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Letter to the editor

Resistance mutations in SARS-CoV-2 omicron variant in patients treated with sotrovimab

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To the Editor,

The SARS-CoV-2 B.1.1.529 (Omicron) variant, divided in five lineages (BA.1/BA.2/BA.3/BA.4/BA.5), harbors numerous spike protein mutations particularly in the receptor-binding domain (RBD). Recent studies showed that the Omicron variant resists the majority of RBD-targeting monoclonal antibodies (mAb) [1]. Sotrovimab, a pan-sarbecovirus neutralizing mAb recently authorized, seems to remain efficient to neutralize the omicron variant [1,2]. However, as it targets a single epitope, the risk of developing resistance mutations is not negligible. We determined the evolution of the virus load, the development of spike mutations, and the evolution of the virus complexity in nasopharyngeal (NP) swabs from sotrovimab-treated ambulatory Omicron-infected patients.

Between 24 January and 21 February 2022, we collected NP samples from Omicron-infected patients, before a single intravenous infusion (day 0) of sotrovimab (500 mg), 7 days after (day 7), and weekly until the viral load reached 31 cycle thresholds (Ct). SARS-CoV-2 RNA was extracted with the MGI extraction system (MGI Tech, Shenzhen, China) and quantified using ThermoFisher (ThermoFisher Scientific, Waltham, MA, USA) TaqPath RT-PCR assay (N-gene) and digital-droplet-RT-PCR. Positive NP samples (N-gene Ct value < 25) were sequenced in the spike-region using the PacBio single-molecule real-time sequencing (SMRT) system (Pacific Biosciences, Menlo Park, CA, USA), as previously described [3]. The haplotypes obtained were aligned on the Wuhan-Hu-1 reference genome (NC_045512.2) to identify BA.1/BA.1.1/BA.2 variants and to detect the following mutations associated with sotrovimab-resistance: P337H/L/R/T, E340A/G/K/V, K356T, S371F [4]. The SARS-CoV-2 spike quasispecies complexity was analysed using the Shannon entropy normalized to the number of reads, the population nucleotide diversity, and the Hill numbers. These analyses were conducted as part of the national SARS-CoV-2 surveillance effort. French law (CSP Art.L1121-1.1) does not require institutional review board approval for anonymous retrospective studies.

Among the 51 patients (45% men; median age, 62 years) treated with sotrovimab at Toulouse University Hospital, 42 were immunocompromised (19 with solid organ transplants), 8 had chronic kidney disease with haemodialysis, and one had severe asthma. 13 patients were infected with BA.1, 30 with BA.1.1, and 5 with BA.2. The SARS-CoV-2 variant infecting 3 patients could not be identified (low viral load). None of these patients had worsening clinical symptoms after sotrovimab-infusion nor required hospitalization. The median SARS-CoV-2 NP viral load decreased from 7.1 (interquartile range (IQR), 6.1–7.8) $\log_{10}$ copies/mL before sotrovimab-infusion to 5.1 (IQR, 3.1–6.5) $\log_{10}$ copies/mL 7 days post-infusion ($p < 0.001$). We found no significant differences in the NP viral load declines between BA.1 (2.1 (IQR, 1.0–4.1) $\log_{10}$ copies/mL), BA.1.1 (2.0 (IQR, 0.6–3.6) $\log_{10}$ copies/mL), and BA.2 (1.3 (IQR, 0.5–4.1) $\log_{10}$ copies/mL) infections ($p > 0.05$). Thirty-four (67%) patients had viral loads sufficiently high to be sequenced before and after infusion. No

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Table 1
Clinical characteristics and viral evolution of sotrovimab-treated patients with resistant spike mutations

Patient number	Clinical characteristics	Vaccination status[a]	Variant	Days after sotrovimab[b]	Mutation acquired	Number of haplotypes	Mutation, % (in the quasispecies)	Viral load evolution[c]
#1	Liver SOT	Boosted	BA.1	14	P337H	1	100	Rebound
#2	IS treatment	Unvaccinated	BA.1.1	21	E340D	1	100	Rebound
#3	Renal SOT	Boosted	BA.1.1	14	E340G	1	100	Rebound
#4	IS treatment	Full	BA.1.1	21	E340K	1	100	Rebound
#5	IS treatment	Boosted	BA.2	7	E340A	1	100	Rebound
#6	IS treatment	Full	BA.1.1	7	P337S	2	86	Slight decline
#7	Renal SOT	Single dose	BA.1.1	14	P337L	1	100	Slight decline
#8	Renal SOT	Boosted	BA.1	14	E340D	1	100	Slight decline
#9	Pulmonary SOT	Boosted	BA.1	14	E340D	1	100	Slight decline
#10	Primary immunode-ficiency	Boosted	BA.1.1	7	E340D	2	20	Slight decline
#11	IS treatment	Boosted	BA.1.1	7	E340K	1	100	Slight decline
#12	Liver SOT	Boosted	BA.1.1	7	E340K	2	100	Slight decline
#13	Renal SOT	Full	BA.1.1	7	S371F	3	16	Slight decline
#14	Chronic kidney disease	Full	BA.1.1	7	P337R	2	57	Decline
#15	Chronic kidney disease	Boosted	BA.1	7	E340D	3	100	Decline
#16	IS treatment	Boosted	BA.1.1	7	E340D	2	44	Decline
#17	IS treatment	Full	BA.1.1	7	E340K	1	100	Decline
#18	IS treatment	Boosted	BA.1	7	K356T	2	27	Decline

Abbreviations: IS, immunosuppressive; SOT, solid organ transplant.
[a] Doses of SARS-CoV-2 vaccine received (single dose, full: two doses, boosted: three doses).
[b] Time after sotrovimab infusion when the mutation was first detected.
[c] Rebound of the viral load after mutation emergence—slight decline means viral load decline <1 $\log_{10}$ copies/mL, decline means viral load decline >1 $\log_{10}$ copies/mL.

sotrovimab-resistant spike mutations were detected before infusion. Of these patients, 53% had acquired sotrovimab-resistant mutations 7 to 21 days post-treatment (Table 1). S:E340A/D/G/K mutations were detected in 12/34 patients, S:P337H/L/R/S mutations in 4/34 patients, S:K356T in one patient, and S:S371F in one patient. SMRT sequencing detected several spike-protein haplotypes in the NP samples of 8 (44%) sotrovimab-treated patients that acquired resistant mutations. The NP viral loads of five patients rebounded after the mutation was first detected, those of eight patients decreased very slowly, and those of only five patients declined without rebound (Table 1, Fig. S1). The spike-protein quasispecies complexity (measured by the Shannon entropy, the population nucleotide diversity index, and the Hill numbers in 24 treated patients) increased significantly 7 days after sotrovimab-infusion compared to day 0 ($p < 0.001$, $p = 0.002$, and $p = 0.001$, respectively).

Our data show the emergence of sotrovimab-resistant spike mutations in half of the patients who remained SARS-CoV-2 RNA positive 7 to 21 days after infusion. The viral load decrease 7 days after infusion was smaller than that observed 7 days after infection in a group of 10 untreated immunocompromised alpha-infected patients (2.5 $\log_{10}$ copies/mL) [3]. Although *in vitro* studies have shown that S309, a precursor of sotrovimab, has less (27-fold) neutralizing activity against the BA.2 variant [5], we found no significant differences in the viral load decreases of patients infected with BA.1, BA.1.1, and BA.2 variants. This lack of significance could be due to the small number of patients infected with BA.2 in our study, for whom viral load decrease was only 1.3 $\log_{10}$ copies/mL.

In vitro studies have shown that sotrovimab can trigger the emergence of SARS-CoV-2 variants with mutation at positions 340, 337, and 356 [4], but we believe ours is the first *in vivo* study showing that sotrovimab exposure induces the emergence of Omicron variants harbouring mutations in these positions and a significant increase in the virus complexity 7 days post infusion. One retrospective study has demonstrated the emergence of mutations in positions 340/337 after sotrovimab infusion in 4 out of 8 Delta-infected outpatients [6]. S:S371F is one of the 8 BA.2-specific spike mutations that induces a 27-fold reduction in the capacity of sotrovimab to neutralize BA.2 [5]. One of our BA.1.1-infected patients had the S:S371F mutation 7 days after infusion; her NP viral load decreased slowly confirming this finding. Sotrovimab binds to a conserved 22-residue epitope (mostly between positions 334–356) in the RBD, outside the receptor-binding motif (RBM). As the mutations that we found were outside the RBM (438–508 positions), they could reduce the neutralization capacity of sotrovimab. The strong selective pressure exerted by sotrovimab was demonstrated by all the measures of SARS-CoV-2 quasispecies complexity.

Our data indicate that mAb-treated patients should be closely monitored to identify the emergence of treatment resistance and so limit the spread of more resistant SARS-CoV-2 variants. Monitoring of patients treated with sotrovimab should also help for future treatment options.

Transparency declaration

The authors declare no conflict of interest.

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Author contributions

CV and JI designed the study. ADB, GMB, PD, NK, CD provided medical care to the participants and collected nasopharyngeal samples; CV, PT and NR collected biological data; JL and NJ processed the data, CV analysed the results, prepared the figures, and performed statistical analyses; CV and JI drafted the initial version of the paper. All the authors revised the manuscript and approved the final version.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cmi.2022.05.002.

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