# AVA6103, a novel Topoisomerase I FAP-enabled pre|CISION® medicine targeted to the tumor microenvironment via FAP cleavage

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## INTRODUCTION

**Antibody drug conjugates have revolutionized treatment of many metastatic cancers, however there are key limitations in this drug class including**

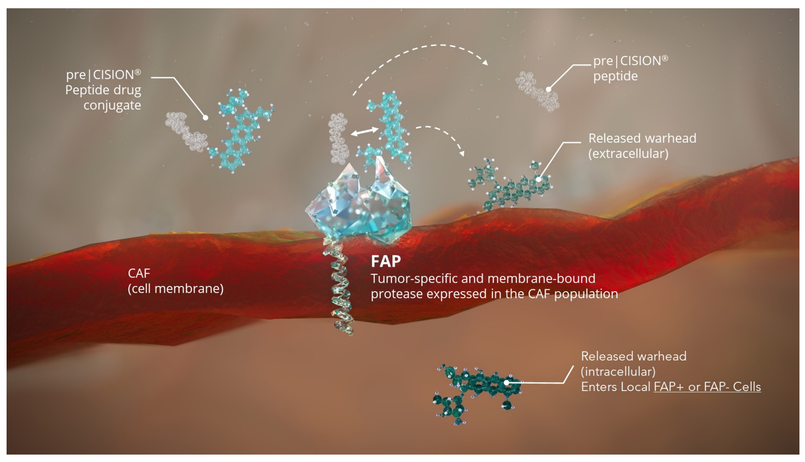
- Non-specific warhead release that results in significant off target toxicities (e.g. interstitial lung disease/pneumonitis)
- Complexity of the bystander effect that targets antigen-negative tumor cells and enables targeting of antigen-low populations
- Complex and costly GMP manufacturing process

**pre|CISION® peptide drug conjugates (PDC) are designed to overcome these limitations by incorporating a peptide that is cleavable only by fibroblast activation protein (FAP) expressed in the cancer associated fibroblast (CAF) population in the tumor microenvironment (TME)**

- The pre|CISION® release mechanism results in 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 Gen One, pre|CISION® doxorubicin Phase 1 results, Banerji et al AACR 2024, Twelves et al ESMO 2024)
- The mechanism of action of the pre|CISION® PDC relies on the bystander effect where the warhead is cleaved from the peptide in the extracellular setting by FAP-positive CAFs
- The GMP manufacturing process of these peptide and warhead conjugates resembles a small molecule process with similar short timelines and costs of clinical drug supply

**Gen Two pre|CISION® PDC (AVA6103) have two key advances in the technology that create a sustainable release mechanism including: (1) adjustable FAP enzyme efficiency (kcat/Km) and (2) extending the half-life of the conjugated molecule**

### CLEAVAGE OF THE pre|CISION® PEPTIDE OCCURS AT FAP+VE CAFS AND CONCENTRATES THE WARHEAD IN THE TME



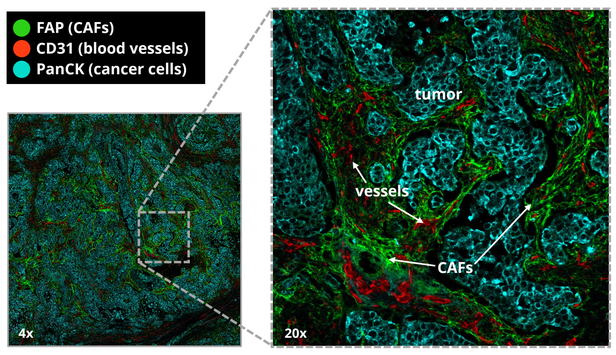

## A pre|CISION® PDC INDUCES DURABLE RESPONSE IN A PATIENT WITH FAP-NEGATIVE TUMOR CELLS

Fibroblast activation protein α (FAP) is **highly expressed** in many tumors where it is expressed on cancer associated fibroblasts (CAFs). To the right shows the spatial arrangement of epithelial cancer cells, CAFs, and blood vessels.

pre|CISION® enabled compounds are designed to allow for FAP specific cleavage and release of the warhead, **directly in the TME**

In the TME, CAFs with the **highest expression of FAP** are concentrated at the tumor-stroma interface and co-located with the blood vessels which delineates the "bystander effect" delivery

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 neighbouring cancer cells.

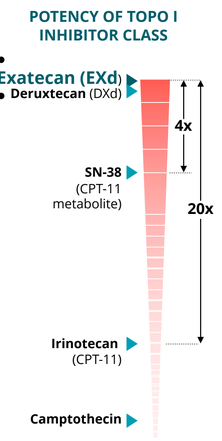



**Case Study :** 79 –year-old male with SGC (ductal histology) with disease progression following first line therapy. Enrolled in the AVA6000 trial (Oct 2023) in the 385 mg/m$^2$ Q3W cohort with durable partial response (duration of response >18 weeks)

In this case, **tumor cells are negative for FAP** (aqua) with prominent FAP-positive Cancer Associated Fibroblast (CAF) populations (green) observed at the tumor-stroma interface with vessels co-localized in stroma (red)

## EXATECAN IS AN IDEAL PAYLOAD FOR THE GENERATION TWO pre|CISION® PLATFORM

1. **Exatecan (EXd) is the most potent topo I inhibitor** with **single agent activity** in Ph 2 trials in several key FAP-positive indications (breast, gastric, small cell lung cancer)
2. **Deruxtecan (DXd) has similar potency but lower membrane permeability** compared with exatecan (EXd) and is a highly **successful ADC warhead**
   - When attached to trastuzumab (Enhertu™), the only ADC shown to have **significant bystander effect** or anti-TROP1 (DATO-DXd)
3. **Exatecan failed in the clinic due to a limited therapeutic index and significant PK issues**
   - Short half-life of ~9 hours which is insufficient for the effective inhibition of the topoisomerase I enzyme
   - The Gen Two pre|CISION® format can optimize both therapeutic index as well as the PK liability




Kumazawa, E., . et al. Potent and broad antitumor effects of DX-8951f, a water-soluble camptothecin derivative, against various human tumors xenografted in nude mice. *Cancer Chemother Pharmacol* 42, 210–220 (1998). https://doi.org/10.1007/s002800050807.

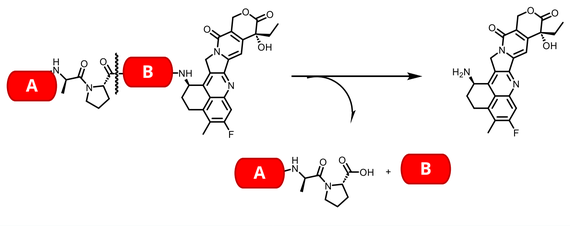
## FAP-EXd (AVA6103): DESIGNING THE OPTIMIZED EXATECAN pre|CISION® MEDICINE

Leverage pre|CISION® technology to **extend the half-life** of released exatecan

- Goal: prolong inhibition of the topo I enzyme for >24 hours in the FAP-EXd development
- **Extend plasma PK** of the conjugated molecule to several hours via capping group (v. 30-90 min, AVA6000 Gen 1)
- **Extend the tumor exposure** by adjusting the release kinetics via linker (alter the kcat/Km of the conjugate)

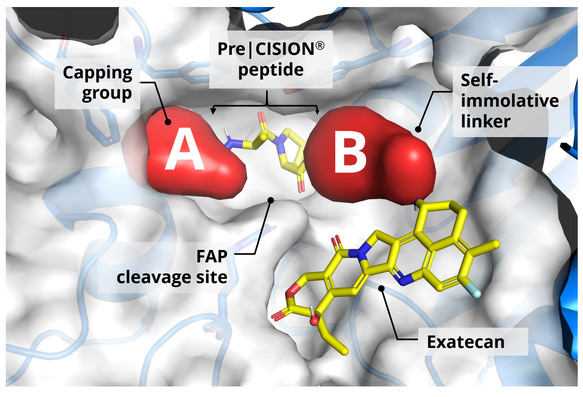
Extend the **Therapeutic Index** of exatecan

- Slower release and extended plasma PK of the conjugate will result in an exatecan sustained release mechanism
- This is expected to result in very low plasma exposure to released exatecan

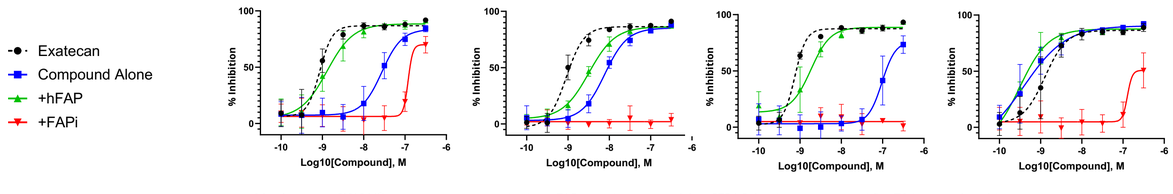



### FAP-EXd (EXATECAN) IN THE FAP ACTIVE SITE

Using both a **Capping Group (A)** and **Linker (B)** to adjust the PK as well as FAP efficiency (kcat/Km) of the drug




| | Compound 1 | Compound 2 | Compound 3 | Compound 4 |
|---|---|---|---|---|
| **R Groups** | A1 / B1 | A2 / B2 | A2 / B3 | A2 / B4 |
| **$k_{cat}$ / $K_m$ ($M^{-1}$ $s^{-1}$)** | 1750 | *T.B.D* | 98 | 58500 |




Multiple pre|CISION® exatecan compounds show FAP-enabled cytotoxicity. CFPAC-1 cells were treated for 72 hours with either exatecan, pre|CISION® exatecan, pre|CISION® exatecan + hFAP, or pre|CISION® exatecan + FAPi. Culture medium contains a low level of FAP activity present in the serum, and hence compounds with varying kcat/Km show different activities; this is used to monitor compound properties. Modulation capping group (A) and self-immolative linker (B) will alter FAP affinity and compound properties, as demonstrated in their respective cytotoxicity plots.

## FAP-EXd (AVA6103): EFFECTIVE KILLING OF FAP-NEGATIVE TUMOR CELLS IN A BYSTANDER ASSAY

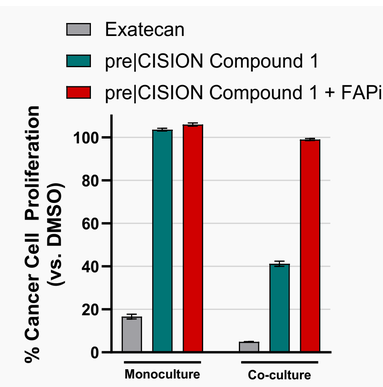
To assess the killing capability effect of pre|CISION® exatecan compounds on cancer cells in a "**closer to physiologically relevant**" model, we employed the use of a bystander (co-culture) assay

In a **co-culture** model, MiaPaCa **pancreatic cancer cells** (FAP-negative) were plated alone, or in combination with **pancreatic fibroblasts** (FAP-positive at physiologic levels similar to human tumors)

Compound 1 (0.001μM) exhibits **no activity in the monoculture** (FAP-negative MiaPaCa alone), proliferation aligns with DMSO-treated cell proliferation (at 120hr post-treatment)

With the **addition of FAP-positive fibroblasts**, compound 1 is cleaved by FAP to release exatecan, **greatly reducing cancer cell proliferation** compared to DMSO-treated cells (at 120hr post-treatment)

Here, the **bystander effect** is achieved, where compound 1 is cleaved by **FAP-positive fibroblasts**, and released exatecan can enter **FAP-negative cancer cells**



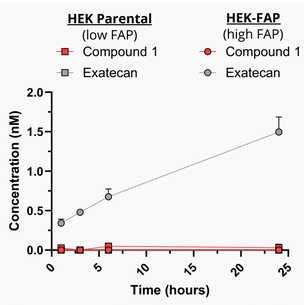

Co-culture with fibroblasts demonstrates FAP-specific cleavage of Compound 1 (0.001μM) at 120hr post-treatment, where Mia PaCa-2-GFP cells were plated either alone, or in combination with pancreatic fibroblasts at a ratio of 28%:72% respectively

## AVA6103 ALLOWS MODULATION OF PROPERTIES TO MAXIMISE TUMOR UPTAKE

In a **cell uptake** experiment, **low FAP** expressing cells (HEK-parental) and **high FAP** expressing cells (HEK-FAP) were treated with compound 1, to assess intracellular levels of the compound 1 itself, and cleaved exatecan warhead

When treating with compound 1, both cells display no intracellular levels over a 24-hour period. **HEK-parental** cells also show **no intracellular exatecan**, due to the absence of FAP and therefore cleavage of compound 1

In the HEK-FAP cells however, exatecan concentration increases over a 24-hour period, as a result of FAP mediated cleavage of compound 1




Compound 1 cannot enter cells, and the exatecan warhead is only released into FAP high cells. Compound 1 cannot enter FAP low or high cells, and only cleaved exatecan is present intracellularly in the FAP high cells.

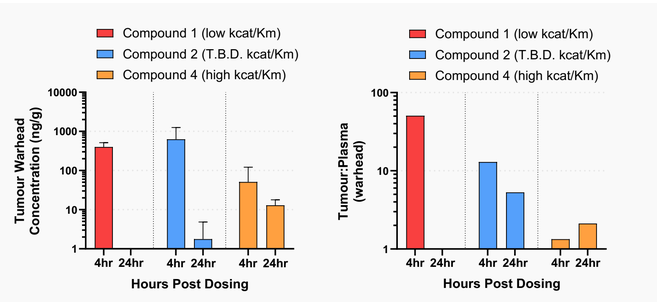
A Patient-derived xenograft model of melanoma (Mel13281) was selected to run a **tumor uptake** experiment, to measure the **intratumoral and plasma levels** of pre|CISION® compounds 1,2 and 4, and the released exatecan warhead

Animals received a single dose of compounds 1,2 and 4 (15mg/kg) and were sampled at either 4 or 24 hours (n=3) post-dosing

At the 4-hour timepoint, cleaved exatecan (left plot) was present at **high levels intratumorally** at 4hr when treating with Compound 1, and again at both 4hr and 24hr when treating with Compound 2 and 4

The ratio of warhead in the tumor vs. plasma was **50-fold** for compound 1 at the 4hr timepoint, and **13-fold** and **5-fold** for compound 2 at the 4hr and 24 timepoints respectively

Through modulation of a compound's kcat/Km, we can alter the intratumoral warhead concentrations, as well as the tumor:plasma ratio




(Left) Cleaved exatecan warhead is present intratumorally at 4hr when treating with Compound 1, and at both 4hr and 24hr when treating with Compound 2 and 4. (Right) Elevated tumour:plasma ratios are seen at 4hr and 24hr for all compounds, with a 50-fold increase for Compound 1 at 4hr.

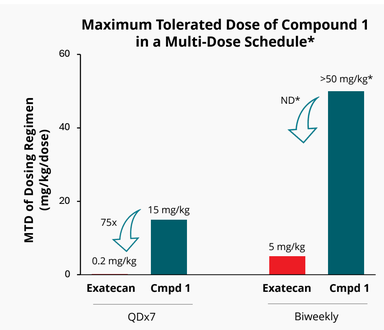
## THE MTD OF AVA6103 IS SIGNIFICANTLY GREATER THAN THAT OF CONVENTIONAL EXATECAN

**Exatecan dosing** in prior clinical trials was designed to optimize inhibition of the topoisomerase I enzyme for multiple cell cycle rounds

To demonstrate the **MTD of pre|CISION® exatecan,** Compound 1 was selected to compare its tolerability vs. conventional exatecan

Dosing in the QDx7 regimen was limited at **0.2 mg/kg** for exatecan alone (which is consistent with previous clinical dosing), whereas the MTD of Compound 1was **15 mg/kg,** 75-fold that of conventional exatecan

Biweekly dosing found exatecan was limited to **5mg/kg**, whereas Compound 1 MTD could not be determined, being well tolerated at the max dose tested at **50 mg/kg**




*Non-tumor bearing mice were dosed in a multi-dose format with the defined schedules using either exatecan or Compound 1 administered s.c. Maximum dose tested of Compound 1 was 50 mg/kg with no toxicity noted, thus the therapeutic index of the biweekly schedule was not determined (ND)

## FAP-EXd (AVA6103) DEMONSTRATES TUMOR GROWTH INHIBITION EQUIVALENT TO EXATECAN IN AN EFFICACY STUDY

**Compound 1 (pre|CISION® exatecan)** was dosed in animals bearing established HEK-FAP tumors, a highly aggressive model of human cancer engineered to express FAP

Compound 1 was compared vs. exatecan alone (both at the established MTD in mice)

Compound 1 demonstrates the ability to achieve **significant tumor growth inhibition** compared to vehicle alone and with equivalent efficacy to exatecan at the MTD. Both compounds demonstrate **tumor regression** in this model




HEK-FAP tumor-bearing NMRI mice (n=6/cohort) were dosed in a multi-dose format with a defined schedule (QDx7, 2 day rest) using AVA6103 - Compound 1, or exatecan administered s.c. Animals were treated when palpable, established tumors were present

## CONCLUSIONS

- **AVA6103 is a novel peptide drug conjugate (PDC) based on proprietary pre|CISION® technology which incorporates a dipeptide that is specifically cleaved by Fibroblast Activation Protein α (FAP) which results in tumor-specific delivery of the exatecan warhead directly to tumor cells**
- **Using AVA6103 Gen Two pre|CISION® compounds, characterized by varying two key pieces of the chemical structure of pre|CISION® exatecan (the capping group [A] and self-immolative linker [B]), we can modulate both FAP enzyme efficiency (kcat/Km) and the PK properties of each compound**
  - The therapeutic index of exatecan is significantly increased by pre|CISION® enabling, specifically the MTD of compound 1 was 75 times that of conventional exatecan in a daily dosing regimen
  - AVA6103 optimizes the bystander effect, where the conjugate is only cleaved by FAP+ fibroblasts, and released exatecan can enter FAP- cancer cells
- **In a Patient-derived xenograft model, high intratumoral warhead concentrations are seen at 4hr and 24hr timepoints for several pre|CISION® exatecan compounds, with up to 50-fold higher warhead concentrations in the tumor vs. plasma**
- **pre|CISION® exatecan demonstrates tumor growth inhibition in an efficacy study, where HEK-FAP tumors treated with Compound 1 displayed no growth over a three-week period, and increased survival vs. vehicle treated tumors**

**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. Presented at the European Society of Medical Oncology Annual Meeting, September 14, 2024, in Barcelona, Spain

Banerjee et al. A Phase I trial of AVA6000, a Fibroblast Activation Protein (FAP)-released and tumor microenvironment (TME)-linked doxorubicin peptide drug conjugate in patients with FAP-positive tumors. Presented at the American Association for Cancer Research Annual General Meeting, April 2024 San Diego, CA USA

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**Poster #173**