Development And Validation Of Affimer-based Adalimumab-TNFα Complex Specific Reagents

POS023

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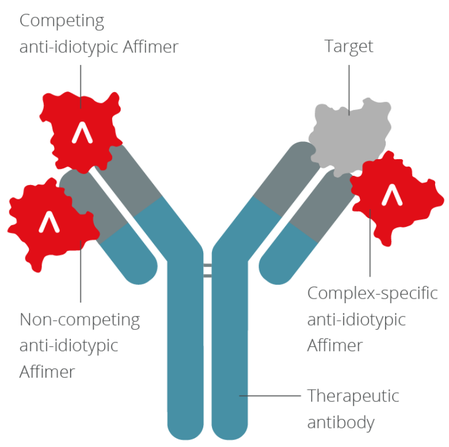
Critical Reagents in TDM Assays

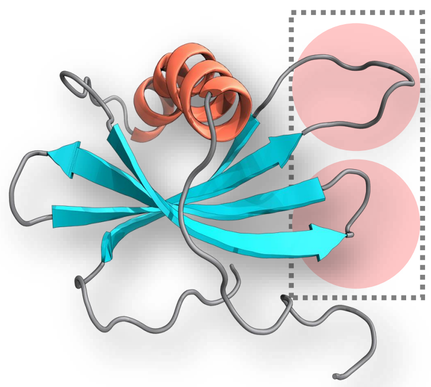
- Here we describe a new generation of reagents specific to antibody-target complexes which can be used to detect and quantify 'target-bound competent drug' in bioassays.
- Accurate quantification of bound drug to its target could be used to monitor the occurrence of interfering events which could affect the drug mode of action (such as the production of ADAs).
- Reliable supply of critical reagents is needed to develop bioanalytical methods that can be used as part of therapeutic drug monitoring (TDM) assays.



Different types of anti-idiotypic reagents to measure bound, free or total antibody concentrations

The Affimer® Scaffold

The Affimer scaffold is based on naturally occurring proteins (cystatins), engineered to stably display two loops creating a binding surface. Avacta has built large Affimer libraries (10^{10} – 10^{11}) which can be screened for binders to a broad range of targets using standard phage display techniques.



Affimers provide significant advantages for bioanalytical applications:

- **Speed of development**: high affinity reagents can be generated in three months without the need for affinity maturation
- **Reproducible supply**: based on robust and high yield recombinant bacterial production process
- **Low matrix effect**: exquisite specificity for three-dimensional conformations allows specific detection in complex background
- ***In-vitro* screening methods**: provide options to design experiments to select binders for protein complexes which are not accessible using classical immunisation approaches

Anti-Idiotypic Affimer Discovery

Phage Display

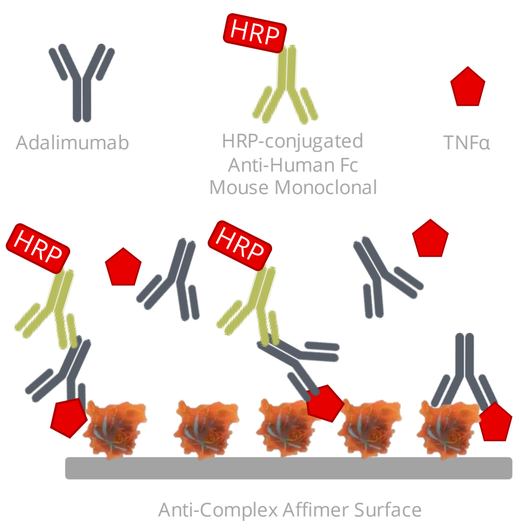
Recombinant antibody containing CDR-regions of Adalimumab were biotinylated, immobilised and submitted to three rounds of phage display in presence of a large excess of TNFα. To remove binders to free antibody, a deselection step was performed using antibody in the absence of TNFα.

Primary Screen and Sequencing

192 clones were sequenced to identify unique clones. They were tested on a high-throughput bead assay for their ability to capture solution phase antibody-TNFα complex and their specificity using a range of irrelevant therapeutic antibodies.

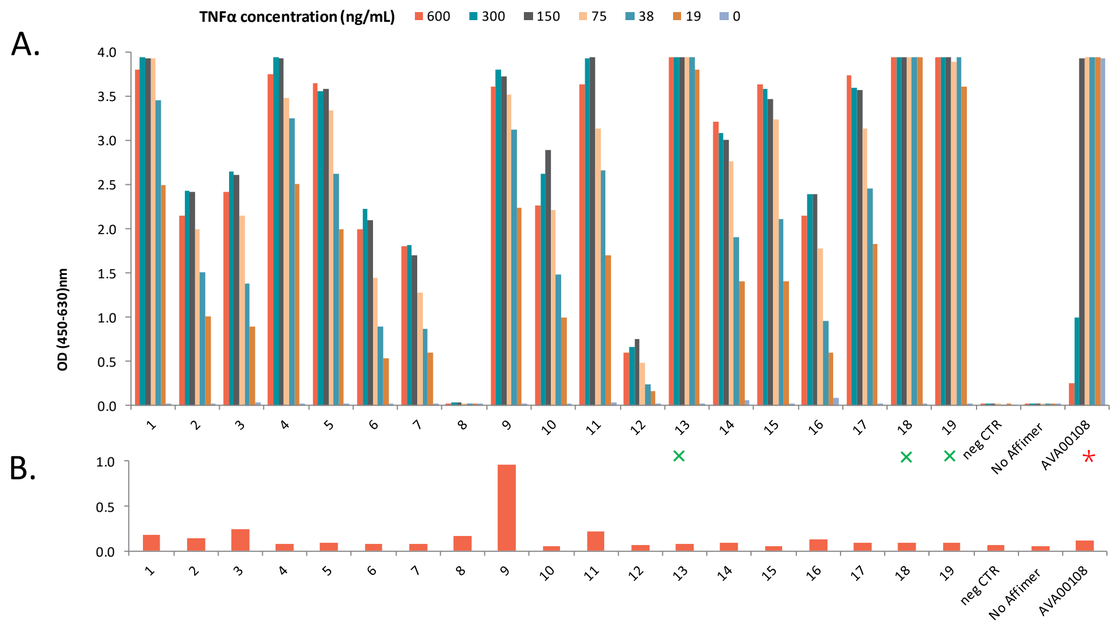
19 unique sequences were taken forward for ELISA validation

ELISA Validation Method



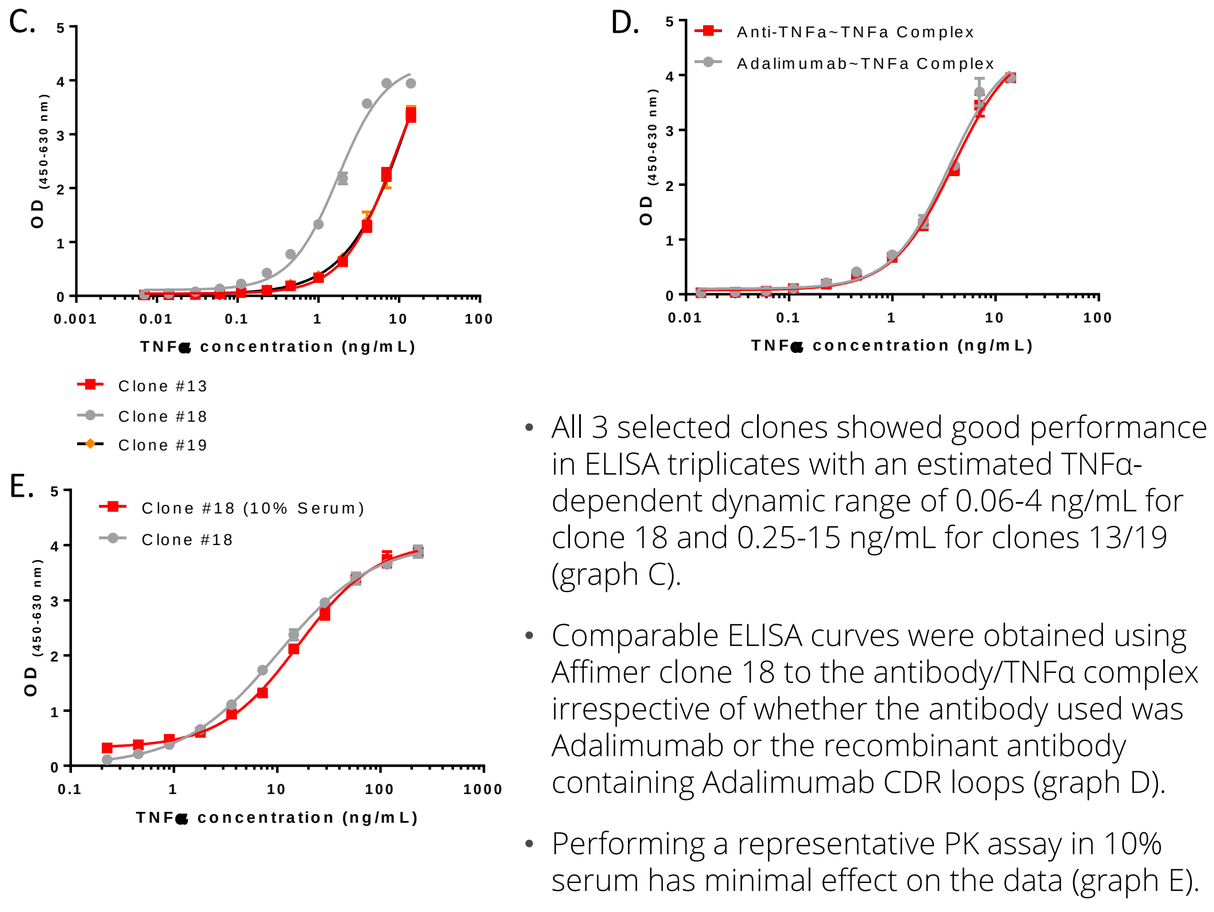
- Affimers coated on solid surface at a concentration of 200nm (2.88 µg /mL)
- Fixed concentration of TNFα antibody (1 µg/mL) and a TNFα titration range
- Detection using HRP conjugated anti-hIgG isotype antibody
- Initial screening was performed on high-throughput bead based assay and top three clones were subsequently evaluated in a plate format

ELISA Screen For Antibody/TNFα-binding Affimers



- Of the 19 clones identified from the primary screen, 18 showed TNFα-dependent binding profiles (graph A). 3 lead clones (13, 18 & 19) were selected for assay development (marked ×).
- An anti-TNFα-Ab competing Affimer (AVA00108) control shows an inverse titration with respect to TNFα concentration (marked *) as expected and no signals were observed when using an irrelevant Affimer control reagent.
- Of the 19 unique Affimer clones tested, only one showed any binding above background to TNFα in capture ELISA using biotinylated TNFα and streptavidin-HRP detection (graph B).

Assay Evaluation Of Lead Clones



- All 3 selected clones showed good performance in ELISA triplicates with an estimated TNFα-dependent dynamic range of 0.06-4 ng/mL for clone 18 and 0.25-15 ng/mL for clones 13/19 (graph C).
- Comparable ELISA curves were obtained using Affimer clone 18 to the antibody/TNFα complex irrespective of whether the antibody used was Adalimumab or the recombinant antibody containing Adalimumab CDR loops (graph D).
- Performing a representative PK assay in 10% serum has minimal effect on the data (graph E).

Affimers As Critical Reagents In PK Assays

- The Affimer platform can be used to reliably develop capture reagents specific to antibody drug-target complexes which are not readily available using classical immunisation strategies.
- These binders can be used in broad range of applications to measure target occupancy, as secondary reagent for Ligand Binding Assays or to monitor the occurrence of binding inhibiting factors (such as ADAs).
- With short development timeline, reliable supply, low batch-to-batch variation and simple assay design, they offer significant advantages for the development of cost-effective PK bioassays.