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SARS-CoV-2 variant evasion of monoclonal antibodies based on in vitro studies

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Abstract

Monoclonal antibodies (mAbs) offer a treatment option for COVID-19 in patients with severe disease and are especially important in high-risk patients where vaccination is not an option. Given the importance of understanding the evolution of resistance to mAbs by SARS-CoV-2, we reviewed the available *in vitro* neutralisation data for mAbs against live variants and viral constructs containing spike mutations of interest. Unfortunately, evasion of mAb induced protection is being reported with new SARS-CoV-2 variants. The magnitude of neutralisation reduction varied greatly between mAb-variant pairs. For example, sotrovimab retained its neutralisation capacity against Omicron/BA.1 but showed reduced efficacy against BA.2, BA.4/BA.5 and BA.2.12.1. At present, only bebtelovimab has been reported to retain its efficacy against all SARS-CoV-2 variants considered here. Resistance to mAb neutralisation was dominated by the action of epitope single amino acid substitutions in the spike protein. While not all observed epitope mutations result in increased mAb evasion, amino acid substitutions at non-epitope positions and combinations of mutations also contribute to evasion of neutralisation. This review highlights the implications for the rational design of viral genomic surveillance and factors to consider for the development of novel mAb therapies.

Introduction

The SARS-CoV-2 pandemic has resulted in nearly 600 million confirmed COVID-19 cases and over 6 million deaths as of August 2022 [1]. Vaccines, antiviral drugs, monoclonal antibodies (mAbs), and non-pharmaceutical interventions such as lockdowns, contact-tracing, isolation, social distancing and intensive care and/or oxygen use have been deployed to control the spread of the virus and mitigate the harms of disease [2-4]. Global efforts to develop and deploy vaccinations have been unprecedented in their speed and scale and have contributed greatly to reducing transmission and severe disease in infected individuals. Antivirals such as remdesivir, molnupiravir, and nirmatrelvir, and mAbs, administered either individually, such as sotrovimab or bebtelovimab, or as combination therapy "cocktails", such as Evusheld (cilgavimab + tixagevimab), have provided much-needed additional treatment options for the clinically vulnerable and those who progress to severe disease [3].

As of August 2022, a dozen mAbs that target the virus's spike protein (Table 1) have been approved for clinical use in treating those infected by, or exposed to, SARS-CoV-2. Note, those mAbs that mediate the immune response by targeting host proteins are outside the scope of this review [5] Virus-targeting mAbs are designed to reduce COVID-19 severity by binding to the SARS-CoV-2 spike glycoprotein leading to direct neutralisation, antibody-dependent cellular phagocytosis, antibody-dependent cell mediated cytotoxicity (ADCC [6]) or complement activation. These prevent the virus from binding to the angiotensin-converting enzyme 2 (ACE2) receptor on the surface of human cells, which is required for infection. The mAbs approved for clinical use and discussed here target the receptor binding domain (RBD) of the spike protein, but some mAbs in the early stages of development target other spike domains [7,8].
The efficacy of mAbs has been threatened by the emergence of SARS-CoV-2 variants resistant to existing treatments [9]. On April 16th 2021, the United States Food and Drug Administration (FDA) ended the Emergency Use Authorisation (EUA) for bamlanivimab due to resistance shown by variants (Iota, Epsilon and others) carrying the L452R and E484K substitutions [10].

Since then, the mAb sotrovimab and the cocktails Ronapreve (casirivimab + imdevemab) and bamlanivimab + etesevimab have also had limitations placed on their use as resistant variants continue to emerge and spread [11-13].Variants of concern (VOCs) Delta and Omicron, BA.1 and BA.2 lineages, have spread globally [14-17], with Gamma and Beta previously associated mainly with regional spread [18](and sequencing bias) in Brazil and South Africa respectively and travel to these regions. At present the Omicron lineages BA.4/BA.5 (defined by the same spike mutations, in particular the RBD substitutions L452R and F486V, deletion 69-70, and the reversion R493Q, relative to BA.2) and BA.2.12.1 sublineage (defined by the additional RBD mutation L452Q, relative to BA.2) are emerging globally and causing localised epidemic waves [19-21](Fig. 1A, Supplementary Fig. 1). Unfortunately all of these VOCs have shown resistance to available mAb treatments *in vitro* and so may be less susceptible to treatment in a clinical setting [22-25].

The acquisition by SARS-CoV-2 of mutations conferring evasion properties to mAbs will almost certainly continue, leading to negative clinical outcomes and impacting the utility and longevity of mAbs in managing the ongoing pandemic. Here, we consider studies in the primary literature presenting data on the neutralisation of globally circulating VOCs by mAbs, with the aim of identifying and characterising the mutations that lead to this resistance. We discuss how knowledge of the dynamics of viral evasion of mAbs can contribute to viral surveillance and the development of novel mAb treatments, as well as inform predictions of resistance that may arise in the future.

SARS-CoV-2 Variant Evasion of mAbs

Neutralisation assays are considered the "gold standard" for high throughput *in vitro* studies of antibody protection against viral infection [26]. The fold-reduction in neutralisation (FRN) is calculated by measuring the concentration of mAb required to prevent infection of cells by virus or pseudovirus carrying a mutated SARS-CoV-2 spike protein and comparing the results to one or more wildtype control sequences (usually the Wuhan-Hu-1 reference sequence sampled early in the pandemic), under the same experimental conditions. This allows comparisons between studies while mitigating the confounding effects of differences in experimental protocol. We reviewed all 118 published studies reporting neutralisation data on clinically approved mAbs against VOCs and other mutants up to 1st August 2022 (Supplementary Data File 1) and calculated the geometric mean FRN (mFRN) for each mAb-variant pair (Fig. 1B, 2, Supplementary Fig. 2, 3) to represent the extent of resistance of a variant relative to that mAbs ability to neutralise wildtype virus. The use of geometric mean is considered the appropriate measure for comparing titers generated by serial dilution [27]. Geometric mean IC50 values were

also calculated (Supplementary Fig. 4) to show the absolute ability of each mAb to neutralise each variant *in vitro*. Below we discuss the main results for historical, i.e., no longer circulating, and contemporary, summer 2022, SARS-CoV-2 VOCs:

As shown in Fig. 1B, the Alpha VOC remains susceptible to most mAbs with mild evasion, relative to wild type of etesevimab neutralisation (mFRN: 9.3; IC50[ng/ml]: 200)(Fig. 1B, Supplementary Fig 4). Delta, despite being generally less resistant to mAbs overall (with mFRN <4 to 13 out of 15 mAbs; IC50[ng/ml] <50 for 12 out of 14 for which IC50 data were available), demonstrated reduced neutralisation by bamlanivimab (mFRN: 650; IC50[ng/ml]: 5000) and regdanvimab (mFRN: 37; IC50[ng/ml]: 67).

Beta and Gamma VOCs exhibited reduced neutralisation for 5 out of 16 mAbs with mFRN >30 (Fig. 1B). This is likely due to both having mutations at the 417, 484, and 501 positions in the spike RBD (Fig. 1A, Supplementary Fig 1). However, considerable variability in FRN results from the different assays underpinning the mFRN values has been observed for these two variants (Supplementary Fig. 2, Supplementary Data File X). For example, several studies reported only minimal or no resistance (FRN <15, n=5) of casirivimab by Beta, whereas others presented very strong evasion (FRN >250, n=13). This may be due to differences in experimental protocols between studies ([28,29]e.g., incubation time, viral system and target cells used). We compared the fold change results for different viral assay systems (virus isolate versus pseudovirus) and found the distribution of results to be similar in each group (Supplementary Fig. 5) indicating that different viral systems do not have a confounding effect on our results. This is in line with the high correlation between neutralisation results using different viral systems observed elsewhere [30] While, details on cell type and other experimental parameters were not readily available for all studies, making it possible that differences in these factors are confounding given that cell type and hACE2 expression have been shown to affect the susceptibility of cells to the virus [30], the over all trends in fold change are likely to be robust.

The Omicron BA.1, BA.2, BA.1.1, BA.2.12.1 and BA.4/BA.5 sublineages were observed to have the strongest, and broadest resistance to neutralisation by mAbs, corresponding to high mFRN values against individual mAbs. Despite this, bebtelovimab, romlusevimab, Evusheld (cilgavimab + tixagevimab) and sotrovimab retained their capacity for neutralisation of BA.1 (mFRN: 1.1, 0.74, 75 and 3.3; IC50 [ng/ml]: 2.6, 240, 270 and 290, respectively)(Fig 1B, Supplementary Fig. 4), remaining viable options for treatment of those infected with BA.1. Sotrovimab also retained its neutralisation capacity against BA.1.1 (mFRN: 2.4; IC50[ng/ml]: 180), but showed decreased potency against BA.2, BA.4/BA.5 and BA.2.12.1 (mFRN: 21, 20 and 18; IC50[ng/ml]: 1400, 790 and 860 respectively). Evusheld retained significant neutralisation capacity against BA.2, BA.4/BA.5 and BA.2.12.1 (mFRN: 7.3, 74 and 21; IC50 [ng/ml]: 37, 180 and 59 respectively), but was more strongly evaded by BA.1.1 (mFRN: 310; IC50 [ng/ml]: 810). Cilgavimab retained its neutralisation capacity against BA.2, BA.4/BA.5 and BA.2.12.1 (mFRN: 2.7, 11, and 3.6; IC50[ng/ml]: 20, 67 and 25 respectively), but was evaded by BA.1, BA.1.1 (mFRN: 220 and 820; IC50[ng/ml]: 2000 and 12000). Only the recently approved bebtelovimab retained its full neutralisation capacity against all Omicron sublineages and the other VOCs, with Evusheld showing high levels of activity against all but BA.1.1. These data on the *in vitro* neutralisation of Omicron variants by bebtelovimab and Evusheld, in combination with large clinical efficacy studies during waves dominated by other variants [31] and smaller retrospective studies [32-34] during Omicron waves, suggest that high doses of both treatments have an important role to play in treating and preventing infection with Omicron variants, although further clinical evidence is required to confirm this alongside the close monitoring of emergent resistance[35].

The high fold change associated with Evusheld against BA.1 (mFRN: 75) (Fig. 1B) compared to bebtelovimab, romlusevimab, and sotrovimab (mFRN: 1.1, 0.74, and 3.3), despite having an IC50 value (IC50 [ng/ml]: 270) (Supplementary Fig 4) within the range of the other antibodies (IC50 [ng/ml]: 2.6, 240, and 290), highlights an important limitation of this metric for comparing the neutralisation capacities of different antibodies. Because fold change compares the mAb neutralsiation of the variant against neutralisation of the WT, mAbs that have very high levels of neutalisation against WT will have higher fold change values against a variant than another mAb that neutralises the WT less but the variant similarly. However, fold change is much better suited to our focus on a given mAbs neutralisation capacity against a variant relative to its neutralisation of other variants.

We did not systematically analyze neutralisation data on the recently emerged BA.2.75 variant, but early studies show it to have some resistance to bebtelovimab but increased sensitivity to tixagevemab and casirivimab [36-38].

Single Mutation Analysis to Identify Key Resistance Mutations

Neutralisation assays using viruses containing the full complement of each VOC's defining mutations cannot conclusively show the impact of specific mutations on mAb efficacy due to interactions between spike mutations in the same variant. Whereas, FRN data from viral constructs that contain single mutations of interest on a wildtype background only assesses the influence of individual mutations on mAb efficacy. For this reason, we compared published FRN values from assays involving viral constructs with single mutations against those using VOC spike proteins (Fig. 2). Interestingly, mutations had similar effects on resistance regardless of whether they were studied individually or in VOC representative sequences (Fig. 2). VOCs were resistant to a mAb if they contained mutations that were resistant to that mAb in isolation, i.e., on the reference/control background.

Imdevimab showed reduction of the neutralising activity in the presence of BA.1 (mFRN: 600) and in the presence of BA.1 RBD mutations, G446S (mFRN: 390) and N440K (nFRN: 92), when studied individually (Fig. 2), and this trend was also seen for the other Omicron sub-lineages. 66Casirivimab was observed to be evaded by Beta (mFRN: 110) and by mutants containing Beta's RBD mutations alone: E484K (mFRN: 15) and K417N (mFRN: 17), and by Gamma (mFRN: 89), and single mutant constructs with K417T and E484K (mFRN: 7.1 and 15, respectively), but not with N501Y (mFRN: 1.1) (Fig. 2). On the other hand, whenever variants lacked mutations that individually reduced neutralisation, the mAbs generally retained their activity against the variants themselves. This was the case for Delta which retained its susceptibility to casirivimab (mFRN: 0.71), as did single mutants containing the Delta substitutions, L452R and T478K (mFRN: 1.4 and 1.0, respectively)(Fig. 2). Additionally, no resistance to bebtelovimab was displayed by any of the variants or any of the single mutant constructs (Fig. 2). In cocktails of mAbs, consisting of non-competing antibodies that target different regions of SARS-CoV-2 spike protein, resistance occurs whenever the combination of mutations in a VOC results in resistance to each of the individual mAbs. For instance, BA.1 displayed strong resistance to Ronapreve (casirivimab + imdevimab) (mFRN: 840) (Supplementary Fig. 3), with K417N and Q493R evading neutralisation by casirivimab (mFRN: 17, and 38 respectively), and N440K, G446S, and S371L resistant to imdevimab (mFRN: 92, 390, and 22 respectively).

The association between the mAb neutralisation levels of single mutant constructs and full variants can also be found in the context of the bamlanivimab and etesevimab cocktail (Supplementary Fig. 3). Beta and Gamma, and mutants containing E484K alone were observed

to be resistant to the cocktail (mFRN: 510 and 410, and 20, respectively) (Supplementary Fig. 3). The greater reduction observed with Beta and Gamma in comparison to E484K taken in isolation, could be due to the combination of E484K with K417N and K417T, found in Beta and Gamma respectively. However, K417N and K417T in isolation showed no loss of neutralisation against the cocktail (mFRN: 1.4, and 0.30). Similarly, the strong evasion of the cocktail by all the Omicron sub-lineage variants is matched by that conferred by E484A (mFRN: 48), with BA.1, BA.1.1, BA.2, and BA.2.12.1 also carrying Q493R (mFRN: 100), and BA.4 and BA.5 carrying F486V (mFRN: 140). In contrast, the neutralisation capacity of the cocktail was retained against Delta (mFRN: 1.1) as it was against the single mutants carrying the Delta RBD mutations L452R (mFRN: 4.2) and T478K (mFRN: 1.5).

For other mAbs too, strong evasion of neutralisation by all Omicron sub-lineage variants aligns with the resistance of viral constructs containing single Omicron RBD mutations. S371L, present in the BA.1 and BA.1.1 variants, conferred resistance to amubarvimab, and etesevimab (mFRN: 17 and 15) (Fig. 2, Supplementary Fig. S3).666 S371F, present in the BA.2, BA.4, BA.5, and BA.2.12.1 variants, conferred resistance to amubarvimab, casirivimab, etesevimab, and regdanvimab (mFRN: 120, 11, 36 and 21). F486V, present in the BA.4 and BA.5 variants, conferred resistance to amubarvimab, casirivimab, etesevimab, and bamlanivimab (mFRN: 12, 660, 11 and 490). Q493R, present in the BA.1, BA.1.1, BA.2, and BA.2.12.1 variants, conferred resistance to amubarvimab, casirivimab, etesevimab, regdanvimab, and bamlanivimab (mFRN: 11, 38, 55, 950, and 760). K417N, present in all the Omicron variants, conferred resistance to casirivimab, and etesevimab (mFRN: 17 and 210). E484A, present in all the Omicron variants, conferred resistance to bamlanivimab (mFRN: 570), indicating it is E484A that causes the Omicron sub-lineages variant resistance to the bamlanivimab + etesevimab cocktail discussed above.

Although these observations support the hypothesis that in general the observed resistance of VOCs to specific mAbs is due to effects of individual resistance mutations acting in isolation [39], in several cases the resistance observed in variants was greater than the sum of the evasive effects of the spike lineage-defining mutations alone. For instance, the neutralising activity of amubarvimab against BA.1 and BA.1.1 was markedly reduced (mFRN: 180 and 220) although S371L and Q493R were individually found to confer only moderate-to-weak evasion of neutralisation (mFRN: 17 and 11, respectively) (Supplementary Fig. 3) . Similarly, BA.1 and BA.1.1 strongly evaded both cilgavimab (mFRN: 220 and 820, respectively) and tixagevimab (mFRN: 360 and 400) administered alone, yet none of the constituent BA.1 RBD mutations conferred mFRN >10 to either mAb. Thus, the strong evasion by BA.1 and BA.1.1 of these mAbs can not be explained by the additive effects of their RBD mutations in isolation, indicating that other mechanisms may be at play. One possibility is that mutations outside the spike RBD contribute to resistance. Another, is that synergistic effects between RBD mutations lead to enhanced resistance when they are present in combination.

On the other hand, there are examples where single mutations of interest show higher reduction of neutralisation than the full variant itself. For example, the mutants bearing the BA.1 and BA.1.1 mutation S371L displayed moderate resistance to sotrovimab (mFRN: 20)(Supplementary Fig. 3), whereas resistance observed with BA.1 and BA.1.1 was only mild (mFRN: 3.3 and 2.4). BA.1 mutations other than S371L, such as G496S [40], might antagonistically dampen the effect of S371L leading to only moderate resistance overall. This possibly explains why BA.2– missing mutations at positions 446 and 496 (relative to BA.1) – was found to be less sensitive to sotrovimab (mFRN: 22 vs 3.3) than BA.1. Alternatively, NTD mutations may allosterically alter the BA.2 RBD leading

to antigenic effects [41]. This mechanistic ambiguity of how mutations interact highlights the need for comparative studies where combinations of mutations are taken into consideration.

Epitope Mutations Drive mAb Evasion

To better understand the effect of single mutations on mAb activity we investigated the role of mutations occurring at epitope and non-epitope positions (S Data File 2) on the reduction of the neutralisation by mAbs. As expected, mutations causing the strongest resistance were found within the binding footprint of the mAb at cognate epitope positions in the spike RBD (Fig. 3A-B). However, not all mutations at epitope positions were observed to cause mAb resistance, and a small number of strongly resistant mutations were found at distal, non-epitope positions in the RBD (Fig. 3A-B) and in the N-terminal domain (NTD) (Supplementary Fig. 6A) able to generate long-range allosteric perturbations. Generally, mutations close to epitope positions, at the +/- 1 proximal positions, but not inside the epitope, were observed to not cause reductions in neutralisation (Supplementary Fig. 6B).

At some sites, the level of evasion depended on the specific amino acid substitution introduced by the mutation. For example, in the case of casirivimab and mutations at 417, the substitution of a Lysine (K) with either Glutamic acid (E) or Arginine (R) was associated with a marked reduction of the neutralisation activity (mFRN: 160 and 61 respectively)(S Data File 3), whereas more moderate evasion was observed with Asparagine (N) (mFRN: 17), with an even smaller effect with Threonine (T) (mFRN: 7.1). Additionally, the single mutant carrying the substitution of Valine (V) with Threonine (T) at 445 showed strong resistance to casirivimab (mFRN: 110), whereas the Alanine substitution (V445A) presented no evasion (mFRN: 1.7)(S Data File 3). Similarly, sotrovimab was strongly evaded by mutants containing P337L (mFRN: 160) and P337R (mFRN: 216) in isolation, but only mildly by those containing P337T and P337H (mFRN: 8.5 and 5.8, respectively), and not at all by the P337S single mutant (mFRN: 1.3)(S Data File 3).

There are some cases in which mutations outside of the epitope contribute to resistance against the mAb, displaying a potential allosteric influence. The S371L single mutant conferred

moderate evasion of Amubarivimab (mFRN: 17), imdevimab (mFRN: 22), sotrovimab (mFRN: 20) and etesevimab (mFRN: 15) despite not being present in the epitope of any of these mAbs (Fig. 3A). (Romlusevimab also displayed reduced neutralisation against S371L, mFRN: 17, but no epitope data is yet available). Similarly, mutants containing E406W alone markedly evaded both casirivimab (mFRN: 84) and imdevimab (mFRN: 110)(S Data File 3); E406D was resistant to casirivimab (mFRN: 51); F377K evaded sotrovimab (mFRN: 300), and P499S imdevimab (mFRN: 210). The broad evasion of S371L and mutations at 406 suggests that, given that each mAb has different epitopes, those mutations might affect the global spike conformation, rather than making local changes to specific epitopes only (Fig. 3C).

Several studies have been carried out to investigate the structural mechanisms through which non-epitope mutations can disrupt antibody binding. The S371L mutation was found to alter the conformation of both the 371-376 loop and the 365-370 helix, leading to disruptive interactions with mAbs at those sites [42,43]. At the same positon, S371F leads to the repositioning of the N343 glycan, potentially causing resistance to sotroviamb [44]. E406W was observed to cause allosteric remodeling of sites contacted by cilgavimab and Ronapreve (casirivimab + imdevimab), reducing binding by these mAbs [45], with allostery being central to spike dynamics more generally [46,47]. G496S may preclude mAb binding through steric clash, which may explain why a different mutation at the same site, G496R, was observed to cause resistance of etesivimab [48]. However, other studies showed G496S to stabilise interactions with mAbs by the formation of hydrogen bonds, indicating

that mutational effects to be mAb specific [40]. Nabel *et al*. found that the D364N, A372S and A372T mutations may cause mAb evasion by supporting N-linked glycosylation at N370 [49]. Addition of a bulky glycan at this site obstructs mAb binding to the epitope directly and through stabilisation of the RBD "down" conformation that hides the epitope (Fig. 3C) [50]. Using an amino acid interaction (AAI) approach, mutations at the non-epitope 373, 440 and 446 positions have been reported to disrupt sotrovimab binding *via* interactions with amino acids that are in the epitope [51]. Molecular dynamics-based approaches have also helped to delineate how residues in the NTD and S2 domain may be able to allosterically affect antigenicity and infectivity [41]. Taken together, these observations show that comprehensive analysis of mAb evasion by SARS-CoV-2 must include non-epitope mutations on top of the more prominent epitope mutation resistance effects.

Protein structure studies have reported on the mechanisms by which specific epitope substitutions affect the interaction of mAbs with the spike protein, shedding light on why some substitutions lead to resistance but others do not. The E484A mutation is likely to cause the loss of salt bridges between spike and class 2 mAbs (e.g bamlanivimab) [40,52], and destabilising energy changes in bonds with class 1 casirivimab [40]. These different effects on bonding to antibodies of different classes may explain why E484A conferred strong evasion of bamlanivimab (class 2, mFRN: 570), but only mild resistance to casirivimab (class 1, mFRN: 9.8). Similarly, explanation of the evasion of bamlanivimab (mFRN: 750), casirivimab (mFRN: 15), tixagevimab (mFRN: 6.8) and regdanvimab (mFRN: 8.7) by mutants containing E484K has been offered by computational modeling that reveals the reversal of the side chain charge to significantly alter the electrostatic complementarity of mAb binding (Fig. 3C) [53]. E484K, E484A and E484Q mutations all drive resistance to bamlanivimab and casirivimab to some extent which, given the different biochemical properties of the amino acid substitutions, suggests in this case it is the loss of Glutamic acid (E) that is important, rather than the amino acid which it is replaced with. This is supported by the identification of E484 as a key binding site for some mAbs [54], and by the loss of hydrogen bonds between E484 and an experimental mAb due to mutation (Fig. 3C) [55]. Structural analysis reveals L452R to disrupt a hydrophobic binding pocket, potentially explaining the loss of neutralisation by antibodies targeting this site in Delta and BA.4/BA.5 variants [21]. BA.4/BA.5 also carries F486V, which involves the loss of phenylalanine from binding site of multiple mAbs [21]. Linking the reduction in neutralisation conferred by a mutation to its structural effects explains observed patterns of neutralisation and may support the prediction of novel evasive mutations based on their putative structural effects.

The structural changes introduced by spike mutations have consequences not only for mAb evasion but also viral infectivity, replication, transmissibility and stability [24,56-59]. Some antigenic mutations decrease infectivity as they affect the ability of RBD to bind the hACE2 receptor required for entry into the human cell [49,60-67] Other mutations can compensate for this detrimental effect by increasing hACE2 binding [52], allowing the virus to retain its infectivity while reducing susceptibility to the antibody response. The BA.1 spike mutational profile demonstrates this as the antigenic evasion mutations G496S, Y505H, K417N, S371L, S373P, S375F alone decrease hACE2 binding affinity, but overall BA.1 infectivity is retained through the compensatory increase in affinity due to N501Y, S477N, G493K, and Q498R [23,52,60]. It is possible that the BA.4/BA.5 affinity for ACE2 is further increased by the electrostatic complementarity of L452R, offering explanation of the transmission advantage of both BA.4/BA.5 and Delta [21]. Other mutations, such as S373P and S375F, do not have immune evasive effects in isolation, but do so in combination [51]. These combinations are able to arise as the individual mutations have positive effects on other aspects of viral fitness and so experience positive selection before the full immune evasive combination is achieved [51]. Thus, the selection of other viral traits can have antigenic effects as some mutations impact multiple viral phenotypes.

Concluding remarks

Care must be taken when extrapolating between neutralisation assays and the clinical efficacy of mAbs. Post-vaccination sera neutralisation titers strongly correlate with protection from symptomatic infection and severe disease [68,69], and mAb neutralisation may be expected to follow a similar trend. A perfect correlation would not be expected due to mechanisms other than neutralisation, such as antibody dependent cellular cytotoxicity, antibody dependent cellular phagocytosis, and complement activation, which are also important to antibodies' role in combating viral infection, and other aspects of the immune response such as T cells that are not captured in neutralisation assays. Such effects likely contribute to the generally strong *in vivo* efficacy of sotrovimab [70], despite it's elevated IC50 values (Supplementary Fig. 4). Some mAbs have their Fc domains altered, meaning they are able to participate in effector functions to different degrees [71,72] . In addition, the variability in results from different studies must be taken into consideration. This is partly explained by the use of different neutralisation assay protocols with alternative viral systems (authentic virus isolates, VSV/lentivirus-based pseudovirus, recombinant chimeric virus), target cells (Vero E6, S-Fuse, 293T ACE2, etc), and other experimental conditions between studies (e.g., incubation time, experimental output, cell type, ACE2/TMPRSS2 expression, etc.) [30,73,74], and highlights the urgent need to build on the WHO's work on standardisation of neutralisation assays [75,76].

Although indications that Omicron infections are less clinically severe are encouraging [77], the reduced susceptibility of Omicron lineages BA.1, BA.2, and BA.4/BA.5 to neutralisation by many of the mAbs available for clinical use highlights the risk that novel variants pose to the efficacy of mAb treatments in the present and future. The currently rapidly emerging BA.4/BA.5 lineages, in particular, show the highest levels of mAb evasion of any VOC to date. Pandemic management strategies must account for the possibility that future variants will evade all currently available mAbs. There are several precautionary steps that can be taken (Fig. 4). Firstly, reliance must not be placed on any single control strategy so that the cost of any strategy failing is reduced. This is being achieved already with the combination of vaccination, monoclonal antibodies, antivirals, and non-pharmaceutical interventions such as self-isolation [2-4]. Secondly, mAb development strategies could minimise resistance to mAbs by targeting conserved epitopes [78] and susceptible epitopes of future variants using knowledge of patterns of resistant mutations, such as those discussed in this review. Maintaining a diverse range of viable drugs will ensure that effective COVID-19 treatments are available, even if some variants are resistant to a subset of drugs. Thirdly, clinical mAb treatment needs to be conducted in a manner that mitigates the risk of directly causing the emergence and spread of resistant variants. Resistance mutations have been observed to emerge when patients are treated with mAbs or convalescent plasma [56,79-81]. These resistant variants threaten to evade both mAb treatment and the polyclonal adaptive immune response as there is considerable overlap in the mutations that drive evasion in each case [35,82].

Desirable clinical outcomes can be supported by virus genome sequencing to determine the variant causing infection, allowing the most appropriate mAb treatment to be selected. Similarly, alignment of regional treatment guidelines with information about which variants are circulating and at what frequencies will support the effective logistical deployment of the available treatments. The FDA have already begun regulating on this basis with EUAs for mAbs including a clause that prohibits a mAb being used in regions where the frequency of resistant variants is above 5% [11-13]. UKHSA recommended longitudinal virus genome sequencing during therapeutic mAb usage to monitor for emerging resistance [83]. Additionally, continued exploitation of combination therapies, selection of appropriate dosages, and preventing the spread of virus from the patient to other individuals will make the development and onwards spread of resistant strains less likely.

Viral surveillance and mAb development efforts will benefit greatly from considering not only single epitope mutations, but also non-epitope mutations alongside combinational and synergistic dynamics. These efforts must extend beyond antigenicity to the full range of viral traits, all of which play a role in determining the antigenic mutations that rise to prominence. The data discussed here depict mAb evasion to be dominated by the isolated effect of single mutations at epitope positions, and therefore that single mutational analysis can provide important insights of the mechanisms of mAb evasion for past and future variants. However, the evasive effects of non-epitope mutations and combinations of mutations mean that a complete understanding of mAb dynamics cannot be achieved by analysis of single epitope mutation alone. Rather, neutralisation data should be combined with structural, combinatorial, molecular dynamic and evolutionary studies to allow the preemption of novel future mutations that may have similar effects. With this in mind, public health agencies should continue to routinely survey sequence data generated locally and worldwide to detect viral mutations and variants that might impact adversely on the efficacy of therapeutics (Fig. 4).

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Competing interests

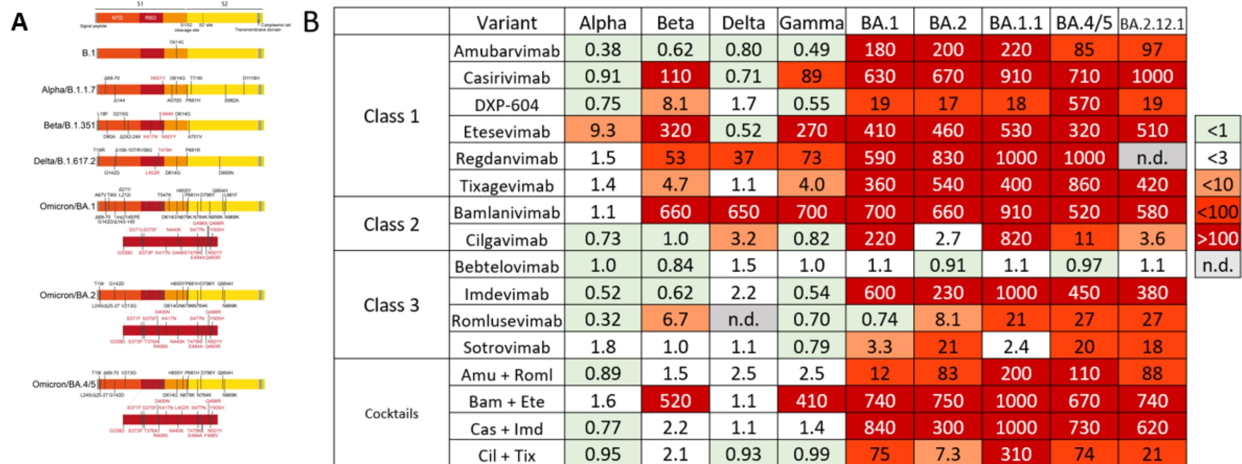
The authors declare no conflict of interest.

Generic name	Other name	Manufacturer	mAb class	Spike epitope	VOC resistance
Amubarvimab+ Romlusevimab [85, 86]	BRII-196+ BRII-198, P2C-1F11+ P2B-1G5,	Brii Biosciences	Amubarvimab: Class 1 Romlusevimab: no data	Amubarivimab: 403, 415, 416, 417, 420, 421, 453, 455, 456, 457, 458, 459, 460, 473, 474, 475, 476, 477, 486, 487,489,493,502,505. Romlusevimab: no data.	Strong: BA.1.1,BA.4/5 Moderate: BA.1, BA.2, BA.2.12.1
Bamlanivimab [10] (withdrawn)	LYCoV555, LY3819253	Eli Lilly	Class 2	351, 449, 450, 452, 455, 456, 470, 472, 481, 482, 483, 484, 485, 486, 487, 488, 489, 490, 492, 493, 494	Strong: Beta, Gamma, Delta, BA.1,BA.1.1, BA.2,BA.2.12.1, BA.4/5
Bamlanivimab+ Etesevimab [13,87,88]	Etesevimab: LY3832479, LY-CoV016. (See bamlanivimab)	Eli Lilly	Etesevimab: Class 1 Bamlanivimab: Class 2	Etesevimab: 403, 405, 406, 408, 409, 415, 416, 417, 420, 421, 455, 456, 457, 458, 459, 460, 473, 474, 475, 476, 477, 486, 487, 489, 493, 494, 495, 500, 501, 502, 504,505. (See bamlanivimab)	Strong: Beta, Gamma,BA.1, BA.1.1, BA.2, BA.2.12.1,BA.4/5
Bebtelovimab [89]	LY-CoV1404, LY3853113	Eli Lilly	Class 3	346, 439, 440, 441, 443, 444, 445, 446, 447, 448, 449, 450, 498, 499, 500, 501, 502, 506, 509	
DXP-604 [90]	n/a	Singlomics + BeiGene	Class 1	403, 415, 416, 417, 420, 421, 453, 455, 456, 457, 458, 459, 460, 473, 474, 475, 476, 477, 486, 487, 489, 493, 496, 498, 500, 501, 502, 503, 505	Moderate: BA.1 Mild: Beta
Regdanvimab [91]	Regkirona, CT-P59	Celltrion Healthcare	Class 1	351, 403, 417, 446, 449, 450, 452, 453, 455, 456, 470, 483, 484, 485, 486, 489, 490, 492, 493, 494, 495, 496, 498, 505	Strong: BA.1,BA.2, BA.1.1 Moderate: Beta, Delta, Gamma
Ronapreve [12,92]	REGEN-CoV2, Casirivimab+ Imdevimab	Regeneron	Casirivimab: Class 1 Imdevimab: Class 3	Casirivimab: 403, 417, 421, 453, 455, 456, 475, 476, 484,485,486,487,488,489,493. Imdevimab: 346, 439, 440, 441, 444, 445, 446, 447, 448, 449, 450, 498, 500.	Strong:BA.1, BA.1.1,BA.2, BA.2.12.1, BA.4/5
Sotrovimab [11,93,94]	Xevudy, VIR-7831, GSK4182136.*Parental antibody: S309*	GlaxoSmith-Kline	Class 3	333, 334, 335, 337, 339, 340, 343, 344, 345, 346, 354, 356, 357, 359, 360, 361, 441, 509	Moderate: BA.2, BA.2.12.1, BA.4/5 Mild: BA.1
Cilgavimab+ Tixagevimab [95,96,97]	Evusheld AZD7442, AZD1061/ AZD8895. *Parental antibodies: COV2-2196, COV2-2130*	AstraZeneca	Tixagevimab: Class 1 Cilgavimab: Class 2	Tixagevimab: 455, 456, 458, 475, 476, 477, 478, 479, 484,485,486,487,488,489,493.	Strong: BA.1.1 Moderate: BA.1, BA.2.12.1,BA.4/5 Mild: BA.2.

Generic name	Other name	Manufacturer	mAb class	Spike epitope	VOC resistance
Amubarvimab+ Romlusevimab [85, 86]	BRII-196+ BRII-198, P2C-1F11+ P2B-1G5,	Brii Biosciences	Amubarvimab: Class 1 Romlusevimab: no data	Amubarivimab: 403, 415, 416, 417, 420, 421, 453, 455, 456, 457, 458, 459, 460, 473, 474, 475, 476, 477, 486, 487,489,493,502,505. Romlusevimab: no data.	Strong: BA.1.1,BA.4/5 Moderate: BA.1, BA.2, BA.2.12.1
Bamlanivimab [10] (withdrawn)	LYCoV555, LY3819253	Eli Lilly	Class 2	351, 449, 450, 452, 455, 456, 470, 472, 481, 482, 483, 484, 485, 486, 487, 488, 489, 490, 492, 493, 494	Strong: Beta, Gamma, Delta, BA.1,BA.1.1, BA.2,BA.2.12.1, BA.4/5
Bamlanivimab+ Etesevimab [13,87,88]	Etesevimab: LY3832479, LY-CoV016. (See bamlanivimab)	Eli Lilly	Etesevimab: Class 1 Bamlanivimab: Class 2	Etesevimab: 403, 405, 406, 408, 409, 415, 416, 417, 420, 421, 455, 456, 457, 458, 459, 460, 473, 474, 475, 476, 477, 486, 487, 489, 493, 494, 495, 500, 501, 502, 504,505. (See bamlanivimab)	Strong: Beta, Gamma,BA.1, BA.1.1, BA.2, BA.2.12.1,BA.4/5
Bebtelovimab [89]	LY-CoV1404, LY3853113	Eli Lilly	Class 3	346, 439, 440, 441, 443, 444, 445, 446, 447, 448, 449, 450, 498, 499, 500, 501, 502, 506, 509	
DXP-604 [90]	n/a	Singlomics + BeiGene	Class 1	403, 415, 416, 417, 420, 421, 453, 455, 456, 457, 458, 459, 460, 473, 474, 475, 476, 477, 486, 487, 489, 493, 496, 498, 500, 501, 502, 503, 505	Moderate: BA.1 Mild: Beta
Regdanvimab [91]	Regkirona, CT-P59	Celltrion Healthcare	Class 1	351, 403, 417, 446, 449, 450, 452, 453, 455, 456, 470, 483, 484, 485, 486, 489, 490, 492, 493, 494, 495, 496, 498, 505	Strong: BA.1,BA.2, BA.1.1 Moderate: Beta, Delta, Gamma
				Cilgavimab: 345, 346, 439, 440, 441, 443, 444, 445, 446, 447, 448, 449, 450, 452, 484, 490, 492, 493, 494, 499.	

Table 1. mAbs approved for clinical use with details on their antibody class their alternative names and known resistance to SARS-CoV-2 variants. VOC strength of resistance is reported as strong (mFRN > 100), moderate (mFRN 10-100), mild (mFRN 3-10), based on the data reported in Figure 1. Although these designators are useful to the extent they assign a qualitative judgment to the level of resistance it must be emphasized that resistance of variants occurs across a continuum. Epitope positions are defined as those within 4.5 angstroms of the mAb when it is bound to spike [84]. Approval amubarvimab and romlusevimab: CN approval National Medical Products Association Nov 2021,National Medical Commission Treatment Guidelines Mar 2022 - bamlanivimab; USA approval Nov 2020, revoked April 2021 - bamlanivimab and etesevimab: USA approval Feb 2021, re-issued Jan 2022 restricting use in areas with resistant variants; EU approval for patient use March 2021, marketing application withdrawn Oct

2021 - bebtelovimab: USA approval Feb 2022, re-issued Jun 2022 - DXP-604: CN approval Nov 2021 compassionate use - regdanvimab: EU approval Dec 2021 - Ronapreve (casirivimab + imdevimab): USA approval Nov 2022 for treatment and prophylactic use, re-issued Jan 2022 restricting use in areas with resistant variants; EU approval for treatment and prophylactic use Nov 2021, updated Mar 2022 [12]; UK approval for treatment and prophylactic use Aug 2021 - sotrovimab: US approval Dec 2021, re-issued Apr 2022 , restricting use in areas with resistant variants; EU approval Dec 2021; UK approval Dec 2021 - Evusheld (cilgavimab + tixagevimab): USA approval for prophylactic use Dec 2021, re-issued Mar 2022 ; EU approval for prophylactic use: Mar 2022; UK approval for prophylactic use Mar 2022.



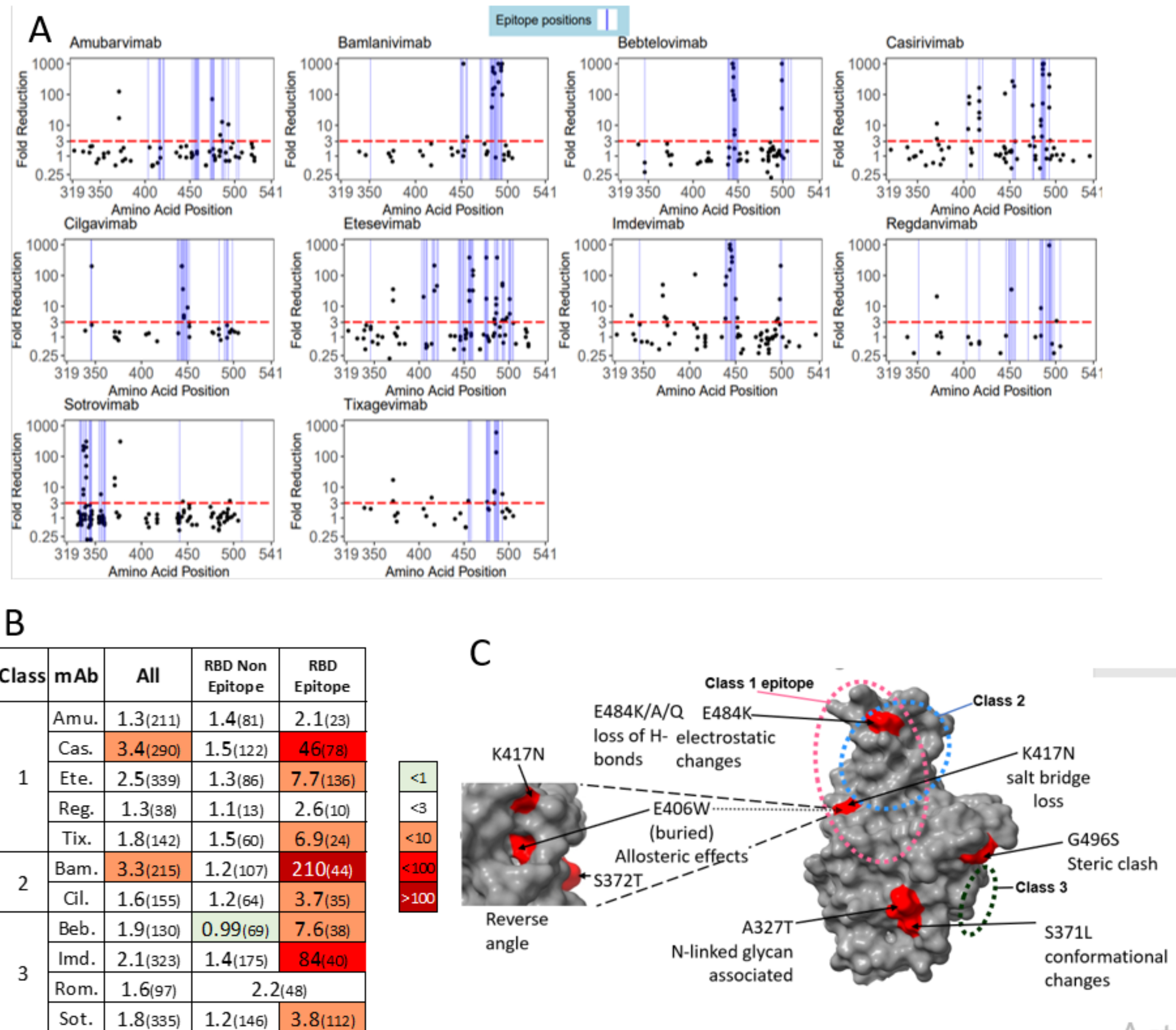
	Variant	Alpha	Beta	Delta	Gamma	BA.1	BA.2	BA.1.1	BA.4/5	BA.2.12.1
Class 1	Amubarvimab	0.38	0.62	0.80	0.49	180	200	220	85	97
	Casirivimab	0.91	110	0.71	89	630	670	910	710	1000
	DXP-604	0.75	8.1	1.7	0.55	19	17	18	570	19
	Etesevimab	9.3	320	0.52	270	410	460	530	320	510
	Regdanvimab	1.5	53	37	73	590	830	1000	1000	n.d.
	Tixagevimab	1.4	4.7	1.1	4.0	360	540	400	860	420
Class 2	Bamlanivimab	1.1	660	650	700	700	660	910	520	580
	Cilgavimab	0.73	1.0	3.2	0.82	220	2.7	820	11	3.6
Class 3	Bebtelovimab	1.0	0.84	1.5	1.0	1.1	0.91	1.1	0.97	1.1
	Imdevimab	0.52	0.62	2.2	0.54	600	230	1000	450	380
	Romlusevimab	0.32	6.7	n.d.	0.70	0.74	8.1	21	27	27
	Sotrovimab	1.8	1.0	1.1	0.79	3.3	21	2.4	20	18
Cocktails	Amu + Roml	0.89	1.5	2.5	2.5	12	83	200	110	88
	Bam + Ete	1.6	520	1.1	410	740	750	1000	670	740
	Cas + Imd	0.77	2.2	1.1	1.4	840	300	1000	730	620
	Cil + Tix	0.95	2.1	0.93	0.99	75	7.3	310	74	21

Legend
<1
<3
<10
<100
>100
n.d.

Figure 1. SARS-CoV-2 variant of concern (VOC) evasion of neutralisation by mAbs. **A)** Spike mutational profiles for each VOC with the receptor binding domain (RBD) also shown for BA.1, BA.2 and BA.4/BA.5 in higher detail; **B)** Geometric mean fold reduction neutralisation (mFRN) values of mAbs against VOCs relative to reference/control variants. Means are calculated from published studies reporting neutralisation data on clinically approved mAbs against VOCs. Data for the associated single studies are shown in Supplementary Fig. 2. Full datasets are available in supplementary data file 3, and confidence statistics in supplementary data file 1. Colours depict the strength of resistance: dark red - strong (mFRN > 100), red - moderate (mFRN 10-100), light red - mild (mFRN 3-10), white - no resistance (mFRN 1-3), light green- increased sensitivity (mFRN <1). "n.d."- no neutralisation data reported for the antibody-variant pair. All mFRN values are given to 2 significant figures. Neutralisation data were not available for all mAb-variant pairs at the time of writing as denoted by cells marked "n.d.".

Full Variants		G339D	R346K	S371L	S371F	S373P	S375F	T376A	D405N	R408S	K417N	K417T	N440K	G446S	L452R	L452Q	S477N	T478K	E484K	E484A	F486V	Q493R	G496S	Q498R	N501Y	Y505H
Casirivimab																										
Alpha	0.91	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1.1	-
Beta	110	-	-	-	-	-	-	-	-	-	17	-	-	-	-	-	-	-	15	-	-	-	-	-	1.1	-
Gamma	89	-	-	-	-	-	-	-	-	-	-	7.1	-	-	-	-	-	-	15	-	-	-	-	-	1.1	-
Delta	0.71	-	-	-	-	-	-	-	-	-	-	-	-	-	1.4	-	-	1.0	-	-	-	-	-	-	-	-
BA.1	630	1.9	-	3.6	-	2.3	0.53	-	-	-	17	-	1.1	1.2	-	-	2.1	1.0	-	9.8	-	38	1.0	1.8	1.1	0.74
BA.1.1	910	1.9	0.95	3.6	-	2.3	0.53	-	-	-	17	-	1.1	1.2	-	-	2.1	1.0	-	9.8	-	38	1.0	1.8	1.1	0.74
BA.2	670	1.9	-	-	11	2.3	0.53	1.6	7.6	1.7	17	-	1.1	-	-	-	2.1	1.0	-	9.8	-	38	-	1.8	1.1	0.74
BA.4/5	710	1.9	-	-	11	2.3	0.53	1.6	7.6	1.7	17	-	1.1	-	1.4	-	2.1	1.0	-	9.8	660	-	-	1.8	1.1	0.74
BA.2.12.1	1000	1.9	-	-	11	2.3	0.53	1.6	7.6	1.7	17	-	1.1	-	-	1.5	2.1	1.0	-	9.8	-	38	-	1.8	1.1	0.74
Imdevimab																										
Alpha	0.52	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1.0	-
Beta	0.62	-	-	-	-	-	-	-	-	-	0.67	-	-	-	-	-	-	-	0.92	-	-	-	-	-	1.0	-
Gamma	0.54	-	-	-	-	-	-	-	-	-	-	1.1	-	-	-	-	-	-	0.92	-	-	-	-	-	1.0	-
Delta	2.2	-	-	-	-	-	-	-	-	-	-	-	-	-	2.1	-	-	1.3	-	-	-	-	-	-	-	-
BA.1	600	1.2	-	22	-	4.5	3.9	-	-	-	0.67	-	92	390	-	-	1.2	1.3	-	1.6	-	1.7	3.9	2.6	1.0	0.49
BA.1.1	1000	1.2	2.6	22	-	4.5	3.9	-	-	-	0.67	-	92	390	-	-	1.2	1.3	-	1.6	-	1.7	3.9	2.6	1.0	0.49
BA.2	230	1.2	-	-	50	4.5	3.9	2.4	2.6	1.9	0.67	-	92	-	-	-	1.2	1.3	-	1.6	-	1.7	-	2.6	1.0	0.49
BA.4/5	450	1.2	-	-	50	4.5	3.9	2.4	2.6	1.9	0.67	-	92	-	2.1	-	1.2	1.3	-	1.6	0.90	-	-	2.6	1.0	0.49
BA.2.12.1	380	1.2	-	-	50	4.5	3.9	2.4	2.6	1.9	0.67	-	92	-	-	4.2	1.2	1.3	-	1.6	-	1.7	-	2.6	1.0	0.49
Etesevimab																										
Alpha	9.3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	5.7	-
Beta	330	-	-	-	-	-	-	-	-	-	210	-	-	-	-	-	-	-	3.1	-	-	-	-	-	5.7	-
Gamma	270	-	-	-	-	-	-	-	-	-	-	32	-	-	-	-	-	-	3.1	-	-	-	-	-	5.7	-
Delta	0.52	-	-	-	-	-	-	-	-	-	-	-	-	-	1.2	-	-	0.77	-	-	-	-	-	-	-	-
BA.1	410	1.7	-	15	-	1.3	1.1	-	-	-	210	-	1.1	1.0	-	-	0.90	0.77	-	3.8	-	55	1.2	4.3	5.7	2.9
BA.1.1	540	1.7	2.2	15	-	1.3	1.1	-	-	-	210	-	1.1	1.0	-	-	0.90	0.77	-	3.8	-	55	1.2	4.3	5.7	2.9
BA.2	450	1.7	-	-	36	1.3	1.1	2.0	20	0.58	210	-	1.1	-	-	-	0.90	0.77	-	3.8	-	55	-	4.3	5.7	2.9
BA.4/5	320	1.7	-	-	36	1.3	1.1	2.0	20	0.58	210	-	1.1	-	1.2	-	0.90	0.77	-	3.8	11	-	-	4.3	5.7	2.9
BA.2.12.1	510	1.7	-	-	36	1.3	1.1	2.0	20	0.58	210	-	1.1	-	-	1.7	0.90	0.77	-	3.8	-	55	-	4.3	5.7	2.9
Bamlanivimab																										
Alpha	1.1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1.3	-
Beta	660	-	-	-	-	-	-	-	-	-	0.50	-	-	-	-	-	-	-	750	-	-	-	-	-	1.3	-
Gamma	700	-	-	-	-	-	-	-	-	-	-	0.10	-	-	-	-	-	-	750	-	-	-	-	-	1.3	-
Delta	650	-	-	-	-	-	-	-	-	-	-	-	-	-	1000	-	-	1.4	-	-	-	-	-	-	-	-
BA.1	700	1.4	-	1.2	-	0.95	0.67	-	-	-	0.50	-	1.1	1.4	-	-	1.1	1.4	-	570	-	760	0.75	2.2	1.3	0.86
BA.1.1	910	1.4	1.1	1.2	-	0.95	0.67	-	-	-	0.50	-	1.1	1.4	-	-	1.1	1.4	-	570	-	760	0.75	2.2	1.3	0.86
BA.2	660	1.4	-	-	1.2	0.95	0.67	1.5	1.5	1.0	0.50	-	1.1	-	-	-	1.1	1.4	-	570	-	760	-	2.2	1.3	0.86
BA.4/5	520	1.4	-	-	1.2	0.95	0.67	1.5	1.5	1.0	0.50	-	1.1	-	1000	-	1.1	1.4	-	570	490	-	-	2.2	1.3	0.86
BA.2.12.1	580	1.4	-	-	1.2	0.95	0.67	1.5	1.5	1.0	0.50	-	1.1	-	-	3.0	1.1	1.4	-	570	-	760	-	2.2	1.3	0.86
Bebtelovimab																										
Alpha	1.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.85	-
Beta	0.84	-	-	-	-	-	-	-	-	-	0.84	-	-	-	-	-	-	-	0.70	-	-	-	-	-	0.85	-
Gamma	1.0	-	-	-	-	-	-	-	-	-	-	0.70	-	-	-	-	-	-	0.70	-	-	-	-	-	0.85	-
Delta	1.5	-	-	-	-	-	-	-	-	-	-	-	-	-	0.74	-	-	0.75	-	-	-	-	-	-	-	-
BA.1	1.1	2.4	-	1.4	-	1.0	0.53	-	-	-	0.84	-	0.91	1.8	-	-	1.6	0.75	-	1.3	-	0.99	1.5	1.0	0.85	1.4
BA.1.1	1.1	2.4	0.61	1.4	-	1.0	0.53	-	-	-	0.84	-	0.91	1.8	-	-	1.6	0.75	-	1.3	-	0.99	1.5	1.0	0.85	1.4
BA.2	0.91	2.4	-	-	2.5	1.0	0.53	1.1	0.57	0.65	0.84	-	0.91	-	-	-	1.6	0.75	-	1.3	-	0.99	-	1.0	0.85	1.4
BA.4/5	0.97	2.4	-	-	2.5	1.0	0.53	1.1	0.57	0.65	0.84	-	0.91	-	0.74	-	1.6	0.75	-	1.3	1.4	-	-	1.0	0.85	1.4
BA.2.12.1	1.1	2.4	-	-	2.5	1.0	0.53	1.1	0.57	0.65	0.84	-	0.91	-	-	1.2	1.6	0.75	-	1.3	-	0.99	-	1.0	0.85	1.4

Figure 2. Influence of individual spike mutations of interest on mAb neutralisation activity compared to mAb resistance of VOCs. Values show geometric mFRN. Pink boxes – epitope positions (S Data File 2). Fill colours depict the strength of resistance: dark red - strong (mFRN > 100), red - moderate (mFRN 10-100), light red - mild (mFRN 3-10), white - no resistance (mFRN 1-3), light green (mFRN <1) - increased sensitivity. "-" mutation not present in the variant. "n.d." mutation present in variant but no neutralisation data available. All defining mutations at RBD positions in VOCs are included. (Supplementary Fig. 3) presents data for other mAbs for which less comprehensive data have been collected. The RBD is defined here as spike positions 319-541 [64].



Class	mAb	All	RBD Non Epitope	RBD Epitope
1	Amu.	1.3(211)	1.4(81)	2.1(23)
	Cas.	3.4(290)	1.5(122)	46(78)
	Ete.	2.5(339)	1.3(86)	7.7(136)
	Reg.	1.3(38)	1.1(13)	2.6(10)
	Tix.	1.8(142)	1.5(60)	6.9(24)
2	Bam.	3.3(215)	1.2(107)	210(44)
	Cil.	1.6(155)	1.2(64)	3.7(35)
3	Beb.	1.9(130)	0.99(69)	7.6(38)
	Imd.	2.1(323)	1.4(175)	84(40)
	Rom.	1.6(97)	2.2(48)	
	Sot.	1.8(335)	1.2(146)	3.8(112)

Legend
<1
<3
<10
<100
>100

Figure 3. Assessment of mAb resistance by mutations of interest occurring at epitope and non-epitope sites. **A**) mFRN data for each mAb against viral constructs containing single mutations in the spike RBD (positions 319-541). Epitope positions (S Data File 2) indicated by the vertical blue lines. The dotted red line shows the mFRN = 3 threshold. Alternative substitutions at the same amino acid position are shown as separate points at the same x coordinate; **B**) pooled mFRN comparison between epitope and non-epitope mutations. "(x)" indicates the number of assays contributing to each geometric mean value. (*) epitope unknown for romlusevimab; **C**) Isolated Omicron spike RBD structure (PDB: 7TGW) [98]. Epitope regions for class 1, 2 and 3 mAbs are circled. Epitope and non-epitope mutations exemplifying mechanisms of mAb evasion are labelled: S371L, conformational changes and N-linked glycosylation[42]; A372T, N-linked glycosylation [49]; E406W, conformational changes in epitope [45]; K417N, abolished salt-bridges between mAb and RBD [40]; E484K/Q/A, loss of H-bonds with mAb [55]; E484K, changes to electrostatic interactions[53]; G496S, steric

clash [99]. Single mutant mFRN data across the full spike protein are shown in Supplementary Fig. 6A and pooled mFRN comparison between epitope, non-epitope mutations, epitope proximal and RBD positions in Supplementary Fig. 6B.

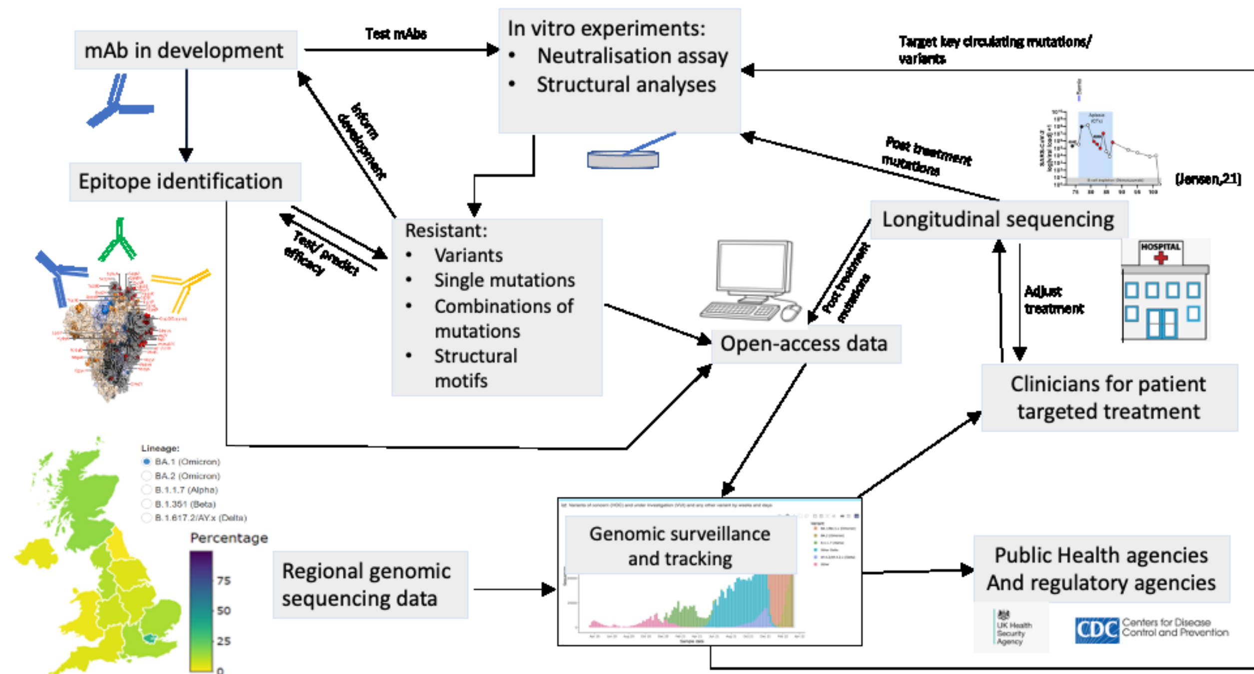


Figure 4. Diagram of a framework for the rational design of viral genomic surveillance for the development of efficient mAb therapies.