A Phase I trial of AVA6000, a Fibroblast Activation Protein (FAP)-released and tumor microenvironment (TME)-targeted doxorubicin peptide drug conjugate in patients with FAP-positive solid tumors

Banerji U[1], Cook N[2], Anthoney A[3], Plummer R[4], Tap WD[5], Evans J[6], Cranmer LD[7], Plummer C[4], Loadman P[8], Lahu G[9], Jones H8, Kinnersley N[10], Mc Laughlin F[10], Twelves C[3]

[1]The Institute for Cancer Research and the Royal Marsden Hospital, [2]The Christie, [3]University of Leeds, [4]University of Newcastle, [5]Memorial Sloan Kettering Cancer Center, [6]University of Glasgow, [7]University of Washington, [8]University of Bradford, UK, [9]Thinkq2, Basel Switzerland, [10]Avacta Life Sciences

BACKGROUND AND PRECLINICAL DATA

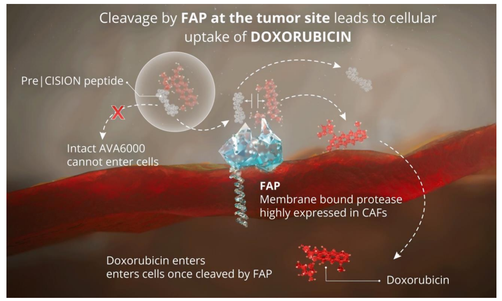
MECHANISM OF ACTION OF AVA6000

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 also observed as a soluble protease 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. FAP CLEAVAGE

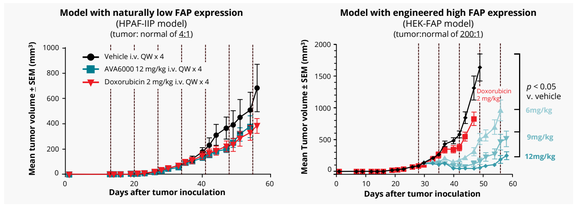


IN VIVO CHARACTERIZATION OF AVA6000

The antitumor activity of AVA6000 was assessed in two in vivo xenograft models, one with low and one with high tumor:plasma FAP activity

As shown in Fig. 2 (left panel), the model with a low activity ratio (HPAF-II) has limited activity of AVA6000, similar to doxorubicin. However, significant antitumor activity with a dose response is demonstrated in the setting of high tumor:plasma FAP activity in Fig. 2 (right panel) suggesting that patients with tumors with high FAP activity will be more sensitive to the AVA6000 mechanism of action

FIGURE 2.
ENHANCED ANTITUMOR ACTIVITY OF AVA6000 IS OBSERVED IN FAP^{high} TUMOR MODELS



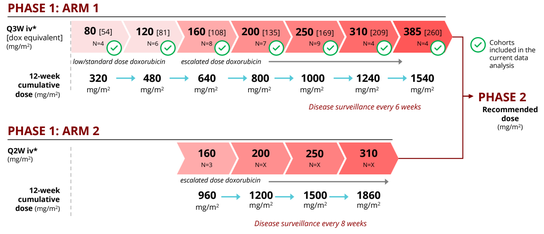
CLINICAL TRIAL METHODS

AVA6000 was assessed in a multi-center, ascending dose first-in-human Phase 1 trial in patients (Data cutoff presented is 11 March 2024). The patient population included the following:

- Patients with a diagnosis of known FAP^{high} cancers, including sarcoma, pancreatic cancer, colorectal cancer, head and neck cancers
- Acceptable performance status (ECOG 0 or 1), adequate organ function and recovery from effects of prior therapies
- Prior therapy with any anthracycline limited to total cumulative dose of less than 350 mg/m^2 doxorubicin or equivalent

For analysis, literature review of FAP expression (Ballal 2021, Kratochwil 2019, Koerber 2021) and IHC of archival tumor samples informed the classification of indications to FAP^{high} and FAP^{mid}. Indications deemed FAP^{low} were excluded from the trial

On-treatment biopsies were obtained (n=11) 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



RESULTS

BASELINE CHARACTERISTICS

TABLE 1. DEMOGRAPHICS AND BASELINE CANCER HISTORY

	AVA6000 (80–385 mg/m² Q3W) N=42
Age, median (range)	64.5 (30-79)
Sex, m/f, n (%)	26 / 16 (61.9/38.1)
ECOG, 0/1. n (%)	14 / 28 (33.3/66.7)
Race	
White, n (%)	34 (81.0)
Asian, n (%)	3 (7.1)
Black or African American, n (%)	1 (2.4)
Other/Not reported, n (%)	4 (9.5)
Ethnicity	
Hispanic/Latino, n (%)	0
Non-Hispanic, non-Latino, n (%)	39 (92.9)
Not reported/unknown, n (%)	3 (7.1)
Cancer diagnosis	
Soft tissue sarcoma, n (%)	14 (33.3)
Colorectal carcinoma, n (%)	11 (26.2)
Pancreatic ductal adenocarcinoma, n (%)	8 (19.0)
Cancers of the biliary tract, n (%)	3 (7.1)
Other[1], n (%)	6 (14.3)[1]
Prior cancer therapy	
No. prior regimens, median (range)	3 (0-7)
Any cytotoxic exposure, n (%)	32 (76.2)
Anthracycline exposure, n (%)	1 (2.4)
Platinum exposure, n (%)	26 (61.9)
Topoisomerase I inhibitor exposure, n (%)	20 (47.6)
Immunotherapy exposure, n (%)	14 (33.3)

[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

SAFETY

SAFETY TABLE 1. TREATMENT-EMERGENT (TE) GRADE 3-4 AVA6000-RELATED AE BY COHORT

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=4	385 mg/m² Q3W n (%) N=4	Total Q3W n (%) N=42	Doxorubicin (75 mg/m² Q3W) N=251 Gr 3-4[A] n (%)
Neutropenia	0	0	0	2 (29)	2 (22)	1 (25)	2 (50)	7 (16.7)	122 (49)
Leukopenia	0	0	0	0	0	1 (25)	2 (50)	3 (7.1)	59 (23.7)
Febrile neutropenia	0	0	0	0	0	0	0	0	41 (16.5)
Anemia	0	0	0	1 (14)	0	2 (50)	0	3 (7.1)	31 (12.4)
Thrombocytopenia	0	0	0	1 (14)	1 (11)	0	0	2 (4.8)	21 (8.4)
Fatigue	0	0	0	0	0	1 (25)	0	1 (2.4)	12 (4.8)
Mucositis	0	0	1 (13)	1 (14)	0	0	0	2 (4.8)	7 (2.8)

Data cutoff 11 March 2024.
[A]Tap WD, et al. 2020. Phase 3 trial of olaratumab with doxorubicin in patients with STS. Data reported from doxorubicin mono arm Grade 3-4 events observed in at least 7 patients

SAFETY TABLE 2. AVA6000-RELATED TEAE BY COHORT, ALL GRADES

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=4	385 mg/m2 Q3W n (%) N=4	Total Q3W n (%) N=42	Doxorubicin (75 mg/m² Q3W) N=251[A] n (%)
Nausea	1 (25)	2 (33)	2 (25)	5 (71)	3 (33)	0	1 (25)	14 (33.3)	166 (67)
Neutropenia	0	1 (17)	0	2 (29)	5 (56)	2 (50)	2 (50)	12 (28.6)	144 (58)
Fatigue	1 (25)	2 (33)	5 (63)	3 (43)	7 (78)	1 (25)	2 (50)	21 (50.0)	147 (59)
Alopecia	0	2 (33)	1 (13)	6 (86)	8 (89)	2 (50)	3 (75)	22 (52.4)	124 (50)
Anemia	1 (25)	1 (17)	1 (13)	1 (14)	6 (67)	2 (50)	2 (50)	14 (33.3)	113 (45)
Mucositis	0	0	2 (25)	0	1 (11)	0	1 (25)	3 (7.1)	101 (41)
Decreased appetite	0	2 (33)	2 (25)	1 (14)	1 (11)	0	1 (25)	7 (16.7)	92 (37)
Constipation	0	0	2 (25)	0	0	0	0	2 (4.8)	87 (35)
Musculoskeletal pain/arthralgia	0	0	1 (13)	1 (14)	0	0	0	2 (4.8)	85 (34)
Leukopenia	0	0	0	0	3 (33)	2 (50)	2 (50)	7 (16.7)	78 (31)
Diarrhea	0	1 (17)	1 (13)	3 (43)	3 (33)	0	0	8 (19.0)	75 (30)

Data cutoff 11 March 2024
[A]Tap WD, et al. 2020. Phase 3 trial of olaratumumab with doxorubicin in patients with STS. Data reported from doxorubicin mono arm , events reported in ≥30% pts (mixed 1L/2L population)

SAFETY TABLE 3. ALL CARDIAC ADVERSE EVENTS

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=4	385 mg/m² Q3W n (%) N=4	Total Q3W n (%) N=42	Doxorubicin (75 mg/m² Q3W) N=251 Gr 3-4[A] n (%)
Cardiac failure[1]	0	1 (17)	0	0	0	0	0	1 (2.4)	14 (5.6)
Troponin increase[2]	0	0	1 (13)	1 (14)	0	0	0	2 (4.8)	*not reported*

Data cutoff 11 March 2024
[A]Tap WD, et al. 2020 and Jones RL, et al. 2019. Phase 3 trial of olaratumab with doxorubicin in patients with STS. Data reported from doxorubicin mono arm all events/any grade
[1] Cardiac failure, grade 2 (DLT) 1 Cardiac failure, grade 2 (DLT)
[2] Troponin increases (n=2) reported as AE, grade 1. Additional grade 1 troponin increases were observed (160 mg/m2 – 250 mg/m^2) considered not related (n=1), or not clinically significant (n=3)

SAFETY TABLE 4. DOSE-LIMITING TOXICITIES

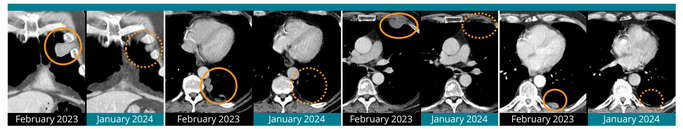
Dose level	Event	Outcome
120 mg/m²	Grade 2 cardiac failure[1]	Cohort expanded and dose escalated to 160 mg/m²
200 mg/m²	Grade 4 neutropenia/ thrombocytopenia	Cohort expanded and dose escalated to 250 mg/m²

Data cutoff 11 March 2024
[1] Grade 2 cardiac failure associated with LVEF reduction from 61% (baseline) to 39% (post-C1)

PRELIMINARY EFFICACY

FIGURE 3. CASE STUDY OF FIRST RESPONSE

Near complete resolution of the multiple pleural metastases



Case Study:
60-year-old male patient with a right-side popliteal mass biopsy diagnosed with a grade 3 undifferentiated pleomorphic sarcoma (UPS). Prior cancer therapy preoperative radiotherapy (May-Jul 2021) followed by surgery (Sept 2021, viable tumour cells in <10% of tumor volume). Stage IV diagnosis (March 2022) with pleural metastases, enrolled in etigilimab + nivolimab (clinical trial June 2022-Jan 2023) with disease progression prior to enrolling in the AVA6000 phase 1 trial

FIGURE 4A. PATIENTS WITH FAP^{high} INDICATIONS[1]

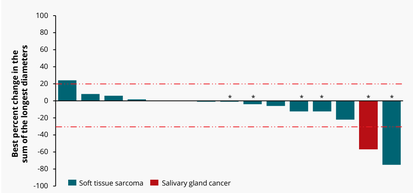
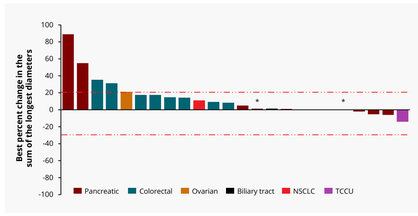


FIGURE 4B. PATIENTS WITH FAP^{mid} INDICATIONS[2]



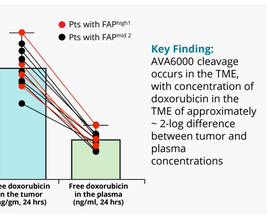
Trial ALS6000-101 | Data cutoff 11 March 2024
[1] FAP^{high} diseases were categorized by FAP expression by IHC of archival tumor samples and literature review and include soft tissue sarcoma and salivary gland cancer, Partial response includes 1 confirmed PR (sarcoma) and 1 unconfirmed PR (salivary gland Ca, both patients ongoing at the time of the data cutoff)
[2] FAP^{mid} diseases include colorectal carcinoma, pancreatic cancer, ovarian cancer, biliary tract cancer, transitional cell cancer of the urethra and lung cancer. Indications categorized as FAP^{low} were excluded from the trial

EFFICACY TABLE 1. BEST OVERALL RESPONSE (FAP^{high} V. FAP^{mid} POPULATION)

	FAP^{high} N=15[1]	FAP^{mid} N=27[2]
Partial response (PR), n[3]	2	0
Minor response (MR), n[4]	3	0
Stable disease (SD)<16 weeks[5]	4	6
SD≥16 weeks[5]	7	10
Progressive disease (PD)	2	11
DCR (PR/MR or SD≥16 weeks), n(%)	10/15 (67)	10/27 (37)

Data cutoff 11 March 2024
[1] FAP^{high} indications were categorized by FAP expression in literature review and archival tumor tissue IHC. FAP^{high} indications include soft tissue sarcoma and salivary gland cancer
[2] FAP^{mid} diseases include colorectal carcinoma, pancreatic cancer, ovarian cancer, biliary tract cancer, transitional cell cancer of the urethra and lung cancer.
[3] PR in FAP^{high} include 1 confirmed PR (sarcoma, duration of response 34 wks) and 1 unconfirmed PR (salivary gland Ca, both patients ongoing at the time of the data cutoff) by RECIST v1.1
[4] Minor response is defined as RECIST v1.1 SD with change in the sum of the longest diameter of -10 to -29% (2 of 3 MR ongoing)
[5] Multiple patients with SD are ongoing at the time of the cutoff: 3 of 4 in FAP^{high} and 2 of 9 in FAP^{mid}

FIGURE 5.
TUMOR DOXORUBICIN V. PLASMA DOXORUBICIN

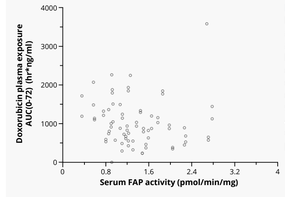


Key Finding:
AVA6000 cleavage occurs in the TME, with concentration of doxorubicin in the TME of approximately ~ 2-log difference between tumor and plasma concentrations

[1] FAP^{high} indications were categorized by FAP expression in literature review and archival tumor tissue IHC. FAP^{high} indications include soft tissue sarcoma and salivary gland cancer. Tumor dox range (206-4396)
[2] FAP^{mid} diseases include colorectal carcinoma, pancreatic cancer, ovarian cancer, biliary tract cancer, transitional cell cancer of the urethra and lung cancer. Tumor dox range (76-2310)

PHARMACOKINETICS/PHARMACODYNAMICS

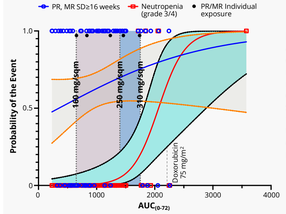
FIGURE 6. HIGH SERUM FAP ACTIVITY DOES NOT TRANSLATE TO HIGH PLASMA DOXORUBICIN



Key Finding:
Higher serum FAP activity is not associated with high plasma exposure of cleaved doxorubicin, suggesting that AVA6000 is not cleaved in the periphery

Figure 6. High Serum FAP Activity does not Translate to High Plasma Doxorubicin Exposure
FAP protease activity was quantitated with a fluorescent D-Ala-Pro substrate to determine the level of enzyme activity in serum. FAP activity was compared to the cleaved doxorubicin plasma exposure [AUC (0-72)]

FIGURE 7. EFFICACY AND SEVERE NEUTROPENIA OBSERVED AT DIFFERENT EXPOSURES



Key Finding:
Responses are observed at AVA6000 doses with doxorubicin exposures much lower than that reported with standard dose doxorubicin. This suggests a more frequent schedule at these doses may enhance efficacy while not increasing toxicity

Figure 7. Efficacy with AVA6000 is Observed at Lower Exposures than Severe Neutropenia
Logistic regression analysis of the relationship between response (PR, MR, SD>16weeks), severe neutropenia and dose indicates high probability of responses at a range of exposures (correlating to 250 – 310 mg/m^2) compared with observation of severe neutropenia. Exposure for individual patients with tumor shrinkage (PR/MR) are included with black dots (1 PR with dose reduction)

PK TABLE 1. PK PARAMETERS FOR RELEASED DOXORUBICIN

AVA6000 dose (n)	Doxorubicin molar equivalent	C_{max} ng/ml, geo.mean	T_{max} (hrs)	$T_{1/2}$ (hrs)	AUC_{last} (h*ng/ml)	AUC_{inf} (hr*ng/ml)	Percent reduction[1] in C_{max}	Pecent reduction[1] in AUC
80 mg/m² (n=4) Analyte, (CV %)	54 mg/m²	190.5 (19)	0.243 (16)	35.73 (26)	488.3 (33)	592.7 (37)	92.5	77
120 mg/m² (n=6) Analyte, (CV %)	81 mg/m²	208.5 (44)	0.351 (35)	44.09 (20)	582.5 (42)	851.7 (55)	91.1	71.7
160 mg/m² (n=8) Analyte, (CV %)	108 mg/m²	216.0 (36)	0.416 (27)	41.56 (35)	615.3 (54)	711.4 (57)	91.2	70.7
200 mg/m² (n=7) Analyte, (CV %)	135 mg/m²	359.5 (27)	0.522 (24)	42.35 (49)	1307 (41)	1474 (28)	85.9	38.9
250 mg/m² (n=9) Analyte, (CV %)	169 mg/m²	456.3 (51)	0.495 (17)	38.5 (65)	1260 (34)	1457 (30)	79.9	44.9
310 mg/m² (n=4) Analyte, (CV %)	209 mg/m²	395.6 (60)	0.629 (20)	48.9 (49)	1748 (84)	2250 (91)	85.2	39.3
385 mg/m² (n=4) Analyte, (CV %)	260 mg/m²	460.8 (45)	0.798 (22)	42.8 (26)	2131 (15)	2465 (15)	77.9	4.8

[1] Percent reduction in C_{max} and AUC(0-72) are calculated using the published reference standard PK of doxorubicin (at 75 mg/m^2) for both C_{max} and AUC (Villalobos, 2019). These PK data from the olatatumab phase 3 trial in combination with doxorubicin published by Tap WD, et al. 2020. PK reference standards were taken from doxorubicin monotherapy arm

CONCLUSIONS

- AVA6000 delivers high concentrations of doxorubicin to the TME relative to plasma, resulting in significant antitumor activity in patients whose tumors have over-expression of FAP
- AVA6000 has a distinct safety profile, with significant reductions in both severe and mild to moderate toxicities associated with standard dose doxorubicin
- Exposure-response modeling suggests that released doxorubicin is generated primarily by cleavage in the TME as opposed to soluble FAP in the bloodstream
- PK/PD modeling demonstrate separation of the probability curves for response v. grade 3-4 neutropenia, supporting the further exploration of the Q2W dosing regimen
- Further development at the recommended dose for expansion (RDE) is planned in 2H 2024 in specific tumor types with high FAP expression and anthracycline sensitivity

REFERENCES

Ballal S, et al. Biodistribution, pharmacokinetics, dosimetry of [68Ga]Ga-DOTA.SA.FAPi, and the head-to-head comparison with [18F]F-FDG PET/CT in patients with various cancers. *Eur J Nucl Med Mol Imaging.* 2021;48(6):1915-1931. doi: 10.1007/s00259-020-05132-y

Kratochwil C, et al. 68Ga-FAPI PET/CT: Tracer Uptake in 28 Different Kinds of Cancer. *J Nucl Med.* 2019;60(6):801-805. doi: 10.2967/jnumed.119.227967

Koerber SA, et al. Novel FAP ligands enable improved imaging contrast in sarcoma patients due to FAPI-PET/CT. *Eur J Nucl Med Mol Imaging.* 2021;48(12):3918-3924. doi:10.1007/s00259-021-05374-4

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. doi: 10.1001/jama.2020.1707

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;9(3):882-893. doi: 10.1002/cam4.2728

Acknowledgements: The investigators and the team at Avacta would like to thank the patients and their families for participating in the trial. Scientific communication support was provided by SlideSource and Random 42.

Presented at the American Association for Cancer Research Annual Meeting, April 2024 San Diego, CA USA