Supporting Information

Radioligand Therapy of Prostate Cancer with a Long-lasting PSMA Targeting Agent ^{90}Y-DOTA-EB-MCG

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Running title: RLT of PCa using ^{90}Y-DOTA-EB-MCG

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Materials and Methods

General

MCG was kindly provided by Dr. Martin Pomper at Johns Hopkins University. DOTA-maleimide was purchased from Macrocyclics (Plano, TX). Synthesis and characterization of DOTA-Maleimide-EB (DMEB) derivative was previously reported by us [1]. Purification and analysis of the conjugated MCG molecules (DOTA-MCG and DOTA-EB-MCG) was done according to the published procedure [2].

Synthesis of DOTA-MCG and EB-DOTA-MCG

MCG (1.05 eq.) was dissolved in 0.1 mL H_2O and added to either DOTA-Maleimide DOTA-Maleimide-EB (1eq) in 0.5 mL in 0.1% Na-ascorbate (w/v) in PBS. The reaction was stirred for 1-2 h at room temperature and purified by HPLC to give the desired conjugated products in a chemical purity >95% and a yield of 70-75%. LC-MS analysis confirmed mass of 819.2 $[M-H]^-$ for DOTA-MCG and 1501.5 $[M-H]^-$ for DOTA-EB-MCG.

Radioisotope Labeling

$^{86}YCl_3$ was purchased from NIH Cyclotron Facility. $^{90}YCl_3$ was purchased from Perkin-Elmer. 10 µL (185-370 MBq) of $^{86}YCl_3$ (370 MBq) and/or $^{90}YCl_3$ were diluted with 0.5 mL 0.4 M ammonium acetate pH 5.6. Then 100 µg of either DOTA-EB-MCG or DOTA-MCG in 10µL of H_2O was added and the reaction mixed for 20 min at 80°C. Purity of the products was assayed by radio-TLC (AR-2000 Bioscan scanner), using iTLC plates (Fisher) and 0.1 M Citric acid pH 5 as the developing solvent for $^{86/90}$Y-DOTA-EB-MCG and normal phase plates and 2% EDTA/NH4OAc (10% w/v) for $^{86/90}$Y- DOTA-MCG. R_f of free $^{86/90}Y \sim 0.9$; R_f of $^{86/90}$Y-DOTA-

EB-MCG and $^{86/90}$Y-DOTA-MCG ~ 0.1. A radiochemical yield of greater than 95% was achieved for both compounds with specific activities of 68-75mCi/μmol.

Table S1. Hematologic analysis of blood samples from untreated mice and mice treated with 7.4 MBq of ^{90}Y-DOTA-MCG (n=3)

	^{90}Y-DOTA-EB-MCG (7.4 MBq)	**Control**
WBC (10^9/L)	3.2 ± 0.5	3.3 ± 0.7
RBC (10^{12}/L	9.3 ± 0.4	9.1 ± 0.7
Hemoglobin (g/L)	118.9 ± 10.9	127.8 ± 13.6
Platelets (10^9/L)	896.3 ± 192.1	932.6 ± 154.3
MCV (fL)	52.1 ± 2.3	50.8 ± 4.1
LYM # (10^9/L)	1.8 ± 0.2	1.6 ± 0.3
MID # (10^9/L)	1.2 ± 0.2	1.3 ± 0.3
GRAN # (10^9/L)	4.5 ± 0.4	5.7 ± 0.8
LYM (%)	47.8 ± 9.3	53.2 ± 8.7
MID (%)	6.8 ± 1.2	6.5 ± 0.7
GRAN (%)	51.2 ± 8.9	53.2 ± 10.6

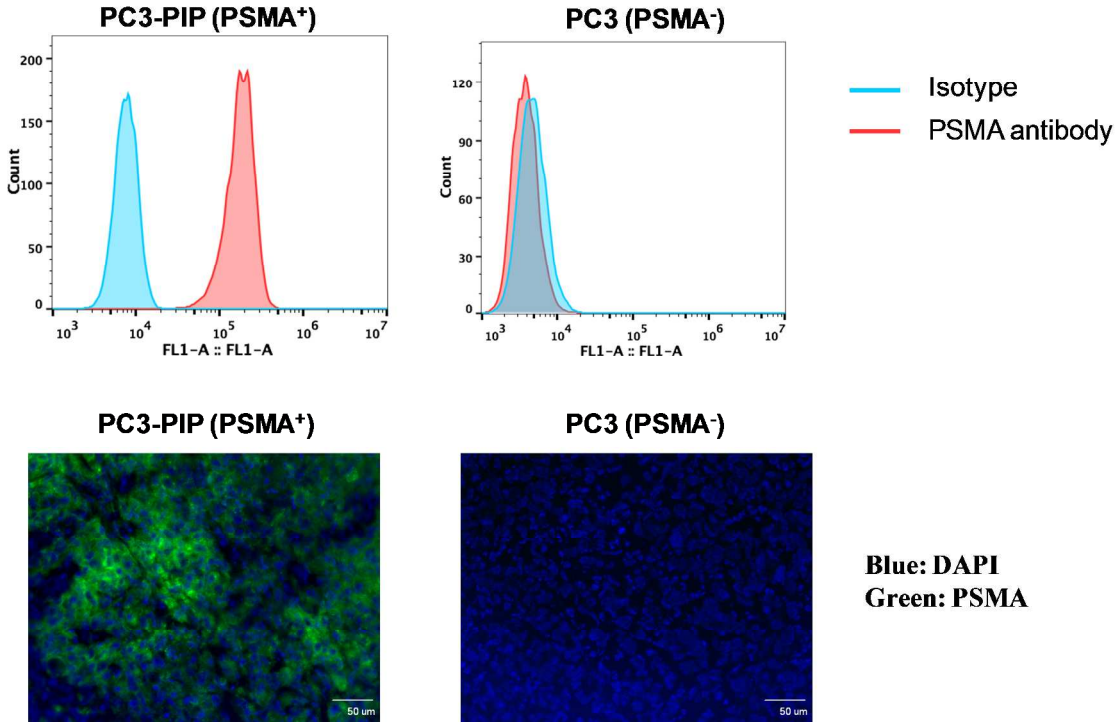

Figure S1. Flowcytometry of PMSA positive PC3-PIP and PMSA negative PC3 cells using antibody against PSMA. Immunofluorescence staining of tumor sections originated from PC3-PIP and PC3 cells.

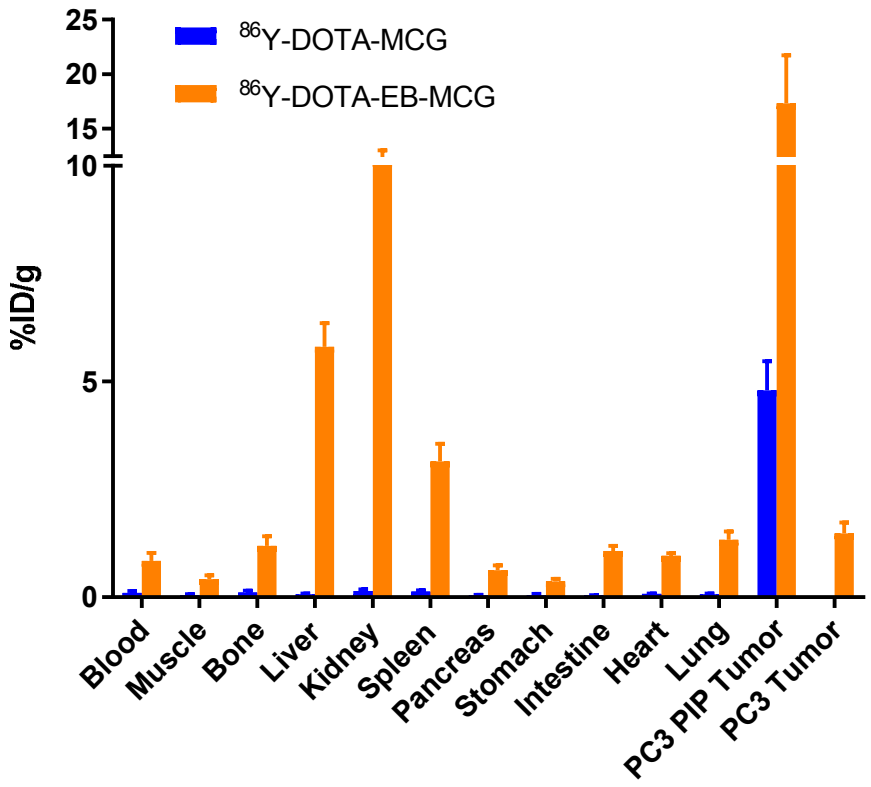

Figure S2. Biodistribution of ^{86}Y-DOTA-MCG and ^{86}Y-EB-DOTA-MCG in normal organs and PSMA positive/negative tumors at 48 hr p.i..

Table S2. PET quantification of tumor, muscle and liver at different time points for ^{86}Y-DOTA-MCG and ^{86}Y-EB-DOTA-MCG.

		1 h	4 h	24 h	48 h
DOTA-MCG	Tumor	17.05±3.17	16.26±3.54	10.94±1.47	0.34±0.06
	Muscle	0.51±0.04	0.25±0.01	0.04±0.01	0.05±0.02
	Liver	0.48±0.01	0.15±0.007	0.08±0.01	0.07±0.01
DOTA-EB-MCG	Tumor	22.03±2.29	32.11±4.15	39.87±4.64	34.42±3.22
	Muscle	3.37±0.29	2.27±0.09	1.24±0.16	1.26±0.12
	Liver	8.51±0.18	7.22±0.37	6.55±0.27	6.28±0.26

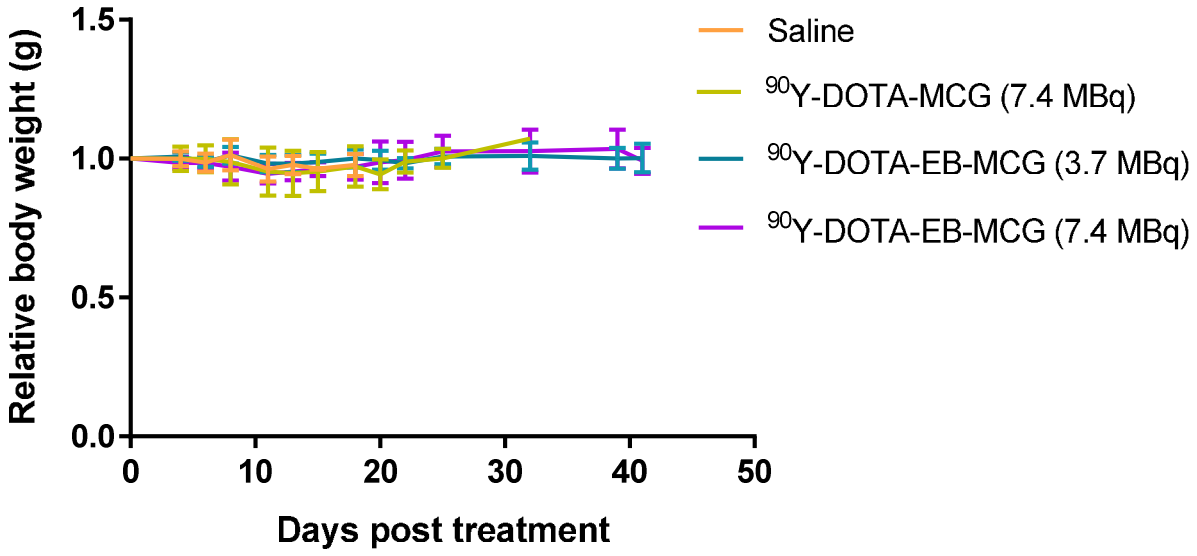

Figure S3. Changes of body weight of PC3 PIP tumor bearing mice after radioligand treatment.

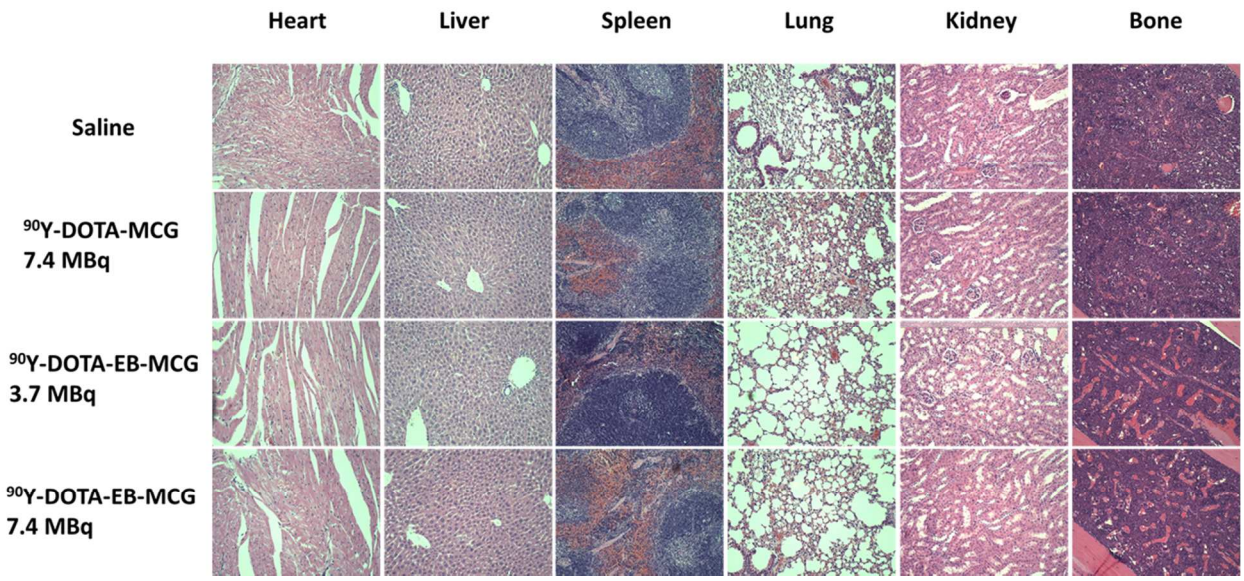

Figure S4. H&E staining of tissue sections from mice treated with saline, 7.4 MBq of ^{90}Y-DOTA-MCG, 3.7 MBq of ^{90}Y-DOTA-EB-MCG and 7.4 MBq of ^{90}Y-DOTA-EB-MCG.

References

(1) Chen, H.; Jacobson, O.; Niu, G.; Weiss, I. D.; Kiesewetter, D. O.; Liu, Y.; Ma, Y.; Wu, H.; Chen, X. Novel "Add-On" Molecule Based on Evans Blue Confers Superior Pharmacokinetics and Transforms Drugs to Theranostic Agents. *J Nucl Med* **2017**, *58*, 590-597.

(2) Tian, R.; Jacobson, O.; Niu, G.; Kiesewetter, D. O.; Wang, Z.; Zhu, G.; Ma, Y.; Liu, G.; Chen, X. Evans Blue Attachment Enhances Somatostatin Receptor Subtype-2 Imaging and Radiotherapy. *Theranostics* **2018**, *8*, 735-745.