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GPC3 targeted radiopharmaceutical therapy for HCC: Preclinical characterization of a novel peptide binder

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Abstract # 279
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Background

Glypican-3 (GPC3) is an attractive target for targeted radiopharmaceutical therapy (RPT)

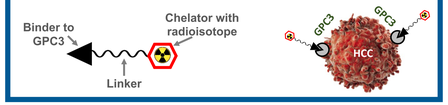
- Absent/minimally expressed in normal adult tissues [1,2]
- Significantly upregulated in up to 75% of hepatocellular carcinomas (HCC), and associated with poor prognosis [3,4,5]
- Clinical evidence of efficacy in HCC established by GPC3-CAR-T [6,7]

Targeting GPC3 could fulfill an urgent unmet medical need

- Liver cancer (HCC accounts for up to 85% of cases) is the 6th most diagnosed cancer and 3rd most common cause of cancer death globally [8]
- Chemotherapy and TKI therapies have limited efficacy and tolerability challenges
- The differential expression of GPC3 between HCC and normal tissues provides opportunity for GPC3-targeted theranostic development

RAYZ-8009 is novel, potent GPC3 binder for RPT

- Comprised of a novel peptide binder to GPC3 and chelator that binds radiometal isotopes
- Demonstrated potent and selective GPC3 binding, fast and efficient internalization, tumor specific uptake and retention, and preclinical anti-tumor efficacy



Results

In vitro binding and internalization

Protein binding by SPR

K_D = 0.7 nM

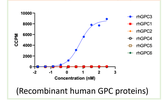
(^{175}Lu labeled binder, human recombinant GPC3)

Comparable binding across isotopes

Label	*Ki (nM)*
^{175}Lu-	9.4
^{139}La-	15.7
^{69}Ga-	13.7
^{63}Cu-	18.3

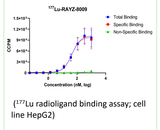
(Cell line HepG2)

No binding to GPC1, 2, 4-6



(Recombinant human GPC proteins)

Cellular binding



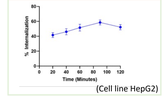
(^{177}Lu radioligand binding assay; cell line HepG2)

Comparable binding across species

Species	*K_D (nM)*
Human	2.1
Mouse	1.4
Cyno	4.0

(^{177}Lu labeled binder; recombinant proteins)

Fast and efficient internalization



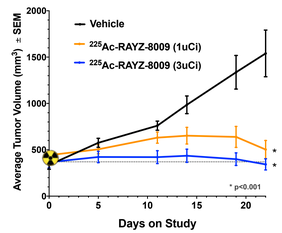
(Cell line HepG2)

PK and stability

	Mean PK Parameters of ^{175}Lu-RAYZ-8009					
Dose Level (mg/kg)	C_0 (ng/mL)	α $T_{1/2}$ (h)	Vd_{ss} (L/kg)	Cl (mL/min/kg)	$AUC_{0\text{-last}}$ (ng•h/mL)	$AUC_{0\text{-inf}}$ (ng•h/mL)
5	50356	0.30	0.172	7.63	10891	10917

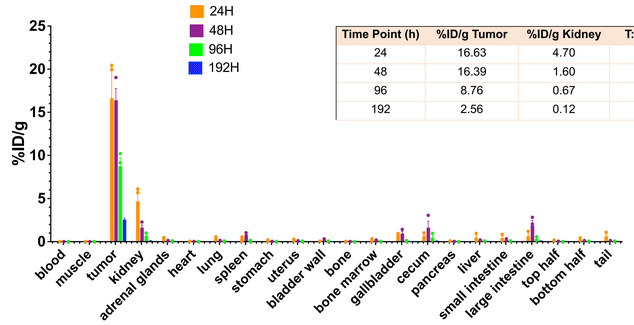
- ^{139}La-RAYZ-8009 was stable in plasma of all tested species with more than 95% remaining after 4 hours of incubation
- Plasma protein binding (PPB) of ^{139}La-RAYZ-8009 was 27%, 54%, 31% and 38% in CD-1 mouse, SD rat, beagle dog and human plasma, respectively

In vivo efficacy



- Single dose 1 µCi (37kBq) and 3 µCi (111kBq)
- Significant and dose (activity)-dependent TGI

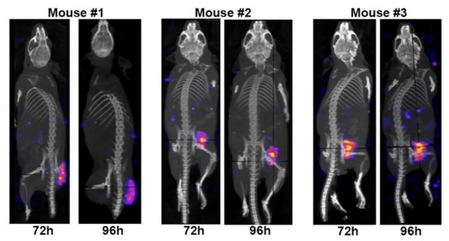
In vivo biodistribution



Time Point (h)	%ID/g Tumor	%ID/g Kidney	T:K Ratio
24	16.63	4.70	**3.69**
48	16.39	1.60	**11.33**
96	8.76	0.67	**15.04**
192	2.56	0.12	**22.29**

- ^{177}Lu-RAYZ-8009, 100 µCi (3.7 MBq), i.v.
- HepG2 tumor bearing (SC) female nude mice, n=3 per time point; cut and gamma count
- High tumor-specific uptake and retention, and fast renal clearance
- Minimal uptake was observed in other normal tissues

SPECT imaging



- ^{177}Lu-RAYZ-8009, 100 µCi (3.7 MBq), i.v.
- HepG2 xenograft model
- High tumor-specific uptake and retention
- No/minimum uptake in other normal tissues

Conclusion

Preclinical pharmacodynamic, pharmacokinetic, biodistribution and efficacy data demonstrate the potential of RAYZ-8009 as a RPT agent for the treatment of patients with GPC3-positive HCC

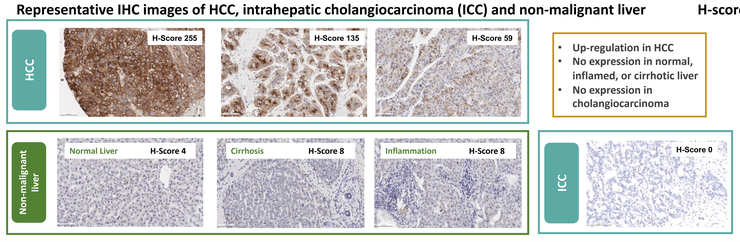
- **RAYZ-8009 is a potent and specific novel peptide binder to GPC3**
- **RAYZ-8009 efficiently internalizes upon binding to GPC3-overexpressing cancer cells**
- **^{177}Lu-RAYZ-8009 demonstrates efficient tumor uptake and retention, fast renal clearance, and minimum normal tissue uptake**
- **^{225}Ac-RAYZ-8009 significantly inhibits tumor growth in GPC3+ HCC xenograft model**

Method

- Binding affinity to recombinant GPC3 protein was determined by SPR
- Binding to HCC cell line HepG2 was assessed by a radioligand binding assay
- Binding affinity of ^{139}La-, ^{69}Ga-, and ^{63}Cu-RAYZ-8009 to HepG2 cells was determined by a competitive radioligand binding (RB) assay
- Cross-species binding was assessed by RB with mouse, cyno, and human GPC3 proteins
- Cross-reactivity to other GPCs was tested by RB assay using recombinant human GPC1-6
- Internalization in HepG2 cells was measured using Microbeta at various time points
- *In vivo* biodistribution studies with ^{177}Lu, and anti-tumor efficacy studies with ^{225}Ac, were performed in GPC3+ tumor-bearing nude mice

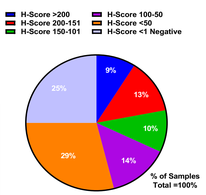
Expression

Representative IHC images of HCC, intrahepatic cholangiocarcinoma (ICC) and non-malignant liver



- Up-regulation in HCC
- No expression in normal, inflamed, or cirrhotic liver
- No expression in cholangiocarcinoma

H-score distribution in HCC cases (N=197)



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References

1. Filmus J, Capurro M, Rast J. Glypicans. Genome Biol. 2008;9:224
2. Haruyama Y, Kataoka H. Glypican-3 is a prognostic factor and an immunotherapeutic target in hepatocellular carcinoma. In World J Gastroenterol. 2016;22(1):275–283
3. Bell MM, Gutsche NT, King AP, et al. Glypican-3-Targeted Alpha Particle Therapy for Hepatocellular Carcinoma. Molecules. 2020;26(1):4
4. Kaseb AO, Hassan M, Lacin S, et al. Evaluating clinical and prognostic implications of Glypican-3 in hepatocellular carcinoma. Oncotarget. 2016;7(43):69916–69926
5. Shirakawa H, Suzuki H, Shimomura M, et al. Glypican-3 expression is correlated with poor prognosis in hepatocellular carcinoma. Cancer Sci. 2009;100(8):1403–1407
6. Sun H, Xing C, Jiang S, et al. Long term complete response of advanced hepatocellular carcinoma to glypican-3 specific chimeric antigen receptor T-Cells plus sorafenib, a case report. Front.Immunol. 2022;13: 963031
7. Steffin DHM, Ghatwai N, Batra S, et al. A Phase 1 Clinical Trial Using Armored GPC3-Car T Cells for Children with Relapsed/ Refractory Liver Tumors. ASGCT Annual Meeting. 2020; Abstract 486
8. Sung H, Ferlay J, Siegel RL, et al. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA Cancer J Clin. 2021;71(3):209–249.

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