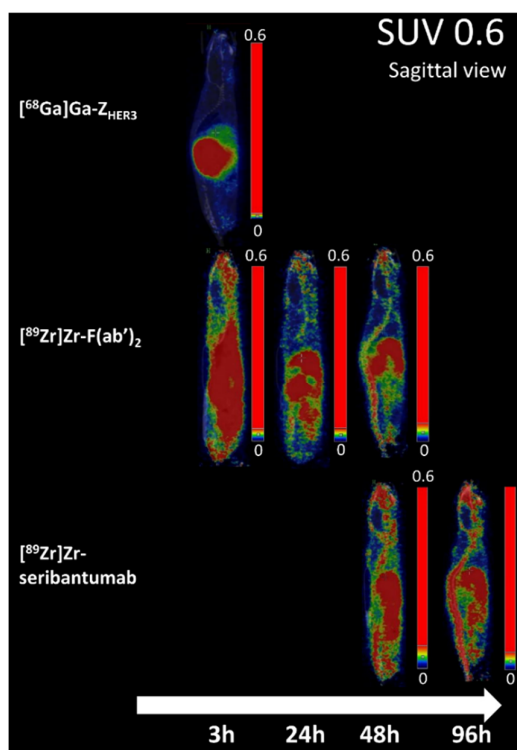


Figure S3: nanoPET/CT images, sagittal view. For imaging mice were injected with 1.18 MBq (27 µg) [⁸⁹Zr]Zr-DFO-seribantumab-F(ab')₂, 1.38 MBq (35 µg) [⁸⁹Zr]Zr-DFO-seribantumab, or 7.05 MBq (2 µg) [⁶⁸Ga]Ga-Z_{HER3}.