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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 molecule shown to play an important role in downregulating the immune system. Affimers (Affimer®) is a novel class of low molecular weight immunological therapeutics, based on the scaffold backbone of a 9 amino acid loop, with equal diversity at each position. Inhibition of the PD-L1 pathway was shown in a Promega IPD-L1/N2 cell-based assay with 49% inhibition of PD-L1 expression through NFKB-mediated luciferase activity.

**Affimer Technology**

**Benefits of Affimer Therapeutics**

- Small size: 14 kDa, 1/10th the size of an antibody.
- High expression: >100 mg/l in flask. (f)
- No post translational modifications: ease of manufacturing and improved stability.
- Ease of formation: internal and inter-chain fusions, potential to generate multi-specific drugs to target multiple disease pathways.
- Time generation: small size gives greater potential of tissue generation for increased efficacy.

**Affimer Discovery Process: Phage Selections**

**Affimer Protein Targeting mPD-L1**

A range of anti-PD-L1 Affimer® proteins demonstrated patent activity in an MLR assay: CD8+ T cells isolated from C57BL/6 mouse were differentiated into effector T cells with anti-PD-L1 fusion Fc. Tumour-specific infiltration was measured based on Thy-1 expression.

**Affimer Therapeutic Targeting hPD-L1**

Purified mouse anti-PD-L1 and mouse IgG2 fusion proteins were characterized by SEC and CD4 binding.

**PK Study of Affimer® 182 hFc1 in Mouse**

Effect of Affimer® 182 hFc1 on mPD-L1 in vivo in the absence of co-expression of FcRn. purified protein was injected SC at a single dose of 10 mg/kg and had a peak of 36.1 pM at 0.5 day with a half-life of 250 days. Data show Affimer® 182 hFc1 had potent activity in the absence of co-expression of FcRn.

**Conclusions**

- Affimers can be formulated as a fusion to both the N and C terminal of the human IgG1.
- Affimers were expressed in E. coli (f) and purified using a p3 eliciting affinity chromatography.
- Affimers were formulated using flexible (f) or rigid (G4S) backbone either on the N or C terminus.
- The position of the Affimer fusion on the Fc did not affect the binding to mPD-L1 by Bicesse.