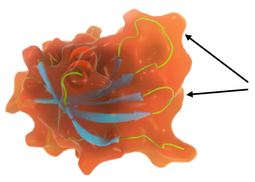
Fc Fusion Affimer® Biotherapeutics: Generation of Potent Human and Mouse PD-L1 Antagonists

POS010

Introduction

Affimer Technology

- The Affimer biotherapeutic protein scaffold is based on human Stefin A
- Two surface loops engineered into the scaffold backbone



Binding loops: Two randomised 9 amino acid loop regions

- Phage display compatible - Large Affimer phage libraries ($1x10^{11}$)

Benefits of Affimer Therapeutics

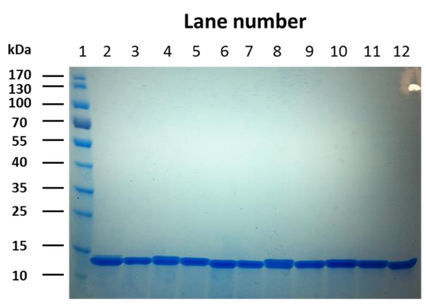
- **Small size:** 14 kDa, $1/10^{th}$ the size of an antibody
- **High expression**: 100's mg/L in flasks (*E. coli*)
- **No post translational modifications**: ease of manufacturing and improved stability
- **Improved tissue penetration:** small size gives greater potential for increased efficacy
- **Ease of formatting:** Fc format and in-line fusions, potential to generate multi-specific drugs to blockade multiple disease pathways

Targeting the Immune Checkpoint PD-L1

- Programmed death-ligand 1 (PD-L1) is clinically validated in oncology and shown to play an important role in down regulating the immune system allowing tumour cells to evade detection and metastasize
- Affimer inhibitors with low single digit nM affinity were identified to either human or mouse PD-L1 (AVA04-182) using phage display.

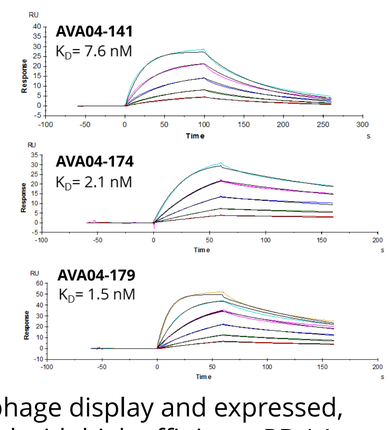
Anti-PD-L1 Affimer Programme (AVA04): Lead Identification

AVA04 Affimer SDS-PAGE Analysis



Lane	Sample	Purified Yield (mg/L culture)
1	Markers	na
2	AVA04-141	300
3	AVA04-142	265
4	AVA04-144	340
5	AVA04-145	323
6	AVA04-146	295
7	AVA04-147	264
8	AVA04-148	231
9	AVA04-149	220
10	AVA04-150	239
11	AVA04-151	44
12	SQT (2 ug)	370

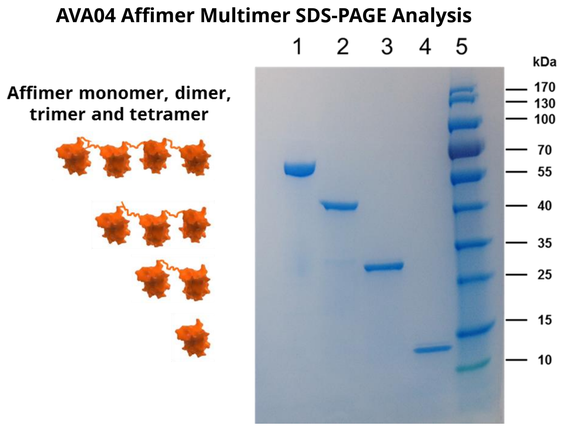
AVA04 Affimer Biacore



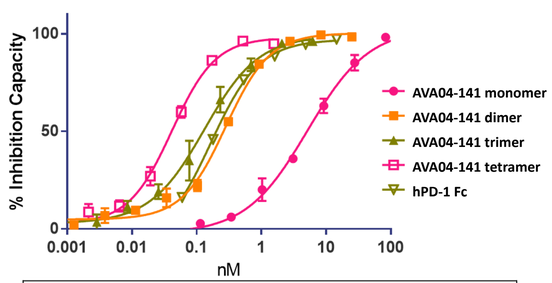
Lead Affimer proteins against human PD-L1 were identified by phage display and expressed, purified from *E. coli.* Over 60 unique Affimer sequences identified with high affinity to PD-L1 as determined by Biacore.

Affimer Formatting : Multimers

AVA04 Affimer Multimer SDS-PAGE Analysis



AVA04-141 Multimers Competition ELISA

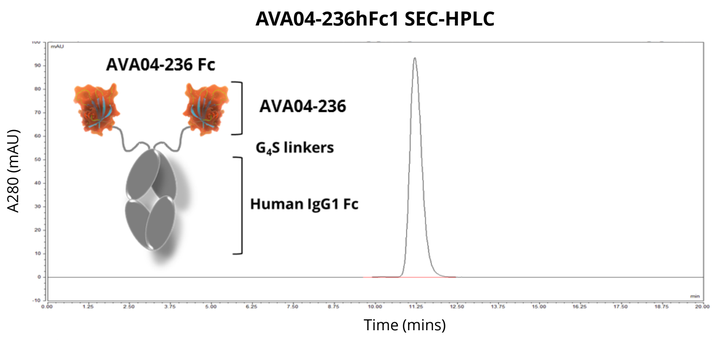


IC_{50} (nM)				
hPD-1 Fc	AVA04-141 monomer	AVA04-141 dimer	AVA04-141 trimer	AVA04-141 tetramer
0.17	5.2	0.28	0.13	0.04

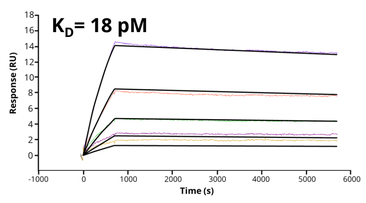
Affimer proteins can be formatted as multimers and expressed in *E. coli* to generate highly potent molecules. Unoptimised yields of purified Affimer multimers from *E. coli* >100mg/L in shake flasks.

Affimer Formatting : Fc Fusions

AVA04-236hFc1 SEC-HPLC



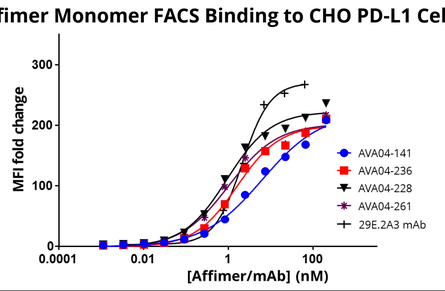
AVA04-236hFc1 Biacore Kinetics



Binder	k_a (1/Ms)	k_d (1/s)	K_D (M)	Chi² (RU^2)
AVA04-236hFc1	9.74E+05	1.73E-05	1.78E-11	0.161

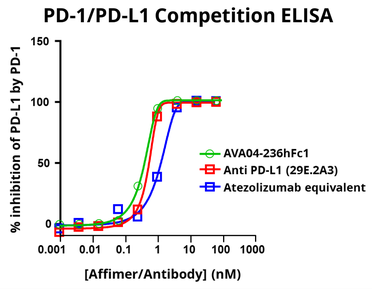
Affimer proteins can be formatted as Fc fusions to add effector function, half-life extension and enhanced affinity. AVA04-236hFc1 gives purified yields of ~200mg/L from transient Expi293F cells and shows similar affinity in the PD-1/PD-L1 competition ELISA to an Atezolizumab surrogate

Affimer Monomer FACS Binding to CHO PD-L1 Cells



EC_{50} (nM)				
AVA04-141	AVA04-236	AVA04-228	AVA04-261	29E.2A3
6.2	1.6	0.93	1.0	2.3

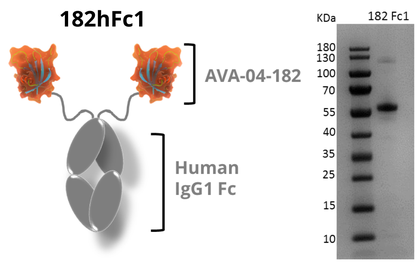
PD-1/PD-L1 Competition ELISA



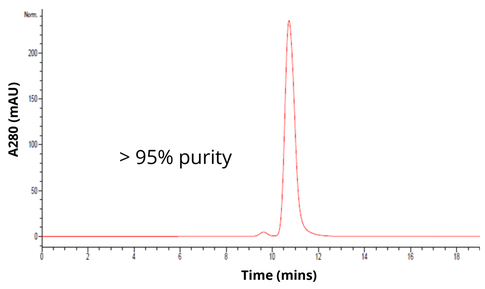
IC_{50} (nM)		
AVA04-236Fc1	29E.2A3	"Atezolizumab"
0.36	0.53	1.24

AVA04-182 Formatting and Characterisation

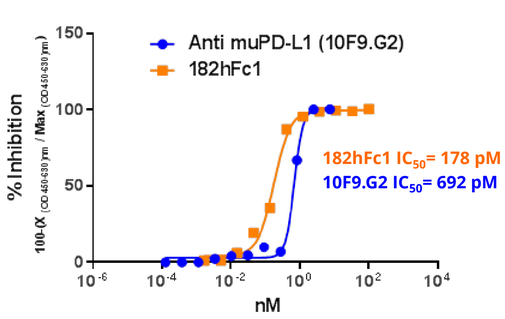
182hFc1



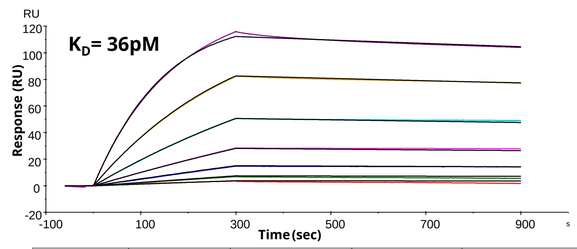
182hFc1 SEC-HPLC



182hFc1 PD-1/PD-L1 competitive ELISA



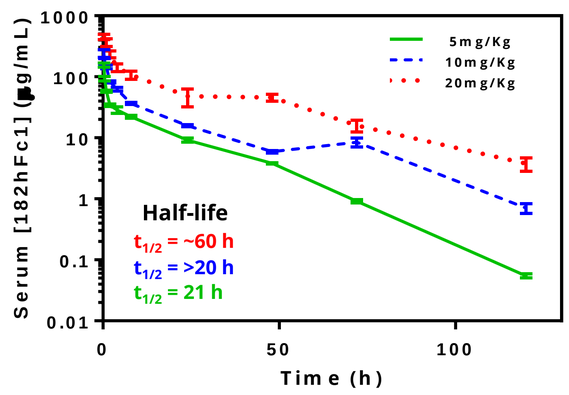
182hFc1 Biacore Kinetics



Binder	k_a (1/Ms)	k_d (1/s)	K_D (M)	Chi² (RU^2)
182hFc1	3.39E+6	1.23E-4	3.6E-11	0.558

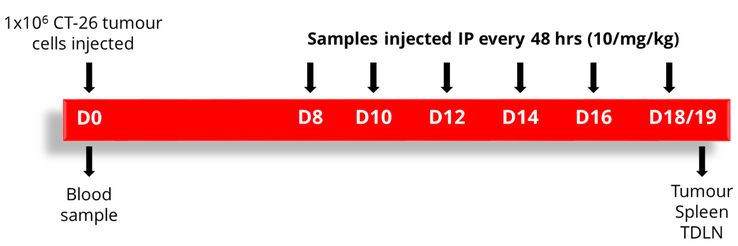
AVA04-182 only binds mouse PD-L1 and was formatted as an Fc fusion (182hFc1) for PK and efficacy models. Expression from Expi293 gave purified yields >100mg/L. 182hFc1 competes against PD-1 for binding to mouse PD-L1 (IC_{50} = 178 pM) and has a K_D of ~40 pM as determined by Biacore.

Pharmacokinetics of 182hFc1 in Mice

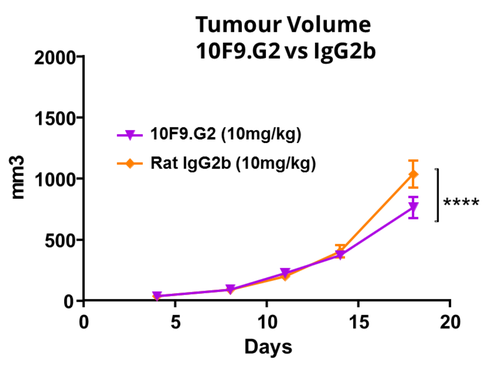


182hFc1 dosed as a bolus IV injection in 3 mice/time point. PK followed for 7 days and serum levels of 182hFc1 determined by binding ELISA. Affimer protein was well tolerated *in vivo* at all doses.

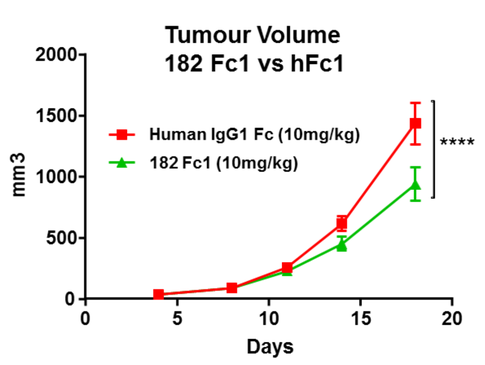
Efficacy of 182hFc1 in a CT26 Mouse Syngeneic Model



Tumour Volume 10F9.G2 vs IgG2b



Tumour Volume 182 Fc1 vs hFc1



Balb/c mice received CT-26 tumour cells ($1x10^6$) subcutaneously at D0. D8 to D18 182hFc1, mAb 10F9.G2 (rat anti-mouse PD-L1), hIgG1 Fc control and rat IgG2b isotype control were injected i.p. at 10 mg/kg every other day. 182hFc1 showed statistically significant inhibition of tumour growth compared to the hIgG1 Fc (hFc1) control.

Conclusions

- **Affimer biotherapeutics can be identified using phage display and easily produced at high levels in bacterial and mammalian expression systems**
- **The human PD-L1 Affimer antagonists can be formatted in a variety of ways to generate high affinity molecules as determined by Biacore and PD-1/PD-L1 competitive ELISA**
- **182hFc1 was shown to be well tolerated in mice, even with repeat dosing at 10 mg/kg in the syngeneic model**
- **182hFc1 inhibited tumour growth in the tumour of a CT-26 syngeneic model**
- **This work demonstrates that the Affimer technology has the necessary properties for a therapeutic platform: generation of high affinity binding proteins that can be formatted to extend the serum half-life and blockade a biologically relevant disease pathway *in vivo***

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