isogenica

Bispecific Discovery:

where do we start – and where are we going?

March 2021

Isogenica

Excellence in Single Domain Biotherapeutics

- Isogenica develops LlamdA® VHH: highly versatile small format antibodies which we use to construct next generation biotherapeutics for the treatment of cancer, inflammation and other serious diseases
- Our experienced team learned their craft at Ablynx, Envigo, Bicycle Therapeutics, Roche and other leading biopharma
- Track record of delivering clinical assets through collaborative partnerships

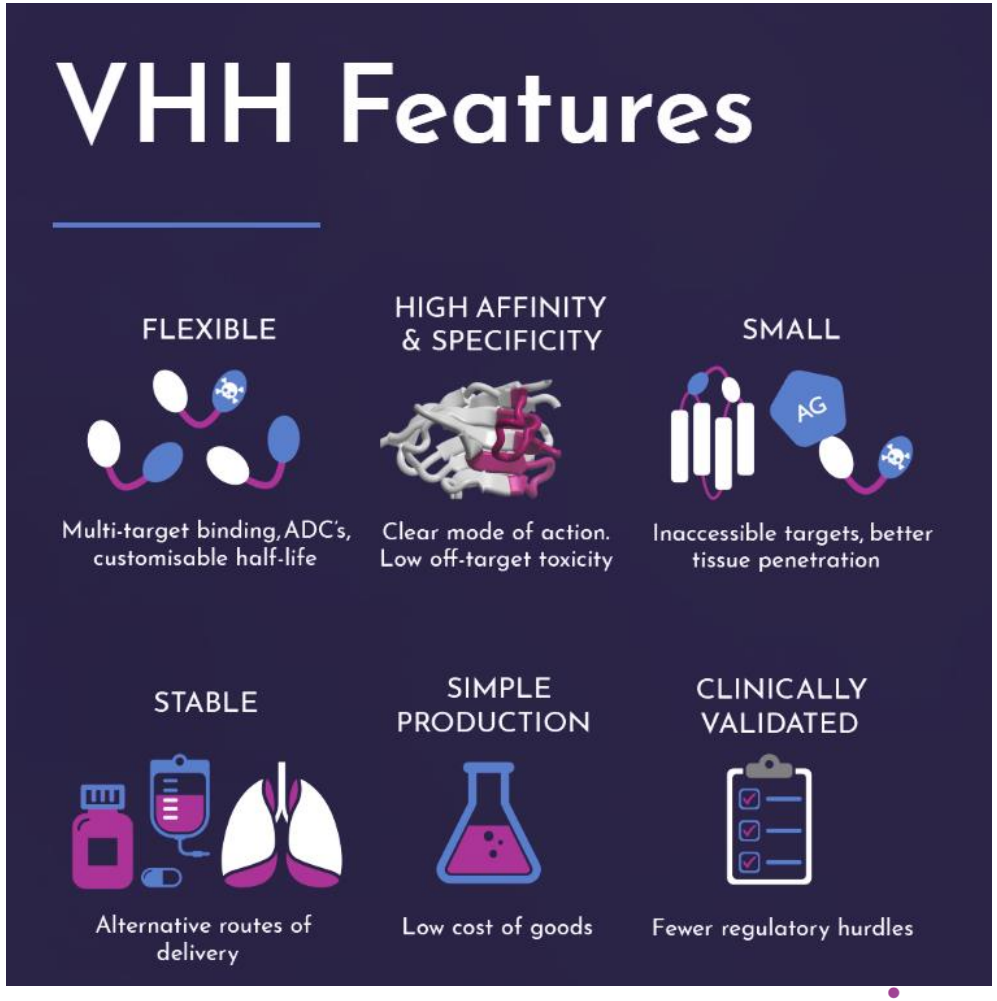
Dr. Ed McGowan
Senior Director, Antibody Discovery and Development

Dr. Mandeep Sehmi
Business Development Manager

LlamdA® Antibodies

LlamdA® VHH are liability-free monovalent antibodies.

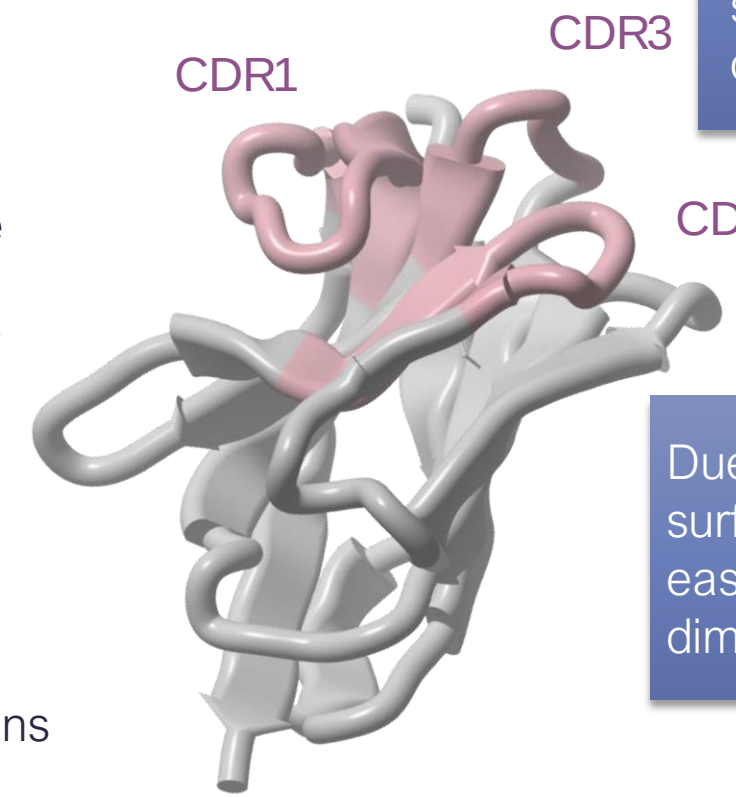
- Small in size (15 kDa)
- Biophysically robust
- Tuneable half-lives
- VHH
  - Targeting inaccessible epitopes
  - Achieve enhanced tissue penetration
  - Multi-valent binding and formatting for payload delivery
- LlamdA® VHH are free from CMC liability motifs (e.g. isomerisation, deamidation, glycosylation, free cysteine, etc.)



VHH are excellent building blocks for novel biotherapeutics

Stability and Engineering

- Small, stable molecules
  - 15kDa
- Considerably easier to engineer
  - Naturally stable and have no quaternary structure
- Easily linked **covalently** to other biologics or pro-drugs (ADC)
- Less immunogenic than other single chain constructs e.g. ScFv
  - high homology with human VH genes
  - absence of exposed hydrophobic antigenic regions

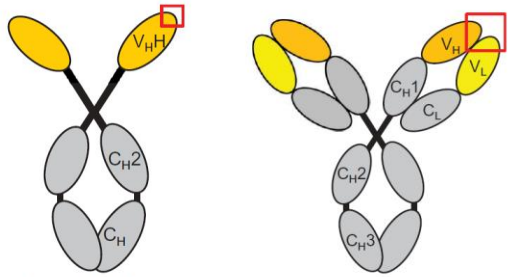


The CDR3 loop of VHH is long and has a prolate shape, exposing a convex paratope

Due to their hydrophilic surfaces, VHH can be easily linked to create dimers or multimers

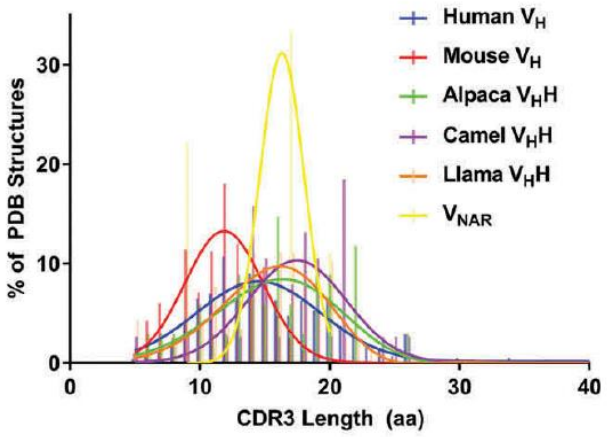
VHH comparative to human VhVl

Similar composition – advantageous size

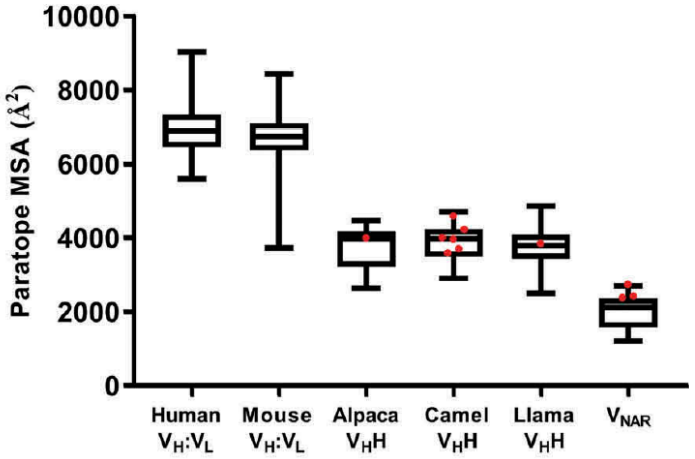


Camelid heavy chain-only IgG

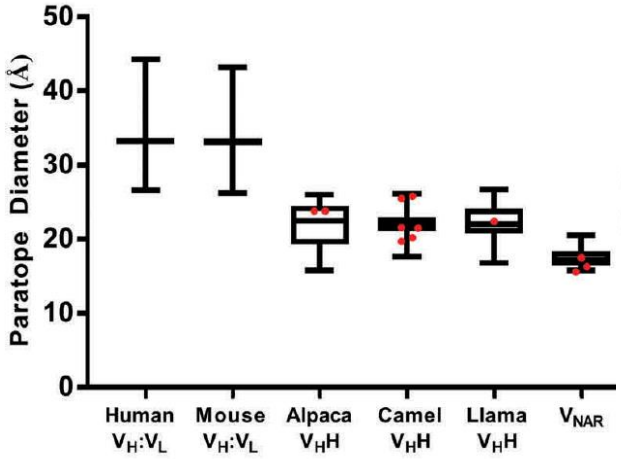
Conventional IgG



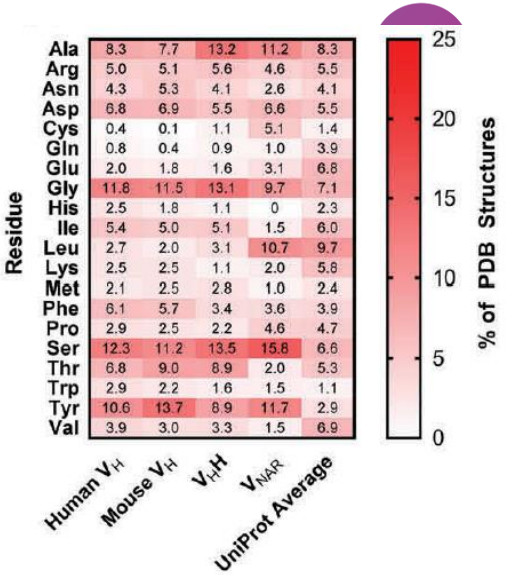
CDR3 length comparable to human VhVl



Smaller paratope area to human VhVl



Smaller paratope diameter to human VhVl



Comparable amino acid constituents to human VhVl

- Comparable composition and features to human paratope
- Smaller paratope by diameter and area

Antigen recognition by single-domain antibodies: structural latitudes and constraints

Kevin A. Henry & C. Roger MacKenzie

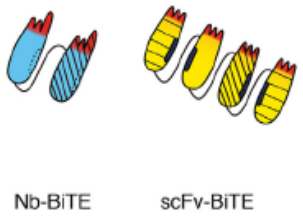
VHH advantages over VhVl

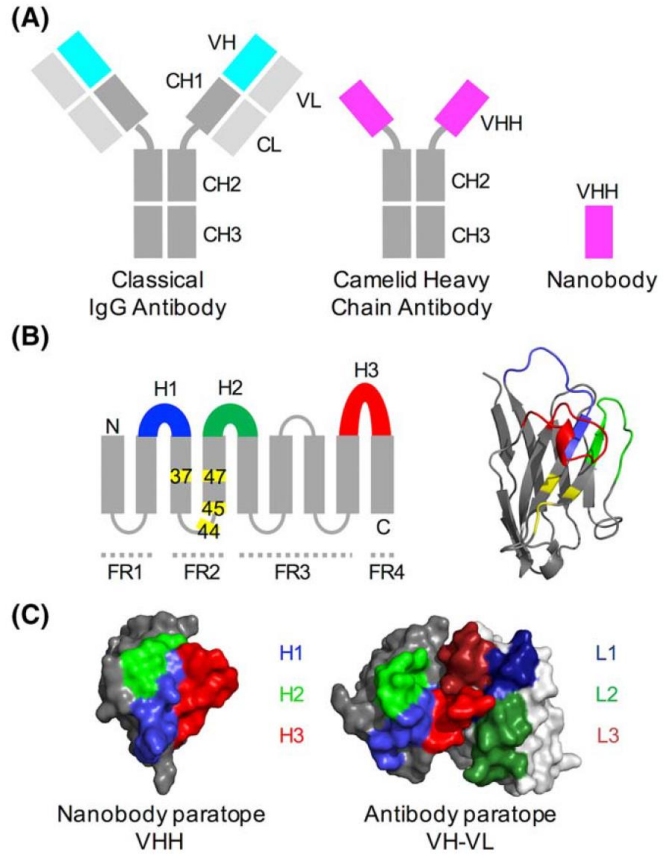
Stability and Engineering

- VHH can be easily engineered
  - No mispairing issues
  - *CDR3 loop is extended allowing identification of hidden / buried epitopes*

- ScFV (VH-VL)
  - Precautions required to prevent mispairing
    - Common light chain
    - Paratope via VH domain
  - Hydrophobic surfaces can dissociate and associate with 'other' hydrophobic surfaces
    - Impact solubility and stability

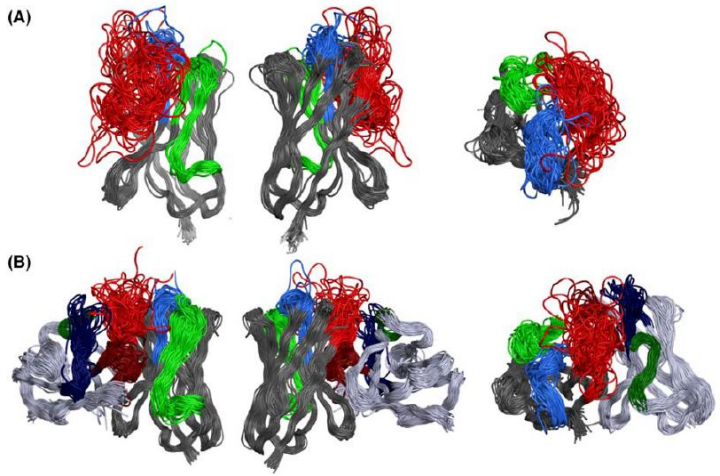
    - VHH considerably easier to engineer
      - Naturally stable
      - No quaternary structure





VHH

VH-VL



Nanobodies and Nanobody-Based Human Heavy Chain Antibodies As Antitumor Therapeutics

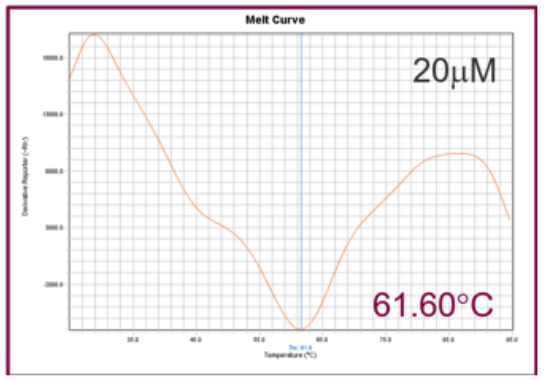
*Peter Bannas[1], Julia Hambach[2] and Friedrich Koch-Nolte[2]**

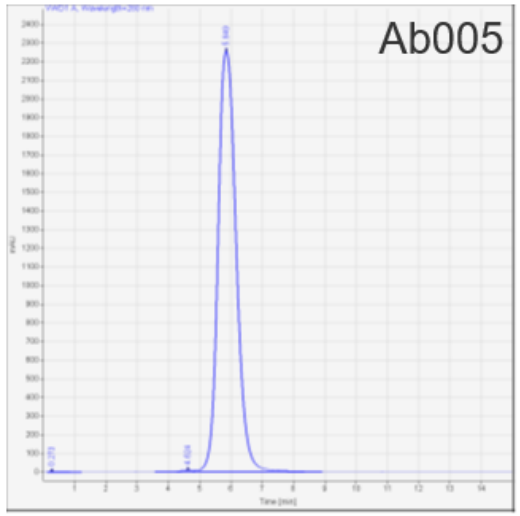
[1]Department of Diagnostic and Interventional Radiology and Nuclear Medicine, Hamburg, Germany, [2]Institute of Immunology, University Medical Center Hamburg-Eppendorf, Hamburg, Germany

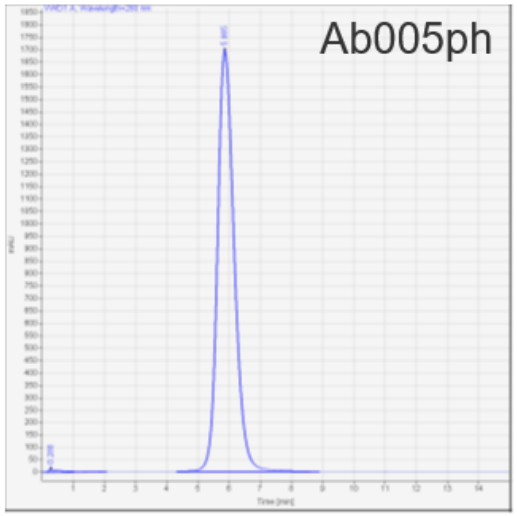
VHH Biophysical Characteristics

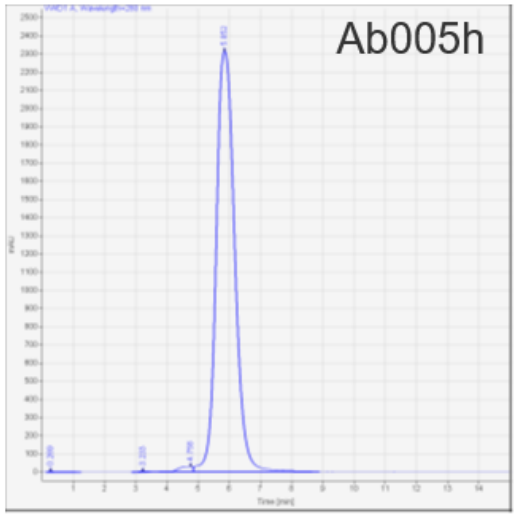
VHH Compare favourably to IgG and ScFv

Name	Tm, °C
Ab005	61.4°C (54.1°C)
Ab005ph	61.3°C (50.2°C)
Ab005h	61.6°C (52.8°C)







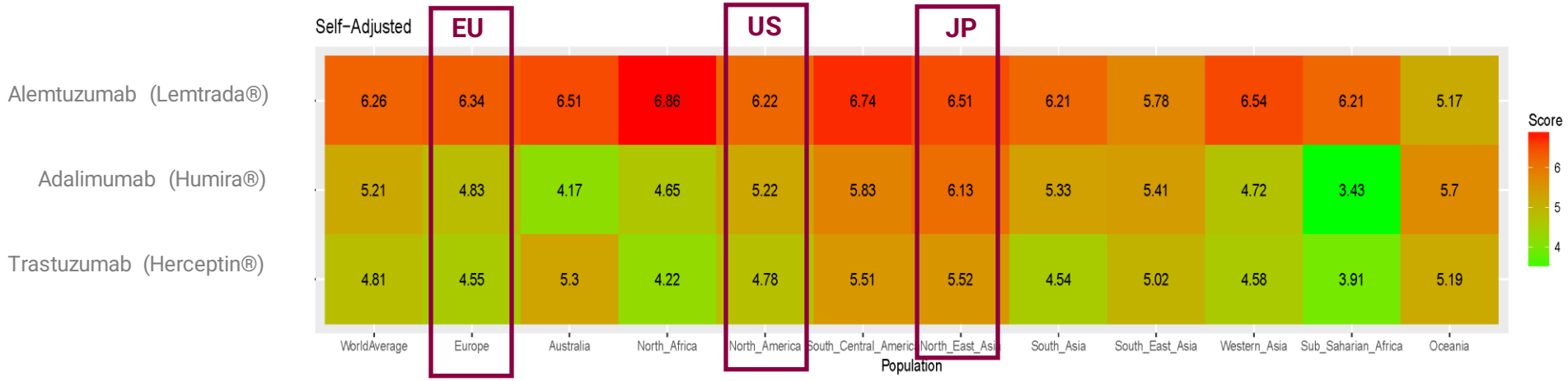


Typical
- Melting curve
- SEC trace

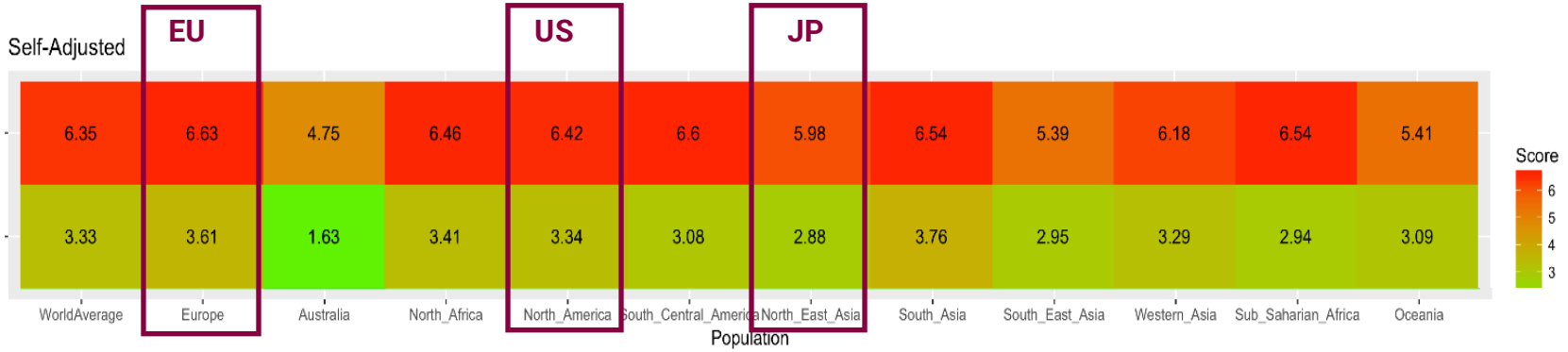
monomeric VHH

llamdA™ library: humanised frameworks reduce immunogenicity risk

Benchmarking VH immunogenicity risk (MHC binding score)



Example VHH immunogenicity risk (MHC binding score)



T-cell Immunogenicity Score

- VHH have low intrinsic immunogenicity potential – hydrophilic surfaces, small size, high human vH homology
- VHH with humanised frameworks are less likely to elicit immunogenicity

LlamdA®

Single domain VHH discovery engine

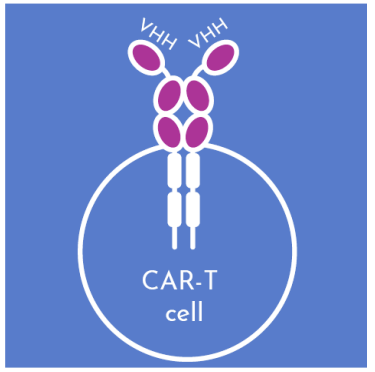
- Incorporating intelligent design with the precision of the COLIBRA® library construction system.
- LlamdA® is a state-of-the-art, highly diverse, synthetic VHH library which, when combined with the power of CIS display, interrogates more than 10^{13} library members: equivalent to the circulating repertoire of one million llamas.
- LlamdA® offers optimal expression of maximised functional diversity.

Applications of VHH Antibodies

VHH – flexible format for new biotherapeutic drugs

DIRECT THERAPEUTICS

VHH's small size makes them advantageous for isolating binders to therapeutically important but challenging targets such as GPCRs and ion channels

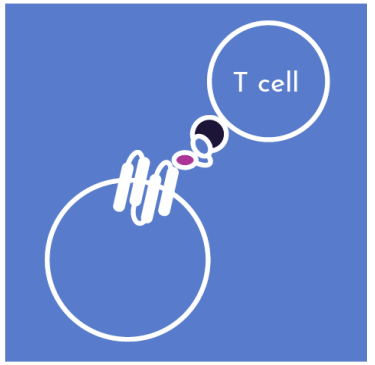


CELL & GENE THERAPIES

VHH offer a small, stable, highly manufacturable alternative to scFv as targeting agents in cell and gene therapies

ANTIBODY DRUG CONJUGATES

VHH enable more effective targeting of pharmaceutical agents (e.g. toxins, RNAi) to diseased tissues reducing side-effects associated with treatment



BISPECIFICS & MULTISPECIFICS

VHH-only bispecifics offer improved targeting and tissue penetration for solid tumours. VHH can also be combined with conventional antibodies to create novel bispecifics

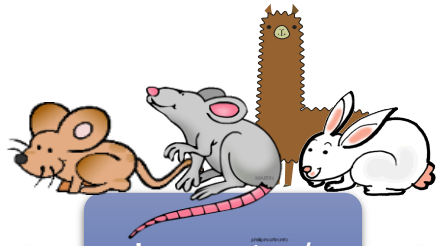
Clinically Validated Single Domain VHH Antibodies

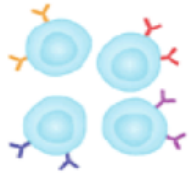
- VHH single domains are one of the most **accepted** next generation antibody therapeutic formats.
- Cablivi (caplacizumab) is the first FDA approved single domain antibody therapeutic.
- So far, VHH are being developed in
  - Monomeric
  - Bispecific
  - Half-life extended
  - CAR-T formats

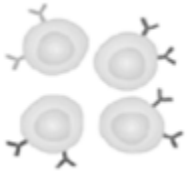
Drug	Sponsor	Domain Properties	Target	Indication	Status
Caplacizumab	Sanofi (Ablynx)	VHH	vWF	aTTP	Approved
Ozoralizumab	Taisho (Ablynx)	VHH	TNF	Rheumatoid Arthritis	Phase III
M1095	Avillion / Merck KGaA (Ablynx)	VHH Bispecific	IL-17A IL-17F	Psoriasis	Phase IIb
LCAR-B38M	Legend/ Janssen	VHH into CAR-T	BCMA	R/R Multiple Myeloma	Phase III (NDA/BLA US filing Dec 2020)
V565	Vhsquared	VHH	TNF	Inflammation	Phase II
M6495	Merck KGaA/ Novartis (Ablynx)	VHH	ADAMTS5	Osteoartheritis	Phase II
BI836880	Boehringer Ingelheim (Ablynx)	VHH Bispecific	VEGF, Ang2	Anal cancer	Phase II
BI655088	Boehringer Ingelheim (Ablynx)	VHH	CX3CR1	Renal Disease	Phase 1

Synthetic VHH Library

Developable therapeutic leads - faster





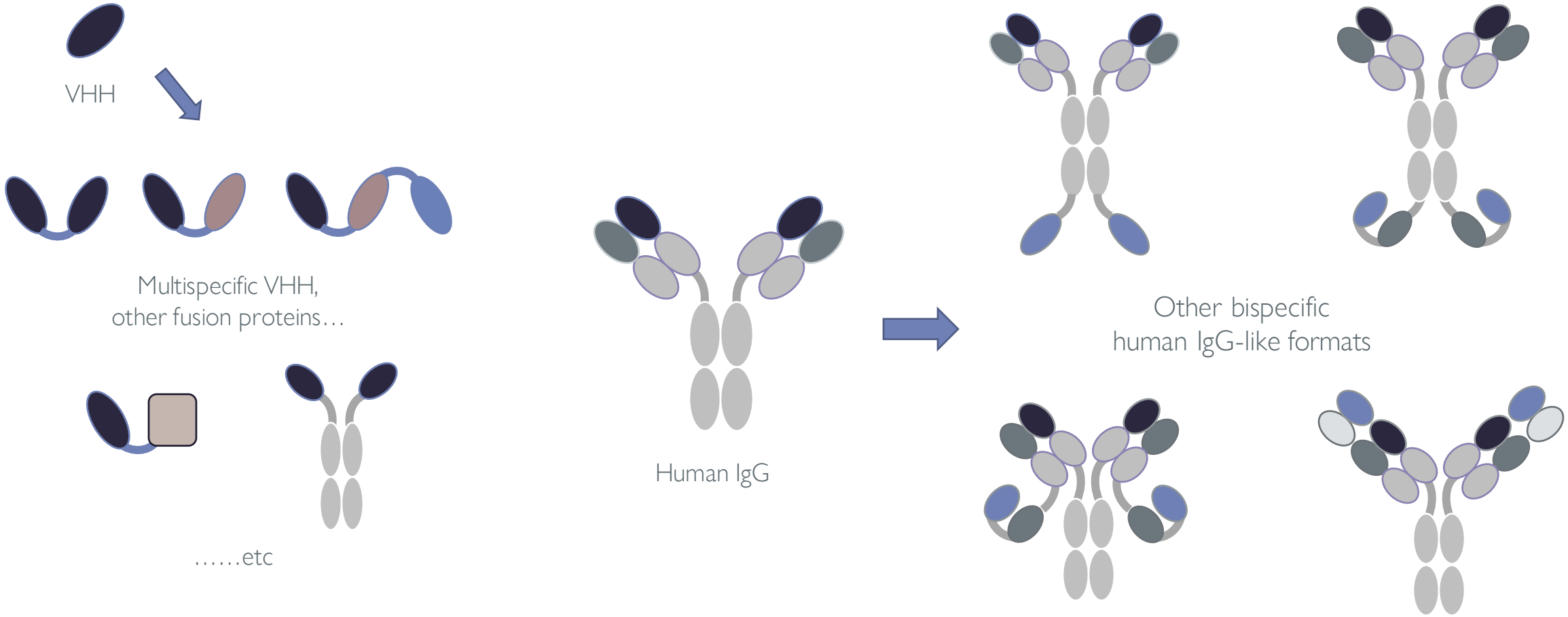


Large scale antigen prep → Immunize/ boost → Sample/ library generation → Selections → Primary/ Secondary screenings → (Humanize) CMC optimize

Large scale antigen prep → Immunize/ boost → Sample/ library generation → Selections → Primary/ Secondary screenings → Humanize/C MC optimize

Next generation biotherapeutics - formatting

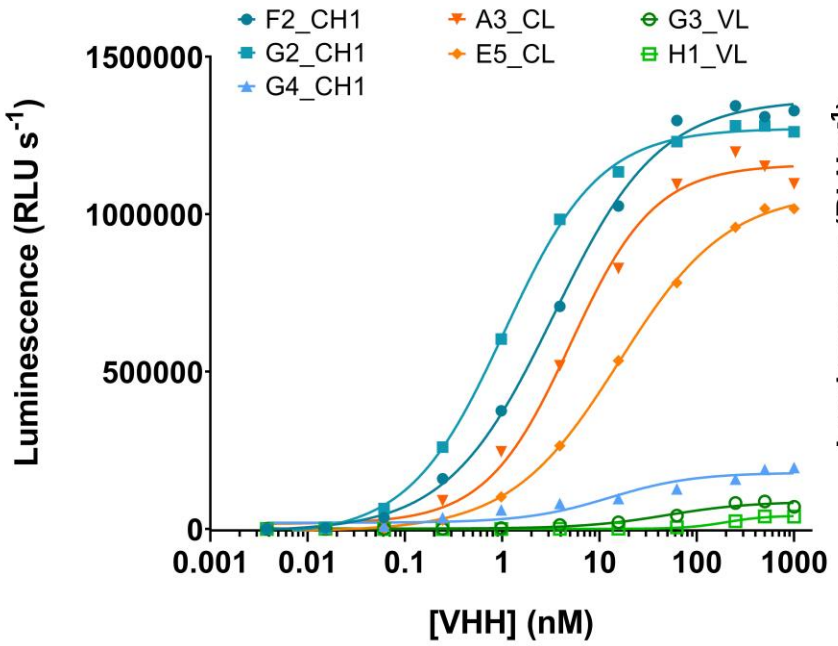
Diversity in formats

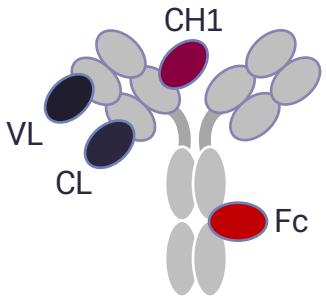


Isogenica is developing novel formatting options to enable multi-valent presentation

Reformatting into multivalent VHHs (multiple clones, different epitopes)

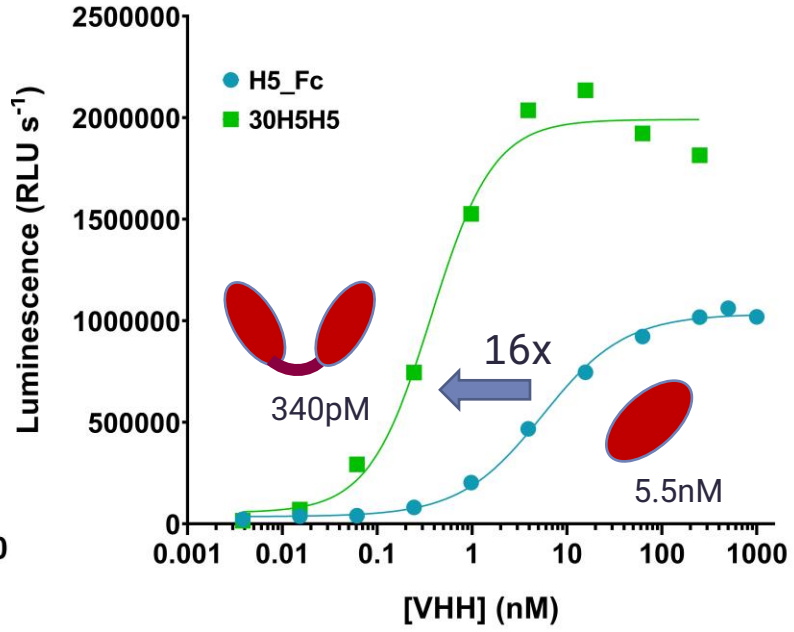
Hu IgG binding VHHs: epitope diversity



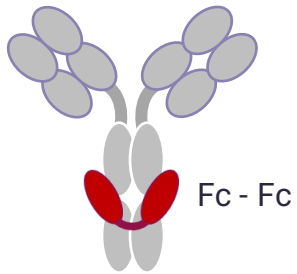


Human IgG

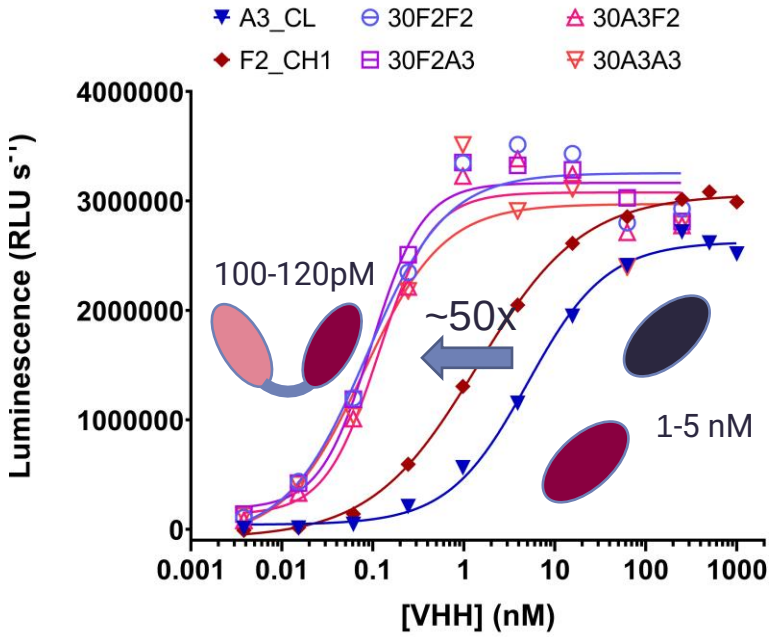
Symmetric bivalent to bivalent epitope



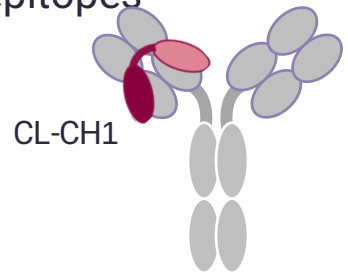
Avidity to repeat epitope



Asymmetric bivalents to different epitopes



Avidity to adjacent epitopes

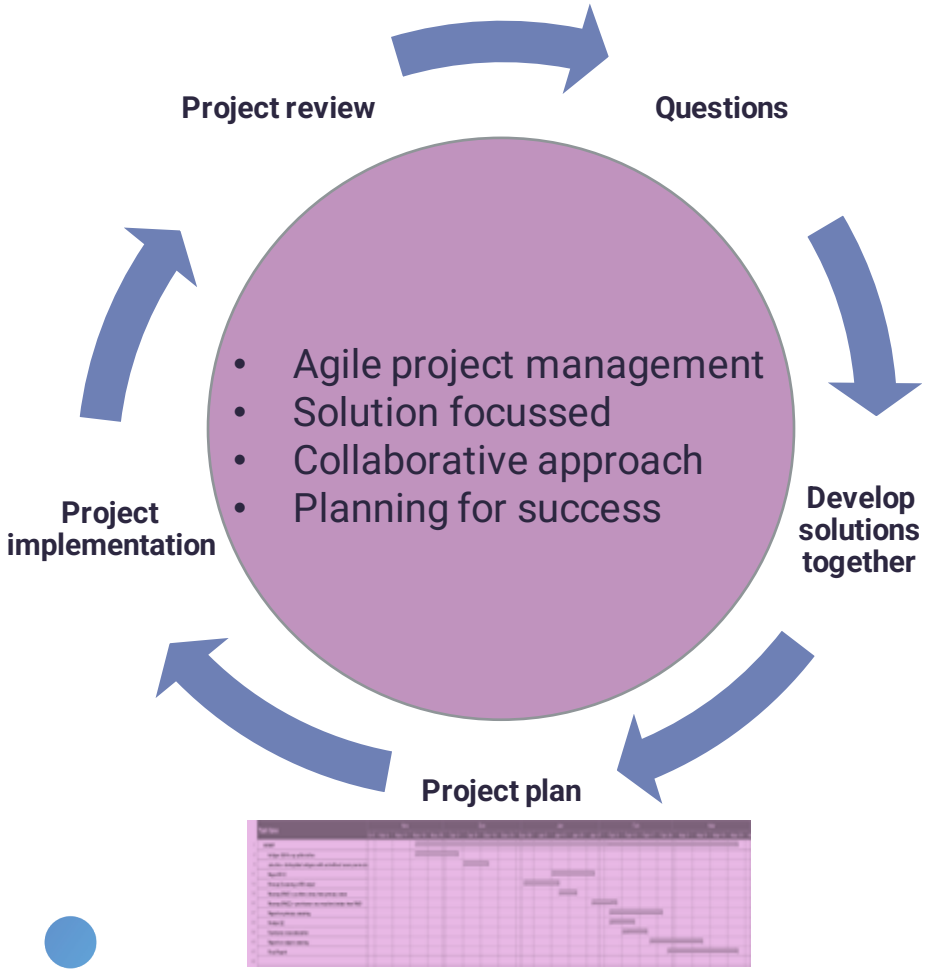


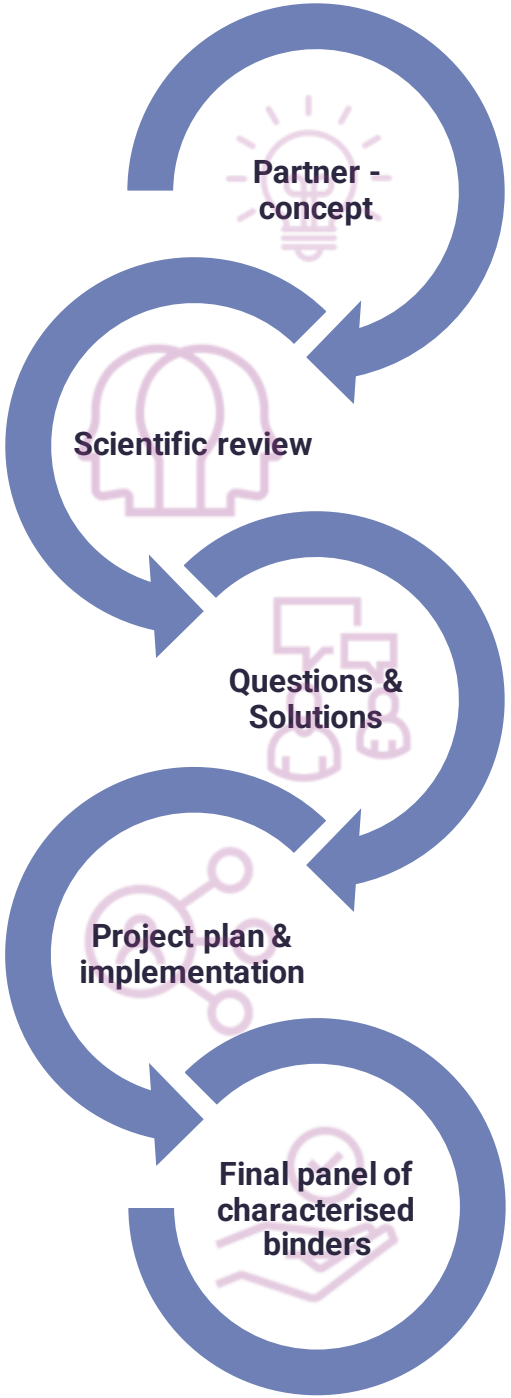
Unpublished data, Isogenica Ltd

Project design

Starting a Partnership with Isogenica

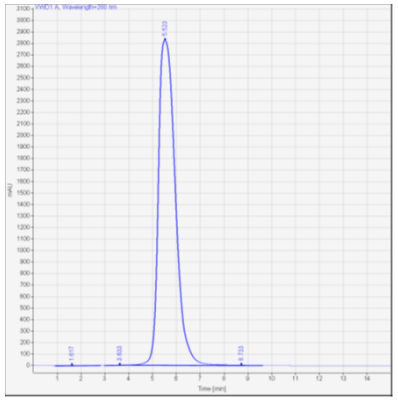
A tailored approach.....

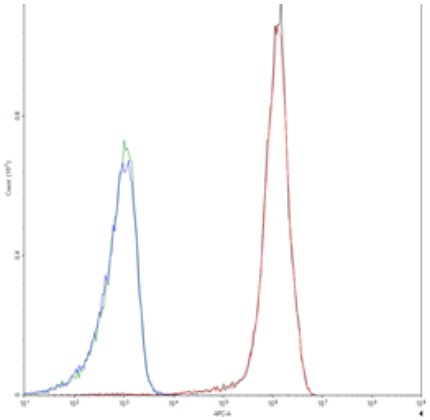


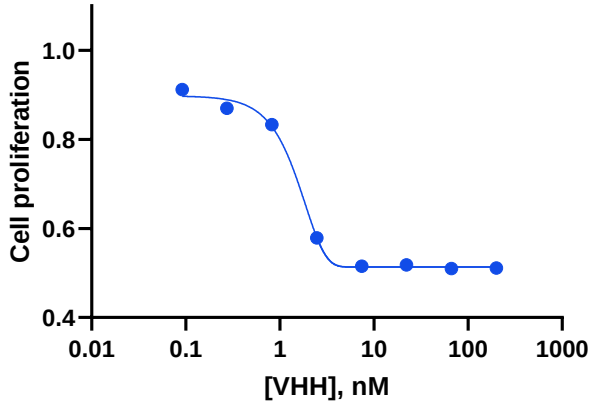


Target to IND

Developing successful candidates







High throughput **primary screens** e.g. HTRF/Alphascreen. Flow cytometry etc.

Hit characterisation e.g. Biophys. SEC, Tm, Tagg, MALDI

Lead optimisation and formatting
e.g. humanization, linker length, orientation, etc.

Functional assays (e.g. Internalisation, cell-killing e.g. PBMC co-cultures. Enzymatic inhibition assays etc.

Safety: Cytokine release, half-life/immunogenicity - tuning

CMC (different expression systems, Purification options)

PK/PD

IND

Summary

- Isogenica's LlamdA® library is a state-of-the-art humanised VHH antibody library
- CIS Display and Colibra® allow interrogation of $>10^{13}$ individual low liability antibody clones.
- Flexibility of VHH facilitate generation of numerous biotherapeutic formats
- Isogenica has over 20 years' experience of successful delivery of biotherapeutic discovery and development campaigns
- Discovery and development partnership with Isogenica is collaborative and builds on our pipeline experience in bispecifics, both as partners and a drug discovery and development company

More info

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