Potent RBD-specific neutralizing rabbit monoclonal antibodies recognize emerging SARS-CoV-2 variants elicited by DNA prime-protein boost vaccination

Yuxin Chen, Liguo Zhu, Weijin Huang, Xin Tong, Hai Wu, Yue Tao, Bei Tong, Haibin Huang, Jiachen Chen, Xianglan Zhao, Yang Lou and Chao Wu

Department of Laboratory Medicine, Nanjing Drum Tower Hospital, Nanjing University Medical School, Nanjing, People’s Republic of China; Jiangsu Provincial Center for Disease Control and Prevention, Nanjing, People’s Republic of China; Division of HIV/AIDS and Sex-Transmitted Virus Vaccines, Institute for Biological Product Control, National Institute for Food and Drug Control, Beijing, People’s Republic of China; Department of Infectious Diseases, Nanjing Drum Tower Hospital, Nanjing University Medical School, Nanjing, People’s Republic of China; Yurogen Biosystem LLC, Worcester, MA, USA; Institute of Botany, Jiangsu Province and Chinese Academy of Sciences, Nanjing, People’s Republic of China; Department of Gastroenterology, Northern Jiangsu People’s Hospital, Clinical Medical College of Yangzhou University, Yangzhou, People’s Republic of China

ABSTRACT

Global concerns arose as the emerged and rapidly spreading SARS-CoV-2 variants might escape host immunity induced by vaccination. In this study, a heterologous prime-boost immunization strategy for COVID-19 was designed to prime with a DNA vaccine encoding wild type (WT) spike protein receptor-binding domain (RBD) followed by S1 protein-based vaccine in rabbits. Four vaccine-elicited rabbit monoclonal antibodies (RmAbs), including 1H1, 9H1, 7G5, and SE1, were isolated for biophysical property, neutralization potency and sequence analysis. All RmAbs recognized RBD or S1 protein with Kd in the low nM or sub nM range. 1H1 and 9H1, but neither 7G5 nor SE1, can bind to all RBD protein variants derived from B.1.351. All four RmAbs were able to neutralize wild type (WT) SARS-CoV-2 strain in pseudovirus assay, and 1H1 and 9H1 could neutralize the SARS-CoV-2 WT authentic virus with IC50 values of 0.136 and 0.026 μg/ml, respectively. Notably, 1H1 was able to neutralize all 6 emerging SARS-CoV-2 variants tested including D614G, B.1.1.7, B.1.429, P.1, B.1.526, and B.1.351 variants, and SE1 could neutralize against the above 5 variants except P.1. Epitope binning analysis revealed that 9H1, SE1 and 1H1 recognized distinct epitopes, while 9H1 and 7G5 may have overlapping but not identical epitope. In conclusion, DNA priming protein boost vaccination was an effective strategy to induce RmAbs with potent neutralization capability against not only SARS-CoV-2 WT strain but also emergent variants, which may provide a new avenue for effective therapeutics and point-of-care diagnostic measures.

INTRODUCTION

The coronavirus disease 2019 (COVID-19) caused by the infection of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was initially identified in December 2019 [1]. The rapid spread of COVID-19 has resulted in more than 156 million confirmed cases and caused around 3.2 million deaths, as of May 8, 2021. The ongoing pandemic continues posing an unprecedented global threat to public health systems. Several promising therapeutic agents including antiviral agents, immunomodulators, glucocorticoids, and convalescent plasma, have been evaluated for treating the COVID-19, generating varying results. However, there are limited approved therapeutic agents against the SARS-CoV-2 available to the general public. SARS-CoV-2 uses the envelope spike (S) glycoprotein, composed of two subunits S1 and S2, to mediate host cell entry. S1 facilitates viral attachment to a cell surface receptor, angiotensin-converting enzyme (ACE2) [2], via its receptor-binding domain (RBD), while S2 is essential for membrane fusion [3]. Disruption of the RBD-ACE2 interaction can block SARS-CoV-2 cell entry, presenting a valid mechanism of clinical interventions.

Due to such an essential role of spike protein during viral entry, recent emerging SARS-CoV-2 variants bearing multiple mutations within spike protein have raised serious concerns. The recent circulating variants include the D614G variant, the B.1.1.7 variant in the United Kingdom, the B.1.351 variant in South America, the B.1.526 variant in the United States, and the B.1.429 variant in South Africa. Some of these variants, such as the B.1.1.7 variant in the United Kingdom, have raised serious concerns due to their increased transmissibility and potential evading host immunity. Therefore, there is an urgent need to develop effective therapeutics and point-of-care diagnostic measures to combat the COVID-19 pandemic.
African, the P.1 variant in Brazil, the B.1.429 variant in California, and the B.1.526 variant in New York. D614G is one of the earliest variants that rapidly emerged and became globally dominant, while convalescent sera demonstrated efficient cross-neutralization for both wild-type strain and D614G variants [4]. B.1.1.7 contains D614G and N510Y in the RBD region, with only slightly neutralization resistance by convalescent individuals and vaccine recipients [5, 6]. The B.1.429 variant, a lineage recently emerged in California, United States, contains 4 missense mutations in spike including a single L452R RBD mutation [7]. The B.1.351 variant (also called 501Y.V2) is one of the greatest variants of concern (VOCs), which was first reported in South Africa and rapidly spreading. B.1.351 bears three RBD mutations (K417N, E484K, and N501Y), in addition to several mutations outside of RBD, leading to substantial or complete loss of neutralization potency from humoral immunity elicited by natural infection and vaccination [7–10]. The P.1 strain in Brazil harboring three RBD mutation has also been demonstrated with effectively escaped neutralization [11, 12]. The B.1.526 variant, another variant currently spreading alarmingly in New York, United States, shares the prevalent D614G and E484K mutation in the RBD region, raising the concerns of escaped immunity for neutralization activity [13]. These fast-spread SARS-CoV-2 variants call for enhanced viral surveillance and efficacy assessment of currently authorized vaccines, therapeutic monoclonal antibodies (mAbs) and convalescent sera against the emerging variants.

Passive administration of monoclonal antibodies (mAbs) has become one of the essential therapeutic agents [14] especially to infectious diseases [15, 16]. A few potent SARS-CoV-2 specific neutralizing antibodies have been identified from infected COVID-19 patients [17–23], immunized mice [24], and llama [25]. Some of these neutralizing antibodies such as bamlanivimab, etesevimab and casirivimab have received Emergency Use Authorization (EUA) by respective regulatory agencies [26]. Accumulating evidence suggested that administration of neutralizing antibodies at the early infection stage could minimize hospitalization and decreases symptom severity among non-hospitalized patients [27], further substantiating neutralizing monoclonal antibodies as valid treatment agents in COVID-19 patients.

Since the rabbit immune system has a unique ontogeny of B cells that is different from mice and other rodents, immunized rabbits usually generate highly diverse antibody repertoires with superior diversity, affinity, and specificity. Improved access to rabbit antibody repertoires by high throughput technologies, such as hybridoma [28], single B cells sorting approach [29, 30] and mass spectrometry [31], has fueled the increased application of rabbit mAbs. Therefore, rabbit monoclonal antibodies (RmAbs) have been widely used for clinical diagnostic and therapeutic applications. Recently, the inhibitor of VEGF-A, Brolucizumab, was the first humanized rabbit ScFv that received FDA approval for patients with wet age-related macular degeneration (AMD) in 2019 [32].

Heterologous prime-boost vaccination strategies using DNA priming vaccine have been adopted in early clinical trials in the context of infectious diseases including HIV [28, 33, 34], influenza [35–37] and malaria [38], eliciting robust and balanced humoral responses and cellular responses. DNA immunization can effectively stimulate both the innate and adaptive immunities to generate high levels of antigen-specific antibody responses, producing functional potent mAbs, in mouse, rabbit and human models [39]. A DNA vaccine (INO-4800) targeting the full-length spike antigen of SARS-CoV-2 has demonstrated an excellent safe and tolerable file, which is immunogenic in 100% vaccinated subjects, as indicated by results from phase 1 clinical trial [40]. A recent COVID-19 vaccine study also suggested that employing a heterologous prime-boost strategy might break the protective immune response bottleneck, inducing balanced neutralizing antibody responses and T cell responses [41].

To analyze the antibody repertoire generated by DNA priming coupled with a protein boost, we utilized SARS-CoV-2 spike protein S1 subunit as the bait to isolate S1-specific single memory B cells from the splenocytes of immunized rabbits. RmAbs were expressed and purified for functional assays. Our data identified at least four highly potent neutralizing rabbit mAbs specific to RBD revealed by either wild-type authentic virus or pseudovirus neutralization assay, indicating a successful vaccination strategy of DNA prime followed by protein boost. More importantly, RmAb 1H1 was able to neutralize all 6 emerging SARS-CoV-2 variants tested including P.1 and B.1.351 variants, two variants of greatest concern, providing a robust basis for developing new therapeutics. Our study sheds light on the development of antibody-based therapeutic treatments and rational vaccine design against the SARS-CoV-2.

**Materials and methods**

**Construction of SARS-CoV-2 DNA vaccine encoding RBD domain and DNA bullet preparation**

The codon-optimized RBD region, corresponding to the genomic positions 22,553 to 23,312 bp in SARS-CoV-2 isolate Wuhan-Hu-1 (GenBank: MN908947.3), was cloned into the pcDNA3.4 expression vector. The expression construct was
propagated in DH5α strain of *Escherichia coli* and purified using the Qiagen Plasmid Mega kit (Cat no. 10023). 36 μg purified expression construct were coated to 100 μL of 100 mg/ml gold powder (Alpha Aesar, Catalog No. 39817) that was precoated with 100 mg/ml spermidine (Sigma, Catalog No. S2626). DNA-coating to gold was facilitated by slowly dripping 200 μl 2.5 M CaCl₂ to the DNA mixed with gold powder, which was washed in absolute ethanol before loading to bullet tubing mounted to bullet maker (Scientz Scientific). Bullet tubing loaded with gold powder was dried by slow N₂ flow at 0.1 MPa for 10 min, and the dried bullet tubing was cut into DNA bullet.

**Recombinant SARS-CoV-2 protein**

Recombinant SARS-CoV-2 S1 protein fused with a Fc tag at C terminal was acquired from Kactus Biosystems, Shanghai (Cat no: COV-VM5S1) for ELISA. Recombinant RBD protein (Kactus Biosystems, Cat no: COV-VM4BD) and SARS-CoV-2 S1 protein fused His tag (Kactus Biosystems, Cat no: COV-VM4S1) were obtained for analyzing ELISA and immunization. Recombinant SARS-CoV-2 ECD protein (Genscript, Cat no: Z03481) and the related RBD variants (RBD N501Y, RBD K417N, RBD E484K, RBD N501Y/K417N/E484K) were used for ELISA binding assay.

**Rabbit immunization**

New Zealand White rabbits (4–6 weeks of age) were purchased and housed in the animal facility. All the procedures were carried out by following animal research guidelines and approved by IACUC (Abclonal, China). The SARS-CoV-2 RBD DNA bullets were loaded into the bullet magazine of the SJ-500 gene gun (Scientz Scientific). The SJ-500 gene gun was fired by 4 MPa helium gas to inject DNA-coated gold powder subcutaneously at shaved abdomen skin (36 μg/immunization) of rabbits. The DNA immunization was performed three times on each rabbit at days 0, 7, and 21. Then, the rabbits were boosted with 100 μg SARS-CoV-2 S1 protein emulsified in incomplete Freund's adjuvant (IFA) twice at day 35 and day 49 via intramuscular (i.m.) route. Two weeks later, the rabbits were boosted subcutaneously (s.c.) with 200 μg S1 protein. Pre and post-immunization sera were collected on days 0, 14, 28, 42 and 69, respectively.

**ELISA for characterization of immunized rabbit sera and monoclonal antibody**

The enzyme-linked immunosorbent assay (ELISA) was performed as previously described [42]. Briefly, 96-well microtiter plates (Corning, Cat no: 9018) were coated with 100 μL of 1 μg/mL ectodomain of spike protein (ECD), S1 protein, RBD protein, RBD variants protein (RBD E484K, RBD K417N, RBD N501Y, RBD N501Y/K417N/E484K), in coating buffer (pH 9.6) overnight at 4°C, respectively. Plates were washed three times in washing buffer (1× PBS supplemented with 0.05% Tween-20 (Sigma, Cat no: P9416)) and blocked with 200 μL blocking buffer (1 × 5% nonfat milk in 1× PBS). Then the plates were incubated with serial diluted rabbit serum samples or monoclonal antibody. After 1-hour incubation at room temperature, plates were washed five times with washing buffer and incubated with anti-rabbit IgG conjugated with HRP (Jackson ImmunoResearch, Cat no: 111-035-045) in blocking buffer at 1:5000 dilution. Plates were washed five times with washing buffer and developed by adding 25 μL TMB substrate (Moss INS, Cat no: TMBHK-1000) for 3 min in the dark at room temperature. Subsequently, the colorimetric reaction of TMB substrate was stopped with 20 μL 1 M H₂SO₄. Optical density (OD) values at 450 nm and 630 nm were measured by Epoch microplate spectrophotometer (Biotek, USA). The final value was obtained using OD₄₅₀ subtracted by OD₆₃₀. The serum titer was calculated as the maximum dilution where the diluted serum produces OD₄₅₀ reading of 2-fold or above than that of the control samples.

**Isolation of S1-specific single B cells from rabbit splenocytes by fluorescence-activated cell sorting (FACS)**

The spleen was harvested from the rabbit with higher titers against the SARS-CoV-2 S1 and RBD domains. Fresh single splenocytes were isolated and cultured overnight in a specialized B cell medium specialized by Yurogen Biosystems (Wuhan, China). A fresh single-cell suspension of splenocytes in PBS supplemented with 2% FBS and 1mM EDTA was prepared before single-cell sorting. Splenocytes were processed using the single B cell SMab® platform [43] at Yurogen Biosystems and sorted one cell per well on a FACS Aria II (BD Biosciences, USA) into 96-well plates. S1-specific primary B cells were cultured in proprietary rabbit B cell complete medium (Yurogen, China) for 10–14 days at 37°C with 5% CO₂.

**Generation of rabbit mAbs**

At the end of primary B cell culture, S1-recognizing B-cell clones were identified by screening of primary B cell culture supernatants against RBD or S1 by direct ELISA. Positive B-cell clones were determined by OD₄₅₀nm values more than 5-fold over background.
noise. The variable region of IgG heavy chain and light chain from top positive clones was recovered by RT-PCR. The full-length IgG heavy and light chains of each clone were co-transfected into HEK293T cells. The supernatants containing recombinant rabbit IgG from transfected HEK293T were screened for their specificity to S1 and RBD by ELISA. The PCR fragments of variable regions of selected clones were cloned into pcDNA3.4 vector for antibody expression in HEK293F cells. Recombinant rabbit mAbs were purified using protein A chromatography for further characterization.

**SARS-CoV-2 pseudotyped virus neutralization assays**

Pseudovirus neutralization assay was performed as previously described with minor modifications [44]. Briefly, SARS-CoV-2 pseudoviruses were prepared using VSV G pseudotyped viruses (G*ΔG-VSV) that package the expression cassette for firefly luciferase instead of VSV-G in the VSV genome. The VSV-based SARS-CoV-2 pseudoviruses contained wild-type, D614G, B.1.351, B.1.1.7, P.1, B.1.429 and B.1.526 variants spike protein. SARS-CoV-2 pseudoviruses were pre-incubated with mAbs starting from B.1.526 variants spike protein. SARS-CoV-2 pseudo-typed, D614G, B.1.351, B.1.1.7, P.1, B.1.429 and B.1.526 variants spike protein. SARS-CoV-2 pseudotyped viruses were double reference subtracted and analyzed to calculate association rate constant, dissociation rate constant, and affinity constants using BIA evaluation 3.2 and 1:1 Langmuir model.

**Surface plasmon resonance analysis**

Surface plasmon resonance (SPR) kinetic analysis was conducted using a BIAcore 3000 instrument with Protein A sensor chip (GE Healthcare, USA). All experiments were performed at 25°C at a flow rate of 40 μL/min. The running buffer was degassed PBS with 0.005% Tween-20. Channel 1 was loaded with a reference antibody without specific binding to the antigens used, and channel 2, 3, and 4 were loaded with the antibody candidates. Typically, 2 μg/mL of antibody and a quick injection for 20–30 s yielded ~150–250 response units (RU) of antibody coupling with high reproducibility. Antigen (S1 or RBD protein) was then injected over all the channel surfaces for 5 min for an association phase followed by a 10-minute dissociation phase by buffer rinse. Multiple association/dissociation cycles were performed using antigen dilution series in the range of 1.2–100 nM, as well as a blank buffer. At the end of each cycle, regeneration was performed by a 30-second injection of glycine buffer (pH 2.0, 10 mM) and antibodies were loaded in each channel again. The kinetic curves were double reference subtracted and analyzed to calculate association rate constant, dissociation rate constant, and affinity constants using BIA evaluation 3.2 and 1:1 Langmuir model.

**Epitope binning assay**

All epitope binning assays were performed on the Gator system (Probe Life, USA) and using the Tandem model for epitope binning of mAbs. Briefly, the anti-His-tag sensor (Probe Life) was pre-wetted in Q buffer (Probe Life), then immobilize 0.5 μg/mL of His-tag SARS-COV2-S1 protein (Kactus Biosystems, Cat. No.COV-VM4S1) to the sensors. The antigen-loaded sensor captured 5 μg/mL of the first rabbit monoclonal antibodies for about 300 s, then the loaded sensor captured 1 μg/mL of the second monoclonal antibodies for about 600 s.
**ELISA assay for ACE2 receptor blocking**

Half maximal effective concentration (EC50) of RBD binding to ACE2 was determined using ELISA. Recombinant ACE2 (Kactus Biosystems, Cat. No. ACE-HM501) was coated at 1 μg/ml on an ELISA plate. B cell supernatant from positive clones with 4 points of 3-fold serial dilution was preincubated with RBD domain protein diluted at EC50 for 1 h at room temperature. B cell supernatant-RBD premix was then deposited to ACE2-coated ELISA plate for 1 h at room temperature.

**Analysis of rabbit mAb heavy chain and light chain sequences**

Sequencing analysis was conducted with the heavy-chain and light-chain genes of rabbit mAbs. Germline V(D)J gene annotation was performed using IMGT/V-QUEST[46]. ClustalW was used and the multiple sequence alignments were performed to construct phylogenetic trees using maximum-likelihood methods. Antibody germline usage, mutation frequency and CDR3 length were determined by IMGT/V-QUEST [47].

**Results**

**Rabbit immunization with RBD DNA prime followed by S1 protein boost**

To produce high-quality SARS-CoV-2 neutralizing antibodies, two female New Zealand white rabbits received 36 μg DNA vaccine encoding SARS-CoV-2 RBD region at day 0, day 7 and day 21. 14 days after primary immunization, rabbits were boosted with two doses of 100 μg recombinant SARS-CoV-2 S1 protein emulsified with incomplete Freund’s adjuvant (IFA) via intramuscular injection on day 35 and day 49. Sera were collected 7 days post each vaccination and subjected to antibody assays (Figure 1(A)). Compared to the pre-bleed serum, post-immunized serum (after three times DNA immunization) showed clear IgG titers against RBD, S1 and ECD, with titers of 2700, 2700 and 900 respectively. Conversely, the post-immunized serum after two protein immunization represented potent and specific serologic activities toward RBD, S1 and ECD binding with a titer of 655,100, 21,800 and 72,900 (Figure 1(B)). Hence, these results indicated that our DNA prime followed by protein boost immunization strategy successfully induced SARS-CoV-2 specific antibodies with virus-neutralizing capability.

**Functional screening of S1-specific single B cells**

To dissect the specificities and binding characteristics of antibody responses, the rabbit with a higher titer against S1 and RBD was subcutaneously injected with 100 μg SARS-CoV-2 S1 protein on day 64 as the final boost. 5 days after the final boost, the rabbit’s spleen was harvested (Figure 1(A)). Single S1-specific binding B cells from splenocytes were isolated by FACS using fluorescent S1 protein probes. A total of 1152...
individual B cells were sorted at 1 cell per well and expanded for 7 days in the rabbit B cell complete medium. The supernatants of the cultured B cell clones were screened using ELISA. Herein, we identified 350 B cell clones that were reactive to S1, and 114 out of 350 were bind to RBD. Among S1-reactive B cell clones, 62 clones were found positive of neutralization activities and 23 blockers of ACE2-RBD interaction (Figure 2 (B)). The top 40 neutralizing clones were cloned and validated for S1 binding, pseudovirus neutralization,

Figure 2. Functional screening of cultured S1-specific single rabbit B cell clones. (A) The sorting strategy for S1-specific B cells from rabbit splenocytes using FACS. (B) High-throughput functional screening of cultured B cells. The number of individually cultured B cell clones (Cultured B cell clones), B cell clones binding SARS-CoV-2 S1 protein (SARS-CoV-2 S1 binding clones), B cell clones binding the SARS-CoV-2 RBD (SARS-CoV-2 RBD binding clones), B cell clones with neutralization potential (Neutralization clones), and B cell clones with the capability of inhibiting RBD and ACE2 interaction (RBD/ACE2 blocking clones) were listed. (C) Representative ELISA screening of cultural supernatants for top 40 B cell clones that can bind to the SARS-CoV-2 S1 protein. (D) Pseudovirus neutralization assay of S1-specific B cell cultural supernatants. (E) ACE2 blocking assay of S1-specific B cell cultural supernatants.
and RBD-ACE2 inhibition (Figure 2(C–E)). Four most potent neutralizing RmAbs clones were selected for recombinant expression and further characterization.

**Binding properties of four RBD-specific RmAbs**

The 4 top B cell clones (1H1, 7G5, 5E1, 9H1) were selected based on their binding epitope and neutralization potency. The naturally paired variable heavy chains (VH) and light chains (VL) were recovered from each cultured B cell clone. The VH and VL fragments were inserted into expression plasmids to express full-length heavy chain and light chain. All four mAbs potently and specifically bind to the RBD, S1 and ECD protein (Figure 3(A)).

Recent SARS-CoV-2 variants emerged in United Kingdom (B.1.1.7) [48], South Africa [49], Brazil [12] and United States [7] have raised concerns on immune escape from antibody recognition. Variant B.1.1.7 has a key mutation N501Y in its spike RBD region and deletion of H69-V70 (S1 del (69-70)) on the spike. Another SARS-CoV-2 lineage, B.1.351, in South Africa (501.V2) had three altered residues in the ACE2 binding site of RBD, including K417N, E484K and N501Y. N501Y mutation leads to enhanced binding to ACE2, whereas K417N/E484K/N501Y in variant 501.V2 increases their infectivity [49]. Subsequently, to determine whether these mutations impact RmAb recognition of RBD, we first compared the antibody binding to wild-type RBD (RBD WT) with that to variants RBD N501Y, RBD K417N, RBD E484K, and RBD N501Y/K417N/E484K. 1H1 and 5E1 recognize RBD WT and RBD variants in a similar manner, but relatively weaker to RBD E484K, RBD K417N, and RBD N501Y/K417N/E484K. 9H1 binds well to RBD WT and most RBD variants except no detectable binding to RBD N501Y. 7G5 binds strongly to RBD WT and RBD E484K, weakly to RBD K417N, and fails to recognize RBD N501Y and RBD N501Y/K417N/E484K (Figure 3(B)). In addition, all four neutralizing RmAbs have a similar binding capacity for S1 WT and S1 69/70 deletion (Figure 3(C)). Thus, the data suggested that despite RBD variants were reported to be capable of immune escape, our vaccine-elicited RmAbs, 1H1 and 5E1, exhibited comparable binding strength to RBD variants. On the contrary, RBD E484K and K417N had little impact on 9H1 recognition with RBD. Moreover, our western blotting analysis revealed that the epitopes of 1H1, 5E1 and 7G5 are linear, while the epitope of 9H1 is likely conformational (Figure 3(D)).

Dissociation constants (K_D) of four RmAbs against the SARS-CoV-2 RBD or S1 protein were measured by surface plasmon resonance (SPR), ranging from 14.0 pM to 6975 pM (Figure 4(A, B)). 9H1 and 7G5 have 4.84-fold and 3.67-fold higher binding affinity to RBD when compared to S1 protein, respectively. 1H1 has a 4.34-fold lower binding affinity to RBD compared to S1 protein, and 5E1 has a comparable binding affinity to both S1 and RBD. The above results suggested that four rabbit mAbs potently and specifically bind to both S1 and RBD.

**Neutralization potency of RmAbs against multiple SARS-CoV-2 variants**

To evaluate the neutralization potency of four rabbit mAbs, both pseudovirus assay and live viral...
neutralization assay were performed. We first determined the neutralization potency of four RmAbs using SARS-CoV-2 wild-type pseudotyped virus assay. The half-maximal inhibitory concentration (IC_{50}) value of 9H1, 1H1, 5E1, and 7G5 for wild type pseudotyped virus assay is 0.014 \mu g/ml, 0.048 \mu g/ml, 0.438 \mu g/ml, and 0.235 \mu g/ml, respectively (Figure 5 (A, B)). Consistent with pseudovirus neutralization, 1H1 and 9H1 could neutralize authentic SARS-CoV-2 virus, with IC_{50} values of 0.136 and 0.026 \mu g/mL, respectively.

To determine whether these RmAbs could neutralize currently circulating SARS-CoV-2 variants, we then tested the neutralization potency of RmAbs against six emerging SARS-CoV-2 variants including the D614G variant, the B.1.1.7 variant, the B.1.429 variant, the P.1 variant, the B.1.526 variant, and the B.1.351 variant (Figure 5 (A, B)). Specifically, we found that neutralization of D614G variant was comparable with wild-type strain by four RmAbs, in line with previous reports [50]. Consistent with ELISA data of 1H1 and 5E1 binding with RBD N501Y, the neutralization potency of 1H1 and 5E1 is similar to that of the wild type and D614G strain, while 9H1 and 7G5 which failed to bind to RBD 501Y also presented neutralization resistance of the B.1.1.7 variant. RBD L452R mutation from B.1.429 variant leads to a slightly increased neutralization resistance of these RmAbs. Since the attenuated neutralizing activity of convalescent sera against emergent variants was largely attributed to the E484K substitution [51], we then tested the other three E484K harboring variants, including P.1, B.1.526, and B.1.351. For Brazil P.1 with three RBD mutations (K417T/E484K/N501Y), only 1H1 was found to mediate neutralization in our study. Despite the neutralization potency against in the B.1.526 strain with D614G and E484K mutation has been slightly reduced by four RmAbs, all four RmAbs isolated from our study were still capable to neutralize B.1.526 variant in presence of E484K mutation. B.1.351, one variant of the greatest concern, was able to be neutralized by two vaccine-elicited RmAbs identified in our study, 1H1 and 5E1, with a comparable IC_{50} as wild-type strain. Notably, 1H1 was able to neutralize all emerging SARS-CoV-2 variants tested at microgram or submicrogram level, 5E1 could neutralize against D614G, B.1.1.7, B.1.429, and B.1.526, B.1.351 except to P.1. 9H1 and 7G5 could neutralize D614G, B.1.429 and B.1.526 variants, but not the other three variants tested.

**Epitope binning and phylogenetic characterization of RmAbs**

To determine the epitope and sequence basis of the four neutralizing RmAbs, epitope binning and phylogenetic characterization were performed. Firstly, we evaluated whether these antibodies could compete for similar epitopes using the Tandem binding
model of antibody pairs by Gator. S1 protein was loaded to Gator optic probes to capture the first antibodies in each antibody pair, followed by the incubation with second RmAbs. As shown in Figure 6 (A), the addition of either 1H1 or 5E1 with 9H1-loaded probes increased signal shift indicative of binding of a second antibody to S1 protein, the addition of 5E1 to 1H1 loaded probe could also increase signal shift. Therefore, the results suggest that 9H1 does not compete with either 1H1 or 5E1, and 1H1 does not compete with 5E1. On the other hand, 7G5 was not able to further elevate the signal shift on the 9H1 loaded probe, suggesting that 9H1 could compete with 7G5 for S1. Our epitope binning results suggested that 9H1, 1H1 and 5E1 recognize distinct unique epitopes on S1, while 9H1 and 7G5 are likely to share a partially overlapped epitope since 9H1 could not recognize linearized S1 protein as 7G5 did in western blotting.

Besides, to define the genetic relationship of these RmAbs, a phylogenetic tree was constructed based on variable region sequences heavy-chain and light chain genes using the maximum-likelihood method (Figure 6(B)). 1H1 and 7G5 heavy chains have less sequence diversification when compared with 9H1 and 5E1, while 1H1 and 5E1 light chains are phylogenetically closer. Finally, we conducted a detailed sequence characterization of the four neutralizing RmAbs (Figure 6(C)). The heavy chain variable region (IGHV) of 1H1 and 7G5 belong to the 1S40*01F family, and IGHV of 9H1 and 5E1 belong to the 1S69*01F family, with the same germline joining segment J4*01F. The corresponding light chain variable regions (IGKV) of RmAbs belong to 1S49*01, 1S32*01, 1S56*01, 1S15*01 families, respectively, but sharing the same joining segment J1-2*01. Somatic hypermutation (SHM) frequency of IGHV for the above four RmAbs ranges from 3.47% to 6.16%, while SHM frequency for IGHJ varies considerably from 0.00% to 25.53%. SHM frequency of IGKV for the above four RmAbs ranges slightly higher from 8.24% to 11.11%, while that of IGKJ is from 5.00% to 7.50%. Even though the length of complementarity-determining region 3 (CDR3) for 1H1, 5E1, 7G5 and 9H1 heavy chains changes widely from 5 to 15 amino acid residues (aa), the CDR3 length of light chains remains narrowly distributed between 12 and 13 aa (Figure 6(C)).
In this study, we described a panel of S1-binding mAbs generated from rabbit primed with a DNA vaccine encoding the SARS-CoV-2 S1 protein. Binding intensity was indicated as the shift in nm over time. Using a single B cell SMab® platform, we identified four neutralizing RBD-specific mAb for further characterization. Our vaccine-induced RBD-specific mAbs, 9H1, 7G5, 5E1 and 1H1, recognized not only RBD and S1 protein with high binding affinity, but also some recently emerging RBD variant proteins including RBD N501Y, RBD 417N, RBD E484K, and RBD N501Y/K477N/E484K. Besides, 9H1, 5E1 and 1H1 recognized distinct epitopes in the RBD domain, while 7G5 shared a partially overlapping epitope with 9H1. Consistent with their capabilities of blocking RBD-ACE2 interaction in ELISA, four RmAbs were able to neutralize SARS-CoV-2 tested by wild-type pseudovirus neutralization assay, two of which were further validated by neutralization assay of wild type live virus strain. Notably, 1H1 was also

**Discussion**

In this study, we described a panel of S1-binding mAbs generated from rabbit primed with a DNA vaccine encoding the SARS-CoV-2 RBD followed by S1 protein boost. Using a single B cell SMab® platform, we identified four neutralizing RBD-specific mAb for further characterization. Our vaccine-induced RBD-specific mAbs, 9H1, 7G5, 5E1 and 1H1, recognized not only RBD and S1 protein with high binding affinity, but also some recently emerging RBD variant proteins including RBD N501Y, RBD 417N, RBD E484K, and RBD N501Y/K477N/E484K. Besides, 9H1, 5E1 and 1H1 recognized distinct epitopes in the RBD domain, while 7G5 shared a partially overlapping epitope with 9H1. Consistent with their capabilities of blocking RBD-ACE2 interaction in ELISA, four RmAbs were able to neutralize SARS-CoV-2 tested by wild-type pseudovirus neutralization assay, two of which were further validated by neutralization assay of wild type live virus strain. Notably, 1H1 was also
able to neutralize all six emerging SARS-CoV-2 mutant variants tested including D614G, B.1.1.7, B.1.429, P.1, B.1.526 and B.1.351 variants.

Understanding the immune response to SARS-CoV-2 is of utmost importance. A recent vaccine study reported that the rabbit animal model, RBD immunogen elicited antibody with 5-fold higher affinity to native spike antigen, compared to S1 and ectodomain (ECD) of spike protein [52]. S1 domain induced a diverse antibody repertoire recognizing the NTD and RBD regions, recombinant RBD induced high-titer antibodies focused on the RBD/receptor binding motif (RBM). Nevertheless, the S2 domain does not appear to elicit as many neutralizing antibodies as RBD or S1. These results suggested that RBD and S1 are ideal immunogens that induce high neutralizing antibodies. In our study, we used a DNA vaccine expressing RBD priming followed by S1 protein boosting, leading to the discovery of potent neutralizing RmAbs specific to RBD.

As demonstrated by ELISA and SPR assays, all 4 anti-RBD rabbit mAbs showed well recognition of three spike protein-related antigens, including RBD, S1 and ECD, despite only S1 and RBD were used for immunization. It is noteworthy that 1H1 has a higher affinity to S1 protein than RBD protein, 9H1 and 7G5 were found to bind RBD with relatively higher affinity if compared to their binding to S1 protein, while 5E1 had a comparable binding affinity for both S1 and RBD. Additionally, the epitope binning assay revealed that 9H1, 5E1 and 1H1 recognize distinct epitopes in the RBD domain, while 7G5 shares likely an overlapping epitope with 9H1. Hence, results propose that these rabbit mAbs recognize diverse epitopes on RBD regions of SARS-CoV-2 spike protein. The data is also consistent with our heterologous prime-boost vaccination strategy, in which the priming DNA vaccine expressing RBD antigen firstly stimulated naïve B cells to generate RBD-specific mAbs and further underwent affinity maturation and somatic hypermutation to give rise to mature antibodies with high affinity and broad diversity. The generally better recognition of RBD than S1 of our RmAbs also reflects the switching of the boosting immunogen to RBD in the prime-boost immunization strategy. Boosting with RBD promoted a more focused immune response and antibody maturation to RBD-specific repertoire.

The best binding affinity does not necessarily confer the highest neutralization capability. 9H1 and 1H1 had potent neutralization capability as demonstrated by both SARS-CoV-2 wild-type pseudovirus neutralization assay and authentic virus neutralization assay. The most potent neutralizing RmAb, 9H1, showed 34.38-fold weaker binding affinity to S1 and 1.63-fold lower binding affinity of RBD when compared to 1H1, whereas 9H1 has 5.23-fold increased neutralizing potency than 1H1 as revealed by authentic virus neutralization assay. A plausible explanation is that the conformational epitope of 9H1 may be better exposed on the trimeric spike protein of SARS-CoV-2 native virions compared to that on S1 or RBD protein. Alternatively, 9H1 could more effectively promote binding avidity to trimeric spike proteins on the virion surface. Nevertheless, consistent with the relative weaker binding affinity with wild-type RBD, 9H1 was found to have a relative limited neutralization breadth, which failed to neutralize the emerging variants, including B.1.1.7, P.1 and B.1.351. The fine epitope of 9H1 and 1H1 remains yet to be defined.

Developing new vaccines to elicit broadly neutralizing antibodies and identifying of therapeutic monoclonal antibodies capable to neutralize circulating SARS-CoV-2 variants are essential to resolve the ongoing COVID-19 pandemic. In our study, in addition to SARS-CoV-2 wild type strain, we tested a panel of emerging SARS-CoV-2 variants using VSV-based SARS-CoV-2 pseudovirus neutralization assay, including the D614G variant, the United Kingdom B.1.1.7 variant, the California B.1.429 variant, the Brazil P.1 variant, the New York B.1.526 variant, and the Brazil B.1.351 variant. Accumulating evidence demonstrated that several key mutations within RBD region could confer potent neutralization escape not only in COVID-19 convalescent plasma [53], but also in vaccinee sera elicited by mRNA vaccination [54], inactivated virus vaccine, or protein-based vaccine [55]. Notably, in our study, 1H1 was able to neutralize all 6 emerging variants tested including B.1.351 and P.1, two VOCs refractory to most of well-characterized mAbs with EUA approval [51, 56]. Moreover, 5E1 could neutralize 5 out of 6 variants tested but not P.1. It is interesting to determine whether such broad neutralization of RmAbs identified in our study is resulted from heterologous DNA prime protein boost vaccination strategy used in our study [41]. Additionally, our result was also assuring that despite of RBD mutations presented in recent circulating SARS-CoV-2 isolates, our RmAbs induced by vaccines formulated from wild-type strain in our study were capable of neutralize against most of emerging variants.

To the best of our knowledge, this is the first panel of RmAbs that neutralize the SARS-CoV-2 wild type and the variants strains. A list of rabbit mAbs has been approved by FDA for diagnostics and therapeutics, encouraging the great clinical potentials of rabbit mAbs. Different from mice, rabbits have a unique B cell ontogeny that generates a highly diverse antibody repertoire. Furthermore, rabbit B cells undergo sophisticated affinity maturation processes which include both somatic hypermutation and gene conversion. Additionally, being genetically diverse and evolutionarily distinct from mice, rabbit
antibodies can be generated against unique epitopes that might be non-immunogenic in mice. This allows the generation of rabbit mAbs with superior sensitivity, affinity and specificity if compared to mouse mAbs. Indeed, in our study, we identified 4 RmAbs with diverse epitope recognition, high affinity, and broad neutralizing activity. Four isolated RmAbs were able to potently neutralize SARS-CoV-2 and most of the emerging SARS-CoV-2 variants, suggesting that RmAbs might target at certain unique epitopes which were cross-reactive with multiple SARS-CoV-2 VOCs including P.1 and B.1.351. In contrast, the P.1 and B.1.351 were refractory to most of EUA approved RBD-specific mAbs [51, 56], which were isolated from convalescent individuals or humanized mice.

Compared to the conventional RmAb discovery approach using hybridoma technology, our strategy of using a high-throughput single-B-cell cloning approach is efficient and robust to identify functional rabbit mAbs. Taking advantage of the single B-cell SMaB* platform, we were able to clone and express the recombinant mAbs within 2 weeks. Our approach enables the accelerated discovery and identification of novel mAbs specific to newly emerged pathogens, resulting in a fast and effective response to challenging clinical needs.

Our study provides promising diagnostic and therapeutic measures against the SARS-CoV-2 but needs further experimental evidence and validation. Although our study identified a panel of rabbit mAbs with potent in vitro neutralization activities, the prophylactic or therapeutic potential of these RmAbs has not been fully validated in animal infection models. The fine epitopes of these RmAbs are yet to be defined for specific mechanisms of action in virus neutralization. Moreover, the humanization of these promising RmAbs is needed to better evaluate their therapeutic potentials.

In summary, we identified four potent neutralizing RmAbs specific to the SARS-CoV-2 spike RBD region from a rabbit immunized with DNA vaccine prime followed by a boost of protein vaccine. These anti-RBD RmAbs induced by the heterologous prime-boost vaccine had broad and potent neutralizing activity against SARS-CoV-2 and most of the currently global circulating SARS-CoV-2 variants. Our newly identified RmAbs may proffer a novel means for versatile, cost-effective therapeutics and point-of-care diagnosis.

Disclosure statement
No potential conflict of interest was reported by the author(s).

Funding
This study was supported by Nanjing Medical Science and Technique Development Foundation [grant number QRX17141], Nanjing Department of Health [grant numbers YKK19056, YKK19078], China Postdoctoral Science Foundation for COVID-19 [grant number 2020T130049ZX], Science & Technology Program of Jiangsu Commission of Health (Grant No. Q2017003), National Natural Science Foundation of China [grant number 81600201] and Yangzhou Key R&D Program (Social Development) [grant number YZ2020101].

References

[1] Huang C, Wang Y, Li X, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet. 2020;395:497–506.
[2] Wu F, Zhao S, Yu B, et al. A new coronavirus associated with human respiratory disease in China. Nature. 2020;579:265–269.
[3] Hoffmann M, Kleine-Weber H, Schroeder S, et al. SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor. Cell. 2020;181:271–280.
[4] Hou YJ, Chiba S, Halfmann P, et al. SARS-CoV-2 D614G variant exhibits efficient replication ex vivo and transmission in vivo. Science. 2020;370:1464–1468.
[5] Davies NG, Abbott S, Barnard RC, et al. Estimated transmissibility and impact of SARS-CoV-2 lineage B.1.1.7 in England. Science. 2021;372(6538):eabg3055.
[6] Wang P, Nair MS, Liu L, et al. Antibody resistance of SARS-CoV-2 variants B.1.351 and B.1.1.7. Nature. 2021. doi:10.1038/s41586-021-03398-2
[7] Shen X, Tang H, Pajon R, et al. Neutralization of SARS-CoV-2 variants B.1.429 and B.1.351. N Engl J Med. 2021. doi:10.1056/NEJMc2103740
[8] Planas D, Bruel T, Grzelak L, et al. Sensitivity of infectious SARS-CoV-2 B.1.1.7 and B.1.351 variants to neutralizing antibodies. Nat Med. 2021. doi:10.1038/s41591-021-01318-5
[9] Wang GL, Wang ZY, Duan LJ, et al. Susceptibility of circulating SARS-CoV-2 variants to neutralization. N Engl J Med. 2021. doi:10.1056/NEJMoa2103022
[10] Li Q, Nie J, Wu J, et al. SARS-CoV-2 501Y.V2 variants lack higher infectivity but do have immune escape. Cell. 2021;184:2362–2371.
[11] Faria NR, Mellan TA, Whitaker C, et al. Genomics and epidemiology of the P.1 SARS-CoV-2 lineage in Manaus, Brazil. Science. 2021. doi:10.1126/science.abb2644
[12] Dejmirattisai W, Zhou D, Supasa P, et al. Antibody evasion by the P.1 strain of SARS-CoV-2. Cell. 2021. doi:10.1016/j.cell.2021.03.055
[13] West AP, Wertheim JO, Wang JC, et al. Detection and characterization of the SARS-CoV-2 lineage B.1.526 in New York. bioRxiv. 2021. doi:10.1101/2021.02.14.431043
[14] Weiner GJ. Building better monoclonal antibody-based therapeutics. Nat Rev Cancer. 2015;15:361–370.
[15] Marston HD, Paules CI, Fauci AS. Monoclonal antibodies for emerging infectious diseases – borrowing from history. N Engl J Med. 2018;378:1469–1472.
[16] Mulangu S, Dodd LE, Davey RT, et al. A randomized, controlled trial of ebola virus disease therapeutics. N Engl J Med. 2019;381:2293–2303.
[17] Chi X, Yan R, Zhang J, et al. A neutralizing human antibody binds to the N-terminal domain of the
spike protein of SARS-CoV-2. Science. 2020. doi:10.1126/science.abc6952

[18] Shi R, Shan C, Duan X, et al. A human neutralizing antibody targets the receptor-binding site of SARS-CoV-2. Nature. 2020;584:120–124.

[19] Liu L, Wang P, Nair MS, et al. Potent neutralizing antibodies against multiple epitopes on SARS-CoV-2 spike. Nature. 2020;584:450–456.

[20] Hansen J, Baum A, Pascal KE, et al. Studies in humanized mice and convalescent humans yield a SARS-CoV-2 antibody cocktail. Science. 2020;369:1010–1014.

[21] Rambaut NL A, Pybus O, Barclay W, et al. (2020). virological.org/t/preliminary-genomic-characterisation-of-an-emergent-sars-cov-2-lineage-in-the-uk-defined-by-a-novel-set-of-spike-mutations. Virological org https://virological.org/t/preliminary-genomic-characterisation-of-an-emergent-sars-cov-2-lineage-in-the-uk-defined-by-a-novel-set-of-spike-mutations/563.

[22] Ju B, Zhang Q, Ge J, et al. Human neutralizing antibodies elicited by SARS-CoV-2 infection. Nature. 2020;584:115–119.

[23] Rogers TF, Zhao F, Huang D, et al. Isolation of potent SARS-CoV-2 neutralizing antibodies and protection from disease in a small animal model. Science. 2020;369:956–963.

[24] Alsoussi WB, Turner JS, Case JB, et al. A potently neutralizing antibody protects mice against SARS-CoV-2 infection. J Immunol. 2020;205:915–922.

[25] Dong J, Huang B, Jia Z, et al. Development of multi-specific humanized llama antibodies blocking SARS-CoV-2/ACE2 interaction with high affinity and avidity. Emerg Microbes Infect. 2020;9:1034–1036.

[26] U.S. Food & Drug Administration. Fact sheet for health care providers emergency use authorization (EUA) of bamlanivimab; 2021. https://www.fda.gov/media/145802/download

[27] Chen P, Nirula A, Heller B, et al. SARS-CoV-2 neutralizing antibody LY-CoV555 in outpatients with covid-19. N Engl J Med. 2020. doi:10.1056/NEJMoa2009849

[28] Chen Y, Vaine M, Wallace A, et al. A novel rabbit monoclonal antibody platform to dissect the diverse repertoire of antibody epitopes for HIV-1 Env immunogen design. J Virol. 2013;87:10232–10243.

[29] Starbke DO, Compson JE, Rapecki S, et al. Generation of recombinant monoclonal antibodies from immunised mice and rabbits via flow cytometry and sorting of antigen-specific IgG+ memory B cells. PLoS One. 2016;11:e0152282.

[30] Seebet S, Ros F, Thorrey I, et al. A robust high throughput platform to generate functional recombinant monoclonal antibodies using rabbit B cells from peripheral blood. PLoS One. 2014;9:e86184.

[31] Cheung WC, Beausoleil SA, Zhang X, et al. A proteomics approach for the identification and cloning of monoclonal antibodies from serum. Nat Biotechnol. 2012;30:447–452.

[32] Markham A. Brolucizumab: first approval. Drugs. 2019;79:1997–2000.

[33] Zolla-Pazner S, Powell R, Yahyaei S, et al. Rationally designed vaccines targeting the V2 region of HIV-1 gp120 induce a focused, cross-clade-reactive, biologically functional antibody response. J Virol. 2016;90:10993–11006.

[34] Chen Y, Wang S, Lu S. DNA immunization for HIV vaccine development. Vaccines. 2014;2:138–159.

[35] Houser KV, Yamshchikov GV, Bellamy AR, et al. DNA vaccine priming for seasonal influenza vaccine in children and adolescents 6 to 17 years of age: A phase 1 randomized clinical trial. PLoS One. 2018;13:e0206837.

[36] Joyce MG, Wheatley AK, Thomas PV, et al. Vaccine-induced antibodies that neutralize Group 1 and Group 2 influenza A viruses. Cell. 2016;166:609–623.

[37] Wang G, Zhou F, Buchy P, et al. DNA prime and virus-like particle boost from a single H5N1 strain elicits broadly neutralizing antibody responses against head region of H5 hemagglutinin. J Infect Dis. 2014;209:676–685.

[38] Obaldia N, Stockelman MG, Otero W, et al. A plasmodium vivax plasmid DNA- and adenosivirus-vectored malaria vaccine encoding blood-stage antigens AMA1 and MSP142 in a prime/boost heterologous immunization regimen partially Protects Aotus monkeys against blood-stage challenge. Clin Vaccine Immunol. 2017;24(4):e00539–16.

[39] Liu S, Wang S, Lu S. Using DNA immunization to elicit monoclonal antibodies in mice, rabbits, and humans. Hum Gene Ther. 2018;29:997–1003.

[40] Tebas P, Yang S, Boyer JD, et al. Safety and immunogenicity of INO-4800 DNA vaccine against SARS-CoV-2: A preliminary report of an open-label, phase 1 clinical trial. EClinicalMedicine. 2021;31:100689.

[41] He Q, Mao Q, An C, et al. Heterologous prime-boost: breaking the protective immune response bottleneck of COVID-19 vaccine candidates. Emerg Microbes Infect. 2021;10:629–637.

[42] Chen Y, Tong X, Li Y, et al. A comprehensive, longitudinal analysis of humoral responses specific to four recombinant antigens of SARS-CoV-2 in severe and non-severe COVID-19 patients. PLoS Pathog. 2020;16:e1008796.

[43] Chu R, Gerstein J, Wu H, et al. Development of rabbit monoclonal antibodies for quantitation of therapeutic human antibodies in preclinical non-human primate studies. Monoclon Antib Immunodiagn Immunother. 2020;39:175–183.

[44] Nie J, Li Q, Wu J, et al. Establishment and validation of a pseudovirus neutralization assay for SARS-CoV-2. Emerg Microbes Infect. 2020;9:680–686.

[45] Hamilton MA, Russo RC, Thurston RV. Trimmed Spearman-Karber method for estimating median lethal concentrations in toxicity bioassays. Environ Sci Technol. 1977;11:6.

[46] Lefranc MP, Giudicelli V, Duroux P, et al. IMG(r), the international ImMunoGeneTics information system(R) 25 years on. Nucleic Acids Res. 2015/43: D413–D422.

[47] Lefranc MP, Giudicelli V, Ginestoux C, et al. IMGT, the international ImMunoGeneTics information system. Nucleic Acids Res. 2009;37:D1006–D1012.

[48] Rambaut NL A, Pybus O, Barclay W, et al. (2020). Preliminary genomic characterisation of an emergent SARS-CoV-2 lineage in the UK defined by a novel set of spike mutations. Virological org https://virological.org/t/preliminary-genomic-characterisation-of-an-emergent-sars-cov-2-lineage-in-the-uk-defined-by-a-novel-set-of-spike-mutations/563.

[49] Tegally H, Wilkinson E, Giovanetti M, et al. Emergence and rapid spread of a new severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2) lineage with multiple spike mutations in South Africa. medRxiv. 2020. doi:10.1101/2020.12.21.20248640.2020.12.21.20248640

[50] Garcia-Beltran WF, Lam EC, St Denis K, et al. Multiple SARS-CoV-2 variants escape neutralization
by vaccine-induced humoral immunity. Cell. 2021. doi:10.1016/j.cell.2021.03.013

[51] Wang P, Nair MS, Liu L, et al. Antibody resistance of SARS-CoV-2 variants B.1.351 and B.1.1.7. Nature. 2021;593:130–135.

[52] Ravichandran S, Coyle EM, Klenow L, et al. Antibody signature induced by SARS-CoV-2 spike protein immunogens in rabbits. Sci Transl Med. 2020;12 (550):eabc3539. doi:10.1126/scitranslmed.abc3539

[53] Wibmer CK, Ayres F, Hermanus T, et al. SARS-CoV-2 501Y.V2 escapes neutralization by South African COVID-19 donor plasma. Nat Med. 2021;27:622–625.

[54] Xie X, Liu Y, Liu J, et al. Neutralization of SARS-CoV-2 spike 69/70 deletion, E484K and N501Y variants by BNT162b2 vaccine-elicited sera. Nat Med. 2021. doi:10.1038/s41591-021-01270-4

[55] Huang B, Dai L, Wang H, et al. Serum sample neutralisation of BBIBP-CorV and ZF2001 vaccines to SARS-CoV-2 501Y.V2. Lancet Microbe. 2021. doi:10.1016/S2666-5247(21)00082-3

[56] Wang P, Casner RG, Nair MS, et al. Increased resistance of SARS-CoV-2 variant P.1 to antibody neutralization. Cell Host Microbe. 2021. doi:10.1016/j.chom.2021.04.007