Innovations in Peptide Targeted Radionuclide Therapies (PTRT) to Target Fibroblast Activation Protein (FAP) in Solid Tumors

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Cancer Associated Fibroblasts (CAFs) have Multiple Tumor Promoting Roles and are Associated With Aggressive Disease

- Cancer associated fibroblasts
  - Are one of the most abundant and heterogeneous components in solid tumors
  - Play an important role in the development and growth of cancer and its response to therapy
  - Have been associated with more aggressive disease and a poor prognosis
  - Can suppress the immune response and have been associated with anti-PD-1 or anti-PD-L1 failure

Figure was adapted from Sahai E et al. Nat Rev Cancer. 2020;20:174–186

FAP is a transmembrane cell surface proteinase that degrades proteins of the extracellular matrix\(^1,2\)

- FAP is highly expressed in activated CAFs abundant in the stroma of most tumors\(^3,4\)
- FAP has limited expression in normal adult tissues\(^5\)
  - Expression is observed at sites of tissue remodelling such as wound healing and fibrosis
- The high levels and restricted expression of FAP on CAFs and tumor cells make it an attractive target for peptide targeted radiotherapy (PTRT)

Fibroblast Activation Protein (FAP) is Highly Expressed in CAFs Found in the Tumor Microenvironment of Solid Tumors

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Pan-tumor Immunohistochemistry (IHC) Screen Confirms Subset of Tumors in Multiple Cancer Types Have High FAP Expression

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; 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. CUP, cancer of unknown primary; HNSCC, head and neck squamous cell carcinoma; NSCLC, non–small cell lung cancer; TNBC, triple-negative breast cancer. Source: Clovis internal data.
Tumor FAP Expression is Detected in Both Primary and Metastatic Sites and is Independent of Grade and Tumor Stage

- High FAP expression was detected in both primary and metastatic samples (left) and was independent of grade (right), or tumor stage (data not shown).
### Examples Highlighting Spectrum of FAP Expression in Pancreatic and Sarcoma Tumors

FAP IHC on tumor microarrays was performed by HistoWiz using the SP325 antibody (Spring Bioscience). Images are 300 μm. The total core was analyzed using ImageScope software and the results were used to calculate H-scores. Source: Clovis internal data.

<table>
<thead>
<tr>
<th>Overall FAP H-score</th>
</tr>
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<tbody>
<tr>
<td>0</td>
</tr>
<tr>
<td>51</td>
</tr>
<tr>
<td>101</td>
</tr>
<tr>
<td>132</td>
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</table>

**Pancreatic**

<table>
<thead>
<tr>
<th>0</th>
<th>49</th>
<th>109</th>
<th>148</th>
<th>221</th>
</tr>
</thead>
</table>

**Sarcoma**

| 0 | 49 | 109 | 148 | 221 |
FAP is Also Expressed on Tumor Cells in Some Cancers

- High FAP expression observed in CAFs in many epithelial tumor types
- Expression of FAP can also be observed in some tumor cells
  - In cancers of epithelial origin, low levels of FAP expression were sometimes observed
  - High FAP expression in tumors can be observed in some cancers of mesenchymal origin

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 bars, 100 μm. Source: Clovis internal data.

HNSCC, head and neck squamous cell carcinoma; NE, not evaluable; NSCLC, non–small cell lung cancer.
• Tumor uptake of $^{68}$Ga-FAPI-04 was evaluated by PET/CT in 80 patients representing 28 primary and metastatic tumor types
  - High and selective uptake observed in many cancers
Discovery efforts at 3B Pharmaceuticals resulted in the identification of FAP-2286. FAP-2286 is a FAP-targeted low molecular weight cyclic polypeptide linked to the chelator DOTA that allows for attachment of radionuclides for imaging and therapeutic use. FAP-2286 is potent and selective for FAP and stable in human plasma.

FAP Targeted Peptide FAP-2286 is Potent and Selective for FAP

FAP-2286 Biochemical and Cellular Characterization

<table>
<thead>
<tr>
<th>Assay Type</th>
<th>Test System</th>
<th>Readout</th>
<th>FAP-2286 (^1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Binding</td>
<td>Recombinant human FAP protein</td>
<td>(K_D)</td>
<td>1.1 ± 0.5 nM</td>
</tr>
<tr>
<td>Binding</td>
<td>Cellular FAP-expressing WI-38 fibroblast</td>
<td>IC(_{50})</td>
<td>2.7 ± 0.9 nM</td>
</tr>
<tr>
<td>Inhibition</td>
<td>Human FAP protease assay</td>
<td>IC(_{50})</td>
<td>3.2 ± 0.6 nM</td>
</tr>
<tr>
<td>Inhibition</td>
<td>Human DPP4 protease assay</td>
<td>IC(_{50})</td>
<td>&gt;10,000 nM</td>
</tr>
<tr>
<td>Inhibition</td>
<td>Human PREP protease assay</td>
<td>IC(_{50})</td>
<td>&gt;3,000 nM</td>
</tr>
<tr>
<td>Stability</td>
<td>Human plasma (24 h)</td>
<td>Remaining compound</td>
<td>106%</td>
</tr>
</tbody>
</table>

Source: Clovis internal data
1. Values are mean ± SD (if indicated).
Key Design Goal of FAP-2286 was Long Tumor Retention Time

- Tumor retention of $^{177}$Lu-FAP-2286 observed up to 120 hours post intravenous injection in mice with HEK-FAP tumors
Sustained Cellular Internalization of $^{177}$Lu-FAP-2286 Drives Long Tumor Retention

- In vitro and in vivo studies suggest sustained tumor retention by $^{177}$Lu-FAP-2286

Female NMRI nu/nu mice were subcutaneously implanted with $5 \times 10^6$ FAP expressing HEK293 cells. A single dose of 10 MBq activity per mouse of the indicated treatments was administered by intravenous injection. 3 mice per group were imaged by SPECT/CT at various time points after injection. HEK-FAP cells were seeded on chambered coverslips and incubated overnight. Cells were incubated with 5 nM fluorescently labeled compound for 1 hour at 37°C. Cells were washed and further incubated at 37°C for the indicated timepoints. Nuclei were stained with Hoechst33342 while lysosomes were stained with LysoTracker Deep Red. Images were taken with a Keyence BZ-X800 microscope. Source: Clovis internal data.
Longer Tumor Retention Results in Better Efficacy

- $^{177}$Lu-FAP-2286 causes tumor regression and maintained tumor inhibition out to day 41
  - No measurable tumors on day 41 in 6/10 mice treated with $^{177}$Lu-FAP-2286

Female NMRI nu/nu mice were subcutaneously implanted with $5 \times 10^6$ FAP expressing HEK293 cells. A single dose of 30 MBq activity per mouse of the indicated treatments was administered by intravenous injection (10 mice per group). Tumor volumes and body weights were measured twice weekly. Source: Clovis internal data.
Efficacy with $^{177}$Lu-FAP-2286 Also Observed in Sarcoma Patient Derived Xenograft (PDX) Model

- Single dose of $^{177}$Lu-FAP-2286 resulted in statistically significant tumor growth in the sarcoma Sarc4809 patient-derived xenograft model with endogenous FAP expression

![Tumor Volume Graph](image1)

![Body Weight Graph](image2)

Zborski D et al. ESMO 2020. Abstract 571P
Clinical Studies with FAP-2286 are Ongoing

- FAP-2286 is being investigated in a Clovis-sponsored Phase 1/2 clinical trial in multiple advanced solid tumors (LuMIERE; NCT04939610)
  - FAP-2286 is being used for imaging ($^{68}$Ga-FAP-2286) to identify FAP-positive patients and as a therapeutic agent ($^{177}$Lu-FAP-2286)
- In addition, FAP-2286 is being investigated to identify FAP-positive tumors in an imaging study of $^{68}$Ga-FAP-2286 PET in patients with solid tumors (NCT04621435)
  - Investigator-initiated study at UCSF (Principal Investigator is Thomas Hope)
LuMIERE: A Phase 1/2, Multicenter, Open-label, Non-Randomized Study to Investigate $^{177}$Lu-FAP-2286 in Patients With Advanced Solid Tumors

Eligibility criteria

**Phase I**
- $\geq$18 years of age
- Advanced or metastatic solid tumor
- Measurable disease per RECIST v1.1
- $^{68}$Ga-FAP-2286 positive solid tumor(s)
- Adequate bone marrow, hepatic and renal function
- ECOG PS 0 or 1
- Life expectancy of 6 months
- No prior systemic radiotherapy
- $^{68}$Ga-FAP-2286 IV for patient selection
- $^{177}$Lu-FAP-2286 IV every 6–8 weeks; maximum 6 doses per patient
- Dose escalation using the BOIN design
- Planar scans at 4, 24, 48, and 168 h; SPECT/CT scans at 24 and 168 h

<table>
<thead>
<tr>
<th>Dose level</th>
<th>$^{177}$Lu-FAP-2286 activity administered</th>
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<tbody>
<tr>
<td>-1</td>
<td>1.85 GBq (50 mCi)</td>
</tr>
<tr>
<td>1 (Starting dose)</td>
<td>3.70 GBq (100 mCi)</td>
</tr>
<tr>
<td>2</td>
<td>5.55 GBq (150 mCi)</td>
</tr>
<tr>
<td>3</td>
<td>7.40 GBq (200 mCi)</td>
</tr>
<tr>
<td>4 (Max dose)</td>
<td>9.25 GBq (250 mCi)</td>
</tr>
</tbody>
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**Primary objectives:** Determination of safety, MTD and/or RP2D  
**Secondary objectives:** Dosimetry, tumor SUV$_{\text{max}}$, PK, and efficacy

**Phase II**
- Tumor-specific cohorts
- Simon 2-stage design for each cohort

<table>
<thead>
<tr>
<th>Treatment</th>
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</table>
| Patients with positive uptake of $^{68}$Ga-FAP-2286 in target lesions will receive the RP2D dose and schedule of $^{177}$Lu-FAP-2286  
**Primary objective:** ORR by RECIST v1.1  
**Secondary objectives:** DoR, PFS, OS, and safety

Source: ClinicalTrials.gov NCT04939610, Clovis internal data. 1. Advanced solid tumor that is refractory to or progressed following prior treatment and have no satisfactory alternative treatment options; 2. Cumulative renal exposure limit = 23 Gy; cumulative bone marrow limit = 2 Gy
Investigator Initiated Study at UCSF Currently Enrolling: Imaging of Solid Tumors Using $^{68}$Ga-FAP-2286 at UCSF

- Principal Investigator: Thomas Hope

Cohort 1 (n=10)
- Agnostic to tumor type
- Dosimetry
  - 3 – 8 mCi $^{68}$Ga-FAP-2286
  - PET Imaging 30, 60, and 120 min

Cohort 2 (n=40)
- Pathologically confirmed breast cancer, pancreatic adenocarcinoma, sarcoma, castrate resistant prostate cancer, bladder cancer, colon cancer, or basket
- Imaging
  - 3 – 8 mCi $^{68}$Ga-FAP-2286
  - PET Imaging 60 min

Cohort 3 (n=30)
- Pathologically confirmed head and neck cancer or bladder cancer
- Imaging
  - 3 – 8 mCi $^{68}$Ga-FAP-2286
  - PET Imaging 60 min

Metastatic disease present on conventional imaging defined as having RECIST 1.1 measurable disease or multiple bone metastases

No evidence of metastatic disease as defined as the absence of RECIST 1.1 measurable disease or bone metastases

NCT04621435
$^{68}$Ga-FAP-2286 Shows High Uptake in Patient with Osteosarcoma

Conclusions

- FAP is highly expressed in multiple tumor types and has limited normal tissue expression, suggesting it is an attractive target for PTRT.
- FAP-2286 potently and selectively binds to FAP.
  - FAP-2286 has a long retention time in the tumor which translates to robust anti-tumor activity of $^{177}$Lu-FAP-2286 in preclinical models.
- Evaluation of $^{68}$Ga-FAP-2286 and $^{177}$Lu-FAP-2286 are ongoing in the Clovis-sponsored Phase 1/2 study LuMIERE.
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