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Development and characterization of SARS-CoV-2 variant-neutralizing monoclonal antibodies

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A B S T R A C T

Vaccination and administration of monoclonal antibody cocktails are effective tools to control the progression of infectious diseases and to terminate pandemics such as COVID-19. However, the emergence of SARS-CoV-2 mutants with enhanced transmissibility and altered antigenicity requires broad-spectrum therapies. Here we developed a panel of SARS-CoV-2 specific mouse monoclonal antibodies (mAbs), and characterized them based on ELISA, Western immunoblot, isotyping, and virus neutralization. Six neutralizing mAbs that exhibited high-affinity binding to SARS-CoV-2 spike protein were identified, and their amino acid sequences were determined by mass spectrometry. Functional assays confirmed that three mAbs, F461G11, F461G15, and F461G16 neutralized four variants of concern (VOC): B.1.1.7 (alpha), B.1.351 (beta), P.1 (gamma) and B.1.617.2 (delta) These mAbs are promising candidates for COVID-19 therapy, and understanding their interactions with virus spike protein should support further vaccine and antibody development.

1. Introduction

Unlike any other outbreaks of an infectious disease in recent history, the coronavirus 2019 (COVID-19) pandemic has changed public health, the global economy, and people's lifestyle. It has resulted in over 192 million confirmed cases and more than 4.1 million deaths worldwide as of 2021 July 23 (https://covid19.who.int/), since its outbreak was recognized in December 2019. It is still not clear when this pandemic will be contained or controlled (Scudellari, 2020).

SARS-CoV-2, the causative agent of COVID-19, is a positive-sense single-strand RNA virus closely related to SARS-CoV (Kim et al., 2020). Approximately 20% of people infected with SARS-CoV-2 remain asymptomatic throughout the infection. In the remaining portion, 80%, exhibit only mild or moderate disease (WHO, 2021) and approximately 15% develop severe disease requiring hospitalization, oxygen support, and other interventions (WHO, 2021; Wu et al., 2020). The case-fatality rates (CFR) of COVID-19 in different regions varies

substantially, from below 1% to up-to-20% (https://www.cebm.net/covid-19/global-covid-19-case-fatality-rates/; Sorci et al., 2020; Zhou et al., 2020; Onder et al., 2020; Kim et al., 2021; Ergonul et al., 2021). Several risk factors are highly correlated with COVID-19 severity and death, including age (>60 yr), obesity, comorbidities such as cardiovascular disease, chronic kidney disease, and diabetes mellitus (Imam et al., 2020; Ergonul et al., 2021; Onder et al., 2020; Zhou et al., 2020; Marin et al., 2021). SARS-CoV-2 has a lower CFR than SARS-CoV and MERS (Ergonul et al., 2021; Hu et al., 2021).

Although SARS-CoV-2 is not as lethal as SARS-CoV and MERS, it is much more contagious, transmitting efficiently through direct contact, respiratory droplets and aerosols through speaking, coughing and sneezing (Wiersinga et al., 2020; Editor, 2020). Keeping a social distance, washing hands, or using hand sanitizer, and wearing masks in enclosed spaces have proven to be effective measures to slow down transmission. Failure to follow these measures can result in a surge of infections followed by exhaustion of medical resources such as hospital

beds, ICUs, and ventilators, and significantly increased deaths (Ergonul et al., 2021), a scenario which many regions have experienced during this pandemic (Onder et al., 2020; Meyerowitz et al., 2021).

Central to controlling the pandemic is vaccination and a number of effective vaccines, developed against the Wuhan wild-type strain, are being administered on a global level (https://www.who.int/emergen cies/diseases/novel-coronavirus-2019/covid-19-vaccines). At this point, all current vaccines target the virus spike protein with the intent of developing neutralizing antibodies against this protein in order to block virus entry into cells (Dong et al., 2020; Krammer, 2020). In addition, vaccines that elicite strong cellular response were also explored (Dong et al., 2020; Krammer, 2020).

As an additional therapeutic measure, neutralizing antibodies, alone or in cocktails, are being assessed. Three SARS-CoV-2-neutralizing monoclonal antibody (mAb) products: REGEN-COV (casirivimab plus imdevimab) (Hansen et al., 2020; Baum et al., 2020a, 2020b), bamlanivimab plus etesevimab, and Sotrovimab were approved for the treatment of mild to moderate COVID-19 in outpatients under Emergency Use Authorizations (EUAs) by the FDA (The U.S. Food and Drug Administration FDA, 2021a; The U.S. Food and Drug Administration (FDA), 2021b; The U.S. Food and Drug Administration (FDA), 2021c). These mAbs were developed through isolated memory B cells from either convalescent patient or immunized mice that contain humanized immunoglobulin genes (Hansen et al., 2020; Shi et al., 2020; Jones et al., 2021; Pinto et al., 2020). All these antibodies showed efficacy in preclinical and clinical studies to SARS-CoV-2 strains isolated at the early stage of this pandemic. However, their neutralization against the most recent SARS-CoV-2 variants has not been fully explored.

Although vaccination and antibody cocktail prophylaxis/therapy seems promising, the emergence of SARS-CoV-2 variants with increased transmissibility, virulence, and antibody-resistance has raised concerns on the success of halting the pandemic (Zhou et al., 2021; Dejnirattisai et al., 2021). Recent studies suggested that several variants such as B.1.1.7 (alpha), B.1.351 (beta), P.1 (gamma) and B.1.617.2 (delta) have displaced the original strain first isolated from samples collected in Wuhan, China in late 2019 (https://www.gisaid.org/hcov19-variants/). These variants contain multiple mutations in the spike protein, demonstrate enhanced transmissibility (Li et al., 2020), and resistance to many antibodies generated during the early stage of this pandemic. For example, variants B.1.351 and P.1 escape both bamlanivimab (LY-CoV555) and etesevimab (LY-CoV016) (Wang et al., 2021; Starr et al., 2021). B.1.351 also showed significant resistance to a panel of mAb cocktails in clinical use or under clinical development, including casirivimab (REGN10933) plus imdevimab (REGN10987), COV2-2196 plus COV2-2130 (Starr et al., 2021; Wang et al., 2021). In addition, B.1.351 resists the neutralization by convalescent plasma and hyperimmune sera from vaccinated volunteers (Wang et al., 2021; Li et al., 2020).

In this study, we report the generation of a panel of murine hybridomas recognizing SARS-CoV-2 spike protein or nucleoprotein. After screening, we identified six neutralizing mAbs with high affinity to SARS-CoV-2 spike protein. Moreover, three of them demonstrated robust neutralization of a broad-spectrum of SARS-CoV-2 variants, including B.1.1.7, B.1.351, P.1, B.1.617.2 and B.1.525, indicating their potential as therapeutic agents.

2. Materials and methods

2.1. SARS-CoV-2 preparation

VeroE6 cells were infected at low moi ($<$0.01) with SARS-CoV-2 (hCoV-19/Canada/ON_ON-VIDO-01-2/2020, GISAID accession# EPI_-ISL_425177) in neat DMEM. After 3 days, supernatant was pooled and cell debris pelleted by centrifugation at $1500 \times g$ for 15 min. Virus was pelleted by centrifugation at 28,000 rpm for 1hr, and washed with PBS. Titer prior to inactivation was determined by standard plaque assay. The

virus was inactivated by treatment with 0.1% formaldehyde solution for 3 days at 4 °C. Inactivation was confirmed by passage of 10-fold serial dilutions of the inactivated virus stock on VeroE6 cells with repassage of the culture supernatants onto fresh VeroE6 cell cultures for another 3 days to confirm lack of cytopathic effect that would be due to virus replication.

2.2. Expression and purification of SARS-CoV-2 spike and NP protein

The coding sequence of the SARS-CoV-2 spike ectodomain amino acids 1–1215 (NCBI Accession # MN908947), was modified (Pallesen et al., 2017), and placed in frame with a Thrombin cleavage site, T4 foldon trimerization motif (Tao et al., 1997), a Strep tag II (Schmidt and Skerra, 2007) and a FLAG tag (Hopp et al., 1988). The sequence was codon optimized for human cell expression, and cloned into pcDNA 3.1. Recombinanat SP was expressed in HEK 293 Expi cells and purified on an AKTA Pure chromatography system (Cytiva, Marlborough, MA, USA). Recombinant NP was expressed and purified using a similar regimen. (**Supplementary Materials and Methods**).

2.3. Immunization of mice and cell fusion

Mice were housed in an animal facility accredited by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) and approved by the Institutional Animal Care and Use Committee (IACUC) of the National Microbiology Laboratory Canada.

Female BALB/c mice (5–6 weeks old) were immunized subcutaneously with 2×10^6 pfu formalin-inactivated SARS-CoV-2 virus in PBS with an equal volume of Emulsigen-D adjuvant (MVP adjuvants. Omaha, NE, USA), and boosted at days 21, 35, 49, and 63, respectively. The mice were further boosted subcutaneously with 3×10^6 pfu formalininactivated SARS-CoV-2 virus three days before cell fusion.

2.4. Cell fusion, generation of mAbs, and immunoassays

Cell fusion and mAb generation were conducted as described before (Berry et al., 2004). Enzyme-linked immunosorbent assay (ELISA), Western immunoblot, and antibody isotyping also followed protocols described previously (Berry et al., 2004).

2.5. Antibody de novo amino acid sequence determination and analysis

Antibody digestion procedures were developed from protocols described in Bandeira et al. (2008) and Tran et al. (2016) (**Supplementary Materials and Methods**).

Full details on mass spectrometry data-dependant aquisistion methods are available in a previous publication (McQueen et al., 2019). Raw mass spectrometry files were analyzed using PEAKS AB software (Bioinformatics Solutions Inc.) (**Supplementary Materials and Methods**). Peptide sequences were aligned against a database of sequences (part of PEAKS AB) to obtain the full amino acid sequence (Shan and Xin, 2013; Tran et al., 2016).

IgBLAST (https://www.ncbi.nlm.nih.gov/igblast/index.cgi) was used for germline gene alignment and allele determination based on protein sequences. Amino acid sequences were also back-translated into DNA sequences via EMBOSS Backtranseq (https://www.ebi.ac. uk/Tools/st/emboss_backtranseq) for further analysis based on nucleic acid sequences using IMGT/V-QUEST (http://www.imgt.org/I MGT_vquest/analysis) and IgBLAST.

2.6. Purified mAb endpoint titer test

Purified mAbs were tested for antigen binding titers to SARS-CoV-2 rSP by ELISA described previously (Berry et al., 2004). Endpoint titers of each sample were determined as the lowest concentrations of the wells at which the optical density (OD) was threefold higher than the

Table 1

Identification and Preliminary characterization of SARS-CoV-2 monoclonal antibodies (hybridoma culture supernatants).

SARS-CoV-2 mAbs	Characterization methods						
	ELISA (O.D) (1/4 dilution of culture supernatants)			Western-blot	Isotype	Surrogate Virus Neutralization Test (sVNT) (% of positive control)	Plaque Reduction Neutralization Test (PRNT)
	rSP (100ng/well)	rNP (100ng/well)	Formalin inactivated Virus (5×10^4 pfu/well)				
F457G1	2.03	0.07	0.45	rSP	IgG1/k	8%	Negative
F457G2	0.07	1.59	0.87	rNP	IgG1/k	1%	Negative
F457G3	0.08	1.79	0.91	rNP	IgG1/k	5%	Negative
F457G4	2.22	0.07	0.24	rSP	IgG1/M/k	14%	Negative
F457G5	0.07	0.07	0.48	N	IgG1/k	4%	Negative
F457G6	0.07	0.88	0.53	rNP	IgG1/k	1%	Negative
F457G7	0.07	0.07	0.35	N	IgG1/k	11%	Negative
F457G8	2.01	0.07	0.43	rSP	IgG1/k	4.3%	Negative
F457G9	0.08	1.18	0.62	rNP	IgG1/k	1%	Negative
F457G10	0.08	1.31	0.54	rNP	IgG1/k	1%	Negative
F457G11	0.08	0.07	0.34	N	IgG1/k	93%	Negative
F457G12	0.08	1.39	0.58	rNP	IgG1/k	(2.62%	Negative
F458G1	N	0.73	0.24	rNP	IgG1/k	15.26%	Negative
F459G1	1.69	N	N	rSP	IgG2a/k	97%	PRNT$_{50}$: ≥640; PRNT$_{90}$: 80
F459G2	2.68	N	N	rSP	IgG2b/k	6.44%	Negative
F459G3	2.9	N	N	rSP	IgG2a/k	11.71%	Negative
F459G4	0.56	N	N	rSP	IgG1/k	10.69	Negative
F459G5	0.6	N	N	rSP	IgG1/k	5.72%	Negative
F459G6	0.5	N	N	rSP	IgG2b/k	7.66%	Negative
F459G7	0.07	2.3	N	rNP	IgG1/k	14.48%	Negative
F459G8	2.1	N	N	rSP	IgG2b/k	11.22%	Negative
F459G9	3.2	N	N	rSP	IgG1/k	6.6%	Negative
F459G10	1.35	N	N	rSP	IgG1/k	10.88%	Negative
F459G11	1.29	N	N	rSP	IgG1/k	15.77%	Negative
F459G12	0.76	N	N	rSP	IgG1/k	15.92%	Negative
F459G13	0.73	N	N	rSP	IgG1/k	4.44%	Negative
F459G14	1.59	N	N	rSP	IgG1/k	7.7%	Negative
F461G1	3.2	N	N	rSP	IgG1/k	3.8%	N
F461G2	3.2	N	N	rSP	IgG1/k	2.8%	N
F461G3	1.28	N	N	rSP	IgG1/k	0.48%	N
F461G4	3	N	N	rSP	IgG2a/k	8.71%	N
F461G5	N	3.5	N	rNP	IgG2a/λ	7.67%	N
F461G6	N	3.4	N	rNP	IgG1/k	5.55%	N
F461G7	N	3.5	N	rNP	IgG1/k	6.61%	N
F461G8	1.00	N	N	rSP	IgG1/k	87%	PRNT$_{50}$: 160; PRNT$_{90}$: negative
F461G9	0.47	N	N	rSP	IgG1/k	15.7%	N
F461G10	3.3	N	N	rSP	IgG2a/k	3.18%	N
F461G11	1.10 (1/2 dilution)			rSP	IgG1/k	92%	PRNT$_{50}$: 160; PRNT$_{90}$: 20
F461G12	N	2.8	N	rNP	IgG1/λ	6.55%	N
F461G13	N	3.3	N	rNP	IgG1/k	2.18%	N
F461G14	2.94	N	N	rSP	IgG1/M/k	93%	PRNT$_{50}$: 40; PRNT$_{90}$: negative
F461G15	1.18	N	N	rSP	IgG1/k	94%	PRNT$_{50}$: 80; PRNT$_{90}$: 20
F461G16	1.33	N	N	rSP	IgG1/k	93%	PRNT$_{50}$: 80; PRNT$_{90}$: negative
F461G17	2.13	N	N	rSP	IgG2b/M/k	31%	Negative

N: no test or not available; rSP: recombinant spike protein; rNP: recombinant nucleoprotein; Negative: below detection limit.

negative control.

2.7. Antibody affinity analysis using surface plasmon resonance (SPR)

Measurement of the affinity of the mAbs for rSP was performed as described (Karlsson et al., 1991) using a Biacore 2000 instrument (Biacore, Uppsala, Sweden) (**Supplementary Materials and Methods**). BIAevaluation 3.2 software was used to measure and plot the k_{on} and k_{off} values directly, which were used to calculate the affinity (K_D).

2.8. Surrogate virus neutralization test (sVNT)

The SARS-CoV-2 Surrogate Virus Neutralization Test Kit (GenScript, Piscataway, NJ, USA) was used to detect neutralizing antibodies against SARS-CoV-2 that block the interaction between the receptor-binding domain (RBD) of the viral spike glycoprotein with the ACE2 cell surface receptor.

2.9. Plaque reduction neutralization test

The hybridoma culture supernatants and six purified neutralizing mAbs were tested at the same antibody concentration (1 mg/mL). The SARS-CoV-2 PRNT was adapted from a previously described method for SARS-CoV-1 (Wang et al., 2005), and with modifications (Valcourt et al., 2021; Papenburg et al., 2021) (**Supplementary Materials and Methods**). PRNT-50 titers and PRNT-90 titers ≥20 were considered positive for SARS-CoV-2 neutralizing antibodies, whereas titers <20 were considered negative for SARS-CoV-2 neutralizing antibodies. The

reference strain and all variants used for the PRNT were sequence confirmed.

3. Results

3.1. Development and screening of SARS-CoV-2 specific monoclonal antibodies

Four mice were immunized and boosted with formalin-inactivated SARS-CoV-2, and the spleens were used for fusion and hybridoma selection.

A panel of forty-four clones were detected based on ELISA screening against purified inactivated SARS-CoV-2, recombinant spike protein (rSP), recombinant nucleoprotein (rNP), and in parallel with negative screening with BSA. Forty-one clones that reacted with rSP or rNP, but not BSA (Table 1), were selected for antigen specificity confirmation by western immunoblot using non-reducing SDS-PAGE condition. Twenty-eight mAbs reacted specifically with SARS-COV-2 spike protein, and thirteen mAbs reacted specifically with nucleoprotein.

Isotype analysis found that most clones are IgG/k type mAbs, while F461G5 and 461G12 contain IgG/λ. F457G4, F461G14, and F461G17 initially contained both IgG and IgM. After two rounds of subcloning, only IgG was selected from F457G4 and F461G14. F461G17 and F458G1 stopped producing antibodies during subcloning and were excluded in the following analyses (Table 1).

Unpurified hybridoma culture supernatants were used for the SARS-CoV-2 surrogate virus neutralization test (sVNT), and eight clones (F457G11, F459G1, F461G8, F461G11, F461G14, F461G15, F461G16, and F461G17) showed potential virus neutralization capability ($\geq$20% inhibition rate of the positive control). Of those, six mAbs were purified and further identified based on plaque reduction neutralization test (PRNT) using a SARS-CoV-2 isolate (Canada/ON_ON-VIDO-01-2/2020, EPI_ISL_42517) (Table 1). All nucleoprotein-specific mAbs didn't show neutralization capacity, and their characteristics is under investigation and will be reported in a future study.

3.2. Characterization of SARS-CoV-2 neutralization antibodies

Based on the preliminary live virus neutralization results, a lead panel of six spike protein-specific mAbs (F459G1, F461G8, F461G11, F461G14, F461G15, and F461G16) were selected for subcloning and production on a large scale for testing antibody sequence, antigen specificity, antigen affinity, surrogate virus neutralization test, and neutralization of multiple SARS-CoV-2 variants.

3.2.1. Antibody sequence analysis

Anti-SARS-CoV-2 antibody amino acid sequences were determined using mass spectrometry based *de-novo* sequencing of purified monoclonal antibody digests. Template sequences for each antibody were exported from PEAKS AB and considered to be the primary amino acid sequence. All six antibody sequences were generated from the results of two replicate digestion sets except for F461G14, which was from the results of a single digestion set. We were able to obtain 100% sequence coverage for all antibodies. Each sequence was evaluated manually for annotation of CDR and amino acid confidence. All the amino acids in F461G1, F461G11, F461G16 heavy and light chain sequences were identified with >95% confidence. All amino acids in F461G14 and F461G15 were identified with >95% confidence except for two amino acids in the CDR3 that were identified with >85% confidence. F461G8 had three unidentified amino acids, two with >85% confidence and one with >95% confidence of the six CDR3 amino acids.

The sequences of the six neutralization mAbs used three heavy-chain-variable genes, with the VH5-9-1*01 gene most frequently used by four mAbs (F461G11, F461G14, F461G15 and F461G16) (Table 2). While the light-chain of the six mAbs belong to two gene families, using three light-chain-variable genes, with the VK4-80*01 used by three

Table 2

Sequence analysis of neutralization antibodies[a] (purified mAbs).

rSP specific mAbs	V gene (Heavy Chain)	Number of mutations and gaps, and identity compared to Germline (Heavy Chain)	V gene (Light Chain)	Number of mutations and gaps and Identity compared to Germline (Light Chain)
F459G1	IGHV1-26*01	15 (84.7%)	IGKV6-20*01	3 (96.8%)
F461G8	IGHV1-53*01	24 (75.5%)	IGKV6-15*01	6 (93.7%)
F461G11	IGHV5-9-1*01	5 (94.9%)	IGKV6-20*01	19 (80.0%)
F461G14	IGHV5-9-1*01	3 (96.9%)	IGKV4-80*01	17 (81.9%)
F461G15	IGHV5-9-1*01	6 (93.9%)	IGKV4-80*01	18 (81.1%)
F461G16	IGHV5-9-1*01	13 (87.7%)	IGKV4-80*01	18 (81.1%)

[a] : the germline genes were based on the highest scores in the alignments; rSP: recombinant spike protein.

mAbs. The sequence identity to germline varied from 75.5% to 96.9% for heavy chains and from 80.0% to 96.8% for light chains, respectively. The broad range of sequence identity to the germline genes is consistent with repeating exposure to the antigen. The same heavy and light chain genes were used by F461G14, F461G15, and F461G16, suggesting they were derived from related hybridoma clones.

3.2.2. Western immunoblot

All six mAbs reacted specifically with the SARS-CoV-2 rSP, but not BSA (negative control) in Western immunoblots. These antibodies reacted with spike protein only in non-reducing conditions, but not in reducing SDS-PAGE (with DTT). This indicated that these mAbs targeted conformational epitopes maintained by disulfide bonds (Supplementary Fig. 1).

3.2.3. The binding affinity of mAbs with SARS-CoV-2 spike protein

Both ELISA and Biacore were used to determine the binding affinity of selected mAbs with rSP.

All six mAbs showed strong binding with spike proteins. Two mAbs, F461G8 and F461G11, had endpoint titers at 156 ng/mL. While the other four mAbs, F459G1, F461G14, F461G15, and F461G16 showed stronger binding with the endpoints at 19.5 ng/mL, 4.9 ng/mL, 4.9 ng/mL, and 3.1 ng/mL, respectively.

Biacore analysis demonstrated a similar trend of rSP-antibody interaction as indicated by endpoint ELISA. Both F461G8 and F461G11 showed binding affinity at the nanomolar level, while the other four mAbs had a stronger affinity to the S protein, with the K_D below 1 nM (Table 3).

3.2.4. Biological activity

Both sVNT and PRNT tests were used for effector function analysis. sVNT is a fast and easy way to screen the antibodies that target the receptor-binding domain (RBD) of the SARS-CoV-2 spike protein. In contrast, the PRNT is a more accurate assay by using the clinical isolated virulent virus. However, it can only be done in a biosafety level 3 (BSL3) lab and is labor-intensive.

The preliminary tests using the hybridoma culture supernatants demonstrated the neutralizing capacity of the six lead mAbs. Purified mAbs were further tested to determine the effects of selected clones. As shown in Table 3, all six mAbs demonstrated strong neutralization capacity using the sVNT, with 96% or more blocking of the RBD binding with ACE2, compared to the positive control. Because of the nature of this test, the results also suggest that the epitope binding domains of the six neutralizing mAbs are located in the receptor-binding domain (RBD).

The six mAbs that showed high blocking values were further

Table 3
Characterization of selected six neutralization mAbs (purified mAbs).

rSP specific mAbs	End point values (ELISA)	Affinity (K_D)	Surrogate Virus Neutralization Test (sVNT) (% of positive control) (1 mg/mL)	PRNT (1 mg/mL)
F459G1	19.5 ng/mL	0.188	96.1%	$PRNT_{50} \geq 640$; $PRNT_{90} = 320$
F461G8	156 ng/mL	8.06	96%	$PRNT_{50} = 320$; $PRNT_{90} = 80$
F461G11	156 ng/mL	9.66	96.2%	$PRNT_{50} \geq 640$; $PRNT_{90} = 320$
F461G14	4.9 ng/mL	0.353	96%	$PRNT_{50} \geq 640$; $PRNT_{90} = 320$
F461G15	4.9 ng/mL	0.212	96%	$PRNT_{50} \geq 640$; $PRNT_{90} = 320$
F461G16	3.1 ng/mL	0.177	96.2%	$PRNT_{50} \geq 640$; $PRNT_{90} = 160$

rSP: recombinant spike protein.

characterized in the PRNT against the SARS-CoV-2 clinical isolate used for hybridoma development. All mAbs demonstrated strong SARS-CoV-2 $PRNT_{90}$ titers ranging from 80 to 320. In summary, all selected six mAbs demonstrate substantial neutralization potency against the SARS-CoV-2 virus.

As the pandemic has progressed, several SARS-CoV-2 variants have emerged and spread in different regions (https://www.gisaid.org/hcov19-variants/). These variants show stronger transmission potency and evasion of mAbs and polyclonal antibodies induced by vaccination (Graham et al., 2021; Leung et al., 2021; World Health Organization, 2021; Public Health England, 2021a). Therefore, we also tested the neutralization activity of these lead mAbs against the variants of concern (VOCs) present in North America (https://www.cdc.gov/coronavirus/2019-ncov/cases-updates/variant-surveillance/variant-info.html#Concern) and other continents.

The neutralization potency of the candidate mAbs to the four VOCs are ascribed into three categories: 1, significantly reduced compared to most of the variants (F461G8 and F461G14, more than a thirty two-fold reduction of the neutralization activity to two (F461G8) to three (F461G14) VOCs, and eight-fold reduction to another VOC (F461G8)), 2, moderately reduced (F459G1, four to sixteen-fold reduction of the neutralization activity to three VOCs), and 3, unchanged or improved neutralization (F461G11, F461G15, and F461G16, up to two-fold decrease and eight-fold increase of the neutralization activity to the VOCs) (Fig. 1).

Both F461G8 and F461G14 showed a substantial reduction of neutralization potency to most of the tested variants. Specifically, the neutralization potency of F461G8 to variants P.1 and B.1.351, decreased up to thirty-two fold, and the potency to variant B.1.1.7 and B.1.617.2 decreased two-fold and eight-fold (by $PRNT_{50}$), respectively. F461G14 showed neutralizing activity at more than thirty-two- to sixty-four–fold reduction to variants P.1, B.1.1.7, and B.1.351, respectively. F459G1 showed sixteen-, four- and eight-fold reduced neutralizing activity to P.1, B.1.1.7, and B.1.351, respectively. In contrast, F461G11, F461G15 and F461G16 showed minor or no change of neutralization (within two-fold reduction or increase of $PRNT_{90}$ titers) to variants P.1, B1.1.7, and B.1.351. Moreover, the three mAb clones exhibited increased neutralizing (four-to eight-fold increase of neutralization by $PRNT_{90}$ titers) variants B.1.617.2, compared to the reference isolate (hCoV-19/Canada/ON_ON-VIDO-01-2/2020, EPI_ISL_425177).

The neutralization of the six mAbs to B.1.525, one variant of interest (VOI) was also consistent with their response to the VOCs. Both F461G8 and F461G14 showed drastic reduction of the neutralization potency, while the other mAbs had only minor or no change of the neutralizing activity.

In summary, F461G11, F461G15, and F461G16 demonstrated strong neutralizing activity to a broad spectrum of clinical isolates, including the current four variants of concern that are prevalent in various
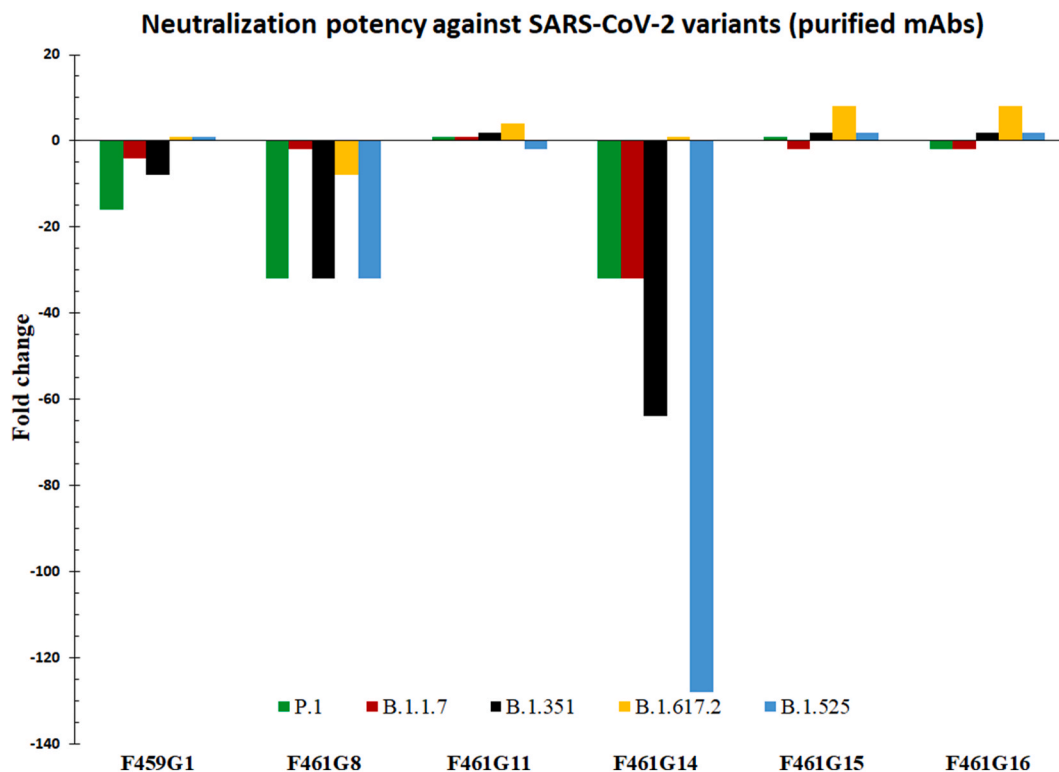

Fig. 1. Neutralization potency changes against SARS-CoV-2 variants by purified mAbs, Six purified neutralizing mAbs were tested against selected variants by Plaque Reduction Neutralization Test (PRNT), and the $PRNT_{90}$ values were compared to the reference strain (hCoV-19/Canada/ON_ON-VIDO-01-2/2020, EPI_ISL_425177) tested at the same time. Increased neutralization potency was positive and reduced potency was designed as negative. F461G8 neutralization potency to B.1.617.2 was based on $PRNT_{50}$, as the $PRNT_{90}$ values were too low to calculate.

geographical regions.

4. Discussion

The COVID-19 pandemic has heavily hit public health and the global economy during the last one and a half years. The vigorous vaccination campaigns in several countries have been crucial in preventing the spread of infections and minimizing severe consequences such as hospitalizations, intensive care admissions or deaths (https://covid19.who.int/table?tableChartType=heat). The development of mAbs and their prophylactic and therapeutic application may also improve the protection of patients or vulnerable individuals, such as the elderly, and people with obesity or comorbidity against more transmissible SARS-CoV-2 variants. However, adaptive evolution of escape mutations in response to the immune pressure and the emergence of immune resistant variants have the potency to hinder efforts to control COVID-19.

Recent studies have shown that several SARS-CoV-2 variants demonstrate resistance to mAbs developed for therapy and polyclonal antibodies induced by vaccine candidates and virus infection. For example, variant B.1.1.7 resists the neutralization of LY-CoV016 (Eli Lilly) (Wang et al., 2021; Dejnirattisai et al., 2021; Starr et al., 2021). Variant P.1 and B.1.351 show significant resistance to both Lilly antibodies (LY-COV555 and LY-CoV016) and REGN10933 (Regeneron) (Wang et al., 2021; Dejnirattisai et al., 2021; Starr et al., 2021). These two variants also demonstrate modest resistance to AstraZeneca's AZD8895 (Dejnirattisai et al., 2021). In addition, the neutralizing activities of convalescent plasma and sera from vaccinated people also decreased (Wang et al., 2021; Dejnirattisai et al., 2021; Starr et al., 2021). In contrast, several mAbs (S309 from Vir, and ADG10, 20 and 30 from Adagio) showed similar neutralization of all variants and early isolates strains (Dejnirattisai et al., 2021; Zhou et al., 2021; Wang et al., 2021).

We generated a panel of spike protein and nucleoprotein specific mAbs by hybridoma fusion and identified six anti-SP mAbs that demonstrate potent inhibition of an early SARS-CoV-2 isolate (hCoV-19/Canada/ON_ON-VIDO-01-2/2020, EPI_ISL_425177). We then tested their neutralization activity to several representative variants, including B.1.1.7 (alpha), B.1.351 (beta), P.1 (gamma), B.1.617.2 (delta) and B.1.525 (eta). B.1.1.7, B.1.351, P.1 and B.1.617.2 are variants with higher transmissibility and are designated as 'Variants of Concern' by the WHO (https://www.who.int/en/activities/tracking-SARS-CoV-2-variants/). B.1.525 belongs to the 'Variants of Interest' category Several candidate mAbs, F461G11, F461G15, and F461G16 demonstrated comparable or increased neutralizing activities to all the variants and the reference strain (hCoV-19/Canada/ON_ON-VIDO-01-2/2020, EPI_ISL_425177, which was isolated at the early stage of this pandemic and belong to the same L clade as the GISAD designated official reference strain WIV04) (Sarkar et al., 2021) indicating that these mAbs have broad-spectrum antiviral potency. Moreover, the three candidate mAbs showed a four-to eight-fold increase of neutralization of B.1.617.2, the variant with over 50% higher transmissibility than B.1.1.7 (Public Health England, 2021b), and increased resistance to tested vaccines (Wall et al., 2021; Bernal et al., 2021). In addition, they exhibited a two-fold increase of neutralization of B.1.351, a variant that resists many neutralizing antibodies (Dejnirattisai et al., 2021).

Both P.1 and B.1.351 contain three similar changes in the RBD of the spike protein (K417 N/T, E484K, and N501Y), while B.1.1.7, B.1.617.2, and B.1.525 have a single mutation at N501Y, L452R, and E484K, respectively. The neutralization titers of F461G11, F461G15and F461G16 are similar across the variants tested, suggesting the epitopes of these three mAbs are not located in these three variant defining amino acid sites. In contrast, F461G8 had a considerable reduction of neutralization of P.1, B.1.351, B.1.617.2 and B.1.525 (up to 32-fold loss of neutralization) but not of B.1.1.7, indicating the epitope of F461G8 contains amino acid sites 452 and 484 of the spike protein. The neutralization of F461G14 to all tested variants except B.1.617.2 was

severely reduced, suggesting that it is interacting with at least two amino acids in sites 484 and 501. Lastly, F459G1 showed moderate loss of neutralization of P.1, B.1.1.7, and B.1.351, implying that the site of interaction may contain asparagine at position 501.

All forty-four mAbs generated in this study were pre-screened using sVNA, and thirty-three were further tested by PRNT. Except for F457G11 and F461G17, all the pre-screening results were consistent with the neutralization of the isolate used for mouse immunization (Table 1), confirming the validity of the sVNT test. F457G11 showed substantial neutralization (93% of the positive control) in the sVNT, but could not inhibit virus infection during virus PRNT. It is possible that the folding of HRP-RBD in sVNT is not the same as that of the wild-type virus spike protein. Thus F457G11 could not access the binding site and neutralize the virus. Although F457G11 showed a positive result in sVNT, the value (31% of the positive control) was substantially lower than the other several neutralizing antibodies (about 96% of the positive control), which may be below the detection limit in the virus PRNT assay.

All the leading six neutralizing antibodies in this study were pre-screened using the surrogate virus neutralization assay, based on their binding to the RBD of SARS-CoV-2 spike protein (Tan et al., 2020). However, it is essential to point out that some antibodies can recognize other domains within the spike protein, such as the NTD of S1, or fusion loop of S2, which may also interfere with the interaction between the RBD and ACE2, or membrane fusion, and inhibit virus entry into the host cell (Jiang et al., 2020; Chi et al., 2020). Therefore, assessment of the other mAbs based on virus neutralization test is needed to fully characterize all spike protein-specific antibodies.

Overall, amino acid sequencing of antibodies using a combination of shotgun proteomic techniques and PEAKS AB *de-novo* sequencing was able to provide high quality sequence information. Most amino acid sequences were determined with >95% confidence while only F461G8 had three unidentified amino acids in the CDR3. CDR3 is a region of high variability so it is natural to assume that challenges would be observed in sequencing this antibody region. Sequencing in this region will largely be dependent on the quality of *de-novo* determined sequences and the amount of variation in this region between homologous sequences. In most cases, we were able to improve sequence information in this region by analysing multiple replicate digests of the same antibody. Ongoing work will continue to further improve the accuracy of amino acid sequences of the antibodies, for example by using different digestion enzymes and/or peptide fragmentation methods.

Four neutralizing antibodies (F461G11, F461G14, F461G15, and F461G16) share the same germline heavy chain V gene (IGHV5-9-1*1) and three of them (F461G14, F461G15, and F461G16) share the same germline light chain V gene (IGKV4-80*01) as well. In contrast, the light chain of F461G11 was suggested from IGKV6-20*01 (80.0% identity). Further analysis found that it is 79.6% identical with IGKV4-80*01, indicating that F461G11 was also derived from hybridoma clone related the other three mAbs. Given the similarity of F461G11, F461G15, and F461G16 in sequences and neutralization potency on the VOCs, it is speculated that they share similar antigen epitopes. It is also intriguing that F461G14, although has similar germline heavy and light chain V genes as the other three, can not neutralize three VOCs (alpha, beta, and gamma variants). Antibody binning and epitope mapping will be conducted to answer these questions.

In summary, we identified three SARS-CoV-2 spike protein-specific murine mAbs that demonstrate broad-spectrum neutralization of the variants of concern. We are working on epitope binning and mapping, and the mAb-spike protein structure determination. Understanding the interactions between these mAbs and the spike protein is expected to shed light on developing a new generation vaccine against a broad spectrum of SARS-CoV-2 variants. In addition, a humanized version of these mAbs, after thorough characterization of the efficacy and safety (European Medicines Agency (EMA), 2016, The U.S. Food and Drug Administration (FDA), 2021d, World Health Organization (WHO), 2016), might also provide more therapy options for COVID-19.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.antiviral.2021.105206.

References

Bandeira, N., Pham, V., Pevzner, P., et al., 2008. Beyond edman degradation: automated de novo protein sequencing of monoclonal antibodies. Nat. Biotechnol. 26, 1336–1338. https://doi.org/10.1038/nbt1208-1336.

Baum, A., Ajithdoss, D., Copin, R., et al., 2020a. REGN-COV2 antibodies prevent and treat SARS-CoV-2 infection in rhesus macaques and hamsters. Science 370, 1110–1115. https://doi.org/10.1126/science.abe2402.

Baum, A., Fulton, B.O., Wloga, E., et al., 2020b. Antibody cocktail to SARS-CoV-2 spike protein prevents rapid mutational escape seen with individual antibodies. Science 369, 1014–1018. https://doi.org/10.1126/science.abd0831.

Bernal, J.L., Andrews, N., Gower, C., et al., 2021. Effectiveness of COVID-19 vaccines against the B.1.617.2 variant. MedRixv. https://doi.org/10.1101/2021.05.22.21257658.

Berry, J.D., Jones, S., Drebot, M.A., et al., 2004. Development and characterization of neutralising monoclonal antibody to the SARS-coronavirus. J. Immunol. Methods 120, 87–96. https://doi.org/10.1016/j.jviromet.2004.04.009.

Chi, X., Yan, R., Zhang, J., et al., 2020. A neutralizing human antibody binds to the N-terminal domain of the spike protein of SARS-CoV-2. Science 369, 650–655. https://doi.org/10.1126/science.abc6952.

Dejnirattisai, W., Zhou, D., Supasa, P., et al., 2021. Antibody evasion by the P.1 strain of SARS-CoV-2. Cell 184, 1–16. https://doi.org/10.1016/j.cell.2021.03.055.

Dong, Y., Dai, T., Wei, Y., et al., 2020. A systematic review of SARS-CoV-2 vaccine candidates. Sig. Transduct. Target Ther. 5, 237. https://doi.org/10.1038/s41392-020-00352-y.

Editorial, 2020. Covid-19 transmission-up in the air. Lancet Respir. Med. 8, 1159. https://doi.org/10.1016/S2213-2600(20)30514-2.

Ergonul, O., Akyol, M., Tanriover, C., et al., 2021. National case fatality rates of the COVID-19 pandemic. Clin. Microbiol. Infect. 27, 118–124. https://doi.org/10.1016/j.cmi.2020.09.024.

European Medicines Agency (EMA), 2016. Guideline on Development, Production, Characterisation and Specification for Monoclonal Antibodies and Related Products. https://www.ema.europa.eu/en/documents/scientific-guideline/guideline-development-production-characterisation-specification-monoclonal-antibodies-related_en.pdf. (Accessed 14 October 2021).

Graham, M.S., Sudre, C.H., May, A., et al., 2021. Changes in symptomatology, reinfection, and transmissibility associated with the SARS-CoV-2 variant B.1.1.7: an ecological study. Lancet Public Health 6, e335–e345. https://doi.org/10.1016/S2468-2667(21)00055-4.

Hansen, J., Baum, A., Pascal, K.E., et al., 2020. Studies in humanized mice and convalescent humans yield a SARS-CoV-2 antibody cocktail. Science 369, 1010–1014. https://doi.org/10.1126/science.abd0827.

Hopp, T.P., Prickett, K.S., Price, V.L., et al., 1988. A short polypeptide marker sequence useful for recombinant protein identification and purification. Bio Technol. 6 https://doi.org/10.1038/nbt1088-1204, 1024-1010.

Hu, B., Guo, H., Zhou, P., et al., 2021. Characteristics of SARS-CoV-2 and COVID-19. Nat. Rev. Microbiol. 19, 141–154. https://doi.org/10.1038/s41579-020-00459-7.

Imam, Z., Odish, F., Gill, I., et al., 2020. Older age and comorbidity are independent mortality predictors in a large cohort of 1305 COVID-19 patients in Michigan, United States. J. Intern. Med. 288, 469–476. https://doi.org/10.1111/joim.13119.

Jiang, S., Hillyer, C., Du, L., 2020. Neutralizing antibodies against SARS-CoV-2 and other human coronaviruses. Trends Immunol. 41, 355–359. https://doi.org/10.1016/j.it.2020.03.007.

Jones, B.E., Brown-Augsburger, P.L., Corbett, K.S., et al., 2021. The neutralizing antibody, LY-CoV555, protects against SARS-CoV-2 infection in non-human primates. Sci. Transl. Med. 13, eabf1906 https://doi.org/10.1126/scitranslmed.abf1906.

Karlsson, R., Michaelsson, A., Mattsson, L., 1991. Kinetic analysis of monoclonal antibody-antigen interactions with a new biosensor based analytical system. J. Immunol. Methods 145, 229–240. https://doi.org/10.1016/0022-1759(91)90331-9.

Kim, B., Kim, S., Jang, W., et al., 2021. Estimation of the case fatality rate based on stratification for the COVID-19 outbreak. PLoS One 16, e0246921. https://doi.org/10.1371/journal.pone.0246921.

Kim, D., Lee, J.Y., Yang, J.S., et al., 2020. The architecture of SARS-CoV-2 Transcriptome. Cell 181, 914–921. https://doi.org/10.1016/j.cell.2020.04.011.

Krammer, F., 2020. SARS-CoV-2 vaccines in development. Nature 586, 516–527. https://doi.org/10.1038/s41586-020-2798-3.

Leung, K., Shum, M.H., Leung, G.M., 2021. Early transmissibility assessment of the N501Y mutant strains of SARS-CoV-2 in the United Kingdom, October to November 2020. Euro Surveill. 26, 2002106 https://doi.org/10.2807/1560-7917.

Li, Q., Wu, J., Nie, J., 2020. The Impact of mutations in SARS-CoV-2 spike on viral infectivity and antigenicity. Cell 182, 1284–1294. https://doi.org/10.1016/j.cell.2020.07.012.

McQueen, P., Busman-Sahay, K., Rieder, F., et al., 2019. Intestinal proteomic analysis of a novel non-human primate model of experimental colitis reveals signatures of mitochondrial and metabolic dysfunction. Mucosal Immunol. 12, 1327–1335. https://doi.org/10.1038/s41385-019-0200-2.

Marin, B.G., Aghagoli, G., Lavine, K., et al., 2021. Predictors of COVID-19 severity : a literature review. Rev. Med. Virol. 31, 1–10. https://doi.org/10.1002/rmv.2146.

Meyerowitz, E.A., Richterman, A., Gandhi, R.T., et al., 2021. Transmission of SARS-CoV-2: a review of viral, host, and environmental factors. Ann. Intern. Med. 174, 69–79. https://doi.org/10.7326/M20-5008.

Onder, G., Rezza, G., Brusaferro, S., 2020. Case-fatality rate and characteristics of patients dying in relation to COVID-19 in Italy. J. Am. Med. Assoc. 323, 1775–1776. http://jamanetwork.com/article.aspx?doi=10.1001/jama.2020.4683.

Pallesen, J., Wang, N., Corbett, K.S., et al., 2017. Immunogenicity and structure of a rationally designed prefusion MERS-CoV spike antigen. Proc. Natl. Acad. Sci. U.S.A. 114, E7348–E7357. https://doi.org/10.1073/pnas.1707304114.

Papenburg, J., Cheng, M.P., Corsini, R., 2021. Evaluation of a commercial culture-free neutralization antibody detection kit for Seere Acute Respiratory Syndrome-Related Coronavirus-2 and comparison with a antireceptor-binding domain enzyme-linked immunosorbent assay. Open Forum Infect. Dis. 8 https://doi.org/10.1093/ofid/ofab220 ofab220.

Pinto, D., Park, Y., Beltramello, M., et al., 2020. Cross-neutralization of SARS-CoV-2 by a human monoclonal SARS-CoV antibody. Nature 583, 290–294. https://doi.org/10.1038/s41586-020-2349-y.

Public Health England, 2021a. SARS-CoV-2 variants of concern and variants under investigation in England. Technical briefing 10. https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/984274/Variants_of_Concern_VOC_Technical_Briefing_10_England.pdf. (Accessed 11 June 2021).

Public Health England, 2021b. SARS-CoV-2 variants of concern and variants under investigation in England. Technical briefing 13. https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/990339/Variants_of_Concern_VOC_Technical_Briefing_13_England.pdf. (Accessed 18 June 2021).

Sarkar, R., Chawla-Sarkar, M., Majumdar, S., 2021. Geographical and temporal distribution of SARS-CoV-2 globally: an attempt to correlate case fatality rate with the circulating dominant SARS-CoV-2 clades. medRxiv, 21257434. https://doi.org/10.1101/2021.05.25.21257434, 2021, 05.25.

Schmidt, T.G.M., Skerra, A., 2007. The Strep-tag system for one-step purification and high-affinity detection or capturing of proteins. Nat. Protoc. 2, 1528–1535. https://doi.org/10.1038/nprot.2007.209.

Scudellari, M., 2020. The pandemic's future. Researchers are developing a host of scenarios to predict how the next few years might look. Nature 584, 22–25. https://doi.org/10.1038/d41586-020-02278-5.

Shan, B., Xin, L., 2013. Integrating de novo sequencing and database search for monoclonal antibody sequencing. J. Biomol. Tech. 24 (Suppl. l), S62–S63.

Shi, R., Shan, C., Duan, X., et al., 2020. A human neutralizing antibody targets the receptor-binding site of SARS-CoV-2. Nature 584, 120–124. https://doi.org/10.1038/s41586-020-2381-y.

Sorci, G., Faivre, B., Morand, S., 2020. Explaining among-country variation in COVID-19 case fatality rate. Sci. Rep. 10, 18909. https://doi.org/10.1038/s41598-020-75848-2.

Starr, T.N., Greaney, A.J., Addetia, A., et al., 2021. Prospective mapping of viral mutations that escape antibodies used to treat COVID-19. Science 371, 850–854. https://doi.org/10.1126/science.abf9302.

Tan, C.W., Chia, W.N., Qin, X., et al., 2020. A SARS-CoV-2 surrogate virus neutralization test based on antibody-mediated blockage of ACE2–spike protein–protein interaction. Nat. Biotechnol. 38, 1073–1078. https://doi.org/10.1038/s41587-020-0631-z.

Tao, Y., Strelkov, S.V., Mesyanzhinov, V.V., et al., 1997. Structure of bacteriophage T4 fibritin: a segmented coiled coil and the role of the C-terminal domain. Structure 5, 789–798. https://doi.org/10.1016/S0969-2126(97)00233-5.

The U.S. Food and Drug Administration (FDA), 2021b. Fact Sheet for Health Care Providers Emergency Use Authorization (EUA) of Bamlanivimab and Etesevimab. https://www.fda.gov/emergency-preparedness-and-response/mcm-legal-regulatory-and-policy-framework/emergency-use-authorization#coviddrugs.

The U.S. Food and Drug Administration (FDA), 2021c. Fact Sheet for Health Care Providers Emergency Use Authorization (EUA) of Sotrovimab. https://www.fda.gov/emergency-preparedness-and-response/mcm-legal-regulatory-and-policy-framework/emergency-use-authorization#coviddrugs.

The U.S. Food and Drug Administration (FDA), 2021a. Fact Sheet for Health Care Providers Emergency Use Authorization (EUA) of Regen-Cov™ (Casirivimab and Imdevimab). FDA. https://www.fda.gov/emergency-preparedness-and-response/mcm-legal-regulatory-and-policy-framework/emergency-use-authorization#coviddrugs.

The U.S. Food and Drug Administration (FDA), 2021d. Development of Monoclonal Antibody Products Targeting SARSCoV-2, Including Addressing the Impact of Emerging Variants, during the COVID-19 Public Health Emergency. FDA. https://www.fda.gov/media/146173/download. (Accessed 14 October 2021).

Tran, N.H., Rahman, Z., He, L., et al., 2016. Complete de novo assembly of monoclonal antibody sequences. Sci. Rep. 6, 31730. https://doi.org/10.1038/srep31730.

Valcourt, E.J., Manguiat, K., Robinson, A., et al., 2021. Evaluation of a commercially-available surrogate virus neutralization test for severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2). Diagn. Microbiol. Infect. Dis. 99, 115294 https://doi.org/10.1016/j.diagmicrobio.2020.115294.

Wall, E.C., Wu, M., Harvey, R., 2021. Neutralising antibody activity against SARS-CoV-2 VOCs B.1.617.2 and B.1.351 by BNT162b2 vaccination. Lancet 397, 19–25. https://doi.org/10.1016/S0140-6736(21)01290-3.

Wang, P., Nair, M.S., Liu, L., 2021. Antibody resistance of SARS-CoV-2 variants B.1.351 and B.1.1.7. Nature 593, 130–135. https://doi.org/10.1038/s41586-021-03398-2.

Wang, S., Sakhatskyy, P., Chou, T.W., et al., 2005. Assays for the assessment of neutralizing antibody activities against Severe Acute Respiratory Syndrome (SARS) associated coronavirus (SCV). J. Immunol. Methods 301, 21–30. https://doi.org/10.1016/j.jim.2005.03.008.

Wiersinga, W.J., Rhodes, A., Cheng, A.C., et al., 2020. Pathophysiology, transmission, diagnosis, and treatment of coronavirus disease 2019 (COVID-19) a review. J. Am. Med. Assoc. 324, 782–793. https://doi.org/10.1001/jama.2020.12839.

World Health Organization (WHO), 2016. Guidelines on Evaluation of Monoclonal Antibodies as Similar Biotherapeutic Products (SBPs). https://www.who.int/biologicals/mAb_1st_draft_KG_IK_1_March_2016_clean.pdf. (Accessed 14 October 2021).

World Health Organization (WHO), 2021a. COVID-19 Clinical Management, Living Guidance. https://www.who.int/publications/i/item/WHO-2019-nCoV-clinical-2021-1. (Accessed 11 June 2021).

World Health Organization (WHO), 2021b. Statement of the WHO Working Group on COVID-19 Animal Models (WHO-COM) about the UK and South African SARS-CoV-2 New Variants.

Wu, Z., McGoogan, J.M., 2020. Characteristics of and important lessons from the coronavirus disease 2019 (COVID-19) outbreak in China summary of a report of 72 314 cases from the Chinese center for disease control and prevention. J. Am. Med. Assoc. 323, 1239–1242. http://jamanetwork.com/article.aspx?doi=10.1001/jama.2020.2648.

Zhou, F., Yu, T., Du, R., et al., 2020. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. Lancet 395, 1054–1062. https://doi.org/10.1016/S0140-6736(20)30566-3.

Zhou, D., Dejnirattisai, W., Supasa, P., et al., 2021. Evidence of escape of SARS-CoV-2 variant B.1.351 from natural and vaccine-induced sera. Cell 189, 2348–2361. https://doi.org/10.1016/j.cell.2021.02.037.