The neutralization potency of anti-SARS-CoV-2 therapeutic human monoclonal antibodies is retained against novel viral variants

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Summary

A wide range of SARS-CoV-2 neutralizing monoclonal antibodies (mAbs) were reported to date, most of which target the spike glycoprotein and in particular its receptor binding domain (RBD) and N-terminal domain (NTD) of the S1 subunit. The therapeutic implementation of these antibodies has been recently challenged by the emerging SARS-CoV-2 variants, harboring an extensively-mutated spike versions. Consequently, the re-assessment of mAbs, previously reported to neutralize the original early-version of the virus, represents an assignment of high priority.

With respect to the evolving mutations in the virus spike RBD, we evaluated the aptitude of four previously selected mAbs, targeting distinct epitopes, to bind RBD versions harboring individual mutations at positions 501, 477, 484, 439, 417 and 453. Mutations of these residues represent the prevailing worldwide distributed modifications of the RBD, previously reported to mediate escape from antibody neutralization. Additionally, the *in vitro* neutralization efficacies of the four RBD-specific mAbs, as well as two NTD-specific mAbs, were evaluated against two frequent SARS-CoV-2 variants of concern (VOCs): (i) the B.1.1.7 variant, emerged in the UK and (ii) the B.1.351 variant, emerged in South Africa. B.1.351, was previously suggested to escape many therapeutic mAbs, including those authorized for clinical use.

The results of the present study, clearly indicate that in spite of mutation accumulation in the spike of the virus, some neutralizing mAbs preserve their potency to combat SARS-CoV-2 emerged variants. In particular, the previously reported highly potent MD65 mAb is shown to retain its ability to bind the prevalent novel viral mutations and to effectively neutralize the B.1.1.7 and B.1.351 variants of high clinical concern.

Introduction

An unprecedented worldwide research and development effort, has resulted in the rapid development of several prophylactic and therapeutic immune tools to combat the COVID-19 pandemic caused by SARS-CoV-2. These tools predominantly target the virus spike glycoprotein which is essential for the attachment of the virus to the target cell and hence plays an essential role in the virus infectivity. Four emergency-authorized vaccines, against the SARS-CoV-2 spike, produced by Pfizer/BioNTech, Moderna, AstraZenica and Johnson&Johnson, respectively (Krammer, 2020), are already being used for mass vaccination campaigns. Additionally, passive immunity was achieved by the administration of convalescent plasma or recombinant neutralizing monoclonal antibodies (mAbs). This therapeutic avenue accelerated the development of many potent neutralizing mAbs, primarily targeting the receptor binding domain (RBD) and the N-terminal domain (NTD) of the spike-S1 subunit. A single therapeutic mAb, generated by Eli Lilly and Company and a dual antibody combination, generated by Regeneron Pharmaceuticals, recently received an emergency use authorization (Chen et al., 2021a; Weinreich et al., 2021).

Prior to its global expansion, SARS-CoV-2 was expected to exhibit a relatively low evolutionary rate of mutations, as compared to many other RNA viruses, since the genome of this virus encodes a proofreading exoribonuclease machinery (Robson et al., 2020). However, the long-term global spread of the SARS-CoV-2, together with selective pressure for immune escape, led to adaptation of the virus to the host and generation of new SARS-CoV-2 variants. Specifically, multiple mutations in the spike glycoprotein are evolving, including mutations that are located in the spike S1 subunit, particularly residing in the

antigenic supersite of the NTD (Cerutti et al., 2021; McCallum et al., 2021; Noy-Porat et al., 2021) or in the RBD (hACE2-binding site) (Baum et al., 2020; Chen et al., 2020; Noy-Porat et al., 2020), sites that represent a major target of potent virus-neutralizing antibodies. The impact of accumulated mutations is closely monitored, yet, only a minor fraction, which are selectively favorable, might spread and reach high frequency, and more importantly, become fixed in the population. Emergence of such genetic variants, has important epidemiological consequences since they may exhibit increased transmissibility or cause disease of enhanced severity. The WHO has recently established the working definitions of "SARS-CoV-2 Variant of Interest" (VOI) and of "SARS-CoV-2 Variant of Concern" (VOC) (https://www.who.int/publications/m/item/weekly-epidemiological-update---23-february-2021). One of the major VOCs identified and monitored recently, is the variant denoted as 20I/501Y.V1 belonging to the B.1.1.7 lineage, which has a total of 18 nonsynonymous mutations relative to the original Wuhan strain, of which 7 replacements and 2 deletions reside in the spike protein (see Supplementary Figure 1 for schematic presentation) (Rambaut et al., 2020b). Since its first emergence in the UK in September 2020 (Rambaut et al., 2020a), the B.1.1.7 variant is rapidly spreading. As of March 2021, the variant has been detected in over 100 countries, with an apparent cumulative prevalence of 26% worldwide (55%, 3% and 28% in the UK, US and Israel, respectively. Furthermore, as of the beginning of March 2021, the worldwide daily prevalence of the B.1.1.7 VOC, was over 75% (https://outbreak.info), and it is anticipated that its fixation within the global contagion is unavoidable. Two additional VOCs were reported in recent months: the B.1.351 lineage (also known as 20H/501Y.V2 variant; schematically depicted in Supplementary Figure 1), identified for the first time in October

2020 in South Africa (Tegally et al., 2020) and the P.1 lineage, (also known as 501Y.V3 variant), first identified in December 2020 in Brazil (Faria et al., 2021). Both variants are less abundant worldwide (up to 1%) and mostly contained in the geographic surrounding of their originating site. Full biological and clinical implications of the SARS-CoV-2 new variants are yet to be determined. Nevertheless, the careful immunological assessment of known mutations, in particular in the receptor binding domain, is essential, due to the possible impact on vaccines and therapeutic measures, such as monoclonal antibodies. Of the multitude of possible genomic loci, mutations at several positions were already reported at relatively high frequency in the ~600,000 sequences available to date (GISAID initiative, https://gisaid.org (Elbe and Buckland-Merrett, 2017)).

Both predictive theoretical and experimental approaches, revealed that escape mutants can rapidly occur when SARS-CoV-2 is exposed to selective pressure mediated by neutralizing polyclonal sera or individual mAbs (Andreano et al., 2020; Liu et al., 2021; Starr et al., 2020; Weisblum et al., 2020). More specifically, escape mutations within the RBD were predicted and experimentally confirmed to affect its function (mainly with respect to hACE2 binding) and recognition by mAbs. Substitutions N501Y, E484K, K417N, Y453F N439K and S477N, were among the most frequent mutations that mediated immune escape and were shown to reduce and even completely abrogate neutralizing activity of several highly potent mAbs, including those which are already in clinical use (Andreano et al., 2020; Chen et al., 2021b; Liu et al., 2021; Starr et al., 2020; Thomson et al., 2021). Similar substitutions naturally occurred in infected individuals and are now represented by SARS-CoV-2 emerged genetic variants which spread worldwide.

We previously reported the isolation of RBD- and NTD-specific mAbs (Barlev-Gross et al., 2021; Noy-Porat et al., 2020; Noy-Porat et al., 2021; Rosenfeld et al., 2021), among which the MD65 mAb showing exceptional neutralization potency, as demonstrated by *in vitro* and *in vivo* experiments. In the current study, we present the re-evaluation of four SARS-CoV-2 neutralizing mAbs (MD65, MD62, MD29 and BL6), directed against 4 distinct epitopes in the spike RBD, for their ability to bind recombinant RBD proteins, individually representing substitutions encountered in the VOCs. Additionally, we assessed the *in vitro* neutralization capacity of these 4 anti-RBD mAbs and two additional anti-NTD mAbs to counteract the SARS-CoV-2 B.1.1.7 and B.1.351 genetic variants.

Results and Discussion

In the current study, we re-evaluated the binding aptitude of the recently reported mAbs, MD65, MD62, MD29 and BL6, targeting 4 distinct epitopes on the RBD. The binding capability of these mAbs, was tested with respect to six individual mutations in the SARS-CoV-2 spike recombinant RBD (rRBD), included in the top 10 most-abundant RBD mutations identified to date (depicted in Figure 1A), some of which are present in viral variants of concerns (VOCs), as detailed below (for complete lineages and mutation reports, see https://outbreak.info. The most frequent mutation, N501Y was first detected in April 2020, and as of March 2021 is present in over 70% of the global cases in more than 100 countries. The N501Y substitution represents the hallmark of all three circulating VOCs (B.1.1.7, B.1.351 and P.1). The mutation S477N, was reported in 4% of the cases worldwide, since its emergence in March 2020. Although it was detected in 67 countries, its frequency is most prominent in Australia (62% as of March 2021). The mutation E484K, was detected in more than 60 countries, exhibiting a worldwide apparent frequency of 1%. This residue replacement was detected in the South African (B.1.351) and Brazilian (P.1) variants, and recently in a UK "B.1.1.7+E484K" fast-spreading variant. The N439K, a sentinel receptor binding motif mutation (Welkers et al., 2021) has an apparent worldwide frequency of 2%, reported in 54 countries. This mutation has emerged in multiple SARS-CoV-2 clades, and is mostly associated with the B.1.258 lineage derivatives, circulating in central Europe. The K417N mutation was reported in over 1% of the cases worldwide in at least 46 countries. This mutation represents one of the hallmarks of the B.1.351 lineage and is exhibited in approximately 50% of South African cases. The replacement Y453F was detected in at least 11 countries, predominantly in Denmark. Recently, this mutation

raised substantial concern, when it was detected in a variant found in the mink population (Welkers et al., 2021).

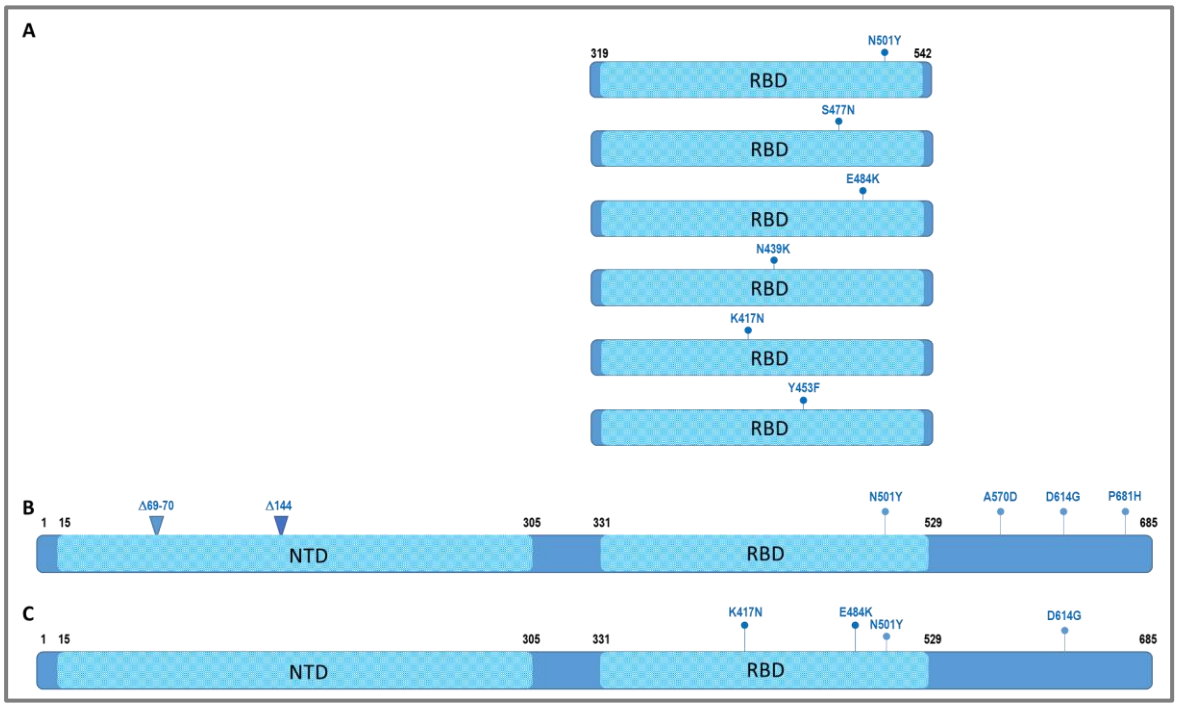


Figure 1. Schematic representation of SARS-CoV-2 RBD and S1 variants. A. Depiction of recombinant RBD variant proteins, each including the indicated single highly frequent replacements reported in the RBD domain. The domain coordinates are according to the recombinant RBD used throughout the study. **B**. Schematic representation of the spike S1 subunit, along with the replacements and deletions characterizing the SARS-CoV-2 B.1.1.7 genetic variant. **C**. Schematic representation of the spike S1 subunit, along with the replacements at the RBD, characterizing the SARS-CoV-2 B.1.351 genetic variant. The numbering is according to the Wuhan reference sequence (Accession no. NC_045512).

Biolayer interferometry (BLI) analysis was applied for evaluating the ability of the four RBD-specific mAbs that we have previously reported to bind the SARS-CoV-2 single mutated-RBD variants. As presented in Figure 2, the binding of the specific mAbs was only slightly affected (5-22% loss of binding) by five of the six substitutions in the RBD. The only significant reduction in binding capacity, compared to the WT rRBD, was

observed for K417N mutant by MD62 mAb (~74% reduction) and to lesser extent by MD65 (17% reduced binding). In light of these results, it is conceivable to anticipate that these mAbs, previously shown to neutralize SARS-CoV-2 by targeting distinct epitopes on the RBD, maintain their efficacy against variant strains carrying these mutations.

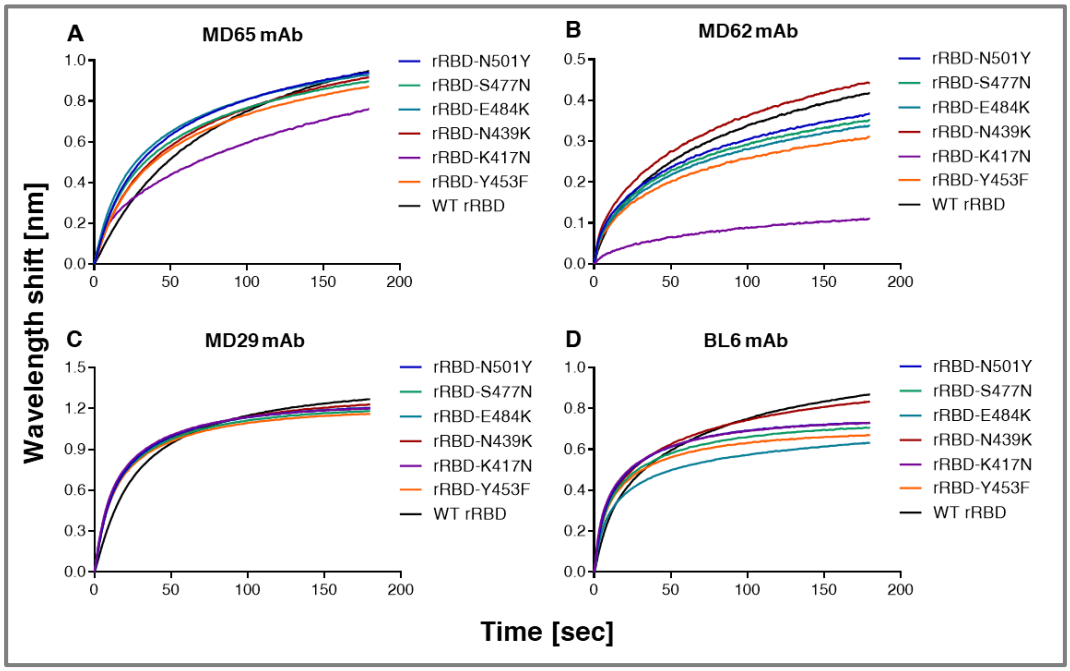


Figure 2. MAbs binding of single-mutated rRBDs. Binding of the WT and the 6 indicated single-mutated recombinant RBDs by MD65 (**A**), MD62 (**B**), MD29 (**C**) and BL6 (**D**) mAbs, was evaluated by Biolayer interferometry (BLI). Each mAb was immobilized on a protein-A sensor and incubated with each of the rRBD mutants [N501Y, S477N, E484K, N439K, K417N and Y453F] or WT rRBD as a control, for 180 sec.

Amongst the 4 RBD-specific mAbs studied here, MD65 is the most effective antibody in terms of *in vitro* neutralization as well as elicitation of *in vivo* post-exposure protection at relatively low doses. MD65 mAb (whose variable regions are encoded by the IGHV3-66 and IGKV3-20 germline heavy and light chain alleles, respectively), belonging to a public clonotype that was extensively characterized in the context of SARS-CoV-2 neutralizing human antibodies (Barnes et al., 2020; Robbiani et al., 2020; Weisblum et al., 2020; Yuan

et al., 2020), specifically targets the receptor binding motif, competing with hACE2 binding. Noteworthy, recent studies showed that binding and neutralization by antibodies, encoded by the IGHV3-66 and IGKV3-20 alleles, are prevented by either the K417N or E484K replacements (Andreano et al., 2020; Fagiani et al., 2020; Yuan et al., 2020). Specifically, the E484K mutation, which as explained above, is present in several SARS-CoV-2 natural isolates (including the B.1.351, P.1 and recently identified "B.1.1.7+E484K" VOCs), and was reported by several studies to be associated with the complete abolishment of neutralization by mAbs and with the reduced neutralization, observed by immunized/vaccinated polyclonal sera (Chen et al., 2021b; Wang et al., 2021b). Taken together, our results, demonstrating that MD65 retained binding even with RBD exhibiting the E484K substitution, further emphasize the importance of evaluating the efficacy of specific mAbs for determining their therapeutic potential against VOCs.

The RBD-specific therapeutic mAb LY-CoV555 (Bamlanivimab) (Chen et al., 2021a), encoded by the germline alleles: IGHV1-69; IGKV1-39, was also shown to block hACE2 binding by SARS-CoV-2 (Jones et al., 2020), and accordingly, possibly compete with MD65 mAb. However, the two mAbs do not share significant sequence similarity and thus, although they may target close epitopes, their recognition pattern may differ. In order to test whether LY-CoV555 functionality is affected in a similar manner as MD65, the commercially available LY-CoV555 mAb was used in binding experiments. First, the binding profile of the LY-CoV555 was tested by ELISA against the SARS-CoV-2 spike protein, and compared to that of MD65 mAb (Figure 3A), demonstrating similar binding profiles. An epitope-binning experiment using BLI, was performed in the presence of MD65 mAb and MD29 (as a negative control). As shown in Figure 3B, LY-CoV555 failed

to bind the rRBD protein, presented in complex with MD65 mAb, while a significant binding was observed when the rRBD was presented in complex with MD29 (which binds a different epitope than MD65). These results clearly indicate that LY-CoV555 and MD65 target overlapping epitopes. Next, the LY-CoV555 binding aptitude towards the panel of RBD mutants was evaluated (Figure 3C), demonstrating equivalent binding of rRBD-N439K, Y453Y, S477N and N501Y, compared to the WT rRBD. However, LY-CoV555 binding was completely obstructed by the E484K substitution. This observation is in line with recently reported studies, suggesting that the E484K substitution is accountable for the abolishment of neutralization of SARS-CoV-2 natural variants, carrying this mutation, by LY-CoV555 mAb (Wang et al., 2021b).

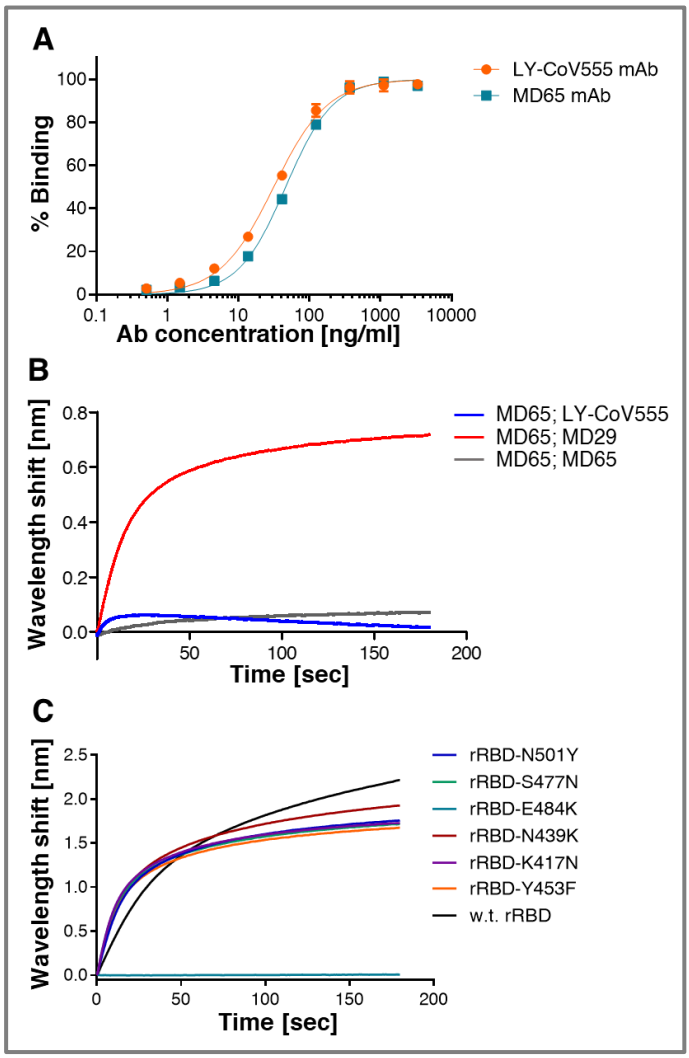


Figure 3. Binding characterization of the LY-CoV555 mAb (Bamlanivimab). A. Binding of the LY-CoV555 mAb was evaluated by ELISA against SARS-CoV-2 spike protein. MD65 mAb was included for comparison. Data shown represent average of triplicates ±SEM **B.** BLI was applied for epitope binning experiments. MD65 antibody was biotinylated, immobilized on streptavidin sensor and saturated with WT rRBD protein. The complex was then incubated for 180 s with LY-CoV555 or MD29 and MD65 as controls. Time 0 represents the binding to the MD65-rRBD complex. **C.** LY-CoV555 binding of the indicated single-mutated rRBDs and the WT rRBD, was evaluated by BLI. LY-CoV555 antibody was immobilized on protein-A sensor and incubated with each indicated rRBD for 180 sec.

The retained binding capabilities, observed for the tested mAbs towards the individual RBD mutations, may not necessarily predict their interaction in the context of multiple mutations, present in emerged VOCs. Therefore, we studied the ability of the four RBD-specific mAbs to bind recombinant mutated spike S1 subunit proteins, representing the RBD accumulated mutations associated with the B.1.1.7 and B.1.351 genetic variants (schematically depicted in Figures 1B and 1C, respectively). BLI analyses (Figure 4) were applied for binding evaluation of recombinant S1:Δ 69-70; Δ 144; N501Y; A570D**;** D614G; P681H, representing all the modifications encountered in the S1 of the B.1.1.7 genetic variant (Figure 4, B.1.1.7 rS1, blue curves) and of recombinant S1:K417N; E484K; N501Y; D614G, representing the RBD-related substitutions of the B.1.351 variant (B.1.351 rS1; red curves). Recombinant WT S1 subunit (WT rS1; black curves) was used for comparison. While binding of B.1.1.7 rS1 by MD65, MD29, BL6 and LY-CoV555 was not impaired by the mutations (Figures 4A, 4C, 4D and 4E, respectively), MD62 binding capacity was reduced by ~45% (Figure 4B). This observation may not be attributed to the minor reduction (8% loss of binding) observed in binding of N501Y-RBD mutant by this antibody (Figure 2B), as it represents the only substitution in the RBD of B.1.1.7. Therefore, it can be speculated that structural changes in the B.1.1.7 rS1 (Figure 1B) involving the mutated NTD, allosterically affected MD62 binding.

The B.1.351 rS1 (schematically depicted in Figure 1C), includes the N501Y substitution, as well as the K417N and E484K replacements that were previously shown to substantially impair binding by various mAbs (Chen et al., 2021b; Cheng et al., 2021; Krammer, 2020; Starr et al., 2020; Wang et al., 2021b). Thus, in line with the individual mutation binding results (see Figure 3C for LY-CoV555 and Figure 2D for BL6), the observed binding

abrogation of the B.1.351 rS1 by LY-CoV555 mAb (Figure 4B) and the mild (18%) reduction observed for BL6 (Figure 4D), can be attributed mainly to the E484K substitution. Similarly, the complete loss of binding by MD62 (Figure 4B) and significant loss of binding by MD65 (~65%; Figure 4A), may have been mediated by the K417N substitution (see Figures 2B and 2A for MD62 and MD65, respectively).

Finally, in order to conclusively determine the potential of the B.1.1.7 and B.1.351 SARS-CoV-2 genetic variants (see Supplementary Figure 1, for their spike mutations) to escape immune-neutralization, we evaluated the ability of the four RBD-specific mAbs, LY-CoV555, and two additional mAbs, targeting separate epitopes of the NTD (BLN14 and BLN12) (Noy-Porat et al., 2021), to countermeasure the B.1.1.7 and B.1.351 live variants. To this end, a plaque reduction neutralization test (PRNT) was applied in which each mAb was challenged by either the B.1.1.7 or B.1.351 variants, or the parental WT SARS-CoV-2 strain.

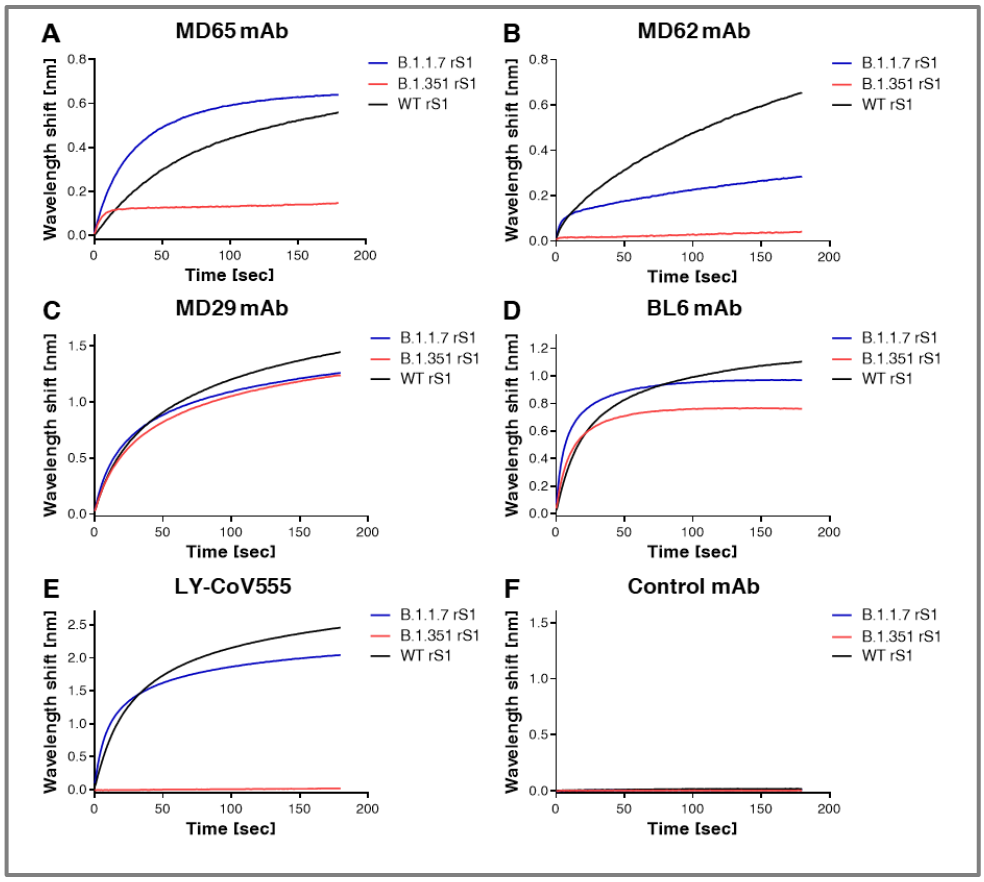


Figure 4. Binding of rS1 variants by RBD-specific mAbs. BLI was applied for evaluating the ability of each tested mAb to bind the indicated recombinant multiple-mutated spike S1 subunit proteins: B.1.1.7 rS1 [Δ 69-70; Δ 144; N501Y; A570D; D614G; P681H] and B.1.351 rS1 [K417N; E484K; N501Y; D614G]. Each of the indicated mAbs (**A**-**E**), was immobilized on protein-A sensor and incubated for 180 sec with B.1.1.7 (blue) or B.1.351 (red) rS1 variants or with the WT rS1 (black). Non-specific control antibody (anti-ricin MH75) was included (**F**). The figure includes representative graphs of at least two independent repeats of each experiment, yielding similar results.

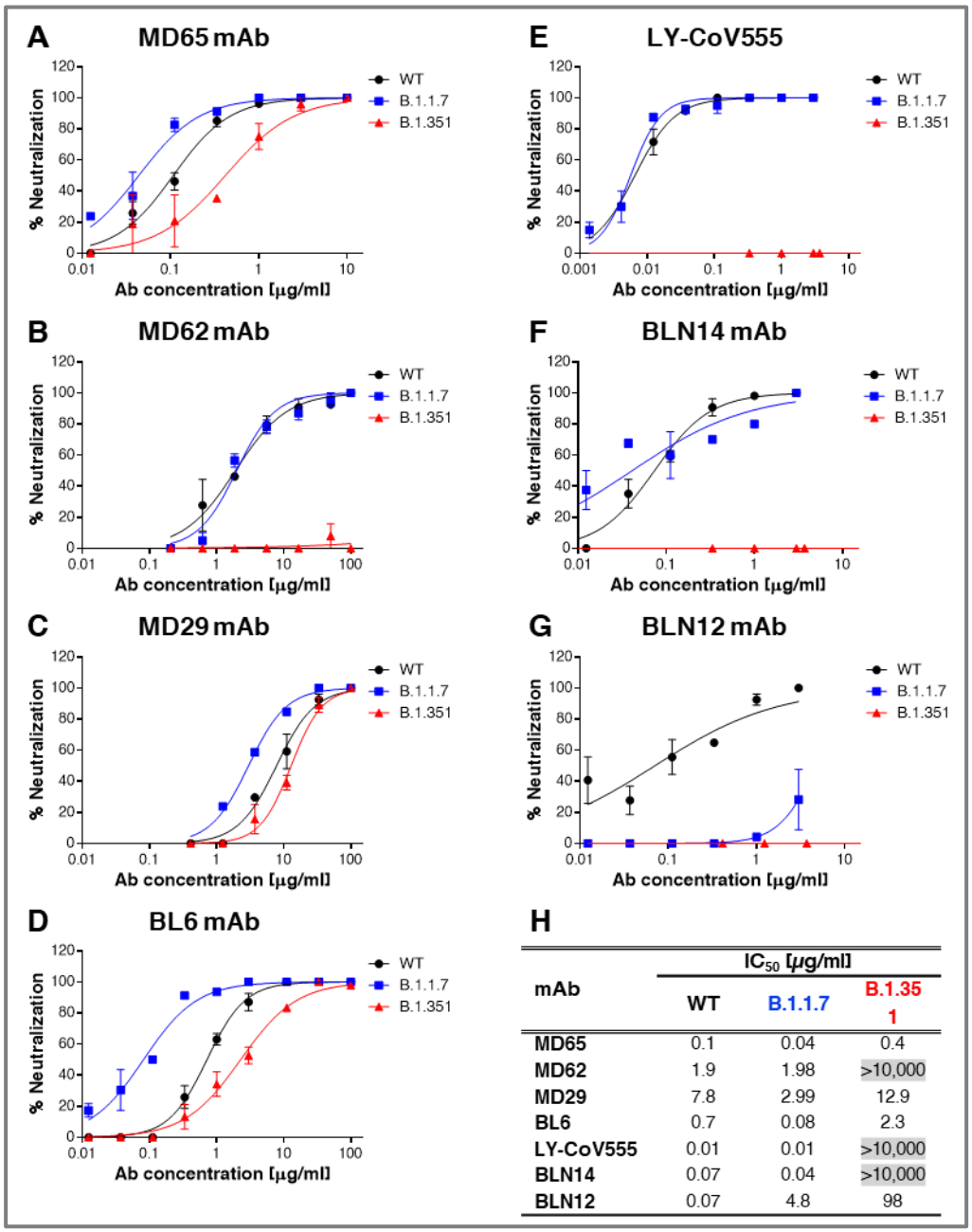
The results, presented in Figure 5, indicate an effective neutralization of the B.1.1.7 variant (blue curves) by anti-RBD MD65 (panel A), MD62 (panel B), MD29 (Panel C), BL6 (Panel D) as well as LY-CoV555 (panel E), a neutralization that was not impaired compared to the WT SARS-CoV-2 strain (black curves). The calculated IC_{50} values, characterizing the neutralization aptitude of each inspected antibody with respect to the

three tested viral strains, are tabulated in Figure 5H. Furthermore, while MD62 and LY-CoV555 manifested similar neutralization of the B.1.1.7 compared to the WT strain, superior neutralization of the variant was demonstrated by MD65, MD29 and BL6. Interestingly, although MD62 mAb revealed reduced binding to the B.1.1.7 rS1, compared to the WT rS1 (Figure 4B), its neutralization capacity of both viral strains was commensurate (Figure 5B). Overall, it can be concluded that all anti-RBD mAbs studied here, fully retained their potency towards the B.1.1.7 variant.

As could be anticipated, the B.1.351 variant (Figure 5, red curves) manifested a higher immune escape potential compared to the B.1.1.7 variant. In line with the complete loss of binding of the respective viral rS1 by MD62 (Figure 4B) and LY-CoV555 (Figure 4E), the neutralization capacity of the two mAbs (MD62 and LY-CoV555) against the B.1.351 variant, was completely abolished (Figures 5B and 5E, respectively). However, in spite of a partial decrease, it is important to note that MD65, MD29 and BL6 effectively neutralized the B.1.351 variant (Figures 5A, 5C and 5D).

Two anti-NTD mAbs, previously shown to potently neutralize SARS-CoV-2, were also tested in the *in vitro* neutralization assay. The two mAbs, BLN14 and BLN12, differed in their efficiency against the B.1.1.7 variant (Figures 5F and 5G, respectively), BLN14 showing comparable neutralization to that of the WT, while BLN12 neutralization activity was eliminated. These results are consistent with binding experiments, showing strong binding of B.1.1.7 rS1 by BLN14 and no binding by BLN12 (Supplementary Figure 2). Epitope mapping previously revealed that BLN12 binds a linear epitope which resides between amino acids 141-155 and also recognizes an N-glycan at position 149 *(Noy-Porat et al., 2021).* It can therefore be speculated that the deletion of a Tyr residue at position

144, in the B.1.1.7 variant, is responsible for the loss of neutralization of this mAb. BLN14 recognizes a conformational epitope which apparently was not significantly altered by the Y144 deletion. However, the neutralization capability of both mAbs was completely abolished in the case of the B.1.351 variant, suggesting a considerable structural change of its NTD. This observation is in agreement with previous studies, which indicated a frequent loss of functionality among NTD-specific mAbs (Andreano et al., 2020; Wang et al., 2021b), especially towards variants containing modifications in NTD supersite associated with significant structural alterations (Cerutti et al., 2021; McCallum et al., 2021).



mAb	IC_{50} [μg/ml]		
	WT	B.1.1.7	B.1.351
MD65	0.1	0.04	0.4
MD62	1.9	1.98	>10,000
MD29	7.8	2.99	12.9
BL6	0.7	0.08	2.3
LY-CoV555	0.01	0.01	>10,000
BLN14	0.07	0.04	>10,000
BLN12	0.07	4.8	98

Figure 5. Neutralization of SARS-CoV-2 B.1.1.7 and B.1.351 genetic variants by RBD and NTD-specific mAbs. Neutralization capacity of the RBD-specific mAbs: MD65 (**A**), MD62 (**B**), MD29 (**C**), BL6 (**D**) and LY-CoV555 (**E**), and of the NTD-specific BLN14 (**F**) and BLN12 (**G**) mAbs, was evaluated by plaque reduction neutralization test (PRNT). The i*n vitro* neutralization of each of the listed mAbs, was assessed against both SARS-CoV-2 B.1.1.7 (blue) and B.1.351 (red) variant, compared to WT SARS-CoV-2 strain (black). Neutralization potency was determined by the ability of each antibody (at indicated concentrations) to reduce plaques formation; results are expressed as percent inhibition of

control without Ab. The figure includes a representative graphs of at least two independent repeats of each experiment, yielding similar results. **H**. Summary of the calculated IC_{50} values [µg/ml]. IC_{50} >10,000 indicates complete loss of neutralization capacity, emphasized by gray shading.

Conclusion

The unprecedented scale of the COVID-19 pandemic, combined with selective pressure for escaping immune responses, boosted the rapid evolution of SARS-CoV-2 virus resulting in antigenic variability which might jeopardize the efficacy of pre- and post-exposure immunotherapies. Consequently, attention must be given to the development of mAb treatments that enable to combat emerging variants. In this perspective, it is of high importance to re-evaluate anti-SARS-CoV-2 mAbs previously shown to exhibit efficiency against the original version of the virus. Furthermore, the impact of individual mutations on the neutralization efficacy of mAbs, may provide important information impacting the preparedness for future anticipated antigenic drifts of the virus.

In the current report, we document the neutralization of the most abundant B.1.1.7 variant (as of today), by four anti-RBD and one anti-NTD mAbs, that we recently generated and determined their therapeutic potential against the original version of the virus. Furthermore, three RBD-specific mAbs (MD65, MD29 and BL6), retained neutralization against the B.1.351 VOC. This conclusion is supported by binding experiments conducted with individual and combined mutations derived from various variants. Among these mutations, the E484K and K417N substitutions, were lately reported to predominantly mediate escape of neutralization by the majority of reported mAbs, including clinically-used LY-CoV555 and REGN10933 as well as by immune post-vaccination sera (Chen et al., 2021b; Cheng et al., 2021; Hu et al., 2021; Rees-Spear et al., 2021; Wang et al., 2021a; Yuan et al., 2021).

Of note, the anti-RBD antibody MD65, shown here to retain its neutralizing potential against emerging variants, was recently suggested by extensive pre-clinical studies to be an important therapeutic product for efficient clinical intervention in COVID-19 cases (Rosenfeld et al., 2021).

Author Contributions

E.M., A.Z., R.A., T.N-P., E.P., A.M., Y.L., E.E., O.M. and R.R. designed, carried out and analyzed the data. M.M., E.M, N.Z., I.N., and L.K isolated, sequenced and provided the SARS-CoV-2 variant strains. N.P., H.T. and T.I. cultured and prepared SARS-CoV-2 viruses for the neutralization experiments. O.Z. and S.W. provided crucial reagents. T.C., S.Y., S.S. added fruitful discussions. R.R., O.M, A.Z., and T. N-P wrote the manuscript. O.M. and R.R. supervised the project. All authors have reviewed and approved the final manuscript.

Competing Interests

Patent application for the described antibodies was filed by the Israel Institute for Biological Research. None of the authors declared any additional competing interests.

Materials and Methods

Resource Availability

Antibodies are available (by contacting Ohad Mazor from the Israel Institute for Biological Research; ohadm@iibr.gov.il) for research purposes only under an MTA, which allows the use of the antibodies for non-commercial purposes but not their disclosure to third parties. All other data and materials are available from the corresponding author upon reasonable requests.

Recombinant Proteins

The SARS-CoV-2 spike (S) stabilized soluble ectodomain, S1 subunit (WT rS1) and receptor binding domain (WT rRBD) were produced as previously described (Noy-Porat et al., 2020).

The following His-tagged recombinant proteins were purchased from Sino Biologicals: B.1.1.7 rS1-SARS-CoV-2 spike S1 [Δ 69-70; Δ 144; N501Y; A570D**;** D614G; P681H], cat#40591-V08H12; B.1.351 rS1-SARS-CoV-2 spike S1 [K417N; E484K; N501Y; D614G], cat#40591-V08H10; spike RBD[N501Y] cat#40592-V08H82; spike RBD[S477N] cat#40592-V08H46; spike RBD[E484K] cat#40592-V08H84; spike RBD[N439K] cat#40592-V08H14; spike RBD[K417N] cat#40592-V08H59; spike RBD[Y453F] cat#40592-V08H80.

All antibodies (except LY-CoV555) were produced as full IgG1 antibodies as described (Barlev-Gross et al., 2021; Rosenfeld et al., 2021), expressed using ExpiCHO™ Expression system (Thermoscientific, USA) and purified on HiTrap Protein-A column (GE

healthcare, UK). The integrity and purity of the antibodies were analyzed using SDS-PAGE. Isolation and characterization of the MD29, MD65 and MD62 mAbs, targeting epitopes I-III on the RBD as previously reported. The BL6 mAb was isolated as described (Barlev-Gross et al., 2021) and is representing epitope IV on the RBD (competing with the MD47 mAb (Noy-Porat et al., 2020). BLN12 and BLN14 mAbs, targeting two distinct epitopes on the NTD, as previously reported (Noy-Porat et al., 2021).

LY-CoV555 (Bamlanivimab) (~2.5 mg Ab/ml in 0.9% Sodium Cholride), was obtained as a remnant from an infusion bag and its set following administration to a COVID-19 patient at Kaplan Medical Center.

ELISA

Direct ELISA (Noy-Porat et al., 2016) consisted of coating microtiter plates with 1 μg/ml of recombinant SARS-CoV-2 spike. ELISA was applied with AP-conjugated Donkey anti-human IgG (Jackson ImmunoResearch, USA, Cat# 709-055-149 lot 130049; used at 1:2000 working dilution) following detection using *p*-nitrophenyl phosphate (*p*NPP) substrate (Sigma, Israel).

Biolayer interferometry

Binding studies were carried out using the Octet system (ForteBio, USA, Version 8.1, 2015) that measures biolayer interferometry (BLI). All steps were performed at 30°C with shaking at 1500 rpm in a black 96-well plate containing 200 μl solution in each well. For assessment of binding to S1 variants or mutated RBD, antibodies were captured on Protein-A or anti-Fab CH1 sensors (FAB2G) and incubated with recombinant S1 (WT, B.1.1.7 or

B.1.351) or recombinant RBD (WT or mutated) for 180 sec and then transferred to buffer containing wells for additional 60 sec. Binding was measured as changes over time in light interference. Parallel measurements from unloaded biosensors were used as control. The anti-ricin MH75 mAb, used as isotype control (Figure 4F). Area under curve (AUC) was calculated for each binding curve, using GraphPad Prism 5, and percent binding was calculated compared to the WT protein, representing 100% binding.

For epitope binning, MD65 antibody was biotinylated, immobilized on streptavidin sensor, incubated with a fixed concentration of WT rS1 (20 µg/ml) to reach saturation, washed and incubated with non-labeled LY-CoV555 for 180 sec. MD29 and MD65 were used as positive and negative controls, respectively.

Cells and virus strains

ExpiCHO-S (Thermoscientific, USA, Cat# A29127) were used for expression of recombinant proteins as described above.

Vero E6 (ATCC® CRL-1586™) were obtained from the American Type Culture Collection. Cells were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS), MEM non-essential amino acids (NEAA), 2 mM L-glutamine, 100 Units/ml penicillin, 0.1 mg/ml streptomycin and 12.5 Units/ml Nystatin (P/S/N) (Biological Industries, Israel). Cells were cultured at 37°C, 5% CO_2 at 95% air atmosphere.

Wild type (WT) SARS-CoV-2 strain (GISAID accession EPI_ISL_406862) was kindly provided by Bundeswehr Institute of Microbiology, Munich, Germany.

SARS-CoV-2 B.1.1.7 (501Y.V1) variant was isolated on Dec 2020 from a person who came back from the UK. The identity of the B.1.1.7 strain was confirmed using NGS. SARS-CoV-2 B.1.351 (501Y.V2) variant was isolated on Jan 2021 from a person who was in contact with a patients who came back from South Africa. The identity of the B.1.351 strain was confirmed using NGS.

Stocks were prepared by infection of Vero E6 cells for two days. When viral cytopathic effect (CPE) was observed, media were collected, clarified by centrifugation, aliquoted and stored at -80°C. Titer of stock was determined by plaque assay using Vero E6 cells.

Handling and working with SARS-CoV-2 was conducted in BL3 facility in accordance with the biosafety guidelines of the IIBR.

Plaque reduction neutralization test (PRNT)

Plaque reduction neutralization test (PRNT), performed essentially as described (Yahalom-Ronen et al., 2020).Vero E6 cells were seeded overnight at a density of 0.5e6 cells/well in 12-well plates. Antibody samples were 3-fold serially diluted (ranging from 200 to 0.002 μg/ml) in 400 μl of MEM supplemented with 2% FBS, MEM non-essential amino acids, 2 mM L-glutamine, 100 Units/ml penicilin, 0.1 mg/ml streptomycin and 12.5 Units/ml Nystatin (Biological Industries, Israel). 400 μl containing 300 PFU/ml of each SARS-CoV-2 strain, were then added to the mAb solution supplemented with 0.25% guinea pig complement sera (Sigma, Israel) and the mixture incubated at 37°C, 5% CO_2 for 1 h. Two hundred μl of each mAb-virus mixture was added in duplicates to the cells for 1 h. Virus mixture w/o mAb was used as control. 2 ml overlay [supplemented MEM containing 0.4% tragacanth (Sigma, Israel)] were added to each well and plates were further incubated at

37°C, 5% CO_2 for 48 h for WT and B.1.351 strains or 5 days for the B.1.1.7 strain. The number of plaques in each well was determined following media aspiration, cells fixation and staining with 1 ml of crystal violet (Biological Industries, Israel). Half-maximum inhibitory concentration (IC_{50}) was defined as mAb concentration at which the plaque number was reduced by 50%, compared to plaque number of the control (in the absence of Ab).

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