Case Study

Discovering VHH antibodies that are reactive toward a cell-expressed target via phage display

The Challenge

Generate a panel of clinically relevant VHH antibody lead candidates against a membrane protein.

The Solution

Immunization of llamas followed by generation of a custom phage library and cell-based pannings.

Immunization of llama glama (n=2) and phage library generation

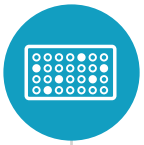
The 94-days protein immunization schedule resulted in differentiating VHH responses in serum to target vs off-target expressing cells

Subsequent library generation yielded a panel with a complexity of 3x10^8.

Phage display with cell-based pannings

Four rounds of panning against target expressing cells were performed; already after the second round, 67% of the clones were specifically reactive with cell-expressed target.

Sequencing yielded 23 VH-CDR3 families.



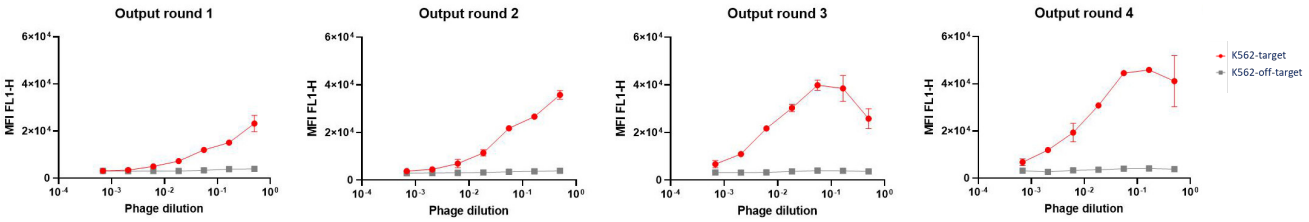
Recombinant production of selected clones

Ten clones were selected for VHH-Fc and VHH-his production. These recombinantly produced and purified VHHs were all specifically reactive with cell-expressed target.

Off-rate analysis of selected clones resulted in the selection of candidates for further validation for designated clinical purpose.

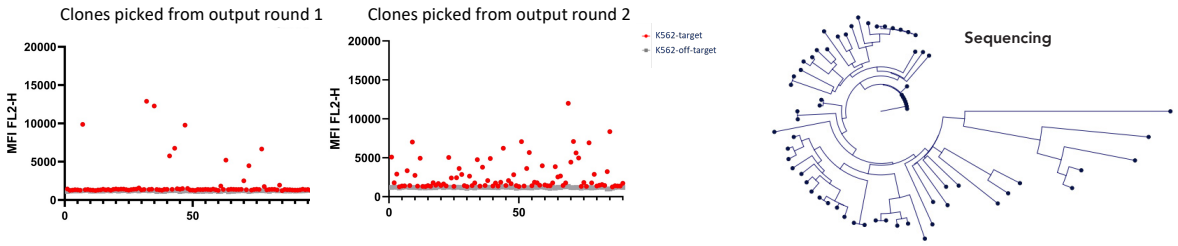
Program Summary

Polyclonal screening – Results of cell-based pannings



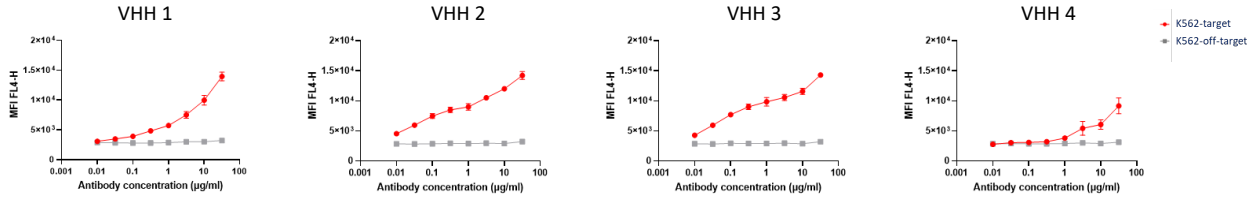
Four rounds of VHH phage library selections were performed applying cell-based selection strategies. Selection outputs were screened against target- and off-target Emerging reactivity toward cell-expressed target after round 1, was obtained by applying a full cell-based selection strategy and increased after each successive round.

Monoclonal screening and sequencing



In order to obtain a sequence diverse, cell-expressed target-specific set of VHH clones, monoclonals were picked from outputs after round 1 and 2 of cell-based selections and were screened against target- and off-target expressing cells and sequenced. 67% of picked monoclonal VHHs after round 2 of cell-based selection are identified as cell-expressed target-specific. Sequencing of 90 monoclonals yielded 23 families based on CDR3 diversity. 66% were sequence unique based on CDR1, 2 and 3.

Recombinant production and purification of VHHs



Ten VHH sequences were selected for recombinant production and purification in eukaryotic expression system for binding and off-rate analysis. The observed reactivities towards cell-expressed target of all ten clones match that of their monoclonal periprep counterparts. Representative data of four antibodies are shown. Octet-based off-rate analysis revealed diverse off-rate profiles of the selected clones, data not shown.

Conclusion

A large, diverse panel of cell-expressed target-reactive and specific VHH clones was obtained by performing cell-based phage display procedures. The designed target-specific discovery program included immunization of llamas with recombinant protein resembling the ECD structure of the transmembrane target protein followed by VHH library generation and cell-based target-specific phage display pannings and screenings. Subsequent monoclonal periprep and eukaryotic-produced and purified VHH screenings resulted in the identification of nine clinically relevant VHH clones.

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