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Supplementary appendix

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Supplementary Appendix

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Materials – Methods

Sequencing strategy and patients inclusion

The routine genomic surveillance at the National Reference Center (NRC) of Respiratory Viruses of Hospices Civils de Lyon (HCL) is based on i) systematic sequencing of hospitalized patients of HCL ii) random sequencing of outpatients and hospitalized patients performed during weekly nationwide surveys where diagnostic laboratories sent a fraction of positive samples to the NRC of HCL and other sequencing centers. Clinical and demographical data were not always available, especially when considering nationwide surveys. Patients harboring a S:337 or S:340 mutation, with several samples including at least one before sotrovimab infusion were retained for follow-up analyses.

Normalized viral load

Normalized viral load kinetics was determined for patients with follow-up samples. Briefly, nucleic acid extraction from nasopharyngeal swab was performed on the automated MGISP-960 workstation using MGI Easy Magnetic Beads Virus DNA/RNA Extraction Kit (MGI Tech, Marupe, Latvia). Normalized viral load was determined on QuantStudio™ 5 Real-Time PCR System (ThermoFisher Scientific, Waltham, Massachusetts, USA) with the QUANTI SARS-CoV-2 R-GENE® kit (bioMérieux, Lyon, France), according to the manufacturer recommendation. This test includes four quantification standards targeting the SARS-CoV-2 N gene and four quantification standards targeting the HPRT1 housekeeping gene in order to normalize the viral load according to the sampling quality, i.e. the number of cells present in the sample.

SARS-CoV-2 Sequencing

After nucleic acid extraction, SARS-CoV-2 sequencing was performed using COVIDSeq-Test™ (Illumina, San Diego, USA) with Artic V4 or V4.1 primers as they became available. Librairies were sequenced with 100 bp paired-end reads using the NovaSeq 6000 Sequencing system SP flow cell.

Bioinformatics

Reads were processed using the in-house bioinformatic pipeline seqmet (available at https://github.com/genepii/seqmet). Trimming of paired reads was performed with cutadapt to remove sequencing adapters and low-quality ends. Alignment to the SARS-CoV-2 reference genome MN908947 was performed by minimap2. Duplicate reads tagged by picard were removed, remaining reads were realigned by abra2 to improve indel detection sensitivity and finally clipped with samtools ampliconclip to remove read ends containing primer sequences. Variant calling with freebayes permitted to obtain variants present at frequencies of 5% or above, which were decomposed and normalized with vt and filtered with bcftools to eliminate false positives.

Ethics

Samples used in this study were collected as part of an approved ongoing surveillance conducted by the NRC of HCL, Lyon, France. The investigations were carried out in accordance with the General Data Protection Regulation (Regulation (EU) 2016/679 and Directive 95/46/EC) and the French data protection law (Law 78–17 on 06 January 1978 and Décret 2019–536 on 29 May 2019). Samples were collected for regular clinical management, with no additional samples for the purpose of this study. Patients were informed of the research and

their nonobjection approval was confirmed. This study was approved by the ethics committee of HCL and registered on the HCL database of RIPHN studies (AGORA N°41).

Acknowledgements:

We would like to thank all the members of the GenEPII sequencing platform who contributed to this investigation. We also thank all the laboratories, clinicians and patients involved in this work. This work was carried out within the framework of the French consortium on surveillance and research on infections with emerging pathogens via microbial genomics (consortium relatif à la surveillance et à la recherche sur les infections à pathogènes EMERgents via la GENomique microbienne EMERGEN; https://www.santepubliquefrance.fr/dossiers/coronavirus-covid-19/consortium-emerg

We thank the authors, the originating and submitting laboratories for their sequence and metadata shared through GISAID on which this research is based.

Tables and figures

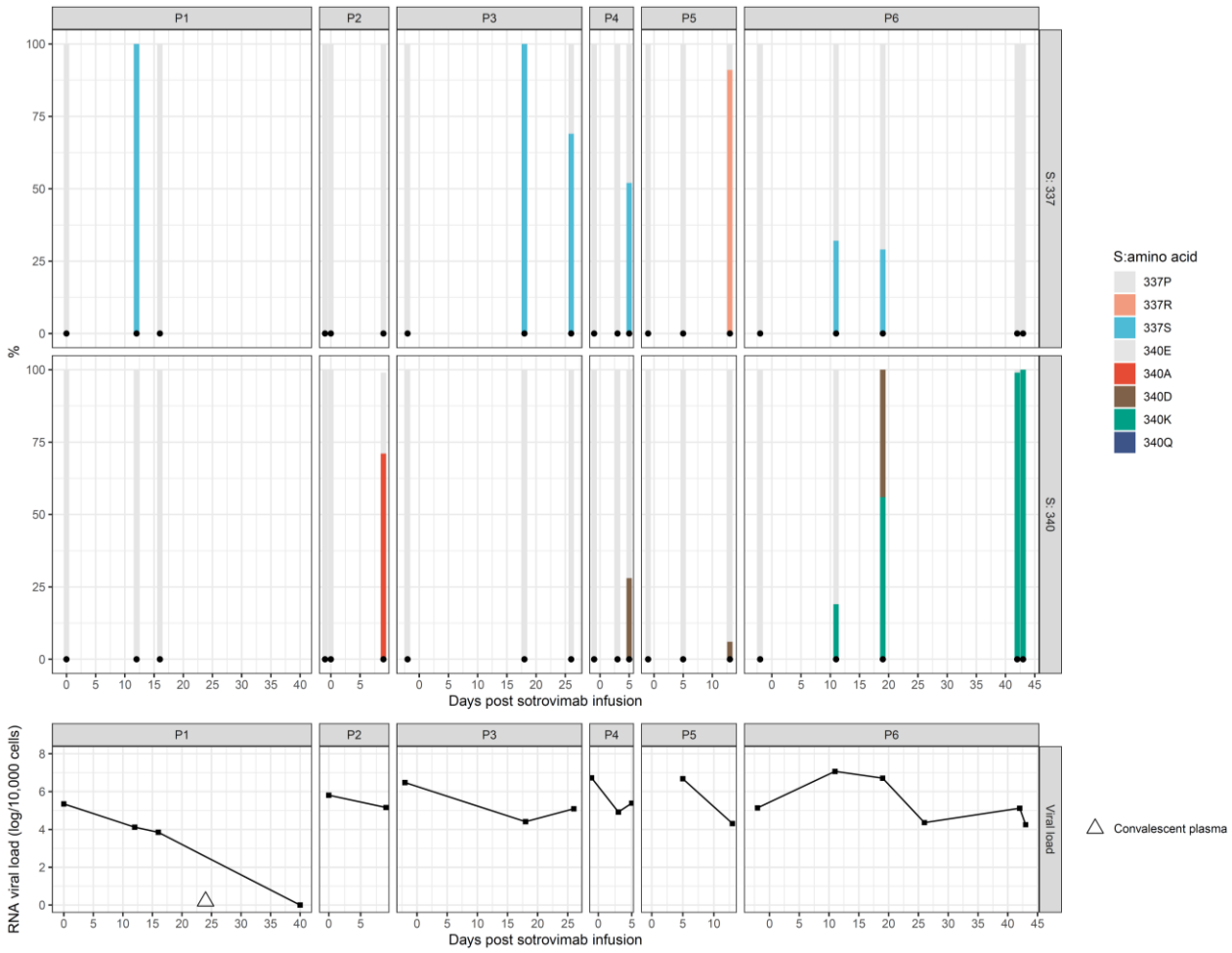

Figure 1. Virological follow-up of immunocompromised patients treated with sotrovimab

The above panel shows bars representing relative frequencies of S:337 and S:340 amino-acid substitutions occurring over time after treatment with sotrovimab in immunocompromised patients (n=6). Each colour represents a different amino-acid substitution. Triangle for patient #1 represents convalescent plasma infusion.

Normalized SARS-CoV-2viral loads expressed as log10(RNA)/10,000 human cells are represented on the bottom panel.

Virus name GISAID	S:337 or S:340 mutation	Patient	available clinical data	Pangolin lineage
hCoV-19/France/ARA-HCL022026019301/2022	S:P337S	P1	yes	BA.1
hCoV-19/France/ARA-HCL022026296401/2022	S:E340A	P2	yes	BA.1
hCoV-19/France/ARA-HCL022033319501/2022	S:P337S	P3	yes	BA.1
hCoV-19/France/ARA-HCL022037698401/2022	S:P337S	P3	yes	BA.1
hCoV-19/France/ARA-HCL122004037701/2022	S:P337S	P4	yes	BA.1
hCoV-19/France/ARA-HCL122005810301/2022	S:P337R	P5	yes	BA.1
hCoV-19/France/ARA-HCL022035008101/2022	S:E340K	P6	yes	BA.1
hCoV-19/France/ARA-HCL022050390701/2022	S:E340K	P6	yes	BA.1
hCoV-19/France/ARA-HCL122009595001/2022	S:E340K	P6	yes	BA.1
hCoV-19/France/ARA-HCL022044350101/2022	S:E340Q	P7	yes	BA.1
hCoV-19/France/NAQ-HCL022053231601/2022	S:E340D	P8	yes	BA.2
hCoV-19/Mayotte/HCL022009868901/2022	S:E340D	P9	no	BA.1
hCoV-19/France/ARA-HCL022026894802/2022	S:P337S	P10	no	BA.1
hCoV-19/France/ARA-HCL022038419401/2022	S:P337R	P11	no	BA.1
hCoV-19/France/ARA-HCL022039160001/2022	S:E340D	P12	no	BA.1
hCoV-19/France/ARA-HCL122010159301/2022	S:E340K	P13	no	BA.1
hCoV-19/France/ARA-HCL122009230601/2022	S:E340K	P13	no	BA.1
hCoV-19/France/ARA-HCL122008542401/2022	S:E340K	P13	no	BA.1
hCoV-19/France/ARA-HCL022005448201/2022	S:P337L	P14	no	BA.1
hCoV-19/France/ARA-HCL722000683101/2022	S:P337L	P14	no	BA.1
hCoV-19/France/COR-HCL722000704801/2022	S:P337S	P15	no	BA.1
hCoV-19/France/NAQ-HCL722000985401/2022	S:E340D	P16	no	BA.1
hCoV-19/France/NAQ-HCL722000782201/2022	S:E340D	P16	no	BA.1
hCoV-19/France/OCC-HCL722001156601/2022	S:E340D	P17	no	BA.1
hCoV-19/France/ARA-HCL722001540001/2022	S:E340K	P18	no	BA.1

Table S1. Omicron viruses sequenced at National Reference Center for respiratory viruses (Lyon, France) harboring S:337 and/or S:440 mutations.

Patient	Age	Sex	GISAID Virus name	clade (Nextstrain)	Pangolin lineage	Days since sotrovimab infusion	S:P337 and/or S:E340 mutations	Number of days between symptoms onset and sotrovimab infusion	Other antiviral treatments	negative result	Comorbidities	Vaccinated	Delay since last dose
P1	60	M	hCoV-19/France/ARA-HCL022018464001/2022	21K	BA.1	0		8	Convalescent plasma (D24)	yes (D40)	Diffuse large B-cell lymphoma R-DHAOx, CAR-T-cells (D26)	3 doses	6 months
			hCoV-19/France/ARA-HCL022026019301/2022			12	S:P337S (100%)						
			hCoV-19/France/ARA-HCL122005337501/2022			16							
P2	70	M	hCoV-19/France/ARA-HCL022019927901/2022	21K	BA.1	-1		10	No	ongoing follow-up	Heart transplant	3 doses	2 weeks
			hCoV-19/France/ARA-HCL022020258601/2022			0							
			hCoV-19/France/ARA-HCL022026296401/2022			9	S:E340A (71%)						
P3	72	F	hCoV-19/France/ARA-HCL022019883201/2022	21K	BA.1	-2		8	No	ongoing follow-up	Kidney transplant	No	ND
			hCoV-19/France/ARA-HCL022033319501/2022			18	S:P337S (100%)						
			hCoV-19/France/ARA-HCL022037698401/2022			26	S:P337S (69%)						
P4	52	F	hCoV-19/France/ARA-HCL022018573901/2022	21K	BA.1	-1		4	No	ongoing follow-up	Gougerot Sjögren, rituximab	3 doses	3 months
			hCoV-19/France/ARA-HCL122003445701/2022			3							
			hCoV-19/France/ARA-HCL122004037701/2022			5	S:P337S (52%) + S:E340D (28%)						
P5	19	M	hCoV-19/France/ARA-HCL122003970901/2022	21K	BA.1	-1		2	No	ongoing follow-up	Germinal embryonal carcinoma	No	ND
			hCoV-19/France/ARA-HCL122004577201/2022			5							
			hCoV-19/France/ARA-HCL122005810301/2022			13	S:P337R (91%) + S:E340D (6%)						
P6	59	M	hCoV-19/France/ARA-HCL022021154301/2022	21K	BA.1	-2		0	No	ongoing follow-up	Systemic scleroderma	3 doses	7 months
			hCoV-19/France/ARA-HCL022029928601/2022			11	S:P337S (32%) + S:E340K (19%)						
			hCoV-19/France/ARA-HCL022035008101/2022			19	S:P337S (29%) + S:E340K (56%);S:E340D (44%)						
			hCoV-19/France/ARA-HCL022050390701/2022			42	S:E340K (100%)						
			hCoV-19/France/ARA-HCL122009595001/2022			43	S:E340K (100%)						
P7	59	M	hCoV-19/France/ARA-HCL022044350101/2022	21K	BA.1	23	S:E340Q (100%)	ND	No	ND	Heart transplant	ND	ND
P8	75	M	hCoV-19/France/NAQ-HCL022053231601/2022	21L	BA.2	6	S:E340D (84%)	0	No	ND	multiple sclerosis		

ND: No Data; R-DHAOx : rituximab, cisplatin, dexamethasone, and high-dose cytarabine

Table S2. Demography, clinical and virological data of patients carrying a S:337 and/or S:340 mutant virus after treatment with sotrovimab.

Sublineage	total	S:337 mutation	%	S:340 mutation only	%	S:337 + S:340 mutations	%	Total of S:337 and/or S:340 mutations	%
BA.1	9300104	261	0·003%	1872	0·020%	88	0·001%	2045	0·021%
BA.2	741958	18	0·002%	703	0·095%	5	0·001%	711	0·095%
BA.3	695	0	0·000%	0	0·000%	0	0·000%	0	0·000%
Total Omicron (BA.1 + BA.2 + BA.3)	10042757	274	0·003%	2575	0·026%	93	0·001%	2756	0·027%

Table S3. Prevalence of S:337 and/or S:340 substitutions in Omicron sequences available on GISAID (Global Initiative on Sharing Avian Influenza Data) Database (on April 5th 2022).

Data availibility

Individual consensus sequences are available at https://www.gisaid.org/ with virus names in supplementary Tables S1 and S2. Raw fastqfiles are available upon request.