A Platform Technology For The Rapid Generation Of Robust Anti-Idiotypic Binders For Clinical PK Assays

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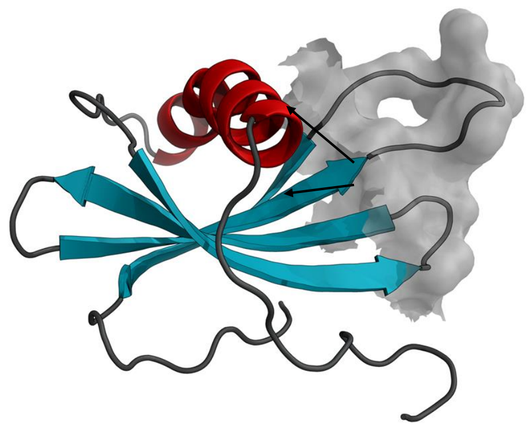
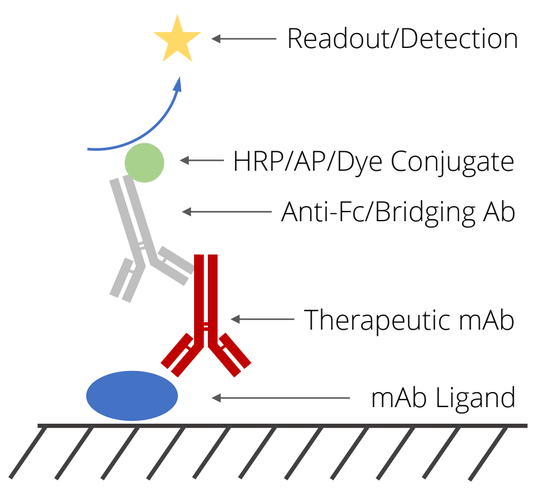
The Affimer Scaffold



- Small single domain proteins (14 kDa) with no disulphide bonds or post translational modifications.
- Derived from the Cystatin family protein fold.
  - 2 scaffolds developed.
  - Mammalian scaffold based on Stefin A.
  - Plant scaffold based on Cystatin A consensus sequence.
- Two surface loops and N-terminus can be engineered to create vast peptide libraries ($1x10^{10}$).
- Utilise phage display to identify binders.

PK Assays for Therapeutic mAbs

- Availability of capture reagents for therapeutic antibody PK assays can be rate limiting in product development.
- Anti-idiotype antibody development can be a long and difficult process.
- Ligand capture reagents are not always reliably available and can be expensive. Specificity of capture reagents can sometimes be problematic (matrix effect).



- Affimer binders can provide a fast, reliable solution for therapeutic antibody capture.

Anti-Idiotypic Affimer Discovery

Target Protein QC → Phage Display → Primary Screen & Sequencing → ELISA Validation

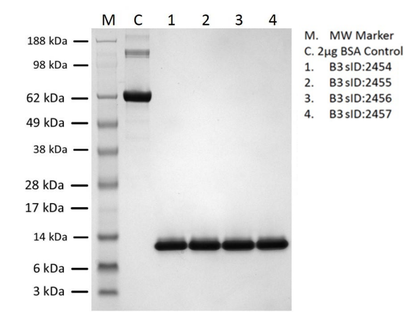
12 – 14 weeks

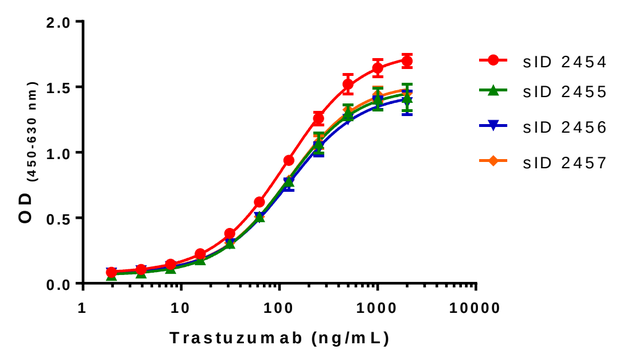
- <u>Target Protein QC:</u> Trastuzumab and recombinant antibodies containing CDR-regions of Ipilimumab, Rituximab and Humira were minimally biotinylated and checked by SDS-PAGE and WB (1wk).
- <u>Phage Display:</u> 3-round phage display used against all 4 targets to identify Affimer repertoires specific for each antibody (2wks).
- <u>Primary Screening:</u> 360 clones per target screened in high-throughput bead assay for specificity and capability to capture solution phase antibody (2wks).
- <u>Sequencing:</u> 96 top hits sequenced to identify unique clones (2wks).

Target	Trastuzumab	Anti-TNFα mAb	Anti-CTLA4 mAb	Anti-CD20 mAb
Unique Clones	17	15	16	17

- <u>Direct Capture ELISA:</u> All clones passively adsorbed to Maxisorp plates and tested for capture of biotinylated-TargetAb with Strep-HRP detection alongside cross-reactivity confirmation (1wk).
- <u>Matrix Test & Cross-reactivity Test:</u> Lead 3 clones per target tested in PBS and 10% serum using Direct Capture to confirm lack of matrix effect (3wks).
- <u>Sandwich Assay Using Fc Detection:</u> Lead clone validated in ELISA using Affimer capture surface, solution-phase unlabelled TargetAb with detection using a HRP-conjugated anti-HumanIgG-Fc Ab. Full titrations in PBS and 10% serum. Intra/Inter-assay CV & %Bias, LLOQ, ULOQ, and Dynamic Range calculated (3wks).

Low Batch-To-Batch Variation

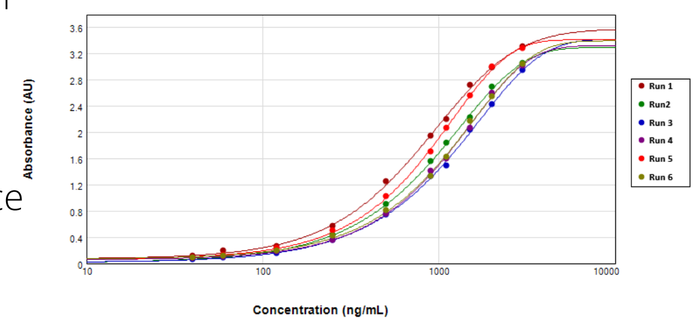




- Four independent batches of anti-Trastuzumab Affimer Clone B3 were produced using cytoplasmic *E. coli* expression and purified by IMAC-SEC.
- Performance of all four batches as a surface in a sandwich ELISA using Fc-detection is highly comparable (EC50 = 122.5 ± 3.8 ng/ml).

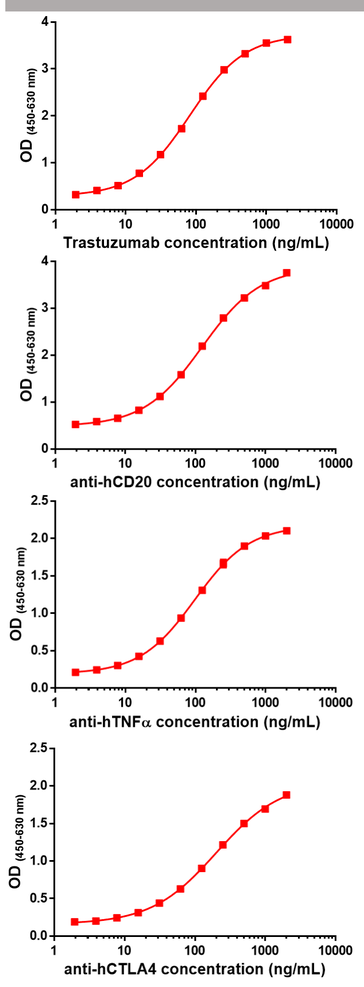
Anti-Trastuzumab Bridging Assay by Covance

- Covance "Bridging" assay with Anti-Trastuzumab Affimer Clone B3 as capture surface.
- Quantitation range (2000 to 60 ng/ml) is greater than twice the working range of current antibody-based PK method.



- Intra-assay precision %CV ≤ 12.4, Intra-assay accuracy %Bias ≤ 10.8
- Inter-assay precision %CV ≤ 19.4, Inter-assay accuracy %Bias ≤ 4.3

Fc-Detection Sandwich Assay Performance



Trastuzmab Assay in 10% Serum					
Quantifiable range (ng/mL)		Inter-assay calibration standard metrics		Intra-assay calibration standard metrics	
LLOQ	ULOQ	% CV	% Recovery	% CV	% Recovery
~30	1600	2.3 - 5.6	98.1 - 104.5	2.8 - 17.3	96.6 - 109.6

Anti-CD20 Recombinant mAb Assay in 10% Serum					
Calibration range (ng/mL)		Inter-assay calibration standard metrics		Intra-assay calibration standard metrics	
LLOQ	ULOQ	% CV	% Recovery	% CV	% Recovery
~2	1000	2.0 - 19.4*	95.8 - 104.6	0.7 - 19.5*	84.0 -122.3*

Anti-CD20-mAb Affimers also bind Rituximab with equivalent performance.

Anti-TNFα Recombinant mAb Assay in 10% Serum					
Calibration range (ng/mL)		Inter-assay calibration standard metrics		Intra-assay calibration standard metrics	
LLOQ	ULOQ	% CV	% Recovery	% CV	% Recovery
~4	1000	1.4 - 7.1	98.4 - 106.1	1.2 - 17.3*	95.4 - 112.7

Anti-TNFα-mAb Affimers also bind Humira with equivalent performance.

Anti-CTLA4 Recombinant mAb Assay in 10% Serum					
Calibration range (ng/mL)		Inter-assay calibration standard metrics		Intra-assay calibration standard metrics	
LLOQ	ULOQ	% CV	% Recovery	% CV	% Recovery
~15	1000	2.7 - 11.4	94.4 - 110.9	0.3 - 12.0	91.7 - 122.6*

Anti-CTLA4-mAb Affimers also bind Ipilimumab with equivalent performance.

* Metrics approaching limits are at LOQ points of the calibration curve

Conclusion

- The Affimer platform can be used to reliably develop anti-idiotypic capture surfaces for the development of immunoassays for measuring concentrations of therapeutic mAbs in clinically relevant ranges.
- Validation of the Affimer platform by Covance[+] demonstrates that Affimers can be qualified as critical reagents in a clinical PK assay.
- Performance improvements: low matrix effect, broader dynamic range, low batch variation, constant supply, ease of use.

[+]Acknowledgments to Sian Estdale, James Munday & Amy Reeves, Covance, Harrogate UK