Machine Learning-Guided Generation of a Combination of Broadly Neutralizing Sarbecovirus Antibodies

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Background

Sarbecoviruses represent a subgenus of coronaviruses including strains mostly circulating in bats and at risk of zoonotic spillover as well as strains that can infect humans (SARS-CoV-1 and SARS-CoV-2). Over the past 20 years, SARS-CoV-1 was responsible for an outbreak in Asia while SARS-CoV-2 initiated a global pandemic and remains a continuous health threat especially for vulnerable populations. There is still the need to develop therapeutic measures that can effectively control human sarbecoviruses and potential zoonotic spillover events, thereby preventing future epidemics/pandemics. Several monoclonal antibodies have been discovered with broad neutralizing activity against sarbecoviruses by targeting conserved regions of the spike proteins such as class 3 and 4 RBD regions and S2 fusion machinery. Viral escape mutations accrued in the SARS-CoV-2 spike protein have rendered most of the antibodies targeting RBD regions ineffective. In addition, antibodies targeting the S2 fusion machinery have relatively low neutralization potency compared to anti-RBD antibodies and therefore their therapeutic utility has not been realized.

Generate:Biomedicines' discovery campaigns for SARS-CoV-2 spike-targeting broadly neutralizing antibodies

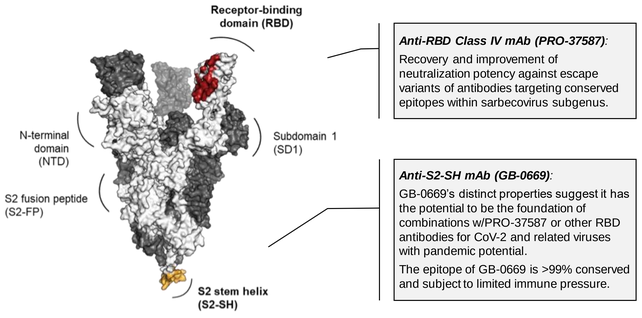


Figure 1: Broadly neutralizing combination targeting non-overlapping epitopes, generated and tested with half-life extending mutations in the Fc region to extend half-life and promote mucosal translocation while preserving Fc effector functions. (PDB ID: 7RA8)

Overview of design/test/learn cycles for the anti-S2-SH campaign

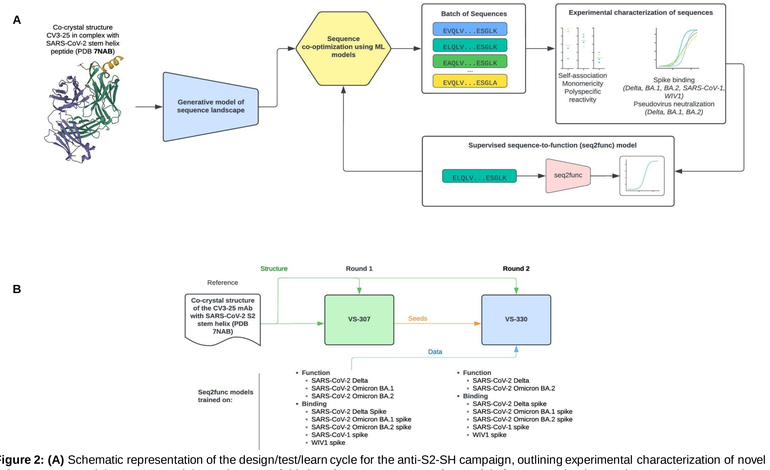


Figure 2: (A) Schematic representation of the design/test/learn cycle for the anti-S2-SH campaign, outlining experimental characterization of novel IgG sequences and the two ML models used as part of this iterative process: generative model of sequence landscape that uses the co-crystal structure of a reference mAb with S2-SH peptide; sequence-to-function model built with data generated as part of the characterization of novel IgG sequences. **(B)** Schematic representation of variant sets (VS) generated and experimental data acquired.

Results

The anti-S2 antibody GB-0669 broadly binds to spike trimers and neutralizes SARS-CoV-2 variants and non-SARS-CoV-2 sarbecoviruses

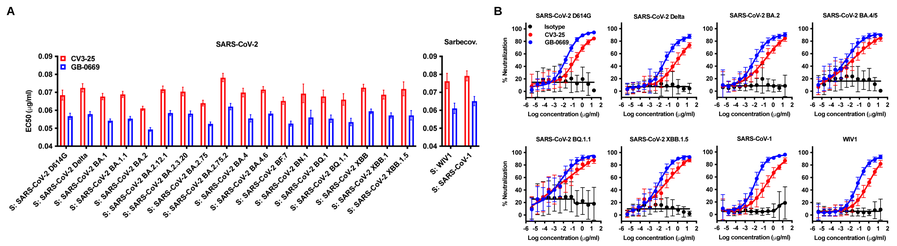


Figure 3: (A) Binding of GB-0669 and reference (CV3-25) mAb to spike trimers (indicated as "S:" followed by the respective virus name) representative of major SARS-CoV-2 variants (SARS-CoV-2) and non-SARS-CoV-2 sarbecoviruses (Sarbecov.) using DELFIA. Results are expressed as EC50 values (mg/mL), shown as mean and 95% confidence interval and representative of two independent experiments, four technical replicates each. **(B)** Neutralization profiles of GB-0669, reference and isotype control mAbs against pseudoviruses representative of SARS-CoV-2 variants and non-SARS-CoV-2 sarbecoviruses. Results are reported as % neutralization and shown as mean ± standard deviation (representative of three to six independent experiments, four technical replicates each).

The class IV anti-RBD antibody PRO-37587 binds to the Spike RBD and neutralizes SARS-CoV-2 variants and non-SARS-CoV-2 sarbecoviruses

Figure 4: (A) Yeast cells expressing RBDs from SARS-CoV-2-related clade 1b, SARS-CoV-related clade 1a, Asia non-ACE2 bat sarbecovirus (sarbecov.) clade 2, and Africa and Europe bat sarbecovirus clade 3 connected to the Aga2 protein via a flexible linker were induced clonally and stained with IgG in an arrayed format. Cells were stained at 1 nM with PRO-37587, a reference or an isotype control in IgG format, together with an antibody targeting a c-terminal MYC epitope tag to assess expression levels. Cells were analyzed via flow cytometry. Median fluorescence of the IgG signal divided by the median MFI of the expression signal was determined for each combination of RBD and PRO-37587, reference or isotype control. Relative signal was plotted in the heat map. **(B)** Neutralization profiles of PRO-37587, reference (S2X259) and isotype control mAbs against pseudoviruses representative of SARS-CoV-2 variants and non-SARS-CoV-2 sarbecoviruses. Results are reported as % neutralization and shown as mean ± standard deviation (representative of three independent experiments, four technical replicates each).

GB-0669 in combination with the anti-RBD class IV mAb PRO-37587 improves neutralization of SARS-CoV-2 variants

Figure 5: (A) Pseudovirus and **(B)** live virus curves displaying the percentage of inhibition of infection of each SARS-CoV-2 variant preincubated with the indicated antibodies over twelve dilutions. Sample values in each plate are normalized to the plate internal controls, where 100% inhibition is derived from the average of the negative control (untreated uninfected) and 0% inhibition is derived from the average of the positive control (untreated infected). The x axes show the concentration (log scale) of each antibody. Results are reported as % neutralization and shown as mean ± standard deviation. The curves represent the best fit of the logarithm of antibody concentration vs. the percentage of inhibition (variable slope, four parameters).

Structural characterization of anti-S2 antibody GB-0669 and the class IV anti-RBD PRO-37587

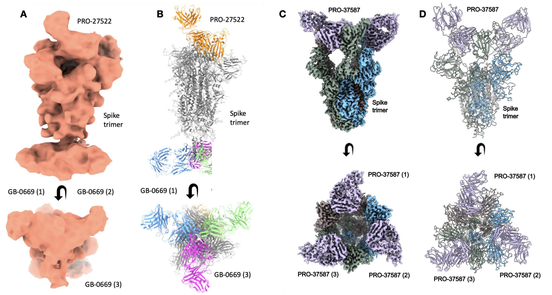


Figure 6: Experimental Cryo-EM Maps of SARS-CoV-2 BA.1 spike trimer in complex with anti S2 **(A)** and anti-RBD Fabs **(C). (B)** Cryo-EM structure of the SARS-CoV-2 BA.1 spike trimer in complex with anti-S2 and anti-RBD Fabs. The final model contains the spike trimer(PDB-ID: 6XR8), the design model for PRO-27522, and the in-house X-ray structure of the S2 peptide bound to GB-0669 (blue, magenta, green). **(D)** Atomic model of the anti-RBD Fab, PRO-37587, bound to SARS-CoV-2 BA.1 spike trimer. Top view of the complex shows three PRO-37587 bound to the trimer.

GB-0669 in combination with the anti-RBD class IV mAb PRO-37587 protect Syrian hamsters from BA.2 challenge

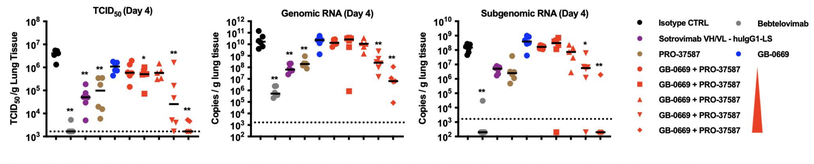


Figure 7: Golden Syrian hamsters were prophylactically administered with the the following antibodies as either single or combination agents: isotype control, Bebtelovimab, Sotrovimab, the anti-RBD Class IV PRO-37587, or the anti-S2 GB-0669 antibodies 1 day prior to challenge with SARS-CoV-2 BA.2 ($2.8x10^3$ $TCID_{50}$/dose). Four days post-challenge the hamsters were euthanized, and the following parameters measured: **(A)** Lungs were harvested on day 4 and the TCID50 determined using the Read-Munch formula. Oral swabs were taken and assessed for **(B)** Genomic RNA **(C)** Subgenomic RNA viral loads using qRT-PCR. The dotted line in indicates assay limit at 50 copies/g tissue. Six hamsters were used per group.

Escape of SARS-CoV-2 Omicron BA.1 from monoclonal and combination antibody therapies in vitro

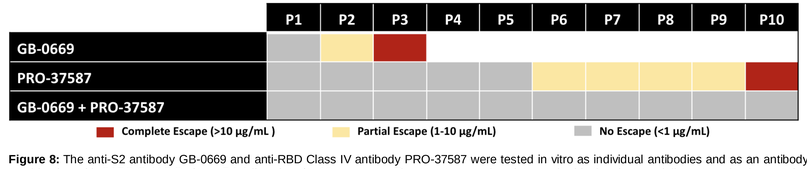


Figure 8: The anti-S2 antibody GB-0669 and anti-RBD Class IV antibody PRO-37587 were tested in vitro as individual antibodies and as an antibody combination with SARS-CoV-2 Omicron BA.1 live virus for 10 passages. The passage number that resulted in the virus partially or completely escaping neutralizing activity of the monoclonal or combination antibody therapies. Complete and partial escape were determined by cytopathic effect (CPE) at each passage. Wells where the CPE was observed and the antibody concentration was 10 mg/mL or above were considered complete escape, while well with the antibody concentration between 1-10 mg/mL were considered partial escape. Wells where either no CPE or CPE at <1 mg/mL were observed were considered no escape.

Conclusions

- We restored the ability of a known class IV anti-RBD antibody against SARS-CoV-2 Omicron variants.
- We improved the neutralization potency and efficacy of a previously described antibody with pan-sarbecovirus activity targeting the S2 stem helix peptide.
- The combination of class IV anti-RBD and anti-S2 antibodies have improved neutralization of both pseudovirus and live SARS-CoV-2 variants.
- In a hamster challenge model, the combination of class IV anti-RBD and anti-S2 antibodies suppressed viral replication.
- The combination of these antibodies showed increased protection against SARS-CoV-2 Omicron BA.1 with no evidence of viral escape through 10 passages.
- This demonstrated successful use of machine learning-guided protein engineering for optimization of anti-viral antibodies.