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

Victoria Juskaite, Tom Clough, Ellen Watts , Alexa Kennedy, Iva Zlatareva, Folake Orafidiya, Hanna Buist, Jannah Jeon, Greg Billenness, Douglas Sammon, Sophie Brown, Curtis Rink, Ruairidh Edwards, Vidicha Chunilal, Manuel Pinto, Dave Liebowitz, Francis Wilson, Michelle Morrow, David Jones

Avacta Life Sciences, Scale Space, White City Imperial College Campus, 58 Wood Lane, London, W12 7RZ, UK

Avacta THERAPEUTICS

Abstract #5656

Introduction

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

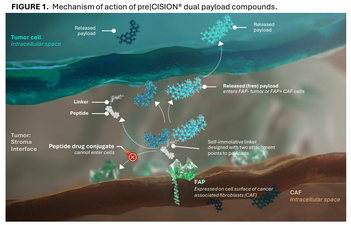
The **pre|CISION® dual payload pipeline** comprises DNA damage response (DDR) agent ATR inhibitor with Topoisomerase I inhibitor (TOP1i): Inhibition of DNA repair potentiates the effect of TOP1i[1, 2]

While dual payload ADCs are in development in oncology[3], **Avacta is the first company to develop Dual Payload Peptide Drug Conjugates** with key advantages compared to traditional ADC

Two clinical stage single payload programs:

1) Faridoxorubicin (FAP-Dox, AVA6000)[4-6] completed Phase 1 testing and currently enrolling expansion cohorts (NCT04969835)
2) FAP-Exd (AVA6103)[7] started Phase 1 in March 2026 (NCT07454642)

FIGURE 1. Mechanism of action of pre|CISION® dual payload compounds.



	Traditional ADC CHALLENGES	pre|CISION® PDC OPPORTUNITIES
Payload release	Non-specific protease cleavable linkers result in **organ toxicity** (e.g. pneumonitis, hepatitis)	**Tumor-specific release** by FAP results in **elimination of organ toxicity** (e.g. loss of cardiac toxicity, no pneumonitis or hepatitis)
DAR/PDR heterogeneity	Single and dual payload platforms demonstrate **heterogeneity of DAR in ADC manufacturing**	Peptide : drug ratio is **stable and controlled in manufacturing** of 1:1 and 1:2 in single and dual payloads
Bystander effect and tumor penetration	**Complex bystander effect** requires antibody tumor penetration, intracellular release and drug extrusion from antigen positive cells	PDC have **small molecule tumor penetration** and simple extracellular release resulting in kill of FAP+ and FAP- cells
Addressable market opportunity	Market opportunity for each ADC is limited by **single antigen expression levels**	**FAP expression in 90% of all solid tumors** with the bystander effect active and release demonstrated in levels to 1+ by IHC

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

See other Avacta posters at this meeting:
AVA6103 is a FAP-enabled pre|CISION® peptide-drug conjugate delivering sustained release of exatecan in the tumor microenvironment with potent antitumor activity (Abstract #5846, Section 17, Board No. 15)
Tuesday, April 21, 2025, 2:00 – 5:00 p.m.

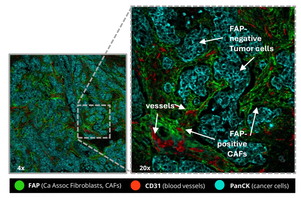
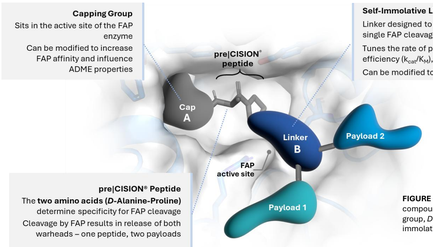


FIGURE 2. Representative 4x and 20x images of merged channels from 3-plex multi-IF staining of human Salivary Ductal Carcinoma. Slides were stained with FAP (Green), CD31 (Red) and PanCK (Cyan) antibodies.

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 increase FAP affinity and influence ADME properties

Self-Immolative Linker
Linker designed to allow two payloads to be released from a single FAP cleavage event
Tunes the rate of peptide cleavage by modulating the enzyme efficiency (k_{cat}/K_M), optimizing delivery of the payload in the TME
Can be modified to influence ADME properties



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 3. Schematic showing a pre|CISION® dual payload compound in the FAP active site, containing a capping group, *D*-Ala-Pro pre|CISION® dipeptide unit, self-immolative linker and two payloads: TOP1i and ATRi.

FAP-TOP1i/ATRi Dual Payload Compounds Elicit FAP-Enabled Killing of Tumor Cells Indicative of Synergy

- FAP-TOP1i/ATRi dual payload compound **activity is FAP-dependent**
- FAP-TOP1i/ATRi dual payload compounds showed **greater FAP-dependent killing** compared to the highly potent TOP1i alone, highlighting **potential synergy**

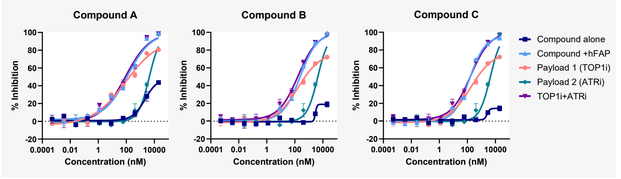
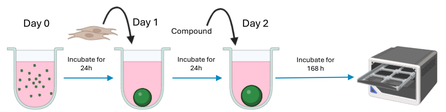


FIGURE 4: MDA-MB-231 cells were treated for 72 h with varying concentrations of Compound (±10 nM hFAP), TOP1i, ATRi, or 1:1 TOP1i:ATRi combination. Cell death was measured by CTG as % luminescence reduction vs. vehicle control.

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

- **Activity** of dual payload compound 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**

FIGURE 5. Assay procedure for 3D spheroid culture.



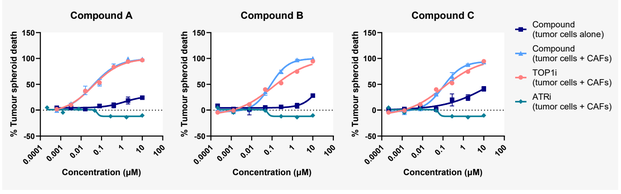


FIGURE 6. 3D spheroids of MDA-MB-231-GFP cells alone or co-cultured with primary human mammary fibroblasts (1:3 ratio) were treated with indicated Compounds. Spheroids were imaged on Incucyte for 168h. Tumor spheroid death was analyzed as % reduction in GFP intensity vs. vehicle control.

FAP-TOP1i/ATRi Dual Payload Compounds Induce Greater FAP-enabled Killing of Tumor Cells with Loss of ATM Function

- **Loss of ATM function** (through pharmacological inhibition) **enhanced the ability of FAP-TOP1i/ATRi to kill tumor cells** in the presence of FAP

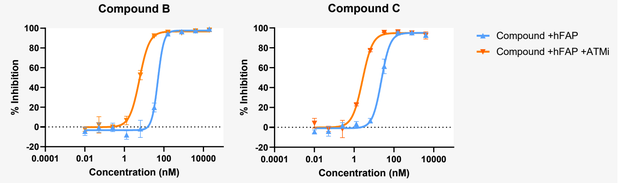
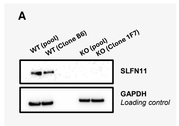


FIGURE 7. MiaPaCa-2 cells were treated for 168 h with varying concentrations of Compound (+10 nM hFAP) in the presence or absence of 30 nM ATMi. Cell death was measured on Incucyte as % confluence reduction vs. vehicle control.

FAP-TOP1i/ATRi Compounds Demonstrate FAP-enabled Biomarker Induction and Tumor Killing, and Synthetic Lethality in SLFN11-deficient cells

- The gene Schlafen 11 **(SLFN11)** has emerged as a **dominant determinant of response to TOP1i** and other DNA damaging agents
- As expected, **SLFN11-proficient wild-type (WT) cells were more sensitive to TOP1i compared to SLFN11 knock-out (KO) cells,** which are more reliant on ATR for survival
- FAP-TOP1i/ATRi **compound C in the presence of FAP reversed TOP1i resistance in SLFN11 KO cells** to similar extent as TOP1i and ATRi free payload combination



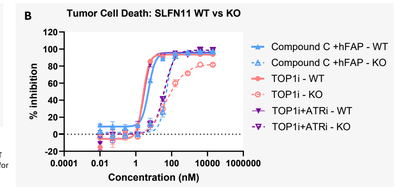
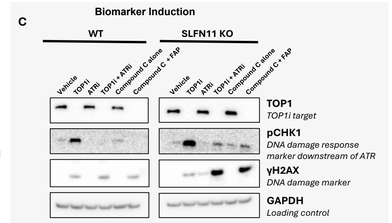


FIGURE 8. A) DU145 WT and SLFN11 KO cells (pool and clonal) analyzed for SLFN11 and GAPDH levels by western blotting. **B)** DU145 WT and SLFN11 KO cells (polyclonal) were treated for 72 h with varying concentrations of Compound (+10 nM hFAP), TOP1i, or 1:1 TOP1i:ATRi combination. Cell death (72h treatment) was measured by CTG as % luminescence reduction vs. vehicle control. **C)** Biomarkers analyzed by Western Blotting after 24h treatment with indicated compounds (500nM) in DU145 WT and SLFN11 KO cells (polyclonal).



- Compound C **reduced TOP1 and TOP1i-induced pCHK1 levels in the presence of FAP,** consistent with **release of both payloads**
- Compound C induced **elevated FAP-dependent DNA damage (γH2AX) in SLFN11 KO** tumor cells compared to single payloads alone
- Compound C **induced replication arrest (loss of EdU incorporation) in SLFN11-proficient WT cells** in a FAP dependent manner, with a much weaker effect in SLFN11 KO cells, consistent with **reduced replication arrest in a SLFN11-deficient background**
- Compound C in the presence of FAP **forced SLFN11 KO cells into premature mitotic entry with unreplicated DNA** (2n<DNA<4n, pH3+), a hallmark of **mitotic catastrophe which leads to cell death**

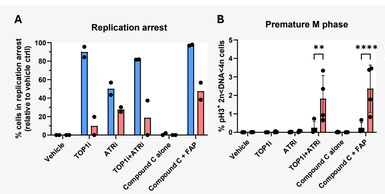


FIGURE 9. Flow Cytometry analysis in DU145 WT or DU145 SLFN11 KO cells. **A)** Flow cytometric analysis of EdU incorporation following a 24h treatment. Data presented as % reduction in EdU incorporation relative to vehicle control. N=2 experimental replicates. **B)** Flow cytometric analysis of pH3 (Ser10) expression and DNA content following a 24h treatment. N=4 experimental replicates. All treatments done with 500nM of each drug. Two-way ANOVA with Tukey's correction. ****p<0.0001, **p<0.01.

Cleavage of pre|CISION® Dual Payload Compound at the Tumor Site Results in High Intratumoral and Low Plasma Concentrations of TOP1i and ATRi

- **Levels of dual payload compound-released-TOP1i and -ATRi were assessed** in the tumor and plasma following a single subcutaneous dose of dual payload compounds in a HEK293T-FAP CDX model
- Compound A and B showed **high intratumoral TOP1i and ATRi concentrations** at 4, 24 and 48h
- Levels of released **TOP1i and ATRi payloads in the plasma were far lower than tumor levels** for both compounds, indicating efficient delivery of payloads by the preCISION® linker
- **Tumor Selectivity Index (TSI):** Ratio of the $AUC_{4\text{-}48hr}$ of released payload in the tumor vs. plasma
- TSI is a straightforward, quantitative measure of how payload preferentially distributes to tumor tissue vs. systemic circulation; **Compound A and B demonstrated high TSI**

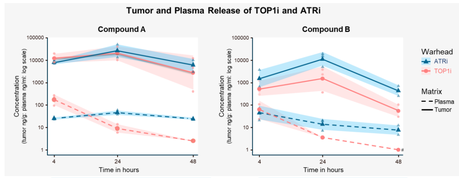


FIGURE 10. HEK293T-FAP tumor-bearing NOG mice were dosed with compound A at 40mg/kg or compound B at 60mg/kg, administered s.c. as single injection (n=3/cohort). Tumors and plasma were collected after 4, 24 and 48h and analysed by LC/MS for released TOP1i and ATRi. LLOQ plasma and tumor were 2ng/ml and 4ng/g, respectively. Values below LLOQ are plotted as LLOQ/2 and indicated with # symbol. Geometric mean and individual data points shown. Shaded ribbons: ± SD.

Compound A TSI: $AUC_{4\text{-}48h}$ [tumor/plasma]		Compound B TSI: $AUC_{4\text{-}48h}$ [tumor/plasma]	
TOP1i	340	TOP1i	64
ATRi	527	ATRi	325

pre|CISION® Dual Payload Compounds Effectively Elicit In Vivo Tumor Growth Inhibition

- Mice inoculated with HEK293T-FAP or Gastric PDX tumors were dosed subcutaneously with **Compound A or B** at maximum tolerated dose (MTD), **or TOP1i or ATRi** at molar equivalent concentrations
- Enhertu comparison was included in the Gastric PDX model, which is FAP-low and HER2+
- FAP-TOP1i/ATRi compounds exhibited **significant tumor growth inhibition** in both models, with **superior activity compared to Enhertu** in the Gastric PDX model

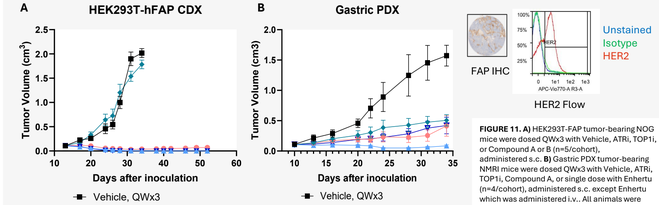


FIGURE 11. A) HEK293T-FAP tumor-bearing NOG mice were dosed QWx3 with Vehicle, ATRi, TOP1i, or Compound A or B (n=5/cohort), administered s.c. **B)** Gastric PDX tumor-bearing NMRI mice were dosed QWx3 with Vehicle, ATRi, TOP1i, Compound A, or single dose with Enhertu (n=4/cohort), administered s.c. except Enhertu which was administered i.v.. 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. Mean ± SEM shown. Inset shows IHC staining for FAP and flow staining for HER2 in previously archived cores of the Gastric PDX model.

CONCLUSIONS

- **pre|CISION® is a payload delivery system based on a tumor-specific protease (FAP) that concentrates potent payloads in the tumor microenvironment: the peptide-linker complex has been adapted to deliver two payloads from a single FAP cleavage event**
- **The AVA6207 dual payload is designed to simultaneously release a topoisomerase I inhibitor (TOP1i) and a DNA Damage Repair (DDR) inhibitor by FAP cleavage, resulting in synthetic lethality in two models of TOP1i resistance: ATM-deficiency and loss of SLFN11. These studies demonstrate the ability to overcome mechanisms of TOP1i resistance and mediate synergistic tumor cell killing**
- **Tumor and plasma PK studies demonstrate robust tumor-selective release of both payloads with compounds having a highly potent Tumor Selectivity Index (TSI: AUC_{tumor}/AUC_{plasma})**
- **FAP-low and HER2+ Gastric PDX model demonstrates optimal activity of the dual payload TOP1i/ATRi compound over the HER2-targeted TOP1i ADC (Enhertu) alone. FAP-high HEK293T-FAP model demonstrates rapid complete responses with the dual payload compounds**
- **Tumor-specific dual payload delivery has the potential to dramatically increase the therapeutic window and reduce systemic toxicities when delivering combination therapy, offering improved outcome for patients with cancer**

References

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Acknowledgements

The investigators and the team at Avacta would like to thank SynthesisMedChem who performed chemical synthesis; Signature Discovery who performed chemical synthesis, cytotoxicity and kinetics assays, and computational modelling; Concept Life Sciences who performed multiplex-immunofluorescence; Experimental Pharmacology & Oncology Berlin who performed tumor uptake and in vivo efficacy studies; SlideSource who supported scientific communication. BioRender was used to generate Figure 5.

Presented at AACR Annual Meeting 2026
April 2026, San Diego, California, USA