

## SUPPORTING INFORMATION

### **[<sup>68</sup>Ga]Ga/[<sup>177</sup>Lu]Lu-BL01, a Novel Theranostic Pair for Targeting C-X-C Chemokine Receptor 4**

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## Synthesis of BL01

Fmoc-Rink Amide MBHA resin (AnaSpec Inc., 0.25 mmol) was deprotected with 20% v/v piperidine in DMF for 1 min at 90°C twice and washed with 3 mL of DMF 5 times. Fmoc-Lys(ivDde)-OH was then conjugated to the Rink Amide MBHA resin using 4/8/4 equiv. of Fmoc-AA-OH/DIC/Oxyma in DMF for 4 min at 90°C. The Fmoc group was removed with 20% v/v piperidine in DMF for 1 min at 90°C. The resin was washed three times with 3 mL DMF after each deprotection. Fmoc-Lys(iPr,Boc)-OH, Fmoc-D-Glu(OAll)-OH, Fmoc-Gly-OH (coupled twice), Fmoc-2Nal-OH (coupled twice), Fmoc-D-Arg-OH (coupled twice), Fmoc-Lys(iPr,Boc)-OH, Fmoc-Tyr(tBu)-OH, and Fmoc-Phe-OH (coupled twice) were sequentially coupled to the peptidyl resin following similar procedures. At a 0.1 mmol scale, the -OAllyl protecting group on D-Glu was removed using Pd(PPh<sub>3</sub>)<sub>4</sub> (25 mg)/Phenylsilane (240 μL) in DCM (5 mL) (2 × 5 min at 35°C). The N<sup>α</sup>-Fmoc on Phe was then removed, and cyclization was performed using DIC/HOBt in DMF (3 × 10 min at 90°C). Following cyclization, the ivDde protecting group was removed by 3% v/v hydrazine in DMF (5 × 5 min at RT). The chelator DOTA tri-*t*-butyl ester (4 equiv.) in DMF was coupled to the ε-amine group on the Lys side-chain with HATU/DIEA (4/7 equiv.) overnight. The peptide was deprotected and simultaneously cleaved from the resin by treating with a cocktail solution of 92.5/5/2.5 TFA/TIS/H<sub>2</sub>O for 2.5 h at 35°C. After filtration, the TFA was removed *in vacuo* and the peptide was precipitated by the addition of cold diethyl ether. The crude peptide was first checked for purity by HPLC using the semi-preparative column eluted with 22% acetonitrile in water with 0.1% TFA at a flow rate of 4.5 mL/min. The crude peptide was purified by preparative HPLC using the preparative column eluted with first 10-18% acetonitrile in water with 0.1% TFA for 0-16 mins, then 18-22% acetonitrile for 16-20 mins, then 22-25% acetonitrile in 20-25 mins at a flow rate of 30 mL/min. The retention times of BL01 were 24.6 min with the semi-

preparative column and 23.3 min with the preparative column. The isolated yield of BL01 was 9%. ESI-MS: calculated  $[M+2H]^{2+}$  for BL01  $C_{84}H_{126}N_{20}O_{18}$  852.5; found  $[M+2H]^{2+}$  852.7.

### **Synthesis of Ga/Lu-BL01**

For Ga-BL01, a solution of BL01 (0.7 mg, 0.342  $\mu$ mol) and  $GaCl_3$  (0.35 mg, 2.0  $\mu$ mol) in 250  $\mu$ L sodium acetate buffer (0.1 M, pH 4.2) was incubated at 80°C for 15 min. The reaction mixture was purified by HPLC using the preparative column eluted with first 10-18% acetonitrile in water with 0.1% TFA for 0-16 min, then 18-22% acetonitrile for 16-20 min, then 22-25% acetonitrile in 20-25 min at a flow rate of 30 mL/min. The retention time of Ga-BL01 was 23.1 min, and the yield of the peptide was 98%. ESI-MS: calculated  $[M+2H]^{2+}$  for Ga-BL01  $C_{84}H_{124}N_{20}O_{18}Ga$  885.9; found  $[M+2H]^{2+}$  886.5. For Lu-BL01, a solution of BL01 (1.3 mg, 0.635  $\mu$ mol) and  $LuCl_3$  (1.0 mg, 3.6  $\mu$ mol) in 250  $\mu$ L sodium acetate buffer (0.1 M, pH 4.2) was incubated at 80°C for 15 min. The reaction mixture was purified by HPLC using the preparative column eluted with first 10-18% acetonitrile in water with 0.1% TFA for 0-16 min, then 18-22% acetonitrile for 16-20 min, then 22-25% acetonitrile in 20-25 min at a flow rate of 30 mL/min. The retention time of Lu-BL01 was 23.0 min, and the yield of the peptide was 71%. ESI-MS: calculated  $[M+2H]^{2+}$  for Lu-BL01  $C_{84}H_{124}N_{20}O_{18}Lu$  938.9; found  $[M+2H]^{2+}$  940.0.

### **SPECT/CT Imaging**

SPECT/CT images were obtained using the MILabs U-SPECT-II/CT scanner. Daudi-bearing NRG mice were injected with 33.8-34.7 MBq of  $[^{177}Lu]Lu$ -BL01 *via* IV injection. Blocking was done *via* IP injection of 7.5  $\mu$ g of LY2510924 15 minutes prior to the administration of the radiotracer. The injected mice were imaged at 1, 4, 24 and 72 hours p.i.. At each time point, the mice were sedated (2% isoflurane in oxygen) and placed into the scanner. The CT scan was performed first, at 60 kV and 615  $\mu$ A. Subsequently, the SPECT scan was performed *via* a 60 min

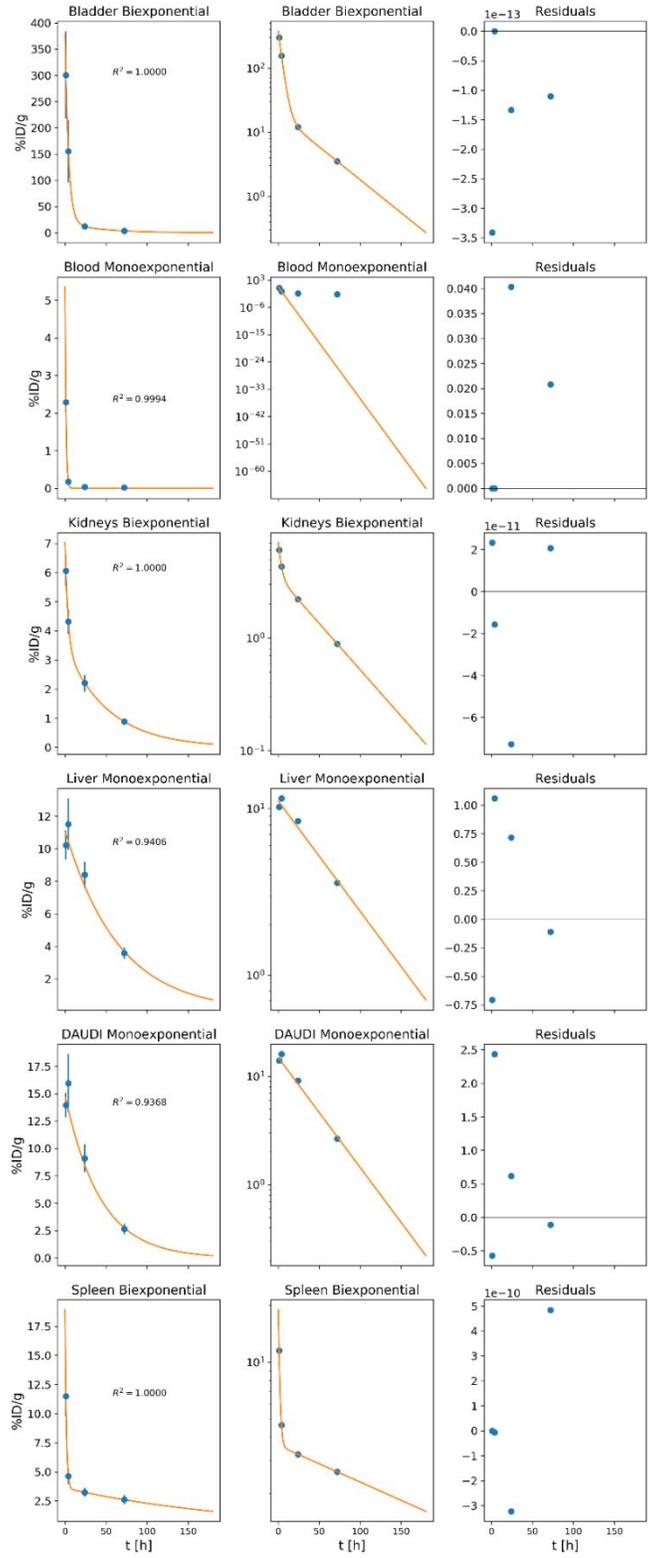
static emission scan acquired in list mode using an ultrahigh-resolution multipinhole rat-mouse (1 mm pinhole size) collimator. The imaging dataset was reconstructed *via* the U-SPECT II software with a 20% window width on three energy windows. The photopeak window was centered at 208 keV, with the lower and upper scatter windows at 187.2 and 228.8 keV, respectively. The images were reconstructed using the ordered subset expectation maximization algorithm (4 iterations, 32 subsets) and a 1 mm post-processing Gaussian filter (collimator dependent calibration factor = 10012.659). Images were decay corrected to the injection time in PMOD (PMOD Technologies) and then converted to DICOM for qualitative visualization in the Inveon Research Workplace software (Siemens Medical Solutions USA, Inc.).

**Supplemental Table 1.** Biodistribution data (%ID/g) of [<sup>68</sup>Ga]Ga-BL01 in Daudi tumor-bearing mice at selected time points. Mice in the 1 h blocked group received an injection of 7.5 μg of LY2510924 (i.p.) 15 min before tracer administration.

<b>[<sup>68</sup>Ga]Ga-BL01</b>	<b>1 h</b>			<b>1 h blocked</b>			<b>2 h</b>		
	<b>Mean</b>	<b>SD</b>	<b>n</b>	<b>Mean</b>	<b>SD</b>	<b>n</b>	<b>Mean</b>	<b>SD</b>	<b>n</b>
<b>Blood</b>	2.55	0.38	6	0.84	0.24	5	1.16	0.12	7
<b>Fat</b>	0.41	0.09	6	0.08	0.03	5	0.21	0.04	7
<b>Testes</b>	0.58	0.10	6	0.21	0.03	5	0.59	0.22	7
<b>Intestines</b>	0.99	0.16	6	0.45	0.16	5	0.65	0.07	7
<b>Stomach</b>	0.29	0.07	6	0.07	0.03	5	0.19	0.05	7
<b>Spleen</b>	12.59	2.36	6	1.90	0.35	5	8.41	0.75	7
<b>Liver</b>	7.05	1.28	6	5.04	0.73	5	9.27	1.04	7
<b>Pancreas</b>	0.70	0.11	6	0.18	0.05	5	0.47	0.04	7
<b>Adrenals</b>	4.71	2.87	6	0.65	0.28	5	2.46	1.00	6
<b>Kidney</b>	5.16	0.69	6	4.52	0.67	5	5.30	0.44	7
<b>Lung</b>	13.16	2.98	6	1.16	0.34	5	7.56	1.51	7
<b>Heart</b>	1.56	0.28	6	0.31	0.08	5	0.96	0.10	7
<b>Muscle</b>	0.45	0.11	6	0.20	0.08	5	0.29	0.04	7
<b>Bone</b>	1.19	0.47	6	0.43	0.25	5	1.20	0.41	7
<b>Brain</b>	0.05	0.01	6	0.02	0.01	5	0.04	0.00	7
<b>Tumor</b>	10.20	2.56	6	1.19	0.35	5	15.29	1.86	7
<b>Ratios</b>									
<b>Tumor-to-Blood</b>	3.97	0.62	6	1.55	0.69	5	13.33	2.14	7
<b>Tumor-to-Muscle</b>	22.67	4.34	6	7.06	3.75	5	52.87	2.26	7
<b>Tumor-to-Liver</b>	1.44	0.23	6	0.24	0.09	5	1.66	0.22	7
<b>Tumor-to-Lung</b>	0.79	0.19	6	1.11	0.46	5	2.12	0.65	7
<b>Tumor-to-Spleen</b>	0.82	0.20	6	0.66	0.27	5	1.83	0.29	7

**Supplemental Table 2.** Biodistribution data (%ID/g) of [<sup>177</sup>Lu]Lu-BL01 in Daudi tumor-bearing mice at selected time points. Mice in the 1 h blocked group received an injection of 7.5 µg of LY2510924 (i.p.) 15 min before tracer administration.

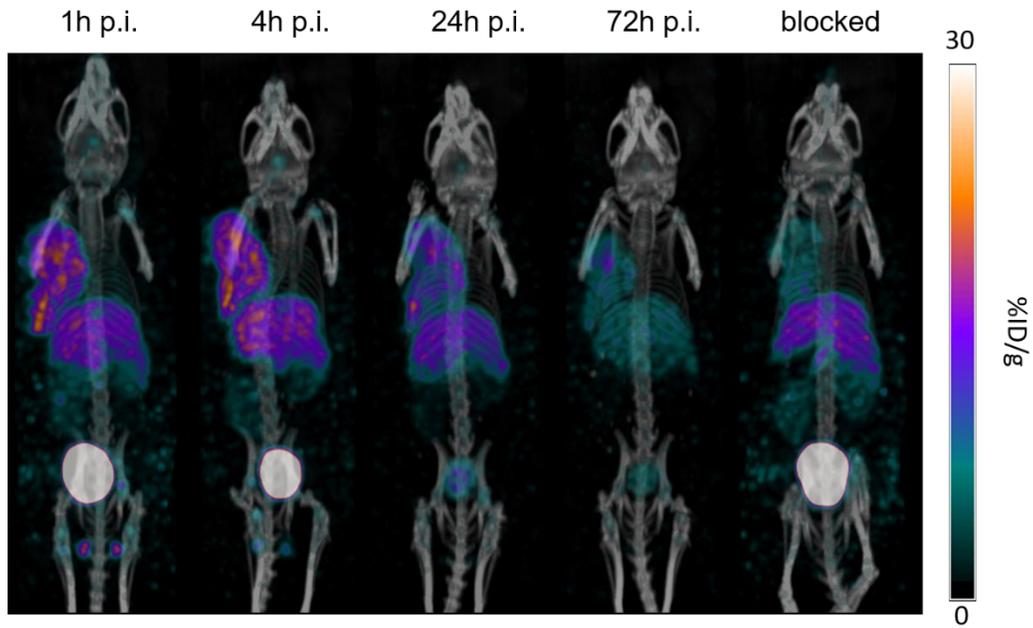
<u>[<sup>177</sup>Lu]Lu-BL01</u>	<u>1 h</u>			<u>1 h blocked</u>			<u>4 h</u>			<u>24 h</u>			<u>72 h</u>		
	Mean	S.Dev	n	Mean	S.Dev	n	Mean	S.Dev	n	Mean	S.Dev	n	Mean	S.Dev	n
<b>Blood</b>	2.30	0.27	7	1.04	0.23	5	0.18	0.04	6	0.04	0.01	5	0.03	0.00	6
<b>Fat</b>	0.43	0.05	7	0.11	0.04	5	0.11	0.02	6	0.08	0.02	5	0.05	0.01	6
<b>Testes</b>	0.64	0.06	7	0.32	0.05	5	0.33	0.05	6	0.22	0.03	5	0.19	0.02	6
<b>Intestines</b>	1.08	0.09	7	0.73	0.15	5	0.37	0.04	6	0.29	0.08	5	0.15	0.04	6
<b>Stomach</b>	0.33	0.10	7	0.11	0.02	5	0.19	0.05	6	0.25	0.10	5	0.15	0.08	6
<b>Spleen</b>	11.55	1.78	7	3.56	0.56	5	4.72	0.67	6	3.59	0.41	5	3.57	0.56	6
<b>Liver</b>	10.27	0.89	7	9.65	0.92	5	11.70	1.62	6	9.33	0.89	5	4.90	0.51	6
<b>Pancreas</b>	0.88	0.19	7	0.29	0.07	5	0.31	0.14	6	0.17	0.05	5	0.09	0.01	6
<b>Adrenals</b>	3.68	1.81	7	0.65	0.38	5	1.32	0.70	6	1.33	0.54	5	1.18	0.35	6
<b>Kidney</b>	6.08	0.54	7	7.18	0.82	5	4.39	0.43	6	2.45	0.31	5	1.21	0.14	6
<b>Lung</b>	12.95	1.27	7	1.85	0.65	5	1.90	0.24	6	0.91	0.13	5	0.45	0.08	6
<b>Heart</b>	1.59	0.20	7	0.44	0.07	5	0.39	0.04	6	0.21	0.04	5	0.11	0.01	5
<b>Muscle</b>	0.44	0.04	7	0.20	0.06	5	0.16	0.03	6	0.08	0.03	5	0.04	0.01	6
<b>Bone</b>	1.80	0.22	7	0.64	0.37	5	0.83	0.19	6	0.60	0.26	5	0.41	0.09	6
<b>Brain</b>	0.06	0.01	7	0.02	0.01	5	0.02	0.01	6	0.01	0.00	5	0.01	0.00	5
<b>Tumor</b>	14.00	1.12	7	1.71	0.80	5	16.24	2.69	6	10.09	1.41	5	3.62	0.68	6
<b>Ratios</b>															
<b>Tumor to Blood</b>	6.14	0.67	7	1.77	1.15	5	92.90	24.74	6	229.12	32.89	5	127.17	17.82	6
<b>Tumor to Muscle</b>	31.63	2.59	7	9.48	6.87	5	104.93	24.77	6	130.81	27.74	5	96.46	22.50	6
<b>Tumor to Liver</b>	1.37	0.08	7	0.17	0.07	5	1.39	0.16	6	1.08	0.10	5	0.74	0.13	6
<b>Tumor to Lung</b>	1.09	0.15	7	1.04	0.68	5	8.56	1.00	6	11.13	0.87	5	8.19	1.83	6
<b>Tumor to Spleen</b>	1.24	0.22	7	0.49	0.23	5	3.45	0.32	6	2.82	0.36	5	1.03	0.22	6



**Supplemental Figure 1.** Uptake of the  $[^{68}\text{Ga}]\text{Ga-BL01}$  as a function of time for tumor and selected organs. The total number of decays per unit injected dose is calculated by multiplying the area under the curve by the phantom organ mass.



**Supplemental Figure 2.** Additional PET/CT and PET alone image of [ $^{68}\text{Ga}$ ]Ga-BL01 in Daudi-tumor bearing acquired at 1 h p.i. The scale bar is in units of %ID/g from 0 to 12.



**Supplemental Figure 3.** SPECT/CT images of [ $^{177}\text{Lu}$ ]Lu-BL01 in Daudi-tumor bearing mice acquired at different time points. The same mouse is shown for 1 to 72 h p.i. A second mouse was used for blocking, with image acquisition being performed at 1 h p.i. The scale bar is in units of %ID/g from 0 to 30.