Pan-Cancer Analysis of Fibroblast Activation Protein Alpha (FAP) Expression to Guide Tumor Selection for the Peptide-Targeted Radionuclide Therapy FAP-2286

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Introduction

- FAP is a membrane-bound protease\(^1\) with limited expression in normal tissues but high expression on cancer-associated fibroblasts abundant in the stroma of most tumors\(^2,3\).
- FAP-2286 is a potent and selective FAP-targeted peptide linked to the chelator DOTA that allows for the attachment of radionuclides for therapeutic (eg, \(^{177}\text{Lu}\)-FAP-2286) and imaging (eg, \(^{68}\text{Ga}\)-FAP-2286) applications.
- Assessing patterns of FAP expression in different tumor types can help guide tumor selection for \(^{177}\text{Lu}\)-FAP-2286 therapy.

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FAP, fibroblast activation protein alpha.

Gene expression screening of multiple tumor types characterized by TCGA data set revealed high FAP mRNA expression in a number of tumor types.

FAP Expression in Publicly Available Data Sets

cBioportal TCGA mRNA Expression, RSEM (batch normalized from Illumina HiSeq_RNASeqV2). Horizontal lines show the median mRNA expression for each tumor type.

FAP, fibroblast activation protein alpha; HNSCC, head and neck squamous cell carcinomas; mRNA, messenger RNA; RSEM, RNA-Seq by Expectation Maximization; TCGA, The Cancer Genome Atlas.
Pan-Tumor IHC Screen of FAP Expression

- Pan-tumor IHC screen revealed that ≥30% of samples in multiple solid tumor types had high FAP expression, including the following:
  - Pancreatic ductal adenocarcinoma (PDAC)
  - Cancer of unknown primary (CUP)
  - Salivary gland
  - Mesothelioma
  - Colon
  - Bladder
  - Sarcoma
  - NSCLC: squamous
  - HNSCC: squamous

Overall FAP H-score in IHC screen

Fraction FAP-high (H-score ≥30) for each tumor type is shown. Horizontal lines show the median H-score for each tumor type; the dashed line shows the cutoff for high FAP expression (H-score of 30). FAP IHC was performed by CellCarta on 360 formalin-fixed paraffin-embedded–preserved whole tumor tissue sections from 16 tumor types using the SP325 antibody (Spring Bioscience) on the Ventana Benchmark XT system. FAP, fibroblast activation protein alpha; HNSCC, head and neck squamous cell carcinoma; IHC, immunohistochemistry; NSCLC, non–small cell lung cancer; TNBC, triple-negative breast cancer.
High FAP expression was detected in both primary and metastatic samples (left) and was independent of grade (right) or tumor stage.

Samples from primary (P) or metastatic (M) sites

Low-grade (L) and high-grade (H) tumors

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Please see data in the accompanying poster, accessible via QR code at the end of the presentation.

Horizontal lines show the median H-score for each tumor type; dashed line shows the cutoff for high FAP expression (H-score of 30). Significant differences within a tumor type are shown; all other comparisons within a tumor type were nonsignificant. P values based on Wilcoxon rank sum test; no multiple hypothesis testing correction was performed.

CUP, cancer of unknown primary; FAP, fibroblast activation protein alpha; H, high grade (grade 3 or 4); HNSCC, head and neck squamous cell carcinoma; IHC, immunohistochemistry; L, low grade (grade 1 or 2); M, metastatic; NA, not available; NSCLC, non–small cell lung cancer; P, primary; PDAC, pancreatic ductal adenocarcinoma; QR, quick response; TNBC, triple-negative breast cancer.
In most tumor types, FAP was predominantly localized to the stroma surrounding the tumor cells.

FAP expression in tumor cells was also observed:
- Tumor-cell FAP expression was rare in cancers of epithelial origin and, when present, appeared weaker than in the adjacent stroma (e.g., esophagus).
- Tumor-cell FAP expression was common, consistent, and strong in cancers of mesenchymal origin (e.g., sarcoma and mesothelioma).

Patterns of FAP Expression Across Tumor Types

Representative images and H-score results for FAP expression

Overall FAP expression H-scores in the whole-tissue sections were calculated using the Visiopharm automated image analysis. FAP expression H-scores specific to the tumor and stroma compartments were calculated for a subset of samples using HALO (Indica Labs) automated image analysis; a trained pathologist validated the scoring results from both automated image analysis approaches. Scale bar, 100 μm. FAP, fibroblast activation protein alpha; HNSCC, head and neck squamous cell carcinoma; NE, not evaluable; NSCLC, non–small cell lung cancer.
A subset of tumors in multiple sarcoma subtypes demonstrated high FAP H-scores, suggesting that FAP expression is not limited to a specific subtype.

Biphasic and sarcomatoid mesothelioma subtypes tended to have higher FAP expression compared to the epithelioid subtype.

Graphs show quantification of FAP expression across different subtypes; horizontal lines show median FAP H-scores. Representative images show high FAP expression in tumor cells of different subtypes. P values based on Wilcoxon rank sum test; no multiple hypothesis testing correction was performed. Scale bars, 200 μm (sarcoma) or 100 μm (mesothelioma). FAP, fibroblast activation protein alpha.
There was significant correlation between FAP expression observed by IHC and FAP-2286 binding as assessed by autoradiography in matched frozen tissues.

Representative images of patient tumors are shown by autoradiography in fresh frozen tissues (top; scale bar, 500 μm) and IHC in matching FFPE-preserved tissue (bottom; scale bar, 100 μm). In the graph, FAP levels by autoradiography demonstrate a correlation with IHC in patient cholangiocarcinoma and sarcoma tumor sections. Autoradiography with $^{111}$In-FAP-2286 was performed on matched frozen tissue sections. Relative optical film density was determined using MCID™ software (InterFocus) and correlated with a calibration curve. CPM, counts per minute; FAP, fibroblast activation protein alpha; FFPE, formalin-fixed paraffin-embedded; IHC, immunohistochemistry.
A pan-tumor IHC screen identified high FAP expression in multiple tumor types that correlated with in vitro FAP-2286 binding, suggesting that FAP is an attractive target for peptide-targeted radionuclide therapy.

The phase 1/2 LuMIERE trial (NCT04939610) is enrolling patients to evaluate FAP-2286 as a therapeutic ($^{177}$Lu-FAP-2286) and imaging ($^{68}$Ga-FAP-2286) agent in multiple solid tumor types.

FAP, fibroblast activation protein alpha; IHC, immunohistochemistry.
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