Affimer® biotherapeutics: Next-generation platform for cancer therapies

SCAN

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Introduction

- Avacta's proprietary Affimer® technology provides a platform for a novel class of protein biotherapeutics.
- Affimer® proteins (15kDa) are based on the small human protease inhibitor, Stefin A (Figure 1A).
- Affimer® proteins are designed to exhibit similar recognition properties to monoclonal antibodies but with 1/10th of its size (Figure 1B).
- Affimer® loops 2 and 4 are engineered to form binding surfaces (Figure 1A).
- Engineered loops can bind to difficult targets with high specificity.
- Affimer® proteins have no disulfide bonds and no post-translational modifications.
- Affimer® proteins can be produced at high levels in *E.coli* or mammalian cells.
- Its small and flexible structure enables the production of modular and multi-specific protein biotherapeutics with tunable pharmacokinetic properties.

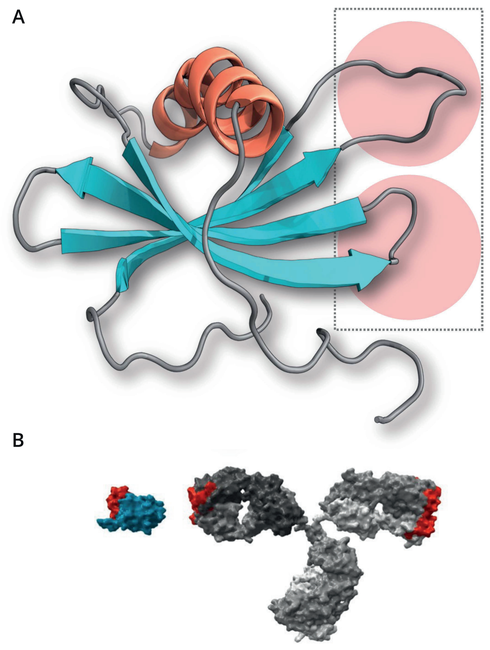


Figure 1. Depiction of Affimer® proteins. A. Structure of an Affimer®. The engineered binding loops, generating unique binding surfaces, are highlighted. B. Size comparison of an Affimer® protein (left) and an antibody (right).

Affimer® libraries design and screening

- Affimer® loops (highlighted in Figure 1A) have been engineered to create large randomized phage-display libraries with sequence diversities of around 10^{10}.
- Libraries are available with randomized sequences (9 amino acid residues) in either both loop 2 and loop 4, or in loop 2 only.
- 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.
- Affimer® libraries have also been designed to remove structural liabilities, sequence distribution bias and to improve solubility and optimize screening efficiency.
- Phage selection rounds of enrichment can be combined with Next-Generation Sequencing (NGS) to maximize depth of library screening, number of hits and hit diversity.
- Structural prediction of Affimer®-antigen interactions using AlphaFold can inform and guide hit screening, characterization and selection process, as well as epitope binning and mapping.

Affimer® protein scaffold shows high thermal stability

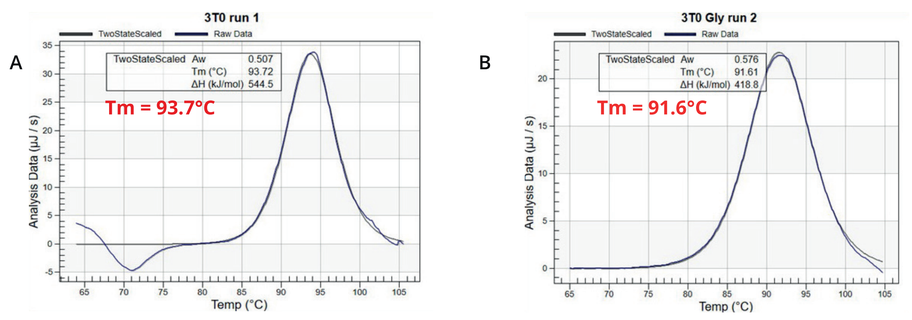
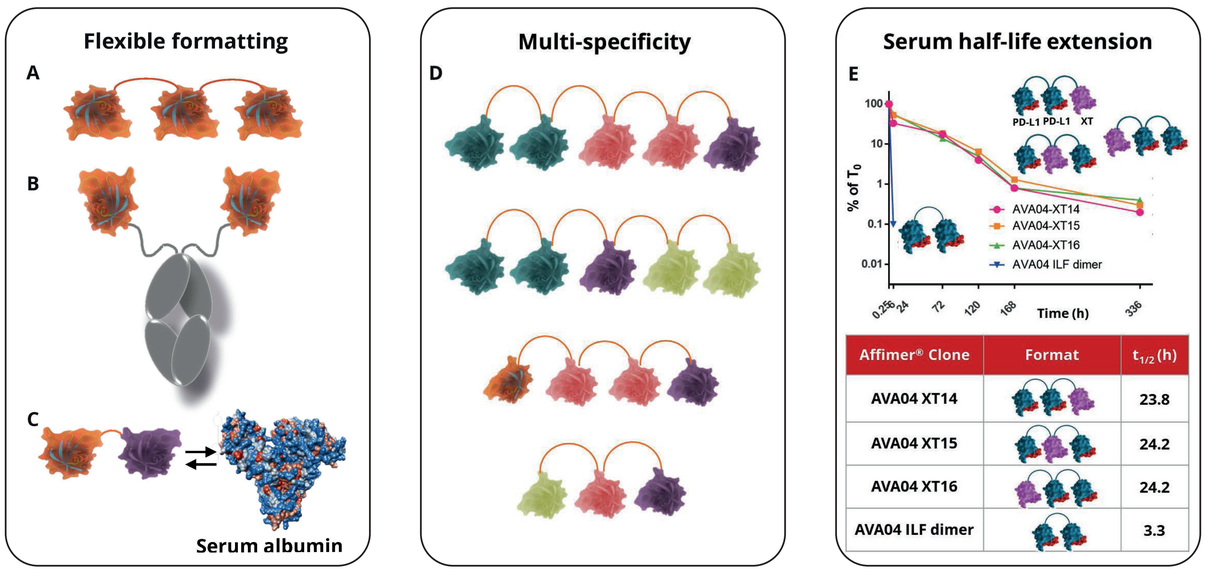


Figure 2. Differential Scanning Calorimetry (DSC) analysis of the melting temperatures (Tm) for Affimer® control proteins containing no loop insertions (A), or nine Glycine residues in both loop 2 and loop 4 (B).

- Affimer® proteins are very stable and exhibit high melting temperatures (Figure 2).
- The high thermal stability and robustness of Affimer® proteins provides an advantage for subsequent processing steps including radiolabeling.

Affimer® formatting options

- Fusions proteins containing multiple Affimer® units can be designed (Figure 3A-C), which retain the properties of the individual monomer Affimer® proteins while introducing avidity.
- Pharmacokinetics/Pharmacodynamics (PK/PD) properties of Affimer® fusion proteins can be modulated using an albumin-binding Affimer® or using an Affimer®-Fc fusion (Figure 3B and 3C, respectively; see also panel E).
- Fusion proteins containing Affimer® units binding to different targets allows modular generation of multi-specific protein biotherapeutics (Figure 3D).



Affimer® Clone	Format	$t_{1/2}$ (h)
AVA04 XT14		23.8
AVA04 XT15		24.2
AVA04 XT16		24.2
AVA04 ILF dimer		3.3

Figure 3. Affimer® proteins offer a variety of formatting options. A-C. Depictions of linear fusions of Affimer® units (A), fusions with an antibody Fc domain (B), and an albumin-binding Affimer® (C) are shown. D. Multi-specific fusion proteins can be designed to contain multiple Affimer® units binding distinct targets, as illustrated here for different in-line fusion (ILF) formats. E. Example of a PK study for Affimer® fusion proteins targeting PD-L1 and containing an albumin-binding Affimer® unit, showing prolonged half-lives in mouse serum.

Affimer® proteins exhibit low immunogenicity

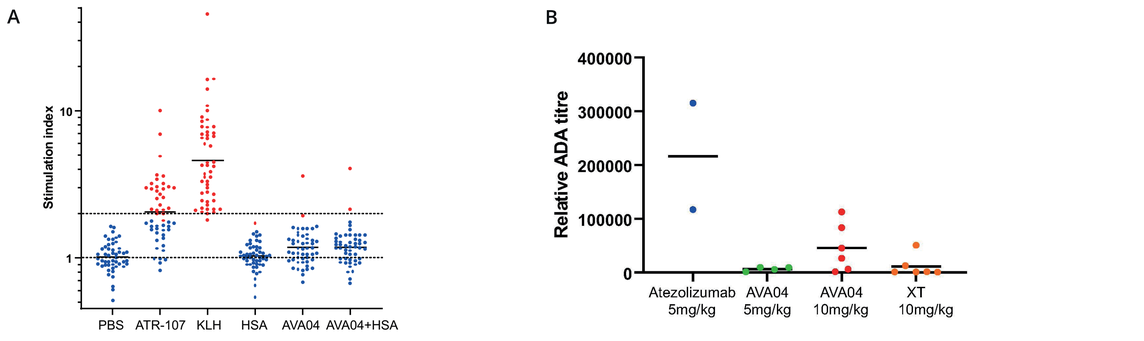


Figure 4. A. Affimer® proteins exhibit low immunogenicity in CD8-depleted PBMC proliferation assay, as measured by CD4+EdU+ cells. Immunogenicity data for Affimer® fusion AVA04, binding PD-L1 and containing an albumin-binding Affimer® unit, ATR-107 antibody and KLH protein is shown. Each dot represents the stimulation index of one donor. B. Affimer® proteins elicit lower levels of anti-drug antibody (ADA) response than commercial antibodies to the same target. The ADA titre for the AVA04 Affimer® fusion, an albumin-binding Affimer® monomer (XT) or Atezolizumab 24 hours after the final dose of 6 administrations is shown.

Affimer® proteins can be linked to cytokines or other biologically active proteins

- Half-life extended Affimer® fusion proteins containing PD-L1 binding Affimer® units fused to human Interleukin 15 (IL-15) receptor alpha sushi domain and human IL-15 (PDL1-IL15 Affimer® fusions) reverse T cell exhaustion in primary human mixed lymphocyte reaction (MLR) assays (Figure 5).
- PDL1-IL15 Affimer® fusion proteins induce higher levels of IFN-γ release than PD-L1 only and non-targeted IL-15 control proteins, anti-PD1 (Nivolumab) or anti-PD-L1 (Atezolizumab).

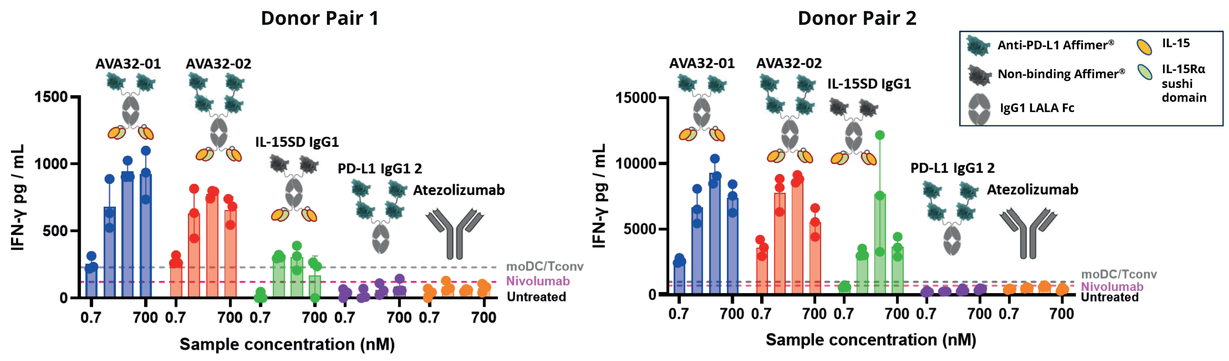


Figure 5. IFN-γ release after exposure with indicated molecules for 5 days in MLR assay. Exhausted T cells (Tex) were generated by isolating pan-T cells (CD3+) from PBMC donors and repeatedly stimulating using CD3/CD28 Dynabeads®. In vitro generated immature monocyte-derived dendritic cells (moDCs) were combined with Tex cells to create MLR pairs (N=2 donor pairs), which were then cultured with indicated molecules, Nivolumab or left untreated for 5 days. As a control, MLR cultures with moDCs and non-exhausted T cells (Tconv) were included. The supernatants were assessed for IFN-γ levels by ELISA.

Affimer® proteins show *in vivo* efficacy

- PDL1-IL15 Affimer® fusion protein AVA32-02 exhibits tumour growth inhibition in the *in vivo* hPD-L1-MC38 tumour model and increases tumour-infiltrating CD8+ T cells (Figure 6).

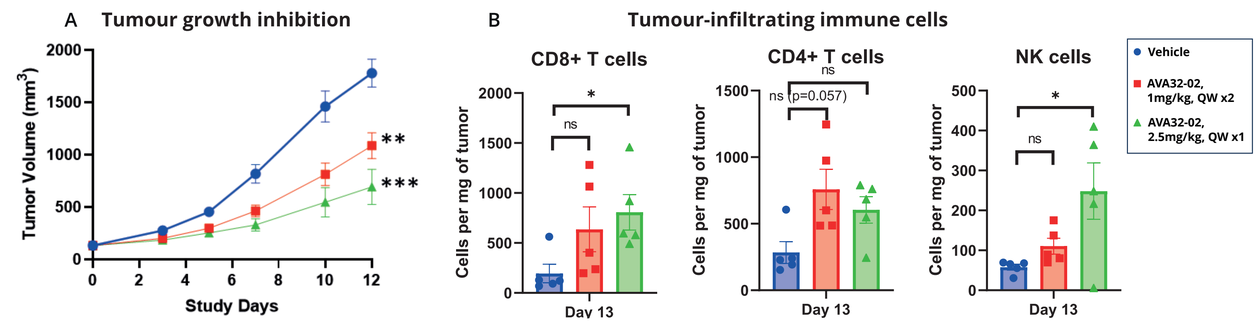


Figure 6. AVA32-02 induces CD8+ T cell and NK cell infiltration into tumour and inhibits growth of MC38 tumours. Female hPD-L1 HuGEMM C57BL/6 mice were subcutaneously injected with hPDL1-MC38 tumour cells. Following tumour formation, mice were administrated intravenously with vehicle control or AVA32-02 at 1, or 2.5 mg/kg at day 0 post randomization. A second dose was administered at day 7 to vehicle and AVA32-02, 1 mg/kg group. Tumour volume (A) is shown as mean -/+ SEM (n= 9-10 mice/group). At day 13, mice were culled and tumours from 5 mice per group were processed for flow cytometry. (B) Cell count of CD4+, CD8+ T cells and NK cells was determined by flow cytometry. Mean -/+ SEM, n=5, is shown. *p<0.05; **p<0.01; ***p<0.001; NS, no significance.

Affimer® proteins offer diverse opportunities

- Tumour microenvironment activated conjugates (TMAC®) bring together Avacta's Affimer® and pre|CISION™ platforms with an Affimer® targeting moiety as well as the FAP-cleavable linker and a biologically active warhead (Figure 7A). *In vivo* tumour growth inhibition of test TMAC® molecules has been observed.
- Affimer® proteins can also be radiolabeled or conjugated to other drugs (Figure 7B).
- Affimer® constructs can also be used in the cell and gene therapy space (e.g., AffyXell Therapeutics, a joint venture with Daewoong Pharmaceutical Co. Ltd). Affimer® proteins targeting CD40L, expressed and secreted by engineered mesenchymal stem cells (MSCs), reduce GvHD in mice (Figure 7C).

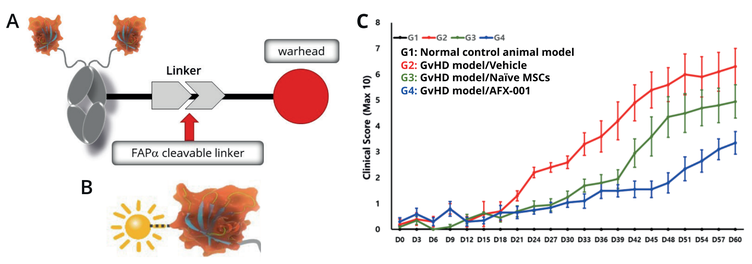


Figure 7. Affimer® proteins offer diverse opportunities.

A. Schematic representation of the TMAC® platform, combining Affimer® and pre|CISION™ technologies.

B. Depiction of a radiolabeled Affimer® protein.

C. MSCs secreting a CD40L-binding Affimer® (AFX-001) show superior *in vivo* efficacy in a GvHD mouse model.

Summary

- Affimer® proteins with specific binding characteristics can be rapidly isolated from phage-display libraries. These can be additionally formatted and modified as multi-specific proteins or other fusion proteins, with modulated PK properties as required.
- Affimer® proteins are based on a human scaffold, exhibit low immunogenicity and have demonstrated *in vivo* activity in different clinical indications.
- Affimer® proteins can be further engineered to various final formats and different therapeutic approaches: from cell and gene therapy to TMAC®, radiolabeling and other drug conjugate options.

For further information please visit www.avacta.com