isogenica

Anti-LRP5/6 VHH inhibits WNT pathway and prevents tumour growth

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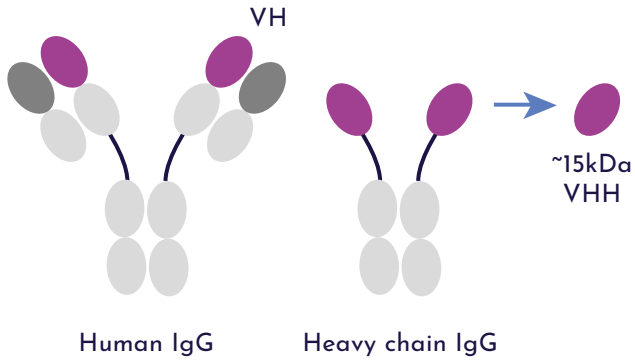
WHAT ARE VHH?

VHH are the variable domain of heavy chain only antibodies. They are small in size (~15 kD) and biophysically robust. With tunable half-lives, these antibodies are ideal for targeting inaccessible epitopes, achieving enhanced tissue penetration, multi-target binding and formatting for payload delivery.[1-3]

In a study performed by the University Medical Center Utrecht, Isogenica's proprietary fully synthetic single domain VHH library, LlamdA®, was screened using our CIS display technology to identify highly potent VHH that bind to the LRP6 Wnt3-binding domain.[4]



WNT-β-CATENIN SIGNALLING AND CANCER

Wnt/β-catenin signalling mediates several biological processes including stem cell proliferation and cell fate determination in development and adult tissue homeostasis. Mutational inactivation of the ubiquitin ligases RNF43/ZNRF3 results in increased Wnt receptor expression, which has recently been identified as an alternative pathway for cancer treatment[5], particularly in colorectal cancer.[6-8]

A major challenge for targeting Wnt signalling in cancer is identifying agents that can effectively reduce Wnt signalling specifically in tumour cells while avoiding damage to normal stem cell function and tissue repair. There are several inhibitors under development for cancer therapy that target the production of all Wnts, however these may cause severe side effects to regeneration and repair mechanisms in normal tissue. More targeted approaches against specific Wnt regulators could be considered to treat Wnt signalling associated cancers.[9]

Low-density lipoprotein receptor-related protein 5 or 6 (LRP5/6) are Wnt receptors involved in Wnt/β-catenin signalling.[10] Domain-dependent Wnt binding to the LRP6 receptor presents an opportunity to selectively block certain classes of Wnts, while leaving other Wnt pathways unaffected.[11] However, currently there are no existing therapeutics for cancer that target LRP5/6 receptors. The small size, high specificity and affinity of VHH makes them an attractive construct for directly targeting LRP5/6 receptors, for the purpose of Wnt-dependant tumour treatments with minimal side effects.

For this reason, Isogenica's LlamdA® VHH library was used in a study by The Oncode Institute at University Medical Center Utrecht identify highly potent VHH that bind to the LRP6 Wnt3-binding domain.

A ROLE FOR ANTI-LRP5/6 VHH

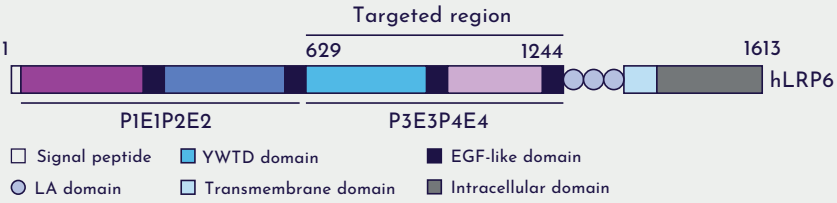


Figure 1. VHHs targeting LRP6^{P3E3P4E4} block cellular responses to Wnt3a. A Schematic representation of LRP6. The P3E3P4E4 module of the extracellular domain was used to generate anti-LRP6 VHH. Recombinant human LRP6 β-propeller-EGF modules P3E3P4E4 (residues UNIPROT 629–1244) were secreted from human embryonic kidney (HEK) 293 cells.

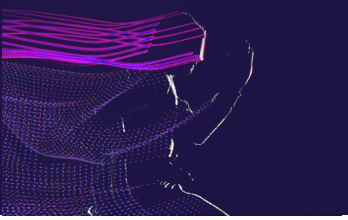
SELECTION

There were 33 unique anti-LRP6 VHH clones were identified. Luciferase Wnt reporter assays confirmed that the vast majority of these clones inhibited Wnt3a-mediated responses. The three most potent VHHs **(LP2-B10, L-P2-D07 and L-P2-H07)** were taken forward for further study.

AFFINITY, ACTIVITY AND SPECIFICITY

The activity and specificity of the VHH were characterised by cellular titration assays. Wnt3a-mediated pathway activation and cellular responses to LRP5 overexpression were also **fully blocked** by anti-LRP6 VHHs in LRP6-/- cells.

These VHH were shown to **selectively inhibit** Wnt3/3a-mediated Wnt/β-catenin pathway activation - treatment with either control VHH or LRP5/6P3-binding VHH did not affect Wnt1-induced reporter activity.



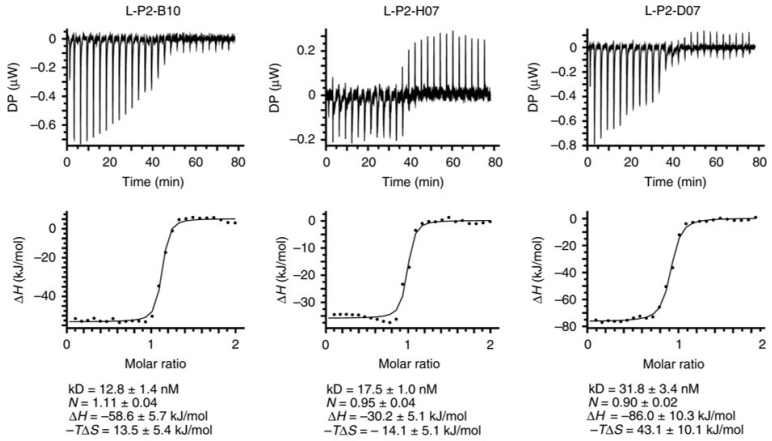


Figure 2. Isothermal titration calorimetry (ITC) showed all three VHH demonstrated low nanomolar binding affinities (<40nM) and a 1:1 binding complex with LRP6^{P3E3P4E4}. DP: differential power, kD: dissociation constant, N: stoichiometry, ΔH: delta enthalpy, –TΔS: temperature delta entropy. Findings validated by quartz crystal microbalance (QCM) analysis.

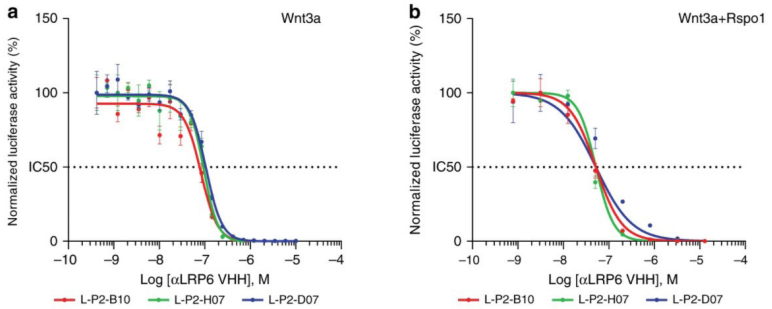
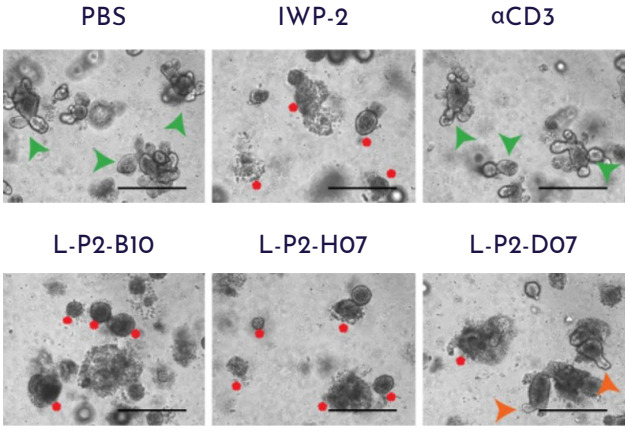


Figure 3. Characterization of anti-LRP5/6^{P3} VHH with highest potency. (a) IC50 calculations of inhibition of cellular responses to Wnt3a by titration in a luciferase reporter assay. Mean luciferase activities ± s.d. (n = 3) are plotted. (b) IC50 calculations by combination of Wnt3a and Rspo1, a strong Wnt pathway agonist. Mean luciferase activities ± s.d. (n = 2) are plotted.

R/Z - mutant intestinal organoids



MUTANT ORGANOID TREATMENT

Tumourigenic R/Z-mutant organoids treated with L-P2-B10 or L-P2-H07 displayed massive cell death, comparable to treatments with IWP-2, a highly potent inhibitor of Wnt secretion.

qRT-PCR and confocal microscopy concluded that the anti-LRP5/6^{P3} VHH block the growth of Wnt-hypersensitive Rnf43/Znrf3-mutant intestinal organoids by removing an essential pathway for renewal of stem-like tumour cells and promoting their terminal differentiation.

Figure 4. Anti-LRP5/6 VHH drive collective differentiation of R/Z-mutant tumour organoids. Anti-LRP5/6P3 VHH block tumorigenic R/Z mutant organoid growth. Organoids were cultured in EN and treated with 10 µM of the indicated anti-LRP5/6P3 VHH for 4 days (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.

VHH: AN OPPORTUNITY FOR TREATING SOLID TUMOURS

These findings demonstrate that anti-LRP5/6P3 VHH treatment not only blocks cellular responses to Wnt3 but also removes the main source of Wnt production. The high affinity and specificity of VHH allows for the selective blockade of Wnt3-mediated cellular responses while leaving other Wnts unaffected. Due to their small size, VHH can also rapidly and deeply penetrate solid tumours.[12] Furthermore, the broad species cross reactivity of these VHH will facilitate future therapeutic proof-of-concept experiments and toxicology studies. This study demonstrates that VHH-mediated interference with selective Wnts involved in β-catenin dependent Wnt signalling branches holds great promise for targeting and treating Wnt-hypersensitive tumours, while potentially limiting side effects.

VHH APPLICATIONS

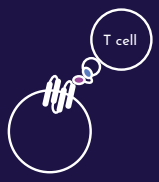
VHH have a broad range of therapeutic applications due to their small size, stability, and low cost of production. VHH can also be easily engineered with carefully designed affinities, valencies and specificities to meet a variety of therapeutic needs, enabling refined cell targeting and modes of action while also minimising off-target toxicity.[13-15]

DIRECT THERAPEUTICS

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

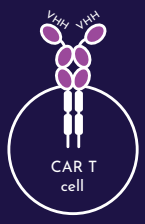
ANTIBODY DRUG CONJUGATES

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



CELL & GENETHERAPIES

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

Our state-of-the-art, highly diverse, synthetic LlamdA® VHH library incorporates intelligent design with the precision of the COLIBRA® library construction system. Offering a diverse library to identify therapeutic candidates for targeted drug delivery of ADC, CAR-T, cell and gene therapies and in the discovery and development of bispecific and multi-specific biotherapeutics.

READY TO START YOUR NEXT PROJECT?

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