VHH drug discovery using synthetic libraries

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Isogenica uses diverse (1e13) fully synthetic camelid domain antibody (VHH) libraries for internal and partnered drug discovery. Humanised synthetic libraries offer several advantages over immunisation or the use of naïve libraries, including diversity and the speed of clone isolation. During the drug discovery process, many clones might meet the desired functional properties but contain liabilities that need to be addressed before the start of the development process. Synthetic libraries include design features that reduce the number and type of liabilities thereby reducing the time of drug development. Here we show an improved library and functional data on two projects with clones generated from the llamdA library.

WHY CHOOSE A SYNTHETIC ANTIBODY LIBRARY FOR YOUR DRUG DISCOVERY CAMPAIGN?

Increased diversity – increased chance of obtaining antibodies meeting the target product profile

- Million-fold greater sequence space covered vs immunisation
- Library built with humanised
- No immunological response biases or self-tolerance gaps

Avoiding liabilities – reducing lead optimisation effort and attrition rate

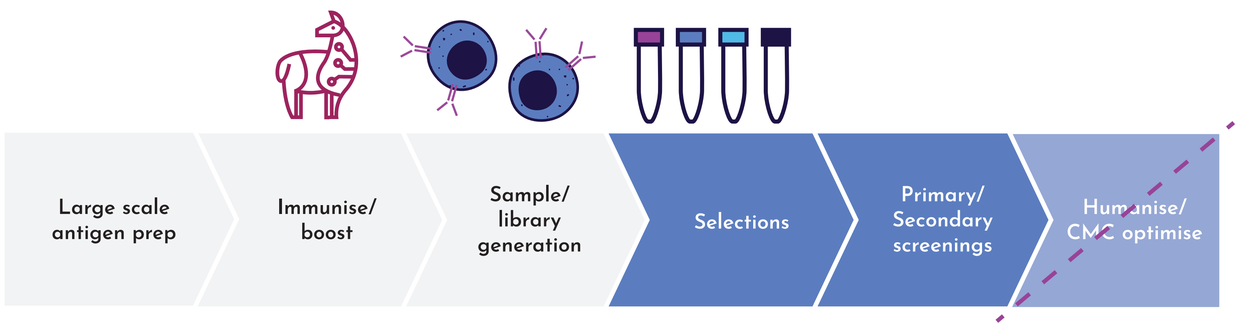
- CMC liability motifs reduced or absent (e.g. isomerisation, deamidation, proteolytic cleavage sites, positively charged patches, glycosylation sites, free cysteine)
- Unusual structures excluded (e.g. highly extended CDR3 loops…)
- No somatic hypermutation in frameworks avoids framework region mutations, which can complicate downstream humanisation

Time to hit identification

- Ready to use Single-pot library - no lead time for immunisation and animal specific library generation prior to initiation of selections
- Lead therapeutic molecule selection time within 3 months

Greater opportunity to find a developable therapeutic antibody candidate within short timeframe

Isogenica route to lead generation



Innovative target lead discovery

- Remove upfront efforts
  - Library ready to use
  - Enable lead discovery to challenging targets
- Reduce or fully remove downstream efforts
  - Pre-humanised leads
  - Majority of clones from library are liability free

Innovative targeting approaches, multispecifics

- Remove upfront efforts
  - Well-characterised lead panels
- Reduce or fully remove downstream efforts
  - Pre-humanised leads
  - Characterised/optimised leads

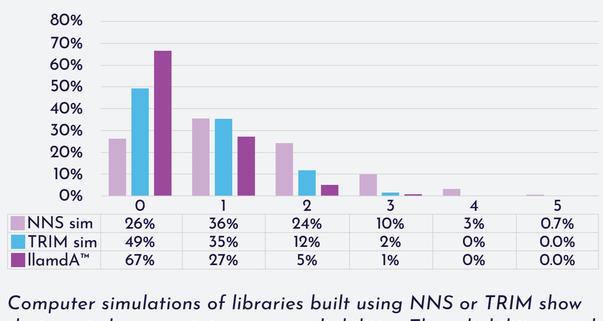
LIBRARY DEVELOPMENT – LIABILITY FREE LIBRARY

Antibodies that enter development must have excellent biophysical and chemical characteristics. Isogenica has built synthetic libraries using human sequences that have specific sequence liabilities completely removed. The libraries were designed to be liability free. We are currently increasing the size of the libraries whilst validating hits from initial selections.

Liability	Motif
Deamidation	NA, NG, NS
Isomerisation	DG, DS
Proteolytic cleavage	DP
N-linked glycosylation	N_S, N_T
Cys/Met	C, M
Positively charged clusters	RK, KR, KK, RR

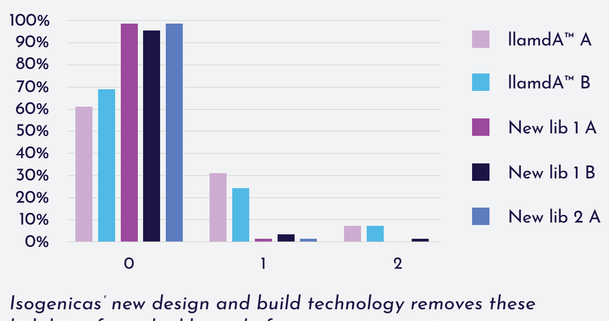
Liabilities reduced or removed from Isogenica's libraries.

Liabilities per clone by synthesis method



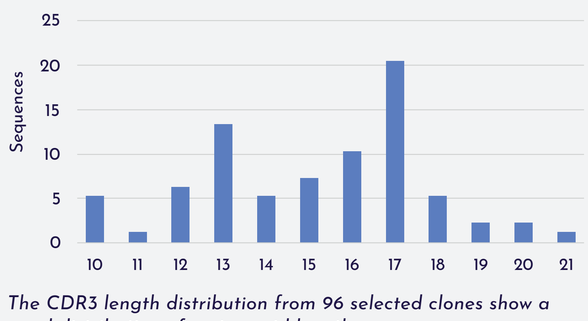
Computer simulations of libraries built using NNS or TRIM show that most clones contain sequence liabilities. These liabilities need to be "fixed" before being taken forward into development.

Number of liabilities per ELISA binder



Isogenicas' new design and build technology removes these liabilities from the library before screening starts.

CDR3 Length

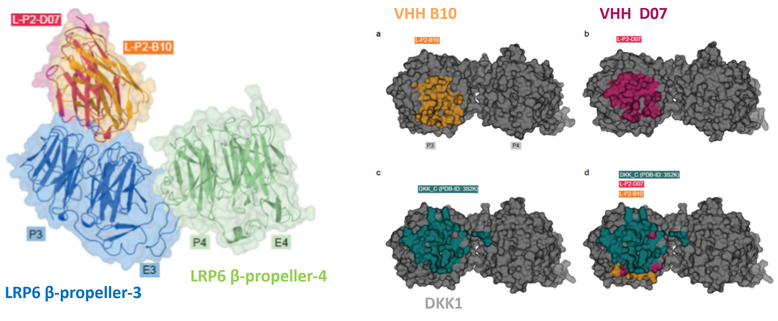


The CDR3 length distribution from 96 selected clones show a good distribution of amino acid lengths.

WHY CHOOSE A SYNTHETIC ANTIBODY LIBRARY FOR YOUR DRUG DISCOVERY CAMPAIGN?

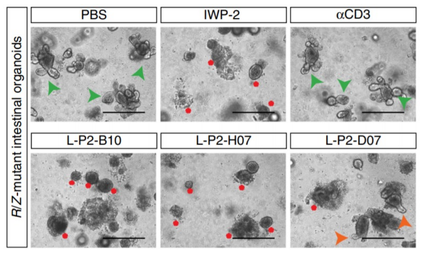
Soluble Wnt ligand trigger signalling through Frizzled/LRP5 signalling. CIS display was used to isolate VHHs that bind to the same epitope as the natural antagonist, DKK1. Unlike anti LRP5/6 IgGs, VHHs do not cause cross-linking of the receptor and are therefore antagonistic. The selected VHHs could be useful therapeutics in several indications, e.g. colon cancer.

Single VHH clones block the functional epitopes of LRP5 and LRP6

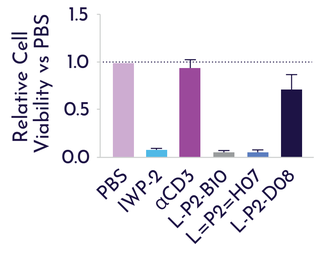


Anti-LRP5/6 VHHs promote differentiation of Wnt-hypersensitive intestinal stem cells
N Fenderico, RC van Scherpenzeel, M Goldflam, D Proverbio, I Jordens, T Kralj, S Stryeck, T Bass, G Hermans, C Ullman, T Aastrup, P Gros & MM Maurice
Nature Communications Vol 10, Article number: 365 (2019) DOI: 10.1038/s41467-018-08172-z

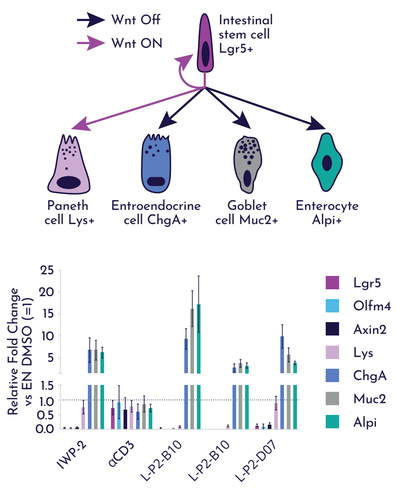
The structures (6H15 and 6H16) of two VHHs co-crystallised with LRP6 bind to the same epitope as DKK1. The orientation of the VHHs was rotated relative to each other. The paratopes are also different, with B10's CDR3 containing six more residues than that of D07.



Organoids were cultured in EN and treated with 10 μM of the indicated anti-LRP5/6P3 VHH for 4 d (Scale bar, 400 μm). Red asterisks indicate cell death; green arrows indicate organoids showing villi and crypts; orange arrows indicate organoids showing a mixed phenotype of cell death and villi crypts structures.



Anti-LRP5/6P3 VHHs strongly diminish cell viability of tumorigenic R/Z mutant organoids. Organoids were cultured in EN and treated with 10 μM of the indicated anti-LRP5/6P3 VHHs for 4 d. Graph represents relative cell viability normalised to PBS treatment. Mean ± s.d. (n = 3) are plotted.

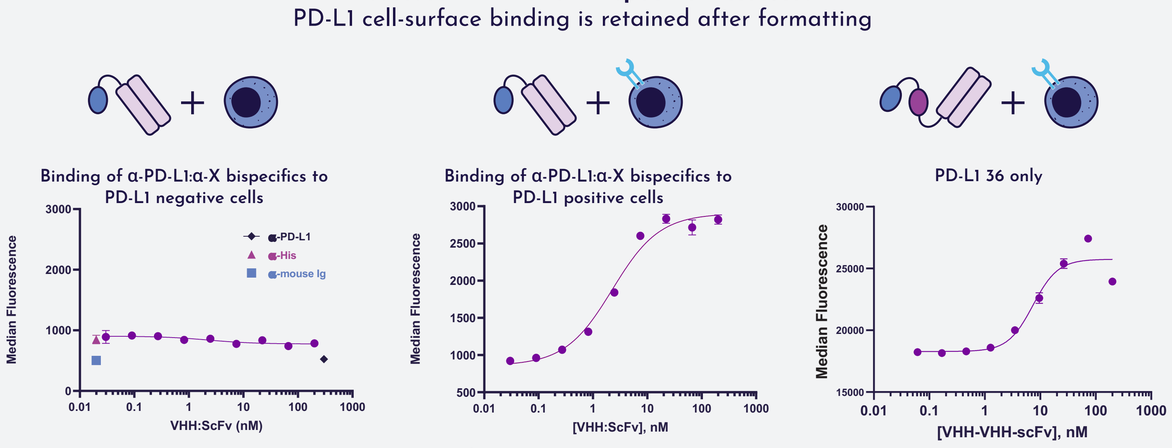


Wnt signaling in maintaining intestinal physiology. qRT-PCR showing anti-LRP5/6P3 VHH treatment (3 d) strongly inhibits the expression of Wnt target genes

ANTAGONISTIC VHHS BINDING TO PD-L1

PD-L1 (programmed death-ligand 1) is a transmembrane protein and ligand for PD-1, which is expressed on the surface of activated T-cells. PD-L1 plays a major role in the suppression of the adaptive immune system, allowing tumours to evade immune destruction by "instructing" the T-cells to leave the tumour cells alone. As a marker of cancer, PD-L1 can be targeted therapeutically through numerous mechanisms. Here we describe the properties of a representative clone that binds antagonistically to PD-L1 and which, like many VHHs, can be reformatted for different purposes.

Assessed in Bi- and Tri-specific molecules
PD-L1 cell-surface binding is retained after formatting

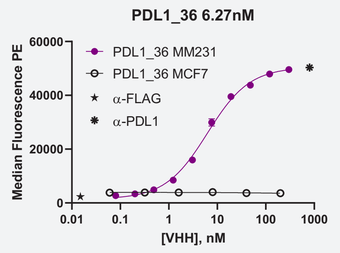


Bi-specific - no binding to PD-L1 negative cells (MCF7)

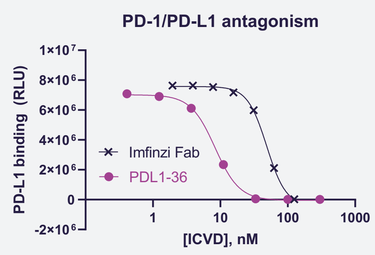
Bi-specific EC50 = 2.3 nM

Tri-specific EC50 = 7.5 nM

Assessed in Bi- and Tri-specific molecules
PD-L1 cell-surface binding is retained after formatting



Specific binding to PD-L1-expressing cells
More potent antagonism than Imfinzi Fab fragment



Full and partial antagonism profiles observed
Panel targets multiple epitopes on PD-L1

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