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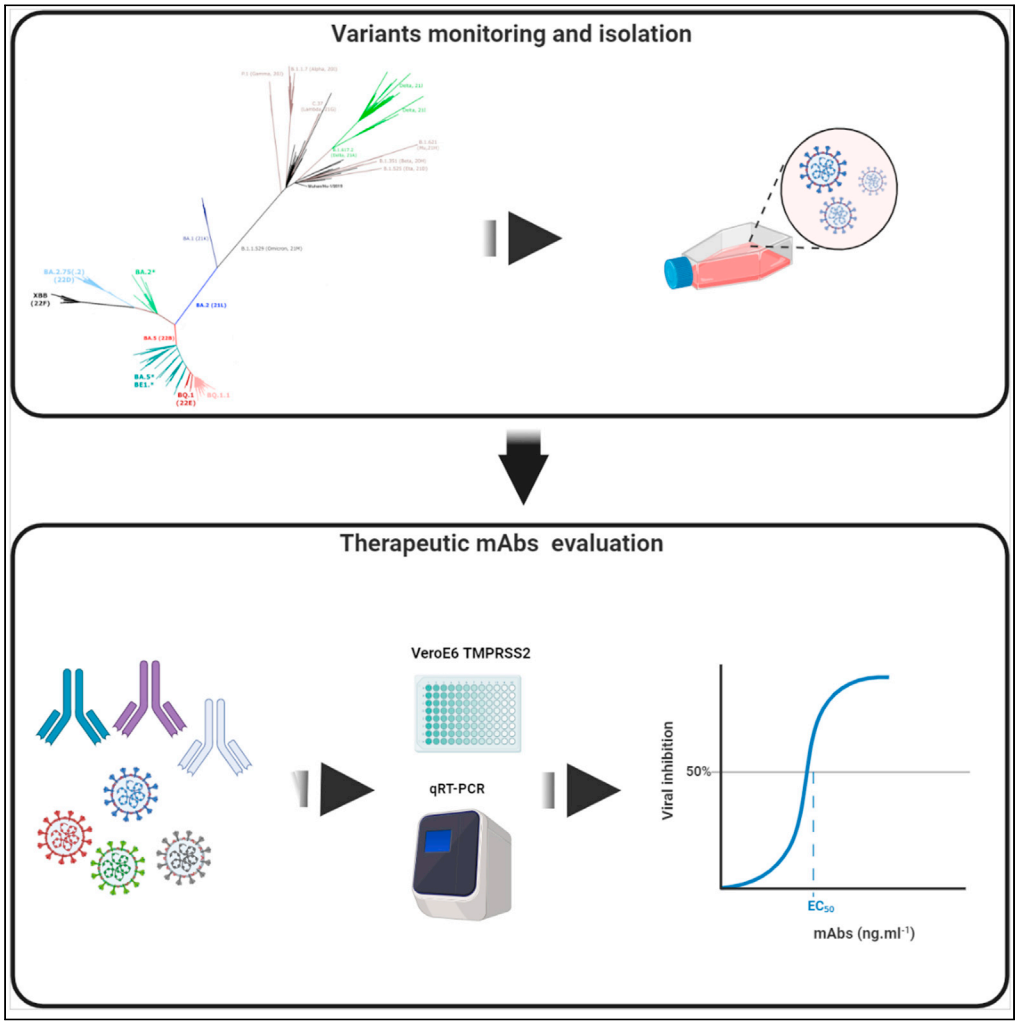
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# Enhanced neutralization escape to therapeutic monoclonal antibodies by SARS-CoV-2 omicron sub-lineages




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## Highlights

SARS-CoV-2 variants have increased with the concomitant rise of many subvariants

Sotrovimab retains some activity against BQ.1, BQ.1.1 and XBB

The Evusheld/AZD7442 cocktail lost all activity against all subvariants tested

Bebtelovimab lost all neutralizing activity against BQ.1, BQ.1.1, and XBB variants

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# Enhanced neutralization escape to therapeutic monoclonal antibodies by SARS-CoV-2 omicron sub-lineages

Franck Touret,[1,5,7,*] Emilie Giraud,[2,5] Jérôme Bourret,[3] Flora Donati,[3] Jaouen Tran-Rajau,[2] Jeanne Chiaravalli,[2] Frédéric Lemoine,[4] Fabrice Agou,[2,6] Etienne Simon-Lorière,[4,6] Sylvie van der Werf,[3,6] and Xavier de Lamballerie[1,6,*]

## SUMMARY

**The landscape of SARS-CoV-2 variants dramatically diversified with the simultaneous appearance of multiple subvariants originating from BA.2, BA.4, and BA.5 Omicron sub-lineages. They harbor a specific set of mutations in the spike that can make them more evasive to therapeutic monoclonal antibodies. In this study, we compared the neutralizing potential of monoclonal antibodies against the Omicron BA.2.75.2, BQ.1, BQ.1.1, and XBB variants, with a pre-Omicron Delta variant as a reference. Sotrovimab retains some activity against BA.2.75.2, BQ.1, and XBB as it did against BA.2/BA.5, but is less active against BQ.1.1. Within the Evusheld/AZD7442 cocktail, Cilgavimab lost all activity against all subvariants studied, resulting in loss of Evusheld activity. Finally, Bebtelovimab, while still active against BA.2.75, also lost all neutralizing activity against BQ.1, BQ.1.1, and XBB variants.**

## INTRODUCTION

Since the emergence of the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) in China in late 2019, vaccines have been the most effective and widely used therapy. However, a fraction of the population does not respond to immunization (*i.e*, immuno-compromised). There monoclonal antibodies (mAbs) have proven a great resource, both for the prevention and treatment of infection.[1] Most of such mAbs have been developed during the early stages of the outbreak and target the original SARS-Cov-2 spike.[1,2] One of them was developed from a SARS-CoV survivor and is a broadly neutralizing antibody.[3] Unfortunately, the most recent circulation of SARS-CoV-2 has been associated with the spread of multiple sub-lineages (*i.e.*, Omicron BA.1, BA.2, BA.4, BA.5 and more recently BA.2.75.2, BQ.1, BQ.1.1 and XBB variants) that combine increased transmissibility and immune escape.[4–9] They harbor different mutations in the spike that can make them more evasive to vaccination and infection-induced antibodies as well as therapeutic monoclonal antibodies.[4]

Specifically, BA.2.75.2 is derived from BA.2 and contains, among others, three major additional mutations in the Receptor-Binding Domain (RBD): R346T, N460K and F486S (Figures 1, S1, and S3) among which N460K and F486S are located in the ACE-2 Receptor Binding Motif (RBM) in the RBD. BQ.1 and BQ.1.1 are direct descendants from BA.5 and therefore contain the F486V mutation.[5] BQ.1 has gained K444T and N460K while BQ.1.1 has in addition to the R346T mutation (Figures 1, S1, and S3). Finally, the XBB variant is the result of a single breakpoint recombination in the RBD between two BA.2 subvariants: BJ.1 which contains the R346T mutation and BM.1.1.1 derived from BA.2.75 with the addition of the F486S and F490S mutations (Figures 1, S1, and S3).

The appearance of recurrent mutations or mutations at recurrent positions in different sub-lineages suggests a convergent evolution of the Omicron RBD as a result of humoral immunity to SARS-CoV-2 in the population.[4,6]

After successive waves of Omicron worldwide with BA.1 then BA.2 in winter and early spring 2022, followed by BA.4/BA.5 during the summer—albeit with regional variations in timing, only few therapeutic monoclonal antibodies neutralizing these variants remained active.[7–9] The convergent RBD mutations observed

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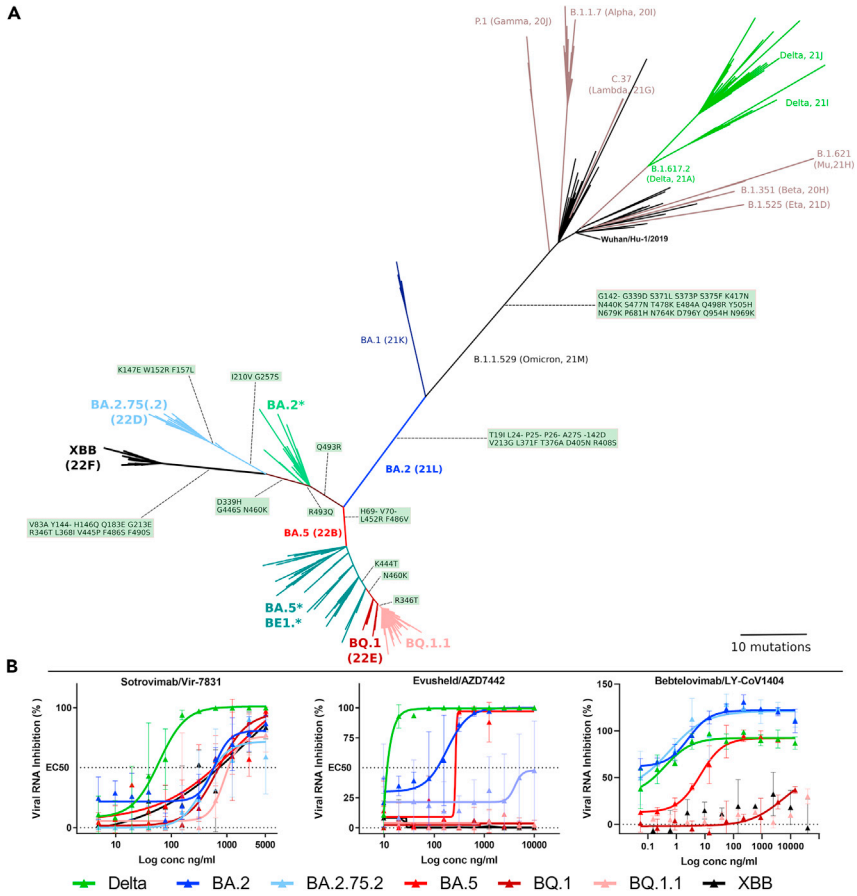



**Figure 1. Omicron subvariants phylogenetic analysis and susceptibility to therapeutic monoclonal antibodies**

(A) Unrooted phylogenetic tree displaying BQ.1.1 and XBB lineages in the context of SARS-CoV-2 main lineages; amino acid mutations in the Spike are displayed on branches of the tree for lineages of interest. The complete set of amino acid mutations is depicted in Figure S3.

(B) dose-response curves reporting the susceptibility of the SARS-CoV-2 Delta pre-omicron variant and Omicron subvariants to a panel of therapeutic monoclonal antibodies. Antibodies tested: Sotrovimab/Vir-7831, Evusheld/AZD7742 cocktail, and Bebtelovimab/Ly-CoV1404. Data presented are from three technical replicates in VeroE6-TMPRSS2 cells, and error bars show mean ± s.d.

in the multiple Omicron subvariants have the potential to further alter the activity of available therapeutic monoclonal antibodies.

## RESULTS AND DISCUSSION

### *In vitro* neutralization of omicron subvariants by therapeutic monoclonal antibodies

In this study, we tested the neutralizing activity of therapeutic antibodies against clinical isolates of the BA.2.75.2, XBB, BQ.1, and BQ.1.1 sub-lineages. We used different sets of clinical isolates as control; for BA.2.75.2 and XBB we used their first progenitor BA.2 and similarly we used BA.5 for BQ.1 and BQ.1.1. The Delta pre-Omicron variant (lineage B.1.617.2) was used as a reference for antibody neutralizing activity.[10]

We mainly tested therapeutic antibodies currently in use that have been shown to retain neutralizing activity against previous Omicron subvariants, namely Sotrovimab, Bebtelovimab, and Cilgavimab which is part, with Tixagevimab, of the Evusheld cocktail.[9] We also tested the Roche Regeneron antibodies Casirivimab (REGN10933) and Imdevimab (REGN10987), which regained activity against BA.2.[11]

**Table 1. Activity of therapeutic antibodies against Delta and Omicron BA.2, BA.5, BA.2.75.2, XBB, BQ.1, and BQ.1.1 variants**

| | | | Strain | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Antibody | | | Delta | BA.2 | BA.2.75.2 | BA.5 | BQ.1 | BQ.1.1 | XBB |
| Regeneron | Casirivimab REGN10933 | $EC_{50}$ | 14.7 | n.n | n.n | n.n | n.n | n.n | n.n |
| | | fold-change | – | – | – | – | – | – | – |
| | Imdevimab REGN10987 | $EC_{50}$ | 20.1 | 345.9 | n.n | 254.4 | n.n | n.n | n.n |
| | | fold-change | – | 17.2 | – | 12.7 | – | – | – |
| GSK/Vir | Sotrovimab (vir-7831) | $EC_{50}$ | 98.6 | 578.9 | 422.3 | 596.5 | 688.2 | 1238.0 | 806.6 |
| | | fold-change | – | 5.9 | 4.3 | 6.0 | 7.0 | 12.6 | 8.2 |
| AstraZeneca | Cilgavimab (AZD1061) | $EC_{50}$ | 21.7 | 184.0 | n.n | 147.4 | n.n | n.n | n.n |
| | | fold-change | – | 8.5 | – | 6.8 | – | – | – |
| | Tixagevimab (AZD8895) | $EC_{50}$ | 12.4 | n.n | n.n | n.n | n.n | n.n | n.n |
| | | fold-change | – | – | – | – | – | – | – |
| | Evusheld (AZD7442) | $EC_{50}$ | 12.5 | 114.9 | n.n | 265.4 | n.n | n.n | n.n |
| | | fold-change | – | 9.2 | – | 21.2 | – | – | – |
| Lilly | Bebtelovimab (LY-Cov1404) | $EC_{50}$ | 0.4 | 2.6 | 0.6 | 6.8 | n.n | n.n | n.n |
| | | fold-change | – | 6.7 | 1.5 | 17.4 | – | – | – |

n.n, non-neutralizing at the highest concentration tested.
Interpolated $EC_{50}$ values are expressed in ng/mL. Sotrovimab $EC_{50}$ values for Delta, BA.2.75.2, BQ.1, BQ1.1, and XBB are the mean of two independent experiments. For the control strain Delta, $EC_{50}$ is the mean of two independent experiments (n.n: non-neutralizing). Fold change reductions were calculated in comparison with the pre-Omicron Delta strain.

All these monoclonal antibodies target the spike Receptor Binding Domain (RBD).[2,12] However, based on analysis of their structure in complex with the RBD showing that they exhibit different binding modes, they were classified into four distinct anti-RBD antibody classes.[13] Sotrovimab/Vir-7831, which is derived from parental antibody S309, and belongs to class 3 neutralizing antibodies, has been isolated and developed from a SARS-CoV survivor and targets the RBD core region, outside the RBM.[3] Like Sotrovimab, Cilgavimab/AZD1061, Imdevimab (REGN10987) and Bebtelovimab (LY-Cov1404) belong to the same structural class and bind outside the RBM.[2,12,14,15] Finally Tixagevimab/AZD8895 and Casirivimab/REGN10933 are targeting the RBM,[2,12,14] and belong to the class 1 Nabs.

We applied a standardized protocol for the evaluation of antiviral compounds based on the reduction of RNA yield,[16,17] which has been applied previously to SARS-CoV-2 antivirals and therapeutic antibodies evaluation.[9,18,19] This assay, based on authentic and replicating viruses, was performed in VeroE6 TMPRSS2 cells; the viral RNA in the supernatant medium was quantified by qRT-PCR at 48h post-infection to determine the 50% effective concentration ($EC_{50}$).

We first observed a complete loss of detectable neutralizing activity for the four subvariants with Imdevimab (REGN10987) (Table 1, Figure S2), and still no activity with Casirivimab which made it impossible to calculate the EC50 (Table 1, Figure S2). This result is in line with previous reports using a pseudo-virus assay,[4,20] live virus[21] and with a study using a fusogenicity reporter assay.[22]

Our results show that Sotrovimab retains some neutralizing activity against BA.2.75.2, XBB, BQ.1, and BQ.1.1 *in vitro*.[4,20,22] Regarding BA.2.75.2, Sotrovimab activity seems unchanged compared to its progenitor BA.2 and a modest decrease in neutralization is observed with the XBB variant (estimated $EC_{50}$ of 806.6 ng/mL). In the case of the BQ.1 variant (Table 1, Figure 1), the $EC_{50}$ increases modestly from 596.5 (BA.5) to 688.2 (BQ.1) ng/mL, which represents a decrease in neutralization activity by a factor of ~7 (Table 1) compared to the Delta strain. For BQ.1.1 there is a further decrease in Sotrovimab activity with an $EC_{50}$ increasing to 1238 ng/mL, corresponding to a 12.6-fold decrease in neutralization activity when compared to Delta. For BQ.1.1 a greater loss of Sotrovimab activity has been recently reported using neutralization with pseudoviruses: a first study reported a decreased in the activity of ~5-folds in comparison to BA.5[4] and a second study reported no detectable neutralizing activity.[20] In a system using replicating virus with a fusogenicity reporter a decrease in neutralization activity by a factor of ~298 for

BA.2.75.2 and ~117 for BQ.1.1 compared to Delta was reported.[22] In a second study, also using replicating virus, with a plaque reduction neutralization test as readout, no neutralizing activity was reported against either XBB or BQ.1.1.[21] The discrepancy between these different results may be explained by the fact that both studies using replicative virus used cell lines overexpressing ACE-2, which have been shown to artefactually underestimate the efficacy of S309 in *in vitro* tests.[23,24]

The neutralizing activity of Tixagevimab is very low against both BA.2 and BA.5 and is not restored in any other tested variants (Figure S2, $EC_{50}$ > 5000 ng/L, see Table 1).

The other antibody of the Evusheld cocktail, Cilgavimab, which had regained neutralizing power against BA.2 and BA.5, completely lost its neutralizing activity against BQ.1 and BQ.1.1 (Figure S2, $EC_{50}$ > 5000 ng/L, see Table 1). The same pattern is observed with both the XBB and BA.2.75.2 variants with no detectable neutralization. This loss of Cilgavimab activity directly affects the Evusheld cocktail with a loss of neutralizing activity against all new subvariants tested (Figure 1, $EC_{50}$ > 10000 ng/L, see Table 1). These results are in line with recent studies aforementioned.[4,20–22]

Mechanistically, the loss of activity against the BA.5 subvariants BQ.1 and BQ.1.1 may be due to the K444T mutation which is located in a region identified as critical for Cilgavimab neutralizing activity.[2] For BA.2.75.2 and XBB the loss of Cilgavimab activity could be due to the presence of two critical mutations: (i) G446S, responsible for the decrease of Cilgavimab activity against BA.1,[18] and (ii) R346T, which, in association with G446S, is responsible for a greater loss of Cilgavimab activity against BA1.1[25]

Finally, Bebtelovimab, the most recently produced antibody which has retained a strong activity against the Omicron BA.2 and BA.5 variants, also kept its activity against BA.2.75.2. However, it has lost all neutralizing activity against BQ.1, BQ.1.1 and XBB. This is likely due to the presence of K444T in BQ.1 and BQ.1.1 and V445P in XBB. These two residue positions have been determined by deep mutational scanning to be the major sites of mutational escape for Bebtelovimab.[26]

Altogether, we conclude that Sotrovimab retains some neutralizing activity against BA.2.75.2, XBB, BQ.1, and BQ.1.1 despite a further decrease in its activity compared to the BA.2 and BA.5 progenitors. This decrease remains limited but should be closely monitored as its clinical impact remains to be documented. Recent reports have demonstrated an *in vivo* antiviral activity of Sotrovimab in a hamster model[27] and the parental antibody (S309) in the KhACE2 mice model.[28] In addition, S309 was also shown to retain its binding properties to B.Q.1.1 as well as its ability to triggers antibody-dependent cell cytotoxicity (ADCC).[28] The combination of ADCC and residual antiviral activity may provide a therapeutic effect, but further investigation *in vivo* should be performed to ensure that the recommended dose of 500 mg is sufficient to provide the best-expected benefit Regarding the Evusheld cocktail, there is a loss of neutralizing activity and it is likely that its *in vivo* activity could be jeopardized against these Omicron subvariants. The emergence of all these subvariants underscores the need for enhanced genomic surveillance to ensure that the given monoclonal antibody, when prescribed, maintains its therapeutic benefit. These results illustrate the need for a strategy that offers a combination of antiviral molecules and therapeutic antibodies that offer a broader spectrum of activity or that effectively accompany the antigenic evolution of SARS-CoV-2.

## Limitation of the study

Our study is performed *in vitro* and the results generated need to be explored *in vivo*. Moreover, in the case of sotrovimab, our assay only measures the direct antiviral effect and not the possible contribution of antibody-dependent cell cytotoxicity (ADCC) which could further increase its therapeutic effect.

## STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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## SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.isci.2023.106413.

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## AUTHOR CONTRIBUTIONS

FT, ESL, and XDL proposed the study. FT, ESL, SVDW, JC, FA, and XDL designed and conceived the experiments. FT, EG, JTR, and JC performed the experiments. FD produced and analyzed some of the viruses. JB, FL, and ESL performed the phylogenetic analysis. FT, EG, and JC analyzed the results. FT and XDL wrote the article. FT, EG, ESL, SVDW, FA, and XDL reviewed and edited the article with input from all authors.

## DECLARATION OF INTERESTS

The authors declare that there is no conflict of interest.

## INCLUSION AND DIVERSITY

We support inclusive, diverse, and equitable conduct of research.

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## STAR★METHODS

### KEY RESOURCES TABLE

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---|---|---|
| Bacterial and virus strains | | |
| SARS-CoV-2 **Delta** variant (B.1.617.2) | European Virus Archive GLOBAL | EPI_ISL_2838050 |
| SARS-CoV-2 **Omicron** BA.2 | National Reference Centre for Respiratory Viruses-Associate Laboratory | EPI_ISL_9426119 |
| SARS-CoV-2 **Omicron** BA.5.3 | National Reference Centre for Respiratory Viruses-Associate Laboratory | EPI_ISL_12852091 |
| SARS-CoV-2 **Omicron** XBB.1 | National Reference Centre for Respiratory Viruses-Associate Laboratory | EPI_ISL_15619797 |
| SARS-CoV-2 **Omicron** BA.2 | National Reference Centre for Respiratory Viruses (Institut Pasteur) | EPI_ISL_9879476 |
| SARS-CoV-2 **Omicron** BA.5.1 | National Reference Centre for Respiratory Viruses(Institut Pasteur) | EPI_ISL_13017789 |
| SARS-CoV-2 **Delta** variant (B.1.617.2) | European Virus Archive GLOBAL | EPI_ISL_2029113 |
| SARS-CoV-2 **Omicron** BA.2.75.2 | National Reference Centre for Respiratory Viruses (Institut Pasteur) | EPI_ISL_15111598 |
| SARS-CoV-2 **Omicron** XBB.3 | National Reference Centre for Respiratory Viruses (Institut Pasteur) | EPI_ISL_15527617 |
| SARS-CoV-2 **Omicron** BQ.1.7 | National Reference Centre for Respiratory Viruses (Institut Pasteur) | EPI_ISL_14778228 |
| SARS-CoV-2 **Omicron** BQ.1.1 | National Reference Centre for Respiratory Viruses (Institut Pasteur) | EPI_ISL_15195982 |
| Antibodies | | |
| Sotrovimab/ Vir-7831 (Vir/GSK) | Hospital pharmacy of the University hospital of La Timone (Marseille, France) | Xevudy |
| Casirivimab (Regeneron pharmaceuticals) | Hospital pharmacy of the University hospital of La Timone (Marseille, France) | Ronapreve |
| Imdevimab (Roche/ Astrazenaca) | Hospital pharmacy of the University hospital of La Timone (Marseille, France) | Ronapreve |
| Cilgavimab (Roche/ Astrazenaca) | Hospital pharmacy of the University hospital of La Timone (Marseille, France) | Evusheld |
| Tixagevimab (Roche/ Astrazenaca) | Hospital pharmacy of the University hospital of La Timone (Marseille, France) | Evusheld |
| Bebtelovimab (AbCellera et Eli Lilly) | Dr. H. Mouquet (Institut Pasteur, Paris, France) see Bruel et al 2022[29] | Bebtelovimab |
| Chemicals, peptides, and recombinant proteins | | |
| Minimal essential medium | ThermoFisher Scientific | Cat#21090022 |
| Non-essential amino acids | ThermoFisher Scientific | Cat#11140035 |
| Penicillin/Streptomycin | ThermoFisher Scientific | Cat#15140122 |
| Heat-inactivated fetal bovine serum | ThermoFisher Scientific | Cat#10270098 |
| Geneticin G-418 | ThermoFisher Scientific | Cat#10131027 |
| Critical commercial assays | | |
| QIAamp 96 DNA kit | Qiagen | Cat#51331 |
| Luna Universal One-Step RT-qPCR Kit | New England Biolabs | Cat#E3005S |

(*Continued on next page*)

*Continued*

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---|---|---|
| GoTaq 1 step RT-qPCR kit | Promega | Cat#A6020 |
| Experimental models: Cell lines | | |
| VeroE6/TMPRSS2 cells | NIBSC | Cat#100978 |
| Oligonucleotides | | |
| Primers SARS-CoV-2 *N* gene<br>Forward: 5′-GGCCGCAAATTGCACAAT-3′<br>Reverse: 5′-CCAATGCGCGACATTCC-3′ | Eurogentec | Figures 1 and S2 |
| Probe SARS-CoV-2 *N* gene<br>5′-FAM-CCCCCAGCGCTTCAGCGTTCT-BHQ1-3′ | Eurogentec | Figures 1 and S2 |
| Primers SARS-CoV-2 *N* gene<br>Forward: 5′- TAATCAGACAAGGAACTGATTA -3′<br>Reverse:5′- CGAAGGTGTGACTTCCATG -3′ | Eurogentec | Figures 1 and S2 |
| Software and algorithms | | |
| GraphPad Prism 9 software | GraphPad software | https://www.graphpad.com/ |
| QuantStudio 12K Flex Real-Time PCR Software v.1.2.3 | Applied Biosystems | https://www.thermofisher.com/fr/fr/home/global/forms/quantstudio-12k-flex-software-download.html |

## RESOURCE AVAILABILITY

### Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Franck Touret (franck.touret@univ-amu.fr).

### Materials availability

This study did not generate new unique reagents.

### Data and code availability

- This study did not generate/analyze any datasets.
- This study did not generate/analyze any code.

## EXPERIMENTAL MODEL

### Cell line

VeroE6/TMPRSS2 cells (ID 100978) were obtained from CFAR and were grown in MEM (Minimal Essential Medium-Life Technologies) with 7 .5% heat-inactivated Fetal Calf Serum (FCS; Life Technologies with 1% penicillin/streptomycin PS, 5000U.mL$^{-1}$ and 5000µg.mL$^{-1}$ respectively (Life Technologies) and supplemented with 1 % non-essential amino acids (Life Technologies) and G-418 (Life Technologies), at 37°C with 5% $CO_2$.

### Antibodies

Sotrovimab/ Vir-7831 was provided by hospital pharmacy of the University hospital of La Timone (Marseille, France). Casirivimab and Imdevimab (Regeneron pharmaceuticals), Cilgavimab and Tixagevimab (AstraZeneca) were obtained from the hospital pharmacy of the University hospital of La Timone (Marseille, France).

Bebtelovimab/LY-Cov1404 (Eli Lilly and Company) was kindly provided by Dr. H. Mouquet (Institut Pasteur, Paris) and its production and purification from Freestyle 293-F suspension cells was already described here.[29]

## Virus isolates

### *Isolates specific to UVE-Marseille*

SARS-CoV-2 **Delta** variant (B.1.617.2) was isolated in May 2021 in Marseille, France. The full genome sequence has been deposited on GISAID: EPI_ISL_2838050. The strain, 2021/FR/0610, is available through EVA GLOBAL (www.european-virus-archive.com, ref: 001V-04282)

SARS-CoV-2 **Omicron** BA.2 strain hCoV-19/France/NAQ-HCL022005338701/2022 was obtained from Pr. B. Lina and the sequence is available on GISAID : EPI_ISL_9426119.

SARS-CoV-2 **Omicron** BA.5.3 strain hCoV-19/France/ARA-HCL022074071401/2022 was obtained from Pr. B. Lina and the sequence is available on GISAID : EPI_ISL_12852091.

SARS-CoV-2 **Omicron** XBB.1 strain hCoV-19/France/PAC-HCL022171892001/2022 was obtained from Pr. B. Lina and the sequence is available on GISAID : EPI_ISL_15619797.

### *Isolates specific to Institut Pasteur-Paris*

SARS-Cov-2 B.1.617.2 (delta) strain hCoV-19/France/HDF-IPP11602i/2021 was supplied by the National Reference Centre for Respiratory Viruses hosted by Institut Pasteur (Paris, France) and headed by Pr. Sylvie van der Werf. The human sample from which strain hCoV-19/France/HDF-IPP11602i/2021 was isolated has been provided by Dr Guiheneuf Raphaël, CH Simone Veil, Beauvais France. Moreover, the strain hCoV-19/France/HDF-IPP11602i/2021 was supplied through the European Virus Archive goes Global (Evag) platform, a project that has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 653316.

SARS-CoV-2 **Omicron** BA.2 (hCoV-19/France/PDL-IPP08031/2022) was isolated by the National Reference Center for Respiratory Viruses hosted by Institut Pasteur (Paris, France) and headed by Pr. Sylvie van der Werf, from a specimen provided by Dr. Bénédicte Lureau, CH Fontenay-le-Comte, 85201 Fontenay-le-Comte, France. The sequence is available on GISAID : EPI_ISL_9879476.

SARS-CoV-2 **Omicron** BA.5.1 (hCoV-19/France/BRE-IPP34319/2022) was isolated by the National Reference Center for Respiratory Viruses hosted by Institut Pasteur (Paris, France) and headed by Pr. Sylvie van der Werf, from a specimen provided by Dr Franck Kerdavid, Laboratoire d'Analyses Médicales, Alliance Anabio, 35520 Melesse, France. The sequence is available on GISAID : EPI_ISL_13017789.

SARS-CoV-2 **Omicron** XBB.3 (hCoV-19/France/HDF-IPP53307/2022) was isolated by the National Reference Center for Respiratory Viruses hosted by Institut Pasteur (Paris, France) and headed by Pr. Sylvie van der Werf, from a specimen provided by Dr Anne Vachée, CH de Roubaix, 59100 Roubaix, France. The sequence is available on GISAID : EPI_ISL_15527617.

### *Isolates common to UVE-Marseille and Institut Pasteur-Paris*

SARS-CoV-2 **Omicron** BA.2.75.2 (hCoV19/France/IDF-IPP50347/2022) was isolated by the National Reference Center for Respiratory Viruses hosted by Institut Pasteur (Paris, France) and headed by Pr. Sylvie van der Werf, from a specimen provided by Dr Laura Djamdjian, CH de Gonesse, 95500 Gonesse, France. The sequence is available on GISAID : EPI_ISL_15111598.

SARS-CoV-2 **Omicron** BQ.1.7 (hCoV-19/France/HDF-IPP49210/2022) was isolated by the National Reference Center for Respiratory Viruses hosted by Institut Pasteur (Paris, France) and headed by Pr. Sylvie van der Werf, from a specimen provided by Dr Arnaud Serpentini, Unilabs Henin Beaumont, 62110, Henin Beaumont, France. The sequence is available on GISAID : EPI_ISL_14778228.

SARS-CoV-2 **Omicron** BQ.1.1 (hCoV-19/France/IDF-IPP50823/2022) was isolated by the National Reference Center for Respiratory Viruses hosted by Institut Pasteur (Paris, France) and headed by Pr. Sylvie van der Werf, from a specimen provided by Dr Beate Heym, Laboratoire des Centres de Santé et d'Hôpitaux d'IDF, 75020 Paris, France. The sequence is available on GISAID : EPI_ISL_15195982.

All viral stocks were prepared by propagation in Vero E6 TMPRSS2 cells or in Vero E6 cells in the presence of TPCK trypsin for the BA.2, BA5.1 viruses used at Institut Pasteur.

All experiments involving live SARS-CoV-2 were performed in Biosafety Level 3 (BSL-3).

## METHOD DETAILS

### $EC_{50}$ determination at UVE-Marseille

#### *1. Experiment conduction*

a. One day prior to infection, $5\times10^4$ VeroE6/TMPRSS2 cells per well were seeded in 100µL assay medium (containing 2.5% FBS) in 96 well culture plates.

b. The next day, antibodies were diluted in PBS with eleven ½ dilutions from 5000 to 4.8 ng/ml for Sotrovimab, Cilgavimab, Tixagevimab, Casirivimab and Imdevimab and its combination Evusheld . Then 25µL/well of the serial dilutions of antibodies were added to the cells in triplicate. Then, 25µL/well of a virus mix diluted in medium was added to the wells. Each well was inoculated with 100 $TCID_{50}$ of virus which correspond here to a MOI at 0.002 as classically used for SARS-CoV-2.[30] Prior to the assay it was verified for each variant that with this MOI, viruses in the cell culture supernatants were harvested during the logarithmic growth phase of viral replication at 48 hours post infection.[17,18] Four virus control wells were supplemented with 25 µL of assay medium.

#### *2. Experimental analysis*

Quantification of the viral genome by real-time RT-qPCR as previously described[30].

a. Nucleic acid from 100 µL of cell supernatant were extracted using QIAamp 96 DNA kit and Qiacube HT robot (both from Qiagen).

b. Viral RNA was quantified by real-time RT-qPCR (GoTaq 1 step RT-qPCR kit, Promega). Quantification was provided by serial dilutions of an appropriate T7-generated synthetic RNA standard. RT-qPCR reactions were performed on QuantStudio 12K Flex Real-Time PCR System (Applied Biosystems) and analyzed using QuantStudio 12K Flex Applied Biosystems software v1.2.3. Primers and probe sequences, which target SARS-CoV-2 N gene, were: Fw: 5′-GGCCGCAAATTGCACAAT-3′ ; Rev : 5′-CCAATGCGCGACATTCC-3′; Probe: 5′-FAM-CCCCCAGCGCTTCAGCGTTCT-BHQ1-3′.

#### *3Interpretation of the results*

Viral inhibition was calculated as follow: 100* (quantity mean VC- sample quantity)/quantity mean VC. The 50% effective concentrations (EC50 compound concentration required to inhibit viral RNA replication by 50%) were determined using logarithmic interpolation after performing a nonlinear regression (log(agonist) vs. response –Variable slope (four parameters)) as previously described.[17–19] All data obtained were analyzed using GraphPad Prism 9 software (Graphpad software).

### $EC_{50}$ determination at Institut Pasteur-Paris

#### *1. Experiment conduction*

a. One day prior to infection, $3\times10^3$ VeroE6/TMPRSS2 cells per well were seeded in 100µL assay medium (containing 2.5% FBS) in Black with clear bottom 384-well plates.

b. individual antibodies were added at indicated concentrations 2 h prior to infection. PBS (2µl) and remdesivir (25 µM; SelleckChem) controls were added in each plate. After the pre-incubation period, the virus inoculum (MOI 0.05 to 0.5 PFU/cell depending on the viral strains) was added to the cells. Following a one-hour adsorption at 37 °C, the supernatant was removed and replaced with 2% FBS/DMEM medium containing the individual antibodies at the indicated concentrations. Cells were incubated at 37 °C for 2 days.

c. Supernatants were harvested and heat-inactivated at 80 °C for 20 min.

### 2. Experimental analysis

Detection of viral genomes from heat-inactivated samples was performed by RT-qPCR using the Luna Universal One-Step RT-qPCR Kit (New England Biolabs) with SARS-CoV-2 specific primers targeting the N gene region (5′-TAATCAGACAAGGAACTGATTA-3′ and 5′-CGAAGGTGTGACTTCCATG-3′) and with the following cycling conditions: 55 °C for 10 min, 95 °C for 1 min, for 1 cycle, followed by 95 °C for 10 s, 60 °C for 1 min, for 40 cycles in an Applied Biosystems QuantStudio 6 thermocycler.

### 3. Interpretation of the results

Curve fits and IC50 values were obtained in Prism.

## Phylogenetic analysis

The full sequence dataset was downloaded from GISAID on Nov. 7th 2022, and annotated using pangolin 4.1.3 and pangolin data v1.16.[31] The dataset was divided in 3 categories: 1) Sequences annotated as BQ.1.1 (7,810 sequences), BA.2.75 (5,491 sequences) and XBB (25 sequences); 2) sequences of interest (lineages close to BQ.1.1 or BA.2.75), annotated as B.1.1.529, BA.2, BE.1, BE.1.1, BE.1.1.1, and BQ.1 (1,047,744 sequences); and 3) all the other sequences (12,971,674 contextual sequences). We then used these categories to build a subsampled dataset using augur 5.0.1, with 25 XBB, 78 BQ.1.1 and 14 BA.2.75 (category 1), 21 sequences of interest (category 2), 250 contextual sequences (category 3), and 45 (resp. 9) additional sequences with genetic proximity to BQ.1.1 (resp BA.2.75). A total of 317 sequences (EPI_SET ID: EPI_SET_221207ws) were then analyzed using augur workflow 5.0.1,[32] and the annotated phylogenetic tree was converted to nexus using gotree v0.4.4a.[33]