Conditionally activated membrane binding probes for improved targeted radiotherapy in metastatic castration resistant prostate cancer.

Apurva Pandey[1], Junyang Qi[1], Fiona Quimby[1], Michael J. Evans[1] and Charles S. Craik[2].

1. *Department of Radiology and Biomedical Imaging, UCSF*
2. *Department of Pharmaceutical Chemistry, UCSF*

Background: Recent FDA approvals and promising trials highlight growing interest in TRT for cancer treatment. However, clinical experiences, such as with Pluvicto for mCRPC, reveal that TRTs are often not curative, with variable and short-lived responses, despite patient preselection based on PET scans. Thus, there is an urgent unmet need to understand the basis of poor tumor responses to empower new strategies to maximize the therapeutic benefit of TRT for cancer patients.
We have approached this challenge by developing a new class of radiopharmaceuticals termed "restricted interaction peptides" (RIPs) that leverage tumor-associated proteases to activate a radiolabeled membrane-binding peptide in the tumor microenvironment. RIPs are small peptides (~4 kDa) with three domains: a membrane-binding antimicrobial peptide (AMP) with a radiolabeled chelator, an endoprotease cleavage site spanning P4-P4', and a masking domain to inhibit membrane binding. Upon cleavage by the target protease, the AMP is released and binds to nearby membranes. Herein, we propose to develop the first radiolabeled RIPs for mCRPC treatment by targeting hK2 (KRIP), an endoprotease specifically overexpressed and secreted by prostate adenocarcinoma, and FAPα (FRIP), a cell surface type II transmembrane endoprotease expressed by CAFs and mCRPC adenocarcinoma and SCNC.

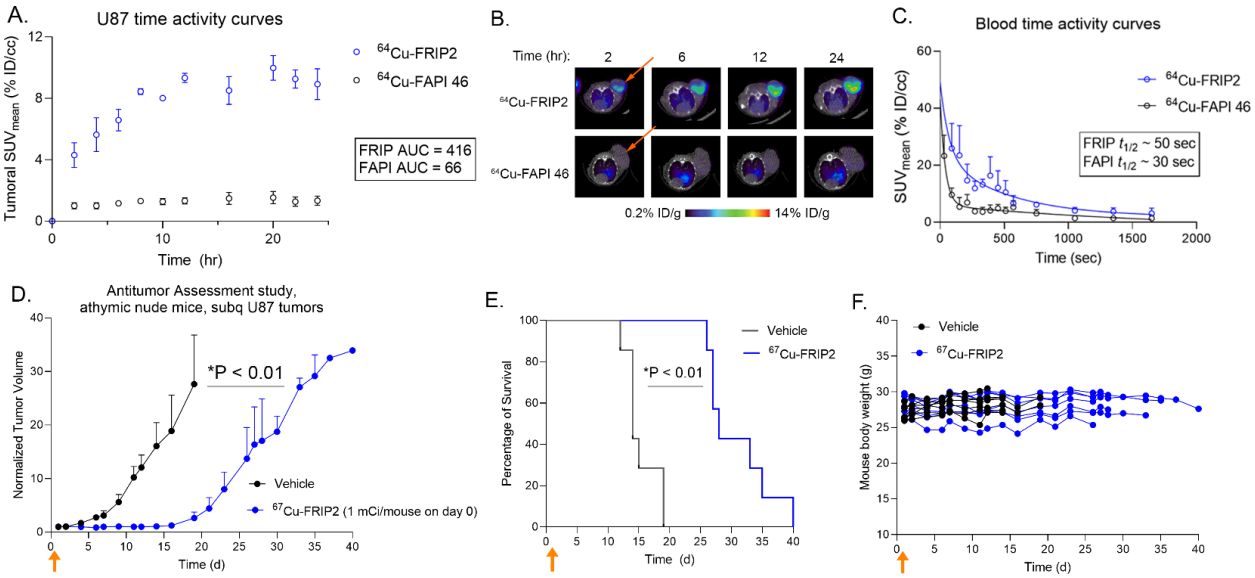
Methods: FRIP and KRIP were radiolabeled with ^{64}Cu and cleavage by FAPα and HK2 was confirmed via HPLC. In vivo selectivity of ^{64}Cu-FRIP2 uptake by FAPα-positive and negative tumors was tested in mouse models with various tumor types. Uptake in U87 (FAPα-positive) tumors was compared with FAPI-46, a clinical FAPαradioligand. Preliminary imaging with KRIP was performed in 22RV1 tumors.

Results: Lead cleavage sequences for FRIP and KRIP were selected using K_{cat}/K_m measurements and enzyme assays. The lead peptides were then labeled with ^{64}Cu, resulting in radiolabeled complexes with over 95% radiochemical yield and 99% purity for imaging experiments. At 24 hours post-injection of ^{64}Cu-FRIP2, U87 tumors showed significantly higher uptake compared to FAPα-negative models. ^{64}Cu-FRIP2 uptake in U87 tumors increased over time, reaching a SUV_{mean} of ~9% ID/cc, much higher than ^{64}Cu-FAPI 46 (~1% ID/cc). A single dose of ^{67}Cu-FRIP2 delayed tumor growth and extended survival in U87 MG and FaDu tumors without affecting body weight. Preliminary studies with KRIP in HK2-positive 22RV1 tumors showed significant, prolonged uptake. Ongoing imaging and therapy studies will be presented

Conclusions: We successfully utilized the RIP platform for imaging and treating cancers expressing FAPα and HK2. The modular nature of RIPs allows targeting of other endoproteases in prostate adenocarcinoma and conjugation with various payloads, including radiolabeling, for preclinical imaging and therapy in different cancer models

Funding Acknowledgements: Jeff and Loyd Zisk PCF YIA (2023) is greatly acknowledged for their funding and support.

Conflicts of Interest Disclosure Statement: CSC and MJE are co-inventors on a patent describing the RIP platform and are co-founders and hold equity in Therapaint, Inc.



A comparison of FRIP and RLT biodistribution suggests advantages for the RIP based approach. A. Time activity curves show that tumoral uptake of FRIP2 is ~5 fold higher than tumoral uptake of FAPI 46. **B.** Longitudinal PET/CT scans in mice with subcutaneous U87 xenografts (orange arrow) shows tumoral uptake of FRIP2 is higher and persists longer than FAPI 46. **C.** Blood curves collected from a dynamic PET scan show the clearance rates of FRIP2 and FAPI 46 are nearly identical. **D.** Tumor volume data showing that a single dose of ^{67}Cu-FRIP2 (1 mCi/mouse on day 0, orange arrow) significantly delays the growth of subcutaneous U87 MG, a tumor with modest and heterogeneous FAPα. **E.** A Kaplan Meier curve showing that ^{67}Cu-FRIP2 significantly extends mouse survival. **F.** Mouse body weight data showing that a single dose of ^{67}Cu-FRIP2 was well tolerated.