Investigating Fibroblast Activation Protein alpha (FAPα) as a Therapeutic Target for Delivery of pre|CISION® Cancer Medicines: Expression, Spatial Localization and Functional Insights

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Avacta THERAPEUTICS

Abstract #2699

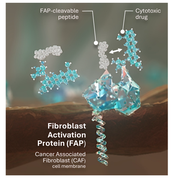
pre|CISION® Medicines Target FAP

Fibroblast activation protein-α (FAP) is a membrane-bound extracellular post-proline protease that is expressed on cancer associated fibroblasts (CAFs). FAP is upregulated in over 90% of solid tumors and largely absent from healthy tissue—making it a compelling oncology target

Avacta is the only company to have developed a dipeptide moiety linker (pre|CISION®) that is enzymatically-cleaved specifically by FAP, enabling targeted cytotoxic delivery via bystander cell killing

Avacta's lead asset **AVA6000 (FAP-Dox)** has **demonstrated tumor-localized delivery of doxorubicin in humans**, validating FAP as a target and pre|CISION® as a delivery strategy

The next generation of **pre|CISION®, AVA6103 (FAP-EXd),** shows exceptional preclinical data and is currently in IND-enabling studies



See other Avacta posters at this meeting:
Comparative pharmacokinetics of pre|CISION® peptide drug conjugates (Abstract #CT15)
Tuesday, April 29, 2025, 9:00 a.m.– 12:00 p.m)

The novel peptide drug conjugate AVA6103 is a pre|CISION® medicine which targets exatecan to the TME following FAP cleavage (Abstract #3139)
Monday, April 28, 2025, 2:00 – 5:00 p.m.

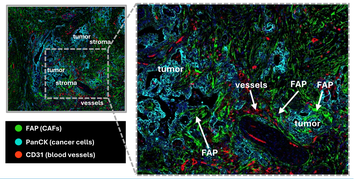


FIGURE 1. Spatial FAP expression
FAP negative tumor cell nests (aqua) are near Cancer Associated Fibroblasts (CAFs) which are positive for FAP (green). Tumor vasculature is close to FAP+ CAFs in the stroma (red)

FAP is Predominantly Expressed on Fibroblasts in Epithelial Tumors

Single-cell RNA-seq from adenoid cystic carcinoma (ACC) and pancreatic ductal adenocarcinoma (PDAC) shows that **FAP is mainly expressed on CAFs**, with lower levels in endothelial and vascular smooth muscle cells

Avacta's pre|CISION® platform exploits this selectivity to **activate therapeutic payloads directly in the tumor**

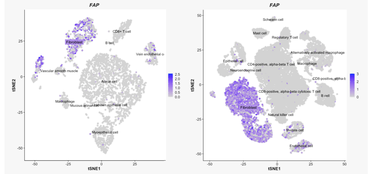


FIGURE 2. FAP expression is predominantly on fibroblasts
tSNE plots of single-cell RNA-sequencing data from adenoid cystic carcinoma (Zhou et al., 2023) and pancreatic ductal adenocarcinoma (Chen et al., 2023) samples. Cell type clustering and FAP expression levels (log normalized) across the annotated cell populations. Color scale represents FAP expression

FAP IHC Shows a Range of Solid Tumors Express FAP

Immunohistochemistry (IHC) was performed on tumor microarrays of **>1000 patient tumor cores** from 11 cancer types

FAP expression was detected in **all indications**, primarily in the stroma, except for sarcoma and ovarian cancer which also showed tumor cell expression

Digital **pathology was used to quantify** FAP + area as a percentage of each entire core

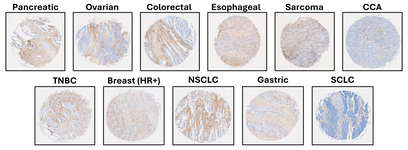


FIGURE 3. FAP protein expression across a range of solid tumors
Representive images and corresponding quantification of FAP+ area (%) across multiple solid tumor types. Digital image analysis was performed on tumor microarrays using automated software to determine mean percentage FAP positive area (Whole core area) per indication. Data are presented as mean, and error bars represent SEM

- To assess the relationship between mRNA and protein levels, we compared **FAP protein IHC data** and **mRNA expression from Tempus AI's LENS database**
- **A strong correlation (Spearman R=0.71)** was observed, **supporting the use of Tempus AI's mRNA dataset to aid indication selection** for pre|CISION® medicines

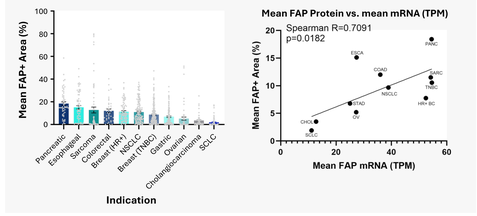
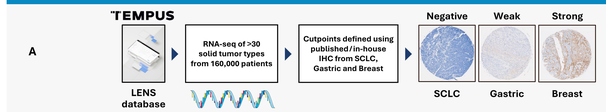


Figure 4. FAP protein expression correlates with FAP mRNA levels across tumor types
Scatter plot showing the relationship between mean FAP mRNA expression (transcripts per million – TPM) and mean FAP positive area (%) across selected solid tumor types. Displayed is the trendline along with Spearman's correlation coefficient (ρ) representing the relationship between FAP mRNA and protein (p=0.7091 p=0.0182)

Pre|CISION® Medicines Are Applicable to Treat a Broad Range of Solid Tumor Types



FAP mRNA expression was found to be strong in a broad range of solid-tumor types demonstrating that **pre|CISION® medicines are applicable in the treatment of many solid-tumors**

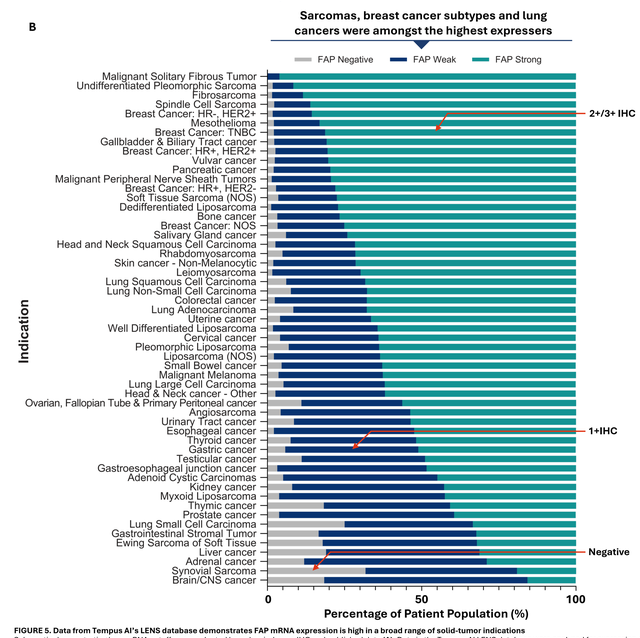


FIGURE 5. Data from Tempus AI's LENS database demonstrates FAP mRNA expression is high in a broad range of solid-tumor indications
Schematic demonstrating how mRNA cutoffs were selected based on in-house IHC and published data **(A).** Data in the Tempus AI LENS database were analyzed for expression of FAP. Cut-points to define negative, weak and strong were the same across the entire database and were set based on known/published positive rates for IHC in 3 diseases: gastric cancer, triple negative cancer and SCLC (Mona et al., 2022; Chen et al., 2022) **(B).** Generally, negative correlates with 0+ stroma staining (<2.115 Log_2(TPM+1)), weak expression correlates with 1+ stroma staining (2.115-4.7 Log_2(TPM+1)), and strong expression correlates with 2-3+ stroma staining (>4.7 Log_2(TPM+1)). No samples were excluded from the analysis, and total N per indication ranged from n=51 to n=21801

FAP mRNA Correlates with Stromal Content

- FAP mRNA levels were compared to ESTIMATE stromal scores which infer stromal and immune cell content based on gene expression signatures
- Strong correlations were observed across solid tumors, especially in breast and pancreatic cancers (R>0.84), which are highly desmoplastic
- This supports the use of **pre|CISION® medicines to target and activate specifically within the TME**, minimizing off-target effects and enhancing efficacy

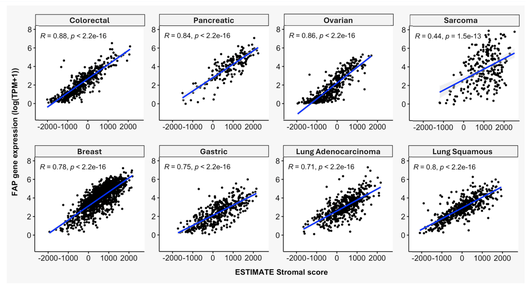
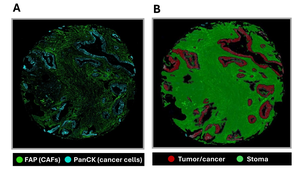
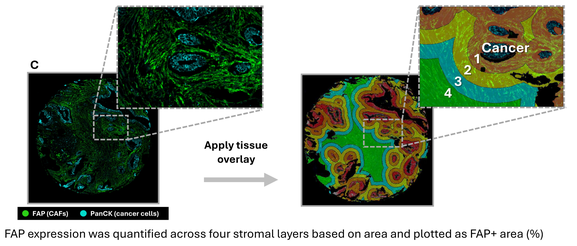


FIGURE 6. FAP expression correlates with stromal score across multiple TCGA cancer types
Scatter plots show the correlation between ESTIMATE-derived stromal scores and FAP mRNA expression (log_2(TPM+1)) in eight TCGA indications. Pearson correlation coefficients (r) and p-values indicated. Each dot represents an individual tumor sample

FAP is Enriched at the Tumor-stroma Interface Providing Optimal Delivery of Pre|CISION® Medicines

- Multi-IF imaging was used to map the **spatial distribution of FAP** in epithelial tumors (colorectal, ovarian, esophageal and pancreatic)
- Digital pathology apps **defined tumor and stromal regions based on pan-cytokeratin (panCK) staining, enabling FAP analysis within the stroma**
- **The stroma was divided into four** 50µm concentric layers from the tumor cell edge:
  Layer 1: CAFs **closest** to tumor cells
  Layer 4: CAFs **farthest** from tumor cells





- FAP expression was quantified across four stromal layers based on area and plotted as FAP+ area (%)
- This demonstrates that **pre|CISION® compounds can be effectively delivered directly to the tumor with close proximity to the tumor cells** themselves which can be killed via the bystander effect

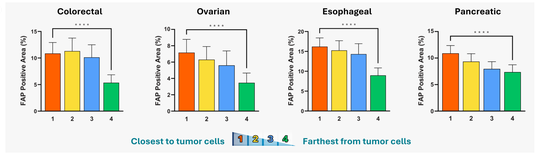


FIGURE 7. Spatial quantification of FAP expression across stromal layers in different tumor types
Tissue microarray cores are processed using multiplex immunofluorescence staining for pan-cytokeratin (PanCK: aqua) to identify tumor epithelial cell regions and FAP (green) to label CAFs **(A)**. A computational algorithm segmented each core into tumor (panCK-positive) and stromal (panCK negative) compartments **(B)**, generating a tumor-stroma mask. Four concentric stromal partitions are generated at 50µm intervals (Layers 1-4), radiating outward from the tumor boundary **(C)**. Bar graphs show the percentage of FAP-positive stromal area in each of the four concentric layers (Layers 1-4) progressing from closest (Layer 1) to farthest (Layer 4) from the panCK positive tumor cells. Colorectal (n=31), Ovarian (n=56), Esophageal (n=60) and Pancreatic (n=56) samples were analyzed. Wilcoxon matched-pairs signed rank test was used to compare layer 1 vs. layer 4 in each tumor type. ****p<0.0001 **(D)**

FAP Colocalizes with Tumor Vasculature for Optimal Pre|CISION® Drug Delivery

- Efficient drug delivery relies on accessing the tumor vasculature to reach target expressing cells
- Multi-IF analysis showed that FAP is frequently localized near blood vessels within the stroma across TNBC, NSCLC, PDAC and CRC
- This proximity may enhance distribution of **pre|CISION® medicines, enabling direct delivery to the tumor via the vasculature**

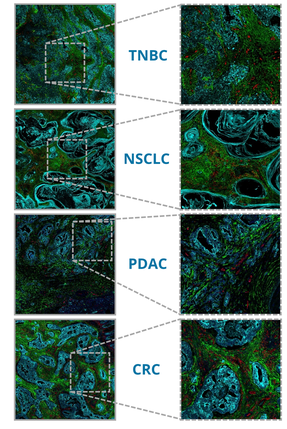


FIGURE 8. Multi-immunofluorescence analysis of FAP and tumor vasculature shows an association between the two
3X tumor samples from TNBC, NSCLC, PDAC and CRC were analyzed for PanCK (Tumor cells – aqua), FAP (CAFs – green) and CD31 (Vasculature – red). Representative images were captured at 4X and 10X magnification to demonstrate association between FAP and CD31

Tempus AI's LENS Database Identifies a FAP-SLFN11 Correlation

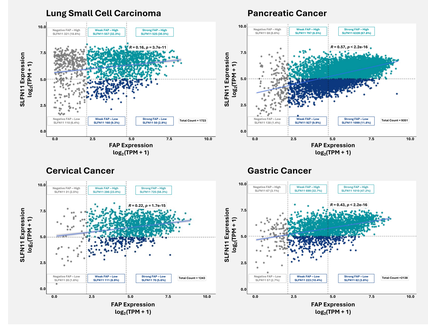


FIGURE 9. FAP expression correlates with SLFN11 expression across multiple tumor types
Scatter plots show the relationship between FAP and SLFN11 mRNA expression (log_2(TPM+1)) in small cell lung, pancreatic, cervical and gastric cancers. Grey indicates FAP negative patients, navy indicates FAP positive, low SLFN11 expressing patients and teal indicates FAP positive high SLFN11 patients. Linear regression analysis with correlation coefficients (R) and p-values are overlaid

- Learnings from the AVA6000 program highlight the importance of payload sensitivity for pre|CISION® medicines
- Using Tempus AI's LENS database, we evaluated co-expression of FAP and SLFN11, a gene linked to replication stress and sensitivity to topoisomerase inhibitors
- SLFN11 has been shown to **confer sensitivity to DNA-damage inducing agents** such as **Topoisomerase inhibitors** (Zhang et al., 2021)
- We observed a positive correlation between FAP and SLFN11 expression across multiple tumor types, supporting the use of AVA6103 (FAP-Exd)

Conventional Therapy Regimens Do Not Impact FAP Expression

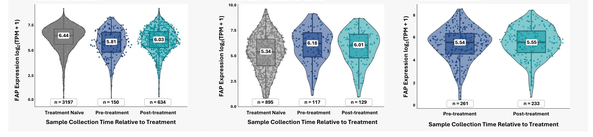


FIGURE 10. Conventional therapy regimens do not significantly impact FAP expression in breast cancer and sarcoma
Violin plots show FAP mRNA expression levels (log_2(TPM+1)) across three biopsy groups – treatment naive, flanking pre-treatment and flanking post-treatment in breast cancer **(A** and **C)** and sarcoma **(B)**. Treatment naive samples were collected before any therapy, while flanking pre-treatment and post-treatment samples were collected within a year before or after doxorubicin or topoisomerase inhibitor treatment respectively and with no intervening therapies. **A** & **B** the treatment was doxorubicin, the treatment in **C** was topoisomerase inhibitors (irinotecan, topotecan, trastuzumab-deruxtecan and Sacituzumab govitecan)

- To assess whether pre|CISION® medicines can be used in pretreated patients, we analyzed **FAP mRNA expression** in treatment-naive, pre-treatment, and post-treatment **tumor biopsies from patients treated with doxorubicin or Topo I inhibitors**
- Across indications, no significant difference in FAP expression was observed between treatment groups
- These findings **support the use of pre|CISION® medicines as second- or third-line therapy** in pretreated patients

CONCLUSIONS

- **FAP is overexpressed across a wide range of solid tumors, with strong correlation between mRNA and IHC data. FAP is spatially enriched at the tumor stroma interface demonstrating an effective mechanism to deliver pre|CISION® drugs to the tumor**
- **FAP+ CAFs are closely associated with tumor vasculature, supporting efficient intratumoral delivery of pre|CISION® medicines from the bloodstream to the tumor microenvironment**
- **In a subset of human tumors, FAP co-expresses with SLFN11, a gene linked to DNA-damaging payload sensitivity, helping identify tumors most likely to respond to pre|CISION®-enabled DNA damaging agents**
- **FAP expression remains consistent after receiving standard chemotherapies doxorubicin and topoisomerase I inhibitors, supporting the use of these pre|CISION®-enabled DNA damaging agents across all lines of therapy from adjuvant to deep metastatic treatment**
- **Together, these findings from a biomarker-driven approach reinforce the potential of Avacta's pre|CISION® platform to deliver potent therapies across multiple solid tumor indications with broad clinical utility**

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