Evasion of neutralizing antibodies by Omicron sublineage BA.2.75

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Abstract

Towards the end of 2021, SARS-CoV-2 vaccine effectiveness was threatened by the emergence of the Omicron clade (B.1.1.529), with more than 30 mutations in spike. Recently, several sublineages of Omicron, including BA.2.12.1, BA.4, and BA.5, have demonstrated even greater immune evasion, and are driving waves of infections across the globe.

One emerging sublineage, BA.2.75, is increasing in frequency in India and has been detected in at least 15 countries as of 19 July 2022. Relative to BA.2, BA.2.75 carries nine additional mutations in spike. Here we report the sensitivity of the BA.2.75 spike to neutralization by a panel of clinically-relevant and pre-clinical monoclonal antibodies, as well as by serum from blood donated in Stockholm, Sweden, before and after the BA.1/BA.2 infection wave.

BA.2.75 does not show greater immune evasion than the currently-dominating BA.5 in our set of serum samples, and exhibits moderate susceptibility to tixagevimab and cilgavimab that form a widely used monoclonal antibody cocktail (Evusheld).

Main Text

Towards the end of 2021, SARS-CoV-2 vaccine effectiveness was threatened by the emergence of the Omicron clade (B.1.1.529), with more than 30 mutations in spike. Recently, several sublineages of Omicron, including BA.2.12.1, BA.4, and BA.5, have demonstrated even greater immune evasion[1–4], and are driving waves of infections across the globe.

One emerging sublineage, BA.2.75, is increasing in frequency in India and has been detected in at least 15 countries as of 19 July 2022. Relative to BA.2, BA.2.75 carries nine additional mutations in spike (Fig. S1): K147E, W152R, F157L, I210V, G257S G339H, G446S, N460K, and a reversion towards the ancestral variant, R493Q. G446S has been predicted to be a site of potential escape from antibodies elicited by current vaccines that still neutralize Omicron[5]. Further, it has been identified as a site of potential escape from LY-CoV1404 (bebtelovimab), which represents one of the last remaining classes of first-generation monoclonal antibodies that are still able to cross-neutralize BA.2 and BA.4/5[1]. As waves of Omicron infections have occurred in many countries, identifying the sensitivity of newly emerging variants to neutralization by sera sampled subsequent to these waves is required to inform public health policy.

Here we report the sensitivity of the BA.2.75 spike to neutralization by a panel of clinically-relevant and pre-clinical monoclonal antibodies, as well as by serum from blood donated in Stockholm, Sweden during week 45, 2021, (N=20) and week 15, 2022, (N=20). This coincides with points before and after a large wave of infections dominated by BA.1 and BA.2 (Dec 2021 - Feb 2022), as well as an expansion of vaccine 'booster' doses.

Cilgavimab had approximately 13-fold reduced potency against BA.2.75 compared to the ancestral B.1 (D614G), in line with its potency against BA.5. While only capable of extremely weak neutralization of BA.2, tixagevimab saw partially restored activity against BA.2.75, possibly due, in part, to the reversion to the ancestral amino acid at spike position 493. While bebtelovimab indeed demonstrated reduced potency against BA.2.75, likely due to G446S, the loss was only around 6-fold, and bebtelovimab still potently neutralizes BA.2.75. Casivirimab, imdevimab, bamlanivimab, and etesevimab all failed to neutralize BA.2.75.

BA.2.75 was neutralized with the lowest geometric mean titer of all variants evaluated by 'pre-wave' sera (Fig. 1). Titers to BA.2.75 were approximately 8-fold reduced compared to ancestral B.1 (D614G), and slightly, but significantly, lower than those against BA.2. Sera sampled following the BA.1/BA.2 infection wave displayed substantially improved neutralization against ancestral B.1 as well as enhanced cross-neutralization of omicron sublineages. Geometric mean titers against BA.2.75 were approximately 6.5-fold higher for 'post-wave' compared to 'pre-wave' sera, likely reflecting a combined contribution of BA.1 and BA.2 infections, as well as 3rd dose booster vaccine rollout, with coverage in Stockholm expanding among persons 18 years or older from 5.1% in week 45 2021 to 59% in week 15 2022[6].

As infection histories become more complex, and a large proportion of infections go undetected, monitoring of population-level immunity from random samples is increasingly critical for understanding and contextualizing the immune evasion properties of new variants. Here we show that the emerging sublineage BA.2.75 does not show greater immune evasion than the currently dominating BA.5 in a set of random samples from Stockholm, and exhibits moderate susceptibility to tixagevimab and cilgavimab that form a widely used monoclonal antibody cocktail (Evusheld).

A

	B.1	BA.2	BA.5	BA.2.75
cilgavimab	6	15	86	83
tixagevimab	<5	>10,000	>10,000	119
S309 (sotrovimab)	78	>1,000	>1,000	>1,000
REGN10933 (casivirimab)	11	>1,000	>1,000	>1,000
REGN10987 (imdevimab)	7	>1,000	>1,000	>1,000
LY-CoV1404 (bebtelovimab)	2	3	2	12
LY-CoV555 (bamlanivimab)	7	>1,000	>1,000	>1,000
LY-CoV016 (etesevimab)	34	>1,000	>1,000	>1,000

B

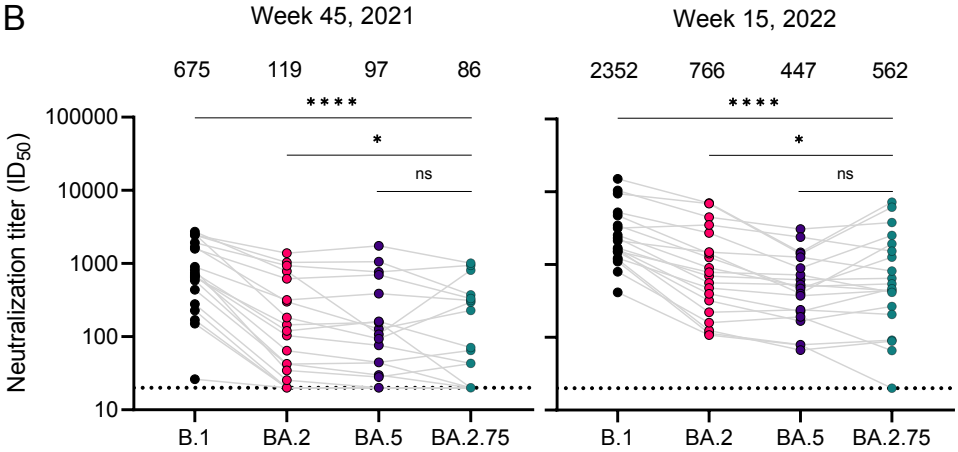

Figure 1. A. IC_{50} titers (ng/µl) for monoclonal antibodies against ancestral B.1 (D614G) and Omicron sublineages BA.2, BA.5 and BA.2.75 in a pseudovirus neutralization assay. **B.** Neutralization of BA.2.75 relative to BA.2, BA.5 and B.1 by serum (N=20) from blood donated in week 45, 2021 (8 Nov - 14 Nov) in Stockholm, Sweden, prior to a wave of infections dominated by BA.1 and BA.2 (**left**). Neutralization by serum (N=20) donated in week 15, 2022 (11 Apr - 17 Apr), after the infection wave (**right**). Depicted above are the geometric mean ID_{50} titres. Serum with an ID_{50} less than the lowest dilution tested (20, dotted line) is plotted as 20.

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Appendix

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Supplementary Figures and Tables

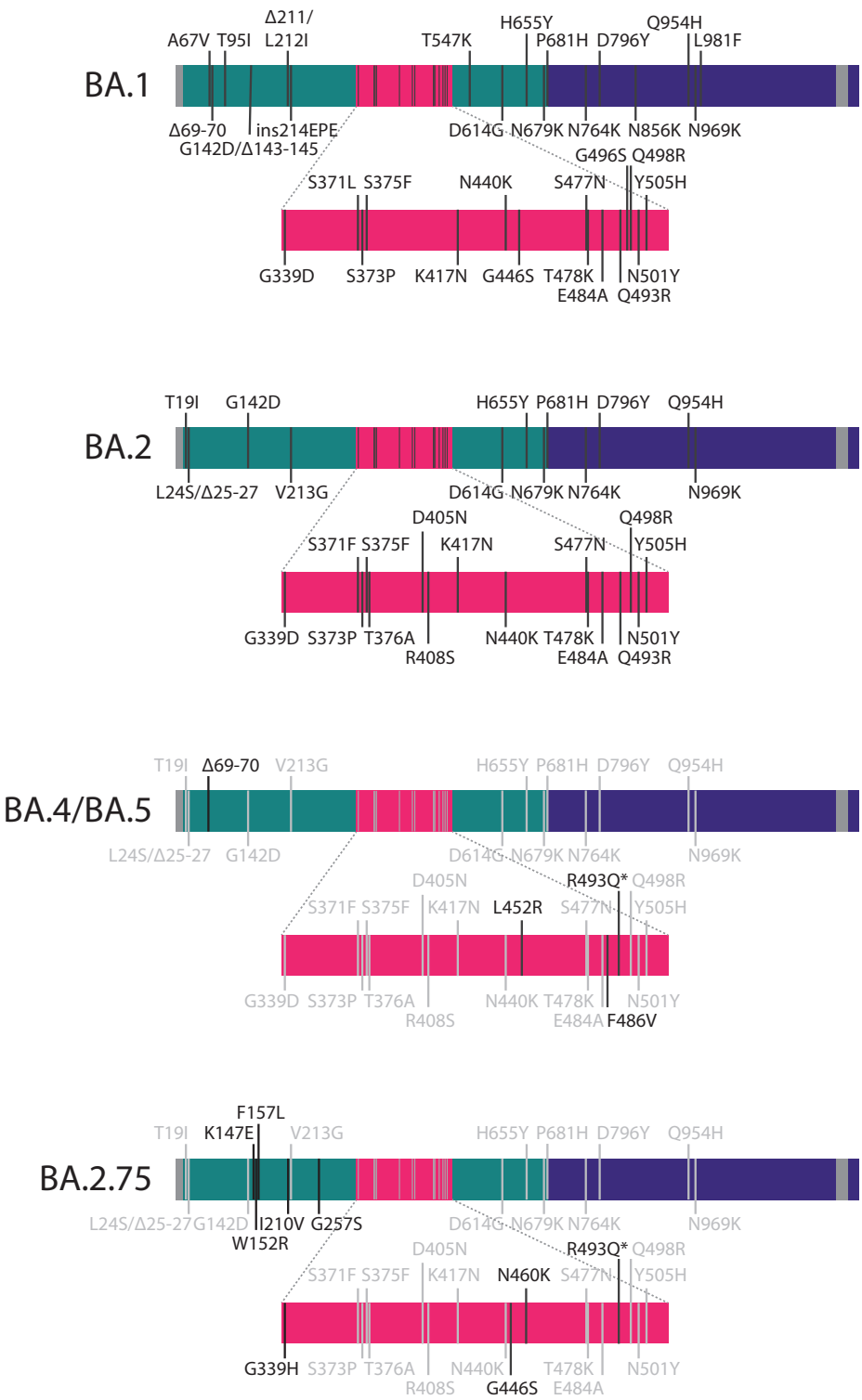

Figure S1. List of spike mutations on Omicron sub-variants BA.1, BA.2, BA.4/5, and BA.2.75. *indicates a reversion to the ancestral amino acid.

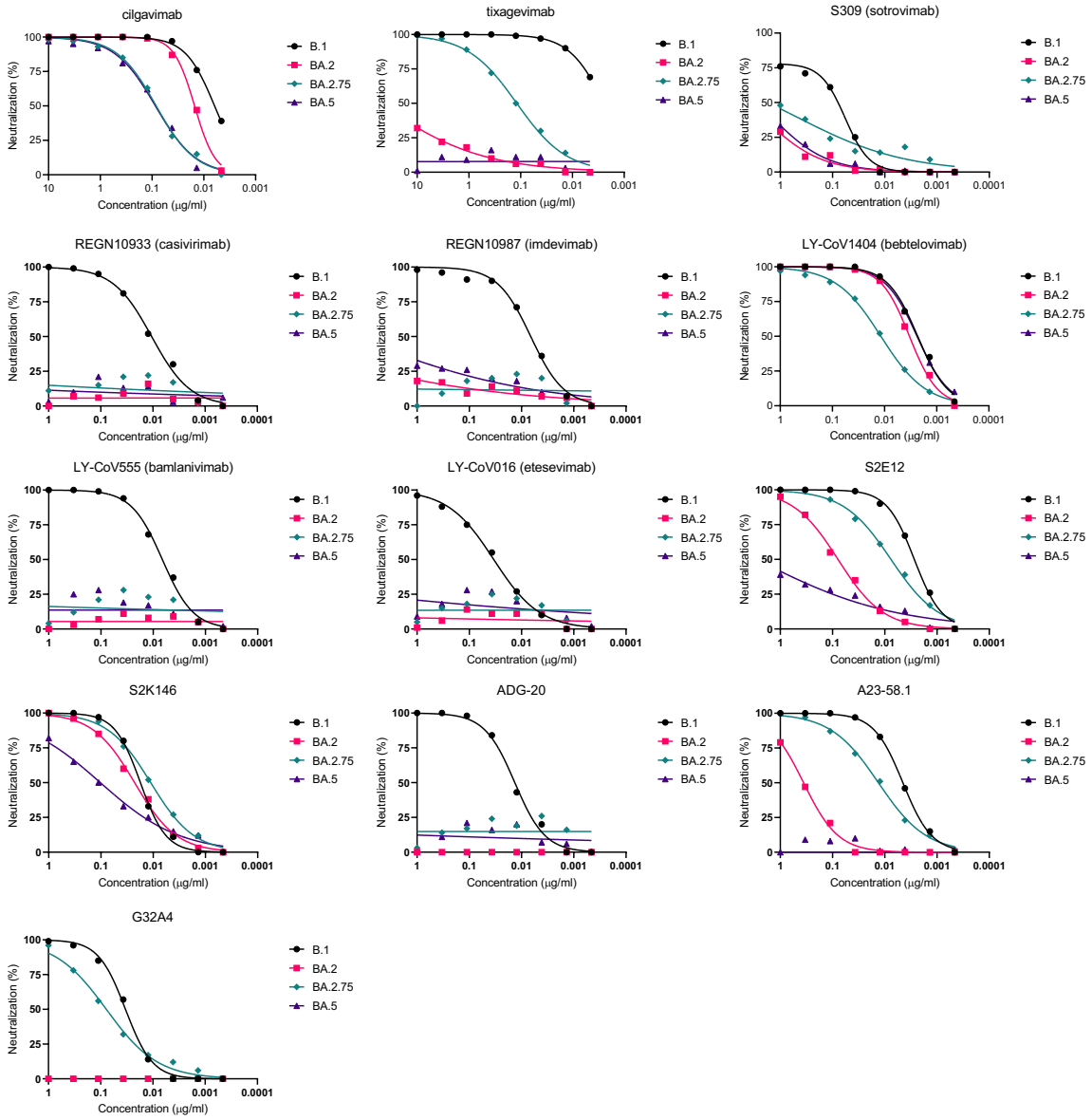

Figure S2. Neutralization curves for monoclonal antibodies against B.1 (D614G), and Omicron sublineages BA.2, BA.5 and BA.2.75.

Table S1. IC_{50} (ng/µl) for monoclonal antibodies against B.1 and Omicron sublineages BA.2, BA.5 and BA.2.75 in a pseudovirus neutralization assay.

	B.1	BA.2	BA.5	BA.2.75
cilgavimab[1]	6	15	86	83
tixagevimab[1]	<5	>10,000	>10,000	119
S309 (sotrovimab)[2]	78	>1,000	>1,000	>1,000
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LY-CoV555 (bamlanivimab)[5]	7	>1,000	>1,000	>1,000
LY-CoV016 (etesevimab)[6]	34	>1,000	>1,000	>1,000
S2E12[7]	3	79	>1,000	8
S2K146[8]	18	23	103	11
ADG20[9]	13	>1,000	>1,000	>1,000
A23-58.1[10]	4	358	>1,000	13
G32A4[11]	34	>1,000	>1,000	78

Methods

Cell culture

HEK293T cells (ATCC CRL-3216) and HEK293T-ACE2 cells (stably expressing human ACE2) were cultured in Dulbecco's Modified Eagle Medium (high glucose, with sodium pyruvate) supplemented with 10% fetal bovine serum, 100 units/ml Penicillin, and 100 µg/ml Streptomycin. Cultures were maintained in a humidified 37°C incubator (5% CO_2).

Pseudovirus Neutralization Assay

Pseudovirus neutralization assay was performed as previously[12]. Briefly, spike-pseudotyped lentivirus particles were generated by co-transfection of HEK293T cells with a relevant spike plasmid, an HIV gag-pol packaging plasmid (Addgene #8455), and a lentiviral transfer plasmid encoding firefly luciferase (Addgene #170674) using polyethylenimine. The BA.2.75 spike plasmid was generated by introducing the following mutations into the BA.2 spike, by multi-site directed mutagenesis: K147E, W152R, F157L, I210V, G257S G339H, G446S, N460K, R493Q, which was subsequently confirmed by sequencing.

Neutralization was assessed in HEK293T-ACE2 cells. Pseudoviruses sufficient to produce ±100,000 RLU were incubated with serial 3-fold dilutions of serum for 60 minutes at 37°C in a black-walled 96-well plate. 10,000 HEK293T-ACE2 cells were then added to each well, and plates were incubated at 37°C for 48 hours. Luminescence was measured using Bright-Glo (Promega) on a GloMax Navigator Luminometer (Promega). Neutralization was calculated relative to the average of 8 control wells infected in the absence of serum. Samples were run against all variants 'head-to-head' using the same dilutions.

Monoclonal antibodies

Cilgavimab and tixagevimab were evaluated as their clinical formulations. For the rest of the monoclonal antibodies evaluated, antibody sequences were extracted from deposited RCSB entries, synthesized as gene fragments, cloned into pTWIST transient expression vectors by Gibson assembly or restriction cloning, expressed and purified, all as previously described[13].

Serum samples

Serum samples from anonymized blood donors from Stockholm, Sweden, were obtained from week 45, 2021 (prior to the BA.1/BA.2 Omicron infection wave), and from week 15, 2022 (after the BA.1/BA.2 Omicron infection wave). 25 serum samples from each time point were pre-screened for detectable neutralization activity against ancestral B.1 (D614G), and 20 samples with detectable activity against B.1 (D614G) for each time point were selected, randomly, for this study. Sera were heat inactivated at 56°C for 60 minutes prior to use in neutralization assays.

Ethical Statement

The blood donor samples were anonymized, and not subject to ethical approvals, as per advisory statement 2020–01807 from the Swedish Ethical Review Authority.

Statistical analysis

Individual ID_{50} values for each sample against each variant were calculated in Prism v9 (GraphPad Software) by fitting a four-parameter logistic curve to neutralization by serial 3-fold

dilutions of serum. P values are summarized as: ns P>0.05; * P<0.05; ** P<0.01; *** P<0.001; **** P<0.0001.

Author contributions

Conceptualization, D.J.S., T.P.P., B.M.;
Formal analysis, D.J.S., B.M.;
Investigation, D.J.S., C.K., J.F., T.P.P.;
Methodology, D.J.S., C.K., R.E., T.P.P, B.M.;
Visualization, D.J.S., T.P.P., B.M.;
Resources, S.M., R.E., S.R, N.K.B., G.B.K.H., J.A., B.M.;
Supervision, D.J.S., G.B.K.H., S.T.R., J.A., B.M;
Funding Acquisition, S.T.R., G.B.K.H., J.A., T.P.P., B.M.
Writing – original draft, D.J.S.;
Writing – review & editing, D.J.S., J.F., G.B.K.H., J.A., T.P.P., B.M.

Competing Interests

STR is a cofounder of and held shares in deepCDR Biologics, which has been acquired by Alloy Therapeutics. All other authors declare no competing interests.

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