Comparative Pharmacokinetics and Tumor Activation of Fibroblast Activation Protein (FAP)-enabled pre|CISION® Peptide Drug Conjugates

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Avacta THERAPEUTICS

Abstract #CT15

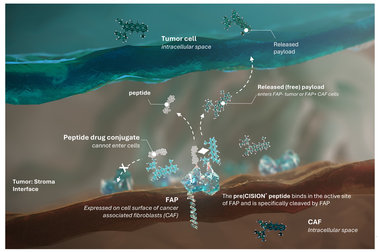
Background

pre|CISION® peptide drug conjugates comprise a peptide moiety bound to an active payload by a linker that is specifically cleaved by FAP, a protease selectively overexpressed in the tumor microenvironment (TME) of many solid tumors on cancer-associated fibroblasts

The pre|CISION® peptide moiety prevents cellular entry of the conjugate unless cleaved by FAP, enabling targeted delivery of released payload directly to tumors. Two payloads are described: doxorubicin and exatecan

- This targeted release mechanism has been validated in both preclinical animal models and clinical biopsy data, demonstrating that the payload is predominantly activated and released in the FAP-rich tumor environment
- Doxorubicin is a widely used and effective chemotherapeutic agent. However, its clinical utility is significantly limited by dose-dependent, cumulative cardiotoxicity and the characteristic bone marrow toxicity observed with many anticancer agents
- Encouraging safety and preliminary efficacy data from the Phase 1 trial with FAP-Dox (AVA6000) have been presented previously (Banerji, AACR 2024, Twelves, ESMO 2024). FAP-EXd (AVA6103, exatecan) is in IND-enabling studies and anticipated to start in Phase 1 in Q1 2026
- It is hypothesized that the tumor-specific release mechanism of pre|CISION® medicines reduces the initial "first pass" cardiac and bone marrow exposure to doxorubicin—an effect sometimes referred to as the "organ extraction effect"

FIGURE 1. The pre|CISION® Bystander Effect



Phase 1 Patient Population and Trial Methods

The AVA6000 Phase 1a dose escalation enrolled patients with a diagnosis of cancers frequently noted as FAP-positive, including sarcoma, pancreatic cancer, colorectal cancer, head and neck cancers. Specific indications were selected for Phase 1b expansion. FAP expression was tested retrospectively. The data cutoff presented is 10 April 2025

- **Prior therapy with any anthracycline was limited to total cumulative dose of less than 350 mg/m².** The lifetime cumulative maximum exposure was limited to 550 mg/m² in the AVA6000 trial based on favorable safety data
- **Trial analyzed for safety (primary endpoint) and efficacy.** Efficacy in patients with a diagnosis of salivary gland cancers treated at the dose of 250 mg/m² and above are presented across doses ranging from 250 mg/m² to 385 mg/m² (n=11)
- **On-treatment biopsies were obtained (n=14) at 24 hours after the dose was administered and assessed for the level of free doxorubicin in the TME.** Plasma samples were obtained at the same time to assess the tumor:plasma ratio of doxorubicin

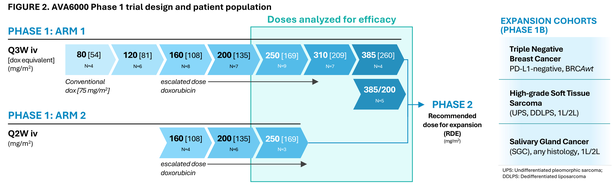
TABLE 1. Patient demographics and baseline characteristics

	AVA6000 (Q3W/Q2W) N=63
Age, median (range)	63 (30-81)
Sex, m/f, n (%)	36 / 27 (57 / 43)
ECOG, 0/1. n (%)	25 / 38 (40 / 60)
Race	
White, n (%)	51 (81)
Asian, n (%)	6 (10)
Black or African American, n (%)	2 (3)
Not reported/unknown, n (%)	4 (6)
Ethnicity	
Hispanic/Latino, n (%)	0
Non-Hispanic, non-Latino, n (%)	59 (94)
Not reported/unknown, n (%)	4 (6)

	AVA6000 (Q3W/Q2W) N=63
Cancer Diagnosis	
Salivary gland cancer, n (%)	17 (27)
Soft tissue sarcoma (other subtype), n (%)	14 (22)
Colorectal carcinoma, n (%)	10 (16)
Pancreatic ducal adenocarcinoma, n (%)	8 (12)
Liposarcoma/high grade UPS, n (%)	6 (10)
Cancers of the biliary tract, n (%)	2 (3)
Other[1], n (%)	6 (10)[1]
Prior systemic cancer therapy	
Number of prior regimens, median (range)	2 (0-7)
Any cytotoxic, n (%)	39 (62)
Anthracycline, n (%)	5 (8)
Platinum, n (%)	36 (57)
Topoisomerase I inhibitor, n (%)	35 (56)
Immunotherapy, n (%)	17 (27)

[1] Cancer types in Other category include (n=1 each): non-small cell lung cancer, prostate cancer, transitional cell cancer of the urethra, ovarian carcinoma, lung cancer (not otherwise specified), esophageal cancer

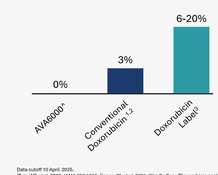
FIGURE 2. AVA6000 Phase 1 trial design and patient population



Results: Preliminary Safety and Efficacy of FAP-Dox (AVA6000)

AVA6000 has Dramatically Reduced the Toxic Effects Associated with Doxorubicin and the ADC Drug Class

- **Bone marrow toxicities are dramatically** reduced when comparing AVA6000 versus conventional dose doxorubicin
- AVA6000 has **no severe cardiac toxicity** despite doses approaching 4x the MTD of conventional doxorubicin (75 mg/m²)
- **Alopecia** is generally limited to hair thinning (grade 1) with infrequent complete hair loss compared with conventional doxorubicin
- Compared with ADCs, there are **no reports of toxicities associated with non-specific release of the payload** with AVA6000 (including no pneumonitis, no ocular toxicity and no liver toxicity)



Data cutoff 10 April. 2025.
[1]Tap, WD et al. 2020. JAMA 323:1266. [2]Jones, RL et al. 2021. Clin Ca Res. †Doxorubicin package insert (at 550mg/m² max) Updated from Twelves et al. 2024 ESMO Annual Meeting

FIGURE 3. FAP-Dox (AVA6000) demonstrates no severe cardiac toxicity (compared to conventional doxorubicin)

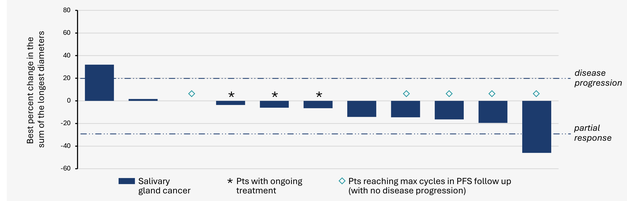
TABLE 2 . Treatment-emergent (TE) Grade 3-4 AVA6000-related AE by cohort observed in >2 pts

Adverse event	80 mg/m² Q3W n (%) N=4	120 mg/m² Q3W n (%) N=6	160 mg/m² Q3W n (%) N=8	200 mg/m² Q3W n (%) N=7	250 mg/m² Q3W n (%) N=9	310 mg/m² Q3W n (%) N=7	385 mg/m² Q3W n (%) N=4	385/200 mg/m² Q3W n (%) N=5	160 mg/m² Q2W n (%) N=4	200 mg/m² Q2W n (%) N=6	250 mg/m² Q2W n (%) N=3	Total n (%) N=63	Doxorubicin (75 mg/m² Q3W) N=251 Gr 3-4* n (%)
Neutropenia	0	0	0	2 (29)	2 (22)	1 (14)	2 (50)	4 (80)	0	0	1 (33)	12 (19)	122 (49)
Leukopenia	0	0	0	1 (14)	0	1 (14)	2 (50)	2 (40)	0	0	0	6 (10)	59 (23.7)
Lymphopenia	0	0	0	2 (29)	0	0	0	1 (20)	0	1 (17)	0	4 (6)	7 (2.8)
Anemia	0	0	0	1 (14)	0	2 (29)	0	0	0	0	0	3 (5)	31 (12.4)
Thrombocytopenia	0	0	0	1 (14)	1 (11)	0	0	0	0	1 (17)	0	3 (5)	21 (8.4)
Fatigue	0	0	0	0	0	1 (14)	0	0	1 (25)	0	0	2 (3)	12 (4.8)

Data cutoff 10 April 2025
*Tap WD, et al. 2020. Phase 3 trial of olaratumumab with doxorubicin in patients with STS. Data reported from doxorubicin mono arm Grade 3-4 events

AVA6000: Preliminary Data Demonstrate Durable Tumor Shrinkage in Patients with Salivary Gland Cancers

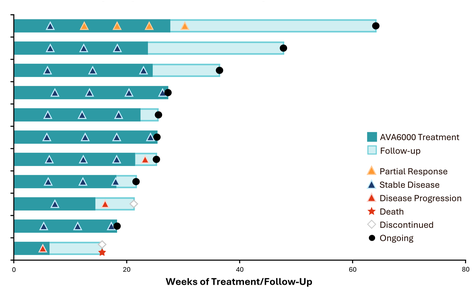
FIGURE 4. Tumor Shrinkage In Patients With Salivary Gland Cancer



Data cutoff 10 April 2025
All patients with the diagnosis of salivary gland cancer treated at or above 250 mg/m² regardless of schedule (n=11)

AVA6000 Demonstrates Multiple Minor Responses, a Disease Control Rate Over 90% and Median PFS Not Reached

FIGURE 5. Efficacy Analysis Set – Subset of Salivary Gland Carcinoma: Phase 1a



Data cutoff 16 February 2024
All pts with the diagnosis of salivary gland cancer treated at or above 250 mg/m² regardless of schedule. Median follow up: 25.3 weeks

PK and PD Effects of FAP-Dox (AVA6000) and FAP-EXd (AVA6103)

PBPK Modelling of FAP-Dox (AVA6000)

Data from two murine animal model studies were used to develop a PK model predicting tissue exposure to released doxorubicin in patients from the Ph I trial of FAP-Dox (AVA6000)

- Both studies showed higher tumor concentrations of released doxorubicin compared to heart and bone marrow

An *in silico*, physiologically based pharmacokinetic (PBPK) model incorporating the murine studies, drug transporters and clinical data was developed. To investigate the extended tumor exposure and low cardiac toxicity observed in the AVA6000 Ph I trial the model structure was applied to AVA6000 and conventional doxorubicin, comparing cardiac and plasma drug concentrations

- Cardiac doxorubicin concentrations ≥1000–5000 ng/ml are associated with cardiotoxicity due to doxorubicin-induced ROS and mitochondrial damage
- In addition to cumulative anthracycline exposure, the heart's low antioxidant capacity and inhibition of TOP2β by doxorubicin also contribute to DNA damage and cardiomyocyte apoptosis (Minotti 2004; Yang 2014)

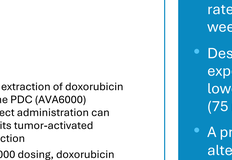


FIGURE 6. Schematic of PBPK Model of Cardiac Exposure with AVA6000

Tumor Uptake and Activation Modelling of Payload Release in Animal Models

- Following administration of pre|CISION® compounds, tumors are exposed to payloads through local release within the tumor microenvironment. We investigated the tumor uptake in standard FAP+ tumor murine models
- Due to differences in catalytic efficiency (K_{cat}/K_m) and variable FAP activity across the tumor uptake models biological compartments, the release rate of exatecan (AVA6103) or doxorubicin (AVA6000) are different. AVA6103 (exatecan) employs a sustained release mechanism (described in Rink et al. Abstract 3139, AACR 2025, April 28th 2-5PM)
- Changes in concentrations over time for the PDC and the released payload are described from the equations below
- Biopsies in patients were taken 24 hours after the first dose was administered with a plasma sample taken at the same time. Both samples were analyzed for released doxorubicin

$$K_{met} = \left(\frac{Kcat[Et]}{Km}\right)$$

$$\frac{d[P]}{dt} = \left(\frac{kcat[Et][P]}{Km+[P]}\right) - Cl_P[P]$$

$$\frac{d[D]}{dt} = \left(\frac{kcat[Et][D]}{Km+[D]}\right) - Cl_D[D]$$

[P] =PDC concentration
[D] = Exatecan concentration
Cl_P and Cl_D = clearances of the parent and the active (Exatecan or Doxorubicin)

K_{met} represents the number of substrate molecules an enzyme converts to product per second when the enzyme is fully saturated and it is dependent upon the Km which can by Precision proprietary technology design tailored to controlled release of thew active moiety

FIGURE 7a. Plasma exposure of released doxorubicin compared to conventional dose doxorubicin

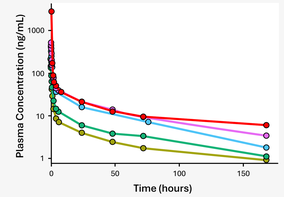
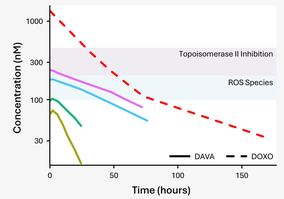


FIGURE 7b. Cardiac tissue exposure (PBPK modeled) of released doxorubicin compared to conventional dose doxorubicin



75 mg/m² conventional dox
Released doxorubicin from 385 mg/m² AVA6000
Released doxorubicin from 310 mg/m² AVA6000
Released doxorubicin from 160 mg/m² AVA6000
Released doxorubicin from 80 mg/m² AVA6000

FIGURE 8a. Tumor uptake model with AVA6000 in a FAP-positive tumor model

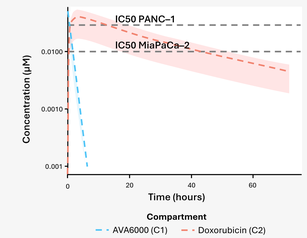


FIGURE 8b. Tumor uptake model with AVA6103 in a FAP-positive tumor model

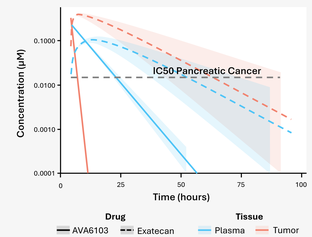
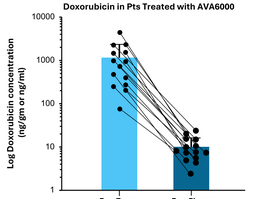


FIGURE 9. Tumor Doxorubicin v. Plasma Doxorubicin in Pts Treated with AVA6000

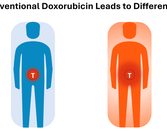


Tumor biopsies from AVA6000 were taken 24 hours post-dose and analyzed for doxorubicin. These samples were compared to the Plasma doxorubicin levels at 24 hours.

Key Finding:
AVA6000 cleavage occurs in the TME, with concentration of doxorubicin in the TME of approximately ~ 2-log difference between tumor and plasma concentrations

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.

FIGURE 10. The First Pass Effect of AVA6000 Versus Conventional Doxorubicin Leads to Different Tissue Distribution



AVA6000
Doxorubicin (red) is released in the tumor (T) and leaks to the bloodstream in limited fashion with a limited V_{dist} limiting cardiac exposure

Conventional Doxorubicin
Doxorubicin (red) is infused in the bloodstream and bathes all tissues within the T_{INF} of 5 minutes, including the heart

- The lower organ extraction of doxorubicin observed with the PDC (AVA6000) compared to direct administration can be explained by its tumor-activated mechanism of action
- Following AVA6000 dosing, doxorubicin is enzymatically released in the tumor microenvironment, and only a fraction of the active drug subsequently enters the systemic circulation, reaching other organs—including the heart—at delayed and lower concentrations
- In contrast, conventional intravenous administration delivers high concentrations of doxorubicin systemically from the outset, resulting in immediate exposure of the heart

See other Avacta posters at this meeting:
The novel peptide drug conjugate AVA6103 is a pre|CISION® medicine which targets exatecan to the TME following FAP cleavage (Abstract #3139)
Monday, April 28, 2025, 2:00 – 5:00 p.m.

CONCLUSIONS

- FAP-Dox (AVA6000) is safe and well-tolerated in both the Q3W and Q2W dosing regimens with, with preliminary evidence of efficacy in patients with salivary gland cancers and no severe cardiac toxicity. No MTD was determined in the trial despite dosing up to 385 mg/m² every 3 weeks (~4x conventional dose doxorubicin)
- Confirmed responses were observed in patients with stromal only expression of FAP with salivary gland cancers. The disease control rate is 91% in this indication with median PFS not reached and >25 weeks median follow up
- Despite dosing up to 4x the dose of conventional doxorubicin, the exposure of released doxorubicin in plasma and normal tissues is lower than that observed with conventional dose doxorubicin (75 mg/m² Q3W) and the median tumor to plasma ratio is 100:1
- A pre|CISION® sustained release chemistry mechanism allows an alternate payload (exatecan) with a short half life to persist longer than doxorubicin in the tumor (for >60 hours) as modeled in animal studies
- Conventional doxorubicin and released dox from AVA6000 have a different first pass effect, limiting the exposure of normal tissues, including the heart to low levels of released doxorubicin. The low exposure in normal tissues explains the reduction in toxicities observed with FAP-Dox versus conventional doxorubicin

References

He H, et al., A Multiscale Physiologically-Based Pharmacokinetic Model for Doxorubicin to Explore its Mechanisms of Cytotoxicity and Cardiotoxicity in Human Physiological Contexts. *Pharm Res*. 2018 Jul 9;35(9):174.

Otter M, Coader S, Keiser M, Oswald S. Expression and Functional Contribution of Different Organic Cation Transporters to the Cellular Uptake of Doxorubicin into Human Breast Cancer and Cardiac Tissue. *Int J Mol Sci*. 2021 Dec 27;23(1):255 .

Minotti G, et al. Anthracyclines: Molecular Advances and Pharmacologic Developments in Antitumor Activity and Cardiotoxicity. *Pharmacol Rev*. 2004;56(2):185–229.

Rink, C. et al. The Novel Peptide Drug Conjugate AVA6103 is a FAP-enabled pre|CISION® Medicine Which Targets Exatecan to the Tumor Microenvironment. AACR Annual Meeting 2025, Abstract 3139. Monday April 28 2-5PM.

Tap WD, et al. Effect of Doxorubicin Plus Olaratumab vs Doxorubicin Plus Placebo on Survival in Patients With Advanced Soft Tissue Sarcomas: The ANNOUNCE Randomized Clinical Trial. *JAMA*. 2020;323(13):1266-1276.

Villalobos VM, et al. Pharmacokinetics of doxorubicin following concomitant intravenous administration of olaratumab (IMC-3G3) to patients with advanced soft tissue sarcoma. *Cancer Med*. 2020 Feb;9(3):882-893.

Wallace KB. Doxorubicin-induced cardiac mitochondrionopathy. *Pharmacol Toxicol*. 2003;93(3): 105–115.

Yang F, et al. Doxorubicin, DNA torsion, and chromatin dynamics. *Biochim Biophys Acta*. 2014;1845(1):84-9.

Zhang S et al. The mitochondrial rein in anthracycline cardiotoxicity. *Circ Res*. 2012;111(6):782–93.

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