Half-life Extension Formatting of Human PD-L1 Antagonist Therapeutic Affimers

POS032

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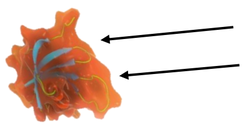
Introduction

Affimer Platform Technology

- The Affimer biotherapeutic protein scaffold is based on human Stefin A protease inhibitor
- Two binding surface loops engineered into the scaffold backbone
- Phage display compatible - large Affimer phage libraries ($1x10^{11}$)



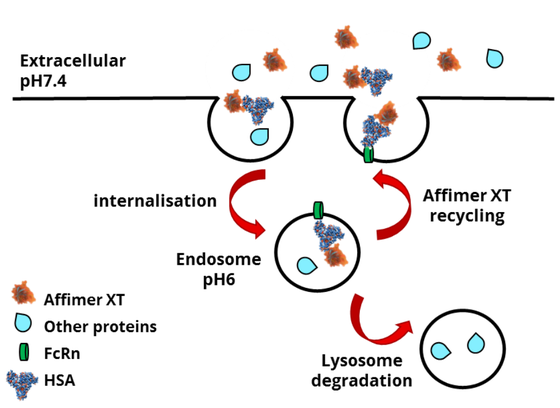
Binding loops: Two randomised x9 amino acid loop regions

Benefits of Affimer Therapeutics

- **Small size:** 14 kDa monomer, $1/10^{th}$ the size of a mAb
- **High expression**: >100 mg/L in flasks
- **Ease of production, manufacturing and improved stability**
- **Ease of formatting:** Fc fusion and in-line fusion (ILF) formatting, potential to generate multi-specific drugs for blockade of multiple disease pathways with half-life extension

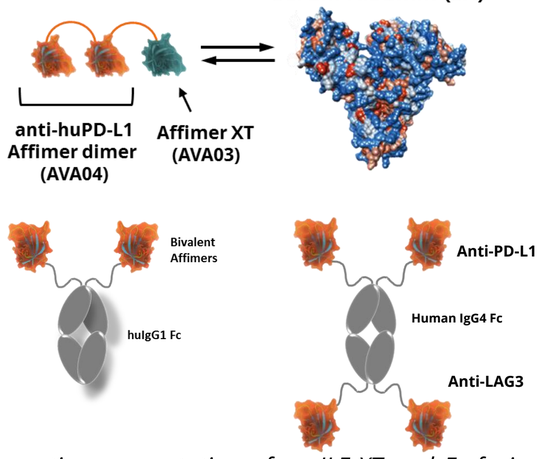
Half-life Extension of huPD-L1 Antagonists

- Human programmed death-ligand 1 (huPD-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 huPD-1/huPD-L1 binding, preventing immune checkpoint inhibition and so reactivating T cells
- Antagonists that specifically bind huPD-L1 with a range of nM affinities were identified following phage selections
- Lead Affimer proteins were genetically fused to hIgG-Fc or formatted as in-line fusion (ILF) molecules fused to human serum albumin binding Affimers (XT) in order to extend the half-life via the FcRn recycling pathway



Schematic representation of the FcRn recycling of a serum albumin binding Affimer (AVA03) monomer protein

Serum Albumin (SA)

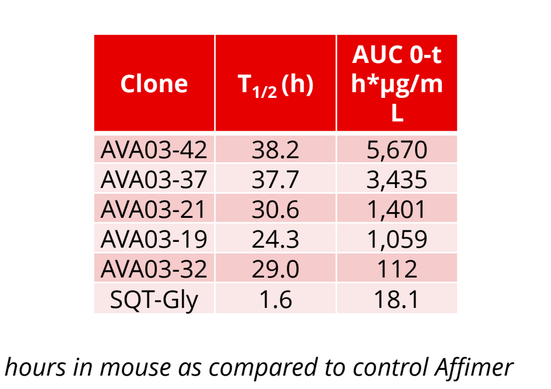


Schematic representation of an ILF XT and Fc fusion formatted Affimer used for half-life extension

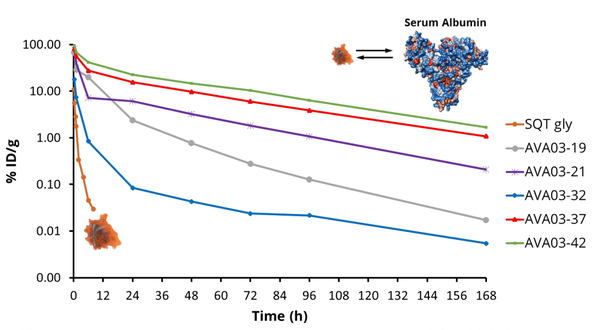
XT Monomer Affimers Characterisation

Ligand	MSA		CSA		huSA	
pH	pH 6.0	pH 7.4	pH 6.0	pH 7.4	pH 6.0	pH 7.4
Affimer	Affinity K_D (nM)					
AVA03-19	618	981	68.7	107	14.9	36.1
AVA03-21	511	622	133	213	23.4	40.5
AVA03-32	3.7	3.3	18.5	83	21.1	15.3
AVA03-37	435	244	1140	2600	132	136
AVA03-42	95.2	47.9	105	71.5	7.1	5.2

AVA03 XT Affimer species cross reactivity demonstrated by kinetic analysis using BioLayer Interferometry (Octet) for MSA, CSA and HSA at both pH6 & 7.4

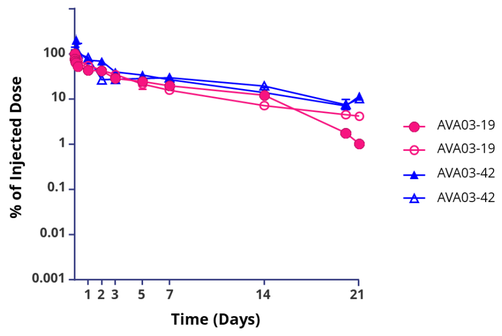


Cross-reactivity of clone AVA03-42 assessed by ELISA



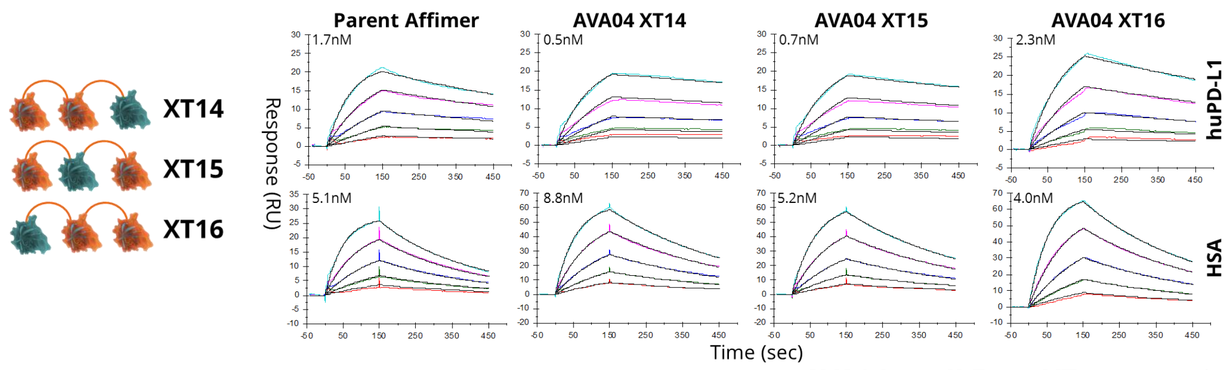
Clone	$T_{1/2}$ (h)	AUC 0-t h*µg/mL
AVA03-42	38.2	5,670
AVA03-37	37.7	3,435
AVA03-21	30.6	1,401
AVA03-19	24.3	1,059
AVA03-32	29.0	112
SQT-Gly	1.6	18.1

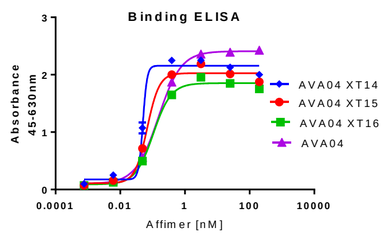
Affimer XT monomers showed increased half-life up to 38.2 hours in mouse as compared to control Affimer

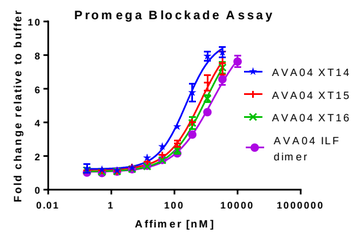


- XT Monomer Affimers 19 and 42 were tested in cynomolgus to generate PK parameters
- XT Affimer 19 binding affinity is ~70x lower for MSA but similar for HSA and CSA
- Cyno PK half-life of XT Affimer 42 was ~7.3 days

ILF XT and Fc Fusion Characterisation

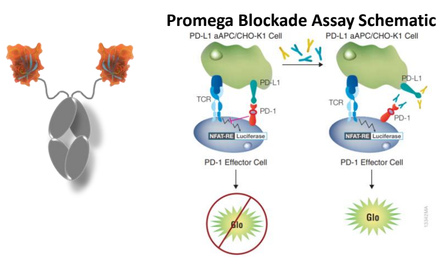


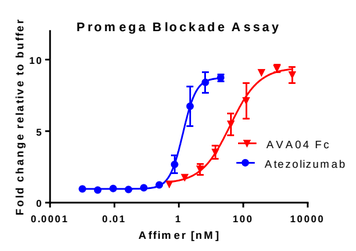


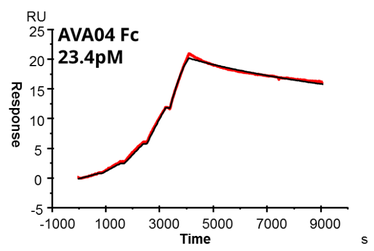


AVA04 ILF XT proteins demonstrate binding to both recombinant target proteins: HSA and huPD-L1 by Biacore and ELISA. Promega cell based blockade assay confirms the AVA04 ILF XT can block the huPD-L1/PD-1 interaction on cells

AVA04 Fc has an affinity of 23.4pM as determined by SPR on Biacore using single cycle kinetic analysis. Promega cell based blockade assay confirms the AVA04 Fc fusion format can block the huPD-L1/PD-1 interaction on cells.



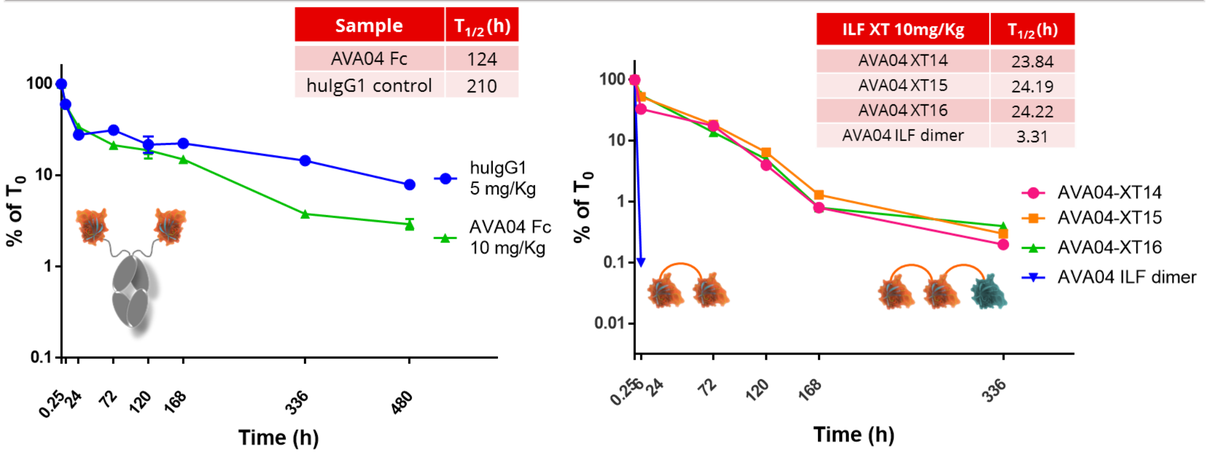




Mouse PK of Half-life Extended Formats

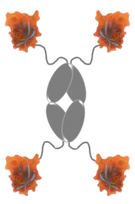
Sample	$T_{1/2}$ (h)
AVA04 Fc	124
huIgG1 control	210

ILF XT 10mg/Kg	$T_{1/2}$ (h)
AVA04 XT14	23.84
AVA04 XT15	24.19
AVA04 XT16	24.22
AVA04 ILF dimer	3.31

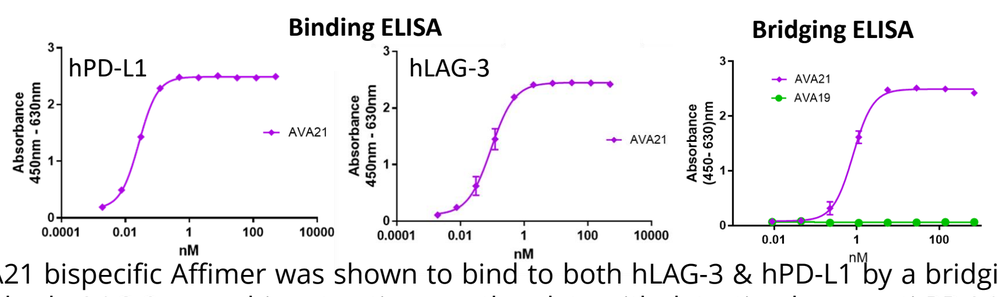


- Mice dosed intravenously with 5mg/kg or 10mg/kg Affimer Fc or 10mg/kg of ILF XT formats. Affimers in serum samples were detected by ELISA at multiple time points. ILF XT and Fc fusion lead AVA04 Affimer formats demonstrate increased half-life PK in mouse

hPD-L1/hLAG-3 Bispecific Affimer format



- Lymphocyte Activation Gene-3 (LAG-3) is expressed on activated T cells, NK cells, DCs and B cells. Both the PD-1/PD-L1 and the LAG-3 pathways are involved in immune suppression, allowing tumor escape and metastasis
- Despite the success of PD-1/PD-L1 targeted immunotherapy, a large proportion of patients fail to respond to treatment. Targeting of both pathways as a combination may lead to more durable antitumor responses



AVA21 bispecific Affimer was shown to bind to both hLAG-3 & hPD-L1 by a bridging ELISA. Method – LAG-3 recombinant antigen on the plate with detection by an anti-PD-L1 mAb

Conclusions

- **Half-life extended Affimer proteins generated novel formats which were shown to maintain binding to their targets, activity in huPD-L1/PD-1 blockade assay, and prolonged half life in vivo**
- **Bispecific hPD-L1/hLAG-3 format showed simultaneous target engagement as determined by bridging ELISA**
- **This data demonstrates that high affinity Affimer therapeutics can be formatted with ease to generate stable binding proteins with biotherapeutic properties to build bispecific molecules or to extend the half-life.**

For further information please contact affimers@avacta.com or visit www.avacta.com

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