BioDrugs

Supplemental section:

Passive immunotherapy against SARS-COV-2: From plasma-based therapy to single potent antibodies in the race to stay ahead of the variants

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S1. Coronaviruses

S1.1. General characteristics of coronaviruses

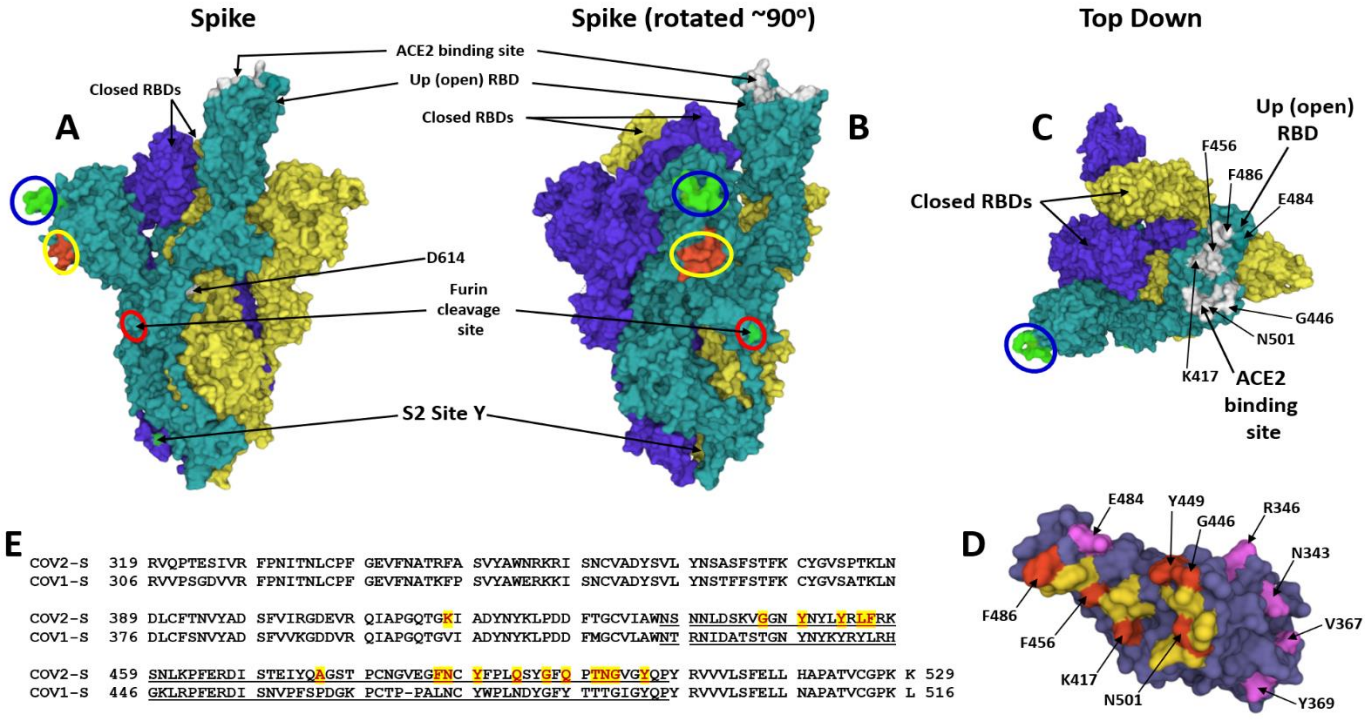
Coronaviruses (CoVs) are enveloped, spherical viruses with a diameter in the range of 100-160 nm [1], enclosing genomes of positive-sense, single stranded RNA ranging from 26-to-32 kilobases (kb), the largest genomes amongst the RNA viruses [2,3]. Currently known CoVs fall into four different genera: Alphacoronaviruses (α-CoV), Betacoronaviruses (β-CoV), Gammacoronaviruses (γ-CoV), and Deltacoronaviruses (δ-CoV), based on serological and genomic differences [1,3]. Additionally, the α-CoV group can be subdivided into two smaller groupings (a, b), and the β-CoV genus is further subdivided into four lineages known as A, B, C, and D [1].

Overall, there are approximately 30 different CoVs capable of infecting mammals and fowl. Thus far, humans have only been infected with CoVs from the α-CoV and β-CoV families. To date, seven CoVs are known to infect humans, including SARS-CoV-1 which caused an epidemic in 2002-2003 [2], Middle East respiratory syndrome (MERS-CoV) which caused an epidemic in 2014-2015 [2,4], and SARS-CoV-2 (also known as 2019-nCoV), the causative agent of COVID-19, starting in late 2019 [5-8], all of which are capable of inflicting serious and sometimes fatal diseases in humans. MERS-CoV, which utilizes receptor CD26 (also known as dipeptidyl peptidase-4 [DPP4]) for cell entry, is in lineage C of β-CoV. SARS-CoV-1 and SARS-CoV-2, which are in lineage B of β-CoV, use angiotensin converting enzyme-2 (ACE-2) as the target receptor for cell entry [9].

Besides MERS-CoV, SARS-CoV-1, and SARS-CoV-2, there are four additional human-infecting CoVs, including the β-CoVs, HKU1 and OC43, and the α-CoVs, NL63 and 229E, which are endemic, mostly less virulent CoVs that cause milder, cold-like symptoms. These endemic CoVs, which account for up to ~30% of human respiratory tract infections, can occasionally progress to more severe respiratory diseases, especially in immunocompromised and elderly patients [10]. It is known that HCoV-OC43, BCoV (presumed to be the zoonotic ancestor of HCoV-OC43), and HCoV-HKU1 specifically target 9-*O*-acetylated-sialic acid-capped sialoglycans to infect target cells [10].

S1.2. SARS-CoV-2 structure and surface targets

The CoV viral envelope is comprised of a phospholipid bilayer into which three key structural proteins are embedded, the envelope (E) protein, a type III transmembrane glycoprotein known as the "membrane" protein (M), and the trimeric spike protein (S), which recognizes ACE2 [9]. Some CoVs also possess a hemagglutinin-esterase (HE) protein as well [11]. Since the spike protein is the target of virtually all anti-SARS-CoV-2 therapeutic antibody programs, our focus will be on the spike protein and its sub-domains (Figure S1).



Supplemental Figure S1. ***A and B****. Two views of the SARS-CoV-2 spike protein. The epitope for NTD-antagonist antibodies such as 4A8 is shown by the blue circle, and the epitope for NTD agonist antibodies is shown by the yellow circle. The red circle identifies the furin cleavage site area.* ***C.*** *Top-down view of the SARS-CoV-2 spike protein showing two receptor binding domains in the down, or closed, conformation, and one RBD in the up, or open, conformation, the latter conformation required for ACE2 binding. The ACE2 binding motif, consisting of 17 amino acid residues, is highlighted in yellow on the single "open" ("up") RBD. Protein Data Bank (PDB) ID 7DX5 and PDB program [12] were used to generate drawings A-C as per Sehnal et al. [13].* ***D.*** *An enlarged figure of SARS-CoV-2 RBD (PDB ID 7CH5)*

showing the ACE2-binding motif and critical amino acids which are involved in binding and which can be mutated in variants to improve transmissibility and potentially evade antibodies. Yellow and red, ACE2 binding site and specific named residues in ACE2 binding site, respectively; light purple, key non-ACE2 binding residues in the receptor binding domain (RBD) core. E. The sequences of SARS-CoV-1 and SARS-CoV-2 RBDs; the RBM sequences are underlined and the 17 residues in the SARS-CoV-2 sequence contacted by human ACE2 are in red/yellow.

The SARS-CoV-2 spike (S) protein, which mediates targeting (and thus host range and species tropism) and cell entry, is a trimer with each ca., 142 kDa monomer comprised of >1100 amino acids [14,15] (Figure S1A, S1B). The S protein has two large subunits, a globular S1 subunit domain at the N-terminus, which contains the receptor binding domain (RBD) and is primarily involved in targeting the receptor, and a membrane-proximal S2 subunit, which possess two heptad repeats (HR1 and HR2) and the transmembrane domain and mediates fusion with the host cell membrane to enable cell entry [2]. The SARS-CoV-2 spike protein has about a 76% sequence identity and a ca. 87% similarity with the SARS-CoV-1 spike protein [16].

The three SARS-CoV-2 RBDs in the trimeric spike protein transition through RBD-closed, or "down" and open, or "up" conformations (see Figure S1) [17]. For an RBD to bind ACE2, it must be in the open or "up" position to make the ACE2 binding site available. Within the RBD is a receptor binding motif (RBM; underlined sequences in Figure S1E), a ca. 70-amino acid residue linear sequence which forms the face directly contacted by the human ACE2 receptor. Based on co-crystal structure data, Shang et al. [9] defined the RBM as residues N437 to P507 (CoV-2 sequence; Figure 2E). When SARS-CoV-2 RBD/RBM binds ACE2, it buries 863Å^2 on the surface of ACE2 and 864Å^2 on the RBD [18] with 17 RBD residues making direct contact with ACE2 [18], all of which are within the RBM except K417 (Figure S1E). From the co-crystal structure of SARS-CoV-2 RBD:HuACE2, the key specific contact residues are found in three subclusters at the interface (SARS-CoV-2 RBD [bold]/ACE2 [italics]) [19]: Cluster 1, also called "the Knob" by Deshpande et al. [20] and "the Mesa" by Hastie et al. [21]): **Q498**/*Q42*; **T500**/*R357*; **N501**/*K353,Y41,R357*; Cluster 2 (called "the Base" by Deshpande et al. [20] and "the Valley" by Hastie et al. [21]): **Y453**/*H34*; **K417**/*D30*; Cluster 3 (called "the Tip" by Deshpande et al. [20] and "the Peak" by Hastie et al. [21]): **Q474**/*Q24*; **F486**/*M82*. Of those 17 residues, only eight are in common with SARS-CoV-1 [18]. These differences result in SARS-CoV-2 binding to human ACE2 with an affinity reported to

be 14.7 nM K_D [15] or 44 nM [9], values which are ca. 4-to-12-fold higher than the affinity of SARS-CoV-1 to ACE2 (ca. 185 nM K_D; [9,14]). This is one of the critical differences between the two viruses and probably plays into the increased ability of SARS-CoV-2 to be transmitted more avidly from person to person.

Another major difference between SARS-CoV-1 and SARS-CoV-2 is the intriguing insertion of a polycationic stretch of amino acids ("PRRAR") at position 681 of the SARS-CoV-2 spike protein, which is located at the junction of the S1 and S2 domains of the spike protein [22] (see Figure S1A). This polycationic sequence of "PRRAR" provides a readily available furin (and other host proteases) cleavage site, which helps promote the binding of the spike protein to the receptor [22,23]. Viral spike proteins change in conformation when bound to their receptor, exposing a proteolytic priming site which, when cleaved, helps the spike protein to adopt a membrane fusion conformation [24]. It has previously been noted that SARS-CoV-1 does not have a readily recognizable furin cleavage site, and was, *in vitro*, relatively resistant to either receptor- or trypsin cleavage-induced conformational changes [14]. Additionally, it has been shown that deletion of the furin cleavage site attenuates the pathogenesis of SARS-CoV-2 [23]. Hoffmann et al. [24] demonstrated, however, that both SARS-CoV-1 and SARS-CoV-2 are processed into fusogenic forms by the serine protease TMPRSS2, at two sites in a process that could be inhibited using a protease inhibitor indicating it's specificity. Additionally, they concluded that the polycationic site present in the SARS-CoV-2 spike protein would make that sequence more labile to proteolytic activity, allowing it to be processed more efficiently, and likely resulting in more efficient S2-domain-driven fusion to the cell membrane [24]. Interestingly, the "PRRAR" furin site sequence present in SARS-CoV-2 has not been found in very few other SARS-related CoVs, including SARS-CoV-1 and those CoVs identified from bats and pangolins [26] or other potential non-human reservoirs [22]; to our knowledge, other than SARS-CoV-2, the only other SARS-CoV to possess a furin site is HCoV-HKU1, which was first found in 2004 in China [27]. Thus, both the ability to bind to the receptor (improved affinity) and the ability to enter the host cells via membrane fusion mechanics (protease processing sites) appear to be significantly improved by amino acid sequence changes in the spike protein in SARS-CoV-2 over SARS-CoV-1.

S2. Evolution of SARS-CoV-2

S2.1. Coronavirus evolution

All viruses evolve and create variants with one or more mutations. Most mutations in SARS-CoV-2 virus will be either neutral or negative to the virus infectivity. However, a small number of mutations have exhibited a strong 'positive' functional effect resulting in more infective and transmissible viruses. Typically, over time, viruses mutate to increase infectivity, irrespective of any increase or decrease in virulence and pathogenicity [28]. These trends lead to more infection, distribution and potentially endemic disease that becomes embedded in a population [29]. It appears that two factors are driving overall evolution of SARS-CoV-2: (i) the mutation rate in the spike protein is significantly higher than expected by mutational models and is higher than in the rest of the virus [30,31], and (ii) there has been a strong natural selection for increased transmissibility of SARS-CoV-2 and antibody resistance, as exemplified by the rapid worldwide spread of the Delta and Omicron variants [32-34]. Interestingly, while many consider that SARS-CoV-2 has evolved to become less virulent, Bhattacharyya et al. [28] argue that the substantially increased transmissibility, i.e., more than 10-fold from ancestral virus to Omicron [34], actually result in inherent greater overall disease burden and severity because so many additional individuals are exposed to infection who may otherwise not been infected.

SARS-CoV-2 is a positive-sense, single-stranded RNA virus, which typically undergoes higher mutation rates compared to double-stranded DNA viruses. While SARS-CoV-2 has a relatively high mutations rate, it was calculated to be only about 10% as high as HIV. Nevertheless, within its genome, the spike protein (S), which is largely responsible for tropism and transmissibility, has a higher mutation rate than the other proteins [31]. SARS-CoV-2 has a large genome of approximately 30,000 bases. Given the large genome and the relatively higher mutation rate of a single stranded RNA virus, there is an apparent requirement for an error correcting function in SARS-CoV-2. Indeed, coronaviruses have proof-reading machinery which reduces the rate of mutation per cycle of replication, so SARS-CoV-2 changes more slowly than HIV or influenza viruses [35]. It is thought that because of the large coronavirus family genomes, the proof-reading mechanism is required to reduce what otherwise would be too many mutations during each cycle, potentially rendering the virus non-pathogenic.

S2.2. Natural evolution from SARS-CoV-1 to SARS-CoV-2

SARS-CoV-2 is relatively similar to the first SARS-CoV-1 virus detected in Guangdong, in southern China in 2002 [36]. The earlier β-coronavirus also caused severe viral pneumonia with 8000 human infections and 774 deaths (9.6% mortality rate) [37]. SARS-CoV-1 circulated in bats as a natural reservoir [38], however the masked palm civet acted as an intermediate host, which in turn transferred the virus to the human population [39]. SARS-CoV-1 has also been detected in various other mammals, including camels, bats, masked palm civets, mice, dogs and cats. Similarly, a related coronavirus known as HKU2 was shown to have caused an outbreak of fatal diarrhea in pigs in 2018 [40].

Numerous SARS-Like viruses (bat-SL-CoV) have been isolated from bats in provinces of China [41]. Two of these SARS-Like viruses, SL-CoV ZXC21 and SL-CoV ZC45, were isolated in Zhoushan, in Zhejiang province in 2015 and 2017, respectively. These viruses are 97% identical to each other, and 81% identical to the SARS-CoV-1 strain isolated from palm civets [41]. Interestingly, these bat-SL-CoV sequences would be the closer homologs of the Wuhan 2019 isolates as they share 90% sequence identity with SARS-CoV-2 [42]. Genome-wide, SARS-CoV-2 shares 79.5% sequence homology with the genome of SARS-CoV-1 [43].

SARS-CoV-2 originated in wild animals, likely starting in horseshoe bats (*Rhinolophus affinis*) [44]. The closest homologs of SARS-CoV-2 are RaTG13, which was isolated from a horseshoe bat in Yunnan province in 2013 [42], and BANAL-52, which was recently isolated in Laos and described [45]. RaTG13 and BANAL-52 share 96.1% and 96.8% sequence identity, respectively, with SARS-CoV-2 [42,45,46]. Significantly, neither RaTG13 [47] nor BANAL-52 [45,46] possess the furin cleavage site in their spike proteins that is present in SARS-CoV-2 spike protein.

When the evolutionary history of SARS-CoV-2 was compared with that of related viruses, including bat-SL-SARS (RaTG13), MERS and pangolin viruses isolated from Guangxi and Guangdong in 2017 and 2019, respectively [48], it appears that SARS-CoV-2 may have diverged from the bat-SL-SARS (RaTG13) decades ago, possibly as early as 1969 [49] and that the pangolin may serve as an intermediate species.

Beyond mutational changes, CoVs are highly recombinogenic, such that large sections of the virus genome can be homologously recombined to create new clades of chimeric virus [49,50]. These large

regions of genomic recombination may have facilitated the origin of SARS-CoV-2, while the point mutations observed during the human pandemic have further optimized the virus for more efficient replication in our species.

S2.3. Occurrence of SARS-CoV-2 in humans

The earliest report of patients with novel coronavirus were described in Wuhan, in the Hubei province of China in late December [5]. This novel coronavirus (first termed 2019-nCoV), became the seventh known coronavirus that infect humans, which includes four viruses associated with common colds (229E, OC43, NL63 and HKU1) plus two zoonotic origin strains having severe pathology and sometimes fatal illness (SARS-CoV-1 and MERS-CoV). While a cluster of SARS-CoV-2 infections occurred at the Wuhan Seafood Market, where ten genomes with 99.98% identity were sequenced from nine patients identified with viral pneumonia in Dec 2019 and Jan 2020, indicating a very recent outbreak with minimal time for variation of the virus [11], there is still uncertainty as to the exact source of the index case [51]. Based on sequencing of viral isolates and use of molecular clock phylogenetic analyses, it is estimated that the first case of SARS-CoV-2 occurred in the Hubei province between mid-October and mid-November 2019 [7]. The first known person to person transmission in the United States was documented in Illinois in January 2020 [52].

There are two critical factors in describing the virulence of SARS-CoV-2. The first is transmissibility, defined by the term R_0 ("R naught"), which stands for "reproductive number", a mathematical indicator of how contagious an infectious disease is. It essentially describes the number of unvaccinated people who will be infected by each infectious individual. For example, seasonal influenza has an R_0 of 1-2, meaning that each infected person will likely infect one to two other non-vaccinated people. On the other end of the spectrum, the most infectious disease, measles, has an R_0 of 16-18. The R_0 for ancestral SARS-CoV-2 is about 2.5 [32] with estimated ranges of 2.2 to 3.9, about two-fold higher than seasonal influenza. The most recent variant, Omicron, has been calculated to have an R_0 in the range of 7-to-14 [53].

T-index has been developed as an alternative, computational approach to calculating relative transmissibility of SARS-CoV-2 variants [53]. The T-index is based on modeling of structural information that takes into consideration the ability of SARS-CoV-2 to bind ACE2-expressing cells, using a

combination of kinetics and affinity parameters based on the structures of RBD, NTD, cell membrane lipid rafts, and ACE-2 [54,55]. As such, this is a molecular approach to compare transmissibility rather than R_0, which is more of a real-world, population-based approach. In any case, the T-index for SARS-CoV-2 Wuhan B.1, Alpha, Beta, and Delta, and Omicron are 2.16, 3.59, 3.82, 10.67, and 3.90, respectively, with the higher numbers indicating enhanced transmissibility [49,51,55], which roughly correlates with the R_0 data on those variants. Omicon BA.1, however, has been demonstrated to be 2.8-fold more transmissible than Delta [32,56], and Omicron BA.2 is currently thought to have a significantly higher transmissibility rate than BA.1, a circumstance that allowed BA.2 to overtake BA.1 in Denmark and quickly become the dominant variant in late December, 2021, to early January, 2022 [57]. Moreover, Omicron has been calculated to be more than 14-fold more likely to be resistant to antibodies raised by a standard two-dose vaccine [32].

The second factor is infection fatality rate (IFR) which tracks the number of infected individuals who die from the disease. The IFR for COVID-19 is much lower than for previous CoV-related diseases such as MERS and SARS [58], and has been estimated in multiple studies to be about 0.75-1.69 (percent of infected individuals who will die) [59]. Weighted by age, however, the IFR for individuals over 80 years of age who become infected was calculated in four studies to be 7.27-15.61 [56]. For younger patients, those numbers declined almost exponentially by age decade. Of significance, recent analyses have demonstrated that the Delta variant, which will be described in more detail below, has an increased virulence over Alpha [60]; as compared with the ancestral virus, Delta was associated with a 108% increase in risk for hospitalization, a 234% increase in risk for intensive care unit (ICU) admission and a 132% increase in risk for death [60]. Thus, R_0, IFR, and increased virulence leading to greater morbidity and risk of hospitalization accentuate the need for prophylactic and therapeutic antibodies that can treat the Delta variant, the Omicron variants, and other potential variants yet to come.

S2.4. Evolution of SARS-CoV-2 in humans: Site specific mutants and Variants of Interest

Both individual mutations and variants with multiple mutations have raised significant concerns for many reasons, including transmissibility, increased virulence, increased mortality rates, resistance to vaccine-induced antibodies, and resistance to therapeutic and prophylactic antibodies. The top ten mutants observed in RBDs were identified from the GISAID database [61] by Deshpande et al. [20], in descending order from 1-10, with frequency of observation in parentheses, as: S477N (2.55%), N501Y

(0.208%), E484K (0.079%), Y453F (0.062%), F486L (0.059%), G446V (0.027%), K417N (0.024%), A475S (0.019%), L455F (0.015%) and G476S (0.012%). While there are many mutations in the various SARS-CoV-2 variants, there are about a half-dozen that rise to the top in terms of their importance to transmission and resistance to vaccine-induced antibodies and therapeutic antibodies. Moreover, as recently modelled by Wang et al. [62], specific co-mutation groupings may confer on the virus a significant transmissibility advantage as well as the ability to escape current vaccines; these include: (i) A411S, L452R, T478K; (ii) L452R, T478K, N501Y; V401L, L452R, T478K; (iii) L452R, T478K, E484K, N501Y; and (iv) P384L, K417N, E484K, N501Y. Note that no current variant, including Omicron BA.1 and BA.2, contain any of these exact combinations, suggesting that further evolution toward greater infectivity and vaccine escape is certainly possible. The mutations D614G, N501Y, E484K/Q, L452R/Q, S477N, T478K, K417N/T, and P681R/H, all of which are significant in both current and potential future variants, will be described in more detail below.

S2.4.1. Specific Mutations of Interest

S2.4.1.1. D614G

Very early in the human history of SARS-CoV-2, and prior to designations being provided to describe variants, SARS-CoV-2 picked up a mutation in the S1 domain of the spike protein in which an aspartic acid at position 614 was replaced with glycine (D614G). Amino acid residue D614 is located in subdomain SD2 of S1 and forms a hydrogen bond with T859 in S2 to stabilize the S1–S2 interface [63]. With the D614G mutation, the S1-S2 interface is destabilized, essentially loosening the S1 domains and favoring an RBD open conformation state, resulting in improved binding to ACE2 and increased infectivity [64,65]. Moreover, the D614G mutant was demonstrated to significantly reduce S1 shedding after the first proteolytic processing step, resulting in more stable and functional S protein for binding ACE2. This mutant function was also shown to contribute to the increase in viral infectivity [66]. These improved entry-related functions make up for the ca. three-fold reduction in affinity of the D614G mutant to human ACE2 [67].

The D614G mutation occurred early in the pandemic (February 2020), emerging in eastern China and spreading rapidly throughout the world, displacing within just a few months most SARS-CoV-2 viruses that did not contain that mutation [66,68]. As such, nearly all of the current Variants of Concern (VOCs)

and Variants of Interest (VOIs) contain the D614G mutation (Figure S2B). The D614G mutation also is present in 29/33 of the variants listed on Stanford's variants website [69], missing only from variants B.1.6.2.1., A.VOI.V2, A.23, and A.27, three variants of which are from the A lineage. D614G does not increase the SARS-CoV-2 disease severity [70], but it does make the virus more fit and transmissible [68,71], likely due to the improved binding as noted above [65].

S2.4.1.2. N501Y

For reference to specific mutations in the SARS-CoV-2 RBD such as N501Y, Figure S2 shows the partial sequence of SARS-CoV-2 RBD from residues K417 to P507. N501Y was first noted in May, 2020, as a mutation present in the Beta (B.1.351) VOC first observed in South Africa in May 2020, again in September, 2020, when the Alpha (B.1.1.7) was isolated in Kent, England, and then again in the Gamma (P.1) variant first observed in Brazil in November, 2020 (Figure S2). By April 2021, N501Y was found in more than 80% of all sequences SARS-CoV-2 genomes [72], and now N501Y is the second highest SARS-CoV-2 RBD mutation observed in global sequencing efforts. The N501Y mutation, which creates a π-π stacking interaction between RBD 501Y and human ACE2 41Y, results in a 10-fold increase over wild-type in affinity of the RBD for ACE2 ([73,74], the largest increase in affinity for any single VOC/VOI mutation in RBD tested [74,75]. Based on a reverse genetics approach, the N501Y variant was demonstrated to induce a greater rate of transmissibility [72], and has been implicated with a higher mortality rate, albeit more likely from the increased transmissibility than increased IFR [20,72,77]. Interestingly, the N501Y mutation has also arisen independently in three other lineages, Beta (B.1.351), Gamma (P.1) and Omicron (B.1.1.529), further suggesting this to be a convergent mutation with the ability to make the virus more fit [8,72]. As such, N501Y is now considered to be one of the most significant mutations to improve SARS-CoV-2 virus fitness [72]. The N501Y mutant does not appear to have a significant effect on resistance to antibodies induced by vaccination or previous infections [76,77].

Supplemental Figure S2. *Partial sequence of SARS-CoV-2 RBD from residues K417 to P507 showing the wild-type sequence on the top line and the mutants in all of the VOCs and a few other variants. The RBM, which encompasses a linear sequence of residues 437-507 within the spike protein, is indicated. Single letter notations for amino acids are used here. On the top line, the 17 residues that are in direct contact with human ACE2, as demonstrated by co-crystallography [9], are shown in underlined, deep red letters. Underlined-blue residues are those demonstrated by mutational analysis also to be critical for ACE2 binding [78]. The mutated resides in VOCs/VOIs are shown by yellow-tinted red letters; green-tinted letters are for those residues that are only sometimes found in the variants. *Mu also has an RBD mutation at R346K, not shown here. Omicron has additional RBD mutations not shown here: G339D, S371L, S373P, S375F [79].*

Importantly for both natural reservoirs as well as the use of experimental animal models, the ancestral SARS-CoV-2 virus (501N) does not efficiently bind mouse ACE2 and thus cannot infect mice under normal circumstances [80]. Mutants with the N501Y mutation, however, and especially those also harboring K417, E484, Q493, and/or Q498 mutations, are able to bind mouse ACE2 ([80-82]. Furthermore, VOCs containing the N501Y mutation, including Alpha [81], Beta [81,83], and Omicron [82,84,85] all have been demonstrated to infect mice, albeit with varying efficiencies. In fact, one of the three major prevailing hypotheses for the origin of Omicron is that a progenitor to Omicron jumped from humans to mice, mutated heavily while in mice, and then jumped back to humans ([84,86], although considering the relatively attenuated infection of Omicron in mice, Halfmann et al. [85] have disputed that hypothesis.

S2.4.1.3. E484K/Q/A

The E484K mutation has arisen independently in multiple variants, including all four VOCs (Beta, Gamma, Delta, and some Alpha variants), as well as most of the VOI variants (Zeta, Eta, Theta, Kappa and some Iota variants) (Figure S2B). E484 forms a salt bridge with K31 of human ACE2, which is lost in the mutation to K484 [87]. Nevertheless, the E484K mutant alone confers a modest increase of about 1.5-fold in the affinity of RBD to human ACE2 [75]. Variants possessing the E484K mutation have been shown to evade antibodies in convalescent patient plasma [76], and there are reports that variants with the E484K mutation were capable of reinfecting individuals who had been previously infected with wild-type of variant SARS-CoV-2 lacking the E484K mutation, i.e., the E484K mutant evaded the immune system of those individuals [77]. Additionally, it has been demonstrated that bamlanivimab does not neutralize variants containing the E484K mutation (essentially all significant variants) [88], resulting in the removal of bamlanivimab from medical use in all US states and the revoking of its EUA for use as a single therapeutic agent [89]. The E484K mutation also has been demonstrated to contribute significantly to reduction in binding of several other SARS-CoV-2 antibodies as well [90].

In the Delta (B.1.617.2) variant, E484Q mutation is paired with the L452R mutation, resulting in the vernacular (but scientifically, incorrect) name “double mutant”. The E484Q mutation found in Delta disrupts the electrostatic bond between E484 with ACE2 K31 and it may confer some level of resistance to antibodies [91], but little is actually known about whether E484Q by itself has much biological effect. Omicron BA.1 and BA.2 both have ~~has~~ the E484A mutation, which is unique amongst VOIs and VOCs (Figure S2). This mutant also would be expected to disrupt the electrostatic bond between E484 with ACE2 K31.

S2.4.1.4. L452R/Q

The L452R mutant was first detected in two sister lineages (B.1.427 and B.1.429; now Epsilon VOI) in California in July 2020 [77], but was also detected independently in October, 2020, in the Delta and Kappa lineages discovered first in India (Figure 2). The L452R mutation also is found in other variants such as A.27, Kappa VOI, and B.1.617.3 (third variant from India) [69]. The L452 residue interacts with L492 and F490 to form a hydrophobic patch on the surface of RBD, which is eliminated in the L452R mutant, which may increase electrostatic interactions with human ACE2 [91]. The L452R mutation has

an approximate two-fold greater affinity for RBD to ACE2 over wild-type virus in 1:1 stoichiometry experiments [20], which may explain its presence in variants that appear to have enhanced human-human transmissibility. This mutation also has been linked to reductions in binding of antibodies present in sera from convalescent patients, particularly when coupled with the E484K mutation [77,92], and also has been shown to decrease the binding of certain therapeutic antibodies and antibody candidates to the SARS-CoV-2 S-RBD [20,93]. The L452R mutation is considered to be one of the most important RBD mutations contributing to Delta transmissibility and immune evasion [94].

L452Q is a unique mutation thus far only found in the Lambda variant. L452Q was first observed in the Lambda (C.37) VOI first detected in Peru in December, 2020 [95]. In concert with the F490S mutation found in Lambda, L452Q conferred an increase in viral infectivity and resistance to antibodies from vaccinated subjects, where F490S alone did not [96]. The increase in infectivity contributed by L452Q was estimated to be about two-fold over wild-type [97].

S2.4.1.5. S477N

As noted above, S477N is by far the most prevalent mutation observed in the genome sequenced samples of SARS-CoV-2. Interestingly, until the emergence of Omicron, S477N was not associated with any current VOC or VOI (Figure 2), although it was found in two lineages, B.1.160 and B.1.1.317 [98]. S477N confers an approximate 2-fold enhancement in affinity of RBD for ACE2 [66,82], forming a new bond with S19 of ACE2, thereby helping to stabilize the RBD:ACE2 complex [98]. Based on its prevalence in natural sequenced variants and its selection during *in vitro* evolution experiments, S477N should be considered to be a prime candidate for potentially increasing, at least incrementally, transmissibility of COVID-19 in future variants [98]. This predictability has played out, as S477N is one of the mutations found in the new VOC, Omicron [99].

S2.4.1.6. T478K

Amongst the current VOCs, T478K is unique to the Delta (B.1.617.2) and Omicron variants (Figure S2). This mutation, however also has been found in about 80% of sequenced viruses of the minor variant B.1.214.3 [100] as well as a minor variant from Mexico, B.1.1.22, showing that it has arisen independently [91]. Together with L452R, T478K has been suggested to increase the stability of the RBD-ACE2 complex [91].

S2.4.1.7. K417N/T

K417 is a residue in the RBD that is outside of the RBM, but still is an important residue due to the fact that it is one of 17 amino acid residues involved in ACE2 binding (Figure S3). Based on 1:1 interactions, the K417N and K417T mutants have approximately 5-fold and 3-fold decreased affinity, respectively, for human ACE2 [75,76]. This is likely due to the loss in either N417 or T417 of the salt bridge that K417 forms with ACE2 D30 [87]. K417N is found in the Beta lineage and is the extra mutation in Delta-plus (but not found in Delta). K417T is present in the Gamma variant [101] (Figure S2). The loss of affinity by K417N/Y is offset by mutations E484K and N501Y in Beta, Gamma, and Omicron [76,90,99].

S2.4.1.8. P681R/H

P681R is present in the Delta and Kappa variants, while P681H is found in the Alpha, P.3 ("Theta"), Mu and Omicron variants (Figure 2). The P681R/H mutations, which alter the furin cleavage site from PRARR to RRARR or HRARR, respectively, enhance basicity of the furin recognition and cleavage site, which is predicted to enhance the cleavage of full-length spike to S1 and S2, and is thought to contribute to the increase in transmission rate and overall fitness of Delta over Alpha [32,91]. It has become clear, however, that variants possessing the P681R mutation exhibit greater ability to enter cells and spread cell-to-cell, resulting in increased transmissibility and virulence over those possessing wildtype P681 or the P681H mutation [32,102].

S2.4.2. Evolution of SARS-CoV-2 in humans: Variants of Interest (VOIs) and other Variants

The Variants of Concern, the most serious SARS-CoV-2 variants, are described in the main text. The named, or formerly named, VOIs, many of which are no longer circulating in significant numbers, will be described as follows. Additionally, variants under monitoring (VUMs) as well as some additional variants that have recently come under scrutiny will be described.

S2.4.2.1. B.1.525 ("Eta")

B.1.525 was first discovered in UK and Nigeria in December 2020 [103]. This variant has the mutations Q52R, A67V, Δ69-70, and Δ144 in the NTD, E484K in the RBD, D614G, Q677H in the S1/S2 region, and F888L in the S2 domain. The incidence of Eta currently is so low that it does not register even 0.1% in the US [104]. B.1.525 was classified as VOI Eta on March 17, 2021, but has since been declassified as a VOI.

S2.4.2.2. B.1.526 ("Iota")

B.1.526 was first observed in New York in November 2020, and was named a VOI on March 24, 2021 [103]. Iota possesses the following mutations: L5F*, T95I, and D253G in the NTD, either S477N or E484K (but typically not both) in the RBD, and D614G and A701V* in the S2 region. Iota was classified as a VOI on March 24, 2021, but has since been declassified as a VOI. There are currently so few SARS-CoV-2 Iota sequences in the US, that it does not even register nationally [104]. On the other hand, Iota appears to be regionally restricted to the New York area, which reported Iota as the second highest variant behind Alpha during the period of February-April, 2021 [105].

S2.4.2.3. B.1.617.1 ("Kappa")

B.1.617.1 which was first found in India in October 2020, harbors the mutations T95I*, G142D, and E154K in the NTD, L452R and E484Q in the RBD, D614G and P681R in the S1/S2 region, and Q1071H in the S2 domain. The kappa variant has a ca. 20% increased affinity to human ACE2 and demonstrates a greater propensity toward the open position as compared with the ancestral virus [67]. Kappa was shown to be more pathogenic in hamsters than the B.1 variant [102], but whether that translates to humans is still not certain. Kappa was classified as a VOI on April 4, 2021, but has since been declassified as a VOI [103].

S2.4.2.4. C.37 ("Lambda")

The C.37 variant was first discovered in Peru in December 2020 and quickly became the dominant variant in that country [79]. Lambda has a series of unique mutations in its spike protein, including

G75V, T76I, and Δ247-253 in the NTD, L452R and F490S in the RBD, and D614G in the S1/S2 region. The combination of L452Q and F490S, as well as the large deletion (Δ247-253) in the "antigenic supersite" region of the NTD domain, conferred resistance to the sera from vaccinated individuals, prompting some concern that this variant could show some resistance to vaccines and possibly therapeutic antibodies [96,106]. Evidence also exists that Lambda may have a higher transmissibility rate [96,106], but not enough cases have been analyzed to calculate an accurate R_0 for it. It appears that the affinity of Lambda RBD for human ACE2 protein is essentially the same as for ancestral virus [107]. Additionally, in an unusual twist, it appears that due to the G75V-T76I mutations, Lambda is more sensitive to infection-enhancing antibodies directed against the NTD domain [80]. These types of antibodies are discussed later in this paper. The WHO designated Lambda as a VOI on June 14, 2021, while the US-CDC has not yet given it that designation.

S2.4.2.4. B.1.621 ("Mu")

B.1.621, first found in Colombia in January 2021, is the newest VOI, having been named by the WHO on August 30, 2021 [103]; note that the US-CDC has not yet given Mu the VOI designation [104]. The Mu spike protein has the mutations T95I, Y144S, and Y145N in the NTD, R346K, E484K, and N501Y in the RBD, D614G and P681H in the S1/S2 region, and D950H in the S2 domain. These result in a nine-fold increase in affinity to human ACE2 protein over ancestral virus [107]. While it is still too early to quantitatively assess the infectivity rate and pathogenicity of this variant, there are already some concerns that, based on its pattern of spike mutations and its rapid transmission through cities nearing theoretical herd immunity to the wild-type virus, Mu may have some level of resistance to vaccines and antibodies [108]. In autumn, 2021, there was an outbreak of Mu in Florida, so it is clearly capable of generating at least a local focused group of infections.

S2.4.2.4. B.1.427 and B.1.429 ("Epsilon")

The B.1.427 and B.1.429 variants, temporarily but no longer the named the "Epsilon" variant, were first observed in California in March, 2020. Both the B.1.427 and B.1.429 spike proteins have S13I and W152C in its NTD, L452R in its RBD, and D614G in the S1/S2 region. These variants are associated with increased viral load and approximately 20% increased transmission [109]. The two variants were

classified as a VOIs under the name Epsilon on March 5, 2021, but have since been declassified as VOIs due to decreasing prevalence in circulation (June 29, 2021) [103].

S2.4.2.5. P.2 (B.1.1.28.2; "Zeta")

The P.2 variant was first detected in Brazil in April 2020 and was classified as a VOI by the WHO on March 17, 2021 due to its potential reduction in neutralization by antibody treatments and vaccine sera [103,104]. The P.2 spike protein has an E484K mutation in its RBD, F565L* and D614G mutations in S1/S2 region, and a V1176F mutation in the S2 domain. This variant has since been declassified as a VOI, since its incidence has now been reduced to near zero [103,104].

S2.4.2.6. P.3 ("Theta")

The P.3 variant was first detected in the Philippines in February, 2021, but has never taken hold internationally [103]. The P.3 spike protein was originally noted to have the Δ141-143 deletion in its NTD (although more recent descriptions have removed these mutations from the lineage, so they are noted as "variable" in Figure 2B), E484K and N501Y in the RBD, D614G and P681H in the S1/S2 region, and E1092K, H1101Y, and V1176F in the S2 domain. Theta was classified as a VOI on March 24, 2021, but by July 2021, the WHO removed the Greek letter designation due to the vanishing incidence of this variant [103].

S2.4.2.7. Other variants of note

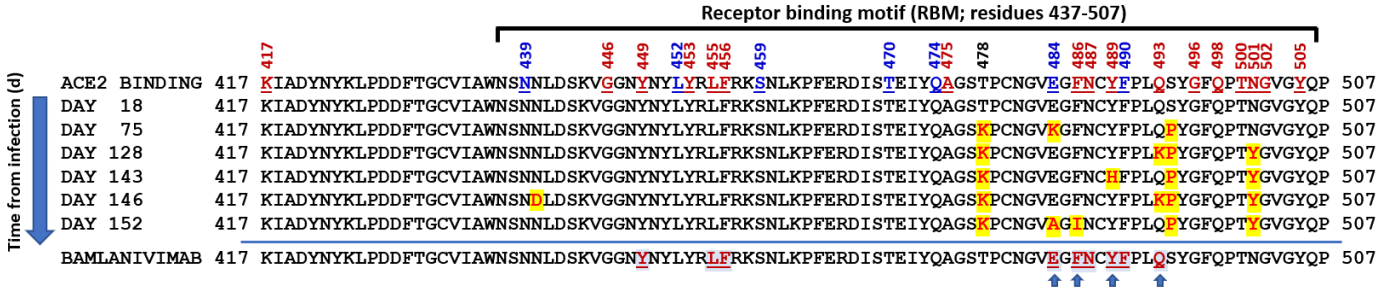
The WHO have a third designation called "variants under monitoring" (VUMs), which are variants that possess mutations that make them potential future threats even though their current status is either unclear or at a low threat level. Currently (February 17, 2022) there are three such VUMs: B.1.1.318, C.1.2, and B.1.640 [103].

Additionally, a few new variants have come onto the radar even though they are not officially recognized. Perhaps the most significant of these is the variant B.1.640.2, dubbed "IHU", because it was identified at the IHU Mediterranee Infection institute in Marseille. This mutant appears to have originated in Cameroon in western Africa, but during November, 2021, it infected several people in southeastern France [110]. While this new IHU variant is not likely to rise to the status of a VOI or VOC,

it does possess key mutations such as N501Y and E484K in RBD, P681H in the furin cleavage site region, and several deletions in the NTD [110].

S2.5. Evolution of SARS-CoV-2 in humans: Evolution of SARS-CoV-2 in an immunocompromised patient

To give an example of how quickly SARS-CoV-2 can evolve, a study was carried out with an immunocompromised patient who developed COVID-19 that became a chronic infection due to the inability of the patient to clear the virus. Figure S4, modified from Choi et al. [111], documents the evolution of the Spike protein receptor binding domain (RBD) sequence of the viruses isolated from a single patient over a 152-day period after initial infection. The epitope for bamlanivimab is also provided in Figure S4, showing that over time, mutations occurred in the patient's SARS-CoV-2 that could have interfered with that therapeutic antibody binding. Additional mutations occurred in other domains of the spike protein as well as in other viral proteins [111]. The take-home message from this study is both the rapidity at which mutations can occur and the potential for mutations that can take a patient from treatable with antibody therapeutics to one who may not be treatable. This rapid mutation within a single patient has recently been dubbed "accelerated evolution" [112].



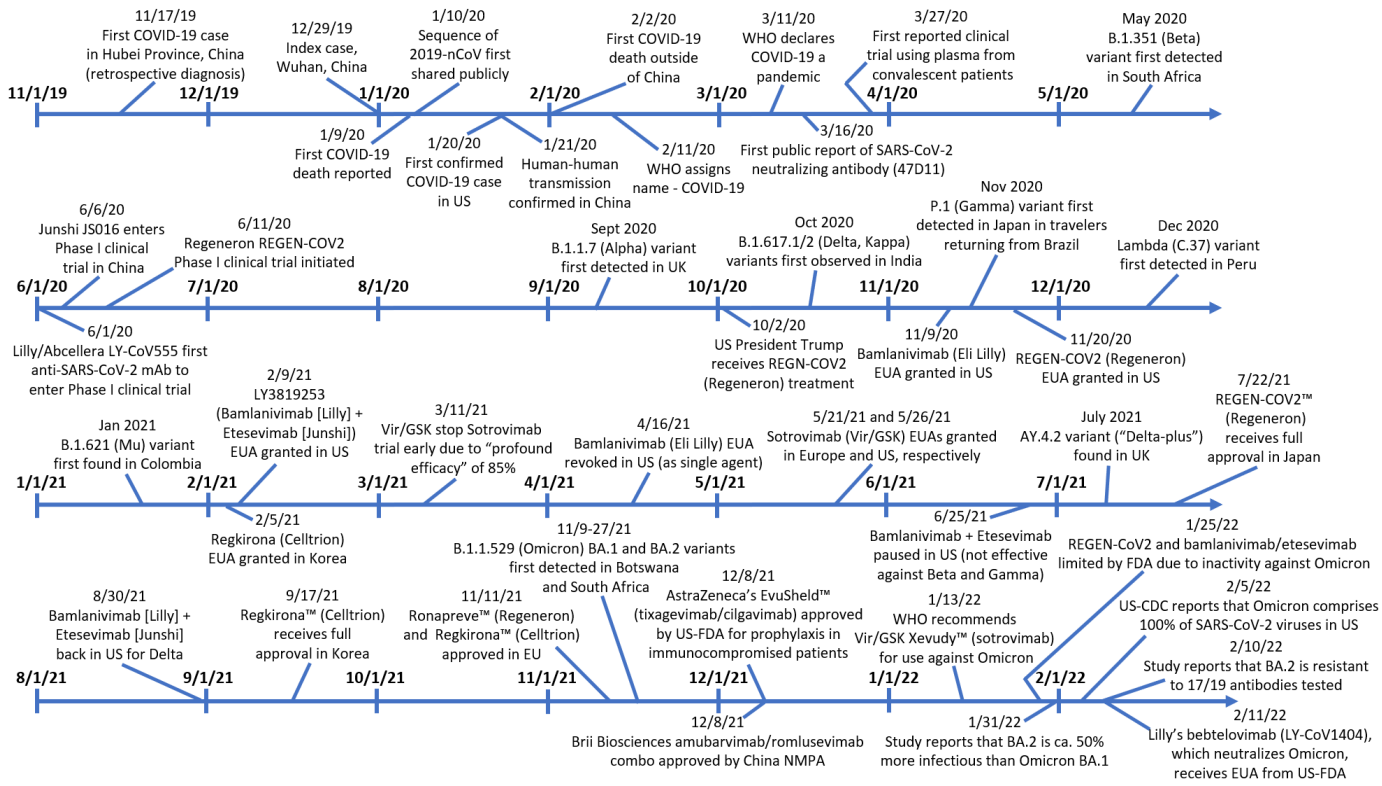
Supplemental Figure S3. *SARS-CoV-2 mutations in an immunocompromised patient over time [111~~18~~]. Sequence of SARS-CoV-2 partial receptor binding domain (RBD) region from residue 417 to 507, which includes the receptor binding motif (RBM). The top sequence shows the 17 residues that are involved directly in binding ACE2 (red underlined) as well as additional residues identified by mutational analysis (blue underlined) to be critical to binding to ACE2. Lines marked by DAY are the sequences of SARS-CoV-2 in an immunocompromised patient from day 18 to day 152 after confirmed infection. The mutated residues are marked by red letters in yellow boxes. The bottom sequence shows the epitope for*

bamlanivimab [113] in red letters, with the arrows pointing out which epitope residues could be affected by mutations arising in the patient's virus.

S3. Brief history of antibodies against SARS-CoV-2

While it appears that the virus that causes COVID-19 was present as early as October, 2019 [7,8], the timelines provided here are based on official notifications that occurred extemporaneously. On December 31, 2019, Chinese authorities notified the World Health Organization (WHO) office in China that a novel coronavirus had caused multiple cases of unusual pneumonia in the city of Wuhan, Hubei Provence, China [5,6] (Figure S4). By January 3rd, China reported that as many as 44 patients had contacted the disease, 11 of which had severe disease; then, on January 9th, the first death, a 61-year-old male, from the disease in Wuhan was reported. The next day after the first known SARS-CoV-2-related death (January 10, 2021), the entire genetic sequence of the novel coronavirus was shared with the WHO, allowing for rapid dissemination of testing and the initiation of development of potential prophylactic and therapeutic treatments.

A high-level timeline of the coronavirus pandemic and the response to generate therapeutic antibodies against it is shown in Figure S4. The first reported case outside of China was on January 13th from Thailand, a woman who had just returned from visiting Wuhan. Over the next week, cases were reported in Japan, South Korea, and the United States, and by the end of January, 2020, more than 20 different countries spanning the entire globe reported the first of what was then known as 2019-nCoV cases. The outbreak was declared an international public health emergency on January 30, 2020, and the disease itself was officially named COVID-19 on February 11, 2020. The WHO declared COVID-19 as a worldwide pandemic on March 11, 2020 (Figure S4~~S5~~), so the disease spread from one cluster of patients in Wuhan to a worldwide pandemic in only 90 days. It is the first time that a coronavirus has led to a pandemic.



Supplemental Figure S4. *Timeline over the past two years for discovery of SARS-CoV-2 and its variants leading to the discovery of antibodies to treat it. These data are from a multitude of sources including press releases from government agencies and companies, as well as from the SARS-CoV-2 tracker continuously updated by Fierce COVID-19 Tracker [114]. Note that while there is some disagreement about the true index case [7], the original index case as originally reported by Chinese researchers [6] is used here.*

Within weeks of the SARS-CoV-2 sequence being available, companies interested in making vaccines and/or antibodies were already designing and expressing constructs based on the released sequences. The historic precedent for developing antibodies as anti-viral drugs had already been established decades ago with the development of Synagis®, which is used as a prophylactic agent against respiratory syncytial virus (RSV) in pre-term infants [115]. In the interim, hundreds of potent neutralizing antibodies have been made against viruses such as HIV [116], MERS-CoV [117,118], SARS-CoV-1 [119,120], dengue virus [121], chikungunya virus [122], Zika virus [123], and influenza virus [124], to name a few. With all of the previous knowledge, especially from MERS and SARS antibody programs, the efforts to generate

anti-SARS-CoV-2 neutralizing therapeutic antibodies started as immediately as it became understood how serious this burgeoning pandemic was becoming. The concept of developing cocktails of multiple antibodies to cover viral variants also had already been set with the use of ZMapp (MB-003), a cocktail of three chimeric antibodies to treat Ebola [125] and, later, the more efficacious fixed dosed cocktail of human antibodies, atoltivimab, maftivimab, and odesivimab-ebgn, from Regeneron [126,127], which was approved by the FDA for treatment of Ebola in October 2020 under the Trade name of Inmazeb®.

The first structures of the SARS-CoV-2 spike and its RBD became available in March 2020 both in the form of models based on cryo-electron microscopy images [15,19,128] and co-crystallography of the SARS-CoV-2 spike protein RBD with its cognate receptor, ACE2 [9,18]. Along with sequence and information on the biology and pathology of SARS-CoV-2, these efforts significantly contributed to the determination of antibody epitopes and the potential for resistant variants, as will be discussed later.

By the end of March, 2020, within two months of the SARS-CoV-2 sequence availability, and only weeks after the WHO's declaration of a world-wide pandemic, at least nine companies or organizations already had reported isolation of neutralizing antibodies against SARS-CoV-2 (Table S1). Several of those have led to approved Emergency Use Applications (EUAs) and late-stage clinical candidates. Amazingly, for multiple programs, from the time that B cells from convalescing patients became available (March 7-8, 2020 for Vanderbilt and Abcellera) [129] to entry into Phase I clinical trials, which included the processes of discovery, cell-line development, IND-enabling toxicology, writing/documentation of the IND and lining up an FPI clinical site, was a record of only about three months [113]. In another equally impressive context, these companies progressed from target sequence availability to the clinical first patient in (FPI) in about six months (Figure S4).

Never before had so many clinical stage therapeutic candidate antibodies been generated so quickly. It is probably fair to say that, along with the improvement in microfluidics and B-cell cloning and sequence retrieval, the COVID-19 pandemic forced academics and companies alike to optimize time for each step of the process, and overlap many processes at risk, in order to meet such incredibly accelerated timelines. Now that this has been done successfully by so many different organizations, it is likely this will change antibody discovery forever.

Supplemental Table S1. Earliest efforts to isolate antibodies targeting SARS-CoV-2[a]

Company	Construct/stage in late March 2020	Platform/ Antibody Source	Date and reference for press release	Clinical trial approximate start date	Current status of lead candidate (February 2022)
Abcellera / Eli Lilly	Pool of 500 MAbs from a screen of over 5 million B cells	Microfluidics coupled with human B cells from convalescent patients	3/12/20 [130]	6/1/20	Bamlanivimab; US-FDA EUA granted 11/9/20; US-FDA EUA granted for combination with JS-016 (etesevimab) 2/9/21
Erasmus MC/ Harbour Biomed	Antibody 47D11 already isolated and tested	H2L2 tg mice	3/16/20 [131]	11/27/20	AbbVie licensed 47D11; Phase I clinical trial completed by 9/2/21; no longer in AbbVie's clinical pipeline, 2/2/22
Regeneron Pharmaceuticals	Pool of hundreds of candidates	VelocImmune™ tg mice and patient B cells from convalescent patients	3/17/20; 3/26/20 [132]	6/11/20	REGEN-COV™ (US), Ronapreve™ (UK); Casirivimab and imdevimab; US-EUA granted 11/20/20; UK and EMA full approval, 8/26/21 and 11/11/21, respectively
Sorrento Therapeutics	STI-4398 cGMP cell line development	COVIDTRAP™ ACE-Fc fusion protein	3/20/21 [134]	NA	COVIDTRAP (STI-4398) no longer in Sorrento's pipeline, 2/2/22
Celltrion	Screening for neutralization	Library of antibodies from human B cells from convalescent patients	3/23/20 [135]	7/18/20	Regkirona™; Korea-EUA 2/5/21; EMA-EUA 3/26/21; Indonesia EUA 7/17/21; Korea approved 9/17/21; EMA approved 11/11/21
Vanderbilt Medical Center	Thousands of "hits"	Rapid identification of B cells from convalescent patients	3/23/20 [136]	8/17/20	EvuSheld™ (AZD-7442, licensed from Vanderbilt); US-FDA EUA 12/8/21 for pre-exposure prophylaxis
Shanghai Junshi Bioscience Ltd / IMCAS	Multiple neutralizing antibodies – both *in vitro* and *in vivo* data	B cells from convalescent patients	3/24/20 [137]	6/5/20	JS016 (etesevimab); US-FDA EUA 2/9/21 in combination with bamlanivimab
Mabpharm Limited / Sorrento	cGMP cell line development	ACE-MAB bispecific protein fusion	3/24/20 [138]	NA	ACE-MAB (STI-4920, CMAB020) no longer in MabPharm or Sorrento's pipelines 2/2/22[b]
Vir Biotechnology, Inc	Lead candidate transferred to CDMO at risk	B cells from convalescent patients after SARS-CoV-1 infection	3/25/20 [139]	8/27/20	Xevudy™ (Sotrovomab); US-FDA EUA 5/26/21; Approved in UK 12/2/21

Brii Biotherapeutics	Multiple candidates in hand	B cells from convalescent patients	3/31/20 [140]	7/12/20 – 7/13/20	Amubarvimab (BRII-196) and romlusevimab (BRII-198) in Phase II/III clinical trials (ACTIV-2); Filed EUA application with US-FDA 10/8/21 based on positive data from Phase III ACTIV-2 clinical trial

Abbreviations: CDMO, Contract Development and Manufacturing Organization; EMA, European Medicines Agency; EUA, Emergency Use Authorization; cGMP, current Good Manufacturing Practices; H2L2, two heavy and two light chains; Mabs, monoclonal antibodies; NA, not applicable; tg, transgenic; UK, United Kingdom; US-FDA, United States Food and Drug Administration.

[a] Based on publicly released information as of 2/2/22.

[b] Likely supplanted in Sorrento's pipeline by STI-2099, an intranasal formulation of STI-2020.

S4. Natural human antibody response to infection and vaccines

In designing potential antibody treatments for a disease like COVID-19, it is important to understand the natural humoral responses by those infected with SARS-CoV-2. A large number of studies have demonstrated the quantitative and qualitative aspects, as well as the kinetics of the humoral response to infection with SARS-CoV-2. Interestingly, the humoral response is clearly not stochastic – in fact, the humoral response was largely focused on certain germlines and several recurring themes that have emerged with other antiviral antibodies.

Antigen-specific humoral immune responses to systemic infection are initiated by primary IgM response, followed by class-switching to memory responses characterized by IgG, IgA and IgE production. The first published study of humoral response to SARS-CoV-2 infection was by Xiao et al. [141], who showed that the serum-based IgM response peaked at three weeks and persisted until the 5- to 6-week range, while the serum IgG response gradually increased from week one after symptoms with a leveling off after four-to-seven weeks in the study. In another study, early virus-specific response to SARS-CoV-2 included, as expected, IgM and increases in IgG and IgA, but perhaps not surprisingly, virus neutralizing IgA dominated the first month of response to SARS-CoV-2, followed by a rapid decline in IgA, giving way to increasing IgG titers [142].

In one study, SARS-CoV-2-specific antibody titers in convalescent patients were quite varied, ranging from as low as <50 (about one-third) to titers in the 50-to-1000 range (46%), and a few with titers >5,000 (1%) [143]. Interestingly, titers alone did not always correlate with protection, as many

convalescent patients generated relatively modest titers of high affinity, neutralizing antibodies [143]. In a separate recent study, significant antibody titers were still present in a large portion of a population of infected individuals nine months after infection with SARS-CoV-2, indicating some level of persistence [144]. In a small portion (15-20%) of infected patients, antibody titers were increased at nine months over that of three months [144]. In a separate study, neutralizing antibodies and antibody-producing memory B cells were found to be relatively stable at both six and 12 months after infection with SARS-CoV-2, but also were significantly improved in those individuals who were subsequently vaccinated after infection [145]. Additionally, over the period of a year post-infection, the pool of non-neutralizing antibodies against SARS-CoV-2 was largely decreased, whereas neutralizing antibodies remained stable and generally increased in affinity over time [145]. Titers against N-protein also were diminished over the period of a year, indicating that the natural immune response became more focused on neutralizing antibodies against RBD [145]. By comparison, it had been determined previously that natural humoral responses against SARS-CoV-1 peaked at about 4-6 months, with 94% and 88-90% of peak response remaining after one and two years, respectively [146].

Liu et al. [147] isolated 252 Ab from five convalescent patients. About half (121/252) of the antibodies targeted S trimer protein but, of those, only 38 were against RBD. Nevertheless, 61 of the antibodies neutralized pseudovirus constructs and 41 neutralized live viruses. Neutralizing affinities can reach sub-nM [147] and, in a separate study, neutralizing IC_{50} values ranged from ca. 3-80 ng/mL [148]. In a separate study, natural human responses to SARS-CoV-2 spike protein were largely represented by antibodies targeting the RBD (ca 65-77% of antibody responses), S2 and S1-C/D domains (ca 4-20%), and epitopes in the N-terminal domain (NTD; ca. 6-20%) of the spike protein [149]. In a separate study, Wec et al. [150] found that 44% of antibodies from convalescent patients targeted NTD in S1, 33% targeted RBD, and 23% targeted S2. Thus, patients produce antibodies against all domains of SARS-CoV-2 spike protein, but only a fraction of which are neutralizing.

Zhou et al. summarized reports from 12 papers on the use of germlines in patients generating neutralizing antibodies after infection with SARS-CoV-2 [148]. They found that, across all of those different studies, the immunoglobulin G heavy-chain variable regions from germlines IGHV1-69, IGHV3-53, and IGHV1-2 were used most frequently to encode SARS-CoV-2 neutralizing antibodies across the patients tested in those studies. Other germlines used frequently include IGHV3-30, IGHV3-66, IGHV3-9, and IGHV1-58, amongst others. In all, 38 different germlines were represented in those analyses [148].

A few germlines have been noted multiple times. For example, in a study of nAbs from six convalescent patients, Robbiani et al. [143] found that the anti-SARS-CoV-2 nAbs were dominated by antibodies of the VH3-30, VH3-53, VH1-69, VH5-51, and VH3-66 families, whereas the overall repertoire was dominated by VH3-23, indicating a significant skewing of the antibody germline families responsible for targeting SARS-CoV-2. In a separate study of multiple convalescent patients, more than 30% of 294 nAbs were of the IGHV3-53 heavy chain (HC) germline, indicating a shared and conserved antibody response to SARS-CoV-2 infection [150,151]. These VH3-53 antibodies possessed high potency in neutralization assays with minimal requirement of affinity maturation [150,151]. The anti-SARS-CoV-2 antibodies possessing IGHV3-53 heavy chain (HC) germline, as well as those possessing the IGHV3-66 and other IGHV3 germlines, tended to have multiple similar properties such as competition with ACE2, similar epitopes and angles of attachment, low rates of somatic mutations, and in many cases long CDR-H3 loops [152,153]. It may be possible that this class of antibodies provides a strong immune pressure on the virus. These studies indicate that selecting a diverse collection of nAbs is critical for mitigating the risk of clinical resistance in developing COVID-19 antibody therapies.

The high frequency of use of the IGVH1-69 antibody germline by infected patients to neutralize SARS-CoV-2 is interesting because this germline usage also has been prevalent in the neutralization of several other viruses [154]. Two hydrophobic residues in the CDR-H2 loop encoded by the IGHV1-69 germline, I53 and F54, have been implicated in binding hydrophobic patches in viral proteins facilitating neutralization of influenza virus [154-156], hepatitis C virus (HCV) [157], and human immunodeficiency virus (HIV) [158]. The anti-SARS-CoV-2 IGVH1-69-derived antibody, 47D1, has an I53/F54 motif in CDR-H2 that interacts with a hydrophobic pocket in SARS-CoV-2 RBD to facilitate neutralization [159]. Additionally, anti-SARS-CoV-2 antibodies ABP-300, bamlanivimab, and S2X259 all are derived from the VH1-69 germline. As stated previously, the high use of IGVH1-69 and demonstration of the CDR-H2 importance in neutralization of SARS-CoV-2 by antibody 47D1 is consistent with the convergence of human immune responses to different viral pathogens [159].

Several studies also have demonstrated that the potency of SARS-CoV-2 neutralizing antibodies did not correlate with CDR-H3 length [159] or the level of somatic hypermutation (SHM) [152,160,161]. Interestingly, some SARS-CoV-2 neutralizing antibodies from convalescing patients were found to have strictly germline VH sequences, formed by the direct joining of the VH and JH chains and containing no somatic hypermutations [152]. Others had as few as a single SHM. These antibody sequences were

found as IgM, IgG1, IgG3, IgG4, and IgA1 isotypes [160-162], indicating that both SARS-CoV-2 neutralizing “natural antibodies” (IgMs; [163]) and class-switched versions could be generated with minimal or no sequence modification. Interestingly, one study uncovered a high preponderance of anti-SARS-CoV-2 IgG3 antibodies in convalescent patients [164]. Potent, near-germline anti-viral antibodies have been reported for anti-Zika, anti-Influenza, anti-Dengue, and anti-MERS antibodies as well [164], supporting the concept that minimally matured antibodies are an important line of defense against SARS-CoV-2 and other viruses [159,160,164].

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