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The Omicron variant of concern: Diversification and convergent evolution in spike protein, and escape from anti-Spike monoclonal antibodies

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\textbf{ABSTRACT}

WHO-defined SARS-CoV-2 variants of concern (VOC) drive therapeutics and vaccine development. The Omicron VOC is dominating the arena since November 2021, but the number of its sublineages is growing in complexity. Omicron represent a galaxy with a myriad of stars that suddenly rise and expand before collapsing into apparent extinction when a more fit sublineage appears. This has already happened with BA.1, BA.2, and BA.4/5 and is happening with BA.2.75. We review here the current PANGO phylogeny, focusing on sublineages with Spike mutations, and show how frequently convergent evolution has occurred in these sublineages. We finally summarize how Omicron evolution has progressively defeated the anti-Spike monoclonal antibodies authorized so far, leaving clinicians to again fall back on COVID19 convalescent plasma from vaccinated donors as the only antibody-based therapy available.

1. Introduction

On November 8, 2021, a novel SARS-CoV-2 lineage named B.1.1.529 in PANGOLIN phylogeny, 21 M in NextStrain, VUI-21NOV-01 in Public Heath England (PHE), and belonging to GISAID clade GR/484 A was reported from 100 cases in South Africa. These strains came primarily from Gauteng, North West and Limpopo regions where it was likely to have been circulating for at least 6 weeks (Yeh and Contreras, 2021). Shortly after the discovery of the Omicron variant in South Africa it was found in numerous countries around the world and it was declared as variant of concern (VOC) Omicron by WHO on November 26, 2021. Omicron initially harbored 32 different Spike mutations (Table 1). Arising from a third level-branch of the PANGOLIN phylogeny (B.1.1.529) it was immediately clear that, according to nomenclature rules, further Omicron sublineages would have been named with aliases, which should occur every fourth branching. BA was selected as the first alias for Omicron. E.g., at the time of this writing, BA.5 stands for B.1.1.529.5, while BF.1.1 stands for BA.1.1.529.5 or B.1.1.529.5.3.4.1. The nomenclature is growing in complexity, and ambiguities can be expected. For example, it is not immediately clear that BF is a descendant of BA rather than an alias of a different lineage. Furthermore, alphabet exhaustion is driving letter addition to names (e.g. BW.\textsuperscript{4} for sublineages and XBB for recombinants).

3. Main Omicron branches

The Omicron VOC members has 5 major sublineages that share a few Spike mutations (K417N, N440K, E484A, and N679K): given that B.1.1.529 had already consumed the three levels of classification allowed by the PANGO nomenclature, the BA alias was introduced for sublineages.

- BA.1 (a.k.a. 21 K in NextStrain) has the hallmark mutations listed in Table 1.
- BA.2 (a.k.a. 21 L in NextStrain, VUI-22JAN-01 in PHE, or colloquially as “Omicron 2”) was first reported in Gauteng on Nov 17, 2021 and carries almost all the spike RBD mutations first noted in Omicron, and both furin cleavage adjacent mutations as well as the NSP6
Occurrence of selected Spike mutations in Omicron VOC and VOC-LUM, and impact of that mutation on efficacy of mAbs authorized (median fold-reduction in in vitro neutralization activity compared to wild-type SARS-CoV-2). BAM: bamlanivimab; ETE: etesevimab; CAS: casirivimab; IMD: imdevimab; TIX: tixagevimab; CIL: cilgavimab; SOT: sotrovimab; BEB: bebtelovimab; ADI: adintrevimab. REG: regdanvimab. = : < 2-fold reduced susceptibility; 2-fold reduced susceptibility; 2–5-fold reduced susceptibility; 5–24.9-fold reduced susceptibility; > 25-fold reduced susceptibility. stanford.edu/search-drdb

| Spike mutation | BA.1 | BA.2 | BA.2.10.4 | BA.2.12.1 | BA.2.75 | BA.4/5 | BAM | ETE | CAS | IMD | CIL | TIX | SOT | BEB | ADI | REG |
|----------------|------|------|-----------|-----------|---------|-------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| T19I           | –    | +    | –         | –         | –       | –     | –   | –   | –   | –   | –   | –   | –   | –   | –   | –   |
| L245/225–27    | –    | –    | +         | –         | –       | –     | –   | –   | –   | –   | –   | –   | –   | –   | –   | –   |
| W64R           | –    | –    | –         | +         | –       | –     | –   | –   | –   | –   | –   | –   | –   | –   | –   | –   |
| A67V           | +    | –    | –         | –         | –       | –     | –   | –   | –   | –   | –   | –   | –   | –   | –   | –   |
| Δ69–70         | +    | –    | –         | –         | –       | –     | –   | –   | –   | –   | –   | –   | –   | –   | –   | –   |
| T95I           | +    | –    | –         | –         | –       | –     | –   | –   | –   | –   | –   | –   | –   | –   | –   | –   |
| Δ141–144       | –    | –    | +         | –         | –       | –     | –   | –   | –   | –   | –   | –   | –   | –   | –   | –   |
| G142D/Δ143–145 | +    | +    | –         | +         | –       | –     | –   | –   | –   | –   | –   | –   | –   | –   | –   | –   |
| K417E          | –    | –    | –         | –         | +       | –     | –   | –   | –   | –   | –   | –   | –   | –   | –   | –   |
| W152R          | –    | –    | –         | –         | +       | –     | –   | –   | –   | –   | –   | –   | –   | –   | –   | –   |
| F157L          | –    | –    | –         | –         | +       | –     | –   | –   | –   | –   | –   | –   | –   | –   | –   | –   |
| I210V          | –    | –    | –         | –         | +       | –     | –   | –   | –   | –   | –   | –   | –   | –   | –   | –   |
| Δ211/L212I     | +    | –    | –         | –         | –       | –     | –   | –   | –   | –   | –   | –   | –   | –   | –   | –   |
| V213G          | –    | –    | +         | –         | –       | –     | –   | –   | –   | –   | –   | –   | –   | –   | –   | –   |
| ins214EPE      | +    | +    | –         | –         | –       | –     | –   | –   | –   | –   | –   | –   | –   | –   | –   | –   |
| G257S          | –    | –    | –         | –         | +       | –     | –   | –   | –   | –   | –   | –   | –   | –   | –   | –   |
| G259x          | D    | D    | –         | D         | H       | –     | –   | –   | –   | –   | –   | –   | –   | –   | –   | –   |
| R346x          | –    | –    | –         | –         | –       | –     | –   | –   | –   | –   | –   | –   | –   | –   | –   | –   |
| S371X          | L    | F    | –         | F         | –       | –     | –   | –   | –   | –   | –   | –   | –   | –   | –   | –   |
| S375P          | +    | +    | –         | +         | –       | –     | –   | –   | –   | –   | –   | –   | –   | –   | –   | –   |
| S375F          | +    | +    | –         | +         | –       | –     | –   | –   | –   | –   | –   | –   | –   | –   | –   | –   |
| T376A          | –    | –    | –         | –         | +       | –     | –   | –   | –   | –   | –   | –   | –   | –   | –   | –   |
| D405N          | –    | +    | –         | –         | +       | –     | –   | –   | –   | –   | –   | –   | –   | –   | –   | –   |
| K417N          | +    | +    | –         | +         | –       | –     | –   | –   | –   | –   | –   | –   | –   | –   | –   | –   |
| D446S          | +    | +    | –         | +         | –       | –     | –   | –   | –   | –   | –   | –   | –   | –   | –   | –   |
| L452x          | –    | –    | –         | Q         | R in BA.2.75.4 | –R    | –   | –   | –   | –   | –   | –   | –   | –   | –   | –   |
| N460K          | –    | –    | –         | –         | –       | –     | –   | –   | –   | –   | –   | –   | –   | –   | –   | –   |
| S477N          | +    | +    | –         | +         | –       | –     | –   | –   | –   | –   | –   | –   | –   | –   | –   | –   |
| S477F          | +    | +    | –         | +         | –       | –     | –   | –   | –   | –   | –   | –   | –   | –   | –   | –   |
| N486K          | +    | +    | –         | +         | –       | –     | –   | –   | –   | –   | –   | –   | –   | –   | –   | –   |
| G464S          | +    | +    | –         | +         | –       | –     | –   | –   | –   | –   | –   | –   | –   | –   | –   | –   |
| Q493R          | +    | +    | –         | R493Q     | –       | –     | –   | –   | –   | –   | –   | –   | –   | –   | –   | –   |
| S494P          | –    | +    | –         | +         | –       | –     | –   | –   | –   | –   | –   | –   | –   | –   | –   | –   |

(continued on next page)
Table 1 (continued)

| Spike mutation | G496S | N501Y | Y505H | H655Y | G614S | G614S in BL.1.1 | D614G | P681H | N764K | T547K | L981F | Q954H | N969K | L11F | N: P151 (WT); synonymous SNPs: A27038G, and C27889T. |
|----------------|-------|-------|-------|-------|-------|-----------------|-------|-------|-------|-------|-------|-------|-------|-----|-------|
| BA.1           | +     | +     |       |       |       |                 |       |       |       |       |       |       |       |     |       |
| BA.2           | +     | +     | +     | +     | +     | +               |       | +     | +     | +     | +     | +     | +     |     |       |
| BA.2.10        |       |       |       |       |       |                 |       |       |       |       |       |       |       |     |       |
| BA.2.10.4      |       |       |       |       |       |                 |       |       |       |       |       |       |       |     |       |
| BA.2.12.1      |       |       |       |       |       |                 |       |       |       |       |       |       |       |     |       |
| BA.2.12.10     |       |       |       |       |       |                 |       |       |       |       |       |       |       |     |       |
| BA.2.75        |       |       |       |       |       |                 |       |       |       |       |       |       |       |     |       |
| BA.4/5         | +     | +     |       |       |       |                 |       |       |       |       |       |       |       |     |       |
| BA.4           |       |       |       |       |       |                 |       |       |       |       |       |       |       |     |       |
| BA.5           |       |       |       |       |       |                 |       |       |       |       |       |       |       |     |       |

4. VOC lineages under monitoring

Among the currently circulating BA.2 and BA.4/5 sublineages, several ones deserve a separate discussion, having been classified by WHO as VOC lineages under monitoring (VOC-LUM: https://www.who.int/activities/tracking-SARS-CoV-2-variants) or listed at https://cov-v-spectrum.org/collections/18 because of their relative growth advantage:

- second-generation BA.2 appeared after BA.4/5 had become dominant over BA.2:
  - BA.2.12.1 (a.k.a. 22 C in NextStrain and V-22MAY-01 in PHE) has been a prevalent lineage in USA in Spring 2022, and carries L452Q and S704F
  - Other lineages first reported from India:
    - BA.2.10 descendants. BA.2.10.4 (nicknamed “Pisces”) harbors W64R, Δ141–144, Δ243-244, G446S, F486P, R493Q and S494P, and P1143L. It was first detected in India in August 2022, showing 10% daily growth advantage over other BA.2. It additionally harbors and is resistant to all mAbs except bebtelovimab (Sheward et al., 2022b). BJ.1 (nicknamed ‘Argus’) harbors R346T, G446S, and F490V; it has recombined with BM.1.1.1 (nicknamed “Mimas”) to generate XBB.1 (nicknamed ‘Gryphon’).
    - BA.2.75 (nicknamed “Centaurus”) was detected in India in May 2022. It carries K147E, W152R, F157L, I210V, G257S, D339H (mutated from G339D), G446S (which causes escape from cilgavimab and imdevimab), N460K, R493Q (reversion). R493Q and N460K cause BA.2.75 to exhibit higher ACE2-binding affinity than BA.2 and BA.4/BA.5 (Cao et al., 2022b; Saito et al., 2022) and the longest evolutionary distance of the S gene (Sugano et al., 2022). BA.2.75 is neutralized by vaccine-elicited and BA.1/2 breakthrough infection-induced sera at the same levels of BA.2, but at lower level from convalescent sera than BA.2 and BA.5. Nirmatrelvir, molnupiravir and ritonavir retain efficacy. Fusogenicity, growth efficiency in human alveolar epithelial cells, and intrinsic pathogenicity in hamsters of BA.2.75 were comparable to those of BA.5 but were greater than those of BA.2 (Saito et al., 2022). Of concern, the replicative ability of BA.2.75 in hamster lungs was higher than that of BA.2 and BA.5, causing focal viral pneumonia (Uraki et al., 2022). Nine sublineages have developed so far. BA.2.75.1 has acquired deletion seen in other VOCs. As shown in Table 1, BA.2 has 7 additional Spike mutations not found in BA.1 and 4 lost compared to BA.1.

- BA.3 harbors R408S and has been a relatively minor sublineage.
- BA.4 (a.k.a. 22 A in NextStrain, V-22APR-03 first and VOC-22APR-03 later in PHE) shares all mutations/deletions with the BA.2 lineage except the following: NSP4: L438F reverted to WT (wild type); S: 69/70 deletion, L452R, F486V, Q493 (WT); ORF 6: D61 (WT); ORF 7b: L11F; N: P151S. The Spike 69/70 deletion produces an undetectable S-gene target (S-gene target failure) in the TaqPath assay. The earliest BA.4 sample in GISAID was from South Africa with a sample collection date of 10 January 2022. BA.4/BA.5 shows the weakest receptor-binding activity due to F486V and R493Q reversion (Cao et al., 2022c). The growth advantages for BA.4 and BA.5 of 0.08 (95% CI: 0.07–0.09) and 0.12 (95% CI: 0.09–0.15) per day respectively over BA.2 in South Africa (Tegally et al. (2022)).
- BA.5 (a.k.a. 22B in NextStrain, V-22APR-04 first and VOC-22APR-04 later in PHE) shares the same mutations/deletions as BA.4 except the following: M: D3N; ORF7b: L11 (WT); N: P151 (WT); synonymous SNPs: A27038G, and C27889T.
R346T to generate BL.1 and BL.2. BA.2.75.2 (nicknamed “Alcyon”, short for “Alcyoneus”, which includes R346T, F486S and D1199N) is the most immune escaping (5-times reduced neutralization by vaccinated donors than BA.5 (Sheward et al., 2022b)). BA.2.75.2 has acquired S494P in CA.2 and L452R in CA.1. BA.2.75.3 has acquired F486S in BM.1 and BM.4, and R346T in BM.2: BM.1.1 (nicknamed “Mimas”) recombined with BJ.1 to create XBB (nicknamed “Griffon”), while the R346T-BM.4 similarly acquired F486S (BM.4.1), R346T (BM.4.1.1), K447T (CH.1), and L452R (CH.1.1). The L452R-harboring BA.2.75.4 has acquired K444M in BR.1, R346T plus F486I in BR.2 and R346T in BR.3. BA.2.75.5 has acquired R346T plus F490N in BM.1 (nicknamed ‘Hydra’), which in turns has acbasiliqueured S494P in BM.1.1. The R346T-harboring BA.2.75.6 has acquired F486S in BY.1, while the R346T-harboring BA.2.75.9 has acquired F486V in CB.1
- BH.1 (i.e., BA.2.38.3.1, nicknamed “Almops”) harbors 144del, F446S, and L452Q
- BA.4/5 descendants. BA.4.6 (nicknamed ‘Aeterna’) harbored R346T and later acquired K444N and N460K in BA.4.6.3, while BA.4.7 harbors R346S. BA.5 has instead many more convergent descendants. BA.5.2 acquired R346I in CE.1, R346S in BF.13, R346T in BA.5.2.6, BA.5.2.13, BF.7 (nicknamed “Minotaur”), and BF.11. BA.5.3 acquired R346T in BE.1.2 and BE.4.1 (which in turn acquired K444R in BE.4.1.1), K447T in BE.1.1.1, and both K444N and N460Kin BE.4.2. BE.1.1.1 is of much interest because it acquired in N460K in BQ.1 (nicknamed “Typhon”), which in turn acquired R346T in both BQ.1.1 (nicknamed “Cerberus”) and BQ.1.17. BA.5.6 acquired K444T in BA.5.6.2, which in turn acquired N460K in BW.1. The R346T-harboring BA.5.1.20 acquired K444M and N460K in BU.1.
- Omicron sublineages with E340 mutations have been exceedingly rare before massive usage of sotrovimab, which is known to induce this escape mutant (Rockett et al., 2021). E340K has been observed in BA.2.77. BA.1.24, first detected in Spain and harboring the E340K Spike mutation, was previously outcompeted by the BA.2 wave.

5. Co-infections and recombinants

Further complicating the landscape, several Delta-Ba.1 (Combes et al., 2022) and BA.1-Ba.2 (Gjorgjievska et al., 2022; Vatteroni et al., 2022) co-infections have been reported.

As expected for coronavirus, multiple recombinants (Focosi and Maggi, 2022) have been detected to date (designed by the PANGOLIN nomenclature with the ‘X’ prefix and progressive alphabet letters, with one more letter added when the alphabet is over), with the left part of the description indicating the Spike donor lineage. The recombinants can be summarized as follows:

- DeltaBa.1: XD (France and Denmark (Zayet et al., 2022)), XF (UK)
- BA.1xBa.2 (Colson et al., 2022):
  - XE (a.k.a. V-22APR-02 in PHE), XG (Denmark), XH (Denmark), XJ (Finland), XK (Belgium), XN (UK), XT (South Africa), XU (India), XV (Denmark and Italy), XW (USA, Germany, England), XY (USA), XAA (USA), XAB (Germany), XAC (Canada and USA), XAF (Costa Rica), XAG (Brazil), XAK (Germany), XAL (Germany), XAQ (Canada), XAR (Reunion)
- BA.1.1xBa.2: XM (Europe), XP (UK), XQ (UK), XR (UK), and XZ
- BA.1.1xBa.2.9: XAM (Panama and USA), XAU (Spain, England, France)
- BA.2xBa.1: XAD (Germany), XAE (USA, Chile), XAH (Slovenia), XAP (USA)
- BA.2.3.13xBa.1: XAT (Japan)
- BA.2xBa.4: BA.2.12.1xBa.4 (England)
- BA.2xBa.5: BA.2.2.12xBa.4 (United States, Scotland)
- BA.2xBa.5.1: XAN (Spain, Denmark)
- BA.2.5xBa.5.1: XAZ (France, Germany, Croatia, Denmark and USA)
- BA.2.76xBa.5.2: (PANGO issue #896)
- BA.2xBaDelta:
  - BA.2xA1.122x: XAW (Russia)
  - BA.2xA1.45: XAV/C1 (South Africa)
  - C2
- BA.5xBa.2: XAS (USA)
- BM.1.1.1xBJ1: XBB

5.1. Convergent evolution across Omicron sublineages

Each Omicron sublineage has tens of descendnt sub-sublineages (see Supplementary Table 1), which often show signs of convergent evolution in the amino acid sequence of the Spike protein (see Fig. 1). A few prominent examples have been selected below, and the reader can find more details on the functional consequences of specific mutations at the references provided. As a primer, it should be considered that the receptor-binding domain (RBD) of Spike consists of amino acid residues from positions 333–527: the specific portion which makes contact with ACE2 is called receptor-binding motif (RBM) and includes amino acids 438–506. In general, mutations that favor increased ACE2 affinity are positively selected, with a few exceptions (e.g., K417T/N) that require compensation by other residues.

- HV69-70 was first seen in VOC Alpha (B.1.1.7), and later detected in linesages B.1.375 (Larsen and Worobey, 2021: Moreno et al., 2021) and B.1.346 reported from USA (Larsen and Worobey, 2021), B.1.1.298, B.1.1.177 (EU1), B.1.1.160 (EU2), and B.1.258Δ (Brejóvá et al., 2021). Among Omicron sublineages, it is a hallmark of both BA.1 and BA.4/5. HV69-Δ70 diminishes protrusion of the 69–76 loop, increasing Spike-mediated infectivity by 2 folds. Interestingly for screening purposes, the deletion causes false negativity in the Spike target (so called S-dropout variant or S-gene target failures (SGTF)) of a 3-target TaqPath® RT-PCR COVID19 assay (Thermo Fischer Scientific) (Bal et al., 2020; Gravagnuolo et al., 2021; Washington et al., 2020). The deletion can also be detected as a positive signal using a pair of molecular beacons paired with loop mediated isothermal amplification (LAMP) (Sherrill-Mix et al., 2021).

- R346 mutations were exceedingly rare before Omicron, but are now hallmarks of various Omicron sublineages (e.g.):
  - R346I in BA.5.9 and CE.1
  - R346K (previously seen in VOC Mu/B.1.621) in BA.1.1
  - R346S in BA.4.7 and BF.13
  - R346T in BA.1.23, BA.2.9.4, BL.1, BL.2 BA.2.75.2, BM.1.1, BM.2, BM.4.1.1, BR.2, BN.1, BP.1, BA.2.75.6, BA.2.75.9, BA.2.80, BA.2.82, BA.4.1.8, BA.4.6, BF.7, BF.11 and BJ
  - BA.4.6, BA.4.7, and BA.5.9 display higher humoral immunity evasion capability than BA.4/BA.5, causing 1.5–1.9-fold decrease in NT50 of the plasma from BA.1 and BA.2 breakthrough-infection convalescents compared to BA.4/BA.5. Importantly, plasma from BA.5 breakthrough-infection convalescents also exhibits significant neutralization activity decrease against BA.4.6, BA.4.7, and BA.5.9 than BA.4/BA.5, showing on average 2.4–2.6-fold decrease in NT50. R346S causes resistance to class 3 antibodies: bnt-Elovimab remains potent, while Evusheld™ is completely escaped by these subvariants (Jian et al., 2022).
• K417T occurs in BA.2.18, BA.2.40.1 and BA.3.1. K417N previously occurred in VOC Beta and Delta “plus” (AY.1 and AY.2 sublineages) and in VOI Mu, while K417T occurred in VOC Gamma. Both mutations eliminate a hydrogen bond in the interaction with ACE2 thus reducing affinity (Villoutreix et al., 2021). Despite the loss in the binding affinity (1.48 kcal/mol, i.e. 6.4-fold drop (Wang et al., 2021b)) between RBD and ACE2 (Laffeber et al., 2021), the K417N/T mutations abolish a buried interfacial salt-bridge between RBD, and escapes neutralization by mAbs etesevimab (Luan and Huynh, 2021; Starr et al., 2021b; Wang et al., 2021b). However, these mutations have only a modest effect on binding by polyclonal antibodies in a few convalescent samples studied (Greaney et al., 2020). K417T leads to resistance to etesevimab (Luan and Huynh, 2021; Starr et al., 2021b; Wang et al., 2021b) and casirivimab (Hoffmann et al., 2021a), while K417R leads to resistance to the REGN-COV2 cocktail (Copin et al., 2021).

• K444 mutations were also exceedingly rare in the pre-Omicron era, while they occur in many Omicron sublineages as:
  o K444M in BR.1.
  o K444N in BA.2.38.1, BA.2.38.2 and BA.4.6.3
  o K444T in BE.1.1.1, BA.5.6.2.1
  o K444R in BF.16, BA.2.3.20 (nicknamed “Basilisk”). BA.5.2.18, and BE.4.1.1.

• L452 mutations have probably been the best example of convergent evolution so far, having been previously detected in multiple VOC (Focosi et al., 2022b).

  o L452R does not have a major impact in ACE2 affinity when tested in the context of recombinant monomeric RBD but presents enhanced binding within the context of full-length membrane-anchored Spike (Geng et al., 2021). L452R was found in A.21, A.2.4, A.2.5, B.1.1.130, B.1.233 (a.k.a. CAL.20 A (Tchesnokova et al., 2021)), B.1.427/B.1.429 (a.k.a. CAL 0.20 C) (Zhang et al., 2021), B.1.617.1 and B.1.617.2 Delta VOCs (Cherin et al., 2021), B.1.362 +L452R, C.16 and C.36. L452R also causes evasion from HLA-A24-restricted CTL response (Motozono et al., 2021). Among Omicron sublineages, L452R is a universal hallmark of BA.4 and BA.5 and occurs in BA.2.11, BA.2.35, BA.2.75.4, BA.2.77, and CA.1

  o L452Q increases ACE2 binding by 3-folds and in vitro infectivity by 2-folds (Tada et al., 2021b). Of interest, the C.37 VOI and a single B.1.74 strain harbors the L452Q mutation (Tchesnokova et al., 2021). Among Omicron sublineages, L452Q occurs in BA.2.12.1, BH.1, BA.2.75.7, BA.2.2.13, BA.2.56, BA.2.74, BP.1 and XBC.1. The effective reproduction numbers of these L452R/M/Q-bearing BA.2-related Omicron variants are greater than the original BA.2. Neutralization experiments revealed that the immunity induced by BA.1 and BA.2 infections is less effective against BA.4/5. Cell culture experiments showed that BA.2.12.1 and BA.4/5 replicate more efficiently in human alveolar epithelial cells than BA.2, and particularly, BA.4/5 is more fusogenic than BA.2. Furthermore, experimental infection in hamsters indicated that BA.4/5 was more pathogenic than BA.2 (Kimura et al., 2022).
• N440K was found in BA.1 and BA.2, and previously in B.1.1.420.

• S371Y is found in both BA.2.43 and BA.2.44.

• K356T is found in BA.2.75.5, BA.2.77 and BA.2.80.

• G339 mutations occur as G339D in BA.1 and BA.2, and as G339H in BA.2.76.

• I68T occurs in BA.2.10.3 and BA.2.36.

• W64 occurs as W64R in BA.2.9.3, BA.2.10.4 and BA.4.5.

• R493Q occurs in BA.2.75, BA.2.77, BA.2.10.4 and BA.4.5. It is called a reversion, because prior to Omicron all previous VOCs had Q493, then BA.1 and BA.2 came with Q493. Q493 mutations were exceedingly rare in other SARS-CoV-2 isolates, suggesting selective pressure rather than spontaneous convergent evolution. Q493E was formerly seen only in the Delta VOC sublineageAY.49, and Q493R was associated with resistance to both bamlanivimab and etesevimab mAbs (Starr et al., 2021a; Starr et al., 2021b; Wang et al., 2021a). The Q493R mutation emerged during treatment with this cocktail (Focosi et al., 2021; Guignon et al., 2021; Lohr et al., 2021; Peiffer-Smadja et al., 2021; Pommeret et al., 2021; Trutfo et al., 2021; Vellas et al., 2021), while the Q493K mutation has emerged in a single patient after treatment with casirivimab plus imdevimab (Choi et al., 2020; Clark et al., 2021). Amazingly, Q493R was found in mice-adapted SARS-CoV-2 lineage, but infection of mice with wild-type SARS-CoV-2 was not possible before Omicron. In this regard, VOC Beta (Ravi et al., 2021) and Gamma (Montagutelli et al., 2021) could establish infection in wild-type mice, but with a milder infection than in human ACE2 transgenic mice (Tarres-Freixas et al., 2021). Omicron, because of its ability to infect mice (Bentley et al., 2021; Liu et al., 2022; Rissmann et al., 2022; Suryawanshi et al., 2022), was hypothesized to emerge from partially fit jump species (Wei et al., 2021), and reviruses after widespread circulation in humans could support such hypothesis. The F486V mutation found in BA.4/5 facilitates escape from certain Class 1 and Class 2 antibodies to the RBD but compromises the Spike affinity for the cellular receptor ACE2, but the R493Q reversion mutation restores receptor affinity, and consequently, the fitness of BA.4/5 (Wang et al., 2022a).

Further examples of convergent evolution that are worth mentioning include:

• W64 occurs as W64R in BA.2.9.3, BA.2.10.4, BA.2.61 and BA.2.71, while W64L occurs in BA.2.14 and BA.2.15.

• I68T occurs in BA.2.10.3 and BA.2.36.

• G181 mutations as G181E in BA.4.5 and as G181A in BF.6.

• L212S occurs in BA.2.50 and in BA.2.72. Δ211/L212F was a defining mutation of BA.1.

• Y248S mutations as Y248H in BA.2.47, Y248S in BA.2.54, and as Y248N in BA.2.76.

• G339 mutations occur as G339D in BA.1 and BA.2, and as G339H in BA.2.75.

• K356T is found in BA.2.75.5, BA.2.77 and BA.2.80.

• S371Y is found in both BA.2.43 and BA.2.44.

• N440K was found in BA.1 and BA.2, and previously in B.1.1.420 (Garcia et al., 2021).

• G446 mutations occur as G446V in BF.3.1 and as G446S in BA.2.75, BA.2.10.4 and BJ.1.

• Y449 mutations as Y449H in BA.2.45 and Y449N in BA.2.53.

• N450D occurs in BA.5.5.1 and BF.14.

• F490 mutations occur as F490V in BJ.1, F490S in BM.1.1.1, BN.1 and BN.2.1.

• S494P occurs in several Alpha VOC sublineages and in BA.2.10.4, BN.1 and CA.2 Omicron sublineages. It causes resistance to bamlanivimab (Chen et al., 2021).

• T547I occurs in BA.5.2.4 and BF.3 (an alias for BA.5.2.4.3).

• Q628K occurs in both BA.1.15.2 and BD.1 (an alias for BA.1.17.2.1).

• S704 mutations occur as S704L in both BA.1.9 and BA.2.12, including BA.2.12.1.

• V1264L occurs in BG.2 (an alias for BA.12.2.1.2) and BA.2.58. It was previously found in AY.4.2.2 and sometimes in VOI kappa.

Cao et al. demonstrated that such convergent mutations partly evade convalescent plasma, including those from BA.5 breakthrough infection, and totally evade authorized mAbs, including Evusheld™ and bebtelovimab. BA.2.75.2, BQ.1.1 and XBB are the most immune evasive strains tested so far. BA.2 and especially BA.5 breakthrough infection caused significant reductions of nAb epitope diversity and increased proportion of non-neutralizing mAbs, which in turn concentrated humoral immune pressure and promoted the convergent RBD evolution (Cao et al., 2022a).

5.2. Escape to anti-Spike monoclonal antibodies

The most concerning implication of Omicron evolution so far has been the progressive escape to vaccine-elicited immunity and anti-Spike monoclonal antibody (mAb) therapies (Tables 1 and 2), including representatives (Focosi et al., 2022c). Models have been developed to predict polyclonal antibody binding to Omicron sublineages remaining after mutating one or more sites in the SARS-CoV-2 RBD, based on deep mutational scanning of RBD targeting antibodies (e.g.: https://jbloomlab.github.io/SARS2_RDB_Ab_escape_maps/escape-calc/ (Greaney et al., 2022)), but to date most interest focuses on mAb efficacy. Eli Lilly’s bamlanivimab and etesevimab never worked against any Omicron VOC or VOC-LUM, as well as Roche’s casirivimab and imdevimab (Ronapreve™). Sotrovimab worked against BA.1, but lost efficacy against BA.2 and BA.4.5. Similarly, the tixagevimab component of the Evusheld™ mAb cocktail has not ever worked against any Omicron VOC or VOC-LUM: the remaining efficacy being based on the cilgavimab component only. Cilgavimab did not work against BA.1, recovered efficacy against BA.2, but there are in vitro evidences of 30-fold reduced efficacy against BA.4/5, and totally lost efficacy against the above-mentioned R346X harboring sublineages (see Tables 1 and 2). Despite attempts at increasing the therapeutic dose (AstraZeneca, 2022; FDA, 2022), to date there is no clearcut evidence of clinical benefit from Evusheld™ at the time of BA.4/5.

No authorized mAb other than bebtelovimab has convincing in vitro activity against BA.4/5 and BA.2.75.2 (Sheward et al., 2022b), and bebtelovimab has anyway reduced activity against BA.2.75 (Yamasoba et al., 2022a): being administered as a monotherapy and based on previous experiences with other mAb monotherapies (Huygens et al., 2022; Rockett et al., 2021), the likelihood of treatment-emergence immune escape is around 10% (Focosi et al., 2022c). Lack of actions by regulatory authorities at withdrawing authorization for mAbs that were no longer effective or troubles in medical education at the time of a pandemic have often driven inappropriate prescription of mAbs (Anderson et al., 2022; Focosi and Tuccori, 2022), with detrimental consequences on both healthcare budgets and public health.

Most importantly, Omicron has severely hit the pipeline of mAbs that were under advanced stages of development, such as amubarvimab and romlusevimab, as well as adintrevimab. So far, among the advanced candidates, only P5C3 and P2G3 retain efficacy against BA.4/5.
| WHO VOC (LUM) | Omicron |
|----------------|---------|
| **NextStrain name** | **UKHSA/PHE name** |
| **GRA (formerly GR/484 A)** | **GISAID name** |
| **PANGOLIN** | **bebtelovimab** (LY-CoV555 / LY3819253) | **etesevimab** (LY-CoV016, CB6 or JS016 / LY3832479) | **imdevimab** (REGN10987) |
| BA.1 | BA.2 | BA.2.12.1 | BA.2.75 | BA.4/B.A.5 | BA.4.6 | BA.4.7 | BA.5.9 |
| 21 K (descendant of 21 M) | 21 L (descendant of 21 M) | 22 C | 22D | 22 A/22B | n.a. | n.a. | n.a. |
| VUI-21NOV-01 | VUI-22JAN-01 | V-22MAY-01 | V-22JUL-01 | VOC-22APR-03/ VOC-22APR-04 | n.a. | n.a. | n.a. |
| **n.a.** | **n.a.** | **n.a.** | **n.a.** | **n.a.** | **n.a.** | **n.a.** | **n.a.** |

**Table 2**

Efficacy of anti-Spike monoclonal antibodies against Omicron sublineages. PHE: Public Health England. VOC: Variant of concern. LUM: lineage under monitoring. = <1-fold reduced susceptibility; ↓ 1-5-fold reduced susceptibility; ↓↓ 5-24.9-fold reduced susceptibility; ↓↓↓ ≥ 25-fold reduced susceptibility.

(continued on next page)
| Table 2 (continued) |
|---------------------|
| **WTR VOC** | **PANGUIN** |
| **name** | **BA.1** | **BA.2** | **BA.2.1.1** | **BA.2.75** | **BA.4** | **BA.4.6** | **BA.4.7** | **BA.4.9** |
| **F486V, BA.2** | (Gruell et al., 2022) | (Gruell et al., 2022) | (Gruell et al., 2022) | (Gruell et al., 2022) | (Gruell et al., 2022) | (Gruell et al., 2022) | (Gruell et al., 2022) | (Gruell et al., 2022) |
| **BA.2 L452Q, BA.2** | (Sheward et al., 2022b) | (Sheward et al., 2022b) | (Sheward et al., 2022b) | (Sheward et al., 2022b) | (Sheward et al., 2022b) | (Sheward et al., 2022b) | (Sheward et al., 2022b) | (Sheward et al., 2022b) |
| **BA.2.10.4** | (Yamasoba et al., 2022b) | (Yamasoba et al., 2022b) | (Yamasoba et al., 2022b) | (Yamasoba et al., 2022b) | (Yamasoba et al., 2022b) | (Yamasoba et al., 2022b) | (Yamasoba et al., 2022b) | (Yamasoba et al., 2022b) |
| **70del, BA.2** | (Kami et al., 2022) | (Kami et al., 2022) | (Kami et al., 2022) | (Kami et al., 2022) | (Kami et al., 2022) | (Kami et al., 2022) | (Kami et al., 2022) | (Kami et al., 2022) |
| **BA.4** | (Sun et al., 2022) | (Sun et al., 2022) | (Sun et al., 2022) | (Sun et al., 2022) | (Sun et al., 2022) | (Sun et al., 2022) | (Sun et al., 2022) | (Sun et al., 2022) |
| **70del, BA.2** | (Kami et al., 2022) | (Kami et al., 2022) | (Kami et al., 2022) | (Kami et al., 2022) | (Kami et al., 2022) | (Kami et al., 2022) | (Kami et al., 2022) | (Kami et al., 2022) |
| **BA.4.6** | (Sun et al., 2022) | (Sun et al., 2022) | (Sun et al., 2022) | (Sun et al., 2022) | (Sun et al., 2022) | (Sun et al., 2022) | (Sun et al., 2022) | (Sun et al., 2022) |
| **BA.4.7** | (Sun et al., 2022) | (Sun et al., 2022) | (Sun et al., 2022) | (Sun et al., 2022) | (Sun et al., 2022) | (Sun et al., 2022) | (Sun et al., 2022) | (Sun et al., 2022) |
| **BA.4.9** | (Sun et al., 2022) | (Sun et al., 2022) | (Sun et al., 2022) | (Sun et al., 2022) | (Sun et al., 2022) | (Sun et al., 2022) | (Sun et al., 2022) | (Sun et al., 2022) |

(Continued on next page)
up with the variants SARS-CoV-2 is uncertain there is one antibody-based therapy that keeps vulnerable to new variants. So, while the future of mAb therapies against the experience with cocktails has shown that they are also fully greatly reduces the incentives of pharmaceutical companies to invest in means that the period of therapeutic usefulness will be short, which contains activity during development time the experience detailed above will be antigenically stable during that time. Even if a new mAb main and given the history of variant generation it is unlikely that the virus nomics. Developing new mAb therapies takes at least several months in theory be targeted by new mAbs. Furthermore, it may still be possible defeated most, if not all, authorized mAb therapies. Whereas the epi-...
7. Conclusions

Omicron sublineages have represented a major turning point in the pandemic (Dhawan et al., 2022). While small molecule antivirals have preserved efficacy, mAbs have been defeated by immune escape, hitting manufacturer confidence with development plans. In the field of passive immunotherapies, CCP is likely to remain a pillar for immunocompromised patients that get infected with novel Omicron sublineages. Additionally, the design of upcoming Omicron-based vaccines (either monovalent or bivalent) will be a hard exercise, given the myriad of diverse sublineages that are circulating at this time and the rapidity with which the landscape is evolving. Ongoing genomic surveillance will be required in the coming years to promptly intercept emerging sublineages with growth advantages and dissect their immune escape abilities, in order to predict waves and update vaccines.

Author contributions

D.F. wrote the firstdraft and curated Tables, S.M. designed Figure, A. C. and S.M. revised themanuscript.

Conflict of Interest

We declare we have no conflict of interest related to this manuscript.

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Appendix A. Supporting information

Supplemental data associated with this article can be found in the online version at doi:10.1016/j.drup.2022.100882.

Appendix B. Supporting information

Supplemental data associated with this article can be found in the online version at doi:10.1016/j.drup.2022.100882.

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