Discovery and characterization of novel pre|CISION® technology compounds delivering complementary dual payloads to the tumor microenvironment following FAP cleavage

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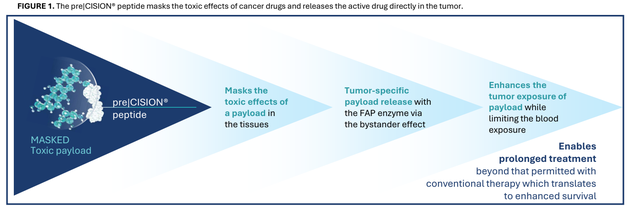
Avacta THERAPEUTICS

Abstract #C123

Introduction

FIGURE 1. The pre|CISION® peptide masks the toxic effects of cancer drugs and releases the active drug directly in the tumor.



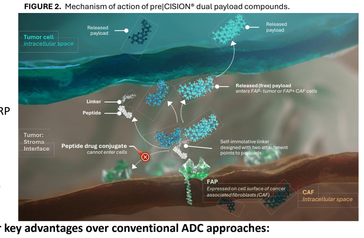
The **pre|CISION® dual payload technology** comprises a single molecule that can simultaneously deliver two payloads in a FAP-selective manner, and offers key advantages:

- Circumvent resistance mechanisms that cancer cells develop against single-drug therapies
- Maximize the therapeutic effect by effectively delivering the combination of payloads to the same cells

The **pre|CISION® dual payload pipeline** currently comprises two approaches:

- Combination of two distinct anti-cancer mechanisms with clinical activity: Microtubule inhibition and Topo I inhibition (MMAE and exatecan)[1]
- DNA damage response (DDR) agents ATR or PARP inhibitors with exatecan: Inhibition of DNA repair potentiates the effect of exatecan[2, 3]

FIGURE 2. Mechanism of action of pre|CISION® dual payload compounds.



While dual payload ADCs are in development in oncology[4], **Avacta is the first company to develop Dual Payload Peptide Drug Conjugates.**

pre|CISION® peptide drug conjugates (PDC) offer key advantages over conventional ADC approaches:

- **Tunable, tumor-specific release** that has been shown in the clinic to have two distinct advantages:
  - (1) Striking concentration of payload in the tumor vs plasma with a concentration of 100:1 (compared with ADC and other PDC that concentrate up to 10X)
  - (2) Dramatic reduction in off-target toxicities associated with the payload (AVA6000, pre|CISION® doxorubicin Phase 1 results[5-7])
- **Optimized bystander MoA** that results in extracellular, rather than intracellular release of the payload, leveraging payloads with excellent membrane permeability[8]
- **Two clinical stage single payload programs:**
  - (1) Faridoxorubicin (FAP-Dox, AVA6000) completed Phase 1 testing and currently enrolling expansion cohorts (NCT04969835)
  - (2) FAP-Exd (AVA6103) with the Phase 1 start scheduled Q1 2026

The pre|CISION® Bystander Effect is Mediated by the Spatial Organization of FAP Expression in Tumors

- pre|CISION® medicines **target a drug specifically to the tumor** by leveraging FAP protease activity
- FAP is highly expressed at the **tumor-stroma interface** and is closely associated with tumor vasculature
- **pre|CISION® medicines** are delivered by vessels to the tumor-stroma interface, cleaved by FAP and active payload is then released to FAP-negative tumor cells

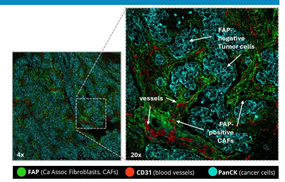


FIGURE 3. Representative 4x and 20x images of merged channels from 3-plex multi-IF staining of human Salivary Ductal Carcinoma. Slides were stained with FAP (Green), CD31 (Red), PanCK (Cyan) antibodies, and DAPI (Blue).

The pre|CISION® Linker Design Enables Dual Payload Release By a Single FAP Cleavage Event

Capping Group

Sits in the active site of the FAP enzyme

Can be modified to extend plasma exposure

Self-Immolative Linker

Fine tunes the rate of peptide cleavage by modulating the enzyme efficiency (k_{cat}/K_M), optimizing delivery of the payload in the TME

Linker designed to allow two payloads to be released from a single FAP cleavage event

pre|CISION® Peptide

The **two amino acids (*D*-Alanine-Proline)** determine specificity for FAP cleavage

Cleavage by FAP results in release of both warheads – one peptide, two payloads

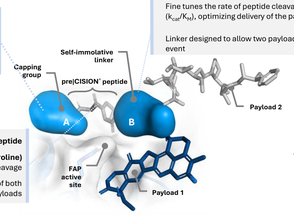
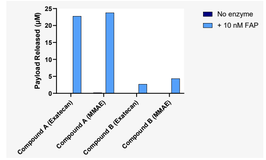


FIGURE 4. pre|CISION® dual peptide compound in the FAP enzyme active site.

Modifications to the Dual Payload Linker Technology Enables Tunable FAP-Dependent Release of Exatecan and MMAE



- **FAP-dependent release** of **multiple payloads** can be detected by LC-MS
- Modifications to the self-immolative linker enable **tunable payload delivery**

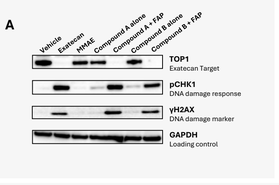
FIGURE 5: FAP-Exd/MMAE compounds (100 µM) were incubated in the presence or absence of 10 nM FAP for a period of 24 hours. The amount of each payload present in the sample after incubation was determined by LC-MS.

FAP-Exd/MMAE Dual Payload Compounds Elicit Tunable FAP-Enabled Biomarker Induction And Cell Cycle Arrest Consistent with Release of Both Payloads

- Target-proximal and downstream **biomarkers specific for each payload are modulated only when FAP is present**

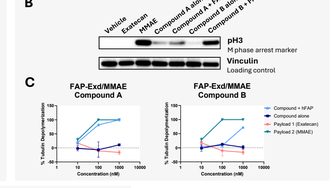
Exatecan-specific activity

Exposure Results in Decreased TOP1 and Increased Markers for DNA damage (γH2AX) and DNA damage response (pCHK1)



MMAE-specific activity

Exposure Results in Tubulin Depolymerization and M Phase Arrest (pH3)

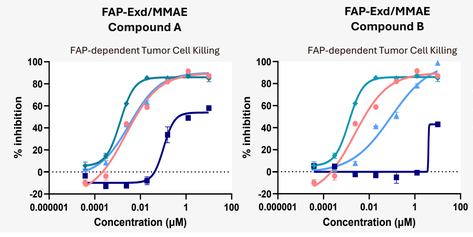


Exatecan + MMAE activity

FIGURE 6. Biomarker and Cell Cycle Analysis following treatment with exatecan, MMAE, or FAP-Exd/MMAE ±10 nM hFAP. **A)** Western blot in HCT116 cells for pCHK1 and γH2AX (1 h treatment), and TOP1 (24 h treatment). **B)** Western blot for pH3 in HCT116 cells after 24 h treatment. **C)** Tubulin depolymerization in HCT116 cells after 6 h treatment, quantified by anti-tubulin staining (% reduction vs. vehicle). **D)** Cell cycle analysis in MDA-MB-231 cells after 24 h treatment with 8 nM of indicated compounds ±10 nM hFAP.

FAP-Exd/MMAE Dual Payload Compounds Elicit FAP-Enabled Killing of Tumor Cells

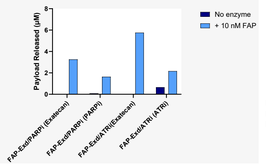
- FAP-Exd/MMAE compound activity is **FAP-dependent, achieving tumor cell kill comparable to free payload**
- **Compound activity** can be **optimized by modification of the linker and capping group**



Conditions	FAP-Exd/MMAE IC_{50} (nM)	
	Compound A	Compound B
Compound + 10 nM FAP	3.3	89.7
Compound alone	104.6	>10000
Payload 1 (Exatecan)	2.3	2.3
Payload 2 (MMAE)	1.3	1.3

FIGURE 7. HCT116 cells were treated for 72 h with varying concentrations of Compound (±10 nM hFAP), Exatecan, or MMAE. Cell death was measured by CTG as % luminescence reduction vs. vehicle control.

Broad Applicability of the FAP-dependent Dual Payload Technology Demonstrated by Release of Two Payloads from FAP-Exd/DDRi Compounds



- Multiple payloads **with synergistic MoAs** can be detected by LC-MS following a single FAP cleavage event
- pre|CISION® peptide can be incorporated into compounds designed to release a **range of payloads**

FIGURE 8: FAP-Exd/PARPi and FAP-Exd/ATRi compounds (100 µM) were incubated in the presence or absence of 10 nM FAP for a period of 24 hours. The amount of each payload present in the sample after incubation was determined by LC-MS.

FAP-Exd/DDRi Dual Payload Compounds Exhibit FAP-Dependent Modulation of Biomarkers Consistent with Release of Both Payloads

Markers are modulated only when FAP is present

- **FAP-Exd/PARPi reduce TOP1 and PAR levels**, consistent with release of both exatecan and PARPi
- **FAP-Exd/ATRi reduce TOP1 and exatecan-induced pCHK1 levels,** consistent with release of both payloads
- **Both compounds induce markers for DNA damage (γH2AX) and apoptosis (cleaved PARP)**

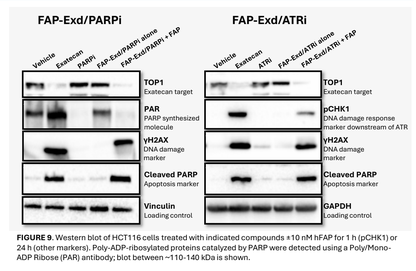


FIGURE 9. Western blot of HCT116 cells treated with indicated compounds ±10 nM hFAP for 1 h (pCHK1) or 24 h (other markers). Poly-ADP-ribosylated proteins catalyzed by PARP were detected using a Poly/Mono-ADP Ribose (PAR) antibody; blot between ~110-140 kDa is shown.

FAP-Exd/DDRi Dual Payload Compounds Demonstrate FAP-Enabled Killing of Tumor Cells and DNA Damage Indicative of Synergy

- Activation of the **DNA Damage Response (DDR) pathway** is a known **key resistance mechanism** to Topo I inhibitors
- **Combining Topo I and DDR inhibition is synergistic**
- **FAP-Exd/DDRi dual payload compounds showed 4-5x greater FAP-dependent killing** compared to the highly potent exatecan alone, highlighting enhanced synergy
- **Elevated γH2AX levels demonstrated with FAP-Exd/PARPi dual payload** compared to single payloads alone

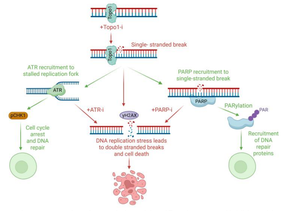
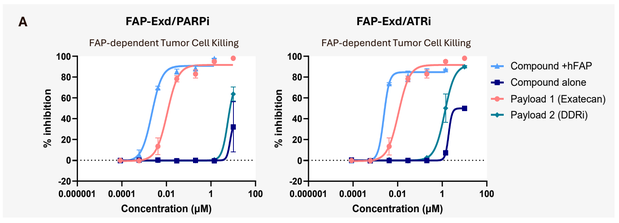


FIGURE 10. Schematic representation of synergistic activity of Topoisomerase I inhibitor with ATR or PARP inhibitors to induce tumor cell death. Figure was generated using BioRender.



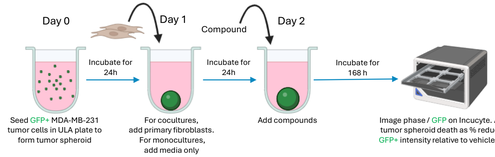
Conditions	IC_{50} (nM)	
	FAP-Exd/PARPi	FAP-Exd/ATRi
Compound + 10nM FAP	2.2	2.4
Compound alone	7209	1942
Payload 1 (Exatecan)	10.9	10.9
Payload 2 (DDRi)	5717	1293

FIGURE 11. A) MiaPaca2 cells treated for 72 h with FAP-Exd/PARPi, FAP-Exd/ATRi (±10 nM hFAP), or free payloads (exatecan, PARPi, ATRi). Cell death was measured on Incucyte as % reduction in confluency vs. vehicle control. **B)** HCT116 cells treated with indicated compounds (800 nM, 24 h), stained for γH2AX and analyzed by flow cytometry. Data from n=2–3 independent experiments.

pre|CISION® Dual Payload Compounds Effectively Kill FAP-Negative Tumor Cells in a 7-day 3D Spheroid Bystander Assay

- Activity of dual payload compounds is **dependent upon the presence of FAP+ fibroblasts** in a **3D tumor/fibroblast co-culture model**
- **Limited activity** observed in the **absence of FAP+ fibroblasts** or with the **addition of FAP inhibitor**

FIGURE 12. Assay procedure for 3D spheroid culture.



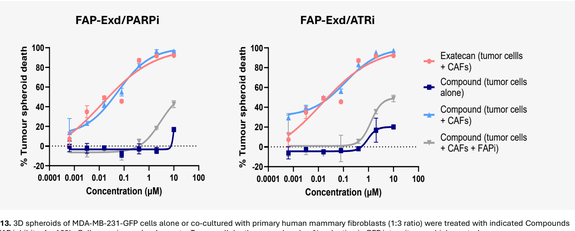


FIGURE 13. 3D spheroids of MDA-MB-231-GFP cells alone or co-cultured with primary human mammary fibroblasts (1:3 ratio) were treated with indicated Compounds ±10 µM FAP inhibitor for 168h. Cells were imaged on Incucyte. Tumor cell death was analyzed as % reduction in GFP intensity vs. vehicle control.

CONCLUSIONS

- FAP-selective pre|CISION® dual payload technology enables simultaneous delivery of two drugs from a single, stable PDC molecule via one FAP-mediated cleavage event
- Tunable kinetics of payload release may be achieved by modifications of the linker and the capping group, as evidenced by comparison of compound A vs B
- pre|CISION® dual payload release was validated through target- and pathway-specific biomarkers
- pre|CISION® dual payloads are released by FAP on cancer associated fibroblasts, concentrating both payloads in the TME and killing tumor cells via the bystander effect
- pre|CISION® dual payload technology allows selective delivery of two payloads, overcoming potential resistance and eliciting synergistic tumor cell kill
- Tumor-specific delivery has the potential to increase the therapeutic window and reduce systemic toxicities, offering improved outcome for patients with cancer

References

1) Stathopoulos et al. Front line chemotherapy in advanced NSCLC with paclitaxel and irinotecan combination, J Clin Oncol, 22, 7127 (2004), doi:10.1200/jco.2004.22.90140.7127

2) Jo et al. TOP1-DNA Trapping by Exatecan and Combination Therapy with ATR Inhibitor, Mol Cancer Ther; 21(7), 1090-1102, (2022), doi:10.1158/1535-7163.MCT-21-1000

3) Cliby WA, Lewis KA, Lilly KK, Kaufmann SH. S phase and G2 arrests induced by topoisomerase I poisons are dependent on ATR kinase function. J Biol Chem. 2002;277(2):1599-1606. doi:10.1074/jbc.M106287200

4) Mullard A. Dual-payload ADCs move into first oncology clinical trials. Nat Rev Drug Discov. 2025;24(8):573-576. doi:10.1038/d41573-025-00121-y

5) 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.

6) 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.

7) Tap et al. A Phase I trial of FAP-Dox (AVA6000), a fibroblast activation protein(FAP)-released and tumor microenvironment (TME)-targeted doxorubicin peptide drug conjugate in patients with FAP-positive solid tumors. European Society of Medical Oncology Annual Meeting 2025.

8) Rink et al. 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 [abstract]. In: Proceedings of the American Association for Cancer Research Annual Meeting 2025; Part 1 (Regular Abstracts); 2025 Apr 25-30; Chicago, IL. Philadelphia (PA): AACR; Cancer Res 2025;85(8_Suppl_1):Abstract nr 3139.

Acknowledgements

The investigators and the team at Avacta would like to thank SynthesisMedChem who performed chemical synthesis; Sygnature Discovery who performed chemical synthesis, cytotoxicity and kinetics assays, and computational modelling; scientific communication support was provided by SlideSource. BioRender was used to generate Figure 10.

Presented at the AACR-NCI-EORTC International Conference on Molecular Targets and Cancer Therapeutics, October 2025 Boston, MA USA