AVA3996, a novel pre|CISION™ medicine, targeted to the tumour microenvironment via Fibroblast Activation Protein-alpha (FAP-α) mediated cleavage

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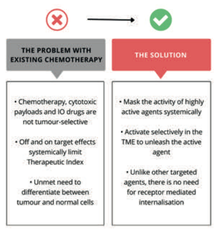
Introduction

- AVA3996 is a therapeutic entity based on Avacta's proprietary pre|CISION™ technology which incorporates a substrate that is sensitive to cleavage by FAP-α
- FAP-α, a post-prolyl endopeptidase, is overexpressed on the surface of activated fibroblastic cells which are abundant in the supporting stroma of over 90% of malignant epithelial cancers.
- The pre|CISION™ substrate can be utilized in a drug conjugate linker or to generate masked warheads that are only activated in the tumour microenvironment.
- AVA3996 consists of a proteasome inhibitor molecule covalently bonded to a peptide containing a cleaving sequence (D-Ala-L-Pro), which is designed to be susceptible to hydrolysis by Fibroblast Activation Protein α (FAP-α) but is resistant to hydrolysis by both closely related and wider mammalian peptidases.
- Proteasome inhibitors are a first line of treatment for certain hematologic indications such as multiple myeloma. However, clinical utility of proteasome inhibitors is limited by severe dose-limiting toxicities, including peripheral neuropathy.
- AVA3996 has the potential to deliver elevated, effective levels of proteasome inhibitor directly to the solid tumour microenvironment while reducing systemic exposure and hence associated toxicities.



FAP mediated hydrolysis of AVA3996 releases free AVA2727D proteasome inhibitor (PI)

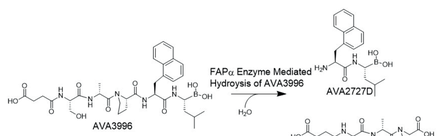


Figure 1a. Structures of AVA3996 and the active warhead AVA2727D which is generated following cleavage by FAP-α

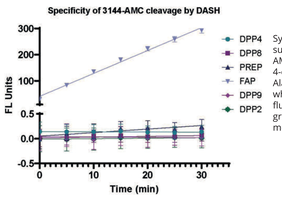


Figure 1b: The linker is exquisitely selective for cleavage by the protease FAP-α and not by closely related proteases.

Cellular proteasome activity is inhibited after AVA3996 cleavage

- In a lysed-cell assay, AVA3996 is cleaved by exogenous FAP-α resulting in release of AVA2727D and subsequent proteasome inhibition.

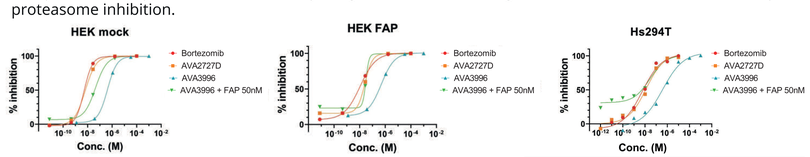


Figure 2. Following cell lysis, proteasome activity is measured by cleavage of an AMC-tagged peptide substrate. Hs294T cells are a melanoma cell line which express very low FAP-α. HEK cells are either transfected with a FAP-α expressing plasmid or with empty vector.

Cell line	Bortezomib	AVA2727D	AVA3996	AVA3996 + FAP
Hs294T	5.6	12.7	373.8	27.31
HEK mock	5.2	6.4	419.6	44.17
HEK FAP	11.0	26.0	492.4	30.8

Table 1. EC_{50} values of proteasome inhibition (nM)

AVA3996 shows greater cell cytotoxicity towards cells which express FAP

- Upon incubation with exogenous FAP-α, the cytotoxicity of AVA3996 increases to show equivalent activity to the active warhead AVA2727D.
- HEK cells which over-express FAP-α (HEK hFAP) are sensitive to AVA3996 in the absence of exogenous FAP-α.

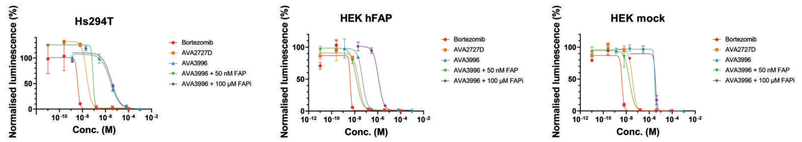


Figure 3 and Table 2. Cell viability (Cell Titer Glo assay) following compound exposure for 48 hours in serum-free conditions.

EC_{50} (nM)	Bortezomib	AVA2727D	AVA3996	AVA3996 + FAP	AVA3996 + FAPi
Hs294T	3.88	23.32	2695	84.16	3283
HEK mock	4.29	34.72	3027	19.2	3482
HEK FAP	4.22	22.75	47.1	14.4	1190

AVA3996 shows greater cell cytotoxicity when cancer cells are co-cultured with fibroblasts

- Stromal regions of cholangiocarcinoma (CCA) typically express high levels of FAP-α
- Co-culture was established with four different CCA cell lines and fibroblast hepatic stellate cells (HSCs); conditions were established for serum-free growth.
- FAP-α in this system is provided by endogenous levels on the HSCs.

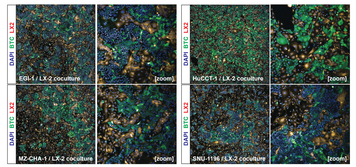


Figure 4. CCA/HSC cocultures.

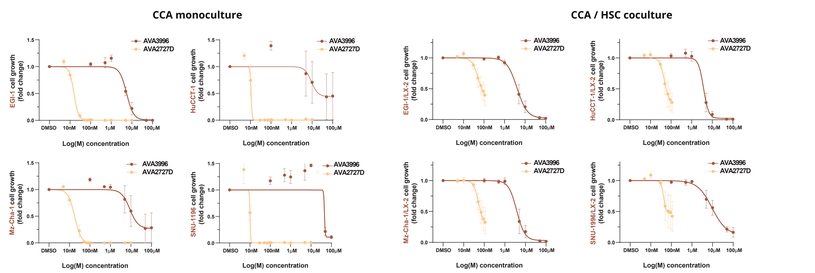


Figure 5 (left) CCA cell death in monoculture; (right) CCA-specific cell death when grown in coculture.

- Activated HSCs contribute to reduced sensitivity of cancer cells to AVA2727D as seen by the increased EC_{50} in coculture vs monoculture (see Table).
- However the presence of the HSCs also enables activation of FAPα-targeted AVA3996 and cell death. This is seen in the reduced EC_{50} of AVA3996 in coculture vs monoculture, and in the narrowing of the distance between AVA2727D and AVA3996 curves.

CCA monoculture

Cell line	AVA3996 EC_{50}	AVA2727 EC_{50}
HuCCT-1 (iCCA)	20.48 μM	10.66 nM
SNU-1196 (pCCA)	46.28 μM	10.11 nM
EGI-1 (dCCA)	5.575 μM	15.58 nM
Mz-Cha-1 (GBC)	12.69 mμM	17.82 nM

CCA / HSC coculture

Cell line	AVA3996 EC_{50}	AVA2727 EC_{50}
HuCCT-1 (iCCA)	3.810 μM	57.75 nM
SNU-1196 (pCCA)	11.68 μM	62.44 nM
EGI-1 (dCCA)	4.165 μM	76.9 nM
Mz-Cha-1 (GBC)	3.855 μM	66.35 nM

AVA3996 shows efficacy in high FAP in vivo PDX models with no toxicity (as observed with Bortezomib)

- Patient-derived xenograft models were selected based on high FAP-α expression and potential sensitivity to proteasome inhibitors.
- Initial studies showed efficacy in three models: melanoma, rhabdomyosarcoma and colorectal (Figure 6).
- In some models (including the melanoma model below), Bortezomib showed toxicity as evidenced by decreased body weight. This was not seen for AVA3996.
- A follow-up study was performed in the melanoma model including standard of care Trametinib (this tumour has a mutated BRAF): significant efficacy was observed for AVA3996 with no toxicity.

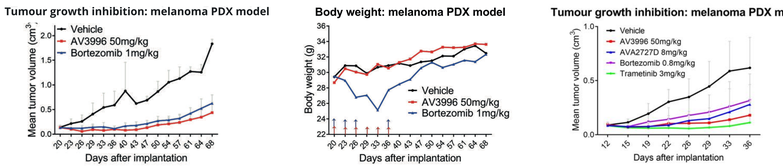
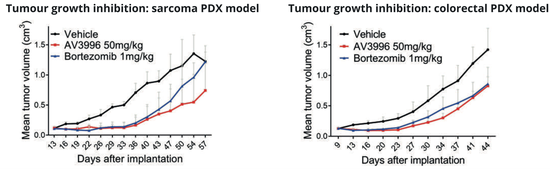


Figure 6. Upper plots show tumour growth in the melanoma model. Bortezomib dosing required a holiday period due to toxicity in the preliminary study: the dose was reduced in the follow-up study. AVA3996, AVA2727D and Bortezomib dosed BIW for 6 doses.
Lower plots show rhabdomyosarcoma and colorectal PDX tumour growth.



Initial pre-clinical safety studies

- Following intravenous administration of 8.3, 25 and 37.5 mg/kg AVA3996 to male and female rats, the following TK parameters were reported. Based on molecular weights 683.56 and 329.19 for AVA3996 and AVA2727D respectively, the equivalent concentration of AVA2727D dosed was 4, 12 and 18 mg/kg

Rat MTD. AVA3996 MTD 37.5mg/kg and was well tolerated at 25mg/kg; AVA2727D MTD 4mg/kg

Dose of AVA3996 administered (mg/kg) [equivalent AVA2727D content]	AVA3996		AVA2727D	
	Cmax (ng/ml)	AUCtlast (ng.h/ml)	Cmax (ng/ml)	AUCtlast (ng.h/ml)
8.3 [4]	18350	2770	117	84
25 [12]	78825	10975	258	237
37.5 [18]	123150	19625	367	394

Summary

- AVA3996 is cleaved by FAP-α with high specificity: no safety issues are apparent with AVA3996 or the active warhead AVA2727D.
- AVA3996 is activated when exposed to cells expressing FAP-α or when exposed to cells in the presence of fibroblasts (which express FAP-α). Activation by FAP-α elicits tumour cell death.
- AVA3996 exhibits tumour growth inhibition in PDX models without the toxicity observed for Bortezomib.
- AVA6000 is the most advanced therapeutic based on Avacta's pre|CISION™ technology, currently in Phase I clinical trials. Further CMC, safety, efficacy and disease positioning studies are ongoing for AVA3996 with the aim to advance this to the clinic.