Introduction to the Affimer® Therapeutic Platform

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Chief Executive, Avacta Group plc

Introduction

Avacta Group plc AIM: AVCT

Affimer®

- Pre-clinical biotech with a proprietary protein scaffold technology – Affimer® platform.
- Public company listed on the London Stock Exchange.
- 80 staff over two sites:
  - 13,000 sqft of bespoke laboratory, production and logistics space in Wetherby, UK.
  - 8,000 sqft of bespoke laboratory space in Cambridge, UK.
  - Business development team: UK, San Diego and Boston.
- Building an in-house pipeline and partnerships with a focus on immuno-oncology.
- Also building towards a profitable business providing bespoke Affimer reagents for non-therapeutic applications with a licensing business model.

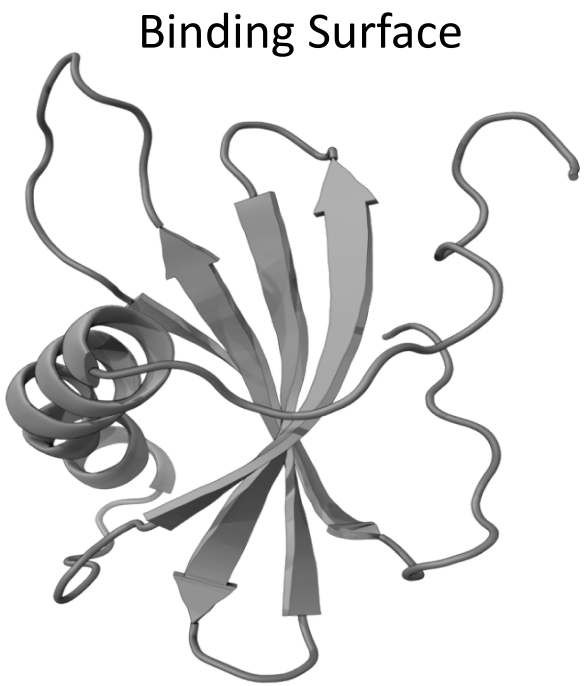
Affimer Technology

Affimer®: A proprietary protein scaffold with key technical benefits

Affimer®

What is an Affimer?

- Based on naturally occurring proteins (cystatins) and engineered to stably display two loops which create a binding surface.
- Loops are randomised to create large libraries of diversity ~10^{10} and Affimers are selected by phage display.



Key Benefits

- Small (14 kDa), simple (no disulphide bridges and no post-translational modifications), robust (thermally and chemically).
- High affinity (single digit nM) Affimers generated for new targets in a few weeks.
- Exquisite specificity demonstrated in numerous examples.
- Easily modified (chemically and as fusion proteins) and easily manufactured in bacterial and mammalian systems with high expression yields.
- Intracellular survival and activity.
- Core Affimer protein is non-immunogenic.

Core Intellectual Property

Broad IP coverage across the cystatin protein family

Affimer®

First Generation

- Acquired from the Medical Research Council and Leeds University UK in 2012.
- Based on human stefin A with multiple mutations to reduce dimerisation and prevent binding to cathepsin.
- Patents granted in EU, US, Asia; Priority date: 2006.
- Current technology for therapeutic programmes.

Second Generation

- Affimer technology based on plant cystatin consensus sequence; high stability suitable for challenging applications in research and diagnostics.
- IP exclusively licensed to Avacta by Leeds University; Priority date: 2014.

Third Generation

- Developed in-house and based on human stefin A with improved biophysical properties and minimal mutations from human sequence for therapeutics; broad claims based on protein engineering and not on a specific sequence.
- Priority date: July 2017.
- New technology for future therapeutic programmes.

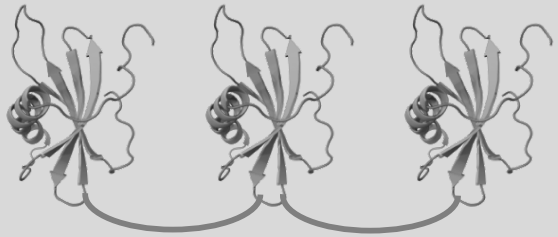
Avacta Therapeutics Strategy

Leveraging Affimer key benefits to create differentiated medicines

Affimer®

Key Benefit of Affimers

Ease of creating and manufacturing "multimers" that combine multiple Affimers

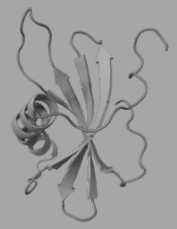


In-house Pipeline

- Immune-checkpoint inhibitors (combinations, bispecifics, biparatopics)
- T-cell engagers
- Agonists

Key Benefits of Affimers

Small size, stability and ease of production by cells



Proof-of-Concept Research Collaborations

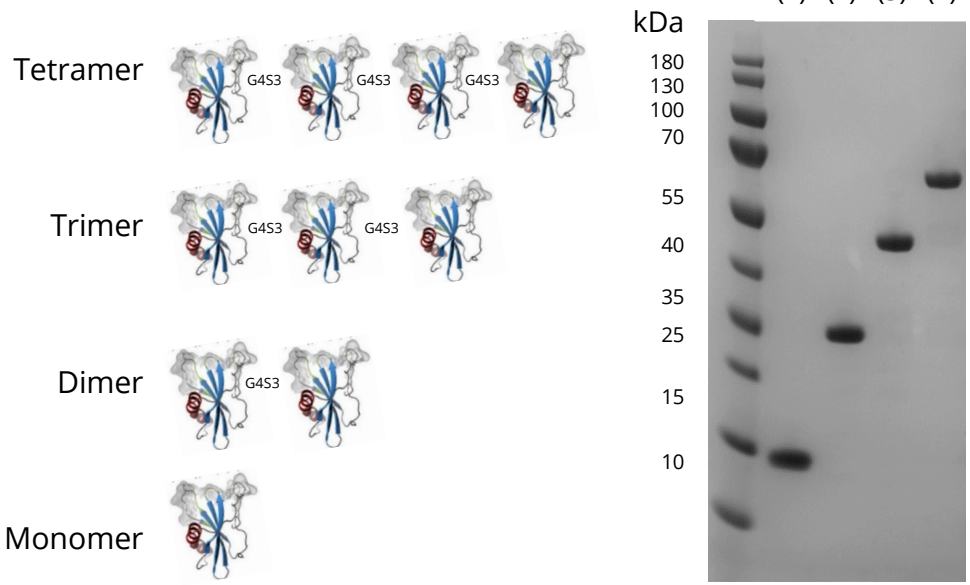
- Gene delivery (Moderna Tx Inc, FIT Biotech)
- CAR-T (Memorial Sloan Kettering)
- Drug conjugates (Glythera)

Formatting: Affimer Multimers

Easily expressed in *E. coli* to generate highly potent molecules

Affimer®

Human PD-L1 Affimer Antagonist

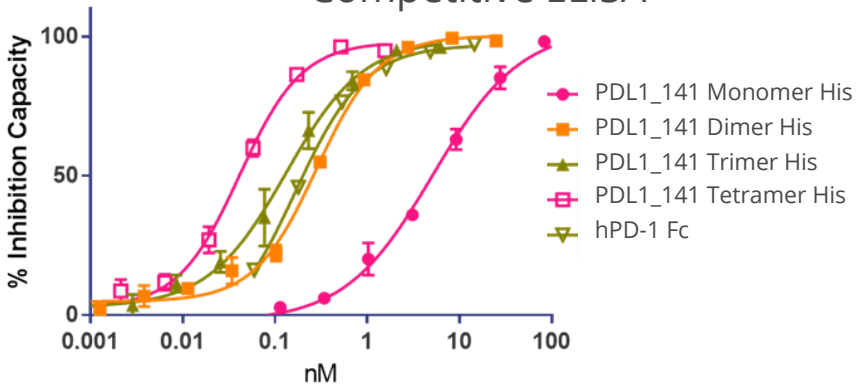


Format of the Affimer protein	Expected MW (kDa)	Yield after one-step purification (mg/L)
Monomer His	14	270
Dimer His	25	278
Trimer His	42	212
Tetramer His	56	205

Biacore

Protein	k_a (1/Ms)	k_d (1/s)	Apparent K_D (M)	Chi^2 (RU^2)
Monomer His	2.81E+06	2.51E-02	8.91E-09	0.0740
Dimer His	1.12E+06	5.35E-04	4.79E-10	0.0251
Trimer His	1.13E+06	4.73E-04	4.18E-10	0.0153
Tetramer His	1.01E+06	3.48E-04	3.44E-10	0.0292

Competitive ELISA

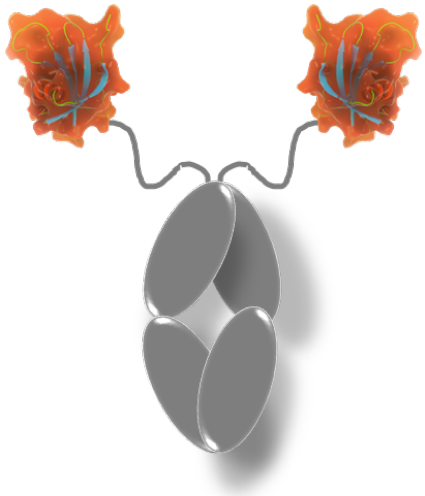


IC_{50} (nM)				
hPD-1 Fc	Monomer His	Dimer His	Trimer His	Tetramer His
0.17	2.5	0.28	0.13	0.04

Formatting: Half-life Extension

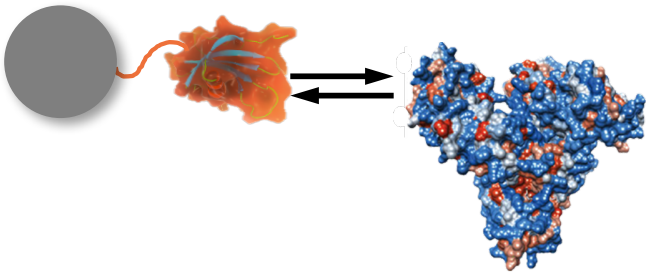
Methods for extending the serum half-life of Affimers

Affimer®



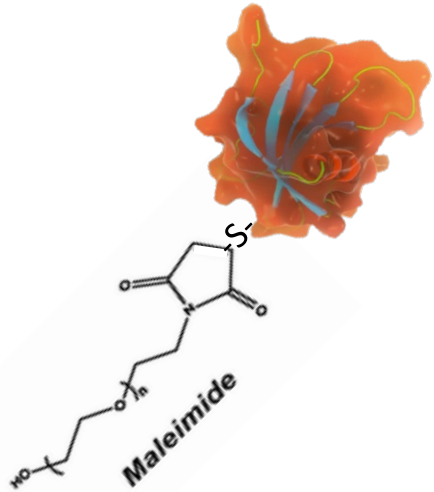
Fc Fusions

Utilising IgG FcRn recycling to maintain high serum levels



Serum Albumin

Affimer binds to SA in the circulation to extend the half-life of a payload



PEGylation

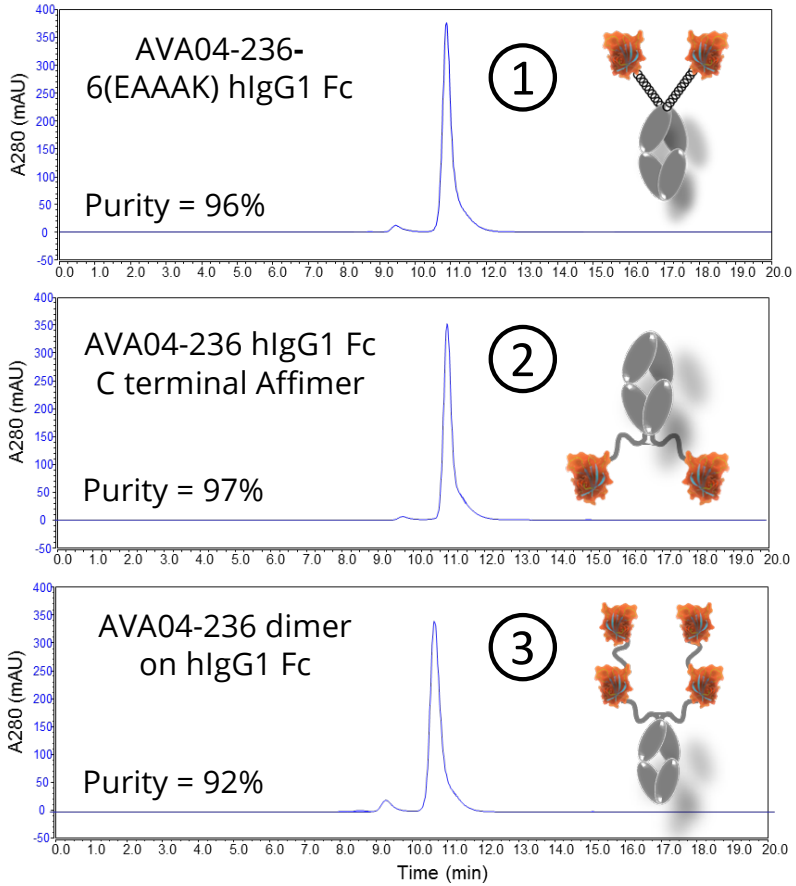
Increases the hydrodynamic size of the protein

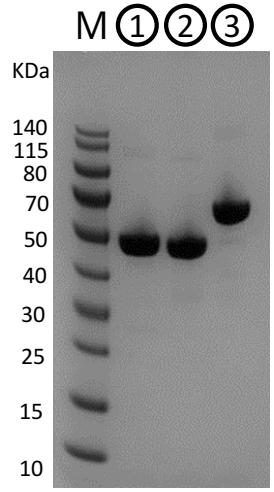
Affimer Fc Fusion Formats

Affimer Fc fusions are expressed easily in mammalian systems

Affimer®

- PoC to demonstrate that Affimers can be formatted at various sites on an Fc, and so should translate to IgG-Affimer fusions.
- Constructs were expressed in HEK239 cells and purified using standard Pr A-sepharose affinity chromatography.
- Typical (unoptimised) expression yields in the range 400-800 mg/l.
- Analytical SEC-HPLC used to assess purity.





Reducing SDS-PAGE

Affimer Fc Fusion Formats

Affimer Fc fusions show expected avidity effects

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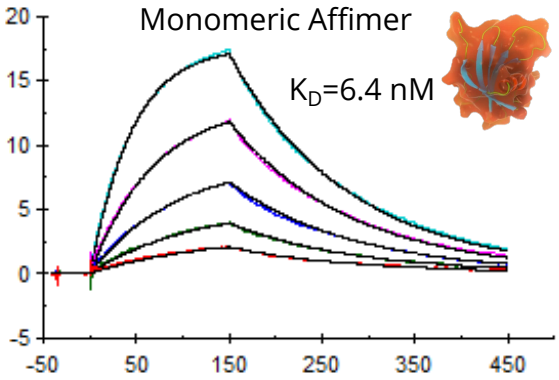
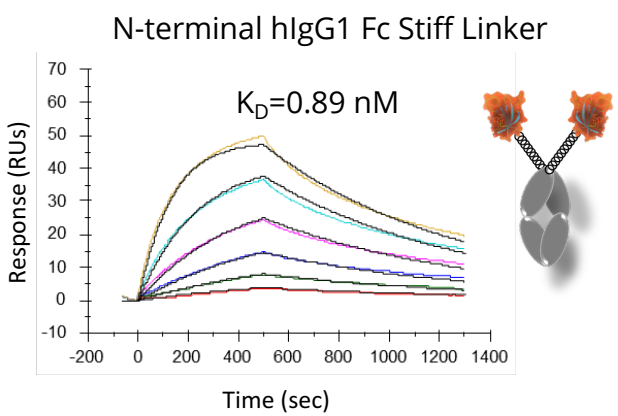
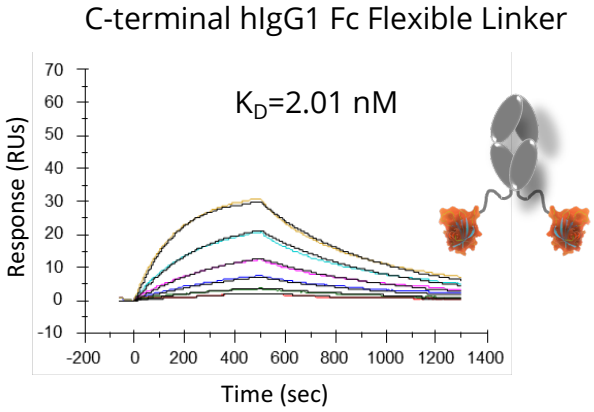
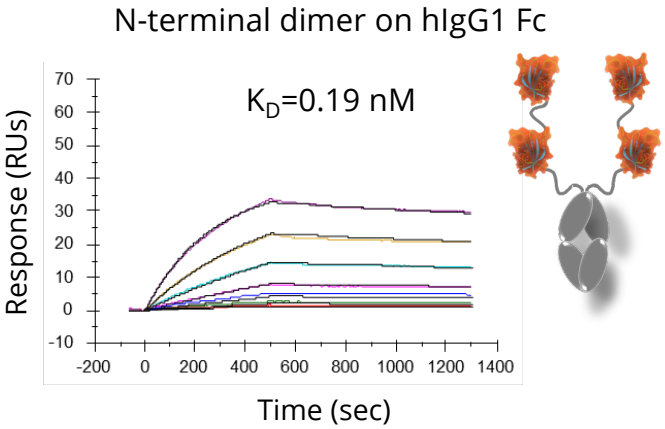
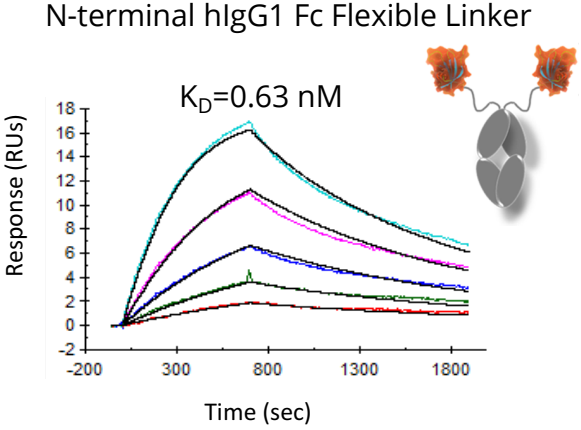
- K_D of several PD-L1 Affimer Fc formats determined using Biacore.
- PD-L1 Fc antigen was immobilised onto the chip surface.
- Avidity effects clearly observed.









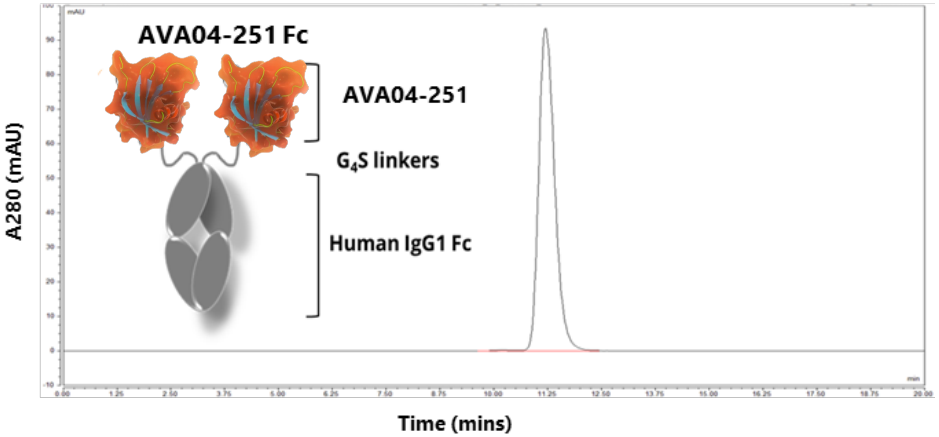


Affimer Fc Fusion Formats

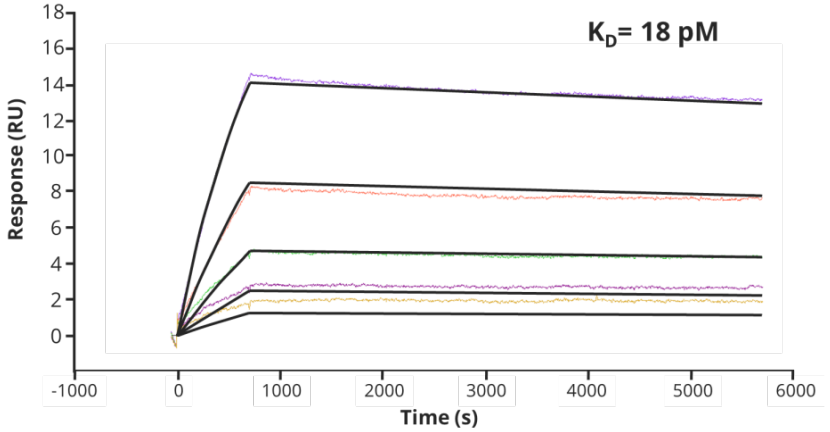
Comparison with monoclonal antibodies

Affimer®

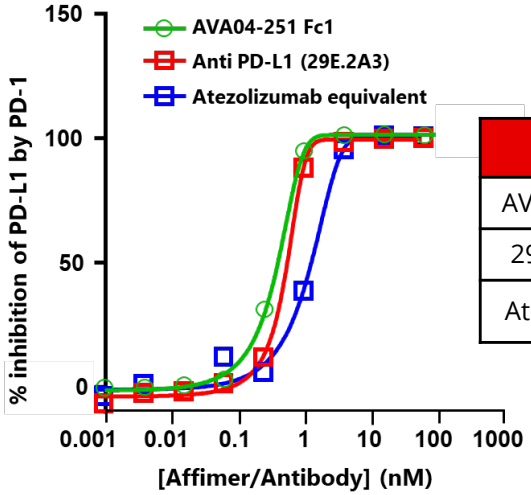
AVA04-251 hFc1 SEC-HPLC



AVA04-251hFc1 Biacore Kinetics



PD-1/PD-L1 Competition ELISA



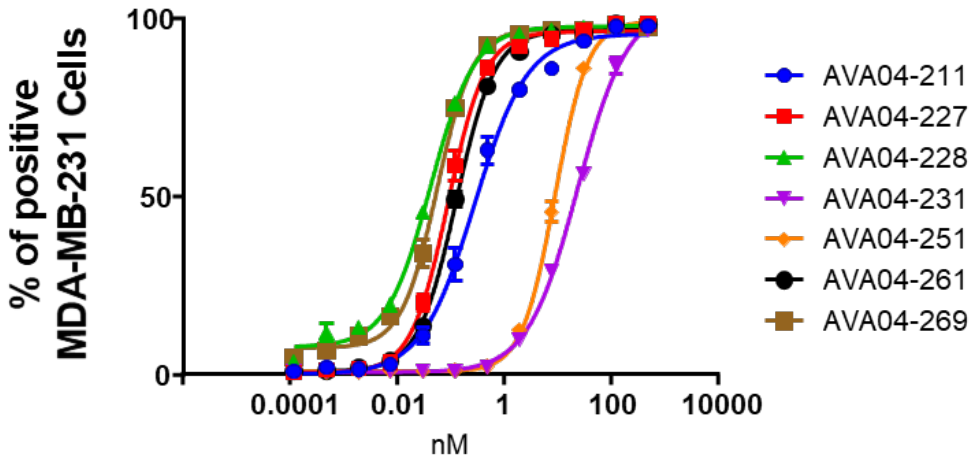
Clone	IC_{50} (nM)
AVA04-251 Fc1	0.4
29E.2A3 mAb	0.5
Atezolizumab”	1.2

PD-L1 Affimer Monomer Cell Binding

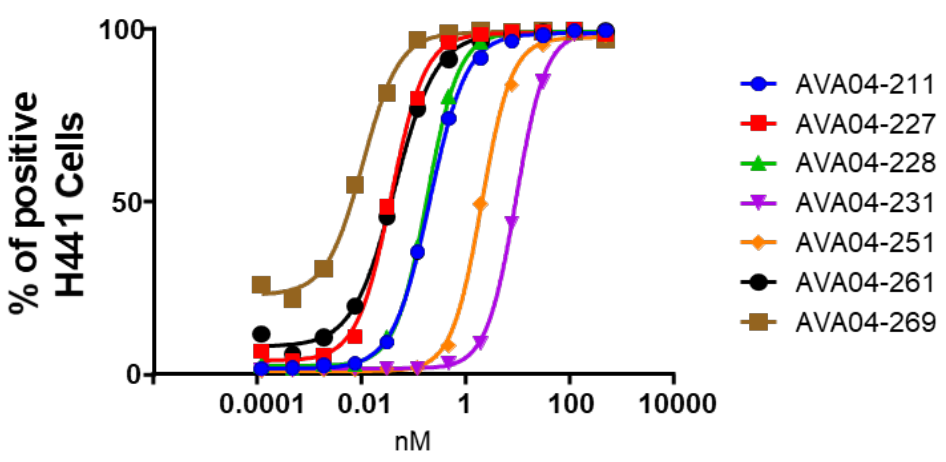
Affimer binding by flow cytometry on two different cancer cell lines

Affimer®

Human Breast Adenocarcinoma Cells



Human Lung Adenocarcinoma Cells



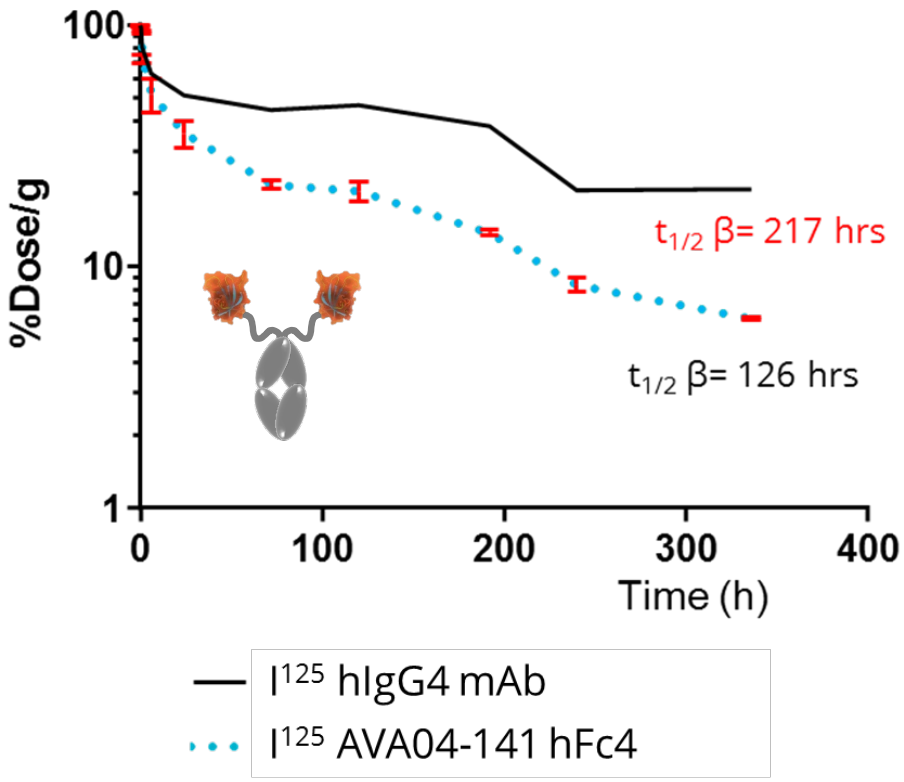
	AVA04-211	AVA04-227	AVA04- 228	AVA04-231	AVA04-251	AVA04-261	AVA04-269
H441 Cells EC_{50} (nM)	0.21	0.04	0.18	9.51	2.06	0.043	0.01
MDA-MB-231 Cells EC_{50} (nM)	0.28	0.09	0.04	24.11	8.75	0.13	0.06

PK of Fc Fusion PD-L1 Affimer in Mouse

Human PD-L1 with human IgG4 fusion

Affimer®

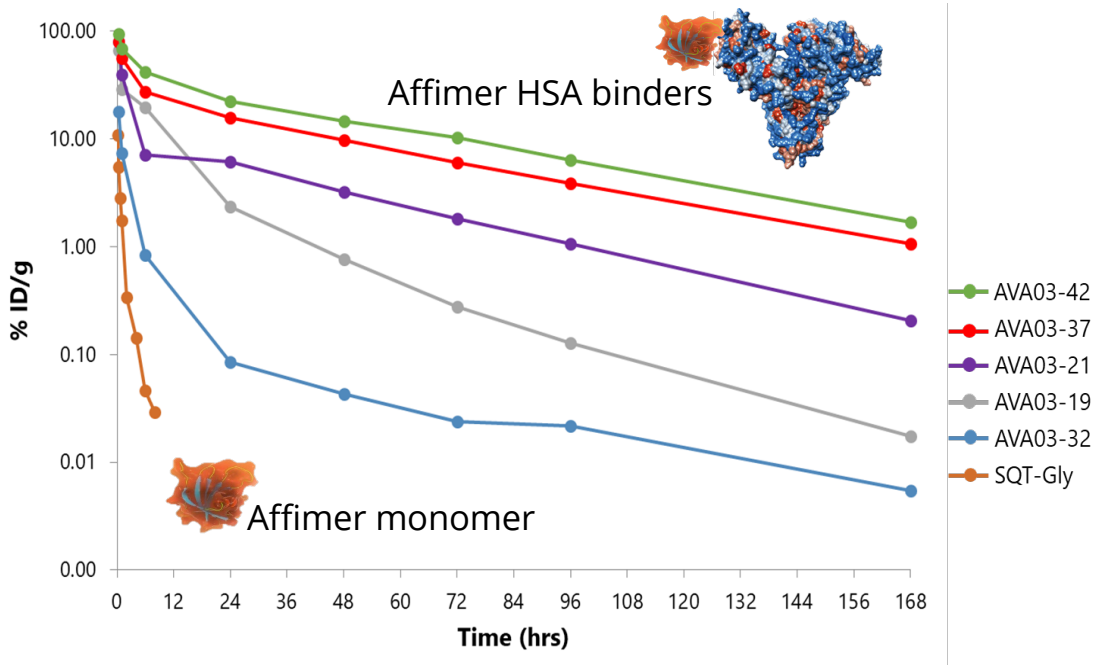
- Mouse PK of hPD-L1 Affimer Fc fusion.
- AVA04-141 hFc4 does not bind mouse PD-L1 and has a human IgG4 Fc.
- Dosed animals via the IV route (10 mg/kg).
- Serum half-life of Affimer Fc fusion ~126 hrs.
- Serum half-life similar to other scaffold-Fc fusions.
- Serum half-life of isotype IgG4 mAb ~217 hrs.



PK of Albumin Binding Affimers

Albumin binding significantly extends the serum half-life

Affimer®



Clone	$t_{1/2}$ (hrs)	AUC 0-t h*µg/mL
AVA03-42	38.2	5,670
AVA03-37	37.7	3,435
AVA03-21	30.6	1,401
AVA03-19	24.3	1,059
AVA03-32	29.0	112
Non-binder	1.6	18.1

- Affimers that bind human serum albumin with a range of affinities have been generated.
- These Affimers also cross react with mouse serum albumin (and cyno).
- Affimers labelled with I^{125} and dosed at 10 mg/kg (IV).
- HSA binding significantly extends the serum half-life ($t_{1/2}$) in mouse.
- The half-life can be tuned via the affinity to albumin.
- Albumin binding Affimers give the option for manufacturing in *E. coli* if the "payload" can be produced in bacteria as well.

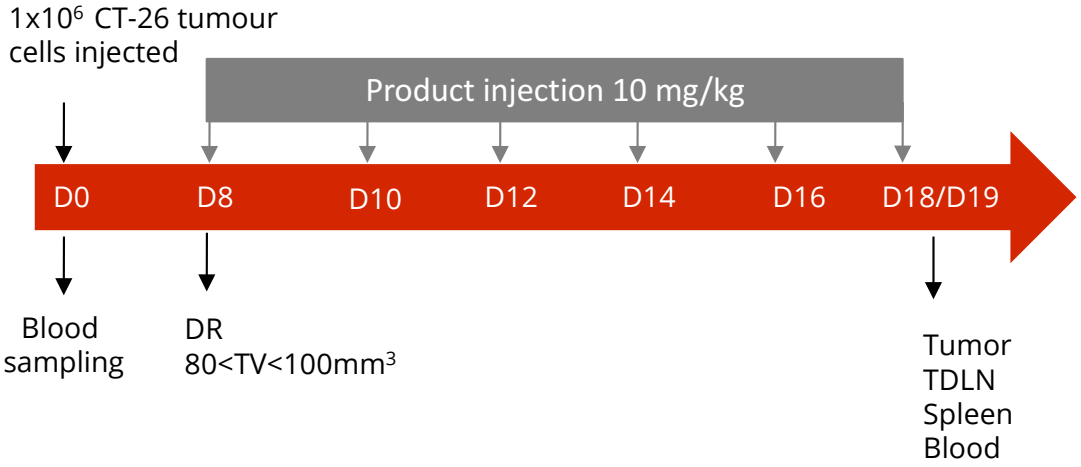
In-vivo Efficacy : PD-L1 Antagonist

CT26 syngeneic model

Affimer®

- Mouse CT26 syngeneic tumour model.
- Fc formatted mPD-L1 Affimer antagonist benchmarked against 10F9.G2 mAb.
- Dosing at 10mg/kg commenced when sub-cutaneous CT26 tumour volume was 80-100mm^3.
- Tumour volume measurements taken from day 8 following randomisation of the animals.
- Tumour, tumour draining lymph nodes, spleen and blood samples taken at day 18/19 for flow cytometry analysis.

Arm	Dose (mg/kg)	Route	N	Strain
Control Human Fc IgG1	10	IP	10	Balb/C
Affimer 182-hFc1	10		10	
mAb (10F9.G2)	10		10	
Isotype Control (Rat IgG2b	10		1	



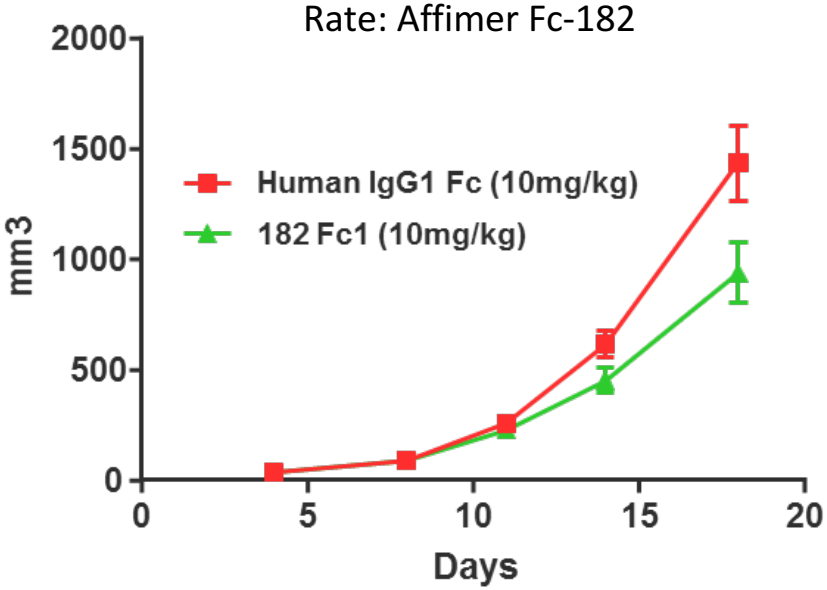
In-vivo Efficacy : PD-L1 Antagonist

CT26 syngeneic model

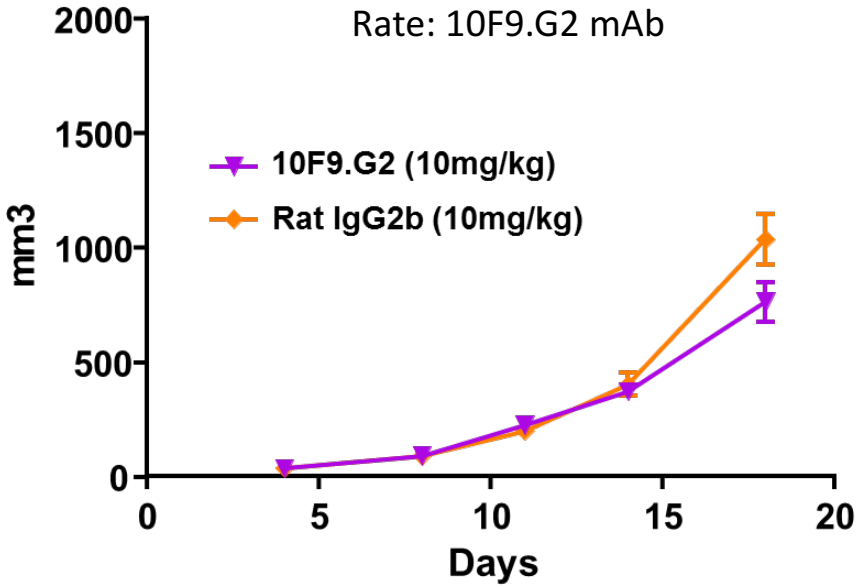
Affimer®

- No macroscopic sign of toxicity or disease dissemination was recorded at the autopsy of mice.
- No significant body weight difference between groups during the D8-D19 period.
- Repeat high dosing of anti-mPDL1 Affimer was well tolerated.
- Anti-tumour effect seen with anti-mPDL1 Affimer comparable to 10F9.G2 mAb.

Reduction in Tumour Growth Rate: Affimer Fc-182



Reduction in Tumour Growth Rate: 10F9.G2 mAb

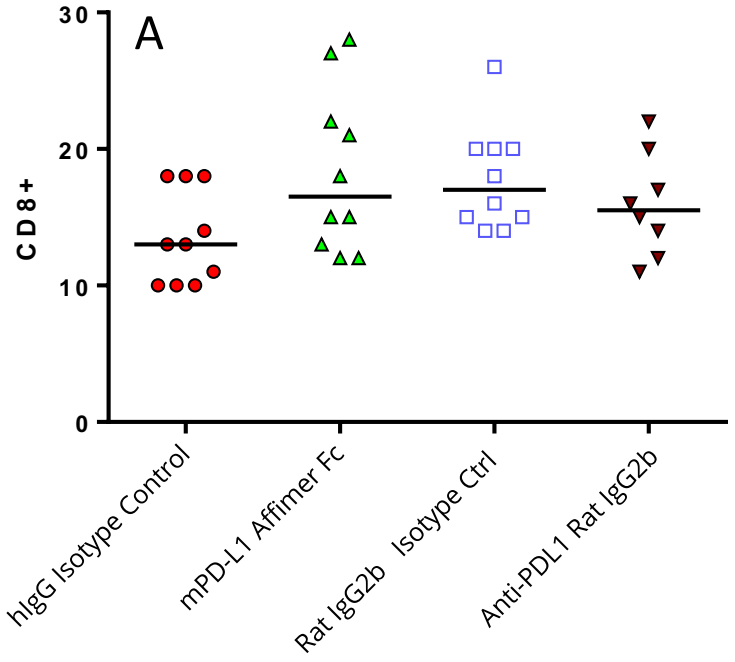


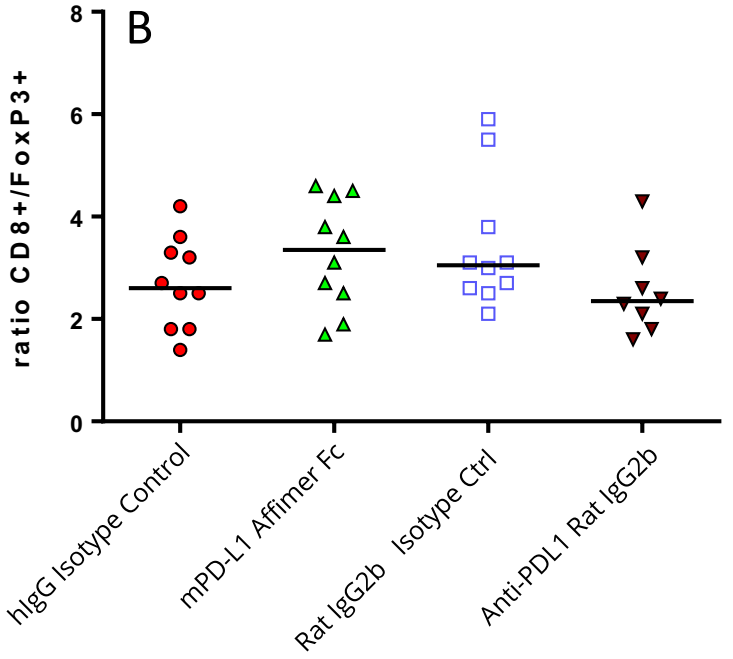
In-vivo Efficacy : PD-L1 Antagonist

CT26 syngeneic model

Affimer®

- In this anti-PD-1 resistant CT-26 mouse syngeneic model mPD-L1 Affimer induces intratumoural :
  - recruitment of $CD8^+$ T-cell (panel A),
  - decrease of $CD4^+$ T-cell (not shown),
  - change in $CD8^+/FOXP3^+$ ratio (panel B),
- This immunological response differentiates the mPD-L1 Affimer Fc fusion from the anti-PD-L1 reference antibody (clone 10F.9G20)
- Antibody response is directed mainly against Fc part of the Affimer Fc fusion.
- No neutralising antibodies detected.



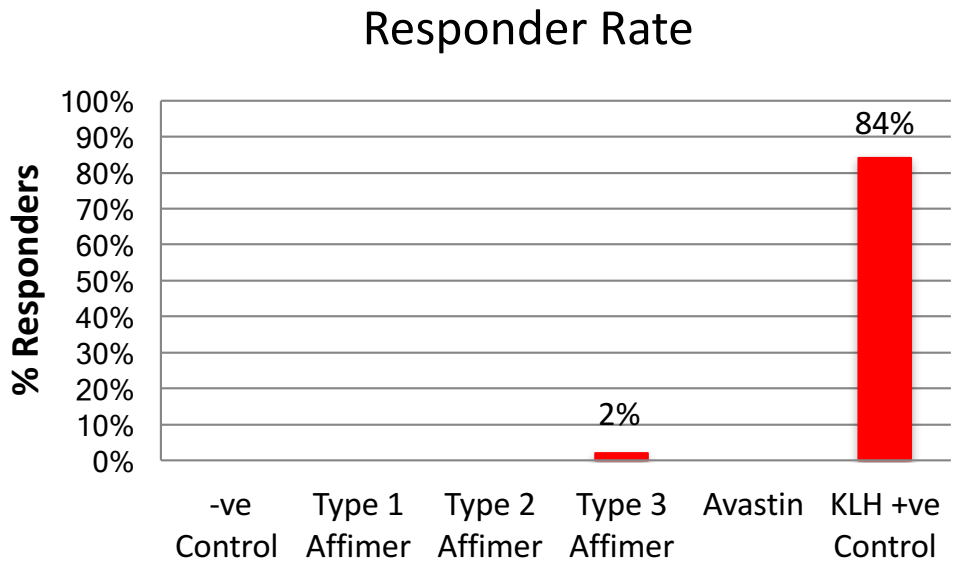


PBMC Study Responder Analysis

No immunogenicity of core Affimer technology

Affimer®

- Cryopreserved PBMC samples from 50 healthy donors selected to represent the different HLA-allotypes in the human population.
- No responders in this set of 50 donors to T1, T2 Affimers or Avastin.
- One responder (2%) to T3 Affimer scaffold just above threshold.
- The Affimer test products were produced in-house under non-GMP conditions.
- The Affimer test products have been tested at five times the concentration of Avastin.



Pipeline

Leveraging the key benefits of the Affimer technology

Affimer®

Programme		Discovery	Lead Optimisation	Pre-clinical	Phase I
Bispecific PD-L1/LAG3					
AVA-004	PD-L1 Antagonist				
AVA-017	LAG-3 Antagonist				
AVA-003	HSA Half-life Extension				
Drug Conjugates					
Glythera	Undisclosed				
AVA-020	5T4				
T-cell Engagers					
AVA-008	CD19				
AVA-002	CD3ε				
AVA-012	CD22				
Agonists					
AVA-014	CD27				
AVA-018	GITR				

Pipeline

Leveraging the key benefits of the Affimer technology

Affimer®

Programme		Discovery	Lead Optimisation	Pre-clinical	Phase I
Gene Delivery					
Moderna	Multiple undisclosed				
FIT Biotech	PD-L1 PoC	Data expected Q2 2018			
Collaborations					
Sloan Kettering	CD19/CAR-T				
Leeds NHS Trust	Fibrinogen/α-2-antiplasmin				

Non-Therapeutic Applications

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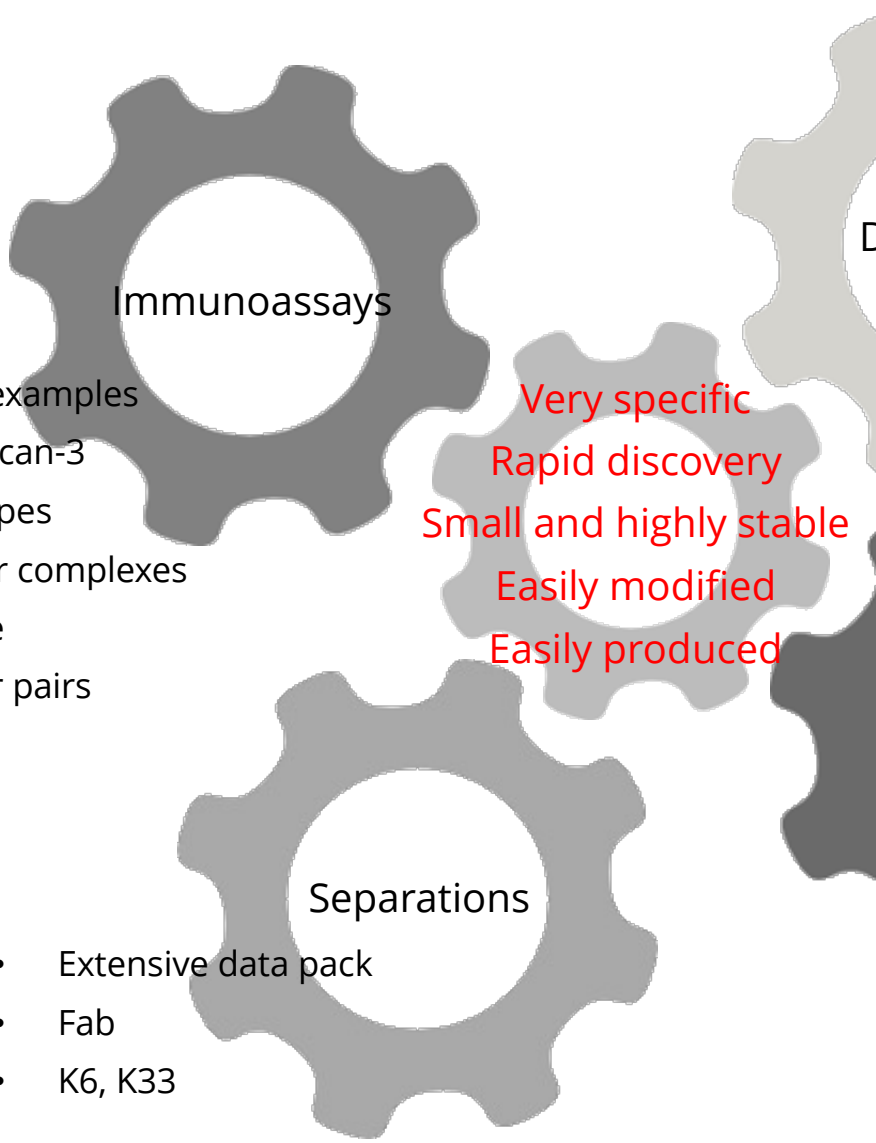


- Wide range of examples
- CRP, ToxB, glypican-3
- Antibody idiotypes
- Ligand-receptor complexes
- Affimer capture
- Affimer-Affimer pairs

Very specific
Rapid discovery
Small and highly stable
Easily modified
Easily produced

- Biosensors
- Lateral flow devices
- ELISA
- Competitive to sandwich assay conversion

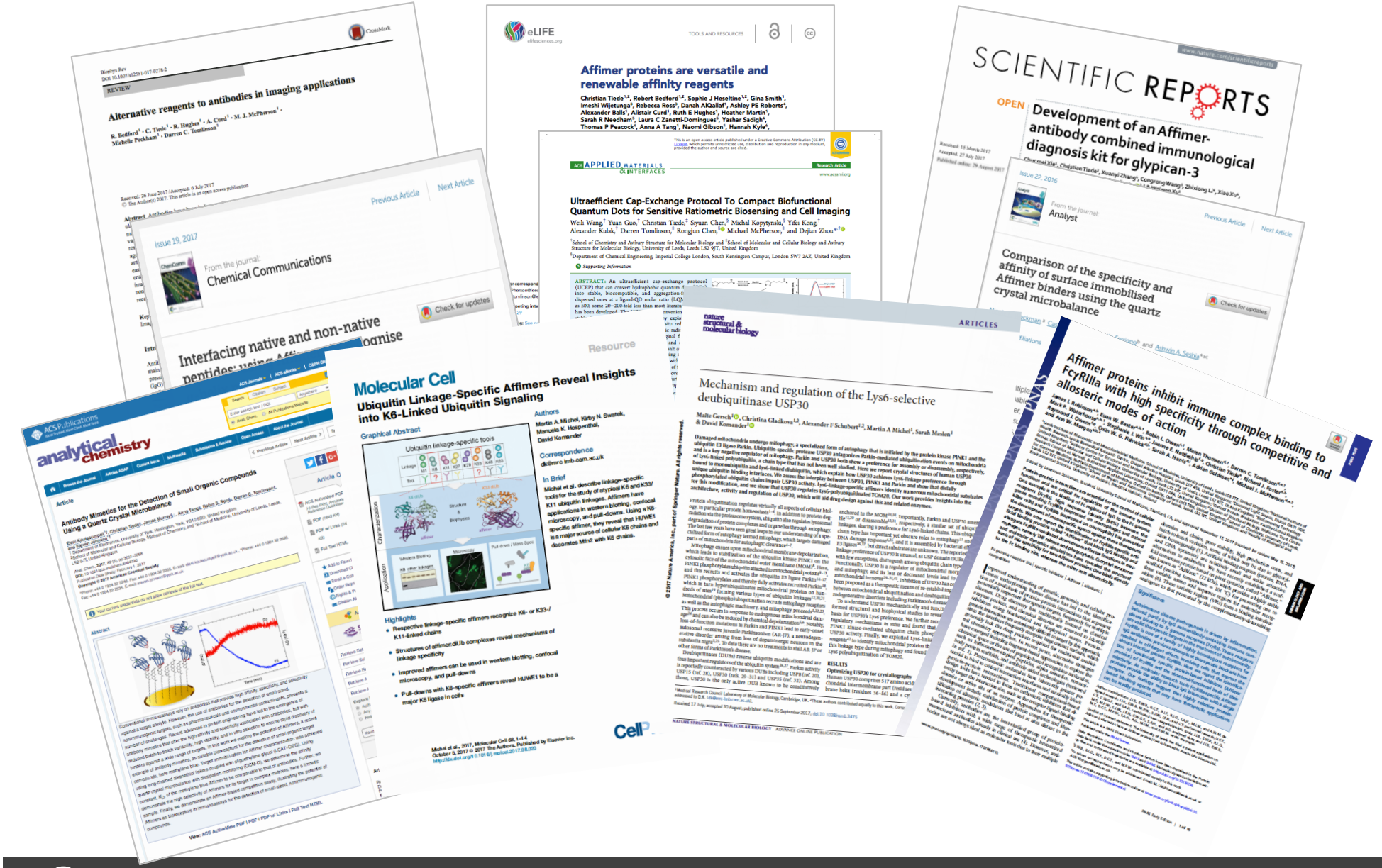
- Extensive data pack
- Fab
- K6, K33

- Wide range of examples
- Crystallisation chaperones
- Intracellular inhibitors
- Imaging reagents
- Immuno-precipitation
- Di-ubiquitin linkage binders

Non-therapeutic Applications

Immunoassays, imaging, cancer diagnostics, microscopy ….

Affimer®



Affimer® Proteins

Affimer®

- Flexible protein scaffold based on the cystatin fold proven to be capable of quickly generating highly specific, single digit nM binders to a broad range of target classes.
- Affimer proteins are easily formatted as multimers and Fc fusions with high expression levels.
- Affimer scaffold is well tolerated in vivo, has a low immunogenicity risk and has demonstrated efficacy in the CT26 model.
- Type 1 and 3 Affimer proteins are a fully human version with low intrinsic immunogenicity, robust and with a simple structure, maintaining excellent expression levels when formatted.
- Type 2 Affimer proteins are based on plant cystatins, have excellent thermal and have been demonstrated in a wide range of non-therapeutic applications.
- Avacta is building a pre-clinical pipeline of Affimer leads with a focus on immuno-oncology with a view to entering the clinic in 2019/2020 and with a strong emphasis on partnering.
- Avacta is also providing Affimer reagents R&D use and licensing by third parties in non-therapeutic applications.

Thank you

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