Viral Load Rebound in Placebo and Nirmatrelvir-Ritonavir Treated COVID-19 Patients is not Associated with Recurrence of Severe Disease or Mutations

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Abstract

Nirmatrelvir-ritonavir was developed for the treatment of COVID-19 and has shown efficacy in a Phase 2/3 study of high risk patients (EPIC-HR1). Monitoring for the emergence of viral resistance and recurrence of symptoms is a critical component of any antiviral drug development. As part of the study of viral resistance, a sub-analysis was conducted to examine viral load rebound (VLR) following nirmatrelvir-ritonavir treatment in the EPIC-HR study. Nasopharyngeal or nasal swabs were collected from all study participants at Day 1, Day 3, Day 5, Day 10, and Day 14, and analyzed for viral RNA load and next generation viral sequencing. Two categories of viral load rebound were considered including present/persistent and transient. In EPIC-HR, the proportion of present/persistent VLR was low, occurring at 1.73% (17/980) vs 2.32% (23/990) and for transient VLR 2.35% (23/980) vs 4.65% (46/990) in placebo vs nirmatrelvir-ritonavir participants, respectively. VLR occurred in both treatment arms and was not associated with low nirmatrelvir exposure, hospitalization or death, severe symptom relapse, serological status, or Mpro gene/cleavage treatment emergent mutations. In summary, viral load rebounds are likely a phenomena COVID-19 disease course and nirmatrelvir-ritonavir continue to be an important treatment option for high risk COVID-19 patients.

Introduction

Nirmatrelvir (PF-07321332) is an orally administered potent antiviral agent targeting the SARS-CoV-2 3-chymotrypsin-like cysteine protease enzyme (Mpro), an essential protein for viral replication. Nirmatrelvir shows antiviral activity across all the SARS-CoV-2 variants of concern (VoCs), and has low likelihood of off-target activity due to lack of a human analogue. Nirmatrelvir-ritonavir was authorized for use in high-risk symptomatic COVID-19 adults and pediatric patients (>12 years weighing >40 kg) via the United States Food and Drug Administration (US FDA)’s Emergency Authorization process. Previous reports of nirmatrelvir-ritonavir efficacy in the EPIC-HR (Evaluation of Protease Inhibition for COVID-19 in High-Risk Patients) trial participants showed an 88% risk reduction of severe outcomes in participants treated within 5 days of symptoms onset (COVID-19 related hospitalization or all-cause death). Nirmatrelvir-ritonavir reduced viral load (VL) at day 5 by an adjusted mean (±SE) of an additional 0.868±0.105 log_{10} copies per milliliter (95% CI, −1.074 to −0.6615; P<0.001) when treatment was initiated within 3 days after symptoms onset, a decrease in viral load by a factor of 10 relative to placebo, and 0.695±0.085 log_{10} copies per milliliter (95% CI, −0.861 to −0.530; P<0.001) when treatment was initiated within 5 days after onset of symptoms.

Here, we report the kinetics and incidence of viral load rebound (VLR) in the EPIC-HR study for nirmatrelvir-ritonavir (treated) and placebo (untreated) groups. In addition, the relationships between VLR and nirmatrelvir exposure, moderate-severe symptom recurrence hospitalization and/or death by Day 28, baseline serological status and treatment emergent mutations (TEMs) in the Mpro gene and cleavage region are explored.
Methods

Study Design and Procedures

The protocol, protocol amendments, Informed Consent Documents, Investigator Brochure, and other relevant documents (e.g., advertisements) were submitted to an Institutional Review Board / Independent Ethics Committee (IRB/IEC) by the investigator and reviewed and approved by the IRB/IEC before the study was initiated.

This study was conducted in accordance with the protocol and consensus ethical principles derived from international guidelines including the Declaration of Helsinki Council and the Council for International Organizations of Medical Sciences International Ethical Guidelines, applicable International Council for Harmonisation Good Clinical Practice Guidelines, applicable International Organization for Standardization 14155 guidelines, medical device guidelines, and other applicable laws and regulations, including privacy laws.

EPIC-HR is a Phase 2/3, randomized, double-blind, placebo-controlled study conducted in 2,224 symptomatic adult participants with COVID-19 who were non-hospitalized and at increased risk for progressing to severe illness. Eligible participants with confirmed diagnosis of SARS-CoV-2 infection and symptom onset within 5 days were randomly assigned in a 1:1 ratio to receive either nirmatrelvir 300 mg co-administered with ritonavir 100 mg or matched placebo orally every 12 hours for 5 days (10 doses total). Randomization was stratified by geographic region and by receipt or expected receipt (based on investigator opinion) of COVID-19 monoclonal antibodies at the time of randomization. Efficacy and safety assessments were conducted through Day 34 visit; a long-term follow-up period continued through Week 24. Further details of the study design have been previously described\(^1\). Participants utilized an electronic diary to capture daily COVID-19 symptoms through Day 28.

Nasopharyngeal (NP) swabs were collected by health care professionals from participants at baseline, Day 1 (D1), Day 3 (D3), Day 5 (D5), Day 10 (D10), and Day 14 (D14). Nasal swabs were self-collected by participants at D3, D10, and D14 if an in-person study visit did not occur (Supplemental Table 1 for collection site details). For analysis, sample collections (e.g., D1, D3, D5, D10, and D14) visits were mapped as baseline (Day -2 to Day 1; within 1 hour post first dose) and post-baseline (>1 hour post first dose). NP/nasal swab samples were shipped on dry ice to the University of Washington Medicine Clinical Virology Laboratory for analysis. Upon receipt, samples were evaluated for quality control/swab deviations and logged into the sample management system. Swabs were placed in Universal Transport Medium and then aliquoted for viral load, viral sequencing, and subsequent infectivity/phenotypic analysis.

Viral Load (VL; RNA) Quantitation
SARS-CoV-2 VL was measured as log$_{10}$ copies/mL using a validated Abbott Real Time Quantitative SARS-CoV-2 assay at the University of Washington Medicine Clinical Virology Laboratory$^5$. In brief, the VL assay is a reverse transcriptase (RT)-PCR assay intended for the quantitative detection of nucleic acid from SARS-CoV-2 in NP or nasal swabs by detecting the RNA-dependent RNA polymerase (RdRp) and nucleocapsid (N) genes using the Abbott m2000 System. The assay has a dynamic range with an upper limit of quantitation (ULoQ) of 8 log$_{10}$ copies/mL and the lower limit of quantitation (LLoQ) of 2.0 log$_{10}$ copies/mL. Data reported as less than 2.0 log$_{10}$ copies/mL was imputed as 1.70 log$_{10}$ copies/mL, and data reported as “not detected” was imputed as 0 log$_{10}$ copies/mL.

**Viral Sequencing**

SARS-CoV-2 genomes from clinical samples were sequenced using the SWIFT biosciences next generation amplicon based sequencing methodology, and sequencing was performed at the University of Washington Medicine Clinical Virology Laboratory$^6$. Virus from D1, D3, D5, D10, and D14 participant samples that meet the sequencing limit of detection (LoD) (viral concentrations $\geq$ 500 copies/mL [$\geq$ 2.70 log$_{10}$ copies/mL]) were sequenced and genotyped by mapping against the reference sequence (NCBI: NC_045512.2). In brief, to ensure quality control of sequence data, the genome acceptability criteria were as defined as positive controls reported with 1 million raw reads, >85% identity to the reference sequence (NCBI: NC_045512.2), 750X average genome coverage, 1000X mean Mpro gene coverage, >90% of 3CL at 100X or higher, and <10% Ns in the consensus genome.

**Viral Load Rebound (VLR)**

The study population for VLR analysis included participants with matched D5 and D10 and/or D14 samples (N=990 for nirmatrelvir/ritonavir and N=980 for placebo). In brief, rebound was classified based on a half log increase in viral load at follow-up (D10 and/or D14) relative to the end of treatment (D5) viral load levels. The lower threshold for rebound was set at 2.7 log$_{10}$ copies/mL (the LLoQ for the viral sequencing assay). For the present study, participants with half-log increases at follow-up were then split into two categories. For the first category, if only one follow-up sample was available and showed a half-log increase, it was classified as present. If the rebound was present at D10 and levels were still elevated by a half log or more by D14, it was classified as persistent. In brief, the statistical definition for present and/or persistent was: If D14 VL was not available, D10 VL change from D5 (CFD5) $\geq$ 0.5 log$_{10}$ copies/mL and D10 VL $\geq$ 2.7 log$_{10}$ copies/mL, or If D14 VL is available, D14 VL CFD5 $\geq$ 0.5 log$_{10}$ copies/mL and D14 VL $\geq$ 2.7 log$_{10}$ copies/mL. If both follow-up samples were available, but the rebound was only evident at D10, it was classified as transient. The classification for “transient” was defined as follows: If D10 VL was $\geq$0.5 log$_{10}$ copies/mL relative to D5 and D10 VL was $\geq$2.7 log$_{10}$ copies/mL AND if D14 VL was <0.5 log$_{10}$ copies/mL relative to D5 or D14 VL was <2.7 log$_{10}$ copies/mL.
Viral Genotyping and Treatment Emergent Mutation Analysis

Virus sequenced from participant samples were genotyped per the University of Washington standard operating procedures. The LLoQ for the sequencing assay was $2.7 \log_{10}$ copies/mL. Mutations resulting in an amino acid (AA) substitution within a sample were called if the amino acid frequency (AAFREQ) was $\geq 1\%$ and the amino acid substitution (AASUB) was different from the reference sequence (NCBI: NC_045512.2). Resequencing was conducted if lineage differences were observed across participant samples. VLR variant calls were cross-examined for strand bias. Mutations with significant strand read bias were detected within the present/persistent VLR population and excluded from the TEM analysis. Any distinct mutation was called TEM if the mutation was absent (AAFREQ <5% or not called) for the participant in their baseline sample, but the same mutation was present (AAFREQ $\geq 5\%$) for the same participant in any post-baseline sample (D3, D5, D10, or D14). Mutations resulting in frameshift (‘fs’) were excluded from the TEM analysis. Full treatment emergent mutation analysis results are described in a separate treatise.

**Lineage classification.** For each participant, the SARS-CoV-2 lineage was called based on classification at D1, or if D1 was not available or had insufficient viral concentrations (<$2.70 \log_{10}$ copies/mL), then at D3, if not, then at D5, if not, then at D10 and if not, then at D14.

Mpro Genomic Regions of Interest

Mutations were grouped into SARS-CoV-2 genomic regions of interest including: 1) Mpro residues within 5 Angstroms of nirmatrelvir-Mpro binding and Mpro cleavage regions external to Mpro gene (Supplemental Tables S2 and S3).

Plasma Assay for Nirmatrelvir

Pharmacokinetic samples were to be collected at D1 between 30 to 90 minutes post-dose, and at D5 preferably as pre-dose or any time post-dose. Additionally, one sample was to be collected at any time post-dose during in-person visit at D2, D3, or D4. Nirmatrelvir concentrations were determined by a specific and sensitive bioanalytical method using liquid chromatography with tandem mass spectroscopy (LC-MS/MS). The calibration curve range for the plasma method was 10.0 to 10,000 ng/mL for nirmatrelvir. The assay validations and study sample analyses were conducted at York Bioanalytical Solutions, York, UK in compliance with the current US FDA and European Medicines Agency Guidance requirements.
Serological Assays

Plasma samples were collected for serological analysis. Roche Elecsys® Anti-SARS-CoV-2 (against N – qualitative) and Elecsys® Anti-SARS-CoV-2 “S” (both quantitative and qualitative) immunoassays were used for the detection of total host immunoglobulins to SARS-CoV-2 N or spike (S) proteins. The immunoassays are set to a sandwich format. Assays were validated following CRO SOPs (PPD) and run per manufacturer’s instructions. Serostatus at baseline was defined as positive in either N or S qualitative assay.

Results

Viral Load Rebound (VLR)

VLR was observed in both placebo and nirmatrelvir-ritonavir participants. Fig. 1A shows VL changes across all of the EPIC-HR population by treatment arm. As reported previously\(^1\), nirmatrelvir-ritonavir significantly reduced VL compared to placebo. Fig. 1B shows VL changes in the present/persistent VLR population. The mean VL at baseline in the present/persistent VLR population was 4.80 (SD=2.26) log\(_{10}\) copies/mL for placebo and 5.21 (SD=2.56) log\(_{10}\) copies/mL for nirmatrelvir-ritonavir, and a decline was observed over time, with mean VL at D5 of 2.44 (SD=1.73) and 2.38 (SD=1.78) for placebo vs the nirmatrelvir-ritonavir participants, respectively. The proportion of present/persistent VLR was 1.73% (17 of 980) for placebo participants and 2.32% (23 of 990) for nirmatrelvir-ritonavir participants (Fig. 1B). Fig. 1C shows VL changes in the “transient” VLR population. The mean VL at baseline in transient VLR groups was 5.98 (SD=1.57) log\(_{10}\) copies/mL for placebo and 5.96 (SD=1.81) log\(_{10}\) copies/mL for nirmatrelvir-ritonavir. A decline was observed over time, with mean VL at D5 of 3.43 (SD=1.38) for placebo and 2.68 (SD=1.40) log\(_{10}\) copies/mL for nirmatrelvir-ritonavir. The proportion of transient VLR was 2.35% (23 of 980) for placebo participants and 4.65% (46 of 990) for nirmatrelvir-ritonavir participants (Fig. 1B).

Combined, the proportion of VLRs was 4.10% (40 of 980) for placebo participants and 6.97% (69 of 990) for nirmatrelvir-ritonavir participants. Thus, VLR was evident in both treatment groups, and the range varied from approximately 2% to 7%.

The VLR subpopulations did not tend to overlap with participants who experienced hospitalization or death. In Fig. 1B, only one nirmatrelvir participant experienced both VLR and hospitalization (blue line in Fig. 1). This participant had already been discharged from the hospital by D8 when the VLR was observed at D10. No placebo participants experienced present and/or persistent VLR and hospitalization or death (Fig. 1B). There were two placebo and no nirmatrelvir-ritonavir participants who experienced transient VLR and hospitalization (Fig. 1C). There were no deaths in either present/persistent or transient VLR groups. Thus, VLR does not appear to be a phenomenon driven solely by nirmatrelvir-ritonavir treatment.
VLR Nirmatrelvir Exposures

The relationship between VLR and nirmatrelvir exposure was examined to assess if there was a relationship of VLR with low exposures. Fig. 2 illustrates the observed nirmatrelvir concentrations over time in present and/or persistent VLR (solid circles) vs all nirmatrelvir-ritonavir EPIC-HR participants. The majority of VLR participants had nirmatrelvir exposures above EC$_{90}$ (292 ng/mL)$^2$. Population pharmacokinetic analyses indicated that the dose of 300/100 mg nirmatrelvir/ritonavir twice daily for 5 days resulted in greater than 98% of the patients achieving trough concentrations of nirmatrelvir above the EC$_{90}$ of 292 ng/mL. Importantly, the median trough concentration across all patients was approximately 5X of EC$_{90}$. There were a few nirmatrelvir concentrations below the EC$_{90}$ but these were noted in both VLR and non-VLR participants, reflecting the variability in pharmacokinetics. Transient VLR exposures also did not differ from overall EPIC-HR exposures (Supplemental Fig. 1). In summary, VLR was not associated with low nirmatrelvir exposures.

Symptom Relapse

Of the EPIC-HR participants who received at least 1 dose of study medication, more in the nirmatrelvir-ritonavir group than in the placebo group achieved symptom-free duration of 1 day or 2, 3, or 4 consecutive days between D1-D8. For those participants with 1 day or 2, 3, or 4 consecutive days of symptom relief through D8, participants treated with nirmatrelvir-ritonavir were less likely to experience severe symptom relapse (Fig. 3A). Of note, out of the 228 or 206 nirmatrelvir-ritonavir participants who achieved 3 or 4 consecutive days of symptom relief, respectively, none experienced symptom relapse (Fig. 3A). Among the present and/or persistent VLR participants, rebounds were noted in asymptomatic participants and in participants with no moderate-severe symptom recurrence (Fig. 3B), suggesting minimal to no association of rebound and moderate to severe symptom recurrence. Similar results were noted in the transient VLR group (data not shown). The data does suggest that achieving sustained symptom relief is important, and the risk of severe symptom relapse is lower in nirmatrelvir-ritonavir treatment group who achieved sustained symptom relief compared to placebo. In brief, VLR was not associated with recurrence of severe symptoms in the EPIC-HR cohort.

VLR vs Geographical Location, symptom initiation, and Serological Status

A preliminary assessment was conducted to determine if VLR differed by geographical region or baseline serological status impacts subsequent VLR phenomena. VLR did not appear to be limited to a geographical location (see Supplemental Tables) or timing of symptom initiation relative to treatment (data not shown). Table 1 depicts the serostatus of VLR groups vs all EPIC-HR. Seropositivity was 51%
and 52% for placebo and nirmatrelvir-ritonavir, respectively, across the EPIC-HR population. In the present and/or persistent VLR population, seropositivity was 59% and 57%, and in the transient VLR population seropositivity was 43% and 28%, for placebo and nirmatrelvir-ritonavir, respectively. In brief, there did not appear to be a strong association with serostatus when entering the study (either positive or negative) and VLR.

Table 1: Baseline serological status in EPIC-HR and VLR groups.

<table>
<thead>
<tr>
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<th>EPIC-HR – All n/N (%)</th>
<th>Present/Persistent VLR n/N (%)</th>
<th>Transient VLR n/N (%)</th>
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<tbody>
<tr>
<td></td>
<td>Seropositive Seronegative</td>
<td>Seropositive Seronegative</td>
<td>Seropositive Seronegative</td>
</tr>
<tr>
<td>Placebo</td>
<td>568/1115 (51%) 534/1115 (48%)</td>
<td>10/17 (59%) 7/17 (41%)</td>
<td>10/23 (43%) 12/23 (52%)</td>
</tr>
<tr>
<td>Nirmatrelvir- Ritonavir</td>
<td>579/1109 (52%) 517/1109 (47%)</td>
<td>13/23 (57%) 10/23 (43%)</td>
<td>13/46 (28%) 33/46 (72%)</td>
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VLR Lineage and TEMs

Next-generation sequencing was utilized to monitor TEMs in the Mpro gene and cleavage regions. Viral sequencing showed that Delta was the dominant variant in the EPIC-HR populations (>98% of study participants; data not shown). Within the VLR populations, Delta was also the dominant variant.

The Mpro gene and cleavage region TEMs were evaluated using the later of D10 and D14 visits with sequencing data (D10/D14 TEM [Fig. 4]). Within the present and/or persistent VLR nirmatrelvir-ritonavir group, 18 out of the 23 participants had sufficient sequencing data to perform TEM analyses on D10 and/or D14 samples. Among those, 12 participants exhibited no Mpro gene or cleavage TEMs, while 6 participants showed one or more Mpro gene or cleavage D10/D14 TEM. Within the VLR placebo participants, 13 out of the 17 had sufficient sequencing data to perform TEM analyses on D10 and/or D14 samples. Among those, 9 contained no Mpro gene or cleavage D10 / D14 TEMs, and 4 had one or more Mpro/cleavage on D10/D14 TEMs. From the available sequence data, there were no single or cluster of Mpro gene or cleavage mutations in participants classified as VLR. In addition, there were no single or cluster of Mpro gene or cleavage mutations predominant in only nirmatrelvir-ritonavir participants. A full analysis of TEMs in the entire EPIC-HR study (including transient VLR) has found no significant association between nirmatrelvir-ritonavir treatment and the Mpro gene or cleavage region mutations occurring with VL ≥4 log<sub>10</sub> copies/mL by D5 (end of treatment) or D14 (data not shown).
Discussion

Emergence of viral resistance is an important concern in the antiviral drug development, and recent reports of recurrence of SARS-CoV-2 infection with symptoms following nirmatrelvir-ritonavir raised questions regarding the emergence of potential resistance\(^7,^8\). During the early part of the pandemic, recurrence of infection and symptoms was described\(^9\), and the recently reported recurrences did not appear to be associated with viral resistance or reinfection with a novel strain\(^7\). However, additional study is needed. To understand reports of recurrence following nirmatrelvir-ritonavir use, the current study utilized data from the EPIC-HR study to characterize the relationship between VLR following treatment and nirmatrelvir-ritonavir administration, nirmatrelvir exposure, hospitalization/death, moderate-severe symptom recurrence, baseline serostatus, geographical occurrence, and the Mpro gene/cleavage treatment emergent mutations. Two definitions of VLR were considered based on probability of resistance risk. It should be noted that no standard for SARS-CoV-2 rebound criteria has yet been established, and the distinction between rebound and re-infection remains gray. In the current treatise, present and persistent rebound was defined if the latter of the D10 and D14 VL was at least \(0.5 \log_{10}\) copies/mL higher than end of treatment (D5) VL and the sample VL remained greater or equal to \(2.7 \log_{10}\) copies/mL (the LLoQ of the viral sequencing assay). The proportion of participants who met the present and/or persistent VLR criteria was low, 2.32% in the nirmatrelvir-ritonavir population and 1.73% in the placebo population, when compared to the total population. A second criteria was defined to capture those participants in which the rebound was known to be transient. The proportion of participants who met the transient VLR criteria was 2.35% (23/980) vs 4.65% (46/990) for placebo vs nirmatrelvir-ritonavir, respectively. Combined, the range of VLR was 2%-7%. Within EPIC-HR, VLR occurred in both placebo and nirmatrelvir-ritonavir treatment arms, and there was no association of VLR with low nirmatrelvir exposure, severe disease recurrence, baseline serological status, geographical occurrence, or the emergence of the Mpro gene/cleavage site resistant mutations in persistent VLR cases.

Early studies suggested that SARS-CoV-2 VL may exhibit both bi- and multi-phasic kinetics as part of the natural course of infection\(^10,^11\). Preliminary VL modeling suggests an important balance between immune activation and actual viral copy number with models predicting rebounds when copy number and immune response are unbalanced\(^12,^13\). The occurrence of VLR in placebo highlights that the phenomenon does occur as a natural course of COVID-19 disease in a small percentage of patients. Modeling also suggests the importance of immune factors in characterizing the probability of recurrence\(^12,^13\), expecting that additional factors would need to be captured to better predict VLR and associated symptom recurrence. Regardless, VLR in EPIC-HR was not associated with recurrence of severe symptoms, and nirmatrelvir-ritonavir does reduce severe outcomes of disease, preventing COVID-19–related hospitalization (88% risk reduction) and all-cause death (100% risk reduction).

There are limitations to the analysis. The EPIC-HR population was comprised mainly of subjects infected with the Delta VoC, who, though many had been exposed to coronavirus, had not been vaccinated with a SARS-CoV-2 vaccine. The current rebound cases are being observed in a mostly vaccinated population...
that are infected with Omicron variants. From the EPIC-HR serostatus data, there is no evidence that VLR is more likely to occur in a seronegative patient, and as many are now seropositive, this seems moot; however, work is still required to quantitatively assess the kinetics of the immune response during infection. Though EPIC-HR efficacy was observed with Delta variant infections, real-world data is also available demonstrating that nirmatrelvir is more effective at preventing severe outcomes of COVID-19 than other oral therapeutics in populations infected with Omicron, highlighting that with current VoCs, the efficacy of nirmatrelvir is not diminished. The rate of VLR in an Omicron-infected population has not been systematically studied; however, Stegger et al. observed that Omicron recurrence with the same subvariant indicative of VLR was observed in an untreated population.

In summary, nirmatrelvir-ritonavir continues to be an effective treatment option for mild-moderate COVID-19 in high-risk adults and pediatric patients (>12 years weighing >40 kg) and an important tool in the ongoing pandemic.

**Declarations**

**Competing Interest Statement**

MLB, RC, HL-T, YZ, SG, CH, WH, ZW, LH, BSP, WB, PC, BD, SM, JH, ASA, HDS are employees of Pfizer and may be shareholders/option holders in Pfizer Inc.

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**References**


Figures
Figure 1

**VLR occurs in both treatment arms.** A) VL changes in EPIC-HR populations with symptoms within 5 days of treatment. Red Line indicates population summary. B) Present and/or persistent VLR. C) Transient VLR. Orange line represents LLoQ of the VL RT-PCR assay ($2 \log_{10}$ copies/mL). Blue lines in B) and C) represent hospitalization.
Figure 2

Nirmatrelvir exposures in present and/or persistent VLR (n=23) did not differ from overall EPIC-HR population exposures. Observed nirmatrelvir plasma concentrations in VLR (solid circles) vs EPIC-HR study population concentrations (gray x).
Figure 3

VLR is not associated with recurrence of severe disease. A) Participants on nirmatrelvir-ritonavir who achieved 3-4 consecutive days of symptom relief showed lower relapse during D8-D28 (0%) compared to placebo (1%). B) Individual moderate-severe symptoms in present and/or persistent VLR over 28 days.
Figure 4

D10/D14 Mpro gene and cleavage region TEMs in present/persistent VLR.

Supplementary Files

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- Supplement.docx