Utilizing the AbNano™ VHH Naïve Library for identifying and characterizing VHH binders to key immuno-oncology targets

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Background

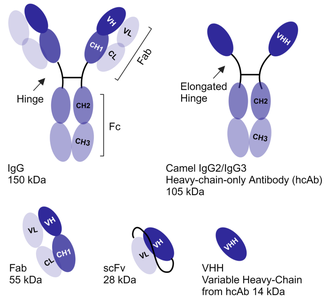

Figure 1: Antibody formats and their approximate molecular weights.

VHH domains, derived from the variable heavy chain of the heavy-chain-only IgG2 and IgG3 domains in camelids, represent a small, single-domain antibody fragment. Discovering novel VHH domains can take place by multiple workflows, including using B-cell sorting and using display libraries. Within display libraries, the library is generally one of three classes of molecule: synthetic molecules, immune-derived molecules, or naïve germline molecules. Here, we present the construction and early validation of a large naïve library from llamas and alpacas. Characterization data suggests that this library, the AbNano™ VHH Naïve Library, may be well-suited for rapid discovery of VHH domains binding to therapeutic targets with varying levels of affinity. In this study we show how the AbNano™ VHH Naïve Library can be used to identify target specific VHHs to PD-L1, including live-cell binding by flow cytometry, while reducing the time and resources necessary to do so.

Library Construction

The library was constructed from 103 naïve animals: 77 llamas and 26 alpacas. A total of 1.51 x 10^{10} PBMC cells were collected and used for library construction. Libraries were constructed using species-specific primers. The total number of transformants for the library was 1.12 x 10^{10} cfu. Transformants were analyzed by Sanger and the individual sublibraries were analyzed by next generation sequencing at a total read count of 1.71 M reads using GeneWiz® Amplicon-EZ services from Azenta Life Sciences. NGS reads from each sublibrary were combined and analyzed using Geneious Biologics. Based on the unique cluster frequency, the in-frame VHH rate, and the number of representative transformants, we estimate the maximum possible library size of the naïve VHH library to be 6.48 x 10^{9} unique clonal VHH.

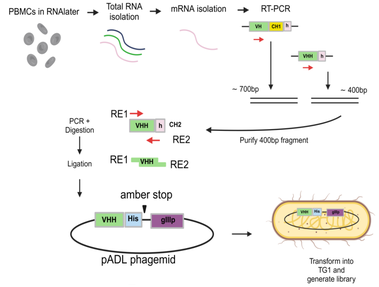

Figure 2: Overview of the library construction process. A total of 1.12 x 10^{10} transformants were collected from 13 sublibraries.

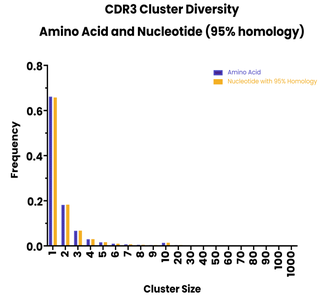

Figure 3: Comparison of cluster diversity for amino acid identity of CDR3 and for 95% homologous nucleotide identity of CDR3.

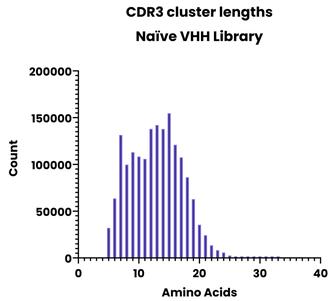

Figure 4: Histogram of length distribution in CDR3 of the VHH domains in the library.

Discovery and Characterization

Phage selections were performed from the pooled phage input. Round 1 panning was performed with approximately 2.5 x 10^{10} pfu of phage. Here, we present campaigns against recombinant PD-L1. Recombinant human PD-L1 (residues F19-Y134 from accession number Q9NZQ7-1 with C-terminal biotinylated Avi tag and poly-His) was incubated with blocked phage before being pulled down by addition of streptavidin-coated magnetic beads. Phage was eluted by triethylamine after panning and PEG-precipitated between rounds. 470 clones were screened by single point ELISA, generating 147 hits above the threshold of A405 > 0.4 with 68 unique sequences identified from the 470 clones screened. This represents a unique hit rate of 14.5%.

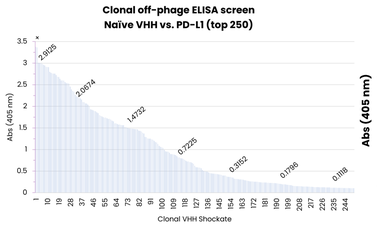

Figure 5. Clonal crude VHH against PD-L1 single-point binding was detected by HRP-anti-VHH conjugate and ABTS.

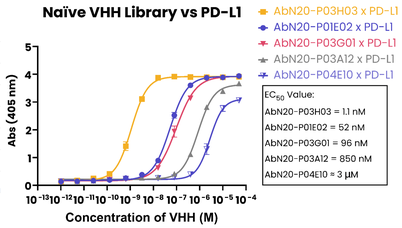

Figure 6. Five clones with varying single-point ELISA signal were purified by Ni-NTA, titrated in duplicate, then visualized with HRP-anti-VHH conjugate and ABTS.

Unique clones identified from the single-point ELISA screening were expressed and purified by Ni-NTA resin, then dialyzed into PBS. For preliminary live-cell flow cytometry, overexpressing cells were generated by transfecting 293F cells with PD-L1 under a CMV promoter with neomycin selection. VHH was applied at a concentration of 1 µg per 10^{6} live cells to either live 293F cells transiently-overexpressing PD-L1 or transfected with corresponding mock vector. Two VHH clones, P03H03 and P03E01, were found to demonstrate live-cell binding of the transient OE over the mock-transfected parent cells after normalization to isotype controls. These two clones were also applied to cell lines HDLM-2, which has endogenous PD-L1 expression, and Ramos, which has near-zero levels of endogenous PD-L1 expression. Only VHH clone P03H03 demonstrated appreciable binding to HDLM-2 cells over the Ramos cells. The single-point ELISA signal for P03H03 was 3.38 while the signal for P03E01 was 1.84.

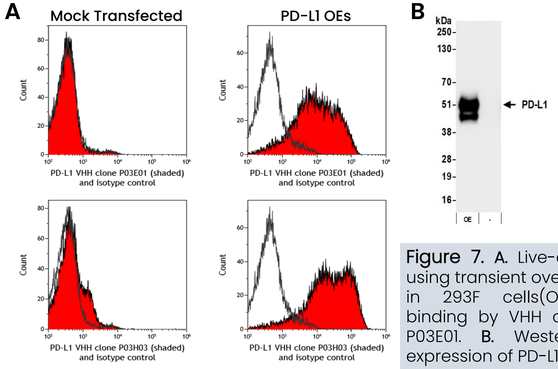

Figure 7. A. Live-cell flow cytometry using transient overexpression of PD-L1 in 293F cells(OEs) demonstrates binding by VHH clones P03H03 and P03E01. B. Western blot showing expression of PD-L1 in OEs.

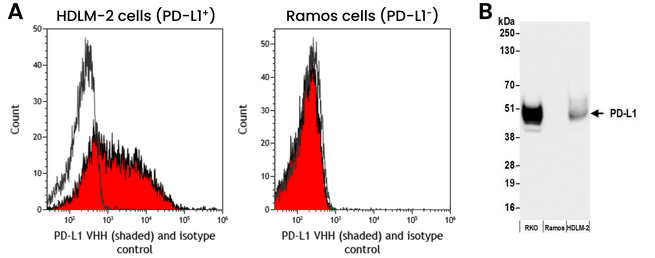

Figure 8. A. Live-cell flow cytometry using a PD-L1 expressing endogenous cell line and a near-zero endogenously-expressing cell line demonstrates PD-L1 binding by VHH clone P03H03. B. Western blot data showing endogenous PD-L1 expression.

Conclusions & Future Directions

Early validation of the AbNano™ VHH Naïve Library suggests that the library is diverse and well-suited for rapid discovery of VHH domains binding to protein targets in a range of panning conditions. Here, we demonstrate that this library may yield individual lead molecules for humanization and affinity maturation workflows. We are utilizing the AbNano™ VHH Naïve Library to isolate, screen, and characterize live cell binders within a 6 month timescale, suggesting that this is an alternative path to rapid and accessible early biologics discovery.

Acknowledgements

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