nature medicine

ARTICLES

https://doi.org/10.1038/s41591-022-01792-5

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Serum neutralization of SARS-CoV-2 Omicron sublineages BA.1 and BA.2 in patients receiving monoclonal antibodies

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The severe acute respiratory syndrome coronavirus 2 Omicron BA.1 sublineage has been supplanted in many countries by the BA.2 sublineage. BA.2 differs from BA.1 by about 21 mutations in its spike. In this study, we first compared the sensitivity of BA.1 and BA.2 to neutralization by nine therapeutic monoclonal antibodies (mAbs). In contrast to BA.1, BA.2 was sensitive to cilgavimab, partly inhibited by imdevimab and resistant to adintrevimab and sotrovimab. We then analyzed sera from 29 immunocompromised individuals up to 1 month after administration of Ronapreve (casirivimab and imdevimab) and/or Evusheld (cilgavimab and tixagevimab) antibody cocktails. All treated individuals displayed elevated antibody levels in their sera, which efficiently neutralized the Delta variant. Sera from Ronapreve recipients did not neutralize BA.1 and weakly inhibited BA.2. Neutralization of BA.1 and BA.2 was detected in 19 and 29 out of 29 Evusheld recipients, respectively. As compared to the Delta variant, neutralizing titers were more markedly decreased against BA.1 (344-fold) than BA.2 (nine-fold). We further report four breakthrough Omicron infections among the 29 individuals, indicating that antibody treatment did not fully prevent infection. Collectively, BA.1 and BA.2 exhibit noticeable differences in their sensitivity to therapeutic mAbs. Anti-Omicron neutralizing activity of Ronapreve and, to a lesser extent, that of Evusheld is reduced in patients' sera.

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) Omicron variant comprises three main sublineages, termed BA.1, BA.2 and BA.3 (ref. [1]). The original BA.1 sublineage (also termed B.1.1.529) was identified in November 2021 and became dominant worldwide in about 2 months. BA.1 demonstrated considerable escape from neutralization by mAbs and sera from vaccinated individuals[2–10]. BA.2 cases have now sharply increased, suggesting that it is more transmissible and possesses a selective advantage over BA.1. As of March 2022, BA.2 was the dominant sublineage in many countries, including Denmark, the Philippines, South Africa, France and Belgium. BA.1 and BA.2 have many mutations in common, but about 21 mutations in the spike protein differentiate the two sublineages (Fig. 1a). Neutralizing activity of sera from Coronavirus Disease 2019 (COVID-19) vaccine recipients is reduced against BA.2 relative to the ancestral strain and prior variants of concern (VOCs), to an extent similar to BA.1 (refs. [11–14]). BA.2 also displays a marked decreased sensitivity to many neutralizing mAbs when compared to previous VOCs[11,12,14–16].

Neutralizing mAbs targeting the receptor-binding domain (RBD) of the SARS-CoV-2 spike protein have been isolated from COVID-19 convalescent individuals and demonstrated efficacy in preventing or treating disease in humans[17,18]. Some mAbs are used in combination, such as Ronapreve (imdevimab and casirivimab) from Regeneron and Evusheld (cilgavimab and tixagevimab) from AstraZeneca. Evusheld mAbs are modified in their Fc regions to improve half-life and decrease Fc effector functions[8]. Post-exposure administration of Ronapreve prevented 84% of infections in a randomized clinical trial, which was conducted before Omicron circulation[19]. In a preclinical model, Evusheld protected macaques from infection with ancestral SARS-CoV-2 (ref. [20]). A press release

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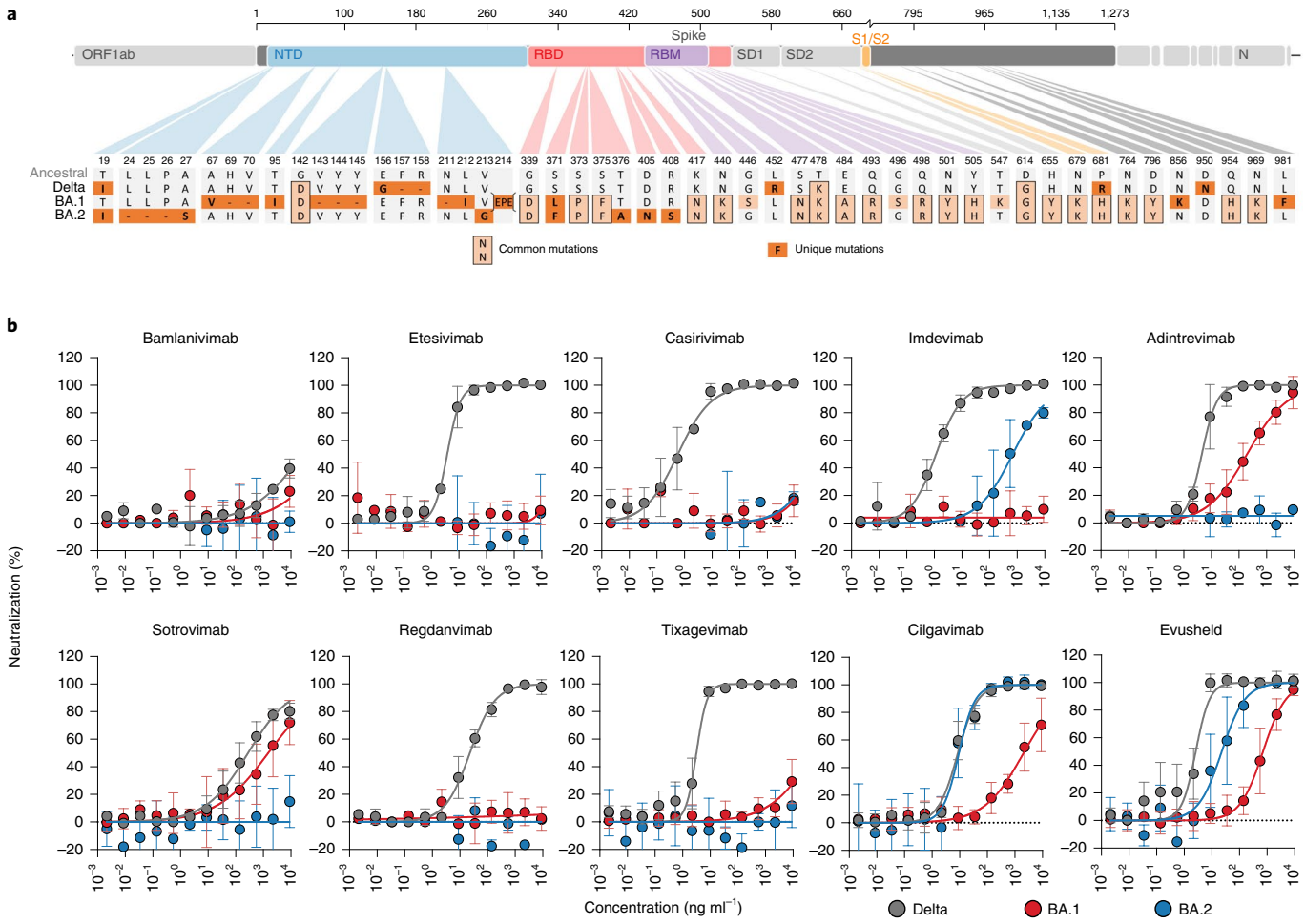

Fig. 1 | Sensitivity of Omicron BA.1 and BA.2 to therapeutic mAbs. a, Mutational landscape of Omicron BA.1 and BA.2 spike proteins. Domains of the protein are color-coded: NTD, N-Terminal Domain; RBD, Receptor-Binding Domain; RBM, Receptor-Binding Motif; SD1, subdomain 1; SD2, subdomain 2, S1/S2, region proximal to the furin cleavage site. Mutations in the amino acid sequence are indicated in comparison to the ancestral Wuhan-Hu-1 sequence (GenBank: NC_045512). Light orange boxes indicate mutations shared by BA.1 and BA.2, and orange boxes indicate mutations unique to BA.1 and BA.2. **b**, Neutralization curves of mAbs. Dose–response analysis of the neutralization by the indicated antibodies and by Evusheld, a combination of cilgavimab and tixagevimab. Data are mean ± s.d. of 2–8 independent experiments. The IC_{50} values for each antibody are presented in Table 1. NTD, N-terminal domain.

from AstraZeneca indicated that intra-muscular administration of Evusheld (300 mg) reduced symptomatic disease by 83%[21]. The efficacy of Evusheld in preventing virus infection is not known. Both Ronapreve and Evushled received emergency use approval for pre-exposure prophylaxis (PrEP) in many countries. However, in cell culture systems, BA.1 is resistant to casirivimab and imdevimab and partially evades cilgavimab and tixagevimab[3,4,6,9]. Different studies have reported an 11–183-fold increase in the 50% inhibitory concentration (IC_{50}) of Evusheld against BA.1 relative to ancestral strains[22]. As BA.1 was becoming predominant, these results motivated the switch of emergency use from Ronapreve to Evusheld for PreP in immunocompromised individuals. Besides Ronapreve and Evusheld, other mAbs are in clinical use. For instance, sotrovimab, a pan-coronavirus antibody, is indicated for treatment of infected individuals at risk for severe disease[23]. The relative capacity of mAbs to neutralize Omicron BA.1 and BA.2 sublineages is poorly characterized, with discordant preliminary results regarding mAbs such as sotrovimab and imdevimab. The clinical significance of the reduced sensitivity of Omicron BA.1 and BA.2 to neutralizing antibodies in cell culture remains unknown. To address this question, we first evaluated the sensitivity of infectious BA.1 and BA.2 isolates to nine therapeutic mAbs in a cell culture system. We then directly measured the neutralizing activity of the antibodies in sera from immunocompromised individuals who had received Ronapreve and/or Evusheld.

Results

We isolated a BA.2 variant from a nasopharyngeal swab that was initially sequenced at the National Reference Center of UZ/KU Leuven (Belgium). The virus was amplified by two passages on Vero E6 cells and re-sequenced (Pango lineage BA.2, 21L (Omicron), according to Nextstrain, GISAID accession ID: EPI_ISL_10654979) (Fig. 1a). When compared to the Delta variant (B.1.617), the BA.2 spike protein contained 30 changes, with 18 modifications that are shared with BA.1 (Fig. 1a). The modifications are dispersed throughout the spike but display a preferential accumulation in the N-terminal domain and the RBD (Fig. 1a). Viral stocks were titrated using S-Fuse cells. These reporter cells become GFP^+ upon infection, allowing rapid measurement of viral infectivity and neutralizing antibody activity[24,25]. Syncytia were observed in BA.2-infected S-Fuse cells, with a size similar to those induced by BA.1 (Extended Data Fig. 1). As previously reported[4], Delta-infected cells formed large syncytia, bigger than BA.1-infected or BA.2-infected cells (Extended Data Fig. 1). This suggests that BA.1 and BA.2 may behave similarly in terms of fusogenicity and fitness.

Table 1 | IC_{50} of therapeutic mAbs against Delta and against Omicron BA.1 and BA.2

	Delta	BA1	BA2
Bamlanivimab	>9,000	>9,000	>9,000
Etesivimab	3.8	>9,000	>9,000
Casirivimab	0.58	>9,000	>9,000
Imdevimab	1.2	>9,000	693
Adintrevimab	4.5	198	>9,000
Regdavimab	23	>9,000	>9,000
Sotrovimab	280	1,508	>9,000
Tixagevimab	3.2	>9,000	>9,000
Cilgavimab	8.5	1,988	9.3
Evusheld	2.6	715	23

IC_{50} (ng ml^{-1}).

We first measured the sensitivity of BA.2 to a panel of nine mAbs that were, or are currently, in clinical use[8,26–31]. These mAbs belong to the four main classes of anti-RBD antibodies, which are defined by their binding site[18,32,33]. In addition to the antibodies present in Ronapreve (casirivimab and imdevimab) and Evusheld (cilgavimab and tixagevimab), we tested the following antibodies: bamlanivimab and etesevimab (class 2 and class 1, respectively) were initially mixed in the Eli Lilly cocktail and are no longer in clinical use; regdanvimab (Regkirona) (Celltrion) is a class 1 antibody; sotrovimab (Xevudy) by GlaxoSmithKline and Vir Biotechnology is a class 3 antibody that targets an epitope outside of the receptor-binding motif (RBM); and adintrevimab (ADG20, Adagio) binds to an epitope located in between class 1 and class 4 sites. We compared the activity of these nine mAbs against the Delta variant and against the Omicron BA.1 and BA.2 sublineages (Fig. 1b).

Seven antibodies (bamlanivimab, etesevimab, casirivimab, sotrovimab, adintrevimab, regdanvimab and tixagevimab) were inactive against BA.2. The two other antibodies (imdevimab and cilgavimab) displayed an IC_{50} of 693 ng ml^{-1} and 9 ng ml^{-1} against BA.2, respectively (Fig. 1b and Table 1), indicating that they were more active against BA.2 than BA.1. The addition of tixagevimab to cilgavimab in the Evusheld cocktail was not more efficient than cilgavimab alone (Fig. 1b and Table 1). These results are in line with recent reports[11,12,14] and highlight substantial differences in the neutralization profiles of BA.1 and BA.2.

We next measured antibody levels and neutralization activity in the sera of 29 immunocompromised individuals before and after administration of Evusheld (Table 2). Some individuals ($n=18$ of 29) were previously treated with Ronapreve 10–49 days (mean, 35 days) before Evusheld administration. The first group of patients was a cohort of eight individuals (six females and two males) from the Centre Hospitalier Regional of Orléans, France, with pre-existing conditions, including rheumatoid arthritis (RA, $n=5$), kidney transplantation ($n=2$) and myelodysplasia ($n=1$). Most patients were receiving anti-CD20 (rituximab) ($n=5$) and prednisone ($n=4$). These treatments were maintained before and after vaccination and at the time of administration of the anti-SARS-CoV-2 mAbs. The patients were previously vaccinated with three doses of BNT162b2 (Pfzier/BioNTech), and three had a 4th dose. Three patients received Ronapreve as PrEP 4–7 weeks before Evusheld. The second group of twenty-one patients (thirteen females and eight males) came from Hôpital Cochin in Paris. They were suffering from autoimmune diseases, including RA ($n=2$), vasculitis ($n=17$), polychondritis ($n=1$) and lupus ($n=1$). They were vaccinated with three doses of BNT162b2, except one who received two doses of ChadOX-1 (AstraZeneca) and one dose of mRNA-1273 (Moderna). Three patients received a 4th dose of BNT162b2, and another had a history of COVID-19. They were mostly treated with rituximab ($n=17$). Fifteen of the 21 individuals were already receiving Ronapreve. None of the 29 individuals elicited antibodies above 264 binding antibody units per milliliter (BAU ml^{-1}) after vaccination and were, thus, eligible to receive Evusheld PreP, according to French health authority guidelines[34].

We first analyzed the eight individuals from the Orléans cohort, as longitudinal samples were available (Fig. 2a). We used the S-Flow assay to quantify anti-spike IgGs in sera collected at days 0, 3, 15 and 30 after Evusheld administration. Day 30 sampling was available

Table 2 | Characteristics of patients

	'Orléans' cohort	'Cochin' cohort	Total (%)
Patient characteristics			
n	8	21	29
Age	58 (42–78)	62 (31–92)	61 (31–92)
Female	6	8	14 (64)
Male	2	13	15 (52)
Obesity	3	2	5 (17)
Diseases			
Rheumatoid arthritis	5	2	7 (24)
Kidney graft	2	0	2 (7)
Ayelodysplasia	1	0	1 (3)
ANCA-associtaed vasculitis	0	17	17 (60)
Polychondritis	0	1	1 (3)
Lupus	0	1	1 (3)
Medications			
Rituximab (anti-CD20)	5	17	22 (76)
Infliximab (anti-TNF)		1	1 (3)
Prednisone	4	10	14 (48)
Mycofenolate mofetil	2	1	3 (10)
Methotrexate	0	3	3 (10)
5-azacytidine	1	0	1 (3)
Tacrolimus	1	0	1 (3)
Cyclosporin	1	0	1 (3)
Vaccines			
1st doses			
Pfizer	8	20	28 (97)
AstraZeneca	0	1	1 (3)
2nd doses			
Pfizer	8	20	28 (97)
AstraZeneca		1	1 (3)
3rd doses			
Pfizer	8	20	28 (97)
Moderna		1	1 (3)
4th doses			
Pfizer	3	3	6 (21)
Previous COVID-19	0	1	1 (3)
PrEP			
Ronapreve	3	15	18 (62)
Evusheld	8	21	29 (100)

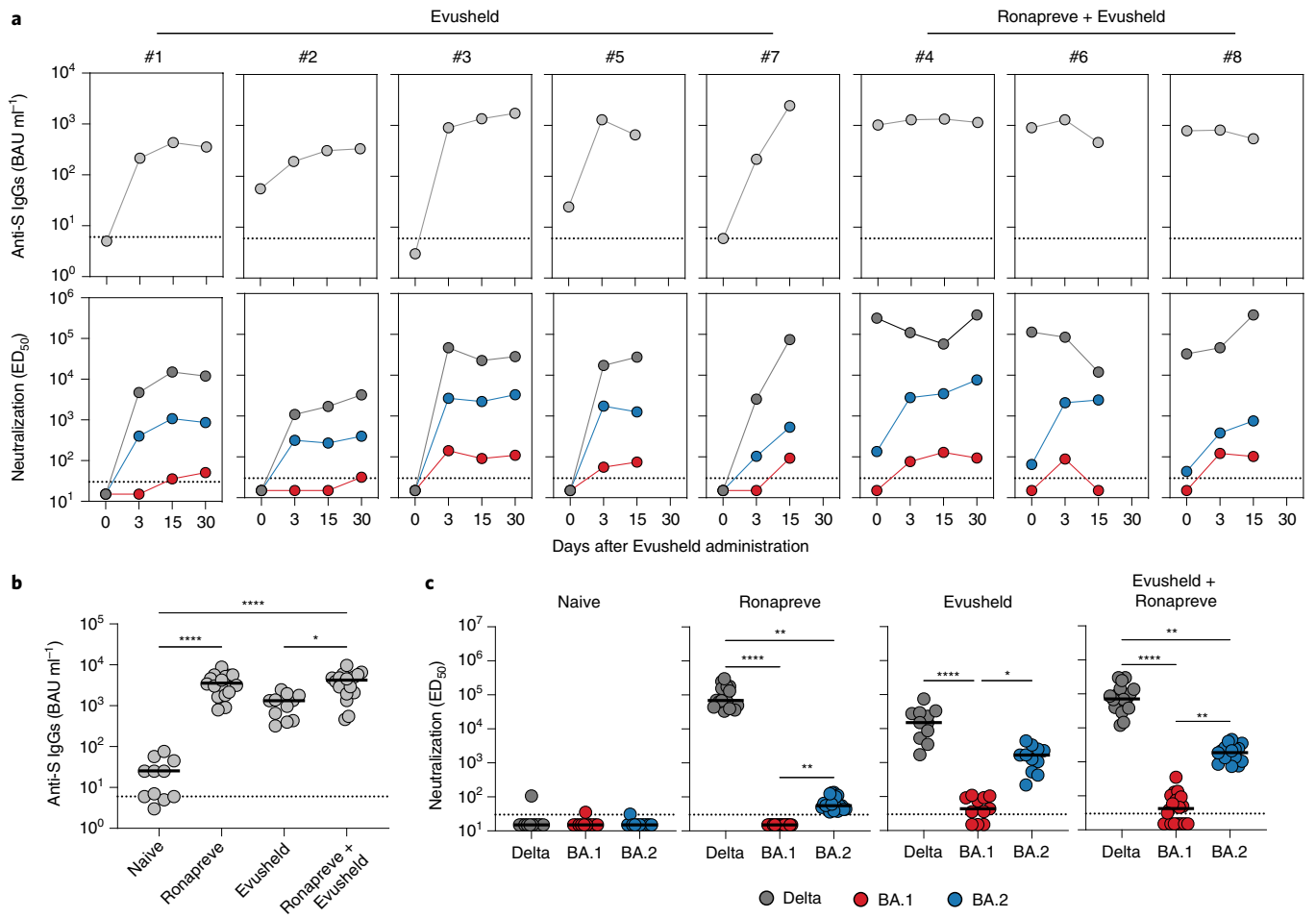

Fig. 2 | Neutralization of Delta and Omicron BA.1 and BA.2 by sera of immunocompromised individuals receiving Ronapreve and/or Evusheld as a pre-exposure prophylaxis. a, Eight individuals from the Orléans cohort were followed longitudinally, before and after Evusheld administration. Anti-S IgGs were measured using the flow cytometry-based S-Flow assay (top panel). Neutralization of Delta and Omicron BA.1 and BA.2 was measured with the S-Fuse assay (bottom panel). The dotted lines indicate the limit of detection of the assays. Three individuals received first Ronapreve and then Evusheld. **b**, Anti-S IgG levels in sera of individuals before PrEP (naive; $n=11$), treated with Ronapreve ($n=18$), treated with Evusheld ($n=11$) or treated with both Ronapreve and Evusheld ($n=18$). Two-sided Kruskall–Wallis test with Dunn's multiple comparison correction. Naive versus Ronapreve ($P<0.0001$), naive versus Ronapreve+Evusheld ($P<0.0001$), Evusheld versus Ronapreve+Evusheld ($P=0.024$). **c**, Sero-neutralization of Delta and Omicron BA.1 and BA.2 in the same individuals as in **b**. Two-sided Friedman tests with Dunn's multiple comparison correction were performed to compare the different groups. Ronapreve: Delta versus BA.1 ($P\leq0.0001$), Delta versus BA.2 ($P\leq0.0081$), BA.1 versus BA.2 ($P\leq0.0081$); Evusheld: Delta versus BA.1 ($P\leq0.0001$), BA.1 versus BA.2 ($P\leq0.031$); Evusheld+Ronapreve: Delta versus BA.1 ($P\leq0.0001$), Delta versus BA.2 ($P\leq0.0081$), BA.1 versus BA.2 ($P\leq0.0081$).

for only four individuals. In the five Ronapreve-naive individuals, administration of Evusheld led to a sharp increase of anti-spike IgGs (from 5–57 BAU ml^{-1} before treatment to 195–1,290 BAU ml^{-1} after treatment) (Fig. 2a). As expected, the three individuals who initially received Ronapreve had anti-spike antibodies (788-1,016 BAU ml^{-1}) at the time of Evusheld administration (day 0), with no detectable effect of Evusheld on antibody levels (Fig. 2a). In all patients, levels of anti-spike antibodies were stable or slightly increasing between days 3 and 30 (Fig. 2a).

We then measured the neutralizing activity of the sera against Delta and Omicron BA.1 and BA.2 by calculating 50% effective dilution (ED_{50}) titers with the S-Fuse assay (Fig. 2a). None of the five Ronapreve-naive individuals had detectable neutralization activity at day 0. Evusheld administration led to a sharp increase of neutralizing activity against Delta, with ED_{50}s between 788 and 1,016. For the three individuals having previously received Ronapreve, Evusheld administration did not increase their levels of neutralization against Delta. In line with in vitro experiments (Fig. 1b and refs. [4,6]), sera from Ronapreve-naive and Ronapreve-treated individuals did not neutralize BA.1. After Evusheld treatment, seven of eight individuals neutralized BA.1 at different time points between days 3 and 30. Titers were, however, very low, ranging from 27 to 128 at day 15. For most of the patients, we observed an increase of antibody levels between days 3 and 15, reflecting the pharmacokinetics of the antibodies. The delayed and low neutralizing activity of the sera at day 3 against BA.1 was likely due to the poor antiviral activity of the mAbs against this viral isolate. A low level of BA.2 neutralizing activity was detectable in the three Ronapreve-treated individuals, in line with the ability of imdevimab to neutralize BA.2 (Fig. 1b). Sera from the five Ronapreve-naive individuals did not neutralize BA.2 at day 0. Evusheld administration raised BA.2 neutralization in all individuals, with titers reaching up to an ED_{50} of 3,534 at day 15 (Fig. 2b). Neutralization titers for the three viral lineages were stable for six of eight individuals, consistent with Evusheld's long half-life[20].

We extended this analysis to the 21 individuals of the second group, who were sampled at a single time point, 15–30 days after Evusheld administration. We combined the results obtained with the first group of eight individuals at day 15 to collectively analyze

Table 3 | Summary of breakthrough cases

Case	Diagnostic	Strain	Days after Evusheld	Anti-S (BAU ml^{-1})	Neutralization BA.1 (ED_{50})	COVID-19
1	PCR$^+$ screening	Omicron	15	9,630	351	Mild
2	PCR$^+$ screening	Omicron	12	5,736	7,5	Mild
3	PCR$^+$ screening	Omicron	21	1,786	36	Mild
4	PCR$^+$ sequencing	BA.1	23	4,536	31	Severe

29 individuals. The nine Ronapreve-naive individuals had low levels of anti-spike antibodies (below 264 BAU ml^{-1}), reflecting the inefficacy of the vaccination (Fig. 2b). Ronapreve or Evusheld therapy strongly and similarly increased anti-spike IgGs in the sera (median of 3,263 BAU ml^{-1} and 1,321 BAU ml^{-1}) (Fig. 2b). These levels were not higher in individuals who successively received the two treatments (Fig. 2b).

We next measured neutralization titers in the 29 sera (Fig. 2c). The untreated individuals did not neutralize any of the three strains. Ronapreve-treated individuals efficiently neutralized Delta, were inactive against BA.1 and poorly neutralized BA.2. Sera from Evusheld-treated and Ronapreve+Evusheld-treated individuals were efficient against Delta (ED_{50} of 15,109 and 71,324, respectively), barely neutralized BA.1 (ED_{50} of 44 and 42, respectively) and quite efficiently neutralized BA.2 (ED_{50} of 1,673 and 1,882, representing a nine-fold and 38-fold decrease, respectively, compared to Delta) (Fig. 2c). After Evusheld administration, eight of 11 individuals, who did not previously receive Ronapreve, had neutralization activity against BA.1 in their sera, and all neutralized BA.2. This confirmed that Evusheld is more active against BA.2 than BA.1. There was no major difference in the neutralization titers in individuals having received only Evusheld or the successive combination of Ronapreve and Evusheld (Fig. 2c). The neutralizing activity against Delta correlated to anti-spike IgG levels, whereas this was not the case for BA.1 and BA.2 (Extended Data Fig. 2). This reflects an uncoupling of the capacity of the antibodies to bind to the spike from the ancestral Wuhan strain and to neutralize Omicron BA.1 and BA.2 strains. Altogether, these data show that administration of Evusheld in immunocompromised individuals elicits poor sera neutralizing activity against BA.1 and better activity against BA.2.

In agreement with the decreased sero-neutralization activity of Evusheld-treated individuals against BA.1, we observed four breakthrough infections among the 29 participants. A summary of the cases is provided in Table 3 and Extended Data Fig. 3, along with the serology and neutralization data of the closest sampling point. The four cases came from the second cohort of patients. Polymerase chain reaction (PCR) screening confirmed Omicron infection for the four cases but did not allow for distinction between BA.1 and BA.2. However, in France, at the time of the sampling, BA.1 represented 90% of sequenced cases, whereas BA.2 was detected in less than 10% of cases. Sequencing was performed only for case 4 and confirmed BA.1 infection in this individual. Three of the four individuals received sotrovimab after diagnosis, according to French guidelines. Three cases were classified as mild disease, whereas case 4 was classified as severe and required hospitalization. Despite detection of high levels of anti-spike antibodies in the sera, the neutralization titers against BA.1 were low and ranged between <7.5 and 351 for the four individuals (Table 3 and Extended Data Fig. 3). These four cases indicate that Evusheld neither protects against Omicron infection nor fully prevents severe disease.

Discussion

We highlight here substantial differences not only between the Delta and Omicron variants but also between BA.1 and BA.2 Omicron sublineages with regard to their sensitivity to therapeutic mAbs. Considering that these variants have sequentially dominated the pandemic in the last few months, and the vulnerability of immunocompromised individuals to both Omicron infection and severe disease, our results support the importance of genomic surveillance. Rapid genotyping or sequencing will need to be introduced in clinical practice to better inform treatment of patients with COVID-19. For pre-exposure prophylaxis, which was the application studied here, it will be important to use mAbs that cover both BA.1 and BA.2 (for example, bebtelovimab)[14,35], especially in regions where both sublineages are prevalent. Our results also show that measuring antibody levels with standard serology assays that currently use an ancestral spike antigen does not inform on protection. Future work will help determine whether adapted, lineage-specific, serological or neutralization assays can be used as a marker of clinical efficacy.

Our study has limitations. The relatively low number of individuals analyzed did not allow us to evaluate the clinical efficacy of Evusheld against BA.2. We did not have access to nasopharyngeal samples of the individuals. Measuring antibody levels and neutralizing activity in these types of samples could provide insights into the capacity of mAbs to neutralize Omicron sublineages at the infection site. We also did not test neutralization of the BA.1.1 and BA.3 sublineages of Omicron. Future experiments with these sublineages are needed to determine the antiviral activity of mAbs against the full landscape of the Omicron clade, which we recently proposed to be considered as a distinct SARS-CoV-2 serotype from ancestral strains and previous variants[36]. We observed that syncytia induced by BA.1 and BA.2 are of similar size and smaller than those formed by Delta-infected cells. Future experiments are warranted to determine affinity to ACE2 and other characteristics of the BA.2 spike. It will also be informative to study the binding of the sera to BA.1 and BA.2 spike proteins to confirm the neutralization results obtained with infectious viral strains.

Although clinical trials that can provide a complete evaluation of the effect of BA.2 on the treatment efficacy of mAbs have yet to be completed, based on our observation of breakthrough infections we expect more frequent treatment failures. It is also possible that the progressive accumulation of further mutations will increase the level of resistance of BA.1 or BA.2 to mAbs during prolonged infection. The low or intermediate sensitivity to Ronapreve and Evusheld, when used as a pre-exposure prophylaxis in immunocompromised individuals at risk for severe disease, is of potential concern. One can speculate that the risk that further escape mutations will arise in these individuals is higher compared to Delta. We, therefore, recommend a close follow-up of these individuals, particularly in case of prolonged infection despite treatment.

Online content

Any methods, additional references, Nature Research reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at https://doi.org/10.1038/s41591-022-01792-5.

Received: 6 March 2022; Accepted: 22 March 2022;
Published online: 23 March 2022

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Methods

No statistical methods were used to predetermine sample size. The experiments were not randomized, and the investigators were not blinded to allocation during experiments and outcome assessment. Our research complies with all relevant ethical regulation, as detailed in the 'Cohorts' section.

Cohorts. Immunocompromised individuals receiving Evusheld were recruited in two centers (CHR d'Orléans and Hôpital Cochin) in the French cities of Orléans and Paris. The 'Orléans' cohort is an ongoing prospective, monocentric, longitudinal, observational cohort clinical study aiming to describe the kinetics of neutralizing antibodies after SARS-CoV-2 infection or vaccination (ClinicalTrials.gov identifier: NCT04750720). This study was approved by the Est II (Besançon) ethical committee. At enrollment, written informed consent was collected, and participants completed a questionnaire that covered sociodemographic characteristics, clinical information and data related to anti-SARS-CoV-2 vaccination. Blood sampling was performed on the day of Evusheld infusion and after 3 days, 15 days and 1 month. The 'Cochin' cohort is a prospective, monocentric, longitudinal, observational clinical study (NCT04870411) enrolling immunocompromised individuals with rheumatic diseases, aiming at describing immunological responses to COVID-19 vaccine in patients with autoimmune and inflammatory diseases treated with immunosuppressants and/or biologics. Ethics approval was obtained from the Comite de Protection des Personnes Nord-Ouest II. Leftover sera from usual care were used from these individuals in the setting of the local biological samples collection (RAPIDEM). A written informed consent was collected for all participants. None of the study participants received compensation.

Viral strains. The Delta strain was isolated from a nasopharyngeal swab of a hospitalized patient returning from India[37]. The swab was provided and sequenced by the virology laboratory of Hopital Européen Georges Pompidou (Assistance Publique–Hopitaux de Paris). The Omicron strain was supplied and sequenced by the NRC UZ/KU Leuven (Belgium)[4]. The BA.2 strain was isolated from a nasopharyngeal swab sampled on 4 January 2022 from a 10-year-old male patient. His legal authorized representative provided written informed consent. The sample was sequenced in the context of active surveillance by the NRC UZ/KU Leuven, showing an average coverage of 989× for the Omicron BA.2 genome, after which it was cultured on Vero E6 cells. We noted an additional mutation in the spike of our BA.2 isolate (R682W) compared to the primary sample from which it was isolated, although this mutation was already present at low frequency in the original swab. We obtained similar neutralization profiles with another BA.2 isolate. Both patients provided informed consent for the use of the biological materials. The sequences of the isolates were deposited on GISAID immediately after their generation, with the following Delta ID: EPI_ISL_2029113; Omicron ID: EPI_ISL_6794907; and Omicron BA.2 GISAID ID: EPI_ISL_10654979. Titration of viral stocks was performed on Vero E6 cells, with a limiting dilution technique allowing a calculation of $TCID_{50}$ or on S-Fuse cells.

mAbs. Bamlanivimab, casirivimab, etesevimab, imdevimab, cilgavimab, tixagevimab and sotrovimab were provided by CHR Orleans. Adintrevimab (ADG20) and regdanvimab (CT-P59) were produced as previously described[4].

S-Fuse neutralization assay. U2OS-ACE2 GFP1-10 or GFP11 cells, also termed S-Fuse cells, become GFP^+ when they are productively infected by SARS-CoV-2. Cells tested negative for mycoplasma. Cells were mixed (ratio 1:1) and plated at 8×10^3 per well in a µClear 96-well plate (Greiner Bio-One). The indicated SARS-CoV-2 strains were incubated with serially diluted mAb or sera for 15 minutes at room temperature and added to S-Fuse cells. The sera were heat-inactivated for 30 minutes at 56 °C before use. Eighteen hours later, cells were fixed with 2% paraformaldehyde (PFA), washed and stained with Hoechst (dilution 1:1,000, Invitrogen). Images were acquired with an Opera Phenix high-content confocal microscope (PerkinElmer). The GFP area and the number of nuclei were quantified using Harmony software version 4.9 (PerkinElmer). The percentage of neutralization was calculated using the number of syncytia as value with the following formula: 100 × (1 − (value with serum − value in 'non-infected') / (value in 'no serum' − value in 'non-infected')). Neutralizing activity of each serum was expressed as the ED_{50}. ED_{50} values (in µg ml^{-1} for mAbs and in dilution values for sera) were calculated with a reconstructed curve using the percentage of the neutralization at the different concentrations. We previously reported correlations between neutralization titers obtained with the S-Fuse assay and both pseudovirus neutralization and microneutralization assays[38,39].

Anti-spike serology. The S-Flow assay uses 293T cells stably expressing the spike protein (293T spike cells) and 293T control cells as control to detect anti-spike antibodies by flow cytometry[40]. In brief, the cells were incubated at 4 °C for 30 minutes with sera (1:300 dilution) in PBS containing 0.5% BSA and 2 mM EDTA. Cells were then washed with PBS and stained with an anti-human IgG Fc Alexa Fluor 647 antibody (109-605-170, Jackson Immuno Research). After 30 minutes at 4 °C, cells were washed with PBS and fixed for 10 minutes using 4% PFA. A standard curve with serial dilutions of a human anti-spike monoclonal antibody (mAb48) was acquired in each assay to standardize the results as a binding Unit (BU). Data were acquired on an Attune NxT instrument using Attune NxT software version 3.2.2 (Life Technologies) and analyzed with FlowJo version 10.7.1 software (see Extended Data Fig. 4 for gating strategy). The sensitivity is 99.2% with a 95% confidence interval of 97.69–99.78%, and the specificity is 100% (98.5–100%)[40]. To determine BAU ml^{-1}, we analyzed a series of vaccinated ($n = 144$), convalescent ($n = 59$) samples and World Health Organization international reference sera (20/136 and 20/130) on S-Flow and on two commercially available ELISAs (Abbott 147 and Beckmann 56). Using this dataset, we performed a Passing–Pablok regression, which shows that the relationship between BU and BAU ml^{-1} is linear, allowing calculation of BAU ml^{-1} using S-Flow data[41].

Statistical analysis. Flow cytometry data were analyzed with FlowJo version 10 software. Calculations were performed using Excel 365 (Microsoft). Figures were drawn on Prism 9 (GraphPad Software). Statistical analysis was conducted using GraphPad Prism 9. Statistical significance between different groups was calculated using Kruskall–Wallis test with Dunn's multiple comparions, Friedman tests with Dunn's multiple comparison correction and Spearman non-parametric correlation test. All tests were two-sided.

Reporting Summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability

All data supporting the findings of this study are available within the article or from the corresponding authors upon reasonable request without any restrictions. Source data are provided for Figs. 1 and 2 and Extended Data Fig. 2. Source data are provided with this paper.

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Acknowledgements

We thank the European Health Emergency Preparedness and Response Authority (HERA) for supporting the work being done at Institut Pasteur and UK Leuven. We thank J. Buchrieser, J. Puech and F. Gambaro for their help with the sequencing data analysis. We thank the patients who participated to this study. We thank Y. Yazdanpanah, members of the Virus and Immunity Unit and other teams for discussions and help, N. Aulner and the UtechS Photonic BioImaging (UPBI) core facility (Institut Pasteur), a member of the France BioImaging network, for image acquisition and analysis. The Opera system was co-funded by Institut Pasteur and the Région Île-de-France (DIM1Health). We thank the KU Leuven University authorities and J. Arnout, B. Lambrecht, C. Van Geet and L. Sels for their support. We thank F. Peira, V. Legros and L. Courtellemont for their help with the cohorts. UZ Leuven, as national reference center for respiratory pathogens, is supported by Sciensano, which is gratefully acknowledged. Work in the O.S. lab is funded by Institut Pasteur, Urgence COVID-19 Fundraising Campaign of Institut Pasteur, Fondation pour la Recherche Médicale (FRM), ANRS, the Vaccine Research Institute (ANR-10-LABX-77), Labex IBEID (ANR-10-LABX-62-IBEID), ANR/FRM Flash Covid PROTEO-SARS-CoV-2, ANR Coronamito and IDISCOVR. Work in the UPBI facility is funded by grant ANR-10-INSB-04-01 and the Région Île-de-France program DIM1Health. D.P. is supported by the Vaccine Research Institute. G.B. acknowledges support from the Internal Funds KU Leuven under grant agreement C14/18/094 and the Research Foundation–Flanders (Fonds voor Wetenschappelijk Onderzoek–Vlaanderen, G0E1420N and G098321N). P.M. acknowledges support from a COVID-19 research grant of 'Fonds Wetenschappelijk Onderzoek'/Research Foundation Flanders (grant G0H4420N). P.M. acknowledges support of a COVID-19 research grant from 'Fonds Wetenschappelijk Onderzoek'/Research Foundation Flanders (grant G0H4420N) and 'Internal Funds KU Leuven' (grant 3M170314). E.S.L. acknowledges funding from the INCEPTION program (Investissements d'Avenir grant ANR-16-CONV-0005). The funders of this study had no role in study design, data collection, analysis and interpretation or writing of the article.

Author contributions

Experimental strategy and design: T.B., D.P., B.T., T.P. and O.S. Laboratory experiments: T.B., D.P., I.S., F.G.B., F.P., W.H.B., C.C., H.P., D.V., M.P., A.B. and C.P. Cohort management and clinical research: T.B., J.H., D.P., A.S., I.S., Y.N., M.C., C.C., H.P., D.V., L.M., L.H., B.T., T.P. and O.S. Viral strains: P.M., F.G.B., H.P., D.V., L.C., G.B. and E.A. Manuscript writing: T.B. and O.S. Manuscript editing: T.B., J.H., P.M., D.P., G.B., L.H., E.S.L., E.A., B.T. and O.S.

Competing interests

T.B., C.P., H.M. and O.S. have a pending patent application for an anti-RBD mAb not used in this study (PCT/FR2021/070522). All other authors declare no conflicts of interest.

Additional information

Extended data is available for this paper at https://doi.org/10.1038/s41591-022-01792-5.

Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41591-022-01792-5.

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Peer review information *Nature Medicine* thanks Ailong Huang, Joshua Tan and the other, anonymous, reviewer(s) for their contribution to the peer review of this work. Saheli Sadanand was the primary editor on this article and managed its editorial process and peer review in collaboration with the rest of the editorial team.

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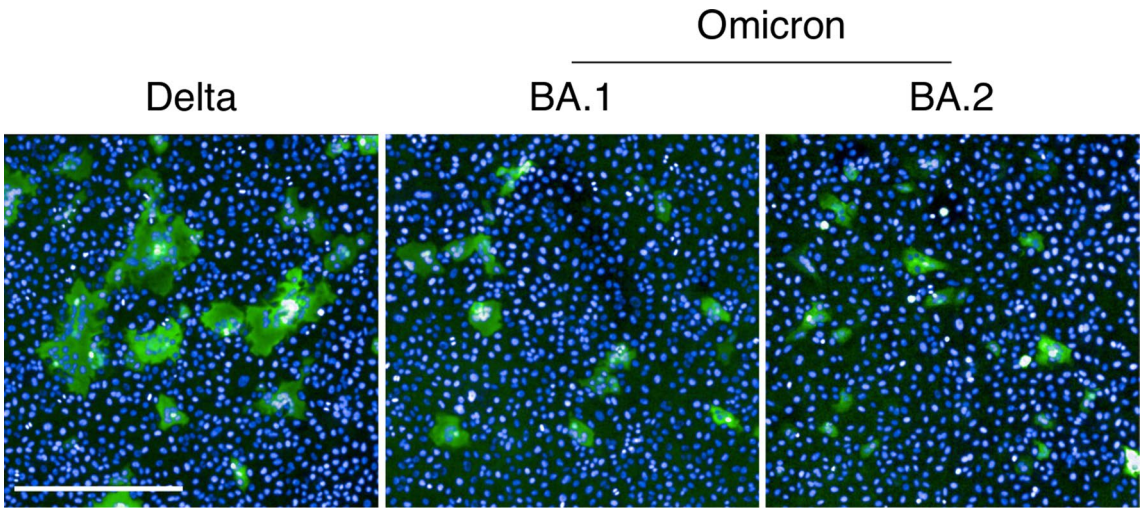
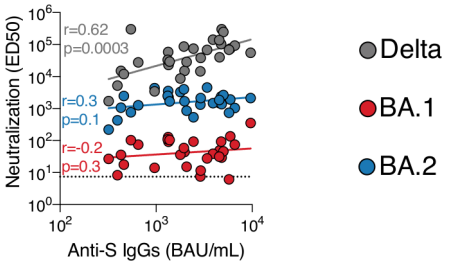
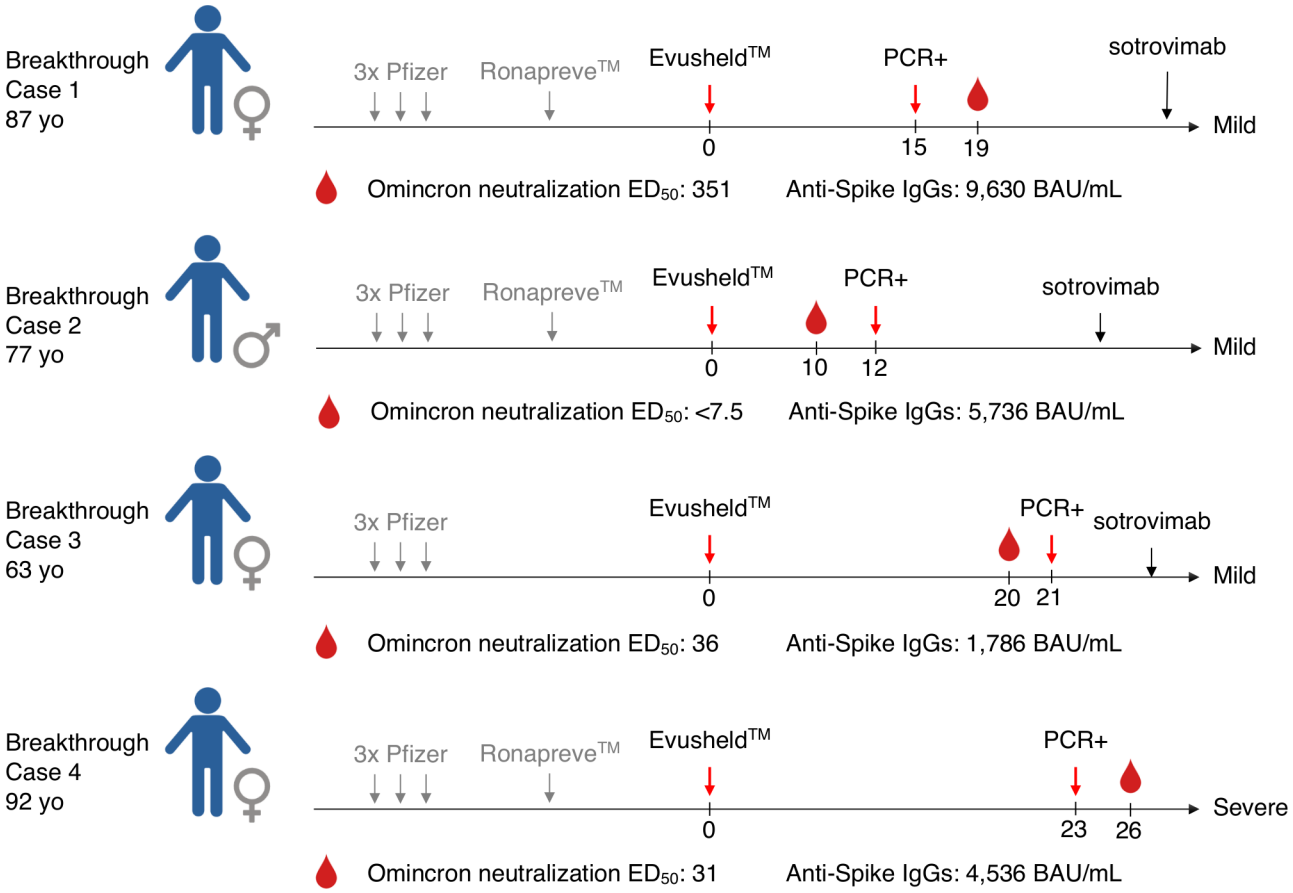


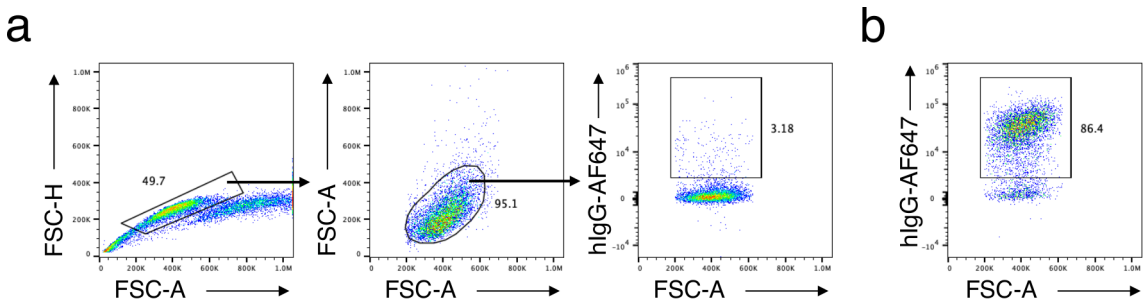
Extended Data Fig. 1 | SARS-CoV-2 variants Delta, BA.1 and BA.2 induce syncytia in S-Fuse cells. S-Fuse cells that become GFP + upon cell-cell fusion were exposed to the indicated SARS-CoV-2 strains. After 20 h, cells were stained with Hoechst to visualize nuclei. Syncytia (green) and nuclei (blue) are shown. Representative images from three independent experiments are shown. Scale bar, 500 µm.



Extended Data Fig. 2 | Correlation of neutralization capacity and anti-S antibody levels in individuals having received Ronapreve and/or Evusheld. Two-sided Spearman non-parametric correlations of neutralizing antibody titers against Delta, Omicron BA.1 and BA.2 and the level of anti-S IgG. R and p-values are indicated.



Extended Data Fig. 3 | Report of four Omicron breakthrough infections in Evusheld treated patients. A timeline indicates the key events for each of the 4 Omicron breakthrough cases. Patients' characteristics and antibody measurement of the closest sampling point are indicated.



Extended Data Fig. 4 | Gating strategy of the S-Flow assay. 293T cells stably expressing the Wuhan Spike were incubated with sera from patient treated with monoclonal antibodies (dilution 1:300), stained with an anti-human secondary antibody and analyzed by flow-cytometry. **a**. One representative example of the gating strategy is shown. Gates are set on cells transfected with a control plasmid not encoding a spike. **b**. An example of the signal obtained by a reactive serum on spike expressing cells is shown.

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☐	☒	A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
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☒	☐	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
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☒	☐	Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	Harmony Software v4.9 (Perkin-Elmer), Attune Nxt Software v3.2.1 (ThermoFischer), Flowjo Software v10.7.1
Data analysis	Excel 365 v16.46 (Microsoft), Prism v9.0.2 (GraphPad Software)

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All data supporting the findings of this study are available within the article or from the corresponding authors upon request without any restrictions. Source data are provided for Figures 1 and 2, and Extended Data Figure 2.

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Life sciences study design

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Sample size	Given the explanatory nature of the study aiming at describing a phenomenon whose frequency has not yet been established it was not possible to use statistical methods were used to predetermine sample size. Thus, we included between 10 and 50 patients per group to allow statistical analysis.
Data exclusions	None.
Replication	All experiments were performed and verified in multiple replicates as indicated in their methods/figure legends.
Randomization	The experiments were not randomized as we tested all available samples. Individuals were included without any selection other than those imposed by the entry criteria. Under these conditions, no particular bias is envisaged.
Blinding	For convenience experiments were not blinded. However, the clinical sampling and biological measurements were performed by different teams. Only the final assembly of the data revealed the global view of the results.

Reporting for specific materials, systems and methods

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Materials & experimental systems

n/a	Involved in the study
☐	☒ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☒	☐ Animals and other organisms
☐	☒ Human research participants
☐	☒ Clinical data
☒	☐ Dual use research of concern

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☐	☒ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used	Adintrevimab (ADG20) and Regdavimab (CT-P59) are human anti-S monoclonal antibodies produced by Hugo Mouquet (Institut Pasteur). Bamlanivimab (LY-CoV555; Lily), Etesivimab (LY-CoV016; Lily), Casirivimab (REGN10933; Regeneron), Imdevimab (REGN10987; Regeneron), Cilgavimab (AZD1061; Astrazeneca), Tixagevimab (AZD8895; AstraZeneca) and Sotrovimab (VIR-7831; GSK) were kind gifts of Thierry Prazuck and Laurent Hocqueloux. The Goat anti-Human IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor 647 (109-605-170) was obtained from Jackson ImmunoResearch.
Validation	The reactivity of Adintrevimab and Regdavimab to the SARS-CoV-2 spike was validated using ELISA binding assays (against the trimeric S, RBD, and S2 proteins) by the team of H.Mouquet. The reactivity of Bamlanivimab, Etesivimab, Casirivimab, Imdevimab, Cilgavimab, Tixagevimab and Sotrovimab to the SARS-CoV-2 spike was validated by measuring their neutralizing activity against SARS-CoV-2. Validation of the goat anti-human IgG has been performed by Jackson ImmunoResearch, using immunoelectrophoresis and/or ELISA to confirm that the antibody reacts with the Fc portion of human antibodies.

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	Vero E6 (ATCC® CRL-1586™), 293T cells (ATCC CRL- 3216) and U2OS cells (ATCC® HTB-96™), all obtained from the ATCC.
Authentication	Cell lines were not authenticated.

Mycoplasma contamination	All cells are negative for mycoplasma contamination. Tests are performed every Monday.
Commonly misidentified lines (See ICLAC register)	None

Human research participants

Policy information about studies involving human research participants

Population characteristics	Given the exploratory design of the study, the characteristics of participants were not pre-established when entering the cohorts. Relevant co-variates (age, sex, obesity, disease, medications, vaccinations and previous COVID-19) are provided in the corresponding supplementary tables. Nasopharyngeal swabs used for viral isoaltion were leftover samples from usual care. All Participants or their legal authorized representatives provided a written informed consent.
Recruitment	Individuals admitted to the hospitals for Evusheld administration were invited to participate. Individuals were included without any selection other than those imposed by the entry criteria. Under these conditions, no particular bias is envisaged. Leftover sera from usual care were used from these individuals in the setting of the local biological samples collection (RAPIDEM).
Ethics oversight	The "Orléans" cohort is an ongoing prospective, monocentric, longitudinal, observational cohort clinical study aiming to describe the kinetic of neutralizing antibodies after SARS-CoV-2 infection or vaccination (ClinicalTrials.gov Identifier: NCT04750720). This study was approved by the Est II (Besançon) ethical committee.The "Cochin" cohort is a prospective, monocentric, longitudinal, observational clinical study (NCT04870411) enrolling immunocompromised individuals with rheumatic diseases, aiming at describing immunological responses to COVID-19 vaccine in patients with autoimmune and inflammatory diseases treated with immunosuppressants and/or biologics. Ethics approval was obtained by Comite de Protection des Personnes Nord-Ouest II. Leftover sera from usual care were used from these individuals in the setting of the local biological samples collection (RAPIDEM).

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Clinical data

Policy information about clinical studies

All manuscripts should comply with the ICMJE guidelines for publication of clinical research and a completed CONSORT checklist must be included with all submissions.

Clinical trial registration	NCT04750720 and NCT04870411
Study protocol	All protocols can be accessed on clinicaltrial.gov
Data collection	The Orléans cohort started on August 2020 in Orléans Hospital (Centre hospitalier Réginal Orléans), and is on-going. The Cochin started on May 2021 in Orléans Hospital (Centre hospitalier Réginal Orléans), and is on-going.
Outcomes	The primary outcome of the study was the presence of antibody to SARS-CoV-2 antibody binding to the spike protein (S-Flow assay). The secondary outcome of the assay was the presence of neutralizing antibodies (S-Fuse assay)

Flow Cytometry

Plots

Confirm that:

☒ The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).

☒ The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).

☒ All plots are contour plots with outliers or pseudocolor plots.

☒ A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation	SARS-CoV-2 infected Vero cells were stained as indicated in the method section. All samples were acquired within 24h.
Instrument	Attune NxT Acoustic Focusing Cytometer, blue/red/violet/yellow (catalog number : 15360667)
Software	AttuneNxT Software v3.2.1
Cell population abundance	At least 10,000 cells were acquired for each condition.

Gating strategy | All gates were set on uninfected Vero cells.

☒ Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.