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

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BACKGROUND AND PRECLINICAL DATA

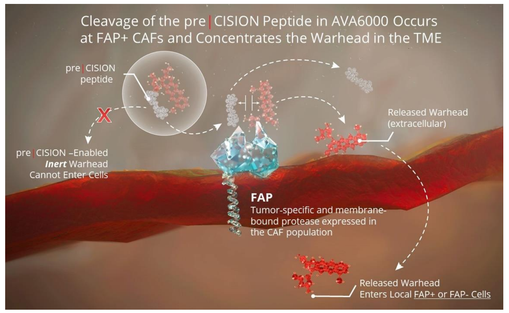
MECHANISM OF ACTION OF AVA6000

Fibroblast activation protein-α (FAP) is an extracellular post-proline protease that is upregulated in many solid tumors in a membrane-bound form on cancer associated fibroblasts as well as tumor cells. FAP activity is only observed to a low degree in plasma

AVA6000 is a **peptide drug conjugate**, that leverages the tumor-specific expression of FAP by linking a peptide moiety that is specifically cleaved by FAP to doxorubicin

The **peptide moiety linker** (pre|CISION™) prevents cellular entry of doxorubicin unless cleaved by FAP, thus enabling targeted delivery of doxorubicin to tumors

FIGURE 1.



CLINICAL TRIAL METHODS

AVA6000 was assessed in a multi-center, ascending dose first-in-human Phase 1 trial in patients. The patient population included the following:

- Patients with a diagnosis of known FAP-positive cancers, including sarcoma, pancreatic cancer, colorectal cancer, head and neck cancers. Indications previously described as FAP low were not enrolled in the trial
- Acceptable performance status (ECOG 0 or 1), adequate organ function and recovery from effects of prior therapies (with a cap on prior anthracycline)

Classification of indications as FAP high and FAP mid was based on literature. In general:

- FAP low are indications in which most patients were negative for FAP expression by IHC and/or FAPI-PET (and patients with these diseases were excluded from the trial)
- FAP mid are diseases in which some patients had reports of FAP expression
- FAP high are diseases in which patients were noted to have extensive FAP expression based on IHC and/or FAPI-PET imaging (Ballal 2021, Kratochwil 2019, Koerber 2021)

Data Cutoff 19 August 2024

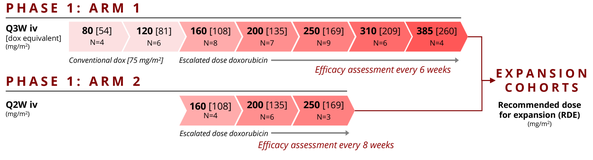
On-treatment biopsies were obtained (n=13) at 24 hours after the dose was administered and assessed for the level of free doxorubicin in the TME by LC/MS and for FAP activity by in vitro fluorescent labeled peptide (D-Ala-Pro) assay. Plasma samples were obtained in parallel to assess the plasma concentration of doxorubicin

Expression of FAP, CD31 and pan-cytokeratin were assessed based on standard immunohistochemistry (IHC, semi-quantitative analysis of FAP DAB staining with hematoxylin counterstain) and multiplex immunofluorescence (IMF, quantitative analysis). Malignant and stromal compartments within the tumor were analyzed separately by IMF. Representative 4X-20X images are shown

FIGURE 2. ANALYSIS SETS

		EVALUABLE FOR EFFICACY ANALYSIS
SAFETY	**Patients treated with at least one dose of AVA6000 (n=57) across two dose regimen arms,** every 3 weeks, Q3W and every 2 weeks, Q2W. AE graded per CTCAEv5.0	
EFFICACY	**Patients with:** (1) **known FAP higher tumor types** (within the FAP+ tumor types) including soft tissue sarcoma and salivary gland cancer, (n=28) or (2) **FAP mid tumor types** which have some evidence of FAP expression, including colorectal cancers, pancreatic cancers, etc. (n=29)	Subset 1: **23** Subset 2: **26**

FIGURE 3. AVA6000 PHASE 1 TRIAL DESIGN



BASELINE CHARACTERISTICS

TABLE 1. DEMOGRAPHICS AND BASELINE CANCER HISTORY

	AVA6000 (Q3W/Q2W) N=57
Age, median (range)	63 (30-81)
Sex, m/f, n (%)	34 / 23 (59.6/40.4)
ECOG, 0/1. n (%)	20 / 37 (35.1/64.9)
Race	
White, n (%)	47 (82.5)
Asian, n (%)	5 (8.8)
Black or African American, n (%)	1 (1.8)
Not reported/unknown, n (%)	4 (5.3)
Ethnicity	
Hispanic/Latino, n (%)	0
Non-Hispanic, non-Latino, n (%)	54 (94.7)
Not reported/unknown, n (%)	3 (5.3)

	AVA6000 (Q3W/Q2W) N=57
Cancer diagnosis	
Soft tissue sarcoma (other subtype), n (%)	13 (22.8)
Colorectal carcinoma, n (%)	11 (19.3)
Salivary gland cancer, n (%)	10 (17.5)
Pancreatic ducal adenocarcinoma, n (%)	8 (14.3)
Liposarcoma, n (%)	5 (8.8)
Cancers of the biliary tract, n (%)	3 (7.1)
Undifferentiated pleomorphic sarcoma, n (%)	1 (1.8)
Other[1], n (%)	6 (10.5)[1]
Prior systemic cancer therapy	
No. prior regimens, median (range)	2 (0-7)
Any cytotoxic exposure, n (%)	37 (64.9)
Anthracycline exposure, n (%)	3 (5.3)
Platinum exposure, n (%)	27 (47.3)
Topoisomerase I inhibitor exposure, n (%)	20 (35.1)
Immunotherapy exposure, n (%)	16 (28.1)

[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

RESULTS

TABLE 2. TREATMENT-EMERGENT (TE) GRADE 3-4 AVA6000-RELATED HEMATOLOGIC AE COMPARED WITH CONVENTIONAL DOXORUBICIN

	Arm 1: Q3W dosing							Arm 2: Q2W dosing				
CTCAE Gr 3-4 Adverse events	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=6	385 mg/m² Q3W n (%) N=4	160 mg/m² Q2W n (%) N=4	200 mg/m² Q2W n (%) N=6	250 mg/m² Q2W n (%) N=3	Total n (%) N=57	Doxorubicin (75 mg/m² Q3W) N= 249 Gr 3-4[1] n (%)
Neutropenia	0	0	0	2 (29)	2 (22)	1 (17)	2 (50)	0	0	1 (33)	8 (14)	122 (49)
Leukopenia	0	0	0	1 (14)	0	1 (17)	2 (50)	0	0	0	4 (7)	59 (23.7)
Febrile neutropenia	0	0	0	0	0	0	0	0	0	0	0	41 (16.5)
Anemia	0	0	0	1 (14)	0	2 (33)	0	0	0	0	3 (5.3)	31 (12.4)
Thrombocytopenia	0	0	0	1 (14)	1 (11)	0	0	0	1 (17)	0	3 (5.3)	21 (8.4)

Data cutoff 19 AUG 2024. [1]Tap, WD *et al.* 2020. Phase 3 trial of olaratumumab with doxorubicin in patients with STS. Data reported from doxorubicin monotherapy arm, Grade 3-4 events

TABLE 3. LVEF CHANGES FROM BASELINE VS. CONVENTIONAL DOXORUBICIN

Observed LVEF Changes from Baseline	AVA6000 80-385 mg/m² Q3W/Q2W N=57 n (%)	Doxorubicin[1] 75 mg/m² Q3W N= 401 n (%)
LVEF, n (%)		
<50%	1 (1.8)	48 (12.0)
>10% decrease from baseline	7 (12.3)	191 (47.6)
<50% and/or >10% decrease from baseline	7 (12.3)	194 (48.4)

Data cutoff 19 AUG 2024
[1]Jones *et al. Clin Ca Res* (2021). Phase 3 ANNOUCE trial cardiac data analysis based on Tap, WD *et al.* 2020.

TABLE 4. DOSE-LIMITING TOXICITIES

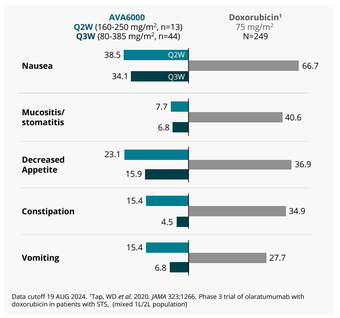
Dose level	Event	Outcome
120 mg/m² Q3W	Grade 2 cardiac failure[1]	Cohort expanded and dose escalated to 160 mg/m²
200 mg/m² Q3W	Grade 4 neutropenia/ thrombocytopenia	Cohort expanded and dose escalated to 250 mg/m²

Data cutoff 19 AUG 2024
[1] Grade 2 cardiac failure associated with LVEF reduction from 61% (baseline) to 39% (post-C1)

SAFETY KEY FINDINGS

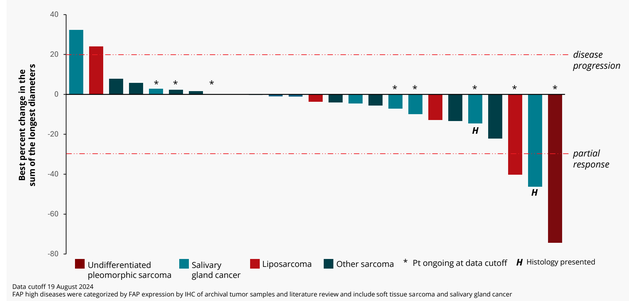
- Favorable safety profile was observed in both the Q2W and Q3W dosing arms, with no MTD identified in either arm
- Limited cardiac signal with low incidence of LVEF changes and no CTCAE grade 3 or 4 severe cardiac events reported in the trial

FIGURE 4. COMPARISON OF SELECT TREATMENT-EMERGENT TOXICITIES OF ANY GRADE IN THE Q2W AND Q3W PHASE 1 ARMS WITH CONVENTIONAL DOXORUBICIN



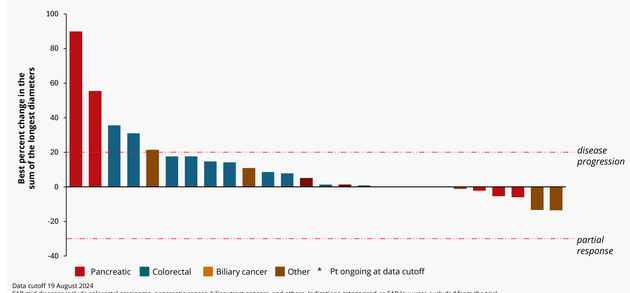
Data cutoff 19 AUG 2024. [1]Tap, WD *et al.* 2020. *JAMA* 323:1266. Phase 3 trial of olaratumumab with doxorubicin in patients with STS, (mixed 1L/2L population)

FIGURE 5. BEST RESPONSE IN PATIENTS IN THE FAP HIGH EFFICACY SET



Data cutoff 19 August 2024
FAP high diseases were categorized by FAP expression by IHC of archival tumor samples and literature review and include soft tissue sarcoma and salivary gland cancer

FIGURE 6. BEST RESPONSE IN PATIENTS IN THE FAP MID EFFICACY SET

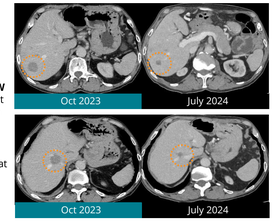


Data cutoff 19 August 2024
FAP mid diseases include colorectal carcinoma, pancreatic cancer, biliary tract cancers, and others. Indications categorized as FAP low were excluded from the trial

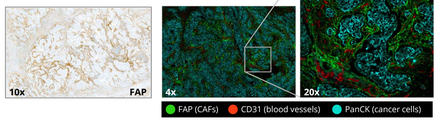
CASE STUDIES

Case Study 1:

- 79 yo male with SGC (ductal histology)
- Prior therapy: triptorelin/ bicalutamide followed by disease progression
- Enrolled in the AVA6000 trial (Oct 2023) in the **385 mg/m² Q3W cohort**; noted to have the highest level of intratumoral doxorubicin of any patient at 24 hours post-dose
- **Partial response** at 12 weeks, following minor response noted at first scan (SLD -22%); duration of response >18 weeks
- Discontinued after reaching lifetime max of doxorubicin exposure; the observed PR is ongoing in follow-up



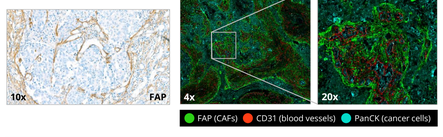
In the first case, tumor cells are **negative for FAP** (aqua) with prominent FAP-positive Cancer Associated Fibroblast (CAF) populations (green) observed at the tumor-stroma interface with vessels co-localized in stroma (red)



Case Study 2:

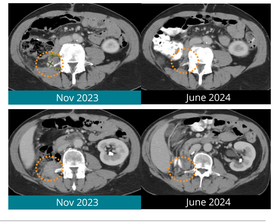
- 65 yo female with salivary gland cancer (ductal histology), diagnosed in 2018, recurrence in Dec 2023
- Prior therapy: bicalutamide/triptorelin with progression prior to study entry
- Treated with AVA6000 in the 2L setting in the **250 mg/m² Q2W cohort** (June 2024)
- **Minor response** at first scan (SLD of -14.6%, week 8); this patient continuing on trial

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



Case Study 3:

- 55 yo male with dedifferentiated liposarcoma (DDLPS), diagnosed in 2012, noted to have a rapidly growing recurrence in 2018
- Two prior lines of therapy in the metastatic setting with progression following both therapies
- Enrolled in AVA6000 trial in the 3L setting in **the 250 mg/m² Q3W cohort** (Nov 2023)
- **Partial response** (SLD –40.5%, June 2024) following a minor response (SLD of –12.5%, Feb 2024). Patient experienced new lesions at the follow-up scan



PHARMACOKINETICS/PHARMACODYNAMICS

The PK of released doxorubicin from AVA6000 demonstrates several fundamental changes compared with conventional dose doxorubicin:

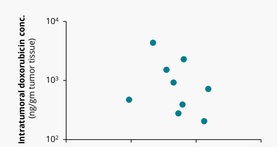
- Extension of the half-life of released doxorubicin v. conventional doxorubicin up to 40-45%
- Approximately 40% reduction in the peripheral volume of distribution of released dox, suggesting a limited distribution into normal tissues of released doxorubicin v. conventional doxorubicin
- Reductions in plasma C_{max} across doses compared with that observed with conventional doxorubicin

TABLE 5. PHARMACOKINETICS OF AVA6000, RELEASED DOXORUBICIN AND CONVENTIONAL DOXORUBICIN

	AVA6000 Peptide drug conjugate PK		AVA-6000 released doxorubicin derived from peptide cleavage		Conventional dose doxorubicin
Dose	310 mg/m² n=6	385 mg/m² n=4	310 mg/m² n=6	385 mg/m² n=4	75 mg/m² n=23[1]
AUC_{0-inf} (ng*hr)/mL Geo mean, (CV%)	12710 (9.2)	12070 (110)	1613 (8.2)	2320 (15.5)	2240 (25)
C_{max} (ng/mL) Geo mean, (CV%)	18650 (20.1)	12410 (197)	305 (37.8)	393.9 (63.0)	2570 (47)
Elimination Half-life (h) Geo mean, (CV%)	0.85 (0.1)	1.36 (105)	52.9 (69.5)	52.1 (33.9)	36.4 (NR)
Peripheral Volume distribution (L)	–	–	1120[2]		1830[3]

Data cutoff 19 AUG 2024. [1]Villalobos *et al. Cancer Medicine* (2020) [2] Data on file, Population Pharmacokinetic Model of Doxorubicin from AVA6000 (Avacta internal reference) [3]Kontny *et al. Cancer Chemother Pharmacol* (2013); Blanco *et al.* (2016) *BJCP*

FIGURE 7. RELEASED DOXORUBICIN CONCENTRATION IN THE TUMOR VERSUS TUMOR FAP PROTEASE ACTIVITY



- The level of released doxorubicin in the tumor at 24 hours post-dose does not correlate with FAP activity level in patients with FAP+ tumor types
- This suggests even a minimal amount of FAP expression is sufficient to release the warhead in the TME
- Even at the lower levels of FAP activity in the tumor, we observe levels of released doxorubicin in the tumor at 24 hours that are substantially higher than in plasma (data not shown)

CONCLUSIONS

- AVA6000 is safe and well-tolerated in both the Q3W and Q2W dosing regimens, with preliminary evidence of efficacy and early limited cardiac safety signal. No MTD was determined in the trial despite dosing up to 385 mg/m² every 3 weeks
- Multiple RECIST responses observed in patients with FAP high and doxorubicin sensitive disease at various doses and both schedules, with durable and ongoing responses
  - Confirmed partial and minor responses were observed in patients with stroma-only expression of FAP [salivary gland] and FAP expression in both stroma and tumor cells [soft tissue sarcoma]
- In tumors expressing FAP, the level of positivity appears not to correlate with the level of released doxorubicin in the TME (n=9), indicating that lower levels of FAP activity are sufficient for warhead release
- pre|CISION-enabling of doxorubicin (AVA6000) results in multiple fundamental changes in the PK of released doxorubicin (compared to conventional doxorubicin administration) including:
  - Extension of the plasma half-life by ~40% and
  - Approximately 40-50% reduction in both C_{max} and peripheral volume of distribution
- The study continues with recommended dose for expansion (RDE) cohorts focusing on salivary gland cancer (SGC) and subsets of soft tissue sarcoma (STS)

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