Isolation of Affimer Therapeutics with Nanomolar Affinity to Human PD-L1 from a Synthetic Phage Display Library

Affimer®

POS015

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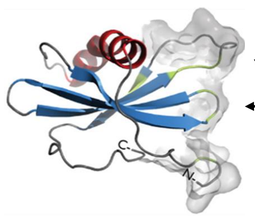
Introduction

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 for blockade of multiple diseases pathways

PD-L1 as a Therapeutic Target

- 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
- This project aims to discover Affimer proteins that block hPD-1/hPD-L1 binding and activate T-cells, preventing checkpoint inhibition

Discovery Process

Synthetic Library
- Affimer library with diversity of 10^{11}
- 9 amino acids in two loops with equal diversity at each position (no Cys, Met or Stop codons)
- Loop regions made from trinucleotide blocks

Phage Display
- Solution selections performed using biotinylated hPD-L1
- Enrichment after each round monitored and confirmed by polyclonal ELISA

Primary Screening
- 2 rounds delivered 20 % hit rate to hPD-L1 and 38 unique sequences
- 3 rounds showed 70 % hit rate and increased panel to 64 unique clones

Protein Production
- Binding clones were subcloned into expression vector and purified using Ni-NTA resin

Characterisation
- Affimer proteins were characterized by SEC-HPLC, Biacore, binding & competitive ELISA and cell based blockade assay

Biacore® SPR Analysis

Figure 1. Biacore® kinetic analysis for AVA04-228 and AVA04-261 binding to human PD-L1

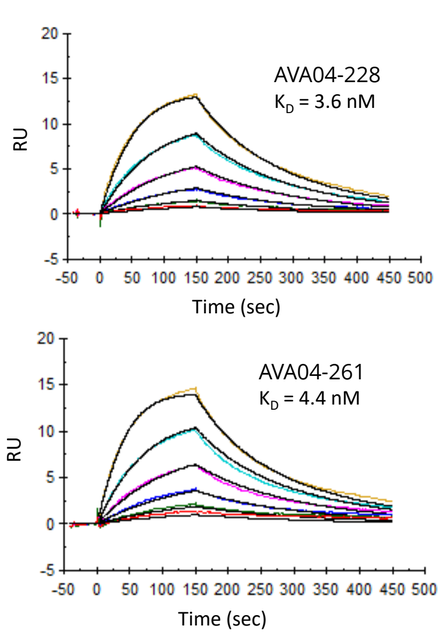


Table 1. Biacore® kinetic analysis for top Affimer proteins binding to human PD-L1

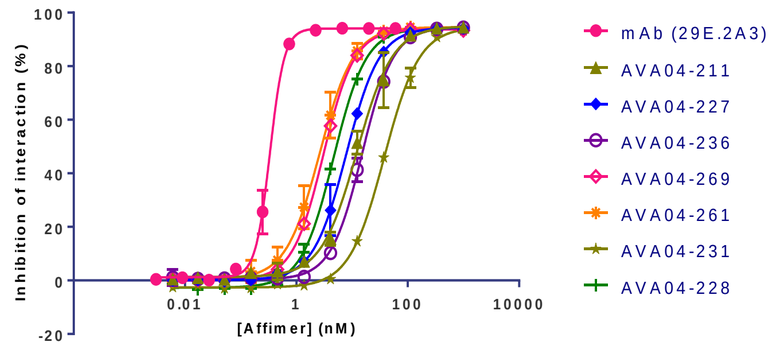
Clone ID	k_a (1/Ms)	k_d (1/s)	Apparent K_D (nM)	Chi^2 (RU^2)
AVA04-228	2.74×10^6	9.78×10^{-3}	3.57	0.0243
AVA04-269	1.43×10^6	5.27×10^{-3}	3.68	0.0249
AVA04-261	2.74×10^6	1.20×10^{-2}	4.38	0.0676
AVA04-227	1.79×10^6	1.48×10^{-2}	6.10	0.0264
AVA04-236	8.69×10^5	9.12×10^{-3}	10.5	0.0141
AVA04-231	1.27×10^7	1.40×10^{-1}	11.0	0.0441
AVA04-211	1.58×10^6	1.94×10^{-2}	12.3	0.148

- Amine coupling of hPD-L1 on a CM5 sensor chip, binding of Affimer analyte to hPD-L1 was measured using a T200 Biacore (GE Healthcare)

- **Top Affimer proteins demonstrate a K_D between 3.6 nM and 12 nM to immobilized hPD-L1 target antigen**

Competitive ELISA hPD-1/hPD-L1

Figure 2. Inhibition of human PD-1 / PD-L1 interaction by competitive ELISA

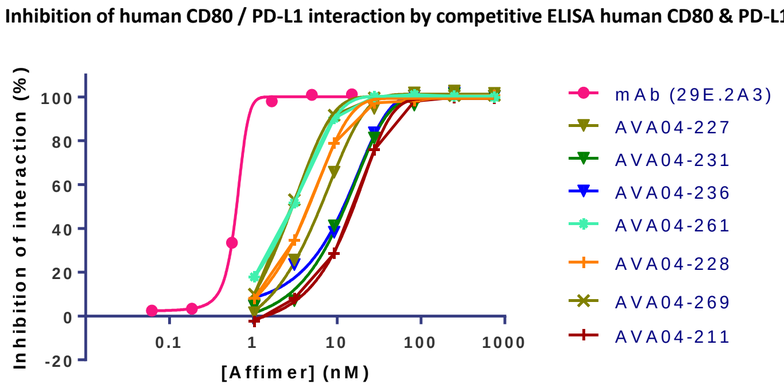


Clone ID	IC_{50} (nM)
AVA04-269	2.58
AVA04-228	2.77
AVA04-261	3.09
AVA04-211	3.89
AVA04-227	5.26
AVA04-231	11.24
AVA04-236	14.06
mAb 29E.2A3	0.12

- **All Affimer proteins tested blocked the interaction between hPD-1/hPD-L1 in a competitive ELISA**

Competitive ELISA hCD80/hPD-L1

Figure 3. . Inhibition of human CD80 / PD-L1 interaction by competitive ELISA human CD80 & PD-L1



Clone ID	IC_{50} (nM)
AVA04-269	2.86
AVA04-261	3.35
AVA04-228	4.5
AVA04-227	5.79
AVA04-231	12.00
AVA04-236	13.13
AVA04-211	14.79
mAb 29E.2A3	0.68

- **All Affimer proteins tested blocked the interaction between hCD80/hPD-L1 in a competitive ELISA**

Promega Blockade Cell Based Assay

- Potency of the selected / unformatted Affimer proteins was determined in a Promega hPD-1/hPD-L1 blockade cell based assay which measured the T-cell signalling through NFAT-mediated luciferase activity

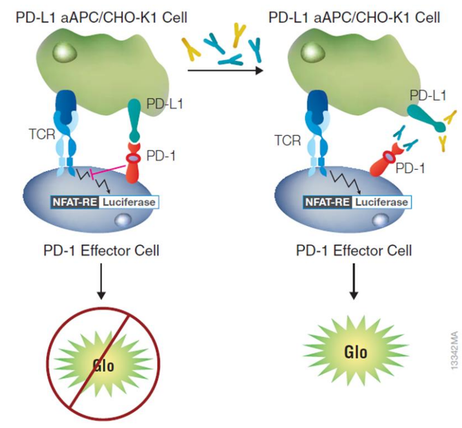
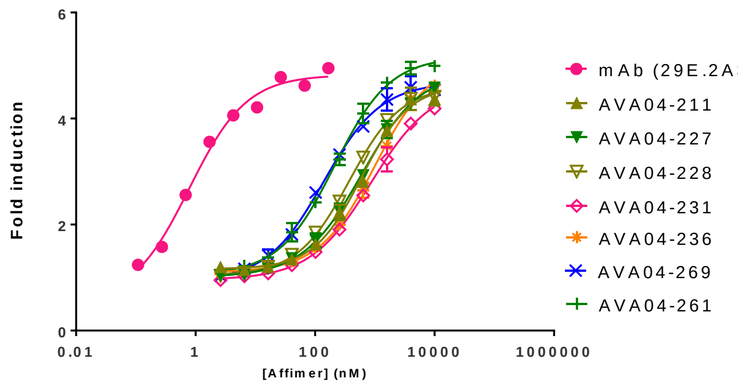


Figure 4. Effect of Top Affimer proteins in the Promega PD-1 / PD-L1 blockade assay



Clone ID	IC_{50} (nM)
AVA04-269	170.1
AVA04-261	430.6
AVA04-231	804.3
AVA04-228	933.4
AVA04-227	1283
AVA04-211	1366
AVA04-236	1402
mAb 29E.2A3	0.845

- **All Affimer proteins blocked the interaction between hPD-1 to hPD-L1 in the Promega blockade cell based assay**

Conclusions

- **Two rounds of phage display selections delivered 20 % hit rate resulting in 38 unique sequences. Round three increased the panel to 64 unique sequences and showed 70 % hit rate**
- **Top Affimer proteins block the interaction between hPD-1 / hPD-L1 in ELISA and in a Promega cell based assay, and between hCD80 / hPD-L1 in competitive ELISA**
- **Avacta's technology shows great potential for the isolation of high affinity Affimers proteins with therapeutic applications.**
- **Further formatting can improve affinity and potency of Affimer therapeutics**