

# Virologic and Immunologic Characterization of COVID-19 Recrudescence after Nirmatrelvir/Ritonavir Treatment

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## Case Report

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# Abstract

We isolated a SARS-CoV-2 BA.2 variant from a person with COVID-19 recrudescence after nirmatrelvir/ritonavir treatment. Antiviral sensitivity and neutralizing antibody testing was performed and compared with parental SARS-CoV-2 and multiple variants of concern. We found that neither NM resistance nor absence of neutralizing immunity were likely causes of the recrudescence.

## Full Text

Early administration of the oral protease inhibitor, Nirmatrelvir, combined with ritonavir (NM/r) (Paxlovid™) can reduce severe disease due to COVID-19<sup>1</sup>, but virologic and symptomatic rebound after NM/r treatment was recently reported<sup>2</sup>. We evaluated if NM resistance or impaired humoral immunity contributed to a case of COVID-19 recrudescence after NM/r treatment.

Three of four boosted adult travelers acquired COVID-19 after returning to the United States from South Africa and were treated with NM/r. All cases resolved quickly except one with rebounding symptoms associated with high viral shedding and culturable virus 5 days after an NM/r course. We isolated a SARS-CoV-2 pango lineage BA.2 virus (PRSD01) from a nasopharyngeal swab following development of worsening symptoms. SARS-CoV-2 full genome sequences isolated from the nasopharyngeal swab sample and culture isolate were assigned as SARS-CoV2 BA.2 lineage. Sequence comparison to the BA.2 reference showed no amino acid difference in any coding region, including ORF1a and Spike protein. Phenotypic analysis of the antiviral susceptibility of BA.2 PRSD01 (isolate), WA1/2020 (parental), B.1.617.2 (delta), BA.1 and BA.2.3 variants to NM and remdesivir were conducted. The half maximal inhibitory concentrations (IC50) of NM against BA.2 PRSD01 were 2.0, 1.8, 1.7, and 2.0 fold lower than the parental, delta, BA.1 and BA.2.3 strains respectively (Figure 1A). As a control, we determined the IC50 for remdesivir, a drug to which this individual was not exposed, for each strain. The remdesivir IC50 for BA.2 PRSD01 was 2.0, 1.8, 1.1, and 1.3 higher than the parental, delta, BA.1 and BA.2.3 strains respectively (Figure 1A).

We next evaluated the susceptibility of the viral panel to the neutralizing antibody response in the plasma from the patient and two controls. Both controls were fully vaccinated and boosted with the BNT162b2 mRNA vaccine (Pfizer–BioNTech), but one had been infected with and recovered from a SARS-CoV-2 infection with symptom onset 3 days prior to the individual who experienced NM/r recrudescence. The average half maximal authentic virus neutralizing antibody concentrations (NT50) against BA.2 PRSD01, BA.1, and parental were, 1668, 1170 and 5239 respectively in the NM/r treated patient. These levels were 8.9, 7.1 and 2.1 times higher than the boosted control but 2.0, 1.7 and 2.6 times lower than the boosted and infected control (Figure 1B).

This study found that neither development of NM resistance or absence of neutralizing antibody were likely causes of the recrudescence. Although we were unable to measure T cell responses or drug levels, we feel the most likely possibility for the observed recrudescence is insufficient drug exposure.

## References

1. Hammond J, Leister-Tebbe H, Gardner A, Abreu P, Bao W, Wisemandle W, Baniecki M, Hendrick VM, Damle B, Simón-Campos A, Pypstra R, Rusnak JM; EPIC-HR Investigators. Oral Nirmatrelvir for High-Risk, Nonhospitalized Adults with Covid-19. *N Engl J Med*. 2022 Apr 14;386(15):1397-1408. doi: 10.1056/NEJMoa2118542. Epub 2022 Feb 16. PMID: 35172054; PMCID: PMC8908851.
2. Gupta K, Strymish J, Stack G, Charness M. Rapid relapse of symptomatic SARS-CoV-2 infection following early suppression with nirmatrelvir/ritonavir. *Research Square*. 2022. Available at: <https://www.researchsquare.com/article/rs-1588371/v1>.

## Supplemental Information

Methods:

### Study Participants

Nasopharyngeal swabs and plasma samples from participants were obtained under UCSD IRB #200236X and #181624.

### SARS-CoV-2 sequencing and sequence analysis

SARS-CoV-2 full genome sequencing was performed using the COVID-19 ARTIC v4 Illumina library construction and sequencing protocol ([https://github.com/CDCgov/SARS-CoV-2\\_Sequencing](https://github.com/CDCgov/SARS-CoV-2_Sequencing)). Amplicons were generated with the NEBNext VarSkip VSS2b Primer kit (<https://github.com/nebiolabs/VarSkip>). PCR conditions were 98°C for 30 s, followed by 24 cycles of 98°C for 15 s and 63°C for 5 min, with a final 65°C extension for 5 min. Libraries were generated using the Nextera DNA Flex library preparation kit with Illumina index adaptors and sequenced on a MiSeq instrument (Illumina, San Diego, CA, USA). Samples were sequenced using 300 × 150-bp paired-end reads. Reads were processed with the CLC Genomics Workbench V22 (Qiagen). Briefly the workflow identifies individual SARS-CoV-2 sample variants by first trimming and mapping high quality reads (>20) to the reference genome and then calling variants to generate a full genome consensus for each sample. All consensus sequences were assigned to lineages by Pangolin (PMID: 34527285). Sequences were further aligned to a set of representative SARS-CoV-2 variants using NextAlign (PMID: 29790939). Amino acid variations across all coding regions of the sample isolates as compared to the reference strain BA.2 were interrogated.

### Cells and Chemicals

Vero-TMPRSS2 cells (Sekisui XenoTech) were maintained in DMEM plus 10% FBS, 1x Penicillin/Streptomycin, and 0.5 mg/ml Geneticin at 37°C and 5% CO<sub>2</sub>. Calu3 cells (ATCC # HTB-55) were maintained in MEM plus 10% FBS, 1x Penicillin/Streptomycin, 1mM sodium pyruvate, and GlutaMAX (Gibco) (Calu-3 media) at 37°C, 5% CO<sub>2</sub>. Nirmatrelvir (Selleckchem PF-07321332) and Remdesivir (GS-

5734, AA Blocks, San Diego, CA) were diluted in DMSO to create concentrated stock solutions at 10mM prior to diluting in Calu-3 media for antiviral assays.

### SARS-CoV-2 isolation and propagation

TMPRSS2-VeroE6 cells (Sekisui XenoTech) were plated in a 96well plate in DMEM + 2% FBS + Penicillin/Streptomycin. Patient sample stored in viral transport medium at 4°C was twofold serially diluted in DMEM + 2x Antibiotic/Antimycotic (anti/anti) and added to the newly-plated cells. After 7h, inoculum was removed and replaced with DMEM + 2% FBS with 1x anti/anti. When CPE became apparent, supernatant and scraped cells were added to fresh confluent cultures of TMPRSS2-VeroE6. The resulting passage 2 viral supernatant was centrifuged at 1000g for 5 min at 4°C, and aliquots were stored at -80°C. Passage two BA.2 PRSD01 was sequenced and found to have a single non-synonymous mutation at position L3829F (ORF1ab/nsp6 codon 260) and used for experiments. WA1 (NR-52281) and B.1.617.2 (NR-55611) strains were obtained from BEI Resources. BA.1 (GISAID # EPI\_ISL\_8186377) and BA.2.3 strains were isolated from clinical samples at UC San Diego under IRB #160524. Viruses from clinical samples were isolated on Calu3 cells (BA.2.3) or TMPRSS2-VeroE6 (BA.1, BA.2 PRSD01). All stocks were propagated on TMPRSS2-VeroE6 cells and verified by deep sequencing.

### SARS-CoV-2 antiviral assay.

Calu3 cells plated 2 days before infection in 96 well plates were pretreated with drugs 60-90min before infection. Cells were infected with a MOI of 0.125 of SARS-CoV-2 variants and incubated 40-44h at 37°C, 5% CO<sub>2</sub>. Cells were then fixed with 4.5% formaldehyde for at least 30 min and stained using anti-SARS-CoV-2 nucleocapsid protein (GeneTex, gtx135357) and anti-rabbit AlexaFluor 594 secondary antibody (Thermo Fisher Scientific) with Sytox Green nuclear counterstain. Five images per well were acquired at 10x magnification with an Incucyte S3 imager. Percent infected cells was calculated using Incucyte software tools and best fit curves created using GraphPad Prism 9. Graphs show averages of 2 independent experiments each done using 2 biological replicates.

### Authentic SARS-CoV-2 neutralizing antibody assay.

Neutralization was determined by focus reduction neutralization test (FRNT). Plasma samples were heat-inactivated at 56°C for 30min, centrifuged at 8000 RPM for 5 min, and then aliquoted and frozen at -80°C until use. Fourfold serial dilutions of plasma samples in DMEM + 1% FBS were incubated with 100-250 focus forming units of authentic SARS-CoV-2 diluted in DMEM for 1h at 37°C. Confluent TMPRSS2-VeroE6 cells in 96 well plates were washed once with PBS then infected with the virus+antibody mixture for 1h with gentle rocking. Inputs were removed, and cells were overlaid with 1% methylcellulose in MEM + 2% FBS and incubated 24h at 37°C. Cells were then fixed and stained for nucleocapsid protein as above, and whole-well images were acquired on an Incucyte S3 imager. Foci were counted using the Incucyte software, and percent neutralization was calculated relative to media-only control wells on each plate. Graphs show averages of 2 independent experiments each done using 2 biological replicates. SARS-CoV-2 neutralization titers were defined as the sample dilution at which a 50% reduction (NT50) in

foci was observed relative to the average of the virus control wells. Best fit curves determining  $NT_{50}$  were generated in GraphPad Prism 9. Graphs show averages of 2 independent experiments each done using 2 biological replicates.

## Declarations

### Acknowledgments

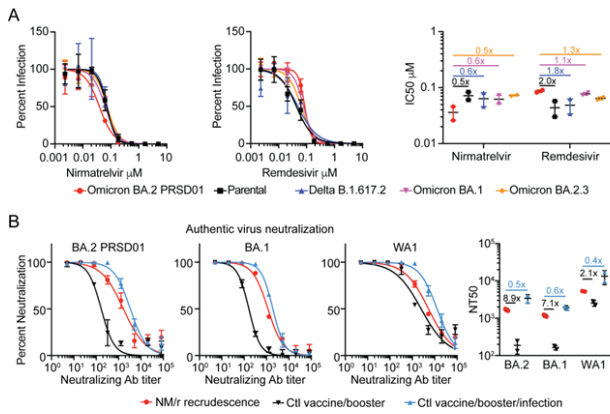
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### Conflicts of Interest:

DMS has served as consultant for FluxErgy Inc, Brio Clinical, Bayer HealthCare, Kiadis, Matrix BioMed, Model Medicines, Linear Therapies and VxBiosciences, and Signant Health

The patient consented to participate and publish their clinical data and images.

## Figures



**Figure 1**

Comparison of antiviral activity and neutralization against BA.2 PRSD01 after NM/r recrudescence. Dose response curves, IC<sub>50</sub> and NT<sub>50</sub> show the averages  $\pm$  standard deviation (SD) from 2 independent experiments with 2 biological replicates.