The Novel Peptide Drug Conjugate AVA6103 is a FAP-enabled pre|CISION® Medicine which Targets Exatecan, a Topoisomerase I Inhibitor, to the Tumor Microenvironment Following FAP Cleavage

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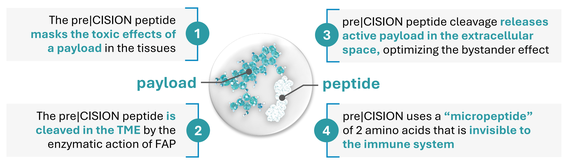
Avacta THERAPEUTICS

Abstract #3139

Introduction

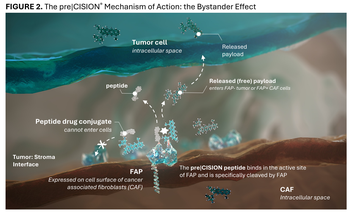
FIGURE 1. The pre|CISION® peptide drug conjugates unleash powerful drugs selectively in the tumor through masking and release



pre|CISION® PDCs have key advantages over conventional ADC approaches:

- **Tunable, tumor-specific release** that has been shown in the clinic to result in a dramatic reduction in off-target toxicities associated with the warhead (AVA6000, pre|CISION® doxorubicin Phase 1 results, *Banerji et al AACR 2024*, *Twelves et al. ESMO 2024*)
- **Optimized bystander MoA** that results in extracellular, rather than intracellular release of the payload (The novel peptide drug conjugate AVA6103, *Watts et al., EORTC 2024*)
- **GMP manufacturing simplicity**, with reduced costs and shortened production timelines versus ADCs

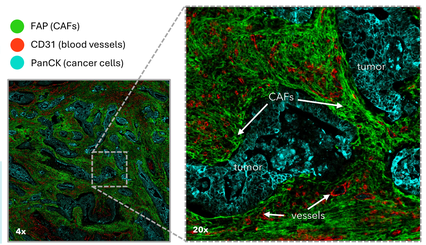
FIGURE 2. The pre|CISION® Mechanism of Action: the Bystander Effect



The second pre|CISION® PDC, AVA6103 (FAP-EXd), is currently in IND-enabling studies with potential Phase 1 start in Q1 2026

Tumor Architecture and FAP Expression is Ideal for Delivery of pre|CISION® PDCs

FIGURE 3. The bystander effect of the pre|CISION® platform warhead delivery: The close proximity of **blood vessels** (●), FAP+ **CAFs** (●), and **cancer cells** (●), allows pre|CISION® compounds to be readily delivered, cleaved, and taken up by neighboring cancer cells



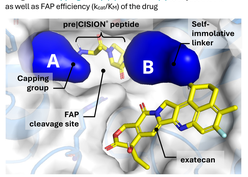
See other Avacta posters at this meeting:

Investigating fibroblast activation protein alpha (FAPα) as a therapeutic target for delivery of pre|CISION® cancer medicines: Expression, spatial localization and functional insights (Abstract #2699)
Monday, April 28, 2025, 2:00 – 5:00 p.m.

AVA6103 is Uniquely Cleaved by FAP to Release the Cytotoxic Warhead Exatecan

- AVA6103 leverages pre|CISION® technology to **extend the half-life** of released exatecan to cover the entire cell cycle
- **Increase tumor exposure to exatecan** by adjusting the release kinetics via modification of the linker (alter the k_{cat}/K_M of the conjugate)
- Extend the **therapeutic index** of exatecan:
  - Slower exatecan release and extended plasma PK of the conjugate will result in a **sustained release mechanism**, lower plasma levels of exatecan and **reduced systemic exposure**

FIGURE 4. AVA6103 in the FAP Active Site
Using both a **Capping Group (A)** and **Linker (B)** to adjust the PK as well as FAP efficiency (k_{cat}/K_M) of the drug



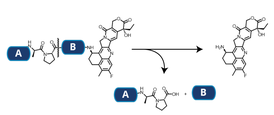


FIGURE 5. Schematic showing a pre|CISION®-exatecan compound containing a capping group (A), *D*-Ala-Pro pre|CISION® dipeptide unit, self-immolative linker (B) and exatecan
After cleavage of the pre|CISION® dipeptide by FAP, the linker undergoes self-immolation and free exatecan is released

Effective Killing of FAP-negative Tumor Cells in a 2D Co-culture Bystander Assay

- Cytotoxicity of AVA6103 was evaluated in a 2D assay in monoculture with FAP-negative LS174T cells, or in **co-culture with additional human colorectal fibroblasts**
- AVA6103 showed minimal activity in monoculture, but **when FAP-positive fibroblasts are present, AVA6103 exhibits cell kill activity similar to exatecan**
- When a FAP inhibitor is added, cytotoxic activity of AVA6103 is reversed **confirming FAP enablement**

FIGURE 6.

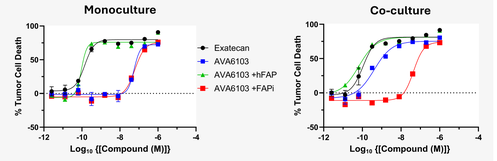


TABLE 1.

Conditions	Monoculture IC_{50}(nM)	Co-culture IC_{50}(nM)
Exatecan	0.1	0.1
AVA6103	55.4	0.4
AVA6103 + 10 nM hFAP	0.1	0.1
AVA6103 + 10 µM FAPi	61.3	44.4

FIGURE 6; TABLE 1. Tumor cell death following treatment with AVA6103. LS174T-GFP cells alone, or in co-culture with primary human colonic fibroblasts, were treated with varying concentration of AVA6103 (+/- recombinant hFAP or FAP inhibitor) or Exatecan warhead for 120h. Tumor cell death was assessed via Incucyte and calculated as % reduction in GFP area relative to vehicle control

Effective Killing of FAP-negative Tumor Cells in a 3D Spheroid Bystander Assay

- In a **3D co-culture** model, MDA-MB-231 **triple negative breast cancer cells** (FAP-negative, GFP-positive) were plated alone, or in combination with primary human **mammary fibroblasts**
- In **monoculture** (cancer cells only), **AVA6103 showed little activity**
- Upon **addition of FAP-positive fibroblasts**, **AVA6103 exhibits cytotoxicity similar to that of exatecan** warhead alone. Cytotoxic activity was reversed in the presence of a FAP inhibitor
- The **activity was maintained** even in co-cultures **with reduced fibroblast** to tumor cell ratios

FIGURE 7. Assay procedure for 3D spheroid culture

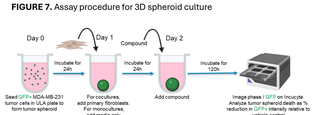
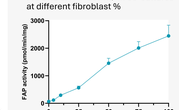


FIGURE 8. FAP activity in co-cultures at different fibroblast %



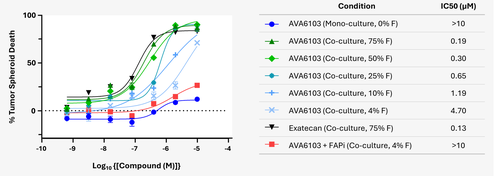


FIGURE 9. Tumor cell death and FAP activity at difference tumor (T) cell to fibroblast (F) ratios. 3D spheroids consisting of MDA-MB-231-GFP cells only or co-cultures with primary human mammary fibroblasts at various T:F ratios were treated with exatecan warhead or AVA6103 +/- 10 µM FAP inhibitor for 120h. Cells were imaged via Incucyte. Tumor cell death was analyzed as % reduction in GFP intensity relative to vehicle control. In parallel, FAP enzymatic activity of untreated co-cultures at Day 2 was measured

Cleavage of AVA6103 at the Tumor Site Results in High Intratumoral and Low Plasma Concentrations of Exatecan

- **Levels of AVA6103-released-exatecan were assessed** in the tumor and plasma following a single subcutaneous dose of AVA6103 in four FAP-expressing tumor models
- All models showed intratumoral exatecan concentrations above the IC_{90} at 4hr at levels relative to FAP activity, and **concentrations above the IC_{90} at 24hr** in the sarcoma and colorectal models
- Levels of **released exatecan in the plasma were far lower** than tumor levels in all models, indicating efficient delivery of warhead by the pre|CISION® linker
- The **tumor:plasma ratio** of **exatecan at 4hr** in the melanoma, sarcoma, and colorectal models was 4-fold, 16-fold, and **85-fold** respectively

TABLE 2. FAP activity in each of the *in vivo* models used at right, and in the efficacy studies

Tumor Model	FAP Activity Measured in Tumor (pmol/min/mg)
Melanoma PDX	1203
Sarcoma PDX	1850
Colorectal CDX (LS174T-FAP)	7136
HEK293T-FAP CDX	68071

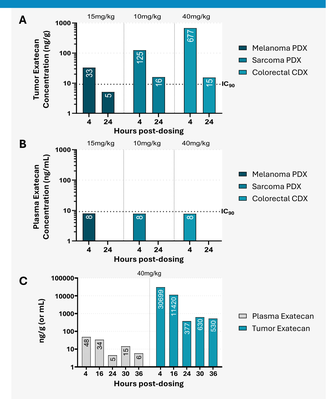


FIGURE 10. (A). High concentrations (>IC_{90}) of released exatecan are present intratumorally at 4hr and 24hr when treating with AVA6103. **(B).** Low concentrations (<IC_{90}) of released exatecan are present in the plasma at 4hr and 24hr when treating with AVA6103. **(C).** In the HEK-FAP model (overexpressing FAP to a very high level) when treating with AVA6103, increased concentrations and extended exposure of released exatecan were observed intratumorally, whilst plasma levels remained much lower

AVA6103 Induces DNA Damage Pathways Consistent with Topo I Inhibitor Mechanism of Action

- The **LS174T-FAP model** used in the tumor uptake evaluation was also analyzed for changes in potential on-target **pharmacodynamic biomarkers**
- Western blot showed **increases in** markers for **DNA damage** (γH2AX), **DNA damage repair** (pKAP1, pCHK2) and **cell cycle arrest** (p21) following administration of AVA6103
- The expression of **DNA damage response and repair** markers increase and persist **up to** and beyond **24 hours**

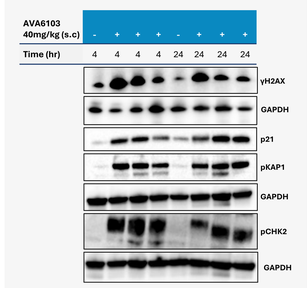


FIGURE 11. Western blot analyses to examine the induction of the DNA damage response pathway after subcutaneous treatment of LS174T-hFAP inoculated NMRI nu/nu tumor mice with 40mg/kg AVA6103 for 4hrs and 24hrs

Tumor and Plasma PK with AVA6103 Demonstrate Sustained Tumor Release Resulting in Multiple Days Above the IC_{50} in the Tumor

- In a high FAP expression model with dosing at the MTD, simulations **suggest prolonged exatecan exposure in the tumor** with low plasma concentration
- A single subcutaneous **dose of AVA6103 (FAP-EXd)** at 40 mg/kg (MTD) is compared with conventional exatecan dosed at 5 mg/kg (MTD) with similar plasma exposure and **dramatic high tumor exposure**

See other Avacta posters at this meeting:

Comparative pharmacokinetics and tumor activation of fibroblast activation protein (FAP)-enabled pre|CISION® peptide drug conjugates (Abstract #CT15)
Tuesday, April 29, 2025, 9:00 a.m. – 12:00 p.m.

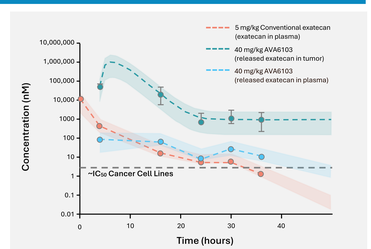
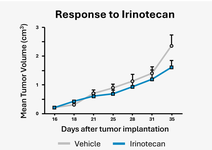


Figure 12. PK Modeling of tumor and plasma concentration following AVA6103 dosing suggests prolonged tumor exposure. PK curves show tumor and plasma concentration of exatecan, AVA6103, and exatecan released from AVA6103 after a single s.c dose. Plotted values are the mean concentration from N=3 mice

AVA6103 Demonstrates Tumor Growth Inhibition and Complete Regressions Superior to Exatecan in Multiple Efficacy Studies

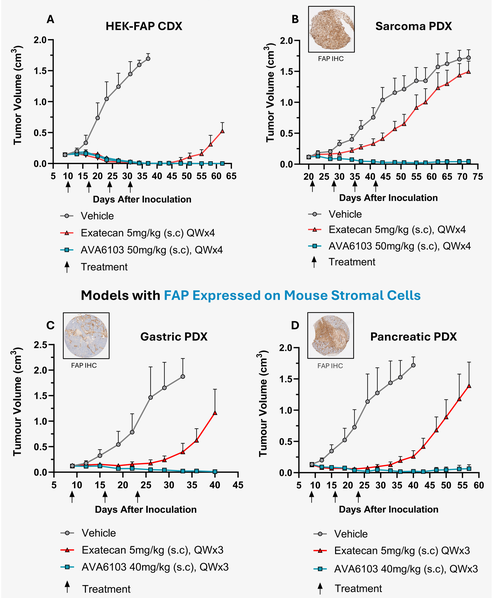
- In a series of **efficacy studies**, mice were dosed subcutaneously with **AVA6103 or exatecan** at maximum tolerated dose (MTD) for all studies
- **HEK-FAP CDX** – 6/6 mice exhibited complete response with AVA6103
- **Sarcoma PDX** – 7/8 mice exhibited complete response with AVA6103
- **Gastric PDX** – 4/4 mice exhibited complete response with AVA6103
- **Pancreatic PDX** – 2/3 mice exhibited complete response with AVA6103



Gastric model previously shown to be **resistant to irinotecan**

FIGURE 13. In a previously conducted efficacy study, Gastric PDX tumor-bearing NMRI mice treated exatecan were found to be refractory to irinotecan (Topo I inhibitor) treatment (data courtesy of EPO, Berlin)

FIGURE 14.
(A). HEK-FAP tumor-bearing NOG mice were dosed QWx4 with AVA6103 (n=6) or exatecan (n=5), administered s.c. **(B).** Sarcoma PDX tumor-bearing NMRI mice (n=8/cohort) were dosed QWx4 with AVA6103 or exatecan, administered s.c. **(C).** Gastric PDX tumor-bearing NMRI mice were dosed QWx3 with AVA6103 (n=4) or exatecan (n=3), administered s.c. **(D).** Pancreatic PDX tumor-bearing NMRI mice (n=3/cohort) were dosed QWx3 with AVA6103 or exatecan, administered s.c. All animals were treated when palpable, established tumors were present, and were terminated at humane endpoints. Terminal tumor volumes continued until termination of last animal in the group



CONCLUSIONS

- AVA6103 (FAP-EXd) is a novel pre|CISION® peptide drug conjugate (PDC) that is specifically cleaved by Fibroblast Activation Protein-α (FAP), resulting in **highly specific delivery of exatecan directly to tumor-stroma interface**
- **The pre|CISION® peptide is cleaved in the presence of membrane-bound FAP** expressed on the cell surface of cancer associated fibroblasts (CAFs), and active exatecan is released in the extracellular space. This mechanism masks the toxic effects of a payload in the tissues and mediates specific delivery of exatecan to the tumor
- **AVA6103 cytotoxicity is mediated via the bystander effect,** where the PDC is cleaved by FAP+ fibroblasts to release exatecan, and released exatecan mediates cytotoxicity against FAP- cancer cells
- The kinetics of **AVA6103 and the release of active exatecan is optimized to provide prolonged tumor exposure** by inserting a capping group [A] and self-immolative linker [B] with optimal FAP enzyme efficiency (k_{cat}/K_M)
- Following administration of **AVA6103 to tumor-bearing mice, exatecan is concentrated in the tumor** at levels up to 85-fold higher than plasma, with extended tumor exposure at concentrations above IC_{90}
- **AVA6103 demonstrates tumor growth inhibition and durable complete responses in multiple efficacy studies.** Biomarker analysis demonstrated on-target cell death mechanism

References

Twelves et al. A Phase I trial of AVA6000, a Fibroblast Activation Protein (FAP)-released, tumor microenvironment (TME)-targeted doxorubicin peptide drug conjugate (PDC) in patients with FAP-positive solid tumors [abstract]. In: The European Society of Medical Oncology Annual Meeting; September 14, 2024; in Barcelona, Spain; *Annals of Oncology* (2024) 35 (suppl_2): S482-S535. 10.1016/annonc/annonc1589.

Banerji et al. A Phase I trial of AVA6000, a Fibroblast Activation Protein (FAP)-released and tumor microenvironment (TME)-targeted doxorubicin peptide drug conjugate in patients with FAP-positive solid tumors [abstract]. In: Proceedings of the American Association for Cancer Research Annual Meeting 2024; Part 2 (Late-Breaking, Clinical Trial, and Invited Abstracts); 2024 Apr 5-10; San Diego, CA. Philadelphia (PA): AACR; *Cancer Res* 2024;84(7_Suppl):Abstract nr CT188.

Watts et al. The novel peptide drug conjugate AVA6103 is a FAP-enabled pre|CISION® medicine which targets Topoisomerase I to the tumor microenvironment via FAP cleavage [abstract]. In: The 36th EORTC-NCI-AACR Symposium on Molecular Targets and Cancer Therapeutics; 2024 Oct 23-25; in Barcelona, Spain; *European Journal of Cancer* 211S1 (2024) 114693; https://10.1016/j.ejca.2024.114693.

Acknowledgements

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