Title: Biological and Clinical Significance of SARS-CoV-2 Spike Mutations

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Acknowledgements: The authors thank Benjamin A. Pinsky (Stanford University), Scott D. Boyd (Stanford University), and Jesse D. Bloom (University of Washington, Seattle, WA)

Related Links: The website https://covdb.stanford.edu maintains a relational database of in vitro SARS-CoV-2 neutralization data. The complete set of experiments described in this review can be downloaded in the form of tab-delimited files from https://covdb.stanford.edu/page/susceptibility-data/.

Competing interests: Robert W. Shafer has received grant funding from Janssen Pharmaceuticals, Vela Diagnostics, and Insilixa, Inc.

Author contributions: KT, PLT, and RWS conceived of the project. KT, PLT, JN, and RWS reviewed the primary literature. KT and PLT created the database software necessary for the project. KT and reviewed the primary data presented in the review. KT, PLT, and RWS analyzed the primary data presented in the manuscript. RWS wrote the manuscript. DF assisted with creating manuscript figures. RKG, TDO, SLK and DF reviewed multiple drafts of the manuscript.

Abstract

An increasing number of studies have described the emergence of SARS-CoV-2 variants containing spike mutations that influence virus transmission and antigenicity. These variants will influence the epidemiological and clinical aspects of the SARS-CoV-2 pandemic, increasing the risk of re-infection and reducing the protection afforded by vaccine-induced immunity and neutralizing monoclonal antibodies. Here we review the prevalence and phenotypic characteristics of the most epidemiologically relevant SARS-CoV-2 spike mutations. We also review the results of approximately 3,500 neutralization experiments in which monoclonal antibodies, convalescent plasma, and plasma from vaccinated persons were assessed for the inhibition of pseudotyped viruses containing the sets of spike mutations present in three variants of concern (B.1.1.7, B.1.351, and P.1), as well as individual S1 receptor binding domain mutations (K417N/T, N439K, L452R, Y453F, E484K, and N501Y), N-terminal domain deletions (Δ69/70, Δ142-144, and Δ242-245), and additional S1 mutations including D614G and mutations proximal to the S1/S2 furin cleavage site. The emergence of SARS-CoV-2 spike variants with increased transmissibility and reduced neutralization sensitivity complicates the response to the COVID-19 pandemic. Understanding the impact of these variants on the risk of re-infection and vaccine failure requires new forms of *in vitro*, epidemiological, and clinical outcome data.

Key points

1. The past few months witnessed the emergence of three SARS-CoV-2 variants of concern (VOCs) associated with increased transmissibility, increased risk of reinfection, and reduced vaccine efficacy.
2. The three VOCs contain spike mutations that enhance ACE2 binding and/or reduce susceptibility to neutralizing antibodies targeting the S1 receptor binding domain (RBD) and S1 N-terminal domain (NTD).
3. Each VOC contains the RBD mutation N501Y, which increases ACE2 binding and virus transmission. Two VOCs contain E484K, which reduces neutralization by monoclonal antibodies and convalescent and vaccine plasma.
4. Spike RBD and NTD mutations that enhance ACE2 binding and/or reduce neutralizing antibody susceptibility have also been reported in additional virus lineages and in persons with prolonged virus shedding.
5. To interpret the significance of *in vitro* neutralization experiments, it is necessary to determine the comparability of data from different laboratories and their correlation with disease protection.
6. To study SARS-CoV-2 molecular epidemiology, it is necessary to link SARS-CoV-2 sequence data with the infection and vaccination history, and ideally past sequences, of persons undergoing virus sequencing.

Introduction

There have been an increasing number of studies describing the emergence of SARS-CoV-2 spike variants containing mutations that influence virus transmission and antigenicity. These variants will likely influence the epidemiological and clinical aspects of the SARS-CoV-2 pandemic, potentially increasing the risk of re-infection and reducing the protection afforded by vaccine-induced immunity and neutralizing monoclonal antibodies (mAbs). Here we review the prevalence and phenotypic characteristics of the most epidemiologically relevant SARS-CoV-2 spike mutations within the context of SARS-CoV-2 evolution, spike structure, and the *in vitro* methods used to study spike mutations.

SARS-CoV-2 Evolution

Coronaviruses mutate less frequently than most RNA viruses because they contain an exonuclease enzyme that reduces their error rate by about 15 to 20-fold *in vitro*[1,2]. Coronaviruses also undergo recombination when variants with different mutations infect the same host[3,4]. Recombination between different SARS-related coronaviruses likely led to the emergence of SARS-CoV-2[5] and, although it can be difficult to detect due to the similarity of most variants, recombination is occurring to some extent among circulating SARS-CoV-2 variants[6–8]. Host-mediated RNA editing by APOBEC and ADAR enzymes, as evidenced by the overwhelming dominance of C to U changes occurring in specific di-nucleotide contexts, may also be contributing to viral diversity[9,10].

However, despite their lower mutation rates, human coronaviruses evolve antigenically[11,12]. Although it had been widely assumed that waning immunity explained the observation that persons were commonly re-infected with endemic common-cold coronaviruses[13], recent studies have suggested that antigenic drift may also contribute to the lack of long-lasting protection from endemic coronaviruses. Phylogenetic analyses of HCoV-229E and HCoV-OC43 sequences over a 30-year period demonstrated a ladder-like tree topology consistent with the repeated emergence of novel variants that swept through

the human population in a manner similar to seasonal influenza infections, albeit at a reduced frequency[11,12]. Moreover, virus isolates from one point in time often evade neutralization by plasma obtained from people who had been infected several years earlier[11].

During the first six to nine months of the pandemic, the rate of SARS-CoV-2 evolution was low with the mean pairwise distance between virus variants well below 0.1%. With few exceptions, there appeared to be little evidence that selective forces were influencing global genetic diversity[8,14–17]. However, towards the end of 2020, several lineages containing one or more spike mutations at sites of neutralizing Ab epitopes were found to rapidly increase in prevalence, suggesting that they resulted from selective evolutionary pressure. These lineages have been referred to as “variants of concern” because accumulating data indicate that they are associated with increased transmission and/or escape from humoral immunity (Fig. 1a). In addition to these variants of concern, several case reports have described the emergence of multiple spike mutations in immunocompromised patients with prolonged virus shedding, thus demonstrating the ability of SARS-CoV-2 to rapidly accumulate mutations within an individual under certain circumstances[18–23] (Fig. 1b). There have also been increasing reports of other lineages containing potentially concerning spike mutations, of which several are shown in Fig. 1c).

The phylogenetic classification of emergent SARS-CoV-2 lineages has been difficult because the large number of published virus sequences differ from one another by just a few nucleotides, which reduces statistical confidence in inferred phylogenetic branching patterns[24,25]. Geographic classification has also been difficult because many variants have been detected in multiple countries and there are dramatic differences in the proportion of viruses that undergo sequencing in different countries. As a result the naming of SARS-CoV-2 lineages has by necessity not been intuitive[26,27].

Although the possibility that synonymous mutations may influence SARS-CoV-2 phenotypic properties cannot be excluded, there have been no reports of this phenomenon occurring within the SARS-CoV-2 spike gene. Therefore, in this review, we use the term mutation to indicate an amino acid change from the Wuhan-Hu-1 reference sequence (GenBank accession: NC_945512.2). We examine the

prevalence of mutations using three online resources: CoV-GLUE[28], COVID-19 CoV Genetics (COVID CG)[29], and NextStrain[30] . Although the data from these sites each include high-quality sequences from the GISAID database, they differ in how mutation prevalences are calculated. The CoV-GLUE and COVID-19 CG sites report the proportions of mutations in all sequences since the start of the pandemic. In contrast, the NextStrain prevalence is based on a subsample of sequences heavily weighted towards recently reported sequences and containing equal numbers of sequences from five continental regions and Oceania.

Spike Structure and Immune Epitopes

The SARS-CoV-2 spike protein is a 1,273 amino acid trimeric glycoprotein responsible for virus host cell entry. Each spike monomer has a largely exposed S1 attachment domain (residues 1-686) and a partially buried S2 fusion domain (residues 687-1273)[31,32]. Part of S1, called the receptor binding domain (RBD; residues 306-534), alternates between a closed/down position and an open/up position. When in the up position, it binds to the human angiotensin converting enzyme 2 (ACE2) receptor[33–36]. Approximately 20 RBD residues form contacts with the human ACE2 receptor. The part of the RBD containing these residues encompasses residues 438 to 506 and is called the receptor-binding motif (RBM), while the remainder of the RBD is called the RBD core. Like the RBD, much of the S1 N-terminal domain (NTD) is also exposed on the S trimer surface. The remainder of S1, contains two subdomains traditionally referred to as subdomain 1 (SD1) and subdomain 2 (SD2). As SD1 and SD2 are downstream of the RBD, we refer to this region as the S1 C-terminal domain (CTD).

S1 displays more amino acid variability than S2 among SARS-related coronaviruses (Fig. 2). Within S1, the RBD and the NTD are more variable than the CTD. Within the RBD, the RBM is more variable than the RBD core. This distribution of variability, however, is not yet apparent within SARS-CoV-2 as reported mutations have been distributed across both S1 and S2. As of February 2021, 16 spike mutations have a global sampled prevalence >1.0% and 90 mutations have a global prevalence between 0.1% and 1.0% (Supplementary Table 1). The spike protein also has 22 glycosylation sites, distributed among both

the S1 and S2 domains. Within S1, eight of the glycans are found in the NTD, two are in the RBD core, three are in one of the C-terminal domains, and nine are in S2[37].

The spike RBD is the main target of neutralizing Abs[38–43]. The presence of neutralizing mAbs targeting the RBD have correlated with protection in animal models and in previously infected and vaccinated persons, although cellular immune responses and potentially non-neutralizing Abs are likely to have contributed to protection in these studies[44–49]. The development of neutralizing Abs early in the course of infection has been associated with lower virus levels and greater protection from severe infection [50–55]. Finally, the passive administration of neutralizing Abs either as part of convalescent plasma or as mAbs reduces the severity of infection when administered early[53,54,56,57].

High resolution X-ray crystallography and cryo-EM structures have been published for more than one hundred mAbs including four with FDA Emergency Use Authorizations and four additional mAbs in phase III clinical trials. Most mAbs target either the RBD RBM or RBD core; several target the NTD. Those targeting the RBM compete with RBD binding to ACE2. Those targeting the RBD core often cross-neutralize other SARS-related coronaviruses, however, on average they have lower SARS-CoV-2 neutralizing activity.

Several broad classification schemes have been developed to describe RBD-binding mAbs[58–60]. Among those mAbs targeting the RBM, one group binds epitopes that overlap extensively with the ACE2 binding site and as a result binds solely when the RBD is in the open state. This group, which has been referred to as class I[58] or receptor binding site (RBS) A[60] mAbs are typically encoded by the closely related IGHV3-53 and IFHV3-66 heavy chain genes and have short complementarity-determining region (CDR) H3 loops. The second main group of RBM-binding mAbs (class II or RBS B) has a smaller ACE2-binding footprint and as a result can often bind the RBD in the closed state. Several other RBM-binding mAbs are more difficult to classify including a class that binds a quaternary epitope involving more than one RBD[58,59].

Abs that target the RBD core form two major clusters, one on the surface-accessible face of the RBD and another whose epitope is buried in the closed state. Antibodies that bind to the surface accessible face of the RBD core can bind in either the open or closed state and those that target the RBD core epitope

bind only in the open state. Fig. 3 displays the epitopes of those mAbs with high-resolution structures that are either in advanced clinical trials or that have been assessed for their activity against viruses with mutant spike proteins. Supplementary Table 2 contains the IC_{50} of each of these mAbs and the references in which they were first reported.

The humoral response to SARS-CoV-2 spike also includes non-neutralizing Abs and neutralizing Abs targeting the S1 CTD and S2[61–64]. Most of these additional Abs bind linear epitopes identified in high-throughput studies using overlapping spike peptide pools. As the S1 CTD and S2 are more conserved than the S1 RBD and NTD, neutralizing Abs targeting these regions are theoretically less likely to be affected by virus mutations and more likely to cross-neutralize other SARS-related coronaviruses. However, the inhibitory activity of these mAbs have been much lower than RBD- and NTD-binding mAbs; high-resolution structures of mAbs bound to regions outside of the RBD and NTD have also been difficult to obtain[61,64]. Although non-neutralizing Abs mitigate disease by eliciting Fc-effector functions including Ab-dependent complement activation, cellular cytotoxicity, and phagocytosis, these processes are more difficult to quantify than neutralization and the clinical importance of non-neutralizing SARS-CoV-2 Abs is uncertain.

Although many studies have documented that SARS-CoV-2 infection and immunization elicits T helper and cytotoxic responses, specific MHC-restricted T cell epitopes have been experimentally validated in a much smaller number of studies[52,65–69] (Supplementary Table 3). At least six cytotoxic T lymphocyte epitopes have been identified in two or more studies. However, few spike mutations have been reported within these epitopes, suggesting that confirmed CTL escape mutations are currently uncommon.

Selection and Neutralization Experiments

In vitro selection experiments have been performed almost entirely using non-replicative pseudotyped or replication-competent chimeric viruses[70–73]. SARS-CoV-2 pseudotyped viruses are produced by co-transfecting a SARS-CoV-2 protein expression vector and a construct encoding the

components required for replication of a different virus that lacks the coding sequence for their own surface protein – most commonly vesicular stomatitis virus (VSV), HIV-1, or murine leukemia virus (MLV). These constructs also encode a reporter gene such as luciferase or GFP. Chimeric viruses contain the SARS-CoV-2 spike sequence in a VSV genome lacking the sequence for the VSV surface protein[72,73]. *In vitro* selection and neutralization experiments using pseudotyped and chimeric viruses provide a sensitive digital readout and do not require a biosafety level 3 laboratory. VSV-based chimeric viruses are particularly useful for selecting mutations because they are able to undergo multiple rounds of replication. Additionally, because they lack an error-correcting mechanism they produce replication errors *in vitro* at a higher rate than SARS-CoV-2, which increases the likelihood of identifying escape mutations.

Although many neutralization studies of wildtype viruses have been performed using full-length infectious viruses, studies on the effects of spike mutations have been performed almost entirely using pseudotyped viruses because it is simpler to introduce mutations into a plasmid encoding just the spike gene than into a system that requires either multiple plasmids or an artificial chromosome containing the complete SARS-CoV-2 genome[74,75]. Neutralization studies compare the ability of a mAb or plasma sample to inhibit the cellular entry of a pseudotyped virus containing one or more spike mutations to pseudotyped viruses lacking these mutations. The plasma samples studied usually include convalescent plasma from SARS-CoV-2 infected persons or plasma from persons receiving a SARS-CoV-2 vaccine. Studies of convalescent plasma provide information relevant to the risk of re-infection with a mutant virus variant. Studies of plasma from immunized persons provide information relevant to vaccine efficacy. In studies using convalescent plasma, the genetic sequence of the initial infecting virus is rarely known – a factor likely to complicate the interpretation of these studies as circulating viruses accumulate spike mutations.

The ability of a virus to escape mAb neutralization can be determined in part by high-throughput binding experiments as mAb binding is a pre-requisite for neutralization. The converse, however, is not necessarily true; binding by an mAb does not ensure neutralization[76–78]. Binding experiments can be performed using enzyme-linked assays, surface plasmon resonance, biolayer interferometry, or flow

cytometry. The effects of nearly all individual RBD mutations on protein expression in yeast (a correlate of protein folding stability), ACE2 binding, and binding to a wide variety of mAbs and plasma samples have been assessed using a method referred to as deep mutational scanning since each yeast cell producing a different RBD mutation is labeled with a distinct genomic sequence[79]. This platform has identified mutations that influence the binding of RBD to ACE2 and to mAbs[40,79–82]. Although this approach does not quantify the effect of RBD mutations on mAb neutralization, it has proved useful as a screening assay to identify mutations that require further study in cell culture.

The replication kinetics of viruses containing spike mutations are better studied using full-length infectious viruses (as opposed to pseudotyped viruses) because this allows the effects of a mutation to be evaluated during multiple rounds of replication and to be evaluated in an animal model. The replication kinetics of a growing number of spike variants are now being studied in this manner [83–88].

Spike Mutations

Fig. 4 illustrates the most biologically and clinically significant spike mutations and their increase in prevalence since mid-2020. Each of the three variants of concern have contained the RBD mutation N501Y and the CTD mutation D614G. Two variants (B.1.351 and P.1) contained RBD mutations at positions 484 (E484K) and 417 (K417N/T). B.1.1.7 and B.1.351 each contained one or more NTD deletions, while B.1.352 and P.1 each contained the NTD mutation L18F. N439K, L452R, Y453F, S477N, F490S, and S494P are additional RBM mutations that have been reported in several recently recognized lineages and/or in SARS-CoV-2-infected persons with prolonged virus shedding.

This section summarizes experimental data associated with these mutations including approximately 1,900 neutralization assays performed using pseudotyped and infectious viruses containing a single spike mutation and 1,600 performed containing a combination of spike mutations. Approximately 29% of these assays evaluated the neutralizing activity of convalescent plasma, 34% evaluated plasma from vaccinated persons, and 37% evaluated a structurally characterized mAb.

D614G

The global prevalence of the D614G mutation began increasing in late February 2020. As of February 2021, D614G is present in more than 95% of circulating SARS-CoV-2 variants. The increased prevalence of D614G resulted from the rapid spread of a single virus lineage containing D614G as well as three other nucleotide changes: a 5'UTR mutation, a silent orf1a mutation, and the RdRp mutation P323L[8]. During its spread, this lineage out-competed ancestral viruses in many regions demonstrating that its dominance resulted from greater transmissibility rather than a founder effect[8]. In the UK, this strain was associated higher levels of viral nucleic acid in the upper respiratory tract but not with higher rates of hospitalization[8,83].

Pseudoviruses with D614G have 3 to 10-fold higher levels of replication compared to those without this mutation[8,89]. Infectious virus clones with D614G had 14-fold higher levels of replication in primary human airway tissue and hamsters infected with this clone produced higher infectious titers in nasal washes and trachea but not in their lungs[84,88,90]. Cryo-EM structures have shown that D614G disrupts one or more interprotomer contacts resulting in a greater likelihood that one or more of the three RBDs were in an open versus closed position[91,92]. D614G may also be responsible for increasing the number of spike proteins per virion[83,89].

Viruses with D614G are somewhat more susceptible to neutralization by mAbs, convalescent plasma, and plasma from vaccinated individuals[52,91,93]. Spike proteins containing the original Wuhan reference sequence and those containing D614G (now the global consensus) are now both used as wildtype comparators for *in vitro* neutralization assays.

N501Y

N501Y is present in each of the variants of concern (Fig. 1a)[94–98]. In the late fall of 2020, the prevalence of B.1.1.7 increased dramatically in several parts of the U.K. This variant has now been

identified in more than 80 countries and in many U.S. states[99]. Epidemiological studies suggest it is about 50% more transmissible than previous circulating variants[95,96,100] and that it is associated with significantly higher upper-airway levels than previous viral variants[101,102] and possibly increased disease severity[100,103]. The rapid expansion of B.1.351 in South Africa and P.1 in Brazil suggest that these variants are also more transmissible. Between July 2020 and February 2021, the global prevalence of N501 increased from 0.03% to 16% while its prevalence in the NextStrain subsample increased to 32%.

Several N501 mutations including N501Y increase ACE2 affinity[79,104,105]. N501 is in the binding epitope of a high proportion of mAbs. Nonetheless, N501Y and the complete B.1.1.7 spike retain susceptibility to most RBD-binding mAbs with the possible exception of sotrovimab, for which there are conflicting data (Table 1)[87,106,107].

In aggregate data from multiple studies, N501Y alone displayed low-level reduced susceptibility (3 to 9.9-fold) for seven (9.9%) of 71 convalescent plasma samples[87,106,108] but retained full susceptibility to 100% of 99 samples from persons immunized with BNT162b2 or mRNA-1273 (Fig. 5)[85,87,106,109,110].

The complete set of B.1.1.7 spike mutations displayed ≥10-fold reduced susceptibility to 1.8% and partially reduced susceptibility to 22% of 109 convalescent plasma samples (Fig. 5)[87,106,108,111]. The complete set of B.1.1.7 spike mutations displayed ≥10-fold reduced susceptibility to 0.8% and partially reduced susceptibility to 15% of 129 plasma samples from persons immunized with BNT162b, mRNA-1273, or NVX-CoV2373 (Fig. 5)[87,106,112–114]. Although plasma from recipients of the ChAdOx1 nCoV-19 (AZD1222) vaccine had 9-fold lower levels of neutralization titers compared with pre-B.1.1.7 variants, the vaccine was equally efficacious at preventing B.1.1.7-induced illness[115]

E484K

E484K is present in two of the variants of concern: B.1.351 (aka 501.V2)[94] and P.1 (aka 501.V3; B.1.1.28[K417T/E484K/N501Y]) (Fig. 1a)[97,98]. It has also been detected in an increasing number of viruses within the B.1.1.7 lineage[112,116] and in several additional recently recognized lineages including one

circulating in both the U.S. and South America (B.1.526)[117]. There have been documented re-infections with viruses containing E484K belonging to the P.1 variant of concern[118]. More importantly, epidemiological data suggests that the resurgence of new infections in Manus Brazil despite the high seroprevalence in that region in mid-2020 may be reflecting widespread reinfection with the P.1 variant[119].

Reinfections with viruses containing this mutation were also observed during the phase 2b trial of the NVX-CoV2373 vaccine performed in South Africa, where approximately one-third of infections in both the vaccine and placebo arms represented reinfections with the B.1.351 variant[120]. Of greater concern was the preliminary observation that the ChAdOx1 nCoV-19 (AZD1222) did not appear to prevent B.1.351 infections among its South Africa trial participants infected with the B.1.351 variant[115] .

E484 is within the epitope of a high proportion of mAbs. It has been selected *in vitro* by casirivimab and other class I and II mAbs including C121 and C144[41]. E484K reduces susceptibility to bamlanivimab by >100-fold [87,121], casirivimab by 10 to 20-fold[70,87], and etesevimab by 4-fold[121] (Table 1). It does not appear to reduce susceptibility to imdevimab or sotrovimab[70,87]. Pseudoviruses containing E484K in combination with K417N and N501Y, the other RBD mutations present in 1.351, displayed greater reductions in susceptibility to mAbs than pseudoviruses containing E484K alone.

In aggregate data from multiple studies, E484K alone displayed ≥10-fold reduced susceptibility to 19% and partially reduced susceptibility to 43% of 71 convalescent plasma samples[40,87,122,123], whereas data from three studies reported ≥10-fold reduced susceptibility to 2.3% and partially reduced susceptibility 24% of 42 samples from persons immunized with BNT162b2 or mRNA-1273 (Fig. 5).

However, pseudoviruses containing the complete set of B.1.351 spike mutations displayed higher levels of reduced susceptibility to convalescent and vaccine plasma. In aggregate data, B.1.351 displayed ≥10-fold reduced susceptibility to 39% and partially reduced susceptibility to 41% of 102 convalescent plasma samples[87,111,124] whereas it displayed ≥10-fold reduced susceptibility to 20% and partially reduced susceptibility to 67% of 30 samples from persons immunized with BNT162b2 or mRNA (Fig. 5)[87,114].

K417N/T

K417N is present in B.1.351 and K417T is present in P.1, each in combination with E484K and N501Y (Fig. 1A)[97]. K417N/T rarely occur in the absence of other RBM mutations, possibly because K417 mutations appear to reduce ACE2 binding[81,112]. K417N confers about 10-fold reduced susceptibility to casirivimab[87] and >100-fold reduced susceptibility to etesevimab[80] but retains susceptibility to bamlanivimab, imdevimab, and sotrovimab (Table 1)[87]. K417N appears to display full susceptibility to plasma samples from persons previously infected with SARS-CoV-2 or immunized with either of the mRNA vaccines (Fig. 5)[87,110].

N439K

Steep increases in the prevalence of two lineages containing the spike mutation N439K were reported in the UK in September 2020[125]. However, the two N439K-containing UK lineages declined in prevalence concurrent with the emergence of B.1.1.7. Nonetheless, between July 2020 and February 2021, the global prevalence of N439K increased from 0.4% to 2.4%. N439K increases ACE2 affinity[79,125,126]. N439 is within the epitope for imdevimab and the RBD core 1 mAb C135 and N439K reduces the susceptibility to these mAbs by ~30-fold[80] and >100-fold[41], respectively (Table 1). N439 reduces binding to a relatively small proportion of convalescent plasma samples[40,106,110,125,127].

L452R

L452R has occurred in the absence of other RBD or previously described spike mutations in a large cluster of sequences designated CAL.20C. In February 2021, this variant accounted for 36% of sequences in Southern California[128]. L452R has been associated with reduced susceptibility to several RBM class II mAbs including P2B-2F6[123,127] and BD-368-2[129]. It has also been shown to escape binding by bamlanivimab

in the previously described widely used yeast display assay [82]. Its susceptibility, however, to most other mAbs has not been reported.

Y453F

Y453F has been reported in viruses from two immunocompromised persons with prolonged viral shedding (Fig. 1b)[21]. It has also emerged independently at least four times in mink lineages, including one that subsequently spread among humans but which is no longer actively spreading[130]. Nonetheless, between July 2020 and February 2021, the global prevalence of Y453F increased from 0.1% to 0.3%. Y453F has also been reported to increase ACE2 binding[79,131]. It is part of the epitope of many neutralizing RBM-directed mAbs and markedly reduces susceptibility to casirivimab[70]. Its susceptibility, however, to most mAbs has not been reported.

Other RBD variants

The prevalence of S477N has increased gradually since the start of the pandemic and it was present in a variant that spread widely in Europe in the summer of 2020[27]. In one study, S477N was associated with reduced susceptibility to a panel of structurally uncharacterized mAbs isolated from immunized mice despite but retained full susceptibility to a panel of four convalescent plasma samples[123]. S477N also did not interfere with binding and/or susceptibility to several other mAbs or convalescent plasma samples in three other studies[40,80,132].

V483A is a rarely occurring mutation within the epitope of many mAbs including bamlanivimab, etesevimab, and CT-459. It was reported in one immunocompromised patient with prolonged SARS-CoV-2 infection[22]. V483A reduces binding to bamlanivimab [82] and is associated with high-level reductions in susceptibility to two other class II mAbs – C121[41] and BD-368[129].

F490S and S494P are uncommon RBM mutations that have recently been reported to arise independently within several B.1.1.7 lineage sequences[116]. F190S is associated with reduced susceptibility

to bamlanivimab[121] while S494P is associated with reduced susceptibility to bamlanivimab, C121, and C144 (**Table 1**)[41,121]. S494P was also reported in an immunocompromised patient with prolonged virus shedding[18].

S1 N-terminal domain (NTD) mutations

Deletions between positions 69 and 70 (ΔH69/V70), 141 and 146 (Δ141/146; most commonly ΔY144), and 242 and 244 (Δ242/244) have frequently been observed in association with RBD mutations. Both Δ69/70 and Δ141/146 are present in the B.1.1.7 variant while Δ243/244 is present in the B.1.351 variant[94]. Δ69/70 and/or Δ141-146 have also been reported in six immunocompromised persons with prolonged virus infections (Fig. 1B)[18–23].

Both Δ69/70 and Δ141/146 alter the conformation of protruding NTD loops. Δ69/70 does not interfere with the neutralizing activity of any mAbs tested including those that bind to the NTD or with the neutralizing activity of convalescent plasma[20,106]. Δ141/146 overlaps the epitope of most neutralizing NTD-binding mAbs[42,133,134] and reduces susceptibility to two NTD-binding mAbs, 4-8 and 4A8[42,87,133,134]. Δ242/244 reduces susceptibility to these and other NTD binding mAbs [87]. Δ144 displayed 4-fold reductions in susceptibility to 2 of 20 convalescent plasma samples but retained full susceptibility to 21 plasma samples from persons receiving an mRNA vaccine (**Fig. 5**)[87].

S1 C-terminal domain (CTD) mutations

Among the S1 C-terminal domain mutations, several occur just upstream of the RRAR polybasic furin cleavage site separating S1 from S2 including Q675H/R, Q677H/P, N679K, and P681H. P681H is present in B.1.1.7 and has also been reported in clusters of viruses in Hawaii[135] and Nigeria[136]. In February 2021, its COVID CG and NextStrain subsample prevalences were 16% and 27%, respectively. P681H has not been shown to reduce susceptibility to any RBD-binding mAb[87]. Q677H and Q677P have been reported in at least seven independent lineages[137]. Q677H has been reported in 5% of global sequences (COVID

CG). The effects of RRAR upstream mutations on spike function and the selective forces responsible for their increasing prevalence are not known.

Implications for COVID-19 Research

The emergence of SARS-CoV-2 spike variants with increased transmissibility and reduced neutralization sensitivity has complicated COVID-19 research because understanding the impact of these variants on the risk of re-infection and vaccine failure now requires new forms of *in vitro*, epidemiological, and clinical outcome data. Based on current epidemiologic trends, it is likely that one or more of the N501Y-containing variants of concern will replace variants without this mutation in most parts of the world. Although the B.1.1.7 variant, which contains N501Y but not E484K, retains neutralization susceptibility to convalescent plasma and plasma from vaccinated persons, this variant provides a platform for the emergence of additional immune escape mutations. Indeed, E484K is increasingly being reported in persons infected with the B.1.1.7 variant.

In vitro selection experiments are performed to identify spike mutations selected by mAbs and immune plasma. *In vitro* neutralization experiments are performed to determine the susceptibility of viruses containing spike mutations to mAbs and to plasma from previously infected and vaccinated persons. To aggregate and synthesize these *in vitro* data it has become essential to determine the reproducibility and comparability of data generated in different laboratories. To determine the clinical implications of these data, it has become essential to learn how well *in vitro* selection experiments identify mutations that are likely to emerge clinically and to determine how well *in vitro* neutralizing data correlate with protection from disease.

Additional epidemiological data is now also required to interpret the relationship between SARS-CoV-2 sequences and clinical outcome. Specifically, it is now essential to know whether a virus sequence was obtained from a person who was vaccinated for SARS-CoV-2 or experiencing a SARS-CoV-2 reinfection. Among vaccinated persons, it is important to know the vaccine used and the time of its

administration. Among persons experiencing re-infection, it will be important to know the time and place of the initial infection and whether a sequence was performed on the primary infection sample.

Protection from illness does not depend entirely on neutralizing antibodies. Therefore, the severity of acute disease and risk of long-term complications will likely be reduced in persons experiencing reinfection or vaccine failure with a neutralization resistant variant. Nonetheless, it will be necessary to characterize the severity and duration of illness associated with these cases. Simply knowing whether a reinfected or vaccinated person required hospitalization or died will be insufficient for weighting the benefits of different strategies for combatting novel variants. Determine the cost-effectiveness of interventions such as booster vaccinations with updated immunogens, continued investment in antiviral therapeutics, and the maintenance of nonpharmaceutical interventions will require the consideration of a wider spectrum of non-hospitalization outcomes including the length of work absenteeism the development of chronic disability.

Conclusion

The rapid development of highly effective vaccines has been the most notable achievement in combatting the COVID-19 pandemic. Simultaneously, researchers isolated of scores of highly potent neutralizing mAb, several of which are in advanced clinical trials. Although the impact of therapeutic and prophylactic mAbs on the global pandemic will be low because of their cost and difficulty to administer in a timely manner, their development has provided unprecedented insight into the targets of humoral immunity. The most striking feature of these mAbs is that they universally required minimal affinity maturation because both their heavy and light chain variable sequences differ minimally from their putative germ line sequences. However, the rapidity with which neutralizing Abs arise in persons infected with or vaccinated for SARS-CoV-2 must be contrasted with the brittle nature of the response, as evidenced by the fact that a small number of mutations or even a single mutation can lead to neutralization escape in many people.

Figure Legends

Figure 1

Graphical depiction of spike mutations in three variants of concern (A), in six variants containing mutations present in immunocompromised persons with prolonged viral shedding (B), and in several other expanding lineages containing potentially concerning spike mutations (C). The variants of concern include B.1.1.7, also referred to as 501Y.V1, which was initially identified in the United Kingdom; B.1.351 also referred to as 501.V2, which was initially identified in South Africa; and P.1 also referred to as 501Y.V3, which was initially identified in Brazil. The variants associated with prolonged virus shedding are labelled with the last name of the first author reporting the variant: Kemp[20]; McCarthy[19]; Choi[18]; Avanzato[22]; Bazykin[21]; and Khatamzas[23]. Genomes are not drawn to scale. NTD: N-terminal domain; RBD: receptor binding domain; RBM: receptor binding motif; SD1 and SD2 comprise the S1 C-terminal domain. S2 is the area in green that lies downstream of the S1/S2 cleavage site.

Figure 2

Position-specific sequence variability and median domain-specific pairwise distances among SARS-related coronaviruses. Results were derived from an alignment of 24 representative sarbecovirus spike sequences having a TN93 distance of $\geq$0.01. Position-specific entropy is superimposed for one of three monomers on a surface representation of trimeric SARS-CoV-2 spike (PDB: 6XR8). Two 90^{o} rotated side views (left and middle panels) and one top view (right panel) of the spike trimer are shown.

Figure 3

Classification of monoclonal antibodies (mAbs) targeting the SARS-CoV-2 spike receptor binding domain (RBD) epitopes. For the two RBM-binding mAbs, 90^{o} rotated side views and one top view of a surface RBD representation are shown. For the two RBD core-binding mAbs, just the 90^{o} rotated side views are shown.

The bold underlined mAbs are in phase 3 clinical trials; the plain text underlined mAbs are in phase 1 clinical trials. The blue intensity is proportional to the number of mAbs binding to the underlying amino acid residues. The receptor binding motif (RBM) refers to the region of the RBD containing the ACE2-binding residues. RBM class 1 mAbs bind the RBD only in its up position while RBM class 2 mAbs can bind the RBD in its up or down position. A third RBM mAb class binds to a quaternary epitope comprising more than one RBD is not shown as it would require the trimeric spike. The epitopes for NTD-binding mAbs are not shown.

Figure 4

The sites of 19 key S1 mutations on the spike trimer including 11 in the receptor binding domain (RBD; green), five in the N-terminal domain (NTD; cyan), and three in the C-terminal domain (CTD; purple) (A) and their changing prevalence (B). S2 Is shown in dark grey. The RBD mutations include three in two or more variants of concern (K417N/T, E484K, and N501Y) and eight in several additional lineages or in persons with prolonged viral shedding. The NTD mutations including three deletions and a mutation (L18F) present in two or more variants of concern. The CTD mutations include D614G which became the consensus amino acid at this position prior to the emergence of the variants of concern, two CTD mutations of increasing prevalence Q677H/P and P681H that are just proximal to the spike furin cleavage site, and a prevalent NTD mutation (A222V). The prevalence of these mutations are shown in Supplementary Table 1. The COVID-19 CG website reports the proportions of mutations in all sequences since the start of the pandemic while NextStrain prevalence is based on a subsample of sequences heavily weighted towards recently reported sequences and containing equal numbers of sequences from five continental regions and Oceania.

Figure 5

Fold-reduced *in vitro* neutralization susceptibility to plasma from previously infected persons (A) and from vaccinated persons (B) associated with three variants of concern (B.1.1.7, B.1.351, and P.1) and several addition spike mutations including the RBD mutations N501Y, E484K, K417N, N439K, and Y453F, and the N-terminal domain deletions Δ69/70 and Δ144. B.1.1.7 contains N501Y plus two S1 N-terminal domain (NTD) deletions (Δ69/70 and Δ144), three S1 C-terminal domain (CTD) mutations, and three S2-mutations. B.1.351 contains the RBD mutations K417E, E484K and N501Y; one S1 NTD deletion and four NTD mutations; D614G, and one S2 mutation. P.1. contains the RBD mutations K417T, E484K and N501Y; five NTD mutations; two S1 C-terminal domain mutations including D614G; and two S2 mutations. The Y-axis indicates the number of plasma units tested. The color scheme indicates the fold-reduction in neutralization: absence of color indicates <3-fold reduced susceptibility; light blue indicates 3 to 9.9-fold reduced susceptibility; dark blue indicates ≥10-fold reduced susceptibility. With few exceptions, the vaccine recipients had received an mRNA vaccine and the samples were obtained one-to-two months following vaccination.

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Table 1. Fold-Reduced Neutralizing Susceptibility to Monoclonal Antibodies (mAbs): Pseudotyped Viruses with Combinations of Spike Mutations Present in Variants of Concern (B.1.1.7, B.1.351, P.1) and with Individual Spike Mutations.

	RBM-I				RBM-II		RBM-III	Core-I		
	CAS	**ETE**	**TIX**	**S2E12**	**BAM**	**CIL**	**C144**	**IMD**	**SOT**	**C135**
B.1.1.7	2.0_3	3.6	1.5	1.1	0.7_3	1.1	-	1.0_3	3.1_2^*	1.4
B.1.351	59_2	$>100_2$	3.5	-	$>100_2$	1	-	1.0_2	0.8_2	0.8
P.1	16	-	-	-	>100	-	-	0.7	-	-
E484K	20_3	4.4_2	8.1_2	-	$>100_2$	2_2	>100	1.7_3	0.4	1.2_2
N501Y	1.4_3	5_2	1.7	-	1	1.3	1.4	1_3	3.1_2^*	2.2_2
K417N	13	$>100_2$	0.4	-	0.1	0.6	-	1.2	0.6	0.3
N439K	0.8_2	0.6	-	0.9	-	-	0.9	36_2	0.9	>100
L452R	-	1.0	-	-	$>10^\dagger$	-	-	-	-	-
Y453F	$>100_3$	-	-	-	-	-	-	2.8_2	-	-
F190S	-	-	-	-	>100	-	4.5	-	-	1.2
S494P	-	0.5	-	0.5	>100	-	45	-	-	0.8

Footnote: RBM-I: mAbs binding to the receptor binding motif - class I; RBM-II: mAbs binding to the RBM - class II; RBM-III: mAbs binding to a quaternary RBM epitope; Core-I: mAbs binding to the RBD core. CAS (casirivimab), ETE (etesevimab), TIX (tixagevimab), BAM (bamlanivimab), CIL (cilgavimab), IMD (imdevimab), and SOT (sotrovimab) are in phase III clinical trials. C144 and C135 are in a phase I clinical trial. CAS + IMD, BAM + ETE, TIX + CIL, SOT + S2E12; and C135 + C144 are being developed for use in combination. B.1.1.7 contains the following spike mutations: Δ69/70, Δ144, N501Y, A570D, D614G, P681H, T716I, S982A, and D1118H. B.1.351 contains L18F, D80A, D215G, Δ242-244, K417N, E484K, N501Y, D614G, and A701V. P.1 contains L18F, T20N, P26S, D138Y, R190S, K417T, E484K, N501Y, D614G, H655Y, T1027I, and V1176F. "-": susceptibility data absent. *indicates conflict between two research groups, with one showing a high-level of reduced susceptibility especially using IC_{80} rather than IC_{50}[106]. †Susceptibility determined using a binding assay that correlates with <10-fold reduced susceptibility[82].

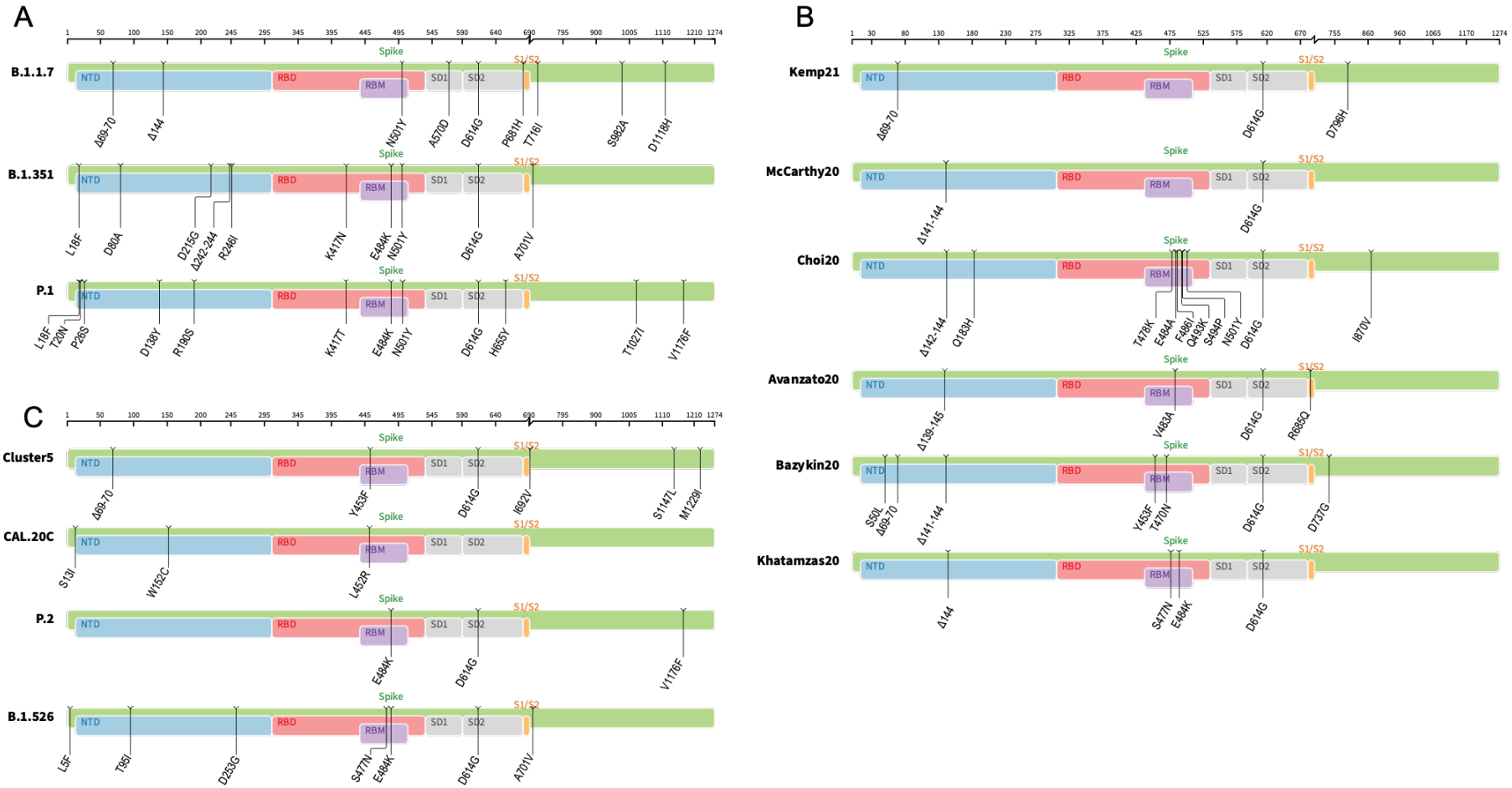

A
B
C
Spike
S1/S2
NTD
RBD
RBM
SD1
SD2
B.1.1.7
Δ69-70
Δ144
N501Y
A570D
D614G
P681H
T716I
S982A
D1118H
B.1.351
L18F
D80A
D215G
Δ242-244
R246I
K417N
E484K
N501Y
D614G
A701V
P.1
L18F
T20N
P26S
D138Y
R190S
K417T
E484K
N501Y
D614G
H655Y
T1027I
V1176F
Cluster5
Δ69-70
Y453F
D614G
I692V
S1147L
M1229I
CAL.20C
S13I
W152C
L452R
P.2
E484K
D614G
V1176F
B.1.526
L5F
T95I
D253G
S477N
E484K
D614G
A701V
Kemp21
Δ69-70
D614G
D796H
McCarthy20
Δ141-144
D614G
Choi20
Δ142-144
Q183H
T478K
E484A
F486I
Q493K
S494P
N501Y
D614G
I870V
Avanzato20
Δ139-145
V483A
D614G
R685Q
Bazykin20
S50L
Δ69-70
Δ141-144
Y453F
T470N
D614G
D737G
Khatamzas20
Δ144
S477N
E484K
D614G

FIGURE 1

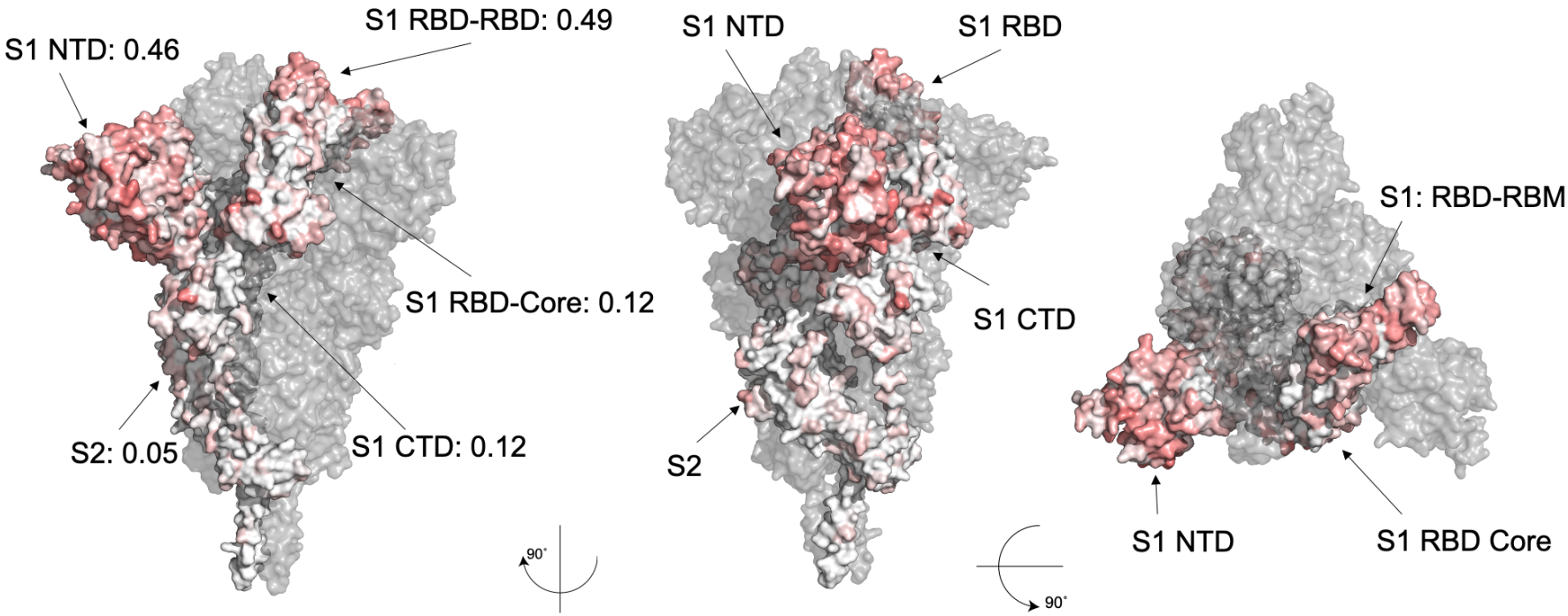

S1 NTD: 0.46
S1 RBD-RBD: 0.49
S1 RBD-Core: 0.12
S2: 0.05
S1 CTD: 0.12
90°
S1 NTD
S1 RBD
S1 CTD
S2
90°
S1: RBD-RBM
S1 NTD
S1 RBD Core

FIGURE 2

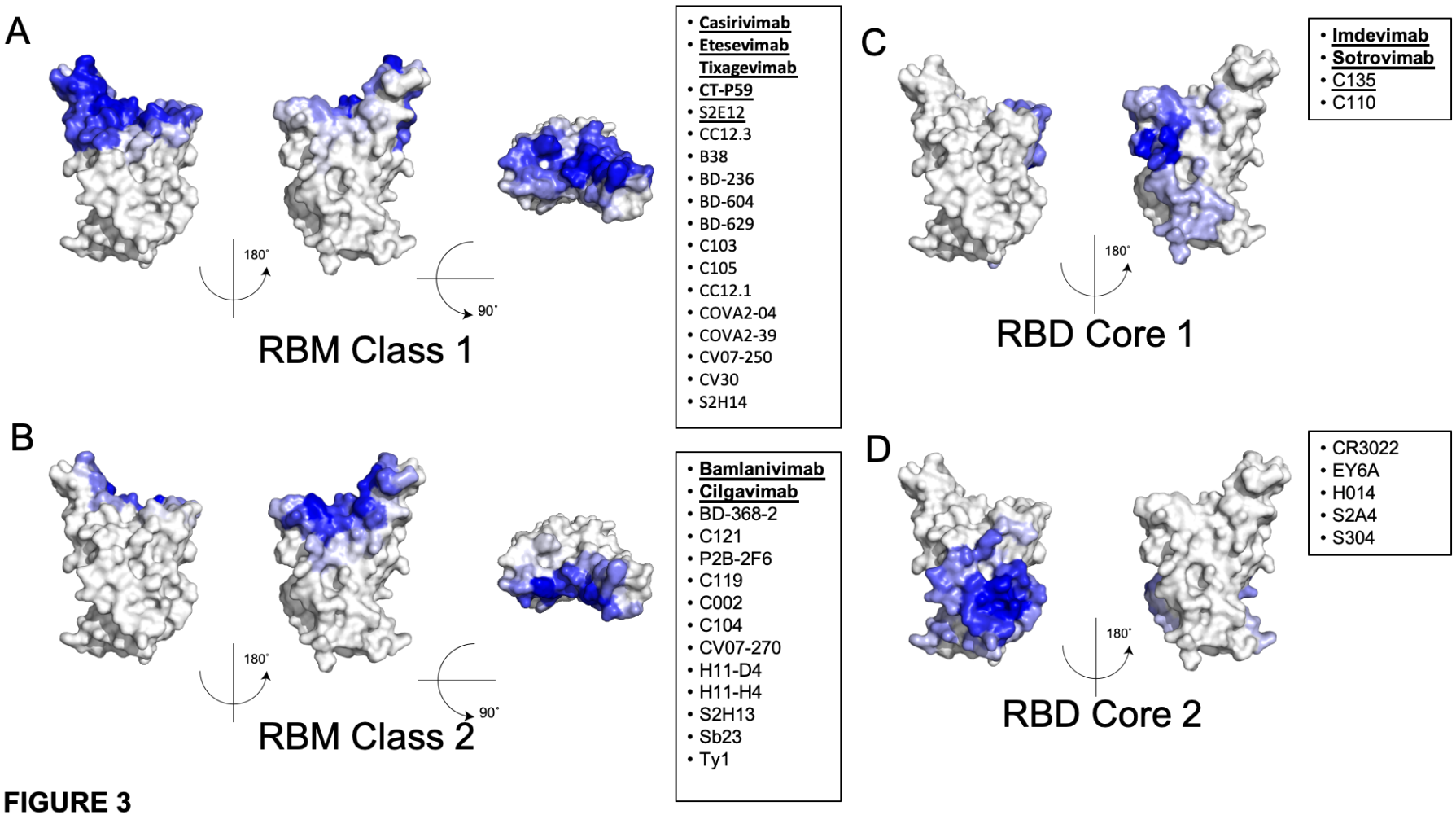
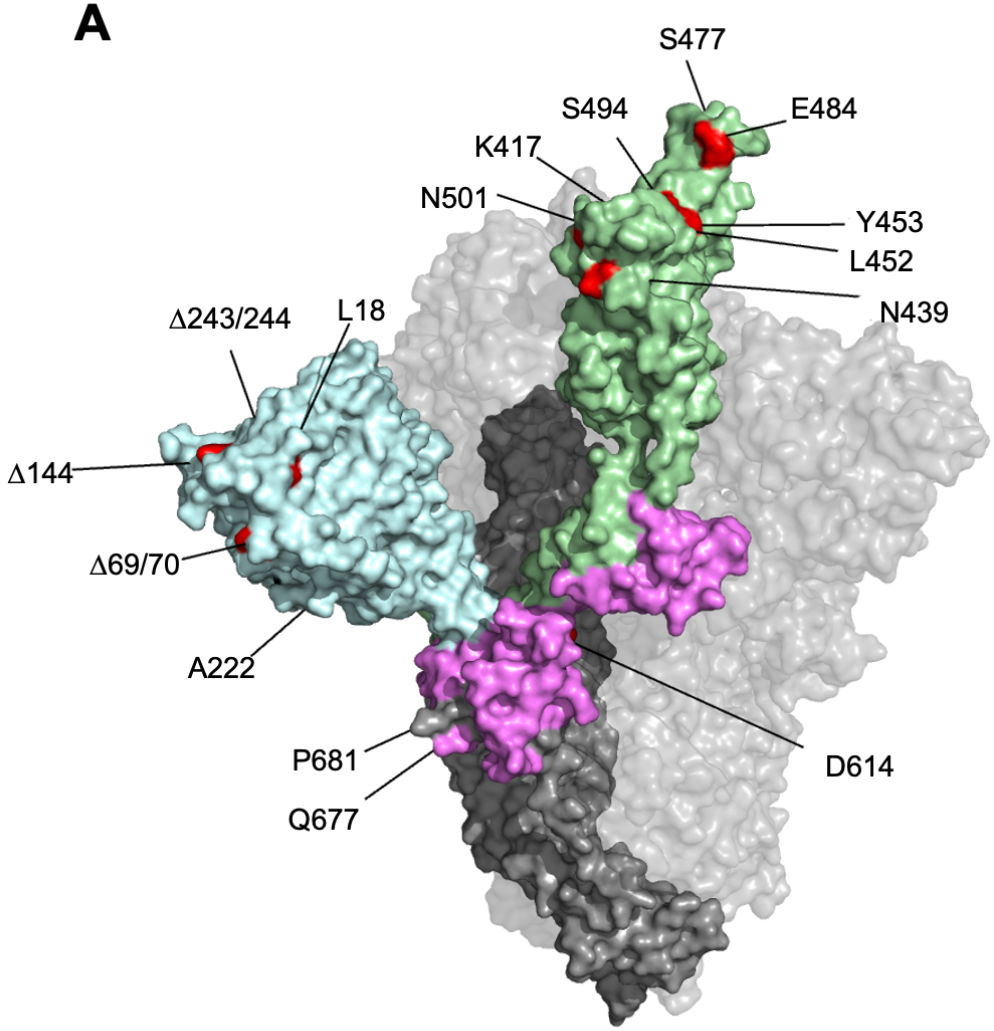

A
180°
90°
RBM Class 1
• Casirivimab
• Etesevimab
Tixagevimab
• CT-P59
• S2E12
• CC12.3
• B38
• BD-236
• BD-604
• BD-629
• C103
• C105
• CC12.1
• COVA2-04
• COVA2-39
• CV07-250
• CV30
• S2H14
B
180°
90°
RBM Class 2
• Bamlanivimab
• Cilgavimab
• BD-368-2
• C121
• P2B-2F6
• C119
• C002
• C104
• CV07-270
• H11-D4
• H11-H4
• S2H13
• Sb23
• Ty1
C
180°
RBD Core 1
• Imdevimab
• Sotrovimab
• C135
• C110
D
180°
RBD Core 2
• CR3022
• EY6A
• H014
• S2A4
• S304

FIGURE 3

A



B

	July 2020 => February 2021	
	COVID CG	**NextStrain**
Spike mutations present in variants of concern		
L18F	0.3 => 9.7	0 => 8.5
Δ69/70	0.01 => 17	0 => 28
Δ144	0.1 => 6.6	0 => 22
Δ243/244	0.02 => 0.01	0 => 4.8
K417N	0 => 0.3	0 => 4.9
K417T	0 => 0	0 => 4.4
E484K	0.01 => 0.6	0.6 => 13
N501Y	0.03 => 16	0 => 32
P681H	0.02 => 16	1.8 => 27
Additional emerging RBD variants		
N439K	0.4 => 2.3	0 => 4.7
L452R	0 => 0.9	0 => 4.7
Y453F	0.1 => 0.3	0 => 0
S477N	0.4 => 5.2	9.2 => 2.5
T478K	0.01 => 0.1	0 => 0.6
V483A	0.04 => 0.01	0 => 0
F490S	0.01 => 0.03	0 => 0
S494P	0.02 => 0.2	0 => 0.9

FIGURE 4

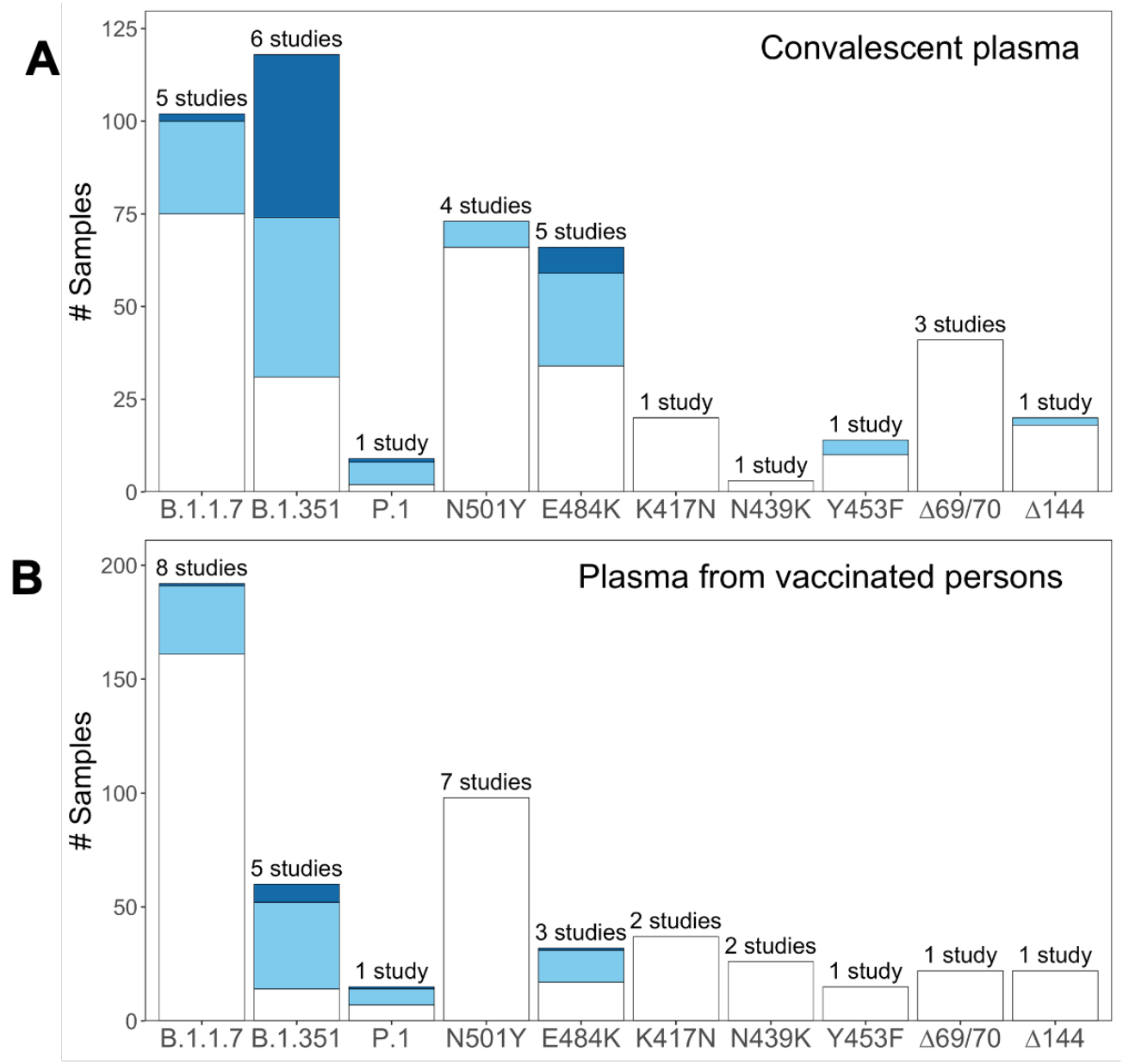

A
Convalescent plasma
Samples
125
100
75
50
25
0
5 studies
6 studies
1 study
4 studies
5 studies
1 study
1 study
1 study
3 studies
1 study
B.1.1.7
B.1.351
P.1
N501Y
E484K
K417N
N439K
Y453F
Δ69/70
Δ144
B
Plasma from vaccinated persons
Samples
200
150
100
50
0
8 studies
5 studies
1 study
7 studies
3 studies
2 studies
2 studies
1 study
1 study
1 study
B.1.1.7
B.1.351
P.1
N501Y
E484K
K417N
N439K
Y453F
Δ69/70
Δ144

FIGURE 5

Supplementary tables

Supplementary Table 1: SARS-CoV-2 Spike Variants with a Global Prevalence Above 0.1% (February 2021)

S1 NTD				S1 RBD				S1 CTD				S2			
Pos	**Ref**	**Mut**	**Pcnt**	**Pos**	**Ref**	**Mut**	**Pcnt**	**Pos**	**Ref**	**Mut**	**Pcnt**	**Pos**	**Ref**	**Mut**	**Pcnt**
222	A	V	23	501	N	Y	11	614	D	G	95	716	T	I	11
69	H	-	13	477	S	N	5.30	570	A	D	11	982	S	A	11
144	Y	-	11	439	N	K	2.40	681	P	H	11	1118	D	H	11
18	L	F	10	452	L	R	0.50	677	Q	H	0.78	1163	D	Y	0.53
5	L	F	1.60	484	E	K	0.37	583	E	D	0.65	1167	G	V	0.45
98	S	F	1.50	453	Y	F	0.31	675	Q	H	0.54	701	A	V	0.34
262	A	S	1.10	417	K	N	0.21	655	H	Y	0.32	1176	V	F	0.34
272	P	L	0.81	520	A	S	0.18	626	A	S	0.24	688	A	V	0.33
153	M	T	0.76	494	S	P	0.16	622	V	F	0.19	936	D	Y	0.32
80	D	Y	0.50	501	N	T	0.11	613	Q	H	0.18	1073	K	N	0.26
13	S	I	0.40					673	S	T	0.15	939	S	F	0.25
21	R	I	0.39					572	T	I	0.13	769	G	V	0.23
152	W	C	0.37					640	S	F	0.13	1078	A	S	0.23
54	L	F	0.36					677	Q	P	0.12	1263	P	L	0.23
176	L	F	0.32					679	N	K	0.12	1219	G	C	0.22
143	V	F	0.27					574	D	Y	0.10	723	T	I	0.21
215	D	H	0.27					675	Q	R	0.10	1020	A	S	0.21
49	H	Y	0.26					681	P	R	0.10	732	T	A	0.20
253	D	G	0.26									1228	V	L	0.17
256	S	L	0.25									859	T	I	0.16
95	T	I	0.24									1252	S	F	0.15
26	P	S	0.23									772	V	I	0.14
138	D	Y	0.23									812	P	S	0.14
80	D	A	0.21									812	P	L	0.14
241	L	-	0.21									845	A	S	0.14
181	G	V	0.20									879	A	S	0.13
215	D	G	0.20									1073	K	T	0.12
255	S	F	0.20									1084	D	Y	0.12
164	N	T	0.19									780	E	Q	0.11
23	Q	H	0.18									929	S	T	0.11
12	S	F	0.15									957	Q	L	0.11
29	T	I	0.15									1122	V	L	0.11
153	M	I	0.15									1191	K	N	0.11
20	T	I	0.14									1219	G	V	0.11

189	L	F	0.14			839	D	Y	0.10
146	H	Y	0.13			1104	V	L	0.10
210	I	-	0.13			1117	T	I	0.10
254	S	F	0.13			1162	P	S	0.10
75	G	R	0.12			1229	M	I	0.10
138	D	H	0.12						
26	P	L	0.11						
245	H	Y	0.11						
22	T	I	0.10						
27	A	S	0.10						
67	A	V	0.10						
233	I	V	0.10						
261	G	V	0.10						

Footnote: NTD – N-terminal domain; RBD – receptor binding domain; CTD – C terminal domain; Mutation prevalences obtained February 2021 from COVID CG

Supplementary Table 2. Neutralizing Monoclonal Antibodies (mAbs) with Published High-Resolution Structures[1]

Name (Synonyms)	PDB Code	Epitope – Class[3]	IC_{50} (ng/ml) (wildtype)[4]	Reference
mAbs in phase 3 trials[2]				
Casirivimab (CAS; REGN-10933)	6XDG	RBM-1	8	[1,2]
Regdanvimab (CT-P59)	7CM4	RBM-1	8	[3]
Tixagevimab (TIX; COV2-2196/AZD8895)	7L7D	RBM-1	15	[4,5]
Etesevimab (ETE; CB6; JS016; LY-CoV16)	7C01	RBM-1	835	[6]
Bamlanivimab (BAM; LY-CoV555)	7KMG	RBM-2	10	[7]
Cilgavimab (CIL; COV2-2130; AZD1061)	7L7E	RBM-2	105	[4,5]
Imdevimab (IMD; REGN10987)	6XDG	RBD-core-1	6	[1,2]
Sotrovimab (S309; VIR-7831; GSK4182136)	6WPS	RBD-core-1	80	[8]
mAbs tested against ≥1 mutant pseudovirus				
BD-629	7CH5	RBM-1	4	[9,10]
CC12.1	6XC2	RBM-1	46	[11,12]
B38	7BZ5	RBM-1	180	[13]
P2C-1F11	7CDI	RBM-1	280	[14]
CV30	6XE1	RBM-1	118	[15,16]
COVA2-39	7JMP	RBM-1	54	[17,18]
BD-368-2	7CHH	RBM-2	15	[9,10]
P2B-2F6	7BWJ	RBM-2	410	[14]
C121	7K8X	RBM-2	2_{PV}	[19,20]
C119	7K8W	RBM-2	-	[19,20]
C144	7K90	RBM-3	3_{PV}	[19,20]
2-4	6XEY	RBM-3	-	[21]
S2M11	7K43	RBM-3	1	[22]
C110	7K85	RBD-core-1	-	[19,20]
C135	7K8Z	RBD-core-1	3_{PV}	[19,20]
COVA1-16	7JMW	RBD-core-2	745	[17,18]
H014	7CAH	RBD-core-2	570	[23]
4A8	7C2L	NTD	610	[24]
4-8	-	NTD	-	[21]
COVA2-04	7JMO	RBM-1	2500	[17,18]
DH1047	7LD1	RBD-core-2	-	[25]
S2E12	7K4N	RBM-1	4.2	[22]

S2H14	7JX3	RBM-1	900_{PV}	[26]
S2X35	7JXE	RBD-core-2	NA	[26]

Footnote: [1]mAbs in phase 3 clinical trials and mAbs that have undergone high-resolution structural studies (XR crystallography or cryo-EM) and been studied for their neutralizing activity against variants with one or more spike mutations. [2]Some of the mAbs in clinical trials have undergone Fc modifications such as changes to result in prolonged half-lives. The following mAbs are being studied only in combination – casirivimab/imdevimab and cilgavimab/tixagevimab. Bamlanivimab has been studied alone and in combination with etesevimab. The FDA has granted emergency use authorization (EUA) for the use bamlanivimab, bamlanivimab/etesevimab, and casirivimab/imdevimab. [3]The classification is derived from Finkelstein [27] which is slightly more granular than Barnes [28]; RBD – receptor binding domain, RBM – receptor binding motif, NTD – N-terminal domain. [4]IC_{50}s were obtained using infectious wildtype viruses (WT) defined as the Wuhan-Hu-1 reference sequence (GenBank accession: NC_945512.2). Results obtained using just pseudotyped viruses (PV) are indicated with a subscript. A dash indicates that susceptibility data was not available.

Supplementary Table 3. Experimentally confirmed cytotoxic T cell epitopes identified in more than one study

Peptide	Positions	Region	HLA	Notes	Mutations (≥0.1%)	Reference
YLQPRTFLL	269-277	NTD	A*02:01	Multiple patients	P272L (0.8%)	[30]
YLQPRTFLL	269-277	NTD	A*02:01	Multiple patients		[31]
YLQPRTFLL	269-277	NTD	A*02:01	NA		[32]
YLQPRTFLL	269-277	NTD	A*02:01	Multiple patients		[33]
YLQPRTFLL	269-277	NTD	A*02:01	Vaccinated persons		[34]
NYNYLYRLF	448-456	RBD	A*24:02	Multiple patients	L452R (0.5%); Y453F (0.3%)	[33]
NYNYLYRLF	448-456	RBD	A*24:02	Vaccinated persons		[34]
RLQSLQTYV	1000-1008	S2	A*02:01	Vaccinated persons	None	[34]
RLQSLQTYV	1000-1008	S2	A*02:01	NA		[32]
VVFLHVTYV	1060-1068	S2	A*02:01	NA	None	[35]
VVFLHVTYV	1060-1068	S2	A*02:01	NA		[32]
QYIKWPWYI	1208-1216	S2	A*24:02	Multiple patients	None	[30]
QYIKWPWYI	1208-1216	S2	A*24:02	Vaccinated persons		[34]
QYIKWPWYI	1208-1216	S2	A*24:02	Multiple patients		[33]
FIAGLIAIV	1220-1229	S2	A*02:01	NA	V1228L (0.2%)	[35]
FIAGLIAIV	1220-1228	S2	A*02:01	NA		[32]

Footnote: FVFLVLLPL (Positions 2-10) was found in one study [35] to be an HLA*02 CTL epitope: L5F is a recurrent mutation that has been hypothesized to possibly represent a CTL escape mutation [36].

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