Supplemental Material*

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Contents

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Supplement Methods

Quantitative Viral Load Assay

Quantification of SARS-CoV-2 viral load was performed as previously described (1). Anterior nasal swabs were placed in viral transport media (VTM), which was then aliquoted in 250 µL and stored at -80^{o}C until testing. Specimens were thawed and mixed with 10 µL of replication-competent avian retrovirus (RCAS) virion as an internal quality control, and the homogenized mixtures were pelleted at 21,000 x g for 2 hours at 4^{o}C. The supernatant was discarded, and 750 µL TRIzol-LS Reagent (ThermoFisher Scientific) was added and vortexed for 30 seconds. Following incubation on ice for 10 minutes, 200 µL of chloroform (MilliporeSigma) was added, and the mixtures were vortexed for 30 seconds. Phase separation was accomplished via centrifugation at 21,000 x g for 15 minutes at 4^{o}C. The aqueous RNA-containing layer was isolated and added to tubes containing 100 µL 3 M Sodium Acetate (Life Technologies) and 1.5 µL GlycoBlue Co-precipitant (ThermoFisher Scientific). 300 µL of Isopropanol (MilliporeSigma) was added and the mixtures were shaken, incubated in dry ice for 15 minutes, and then centrifuged at 21,000 x g for 45 minutes at 4^{o}C to precipitate RNA pellets. Afterwards the supernatant was discarded, and RNA pellets were washed with 900 µL cold 70% ethanol. RNA pellets were resuspended in diethylpyrocarbonate-treated Water (ThermoFisher Scientific) and used for RT-qPCR with the US CDC 2019-nCoV_N1 primer and probe set (Integrated DNA Technologies). Absolute quantification of viral load was achieved via comparison to a standard curve generated by a 16-fold serial dilution of N1 RNA run on the same plate. All plates contained two non-template control wells and a positive and negative control for N1. The efficiency of the RNA extraction and RT-qPCR amplification was evaluated by quantifying the RCAS RNA recovered from each sample and the two N1 controls. The importin-8 (IPO8) human

housekeeping gene was also amplified and evaluated as a measure of sample collection quality. Samples were run in triplicate wells for N1, and in duplicate wells for RCAS and IPO8.

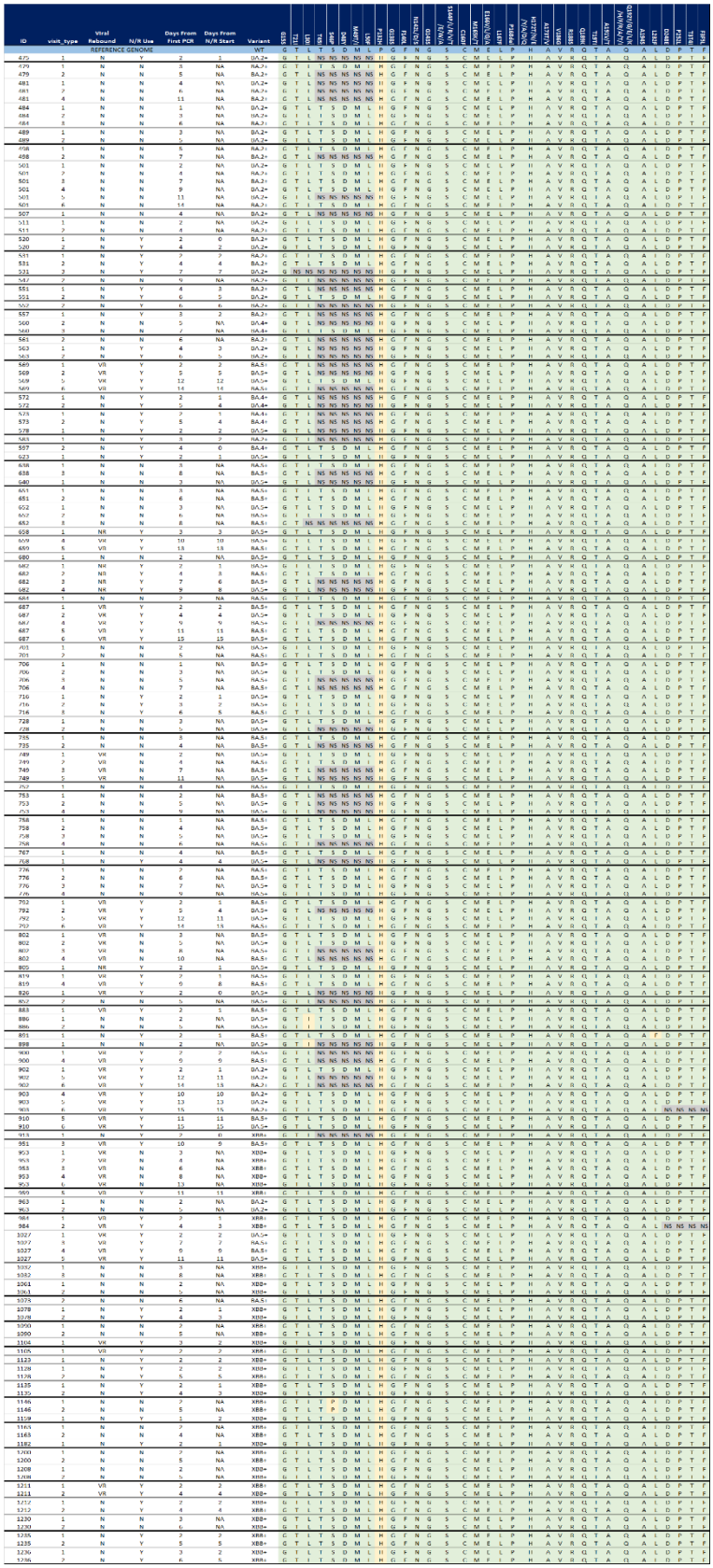
Viral Culture

Semi-quantitative viral culture was performed in the BSL3 laboratory of the Ragon Institute of MGH, MIT, and Harvard as previously reported (2). Vero-E6 cells (ATCC) were maintained in DMEM (Corning) supplemented with HEPES (Corning), 1X Penicillin/Streptomycin (Corning), 1X Glutamine (Glutamax, ThermoFisher Scientific), and 10% Fetal Bovine serum (FBS) (Sigma), harvested using Trypsin-EDTA (Fisher Scientific) and plated at 20,000 cells per well in 96w plates 16-20 hours before infection. Aliquoted VTM specimens were thawed on ice and filtered through either Spin-X 0.45 µm or 0.65 µm filters (Corning) at 10,000 x g for 5 minutes. 2 µL of the undiluted filtrate was added to four wells of a 96w plate and serially diluted (1:5) in media containing 5 µg/milliliter (mL) of polybrene (Santa Cruz Biotechnology) before spinfection for 1 hour at 2000 x g at 37^{o}C. Each 96w plate contained wells inoculated with SARS-CoV-2 isolate USA-WA1/2020 strain (BEI Resources) as a positive control and medium only as a negative control. The viral culture plates were scored 7 days post-infection by observation under a light microscope and wells showing cytopathic effect (CPE) counted as positive. A median tissue culture infectious dose (TCID50) was calculated using the Spearman-Karber method. For each well showing CPE, the culture supernatant was harvested for virus expansion and RNA isolation using QIAamp Viral RNA Mini kit (QIAGEN) for confirmation of the viral sequence.

SARS-CoV-2 Whole Genome Sequencing

Whole genome sequencing was carried out using the Illumina COVIDSeq Test protocol as previously described (3). Briefly, DNA libraries were constructed using the Illumina COVIDSeq Test Kit, pooled together, and then quantified with a Qubit High Sensitivity dsDNA kit (Invitrogen). Afterwards, genomic sequencing was performed on an Illumina NextSeq 2000 instrument. Sequenced genomes were demultiplexed and assembled on the Terra platform (app.terra.bio). Complete genomes (sequence assembly length greater than 24000 base pairs) were assigned a Pango lineage (https://github.com/cov-lineages/pangolin-data) and deposited to NCBI GenBank under Project Accession PRJNA759255.

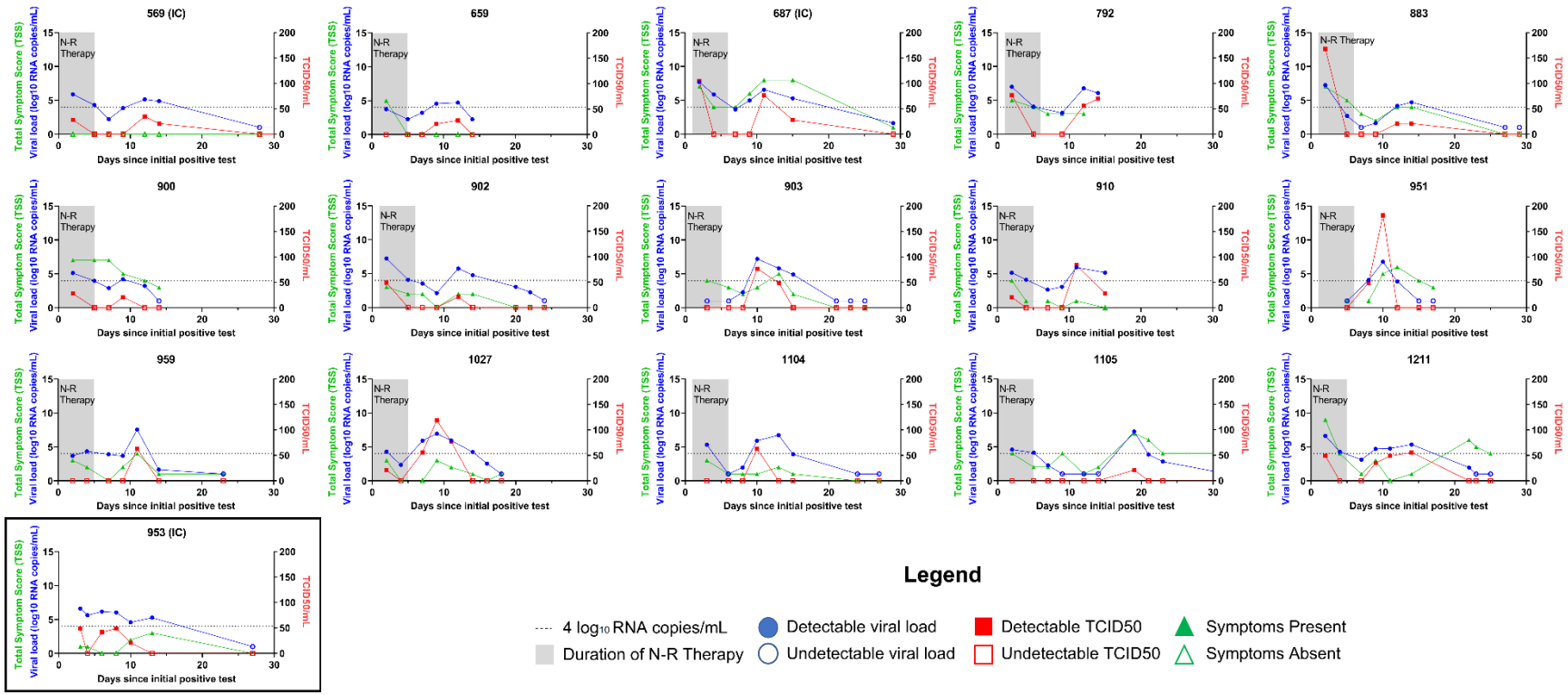
Supplement Figure 1. Sequencing of NSP5 for nirmatrelvir resistance mutations.



Wildtype
Not Sequenced
Polymorphism
Drug-resistance Mutation

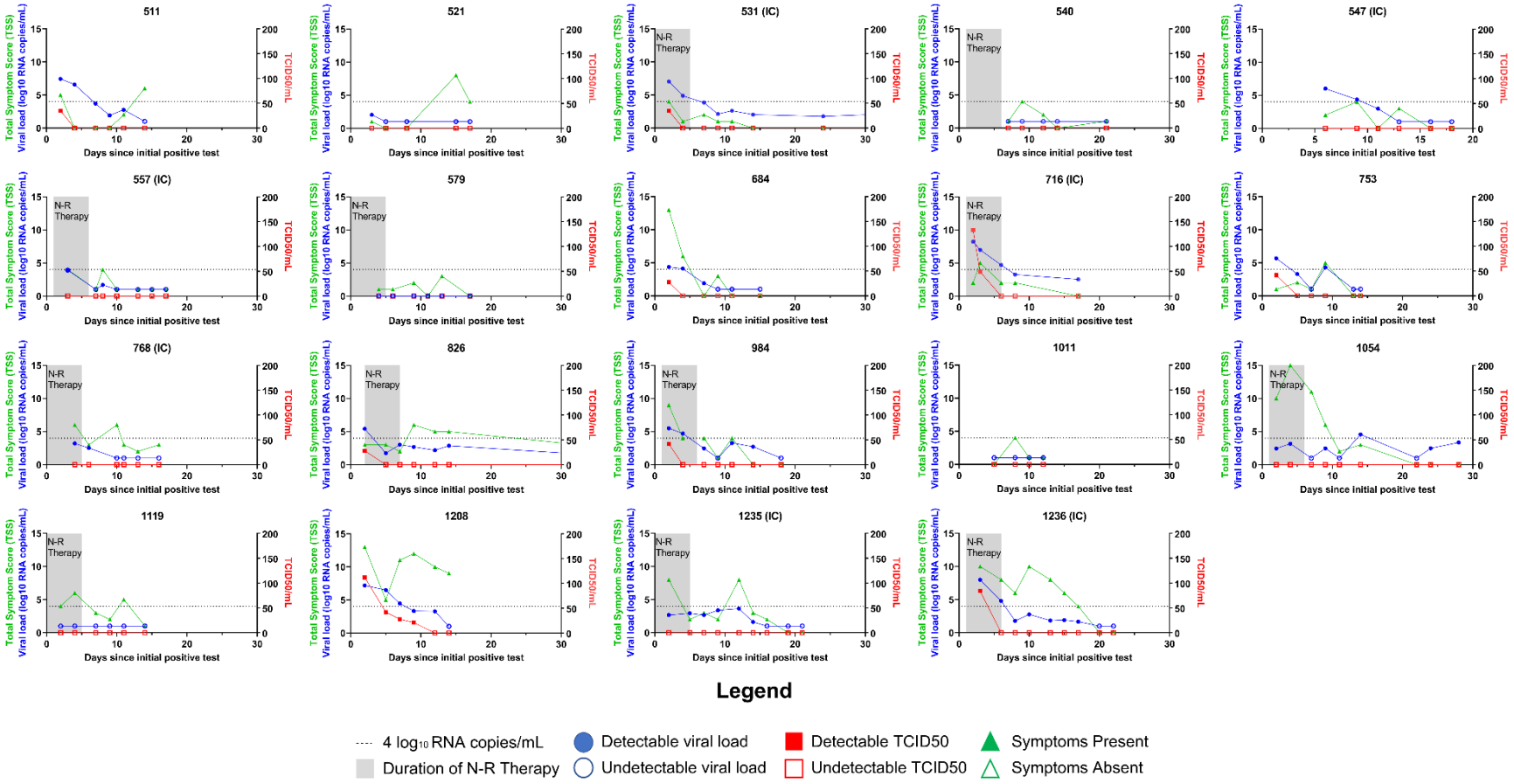
Supplement Figure 2. Decay curves for individuals with virologic rebound.

The number above each graph corresponds to the participant's study ID. ID 953 (black box) did not receive nirmatrelvir-ritonavir. Abbreviations: TCID50, median tissue culture infectious dose; N-R, nirmatrelvir-ritonavir; IC, immunocompromised (full details are available in Supplement Table 1).



Supplement Figure 3. Decay curves for individuals who experienced symptomatic rebound but not virologic rebound.

The number above each graph corresponds to the participant's study ID. Abbreviations: TCID50, median tissue culture infectious dose; N-R, nirmatrelvir-ritonavir; IC, immunocompromised (full details are available in Supplement Table 1).



Supplement Table 1. Clinical characteristics of individuals with immunosuppressing conditions or therapies in the cohort.

ID	Diagnosis	Treatment	COVID-19 Therapy
475	Multiple sclerosis	Rituximab within 12 months of COVID-19	Nirmatrelvir-Ritonavir
531	Sarcoidosis	Infliximab	Nirmatrelvir-Ritonavir
547	Rheumatoid arthritis	Tocilizumab, Methotrexate	Untreated
549	Bechet's disease	Azathioprine	Nirmatrelvir-Ritonavir
550	Rheumatoid arthritis	Methotrexate, Hydroxychloroquine	Nirmatrelvir-Ritonavir
551	Psoriatic arthritis	Infliximab	Nirmatrelvir-Ritonavir
552	Seronegative spondyloarthropathy	Adalimumab, Methotrexate	Nirmatrelvir-Ritonavir
557	Rheumatoid arthritis, systemic lupus erythematosus	Methotrexate	Nirmatrelvir-Ritonavir
563	Rheumatoid arthritis	Adalimumab	Nirmatrelvir-Ritonavir
569	Systemic lupus erythematosus	Hydroxychloroquine, Methylprednisolone daily	Nirmatrelvir-Ritonavir
573	Inflammatory arthritis	Adalimumab, Hydroxychloroquine	Nirmatrelvir-Ritonavir
597	Giant cell arteritis, polymyalgia rheumatica	Tocilizumab, Prednisone daily	Nirmatrelvir-Ritonavir
658	Rheumatoid arthritis	Tocilizumab	Nirmatrelvir-Ritonavir
678	Rheumatoid arthritis	Tofacitinib	Nirmatrelvir-Ritonavir
687	Systemic lupus erythematosus, rheumatoid arthritis	Hydroxychloroquine, methotrexate	Nirmatrelvir-Ritonavir
691	Rheumatoid arthritis	Rituximab	Nirmatrelvir-Ritonavir
716	Rheumatoid arthritis	Methotrexate	Nirmatrelvir-Ritonavir
723	Multiple sclerosis, acquired hypogammaglobulinemia	IVIG every 4 weeks; Ocrelizumab within 12 months	Untreated
725	Rheumatoid arthritis	Infliximab, methotrexate, hydroxychloroquine	Nirmatrelvir-Ritonavir
735	Psoriatic arthritis	Adalimumab	Untreated
768	Ankylosing spondylitis	Secukinumab	Nirmatrelvir-Ritonavir
805	Ulcerative colitis, inflammatory arthritis	Golimumab, methotrexate	Nirmatrelvir-Ritonavir
892	HIV infection	N/A, on antiretroviral therapy, CD4 cell count>200	Untreated
945	IgG4 related disease	Rituximab	Nirmatrelvir-Ritonavir
952	Inflammatory arthritis	Adalimumab	Nirmatrelvir-Ritonavir
953	Rheumatoid arthritis	Infliximab, methotrexate, prednisone	Untreated
1235	Psoriatic arthritis	Etanercept, methotrexate	Nirmatrelvir-Ritonavir
1236	Systemic lupus erythematosus	Belimumab, methotrexate, prednisone	Nirmatrelvir-Ritonavir

Supplement Table 2. Characteristics of eligible individuals included in the analytic cohort and those excluded from the analytic cohort.

Individuals were excluded due to receipt of alternate therapies after enrollment (n=2), receipt of less or more than 5 days of N-R (n=2), or lack of a nasal swab collection more than 11 days from their index diagnostic test (n=11).

Characteristic	Included in Analytic Cohort (n=127)	Excluded from Analytic Cohort (n=15)	Standardized difference
Age (median/IQR)	53 (35-66)	49 (36-59)	-0.08
Missing	0	0	
Gender (n, %)			0.02
Female	96 (76)	8 (53)	
Male	31 (24)	7 (47)	
Missing	0	0	
Race (n, %)			0.94
White	97 (76)	6 (40)	
Black/AA	12 (9)	1 (7)	
Asian	5 (4)	1 (7)	
Other	7 (6)	3 (20)	
Unknown/Missing	6 (5)	4 (27)	
Ethnicity (n, %)			0.77
Hispanic/Latino	10 (8)	3 (20)	
Non-Hispanic/Latino	103 (81)	7 (47)	
Other/Unknown/Missing	14 (11)	5 (33)	
COVID-19 Vaccines (median/IQR)	4 (3-4)	3 (0-3)	-0.85
Missing	0	0	
Days since last vaccine (median/IQR)	163 (79-253)	223 (105-371)	0.40
Missing	0	0	
Immunosuppression[a] (n, %)			0.11
Absent	99 (78)	11 (73)	
Present	28 (22)	4 (27)	
Missing	0	0	
COVID-19 Variant (n, %)			0.32
BA.2[b]	20 (16)	3 (20)	
BA.5[c]	39 (31)	4 (27)	
XBB[d]	24 (19)	4 (27)	
Other	5 (4)	1 (7)	
Incomplete[e]	39 (31)	3 (20)	

Reason for Baseline Test[f]			0.23
Symptoms	110 (87)	12 (80)	
Exposure	13 (10)	2 (13)	
Screening	3 (2)	1 (7)	
Other	1 (1)	0 (0)	
Missing	0	0	
Baseline Test Type (n, %)			0.08
PCR	80 (63)	10 (67)	
Rapid Antigen	47 (37)	5 (33)	
Missing	0	0	
Baseline Test Ct Value (median/IQR)	22.7 (18.5-30.6)	26.8 (19.6-29.3)	-0.01
Missing	71 (56)	11 (73)	
First study viral load in copies/mL (median/IQR)	5.1 (3.2-6.3)	5.0 (3.7-6.0)	0.12
Missing	0	0	
Days from index PCR to first study viral load (median/IQR)	2 (2-3)	2 (2-4)	0.28
Missing	0	0	
Days from Symptom Onset to Baseline Test (median/IQR)	1 (1-2)	2 (0-3)	0.10
Asymptomatic/Missing	17 (13)	3 (20)	

[a] Immunosuppression defined as presence of an immunosuppressing condition or use of an immunosuppressing medication, as determined by physician chart review. Full details of these conditions are available in Supplement Table 1.

[b] Includes BA.2 subvariants

[c] Includes BA.5 subvariants

[d] Includes XBB subvariants

[e] Only genomes with ≥24000 base pair sequence lengths were considered complete

[f] Participants could select multiple reasons for testing. We categorized them such that symptoms took precedence, followed by exposure, and then asymptomatic screening

Supplement Table 3. Sensitivity analyses with inclusion of those who received non-standard N-R therapy, those who received alternate therapies and/or dropped out of the study before 12 days of observation.

	N-R Rebound Frequency	No Therapy Rebound Frequency	Absolute Difference (95%CI)	P-value
Primary Analysis	15/72 (20.8%)	1/55 (1.8%)	19.0% (9.0-29.0%)	0.001
Inclusion of individuals with non-standard N-R regimens*	15/74 (20.3%)	1/55 (1.8%)	18.5% (8.6-28.3%)	0.002
Inclusion of individuals with non-standard N-R regimens, initiation of other antivirals during observation, and/or discontinuation of swab collection before 12 days	15/76 (19.7%)	2/66 (3.0%)	16.7% (6.8-26.6%)	0.002

* Includes one individual with 3 days of N-R treatment and one with 15 days N-R of treatment that were not included in the primary analysis

Supplement Table 4. Median number of days to first culture conversion and to final culture conversion.

	Median (IQR) days to first negative viral culture	P-value (compared to no therapy group)	Median (IQR) days to final negative viral culture	P-value (compared to no therapy group)
No therapy group	4 (3-6)	REF	4 (3-6)	REF
All N/R users	3 (2-4)	<0.001	4 (2-6)	0.294
N/R rebound	3 (3-4)	0.022	14 (13-20)*	<0.001
N/R no rebound	3 (2-4)	<0.001	3 (2-4)	<0.001

* Two participants with virologic rebound were culture-positive at their last study timepoint

Supplement Table 5. Virologic characteristics of individuals experiencing virologic rebound.

Rebound after nirmatrelvir-ritonavir use										
ID	Initial viral load nadir (log_{10} RNA copies/mL)[a]	Days to initial nadir*	Days to detection of virologic rebound*	Days from end of N-R therapy to detection of rebound*	Viral load peak during rebound (log_{10} RNA copies/mL)	Culturable virus during rebound	Any symptoms during rebound	Symptom rebound (TSS ≥3)	Days to final negative viral load*	Days to final negative viral culture*
569	2.2	7	12	7	5.1	Yes	No	No	21	21
659	2.3	6	10	6	4.7	Yes	No	No	15[b]	14
687	3.7	7	9	4	6.6	Yes	Yes	Yes	29[b]	22
792	3.1	9	12	6	6.8	Yes	Yes	No	14[b]	14[b]
883	1.0	7	12	6	4.7	Yes	Yes	No	21	21
900	2.9	7	9	4	4.2	Yes	Yes	No	13	11
902	2.1	9	12	6	5.8	Yes	Yes	No	23	13
903	1.0	3	10	5	7.2	Yes	Yes	Yes	18	14
910	2.7	7	11	6	6.0	Yes	Yes	No	15[b]	15[b]
951	1.0	5	8	2	6.8	Yes	Yes	Yes	14	11
959	3.7	9	11	6	7.6	Yes	Yes	Yes	19	13
1027	2.3	4	7	2	6.9	Yes	Yes	Yes	17	13
1104	1.0	6	10	4	6.7	Yes	Yes	No	20	12
1105	1.0	9	19	14	7.3	Yes	Yes	Yes	28	20
1211	3.1	7	9	4	5.4	Yes	Yes	Yes	23	18
	Median/IQR 2.3 (1.0-3.1)	Median/IQR 7 (6-9)	Median/IQR 10 (9-12)	Median/IQR 6 (4-6)	Median/IQR 6.6 (5.1-6.9)	n, % 15 (100%)	n, % 13 (87%)	n, % 7 (47%)	Median/IQR 19 (15-23)	Median/IQR 14 (13-20)

Rebound after no therapy									
ID	Initial viral load nadir (log_{10} RNA copies/mL)[a]	Days to initial nadir*	Days to detection of virologic rebound*	Viral load peak during rebound (log_{10} RNA copies/mL)	Culturable virus during rebound	Any symptoms during rebound	Symptom rebound (TSS ≥3)	Days to final negative viral load*	Days to final negative viral culture*
953	5.6	4	6	6.2	Yes	Yes	Yes	20	12

* Days are from initial positive COVID-19 test

[a] Undetectable viral loads imputed as 1.0 log_{10} RNA copies/mL

[b] Final study specimen with detectable viral load or positive viral culture

Supplement Table 6A. Validity of symptom rebound to detect virologic rebound in total cohort.

	Symptomatic Rebound	No Symptomatic Rebound	Total
Virologic Rebound	8	8	16
No Virologic Rebound	19	92	111
Total	27	100	127

	Measure	**Estimate**	**95%CI**
	Sensitivity	50% (8/16)	25-75%
	Positive predictive value	30% (8/27)	14-50%
	Specificity	83% (92/111)	75-89%
	Negative predictive value	92% (92/100)	85-96%

Supplement Table 6B. Validity of symptom rebound to detect virologic rebound among individuals receiving nirmatrelvir-ritonavir.

	Symptomatic Rebound	No Symptomatic Rebound	Total
Virologic Rebound	7	8	15
No Virologic Rebound	12	45	57
Total	19	53	72

	Measure	**Estimate**	**95%CI**
	Sensitivity	47% (7/15)	21-73%
	Positive predictive value	37% (7/19)	16-62%
	Specificity	79% (45/57)	66-89%
	Negative predictive value	85% (45/53)	72-93%

Supplement References

1. North CM, Barczak A, Goldstein RH, et al. Determining the Incidence of Asymptomatic SARS-CoV-2 Among Early Recipients of COVID-19 Vaccines (DISCOVER-COVID-19): A Prospective Cohort Study of Healthcare Workers Before, During and After Vaccination. Clin Infect Dis. 2022 Apr 9;74(7):1275–8.

2. Boucau J, Marino C, Regan J, et al. Duration of Shedding of Culturable Virus in SARS-CoV-2 Omicron (BA.1) Infection. N Engl J Med. 2022 Jul 21;387(3):275–7.

3. Boucau J, Uddin R, Marino C, et al. Characterization of Virologic Rebound Following Nirmatrelvir-Ritonavir Treatment for Coronavirus Disease 2019 (COVID-19). Clin Infect Dis. 2023 Feb 8;76(3):e526–9.