Delta-Omicron recombinant SARS-CoV-2 in a transplant patient treated with Sotrovimab

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Summary

We identified a Delta-Omicron SARS-CoV-2 recombinant in an unvaccinated, immunosuppressed kidney transplant recipient who had positive COVID-19 tests in December 2021 and February 2022 and was initially treated with Sotrovimab. Viral sequencing in February 2022 revealed a 5' Delta AY.45 portion and a 3' Omicron BA.1 portion with a recombination breakpoint in the spike N-terminal domain, adjacent to the Sotrovimab quaternary binding site. The recombinant virus induced cytopathic effects with characteristics of both Delta (large cells) and Omicron (cell rounding/detachment). Monitoring of immunosuppressed COVID-19 patients treated with antiviral monoclonal antibodies is crucial to detect potential selection of recombinant variants.

Introduction

Recombination occurs by template switching during viral RNA synthesis in individuals who are simultaneously infected with more than one viral strain[1]. Recombination can accelerate viral evolution and result in selective advantages like enhanced transmission or immune escape. Recombination events have been described in seasonal coronaviruses and MERS-CoV, and are a plausible cause for the emergence of SARS-CoV-2[2]. In contrast, there was little evidence of recombination in the SARS-CoV-1 epidemic or within the first year of the SARS-CoV-2 pandemic[3]. Recent high case numbers and the co-circulation of Delta and Omicron increased the likelihood of coinfections[4,5] and thus the possibility of inter-variant recombination[4,6,7]. Delta was the dominant variant in the second half of 2021 and remained in circulation through early 2022. The independent emergence of Omicron, potentially in an immunosuppressed individual, caused a global wave beginning in November 2021, that overlapped with Delta for many weeks[8]. Omicron arrived in NYC when the only circulating variant was Delta and its many sublineages[9]. Recently, other Delta-Omicron recombinants have been identified in Europe and the US[4,6,7]. Despite their sequence differences, the recombination events frequently occur in the N-terminal domain (NTD) of the spike gene, combining non-structural ORF1a/b genes from Delta with Omicron C-terminal spike regions, including the receptor-binding domain (RBD). So far, there is no data on the triggers of recombination or its consequences on selective adaptation, virulence, transmission, resistance to neutralizing antibodies (nAb), and whether viral, host, or therapeutic factors are associated with the emergence or outgrowth of recombinants. Here, we describe a novel Delta-Omicron recombinant, detected in an immunosuppressed, unvaccinated patient, treated with Sotrovimab, a lead nAb against the BA.1 Omicron lineage.

Methods

SARS-CoV-2 sequencing and bioinformatic analysis

SARS-CoV-2 full-genome sequencing was performed in two independent laboratories using four different approaches to confirm the identity of the recombinant and to exclude artifacts (e.g. by mixed reads) as reason for the detection of recombinant sequences. The methods included xGen Amplicon[10] and metagenomics sequencing (NYU) as well as AmpliSeq Insight and ARTIC Amplicon sequencing (NY State DOH).

Virus isolation and culture

VeroE6/TMPRSS2 cells[11] were used for virus isolation, obtained from the Japanese Collection of Research Bioresources (JCRB Cell Bank) cell number JCRB1819, through Sekisui Xenotech, LLC (Kansas City, KS), agreement # A2000230. VeroE6/TMPRSS2 cells were seeded three days prior to infection, to reach 85-90% confluency. Infected monolayers were checked daily for cytopathic effect (CPE), and 110 µL of supernatant was removed at 24, 48, 72, and 96 hours post-infection (hpi) for further analysis. At 96 hpi, cells and supernatant were harvested together.

Mutation and phylogenetic analysis

Highlighter analyses were performed on MAFFT-aligned SARS-CoV-2 full-genome sequences using the Highlighter tool provided by the Los Alamos HIV sequence database[12]. Phylogenetic analyses were done using the Nextstrain CLI package using a New York, New Jersey, and Connecticut-focused subsampling of global SARS-CoV-2 sequences[8].

Structural analysis

Molecular graphics and analyses were performed with UCSF ChimeraX 1.3[13]. Homology models of the recombinant spike were generated with the protein structure homology-modelling server SWISS-MODEL[14].

Study approval

This study was approved by the NYULH Institutional Review Board, protocol numbers i21-00493 and i21-00561.

Detailed methods can be found in the Supplementary Appendix.

Results

Clinical background. The patient (male in the late twenties) had end-stage renal disease due to hypertensive nephrosclerosis and received a deceased donor kidney transplant in June 2021. He was unvaccinated against COVID-19. His immunosuppressive regimen consisted of thymoglobulin induction at transplant and Tacrolimus, prednisone, and mycophenolate mofetil as maintenance (**Table 1**). His concurrent medications included labetalol, nifedipine, pantoprazole and entecavir.

Diagnosis and treatment.

1st COVID-19 episode: In late December, he developed cough and fever and tested positive for COVID-19 by rapid antigen test on the same day. No PCR test was done.

He received an infusion of Sotrovimab 500 mg two days after onset of symptoms which resolved the next day. He did not require hospitalization at this time.

2nd COVID-19 episode:

In the middle of February 2022, he presented with a fever up to 102^0F, chills, body aches, and severe fatigue for one day, requiring hospitalization. He had no cough, shortness of breath, or chest pain. A chest X-ray showed left-sided basilar opacities. A CT scan of the chest showed patchy airspace opacities with consolidation in the left lower lobe consistent with pneumonia. A respiratory viral pathogen panel multiplex PCR on a nasopharyngeal swab specimen was positive for SARS-CoV-2 (Ct 26.4). He did not receive antiviral medications during or after this hospitalization. He received antibiotics for a presumed bacterial superinfection for seven days. His immunosuppressive regimen was modified by halving the dose of mycophenolate and continuing his usual dose of tacrolimus and prednisone. The patient became afebrile two days into hospitalization, and was discharged two days later. No hypoxia was observed during the clinical course (**Table 1**).

Identification of a Delta-Omicron recombinant virus

Our genomic surveillance in the greater New York City area revealed an unusual SARS-CoV-2 sequence in late February 2022, based on its outlier Omicron BA.1 placement in a global phylogenetic tree and its high number of mutations (**Figure 1**). PANGO did not assign a lineage[15], and further inspection of each individual mutation and comparison with the mutations from different lineages revealed that the entire ORF1ab genomic region and the beginning of spike up to position 22035 contained Delta-specific mutations, most similar to Delta sublineage AY.45, and no Omicron-specific mutations. In contrast, the remainder of the genome, specifically after position 22193 contained Omicron BA.1-specific mutations and no Delta-specific mutations, indicative of a Delta-Omicron recombination event with a single breakpoint in the 5' region of spike (**Figure 1A**). Phylogenetic analyses of the separate subregions substantiated the relatedness of the 5' and 3' segments with Delta and Omicron clades, respectively (**Figure 1B**). We performed a re-extraction and re-sequencing from the original swab, processed in the same manner, which revealed the exact same 5' Delta and 3' Omicron-specific mutations with a breakpoint between bp 22035-22193 in the near full-length high-quality sequence (**Table S1**). No mixed bases were observed, suggesting that this was not a co-infection or technical artifact.

For further confirmation, we prepared and sequenced libraries from the same sample using a ribodepletion shotgun metagenomics approach. GATK variant detection revealed a 100% concordance between mutations called with both the amplicon and independent shotgun metagenomics approach. The metagenomics approach yielded a single additional Omicron-specific change at the beginning of the Omicron-like portion of the genome, i.e., a 9 bp insertion at position 22204 (spike 214EPE). The specimen was also separately extracted and sequenced by AmpliSeq Insight at a second site, further confirming the sequencing results.
Additionally, we grew the virus in VeroE6/TMPRSS2 cells and sequenced the progeny using another amplicon-based method, ARTIC V4, producing a fourth confirmation of the recombinant identity (NY State DOH). Delta and Omicron-derived mutations were supported by 99-100% of the reads, consistent with the presence of a single recombinant. ARTIC sequencing confirmed the spike 214EPE insertion, but did not cover three spike mutations (K417N, N440K, G446S), detected by the previous approaches.
In summary, xGen amplicon sequencing, metagenomics, and *in vitro* virus growth with Ion AmpliSeq Insight and ARTIC sequencing confirmed the identity of a Delta-Omicron recombinant. The sequence was deposited in GISAID (EPI_ISL_10792641), flagged as a recombinant. **Table S1** summarizes all mutations detected by nine multi-method sequencing runs on different specimens.

Cytopathic effects and viral load monitoring
Cytopathic effects (CPE) were first observed at 48 hpi of VeroE6/TMPRSS2 cells, with minor cell rounding in small areas of the monolayer, progressing to widespread cell rounding, detachment, debris, and cell death by 96 hpi (**Figure S1**), consistent with Omicron. However, affected cells appeared larger and more prominent in the CPE produced by the recombinant virus, and cell detachment was more advanced. As with Omicron, syncytia formation was not apparent in the recombinant virus cultures. Overall, the recombinant virus induced CPE more similar to those seen with Omicron than Delta (**Figure 1C**). Virus growth was monitored by RT-qPCR and the results are summarized in **Table S2**. By 96 hpi (Ct 13.5), viral production had increased more than 5 $\log_{10}$ from the 24h timepoint in the culture supernatant.

Structural analysis of the recombinant spike protein and the Sotrovimab binding epitope.
Since recombination occurred in the last portion of the spike NTD, within a region that is identical in Delta AY.45 and BA.1 (bp 22035-22193) (**Figure 1A**), most of NTD in the recombinant is composed of Delta, while RBD and the C-terminal regions of spike are Omicron (**Figure 2**). Notably, during the first symptomatic COVID-19 episode in December 2021, the patient was treated with Sotrovimab, a class 4 anti-spike RBD nAb with broad antiviral activity against SARS-CoV-2, including BA.1[16-18]. The spike binding epitope for Sotrovimab involves the N-terminus of RBD, and in a 3D structural model, the Fab moiety engages the space between RBD and the neighboring NTD (**Figure 2**). The recombinant virus sequence harbors one atypical spike mutation (E340D) that is not related to Delta or Omicron and is rarely found in global SARS-CoV-2 sequences with a frequency of less than 3 E-5[8,19]. Interestingly, E340 is in the middle of the Sotrovimab binding epitope and is the primary site of resistance against Sotrovimab[16,20]. E340D is a conservative replacement without a change in charge, in contrast to the more abundant

resistance mutations E340A/K[16,20], and its impact on Sotrovimab resistance remains to be determined, particularly in the context of the recombinant sequence.

Discussion

Monitoring for recombinant SARS-CoV-2 is critical as recombination can cause evolutionary leaps potentially altering transmission dynamics, immune evasion, or clinical impact. Few cases of SARS-CoV-2 recombinants have been reported so far[4,6,7,21], and here, we describe the first detected in an unvaccinated, immunosuppressed transplant patient after treatment with a nAb. Although we do not have a causative link, immunosuppression presumably contributed to its emergence or outgrowth. Immunocompromised patients are a primary source for the development of new variants due to delayed viral clearance and prolonged viral replication/evolution, particularly under selective pressure by nAb treatment[22-25].
Among the reported recombinant SARS-CoV-2 sequences, ours and others show a single breakpoint splitting the genome into a 5' Delta and a 3' Omicron segment[4,6]. A three-case cluster in France was caused by a recombinant with a smaller Omicron insertion, principally spike, flanked by 5' and 3' Delta regions[7]. All cases share an ORF1a/b Delta region and an Omicron region encompassing spike's RBD and C-terminal regions. These data suggest that either a recombination breakpoint in spike NTD is favored, or Delta's non-structural proteins, or Omicron's C-terminal spike regions, including RBD, have selective advantages.

Notably, we detected the recombinant in a patient after treatment with Sotrovimab, a nAb binding SARS-CoV-2 spike near the recombination site. At this time, it is not known whether Sotrovimab treatment triggered selection of the recombinant, or whether the recombinant is resistant to Sotrovimab. Since Delta had disappeared from circulation by February 2022 in the NYC metro area, and presuming that Sotrovimab exerted immune pressure leading to E340D selection within the Omicron part of spike, we speculate that the patient became co-infected with Delta and Omicron in December 2021 when Delta and Omicron were at ~10% and 90% prevalence in New York, respectively. An *in vitro* study suggests that E340D decreases Sotrovimab neutralization <10-fold compared to the more impactful E340A/K mutations[20]. Since spike aa340 is in the center of the Sotrovimab binding epitope and E340D is extremely rare, making random occurrences or community spread less likely, E340D might be an indicator of exerted Sotrovimab immune pressure. Since the recombinant was the only variant detected in the February 2022 specimen, without detectable parental Delta or Omicron lineages, either the recombinant escaped Sotrovimab treatment in a Delta/Omicron co-infection, the recombinant had replication advantages, or the patient was initially infected with the recombinant from an unidentified source. Whereas recombinants are still rare and have not yet caused significant community transmissions, these early cases highlight the importance of genomic surveillance and further functional testing. Monitoring of SARS-CoV-2 infections in immunocompromised individuals treated with nAbs remains a priority for pandemic preparedness.

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Tables

Table 1. Demographic and clinical features of a patient infected with a Delta-Omicron recombinant SARS-CoV-2 virus.

Demographics	
Age range	25-29
Sex	Male
Clinical features	
COVID-19 vaccination status	Unvaccinated
Immune status	Immunosuppressed
Comorbidities	Hypertension,
	End-Stage Renal Disease (ESRD) due to hypertensive nephrosclerosis
	Deceased donor kidney transplant recipient in 2021
Immunosuppression at baseline	Induction (at transplant): Thymoglobulin
Maintenance immunosuppression	Tacrolimus 2mg q AM / 3 mg q PM, prednisone 5 mg daily, mycophenolate mofetil 1000 mg q12h
COVID-19 clinical course	
1) December 2021	COVID-19 positive by rapid antigen test (no PCR done)
	Cough and fever
	Not hospitalized

	Received Sotrovimab 500 mg infusion once
	Symptoms resolved the next day
2) February 2022	Fever (up to 102 ^{o}F), chills, body aches, and fatigue for 4 days
	Hospitalized
	No cough, shortness of breath, hypoxia, or chest pain. Not on supplemental oxygen
	Chest X ray: right basilar opacities
	Chest CT: left lower lobe pneumonia
Tests for potential pathogens	Respiratory viral pathogen panel multiplex PCR positive for SARS-CoV-2 (Ct 26.4)
	Negative for blood cultures, *Legionella* and *Streptococcus pneumoniae* urinary antigens, serum cryptococcal antigen, beta-D- glucan, and Aspergillus galactomannan.
	Sputum culture was not performed due to lack of a good quality specimen.
Treatment	No antivirals given
	Antibiotics for 7 days (2 days Piperacillin-tazobactam + vancomycin, then 2 days piperacillin-tazobactam + azithromycin, then 3 days Levofloxacin)
	Immunosuppression reduced by halving mycophenolate dose, and maintaining the doses of tacrolimus and prednisone
Outcome	Afebrile after 2 days of antibiotic treatment
	Discharged after 4 days

Figures

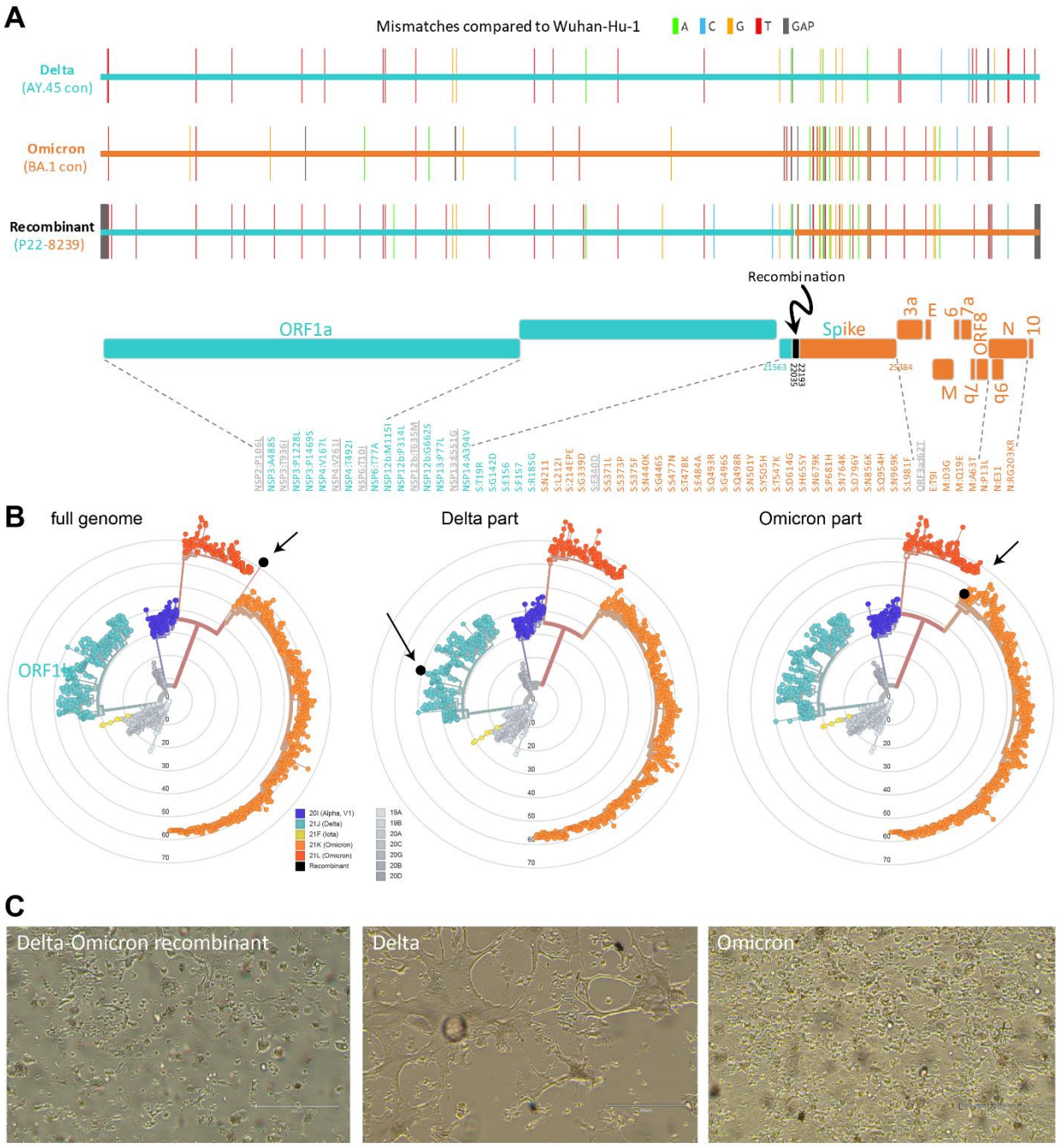

Figure 1. Genomic mutations, phylogenetic characterization, and cytopathic effects of the Delta-Omicron recombinant SARS-CoV-2 virus.

A. Full genome mutations of the SARS-CoV-2 Delta-Omicron recombinant, a Delta AY.45 consensus (con), and an Omicron BA.1 consensus sequence compared to the Wuhan-Hu-1 reference. Mutations are shown as ticks, color-coded according to the legend on the top. A schematic of the Delta and Omicron portions as well as the recombinant breakpoint region of

the recombinant strain are shown. All non-synonymous mutations are listed and shown in teal (Delta mutation), orange (Omicron mutations), or gray underlined (not related to Delta or Omicron). **B.** Maximum likelihood (IQ) trees of 1557 global SARS-CoV-2 full genome sequences based on a New York, New Jersey, and Connecticut focused subsampling. Nextstrain clades are colored according to the legend and the recombinant variant is shown in black and highlighted by arrows. The phylogenetic analysis was done over the recombinant's full genome (left) and separately for the Delta or Omicron parts only (middle and right), the latter by masking the complementary Omicron or Delta genomic regions, respectively. Concentrical rings indicate the number of mutations compared to the root Wuhan-Hu-1 sequence. **C.** Cytopathic effects observed in various lineages of SARS-CoV-2 on VeroE6/TMPRSS2 cells. Omicron (BA.1, right) induces cell rounding and partial detachment from the monolayer, while Delta (AY.119, middle) induces large syncytia with extreme morphological changes and complete detachment. The Delta-Omicron recombinant (left) displayed cell rounding with eventual detachment from the monolayer, with large cells observed. Images were taken with an EVOS M5000 inverted microscope (ThermoFisher Scientific, Waltham, MA); 10X magnification.

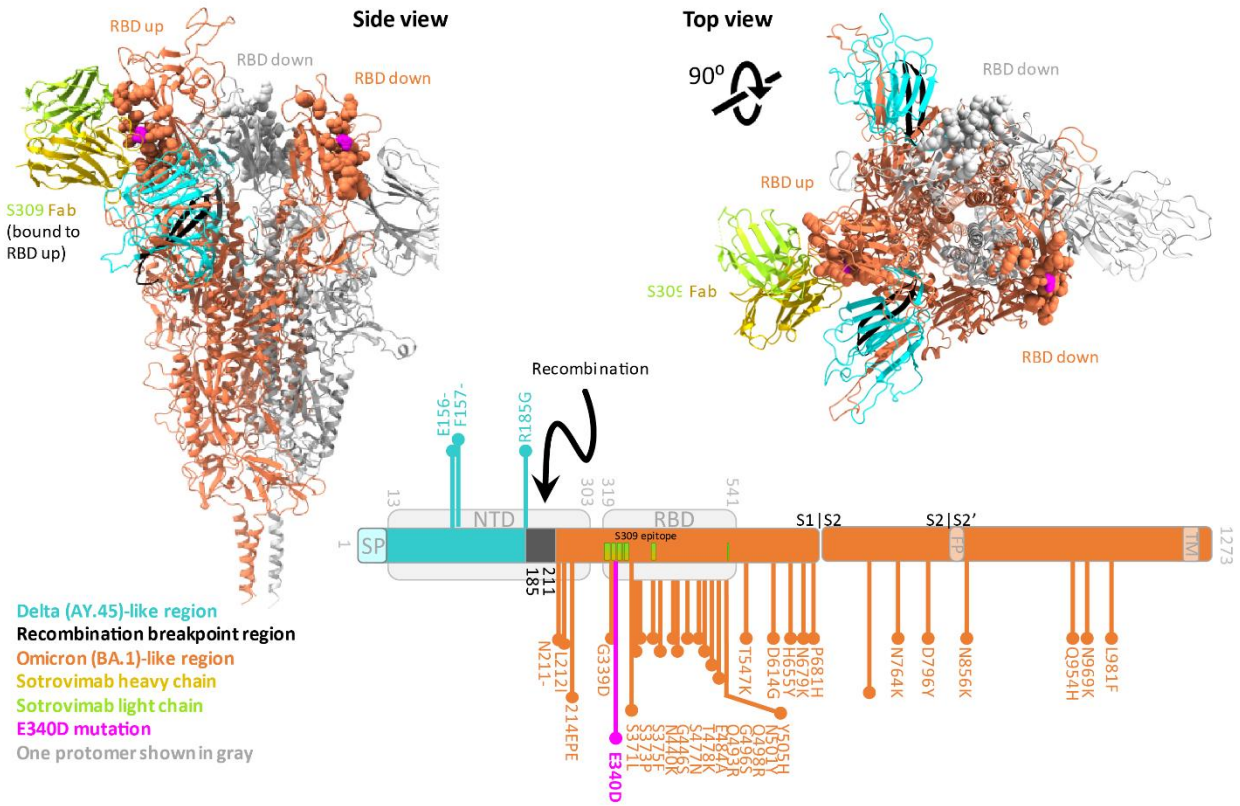

Figure 2. Quaternary structural analysis of the Delta-Omicron recombinant spike and its breakpoint region in relation to the Sotrovimab (S309) binding epitope.
Trimer spike structure of the Delta-Omicron recombinant in the open, one RBD-up conformation with one Sotrovimab Fab molecule bound to the RBD in up-position. Sotrovimab

is a slightly refined, engineered version of its precursor S309 that has been used in the structures. The spike portions are shown as ribbons and the Sotrovimab epitopes as spheres. One spike protomer is shown in gray; the other two protomers are colored according to the legend. Lower right: schematic of the location of the Delta (teal) and Omicron (orange) spike portions as well as the recombinant breakpoint region (black) including the mutations related to Delta (teal, facing up) or Omicron (orange, facing down). The mutation E340D, which is not related to Delta or Omicron, is highlighted in pink. The recombinant spike structure is a homology model of the recombinant spike based on pdb 7TO4 (1 RBD-up Omicron spike trimer). The S309 (Sotrovimab) molecule was added by structural overlay of a S309-bound spike co-structure in 1 RBD-up position (pdb 7TM0). FP: fusion peptide, NTD: N-terminal domain, RBD: receptor-binding domain, SP: signal peptide, TM: transmembrane domain.

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