Development and Characterisation of a LAG-3/ PD-L1 Bispecific Affimer® Biotherapeutic

POS034

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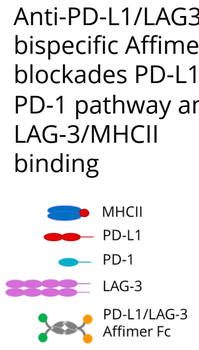
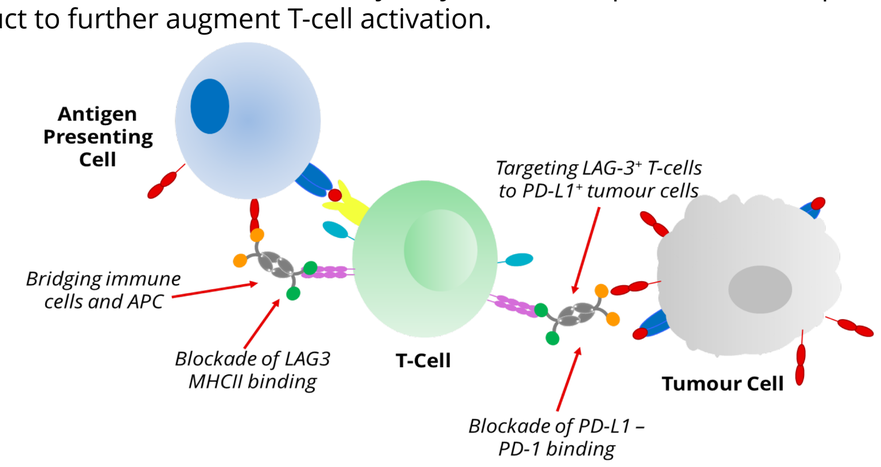
Introduction

The combination of PD-L1 & LAG-3 blockade was shown to be superior compared to a single agent in a cell-based assay using either anti-PD-L1 or anti-LAG-3. Achieving the full potential of PD-L1/LAG-3 dual blockade would justify the development of a bispecific biotherapeutic product to further augment T-cell activation.

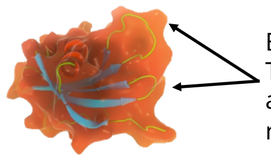
Anti-PD-L1/LAG3 bispecific Affimer blockades PD-L1/ PD-1 pathway and LAG-3/MHCII binding





Affimer® Therapeutic Technology

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

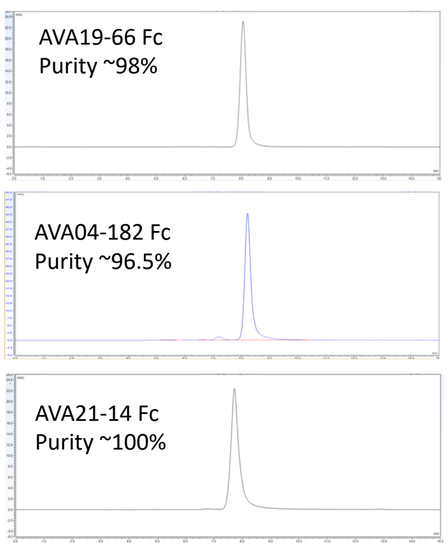


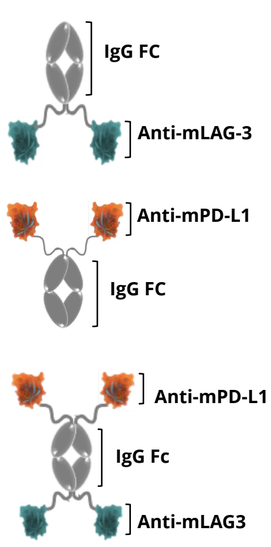
Binding loops: Two randomised 9 amino acid loop regions

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

- **Small size:** 14 kDa, $1/10^{th}$ the size of an antibody.
- **High expression**: >200 mg/L in flasks.
- **No post translational modifications**: ease of manufacturing and improved stability.
- **Ease of formatting:** Fc format and in-line fusions, potential to generate multi-specific drugs to blockade multiple disease pathways.
- **Tissue penetration:** small size gives greater potential of tissue penetration for increased efficacy.

Affimer® Protein Formatting and Production



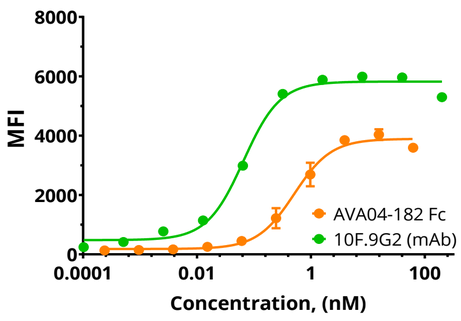


Anti-mLAG-3 and Anti-mPD-L1 Affimer proteins were formatted as Fc fusions, transiently expressed in Expi293F (HEK293) cells and purified by protein A (Pr-A) affinity chromatography and Size Exclusion Chromatography (SEC-HPLC), resulting in a high quality Affimer protein product.

To investigate the anti-tumour potential of bispecific Affimer therapeutics in a mouse model, we generated a surrogate Affimer protein. We have identified the clone AVA04-182 which was formatted to a Fc portion of human IgG1 (AVA04-182 Fc). AVA04-182 was characterized by Biacore® and flow cytometry in a cell binding assay. Competitive binding to mPD-L1 was assessed by ELISA and the Affimer tested *in vivo* for anti-tumour activity. Potent Affimers targeting mLAG-3 with affinities of nM to pM when Fc formatted were identified. mLAG-3 binding Affimers were characterised by competitive ELISA and in a cell-based assay, both as single agents or in combination with a PD-L1 antagonist. Bispecific PD-L1/LAG-3 antagonist Affimers formatted as Fc fusions bound to both targets simultaneously and demonstrated very high potency in a cell-based assay. The results substantiate the evaluation of this surrogate bispecific Affimer Fc in a CT-26 syngeneic model to demonstrate efficacy.

Affimer® Therapeutic Targeting mPD-L1

Binding on mPD-L1 LK35.2

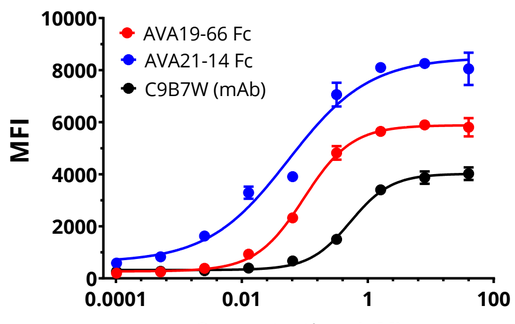


Clone/Format	EC_{50} (nM)
AVA04-182 Fc	0.51
10F.9G2 (mAb)	0.066

Anti-mPD-L1 Affimer AVA04-182 Fc binds to mPD-L1 overexpressing LK35.2 cells.

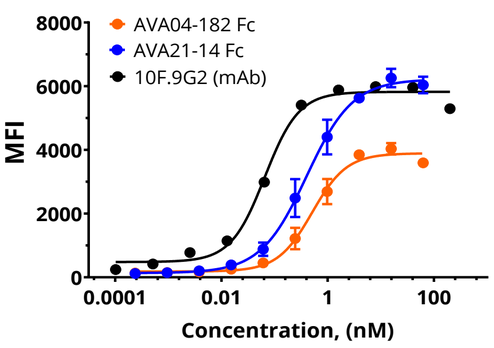
An Affimer Bispecific Targeting mPD-L1/mLAG-3

A. Binding on mLAG-3 DO-11-10 Cells



Clone/Format	EC_{50} (nM)
AVA19-66 Fc	0.097
AVA21-14 Fc (Bispecific)	0.055
10F.9G2 (mAb)	0.53

B. Binding on mPD-L1 LK35.2 Cells

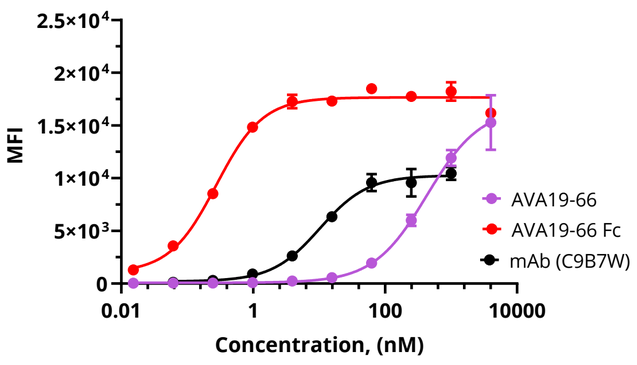


Clone/Format	EC_{50} (nM)
AVA04-182 Fc	0.51
AVA21-14 Fc (Bispecific)	0.40
10F.9G2 (mAb)	0.066

Bispecific anti-PD-L1/LAG-3 Affimer binds to mLAG-3 DO-11-10 (A) or mPD-L1 Lk35.2 cells (B) with affinities similar to the individual Fc fusion dimeric forms, demonstrating that the formatting has not negatively impacted Affimer affinity to the individual targets.

Affimer® Therapeutic Targeting mLAG-3

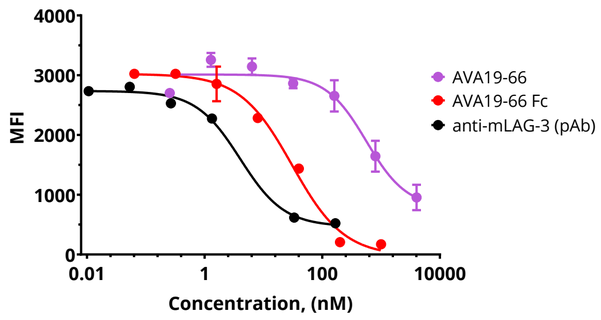
Binding to mLAG-3 overexpressing cells



Clone/Format	EC_{50} (nM)
AVA19-66	432*
AVA19-66 Fc	0.28
C9B7W (mAb)	10.24

Anti-mLAG-3 Affimer AVA19-66 binds to mLAG-3 overexpressing DO-11-10 Cells. Formatting as an Fc fusion protein significantly increases the binding affinity to mLAG-3 on the cell surface demonstrating that this Affimer is suitable for an Fc formatted bispecific.

mLag-3 / MHCII Competition Cell Binding

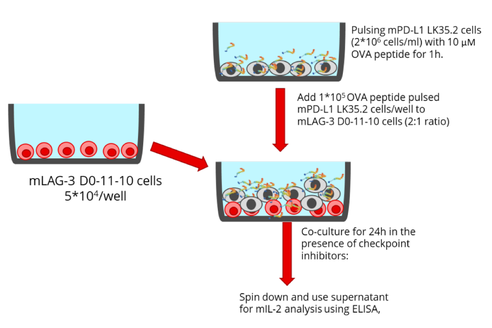


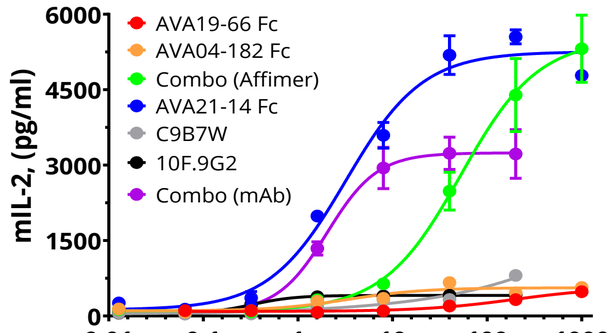
Clone/Format	IC_{50} (nM)
AVA19-66	560*
AVA19-66 Fc	30.7
Anti-mLAG-3 (pAb)	4.1

AVA19-66 inhibits the binding of mLAG-3 Fc to MHCII on the surface of LK35.2 cells. Formatting as an Fc fusion protein significantly increases the inhibitory potency.

- AVA19-66 binds mLAG-3 expressed on cell surface and also blockades the binding of mLAG-3 to MHCII on the surface of APC (LK35.2), demonstrating that the AVA19-66 can be used as an MHCII mediated checkpoint antagonist.
- AVA19-66 demonstrated significantly increased potency when Fc formatted and was selected as the lead clone to generate the PDL1/LAG3 bispecific AVA21-14.

T cell Exhaustion Assay





Clone/Format	EC_{50} (nM)
AVA19-66 Fc	195.4
AVA04-182 Fc	3.4
Combo (Affimer) AVA19-66 Fc + AVA04-182 Fc	52.7
AVA21-14 Fc (Bispecific)	3.2
α-mLAG-3 mAb (C9B7W)	NA
α-mPD-L1mAb (10F.9G2)	0.32
Combo (mAb)	2.0

- mLAG-3 or mPD-L1 antagonists increased the IL-2 production by 5-6 fold. An approx. 50-fold increase was achieved when using the combination or bispecific.
- IL-2 release reaches the same maximum for both the Affimer combination and the bispecific, however the bispecific is more potent.

Conclusions

- We have identified potent mLAG-3 and mPD-L1 antagonist Affimers that can be formatted as Fc fusion proteins to significantly improve affinity/avidity.
- The formatted Affimers maintain significantly improved functionality demonstrated by binding to targets on cell surface and competing against endogenous ligands/targets.
- The bispecific Affimer AVA21-14 Fc blocks the PD-L1/PD-1 and LAG-3/MHCII immune checkpoint pathway and reverses the inhibition of IL-2 production in a surrogate T cell based assay.

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