Comparative Biodistribution and Radiotherapeutic Efficacy of the Fibroblast Activation Protein (FAP)–Targeting Agents FAP-2286 and FAPI-46

Abstract 3317

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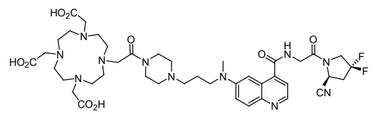
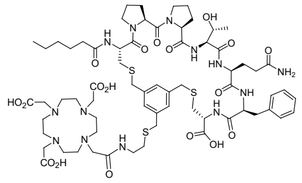
INTRODUCTION

- Fibroblast activation protein (FAP) is a membrane-bound protease under investigation as a pan-cancer imaging and therapeutic target given its limited expression in normal adult tissues but high expression on cancer-associated fibroblasts[1]
- FAP-targeting agents, FAP-2286 and FAPI-46, have shown great promise as positron emission tomography (PET) imaging agents when chelated to Gallium-68 (^{68}Ga)
- FAPI-46 is a quinoline-based, FAP-targeting, small-molecule tetraazacyclododecane tetraacetic acid (DOTA) conjugate and has been shown to yield high tumor-to-background ratios in patients with various cancers[2]
- FAP-2286, a FAP-binding peptidic macrocycle coupled to the radionuclide chelator DOTA, constitutes a new class of FAP-targeting modalities. PET scans of ^{68}Ga-FAP-2286 demonstrated high uptake in neoplastic lesions and low uptake in normal tissues of patients with solid cancers[3]
- The goals of these studies were to evaluate the biodistribution of FAP-2286 and FAPI-46, when chelated to the beta emitter Lutetium-177 (^{177}Lu), and to correlate these results with the efficacy observed in the HEK293 FAP-overexpressing tumor model HEK-FAP

FAPI-46



FAP-2286



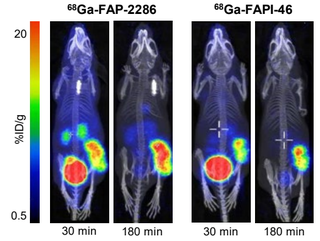
RESULTS

Biochemical and Cellular Characterization Showed High FAP Affinity

Test Systems	Readout (Units)	FAP-2286	FAPI-46
Recombinant human FAP protein	k_D (nM, mean ± SD)	1.1 ± 0.5	0.04 ± 0.01
Cellular FAP-positive WI-38 fibroblast	IC_{50} (nM, mean ± SD)	2.7 ± 0.9	1.3 ± 0.2
Recombinant human FAP protease assay	IC_{50} (nM, mean ± SD)	3.2 ± 0.6	1.2 ± 0.4

IC_{50}, half maximal inhibitory concentration; k_D, equilibrium dissociation constant; SD, standard deviation

PET/CT Imaging of ^{68}Ga-FAP-2286 and ^{68}Ga-FAPI-46 Demonstrated Comparable Uptake in HEK-FAP Tumors



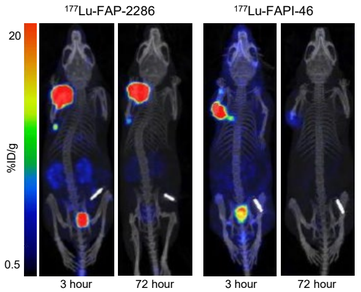
In Vivo Biodistribution of ^{68}Ga-FAP-2286 and ^{68}Ga-FAPI-46 in HEK-FAP Tumor-Bearing Mice by PET/CT Imaging

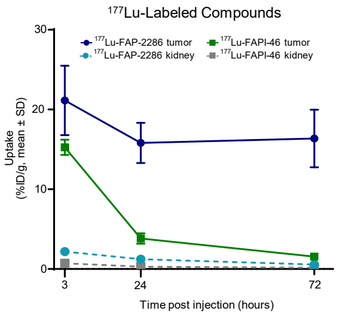
- *In vivo* imaging with ^{68}Ga-FAP-2286 and ^{68}Ga-FAPI-46 showed similar tumor uptake with 9.8 ± 0.7 and 9.3 ± 1.8 %ID/g at 30 minutes post injection (p.i.), respectively; and 10.8 ± 0.7 and 9.2 ± 1.7 %ID/g at 180 minutes p.i., respectively (n=3, *P*>0.05)

%ID/g, percent injected dose of test article per gram of tissue; CT, computerized tomography; PET, positron emission tomography

RESULTS

SPECT/CT Imaging Showed Longer HEK-FAP Tumor Retention of ^{177}Lu-FAP-2286 Compared with ^{177}Lu-FAPI-46





In Vivo Biodistribution of ^{177}Lu-FAP-2286 and ^{177}Lu-FAPI-46 in HEK-FAP Tumor-Bearing Mice by SPECT/CT Imaging

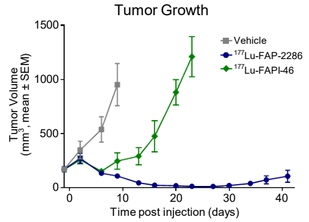
- ^{177}Lu-FAP-2286 maintained tumor levels of 15.8 ± 2.5 and 16.4 ± 3.6 %ID/g at 24 and 72 hours p.i., respectively, while imaging with ^{177}Lu-FAPI-46 showed shorter tumor retention time with 3.8 ± 0.7 (*P*=0.001) and 1.6 ± 0.4 %ID/g (*P*=0.002) at 24 and 72 hours p.i., respectively
- Longer tumor retention of FAP-2286 as compared to FAPI-46 resulted in 9- and 8-fold higher TIAC and absorbed dose delivered to the tumors

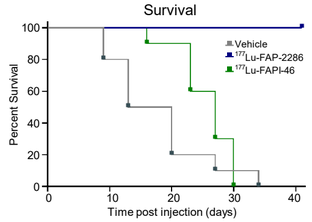
Tumor Uptake in HEK-FAP Xenograft Model

Compound	Tumor uptake (%ID/g, mean ± SD)			TIAC (MBq*h/MBq)	Absorbed dose (Gy/MBq)
	3 hour	24 hour	72 hour		
^{177}Lu-FAP-2286	21.1 ± 4.4	15.8 ± 2.5	16.4 ± 3.6	6.6	1.6
^{177}Lu-FAPI-46	15.3 ± 1.0	3.8 ± 0.7	1.6 ± 0.4	0.7	0.2

%ID/g, percent injected dose of test article per gram of tissue; CT, computerized tomography; p.i., post injection; SD, standard deviation; SPECT, single-photon emission computerized tomography; TIAC, time-integrated activity coefficient

^{177}Lu-FAP-2286 Demonstrated Greater HEK-FAP Tumor Growth Inhibition Compared with ^{177}Lu-FAPI-46





Antitumor Activity of ^{177}Lu-FAP-2286 and ^{177}Lu-FAPI-46 in FAP-expressing HEK-FAP Xenograft Model

- Treatment with ^{177}Lu-FAP-2286 and ^{177}Lu-FAPI-46 (30 MBq) resulted in 108% and 90% tumor growth inhibition (TGI) on day 9, respectively (*P*<0.001)
- Suppression of ^{177}Lu-FAPI-46 treated tumors was short-lived and tumors continued to grow, while ^{177}Lu-FAP-2286 treatment maintained tumor regression on day 23 when MTVs were significantly different between ^{177}Lu-FAP-2286 and ^{177}Lu-FAPI-46 (12 and 1210 mm^3, respectively)
- The median survival time (MST) for the ^{177}Lu-FAP-2286 group was not reached, while the MST for the vehicle control and ^{177}Lu-FAPI-46 groups were 16.5 and 27.5 days, respectively

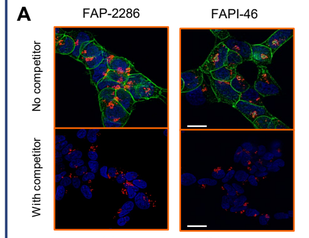
Tumor Efficacy in HEK-FAP Xenograft Model

Compound	MTV ± SEM (mm³, day 0)	MTV ± SEM (mm³, *P* value, day 9)	MTV ± SEM (mm³, day 23)	TGI (%, day 9)	MST (day)
Vehicle	169 ± 21	952 ± 195	NA	NA	16.5
^{177}Lu-FAP-2286	169 ± 23	107 ± 15 (*P*<0.0001)*	12 ± 4	108	undefined
^{177}Lu-FAPI-46	168 ± 22	245 ± 76 (*P*=0.0006)*	1210 ± 185 (*P*<0.0001)*	90	27.5

MST, median survival time; MTV, mean tumor volume; TGI, tumor growth inhibition

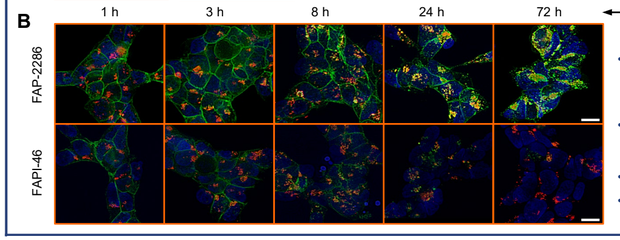
*P value was determined for day 9 comparisons to the vehicle group (n=10 for all groups), while day 23 comparison was between ^{177}Lu-FAP-2286 (n=10) and ^{177}Lu-FAPI-46 (n=9)

FAP-2286 Showed Durable Cellular Retention Compared to FAPI-46 in HEK-FAP cells



Live Cell Fluorescence Microscopy with AlexaFluor488-labeled FAP-2286 and FAPI-46

- FAP-2286 and FAPI-46 showed similar extracellular binding to HEK-FAP cells that was completely blocked by unlabeled competitor
- FAP-2286 showed greater cell surface and intracellular fluorescence compared to FAPI-46 starting at 8 hours of incubation, and by 72 hours, FAPI-46 was not detectable while FAP-2286 retained intracellular signal with minimal cell surface fluorescence



- Green: AlexaFluor488-labeled test compounds
- Red-orange: Lysosomes or late endosomes
- Blue: nucleus
- Scale bar 10 µm

METHODS

- **Biochemical and cellular assays:** The binding kinetics (k_D) of the test compounds to antibody-immobilized human FAP protein was measured by surface plasmon resonance. Human recombinant FAP was incubated with test compounds before a fluorophore-labelled FAP substrate was added, and fluorescence output was measured in a kinetic mode. FAP-expressing WI-38 fibroblasts were co-incubated with Cy5-labelled FAP-binding competitor peptide and test compounds. Median fluorescence intensity was measured by flow cytometry
- **In vivo biodistribution and efficacy studies:** Female NMRI nu/nu mice were subcutaneously implanted with 5 × 10^6 human FAP-transfected HEK293 cells (HEK-FAP). For PET imaging,10 MBq (1 nmol) ^{68}Ga-FAP-2286 or ^{68}Ga-FAPI-46 single dose was administered by intravenous injection, while for single-photon emission computed tomography (SPECT) imaging and tumor efficacy, 30 MBq (1 nmol) ^{177}Lu-FAP-2286 or ^{177}Lu-FAPI-46 single dose was administered. Studies were performed at Minerva Imaging ApS, Denmark
- **Internalization assay**: HEK-FAP cells seeded on chamber slides were incubated with 5 nM of AlexaFluor488-labeled FAP-2286 or FAPI-46 for 1 h at 37°C. Cells were then washed and further incubated for various time points. Cells were stained with Hoechst 33342 dye and LysoTracker Deep Red. Images were acquired by using a Keyence BZ X800E fluorescence microscope at 40× magnification

SUMMARY

- **FAP-2286 demonstrated potent affinity for human FAP by biochemical and cell-based assays**
- **^{177}Lu-FAP-2286 showed longer tumor retention, resulting in greater tumor growth inhibition as compared to ^{177}Lu-FAPI-46**
- **The prolonged tumor retention of FAP-2286 correlated with a higher intracellular accumulation**
- **The phase 1/2 LuMIERE clinical trial (NCT04939610) is evaluating FAP-2286 as a therapeutic (^{177}Lu-FAP-2286) and imaging (^{68}Ga-FAP-2286) agent in multiple FAP-expressing tumor types**

AUTHOR DISCLOSURES

Dirk Zboralski, Aileen Hoehne, Anne Bredenbeck, Matthias Paschke, Jan Lennart von Hacht, and Frank Osterkamp are employees of 3B Pharmaceuticals GmbH and may own stock or have stock options in that company. Andrew Simmons, Minh Nguyen, Jim Xiao, and Thomas Harding are or were employees of Clovis Oncology, Inc. and may own stock or have stock options in that company.

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1. Pure et al. *Oncogene.* 2018;37:4343-57. **2.** Loktev et al. *J Nucl Med.* 2019; 60:1421-29. **3.** Baum et al. *J Nucl Med.* 2021;62:1571-81.

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