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Supplementary appendix

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Supplement to: Arora P, Kempf A, Nehlmeier I, et al. Omicron sublineage BQ.1.1 resistance to monoclonal antibodies. *Lancet Infect Dis* 2022; published online Nov 18. https://doi.org/10.1016/S1473-3099(22)00733-2.

Appendix

Omicron sublineage BQ.1.1 resistance to monoclonal antibodies

Content

Table

Table: Antibody information

Target	Mode of action	Authorisation status	Recommended use	Ref.
RBD (RBM)	Abrogates S protein/ACE2 interaction	*Monotherapy* • FDA: not approved • EMA: not approved *Combi-therapy with Imdevimab* • FDA: authorised for clinical use (EUA); restricted (24.01.2022) • EMA: authorised for clinical use	*Monotherapy* • FDA: n/a • EMA: n/a *Combi-therapy with Imdevimab* • FDA: early treatment in outpatients (risk patients) • EMA: early treatment in outpatients and post exposure prophylaxis (risk patients)	1-3
RBD (not RBM)	Abrogates S protein/ACE2 interaction	*Monotherapy* • FDA: not approved • EMA: not approved *Combi-therapy with Casirivimab* • FDA: authorised for clinical use (EUA); restricted (24.01.2022) • EMA: authorised for clinical use	*Monotherapy* • FDA: n/a • EMA: n/a *Combi-therapy with Casirivimab* • FDA: early treatment in outpatients (risk patients) • EMA: early treatment in outpatients and post exposure prophylaxis (risk patients)	1-3
RBD (RBM)	Abrogates S protein/ACE2 interaction	*Monotherapy* • FDA: authorised for clinical use (EUA); revoked (15.01.2021) • EMA: not approved *Combi-therapy with Etesevimab* • FDA: authorised for clinical use (EUA); restricted (24.01.2022) • EMA: not approved	*Monotherapy* • FDA: early treatment in outpatients (risk patients) • EMA: n/a *Combi-therapy with Etesevimab* • FDA: early treatment in outpatients (risk patients) • EMA: early treatment in outpatients (risk patients)	3-6
RBD (RBM)	Abrogates S protein/ACE2 interaction	*Monotherapy* • FDA: not approved • EMA: not approved *Combi-therapy with Bamlanivimab* • FDA: authorised for clinical use (EUA); restricted (24.01.2022) • EMA: not approved	*Monotherapy* • FDA: n/a • EMA: n/a *Combi-therapy with Bamlanivimab* • FDA: early treatment in outpatients (risk patients) • EMA: early treatment in outpatients (risk patients)	3-6
RBD (RBM)	Abrogates S protein/ACE2 interaction	*Monotherapy* • FDA: not approved • EMA: not approved *Combi-therapy with Tixagevimab* • FDA: authorised for clinical use (EUA) • EMA: authorised for clinical use	*Monotherapy* • FDA: n/a • EMA: n/a *Combi-therapy with Tixagevimab* • FDA: pre-exposure prophylaxis • EMA: pre-exposure prophylaxis	3, 7-9
RBD (RBM)	Abrogates S protein/ACE2 interaction	*Monotherapy* • FDA: not approved • EMA: not approved *Combi-therapy with Cilgavimab* • FDA: authorised for clinical use (EUA) • EMA: authorised for clinical use	*Monotherapy* • FDA: n/a • EMA: n/a *Combi-therapy with Cilgavimab* • FDA: pre-exposure prophylaxis • EMA: pre-exposure prophylaxis	3, 7-9
RBD (RBM)	Abrogates S protein/ACE2 interaction	*Monotherapy* • FDA: not approved • EMA: not approved *Combi-therapy with Romlusevimab* • FDA: not approved (EUA pending) • EMA: not approved	*Monotherapy* • FDA: n/a • EMA: n/a *Combi-therapy with Romlusevimab* • FDA: n/a • EMA: n/a	3, 10, 11
RBD (not RBM)	Abrogates S protein/ACE2 interaction	*Monotherapy* • FDA: not approved • EMA: not approved *Combi-therapy with Amubarvimab* • FDA: not approved (EUA pending) • EMA: not approved	*Monotherapy* • FDA: n/a • EMA: n/a *Combi-therapy with Amubarvimab* • FDA: n/a • EMA: n/a	3, 10, 11
RBD (RBM)	Abrogates S protein/ACE2 interaction	*Monotherapy* • FDA: not approved • EMA: not approved	*Monotherapy* • FDA: n/a • EMA: n/a	12
RBD (RBM)	Abrogates S protein/ACE2 interaction	*Monotherapy* • FDA: not approved • EMA: authorised for clinical use	*Monotherapy* • FDA: n/a • EMA: early treatment in outpatients (risk patients)	3, 13, 14
RBD (not RBM)	Abrogates S protein/ACE2 interaction	*Monotherapy* • FDA: authorised for clinical use (EUA) • EMA: not approved	*Monotherapy* • FDA: early treatment in outpatients (risk patients) • EMA: n/a	3, 7, 15-19
RBD (not RBM)	Abrogates S protein-driven entry at a post attachment step	*Monotherapy* • FDA: authorised for clinical use; revoked (05.04.2022) • EMA: authorised for clinical use	*Monotherapy* • FDA: early treatment in outpatients (risk patients) • EMA: early treatment in outpatients (risk patients)	3, 17, 18, 20, 21

mAb	Alternative names	Company
Casirivimab	REGN10933, Ronapreve (cocktail with Imdevimab), REGN-COV2 (cocktail with Imdevimab), REGEN-COV (cocktail with Imdevimab)	Regeneron Pharmaceuticals, Hoffmann-La Roche
Imdevimab	REGN10987, Ronapreve (cocktail with Casirivimab), REGN-COV2 (cocktail with Casirivimab), REGEN-COV (cocktail with Casirivimab)	Regeneron Pharmaceuticals, Hoffmann-La Roche
Bamlanivimab	LY-CoV555, LY3819253	Eli Lilly
Etesevimab	CB6, JS016, LY3832479, LY-CoV016	Eli Lilly
Cilgavimab	AZD1061, COV2-2130, Evusheld (cocktail with Tixagevimab), AZD7442 (cocktail with Tixagevimab)	AstraZeneca
Tixagevimab	AZD8895, COV2-2196, Evusheld (cocktail with Cilgavimab), AZD7442 (cocktail with Cilgavimab)	AstraZeneca
Amubarvimab	BRII-196, P2C-1F11	Brii Biosciences
Romlusevimab	BRII-198, P2B-1G5	Brii Biosciences
Adintrevimab	ADG20	Adagio Therapeutics
Regdanvimab	Regkirona, CT-P59	Celltrion Healthcare
Bebtelovimab	LY-CoV1404, LY3853113	AbCellera, Eli Lilly
Sotrovimab	Xevudy, VIR-7831, GSK4182136, S309	VIR Biotechnology, GlaxoSmithKline

Abbreviations: RBD, receptor-binding domain; RBM, receptor-binding motif; FDA, United States Food and Drug Administration; EMA, European Medicines Agency; EUA, emergency use authorization; Ref., references; n/a, not applicable.

Methods

Cell culture

Vero cells (African green monkey kidney, female, kidney; CRL-1586, ATCC; RRID: CVCL 0574, kindly provided by Andrea Maisner) and 293T (human, female, kidney; ACC-635, DSMZ; RRID: CVCL 0063) were cultivated at 37 °C in a humidified atmosphere containing 5 % CO_2 using Dulbecco's modified Eagle medium (PAN-Biotech), supplemented with 10% fetal bovine serum (Biochrom), 100 U/ml of penicillin, and 0.1 mg/ml of streptomycin (PAN-Biotech). Cell lines were validated by STR analysis, amplification and sequencing of a cytochrome c oxidase gene fragment, microscopic examination, and/or their specific growth characteristics. In addition, cell lines were routinely screened for mycoplasma contamination. Transfection of 293T cells was performed by calcium phosphate precipitation.

Expression plasmids and sequence analysis

Expression plasmids pCAGGS-DsRed[22], pCG1-SARS-CoV-2 B.1 SΔ18 (codon-optimised, C-terminal truncation of 18 amino acid residues, GISAID Accession ID: EPI_ISL_425259)[23], pCG1-SARS-CoV-2 BA.1 SΔ18 (codon-optimised, C-terminal truncation of 18 amino acid residues, GISAID Accession ID: EPI_ISL_6640919)[24] and pCG1-SARS-CoV-2 BA.4/5 SΔ18 (codon-optimised, C-terminal truncation of 18 amino acid residues, GISAID Accession ID: EPI_ISL_11550739 and EPI_ISL_12029894)[25] have been described before. For the generation of expression plasmids for SARS-CoV-2 BA.2.75.2 SΔ18 (GISAID Accession ID: EPI_ISL_15019960), SARS-CoV-2 BJ.1 SΔ18 (GISAID Accession ID: EPI_ISL_14913530) and SARS-CoV-2 BQ.1.1 SΔ18 (GISAID Accession ID: EPI_ISL_14752457), overlapping DNA strings were purchased (five strings for each S protein; Thermo Fisher Scientific, sequences available upon request), and assembled into linearised (BamHI/XbaI digest) pCG1 plasmid (a kind gift of Roberto Cattaneo, Mayo Clinic College of Medicine, Rochester, MN, USA) by Gibson assembly using the GeneArt™ Gibson Assembly HiFi Master Mix (Thermo Fisher Scientific) according to manufacturer's instructions. In addition, the expression plasmids for SARS-CoV-2 BA.4.6 SΔ18 (GISAID Accession ID: EPI_ISL_14429885) was obtained by introducing R346T and N658S mutations into plasmid pCG1-SARS-CoV-2 BA.4/5 SΔ18 through overlap-extension PCR,

using overlapping primers that harbour the respective mutations, followed by restriction digest (BamHI/XbaI) and ligation of the S protein open reading frame into the pCG1 plasmid. Integrity of all S protein sequences was confirmed by Sanger sequencing (Microsynth SeqLab). Information on S protein sequences and Omicron sublineage distribution was obtained from GISAID (Global Initiative on Sharing All Influenza Data) (https://gisaid.org/) and CoV-Spectrum (https://cov-spectrum.org/) databases.

Pseudovirus particle production

We employed a previously published protocol to generate particles pseudotyped with SARS-CoV-2 S proteins[26]. First, 293T cells transfected to express the respective S protein or DsRed (control) were inoculated with VSV-G-transcomplemented VSV*G(FLuc) (kindly provided by Gert Zimmer)[27] at a multiplicity of infection of 3. After 1h of incubation, the inoculum was aspirated and cells were washed with PBS, before medium containing anti-VSV-G antibody (culture supernatant from I1-hybridoma cells; ATCC no. CRL-2700) was added and cells were further incubated for 16-18h. Next, cell culture supernatants were collected, clarified by centrifugation (4,000 x g, 10 min), and stored at -80 °C until further use.

Neutralisation assay

Neutralisation assays were performed according to a previously published protocol[28]. One day before the neutralisation assay, Vero cells were seeded into 96-well plates and allowed to reach confluency the next day. Pseudotype particles were pre-incubated (30 min at 37°C) with different concentrations (5, 0.5, 0.05, 0.005, 0.0005 µg/ml) of monoclonal antibody (mAb; Casirivimab, Imdevimab, Bamlanivimab, Etesevimab, Cilgavimab, Tixagevimab, Adintrevimab, Amubarvimab, Romlusevimab, Regdanvimab, Bebtelovimab, Sotrovimab, or an unrelated human control antibody) or antibody cocktails (Casirivimab/Imdevimab, Bamlanivimab/Etesevimab, Cilgavimab/Tixagevimab, Amubarvimab/Romlusevimab; in case of mAb cocktails each antibody was used at half the concentration to keep total antibody concentrations constant). Next, pseudovirus/mAb-mixtures were then inoculated onto Vero cells and further incubated. After an incubation phase of 16–18 h, cell culture supernatants were aspirated and cells were lysed using PBS containing 0.5 % Triton X-100 (Carl Roth).

Following 30 min of incubation at room temperature, cell lysates were transferred into white 96-well plates, firefly luciferase substrate (Beetle-Juice, PJK) was added, and luminescence was recorded using a Hidex Sense plate luminometer (Hidex). Neutralisation efficiency was calculated based on the relative inhibition of pseudovirus entry, for which pseudovirus particles incubated with medium without mAb served as reference (=0% inhibition).

Information on epitopes of monoclonal antibodies

Information on epitopes of monoclonal antibodies was retrieved from literature[2, 6, 12, 15, 21, 29-32] and published protein structures (PDB_6XDG, PDB_7L3N, PDB_7C01, PDB_6WPS, PDB_7L7E, PDB_7CM4, PDB_8GX9, PDB_7U2D, PDB_7MMO).

Data analysis

For data analysis Microsoft Excel (part of Microsoft Office Professional Plus, version 2016, Microsoft Corporation) and GraphPad Prism version 8.3.0 (GraphPad Software) were used. Antibody concentrations resulting to a half-maximal inhibition (effective concentration 50, EC50) were calculated using a non-linear regression model.

Limitations of the study

This study has limitations. First, we utilised pseudotype particles bearing SARS-CoV-2 S protein to study SARS-CoV-2 neutralisation. Although pseudovirus particles have been demonstrated to faithfully recapitulate SARS-CoV-2 host cell entry and its neutralisation, our data await formal confirmation with clinical SARS-CoV-2 isolates. Second, the highest mAb concentration tested was 5 µg/ml, which is below the typical concentrations for treatment with respect to the total blood volume (e.g., 600 mg of Casirivimab and 600 mg of Imdevimab per patient, which roughly translates into a blood concentration of 100-150 µg/ml for adults based on a total blood volume of 4-6 l). Third, this calculation does not factor in additional neutralisation due to vaccination- or infection-induced antibodies.

Acknowledgements

We gratefully acknowledge the originating laboratories responsible for obtaining the specimens, as well as the submitting laboratories where the genome data were generated and shared via GISAID, on which this research is based.

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Supplementary figures

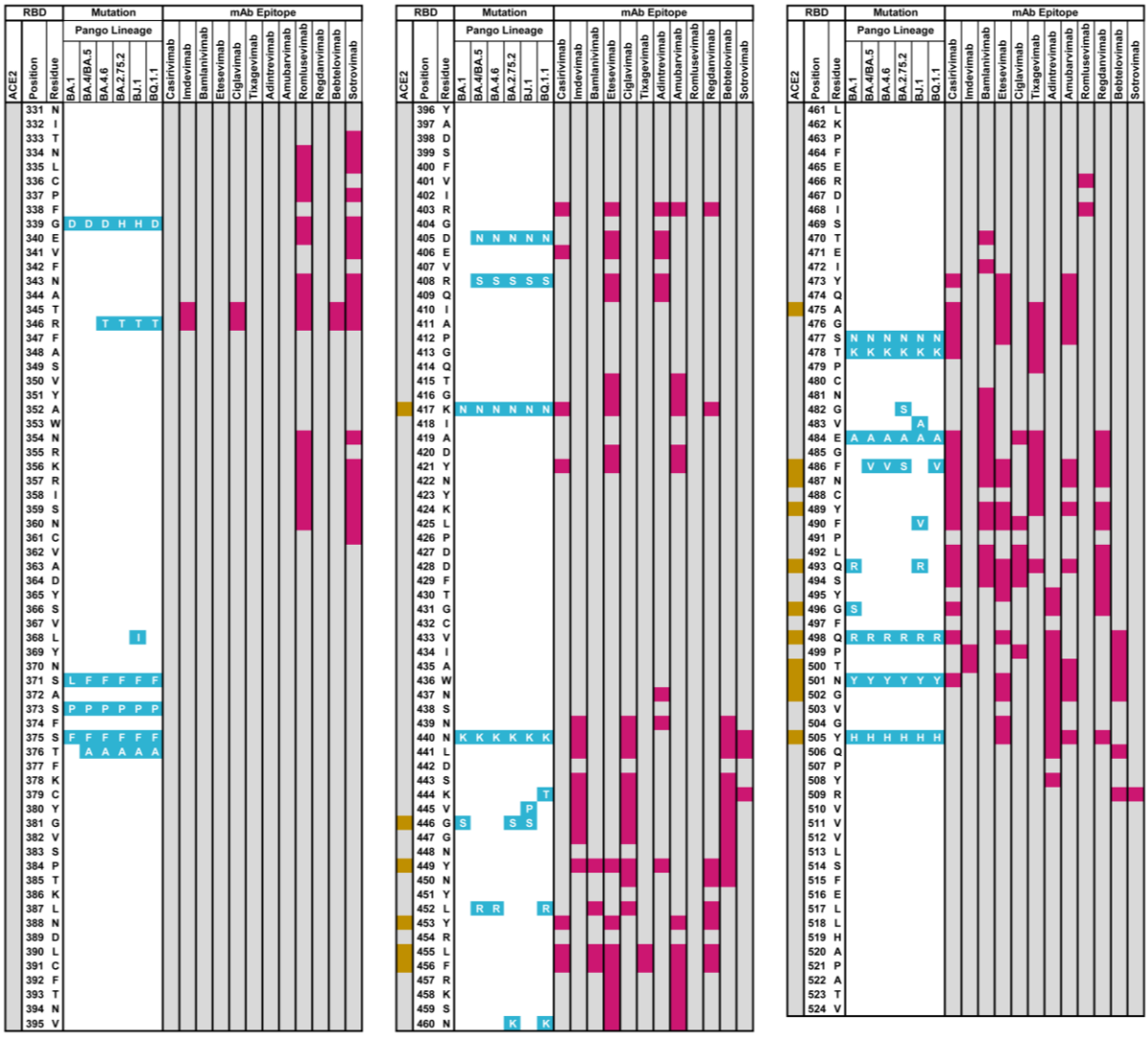

Figure S1: Location of mAb epitopes and Omicron sublineage-specific RBD mutations.

Omicron sublineage-specific RBD mutations are highlighted in blue (numbering according to SARS-CoV-2 Wuhan-Hu-01). RBD residues that interact with ACE2 (yellow) or that are part of the epitopes that are recognised by mAbs (pink) are indicated.

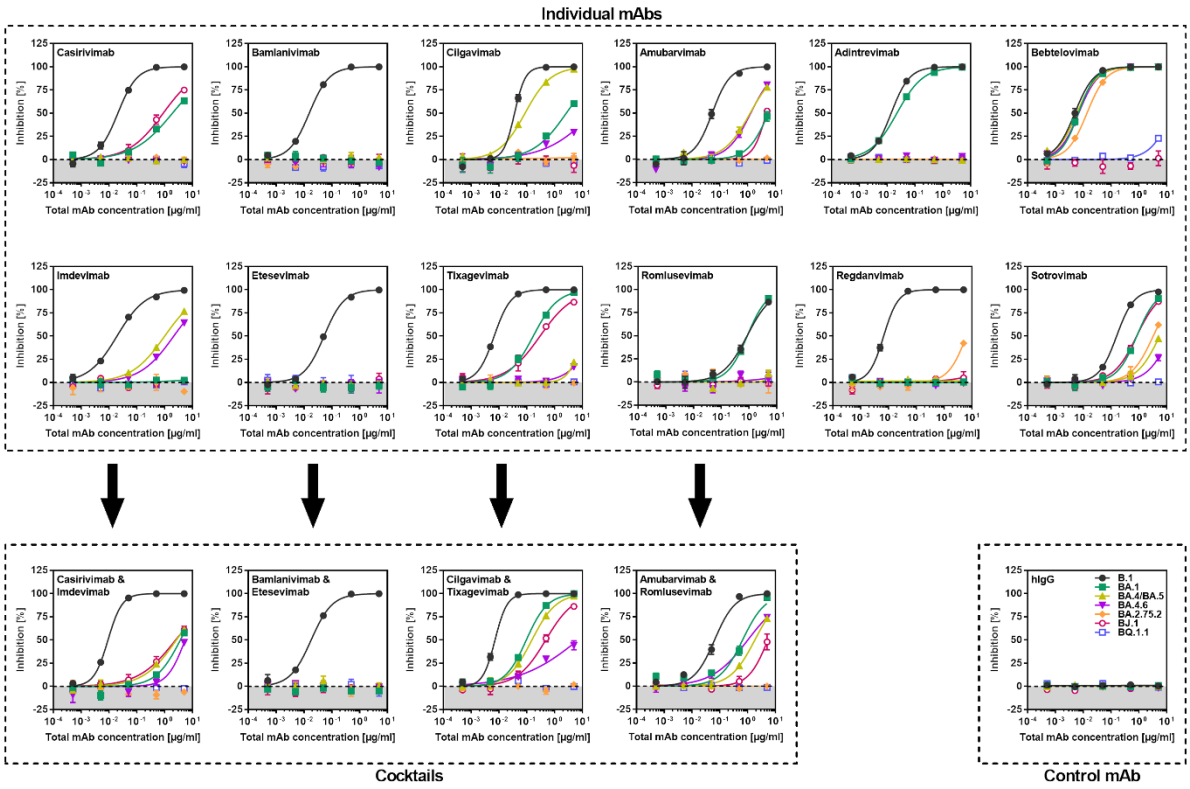

Figure S2: Individual mAb neutralisation data.

Pseudovirus particles harbouring the indicated S proteins were preincubated with different concentrations of single mAbs or mAb cocktails before being inoculated onto Vero cells (of note, for mAb cocktails, each antibody was used at half concentration to keep total antibody concentrations identical). Pseudovirus entry was analysed and normalised to samples containing no antibody (= 0% inhibition). Further, data for a human control antibody that does not target the S protein (hIgG) are shown. Presented are the mean data of three biological replicates (performed with four technical replicates). Error bars represent the standard error of the mean.