Fc fusion Affimer® Biotherapeutic Targeting PD-L1 Inhibits Tumour Growth in Mouse

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Introduction

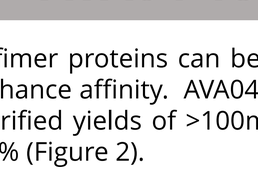
Monoclonal antibodies e.g. Ipilimumab, Atezolizumab, have successfully shown that blocking cellular interactions that negatively regulate T-cell immune responses can amplify pre-existing immunity to cancer. Programmed death-ligand 1 (PD-L1) is a clinically validated oncology target shown to play an important role in down-regulating the immune system allowing tumour cells to evade detection and metastasize.

Affimer molecules were selected against mouse PD-L1 (AVA04 programme) using phage display. AVA04-182, a lead mouse PD-L1 antagonist, has been identified as a surrogate for *in vivo* mouse studies. The molecule was fused to the Fc moiety of a human IgG1 (AVA04-182hFc1) for half life extension. Competitive ELISAs have shown AVA04-182hFc1 to compete with the binding of PD-L1 to both PD-1 and a commercially approved immunotherapy, confirming the potential of the Affimer technology for immuno-oncology therapeutics.

Affimer Technology

Affimer 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 ($1x10^{11}$)

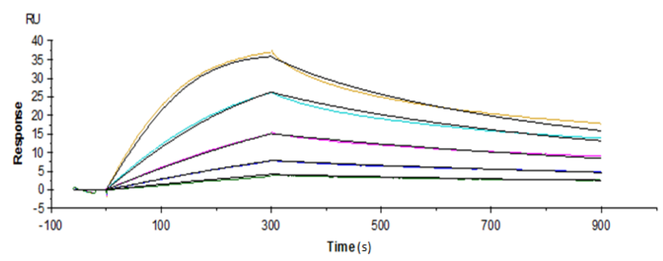
Benefits of Affimer Therapeutics

- **Small size:** 14 kDa, $1/10^{th}$ the size of an antibody
- **High expression**: >100 mg/L in flasks (*E. coli*)
- **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 for increased efficacy

Affimer AVA04-182 Binds PD-L1

Affimer proteins against PD-L1 were selected by phage display, then expressed and purified from *E. coli*. AVA04-182 was identified as a high affinity binder to mouse PD-L1 by Biacore® (Figure 1). No interaction was observed between AVA04-182 and human PD-L1.

Figure 1: Biacore® kinetics for AVA04-182 binding mouse PD-L1



Affimer	k_a (1/Ms)	k_d (1/s)	App K_D
AVA04-182	$8.99x10^{6}$	$2.3x10^{-3}$	315.7pM

AVA04-182 Formatting and Characterisation

Affimer proteins can be formatted as Fc fusions to add effector functions, improve half-life and enhance affinity. AVA04-182 was formatted as a human IgG1 Fc fusion (AVA04-182hFc1) and gave purified yields of >100mg/L from transient expression in Expi293F cells with purity greater than 95% (Figure 2).

AVA04-182hFc1 schematic

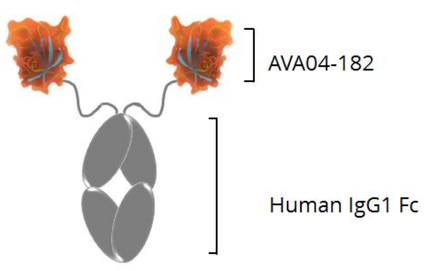
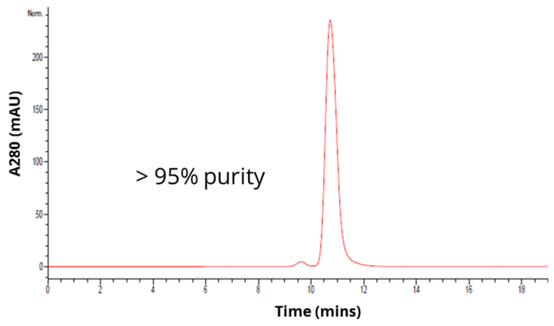
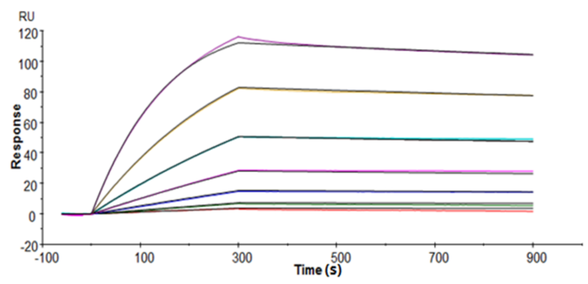


Figure 2: AVA04-182hFc1 SEC-HPLC



AVA04-182hFc1 binds mouse PD-L1 with greater affinity than AVA04-182 as determined by Biacore® (Figure 3).

Figure 3: Biacore® Kinetics for AVA04-182hFc1 binding mouse PD-L1



Affimer	k_a (1/Ms)	k_d (1/s)	App K_D
AVA04-182hFc1	$3.39x10^{6}$	$1.23x10^{-4}$	36.1pM

Figure 4: Effect of AVA04-182hFc1 on PD-L1/PD-1 interaction by ELISA

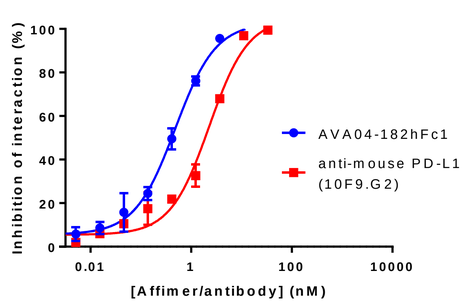
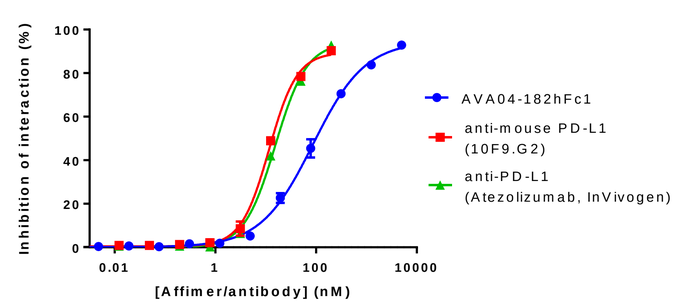


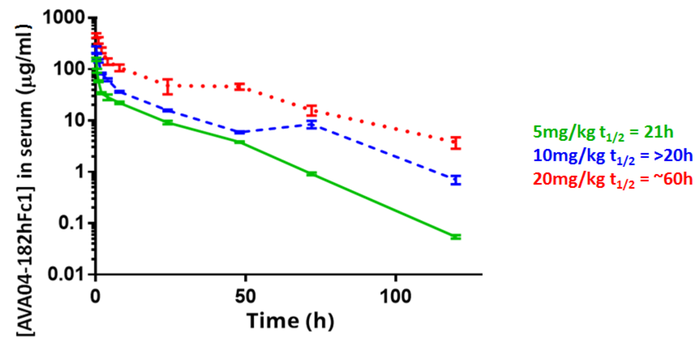
Figure 5: Effect of AVA04-182hFc1 on PD-L1/Atezolizumab interaction by ELISA



Competitive ELISA confirmed AVA04-182hFc1 interaction with mouse PD-L1 disrupts mouse PD-L1/PD-1 interaction with similar affinity to anti-mouse PD-L1 antibody clone 10F9.G2 (Figure 4). Binding of AVA04-182hFc1 to a PD-L1 expressing mouse cell line was confirmed by flow cytometry (data not shown). Competitive ELISA showed AVA04-182hFc1 to recognise the same epitope region as the anti-mouse PD-L1 antibody (clone 10F9.G2) and the commercially approved monoclonal anti-human PD-L1 antibody, Atezolizumab from InVivogen (Figure 5).

Pharmacokinetics of AVA04-182hFc1 in Mouse

Figure 6: Measurement of serum concentration over time by ELISA



AVA04-182hFc1 was dosed as a bolus IV injection, 3 mice per time point. PK was followed for 7 days and serum levels of AVA04-182hFc1 determined by sandwich ELISA (Figure 6). AVA04-182hFc1 was well tolerated *in vivo* at all doses.

Efficacy of AVA04-182hFc1 in a Syngeneic Model

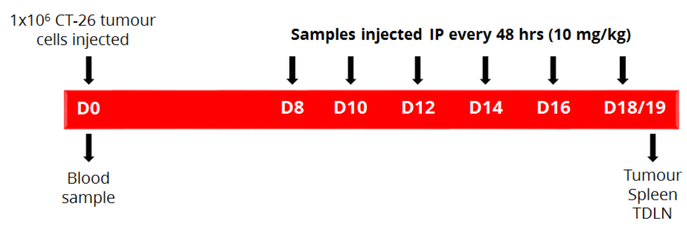
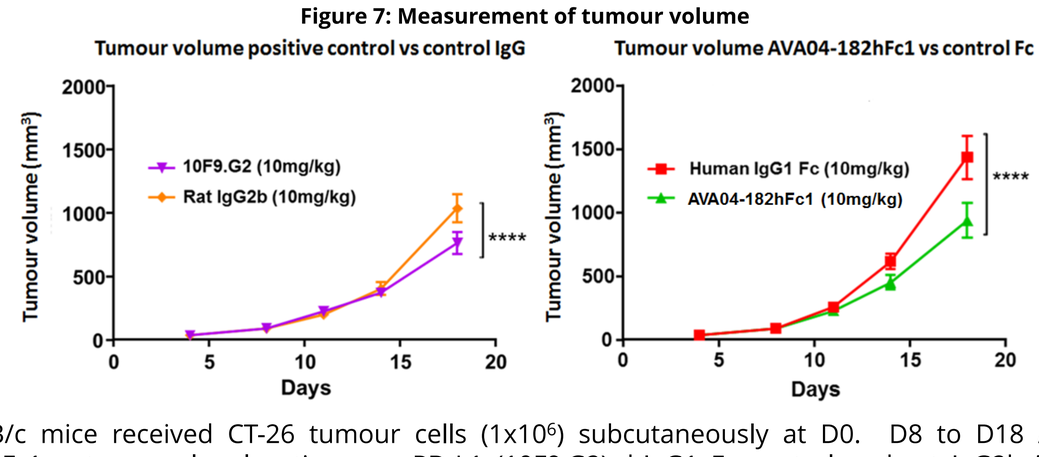


Figure 7: Measurement of tumour volume



BALB/c mice received CT-26 tumour cells ($1x10^{6}$) subcutaneously at D0. D8 to D18 AVA04-182hFc1, rat monoclonal anti-mouse PD-L1 (10F9.G2), hIgG1 Fc control and rat IgG2b isotype control were injected IP at 10 mg/kg every other day. AVA04-182hFc1 and anti-mouse PD-L1 (clone 10F9.G2) significantly increased the regulatory T cell population in the tumour relative to respective controls (data not shown). AVA04-182hFc1 showed statistically significant inhibition of tumour growth compared to the hIgG1 Fc (hFc1) control (Figure 7).

Conclusions

- **Affimer biotherapeutics can be identified using phage display and easily produced at high levels in bacterial and mammalian expression systems**
- **The 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**
- **AVA04-182hFc1 was shown to be well tolerated in mice, even with repeat dosing at 10 mg/kg in the syngeneic model**
- **AVA04-182hFc1 inhibited tumour growth in the 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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