The Production and Characterisation of Affimer® In-Line Fusion Formats with High Affinity hPD-L1 Blockade and Half-life Extension for Immunotherapy

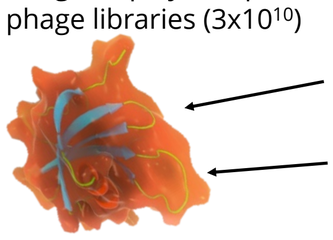
POS033

Wood T., Jenkins E., Adam E., Writer M., D'Avino C., Wall H., Hillman J., Strong L., West M., Chiang I., Wicks A., Sherlock F., Basran A.
Avacta Life Sciences, Cambridge, UK

Introduction

Affimer® Platform Technology

- The Affimer biotherapeutic protein scaffold is based on human Stefin A, a protease inhibitor
- Two binding surface 9aa loops engineered into the scaffold backbone
- Phage display compatible - large Affimer phage libraries ($3x10^{10}$)



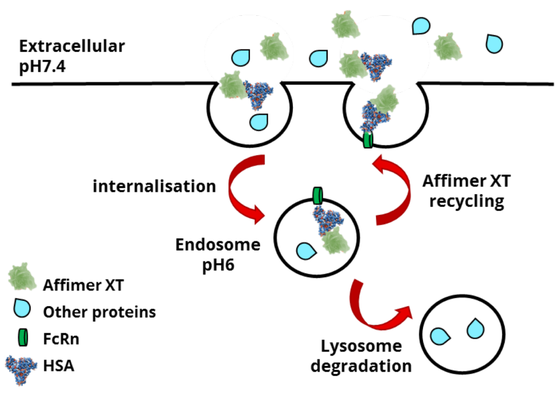
Binding loops: Two randomised x9 amino acid loop regions

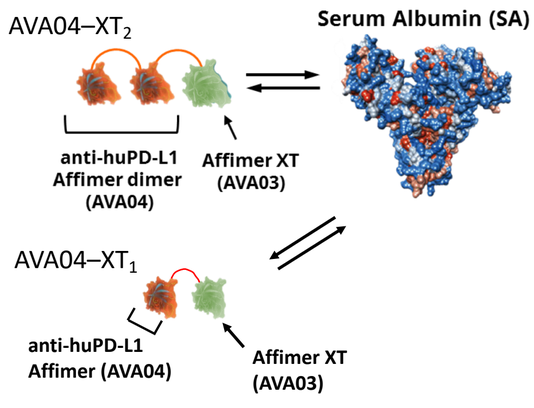
Benefits of Affimer® Therapeutics

- **Small size:** 15 kDa monomer, 1/10th the size of a mAb
- **High expression**: >200 mg/L in shake flasks from *E.coli*
- **Ease of production and formatting:** In-line fusion (ILF) formatting gives the potential to generate multi-specific drugs for blockade of multiple disease pathways with half-life extension

Half-life Extension of hPD-L1 Antagonists

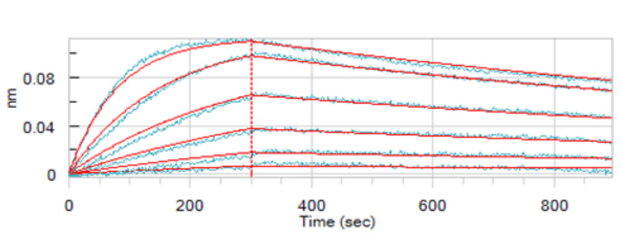
- Human programmed death-ligand 1 (hPD-L1) plays an important role in down-regulating the immune system allowing tumour cells to evade destruction and metastasize. This project aims to discover Affimer proteins that block hPD-1/hPD-L1 binding, preventing immune checkpoint inhibition and so reactivating T cells
- An antagonist that specifically binds hPD-L1 with nanomolar affinity was identified following phage selections (AVA04)
- The Lead Affimer protein was formatted as in-line fusions (ILF) genetically fused to a human serum albumin (HSA) binding Affimer (XT) in order to extend the half-life *in vivo* via the FcRn recycling pathway. AVA04-XT_1 and AVA04-XT_2 schematics are represented below

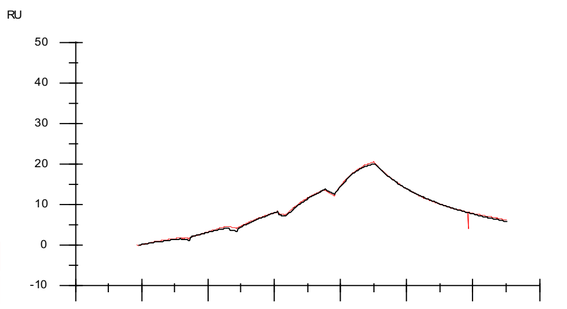




- *Schematic representation of the FcRn recycling of a serum albumin binding Affimer (AVA03) monomer protein*
- *Schematic representation of ILF AVA04-XT_1 and AVA04-XT_2 formatted with one AVA03-42 Affimer used for half-life extension*

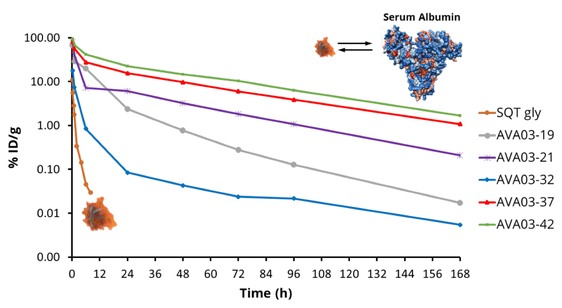
Monomer Affimer Characterisation





Affimer	k_a (1/Ms)	k_d (1/s)	K_D (M)	Chi² (RU^2)
Biacore - AVA04	2.58E+06	1.40E-03	5.40E-10	0.0681
Octet - AVA04	5.95E+05	5.86E-04	9.84E-10	0.9951

- PD-L1 binding by AVA04 monomer was shown to have similar K_D(M) in the sub-nanomolar range using Octet and Biacore

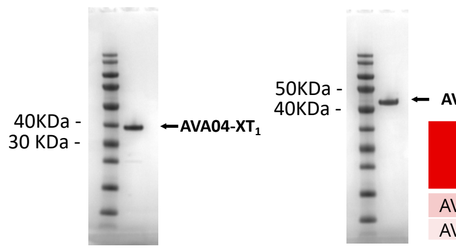


Mouse PK study with AVA03 clones		
Affimer	**$T_{1/2}$ (h)**	**AUC 0-t h*µg/mL**
AVA03-42	38.2	5,670
AVA03-37	37.7	3,435
AVA03-21	30.6	1,401
AVA03-19	24.3	1,059
AVA03-32	29.0	112
SQT-Gly	1.6	18.1

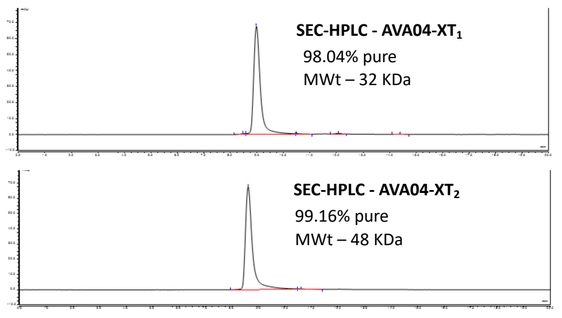
- Anti-serum albumin Affimer proteins were radiolabelled using I-125 and dosed at 10 mg/kg as a bolus IV injection, 3 mice per time point
- Serum concentration of Affimer proteins was determined over 7 days by measurement of radioactivity
- All Affimer proteins tested were well tolerated *in vivo*
- AVA03-42 Affimer monomer protein was determined to be the longest half-life extension clone for ILF formatting

ILF XT - Expression and Purification

SDS-PAGE following 2 stage purification - NiNTA & preparative SEC

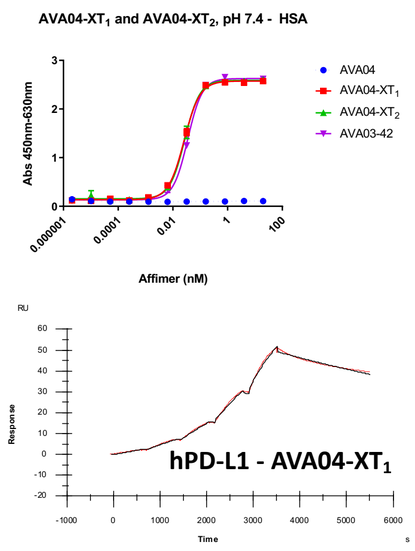


ILF	NiNTA Yield /L
AVA04-XT_1	342 mg
AVA04-XT_2	332 mg



- Eluted protein was buffer exchanged into PBS and purified with a preparative SEC 26/600 200pg column. The final protein batch purity was analysed by SEC-HPLC using an Acclaim-SEC 300 column (Thermo). High purity of AVA04-XT_1 and AVA04-XT_2 following a two stage purification strategy

ILF XT - ELISA HSA & hPD-L1 SPR



- ELISA results confirm the binding at both pH 7.4 and pH 6.0 for both XT ILFs which is comparable to AVA03-42 monomer.
- This recreates the conditions seen throughout the FcRn recycling pathway
- Protein A capture of hPD-L1-Fc antigen (R&D Systems), Affimers run in solution, analysed using single cycle kinetics, data blank subtracted and fitted to a 1:1 binding model

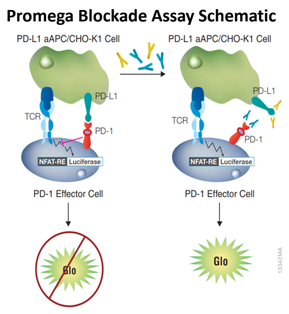
ILF AVA04-XT_1 & AVA04-XT_2 Binding to hPD-L1 by SPR

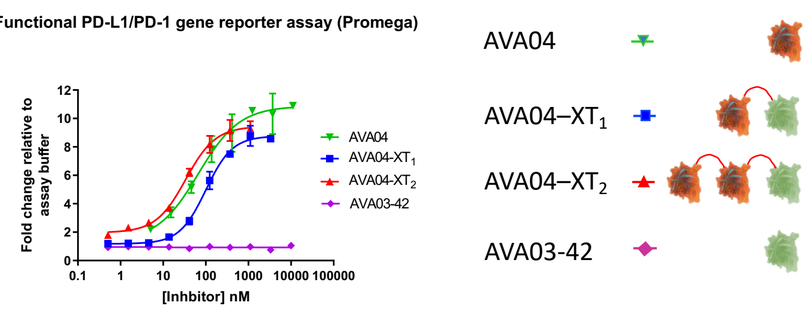
Affimer	ka (1/Ms)	kd (1/s)	K_D (M)	Chi² (RU^2)
AVA04-XT_1	4.57E+05	1.30E-04	**2.84E-10**	0.264
AVA04-XT_2	1.41E+07	4.23E-05	**3.01E-12**	0.584

- No change is seen with binding to target proteins after ILF formatting AVA04-XT_1 or XT_2
- Increased avidity to hPD-L1 is seen with the AVA04-XT_2 format

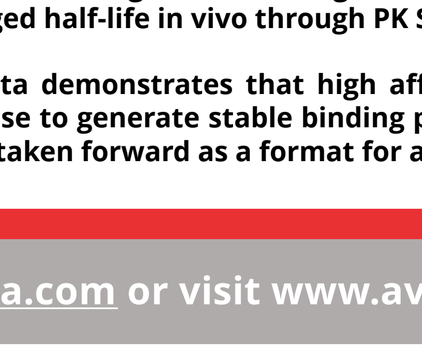
ILF XT Promega cell based assay

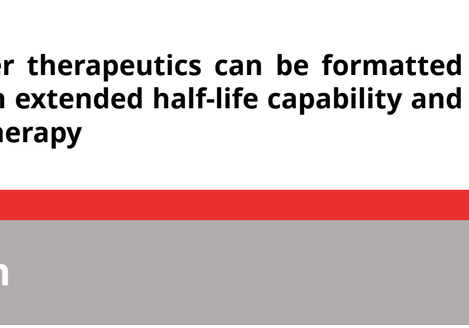
- Promega cell based blockade assay confirms the AVA04-XT_1 and AVA04-XT_2 can block the hPD-L1/PD-1 interaction on cells as well as the AVA04 monomer protein





Half-life Extended Formats - Mouse PK





- Mice dosed intravenously with 5mg/kg of Affimer and ILF XT formats. Affimer in serum samples were detected by ELISA at multiple time points. PD-L1 ILF XT Affimer formats demonstrate half-life extension in PK mouse study with AVA04-XT_1 and AVA04-XT_2 half-life being over 28 hours (*Note: non-specific Affimer SQT gly control data taken from previous PK study)

Conclusions

- **Half-life extended ILF Affimer proteins generate novel formats which were shown to maintain binding to their targets, activity in hPD-L1/PD-1 blockade assay and prolonged half-life in vivo through PK Studies**
- **This data demonstrates that high affinity Affimer therapeutics can be formatted with ease to generate stable binding proteins with extended half-life capability and can be taken forward as a format for an immunotherapy**