



UK Health
Security
Agency

SARS-CoV-2 therapeutics technical briefing 3

Genomic surveillance

10 May 2022

This report provides an update on previous [briefings](#) up to 3 March 2022

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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 18 April 2022 to allow time for analyses.

Surveillance sampling

Surveillance sampling has been updated in line with the changing clinical policy and to encompass community settings. The main updates are:

- all patients treated with neutralising monoclonal antibodies (nMABs) and directly acting antivirals (AVs) are included for surveillance, including community settings and patients of all ages
- patients in hospital should have samples sequenced pre-treatment, at day 5 after start of treatment or discharge, and at day 14 if in a high-risk group as directed by NHS England/Improvement (NHSE/I) clinical policies
- patients in the community should have samples sequenced pre-treatment, and at approximately day 5 after the start of treatment
- addition of experimental pathogen genomic analyses for new variants of concern
- addition of equitable use analyses to support national antimicrobial stewardship

UK genomic data set mutation scanning

The whole 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.

VOC-21NOV-01 (Omicron, BA.1)

Of the acquired spike mutations (additional to those mutations that define the lineage) occurring at over 1% prevalence in BA.1, R346K is at a predicted contact residue site for sotrovimab. The other mutations over 1% are L5F, I68L, G181A, N211S, A263V, L368I, H625R, Q628K, and A701V. These were not associated with predicted contact residue site for sotrovimab. There are 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, remdesivir or molnupiravir.

VOC-22JAN-01 (Omicron, BA.2)

There are no acquired spike mutations occurring at over 1% prevalence in BA.2. There are no mutations observed in NSPs 5-14 at contact residue sites associated with nirmatrelvir plus ritonavir, remdesivir or molnupiravir.

Structural modelling assessment on therapeutics

V-22APR-01 (XD) is a double break point recombinant (21,643 and 25,581) that consists of a V-21APR-02 (Delta) backbone with a BA.1 spike gene. Therefore, spike protein modelling and effect on sotrovimab remains consistent with BA.1 whilst NSP5, NSP10, NSP12, and NSP14 are comparable to Delta. Of the lineage defining spike mutations in BA.1, N440K is at a predicted contact residue site for sotrovimab. No lineage defining Delta mutations are associated with NSP5, NSP10, NSP12, and NSP14 contact sites.

V-22APR-02 (XE) is a single break point Omicron recombinant that consists of a BA.1 5' and a BA.2 3' section, with a break point at nucleotide position 11,537). Therefore, NSP5, NSP10 mutations will be equivalent to BA.1 whilst Spike, NSP12, and NSP14 mutations will be equivalent to BA.2.

V-22APR-03 (BA.4)/V-22APR-04 (BA.5)

Based on structural models, the F486V mutation seen in both BA.4 and BA.5 is likely to impact the casirivimab component of casirivimab and imdevimab and the tixagevimab component of tixagevimab/cilgavimab (Evusheld®); sotrovimab is not expected to be further affected compared to BA.2; molnupiravir, remdesivir and paxlovid are not expected to be impacted.

Post-treatment viral sequences

Eleven amino acid residues exhibited a significant change in frequency in post-treatment sequences compared to pre-treatment sequences, suggesting possible evidence of selection: S:E406D/Q, S:G446V, Y453F and L455F/S in patients infected with Delta and treated with casirivimab and imdevimab; S:P337R/S and S:E340A/D/K/V, S:K356T and R493Q in patients infected with BA.1 and treated with sotrovimab; S:E340K in patients infected with BA.2 and treated with sotrovimab; and NSP12:E136A/D, NSP12:V166A/L and NSP12:V792I in patients infected with neither Delta nor Omicron (most were Alpha) and treated with remdesivir. In a small number of patients (fewer than 20), mutations were found in combination. There are no amino acid residues displaying change in post-treatment sequences in NSPs 7 to 14 for molnupiravir or NSP5 for nirmatrelvir plus ritonavir.

The frequency of post treatment mutations within the UK genomic data set for mutations associated with casirivimab and imdevimab in Delta sequences are: 6 S:E406D; 7 S:E406Q; 1,946 S:G446V, 12 S:Y453F; 179 S:L455F; 1 S:L455S. Frequency of mutations post sotrovimab treatment with the BA.1 variant since 6 September 2022: 11 S:P337R; 32 S:P337S; 39 S:E340A; 82 S:E340D; 52 S:E340K; 5 S:E340V; 57 S:K356T; S:R493Q. Frequency of mutations post sotrovimab treatment with the BA.1 variant since 6 September 2022: 10 S:E340K. There were no samples observed for the remdesivir mutations listed in non-Delta and non-Omicron samples since 6 September 2021.

These mutations are undergoing assessment through structural modelling, generation of pseudovirus models and further laboratory testing to confirm this hypothesis.

Longitudinal host analyses

Differences in pre- and post-treatment sequences were analysed with a range of cut-offs between day of treatment and the post-treatment sequence (0 days, 5 days, 10 days). Using this stratification, while the number of post-treatment sequences reduced, mutation sites associated with post-treatment sequences became more pronounced.

Transmission of post-treatment mutations in UK genomic data set

There was no evidence within the UK genomic data set of transmission of acquired mutations post treatment. A potential transmission of a mutation can only be identified if both the source and the recipient can be sequenced. As the sequencing coverage in the UK is reduced, observing such a pair 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 widely.

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

In preliminary viral neutralisation assays, both BA.1 and BA.2 showed reduced neutralisation by sotrovimab, compared to the Victoria isolate (Wuhan) control. Neutralisation of BA.2 was reduced approximately 5-fold relative to BA.1. Further studies are required to determine the IC50.

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

In an observational cohort study based on routine clinical data, there was no significant difference observed in the hazard ratio (HR) for hospital admission as an inpatient or hospital attendance after sotrovimab use between BA.1 and BA.2, when adjusted for age, linear effect in age and vaccination status.

2. Published information on therapeutics

The UK Health Security Agency's (UKHSA) coronavirus (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 genomic 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 - COVID-19 alerts](#). These were outlined in [therapeutics technical briefing 2](#). In addition, casirivimab and imdevimab ceased to be available in clinical policies from February 2022 and nirmatrelvir plus ritonavir (Paxlovid®) has been available to both non-hospitalised patients at highest risk and those with hospital-onset COVID-19 infection since February 2022.

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 4 April 2022, 2.44% of sequences were BA.1 (325), 97.00% were from BA.2 (including sub-lineages) (12,921), and 0.56% were unclassified (75). The latest data for the prevalence of different variants amongst sequenced episodes is presented in Technical Briefing 40. BA.2 rarely contains the spike gene deletion at position 69 to 70 and is S-gene target positive (SGTP) on polymerase chain reaction (PCR) diagnostic assays with targets in this area. SGTP is now a reasonable proxy for BA.2, accounting for 97% of sequenced SGTP cases with an increasing trend.

The whole 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 casirivimab, imdevimab, and 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.

3.1 VOC-21NOV-01 (Omicron BA.1)

Of the lineage defining spike mutations in BA.1, N440K is at a predicted contact residue site for sotrovimab. The acquired spike mutations occurring at over 1% prevalence in BA.1 between the 4 and 12 of April (N=325) are L5F (5), I68L (1), G181A (6), N211S (1), A263V (3), R346K (262), L368I (6), H625R (7), Q628K (12), and A701V (23). R346K is the lineage defining mutation for BA.1.1 (a sub-lineage of BA.1) representing 82% of BA.1 samples and is a predicted contact residue site for sotrovimab. The remaining mutations were not associated with predicted contact residue sites for sotrovimab.

Contact residue sites in NSPs 7-14 are associated with remdesivir or molnupiravir and NSP5 with nirmatrelvir plus ritonavir. The NSP12 mutations occurring at over 1% prevalence are G44S (7), K91E (4), D140G (8), I223V (7), T226M (9), K263R (4), P323L (322), F694Y (14), and Q875R (8). There are no mutations observed in NSP12 at contact residue sites associated with remdesivir or molnupiravir. No NSP6, NSP7, NSP8, NSP9, NSP10 or NSP14 acquired mutations were observed over 1% prevalence. None of the lineage defining or acquired NSP5

mutations occurring at over 1% prevalence observed in BA.1 were associated with contact sites for paxlovid.

3.2 VOC-22JAN-01 (Omicron BA.2)

Of the lineage defining spike mutations in BA.2, N440K is at a contact residue site for sotrovimab. The acquired spike mutations occurring at over 1% prevalence in BA.2 between the 4 and 12 of April (N=12,921) are L5F (154), I68T (141), and K417T (158). T35I (317) was the only acquired NSP9 mutation occurring at over 1% prevalence. A32V (13), T58I (19), T102I (45), and A104V (26) were the acquired NSP10 mutations occurring at over 1% prevalence. NSP10 is a co-activator of the exonuclease (NSP14). Mutations which influence the activity of the exonuclease have the potential to influence antiviral treatments (molnupiravir and remdesivir). A32V and T58I were predicted to be contact sites between NSP10 and NSP14, however *in silico* models indicated mutations at these positions have a low impact on the interactions between NSP10 and NSP14, and therefore, are unlikely to influence molnupiravir and remdesivir efficacy without additional mutations. F694Y (275) was the only acquired NSP12 mutations occurring at over 1% prevalence. None of these were highlighted as concerning contact sites *in silico*. No NSP5, 6, NSP7, NSP8, or NSP14 acquired mutations were observed to occur at over 1% prevalence in BA.2.

Figure 1 and Figure 2 show mutation heatmaps of non-synonymous changes accruing on top of the BA.1 lineage defining mutations. Figure 3 and Figure 4 show mutation heatmaps of non-synonymous changes accruing on top of the BA.2 lineage defining mutations.

Casirivimab/imdevimab and sotrovimab are nMABs targeting the spike gene. NSP12 contains the predicted contact sites for remdesivir and molnupiravir. Each tile shows the proportion of sequences with each mutation per week. The total number of sequences is shown within the box. In NSP12, F694Y is reported to be an artifact in sequences using the Artic V4 primers as reported by [Sanderson and others \(2021\)](#).

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

Supplementary data is not available. It should be noted all mutations in the sequence alignment are reported in these plots for review purposes. Those reported here at positions 145 and 211 arise due to base deletions affecting the sequence alignment and are not true, acquired mutations and are artifactual.

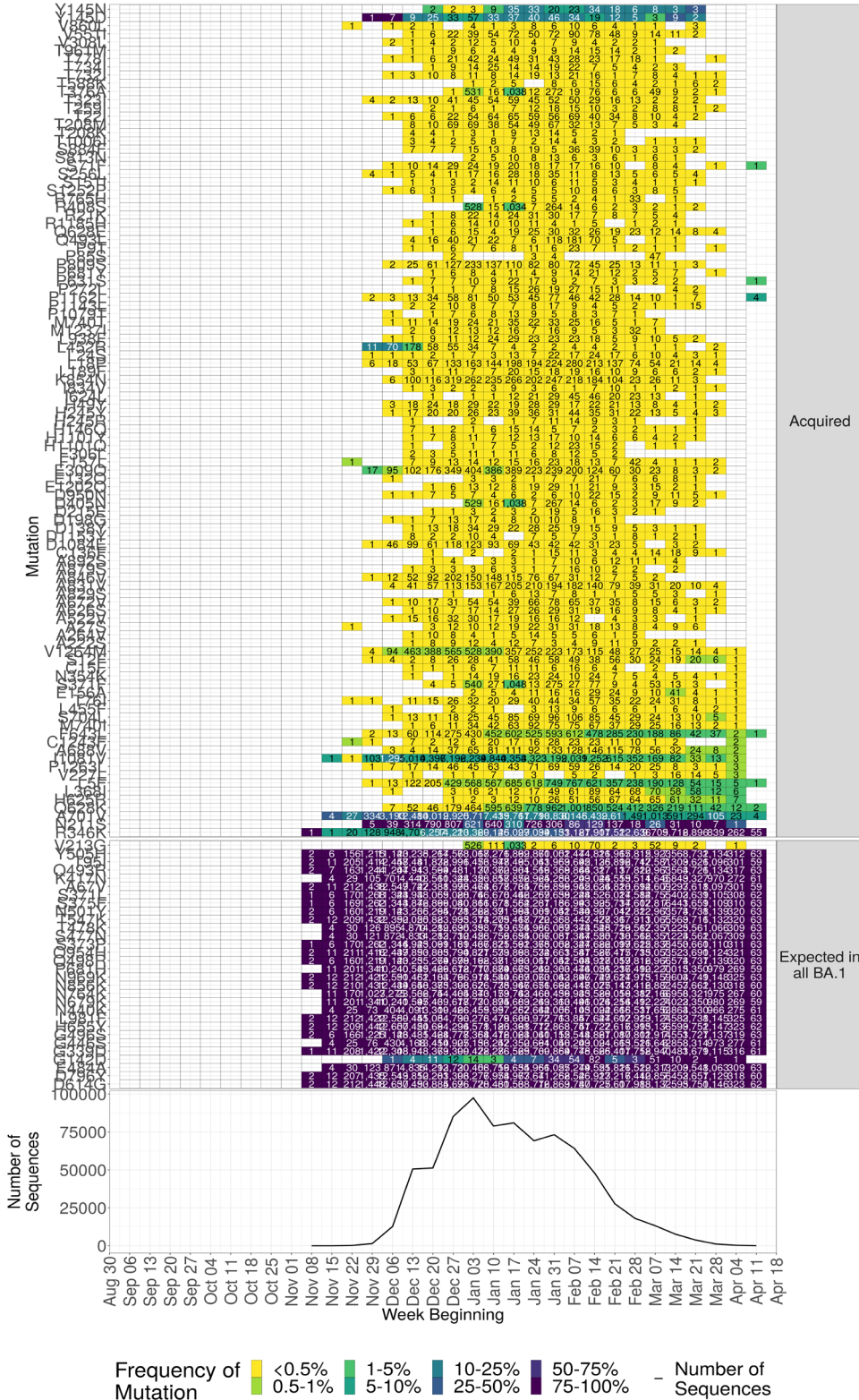


Figure 2. NSP12 mutations found in BA.1 genomes in the UK data set relative to the Wuhan sequence NC_045512.2

Supplementary data is not available.

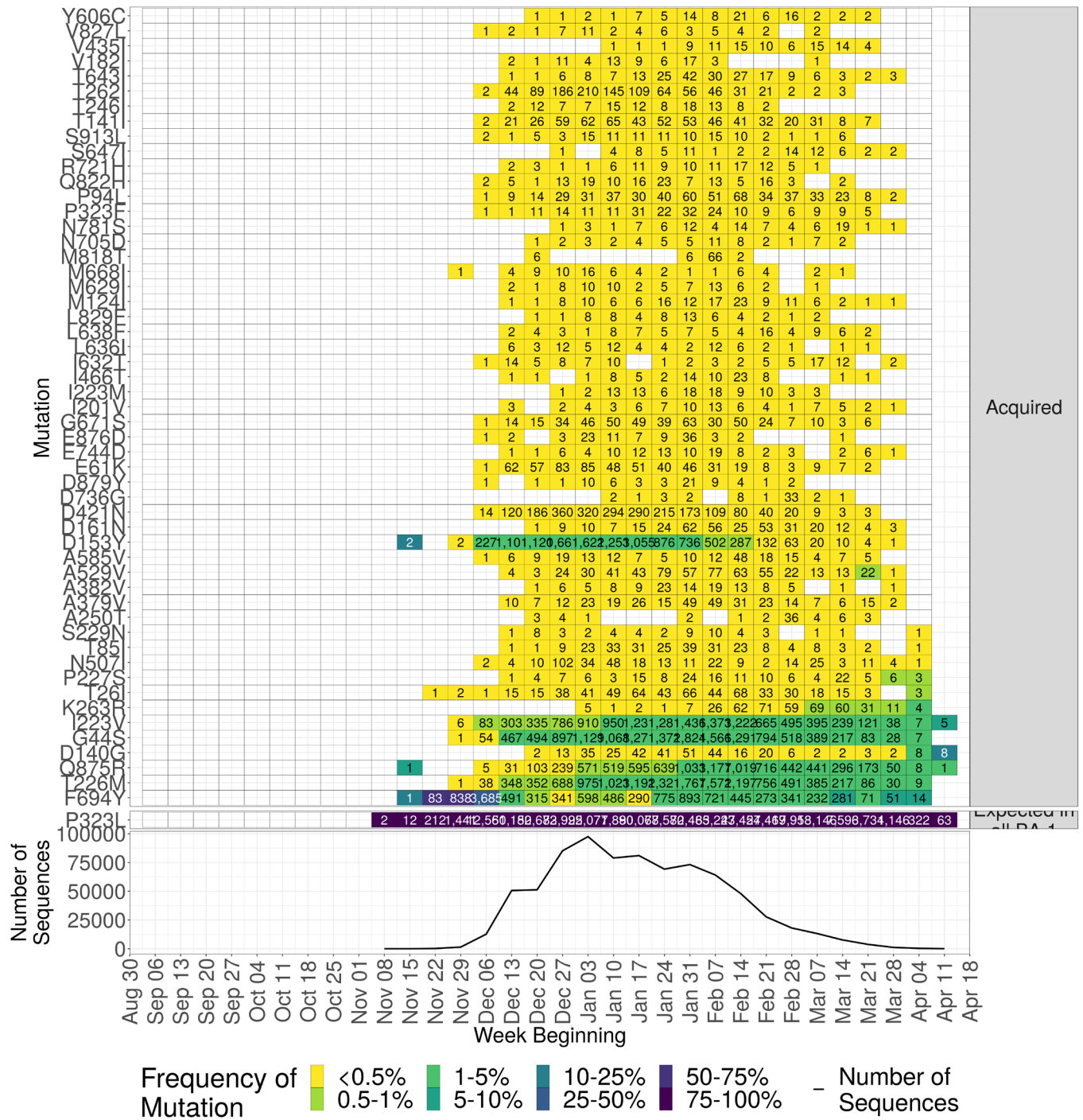
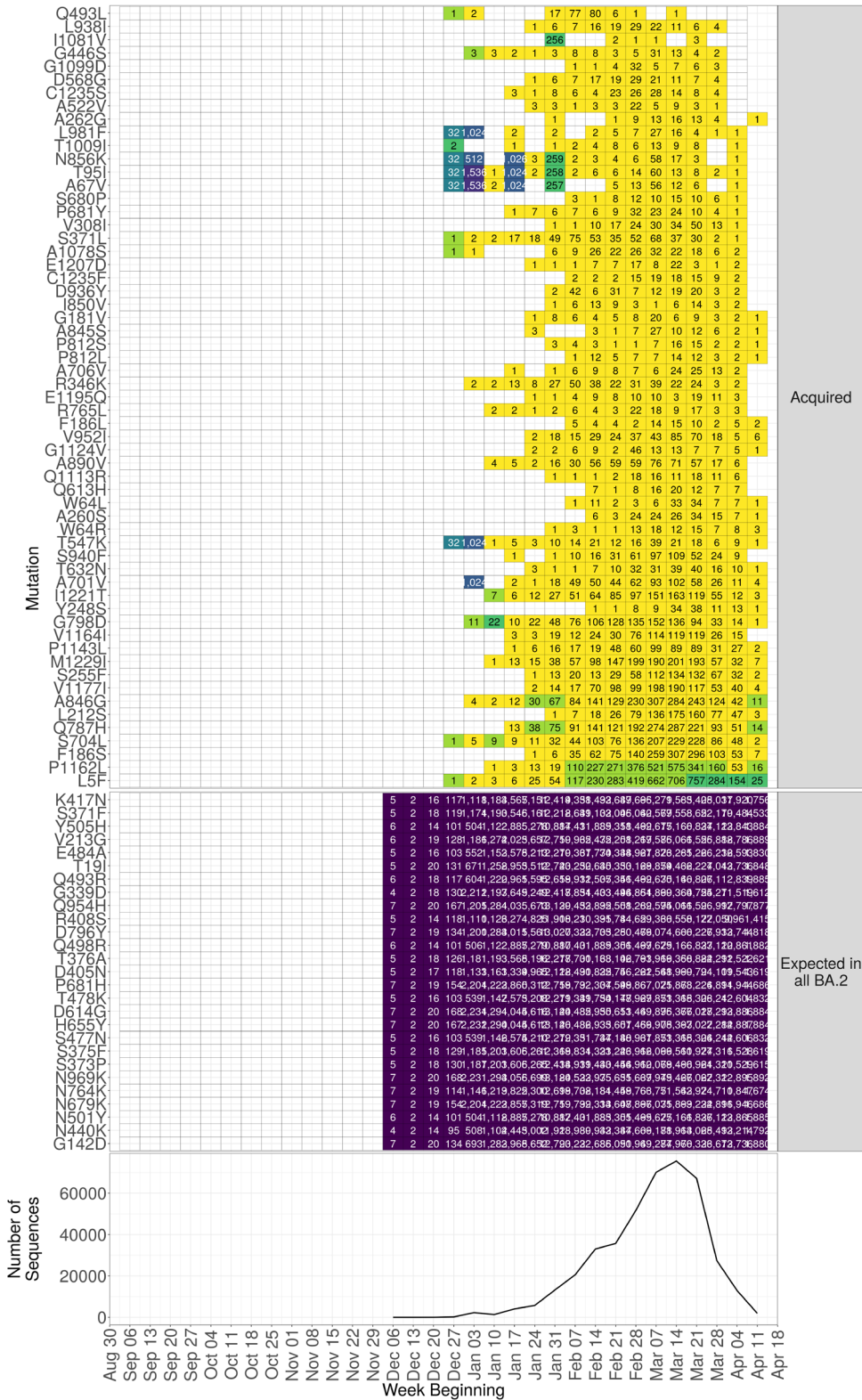


Figure 3. Spike mutations found in BA.2 genomes in the UK data set relative to the Wuhan sequence NC_045512.2

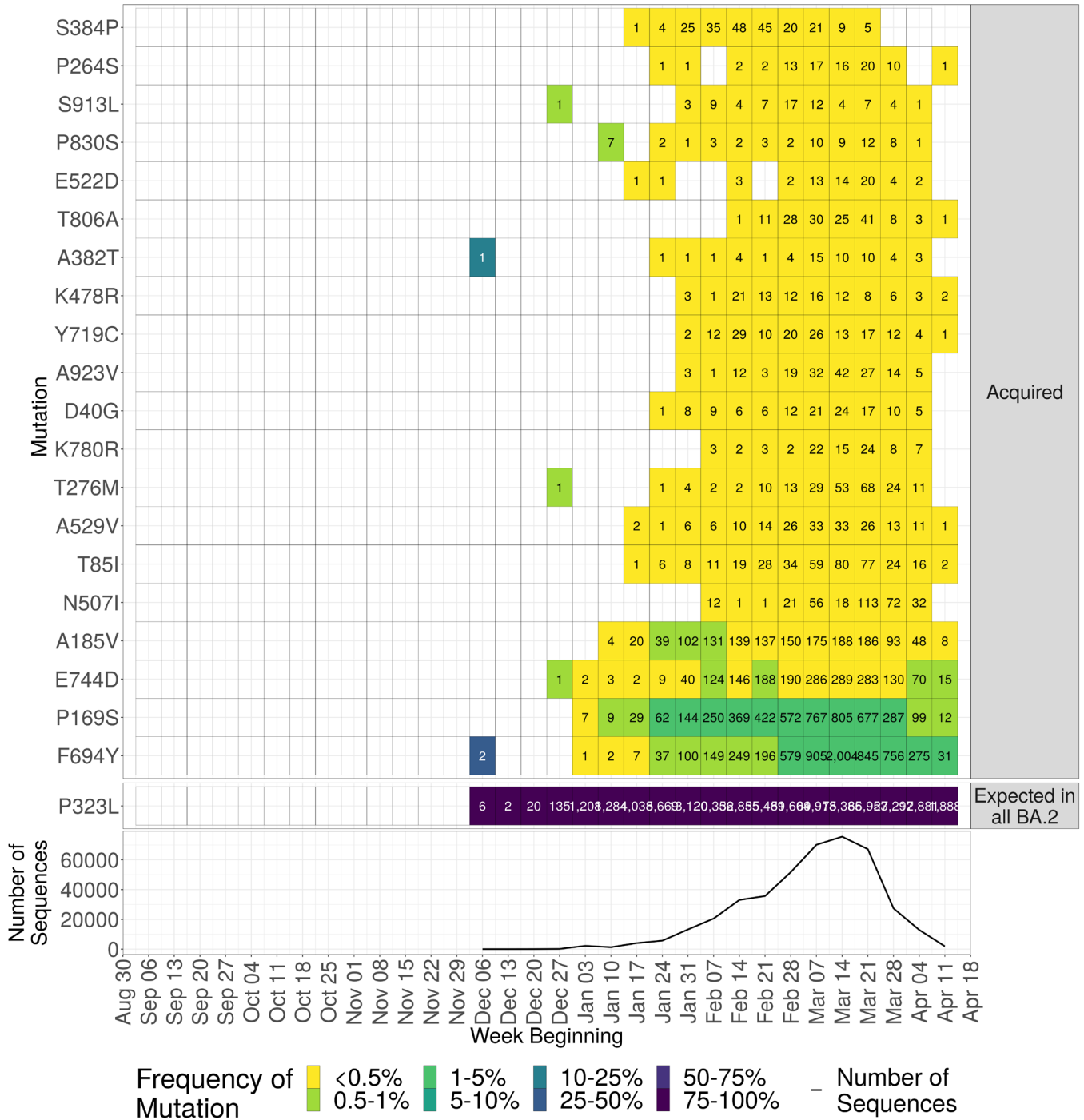
Supplementary data is not available.



Frequency of Mutation: <0.5% (lightest), 0.5-1%, 1-5%, 5-10%, 10-25%, 25-50%, 50-75%, 75-100% (darkest). - Number of Sequences

Figure 4. NSP12 mutations found in BA.2 genomes in the UK data set relative to the Wuhan sequence NC_045512.2

Supplementary data is not available.



3.3 Structural modelling assessment of V-22APR-03 (BA.4) and V-22APR-04 (BA.5) on therapeutics

V-22APR-03 (Omicron, BA.4) and V-22APR-04 (Omicron, BA.5) are novel Omicron sub-lineages identified in April 2022, and were both predominantly associated with South Africa, however both BA.4 and BA.5 have now been observed in multiple countries. As of 26 April 2022, 12 BA.4 and 5 BA.5 sequences have been detected in the UK.

BA.4 and BA.5 are most closely related to BA.2. Structural modelling indicates there is likely to be antigenic change related to L452R (found in Delta) and F486V (a more radical version of the F486L found in some mink adapted viruses), both of which may affect the binding of neutralising antibodies. In addition, the differences between BA.2 and BA.4/5 at position 493 and 408 may have some effect, as well as the 2-residue deletion in the N-terminal domain in BA.4/5 compared to BA.2.

Based on structural modelling data, the F486V mutation is likely to impact the casirivimab component of casirivimab and imdevimab and the tixagevimab component of tixagevimab/cilgavimab (Evusheld®), however this would require confirmation using laboratory studies. Based on existing structural models of sotrovimab bound to the RBD, the mutation profiles for BA.4/BA.5 are not expected to further impact this therapeutic, since both L452R and F486V are located distal to the sotrovimab binding interface.

The therapeutics molnupiravir and remdesivir targeting NSP12 are also unlikely to be impacted by the mutation profiles for BA.4/BA.5 with NSP12. The P323L mutation in BA.4/BA.5 is the same as observed in the ancestral Omicron lineage (B.1.1.529) and located in a position distal to the RNA tunnel of NSP12. No additional mutations are observed in BA.4/BA.5 at contact residue sites associated with molnupiravir/remdesivir. As no additional mutations are observed in BA.4/BA.5 at NSP5, nirmatrelvir plus ritonavir are also unlikely to be impacted.

3.4 Structural modelling assessment of V-22APR-01 (XD) and V-22APR-02 (XE) on therapeutics

V-22APR-01 (XD) is a double break point recombinant (21,643 and 25,581) that consists of a V-21APR-02 (Delta) backbone with a VOC-22JAN-01 (BA.1) spike gene. Therefore, spike protein modelling remains consistent with BA.1 whilst NSP5, NSP10, NSP12, and NSP14 are comparable to Delta. It should be noted that XD has yet to be observed in the UK sequence data. V-22APR-02 (XE) is a single break point Omicron recombinant that consists of a VOC-21NOV-01 (BA.1) 3' and a VOC-22JAN-01 (BA.2) 5' section, with a break point at nucleotide position 11,537). Therefore, NSP5, NSP10 mutations will be equivalent to BA.1 whilst Spike, NSP12, and NSP14 mutations will be equivalent to BA.2. Currently, there are 1,636 sequenced genomes associated with V-22APR-02.

4. Post-treatment viral sequences

If a particular mutation is selected for by 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 1 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 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 1. Number of pre-and post-treatment full genome sequences, broken down by treatment and by variant

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.

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	181	116	94	80
Casirivimab and imdevimab	BA.2	0	14	14	14
Casirivimab and imdevimab	Delta	2,173	298	138	82
Casirivimab and imdevimab	Other	156	72	49	37
Molnupiravir	BA.1	1,642	181	109	70
Molnupiravir	BA.2	285	17	11	8
Molnupiravir	Delta	29	8	7	3
Molnupiravir	Other	21	12	10	6

Treatment	Variant	Pre-treatment sequences	Post-treatment sequences	Post-treatment sequences (more than 5 days)	Post-treatment sequences (more than 10 days)
Paxlovid	BA.1	329	19	8	4
Paxlovid	BA.2	698	41	15	10
Paxlovid	Delta	0	2	2	2
Paxlovid	Other	10	7	5	2
Remdesivir	BA.1	1,268	529	371	307
Remdesivir	BA.2	249	106	83	75
Remdesivir	Delta	4,779	955	402	224
Remdesivir	Other	3,776	1,290	735	470
Sotrovimab	BA.1	3,959	531	318	211
Sotrovimab	BA.2	1,656	130	49	25
Sotrovimab	Delta	36	8	6	3
Sotrovimab	Other	65	37	30	20

Eleven amino acid residues displayed a significant frequency change in post-treatment sequences compared to pre-treatment sequences, suggesting possible evidence of selection: S:E406D/Q, S:G446V, Y453F and L455F/S in patients infected with Delta and treated with casirivimab and imdevimab; S:P337R/S and S:E340A/D/K/V, S:K356T and S:R493Q in patients infected with BA.1 and treated with sotrovimab; S:E340K in patients infected with BA.2 and treated with sotrovimab; and NSP12:E136A/D, NSP12:V166A/L and NSP12:V792I in patients infected with neither Delta nor Omicron and treated with remdesivir (Figures 5, 6, 7 and 8). Five patients infected with Delta treated with casirivimab and imdevimab had 2 of the mutations identified and 11 BA.1 patients treated with sotrovimab had 2 of the mutations identified.

For molnupiravir and nirmatrelvir plus ritonavir, no significant mutations were observed in the available data.

The prevalence of mutations associated in post treatment samples for casirivimab and imdevimab in Delta sequences are: 6 S:E406D; 7 S:E406Q; 1,946 S:G446V, 12 S:Y453F; 179 S:L455F; 1 S:L455S. Prevalence of mutations post sotrovimab with the BA.1 variant since 06/09/2022: 11 S:P337R; 32 S:P337S; 39 S:E340A; 82 S:E340D; 52 S:E340K; 5 S:E340V; 57 S:K356T; S:R493Q. Prevalence of mutations post sotrovimab with the BA.1 variant since 6 September 2022: 10 S:E340K. There were no samples observed for the remdesivir mutations listed in non-Delta and non-Omicron samples since 6 September 2021.

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

Figure 5. 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 inverted 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. Four sites displayed diverging frequencies at the 0.001 level between pre-and post-treatment sequence: E406D/Q, G446S/V, Y453F and L455F/S. Residues 406, 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 1 because not all genomic data was sufficiently high quality for downstream sequence analysis. Supplementary data is not available.

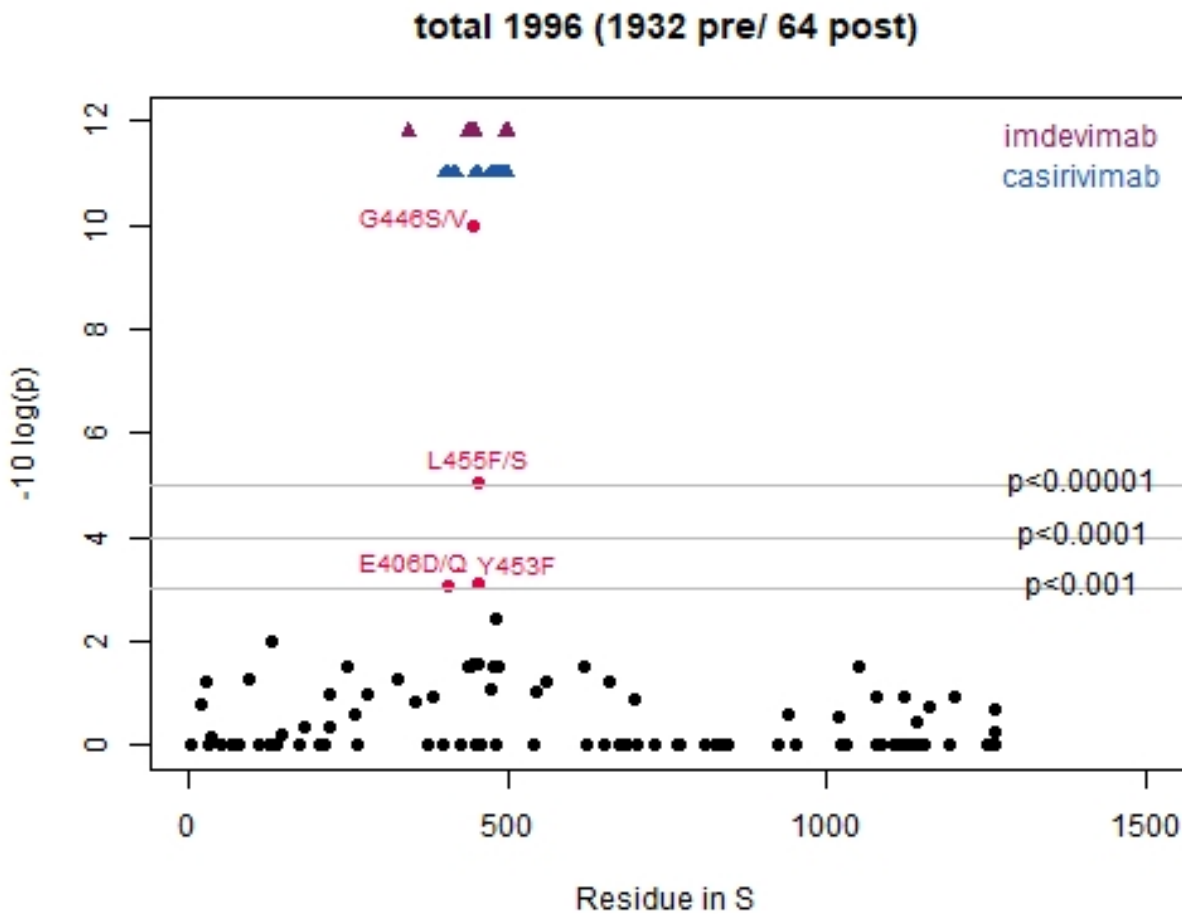


Figure 6. P-values for differences in spike amino acid frequencies between pre- and post-Sotrovimab treatment sequences of patients infected with BA.1

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. Four sites displayed diverging frequencies at the 0.001 level between pre-and post-treatment sequence: P337R/S and E340A/D/K/V, K356T and R493Q. Residues 337, 340 and 356 are known contact residues for sotrovimab. The numbers differ slightly from those in Table 1 because not all genomic data was sufficiently high quality for downstream sequence analysis. Supplementary data is not available.

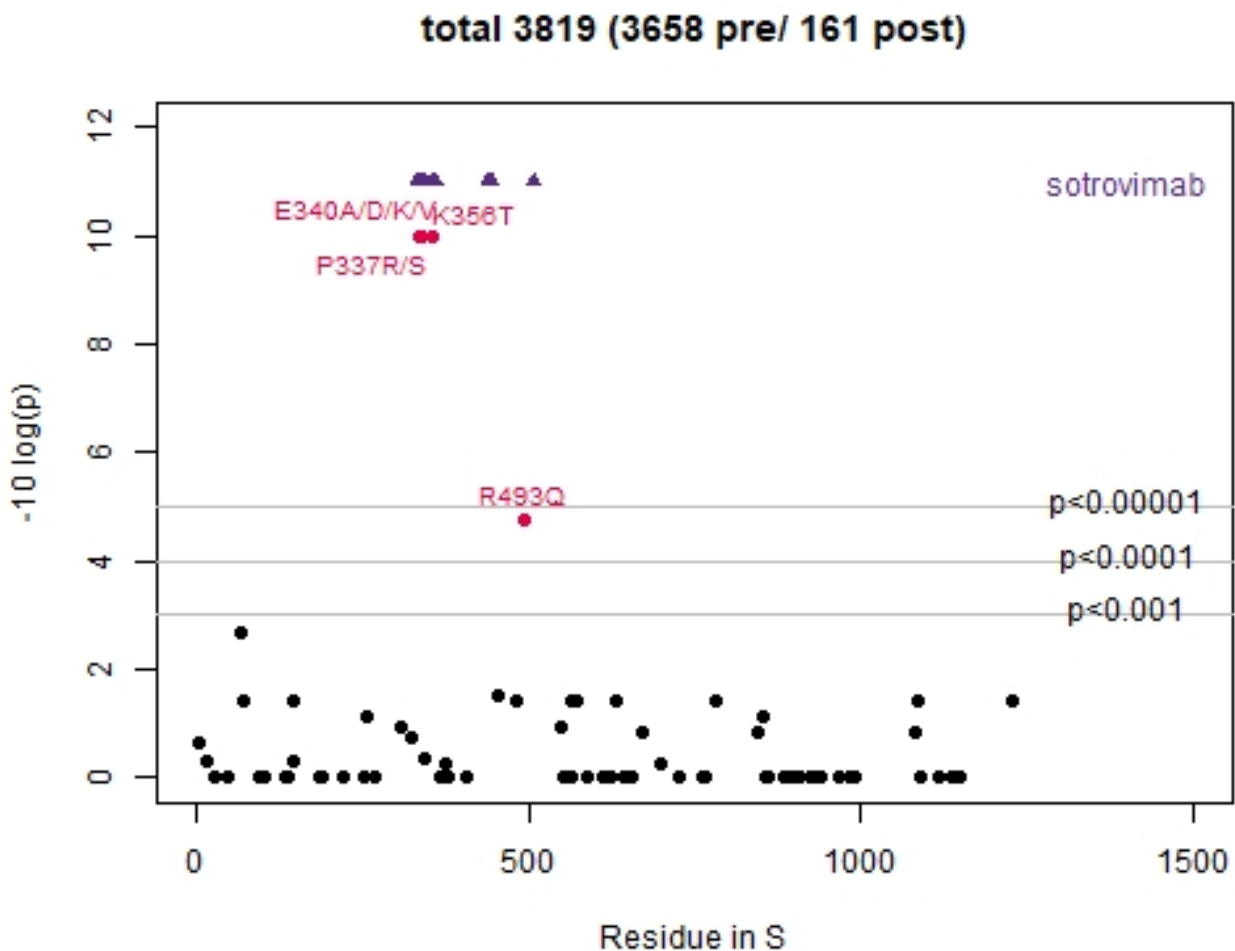


Figure 7. P-values for differences in spike amino acid frequencies between pre- and post-Sotrovimab treatment sequences of patients infected with BA.2

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. One site displayed diverging frequencies at the 0.001 level between pre-and post-treatment sequence: E340K, a known contact residues for sotrovimab. The numbers differ slightly from those in Table 1 because not all genomic data was sufficiently high quality for downstream sequence analysis. Supplementary data is not available.

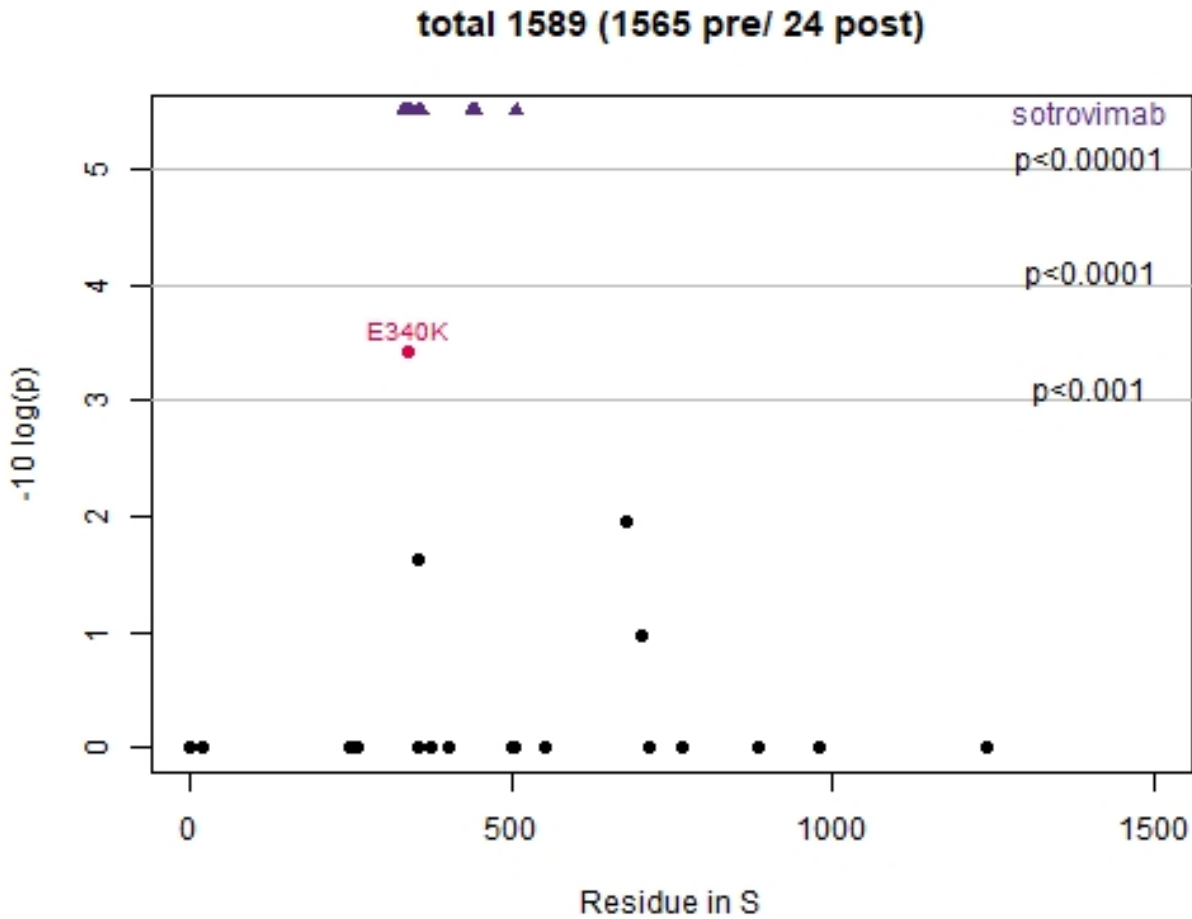
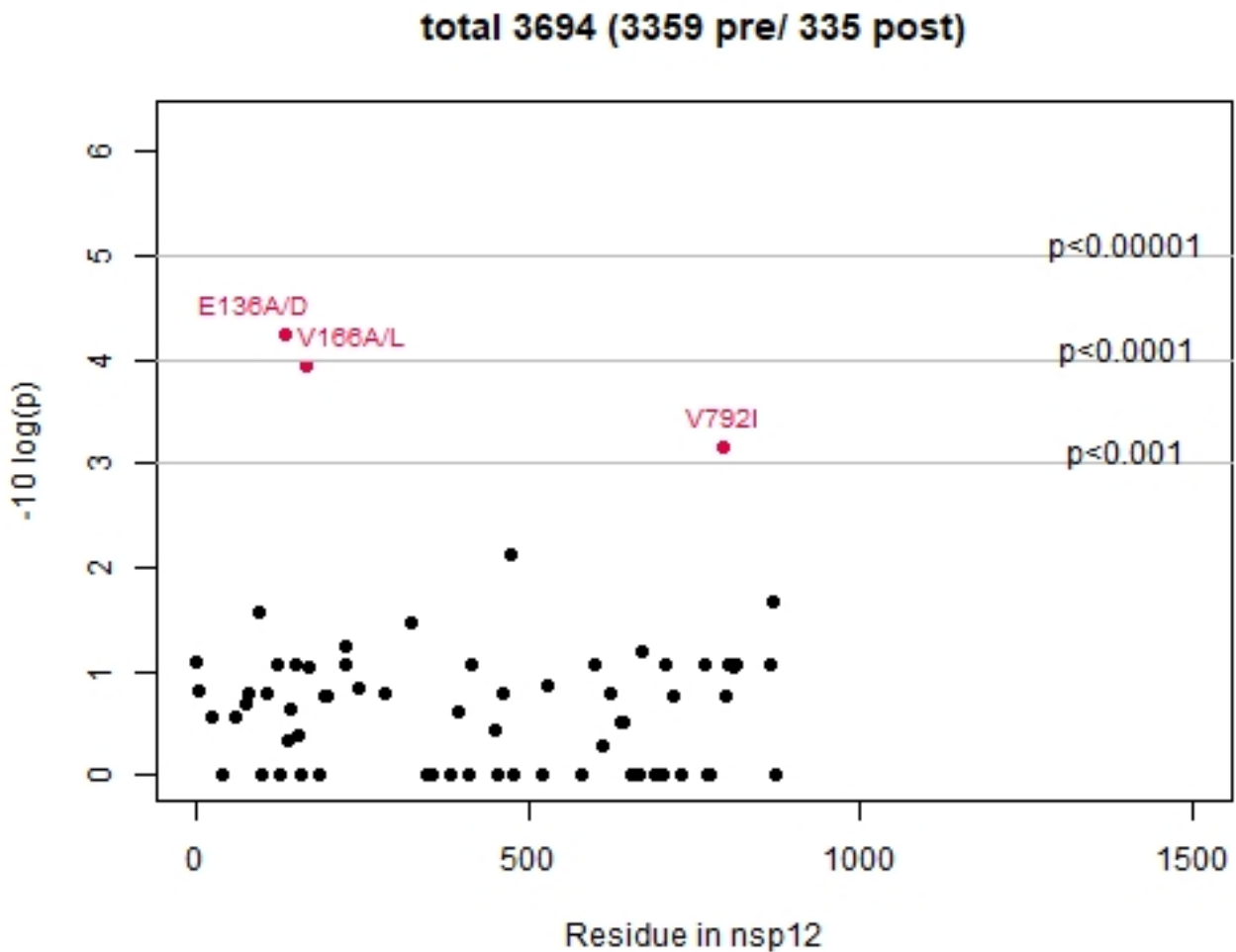


Figure 8. P-values for differences in NSP12 amino acid frequencies between pre- and post- Remdesivir treatment sequences of patients infected with non-Delta, non-Omicron
Amino acid frequencies were compared between pre-and post-treated patients (at least 10 days after treatment), for each site in the NSP12 sequence alignment. P-values for each site were calculated using a Fisher's test, and p-values were log-transformed and inverted 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 0.001 level between pre-and post-treated sequences: E136A/D, V166A/L and V792I. None are known interaction sites for Remdesivir. Given the large numbers of sites independently tested, lower p thresholds are necessary to demonstrate selection. The numbers differ slightly from those in Table 1 because not all genomic data was sufficiently high quality for downstream sequence analysis. Supplementary data is not available.



4.1 Analysis by time post-treatment

In the main analysis, sequences were included in the post-treatment group if there was a delay of at least 10 days between treatment and the post-treatment sequence. The analysis was repeated without a cut-off ('0 days') and with a 5-day cut-off ([Figure 9](#)). While the number of post-treatment sequences was smaller as the cut-off was increased, the sites identified remained consistent, and the support for associations between residues identified and post-treatment sequences increased. For example, in patients infected with BA.1 and treated with Sotrovimab, the K356T and R493Q mutations became more significant with increased time between treatment and the post-treatment sequence ([Figure 9](#)).

Figure 9A. Time restricted analysis of post-treatment Spike sequences for patients infected with Delta and treated with Casirivimab and imdevimab

For each variant, gene and treatment combination where we found significant sites in our preliminary analysis (with a 10-day cut-off between treatment and post-treatment sequence), we repeated the analysis with 2 additional cut-offs (0 and 5 days). Amino acid frequencies were compared between pre-and post-treated patients, for each site in the 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 ($p < 0.01$) are highlighted in red, and where $p < 0.001$, the observed amino acid change is indicated in text. As a general pattern, support for findings increased, with p values associated with significant sites decreasing, despite smaller data sets. Supplementary data is not available.

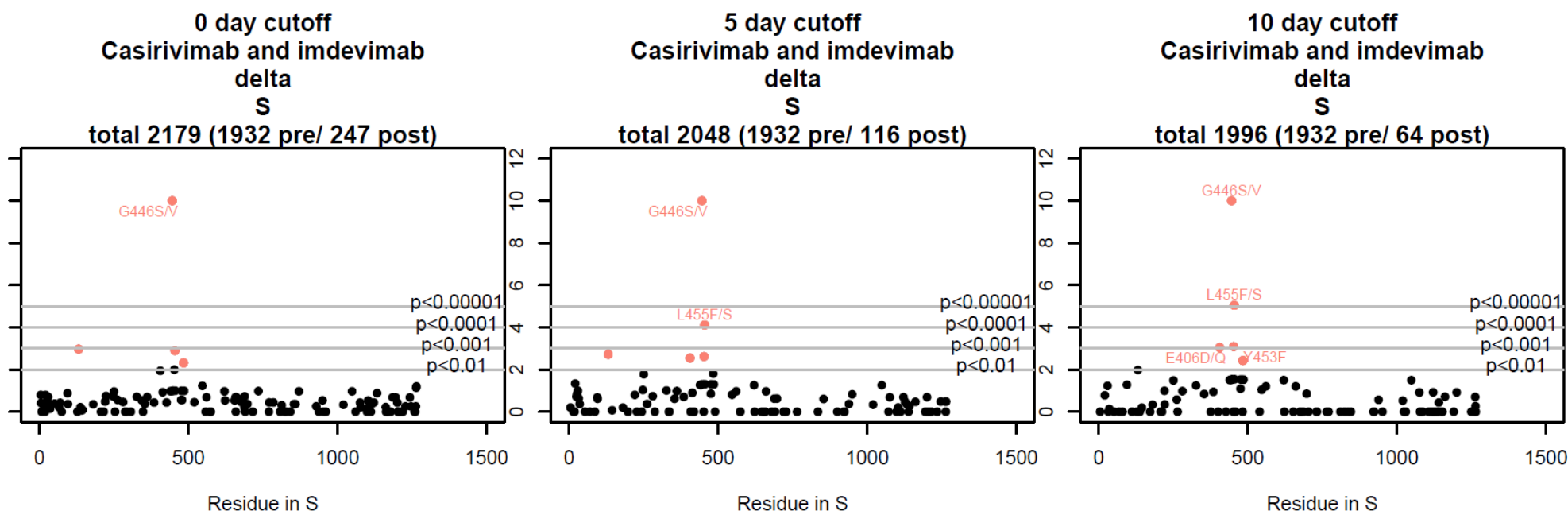


Figure 9B. Time restricted analysis of post-treatment Spike sequences for patients infected with BA.1 and treated with Sotrovimab

For each variant, gene and treatment combination where we found significant sites in our preliminary analysis (with a 10-day cut-off between treatment and post-treatment sequence), we repeated the analysis with 2 additional cut-offs (0 and 5 days). Amino acid frequencies were compared between pre- and post-treated patients, for each site in the 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 ($p < 0.01$) are highlighted in red, and where $p < 0.001$, the observed amino acid change is indicated in text. As a general pattern, support for findings increased, with p values associated with significant sites decreasing, despite smaller data sets. Supplementary data is not available.

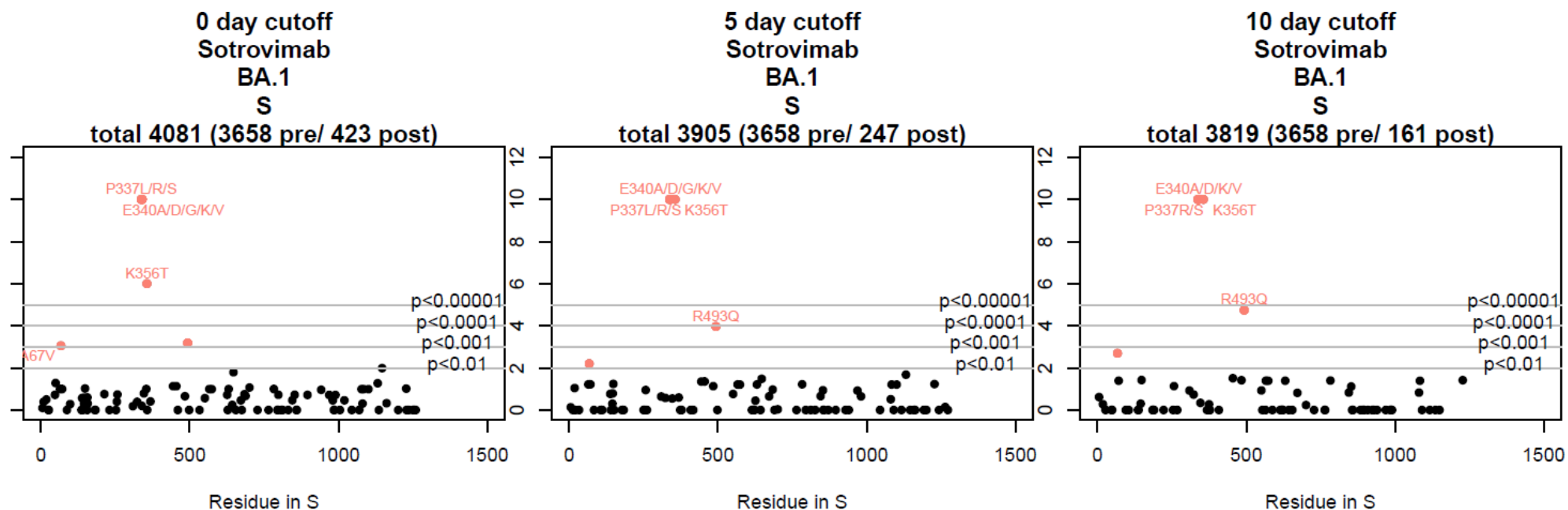


Figure 9C. Time restricted analysis of post-treatment Spike sequences for patients infected with BA.2 and treated with Sotrovimab

For each variant, gene and treatment combination where we found significant sites in our preliminary analysis (with a 10-day cut-off between treatment and post-treatment sequence), we repeated the analysis with 2 additional cut-offs (0 and 5 days). Amino acid frequencies were compared between pre-and post-treated patients, for each site in the 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 ($p < 0.01$) are highlighted in red, and where $p < 0.001$, the observed amino acid change is indicated in text. As a general pattern, support for findings increased, with p values associated with significant sites decreasing, despite smaller data sets. Supplementary data is not available.

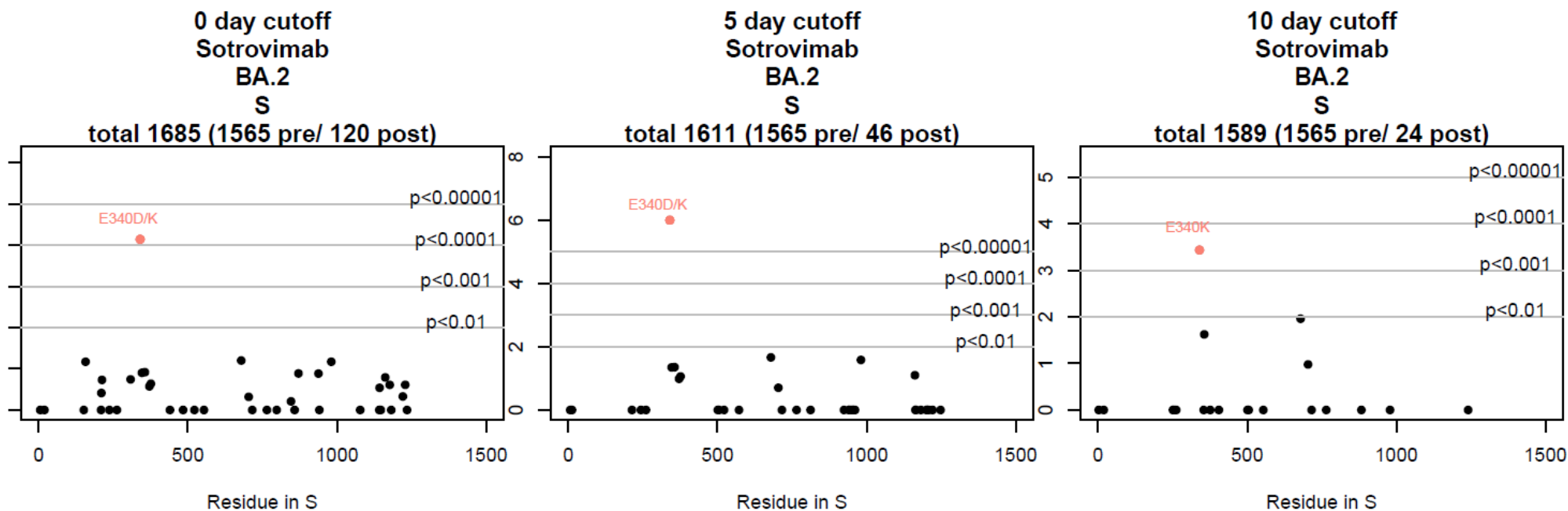
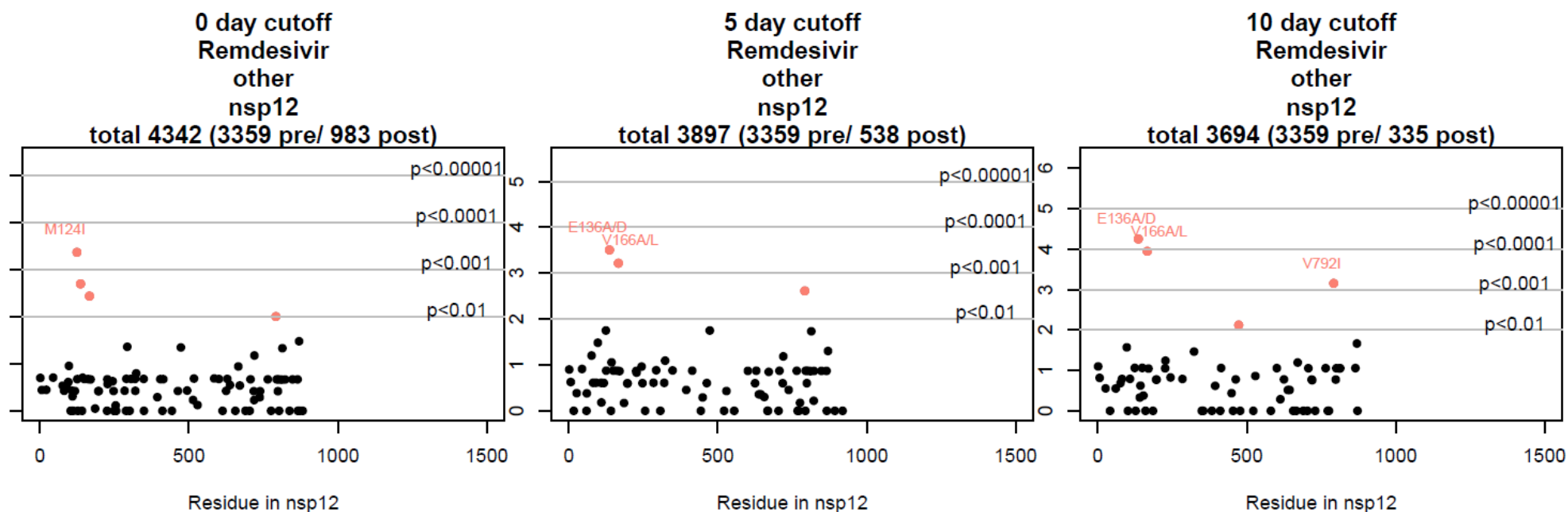


Figure 9D. Time restricted analysis of post-treatment Spike sequences for patients infected with neither Delta nor Omicron and treated with Remdesivir

For each variant, gene and treatment combination where we found significant sites in our preliminary analysis (with a 10-day cut-off between treatment and post-treatment sequence), we repeated the analysis with 2 additional cut-offs (0 and 5 days). Amino acid frequencies were compared between pre-and post-treated patients, for each site in the 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 ($p < 0.01$) are highlighted in red, and where $p < 0.001$, the observed amino acid change is indicated in text. As a general pattern, support for findings increased, with p values associated with significant sites decreasing, despite smaller data sets. Supplementary data is not available.



The pre-post treatment data set was interrogated by setting the threshold at more than 3 sequences across treatment per unique identifier where at least one sequence was pre-treatment, yielding n=1,132/37,486 total sequences. There were 262 individual patients with 1,132 sequences. Of these, 729 were post-treatment sequences. 568 of the post treatment sequences were less than 21 days post-treatment while 4 sequences were more than 100 days post-treatment. Patients with multiple treatments listed have been treated as separate individuals in this analysis. Follow up analysis with mutation analysis, timeline of mutation profiles pre- and post-treatment with a summary of where amplicons fit against regions of interest as well as acquired mutations across groups are in progress.

4.2 Transmission of post-treatment mutations in UK genomic data set

For all BA.1 and Delta–infected patients with at least 2 of the identified post treatment mutations (n=11 and n=5, respectively), and for BA.2-infected patients with the single identified BA.2 mutation (E340D/K; n=5), we searched for potential transmission partners within the UK genomic data set using [CIVET](#) to determine whether mutations might have been transmitted. For each sequence with a mutation, sequences that were genetically close (<2 SNPs) were identified. Those sequences were verified to come from a different patient than the source sequence. The sequences were examined for presence or absence of the mutations in the source sequence. There was no evidence of transmission of mutations. One limitation of this methodology is that it can only identify a potential transmission of a mutation if both the source and recipient have been sequenced. As the sequencing coverage in the UK is reduced, observing such a pair becomes increasingly unlikely, but surveillance scanning of mutations in all UK genomic sequences is ongoing to detect wider community transmission.

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 596 ng/ml. In previous studies, the IC50 for Victoria was measured as 6.6 ng/ml, but this used the originally published staining protocol, so estimates of fold reduction in IC50 should be treated with caution. This data is in line with [other published studies](#), notably Duty and others (2022) where an IC50 for Sotrovimab against Omicron (BA.1) was 393 ng/ml. Preliminary data with BA.2, suggest that neutralisation is further reduced compared to BA.1 by approximately 5-fold, but additional repeats may be required to calculate the IC50. Data will be reported in future SARS-CoV-2 therapeutics technical briefings.

5.2 Epidemiological assessment of sotrovimab with BA.1 and BA.2

An observational cohort analysis using routinely collected data of people treated with sotrovimab in the community who had BA.1 or BA.2 infection has been undertaken to assess whether sotrovimab remains effective in preventing severe outcomes against BA.2, compared to BA.1. Data from these episodes of infection were linked to routine health records on hospitalisation and presentation to emergency care that resulted in admission, transfer or death. A total of 4,167 BA.1 episodes and 3,197 BA.2 episodes were linked to presentation.

In a Cox proportional hazards regression with stratification by specimen week and adjusting for age group, linear effect in age and vaccination status, there was no evidence for an increase in admission as an inpatient (HR 0.8, 95% CI:0.62-1.04) and no evidence for an increase in attendance to hospital (either emergency care presentation or admission as an inpatient, HR 1.02, 95% CI: 0.94-1.12). This analysis of community treatments was limited by a lack of information on co-morbidities of cases, and insufficient information at the time of analysis on

alternative endpoints of severity such as patients requiring ventilatory support. This analysis was also limited by overall numbers of case episodes available for inclusion, with the latter end of the study period potentially affected by changes in community testing.

It remains possible both BA.1 and BA.2 may be refractory to sotrovimab, compared to previously circulating variants such as Delta variant. However, due the lack of cases of BA.2 and Delta occurring at the same dates, it is not possible to compare severe outcomes following sotrovimab use between BA.2 and Delta cases.

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