UK Health Security Agency

SARS-CoV-2 Therapeutics Technical Briefing 4

Genomic surveillance

11 July 2022

This report provides an update on previous briefings up to 10 May 2022

Contents

1. Summary

This specialist technical briefing contains early data and analyses, and findings have a high level of uncertainty. Unless stated otherwise, this technical briefing uses a data cut-off of 21 June 2022 to allow time for analyses. Surveillance sampling has been updated in line with NHS England/Improvement (NHSE/I) clinical policy (can also be accessed on the CAS alert page) to encompass all patients treated with neutralising monoclonal antibodies (nMABs) and directly acting antivirals (AVs), including patients of all ages in hospital and community settings.

UK genomic dataset mutation scanning

The United Kingdom (UK) SARS-CoV-2 genomic data were screened for changes in mutation prevalence for each lineage over the last 12 weeks (data cut off 21 June 2022). Mutations that emerge and are not part of the lineage definition are termed acquired mutations. These mutations are reviewed against potential drug contact sites predicted by *in silico* analysis.

VOC-JAN22-01 (Omicron BA.2)

Of the acquired spike mutations occurring at over 1% prevalence in BA.2, G339H and K417T are at drug contact residue sites, and are predicted to interfere with the binding of sotrovimab. There were no mutations observed in non-structural proteins 5, 6, 7, 8, 9, 10, 12 and 14 (NSP 5-14) at contact residue sites associated with nirmatrelvir plus ritonavir (Paxlovid), remdesivir or molnupiravir.

VOC-22APR-03 (Omicron BA.4)

Of the acquired mutations in BA.4 at over 1% prevalence identified the spike mutation V445F, which is predicted to interfere with the binding of sotrovimab. There were no mutations observed in non-structural proteins 5, 6, 7, 8, 9, 10, 12 and 14 (NSP 5-14) at contact residue sites associated with nirmatrelvir plus ritonavir (Paxlovid), remdesivir or molnupiravir.

VOC-22APR-04 (Omicron BA.5)

Of the acquired mutations in BA.5 at over 1% prevalence, the NSP5 mutation H172L (1.43%; n=1), is adjacent to the binding site of nirmatrelvir plus ritonavir (Paxlovid), where a mutation could re-arrange the conformation of the binding site, hypothetically impacting efficacy. This mutation requires further characterisation through laboratory assessments.

Structural modelling assessment on therapeutics

BA.2.18 (prevalence 0.5%, as of week commencing 13 June 2022) is distinguished from BA.2 by an asparagine to threonine mutation at residue 417 in the spike receptor-binding-domain (RBD). This is not expected to be of therapeutic relevance.

V-22MAY-01 (BA.2.12.1) (prevalence 10.56%, as of week commencing 13 June 2022) has the L452Q mutation, which may affect binding of neutralising antibodies since this position sits on the edge of the angiotensin converting enzyme –2 (ACE2) receptor binding site. The E484A mutation in BA.2.12.1 could have an impact on the casirivimab and the tixagevimab component of Evusheld. BA.2.12.1 also contains the mutations K417N and N440K, which impact casirivimab, imdevimab and sotrovimab binding.

BA.3 (as of week commencing 13 June 2022, not reported) is predicted to escape neutralisation by casirivimab and imdevimab, has reduced susceptibility to tixagevimab, and reduced susceptibility to cilgavimab, based on structural modelling analyses.

Post-treatment viral sequences

Fifteen amino acid residues exhibited a significant change in frequency in post-treatment sequences compared to pre-treatment sequences, suggesting possible evidence of selection: S:G446V, Y453F and L455F/S in patients infected with Delta and treated with casirivimab and imdevimab; S:P337L/R/S and S:E340A/D/G/K/Q/V, S:K356T, L455S/W and R493L/Q in patients infected with BA.1 and treated with sotrovimab; S:E340D/K/Q and K356R/T in patients infected with BA.2 and treated with sotrovimab; and NSP12:E136A/D, NSP12:V166A/L, NSP12:V792I, NSP14:I42V in patients infected with neither Delta nor Omicron (most were Alpha) and treated with remdesivir. In a small number of patients (n=13), mutations were found in combination (Table 5). There are no amino acid residues displaying change in post-treatment sequences in sequences from patients treated with nirmatrelvir plus ritonavir.

The prevalence of treatment-emergent substitutions in post treatment samples for casirivimab and imdevimab in Delta sequences (n=353) are: 9 S: G446V, 2 S: Y453F; 4 S: L455F/S. The prevalence of mutations post-sotrovimab treatment in patients with BA.1 (n=614): 16 S: PS: P337L/R/S; 49 S: E340A/D/G/K/Q/V, 8 S: K356T; 3 S: L455S/W and 4 S: R493Q. The prevalence of mutations post-sotrovimab treatment in patients infected with BA.2 (n=656): 23 S: E340D/K/Q and 10 S: K356R/T. The prevalence of mutations post-remdesivir in patients infected with neither Delta nor Omicron are 3 NSP12:E136A/D, 4 NSP12:V166A/L, 3 NSP12:V792I, 25 NSP14:I42V.

Characterisation of treatment-emergent mutations using pseudovirus models

Mutations selected for following treatment with directly acting antiviral agents (including nMAB and AVs) are identified by comparing pre- and post-treatment SARS-CoV-2 genomic sequences. These treatment-emergent mutations are described in the post-treatment viral sequences section and are now undergoing assessment through structural modelling, generation of pseudovirus models and further laboratory testing to confirm this hypothesis.

Pseudoviral neutralisation assays show that Delta+G446V mutant abolishes neutralisation ability of imdevimab, whilst casirivimab showed more than 10-fold reductions in neutralization when tested against Delta+Y453F (12-fold), Delta+L455F (16-fold) and Delta+L455S (65-fold), compared to the wild-type Delta variant.

The neutralisation titre of casirivimab+imdevimab was reduced 1032-fold against Delta+G446V+Y453F and 276-fold against the Delta+G446V+L455F.

P337R/S, E340A/D/K/V, K356T treatment-emergent mutations in generated pseudovirus assays led to complete knock out of neutralisation by sotrovimab.

The affinity of sotrovimab binding with the RBD of BA.1+P337R/S and BA.1+E340A/D/K/V showed a 1951-fold to 20241-fold reduction compared to the wild-type BA.1, when using SPR to examine binding.

Transmission of post-treatment mutations in United Kingdom genomic dataset

There was one example of limited transmission of a potential treatment-emergent mutation within the UK genomic dataset, involving 2 patients, one of whom was treated with casirivimab and imdevimab. This example involved a G to V substitution at position 446, observed in both patients. Importantly, the mutation exists among untreated coronavirus (COVID-19) patients, and therefore it is unclear whether the mutation was induced by treatment in this case or whether the potential transmission event occurred independently of treatment. The mutation existed in Delta genomes before the introduction of casirivimab and imdevimab and did not increase after that introduction.

As the sequencing coverage in the UK is reduced, observing such pairs becomes increasingly unlikely. However, we would still expect to observe sequenced cases in the wider population if there was a variant with treatment resistance mutations spreading more widely.

Longitudinal analysis of pre/post-treatment serial samples

These analyses describe changes in aggregated mutations over time which may occur due to persistent SARS-CoV-2 positivity or use of therapeutic agents. SARS-CoV-2 samples with at least one pre- and 2 post-treatment sequences, over a period of more than or equal to 90 days, the time definition of SARS-CoV-2 reinfections, were analysed. The UKHSA genomic dataset was processed to yield comparator mutation changes across all gene regions matched (ORF1ab, NSP1-10, NSP12-16, ORF3a, ORF6, ORF7a, ORF8, ORF10, S gene, E, M, and N) and stratified by treatment. A mutation score was determined to define the sum of additional mutations gained by comparing the pre- and post-treatment amino acid changes.

Across all treatments, mutations appear to rise during 21 to 25 days post treatment. This data supports the hypothesis that most individuals who clear their SARS-CoV-2 infection by day 10, are unlikely to drive viral variant selection. Further assessment on mitigating risk of viral evolution in individuals with persistent SARS-CoV-2 positivity should be undertaken.

Laboratory assessment of sotrovimab and BA.1 and BA.2

In live virus neutralisation assays, in vitro, both BA.1 and BA.2 showed reduced neutralisation by sotrovimab, compared to the Victoria isolate (Wuhan-like virus) control. The IC50 (the concentration of antibody which reduces infection by 50%) for sotrovimab against BA.2 was higher (1,207 ng/ml) than that seen for BA.1 (666 ng/ml).

Risk assessment of treatment-emergent mutations

The UK data suggest that whilst mutations are emerging in patients with longer term infections after treatment, the viruses with these mutations are not transmitting widely in the population at present. Patients affected have persistent infections and there may be interactions with the effect of immunocompromise. Whilst the current findings do not pose immediate risks to the national clinical treatment policy, the changes observed highlight that this could change quickly.

2. Published information on therapeutics

The UK Health Security Agency's (UKHSA) COVID-19 therapeutics programme of work aims to support rapid deployment of specific COVID-19 therapeutics including nMABs and AVs by undertaking genomic, virological, and epidemiologic surveillance. This report is produced to share surveillance information with partner organisations.

Clinical access policies for the use of nMABs and AVs for the UK are published via the therapeutic central alert system (CAS alerts), following agreement by the Chief Medical Officer. The latest COVID-19 therapeutic access policies can be found in the CAS – Coronavirus (COVID-19) Alerts (mhra.gov.uk). These were outlined in therapeutics technical briefing 2.

Detailed variant surveillance analysis is published in the SARS-CoV-2 variant technical briefings.

3. Genomic surveillance analyses

For the purpose of this analysis, in the week beginning 13 June 2022, 50.04% of sequences were BA.2 (n=4,289), 33.16% were from BA.5 (n=2,842), 16.00% were from BA.4 (n=1,371), 0.29% were from BA.1 (n=25), 0.23% were from XE (n = 20), 0.15% were from B.1.617.2 (Delta) (n=13), and 0.13% were unclassified (n=11). The latest data for the prevalence of different variants amongst sequenced episodes is presented in Technical Briefing 43.

The UK SARS-CoV-2 genomic data set was screened for changes in mutation prevalence for each lineage over the last 12 weeks. Mutations that emerge and are not part of the lineage definition are termed acquired mutations. These mutations are reviewed against potential therapeutic contact sites predicted by in silico analysis.

Genomic surveillance analyses utilise information on residues which are involved in drug binding. These residues of interest have been identified by selecting structural models of SARS-CoV-2 proteins of interest in complex with either therapeutic antibodies or small molecule inhibitors, as described in the first therapeutics technical briefing. Mutations at residues of interest are hypothesised to predict changes in drug-virus complexes, providing preliminary data on the potential phenotypic effects of mutations. Residues on the spike gene are monitored with respect to sotrovimab. Contact residue sites in NSPs 6 to 14 are monitored with respect to molnupiravir and remdesivir and residue sites in NSP5 are monitored with respect to nirmatrelvir plus ritonavir (Paxlovid). F694Y in NSP12 has been reported to be an artefact by Sanderson and others (2021) and has therefore been removed from the analysis.

3.1 VUI-22JAN-01 (Omicron BA.2)

Of the lineage defining spike mutations in BA.2, the following mutations are at drug contact sites for therapeutic agents which are currently deployed: N440K and G339D (sotrovimab). Other lineage defining spike mutations in BA.2 for therapeutic agents previously in use are: E484A (casirivimab), K417N (casirivimab), N501Y (imdevimab, casirivimab), Q493R (casirivimab), Q498R (imdevimab, casirivimab), S477N (casirivimab), and T478K (casirivimab). None of the other lineage defining mutations were predicted to be of therapeutic relevance.

The prevalence of acquired mutations detected at over 1% in BA.2 during the week beginning with 13 June are summarised in Table 1. Of the mutations listed, G339H (1.92% prevalence) and K417T (5% prevalence) in spike were predicted to interfere with the binding of sotrovimab and casirivimab, respectively.

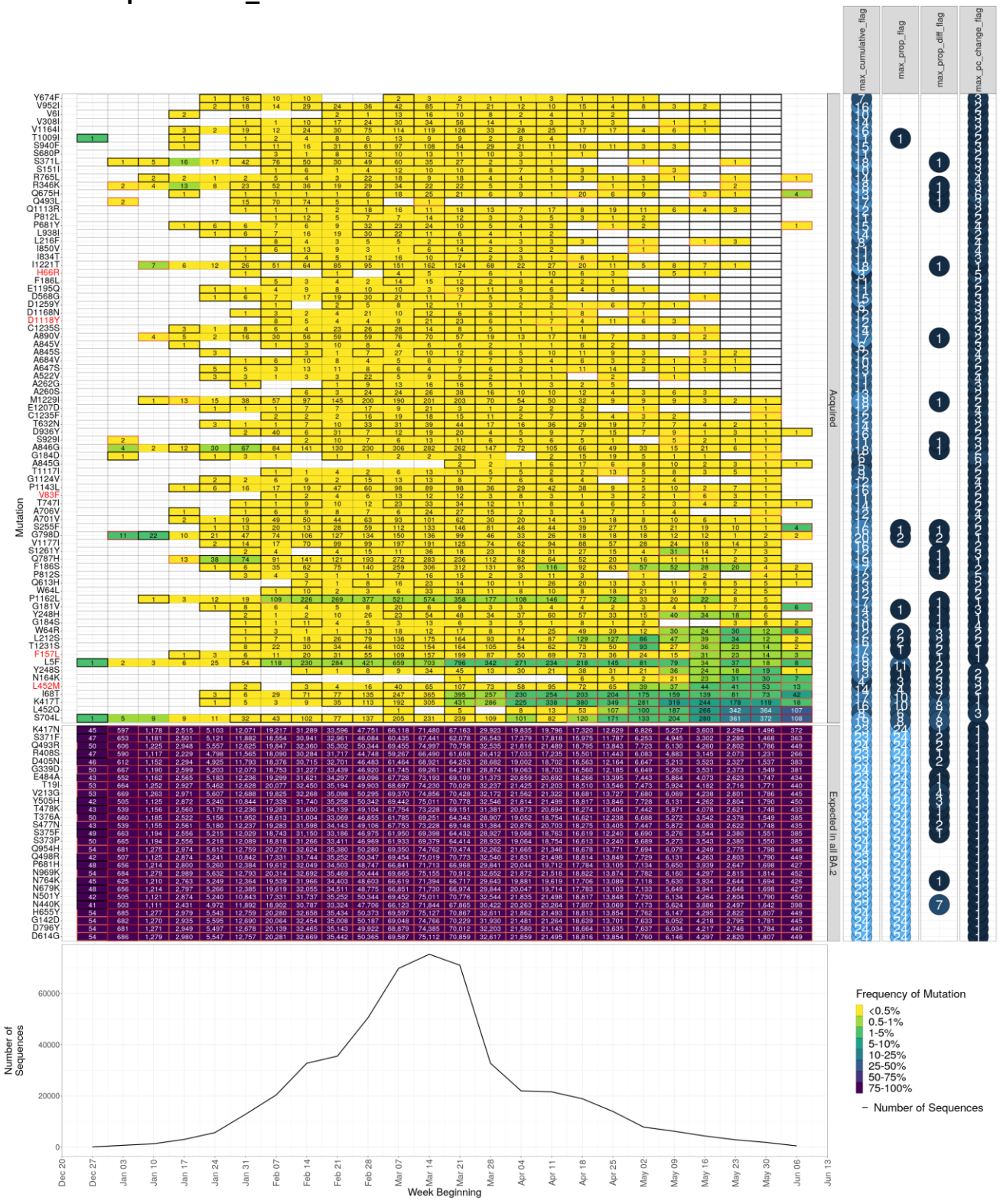
None of the acquired mutations (above 1% prevalence) in any of the other therapeutic targets (NSP5 – 14) were predicted to be of therapeutic relevance.

Figure 1 shows a mutation heatmap of non-synonymous changes in spike accruing on top of the BA.2 lineage defining mutations.

Table 1. Prevalence of acquired mutations in BA.2 during the week starting with 13 June 2022

SARS-CoV-2 Site	Mutations (prevalence)
Spike	L452Q (30.01%), S704L (29.90%), K417T (5%), Y248H (3.38%), L452M (3.33%), F186S (3.22%), I68T (3.17%), L5F (3.00%), G339H (1.92%), A688V (1.75%), L212S (1.64%), G184S (1.61%), E1144Q (1.51%), A1020V (1.51%), E1207D (1.51%), T1231S (1.51%), V1177I (1.51%), W64L (1.51%), W64R (1.51%), A879V (1.49%), E990D (1.49%), H1058Y (1.49%), L10V (1.49%), Q1071R (1.49%), Q607K (1.49%), R21T (1.49%)
NSP5	L75F (1.54%), N277S (1.54%), P132Y (1.54%), P96H (1.54%)
NSP6	L260F (1.69%), A128V (1.51%), F184V (1.49%), T180I (1.49%)
NSP8	A13S (1.51%), P178S (3.03%), Q24H (3.03%)
NSP12	A16V (1.54%), C93S (1.75%), L329I (1.51%), N743D (1.49%), P94S (1.75%)
NSP14	L493F (1.49%) and Q22H (3.45%)

Figure 1. Spike mutations found in BA.2 genomes in the UK dataset relative to the Wuhan sequence NC_045512.2



Each tile shows the proportion of sequences with each mutation per week. The total number of sequences is shown within the box. Supplementary data is not available. It should be noted all mutations in the sequence alignment are reported in these plots for review purposes.

3.2 VOC-22APR-03 (Omicron BA.4)

Of the lineage defining spike mutations in BA.4, the following mutations are at drug contact sites for therapeutic agents which are currently deployed: N440K (sotrovimab), G339D (sotrovimab). Other lineage defining spike mutations in BA.2 for therapeutic agents previously in use are: N440K (imdevimab), E484A (casirivimab), F486V (casirivimab), K417N (casirivimab), N501Y (imdevimab, casirivimab), Q498R (imdevimab, casirivimab), S477N (casirivimab), T478K (casirivimab). None of the other lineage defining mutations were predicted to be of therapeutic relevance.

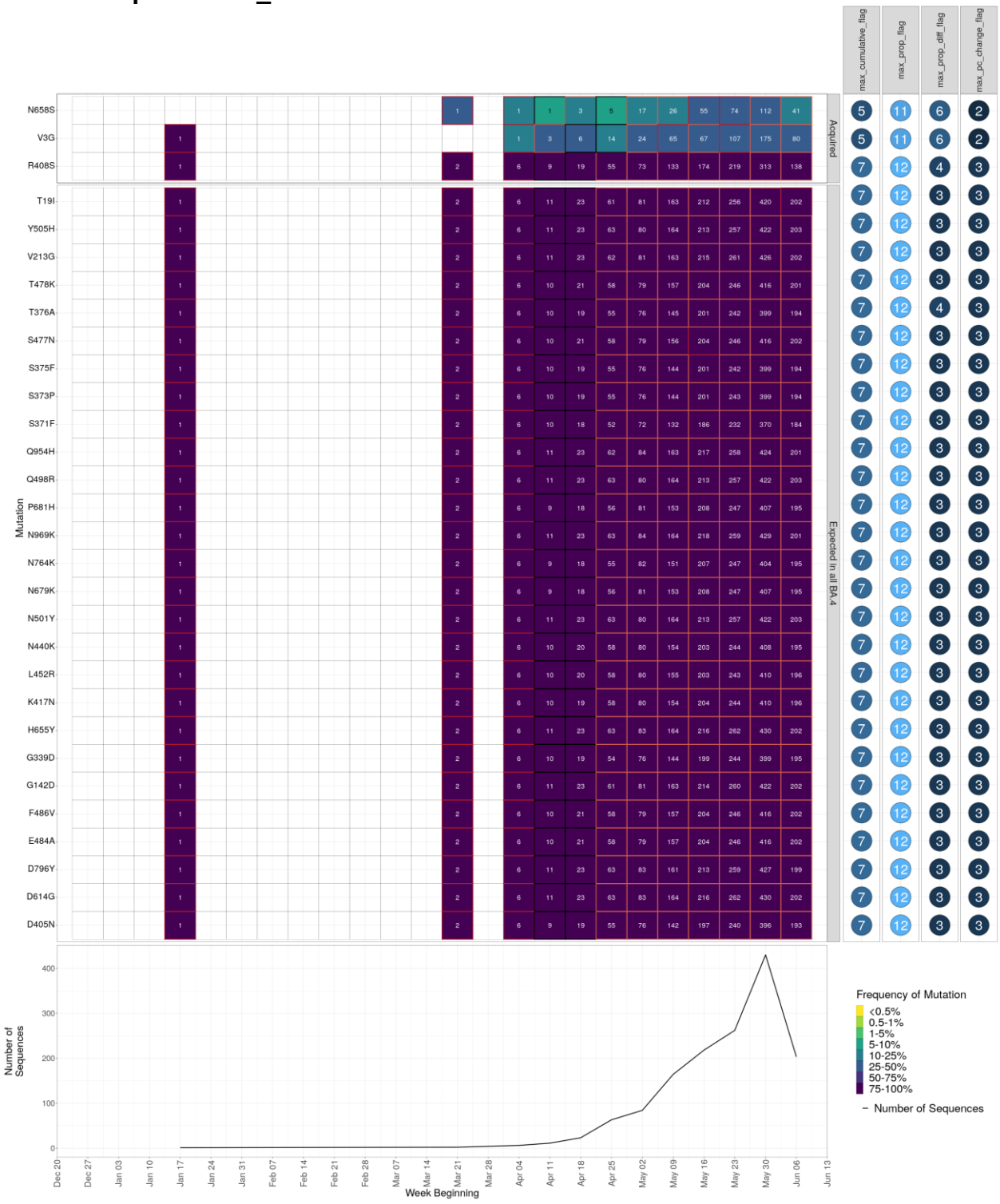
The prevalence of acquired mutations detected at over 1% in BA.4 during the week beginning with 13 June are summarised in Table 2. Of these, only V445F (3.33%) in spike protein was predicted to interfere with the binding of sotrovimab (and imdevimab). None of the acquired mutations (above 1% prevalence) in any of the other therapeutic targets (NSP5 – 14) were predicted to be of therapeutic relevance.

Figure 2 shows a mutation heatmap of non-synonymous changes in spike accruing on top of the BA.4 lineage defining mutations.

Table 2. Prevalence of acquired mutations in BA.4 during the week starting with 13 June 2022

SARS-CoV-2 Site	Mutations (prevalence)
Spike	V3G (29.03%), N658S (25.81%), S255F (3.45%), V445F (3.33%), I1225T (3.22%), K97N (3.22%)
NSP5	K90R (6.45%)
NSP6	M183I (3.22%), R233C (3.22%)
NSP8	-
NSP12	A529V (3.22%), E744D (3.22%)
NSP14	M501I (6.45%)

Figure 2. Spike mutations found in BA.4 genomes in the UK dataset relative to the Wuhan sequence NC_045512.2



Each tile shows the proportion of sequences with each mutation per week. The total number of sequences is shown within the box. Supplementary data is not available. It should be noted all mutations in the sequence alignment are reported in these plots for review purposes.

3.3 VOC-22APR-04 (Omicron BA.5)

BA.5 shares all the lineage defining spike mutations that are also predicted therapeutic contact sites with BA.4. None of the other lineage defining mutations were predicted to be of therapeutic relevance.

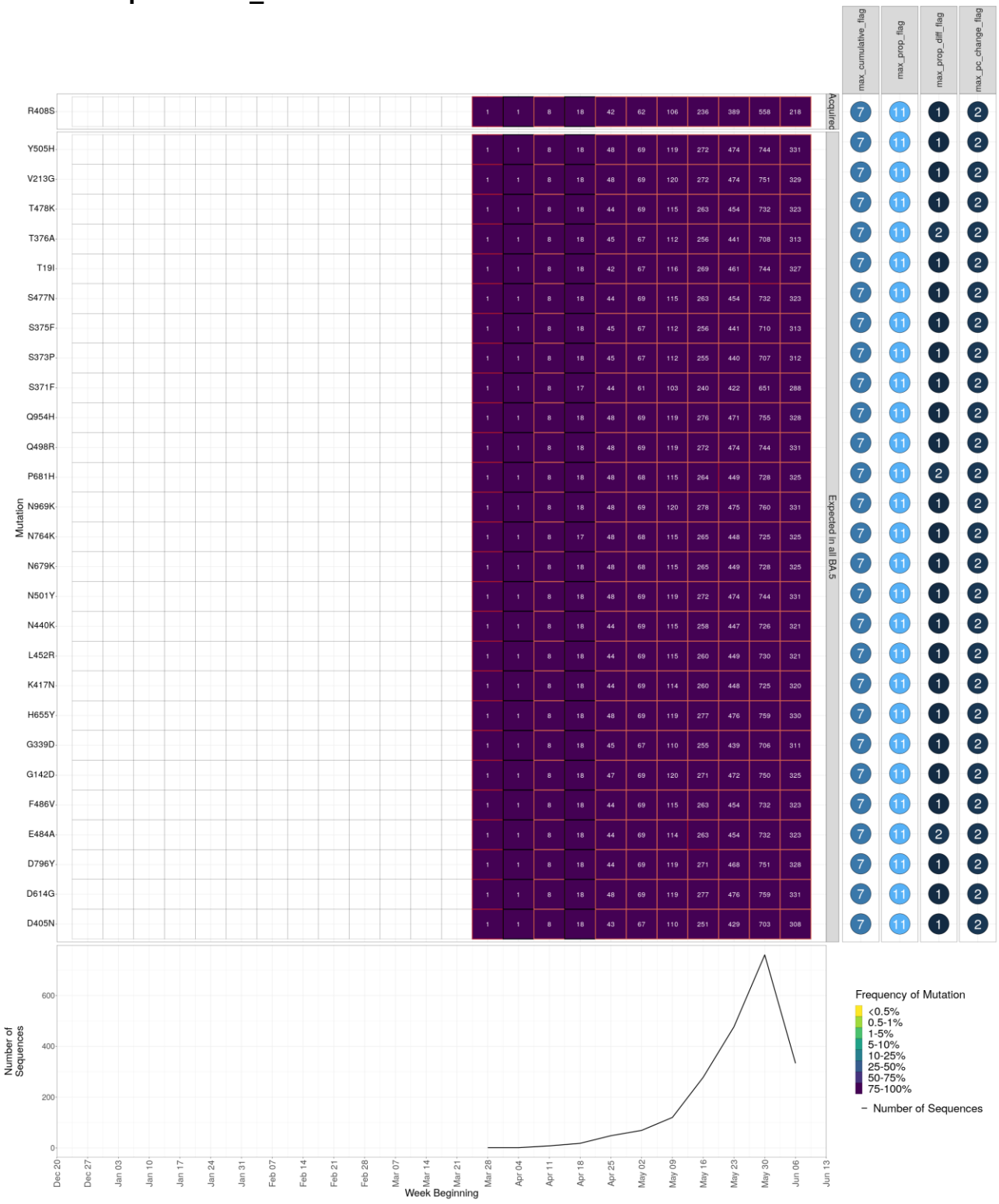
The prevalence of acquired mutations detected at over 1%in BA.5 during the week beginning with 13 June are summarised in Table 3. Of the mutations listed, H172L in NSP5 (1.43%, n = 1) could potentially be of therapeutic relevance. Position 172 in NSP5 is adjacent to the binding site of nirmatrelvir plus ritonavir (Paxlovid), where a mutation could re-arrange the conformation of the binding site, hypothetically impacting efficacy of nirmatrelvir plus ritonavir. Phenotypic assessments conducted by the United States Federal Drug Administration (FDA) to characterize the impact of naturally occurring SARS-CoV-2 Mpro polymorphisms on the activity of nirmatrelvir in a biochemical assay using recombinant Mpro enzyme, identified that H172Y mutations result in a 233-fold reduction in activity. The clinical significance of these polymorphisms is unknown, and it is also unknown if results from the biochemical assay are predictive of antiviral activity in cell.

Figure 3 shows a mutation heatmap of non-synonymous changes in spike accruing on top of the BA.5 lineage defining mutations.

Table 3. Prevalence of acquired mutations in BA.5 during the week starting with 13 June 2022

SARS-CoV-2 Site	Mutations (prevalence)
Spike	R408S (100%), L5F (2.78%), A1020S (1.40%), D198G (1.40%), G181A (1.40%), K182E (1.40%), T547I (1.40%), T76I (1.40%)
NSP5	H172L (1.43%), L50F (1.40%), R279C (5.63%)
NSP6	A128V (1.43%), L239F (1.43%), T181I (1.43%), V289L (7.60%)
NSP8	A126V (1.41%), S7N (1.40%)
NSP12	N88S (1.70%), S451R (1.70%), V128I (1.41%)
NSP14	A308S (1.41%), D41N (1.70%), V263I (1.41%)

Figure 3. Spike mutations found in BA.5 genomes in the UK dataset relative to the Wuhan sequence NC_045512.2



Each tile shows the proportion of sequences with each mutation per week. The total number of sequences is shown within the box. Supplementary data is not available. It should be noted all mutations in the sequence alignment are reported in these plots for review purposes.

3.4 Structural modelling assessment of BA.2.18, V-22MAY-01 (BA.2.12.1), and BA.3 on therapeutics

BA.2.18, BA.2.12.1 and BA.3 are all sub-lineages of the Omicron variant. Assessment of the BA.4 and BA.5 variant was described in Therapeutics Technical Briefing 3.

BA.2.18

The prevalence of BA.2.18 in the UK as of the week starting with 13 June 2022 was 0.5% (n = 2). BA.2.18 is very similar to BA.2 with the only significant difference between them being the BA.2.18 K417T mutation in the spike RBD, compared to the K417N mutation in BA.2. It is not expected that this change would produce major escape from any therapeutic antibody currently in clinical use.

V-22-MAY-01 (Omicron BA.2.12.1)

The prevalence of BA.2.12.1 in the UK as of the week starting with 13 June 2022 was 10.56% (n = 47). BA.2.12.1 is genetically very similar to BA.2 in the RBD, differentiated only by a mutation at residue 452 (L452Q in BA.2.12.1). Structural modelling suggests that mutations at this residue could have an impact on binding of cilgavimab component of Evusheld and, to a lesser extent, the casirivimab and imdevimab components of Ronapreve. The L452Q mutation carried by BA.2.12.1 is expected to be less severe than the L452R mutation in BA.4/5.

BA.2.12.1 carries the wild-type phenylalanine residue in position 486, which has an impact on binding of the casirivimab component of Ronapreve and the tixagevimab component of Evusheld.

BA.2.12.1 is likely to remain susceptible to sotrovimab to the same extent as BA.2 as there are no significant changes in its mutation profiles at the sotrovimab binding site.
BA.2.12.1 is likely to remain susceptible to the AVs molnupiravir and remdesivir, which target RdRp (NSP12). BA.2.12.1 carries the P323L mutation in NSP12, the impact of which on the function of NSP12 is poorly understood, but unlikely to interfere with the aforementioned antivirals. Although BA.2.12.1 carries the P132H mutation in NSP5, the target of nirmatrelvir plus ritonavir (Paxlovid), the mutation is unlikely to have an impact on susceptibility to the therapeutic.

BA.3

There were no samples assigned to BA.3 during the week starting with 13 June 2022. Compared to BA.2.12.1, BA.3 is genetically more distant from BA.2. As reported in Tuekprakhon and others. (2022), BA.3 is susceptible to sotrovimab to a similar level as BA.2,

but escapes neutralisation by casirivimab and imdevimab. There is some reduction in susceptibility to both the tixagevimab and cilgavimab components of Evusheld.

BA.3 is likely to remain susceptible to molnupiravir and remdesivir, but carries the NSP12 mutation P323L, like BA.2.12.1. Similarly, BA.3 carries the P132H mutation in NSP5, which is unlikely to have an impact on nirmatrelvir plus ritonavir (Paxlovid) susceptibility.

4. Post-treatment viral sequences

If a particular mutation is selected for as a result of treatment, it is expected to increase in frequency in viral genomes from treated patients. Residues in the spike, NSP5, NSP7, NSP8, NSP9, NSP10, NSP12 and NSP14 proteins that displayed distinct amino acid frequencies between pre- and post-treatment sequences were identified. These proteins were selected because they are theorised to interact with treatments currently, or were recently, in use (casirivimab/imdevimab, sotrovimab, molnupiravir, nirmatrelvir plus ritonavir and remdesivir). This analysis is run weekly to scan for mutations which require further assessment.

Pre-treatment sequences are those obtained from patients with a sequenced sample within one week prior to treatment initiation (including the day of treatment initiation). In the main analysis, sequences sampled at least 10 days after treatment initiation were defined as post-treatment sequences. Table 4 shows the number of available full genome sequences pre- and post-treatment, for each treatment. Sequences were translated to amino acids for analysis. Analyses were split by SARS-CoV-2 variant (Delta, BA.1, BA.2, BA.4, BA.5 and non-Delta/non-Omicron) and were conducted separately for each gene region (spike, NSP5, NSP7, NSP8, NSP9, NSP10, NSP12 and NSP14) and for each treatment.

Table 4. Number of pre-and post-treatment full genome sequences, broken down by treatment and by variant

Treatment	Variant	Pre-treatment sequences	Post-treatment sequences	Post-treatment sequences (more than 5 days)	Post-treatment sequences (more than 10 days)
Casirivimab and imdevimab	BA.1	190	121	98	83
Casirivimab and imdevimab	BA.2	0	50	50	50
Casirivimab and imdevimab	BA.4	0	2	2	2
Casirivimab and imdevimab	BA.5	0	2	2	2
Casirivimab and imdevimab	Delta	2,343	353	175	108
Casirivimab and imdevimab	Other	54	39	32	31
Molnupiravir	BA.1	1,669	206	128	86
Molnupiravir	BA.2	736	121	88	63
Molnupiravir	BA.4	3	1	1	0
Molnupiravir	BA.5	13	6	6	6
Molnupiravir	Delta	30	8	7	3
Molnupiravir	Other	43	45	35	26
Paxlovid	BA.1	381	39	22	15
Paxlovid	BA.2	2,286	293	173	134
Paxlovid	BA.4	34	5	2	2
Paxlovid	BA.5	55	8	8	6
Paxlovid	Delta	0	2	2	2
Paxlovid	Other	71	75	53	33
Remdesivir	BA.1	1,415	626	436	363
Remdesivir	BA.2	1,271	473	343	282
Remdesivir	BA.4	10	2	2	2
Remdesivir	BA.5	13	4	1	1
Remdesivir	Delta	5,090	1,052	452	263
Remdesivir	Other	3,719	1,321	789	517
Sotrovimab	BA.1	4,123	614	389	270
Sotrovimab	BA.2	3,913	656	333	202
Sotrovimab	BA.4	25	11	6	6
Sotrovimab	BA.5	75	16	10	10
Sotrovimab	Delta	37	8	6	3
Sotrovimab	Other	221	147	100	65

Note that counts reflect sequences rather than patients, a single patient may have more than one sequence and may be on more than one treatment and will be counted multiple times.

Fifteen amino acid residues exhibited a significant change in frequency in post-treatment sequences compared to pre-treatment sequences, suggesting possible evidence of selection: S:G446V, Y453F and L455F/S in patients infected with Delta and treated with casirivimab and imdevimab; S:P337L/R/S and S:E340A/D/G/K/Q/V, S:K356T, S:L455S/W and R493L/Q in patients infected with BA.1 and treated with sotrovimab; S:E340D/K/Q and K356R/T in patients infected with BA.2 and treated with sotrovimab; and NSP12:E136A/D, NSP12:V166A/L, NSP12:V792I, NSP14:I42V in patients infected with neither Delta nor Omicron (most were Alpha) and treated with remdesivir. NSP12:F694Y is identified among patients infected with neither Delta nor Omicron (most were Alpha) and treated with remdesivir, and among patients infected with BA.2 and treated with molnupiravir but it is a sequencing artefact. In a small number of patients (n=13, 5 on casirivimab and imdevimab, 8 on sotrovimab), mutations were found in combination (Table 5). There are no amino acid residues displaying change in post-treatment sequences in sequences from patients treated with nirmatrelvir plus ritonavir.

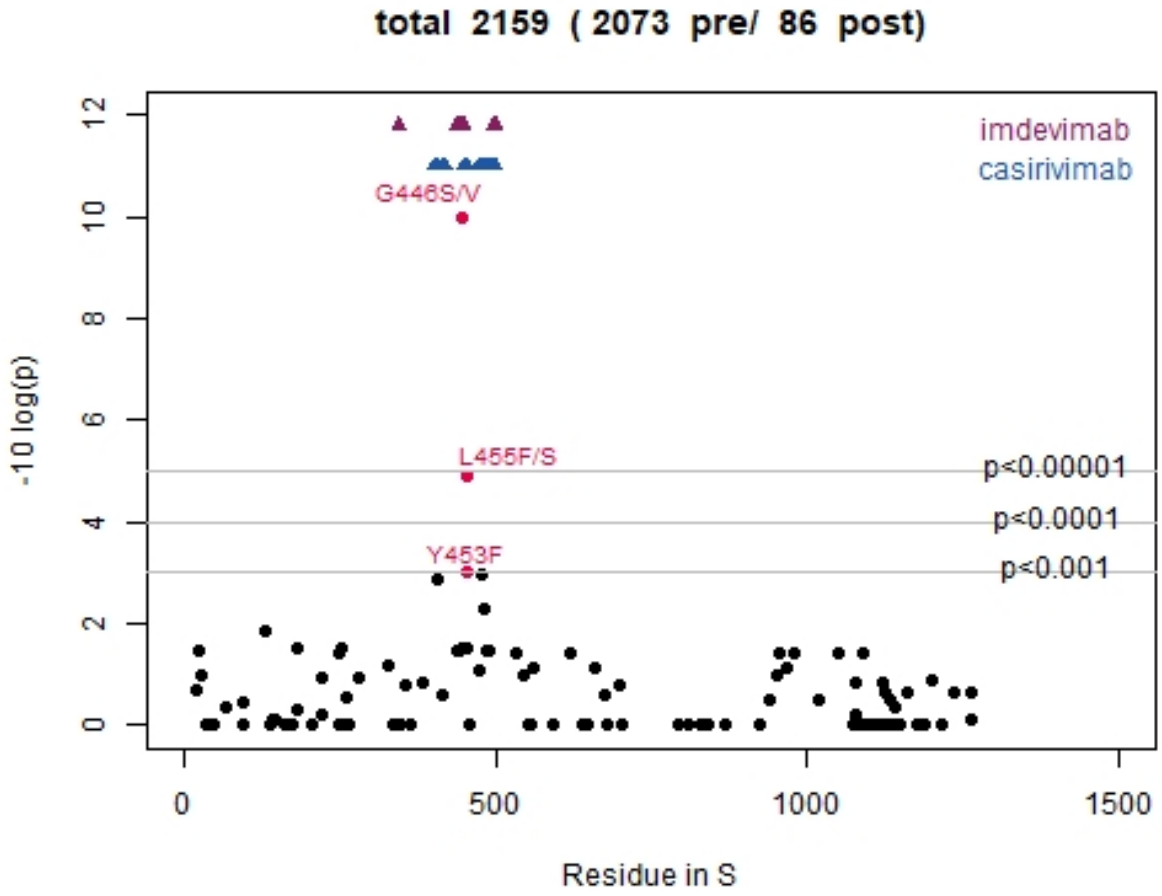
Table 5. Combination of mutations found in 13 patients with >1 treatment-emergent substitution

Treatment	Variant	Combination of mutations
Casirivimab and imdevimab	Delta	1 G446S, L455S 3 G446V, L455F 1 G446V, Y453F
Sotrovimab	BA.1	2 E340A, R493Q 1 E340D, L455W 1 E340D, R493Q 1 E340K, L455S 1 E340Q, L455S 1 K356T, R493Q
Sotrovimab	BA.2	1 E340Q, K356T

The prevalence of mutations associated in post treatment samples for casirivimab and imdevimab in Delta sequences (n=353) are: 9 S:G446V, 2 S:Y453F; 4 S:L455F/S. Prevalence of mutations post sotrovimab with the BA.1 variant (n=614): 16 S:P337L/R/S; 49 S:E340A/D/G/K/Q/V; 8 S:K356T; 3 S:L455S/W and 4 S:R493Q. Prevalence of mutations post sotrovimab with the BA.2 variant (n=656) : 23 S:E340D/K/Q and 10 S:K356R/T. The prevalence of mutations post-remdesivir in patients infected with neither Delta nor Omicron are 3 NSP12:E136A/D, 4 NSP12:V166A/L, 3 NSP12:V792I, 25 NSP14:I42V.

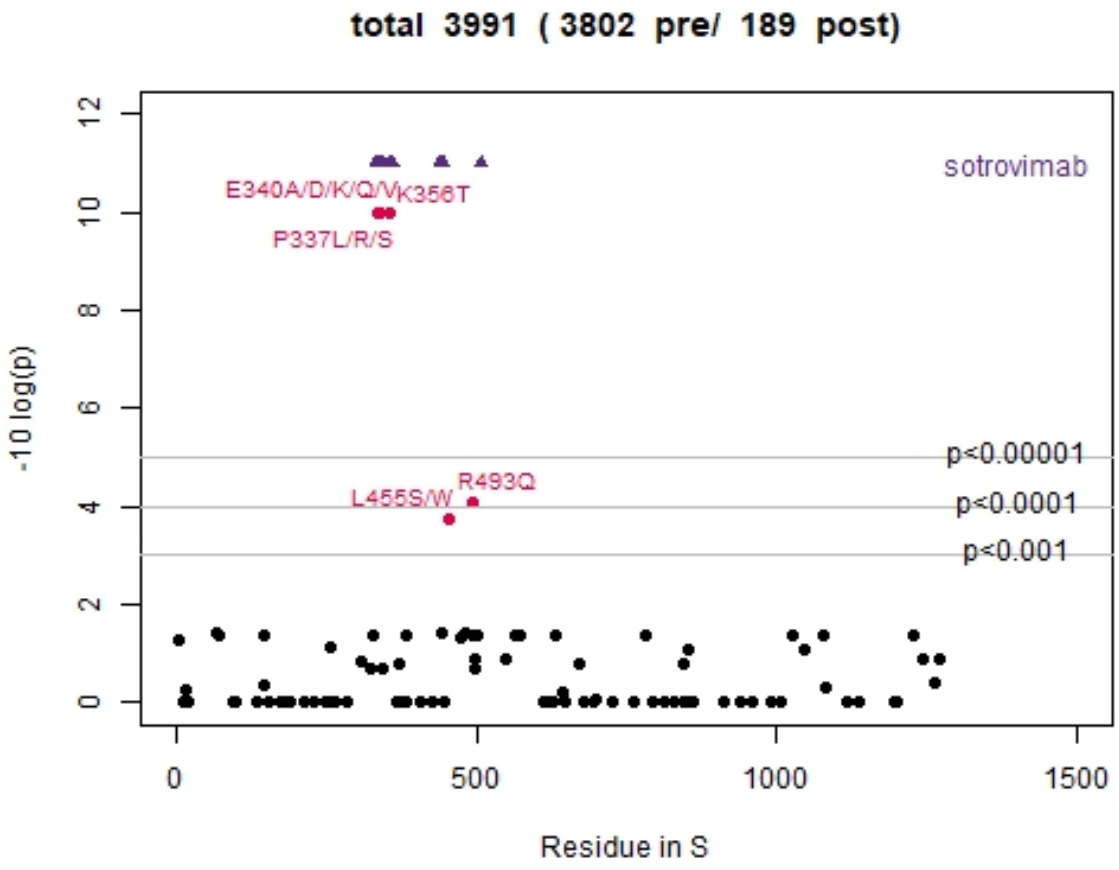
Further analyses of the mutations with increased frequency post-treatment are underway, including in silico modelling and laboratory assessments.

Figure 4. Calculated p-values for differences in spike amino acid frequencies between pre- and post-casirivimab/imdevimab treatment sequences of patients infected with Delta



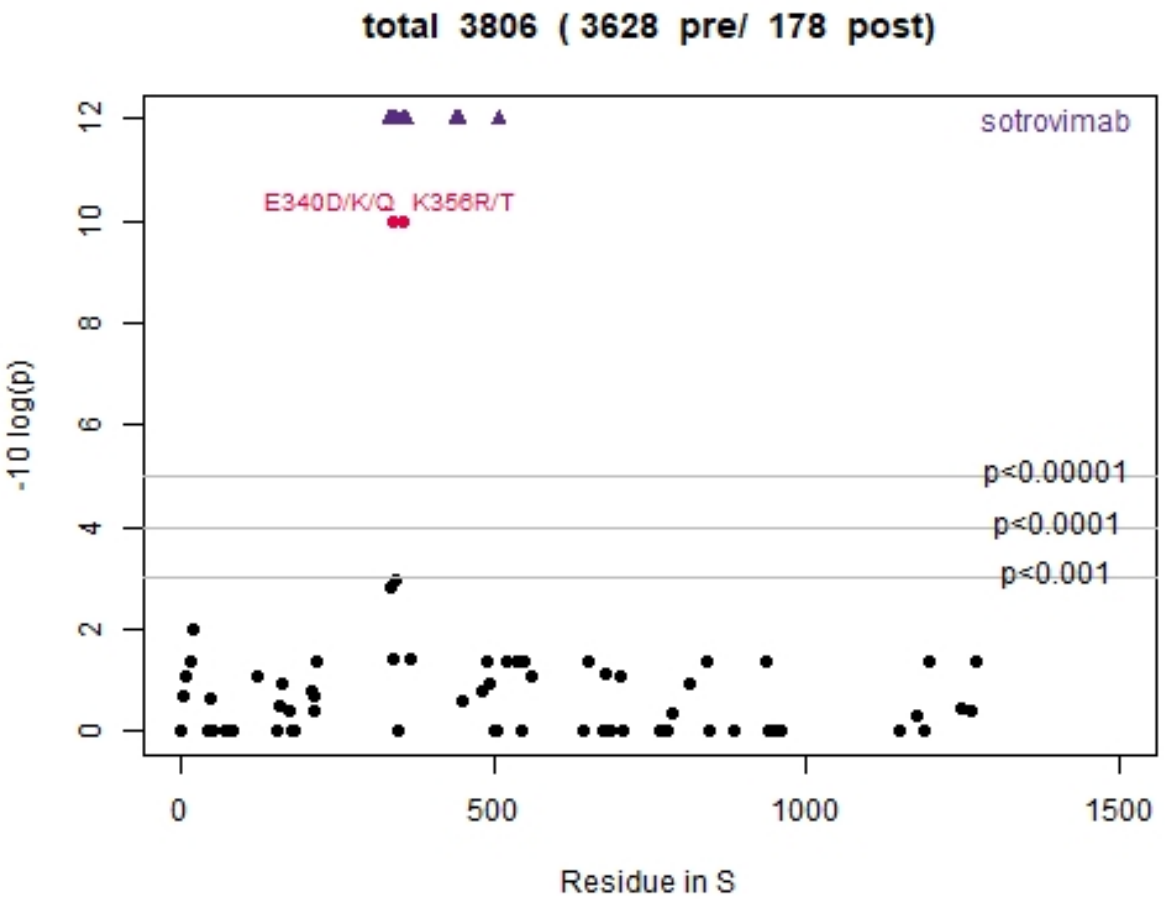
Amino acid frequencies were compared between pre-and post-treatment samples (at least 10 days after treatment) at each site in the spike sequence alignment. P-values for each site were calculated using a Fisher's test, and p-values were log-transformed and inversed for visualisation so that sites with diverging values appear higher up on the figure. Only sites with some variability (>1 amino acid) are shown on the figure. The horizontal lines indicate p-value thresholds of p<0.001, p<0.0001 and so on. Residues with diverging frequencies (p<0.001) are highlighted in red, with the observed amino acid change indicated in text. Three sites displayed diverging frequencies at the p<0.001 level between pre-and post-treatment sequence: G446S/V, Y453F and L455F/S. Residues 453 and 455 are known contact residues of casirivimab and residue 446 is a known contact residue for imdevimab. Given the large numbers of sites independently tested, lower p thresholds are necessary to demonstrate selection. Residues known to interact with each drug are indicated in blue and purple at the top of the figure. The numbers differ slightly from those in Table 4 because a single sequence per timepoint and per patient is used in this analysis and not all genomic data was sufficiently high quality for downstream sequence analysis. Supplementary data is not available.

Figure 5. Calculated p-values for differences in spike amino acid frequencies between pre- and post-sotrovimab treatment sequences of patients infected with BA.1



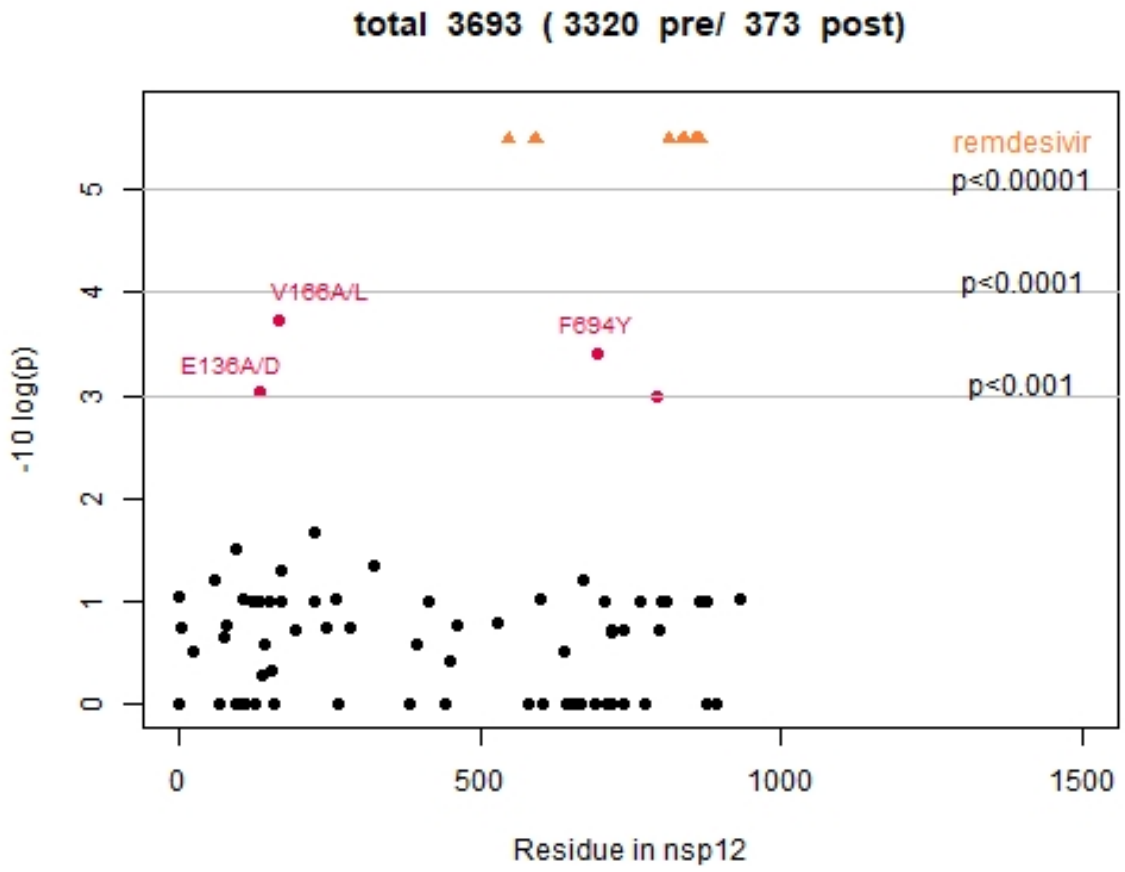
Amino acid frequencies were compared between pre-and post-treated samples (at least 10 says after treatment), for each site in the spike sequence alignment. P-values for each site were calculated using a Fisher's test, and p-values were log-transformed and inversed for visualisation so that sites with diverging values appear higher up on the figure. Only sites with some variability (>1 amino acid) are shown on the figure. The horizontal lines indicate p-value thresholds of p<0.001, p<0.0001 and so on. Residues with diverging frequencies (p<0.001) are highlighted in red, with the observed amino acid change indicated in text. Given the large numbers of sites independently tested, lower p thresholds are necessary to demonstrate selection. Five sites displayed diverging frequencies at the p<0.001 level between pre-and post-treatment sequence: P337R/S and E340A/D/K/V, L455S/W, K356T and R493Q. Residues 337, 340 and 356 are known contact residues for sotrovimab. The numbers differ slightly from those in Table 4 because a single sequence per timepoint and per patient is used in this analysis and not all genomic data was sufficiently high quality for downstream sequence analysis. Supplementary data is not available.

Figure 6. Calculated p-values for differences in spike amino acid frequencies between pre- and post-sotrovimab treatment sequences of patients infected with BA.2



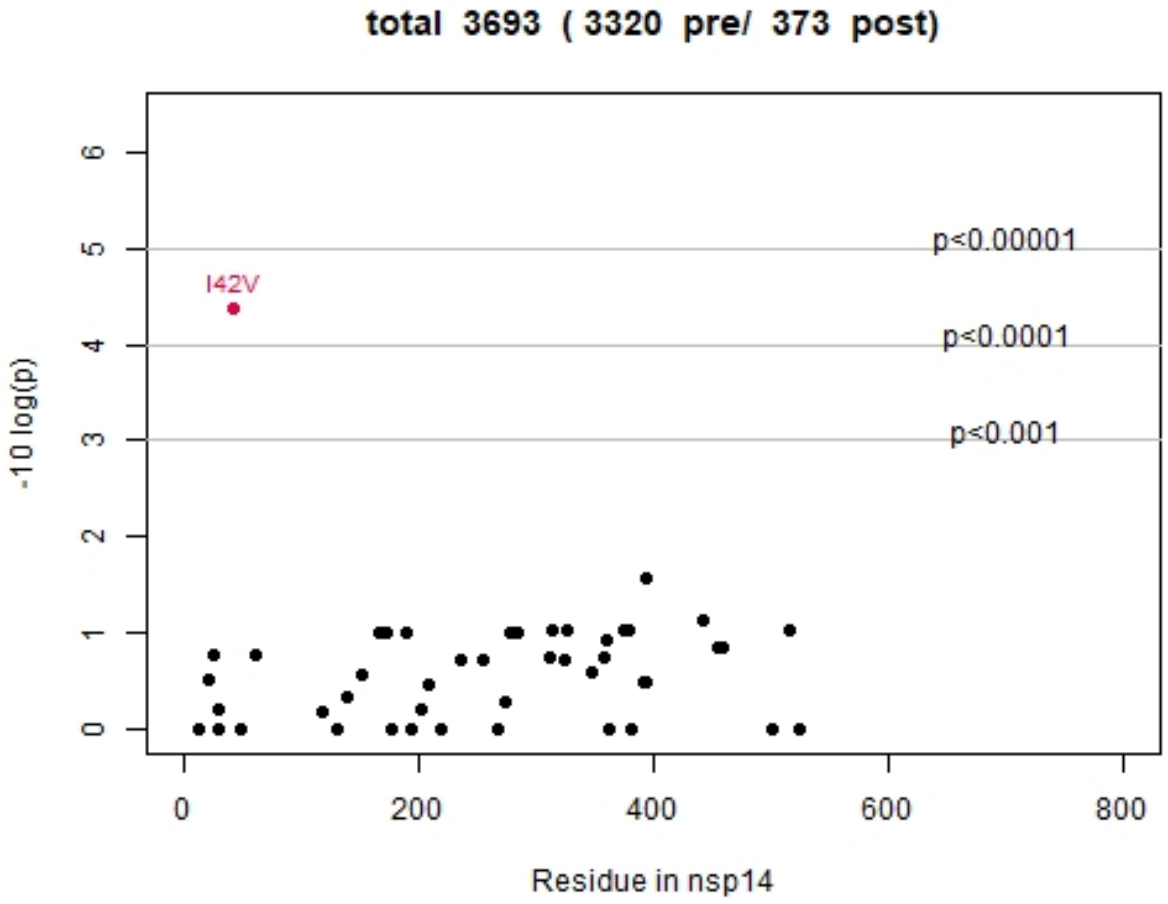
Amino acid frequencies were compared between pre-and post-treated samples (at least 10 says after treatment), for each site in the spike sequence alignment. P-values for each site were calculated using a Fisher's test, and p-values were log-transformed and inversed for visualisation so that sites with diverging values appear higher up on the figure. Only sites with some variability (>1 amino acid) are shown on the figure. The horizontal lines indicate p-value thresholds of p<0.001, p<0.0001 and so on. Residues with diverging frequencies (p<0.001) are highlighted in red, with the observed amino acid change indicated in text. Given the large numbers of sites independently tested, lower p thresholds are necessary to demonstrate selection. Two sites displayed diverging frequencies at the p<0.001 level between pre-and post-treatment sequence: E340D/K/Q and K356T, known contact residues for sotrovimab. The numbers differ slightly from those in Table 4 because a single sequence per timepoint and per patient is used in this analysis and not all genomic data was sufficiently high quality for downstream sequence analysis. Supplementary data is not available.

Figure 7. Calculated p-values for differences in NSP12 amino acid frequencies between pre- and post-remdesivir treatment sequences of patients infected with non-Delta, non-Omicron



Amino acid frequencies were compared between pre-and post-treated patients (at least 10 days after treatment), for each site in the NSP12 sequence alignment. P-values for each site were calculated using a Fisher's test, and p-values were log-transformed and inversed for visualisation so that sites with diverging values appear higher up on the figure. Only sites with some variability (>1 amino acid) are shown on the figure. The horizontal lines indicate p-value thresholds of p<0.001, p<0.0001 and so on. Residues with diverging frequencies (p<0.001) are highlighted in red, with the observed amino acid change indicated in text. Three sites displayed diverging frequencies at the p<0.001 level between pre-and post-treated sequences: E136A/D, V166A/L and F694Y. None are known interaction sites for remdesivir. F694Y is a sequencing artefact. Given the large numbers of sites independently tested, lower p thresholds are necessary to demonstrate selection. The numbers differ from those in Table 4 because a single sequence per timepoint and per patient is used in this analysis and not all genomic data was sufficiently high quality for downstream sequence analysis. Supplementary data is not available.

Figure 8. Calculated p-values for differences in NSP14 amino acid frequencies between pre- and post-remdesivir treatment sequences of patients infected with non-Delta, non-Omicron



Amino acid frequencies were compared between pre- and post-treated patients (at least 10 days after treatment), for each site in the NSP14 sequence alignment. P-values for each site were calculated using a Fisher's test, and p-values were log-transformed and inversed for visualisation so that sites with diverging values appear higher up on the figure. Only sites with some variability (>1 amino acid) are shown on the figure. The horizontal lines indicate p-value thresholds of $p<0.001$, $p<0.0001$ and so on. Residues with diverging frequencies ($p<0.001$) are highlighted in red, with the observed amino acid change indicated in text. One site displayed diverging frequencies at the $p<0.001$ level between pre-and post-treated sequences: I42V. There are no known interaction sites in NSP14 for remdesivir. Given the large numbers of sites independently tested, lower p thresholds are necessary to demonstrate selection. The numbers differ from those in Table 4 because a single sequence per timepoint and per patient is used in this analysis and not all genomic data was sufficiently high quality for downstream sequence analysis. Supplementary data is not available.

4.1 Characterisation of treatment-emergent mutations using pseudovirus models

Academic partners (University of Oxford) evaluated the binding behaviour of treatment-emergent mutations using surface plasmon resonance (SPR) and examined their impact on the neutralising activity of therapeutic antibodies using pseudoviral assays.

Casirivimab and imdevimab and Delta

Pseudotyped lentiviruses expressing the spike protein with the identified treatment-emergent mutations (S: E406D/Q, S: G446V, Y453F and L455F/S) were generated. Pseudoviral neutralisation assays showed that activity of imdevimab against the Delta+G446V mutant was completely knocked out, whilst casirivimab showed more than10 fold reductions in the neutralisation titre of Delta+Y453F (12-fold), Delta+L455F (16-fold) and Delta+L455S (65-fold), compared to the wild-type Delta variant (Figure 9A and Table 6A).

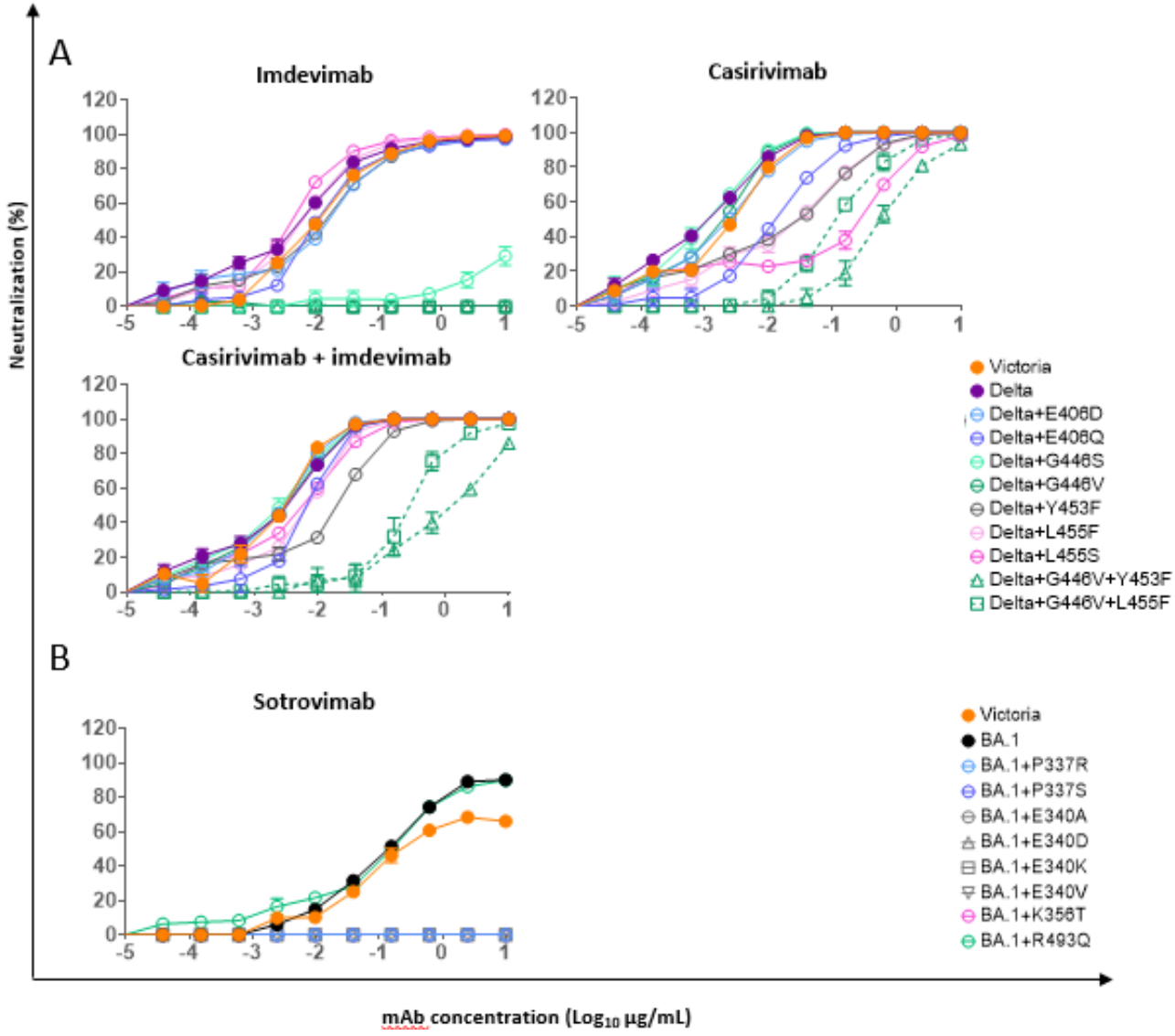
As casirivimab remained fully active against the Delta+G446V mutant, and imdevimab was still able to potently neutralise the Delta+Y453F and Delta+L455F/S mutants, the combination of casirivimab and imdevimab retained neutralization potency against all these single mutants. However, the combined mutations of Delta+G446V+Y453F and Delta+G446V+L455F led to complete knock-out of the neutralising activity of imdevimab, but also severe knock-down of casirivimab activity. As a result, the neutralisation titre of casirivimab+imdevimab was reduced 1,032-fold against Delta+G446V+Y453F and 276-fold against the Delta+G446V+L455F (Figure 9A and Table 6A). The observed effects on neutralisation were confirmed by using surface plasmon resonance to be directly attributable to the change in affinity of nMAB and RBD mutant interaction (Table 7A).

Sotrovimab and BA.1

Pseudotyped lentiviruses expressing the spike protein with the identified post sotrovimab treatment-emergent mutations (S: P337R/S and S: E340A/D/K/V, S: K356T and S: R493Q) were generated. P337R/S, E340A/D/K/V, K356T treatment-emergent mutations led to complete knock out of neutralisation by sotrovimab. Although the BA.1+R493Q (reversion to Wuhan wild type) was also identified as a post-treatment emergent mutation, no obvious effect on the neutralising activity of sotrovimab was observed (Figure 9b and Table 6B).

Using SPR, the RBD of BA.1+P337R/S and BA.1+E340A/D/K/V was expressed to allow examination of their binding with sotrovimab (Table 7B). The affinity of sotrovimab was reduced by 1951-fold to 20241-fold compared to the wild-type BA.1 RBD, explaining why these mutants were resistant to sotrovimab neutralisation.

Figure 9. Neutralisation escape of RBD mutations (A) Pseudoviral neutralisation curves and IC50 of the indicated Delta variants from casirivimab and imdevimab (B) Pseudovirus neutralisation curves and IC50 for BA.1 sotrovimab mutants

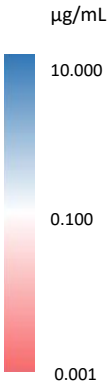


Supplementary data is not available.

Table 6. Neutralisation escape of RBD mutations (A) Pseudoviral neutralisation curves and IC50 of the indicated Delta variants from casirivimab and imdevimab (B) Pseudovirus neutralisation curves and IC50 for BA.1 sotrovimab mutants

A)

Pseudovirus	IC50 (Log10 µg/ml)		
	Commercial mAbs		
	Casirivimab	Imdevimab	Casirivimab + imdevimab
Victoria	0.002	0.012	0.002
Delta	0.001	0.004	0.001
Delta+E406D	0.002	0.009	0.002
Delta+E406Q	0.012	0.015	0.005
Delta+G446S	0.001	10.000	0.002
Delta+G446V	0.001	10.000	0.002
Delta+Y453F	0.012	0.01	0.008
Delta+L455F	0.016	0.005	0.004
Delta+L455S	0.065	0.004	0.003
Delta+G446V+Y453F	0.626	10.000	1.032
Delta+G446V+L455F	0.129	10.000	0.276



B)

Pseudovirus	IC50 (Log10 µg/ml)
	Commercial mAbs
	Sotrovimab
Victoria	0.219
BA.1	0.14
BA.1+P337R	10.000
BA.1+P337S	10.000
BA.1+E340A	10.000
BA.1+E340D	10.000
BA.1+E340K	10.000
BA.1+E340V	10.000
BA.1+K356T	10.000
BA.1+R493Q	0.151

Table 7. Affinity of RBD mutations using SPR analysis (A) SPR analysis of Delta RBD/ mAb (imdevimab/casirivimab) interaction (B) SPR analysis of RBD/sotrovimab interactions

A)

	Casirivimab		Imdevimab	
RBD	**K_D (nM)**	**Fold reduction**	**K_D (nM)**	**Fold reduction**
Delta RBD WT	0.36	-	9.4	-
Delta RBD+E406D	7.1	20	15	1.6
Delta RBD+E406Q	14	38	8.9	1.1
Delta RBD+G446V	0.64	1.8	Very weak binding	
Delta RBD+Y453F	67	186	9.8	1.0
Delta RBD+L455F	44	122	11	1.2
Delta RBD+L455S	133	369	9.1	1.0
Delta RBD+G446V+Y453F	125	347	Very weak binding	
Delta RBD+G446V+L455F	69	192	Very weak binding	

B)

	Sotrovimab	
RBD	**K_D (nM)**	**Fold reduction**
BA.1 RBD WT	0.17	-
BA.1 RBD+P337R	753	4,428
BA.1 RBD+P337S	332	1,951
BA.1 RBD+E340A	3,415	20,088
BA.1 RBD+E340D	764	4,494
BA.1 RBD+E340K	3,441	20,241
BA.1 RBD+E340V	345	2,027

4.2 Transmission of post-treatment mutations in United Kingdom genomic dataset

There was some evidence of potential limited transmission of treatment-emergent mutations within the UK genomic dataset. Here, a potential transmission event is defined as a case where:

- very closely related samples were sampled from 2 patients (less than 4x10-5 nucleotide substitutions per site)
- at least one of these samples was taken from a treated patient, post-treatment
- samples from both patients have the same combination of relevant amino acids at positions of interest in the spike protein (positions: 337, 340, 356, 455, 493 or 446, 453, 455)

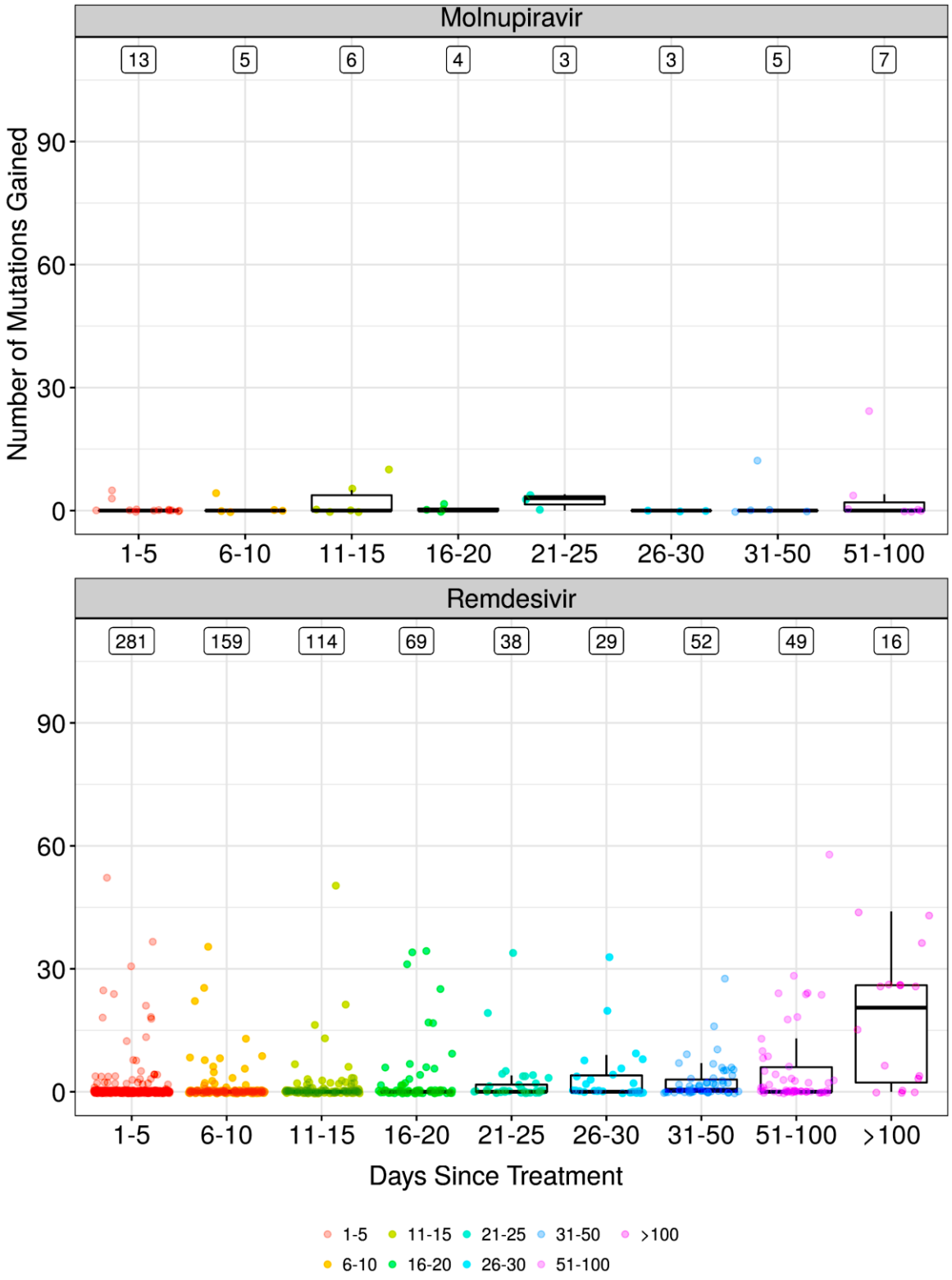
There was one example of such an event, involving a G to V substitution at position 446. The mutation exists among untreated COVID-19 patients, and it is unclear whether the mutation was induced by treatment in this case or whether the potential transmission event occurred post-treatment with casirivimab and imdevimab. The mutation existed in Delta genomes before the introduction of casirivimab and imdevimab and did not increase after that introduction.

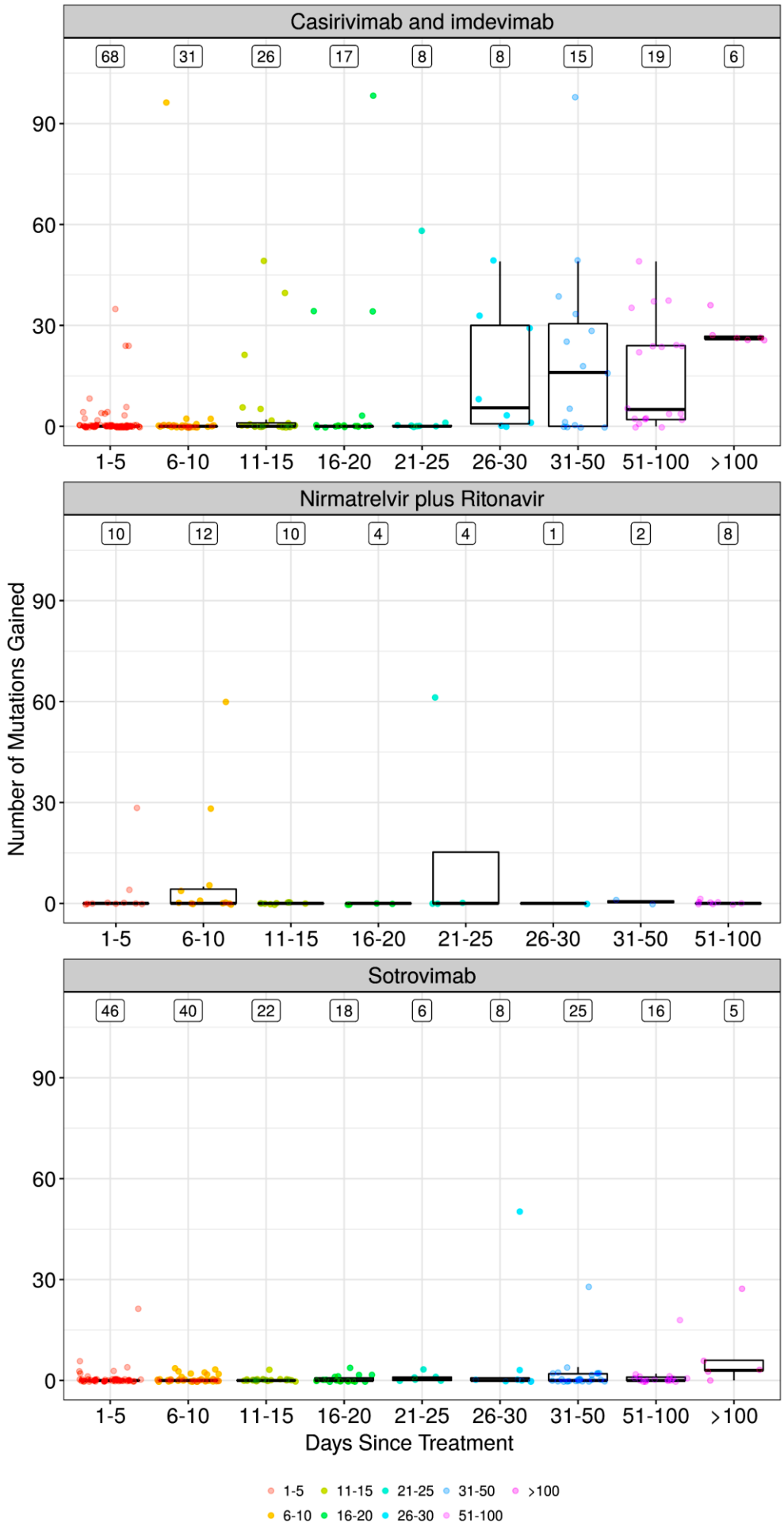
As the sequencing coverage in the UK is reduced, observing such pairs becomes increasingly unlikely. However, we would still expect to observe sequenced cases in the wider population if there was a variant with post treatment mutations spreading more widely.

Longitudinal analysis of pre/post-treatment serial samples

These analyses describe changes in aggregated mutations over time which may occur due to persistent infection or use of therapeutic agents. SARS-CoV-2 samples with at least one pre- and 2 post-treatment sequences, over a period of more than or equal to 90 days, the time definition of SARS-CoV-2 reinfections, were analysed. A single pre-treatment sample was selected per patient, based on proximity to treatment date. The full UKHSA genomic dataset was processed to yield comparator mutation changes across all gene regions matched (ORF1ab, NSP1-10, NSP12-16, ORF3a, ORF6, ORF7a, ORF8, ORF10, S gene, E, M, and N) and stratified by treatment. A mutation score was determined to define the sum of additional mutations gained by comparing the pre- and post-treatment amino acid changes. However, this dataset currently ignores insertion-deletion mutation types and does not record mutation losses following treatment. These analyses describe changes in aggregated mutations over time which may occur due to chronicity of infection or use of therapeutic agents.

Figure 11. Longitudinal pre/post-treatment mutation analysis from serial sampling
Each plot is stratified by treatment drug with the bottom x-axis defining days since treatment in groups. Total sample numbers are denoted on the top x-axis. Timeline specimen/treatment days range from June 2020 to June 2022. The total sample size includes 810 patients with n=3,451 total sequences. Supplementary data is not available.




Casirivimab and imdevimab
68
31
26
17
8
8
15
19
6
Nirmatrelvir plus Ritonavir
10
12
10
4
4
1
2
8
Sotrovimab
46
40
22
18
6
8
25
16
5
Number of Mutations Gained
0
30
60
90
1-5
6-10
11-15
16-20
21-25
26-30
31-50
51-100
>100
Days Since Treatment
1-5
6-10
11-15
16-20
21-25
26-30
31-50
51-100
>100

Across all treatments, mutations appear to rise approximately 21 to 25 days post treatment. Casirivimab and imdevimab show a greater increase of mutations at the same time point whereas remdesivir exhibits a peak in the number of mutations at the longest time window (more than 100 days since treatment). It should be noted that serial sample numbers are highly variable across treatments. Samples lacking at least one amino acid positional change are excluded (that is if an amino acid mutation does not differ before and after treatment).

This data supports the hypothesis that most individuals who clear their SARS-CoV-2 infection by day 10 are unlikely to drive viral variant selection. Further assessment on mitigating risk of viral evolution in individuals with persistent SARS-CoV-2 positivity should be undertaken.

Future analyses are underway to include a control group, who have not received treatment, to assess mutation gains and drop offs over time and to stratify analyses by gene regions with therapeutic implications from structural modelling analyses, including S gene for nMABs and NSP5 – 14 for AVs.

5. Assessment of sotrovimab with BA.1 and BA.2

5.1 Laboratory assessment of sotrovimab BA.1 and BA.2

A micro-neutralisation assay based on a protocol developed by Bewley and others, 2021 was used to assess the neutralising activity of sotrovimab, against both BA.1 and BA.2. The mutations within the spike gene of these variants, necessitated a change in the immunostaining protocol, with a nucleocapsid antibody used with Triton permeabilized cells to measure the formation of foci of infection. To provide a direct comparison, data was generated with the Victoria isolate (hCoV-19/Australia/VIC01/2020) of the original Wuhan strain, using the same staining protocol. BA.1 showed reduced neutralisation compared to Victoria, with a geometric mean IC50 (concentration of antibody which reduces infection by 50%) of 666 ng/ml, whereas the IC50 for Victoria was 54 ng/ml. This data is in line with other published studies, notably Duty and others (2022), Cathcart and others (2022), Case and others (2022) and Ohashi and others (2022) where an IC50 for sotrovimab against Omicron (BA.1) was 373 ng/ml, 169 ng/ml, 452 ng/ml and 958 ng/ml respectively.

The IC50 for sotrovimab against BA.2 was higher (1,207 ng/ml) than that seen for BA.1 (666 ng/ml). Higher IC50 values for BA.2 have also been reported elsewhere (973 ng/ml (Cathcart and others 2022), 1358 ng/ml Ohashi and others (2022) and 5,885 ng/ml Case and others (2022).

6. Risk assessment of treatment-emergent mutations

Data presented in this report show that SARS-CoV-2 may evolve in patients who are treated, leading to the selection of mutations which reduce the effect of the drug in laboratory tests. Drug resistance mutations may come with a fitness cost to the virus, and these mutations only pose a wider risk to public health if the virus remains highly fit and transmissible. The UK data suggest that whilst mutations are emerging in patients with longer term infections after treatment, the viruses with these mutations are not transmitting widely in the population at present. Patients affected have persistent

infections and there may be interactions with the effect of immunocompromise. Whilst the current findings do not pose immediate risks to the national clinical treatment policy, the changes observed highlight that this could change quickly. Genomic surveillance of treated patients and the wider population is required to continue to monitor this risk.

Sources and acknowledgments

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About the UK Health Security Agency

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