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Sandia National Laboratories

DISCOVERY, DESIGN, AND ENGINEERING OF NANOBODY-BASED THERAPEUTICS FOR SARS-COV-2

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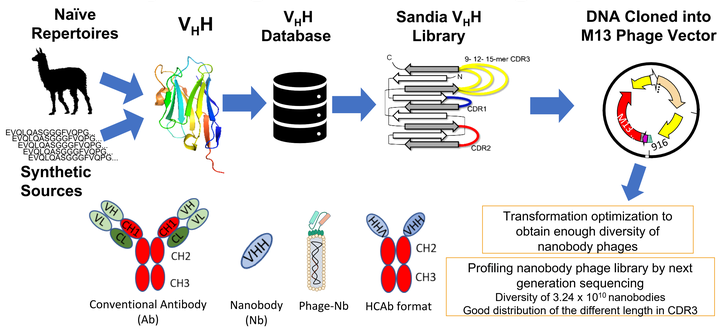
Abstract

At Sandia National Laboratories, we have designed a highly diverse synthetic, humanized nanobody phage display library to swiftly identify nanobodies with high binding affinity for any target of interest. Nanobodies, are the variable antigen binding domain of heavy chain only antibodies (HCAbs) found in camelids. Nanobodies, naturally have high sequence identity with variable domains of human IgG antibodies. In addition, to reduce potential immunogenicity we used previously established methods to humanize our nanobody library. We have previously demonstrated that this library can be screened to rapidly identify highly specific and strongly neutralizing humanized HCAbs with prophylactic and therapeutic efficacy in vivo against fully virulent SARS-CoV-2 in a rodent model of severe infection and disease (Stefan, Light et al. 2021). Recently we have developed multi-valent humanized HCAbs that bind to more than one epitope of the of SARS-CoV-2 Spike. These multivalent constructs increase the spectrum of virus variants neutralized and decrease the ability of the virus to generate escape mutants.

Although, our original multivalent constructs are effective against the Delta variant, the parental nanobodies identified by Sandia have the highest affinity for the Wuhan strain of SARS-CoV-2. Now that there are several circulating SARS-CoV-2 variants of interest, we have developed a screening strategy to identify broad-spectrum nanobodies that neutralize divergent ACE2-binding coronaviruses. Thus far we have identified several promising nanobodies that neutralize the original SARS-CoV-2 and several of its variants, including Omicron and Delta. Binning assays and affinity maturation are underway to inform development of highly effective broad spectrum humanized multivalent HCAbs.

Library Design and Screening Pipeline



Screening Against Multiple Variant RBDs Identified Nanobodies With Efficacy Against New Variants (Omicron)

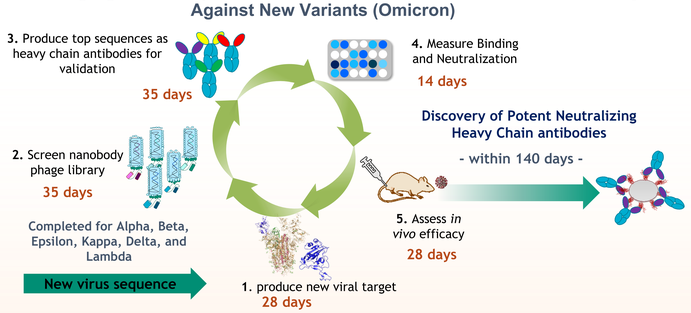


Figure 1: Nb library design and screening campaign to identify broad-spectrum anti-SARS-CoV-2 neutralizing HCAbs. A) The library was designed based of the sequence diversity of 670 nanobodies deposited on the sdAb-DB. The resulting nanobody library has a high diversity CDR3 with three different lengths (9-, 12-, and 15-mer). **B)** Schematic of screening pipeline which includes 1. rapid production of the antigen sequence; 2. three rounds of panning against each variant RBD, ELISA and Neutralization with single phage clones, sequencing and bioinformatics to select the top hits for further validation 3. Production of HCAbs with Fc from human IgG1 and sequences of top nanobodies; 4. Characterization of HCAbs in vitro; 5. Test efficacy of best HCAbs in vivo (not done yet for broad-spectrum candidates)

Original HCAb Preclinical Evaluation

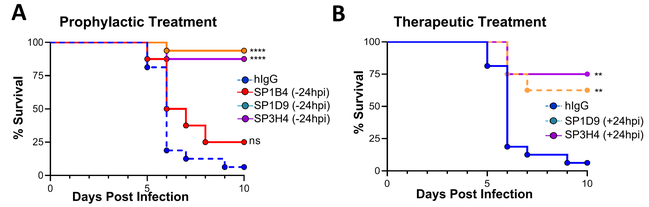
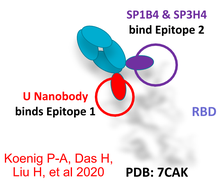


Figure 2: Top nanobody-huFc candidates provide protection from lethal SARS-2 infection *in vivo*. Kaplan-Meier curve illustrating percentage survival of K18-hACE2 mice infected intranasally with 2.5 x 10^4 PFU SARS-CoV-2 and dosed with 10 mg/kg nanobody-huFc via intraperitoneal injection prophylactically **(A)** at 24 hours prior to infection or therapeutically (**B**) at 24 hpi. **Stefan** et al, *mAbs,* **2021**

Asymmetric Bispecific HCAbs Comparable to Approved Therapeutics



Company	Therapeutic	Binding Affinity K_D (nM)	Neutralization EC_{50} (nM)
Sandia	SP1B4-U	2.2	0.025
Sandia	SP3H4-U	0.79	0.023
Eli Lilly and Company	LY-CoV555	3.5[†]	0.080[†]
Regeneron	REGN10933	3.4[‡]	0.043[‡]
Regeneron	REGN10987	45.2[‡]	0.041[‡]
Regeneron	REGN10987/ 10933	N/A	0.033[‡]

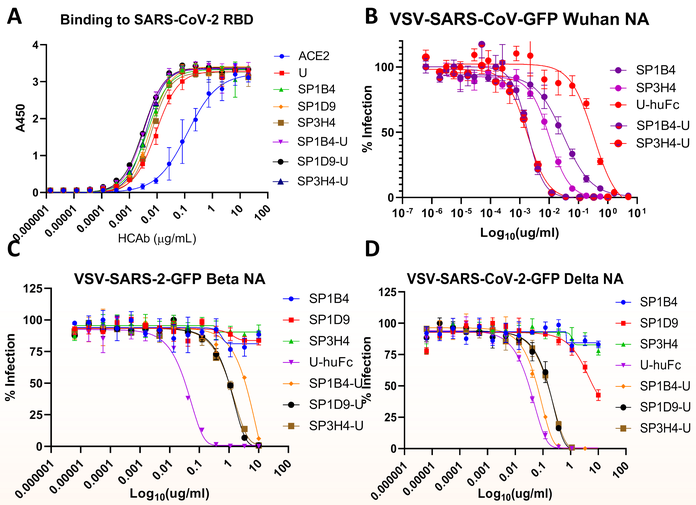


Figure 3. Improving Therapeutic Potential by Targeting Multiple Non-overlapping Epitopes. Construction of multivalent anti-SARS-CoV-2 nanobodies with two distinct antigen binding sites on RBD, shown in purple and red circles (Koenig P-A, Das H, Liu H, et al 2020), linked to human IgG Fc (blue) resulted in a synergistic increase in neutralization to levels comparable to currently deployed therapeutics. (A) ELISA (B) Neutralization assay with monovalent and biparatopic nanobody-huFc constructs and VSV-SARS-CoV-2 GFP expressing Spike from original WT strain (table), (C) Beta and (D) Delta. Similar results were observed for Gamma, Epsilon and Lambda. Data are mean ± the standard deviation performed in triplicate;

Binding of Broad-Spectrum HCAbs

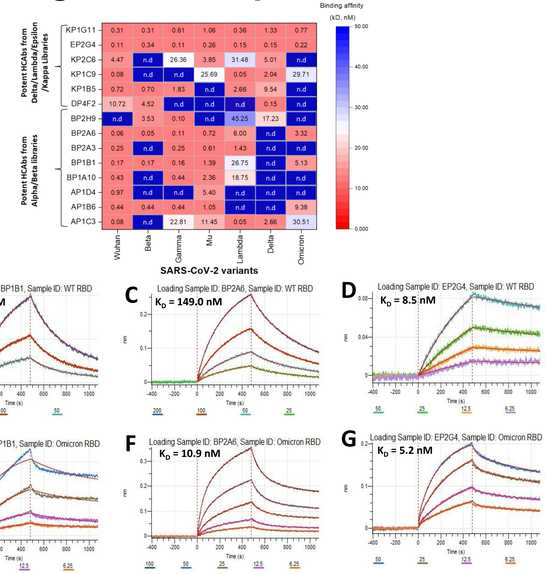


Figure 4: Evaluation of top HCAb hits by ELISA and BLI. Titration ELISA of top ten candidates binding to SARS-CoV-2 RBD variants. The data are from experimental conditions performed in triplicate, the error is the standard deviation from the mean. BLI sensorgrams show binding to SARS-CoV-2 RBD to SARS-CoV-2 Wuhan RBD and SARS -CoV-2 Omicron RBD by top candidates BP1B1 (B, E); BP2A6 (C, F) and EP2G4 (D, G). in 10 mM phosphate (pH 7.4), 300 mM NaCl, 1 mg/mL BSA, 0.1% NP-40 (Thermo, 28,324). VHH-huFc ligands were immobilized on human Fc capturing sensors. SARS-CoV-2 RBD was used as the analyte and sensorgrams were fit to a global 1:1 fit.

Neutralization and Competition Assay with Broad-Spectrum HCAbs

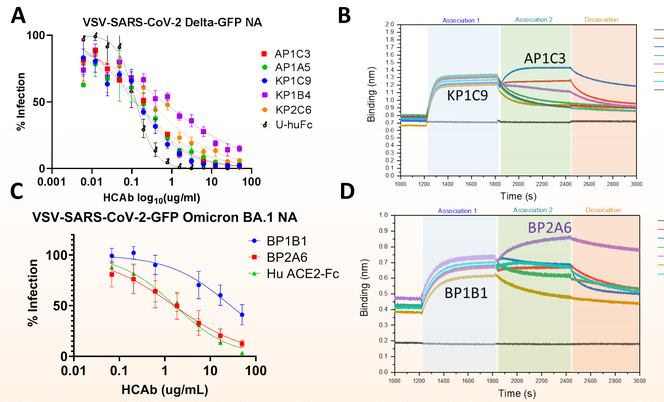


Figure 5. Evaluation of HCAb Neutralization and Synergy. *In vitro* neutralization assay of top candidates against (A) VSV-SARS-CoV-2 Delta, or (C) VSV-SARS-CoV-2 Omicron. Data are the mean ± the standard deviation performed in triplicate. Combinations of (B) KP1C9 and AP1C3 or (D) BP2A6 and BP1B1 shows synergistic increase in binding against two epitopes on SARS-CoV-2 RBD variants.

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