

Emerging SARS-CoV-2 variant BA.3.2 is more poorly neutralized by responses focused on other contemporary strains

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Abstract

BA.3.2, a saltation variant of SARS-CoV-2 has 73 mutations in the spike sequence compared to ancestral Wuhan spike. BA.3, isolated from South Africa in November 2021 at a similar time to the BA.1 and BA.2 Omicron sub-lineages, appeared to fade out, however BA.3.2 was identified in South Africa in late 2024 and subsequently detected in other countries, maintaining a low-level presence. More recently derivatives of BA.3.2, largely BA.3.2.2, have markedly increased, comprising 13% (272/2099) of sequences deposited in March 2026 and have become dominant in several countries. Here we compare the neutralization capacity of serum collected a year ago from vaccinated volunteers and find that BA.3.2.2 is not more difficult to neutralize than other contemporaneously circulating strains such as XFG. However, we find that BA.3.2.2 is not neutralized by most of a panel of mAbs which potently neutralize the JN.1 variant. We explain this by structural analyses and suggest that the large evolutionary distance between JN.1-related variants and BA.3.2.2 is skewing the immune responses, increasing susceptibility to BA.3.2.2 accounting for its current uptick. Furthermore, structure and affinity measurements reveal a significantly remodelled RBD in the down configuration and increased affinity for ACE2. Finally, we looked at escape of BA.3.2.2 from commercially produced therapeutic mAbs and find that BA.3.2.2 has regained susceptibility to two mAbs produced by AstraZeneca.

INTRODUCTION

The COVID-19 pandemic and the ongoing endemic have been characterised by the continuous emergence of novel SARS-CoV-2 variants, each showing mutations in the viral spike (S) protein conferring further antibody evasion¹. New variants usually harbour a small number of S protein mutations, mainly in the receptor binding domain (RBD), where mutations can also increase the affinity to the host receptor angiotensin converting enzyme 2 (ACE2)², potentially giving the virus a transmission advantage³. Mutations at the binding sites for neutralizing anti-RBD antibodies can lead to a reduction in the neutralizing titres of immune serum, promoting immune escape and reinfection⁴. In addition, quantum leaps of SARS-CoV-2 evolution have been observed on three occasions, which are represented by long branch lengths in Fig. 1a. The first occurred at the end of 2021, when the highly mutated Omicron variant (B.1.1.529 or BA.1) became dominant, showing unprecedented antibody evasion⁵. The second was XBB, the first recombinant variant of BA.2 lineages⁶ (Fig. 1a), (BJ.1 and BA.2.75), in late 2022, which was very antibody-evasive but failed to spread fast until it acquired the F486P mutation, which increased affinity to ACE2, and proceeded to sweep the globe as XBB.1.5⁷. The third major evolutionary shift was observed in July 2023, when the BA.2.86, saltation variant of the BA.2 lineage, acquired the L455S mutation and replaced the XBB lineage⁸ (Fig. 1a).

A fourth major evolutionary shift in SARS-CoV-2 evolution seems to be unfolding, which began in late 2024, although this is difficult to track fully due to fewer sequences being submitted to GISAID (~ 10,000 in November 2025, compared to ~ 28,000 sequences in November 2024, GISAID EpiCoV database, <https://gisaid.org/>), (Fig. 1b). The first sequence of a highly mutated descendant of the Omicron

subvariant BA.3⁹ was collected at the end of November 2024 in South Africa and submitted to GISAID in March 2025 (EPI_ISL_19771107), (GISAID EpiCoV database, <https://gisaid.org/>). In Spring 2025, the variant was designated as BA.3.2, with additional sequences submitted from other countries, including Australia, the Netherlands, the USA and Germany. The first BA.3.2 sequence had 73 mutations in S and, according to subsequent sequences, has been allocated two sub-lineages based on signature mutations: BA.3.2.1 (H621R and P1162R) and BA.3.2.2 (K356T and A575S, a total of 75 mutations in S compared to Wuhan), (Fig. 1c). The shift to BA.3.2 is probably larger than the first major shift from Delta (11 S mutations) to Omicron (37 S mutations). It was included in the WHO list of “Currently circulating variants under monitoring” on 5th of December 2025 (<https://www.who.int/activities/tracking-SARS-CoV-2-variants>).

We estimate that the density of sequencing is now hundreds of fold lower than the point at which Omicron emerged, making it harder to reliably understand the infection dynamics, however according to GISAID, the most dominant variant globally is currently XFG, aka Stratus, followed by NB.1.8.1, aka Nimbus¹⁰. BA.3.2, nicknamed “Cicada,” after the insect’s ability to re-emerge after years underground, now appears to be spreading with 2281 sequences submitted for BA.3.2 and its derivatives from four continents, as of 9th of April 2026 (Fig. 1b). It showed a sharp increase to 5.8% (622/ 10747) of all reported sequences in December 2025, which has steadily risen to 13% (272/ 2099) in March 2026, and now dominates in several countries (Fig. 1b). BA.3.2 has been allocated many names (**Supplementary Figure S1**), making it difficult to track and count the spread.

Here we investigate the immune escape conferred by the numerous mutations in BA.3.2 using a panel of sera collected from the end of 2024 to spring 2025 following vaccination (mainly with the JN.1 vaccine) (**Supplementary Table S1**). We report that BA.3.2 shows less antibody evasion than XFG, but this may now be changing. We also assessed the ability of commercial monoclonal antibodies to neutralize BA.3.2 and discovered that AstraZeneca antibodies AZD8895 and AZD3152 are effective against it. We find that some of our previously isolated antibody panels against XBB.1.5⁸ and Omicron¹¹ work well against this variant, however looking at a panel of human mAbs isolated following infection with contemporary SARS-CoV-2 strains and selected to potently neutralize JN.1, the majority cannot neutralize BA.3.2, but work well against XFG and NB.1.8.1. Finally, we determine structures of representative antibodies, to provide structural explanations to the serological results. The mutations acquired by BA.3.2 suggest evasion from only a subset of B-cell epitopes, while one or two further mutations would probably be sufficient to significantly increase virus escape. Furthermore, the resistance to many anti-JN.1 antibodies suggests that even without further evolution BA.3.2 may acquire an advantage as exposure and vaccines amplify responses which are effective against contemporary viruses such as XFG but not against BA.3.2. Finally, we solve the structure of BA.3.2.2 spike in which the highly mutated RBDs are in the down conformation and show increased electrostatic complementarity to ACE2 which corresponds to a higher affinity interaction which may be helping to generate a competitive advantage for BA.3.2.2.

RESULTS

Initial characterisation of BA.3.2.2

We selected the BA.3.2 sequence from GISAID (EPI_ISL_19893762) and produced a pseudo-virus containing the corresponding 75 mutations in S. This sequence was subsequently renamed BA.3.2.2 and then RE.2.2 to represent the BA.3.2 variant with key spike mutations: K356T and A575S, in addition to those present in BA.3.2 (Fig. 1c). This variant is currently driving the BA.3.2 submissions to GISAID, with sequences from Australia, the Netherlands, Germany and the United Kingdom having the most entries (Fig. 1b, **Supplementary Figure S9**). The lag in depositions makes it hard to assess current trends, however in the GISAID release of 9th April 2026, for several countries, this variant made up over 40% of sequences for viruses gathered in February 2026 (Ireland, 64.5%; Denmark, 42.1%; Luxembourg, 48.3%; Norway, 66.3%; and Spain, 48.5%). Early submissions for March already show sharp increases in a number of countries, including France (45.9%), Italy (40%) and Spain (69.9%). We tested neutralization of BA.3.2.2 pseudo-virus along with pseudo-viruses expressing S from Victoria (ancestral strain Wuhan plus S247R), JN.1, NB.1.8.1 ("Nimbus") and XFG ("Stratus"), the current dominant variants globally. Neutralization assays were performed using sera obtained following vaccination from 41 volunteers collected between the end of 2024 and spring 2025 (for details see **Supplementary Table S1**) (Fig. 1d). Compared to the ancestral Victoria strain, all four variants JN.1, NB.1.8.1, XFG and BA.3.2.2 show immune evasion against serum: 3.5-fold ($p < 0.0001$), 3.6-fold ($p < 0.0001$), 11.4-fold ($p < 0.0001$), 4.9-fold ($p < 0.0001$) respectively, however BA.3.2.2 is easier to neutralize than XFG, with an increase in PVNT50 from 1/190 (XFG) to 1/440 (BA.3.2.2).

Next, we tested antibodies developed for clinical use against the same variants by neutralization assay (Fig. 1e). This set of mAbs is very potent against Victoria, but failed against JN.1, NB.1.8.1 and XFG. However, because BA.3.2.2 lacks the F456L and F486P mutations, it is well neutralized by AstraZeneca antibodies AZD8895 and AZD3152 (as well as AZD7442, which is a cocktail of AZD1061 and AZD8895) (Fig. 1d).

Isolation of mAbs from vaccinees suffering from breakthrough infections

Blood samples were obtained from 4 volunteers (**Supplementary Table S2**), three of whom had been vaccinated 5 times. In line with the rise to dominance of JN.1 at that time, one volunteer was infected by GE.1.2 (XBB.1.5 + N184T+N185del+F186I-G252V+D253G+T478R+P521S), one by EG.5 (XBB.1.5.10), and two by JN.1 (**Supplementary Table S2**). Single immunoglobulin G (IgG)-positive memory B cells were stained and sorted using a combination of JN.1 and KP.3 S trimers (Fig. 2a). In total, 690 S specific memory B cells were sorted, and 375 monoclonal antibodies (mAb) were recovered. After RT-PCR and Nested-PCR, heavy and light chain expression plasmids were constructed by Gibson assembly, and mAbs were expressed using 293T cells. Expressed mAbs were screened by ELISA using JN.1 and KP.3 S

trimers and further tested in a neutralization assay against JN.1 and KP.3 pseudo-viruses. 11 anti-RBD mAbs, with IC₅₀ neutralization titres < 100 ng/mL against both JN.1 and KP.3, were selected for further study, which we refer to as JN.1–1 to JN.1–13 (**Supplementary Table S3**), note JN.1–2 and JN.1–7 were essentially duplicates and removed.

Functional assessment of JN.1 mAbs

To assess the neutralizing ability of JN.1–1 to JN.1–13, we constructed a panel of pseudo-viruses, including SARS-CoV-2 variants Victoria, BA.2.86, JN.1, KP.3, NB.1.8.1, XFG and BA.3.2.2. Based on the neutralization profile against SARS-CoV-2 variants, the 11 JN.1 mAbs can be categorised into 4 groups (Fig. 2b):

Group 1: JN.1–1 and JN.1–3. There are similarities in the sequences of these mAbs (**Supplementary Table S3**) and also in the antibody neutralization patterns, which suggest they share similar binding poses. They neutralize BA.3.2.2 but not XFG, LP.8.1, KP.2 and LB.1, which suggests that they may contact R346, since the mutation R346T is not present in any other variant tested (**Supplementary Table S4**).

Group 2: JN.1–6. This antibody neutralizes all variants that appeared after the emergence of XBB.1.5 but poorly neutralizes the early variants predating BA.4 as well as BA.3.2.2 (**Supplementary Table S4**). We infer that the potential binding site of JN.1–6 on the RBD includes residue 486, which is a Pro on variants before BA.4/5, a Val for BA.4/5 and a Phe for later viruses. This would explain the reduced neutralizing ability against BA.4/5, and the complete loss of neutralization against Victoria and BA.2. It may also be affected by the N487D mutation present in LP.8.1.2 and XFG but missing from LP.8.1 and LP.8.1.1 and/or mutations at residue 478 (T478I in NB.1.81, T478N in BA.3.2.2, otherwise mainly T478K).

Group 3: JN.1–8 to JN.1–13. This set of mAbs has little or no neutralizing ability against Victoria, Alpha, Gamma, and Delta (data not shown), but are very potent against Omicron variants, which suggests that they bind at sites mutated in Omicron. JN.1–8, -10 and -12 likely contact residue 435, which has not undergone further mutation in Omicron variants, whereas the A435S mutation is present in NB1.8.1 and BA.3.2.2 against which these antibodies are ineffective (**Supplementary Table S4**).

Group 4: JN.1–4 and JN.1–5. This pair of mAbs has an unusually high percentage of mutations in their VH regions, 12.8% (21AA changes) in JN.1–4 and 35.1% (44AA changes) in JN.1–5, and their CDR2s harbour an amino acid deletion which is very rare (**Supplementary Table S3**). Both show unusually broad neutralizing ability, being effective against all variants tested in the assay with IC₅₀ < 1 µg/mL, and IC₅₀ < 0.1 µg/mL against BA.2.86, JN.1, KP.2, LB.1, KP.3, KP.3.1.1, LP.8.1 family and XFG (**Supplementary Table S4**).

To investigate the mechanism of neutralization of this broadly neutralizing group, we tested the ability of full-length JN.1–4 and JN.1–5 and their Fabs to antagonise ACE2 binding to XBB.1.5.10 S using an ELISA-based assay (**Supplementary Figure S2**). JN.1–9 was also included as a representative of Group 3

mAbs (above), XBB-9 as a positive control, and SD1-1, which does not contact the RBD¹², as a negative control. As expected, JN.1-9, which binds at the receptor binding site (below), blocked ACE2 binding effectively, whilst its Fab showed a slight drop in blocking ability. Interestingly, JN.1-4 and JN.1-5, although binding distant from the ACE2 footprint (see below), were also able to block the ACE2-S interaction, although less completely than JN.1-9 or XBB-9, achieving 80% blocking at 2.5 µg/mL. Furthermore, the reduction of blocking ability on conversion of IgG to Fabs was more significant for JN.1-4 and JN.1-5 than for JN.1-9, consistent with bivalent attachment of JN.1-4 and JN.1-5 to S enhancing blocking.

In summary, BA.3.2.2 was not neutralized by the majority of JN.1 neutralizing mAbs (JN.1-6 to JN.1-13 i.e. 7 out of 11) but was potently neutralized by group 1 (which did not neutralize XFG) and by the unusually broadly neutralizing group 4 antibodies.

Functional assessment of previously reported Omicron and XBB mAbs

We went on to test if mAbs we had previously isolated from earlier timepoints were able to neutralize BA.3.2.2 (Fig. 2c and 2d). We used a panel of mAbs that potently neutralize BA.1, and XBB.1.5^{8,11}. All XBB antibodies, except XBB-4, neutralized BA.3.2.2 with several retaining potent activity. Likewise for the BA.1 mAb panel (Omi) 16/26 retained some neutralizing activity against BA.3.2.2 with 6/26 showing NT50 values < 100 mg/ml¹¹. The substitutions on the RBD between BA.1 and BA.3.2.2 are shown in Fig. 2e. They are broadly spread across the surface but there is a region on the upper left of the back of the RBD that is relatively unchanged (nomenclature defined by Dejnirattisai et al.¹³). Omi antibodies that remain potent against BA.3.2.2 bind in a cluster in this region (Fig. 2f). Inspection of their footprints on the RBD shows that whilst the bulk of the interactions are with unchanged regions there are frequently substitutions on the periphery of the epitope that are tolerated (Fig. 2g).

Structural analysis of JN.1 mAb spike/RBD complexes

To visualize the detailed interactions of the anti-JN.1 mAbs with spike/RBD and the mechanism of neutralization, we selected five examples for structural studies covering all four categories of JN.1 mAb: (1) JN.1-3, (2) JN.1-6, (3) JN.1-9 and (4) JN.1-10 Fabs, by cryo-EM, each complexed with KP.3-RBD and, by crystallography, (5) JN.1-4 Fab complexed with Delta-RBD (**Supplementary Table S6**).

JN.1-3 (IGHV5-51, group 1, neutralizes BA.3.2.2) binds higher on the RBD, at the upper-right flank, centred at residues 343–346 and 439–445 of the RBD. Approaching from the right-front, JN.1-3 uses all three HC CDRs and CDR-L3 for binding, shielding about 780 Å² of the RBD solvent accessible surface. Although the tip of CDR-H3 nestles in an indentation composed of charged and hydrophilic RBD residues, nevertheless the contacts are mainly hydrophobic, making only two hydrogen bonds from the carbonyl oxygens of V103 and T104 to the side chains of N448 and R509, respectively. CDR-L3 interacts

with RBD residues E340-T345, hydrogen bonding to both the main chain and side chain of T345 (Fig. 3). JN.1-1 and JN.1-2 (whose sequences are almost identical) have the same genotypes in both heavy and light chains as JN.1-3, therefore, the three antibodies would be expected to bind in a similar mode. RBD residues R346 and V445H are both at the edge of the epitope, contacting CDR-H3 and H1, respectively. R346T and V445P/H/R mutations are frequently seen in the post-omicron SARS-CoV-2 variants. The R346T mutation abolishes neutralization by JN.1-1 irrespective of the mutation at V445, however, neutralization of JN.1-3 is rescued when V445 is mutated to a proline, as seen for the XBB variants (**Supplementary Table S4 and Fig. 3d, f**).

JN.1-6 (IGHV3-49, group 3, does not neutralize BA.3.2.2), spans the top of the RBD, making an interface of $920 \text{ \AA}^2$, with the heavy chain on the top of the neck of the RBD and the light chain on top of the left shoulder, slightly towards the front of the RBD. All six CDRs of JN.1-6 participate in binding. CDR-H3 extends to the back of the RBD left shoulder, contacting residues F486P, N487, Y489 and L456 and making a network of hydrogen bonds (Fig. 3h). CDR-H2 interacts with Q493E, S494 and Y449. Y31 of CDR-L1 makes extensive hydrophobic interactions with S477N, T478K, F486P and N487, as well as hydrogen bond interactions to the latter three residues (Fig. 3j). Residues on the JN.1-6 footprint are heavily mutated. In line with this, JN.1-6 does not neutralize the earlier SARS-CoV-2 variants or BA.3.2.2 (amongst these, only kappa is mutated in the JN.1-6 footprint, with a single mutation, T478K). JN.1-6 binding is sensitive to mutations Q493R in BA.1 and BA.2, F486V in BA.4/5 and N487D in LP.8.1.2, which lie within the antibody footprint (Fig. 2b and 3g-j).

JN.1-9 and JN.1-10 are from the largest set of IGHV4-30 JN.1 mAbs, group 3, none of which neutralize BA.3.2.2. Both Fabs bind at the side of the right shoulder tilting towards the back of the RBD in an almost identical pose (Fig. 4), making very small footprints (JN.1-9: $670 \text{ \AA}^2$, JN.1-10: $640 \text{ \AA}^2$). RBD residues 500-505 comprise the central binding epitope sandwiched between CDR-H3 and CDR-L3. The CDR-H3 of JN.1-9 is two residues longer than that of JN.1-10 and therefore makes more interactions. Y505H of the RBD makes extensive contacts to residues from all three HC CDRs of JN.1-9, while it only contacts residues from CDR-H3 and CDR-H1 of JN.1-10 (Fig. 4e and 4i). The earlier variants and BA.3.2.2, which don't contain the Y505H mutation, cannot be neutralized by JN.1-9 and JN.1-10 (**Supplementary Table S4**). The A435S mutation in variant NB.1.8.1 significantly reduces the neutralization ability of JN.1-10 (**Supplementary Table S4**). Residue A435 is in the hydrophobic core between the RBD beta-sheet and the $\alpha 4$ helix (residues 405-411) and is about $10 \text{ \AA}$ from the nearest JN.1-10 residue, I58, which contacts the N-terminus of $\alpha 4$ (Fig. 4j). The A435S mutation could disturb the $\alpha 4$ helix, and therefore the interactions with I58. The residue corresponding to I58 in JN.1-9 is a smaller serine, which does not affect the activity of JN.1-9 (**Supplementary Table S4**).

JN.1-4 (gene family IGHV4-61) is a member of group 4 which neutralize all the SARS-CoV-2 variants tested, including BA.3.2.2) and bind in a highly unusual way, tilted backwards to the lower-right flank of the RBD. All six CDRs interact with the RBD to form a substantial interface of $1140 \text{ \AA}^2$. The footprint has some overlap with that of mAb EY6A¹⁴, but is separated by the N343 glycan from the footprint of S309⁴ (Fig. 5 and **Supplementary Figure S3**). Remarkably, JN.1-4 binding causes a significant conformational

change in the RBD, displacing and unwinding the RBD $\alpha 2$ helix (residues 365–369) to create a pocket into which the relatively long (15-residue) and hydrophobic CDR-H3 is inserted. Residues lining the pocket are all aromatic apart from I387 and L434, and these residues are strictly conserved in all SARS-CoV-2 variants. Intriguingly, this pocket has been observed in the locked-down RBD conformation where it is occupied by a bound lipid^{15,16}, but in that case, the $\alpha 2$ helix is not unwound (Fig. 5). RBD residue L368 in the unwound helix makes tight hydrophobic interactions with residues from three HC CDRs (Fig. 5g). Some mutations outside the pocket modulate the effectiveness of this group of antibodies, thus the L368I mutation found in the XBB variants likely creates unfavourable contacts, explaining their weaker neutralization by JN.1–4 (**Supplementary Table S4**). Conversely, the N96-G97 peptide of the CDR-L3 packs against S373 of the RBD, and the S373P mutation found in all post-omicron variants probably makes this interaction more favourable (Fig. 5h). JN.1–5 has the same genotype and high sequence identity to JN.1–4. A low-resolution crystal structure of the Delta-RBD/JN.1–5 complex confirms that it binds in the same position as JN.1–4 (crystals were isomorphous with JN.1–4 but yielded data too weak to reliably refine the structure).

Mechanisms of neutralization of the anti-JN.1 antibodies

The footprints of JN.1–6, JN.1–9 and JN.1–10 partially overlap with that of ACE2, so that binding of these antibodies directly blocks interaction with the receptor. The mechanisms for the other antibodies are less obvious; JN.1–4 and JN.1–5 may lock the RBD down, preventing ACE2 engagement or since incomplete ACE2 blocking is observed, bivalent attachment may be required for them to be effective.

A set of commercial mAbs regain potency against BA.3.2.2

We noted above that BA.3.2.2 lacks the F456L and F486P mutations, which knocked out the AstraZeneca therapeutic antibodies AZD1061, AZD8895 and AZD3152, however, there are many other mutations in BA.3.2.2, some of which impinge on the footprints of AZs three therapeutic mAbs (Fig. 6). AZD1061 is the only AZ antibody to bind to the front of the RBD and does not neutralize BA.3.2.2. In line with this, there are several mutations within the antibody footprint, most notably G446D, which is in close contact with light chain aromatic residues Y55 and W56. Insertion of the relatively bulky charged aspartic acid sidechain would disrupt the binding interface to abrogate binding (Fig. 6a). AZD8895 binds at the very top of the left shoulder, with no mutations within its footprint that would interfere with binding (Fig. 6b), explaining its undiminished potency. AZD3152, which binds towards the top of the back of RBD, within the cluster of Omi antibodies that remain potent, has some mutations within the footprint, however, these are not in key residues, explaining the continuing effectiveness of this antibody against BA.3.2.2 (Fig. 6c).

Structural analysis of BA.3.2.2 spike

To confirm the structural inferences above we determined the structure of the BA.3.2.2 spike by cryo-EM at a resolution of 2.9 Å (Fig. 7a). Despite the large number of mutations, the overall structure resembles other versions of the SARS-CoV-2 spike. In all three subunits, the RBDs are in a 'down' conformation, however, there are slight differences in the RBD positioning between the spike particles seen in cryo-EM. Nevertheless, it was possible to define a subset of particles where the RBD configurations were sufficiently similar to obtain good electron density for one RBD, allowing its structure to be reliably determined. This reveals that there are considerable changes in the structure of the BA.3.2.2 RBD compared to all other variants. In particular, the receptor binding motif at the top of the RBD shows substantial rearrangements of the polypeptide chain towards the outside of both shoulders, leading to topological and electrostatic changes on that part of the surface that engages the ACE2 receptor (Fig. 7b, c). The conformational changes in the structure of the BA.3.2.2 reinforce the observations above, for instance the Omi antibodies which remain effective tend to cluster in a region at the back of the RBD where there is minimal conformation change (Fig. 7c), whilst the single AZ therapeutic antibody which is knocked out (AZD1061) binds at the front of the RBD, where the structure is more perturbed.

The electrostatic changes in the RBD improve its complementarity with the ACE2 epitope (Fig. 7b) whilst shape changes are towards the perimeter of the epitope, so that receptor affinity is not expected to be compromised. To investigate the effect on ACE2 affinity, we performed BLI binding assays for ACE2 with 4 different RBDs, Wuhan, BA.1, KP.3 and BA.3.2.2. The results are shown in (Supplementary Figure S4, Table S5). Although the absolute values for such measurements are hard to relate to the in vivo situation, the comparative values show a steady increase in binding affinity from Wuhan, to BA.1 to KP.3, to BA.3.2.2 (KDs 216, 182, 93 and 66 nM, respectively) with a corresponding slowing of the off-rate for the interaction. This suggests that BA.3.2.2 may have a slight advantage over the other viruses in terms of infectivity. Although some of this advantage may be sacrificed by the downwards disposition of the RBDs in the BA.3.2.2 spike, there may be a compensating advantage from reducing the exposure of major epitopes to antibodies.

DISCUSSION

Despite remaining at a relatively low level for months the number of sequences reported for BA.3.2 has continued to grow and it is now important to consider what level of threat this very highly mutated variant represents in terms of its susceptibility to circulating neutralizing antibodies, which themselves are likely to have changed since BA.3.2 first appeared, driven by infection or vaccination with the most contemporary strains.

The limited studies to date are not in apparent agreement. The first published study of BA.3.2¹⁷ used BA.3.2.1 pseudo-virus lacking the 214ASDT insertion and the K356T and A575S mutations in the spike. NT₅₀ values (from convalescent plasma collected in China from individuals reinfected with JN.1) were worse than titres against XFG and NB.1.8.1, however subsequent studies are broadly in line with the results presented here with escape from immune serum XFG > BA.3.2 > NB.1.8.1^{18–21}.

Neutralization studies of SARS-CoV-2 variants have become naturally more complex as individuals' vaccination and infection histories have become complicated and divergent, mirroring the increasing complexity of virus lineages. Comparative studies are also caveated by the inevitable slight variations in the mutations selected to define circulating viruses. However, it remains important to monitor SARS-CoV-2 evolution to assess the immunity provided by current vaccine strains and to maintain preparedness and update vaccines when required. The very recent rise in the proportion of BA.3.2 submitted sequences (13% in March 2026) may indicate that it is about to start replacing other strains. Especially concerning are recent data suggesting that children between 3 and 15 years old are about five times more likely to contract BA.3.2 than any other variant

(<https://edition.cnn.com/2026/04/02/health/new-covid-variant-cicada>), this is in-line with our observations since any responses they have against SARS-CoV-2 are likely to be skewed towards the alternative lineage of JN.1 derived variants and less effective against BA.3.2.2. Furthermore, the variant will continue to acquire further mutations, and we suggest that a small number of correctly placed mutations could significantly enhance its escape from existing responses, as occurred on the path to JN.1.

There is a question as to how BA.3.2 has maintained a foothold and now seems to be increasing relative to XFG, when in our neutralization assays and those of others, it appears easier to neutralize than XFG, although it must be remembered that the sera used in these assays was collected a while ago and immunity today is being driven by the currently dominant lineages such as XFG and current vaccine strains which show marked antigenic differences to BA.3.2. XFG, and other JN.1 sub lineages, evade antibodies targeting diverse epitopes on the spike protein, however we show that the antibody responses to these JN.1 derived strains have developed a major blind spot for BA.3.2.2 which maintains some of the antigenic characteristics of much earlier viruses from which it is derived, in particular it lacks the highly disruptive mutations R346T, L455S, F456L, N487D, and Q493E present in XFG. Interestingly, whilst many anti-JN.1 antibodies were ineffective against BA.3.2.2, we discovered a small group of antibodies targeting the conserved RBD lipid-binding pocket, which are broadly reactive including against BA.3.2.2.

The fact that most of the potent JN.1 neutralizing mAbs we describe are ineffective against BA.3.2.2 suggests that BA.3.2.2 may develop a significant advantage as antibody responses induced by natural infection or vaccination to earlier lineages such as BA.1 or XBB wane, being replaced by antibodies focussed on epitopes present in the more contemporary JN.1 sub lineages but absent from BA.3.2.2. This waning of early immunity and skewing to the more contemporary antigenic variants may be allowing BA.3.2.2 to get a foothold. The mutations in the ACE2 binding site have maintained a relatively high affinity interaction between BA.3.2.2 RBD and ACE2, which may also be contributing to the maintenance and rise of the BA.3.2.2 lineage in a number of areas.

As the endemic period of SARS CoV-2 evolves, we may see more emergence of throwback variants which exploit the waning of responses to earlier variants. Finally, we note that a very small number of further mutations, such as A475V²², G476S²³, or F490S,²⁴ could allow BA.3.2.2 to achieve significant

escape from many of the antibodies it is currently susceptible to, which could allow it to outcompete the currently dominant JN.1-derived strains.

METHODS

Bacteria strains and cell culture

HEK293T (ATCC CRL-11268) cells were cultured in DMEM low glucose (Sigma-Aldrich, #D5523) supplemented with 10% FBS, and 100 U/ml of penicillin–streptomycin (Sigma, #G6784) at 37°C with 5% CO₂. Human mAbs were expressed in HEK293T cells cultured in FreeStyle™ 293 Expression Medium (Gibco, #12338-018) at 37°C with 5% CO₂. JN.1 and KP.3 soluble spikes and ACE2 were expressed in HEK293T cells cultured in FreeStyle™ 293 Expression Medium at 37°C with 5% CO₂. *E. coli* DH5α bacteria were used for transformation and preparation of plasmids. A single colony was picked and cultured in LB broth overnight in a shaker set up at 37°C at 200 rpm. For pseudotyped lentivirus production and test, HEK293T/17 cells were cultured in Dulbecco's Modified Eagle medium (DMEM) high glucose (Sigma-Aldrich) supplemented with 10% FBS, 2 mM GlutaMAX (Gibco), and 100 U/ml of penicillin–streptomycin (Sigma-Aldrich) at 37°C.

Sources of commercial antibodies

AstraZeneca antibodies, AZD1061, AZD8895, AZD7442 and AZD3152, and Regeneron antibodies, REGN10933 and REGN10987, were provided by AstraZeneca. S309, ADG20 and Ly-CoV1404 were provided by Adagio.

Sera and PBMC from breakthrough infections

Following informed consent, individuals with omicron GE1.2, EG.5 and JN.1 breakthrough infections were co-enrolled into one or more of the following three studies: the ISARIC/WHO Clinical Characterization Protocol for Severe Emerging Infections [Oxford REC C, reference 13/SC/0149], the “Innate and adaptive immunity against SARS-CoV-2 in healthcare worker family and household members” protocol (approved by the University of Oxford Central University Research Ethics Committee), or the Gastro-intestinal illness in Oxford: COVID sub-study [Sheffield REC, reference 16/YH/0247]. The diagnosis was confirmed through reporting of symptoms consistent with COVID-19, hospital presentation, and a positive test for SARS-CoV-2 using reverse transcriptase polymerase chain reaction (RT-PCR) from an upper respiratory tract (nose/throat) swab tested in accredited laboratories and lineage sequence confirmed through national reference laboratories in the United Kingdom.

For the serum samples collected between October 2024 and May 2025, a blood sample was taken following consent at least 26 days after the last vaccination. Clinical information, including times between last vaccination and sampling, infection history, and age of participant was captured for all

individuals at the time of sampling. Sex and gender were not considered in the study design. The sex and gender of participants were determined based on self-report.

Isolation of JN.1/KP.3-S-specific single B cells by FACS

To isolate JN.1/KP.3-S-specific single memory B cells, PBMCs from GE.1.2, EG.5 or JN.1 breakthrough infection cases were stained with LIVE/DEAD Fixable Aqua dye (Invitrogen, #L34957) before adding recombinant trimeric S-twin-Strep of JN.1 and KP.3. Cells were then stained with a mixture of CD3-FITC (7.5 µl in 100 µl sorting buffer, BD, #555332), CD14-FITC (7.5 µl in 100 µl sorting buffer, BD, #555397), CD16-FITC (7.5 µl in 100 µl sorting buffer, BD, #555406), CD56-FITC (1.5 µl in 100 µl sorting buffer, BD, #562794), IgM-FITC (7.5 µl in 100 µl sorting buffer, BD, #555782), IgA-FITC (1.5 µl in 100 µl sorting buffer, Dako, #F0188), IgD-FITC (1.5 µl in 100 µl sorting buffer, Dako, #F0189), IgG-BV786 (1.5 µl in 100 µl sorting buffer, BD, #564230) and CD19-BUV395 (1.5 µl in 100 µl sorting buffer, BD, #563549), along with Strep-MAB-DY549 (1.5 µl in 100 µl sorting buffer, iba, #2-1566-050) to stain the twin-strep tag of the S protein. IgG⁺ memory B cells were gated as CD19⁺, IgG⁺, CD3⁻, CD14⁻, CD56⁻, CD16⁻, IgM⁻, IgA⁻, and IgD⁻, and S⁺ was further selected, and single cells were sorted into 96-well PCR plates with 10 µl of catching buffer (Tris, Nuclease free-H₂O and RNase inhibitor). Plates were briefly centrifuged at 2000 × g for 1 min and left on dry ice before being stored at - 80°C.

Cloning and expression of human mAbs

JN.1/KP.3 S-specific human mAbs were cloned and expressed as described previously¹³. Briefly, RT-PCR and Nested-PCR were performed to amplify genes for Ig IGHV, IgVk, and IgVλ from sorted single memory B cells, using a cocktail of primers specific to human IgG. Nested-PCR products were ligated into the expression vectors of human IgG1 or Ig κ-chain or λ-chain by Gibson assembly. To express mAbs, plasmids encoding HCs and LCs were co-transfected by PEI-transfection into a HEK293T cell line, and supernatants containing mAbs were collected and filtered 4–5 days after transfection. The supernatants were further characterized or purified by protein A/G beads.

Fab production

Plasmids for expressing heavy chains of the Fabs were constructed by amplifying the corresponding IgG heavy chain expression vectors using the primers Fin_dbST_Hvec (CATCCACAGTTCGAGAAATAGGTGCGACGGCCGCAAG) and Rin_CH1_SC_G1_TB (GTGGGACCAGGCACTACTTCCGCGTGGCACCAGACAAGATTTGGGCTCAACTCTCTTG) to get rid of the CH2 and CH3 parts of the heavy chains. In the meantime, the twin-strep tag was amplified by using the primers Fin_CH1_SC_G1_TB (CAAGAGAGTTGAGCCCAAATCTTGTCTGGTGCCACGCGGAAGTAGTGCCTGGTCCCAC) and Rin_dbST_Hvec (CTTGCCGGCCGTCGCACCTATTTCTCGAACTGTGGATG). The two PCR fragments were

joint by Gibson assembly. Fabs were expressed by co-transfecting plasmids encoding HCs and LCs using PEI-transfection into a HEK293T cell line, and supernatants containing Fabs were collected and filtered 4–5 days after transfection. The Fabs were further purified from the supernatant with Strep-Tactin XT resin (IBA lifesciences).

ACE2 binding inhibition assay by ELISA

MAXISORP immunoplates were coated with 5 µg/mL of purified ACE2-His protein overnight at 4°C and then blocked by 2% BSA in PBS. Meanwhile, mAbs were serially diluted and mixed with 2.5 µg/mL of recombinant XBB.1.5.10 trimeric S-twin-Strep. Antibody-S protein mixtures were incubated at 37°C for 1 h. After incubation, the mixtures were transferred into the ACE2-coated plates and incubated for 1 h at 37°C. After wash, StrepMAB-Classic (2-1507-001, iba) was diluted to 0.2 µg/mL by 2% BSA and used as primary antibody, followed by goat anti-mouse IgG-AP (#A16093, Invitrogen) at 1:2000 dilution. The reaction was developed by adding PNPP substrate, and the absorbance was measured at 405nm after 1 h. The ACE2/S binding inhibition was calculated by comparing to the antibody-free control wells. IC₅₀ was determined using the Probit program from the SPSS package.

Pseudovirus plasmid construction and lentiviral particle production

Pseudotyped lentivirus expressing SARS-CoV-2 S proteins from Victoria, BA.1, BA.2, BA.4, BA.4.6, BQ.1.1, XBB, XBB.1, XBB.1.5, XBB.1.5.10, XBB.1.5.70, BA.2.86 and JN.1 were constructed as described previously²⁵ with some modifications^{8,11,23,26,27}. The same method was used to construct BA.3, BA.1 (Omicron) was used as a template, while primers were used to remove ins214EPE, G496S, T547K, N856K and L981F, but add S317F and D405N mutations. KP.2 was made by introducing R346T+F456L+V1104L into JN.1. LB.1 by introducing S31del+Q183H+R346T+F456L into JN.1. KP.3 by introducing F456L+Q493E+V1104L into JN.1, then addition of S31del and of KP.3 + T22N+F59S was used to make KP.3.1.1 and XEC, respectively. LP.8.1 by adding F186L+R190S+R346T+V445R+K1086R to KP.3.1.1, while K679R and N487D we added individually to make LP.8.1.1 and LP.8.1.2. “Nimbus”, also known as NB.1.8.1 was created by introducing G184S+A435S+T478I-V1104L in XEC, and “Stratus”, also known as XFG, was created by introducing T22N+S31P+K182R +R190S+R346T+K444R+V445R+N487D+T572I-V1104L in KP.3. The plasmid to create BA.3.2.2 PV was custom synthesized by Integrated DNA Technologies based on the wild-type SARS-CoV-2 (EPI_ISL_19893762) and cloned into pcDNA3.1 plasmid²⁸. This plasmid carries the S gene and was used for generating pseudoviral particles together with the lentiviral packaging vector and transfer vector encoding a luciferase reporter. A BA.3.2.2 plasmid containing the following mutations was produced: P9L, R21T, P26L, A67V, H69del, V70del, T95I, I101T, C136del, N137del, D138del, P139del, F140del, L141del, G142del, V143del, Y144del, Y145del, H146del, K147del, F157S, N164K, S172F, K187T, N211del, L212I, ins214ASDT, A243del, L244del, P251S, I326V, G339Y, A348P, K356T, S371F, S373P, S375F, R403K, D405N, R408S, K417N, A435S, N440R, V445A,

G446D, L452W, N460K, S477N, T478N, E484K, G496S, Q498R, N501Y, K529N, E554D, A575S, E583D, D614G, H625R, N641K, V642G, E654K, H655Y, N679R, P681H, A688D, S704L, N764K, K795T, D796Y, A852K, S939F, Q954H, N969K, D1184E. All the constructs were sequence confirmed.

Pseudovirus neutralization test

The pseudoviral neutralization test has been described previously²⁹. Briefly, 4-fold serial diluted mAbs were incubated with pseudoviral particles at 37°C, 5% CO₂ for 1 h. Stable HEK293T/17 cells expressing human ACE2 were then added to the mixture at 1.5×10^4 cells/well. 48 h post infection, culture supernatants were removed, and 50 µL of 1:2 Bright-Glo™ Luciferase assay system (Promega, USA) in 1 × PBS was added to each well. The reaction was incubated at room temperature for 5 min, and firefly luciferase activity was measured using CLARIOstar (BMG Labtech, Ortenberg, Germany). The percentage neutralization was calculated relative to the control. Probit analysis was used to estimate the dilution that inhibited half maximum pseudotyped lentivirus infection (PVNT50).

To determine the neutralizing activity of patient serum samples, 3-fold serial dilutions of each sample were incubated with pseudoviral particles for 1 h and the same strategy as mAb was applied.

Construction of trimeric spike of SARS-CoV-2 JN.1, KP.3 and BA.3.2.2

Expression plasmids of XBB.1.5.10, and JN.1 spikes were constructed encoding for human codon-optimised sequences from wild-type SARS-CoV-2 (MN908947), XBB.1.5.10 (EPI_ISL_16917117) and JN.1 (EPI_ISL_18300149) as previously described⁸. Briefly, fragments were cloned into pHLsec vectors downstream of the chicken β-actin/rabbit β-globin hybrid promoter and followed by a T4 fibrin trimerization domain, an HRV 3C cleavage site, a His-8 tag and a Twin-Strep-tag at the C terminus as previously reported by Wrapp et al.³⁰. To construct KP.3 (EPI_ISL_18993074) spike, primers were used to insert F456L, Q493E and V1104L mutations into a pHLsec vector expressing JN.1 spike. The plasmid to create BA.3.2.2 S was custom synthesized by Integrated DNA Technologies based on the wild-type SARS-CoV-2 (EPI_ISL_19893762) and cloned into pHLsec vector. A BA.3.2.2 plasmid containing the following mutations was produced: P9L, R21T, P26L, A67V, H69del, V70del, T95I, I101T, C136del, N137del, D138del, P139del, F140del, L141del, G142del, V143del, Y144del, Y145del, H146del, K147del, F157S, N164K, S172F, K187T, N211del, L212I, ins214ASDT, A243del, L244del, P251S, I326V, G339Y, A348P, K356T, S371F, S373P, S375F, R403K, D405N, R408S, K417N, A435S, N440R, V445A, G446D, L452W, N460K, S477N, T478N, E484K, G496S, Q498R, N501Y, K529N, E554D, A575S, E583D, D614G, H625R, N641K, V642G, E654K, H655Y, N679R, P681H, A688D, S704L, N764K, K795T, D796Y, A852K, S939F, Q954H, N969K, D1184E. All the constructs were sequence confirmed.

Cloning of RBDs

Wuhan and BA.1 RBD constructs were created as previously described^{11, 13}. To construct the expression plasmid of Delta RBD, L452R and T478K mutations were introduced into the plasmid of pNeo-Wuhan RBD by PCR and ligation. To construct the expression plasmid of KP.3 and BA.3.2.2 RBD, the RBD fragments were amplified from KP.3 and BA.3.2.2 SS plasmids and ligated into pHLSec vector using Gibson assembly. All constructs were verified by sequencing.

Viral glycoprotein production

Protein expression and purification were as reported previously. Briefly, Twin-strep tagged JN.1, KP.3 and BA.3.2.2 spikes, all with the 2P mutation and deletion of the furin cleavage site, were transiently transfected in HEK293T cells and purified with Strep-Tactin XT resin (IBA lifesciences). Purified protein was validated by SDS-PAGE and concentrated using a 100 kDa Amicon Centrifugal Filter.

Plasmids encoding WH, Delta and BA.1 RBD were transiently transfected into Expi293F™ cells. Four days after transfection, the conditioned medium was harvested, filtered and buffer-exchanged using QuixStand benchtop system (Amersham Biosciences). The sample was purified with a 5 mL HisTrap nickel column (Cytiva) and further purified using a Superdex 75 HiLoad 16/60 gel filtration column (Cytiva). SDS-PAGE was used to validate the proteins followed by concentrating the proteins using a 10 kDa Amicon Centrifugal Filter. Plasmids encoding KP.3 and BA.3.2.2 RBD were transiently transfected in HEK293T cells and purified with Strep-Tactin XT resin (IBA lifesciences). Purified protein was validated by SDS-PAGE and concentrated using a 100 kDa Amicon Centrifugal Filter.

X-ray Crystallography

Delta RBD was mixed with JN.1–4 Fab in a 1:1 molar ratio, with a final concentration of 9.8 and 4.4 mg/mL and incubated at room temperature for 20 minutes. Crystalquick 96-well X plates (Greiner Bio-One) were used to prepare screens with a Cartesian Robot using the nanoliter sitting-drop vapour-diffusion method, with 100 nL protein with 100 nL reservoir in each drop, as previously described³¹. Crystals formed in a PEGRx (Hampton Research) condition with sodium citrate tribasic dihydrate pH5.5 and 18% w/v PEG 3350.

Crystals were loop mounted and frozen in liquid nitrogen. Diffraction data were collected at 100K at beamline I03, Diamond Light Source, UK as a part of unattended automated data collection at I03 (mx34263-124).

Processing used the automated pipeline at Diamond³², the RBD and Fab were located by molecular replacement³³ and refined using Phenix³⁴. Refinement statistics may be found in table S3.

Cryo-Electron Microscopy

A 3 μ L aliquot of purified BA.3.2.2 spike in PBS or either the JN.1 or KP.3 spikes mixed with Fab (1:6 molar ratio) was prepared, aspirated and almost immediately applied to a freshly glow-discharged (20 s, Plasma Cleaner PDC-002-CE, Harrick Plasma) copper C-flat 2/1-200 mesh holey grid (Protochips). Excess liquid was removed by blotting for 5 s with a force of -1 using vitrobot filter paper (grade 595, Ted Pella Inc.) at 5.5°C, 100% reported humidity before plunge freezing into liquid ethane using a Vitrobot Mark IV (Thermo Fisher). Fab/Spike complexes were prepared immediately prior to application to grids and plunge freezing.

For JN.1–9, JN.1–10 and JN.1–6 with KP.3 spike, data were collected in EER format using EPU acquisition software (ThermoFisher) on a 300 kV Titan Krios G3i microscope equipped with a Falcon-4i detector with selectris-X energy filter and 50 μ m aperture and 100 μ m objective aperture, with a total dose of 40 e/ $\text{\AA}^2$ with a magnification of 165kX corresponding to a 0.7303 $\text{\AA}/\text{pix}$ calibrated pixel size with fringe free illumination in EER format. For the JN.1–3/KP.3 spike complex, data were collected in EER format as above except using a Titan Krios at eBIC (BI34631-29). For the BA.3.2.2 spike, movies were collected with a 50 e/ $\text{\AA}^2$ total dose using EPU on a Titan Krios G4i with SelectrisX/FalconIV in tiff format.

All processing was performed with cryoSPARC v4 with all preprocessing (movie alignment, CTF and motion correction, particle picking and extraction) using cryoSPARC live interface³⁵. Picked particles were classified in 2D into 350 classes (150 in the case of JN.1–10 complex) with 'side view' Spike particles being selected for further processing. For JN1-9, significant preferential orientation was observed, which was mitigated by two cycles of Ab initio processing. In the case of all complexes except for JN.1–3, it was necessary, due to the heterogeneity of RBD poses, to run a local refinement job focussing on a single RBD/Fab (in the case of JN.1–3, no improvement was achieved with such focused refinement). For the BA.3.2.2 spike, 663729 particles were picked and binned four times. This particle set was classified in 2D (250 classes) and the 14 classes showing clear secondary structure and not top views were selected (142112 particles) and used to generate three ab initio models. After classification the ca 100K particles then underwent non-uniform refinement³⁶ before unbinning and further refinement, resulting in a structure to 2.8 $\text{\AA}$ resolution. Although the main body of the spike was well resolved (S2 region and latter part of S1), the RBDs were relatively poorly resolved, with two RBDs being slightly better resolved out of the three and slight deviation from C3 symmetry (consistent with the decision to do all the refinements in C1 symmetry). After trialling various masks around the RBDs/NTDs), the best alignment-free classification was achieved with a mask encompassing the two best resolved RBDs and classification into five classes. There was one class where the RBD density was clearest (28786 particles) and this was further refined resulting in a reconstruction with gold-standard resolution of 3 $\text{\AA}$, B-factor 41 $\text{\AA}^2$. Processing workflows and numbers of particles filtered at each stage can be found in Supplementary Figs, S5-8.

Initial models for each of the fabs were generated using AlphaFold 3³⁷ while the RBD from PDB 8CIM³⁸ was used as a basis for the RBD. Both models were initially rigid body fitted into their respective final

maps using ChimeraX³⁹. The model was then refined using PHENIX³⁴ and rebuilt using COOT⁴⁰ (Supplementary Table S6). Molecular graphics and analyses were performed with ChimeraX³⁹. Alphafold3³⁷ was used to generate an initial model of the BA.3.2.2 spike. However, it was found that the positions of the NTD and RBDs were incorrect, so these domains were rigid body fitted separately before rebuilding of some of the RBD 'shoulder' loops that were also incorrectly predicted using COOT⁴⁰ and refined with PHENIX³⁴.

Affinity determination using Biolayer Interferometry Assay (BLI)

For affinity determination, Octet RED 96e (ForteBio) and Amine Reactive 2nd Generation (AR2G) Biosensors (ForteBio, 18-5092) were used to determine the binding affinities of the receptor binding domain (RBD) of different SARS-CoV-2 variants with ACE2. Before performing kinetic assays, pH and RBD loading concentration was optimised. All measurements were performed at 30°C with a shaking speed of 1000 rpm. In brief, 17.5 µg/mL RBDs were first immobilised onto activated AR2G sensors in immobilising buffer (10 mM Sodium Acetate, pH 6.0) for 300 s; after quenching (150 s) and baseline (120 s), the sensors were dipped into wells containing ACE2 with 2-fold serial dilutions in kinetic buffer (DPBS, 1% BSA, 0.002% Tween-20, 0.6 M Sucrose) for association (200 s), followed by dipping into kinetic buffer again for dissociation (200 s). Note the addition of 0.6M sucrose to the kinetic buffer was to prevent non-specific binding⁴¹. Kinetic parameters (K_d, k_{off}) were calculated using Data Analysis HT 11.1 Software (ForteBio) with a 1:1 global fitting model. Three independent kinetic measurements were conducted for each RBD sample.

Data Availability

The data that support this study are available from the corresponding author upon request. The atomic models and cryo-EM density maps have been deposited into the Protein Data Bank (PDB) and Electron Microscopy Data Bank (EMDB) as follows: Delta-RBD/JN.1–4 Fab: PDB 9TXF, KP.3-RBD/JN.1–9 Fab local refinement: PDB 9SF7/ EMD-54815, RBD/JN.1–10 Fab local refinement: PDB 29RE/ EMD-57321, KP.3-RBD/JN.1–6 Fab local refinement: PDB 9SF5/ EMD-54814, KP.3-RBD/JN.1–3 Fab: PDB 9SHB/ EMD-54894, BA.3.2.2 Spike: PDB 29SU/ EMD-57358. Source data are provided with this paper.

Limitations of the study

Limitations of this study are that the in vitro neutralization assays we used do not probe the full function of the antibody response as they do not measure the effects of complement or antibody dependent cell mediated cytotoxicity which operate in vivo. In addition, as live BA.3.2.2 virus was not available in our laboratory we relied on lentiviral pseudo-neutralization assays for characterization. Furthermore, they do

not take account of T cell responses, which have been shown to be more resilient to the mutations expressed by VoC.

Declarations

CONFLICT OF INTEREST STATEMENT

Declaration of interests G.R.S. sits as a NED on the GSK Board and is a founder member of RQ Biotechnology. Oxford University holds intellectual property related to SARS-CoV-2 mAbs discovered in G.R.S.'s laboratory.

Author contributions

A.D.-G., C.L., P.S., G.R.S., and J.M. generated and characterized mAbs. A.D.-G., C.L., and P.S. generated pseudoviruses and performed and analysed neutralization tests. Z.P.L. performed BLI experiments. H.M.E.D. performed cryo-EM analyses and with J.R. built the models and refined the structures and with E.E.F. and D.I.S. analysed the structural results. B.K., K.J., L.S., P.K. and S.J.D. with the PITCH consortium assisted with patient samples and vaccine studies in the OPTIC Healthcare Worker and the Sheffield STH-Obs studies. S.J.D., and P.K. conceived the study of vaccinated healthcare workers and oversaw study and sample collection/processing. G.R.S., and D.I.S. conceived the study and wrote the initial manuscript draft with other authors providing editorial comments. All authors read and approved the manuscript.

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Figures

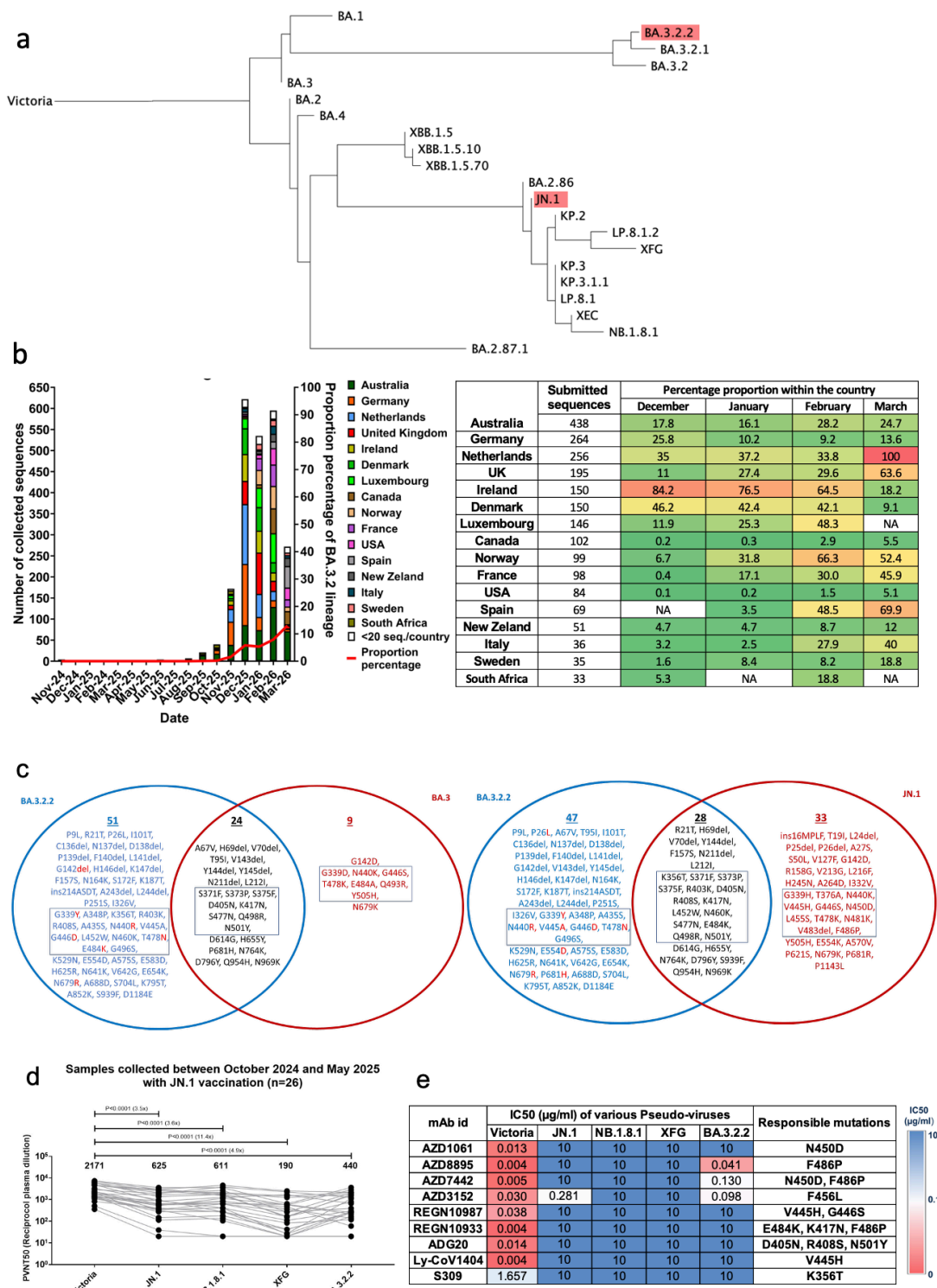


Figure 1

Figure 1

Characteristics of BA.3.2.2. (a) Tree representing divergence in Spike sequences. Pathlengths are the expected amino-acid substitutions per site. Sequence alignment was done with MAFFT⁴² and the signal sequence was removed prior to calculating the tree with IQTREE2⁴³. The tree was visualized with FIGTREE (<http://tree.bio.ed.ac.uk/software/figtree/>). (b) Increasing numbers of submitted BA.3.2 lineage sequences over time that now include four continents. Left Y axis shows the number of sequences per

month designated as BA.3.2 lineage, while the right Y axis shows percentage proportion of BA.3.2 lineage within all submitted sequences to GISAID. On the right: 16 countries which submitted more than 20 sequences to date to GISAID from BA.3.2 lineage. Heat map showing the percentage proportion of BA.3.2 lineage for that country starting December 2025 and finishing March 2026. Information from GISAID database was used to create this heat map on 09.04.2026.

(c) Venn Diagram of shared and unique mutations between BA.3.2.2 (EPI_ISL_19893762) and BA.3 (EPI_ISL_7605589) on the left and between BA.3.2.2 and JN.1 (EPI_ISL_18300149) on the right. BA.3.2.2 has 75 S mutations compared to 33 in BA.3 and 61 in JN.1. Of the 33, 24 are shared, while 51 are unique. With JN.1, 28 are shared, while 33 mutations are unique, including L455S and F486P, but BA.3.2.2 has 47 unique S mutations and of them, 9 are changes to already existing mutations in JN.1 (positions 26, 339, 440, 445, 446, 478, 554, 679 and 681), they are highlighted in red. (d) Geometric mean PVNT50 values for the indicated viruses using serum obtained from vaccinated volunteers from late 2024 to May in 2025 (n = 41). Sera samples were collected in the UK prior to the 1st sequence of BA.3.2.2 which was submitted on 7th of November 2025. (e) Heatmap of IC50 neutralization titers of mAbs developed for commercial use.

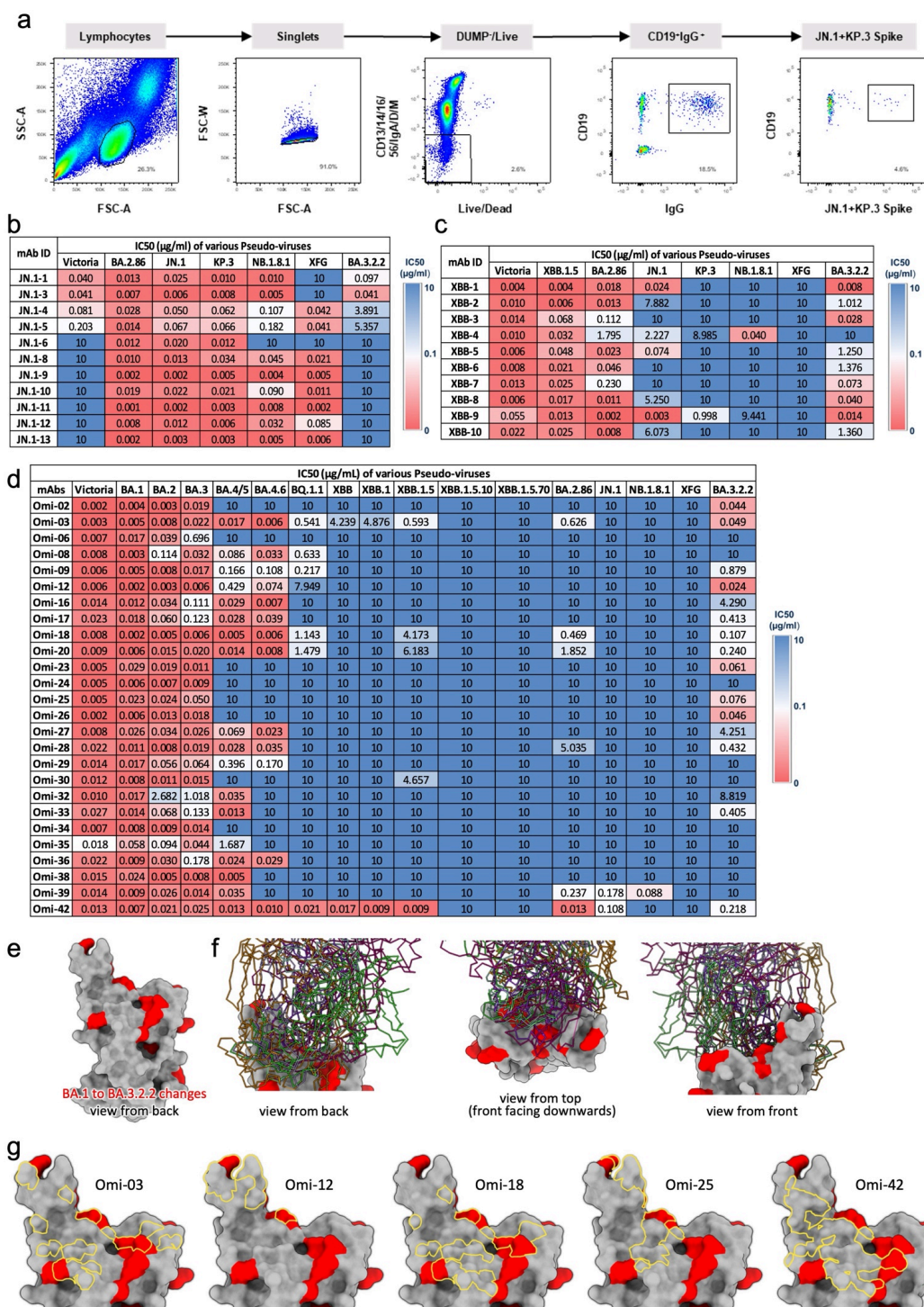


Figure 2

Figure 2

Generation and Pseudoviral neutralization assays. (a) Sorting of JN.1 and KP.3 specific B cells. (b) Heatmap of IC₅₀ neutralization titers of mAbs developed by sorting them against JN.1 and KP.3 spikes. (c) Heatmap of IC₅₀ neutralization titers of mAbs previously developed and already published using XBB.1.5 spike. IC₅₀ values of XBB.1.5, BA.2.86 and JN.1 have been reported previously^{8,11,23,26,27}. (d) Heatmap of IC₅₀ neutralization titers of mAbs developed and already published using Omicron (BA.1)

spike. IC50 values of BA.1^{8,11,23,26,27}, Victoria, BA.2, BA.4, BA.4.6, BQ.1.1, XBB and XBB.1 have been reported previously^{8,11,23,26,27}. (e) surface of BA.1 RBD showing residues mutated in BA.3.2.2 in red. (f) Orthogonal views of the RBD surface, coloured as in (e), showing Ca traces for the Fabs of those five antibodies which retain neutralization ability against BA.3.2.2 and for which structures are available. (g) The footprints of the five antibodies superimposed in (f) are shown in yellow outline.

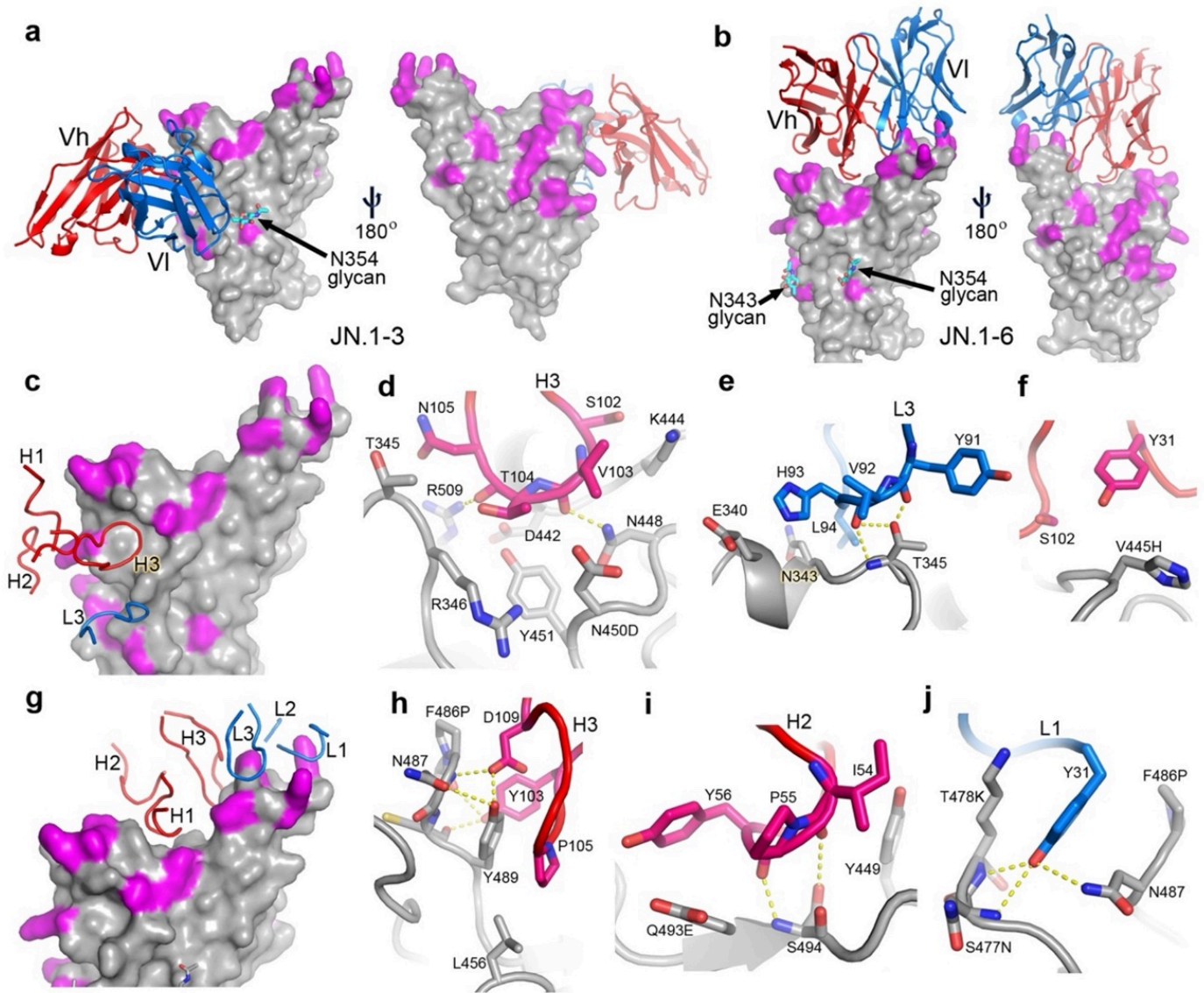


Figure 3

Figure 3

Structures of KP.3-RBD/JN.1-3 and KP.3-RBD/JN.1-6 complexes. (a) and (b) Front and back views of KP.3-RBD bound with JN.1-3 and JN.1-6, respectively. (c-f) CDR positions and details of interactions for KP.3-RBD/JN.1-3 complex, and (g-j) for KP.3-RBD/JN.1-6 complex. The drawing style and colour scheme are as in Fig. 3 with JN.1 mutations labelled explicitly in the close-up panels. The yellow broken sticks represent H-bonds.

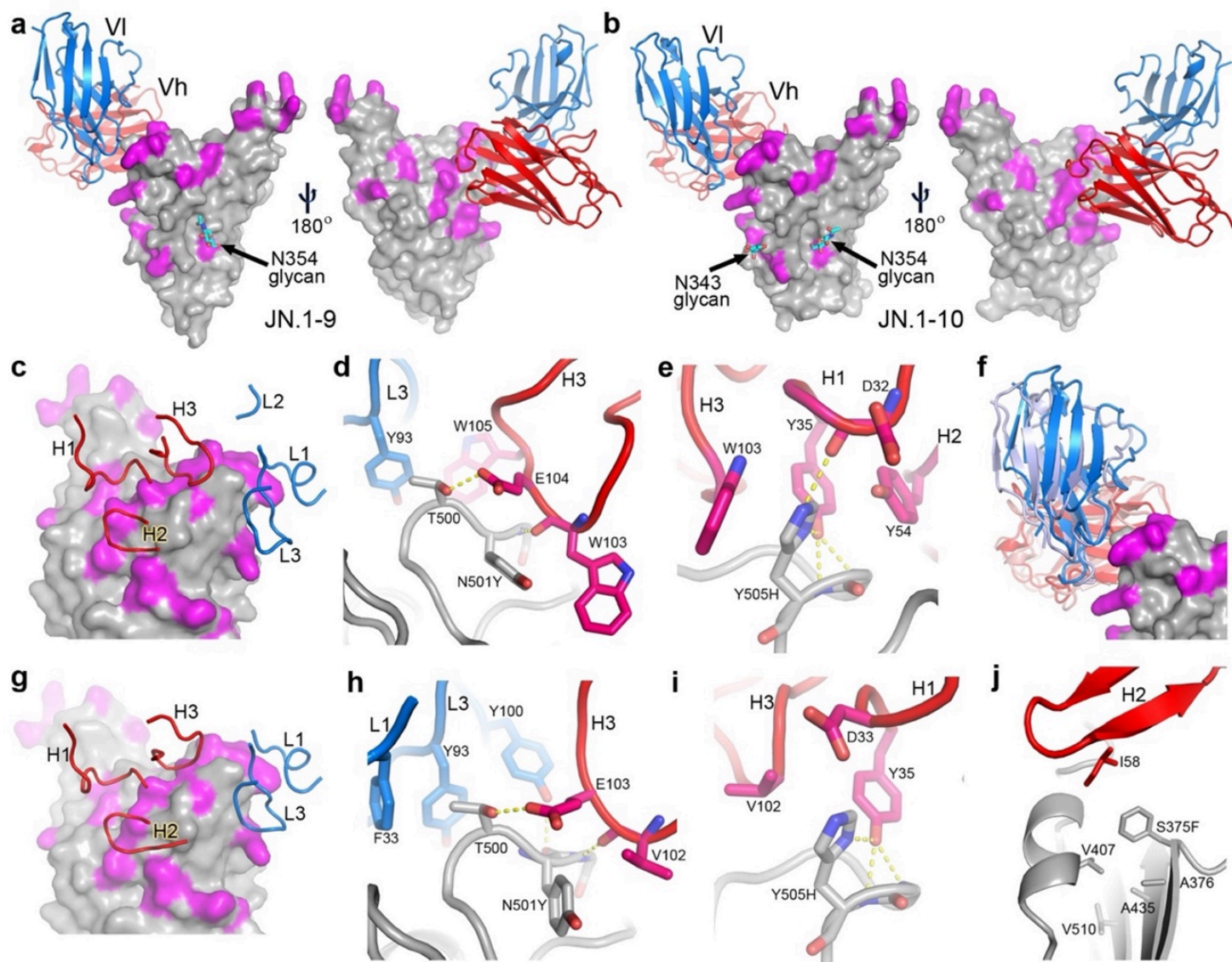


Figure 4

Figure 4

Structures of KP.3-RBD/JN.1-9 and KP.3-RBD/JN.1-10 complexes. (a) and (b) Front and back views of KP.3-RBD bound with JN.1-9 and JN.1-10, respectively. (c-e) CDR positions and details of interactions for KP.3-RBD/JN.1-9 complex. (f) Overlay of KP.3-RBD/JN.1-9 (bright red and blue) and KP.3-RBD/JN.1-10 (salmon and pale blue) complexes by superimposing the RBD. (g-j) CDR positions and details of interactions for KP.3-RBD/JN.1-10 complex. The drawing style and colour scheme are as in Fig. 3 with JN.1 mutations labelled explicitly in the close-up panels.

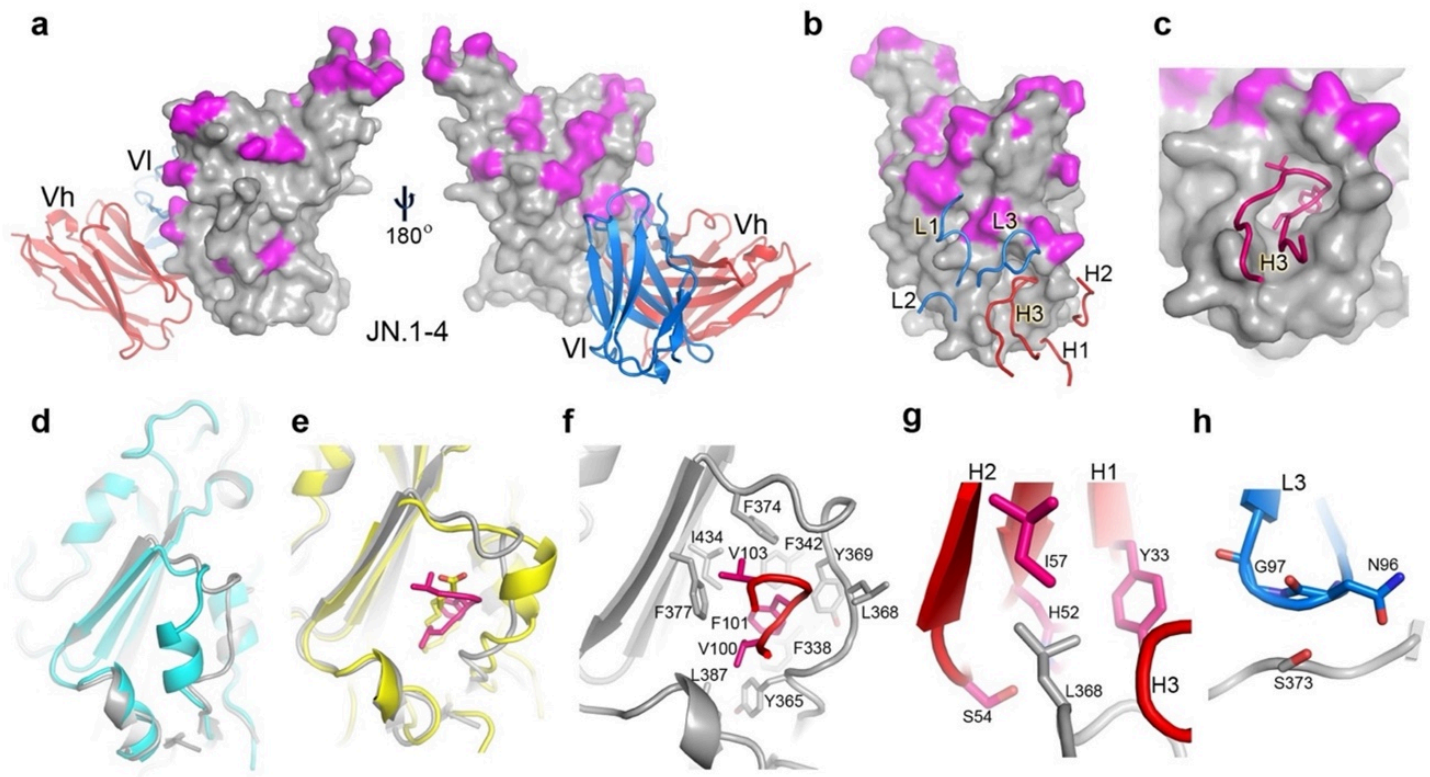


Figure 5

Figure 5

Structure of Delta RBD/JN.1-4 Fab complex. (a) Front and back views of Delta-RBD/JN.1-4 Fab complex. The RBD is shown as a grey surface representation with mutation sites of JN.1 variant coloured in magenta, and the Fab as ribbons with heavy chain in red and light chain in blue (ChCI domains are not shown for clarity). (b) CDR positions of the bound JN.1-4 Fab. (c) CDR-H3 binding pocket. (d) and (e) Cartoon representations of overlays of JN.1-4 bound Delta-RBD (grey) with XBB-2 bound Delta-RBD (cyan, PDB 8QRG) and the lipid (cyan sticks) bound RBD (yellow, PDB 7Z3Z) of the spike in the locked-down conformation, respectively. (f-h) Details of JN.1-4 and RBD interactions. The main chain backbones are drawn as either ribbons and coils or sticks, and side chains as sticks with RBD in grey, heavy chain in red and light chain in blue.

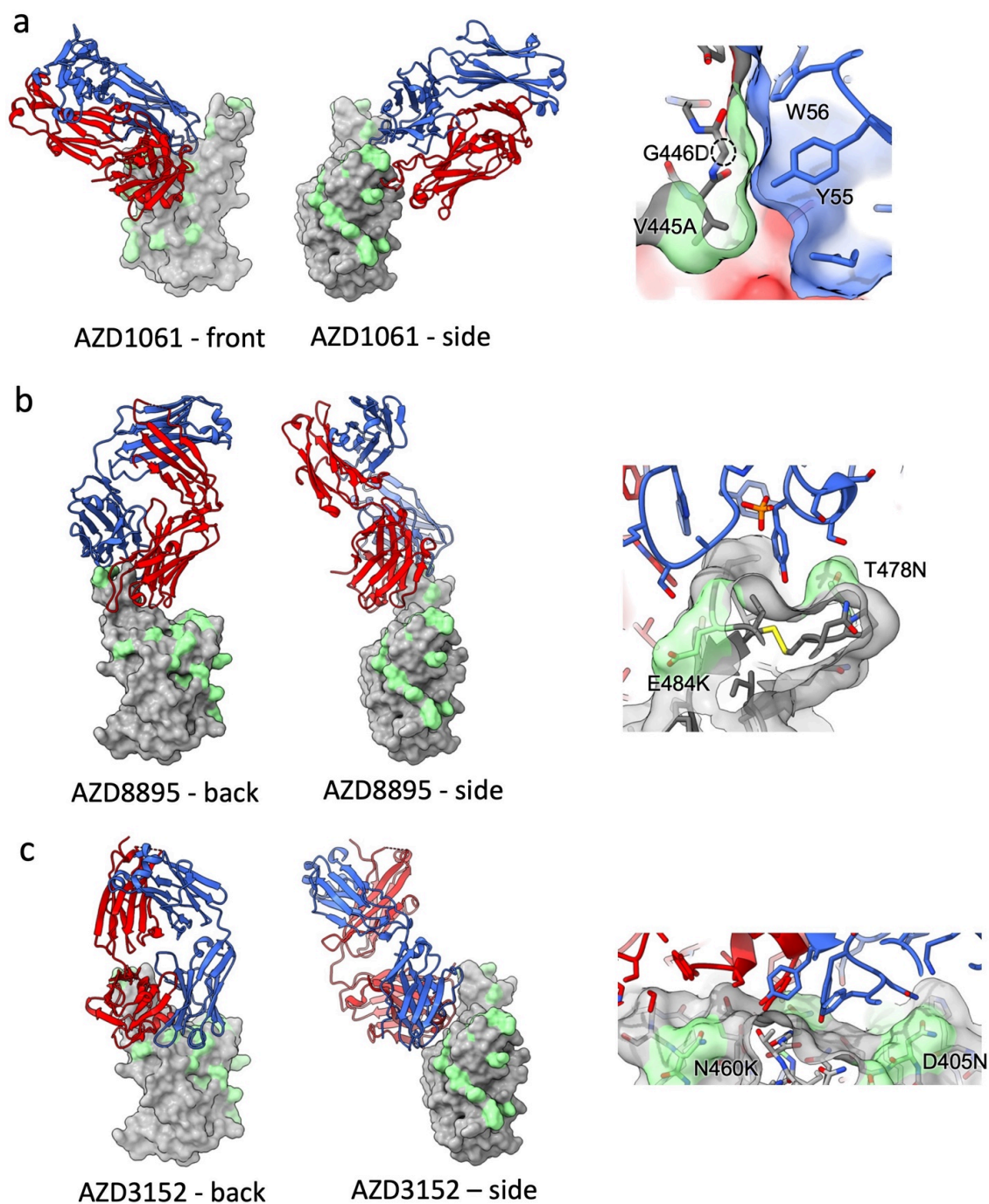


Figure 6

Figure 6

Astra Zeneca therapeutic antibody RBD binding with respect to BA.3.2.2 mutations. (a) AZD1061, (b) AZD8895 and (c) AZD3152. The RBD is shown as a grey molecular surface with the residues mutated from early SARS-Co-2 shown in light green. The Fab light chain is shown in blue and the heavy chain in red. Close-ups of part of the footprints are shown to the right. Changes within the footprints of AZD1061 (a) and AZD3152 (c) explain the complete and slight reduction in potencies, respectively.

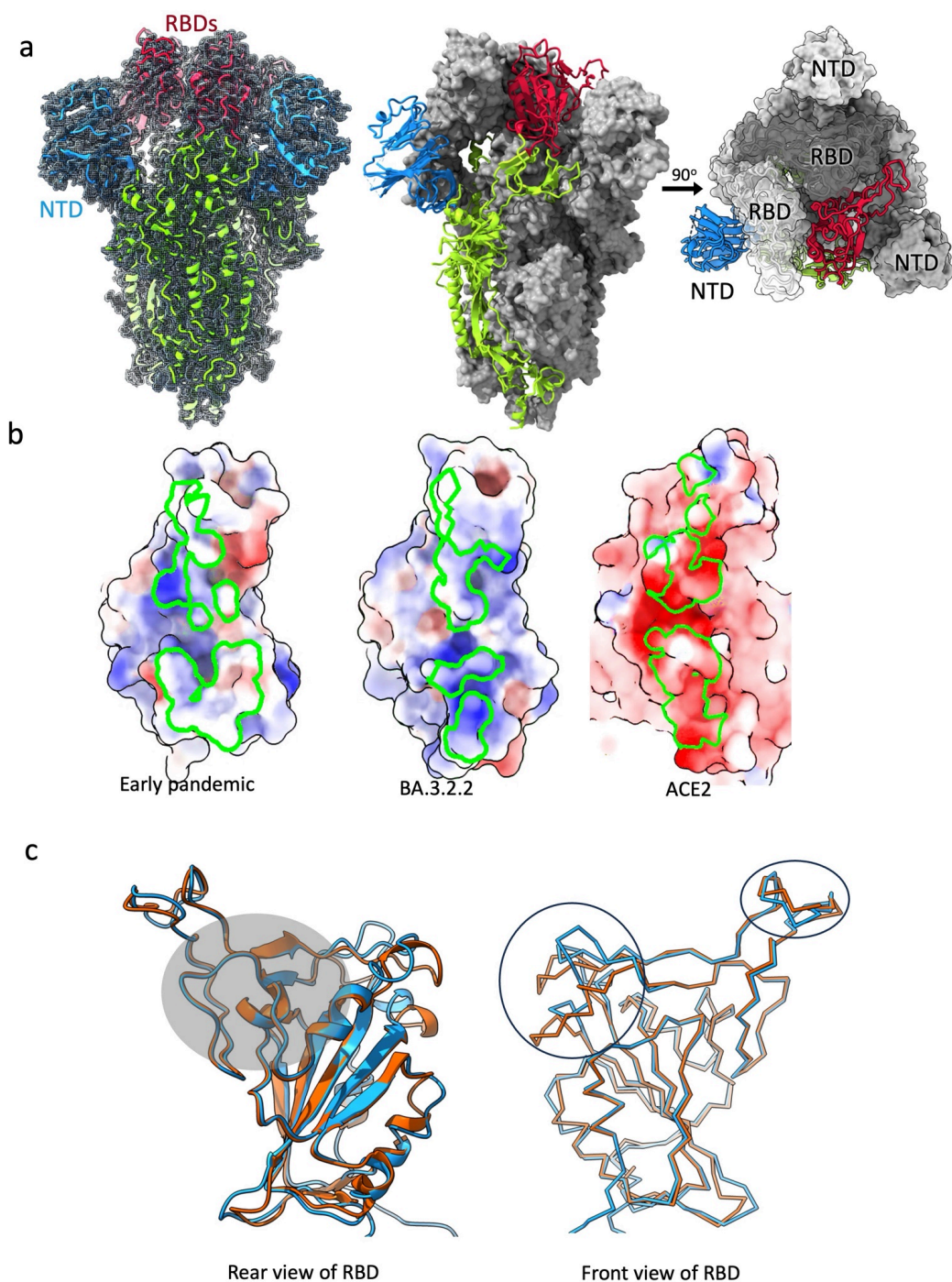


Figure 7

Figure 7

The structure of BA.3.2.2 spike. (a) Schematic of the trimeric spike, the left panel shows a cartoon representation embedded in the electron potential of the cryoEM reconstruction, NTDs are shown in blue and RBDs in red. The middle panel show two subunits in surface representation and one as a cartoon, to the right there is a top view, showing the close packing of the RBDs in a down configuration (b) Comparison of the electrostatic surface of the early pandemic RBD with that of BA.3.2.2. The view if

form the top of the RBD looking down onto the ACE-2 binding surface. The footprint of ACE-2 (residues within 4 Å of ACE-2) is outlined in green. The footprint on BA.3.2.2 is inferred by superimposition of the BA.3.2.2 RBD upon an RBD complexed with ACE-2. The right-hand panel shows the corresponding footprint of BA.3.2.2 on ACE-2 (shown in an 'open-book' relationship with the BA.3.2.2 to its left). Note the improved electrostatic complementarity for BA.3.2.2 compared to the earlier RBD. (c) The left-hand panel compares the BA.3.2.2 RBD (red) with the early pandemic RBD (blue). The view is onto the back of the RBD. The approximate region where the Omi antibodies, which retain potency against BA.3.2.2 (see Fig. 2 f, g), is shown as a grey oval, note that this region is strongly structurally conserved. The right-hand panel is the view from the front of the RBD (similarly coloured). The black ovals highlight the regions at the top of both the left and right shoulders where there is a substantial structural difference in BA.3.2.2.

Supplementary Files

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