CORRESPONDENCE

Resistance Mutations in SARS-CoV-2 Delta Variant after Sotrovimab Use

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TO THE EDITOR: Sotrovimab is a monoclonal antibody that is available under emergency use authorization for the treatment of patients who are at risk for progression of coronavirus disease 2019 (Covid-19) to severe disease.[1] Sotrovimab is thought to neutralize all sarbecoviruses, including severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), by binding to a highly conserved epitope within the receptor-binding domain.[2] However, the use of SARS-CoV-2–specific monoclonal antibodies to target a single viral epitope warrants caution because of the risk of rapid development of mutations that confer resistance after exposure to these antibodies.[2-4] Mutations at positions S:E340K/A/V and S:P337L/T (Fig. 1A) have been associated with a reduction by a factor of 100 to 297 in neutralization by sotrovimab.[5]

We reviewed the first 100 consecutive patients who received sotrovimab at health care facilities in the Western Sydney Local Health District in New South Wales, Australia, during the B.1.617.2 (delta) variant outbreak between August and November 2021 (Fig. S1 in the Supplementary Appendix, available with the full text of this letter at NEJM.org). We identified 8 patients (Patients R001 through R008) with reverse transcriptase–polymerase-chain-reaction (RT-PCR) assays that were persistently positive for SARS-CoV-2 and for whom respiratory tract specimens obtained before and after the use of sotrovimab were available.

Genomic analysis showed that 4 of these 8 patients (Patients R001 through R004) acquired previously defined receptor-binding domain mutations within 6 to 13 days after they received sotrovimab (Fig. 1C and Table S1). Mutations in S:E340 developed in all 4 patients, findings that are concordant with those in the Covid-19 Monoclonal Antibody Efficacy Trial–Intent to Care Early (COMET-ICE).[2] Cultures obtained from these patients remained positive for 23, 24, 12, and 15 days, respectively, after they received sotrovimab (Table S2). Read frequencies of S:E340K/A/V mutations increased over the course of infection; the proportion of the viral population carrying S:E340K/A/V exceeded 75% by day 7 in Patient R002, by day 13 in Patient R003, and by day 37 in Patient R004 (Fig. 1C and Table S2). In addition, a minority variant developed in Patient R002 at position P337L after fixation of the S:E340K mutation. A retrospective review of 11,841 SARS-CoV-2 genomes in the Global Initiative on Sharing All Influenza Data database (a site for compiling sequence data on viruses) (Table S3) and reported in New South Wales, Australia, identified 4 additional patients with S:E340 mutations. In 1 patient, the SARS-CoV-2 genome was detected 5 days after sotrovimab treatment, and in another it was detected 11 days after treatment.

These data show the persistence of viable SARS-CoV-2 in patients after sotrovimab infusions and the rapid development of spike gene mutations associated with high-level sotrovimab resistance in vitro. These findings underscore the importance of stewardship of monoclonal antibodies, particularly because sotrovimab is one of the few monoclonal antibodies with retained activity against the B.1.1.529 (omicron) variant.[1] Postmarketing genomic surveillance of patients who receive monoclonal antibodies for the treatment of SARS-CoV-2 infection is prudent in order to minimize the risk of both treatment failure and the transmission of potentially resistant SARS-CoV-2 variants in health care settings and the community, given that SARS-CoV-2 may be isolated up to 24 days after sotrovimab treatment.

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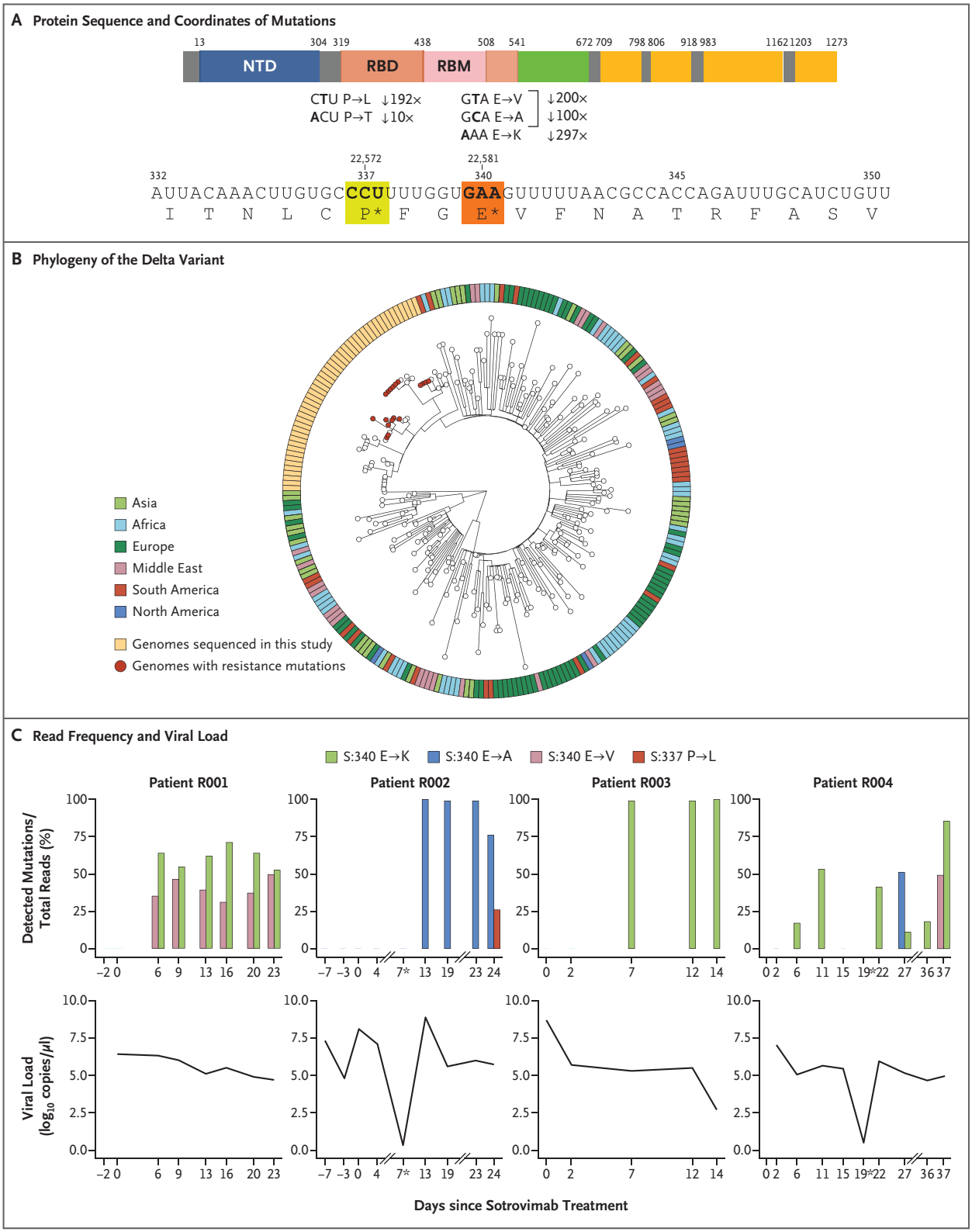

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Figure 1 (facing page). SARS-CoV-2 Viral-Load Dynamics and Acquisition of Resistance Mutations after Sotrovimab Treatment.

The acquisition of mutations conferring a high level of resistance to sotrovimab and the dynamics of the viral load of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) are shown. Panel A shows the N-terminal domain (NTD), receptor-binding domain (RBD), and receptor-binding motif (RBM) of the SARS-CoV-2 spike protein and the protein sequence and coordinates of mutations that were acquired in the RBD of the spike protein after sotrovimab treatment. We found five mutations (S:E340K/A/V and S:P337L/T) that have been reported to reduce susceptibility to sotrovimab by factors of 297, 100, 200, 192, and 10, respectively.[1,5] Panel B shows the global phylogeny of subsampled isolates of the SARS-CoV-2 delta variant, a variant of concern, with the geographic region of sequences indicated in the outer ring. Panel C shows the findings in four patients with SARS-CoV-2 infection who received sotrovimab. The acquisition and read frequency of mutations conferring high levels of resistance to sotrovimab and the SARS-CoV-2 load at each sampling point are shown. The asterisks indicate two sampling time points in which a high-quality SARS-CoV-2 genome could not be recovered (in Patient R002 on day 7 and in Patient R004 on day 19) and potential resistance mutations could not be revealed. All the patients in whom resistance mutations developed (Patients R001 through R004) were hospitalized during the sampling periods.

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Supplementary Appendix

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Supplement to: Rockett R, Basile K, Maddocks S, et al. Resistance mutations in SARS-CoV-2 delta variant after sotrovimab use. N Engl J Med. DOI: 10.1056/NEJMc2120219

This appendix has been provided by the authors to give readers additional information about the work.

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STUDY METHODS

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Patient cohort

A total of 100 patients received sotrovimab between 22nd August 2021 and 13th November 2021 at a single center in Australia. Sotrovimab was provisionally registered for use by the Australian Therapeutics Goods Administration in August 2021, with limited supply. Treatment, a single 500mg infusion, was targeted at patients within five days of symptom onset who presented with risk factors for progression to severe disease.[1]

Of the 100 patients that received sotrovimab, 23 had persistent SARS-CoV-2 RNA detected by reverse transcriptase – real time polymerase chain reaction (RT-PCR) for more than 10 days post-infusion (Supplementary Figure S1). A total of 68 patients did not have a follow-up SARS-CoV-2 RT-PCR test following treatment. Longitudinally-collected respiratory tract specimens pre- and post-sotrovimab were available for eight patients (35%) of the persistently RT-PCR positive patients and were thus investigated in this study (R001-R008).

Case demographic and clinical (co-morbidities, treatment and COVID-19 vaccine status) information was compiled for the study cohort (Table 1, Supplementary Table S1). Complete COVID-19 vaccination was defined as two doses of BNT162b2 (Comirnaty, Pfizer/BioNTech) received at least 7 days before testing positive to SARS-CoV-2 and in accordance with COVID-19 local vaccination recommendations.[2] Partial vaccination was defined as either one dose of vaccine or receipt of the second dose <7 days from SARS-CoV-2 RNA detection by RT-PCR. Ethical and governance approval for the study was granted by the Western Sydney Local Health District Human Research Ethics Committee (2020/ETH02426) and (2020/ETH00786)

SARS-CoV-2 culture

Respiratory tract specimens that had detectable SARS-CoV-2 RNA by RT-PCR were cultured in vero E6 cells expressing transmembrane serine protease 2 (VeroE6/TMPRSS2; JCRB1819) as previously outlined (Supplementary Figure S2).[3] Briefly, cell cultures were seeded at 1-3x10^4 cells/cm^2 in Dulbecco's minimal essential medium (DMEM, Lonza, Basel, Switzerland) supplemented with 9% foetal bovine serum (FBS, HyClone). Media was replaced within 12 hours with inoculation media containing 1% FBS with the addition of penicillin (10,000 U/mL), streptomycin (10,000 µg/mL) and amphotericin B deoxycholate (25 µg/mL) (Lonza) to prevent microbial overgrowth and then inoculated with 100-500 µL of SARS-CoV-2 positive respiratory sample. The inoculated cultures were incubated at 37˚C in 5% CO_2 for 4 days and observed daily for cytopathic effect (CPE). Routine mycoplasma testing using RT-PCR was performed to exclude cell line mycoplasma contamination and culture work was undertaken under physical containment laboratory level 3 (PC3) biosafety conditions. The presence of CPE and increasing viral load was indicative of positive SARS-CoV-2 culture. Culture supernatant was harvested four days after inoculation and stored at -80˚C.

SARS-CoV-2 metagenomics

RNA extracts from case R004 (day 2, 6, 11 and 15) were rRNA depleted using the NEBNext rRNA Depletion Kit (Human/Mouse/Rat) (New England Biolabs, Notting Hill, Australia). The rRNA-depleted RNA was then converted to cDNA using LunaScript RT SuperMix Kit (New England Biolabs) and the second strand was synthesised with NEBNext Ultra II Non-Direction RNA Second Strand Synthesis Module (New England Biolabs). Libraries were prepared in duplicate using Nextera XT (Illumina, Melbourne, Australia) according to manufacturer's instructions and sequenced with paired end 100 bp chemistry on the NextSeq 2000 (Illumina).

SARS-CoV-2 genomics

Tiling PCR was used to amplify the entire SARS-CoV-2 genome from RNA extracts of clinical specimens using primers outlined in the Midnight sequencing protocol.[4] Each PCR included 12.5µL Q5 High Fidelity 2x Master Mix (New England Biolabs), 1.1µL of either pool 1 or pool 2 10µM primer master mix, 2.5µL of template RNA and molecular grade water was added to generate a total volume of 25µL. Cycling conditions were initial denaturation at 95°C for 2 min, then 35 cycles of: 95°C for 30s, 65°C for 2 min 45s, and a final extension step of 75°C for 10 min. Pool 1 and pool 2 amplicons were combined and purified with a 1:1 ratio of AMPureXP beads (Beckman Coulter) and eluted in 30µL of RNAase free water. Purified products were quantified using Qubit™ 1x dsDNA HS Assay Kit (Thermo Fisher Scientific) and diluted to the desired input concentration for library preparation. Sequencing libraries were prepared using Nextera XT (Illumina) according to the manufacturer's respective instructions and pooled with the aim of producing $1x10^6$ reads per library. Sequencing libraries were then sequenced with paired end 76 bp chemistry on the iSeq or MiniSeq (Illumina) platforms.

Bioinformatic analysis

Raw sequence data were processed using an in-house quality control procedure prior to further analysis as described previously.[5,6] De-multiplexed reads were quality trimmed using Trimmomatic v0.36 (sliding window of 4, minimum read quality score of 20, leading/trailing quality of 5 and minimum length of 36 after trimming).[7] Briefly, reads were mapped to the reference SARS-CoV-2 genome (NCBI GenBank accession MN908947.3) using Burrows-Wheeler Aligner (BWA)-mem version 0.7.17[8], with unmapped reads discarded. Average genome coverage was estimated by determining the number of missing bases (Ns) in each sequenced genome. Variant calling and the generation of consensus sequences was conducted using iVar[9], with soft clipping over primer regions (version 1.2.1, min. read depth >10x, quality >20, min frequency threshold of 0.1). Single nucleotide polymorphisms (SNP) were defined based on an alternative frequency >0.75 whereas Minority allele Frequency Variants (MFV) were defined by an alternative frequency between 0.1 and 0.75. Variants falling in the 5' and 3'UTR regions were excluded. Polymorphic sites that have previously been highlighted as problematic were monitored.[10] To ensure the accuracy of variant calls only high-quality genomes with >90% genome coverage and a mean depth of >1000x were included. The MFV calls were

excluded in the base pair either side of the 5' or 3'-end of indels due to potential mis-mapping. SARS-CoV-2 lineages were inferred using Phylogenetic Assignment of Named Global Outbreak LINeages v1.2.86 (PANGO and PLEARN).[11,12] Representative SARS-CoV-2 genomes collected between July to November 2021 (n=1,300, ≥27,000bp in length) were downloaded from Global initiative on sharing all influenza data (GISAID)[13] EpiCoV, using a global subsampling strategy developed by Nextstrain[14] Phylogenetic inference and visualisation of the 1,133 high quality consensus SARS-CoV-2 FASTA sequences (GISAID, n = 1,084; study, n = 50) was performed using the Nextstrain conda environment, Augur v13.0.3 (https://github.com/nextstrain/augur), and Auspice (open-source visualisation tool) v2.32.0 (https://github.com/nextstrain/auspice). Wuhan/Hu-1/2019 was used to root the phylogram and Auspice was used to visualise the resulting phylogeny (Supplementary Figure S2). Genomes defined by PANGO as the Delta lineage (n=205) were used to contextualise sotrovimab resistant specimens generated in this study (n=50) (Figure 1B). The GISAID and New South Wales (NSW) genomes were aligned with MAFFT v7.402 (FFT-NS-2, progressive method).[15] Phylogenetic analysis was performed using the maximum likelihood approach (IQTree v1.6.7 (substitution model: GTR+F+R2) with 1,000 bootstrap replicates.[16] Graphs were generated using RStudio (version 3.6.1) and phylogenetic trees were constructed using the R package ggtree.[17]

Prevalence of S:E340K/A/V and S:P337L mutations

Local and international genomes were interrogated for mutations S:E340K/A/V and S:P337L reported to confer sotrovimab resistance. A total of 11,841 SARS-CoV-2 genomes from NSW, Australia which were collected between 1 June - 11 November 2021 were downloaded from GISAID. Raw sequencing data was available for 17,303 local SARS-CoV-2 genomes generated at the Microbial Genomic Reference Laboratory, New South Wales Health Pathology-Institute of Clinical Pathology and Medical Research (ICPMR). The presence of mutations conferring resistance in viral sub-populations was investigated in variant calling for sequences generated at the ICPMR. International genomes were assessed for the presence of mutations using covSPECTRUM[18], with all international genomes between 6 January 2020 and 28 November 2021 included (accessed November 28th 2021 https://cov-spectrum.ethz.ch/)

Respiratory virus detection by RT-PCR

The RNA was extracted using the Viral NA Small volume kit on the MagNA Pure 96 system (Roche Diagnostics GmbH). A previously described RT-PCR[18], targeting the nucleocapsid gene was employed to estimate the viral load of clinical specimens. A commercially available synthetic RNA control (Wuhan-1 strain, TWIST Biosciences NCBI GenBank accession MN908947.3) was used in 10-fold dilutions starting at 20,000 copies/µL to 2 copies/µL to generate a standard curve and quantify the viral load of each specimen in duplicate.

Statistical analysis

Mann-Whitney two tailed *t*-tests were used to assess differences in SARS-CoV-2 RT-PCR cycle threshold values. A significance threshold of <0.01 was used.

RESULTS

SARS-CoV-2 viral load before and after treatment

A median viral load of 142,619 copies/µL of SARS-CoV-2 RNA extract (range 2-8.2x10^8 copies/µl) was detected in longitudinal study cohort specimens collected between seven days pre- and 37 days post-sotrovimab infusion.

Longitudinal fluctuations in the viral load were observed for all cohort members (Figure 1C, Supplementary Table S2). A significant difference was noted in the cycle threshold values of cases with (n=27 timepoints) and without (n=20 timepoints) resistant mutations after sotrovimab treatment (2 - 37 days post-sotrovimab *p*=0.0084) (Figure 1C, Supplementary S2). A substantial drop in viral load was noted in two cases (R004 & R002) that subsequently rebounded after resistance mutations were detected.

Development of sotrovimab resistance conferring mutations in SARS-CoV-2 populations

Consensus genomes were recovered for 50/59 clinical specimens with median genome coverage and depth of 98.2% and 5,035.5x respectively (sequencing coverage min 92.0% max 99.9%, depth min 1,784.2 max 6,573.0) (Supplementary Table S5). All genomes were found to belong to Pangolin lineage AY.39.1, a sub-lineage of the Delta VOC that predominates in Australia and globally during the study period (Figure 1B). Specimens from which high-quality SARS-CoV-2 genomes were unable to be obtained had significantly lower viral loads (cohort median Ct 24.79 compared to Ct 34.17 for failed genomic specimens *p*=<0.0000.1) (Figure 1. Supplementary Table S2). Four of the eight patients in this study acquired previously defined RBD mutations between 6-13 days after sotrovimab treatment (Table 1; Figure 1C). All but one case developed the S:E340K mutation which has previously demonstrated the highest resistance to sotrovimab. Read frequencies of S:E340K/A/V mutations generally increased over the course of infection, in two cases (R002 & R003) the proportion of the viral population carrying these mutations exceeded 75% at days 7 and 13, respectively, and remained fixed at subsequent time-points. In cases R002 and R004, S:E340K was initially detected in increasing frequency, subsequently interchanging between S:E340A and S:E340V. In addition, R002 developed a MFV at P337L after fixation of the S:E340K mutation 24 days after sotrovimab. R004 died 37 days after sotrovimab treatment due to non-COVID-19 related underlying conditions. R002 was the only case to receive concurrent treatment with dexamethasone and remdesivir, from day 2 to 6 following sotrovimab. The S:E340K mutation was detected on Day 13 which corresponded to peak viral load for this case. In contrast, a resistance mutation was detected at day 7 for R003 followed by a gradual decline in viral load without additional COVID-19 treatment.

RNA extracted from specimens collected from case R004 underwent confirmatory metagenomic sequencing, which removes bias from SARS-CoV-2 RNA amplification. Between 49 and 577 million reads were generated per specimen which were collected 2, 6, 11 and 15 days after sotrovimab. These reads produced consensus SARS-CoV-2 genomes with >99.9% coverage with 579-55,767X average depth. The results confirmed the presence of the S:E340K mutation six and 11 days after sotrovimab, but was not detected 15 days post-infusion.

SARS-CoV-2 culture positivity following sotrovimab therapy

All cases that developed mutations conferring sotrovimab resistance had at least one culture-positive specimen after the acquisition of resistant mutations (Supplementary Table S2). Cases R001, R002, R003 and R004 remained culture-positive for 23, 24, 12 and 15 days after sotrovimab treatment, respectively.

Prevalence of spike RDB E340K/A/V and P337L

Four of the 11841 local SARS-CoV-2 genomes generated in NSW and available on GISAID contained a consensus mutation at position E340K (Supplementary Table S3). Clinical information was only available for two cases, both of which had been treated with sotrovimab. SARS-CoV-2 genomes were generated 5 and 11 days after sotrovimab treatment, respectively.

Resistance mutations S:E340K/A/V and P337L/T/S were detected in the raw variant calling files of an additional six cases. Three cases contained the mutation S:E340K as a consensus mutation in the genome, one case had received sotrovimab 15 days prior, one case had no reported treatment for COVID-19, and clinical information was unavailable for one case. The remaining three genomes contained mutations detected at position P337T/L/S, but no cases had been treated with sotrovimab (Supplementary Table S4).

Of the 527,931 international SAR-CoV-2 genomes 130, 101 and 24 contained the mutation E340K/A/V, respectively. A further 65 genomes contained the mutation S:P337L. The overall prevalence of these mutations was initially very low in March 2020, however the proportion of international sequences carrying all three mutations increased by September 2021 (Supplementary Figures S4-7).

DATA AVALIBILITY

Fastq files have been deposited in BioProject PRJNA633948 for all 50 genomes produced in this study. Individual SRA accessions are available in Supplementary Table S5.

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Table S1. Characteristics of SARS-CoV-2 positive cases treated with sotrovimab

Case	Age Range (yrs)/Gender	Vaccination Status	Co-morbidities and risk factors for severe COVID-19	Hospital admission	Other antiviral therapy	Spike mutations detected following Sotrovimab therapy
R001	30-40/F	Complete (Pfizer/BioNTech)	Lung Tx	Ward	Remdesivir	S:E340K S:E340A
R002*	30-40/F	Partial (Pfizer/BioNTech)	CVID Bronchiectasis	ICU - ECMO,	Sarilumab, Remdesivir	S:E340A S:P337L
R003	20-30/M	Partial (Pfizer/BioNTech)	Renal Tx, PCKD, CMV, BK viraemia	Ward		S:E340K
R004	70-80/F	Not vaccinated	MDS/myeloproliferative disorder †	Ward		S:E340K S:E340A S:E340V
R005	70-80/F	Not vaccinated	Interstitial lung disease Hypogammaglobulinaemia Amyloidosis †	Ward		Not detected
R006	50-60/M	Not vaccinated	Renal Tx, MM, T2 DM	Ward	Remdesivir	Not detected

R007	60-70/F	Partial (Pfizer/BioNTech)	ESRF on HD/T2DM	Ward	Sotrovimab	Not detected
R008	70-80/M	Complete (Pfizer/BioNTech)	CLL, ESRF on HD, T2 DM, Hyperlipidemia.	Ward		Not detected

***** Off-label use of sotrovimab 15 days after symptom onset. **Key:** CLL – chronic lymphocytic leukaemia treated with intragram; Complete - two doses of BNT162b2 (Comirnaty, Pfizer/BioNTech) received 7 days before testing positive to SARS-CoV-2 in accordance with COVID-19 local vaccination recommendations[14]; CMV – cytomegalovirus; CVID – Common variable Immunodeficiency, receives weekly IVIg; ECMO – extracorporeal membrane oxygenation, without intubation; ESRF- end stage renal failure; F – female; HD – haemodialysis; ICU – intensive care unit; IVIg- intravenous immunoglobulin; M – male; MDS – myelodysplastic syndrome; MM – multiple myeloma; N – No; Partial – either one dose of vaccine or receipt of the second dose <7 days from SARS-CoV-2 RNA detection by RT-PCR; PCKD – polycystic kidney disease; T2 DM – Type 2 Diabetes Mellitus; Tx – transplant; † - passed away.

Table S2. *Details of SARS-CoV-2 infection dynamics and acquisition of resistance mutation in longitudinally collected specimens*

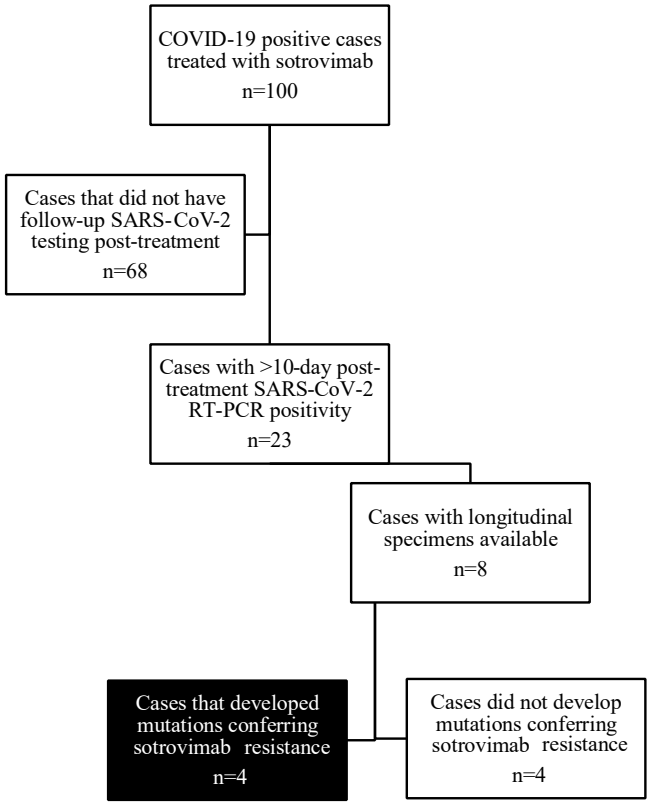
Case ID	Days post-sotrovimab treatment	Resistance mutation (read frequency)	Viral load (cycle threshold value)	Viral Isolation
R001	0	ND	6.1 (22.2)	Not performed
	6*	S:E340K (62%), S:E340V (34%)	6.0 (22.5)	Positive
	9	S:E340K (51%), S:E340V (49%)	5.7 (23.1)	Positive
	13	S:E340K (60%), S:E340V (38%)	4.8 (25.6)	Positive
	16	S:E340K (69%), S:E340V (30%)	5.2 (24.6)	Positive
	20	S:E340K (62%), S:E340V (36%)	4.6 (26.1)	Positive
	23	S:E340K (51%), S:E340V (48%)	4.4 (26.6)	Positive
R002	-7	ND	7.4 (18.8)	Positive
	-3	ND	4.8 (25.6)	Positive
	0	ND	8.0 (17.2)	Positive
	4	ND	7.1 (19.6)	Positive
	7	Failed	0.3 (37.1)	Negative
	13	S:E340A (100%)	8.9 (15.0)	Positive
	19*	S:E340A (99%)	5.6 (23.4)	Positive
	23	S:E340A (99%)	6.0 (22.5)	Positive
	24	S:E340A (76%), S:P337L (26%)	5.7 (23.3)	Positive
R003	0	ND	8.7 (15.5)	Not performed
	2	ND	5.7 (23.3)	Negative
	7	S:E340K (99%)	5.3 (24.1)	Not performed
	12	S:E340K (99%)	5.5 (23.7)	Positive
	14	S:E340K (100%)	2.7 (30.9)	Negative
R004	2	ND$^{\lozenge}$	7.3 (19.1)	Negative
	6	S:E340K (17%)$^{\lozenge}$	5.3 (24.3)	Not performed
	11	S:E340K (53%)$^{\lozenge}$	5.9 (22.8)	Positive
	15	ND$^{\lozenge}$	5.7 (23.2)	Positive
	19	Failed	0.7 (36.1)	Negative

	22	S:E340K (41%)	6.2 (21.9)	Negative
	27	S:E340K (11%), S:E340A(51%)	5.4 (24.1)	Not performed
	36	S:E340K (18%), S:E340V(49%)	4.9 (25.3)	Negative
	37	S:E340K (85%)	5.2 (24.6)	Negative
R005	-2	Failed	0.5 (36.5)	Negative
	3	ND	6.1 (22.2)	Positive
R006	-3	Failed	4.4 (26.4)	Not performed
	4	ND	2.9 (30.4)	Not performed
	11	Failed	1.4 (34.1)	Not performed
	13	Failed	0.6 (36.3)	Not performed
	15	ND	4.5 (26.4)	Not performed
	18	ND	7.7 (18.2)	Not performed
	20	ND	6.4 (21.3)	Not performed
R007	6	ND	4.4 (26.6)	Negative
	7	ND	3.7 (28.3)	Negative
	8	ND	2.8 (30.7)	Not performed
	11	Failed	1.4 (34.2)	Negative
R008	-5	ND	8.1 (17.0)	Not performed
	-2	ND	5.1 (25.0)	Not performed
	0	ND	4.4 (26.5)	Positive
	6	ND	5.0 (25.1)	Positive
	9	ND	4.2 (26.9)	Positive
	11	ND	5.4 (24.1)	Positive
	14	ND	3.5 (29.0)	Negative
	19	ND	5.6 (23.4)	Positive
	25	ND	4.6 (26.1)	Negative
	26	ND	3.4 (29.1)	Negative
	28	ND	4.2 (27.1)	Intermediate
	29	ND	4.9 (25.2)	Not performed

Key - Duplicate specimens collected on the same day are denoted with an astricks only a single case was included in the final results*

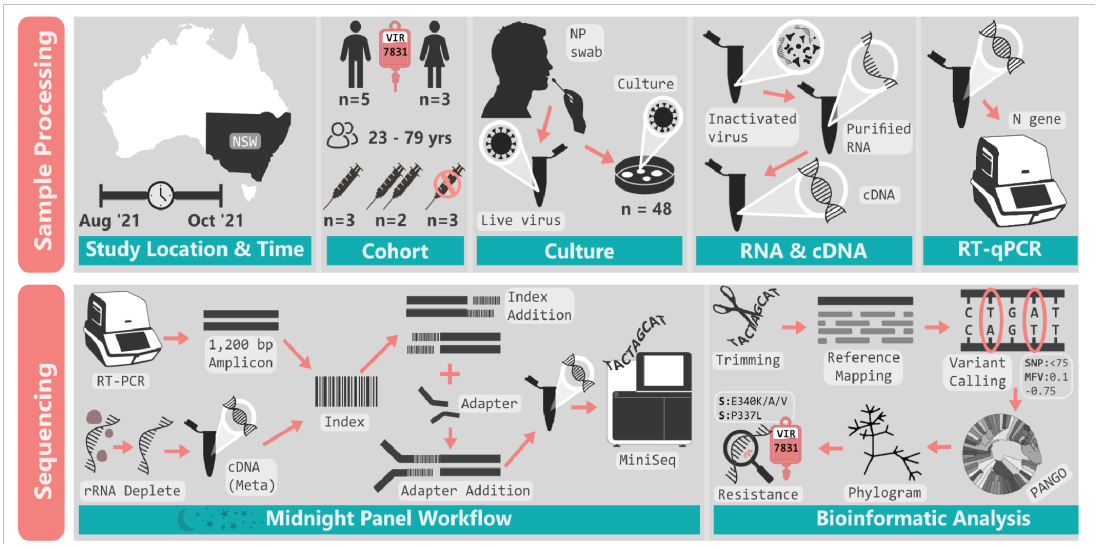
◊ Detection of resistance mutations was confirmed with minimal biased metagenomic sequencing (R004, Day 2 post-sotrovimab - no resistance detected, R004, Day 6 S:E340K (20%), R004, Day 11 S:E340K (55%), R004, Day 15 – no resistance detected).

Figure S1. Outline of testing details for patients treated with sotrovimab



Key: COVID-19 – Coronavirus disease 2019 (COVID-19); RT-PCR – reverse transcriptase real-time polymerase chain reaction; SARSCoV-2 – Severe Acute Respiratory Syndrome Coronavirus 2.

Figure S2. Outline of molecular, phenotypic and next generation sequencing methodology



Key: NP swab – Nasopharyngeal swab; RT-PCR – reverse transcriptase real time polymerase chain reaction; N-gene – qRT-PCR amplifying the SARS-CoV-2 Nucelocapsid gene; rRNA deplete –rRNA Depletion (Human/Mouse/Rat), SNP – Single Nucelotide Polymorphism; MFV – Minority allele Frequency Variant; PANGO - Phylogenetic Assignment of Named Global Outbreak LINeages.

Figure S3. Subsampled global phylogeny of SARS-CoV-2 highlighting Delta VOC genomes sequenced in this study

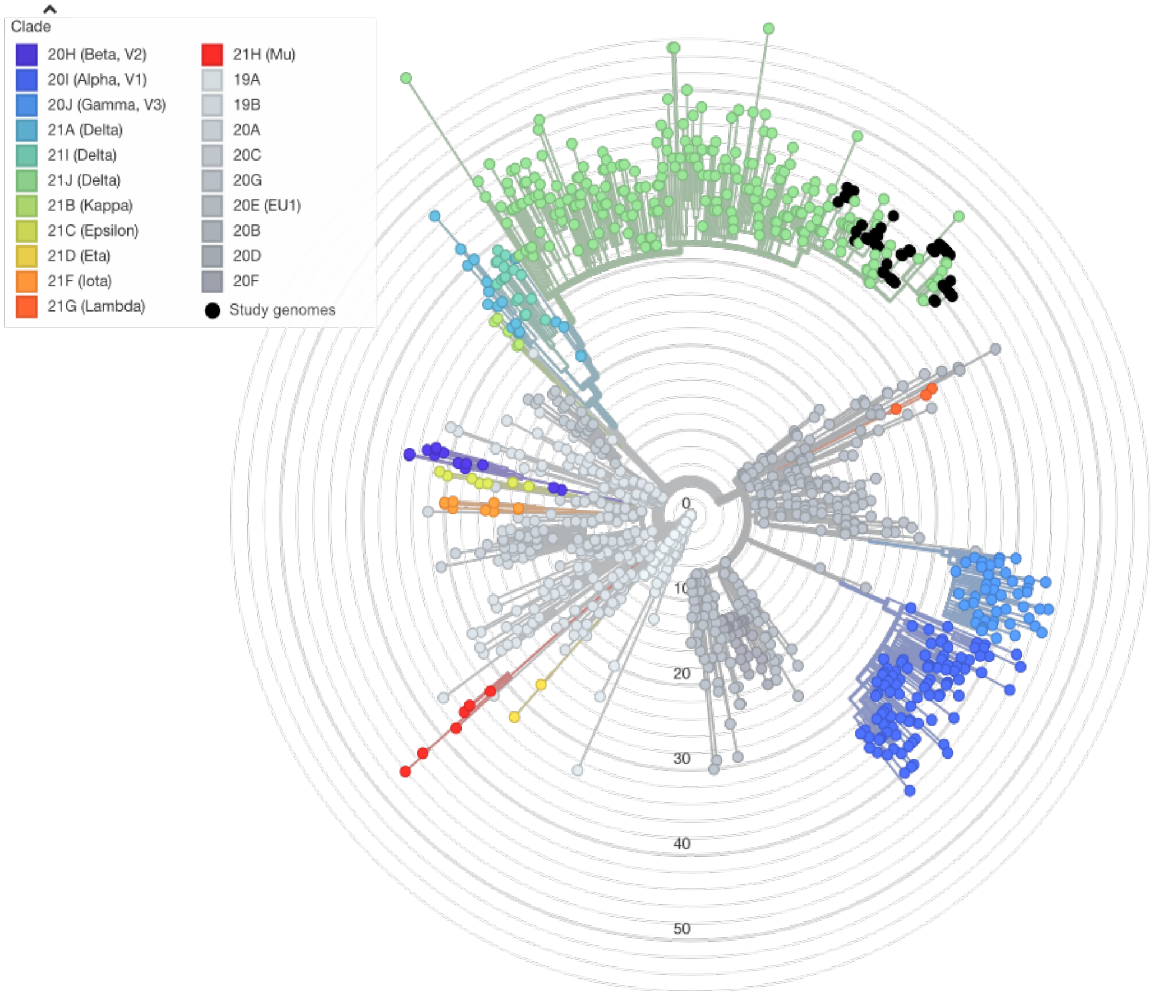


Figure S3. A maximum likelihood phylogeny representing the global diversity of SARSCoV-2 genomes during the study period (n= 1,084). Genomes generated as part of this study are highlighted with back nodes and are part of the Delta VOC (GISAD clade 21J) (n=50). Node colours depict SARS-CoV-2 lineages circulating globally.

Table S3. SARS-CoV-2 genomes containing consensus mutations at E340K in NSW, Australia

Virus name	Accession ID	Lineage	COVID-19 treatments
hCoV-19/Australia/NSWICPMR-7006/2021	EPI_ISL_4205292	AY.39.1	Sotrovimab
hCoV-19/Australia/NSWICPMR-9723/2021	EPI_ISL_4968481	AY.39.1	Sotrovimab
hCoV-19/Australia/NSWSAVID-6825/2021	EPI_ISL_5321824	AY.39.1	Unknown
hCoV-19/Australia/NSWSAVID-6814/2021	EPI_ISL_5321822	AY.39.1	Unknown

Table S4. SARS-CoV-2 genomes containing mutations conferring sotrovimab resistance in NSW, Australia

Genome ID	Treatment	Conferring mutation (read frequency)	GISAID Virus name
R013	Sotrovimab	S:E340K (0.99)	Not uploaded (incomplete)
R017	Unknown	S:E340K (0.99)	Not uploaded (incomplete)
R018	Nil	S:E340K (0.99)	Not uploaded
R019	Nil	S:P337T (0.99)	hCoV-19/Australia/NSW-ICPMR-11885
R020	Nil	S:P337L (0.11)	hCoV-19/Australia/NSW-NSW1877
R021	Nil	S:P337S (0.16)	hCoV-19/Australia/NSW-NSW4424

Figure S4. International prevalence of the mutation S:E340K which has been detected in a total of 130 genomes

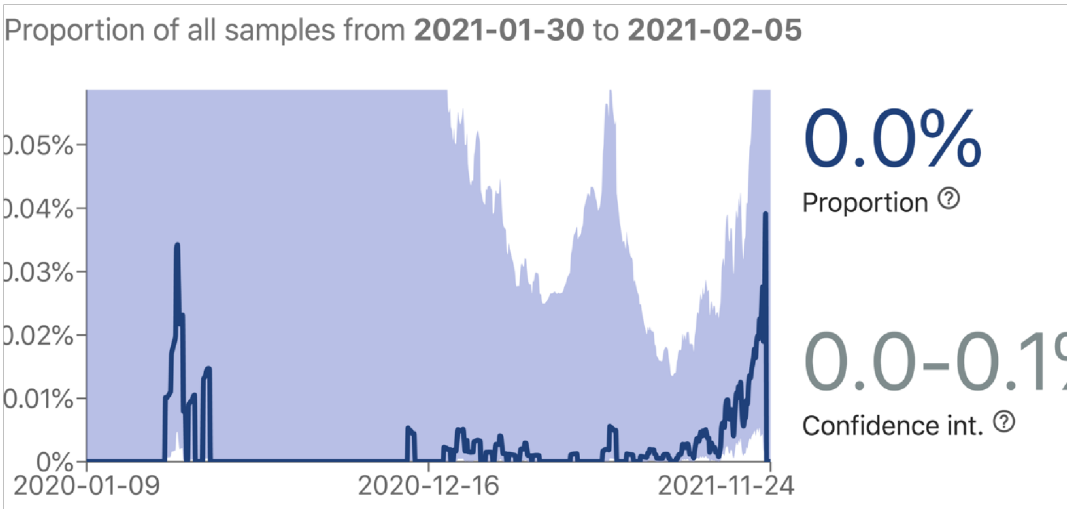


Figure S5. International prevalence of the mutation S:E340A which has been detected in a total of 101 genomes

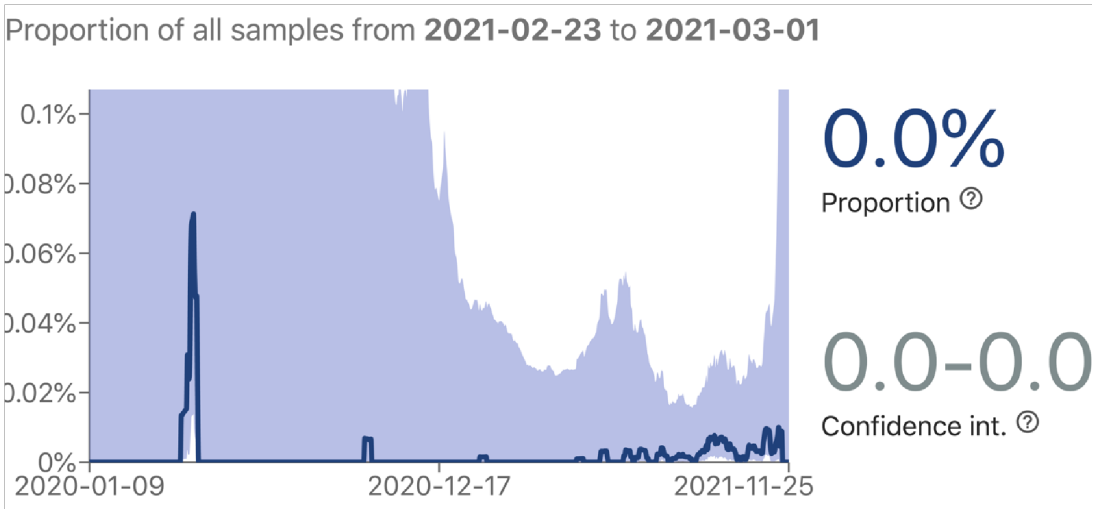


Figure S6. International prevalence of the mutation S:E340V which has been detected in a total of 24 genomes

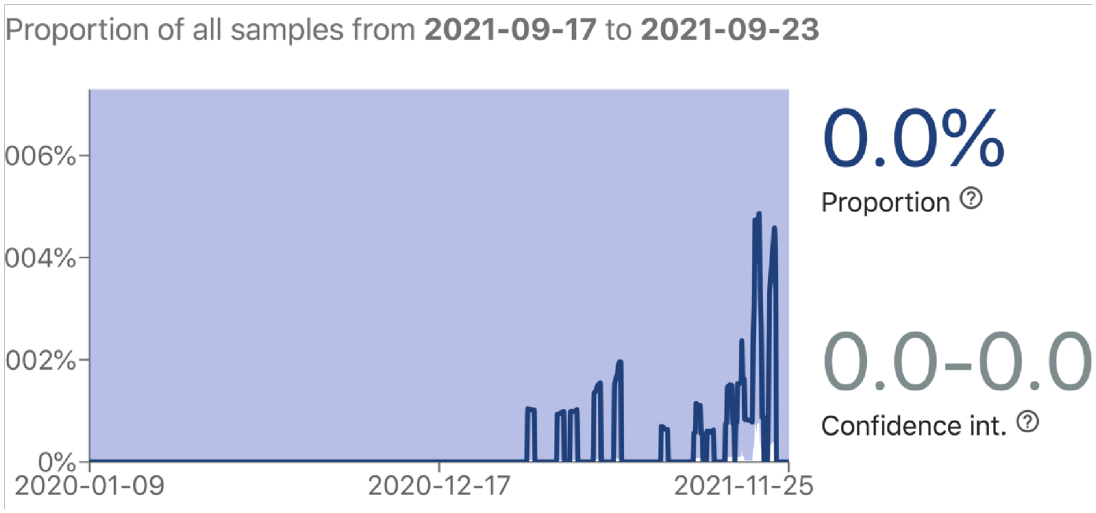


Figure S7. International prevalence of the mutation S:P337L which has been detected in a total of 65 genomes

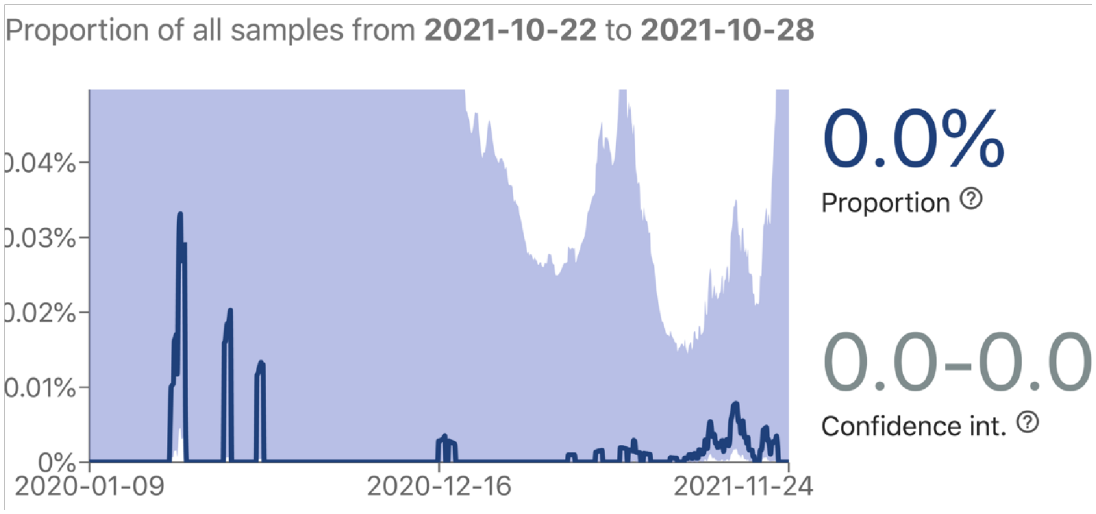


Table S5. Bioinformatic details of SARS-CoV-2 genomes produced in this study

Genome ID	Days post sotrovimab	% coverage	lineage	Scorpio call	Depth	Accession
21-R001-001	0	99.9	AY.39.1	Delta (B.1.617.2-like)	2139x	SRR17088998
21-R001-002	6	99.9	AY.39.1	Delta (B.1.617.2-like)	1784x	SRR17088997
21-R001-003	6	99.9	AY.39.1	Delta (B.1.617.2-like)	2243x	SRR17088985
21-R001-004	9	99.9	AY.39.1	Delta (B.1.617.2-like)	2267x	SRR17088977
21-R001-005	13	99.9	AY.39.1	Delta (B.1.617.2-like)	3048x	SRR17088955
21-R001-006	16	99.9	AY.39.1	Delta (B.1.617.2-like)	3537x	SRR17088972
21-R001-007	20	99.8	AY.39.1	Delta (B.1.617.2-like)	3539x	SRR17088971
21-R001-008	23	99.8	AY.39.1	Delta (B.1.617.2-like)	3730x	SRR17088970
21-R002-001-MI	-7	98.2	AY.39.1	Delta (B.1.617.2-like)	5045x	SRR17088969
21-R002-002-MI	-3	97.2	AY.39.1	Delta (B.1.617.2-like)	5194x	SRR17088968
21-R002-003-MI	0	98.2	AY.39.1	Delta (B.1.617.2-like)	5259x	SRR17088996
21-R002-004-MI	4	98.0	AY.39.1	Delta (B.1.617.2-like)	5070x	SRR17088995
21-R002-006-MI	19	94.8	AY.39.1	Delta (B.1.617.2-like)	5763x	SRR17088993
21-R002-007-MI	13	98.2	AY.39.1	Delta (B.1.617.2-like)	5033x	SRR17088992
21-R002-008-MI	19	98.2	AY.39.1	Delta (B.1.617.2-like)	5293x	SRR17088991
21-R002-009-MI	23	98.2	AY.39.1	Delta (B.1.617.2-like)	5108x	SRR17088994
21-R002-010-MI	24	98.2	AY.39.1	Delta (B.1.617.2-like)	4906x	SRR17088990
21-R003-001-MI	0	98.2	AY.39.1	Delta (B.1.617.2-like)	5041x	SRR17088989
21-R003-002-MI	2	98.2	AY.39.1	Delta (B.1.617.2-like)	4406x	SRR17088988

21-R003-003-MI	7	98.2	AY.39.1	Delta (B.1.617.2-like)	5168x	SRR17088987
21-R003-004-MI	12	98.2	AY.39.1	Delta (B.1.617.2-like)	4834x	SRR17088984
21-R004-001-MI	6	98.2	AY.39.1	Delta (B.1.617.2-like)	5882x	SRR17088983
21-R004-002-MI	11	98.2	AY.39.1	Delta (B.1.617.2-like)	5059x	SRR17088986
21-R004-003-MI	15	98.2	AY.39.1	Delta (B.1.617.2-like)	5074x	SRR17088982
21-R004-005-MI	22	98.2	AY.39.1	Delta (B.1.617.2-like)	4688x	SRR17088981
21-R004-006-MI	27	98.2	AY.39.1	Delta (B.1.617.2-like)	5058x	SRR17088980
21-R004-007-MI	36	92.4	AY.39.1	Delta (B.1.617.2-like)	5092x	SRR17088979
21-R004-008-MI	37	94.7	AY.39.1	Delta (B.1.617.2-like)	4402x	SRR17088976
21-R004-009-MI	2	98.2	AY.39.1	Delta (B.1.617.2-like)	4437x	SRR17088967
21-R005-002-MI	3	98.2	AY.39.1	Delta (B.1.617.2-like)	4640x	SRR17088966
21-R006-001-MI	4	92.2	AY.39.1	Delta (B.1.617.2-like)	4296x	SRR17088965
21-R006-004-MI	15	98.2	AY.39.1	Delta (B.1.617.2-like)	5647x	SRR17088964
21-R006-005-MI	18	98.2	AY.39.1	Delta (B.1.617.2-like)	5321x	SRR17088963
21-R006-006-MI	20	98.2	AY.39.1	Delta (B.1.617.2-like)	4723x	SRR17088962
21-R007-001-MI	6	98.2	AY.39.1	Delta (B.1.617.2-like)	5042x	SRR17088961
21-R007-002-MI	7	96.3	AY.39.1	Delta (B.1.617.2-like)	4860x	SRR17088960
21-R008-001-MI	-5	98.2	AY.39.1	Delta (B.1.617.2-like)	5038x	SRR17088959
21-R008-002-MI	-2	98.2	AY.39.1	Delta (B.1.617.2-like)	6573x	SRR17088958
21-R008-010-MI	0	98.2	AY.39.1	Delta (B.1.617.2-like)	5327x	SRR17088949
21-R008-011-MI	6	98.2	AY.39.1	Delta (B.1.617.2-like)	5190x	SRR17088978
21-R008-003-MI	9	98.2	AY.39.1	Delta (B.1.617.2-like)	5683x	SRR17088957
21-R008-012-MI	11	98.2	AY.39.1	Delta (B.1.617.2-like)	4621x	SRR17088975
21-R008-004-MI	14	93.7	AY.39.1	Delta (B.1.617.2-like)	4469x	SRR17088956
21-R008-005-MI	19	98.2	AY.39.1	Delta (B.1.617.2-like)	4571x	SRR17088954
21-R008-006-MI	25	96.8	AY.39.1	Delta (B.1.617.2-like)	4055x	SRR17088953
21-R008-007-MI	26	92.0	AY.39.1	Delta (B.1.617.2-like)	5111x	SRR17088952
21-R008-008-MI	28	96.9	AY.39.1	Delta (B.1.617.2-like)	5208x	SRR17088951
21-R008-009-MI	29	98.2	AY.39.1	Delta (B.1.617.2-like)	4669x	SRR17088950

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