APPLICATION NOTE

Rapid Discovery of Therapeutic Antibodies for the SARS-CoV-2 Pandemic Virus Using the Opto™ Viral Neutralization 1.0 Workflow

APPLICATION SPOTLIGHT

- Discover blocking antibodies against SARS-CoV-2 in 1 day
- Find cross-reactive antibodies against SARS-CoV-1 and SARS-CoV-2
- Confirm binding and blocking of SARS-CoV-2 in plate-based functional assays

INTRODUCTION

Humans are susceptible to a myriad of different pathogenic viruses, including influenza, HIV, Zika virus, Ebola and the severe acute respiratory syndrome coronavirus 2, known as SARS-CoV-2. These viruses can result in a wide range of diseases with symptoms ranging from the common flu to death. Development of therapeutics against each virus has its unique set of challenges related to, for example, rapid transmission, high mutation rates, and delayed onset of symptoms due to long viral latency times.

One common and promising approach to developing an anti-viral therapeutic is to take advantage of the adaptive immune system. In response to infection, the immune system produces B cells that secrete antibodies that can bind viruses and block their entry into cells essentially neutralizing the virus. Rapid discovery of such "neutralizing" antibodies is therefore a promising strategy for developing drugs that may protect individuals from viral infection or enable them to recover post-infection.

The 2020 COVID-19 pandemic is a dramatic example of the challenge of curtailing the rapid transmission of the SARS-CoV-2 virus. In a matter of months, COVID-19 turned from a regional disease in Hubei province, China to a global pandemic that by the time of this publication has infected more than 9 million people and killed over 920k people[1]. The continued explosion in cases and deaths highlight the urgent need for the rapid development of safe, effective therapies for COVID-19 (**Figure 1A**). For the SARS-CoV-2 virus, antibodies targeting the Spike (S1) protein may prevent binding of the virus to the human angiotensin-converting enzyme 2 (ACE-2) receptor and prevent infection (**Figure 1B**).

Antibody discovery using the traditional hybridoma approach, however, is a slow and inefficient process that requires immortalization of B cells by cell fusion, followed by a lengthy process of expansion, sub-cloning, and screening. The immortalization step often causes the death of >90% of B cells, resulting in significant loss of B cell diversity[2]. This means that promising therapeutic candidates can be missed.

The Opto™ Viral Neutralization 1.0 workflow on the Beacon® optofluidic system leverages our Opto™ Plasma B Discovery process to bypass inefficient hybridoma fusion and lets you directly screen plasma B cells from infected human donors or immunized animals (**Figure 2**). Plasma

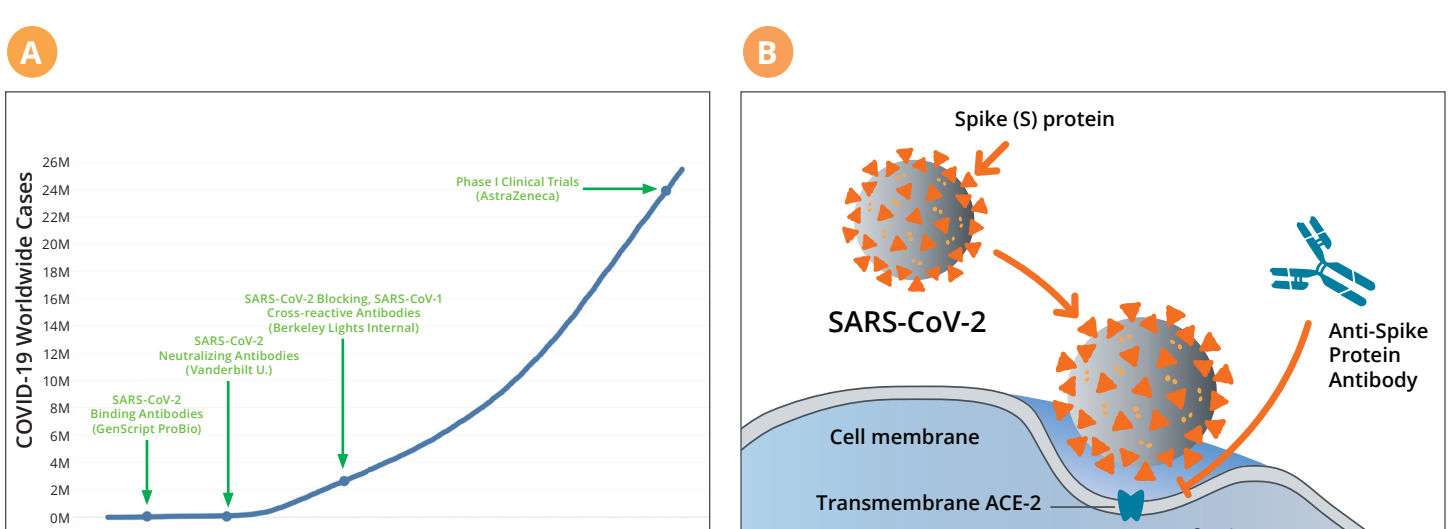

Figure 1. COVID-19 is a rapidly expanding pandemic caused by the SARS-CoV-2 virus. A. The Opto Viral Neutralization 1.0 workfow enabled rapid discovery of antibodies against the SARS-CoV-2 virus early in the pandemic. **B.** Antibodies that block the interaction of Spike protein and the human ACE-2 cell surface receptor can prevent SARS-CoV-2 infection.

B cells can be harvested and purified from diverse B cell compartments, including peripheral blood, spleen, and bone marrow, and tens of thousands of single B cells can be functionally screened in a single day. Antigen-specific clones can then be recovered for sequencing. Furthermore, this entire process, from sample loading to screening and cDNA synthesis, is fully automated, further increasing the efficiency and speed with which plasma B cells can be screened.

Using the Opto Viral Neutralization 1.0 workflow, cells are assayed in sub-nanoliter-sized NanoPen™ chambers on a microfluidic OptoSelect™ chip. The small volumes of these reaction chambers enable antibody concentrations to reach detectable levels within minutes. Culture media and assay reagents can be perfused in and out enabling rapid down-selection of lead candidates through assays for antigen specificity, cross-reactivity, and function which can be performed either simultaneously or sequentially. The ability to perform multiple assays is critical for identifying anti-SARS-CoV-2 antibodies as well as down-selecting blocking and neutralizing antibodies, which constitute only a fraction of the antibodies that bind the virus.

The Opto Viral Neutralization 1.0 workflow has enabled the discovery of antibodies against SARS-CoV-2 isolated from both the B cells of immunized mice and B cells isolated from convalescent human patients (Figure 1A). In the early days of the COVID-19 pandemic, GenScript ProBio demonstrated successful use of this workflow by screening immunized mice in under 24 hours and identifying antibodies that bind SARS-CoV-2 viral proteins[3]. Focusing on convalescent human patients, a team of researchers at the Vanderbilt University Medical Centre successfully transferred SARS-CoV-2 neutralizing antibody sequences to manufacturing partners a mere 18 days after isolation of human B cells from some of the earliest identified patients with SARS-CoV-2 infection in North America[4]. The panel of antibody sequences discovered by the Vanderbilt team led to the identification of lead antibody molecules that protect mice and rhesus macaques in preclinical models of SARS-CoV-2 infection[5]. A therapeutic antibody cocktail consisting of these lead molecule(s) has entered Phase 1 human clinical trials for COVID-19 that was initiated by AstraZeneca in August 2020.

In this application note, we describe how the Opto Viral Neutralization 1.0 workflow can be used to rapidly select functional antibodies against SARS-CoV-2. We screened plasma B cells from immunized mice to identify antibodies that bind soluble forms of the Receptor Binding Domain (RBD) and S1 domains of the SARS-CoV-2 Spike protein, as

Figure 2. The Opto Viral Neutralization 1.0 workflow enables rapid identification and selection of antibody lead candidates by directly screening B cells in a single day. B cells can be screened from immunized animals or after activation of B cells obtained from the peripheral blood of human donors. Antibodies can be functionally screened in a single day using both bead- and cell-based assays, enabling functional characterization in a single integrated workflow.

well as the S1 domain of the SARS-CoV-1 Spike protein. We also performed an on-chip blocking assay to down-select antibodies that block the interaction between SARS-CoV-2 RBD and ACE-2. We then recovered antigen-specific antibody sequences for these antibodies and confirmed the function of re-expressed antibodies using plate-based assays, validating the accuracy of these on-chip binding assays. These workflows identified 3 antibodies that cross-react with both SARS-CoV-1 and SARS-CoV-2 and also block the interaction between SARS-CoV-2 RBD and ACE-2, making them exciting examples of antibodies that may have broad neutralization capacity across the coronavirus family.

MATERIALS AND METHODS

Upstream plasma B cell isolation

We harvested the spleens and lymph nodes from mice immunized with DNA encoding the SARS-CoV-2 Spike protein S1 subdomain and enriched plasma B cells according to the Plasma B Cell Isolation for Mouse Tissues protocol (Berkeley Lights) of the Opto™ Plasma B Discovery Sample Prep Kit (Berkeley Lights). Briefly, we treated organs with DNase and strained them into RPMI 1640 + 10% FBS. Next, we isolated and collected the mononuclear cell layer from the cell suspension using density-gradient centrifugation. We then counted the cells and sorted the mononuclear cell layer based on the CD138 surface marker using a commercially available magnetic-activated cell sorting (MACS) protocol for plasma B cells using positive isolation (Miltenyi Biotec). Once enriched, we split the plasma B cells into two groups: (1) cells to be screened the day of enrichment (Day 0); and (2) cells to be screened the following day (Day 1). For the Day 1 workflow, we cultured cells overnight in a well plate with plasma B cell culture media (Berkeley Lights) in a commercial incubator.

Plasma B cell screening on the Beacon system

We loaded plasma B cells on multiple Beacon systems (Berkeley Lights) for antibody screening using the Opto Plasma B Discovery 2.0 workflow (Berkeley Lights) and ran four OptoSelect™ 11k chips (Berkeley Lights) in parallel for each workflow.

After cells were imported into the main channels of a chip, Cell Analysis Suite (CAS™) software identified, selected, and moved individual B cells into sub-nanoliter-sized NanoPen chambers using opto-electropositioning (OEP™) technology and counted the number of cells in each

pen. We then performed SARS-CoV-2 specific assays to detect and characterize the specificity, cross-reactivity and function of anti-SARS-CoV-2 antibodies. The assays included assays for identifying antibodies that bind SARS-CoV-1 S1, SARS-CoV-2 S1, and SARS-CoV-2 Receptor Binding Domain (RBD), and one blocking assay for SARS-CoV-2 RBD and human ACE-2. The combination of our Opto™ Plasma B Discovery 2.0 workflow and these virus-specific assays constitute our Opto Viral Neutralization 1.0 workflow.

SARS-CoV-2 RBD / ACE-2 Blocking assay

We designed the blocking assay as an in-pen assay that used bead-bound ACE-2 and soluble mCherry-tagged SARS-CoV-2 RBD. To generate these reagents, we fused amino acid residues 319 to 514 of SARS-CoV-2 S1 with mCherry (RBD-mCherry) and coated streptavidin-coated beads (Berkeley Lights) with Avi-tagged ACE-2 (amino acid residues 18-740) (Acro Biosystems) using standard protocols. To perform the assay, we first loaded ACE-2-coated beads into NanoPen chambers by importing the beads into the main channels of the chips, stopping the fluid flow, removing the chips from the Beacon system, and tilting the chips for approximately 10 minutes to allow the beads to settle into the pens. After the bead load, we remounted the chips on the Beacon system and loaded single plasma B cells into the pens using OEP.

The plasma B cells were then incubated with the ACE-2 coated beads for approximately 60 minutes to increase the concentration of secreted antibodies in each of the pens. We then flowed soluble RBD-mCherry into the main channels of the chip, stopped fluid flow, and allowed the RBD-mCherry to diffuse into the pens to start the assay.

We defined non-blockers as antibodies in pens where RBD-mCherry was able to bind ACE-2, resulting in RBD-mCherry accumulation on the surface of the ACE-2-coated beads and a fluorescence readout in the Texas Red channel. We defined antibodies as blockers if the RBD-mCherry was blocked from binding to the ACE-2-coated beads, resulting in insignificant bead-associated fluorescence in the Texas Red channel.

SARS-CoV-1 S1 and SARS-CoV-2 RBD Binding Assays

We performed a second multiplexed SARS-CoV-1 S1 and SARS-CoV-2 RBD binding assay which comprised human IgG capture beads (Spherotech, Inc), soluble SARS-CoV-2 RBD-mCherry, and SARS-CoV-1 S1 (Acro Biosystems) labeled with Alexa Fluor 647 (AF647) NHS Ester (Thermo Fisher Scientific) using standard protocols. In this assay, we imported the human IgG capture beads into the main channels of the chips in a solution containing SARS-CoV-2 RBD-mCherry and SARS-CoV-1 S1 (AF647). We stopped fluid flow and allowed secreted antibodies to diffuse from the pens to the main channels, where they could be captured on the IgG capture beads. We identified antigen-specific antibodies by detecting accumulation of fluorescently-labeled antigen on the surface of the IgG capture beads, resulting in a fluorescence "halo" at the opening of the pen from which the antibodies were secreted. Antibodies were identified as SARS-CoV-2 RBD binders and/or SARS-CoV-1 S1 binders if fluorescence was detected in the TRED and CY5 channels, respectively.

SARS-CoV-2 S1 Binding Assay

We performed a third assay using soluble SARS-CoV-2 S1 coated on the surface of beads and anti-human IgG secondary antibodies labeled with AF488 (Jackson ImmunoResearch Laboratories, Inc.). We coated soluble SARS-CoV-2 S1 onto streptavidin-coated beads (Berkeley Lights, Inc.) using standard protocols and imported SARS-CoV-2 S1-coated beads into the main channels of the chips in a solution containing the anti-human IgG (AF488) secondary antibody. We stopped fluid flow to allow secreted antibodies to diffuse from the pens into the main channels. We classified antibodies as SARS-CoV-2 S1 binders if the secreted antibodies bound to the SARS-CoV-2 S1-coated beads and generated a fluorescent halo at the opening of the pen detected in the FITC channel.

Antibody Sequence Recovery

After we completed the blocking and binding assays, we exported plasma B cells secreting antigen-specific antibodies into individual wells of 96-well plates pre-filled with lysis buffer and mineral oil to prevent evaporation of the samples.

We synthesized mRNA from the single B cell exports using the cDNA Recovery for Cell Exports protocol (Berkeley Lights, MAN-000047) included with the Opto™ Plasma B Discovery cDNA Synthesis Kit. We then used the Opto™ Plasma B Discovery Sanger Kit, Human (Berkeley Lights) to amplify antibody heavy/light chain genes and performed Sanger sequencing of the amplicons. Sequences were aligned using IgBlast to confirm that the amplicon contained a complete VDJ assembly.

Antibody re-expression and binding confirmation

To confirm binding, we re-expressed a subset of antibodies in HEK293 cells. We cultured transfected cells for 3 days and harvested the IgG supernatant for testing in ELISA plates coated with SARS-CoV-1 S1, SARS-CoV-2 S1, or SARS-CoV-2 RBD (Acro Biosystems). As a control, we used an antibody known to bind the RBD domain and S1 protein of SARS-CoV-2 (Acro Biosystems) and block the binding of ACE-2.

To assay for blocking, we used a commercial kit (Acro Biosystems). We incubated supernatant from HEK cells transiently transfected with rapid re-expression constructs in ELISA plates coated with RBD from SARS-CoV-2. After incubation, we washed the ELISA plates and serially incubated them with ACE-2-biotin and Streptavidin-HRP. We used non-transfected controls to determine signal without blocking activity and set a threshold using the lowest signal generated from these control wells. HRP signals below this threshold were scored as blocking antibodies.

RESULTS AND DISCUSSION

Running multiple functional assays allows rapid discovery of binding and blocking antibodies against SARS-CoV-1 and SARS-CoV-2

The design of OptoSelect chips and NanoPen chambers allows assay reagents to be exchanged on-chip so that multiple assays can be performed on the same population of B cells. This enabled us to screen secreted antibodies for binding to SARS-CoV-2 RBD and S1, binding to SARS-CoV-1 S1, and blocking of SARS CoV-2 RBD to ACE-2.

We first performed a blocking assay by co-incubating plasma B cells with bead-bound ACE-2 in NanoPen chambers, then diffusing in soluble mCherry-tagged SARS-CoV-2 RBD. Plasma B cells that secreted antibodies that blocked the interaction between SARS-CoV-2 and ACE-2 resulted in pens with no significant bead-associated mCherry fluorescence (**Figure 3 A-C**).

After performing the blocking assay, we exchanged blocking assay reagents with binding assay reagents to assess the specificity and cross-reactivity of antibodies against subdomains of the viral Spike proteins from the SARS-CoV-1 and SARS-CoV-2 viruses. In these assays, antibodies secreted by single plasma B cells in NanoPen chambers diffused into the chip channel and were captured on beads coated either with antigen or anti-IgG antibodies. The capture of secreted antibodies recruited the binding of fluorescently-labeled detection reagents, resulting in fluorescence "halo" signals at the mouths of NanoPen chambers that contained antigen-specific antibodies (**Figure 3 D-G**).

By running multiple assays on the same plasma B cells, we were able to both identify and characterize antibodies with multiple different functional profiles. In total, we screened 129,014 plasma B cells from which 698 antibodies (0.5%) were identified as specific to subdomains of the SARS-CoV-1 and/or SARS-CoV-2 viral Spike proteins (**Table 1**). Of these 698 antibodies, we identified 668 SARS-CoV-2 S1 binders, 267 SARS-CoV-2 RBD binders, and 78 SARS-CoV-2 RBD/ACE-2 blockers. We also identified 32 antibodies that cross-reacted with the S1 subdomains of both SARS-CoV-1 and SARS-CoV-2 Spike proteins. Most significantly, in single-day workflows, we identified 3 antibodies that bound SARS-CoV-2 S1 and RBD protein subunits, blocked the CoV-2 RBD/ACE 2 interaction, and cross-reacted with SARS-CoV-1 S1 (**Table 1 and Figure 3**).

Sequence recovery and confirmation of on-chip phenotypes

After we identified plasma B cells secreting antibodies of interest, we exported plasma B cells into 96-well plates

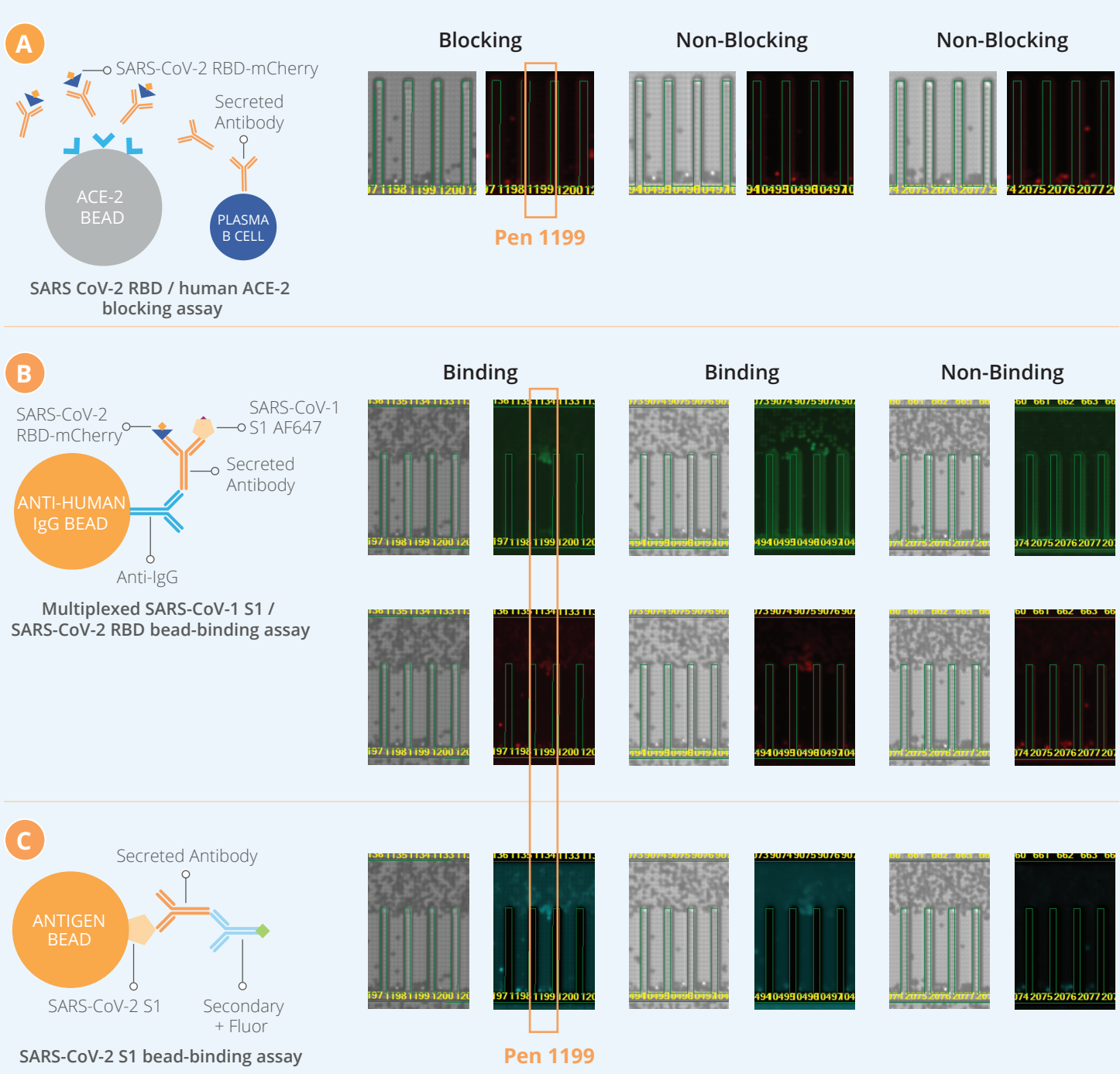

Figure 3. Multiple binding and blocking assays run on the same population of plasma B cells enable selection of antibodies that both bind SARS-CoV-1 and SARS-CoV-2 S1 and RBD Spike protein subdomains and block binding of SARS-CoV-2 RBD to human ACE-2. A. A blocking assay using fluorescent SARS-CoV-2 RBD-mCherry and human ACE-2 coupled to beads. **B.** A multiplexed binding assay for simultaneously detecting antibodies that are specific and cross-reactive against SARS CoV-2 RBD and SARS-CoV-1 S1. **C.** A binding assay for detecting antibodies that bind SARS-CoV-2 S1. Pen 1199 contains a plasma B cell secreting an antibody that binds SARS-CoV-2 RBD and S1, cross-reacts with the SARS-CoV-1 S1, and blocks the interaction between ACE-2 and SARS-CoV-2 RBD.

Antibody Functional Profile				Number of Plasma B Cells Identified	Fraction of Plasma B Cell Population (%)
SARS-CoV-2 S1 Binder	SARS-CoV-2 RBD Binder	SARS-CoV-1 S1 Binder	SARS-CoV-2 RBD / ACE-2 Blocker		
+	–	–	–	409	0.317%
+	+	–	–	163	0.126%
+	+	+	–	11	0.009%
+	+	–	+	65	0.050%
+	–	+	–	17	0.013%
+	–	+	+	0	0.000%
+	–	–	+	0	0.000%
+	**+**	**+**	**+**	**3**	**0.002%**
–	+	–	–	15	0.012%
–	+	+	–	0	0.000%
–	+	+	+	1	0.001%
–	+	–	+	9	0.007%
–	–	+	–	5	0.004%
–	–	+	+	0	0.000%
–	–	–	+	0	0.000%
–	–	–	–	128,316	99.459%
Total Number of Cells Screened				**129,014**	**100.000%**

Table 1. Functional profile of mouse plasma B cells assayed for binding and blocking to Spike protein subdomains from SARS-CoV-1 and SARS-CoV-2. 3 antibodies were discovered that bind SARS-CoV2 S1 and RBD domains, cross-react with SARS-CoV-1 and block the SARS CoV-2 RBD/ACE-2 interaction. + and - signify positive and negative assay scores, respectively.

for cDNA recovery and amplification of antibody heavy/light chain genes. We recovered paired heavy/light chain sequences from >60% (411 of 658) of plasma B cells exported from the chips. From these sequences, we selected 53 SARS CoV-2 RBD/ACE-2 blocking antibodies for re-expression. As measured in plate-based ELISA assays, 100% of re-expressed antibodies (53 of 53) bound to the subdomains (S1 and/or RBD) of the SARS-CoV-2 Spike protein. Importantly, the ELISA measurements confirmed the function of an antibody that

bound both S1 and RBD domains of the SARS-CoV-2 Spike protein, cross-reacted with SARS-CoV-1 S1 domain, and blocked the SARS CoV-2 RBD/ACE-2 interaction.

CONCLUSIONS

Here we have demonstrated how the Opto Viral Neutralization 1.0 workflow can be used to run multiple functional assays to deeply characterize and down-select functional antibody lead candidates against SARS-CoV-2 in a single day. This workflow allowed us to identify nearly 700 antibodies specific to SARS-CoV-1 and SARS-CoV-2 viral proteins, of which 32 antibodies were cross-reactive to both viral strains. We also confirmed that all recovered antibody sequences resulted in re-expressed antibodies that bound the S1 and/or RBD domains of the SARS-CoV-2 Spike protein. Furthermore, we identified and confirmed the function of an antibody lead candidate that bound SARS CoV-2 S1 and RBD domains, cross-reacted with the SARS-CoV-1 S1 domain, and blocked the interaction between SARS-CoV-2 RBD and the human ACE-2 receptor. Such antibodies are particularly desirable because they are presumed to bind conserved parts of the virus and thus may be potentially effective against future coronavirus strains[6].

Rapid discovery of antibodies that bind and block the interaction of viral proteins with human cell surface receptors is a key goal to the development of anti-viral therapies against pandemic viruses. Within months of the onset of the worldwide COVID-19 pandemic, the Opto Viral Neutralization 1.0 workflow was used to identify lead molecules that successfully prevent infection in preclinical animal models, resulting in a therapeutic cocktail in Phase 1 clinical trials[5-7]. Thus, the Opto Viral Neutralization 1.0 workflow could facilitate a global strategy to combat future pandemics by enabling early identification of promising therapeutic candidates.

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