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# Independent FDA Analyses of Nirmatrelvir/Ritonavir Resistance in the Phase 2/3 Trials EPIC-HR and EPIC-SR

**Jonathan M.O. Rawson, Eric F. Donaldson, Julian J. O'Rear, and Patrick R. Harrington**
Division of Antivirals (DAV)/OID/OND/CDER, 10903 New Hampshire Ave, Silver Spring, Maryland 20993

## Abstract

**Introduction:** PAXLOVID consists of nirmatrelvir, an inhibitor of SARS-CoV-2 main protease (Mpro), copackaged with ritonavir, a pharmacokinetic enhancer. Nirmatrelvir/ritonavir received emergency use authorization in the United States in 2021 and was approved in 2023. However, there is limited published information on SARS-CoV-2 clinical resistance to nirmatrelvir/ritonavir. **Methods:** To investigate SARS-CoV-2 resistance development to nirmatrelvir/ritonavir in treated patients, we analyzed baseline and matching post-baseline SARS-CoV-2 next-generation sequencing data from 1,862 participants (912 nirmatrelvir/ritonavir, 950 placebo) in EPIC-HR and EPIC-SR, which were Phase 2/3, randomized, double-blind, placebo-controlled trials in participants with mild-to-moderate COVID-19. Potential resistance-associated substitutions (RAS) were defined as those that were enriched in nirmatrelvir/ritonavir-treated participants or occurred at Mpro positions of interest, defined using nonclinical data. SARS-CoV-2 sequence databases were analyzed to characterize temporal frequencies of nirmatrelvir/ritonavir RAS in circulating viruses. **Results:** In EPIC-HR, nirmatrelvir/ritonavir RAS included Mpro T21I (n=1), E166V (n=3), A173T (n=1), and T304I (n=1), with E166V being the clearest RAS observed. In EPIC-SR, no RAS were detected. Nirmatrelvir/ritonavir RAS were not associated with hospitalization or death. Analyses of SARS-CoV-2 sequence databases did not reveal concerning increases in the frequencies of nirmatrelvir/ritonavir RAS over time. **Conclusions:** In clinical trials, emergence of SARS-CoV-2 resistance to nirmatrelvir/ritonavir was infrequent (<0.3%-1.1%). Surveillance data currently indicate a low frequency of circulating SARS-CoV-2 variants with nirmatrelvir/ritonavir RAS. Collectively, these results provide the most comprehensive analysis of SARS-CoV-2 resistance to nirmatrelvir/ritonavir in the clinical setting to date. Viral sequences should continue to be closely monitored to identify the potential emergence of nirmatrelvir/ritonavir-resistant variants.

## Introduction

- PAXLOVID consists of nirmatrelvir (SARS-CoV-2 Mpro inhibitor) co-packaged with ritonavir (HIV-1 protease inhibitor and CYP3A inhibitor).
- PAXLOVID was authorized for emergency use by the FDA on 12/22/2021 and approved by the FDA on 5/25/2023 for the treatment of mild-to-moderate COVID-19 in high-risk adults.
- SARS-CoV-2 resistance to nirmatrelvir has been well characterized in nonclinical studies, e.g., using biochemical assays, cell-based Mpro reporter assays, recombinant viruses/replicons, and cell culture resistance selection experiments using authentic SARS-CoV-2.
- There is currently little published information on clinical resistance to nirmatrelvir/ritonavir; thus, the frequency, mechanisms, and consequences of clinical resistance to nirmatrelvir/ritonavir are unclear.

## Methods

- We analyzed SARS-CoV-2 whole-genome next-generation sequencing (NGS) data from 1,862 participants (912 nirmatrelvir/ritonavir, 950 placebo) with matching baseline and post-baseline results from the Phase 2/3 trials EPIC-HR and EPIC-SR.
- We analyzed both the sponsor's amino acid substitution frequency table and the sponsor's raw NGS data (fastq files) using two independent, FDA-established, bioinformatics pipelines (HIVE and CLC Genomics). These data were submitted to the FDA as part of the PAXLOVID NDA.
- We identified nirmatrelvir/ritonavir treatment-emergent substitutions (TES) and potential resistance-associated substitutions (RAS), which were defined as TES that were enriched in the nirmatrelvir/ritonavir arm (relative to placebo) or occurred at specific Mpro positions of interest based on nonclinical studies.

## Results

| Mpro Amino Acid Change(s) | Close Proximity to Nirmatrelvir[a] | Emerged in Cell Culture[b] | $IC_{50}$/K, Fold-Change (Biochemical Assay)[c,e] | $EC_{50}$ Fold-Change (Cell Culture)[d,e] | | | |
|---|---|---|---|---|---|---|---|
| | | | | Selected Virus | Recombinant Virus | Replicon | Mpro Reporter |
| T21I | | X | 0.8, 0.9, 1.6 | 2.3, 17 | 1.1-1.5, 4.6 | 2.4 | 1.2 |
| S46F | | X | 1.0, 1.3 | | | | <2 |
| L50F | | X | 0.2, 0.5, 0.9, 1.1 | | 1.5, 1.5-2.1, 1.2-2.3, 4.2 | | 2.0 |
| P108S | | X | 1.7, 2.9 | | | | |
| T135I | | X | 1.2, 2.2 | | | | |
| F140L | X | X | 1.2, 7.6 | | 4.1 | | |
| S144A | X | X | 1.2, 14, 19, 46 | | 2.2, 5.0, 5.3 | | 8.0, 12 |
| C160F | | X | 0.6, 1.0 | | 2.1 | | |
| E166A | X | X | 10, 35, 39, 47 | | 1.3, 3.3 | | 21 |
| E166V | | X | 187, 219, 709, >1,538, 5,523, 7,700 | | Failed QC, 100, 25-288 | 132 | >300 |
| L167F | X | X | 1.4, <1.5, 4.4, 4.5 | 2.5 | 1.9-2.4 | | 3.5-8.5, 9.6 |
| T169I | | X | <1.4 | | | | <2 |
| H172Y | X | X | 31, 55, 146, 250 | | Failed QC, 15 | | |
| A173V | | X | 6.5, 16, 52 | 0.9 | 1.7, 1.8-2.3, 8.1 | | 9.8, 7.4-12, 16 |
| V186A | X | X | <0.8 | | | | |
| R188G | X | X | 38 | | | | |
| A191V | X | X | <0.8, 1.1, 1.4, 2.6 | 1.4, 0.7-1.5 | Failed QC | | |
| A193P | | X | 0.9, 1.3 | | | | |
| P252L | | X | <0.9 | | 5.9 | | 2.6 |
| S301P | | X | 0.2 | | | | |
| T304I | | X | 1.0 | 3.4, 3.5-4.5 | 2.1-2.5, 5.5 | | 1.4 |

QC, quality control.

**Table 1. Summary of nirmatrelvir nonclinical resistance data.** To inform our analyses of clinical resistance to nirmatrelvir/ritonavir, we first summarized all available data on nonclinical resistance to nirmatrelvir. The table above represents a small section of the nonclinical resistance table we compiled based on data from the sponsor and others. The table indicates SARS-CoV-2 Mpro residues that were located in close proximity of (≤5 Å based on analysis with PyMol) nirmatrelvir in cocrystal structures (a), Mpro substitutions that were selected by nirmatrelvir in cell culture (b), and nirmatrelvir activity fold-changes (relative to wild-type) in biochemical (c) and cell culture (d) assays. (e) Orange and red shading indicate substitutions that resulted in 3-<10-fold or ≥10-fold decreases in nirmatrelvir activity, respectively.

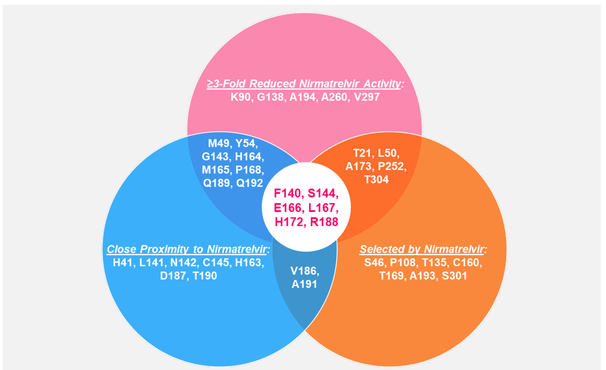



**Figure 1. SARS-CoV-2 Mpro positions potentially associated with nirmatrelvir resistance based on nonclinical studies.** The figure indicates SARS-CoV-2 Mpro residues that directly contacted or were located in close proximity (≤5 Å; PyMol analysis) of nirmatrelvir in cocrystal structures (left), were selected by nirmatrelvir in cell culture (right), or were associated with ≥3-fold reduced nirmatrelvir activity in biochemical and/or cell culture assays (top). In total, 40/306 (13%) SARS-CoV-2 Mpro residues fell into at least one of these groups, with 21/306 (6.8%) in at least two groups and 6/306 (2.0%) in all three groups: F140, S144, E166, L167, H172, and R188. These 40 residues were subjected to more intense clinical resistance analyses.

| Amino Acid Substitution | Detected by Applicant or FDA | Reporting Criteria | # NIR/r-Tx Participants (n=530) | # Placebo-Tx Participants (n=548) | AA Frequency in NIR/r-Tx Participants | Treatment Failure? (NIR/r-Tx Participants) | Nonclinical NIR Activity Fold-Change (AA) |
|---|---|---|---|---|---|---|---|
| **Mpro Substitutions** | | | | | | | |
| G11C/S/V | Applicant | TE | 3 | 0 | 10-16% | No | Inactive[b] (V) |
| T21I | FDA | RA, S | 1 | 0 | 11% | No | 0.8-1.6[b], 1.1-17[c] |
| K90R | Applicant, FDA | RA | 2 | 2 | 12-13% | No | 0.8-2.5[b], 1.2-3.7[c] |
| T111I | Applicant | TE | 3 | 1 | 11-14% | No | ND |
| C145F/R/Y | Applicant, FDA (F) | CP | 1 | 0 | 13-16% | No | Inactive[b] (F) |
| C160R | Applicant, FDA | S | 1 | 0 | 15% | No | ND |
| E166V | Applicant, FDA | CP, RA, S, TE | 3 | 0 | 24-94% | No | 187-7,700[c], 25->300[d] |
| P168S | Applicant | CP, RA | 1 | 0 | 12% | No | 0.6[b], <2[c] |
| A173T | Applicant, FDA | RA, S | 1 | 0 | 20% | No | 1.8[b], 4.0-4.1[c] |
| V186G | Applicant | CP, S | 6 | 4 | 14-18% | No | 1.3[b] |
| R188M | Applicant | CP, RA, S | 1 | 0 | 13% | No | 1.0[b] |
| Q189K | Applicant | CP, RA | 5 | 4 | 14-32% | No | <1.6-16[b], 0.2[c] |
| T190I | Applicant | CP | 1 | 1 | 24% | No | 0.7-2.0[b] |
| A260S/T/V | Applicant | RA, TE | 7 (1S, 4T, 2V) | 2 (1S, 1V) | 11-20% | Yes (n=1), No (n=6) | <0.3[b] (S), <0.5[b] (T), 0.6-3.3[b] (V) |
| V297F/del | Applicant, FDA (F) | RA | 2 | 0 | 14-15% | No | <2.8[b] (F) |
| T304I | Applicant, FDA | RA, S | 1 | 0 | 24% | No | 1.0[b], 1.4-5.5[c] |
| **Mpro Cleavage Site Substitutions** | | | | | | | |
| CS#2 A357I/V | Applicant, FDA | TE | 3 | 0 | 100% | No | ND |
| CS#8 A5328S/T | Applicant | TE | 3 (S) | 1 (T) | 10-34% | No | 0.3[c] (S) |
| CS#11 S6799A/Y | Applicant | TE | 3 (2A, 1Y) | 0 | 10-15% | No | 0.7[c] (A) |

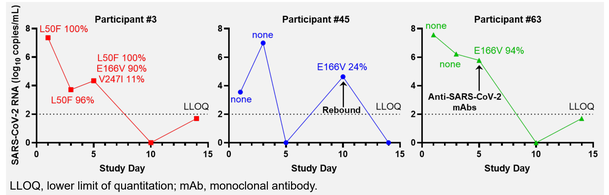
AA, amino acid; b, biochemical assay; c, cell culture assay; CP, residue located in close proximity to nirmatrelvir; ND, no data; NIR/r, nirmatrelvir/ritonavir; RA, residue associated with ≥3-fold reduced activity of nirmatrelvir in nonclinical assays; S, selected by nirmatrelvir in cell culture; TE, treatment-emergent; Tx, treated.

**Table 2. Nirmatrelvir/ritonavir potential RAS observed in EPIC-HR.** Mpro and Mpro cleavage site potential RAS were defined as: a) TES identified in ≥3 nirmatrelvir/ritonavir-treated participants and with a nirmatrelvir/ritonavir-to-placebo ratio ≥2, or b) TES identified at one of the 40 Mpro positions of interest based on nonclinical studies. Mpro E166V (n=3, dark pink shading) was the clearest RAS, as it was identified in multiple participants and led to 25->300-fold reduced nirmatrelvir activity in cell culture. T21I, A173T, and T304I (n=1 each, light pink shading) were also identified as true RAS because they were observed only in nirmatrelvir/ritonavir-treated participants and led to ≥3-fold reduced nirmatrelvir activity in cell culture. The other substitutions were not identified as true RAS because they: a) were observed only at low frequencies, b) occurred at a similar rate in the placebo arm, c) did not affect nirmatrelvir activity in nonclinical assays, and/or d) were located distant from the nirmatrelvir binding site. The clinical resistance frequency was determined to be 1.1% (6/530), and importantly, resistance was not associated with treatment failure, i.e., hospitalization or death through Day 28.

| Amino Acid Substitution | Detected by Applicant or FDA | Reporting Criteria | # NIR/r-Tx Participants (n=382) | # Placebo-Tx Participants (n=402) | AA Frequency in NIR/r-Tx Participants | Treatment Failure? (NIR/r-Tx Participants) | Nonclinical NIR Activity Fold-Change (AA) |
|---|---|---|---|---|---|---|---|
| **Mpro Substitutions** | | | | | | | |
| L50F | Applicant | RA, S | 1 | 0 | 11% | No | 0.2-1.1[b], 1.5-4.2[c] |
| K90R | Applicant, FDA | RA | 2 | 1 | 19-20% | No | 0.8-2.5[b], 1.2-3.7[c] |
| T98I/R | Applicant | TE | 3 (2I, 1R) | 0 | 12-15% | No | <0.3[b] (I) |
| P108S | Applicant | S | 1 | 0 | 24% | No | 1.7-2.9[b] |
| H172del | Applicant | CP, RA, S | 1 | 0 | 13% | No | ND |
| Q189K | Applicant, FDA | CP, RA | 7 | 11 | 10-54% | No | 1.6-16[b], 0.2[c] |
| S301L | Applicant | S | 1 | 0 | 11% | No | ND |
| **Mpro Cleavage Site Substitutions** | | | | | | | |
| CS#8 A5328S/V | Applicant | TE | 4 (2S, 2V) | 0 | 11-37% | No | 0.3[c] (S), 0.2[c] (V) |

AA, amino acid; b, biochemical assay; c, cell culture assay; CP, residue located in close proximity to nirmatrelvir; ND, no data; NIR/r, nirmatrelvir/ritonavir; RA, residue associated with ≥3-fold reduced activity of nirmatrelvir in nonclinical assays; S, selected by nirmatrelvir in cell culture; TE, treatment-emergent; Tx, treated.

**Table 3. Nirmatrelvir/ritonavir potential RAS observed in EPIC-SR.** In EPIC-SR, which included participants infected with the SARS-CoV-2 Omicron variant (mainly BA.2), none of the Mpro and Mpro cleavage site substitutions listed were identified as true RAS for the same reasons listed under Table 2. The clinical resistance frequency was determined to be <0.3% (<1/382).




LLOQ, lower limit of quantitation; mAb, monoclonal antibody.

**Figure 2. Nirmatrelvir/ritonavir-treated participants with the SARS-CoV-2 Mpro E166V substitution in EPIC-HR.** Participants with E166V were characterized in greater detail. The data above represent SARS-CoV-2 RNA levels in nasopharyngeal swab samples collected on Days 1-14, with the labels indicating Mpro substitutions detected (≥10% frequency) at each timepoint. Missing labels indicate no data. All 3 participants with E166V were anti-SARS-CoV-2 seronegative at baseline and infected with the Delta variant. None of the participants were vaccinated against COVID-19 or were immunocompromised. Participant #3 had the L50F substitution (a known compensatory substitution for E166V) on Day 1 and acquired E166V on Day 5. Participant #45 had E166V and viral RNA rebound on Day 10. Participant #63 had E166V on Day 5 and was also treated with anti-SARS-CoV-2 mAbs.

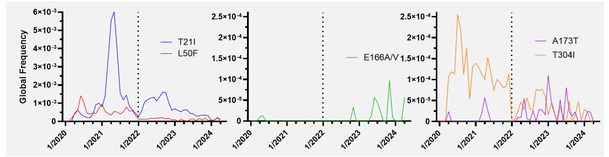



**Figure 3. Global monthly frequencies of clinical nirmatrelvir/ritonavir Mpro RAS.** Global monthly frequencies of Mpro RAS were determined using covSPECTRUM. The dashed line indicates the date that the FDA authorized PAXLOVID (12/22/2021). No concerning trends in frequencies were identified.

## Conclusions

- Clinical resistance to nirmatrelvir/ritonavir was infrequent, occurring in 0.7% of participants overall across EPIC-HR and EPIC-SR.
- Clinical resistance was due to the Mpro T21I (n=1), E166V (n=3), A173T (n=1), and T304I (n=1) substitutions. Based on published literature, Mpro L50F and E166A should also be considered clinical RAS.
- Clinical resistance was not associated with hospitalization or death. In some cases (one participant each with E166V or T304I), resistance was associated with viral RNA rebound, but we previously demonstrated that viral RNA rebound was not usually associated with resistance.
- Surveillance data did not reveal sustained increases or other concerning trends in the frequencies of nirmatrelvir/ritonavir clinical RAS. Clinical resistance to nirmatrelvir/ritonavir may be infrequent because: a) clinical Mpro RAS result in fitness defects, b) SARS-CoV-2 infections are typically acute, and/or c) SARS-CoV-2 variants are continually being displaced by novel variants encoding changes in spike.

## Acknowledgements

We would like to acknowledge the sponsor (Pfizer), the FDA PAXLOVID review team, and the EPIC-HR/EPIC-SR investigators and participants.