A novel Carbonic Anhydrase IX targeting radiopeptide, ^{64}Cu-PD-32766 and ^{177}Lu-PD-32766, exhibit promising theranostic potential in ccRCC tumors.

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Introduction

- Carbonic Anhydrase IX (CA9) is a zinc metalloenzyme that regulates the pH for cell growth[1].
- CA9 is considered an attractive theranostic target and is upregulated in a variety of cancers, especially, in 95% of clear cell renal cell carcinoma (ccRCC) due to von Hippel-Lindau (VHL) loss of function[2].
- PD-32766, a selective CA9 binding macrocyclic peptide with DOTA, was identified by PeptiDream's proprietary PDPS (Peptide Discovery Platform System) technology, which displays peptides with huge diversity (>10^{13}).
- Here we demonstrate the promising theranostic translational potency of PD-32766 for CA9 expressing tumors including ccRCC.

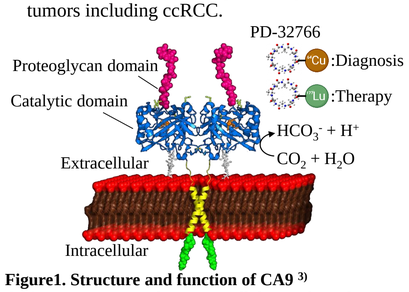


Figure1. Structure and function of CA9 [3]
CA9 is an enzyme with an extracellular catalytic domain that converts carbon dioxide to bicarbonate ions.

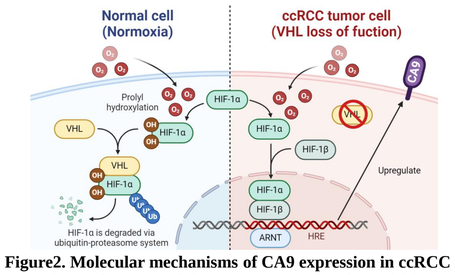
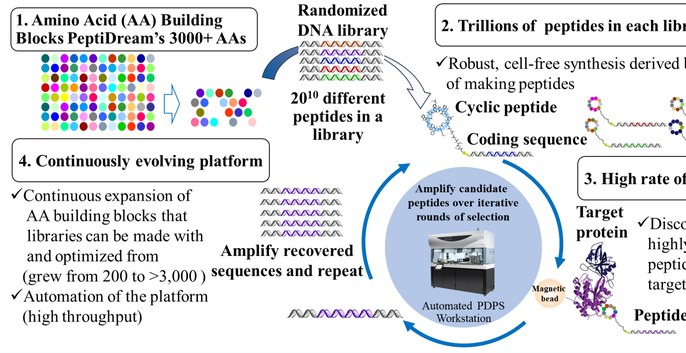


Figure2. Molecular mechanisms of CA9 expression in ccRCC [4]
Loss of VHL function stabilizes HIF1α, which induces CA9 expression in ccRCC even under normoxia.

PDPS: A Powerful Peptide Discovery Platform



CA9 is highly expressed in majority of ccRCC, also in VMRC-RCW.

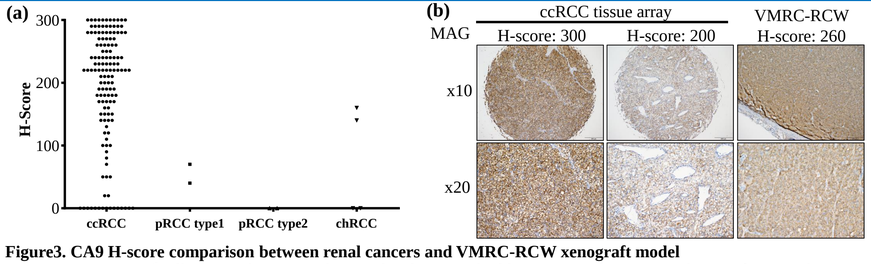


Figure3. CA9 H-score comparison between renal cancers and VMRC-RCW xenograft model
VMRC-RCW (5 x 10^6 cells) was subcutaneously inoculated into 6-wk old BALB/c nude mice. On day 17 after inoculation, the tumors were collected. VMRC-RCW tumors and Human kidney tumor tissue array (KD2001, TissueArray.Com LLC) were immuno-stained with the anti-CAIX antibody (mouse clone M76) at 0.38 μg/ml. (a) The H-score was calculated according to the following formula: H-score = [(0 x % negative cells) + (1 x % weak positive cells) + (2 x % moderate positive cells) + (3 x % strong positive cells). (b) Representative IHC staining images of ccRCC tissue array and VMRC-RCW tumors. pRCC = papillary renal cell carcinoma, chRCC = Chromophobe renal cell carcinoma, MAG = magnification. Similar CA9 H-score indicates VMRC-RCW xenograft model is a clinically relevant system.

PD-32766 shows selective and strong binding to CA9.

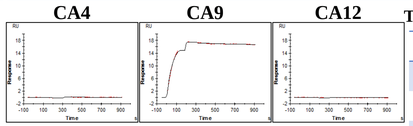


Figure4. Cross-reactivity to other CA family proteins

Table1. CA9 binding affinity of metal-conjugate PD-32766

	Ka(1/Ms)	kd(1/s)	KD (M)
PD-32766	7.51 x 10^5	4.57 x 10^{-5}	6.09 x 10^{-11}
^{nat}Cu-PD-32766	3.78 x 10^5	6.11 x 10^{-5}	1.11 x 10^{-10}
^{nat}Lu-PD-32766	8.44 x 10^5	4.76 x 10^{-5}	5.64 x 10^{-11}

Table2. Species cross-reactivity of PD-32766 analysis (KD (M))

	Human CA9	Mouse CA9	Rat CA9	Monkey CA9	Dog CA9
PD-32766	6.09 x 10^{-11}	4.97 x 10^{-8}	4.63 x 10^{-8}	3.67 x 10^{-10}	4.37 x 10^{-10}

Surface plasmon resonance (SPR) analysis of PD-32766
To analyze cross-reactivity of PD-32766 to other CA family proteins, His tagged human CA4, CA9 and CA12 were captured through His antibody on a sensor chip. To analyze species cross-reactivity of PD-32766, Fc tagged human, mouse, rat, monkey, dog CA9 proteins were captured through Protein A on a sensor chip. PD-32766, ^{nat}Cu-PD-32766 or ^{nat}Lu-PD-32766 was flowed onto a sensor chip in which protein was captured, and the association and disassociation constants were analyzed.

PD-32766 clearly detects ccRCC tumor with rapid renal clearance.

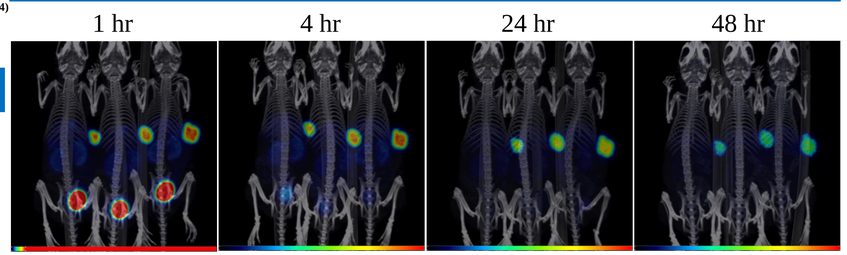


Figure5. PET-CT imaging of ^{64}Cu-PD-32766 in VMRC-RCW xenograft mouse
^{64}Cu-PD-32766 (4.3 MBq) was injected intravenously to xenografted mouse (N=3). PET-CT imaging was performed at 1-, 4-, 24-, and 48-hours post-dosing. PET-CT= positron emission tomography- computed tomography.

PD-32766 showed strong and specific distribution in the tumor.

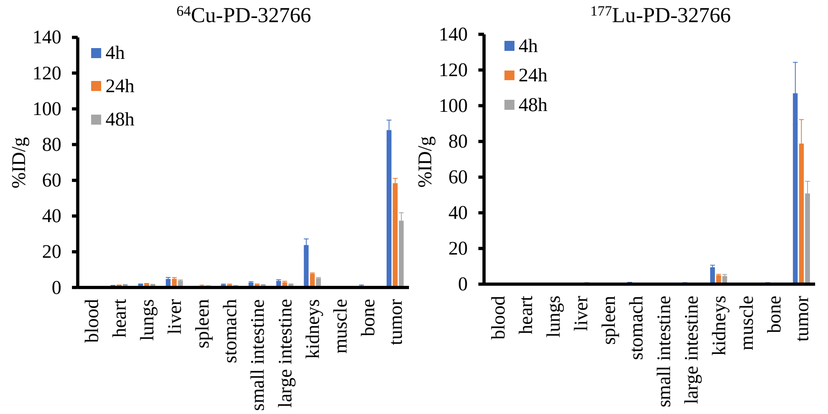


Figure6. Biodistribution of ^{64}Cu-PD-32766 and ^{177}Lu-PD-32766 in VMRC-RCW xenograft model
VMRC-RCW xenograft mice were prepared in the same method as for Figure 3. Mice were injected intravenously with 4.3 MBq of ^{64}Cu-PD-32766 or 2.8 MBq of ^{177}Lu-PD-32766. At 4, 24, and 48 h post-injection, mice (N=3, each time point) were sacrificed. The percentage injected dose per gram (%ID/g) was then calculated by weighing the organ and counting the radioactivity with a gamma counter. Data are shown as mean ± SD.

^{177}Lu-PD-32766 showed robust *in vivo* anti-tumor effects.

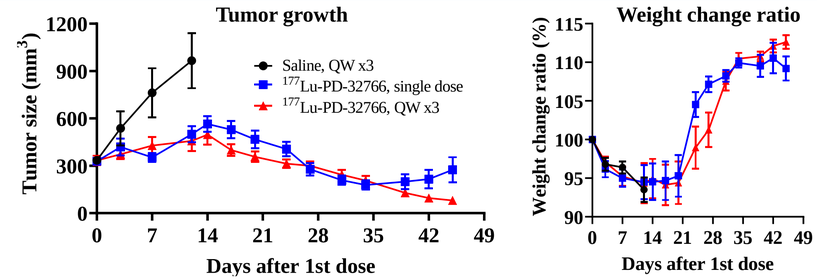


Figure7. *In vivo* efficacy of ^{177}Lu-PD-32766 in VMRC-RCW xenograft mice.
At 14 day after VMRC-RCW inoculation, mice were divided into 3 groups with 6 mice per group: (1) saline (2) single dose of ^{177}Lu-PD-32766 (30 MBq) (3) repeated dose of ^{177}Lu-PD-32766(30 MBq, QW 3times). A 30 MBq dose to mice is equivalent to 7.32 GBq in human, which is a clinically used dose. (cf: Lutathera® is administered at 7.4 GBq/dose in human[5]). Data are shown as mean ± SEM.

PD-32766 has rapid blood clearance and large safety margin.

Figure8. Plasma concentration of PD-32766 in Rats

Table3. PK parameter of PD-32766 in rats

	Male	Female
$T_{1/2}$(h)	0.48 ± 0.04	0.57 ± 0.10
C_0 (ng/mL)	9112 ± 334	7372 ± 2126
AUC_{last} (h*ng/mL)	4353 ± 320	4181 ± 465

Table4. Predicted human ^{64}Cu-PD-32766 dosimetry based on rat biodistribution of ^{64}Cu-PD-32766

Organ	Absorbed dose (mGy/MBq)	EBRT limit (Gy)	Allowed dose (GBq)
Kidneys	0.159	23[6]	145
Urinary bladder wall	0.060	65[7]	1078
Lower Large Intestine	0.015	45[7]	2961
Small intestine	0.013	40[7]	2985
Liver	0.010	30[6]	3036
Stomach wall	0.008	45[6]	5747
Upper Large Intestine	0.006	45[7]	7732
Spleen	0.005	40[6]	8097
Heart wall	0.004	26[6]	7242
Red Marrow	0.003	2[6]	671
Lungs	0.002	20[8]	8969

Predicted clinical dose of ^{64}Cu-PD-32766 is 148 MBq.
Human estimated effective dose (male): 0.0118 mSv/MBq.

PK parameter and dosimetry test in rat
To evaluate PK parameter in rat, male and female SD rats (n=3 each) were injected intravenously with 2.5 mg/kg of PD-32766. At 0.5-, 1-, 2-, 4-, 6-, 24-hours post-dose, the plasma concentration of PD-32766 was measured by LC-MS. At 6-, 24-hours post-dose, the plasma concentration of PD-32766 was below the detection limit.
For dosimetry, SD rats were injected intravenously with 2.3 MBq of ^{64}Cu-PD-32766. At 0.082-, 1-, 2- , 4-, 6-, 20-, 24-hours post-dose, radioactivity levels were measured in each organ by cut-and-count method (N=3, each time point). Predicted human dosimetry and human estimated effective dose were calculated using OLINDA/EXM version 1.0. .
BLOQ= Below quantifiable limit, EBRT= External beam radiotherapy.

Conclusion

- PD-32766 selectively and strongly binds to CA9 regardless to metal ions chelating.
- ^{64}Cu-PD-32766 highly specifically detected CA9 expressing tumors in PET-CT, which is similar to the biodistribution of ^{177}Lu-PD-32766, indicating that ^{64}Cu-PD-32766 PET imaging is valuable to select patients with CA9 positive tumors, and evaluation of the efficacy of ^{177}Lu-PD-32766 treatment.
- ^{177}Lu-PD-32766 showed evident *in vivo* anti-tumor effect and good tolerability in a clinically relevant ccRCC xenograft mouse model at a clinical dose.
- Taken together, PD-32766 is a potential Best-in-class theranostic drug for CA9 expressing tumors including ccRCC.
- Clinical imaging study of ^{64}Cu-PD-32766 in ccRCC patients is on-going in Japan.

Reference

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