Fibroblast Activation Protein (FAP)-Targeted Radiotherapy Increases Tumor CD8⁺ T Cell Infiltration and Enhances Response to PD-1 Immune Checkpoint Blockade

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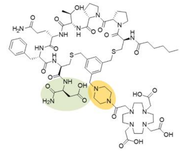
INTRODUCTION

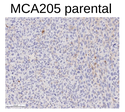
- Fibroblast activation protein (FAP) is a membrane-bound protease under investigation as a pan-cancer target, given its high levels in tumors but limited expression in normal tissues.[1]
- FAP-2286 is a radiopharmaceutical in clinical development for solid tumors that consists of two functional elements: a FAP-targeting cyclic peptide and a DOTA chelator used to attach radioisotopes.[2]
- Preclinically, we evaluated the immune modulation and anti-tumor efficacy of FAP-2287, a murine surrogate for FAP-2286, conjugated to the beta-particle emitting radionuclide lutetium-177 (^{177}Lu) as a monotherapy and in combination with a PD-1 targeting antibody in a syngeneic mouse model of sarcoma (MCA205-mFAP).

FAP-2287 structure

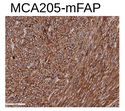


MCA205 mouse model

MCA205 parental



MCA205-mFAP



METHODS

- **Biochemical and cellular FAP assays:** The binding kinetics (k_D) were measured by surface plasmon resonance (SPR). Inhibition of enzyme activity was measured in a FAP endopeptidase assay.[2]
- ***In vivo* biodistribution and efficacy study:** Female C57BL/6 mice were subcutaneously implanted with 1 × 10^6 MCA205-mFAP cells. For SPECT imaging and tumor efficacy, a single dose of 30 MBq and 60 MBq (1 nmol) ^{177}Lu-FAP-2287, respectively, was administered by intravenous injection while 10 mg/kg anti-PD-1 (RPM1-14) was given intraperitoneal injection twice weekly for 6 doses. The study was performed at Minerva Imaging ApS, Denmark.
- **Flow cytometric immune cell characterization**: MCA205-mFAP tumor bearing mice were treated with 60 MBq (1 nmol) ^{177}Lu-FAP-2287 single dose and 10 mg/kg anti-PD-1 (RPM1-14) twice weekly. Tumors were collected at days 8 and 13, dissected into small pieces and digested using a tumor dissociation enzyme mix (Miltenyi Biotec). Cells were analyzed by flow cytometry. The study was performed at Minerva Imaging ApS, Denmark. Significant changes was determined by 2-way ANOVA for multiple comparison between groups in table or graphs and noted as *P<0.05, **P<0.01, ***P<0.001 and ****P<0.0001.
- **RNA expression analysis**: Total RNA was isolated from dissected tumors using the PureLink RNA Mini kit (Thermo Fisher Scientific). The nCounter PanCancer Immune Profiling (mouse) panel (NanoString Technologies) was used. Following hybridization, transcripts were quantitated and analyzed using the nCounter Digital Analyzer and nCounter Advanced Analysis Software v4.0 (NanoString Technologies).

REFERENCES

1. Pure et al. *Oncogene*. 2018;37:4343-57
2. Zboralski et al. *Eur J Nucl Med Mol Imaging*. 2022;49(11):3651-3667

RESULTS

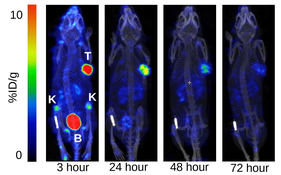
FAP-2287 has High Affinity to Human and Mouse FAP

Test Systems (Readout)	FAP-2287 (mean ± SD)	^{nat}Lu-FAP-2287 (mean ± SD)
SPR - Human FAP (K_D, nM)	0.4 ± 0.1	0.1 ± 0.1
SPR - Mouse FAP (K_D, nM)	1.2 ± 0.2	0.5 ± 0.1
Activity - Human FAP (IC_{50}, nM)	1.4 ± 0.3	1.3 ± 0.3
Activity - Mouse FAP (IC_{50}, nM)	5.1 ± 0.5	3.3 ± 0.4

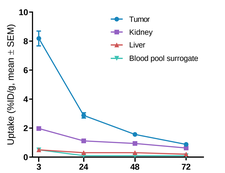
SPR, surface plasmon resonance; IC_{50}, half maximal inhibitory concentration; K_D, equilibrium dissociation constant; SD, standard deviation

In Vivo Biodistribution of ^{177}Lu-FAP-2287 by SPECT Imaging

^{177}Lu-FAP-2287 SPECT/CT imaging



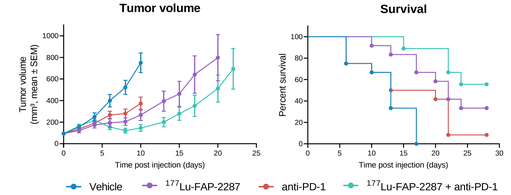
Quantification of SPECT/CT imaging



- ^{177}Lu-FAP-2287 accumulated higher in MCA205-mFAP tumors than in organs

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

Anti-tumor Activity of ^{177}Lu-FAP-2287 plus anti-PD-1



- Significant efficacy with ^{177}Lu-FAP-2287 and the combination with anti-PD-1

Compound	MTV ± SEM, mm³ (*P* value)	TGI (%)	MST, day (*P* value)
Vehicle	750 ± 91	NA	13.0
^{177}Lu-FAP-2287	266 ± 53 (*P*=0.0025)	74	22.0 (*P*=0.0006)
Anti-PD-1	373 ± 60 (*P*=0.0182)	57	16.5 (*P*=0.0606)
^{177}Lu-FAP-2287 + anti-PD-1	145 ± 31 (*P*=0.0005)	92	27.0 (*P*<0.0001)

MTV, mean tumor volume on day 10; TGI, tumor growth inhibition on day 10; MST, median survival time; *P* value for MTV vs vehicle group by 2-way ANOVA for multiple comparison on day 10 when >75% of mice were on study

ACKNOWLEDGEMENTS

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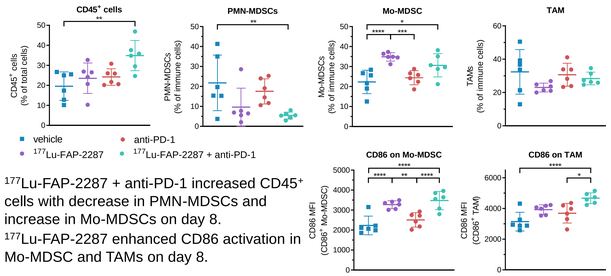
Immune Profiling of MCA205-mFAP Tumors by Flow Cytometry

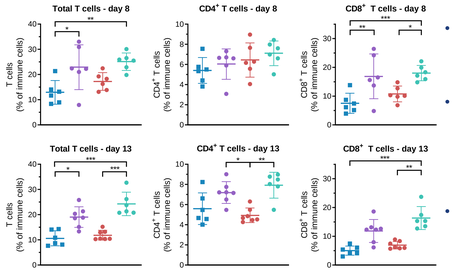
^{177}Lu-FAP-2287 + anti-PD-1 Enhanced CD86 Activation in Mo-MDSCs and TAMs



- ^{177}Lu-FAP-2287 + anti-PD-1 increased CD45⁺ cells with decrease in PMN-MDSCs and increase in Mo-MDSCs on day 8.
- ^{177}Lu-FAP-2287 enhanced CD86 activation in Mo-MDSC and TAMs on day 8.

MDSCs, myeloid-derived suppressor cells; Mo-MDSC, monocytic MDSCs; PMN-MDSCs, polymorphonuclear MDSCs; TAM, tumor associated macrophages

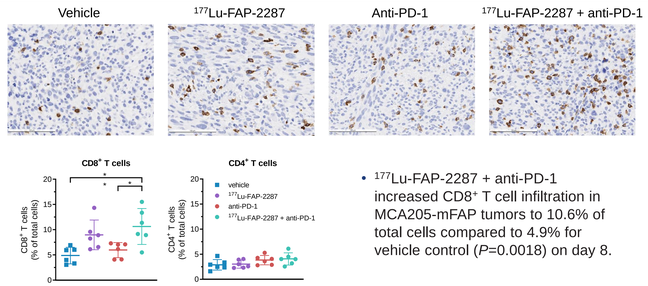
^{177}Lu-FAP-2287 + anti-PD-1 Increased CD8⁺ T cell Tumor Infiltration



- ^{177}Lu-FAP-2287 alone and in combination with anti-PD-1 increased T cells on days 8 and 13.
- ^{177}Lu-FAP-2287 alone and in combination with anti-PD-1 increased CD8⁺ T cells on day 8, but CD8+ levels were maintained on day 13 with combination.
- No significant change was observed in CD4⁺ T cells on days 8 and 13.

CD8⁺ T cell infiltration by Immunohistochemistry

Confirmation of increased CD8⁺ T cell infiltration by IHC



- ^{177}Lu-FAP-2287 + anti-PD-1 increased CD8⁺ T cell infiltration in MCA205-mFAP tumors to 10.6% of total cells compared to 4.9% for vehicle control (*P*=0.0018) on day 8.

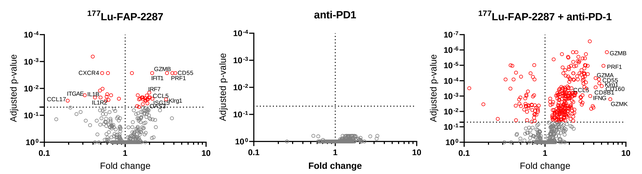
Representative images of tumor from one mouse for each treatment; **P* value was determined by 2-way ANOVA for multiple comparison

AUTHOR DISCLOSURES

D. Zboralski, F. Osterkamp, M. Paschke and A. Hoehne are employees of 3B Pharmaceuticals GmbH and are named inventors of FAP-2287. E. Christiansen is an employee of Minerva Imaging ApS. A. D. Simmons, L. Robillard, M. Nguyen and T. C. Harding are or were employees of Clovis Oncology, Inc and may own stock or have stock options in that company.

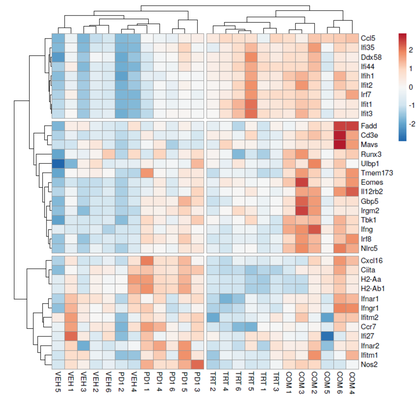
RNA Expression Profiling of MCA205-mFAP Tumors

^{177}Lu-FAP-2287 + anti-PD-1 Increased mRNA levels of many immune-related genes



- Differential expression analysis compared to vehicle control showed ^{177}Lu-FAP-2287 increased RNA levels of 28 genes involved in interferon response and natural killer cell biology, while combined with anti-PD-1 had more dramatic changes with transcript levels of 238 genes increasing and 52 decreasing.

Upregulation of STING pathway inducible genes in MCA205-mFAP tumors



- Several genes clustering together had increased levels in tumors treated with ^{177}Lu-FAP-2287 or combined with anti-PD-1 including STING pathway inducible genes.
- Another gene subset showed higher expression in combination treated tumors than with either monotherapy.
- The last gene subgroup appeared to be more involved with anti-PD-1 efficacy than with ^{177}Lu-FAP-2287 treatment.

VEH, vehicle; TRT, targeted receptor therapy (^{177}Lu-FAP-2287), COM, combination (^{177}Lu-FAP-2287 + anti-PD-1)

SUMMARY

- **FAP-targeted radionuclide therapy induces an immunogenic tumor microenvironment through infiltration and activation of immune cells resulting in enhanced tumor efficacy when combined with PD-1 checkpoint inhibition.**
- **Combination of ^{177}Lu-FAP-2287 with anti-PD-1 increased expression of immune-related genes in MCA205-mFAP tumors including STING pathway inducible genes.**
- **These findings provide a rationale for clinical studies of combined ^{177}Lu-FAP-2286 radiotherapy and immune checkpoint inhibition in FAP-positive tumors.**

Poster Download

Also available at: https://3b-pharma.com/wp/wp-content/uploads/2023/03/AACR_2023_Zboralski_a2835.pdf

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