Affimer® proteins: A novel therapeutic platform

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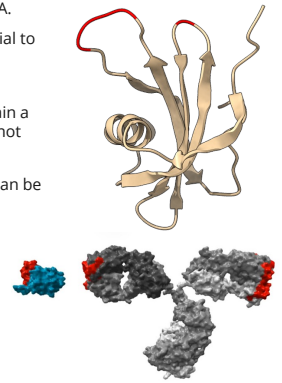
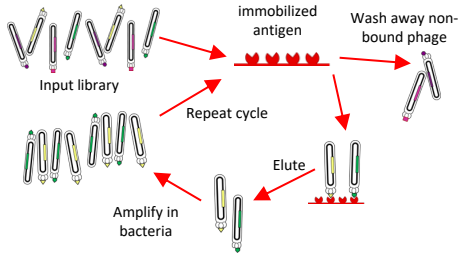
Introduction

- Affimer® proteins are based on the human protease inhibitor, Stefin A.
- Loops may be inserted in the positions shown: these have the potential to bind target proteins with high affinity and high specificity. Insertions eliminate normal biological activity.
- Stefin A was selected as a scaffold as it is sufficiently stable to constrain a broad range of inserted peptides, and flexible enough that folding is not affected by the inserted peptides.
- Stefin A is highly stable, has no post-translational modifications and can be produced at high levels in E.coli or mammalian cells.
- Stefin A is 11kDa, increasing to around 14kDa when Affimer® loops are present. This compares to 150kDa for an IgG antibody, and provides potential benefits for tissue penetration and clearance from the body.
- Production of multiple Affimer® molecules linked in series provides opportunities to generate multi-specific proteins and to modify pharmacokinetic properties.



Generation of Affimer® libraries and selections

- Libraries have been generated with all structural liabilities eliminated, no bias in amino acid distribution and with optimized sequences for efficient screening processes.
- A loop size frequently utilised is 9 amino acids. Libraries are available with 9 amino acid loops at either both loop 2 and loop 4, or at loop 2 only. Libraries have a sequence diversity of around 10^{10}.
- Libraries with longer loop sizes have also been generated. These may offer access to hidden or novel epitopes; Affimer® molecules may be able to bind epitopes inaccessible to larger biologics.

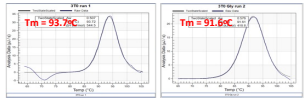


Selections

- Input library binds to immobilized antigen.
- Non-binding phage are washed away; phage which remain bound are eluted and bacteria infected with these phage to amplify.
- Multiple rounds of selection are performed.
- Isolation of specific phage (in parallel to next generation sequencing of pools) results in identification of Affimer® molecules which bind to the target protein.

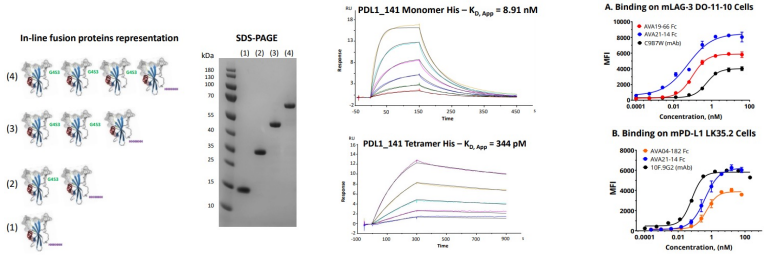
Affimer® molecules show thermal stability similar to Stefin A

- Stefin A exhibits a high melting temperature (Tm); Affimer® molecules with binding loops also exhibit high Tm's.
- This provides advantages for subsequent processing steps including radiolabelling.



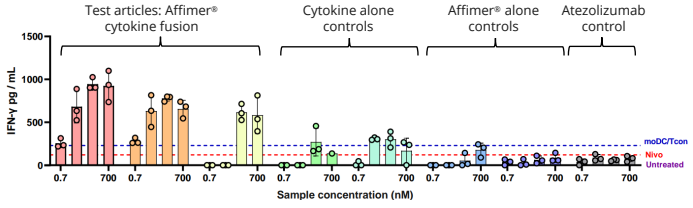
Formatting options

- Multiple Affimer® molecules can be covalently linked into a single protein: characteristics can be retained and productivity of the resulting protein remains high.
- Linking repeat units enables modification of avidity. When an Affimer® is included which binds albumin, PK properties may be modulated.
- Incorporation of Affimer® molecules to different targets allows simple generation of multi-specific molecules.



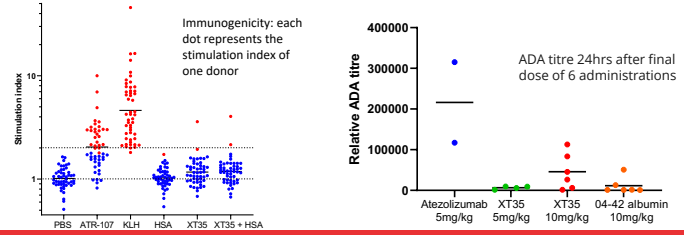
Affimer® molecules may be linked to cytokines or other biologically active proteins

- Activity of an Affimer® - cytokine fusion protein is demonstrated in a mixed lymphocyte reaction. T cell activity is observed in a dose dependent manner for both Fc- and Affimer® PK extended molecules.



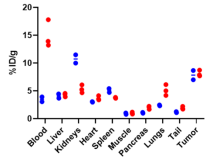
Affimer® molecules exhibit low immunogenicity

- Affimer® molecules exhibit low immunogenicity in CD8+ depleted PBMC proliferation measured by CD4+EdU+ cells.
- Affimer® molecules have also been demonstrated to elicit lower levels of anti-drug antibody response than commercial antibodies to the same target.

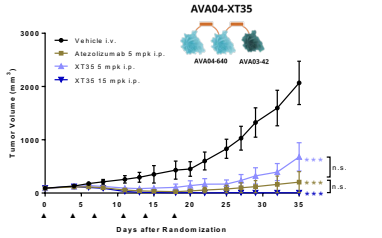


Biodistribution and in vivo activity

- Affimer® molecules exhibit tumour localisation in mice similar to that seen for commercial antibodies. Affimer® molecules may also be engineered to have distinct clearance compared to antibodies which has potential benefit in certain clinical settings.
- Affimer® molecules (both Fc-linked and with PK-extending engineering) have shown activity in multiple in vivo tumour growth inhibition studies.

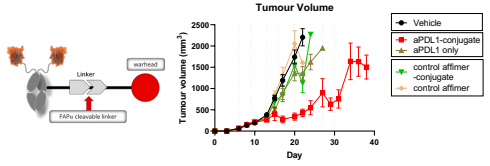


Biodistribution at 72hrs. Percentage of injected dose per gram in mice bearing subcutaneous A375 tumours.



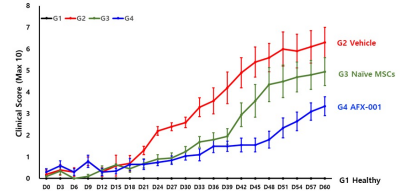
Tumour microenvironment activated conjugates: TMACs

- TMAC can bring together the preCISION™ and the Affimer® platforms with a targeting moiety as well as the FAP-cleavable linker and a biologically active warhead.
- In vivo tumour growth inhibition of test TMACs has been observed.



Diverse opportunities: AffyXell and MSCs

- Affimer® molecules antagonistic to CD40L may be expressed on the surface of mesenchymal stem cells with no change to MSC gene expression or other characteristics.
- These engineered MSCs have the potential to modulate activity of host B cells.
- These Affimer® -expressing MSCs have been shown to reduce GvHD in mice.



Conclusions

- Affimer® molecules with specific binding characteristics may be rapidly isolated from phage display libraries. These may be further modified as multi-specific proteins or other fusion proteins, and PK properties modified as required.
- Affimer® molecules are based on a human scaffold, exhibit low immunogenicity and have demonstrated *in vivo* activity in different clinical indications.
- Affimer® molecules may be engineered to various final formats: TMAC provides one opportunity, while radiolabelled or ADC options are also feasible.