Preclinical characterization of novel peptide binders for EphA2-targeted radiopharmaceutical therapy

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BACKGROUND

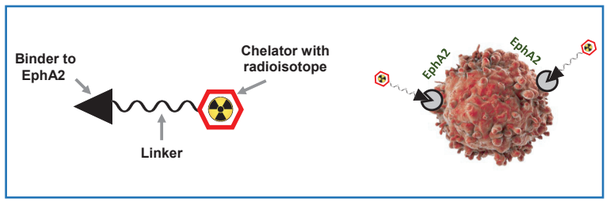
EphA2 is an attractive target for targeted radiopharmaceutical therapy

- EphA2 is a transmembrane glycoprotein that is primarily involved in tissue patterning during embryonic development.[1]
- Expression of EphA2 is low or absent in normal adult tissues, but it is overexpressed in multiple cancers, where it is often associated with poor prognosis.[1–3]
- The clinical validity of targeting EphA2 in cancers has been established using peptide-drug conjugates.[4–6]
- Broad overexpression in solid tumors and relatively low expression in normal adult tissues make EphA2 attractive for targeted radiopharmaceutical therapy (RPT).

RAYZ-6114 and RAYZ-6283 are novel, potent EphA2 binders for RPT

- RAYZ-6114 and RAYZ-6283 are made up of a novel macrocyclic peptide binder to EphA2, a linker and a chelator that binds radiometals (**Figure 1**).
- This poster describes the preclinical characterization of RAYZ-6114 and RAYZ-6283.

FIGURE 1. EphA2-targeted radiopharmaceutical therapy



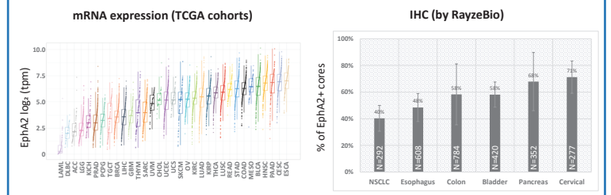
METHODS

- Immunohistochemistry (IHC) of human EphA2 was performed on human tissue microarrays representing diverse tumor types.
- RAYZ-6114 comprises a peptide binder to EphA2, a linker, and DOTA chelator that can be complexed with different isotopes:
  - RAYZ-6283 differs from RAYZ-6114 only in the linker.
- Binding affinity, selectivity and cross-species reactivity of the peptide binders to EphA2 and other ephrin proteins were determined by surface plasmon resonance (SPR).
- Internalization was measured using flow cytometry.
- *In vivo* biodistribution and anti-tumor efficacy studies were performed in PC3 tumor-bearing nude mice.
- Coagulopathy study was performed in Sprague Dawley rats.

RESULTS

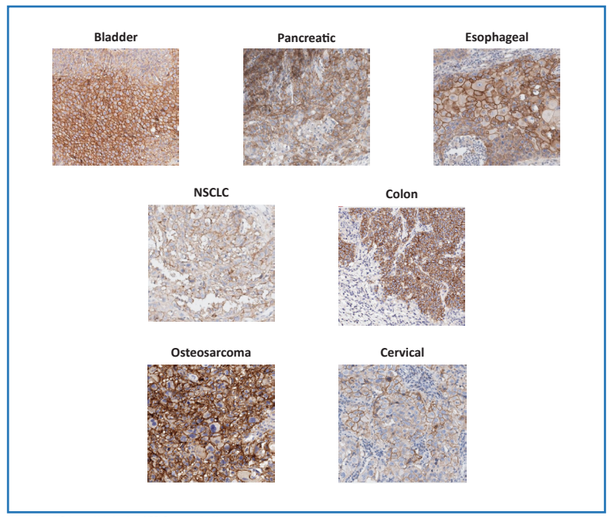
- IHC confirmed EphA2 expression in a multitude of solid tumors, with the highest positivity rates observed in cervical, pancreatic, bladder, colorectal, esophageal, osteosarcoma, and lung cancers (**Figure 2** and **Figure 3**).
- A final core binder was developed that retained tumor uptake while reducing kidney retention (**Figure 4**).
- RAYZ-6114 showed potent binding to human EphA2 (KD=0.03 nM), and equal potency against mouse and cynomolgus monkey EphA2 (**Figure 5**). RAYZ-6114 showed no cross-reactive binding to other ephrin type-A or ephrin type-B proteins (**Figure 5**).
- RAYZ-6114 exhibited potent binding to EphA2+ cells and no binding to EphA2-null cells (**Figure 6**). RAYZ-6114 was rapidly and efficiently internalized in EphA2+ cells, with ~75% internalized by 1 hour (**Figure 6**).
- In PC3 xenograft mice, ^{177}Lu-RAYZ-6283 showed sustained tumor uptake (~25% ID/g) for up to 48 hours (**Figure 7**) and tumor/kidney ratios of 2.7, 3.3, and 5.9 at 24 hours, 48 hours, and on Day 7, respectively (**Figure 7**). Low uptake was observed in normal tissues (**Figure 7**).
- Both ^{177}Lu- and ^{225}Ac-RAYZ-6114 significantly inhibited tumor growth in the PC3 xenograft model (Figure 8). Durable tumor regression and survival benefit was achieved by a single dose of ^{225}Ac-RAYZ-6114 (3 µCi), outperforming ^{177}Lu that was dosed at 1,000x higher activity (**Figure 8**).
- A coagulopathy study in Sprague Dawley rats showed no difference between ^{225}Ac-RAYZ-6283 (5uCi) and La-RAYZ-6283 (non-radioactive control) in blood clotting by prothrombin time test and activated partial thromboplastin time test (**Figure 9**). No bleeding was observed across multiple PK, biodistribution and efficacy studies in the mice using various radioisotopes.

FIGURE 2. EphA2-targeted radiopharmaceutical therapy



Abbreviations: IHC, immunohistochemistry; mRNA, messenger ribonucleic acid; NSCLC, non-small cell lung cancer; TCGA, The Cancer Genome Atlas.

FIGURE 3. Representative IHC of EphA2 in solid tumors



Abbreviations: IHC, immunohistochemistry; NSCLC, non-small cell lung cancer.

FIGURE 4. Evolution of EphA2 lead binder

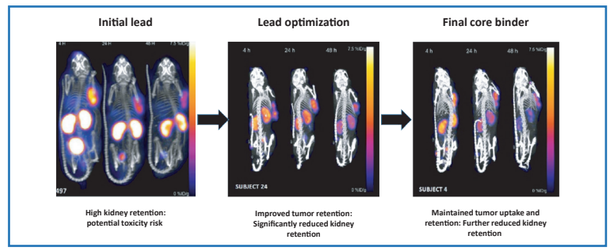


FIGURE 5. RAYZ-6114 binding potency and selectivity

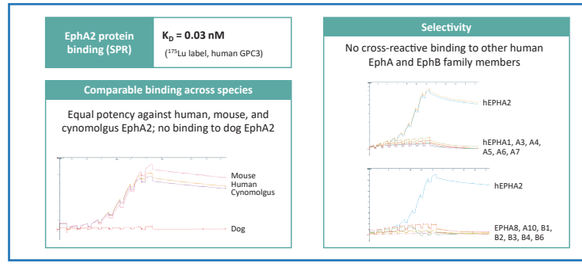


FIGURE 6. RAYZ-6114 cell binding and internalization

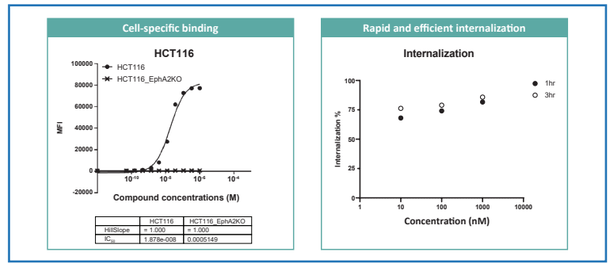


FIGURE 7. Biodistribution of RAYZ-6283 in PC3 tumor-bearing mice

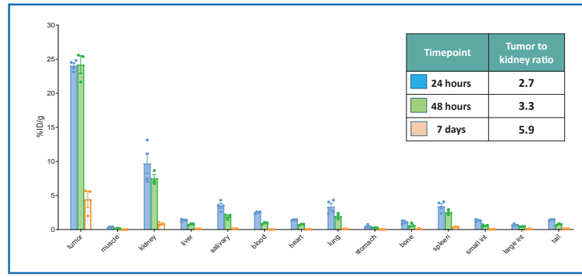


FIGURE 8. Efficacy of RAYZ-6114 in the PC3 xenograft model

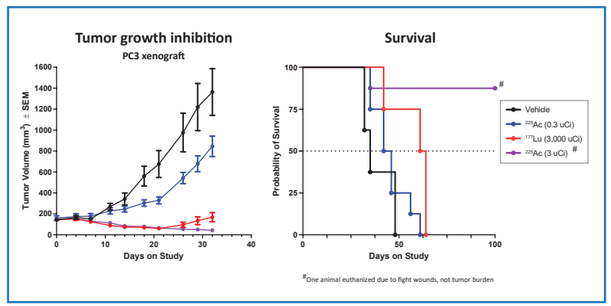
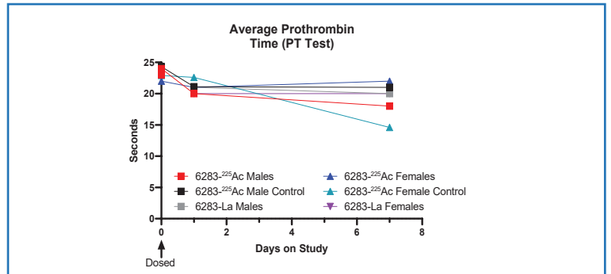


FIGURE 9. Coagulopathy in Sprague Dawley rats



CONCLUSIONS

- EphA2 is a promising target for radiopharmaceutical therapy to treat multiple cancer types.
- RAYZ-6114 and RAYZ-6283 are novel, potent EphA2 binders for radiopharmaceutical therapy.
- Preclinical characterization of RAYZ-6114 and RAYZ-6283 demonstrated highly potent and selective EphA2 binding, efficient internalization, and tumor specific uptake and retention.
- Single treatment of RAYZ-6114 in preclinical models using ^{225}Ac and ^{177}Lu resulted in notable tumor regression with ^{225}Ac showing better efficacy.

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- Renee Clift, Samantha Richardson, Ivy Hung, Zaid Amso, Katrina Salvador, Matt Guest, Guangzhou Han, Abhijit Bhat, Derek Cole, Eric Bischoff and Gary Li performed the studies as employees of RayzeBio, Inc.
- Takeru Ehara and Hayato Yanagida are employees of PeptiDream, Inc.

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