AVA6207 is a novel pre|CISION® technology compound delivering complementary TOPO1i and ATRi payloads to the tumor microenvironment following FAP cleavage

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Poster #161

Introduction

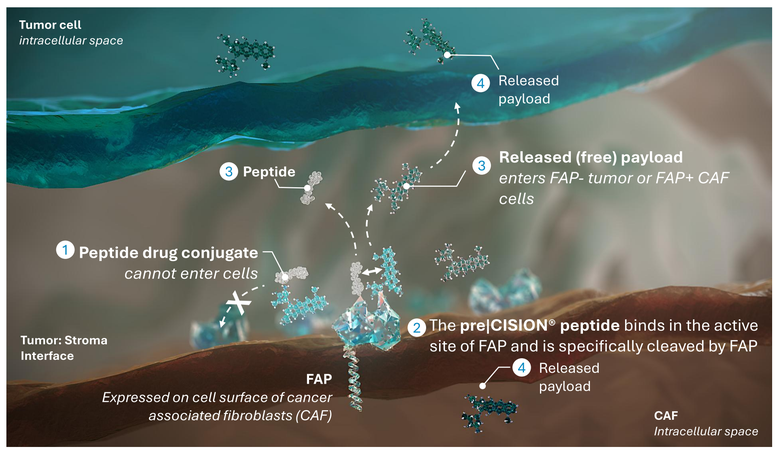


FIGURE 1. The pre|CISION® peptide masks toxic cancer drugs in the bloodstream and enables specific release of the active drugs at the tumor site. Figure shows mechanism of action at the tumor site. *CAF = Cancer-Associated Fibroblast*

- **pre|CISION® dual payload technology** comprises a single molecule peptide drug conjugate (PDC), consisting of a FAP-cleavable peptide, linker, and two locations for attachment of payloads – both released from a single cleavage event to **deliver two payloads to the same cells**
- pre|CISION® AVA6207 dual payload comprises the DNA damage response agent **ATR inhibitor berzosertib** with the **topoisomerase I inhibitor exatecan**: inhibition of DNA repair potentiates the effect of TOP1i
- Selective expression of the protease FAP by CAFs ensures **payload is released in the TME**, minimising exposure to healthy tissues
- pre|CISION® PDCs have the potential to **improve therapeutic windows** and **maximise efficacy; reduce adverse effects** compared to treatment with payload alone; and **circumvent key known resistance mechanisms** to single-drug therapies

- Two settings are investigated: **ATM loss** and **SLFN11 loss**. Both biomarkers identify areas of high unmet need for the clinical development of AVA6207
- Avacta is the first company to develop Dual Payload PDCs, with key advantages over conventional ADC approaches including tuneable **tumor-specific release, optimisation of bystander MoA** and **reduced manufacturing costs**
- **Avacta's two clinical stage programs:**
  (1) Faridoxorubicin (FAP-Dox, AVA6000) completed Phase 1 testing (NCT04969835)[1];
  (2) FAP-Exd (AVA6103) Phase 1 commenced Q1 2026 (FOCUS-01, NCT07454642)[2]

	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 with minimal organ toxicity (e.g. no cardiac toxicity, no pneumonitis or hepatitis)
Dose density to treat resistant disease	Increasing the dose density of an ADC is limited by **the extended PK of the antibody** and **safety of payload in plasma**	**Dose density can be increased** to treat resistant disease given the lack of peptide accumulation and low release of payload in plasma
Internalization dilemma	**Payload is released following slow internalization** and the bystander effect to treat Ag-negative cells is complex	PDCs have **simple extracellular release** resulting in kill of FAP+ and FAP- cells
Tumor penetration	**Slow uptake kinetics** (>24 hours) into tumor cells for payload to be released intracellularly	**Payload release observed in minutes** with T_{max} in the tumor less than 1 hour

FIGURE 2. Advantages of pre|CISION® over antibody-drug conjugates (ADCs)

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

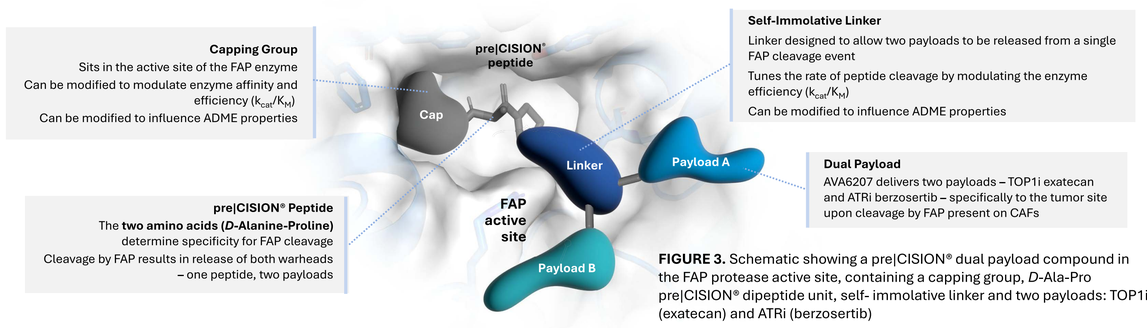


FIGURE 3. Schematic showing a pre|CISION® dual payload compound in the FAP protease active site, containing a capping group, *D*-Ala-Pro pre|CISION® dipeptide unit, self- immolative linker and two payloads: TOP1i (exatecan) and ATRi (berzosertib)

pre|CISION® Dual Payload Compound Activity is FAP-dependent and Effective in *In Vitro* Models

- **Activity** of pre|CISION® dual payload compounds is **dependent upon the presence of FAP+ fibroblasts** in a **3D tumor/fibroblast co-culture model. Limited activity** is observed in the **absence of FAP+ fibroblasts (Fig 4A)**
- The measured FAP activity in the 3D co-culture model (tumor:fibroblast ratio of 1:3) lies within the **physiological range** of FAP activity observed across the AVA6000 clinical trial[1]
- **Immunofluorescence** confirms **FAP-dependent MoA**, namely increase in DNA damage measured by γH2AX fluorescence intensity, and reduction in the percentage of EdU-incorporating proliferating cells **(Fig 4B)**

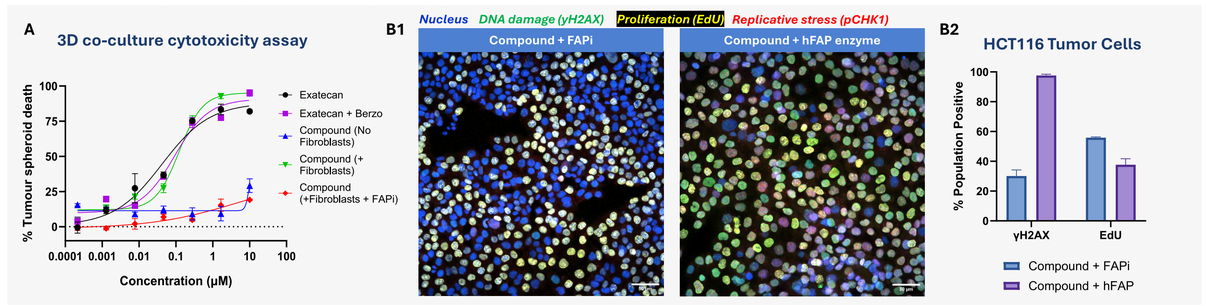


FIGURE 4. The MoA of of pre|CISION® dual payload compounds is FAP-dependent. A 3D spheroids of MDA-MB-231-GFP cells alone or co-cultured with primary human mammary fibroblasts (1:3 ratio) treated with Compound D. Spheroids were imaged on Incucyte for 120hr. Tumor spheroid death was analyzed as % reduction in GFP intensity vs. vehicle control. **B** HCT116 cells were treated with 1.28nM Compound A (delivering TOP1i exatecan and ATRi berzosertib) for 24 hours, prior to EdU pulsing, fixation and staining for γH2AX Ser139 (green), phospho-CHK1 Ser345 (red), EdU (yellow) and Hoechst (blue). **B1** Four-channel overlays of cells treated with Compound A and FAP inhibitor (FAPi) or human FAP enzyme (hFAP) respectively. **B2** Quantification of marker-positive populations. Images were acquired at 60x magnification. Scale = 50μm. Image analysis was performed in Fiji and QuPath.

pre|CISION® Dual Payload Compounds Rescue TOP1i Resistance in SLFN11-Deficient Cells

- **SLFN11** has emerged as a **dominant determinant of response to TOP1i** and other DNA damaging agents[4]. Here, SLFN11 WT were **more sensitive to TOP1i** compared with SLFN11 KO, which are more reliant on ATR for survival **(Fig 5A)**
- pre|CISION® dual payload compound in the presence of FAP **reversed TOP1i resistance in SLFN11 KO cells** to a similar extent as TOP1i and ATRi (berzosertib) free payload combination **(Fig 5A)**

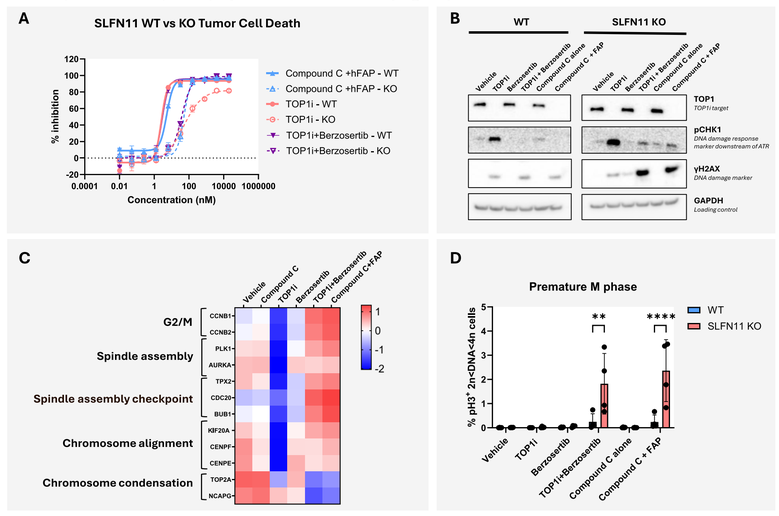


FIGURE 5. A DU145 wild-type (WT) and SLFN11 knock-out (KO) cells (polyclonal) were treated for 72 h with varying concentrations of Compound C (+10 nM hFAP), TOP1i, or 1:1 TOP1i:ATRi (berzosertib) combination. Cell death was measured by CellTiter-Glo assay as % luminescence reduction vs. vehicle control. Data shown is for compound-matched TOP1i SN-38.

B Biomarkers analyzed by Western Blotting after 24h treatment with indicated compounds (500nM) in DU145 WT and SLFN11 KO cells (polyclonal).

C Heatmap showing Z-score normalized expression of indicated genes in NCI-H82 cells (a SLFN11-low cell line) following exposure to indicated compounds for 24h and subsequent RNAseq analysis.

D Flow cytometric analysis in DU145 WT or DU145 SLFN11 KO cells. Flow cytometric analysis of pH3 (Ser10) expression and DNA content following 24h treatment with 500nM of indicated compounds. N=4 experimental replicates. Two-way ANOVA with Tukey's correction. ****p<0.0001, **p<0.01.

- pre|CISION® dual payload compound **reduced TOP1** and **exatecan-induced pCHK1 levels in the presence of FAP,** consistent with **release of both payloads.** SLFN11 KO tumor cells demonstrated **increased DNA damage (γH2AX)** compared with SLFN11 WT cells **(Fig 5B)**
- NCI-H82, a SLFN11-low cell line, exposed to pre|CISION® dual payload compound in the presence of FAP showed the **same changes in gene expression as exposure to both free payloads**, including higher than average expression of genes associated with mitotic entry **(Fig 5C)**
- pre|CISION® dual payload compound 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 (Fig 5D)**

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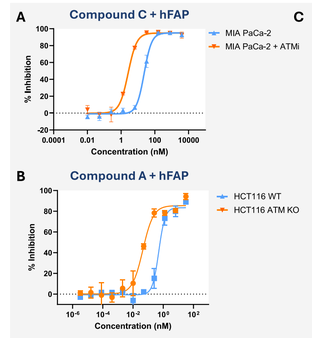
Acknowledgements

The investigators and the team at Avacta would like to thank SynthesisMedChem who performed chemical synthesis; Sygnature Discovery who performed bioanalysis; Concept Life Sciences who performed immunohistochemistry; Experimental Pharmacology & Oncology Berlin who performed tumor uptake and *in vivo* efficacy studies; and SlideSource who supported figure generation.

Presented at EORTC-NCI-AACR Symposium on Molecular Targets and Cancer Therapeutics. November 18-20 2026, Barcelona, Spain.

ATM Deficiency Sensitizes Tumor Cells to pre|CISION® Dual Payload Treatment

- **ATM** serves as a master regulator of the DNA damage response and is frequently mutated or lost in solid tumors[5]
- ATM deficiency impairs DNA damage sensing and repair, increasing **reliance on ATR-mediated** replication stress responses[5]
- **Loss of ATM function** (through inhibition or knock-out) led to an approximate **10-fold increase** in sensitivity of tumor cells to preCISION ® dual payload compounds (**Fig 6**)
- **ATM KO tumor cells** are more sensitive to pre|CISION® dual payload compound at lower doses than WT cells **(Fig 7A)**, demonstrated by a greater fold-change increase in γH2AX intensity vs vehicle-treated control **(Fig 7B)**



C

	pre|CISION® dual payload compound(s) IC_{50} (nM)		Sensitivity fold-change (n=2)
MIA PaCa-2	- ATMi	+ ATMi	
	23.4	2.6	9-fold
HCT116	WT	ATM K/O	
	0.53	0.042	12-fold

FIGURE 6. A MIA PaCa-2 cells were treated for 7 days with varying concentrations of pre|CISION® Dual Payload Compound C (+10 nM hFAP) in the presence or absence of 30 nM ATM inhibitor (ATMi). Cell death was measured on Incucyte as % confluence reduction vs. vehicle control (n=2). **B** Isogenic HCT116 wild-type (WT) and ATM knock-out (KO) cells were treated for 5 days with Compound A in the presence of hFAP, and CellTiter-Glo readout used to measure cell death as % luminescence reduction vs vehicle control (n=2). **C** Summary of IC_{50} values obtained and corresponding shift in sensitivity to pre|CISION® compounds tested.

- **Greater DNA damage** is induced in ATM KO cells by pre|CISION® dual payload compound vs either exatecan or berzosertib alone **(Fig 7B)**

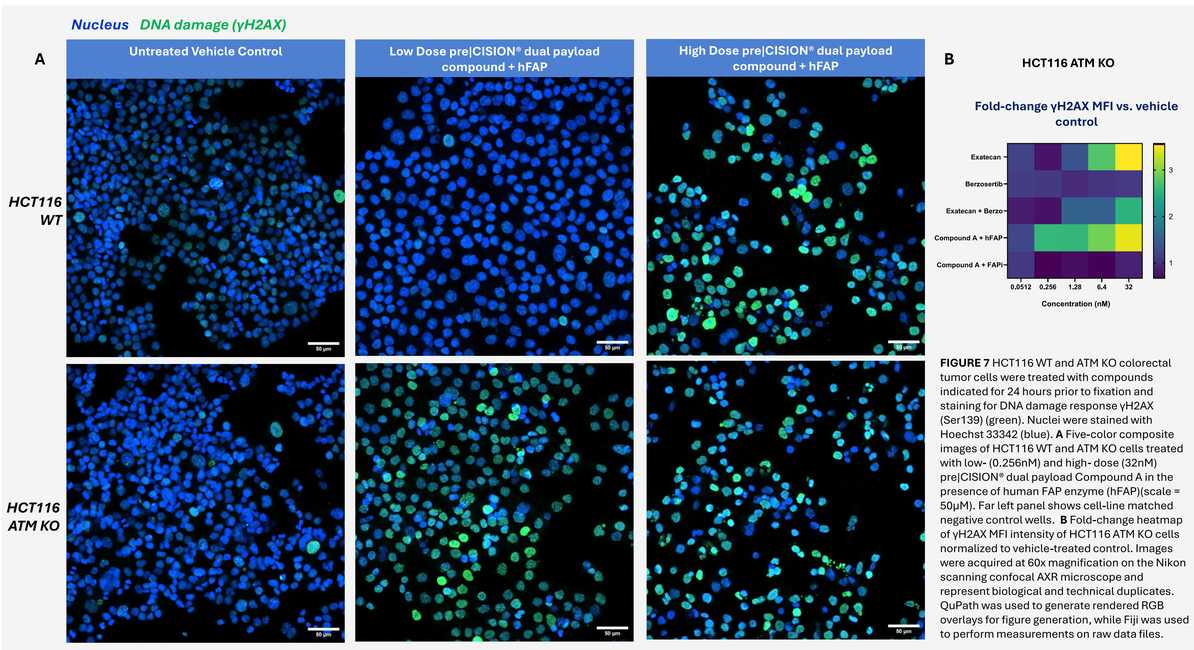


FIGURE 7 HCT116 WT and ATM KO colorectal tumor cells were treated with compounds indicated for 24 hours prior to fixation and staining for DNA damage response γH2AX (Ser139) (green). Nuclei were stained with Hoechst 33342 (blue). **A** Five-color composite images of HCT116 WT and ATM KO cells treated with low- (0.256nM) and high- dose (32nM) pre|CISION® dual payload Compound A in the presence of human FAP enzyme (hFAP)(scale = 50μM). Far left panel shows cell-line matched negative control wells. **B** Fold-change heatmap of γH2AX MFI intensity of HCT116 ATM KO cells normalized to vehicle-treated control. Images were acquired at 60x magnification on the Nikon scanning confocal AXR microscope and represent biological and technical duplicates. QuPath was used to generate rendered RGB overlays for figure generation, while Fiji was used to perform measurements on raw data files.

Cleavage of pre|CISION® Dual Payload Compound at the Tumor Site Results in High Intratumoral and Low Plasma Concentrations of TOP1i and ATRi, with Expected On-Target Changes to PD Biomarkers

- pre|CISION® dual payload compound showed **sustained, high intratumoral TOP1i (exatecan) and ATRi (berzosertib)** concentrations in a HEK293T-FAP mouse model with **far lower concentrations in plasma (Fig 8A)**
- Preferential payload distribution to the tumor can be quantified by the tumor selectivity index (TSI), which shows limited systemic exposure
- **Pharmacodynamic biomarkers of MoA** were assessed in harvested tumors which demonstrate simultaneous release of both payloads in the tumor
- Western blot showed reduction in TOP1 levels, consistent with release and **target engagement with a TOP1 inhibitor** and increases in markers for DNA damage (γH2AX), DNA damage repair (pKAP1), and apoptosis (cleaved PARP)
- The expression of **DNA damage response and repair, and apoptosis markers** increase and persist at 48 hours **(Fig 8B)**

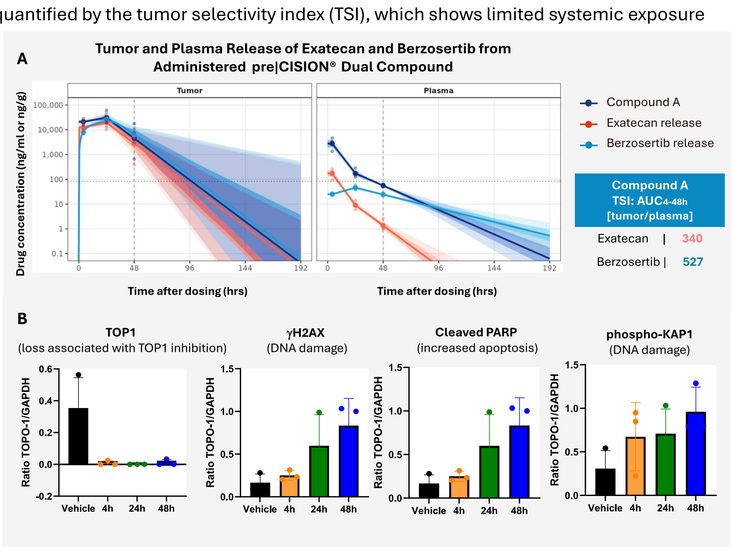


FIGURE 8. HEK293T-FAP tumor-bearing NOG mice were dosed with Compound A at 40mg/kg, administered s.c. as single injection (n=3/cohort). **A** Tumors and plasma were collected after 4, 24 and 48h and analyzed by LC/MS for released exatecan and berzosertib. LLOQ plasma and tumor were 2ng/ml and 4ng/g, respectively. Modeling used to predict exposure following the 48-hour timepoint. Tumor selectivity index (TSI) was calculated as the ratio of AUC (tumor)/AUC (plasma). Bootstrapped values up to 48 hours used with 2000 simulations to generate terminal data. **B** Quantification of Western Blots of tumors from mice exposed to Compound A relative to GAPDH loading control (n=3 mice/group).

pre|CISION® Dual Payload Compounds Effectively Elicit *In Vivo* Tumor Growth Inhibition including in PDX Models with Low ATM and Low SLFN11

- Mice implanted with **low ATM and low SLFN11** ovarian or NSCLC lung PDX tumors were dosed subcutaneously with **pre|CISION dual payload compound** at maximum tolerated dose (MTD)
- pre|CISION® dual payload compound **exhibited significant tumor growth inhibition** in both models **(Fig 9A1, B1)**
- In the NSCLC lung model, 4/5 animals showed **complete responses** to pre|CISION® compound and survival until study termination **(Fig 9A2, A3)**
- In comparison, only one animal from the **exatecan-treated cohort** showed partial response in the NSLC model
- All animals in the ovarian model demonstrated **complete responses (Fig 9B2)** to pre|CISION® compound, compared with two animals from the exatecan-treated cohort

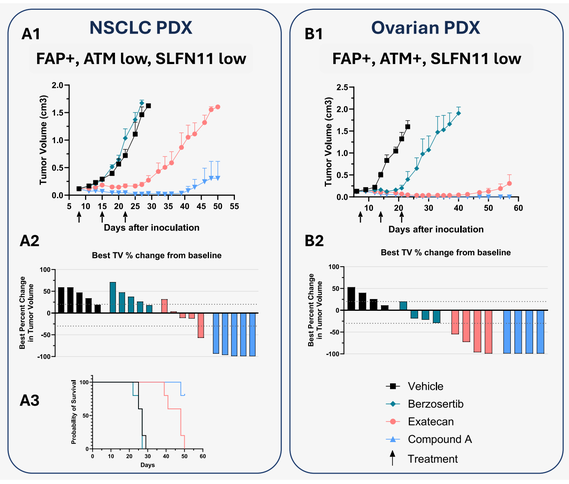


FIGURE 9. PDX tumor-bearing NMRI nu/nu mice were dosed QWx3 with vehicle, ATRi berzosertib (10mg/kg), TOP1i exatecan (5mg/kg), or pre|CISION® exatecan/berzosertib dual payload Compound A (40mg/kg) administered s.c. **A1,B1** Efficacy of indicated compounds as measured by tumor growth for NSCLC PDX (n=5/cohort) and ovarian PDX (n=4/cohort), respectively. **A2, B2** Best percentage change in tumor volume from baseline per mouse, per cohort. **A3** Kaplan-Meier survival curve for NSCLC PDX. 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.

Clinical Significance and Potential Biomarker-Defined Populations for AVA6207

Combined TOP1i and ATRi may **overcome resistance** to single-agent TOP1i used in the clinic by combining TOP1i-induced DNA damage with ATR-induced inhibition of DNA damage repair and replication stress responses[3]. **Low SLFN11 expression** or promoter methylation, and tumors with **ATM loss of function** (LoF), and therefore ATR pathway dependency, may identify patients who stand to gain **additional clinical benefit from AVA6207** treatment.

Indications	ATM Loss/LoF		SLFN11 Low	
	Estimated Prevalence (%)	Sources	Estimated Prevalence (%)	Sources
Non-Small Cell Lung Cancer (NSCLC)	**10-46%**	Peterson et al., 2017; Weber et al., 2016	**18.2%** methylation	Markou et al., 2022
Colorectal Cancer (CRC)	**4-20%**	Grabsch et al., 2006; Sundar et al., 2018	**29*-55%** methylation **95%** low	Kaczorowski et al., 2024, 2025; He et al 2017
Ovarian Cancer	**11-25%**	Konstantopoulos et al., 2021; Ye et al., 2014	**31-39%** methylation/loss (serous ovarian cancer)	Nogales et al., 2015; Kaczorowski et al., 2024;
Esophageal Cancer	**2-10%** mutation (esophageal/esophagogastric)	Lee et al., 2023; Trybala et al., 2026	**25.6%** methylation **33-53%** low	Zhou et al., 2024; Kagami et al., 2020

TABLE 1. Prevalence of ATM loss/loss of function (LoF) and SLFN11 low/deficient biomarkers within AVA6207 target solid tumor indications. ATM loss was assessed at the protein level by IHC for all indications except esophageal tumors, where values represent mutation rate detected by pan-TCGA analysis. *SLFN11* methylation was assessed by methylation-specific PCR (MSP), and loss was detected at the protein level by IHC. Values gathered from sources indicated and *internal Avacta reports.

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 with AVA6207 – the **TOP1i exatecan** and the **ATRi berzosertib** - from a single FAP cleavage event.
- Next-generation AVA6207 compounds **are designed to target TOP1i resistance** in SLFN11-deficient models, and capitalize on synthetic lethality in ATM-deficient models as demonstrated by killing and immunofluorescence assays.
- 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}). PK/PD analysis confirms on-target activity.
- PDX models in both NSCLC and ovarian cancer with low ATM and low SLFN11 are highly sensitive to dual payload administration. **SLFN11-low** and **ATM-deficient tumors** represent a biomarker-defined population **especially vulnerable** to dual TOP1 and ATR inhibition delivered by AVA6207.
- Tumor-specific dual payload delivery has the potential to dramatically **increase the therapeutic window, reduce systemic toxicities** and **overcome TOP1i resistance** when delivering combination therapy, an area of **growing unmet need in oncology.**