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

POS009

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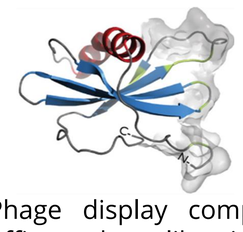
Introduction

Affimer Technology

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



Binding loops: Two randomised 9 aa loop regions

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

Benefits of Affimer Therapeutics

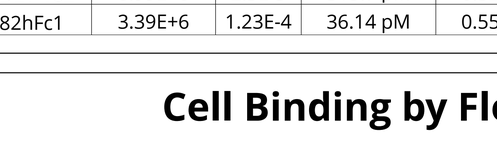
- **Small size:** 14 kDa, $1/10^{th}$ the size of an antibody
- **High expression**: 100's mg/L in flasks (*E. coli*)
- **No post translational modifications**: ease of manufacturing and improved stability
- **Improved tissue penetration:** small size gives greater potential for increased efficacy
-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

Proof of Concept Study - PD-L1

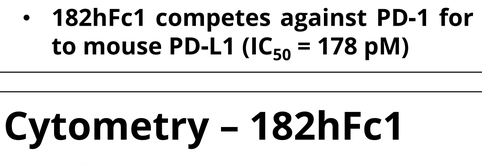
- Programmed death-ligand 1 (PD-L1) is clinically validated in oncology and shown to play an important role in down regulating the immune system allowing tumour cells to evade detection and metastasize.
- Affimer inhibitors with low single digit nM affinity were identified to either human or mouse PD-L1 using phage display. A lead mouse PD-L1 antagonist, PDL1-182 has been identified.

Affimer Formatting and Characterisation

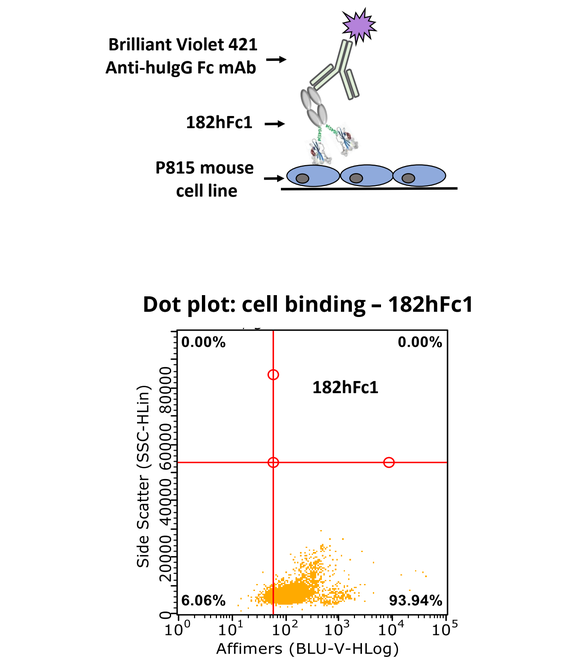
Affimer lead candidate – PDL1-182



Affimer lead candidate Fc formatted – 182hFc1

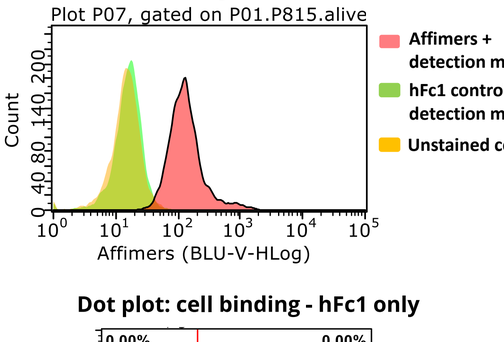


Biacore sensograms of PDL1-182 and 182hFc1



Binder	ka (1/Ms)	kd (1/s)	Apparent K_D	Chi² (RU²)
PDL1-182	8.99E+6	2.3E-3	315.7 pM	0.325
182hFc1	3.39E+6	1.23E-4	36.14 pM	0.558

PD-1/PD-L1 competitive ELISA – Affimer vs tool mAb



Key results:

- **182hFc1 expressed from Expi293F cells transiently and purified to yields >100 mg/L**
- **182hFc1 has an apparent K_D 10 fold higher than the parent PDL1-182**
- **182hFc1 competes against PD-1 for binding to mouse PD-L1 (IC_{50} = 178 pM)**

Cell Binding by Flow Cytometry – 182hFc1

Schematic – Fc binding on mouse P815 cells

Flow cytometry histogram - 182hFc1 binding to live cells

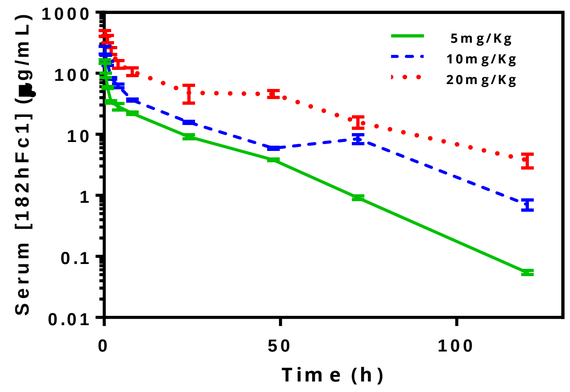
Dot plot: cell binding – 182hFc1

Dot plot: cell binding - hFc1 only

Key result:

- **182hFc1 shows specific binding to mouse mastocytoma cells (93.94% of cells) compared to the hFc1 negative control (1.54% of cells)**

Pharmacokinetics of 182hFc1 in mouse



Product	Dose (mg/kg)	Cmax (µg/ml)	AUC min*µg/mL	Terminal Half-life (h)
182hFc1	5	155±10.5	3313.8	20.9±1.3
	10	241±36	5964.6	19.2*
	20	462±46	9852	59.9±5.3

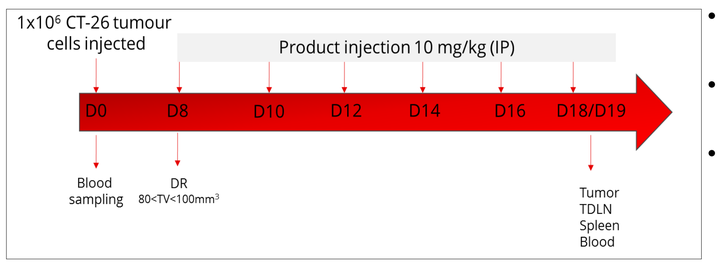
PK parameters of 182hFc1. Parameters were calculated with a non linear fit at 2 phases decay. *error could not be determined

- C57BL/6 mice dosing - single i.v. injection of 182hFc1 at 5, 10, or 20 mg/kg
- Blood samples were collected over 7 days
- The concentration of 182hFc1 in serum was measured by a fluorescent ELISA

Key results:

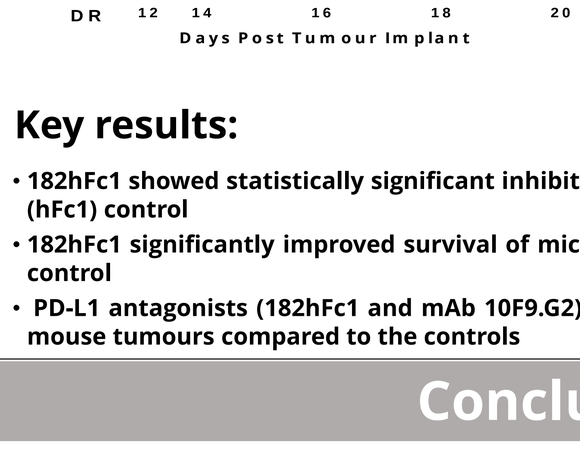
- **The serum half-life of the Affimer Fc fusion protein was successfully extended**
- **182hFc1 was well tolerated at all doses administered**

CT-26 Syngeneic Mouse Model

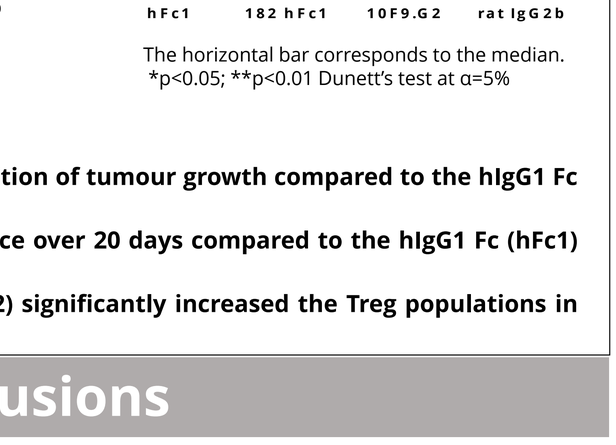


- Balb/c mice received CT-26 tumour cells ($1x10^6$) subcutaneously at D0
- Randomization of groups at D8 Mean tumor volume 80-100 mm^3
- D8 to D18 182hFc1, mAb 10F9.G2, hIgG1 Fc control and rat IgG2b isotype control were injected i.p. at 10 mg/kg every other day

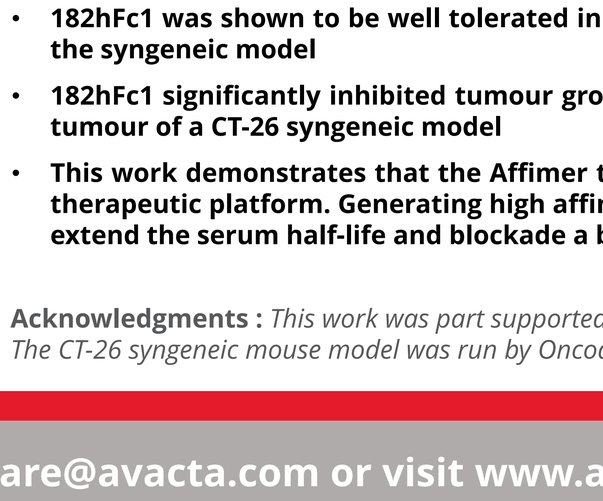
Individual tumour volume curves 182hFc1 and hIgG1 control



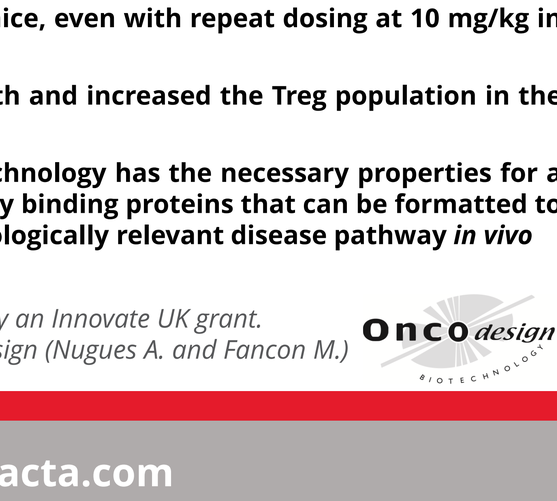
Individual tumour volume curves mAb 10F9.G2 and rat IgG2b control



Time to reach tumour vol. 800 mm^3



Percentage of Treg in the tumour



The horizontal bar corresponds to the median.
*p<0.05; **p<0.01 Dunett's test at α=5%

Key results:

- **182hFc1 showed statistically significant inhibition of tumour growth compared to the hIgG1 Fc (hFc1) control**
- **182hFc1 significantly improved survival of mice over 20 days compared to the hIgG1 Fc (hFc1) control**
- **PD-L1 antagonists (182hFc1 and mAb 10F9.G2) significantly increased the Treg populations in mouse tumours compared to the controls**

Conclusions

- **PDL1-182 showed a 10 fold increase in apparent K_D when formatted as an Fc fusion (182hFc1) and specifically bound to PD-L1 expressed on a mouse cell line surface**
- **182hFc1 was shown to be well tolerated in mice, even with repeat dosing at 10 mg/kg in the syngeneic model**
- **182hFc1 significantly inhibited tumour growth and increased the Treg population in the tumour of a CT-26 syngeneic model**
- **This work demonstrates that the Affimer technology has the necessary properties for a therapeutic platform. Generating 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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