Xocova – Resistance

This medical response contains information in addition to that provided in the XOCOVA® (ensitrelvir) tablets, for oral use Full Prescribing Information approved by the U.S. Food and Drug Administration (FDA). Shionogi does not recommend the use of XOCOVA in any manner other than as described in the Full Prescribing Information. **Please read the attached XOCOVA Full Prescribing Information.**

1. SUMMARY

- Resistance to ensitrelvir is thought to be due to mutations in the virus on the *nsp5* gene, which encodes for the targeted $3CL^{pro}$.[1,2] Amino acid substitutions (AASs) in this gene can affect antiviral activity[3-9]

Clinical Trials

- **Hayden et al. (2026)** conducted a post-hoc analysis of the SCORPIO-PEP clinical study which showed TEAASs in 20/1030 (1.9%) mITT and 20/114 (14.9%) ITT ensitrelvir-treated HHCs by Day 28. The most common mutation was M49L with 9/1030 in the mITT group and 11/114 in the ITT group.[10]
- **Choudhary et al. (2025)** conducted an exploratory analysis of the SCORPIO-HR clinical study that examined the emergence of mutations associated with treatment resistance after ensitrelvir treatment.[11]
  - T25A/N and M49I/L were found more frequently in the ensitrelvir group (n=18; T25A/N: n=4, M49I/L: n=14) than in the placebo group (n=0).[11]
- **Uehara et al. (2025)** presented an analysis of the SCORPIO-SR clinical study to assess the impact of SARS-CoV-2 *nsp5* TEAASs on virological and clinical outcomes in patients treated with ensitrelvir.[12]
  - In the enstirelvir 125 mg arm, 204/345 patients from the ITT population had paired sequence data and 19 showed treatment-emergent amino acid substitutions (TEAASs). The most commonly observed TEAASs were M49L (n=12; 5.9%), M49I (n=3; 1.5%), and S144A (n=2; 1%).[12]
  - The median time to resolution of the 5 COVID-19 symptoms was comparable between patients with and without M49L, M49, or S144A (158.8 hours [95%CI 112.1–281.9] vs 189.7 hours [95%CI 151.4–234.4], respectively).[12]
  - In patients with M49I, M49L or S144A, the decrease in viral RNA was slower compared to those without TEAASs, but the decrease in viral titers were comparable.[12]

Surveillance Studies

- **Inoue et al. (2025)** conducted a retrospective study to identify gene mutations resulting in resistance to 3CL protease inhibitors in patients at Kansai Medical University General Medical Center in Japan.[13]
  - In samples randomly selected for genomic analysis, T25A, T25I, and T45N mutations were found in 1/1156 cases each, and M49L mutations were found in 6/1156 cases; 5/9 of the patients had previously received ensitrelvir treatment and all 5 had underlying medical history of either malignant lymphoma or acute myeloid leukemia.[13]
  - In patients with a viral load ≥5000 copies/μL 10 days after ensitrelvir administration, T25A, T25N, M49I, and M49L mutations were found in 19% (3/16), 13% (2/16), 31% (5/16), and 63% (10/16) of cases, respectively.[13]

    - Genomic analysis of six immunocompromised patients with persistent infection showed that M49I and D48G mutations emerged by Day 5 and M49L by Day 6 after starting ensitrelvir. Over time, M49L became the dominant mutation, while others declined.[13]
- **Doi et al. (2024)** conducted an analysis using data from the GISAID EpiCoV™ database to detect amino acid substitutions (AAS) mutations in *nsp5*, which is associated with conferred resistance to ensitrelvir.[14]
    - Most cases of *nsp5* AAS were not associated with ensitrelvir treatment, although certain AASs were observed as having a regional bias.[14]
    - M49L was reported in 277/845,796 (0.03%) of cases globally, 92.4% (256/277) of which were found in Japan.[14]
    - M49I was reported in 90 cases, most of which were reported in May 2023 in the US.[14]

In Vitro Studies

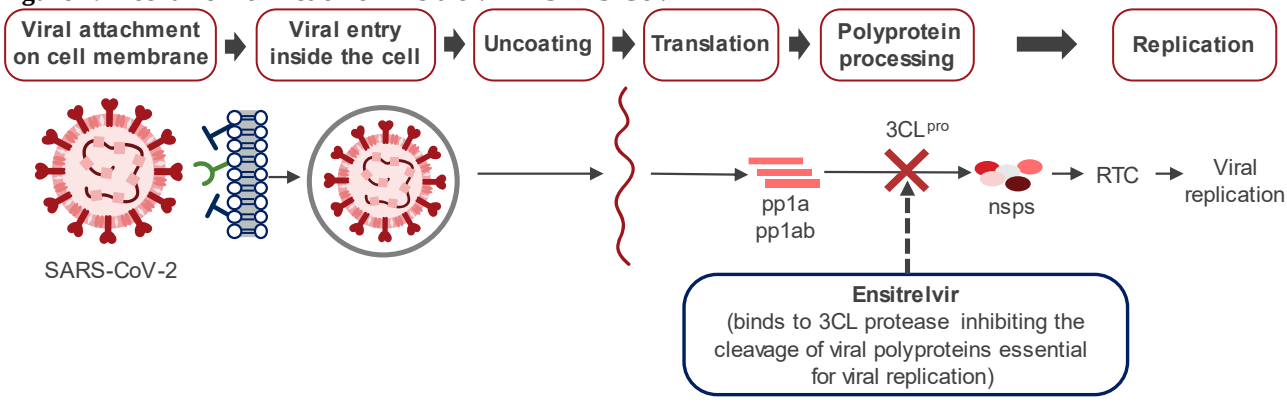
- **Baba et al (2024)** conducted *in vitro* viral passage studies showed AAS-containing viruses similar to the TEAASs identified in SCORPIO-SR, which had reduced susceptibility to ensitrelvir but did not have higher replicative fitness compared with wild-type virus.[3,4]
- **Rauch et al (2024)** conducted a study in a VSV-based system that analyzed a wide range of $3CL^{pro}$ mutations for effect on viral fitness, proximity to antiviral binding sites, and effect on susceptibility to ensitrelvir and nirmatrelvir.[15]

2. BACKGROUND

The active site of $3CL^{pro}$, to which ensitrelvir binds as seen in **Figure 1**, is highly conserved among viruses in the Coronaviridae family and $3CL^{pro}$ is less prone to spontaneous mutations when compared with the spike protein. This suggests that, aside from drug-induced mutations, significant naturally occurring genetic mutations that may affect the activity of $3CL^{pro}$ inhibitors are not likely to be common.[1,2] As $3CL^{pro}$ and the spike protein are distinct proteins encoded in different regions of the viral genome, the antiviral efficacy of a $3CL^{pro}$ inhibitor is unlikely to be affected by mutations within the spike protein and is also unlikely to induce mutations within the spike protein.[1]

Certain mutations in the virus on the *nsp5* gene can cause substitutions in the amino acids on the targeted $3CL^{pro}$ protein. These AASs can affect the antiviral activity of $3CL^{pro}$ inhibitors.[3-9]

Figure 1. Mechanism of Action of Ensitrelvir in SARS-CoV-2[1,15,16]



This image is adapted under a Creative Commons CC BY 4.0 International License specific to the article published by Frontiers Media S.A.: Jeong GU et al. Front Microbiol. 2020;11:1723. doi:10.3389/fmicb.2020.01723

Abbreviations: $3CL^{pro}$, 3-chymotrypsin-like protease; nsps, nonstructural proteins; pp1a/pp1ab, polyproteins 1a/1ab; RTC, replicase-transcriptase complex; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

3. CLINICAL TRIALS

SCORPIO-PEP (Phase 3 Study)

SCORPIO-PEP (NCT05897541) is a completed, global Phase 3 study that evaluated the safety and efficacy of ensitrelvir vs. placebo in the prevention of symptomatic SARS-CoV-2 infection in HHCs of people who tested positive for COVID-19 conducted from June 2023 to September 2024 (see **Appendix-SCORPIO-PEP** for more information on study design).[10,17]

HHC participants with a negative screening test for SARS-CoV-2 infection and no symptoms, who were exposed to a person living in their household with symptomatic COVID-19, were randomly assigned in a 1:1 ratio to receive ensitrelvir 125 mg (375 mg on Day 1 and 125 mg on Days 2-5) or placebo once daily for 5 days. Study participants began treatment within 3 days of when the household member with COVID-19 began showing symptoms.[10]

The primary endpoint was the proportion of HHCs with COVID-19 development (central laboratory–confirmed RT-PCR positivity of SARS-CoV-2 and ≥1 of the 14 COVID-19 symptoms lasting ≥48 hours) through Day 10 in the mITT population.[10]

- The ITT population was comprised of HHCs with a local negative test for SARS-CoV-2 at baseline who were enrolled within 72 hours following symptom onset in the index patient .[10]
- The mITT population included HHCs who were randomized, confirmed SARS-CoV-2 negative by central RT-PCR at baseline, and received ≥1 dose of study treatment.[10]
- The ITT-BP included randomized HCCs who tested positive for SARS-CoV-2 by central RT-PCR at baseline.[10,18]

In a post-hoc analysis of the mITT and ITT-BP groups treated with ensitrelvir, whole-genome sequencing showed treatment-emergent amino acid substitutions (TEAASs) in 20 (1.9%) and 17 (14.9%) HHCs by Day 28, respectively (**Table 1**).[10,18] Among the related index patients, 20 had no history of antiviral therapy, while 10 were given ensitrelvir, 2 received molnupiravir, and 3 were treated with nirmatrelvir/ritonavir. This distribution was comparable to that observed in index patients linked to household contacts without TEAAS. No TEAAS associated with ensitrelvir were found in placebo recipients, including 101 individuals exposed to ensitrelvir-treated index patients. Of those with detected TEAAS, 25 reported COVID-19 symptoms.[10]

Table 1: Summary of 3CL Substitutions Associated with Reduced Susceptibility to Ensitrelvir in HHCs.[18]

Substitution	mITT (N=1,030)	ITTBP (N=114)
Number with sequencing[a]	51	26
M49L	9	11
M49I	2	0
T25A	3	1
T25I	1	0
P52S	3	1
T25A/M49L	1[b]	1[c]
M49I/P52S	0	1[c]
M49I/S144A	0	1
T25I/M49I	1[b]	0
T25N/M49I	0	1[b]
Total	**20**	**17**

Isolates reported in the publication were collected through Day 28. Additional isolates were included in the XOCOVA U.S. Prescribing Information.

Abbreviations: 3CL, 3C-like protease; EC_{50}, 50% efficacy concentration; HHC, household contact; ITTBP, intention-to-treat with baseline RT-PCR–positive; mITT, modified intention to treat; NGS, next-generation sequencing; RNA, ribonucleic acid.

[a]Number of participants with post-dose sequence data available. Samples containing ≥4.0 $\log_{10}$ copies/mL. Viral RNA was analyzed using NGS sequencing.

[b]Multiple substitutions detected in viruses from the same sample.

[c]Substitutions were not detected in the same sample; instead, different substitutions were detected at different timepoints in a single participant.

Fold susceptibility changes of ensitrelvir in EC_{50}: T25A (14.4), T25I (2.9), M49I (5.3), M49L (33.0), P52S (4.4), S144A (8.2) [12], T25N (4.8)

Table adapted from Hayden et al. NEJM 2026.

Additional isolates were reported in **XOCOVA Full Prescribing Information Section 12.4 Microbiology** from the SCORPIO-PEP clinical trial, as seen below:

> In Trial SCORPIO-PEP, post-baseline sequencing data were provided for 29% (93/318) of XOCOVA-treated ITT subjects who had at least one SARS-CoV-2 RT-PCR-positive post-baseline sample. SARS-CoV-2 M^{pro} amino acid changes (relative to the Wuhan-Hu-1 reference sequence) were classified as XOCOVA resistance-associated substitutions (RAS) based on the number of subjects in each arm with the substitution, the frequency of the substitution within the samples, and the impact of the substitution on ensitrelvir susceptibility in cell culture (generally a ≥3-fold decrease). The following M^{pro} amino acid changes were considered XOCOVA RAS (n: number of XOCOVA-treated subjects): T25A/I/N (n=9), D48G (n=2), M49I/L (n=29), P52S (n=5), S144A (n=1), and E166A (n=1). In some subjects, multiple M^{pro} substitutions were observed, either as single substitutions in different viruses or as combinations in the same virus, with T25A+E166A and M49I+S144A detected as combinations in the same virus. All of the single M^{pro} substitutions except T25I reduced ensitrelvir activity ≥3-fold in cell culture, whereas the combinations have not been tested. The proportion of XOCOVA-treated subjects with XOCOVA RAS was 3.4% (40/1,190) in the ITT, 1.9% (20/1,030) in the mITT, and 18% (20/114) in the ITT baseline positive populations. There were insufficient numbers of subjects to determine the impact of XOCOVA RAS on viral RNA shedding and clinical outcomes.

SCORPIO-HR (Global Phase 3 Study)

SCORPIO-HR (NCT05305547) is a global Phase 3, multicenter, randomized, double-blind, parallel-group, 24-week clinical study that assessed the clinical and antiviral effects of ensitrelvir (oral once daily for 5 days) vs placebo in nonhospitalized patients with COVID-19 with or without high-risk factors for severe COVID-19 (see **Appendix-SCORPIO-HR** for more information on study design and results).[19,20]

This study was conducted across North America, South America, Europe, Asia, and Africa. It is a part of the Accelerating COVID-19 Therapeutic Interventions and Vaccines (ACTIV)-2 clinical trials portfolio, overseen by the AIDS Clinical Trials Group (ACTG) and supported by the National Institutes of Health (NIH). This study was conducted when Omicron variant infection was predominant and among a mostly vaccinated population.[19,20]

Choudhary et al. (2025) conducted an exploratory analysis of the SCORPIO-HR study that examined the emergence of mutations associated with treatment resistance after ensitrelvir treatment. Whole genome and *nsp5* sequencing were done for SARS-CoV-2 samples from nasopharyngeal swabs collected on Days 1, 4, 8, and 16 from 1888 participants (ensitrelvir n=945 vs placebo n=943) in the mITT population. Changes that reduced susceptibility by ≥3-fold were considered treatment-emergent resistance-associated mutations.[11]

Resistance Mutations:

- Baseline characteristics of participants in the ensitrelvir group vs the placebo group were generally balanced. The distribution of SARS-CoV-2 variants was consistent across the study groups.[11]
- T25A/N and M49I/L were found more frequently in the ensitrelvir group (n=18; T25A/N: n=4, M49I/L: n=14) than in the placebo group (n=0).[11]
- GISAID surveillance data shows that the frequency of M49L substitutions increased around September 2023 in Japan to 1.5% (14/945) and then consistently declined to approximately 0.5% (**Figure 2**).[11]
- E166A, which has been reported to reduce susceptibility to ensitrelvir, was reported in one patient.[11]
- When compared to those without mutations (n=917) and the placebo group (n=934), the patients with treatment-emergent resistance-associated mutations in the ensitrelvir group (n=18) had higher SARS-CoV-2 RNA viral load at baseline, Day 4, and Day 8.[11]
- This exploratory analysis includes a small number of participants with resistance mutations, and findings should be interpreted with caution.

Figure 2. GISAID Surveillance Data[11]

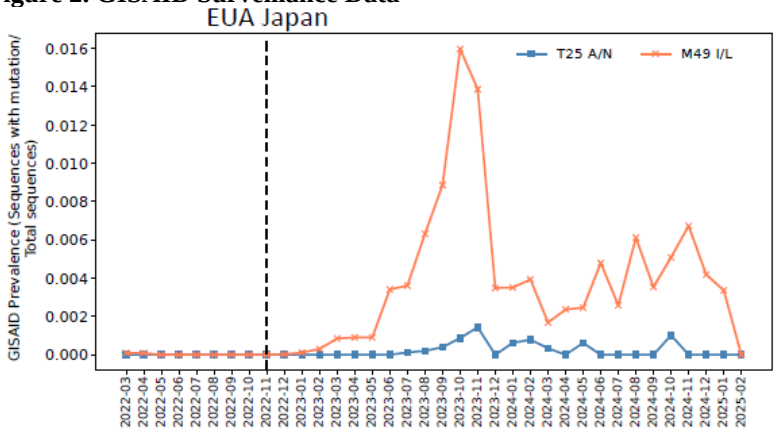


Figure adopted from Choudhary et al. CROI. 2025.
Abbreviations: EAU, Emergency Use Authorization.

SCORPIO-SR (Phase 3 Study)

The SCORPIO-SR (Phase 3 part) trial is part of the Phase 2/3, multicenter, randomized, double-blind, placebo-controlled clinical study and evaluated the efficacy (clinical symptom improvement and antiviral effect) and safety of ensitrelvir administered orally, once daily for 5 days in patients with SARS-CoV-2 infection (see **Appendix-SCORPIO-SR** for more information on study design and results). SCORPIO-SR was conducted in Japan, South Korea, and Vietnam.[21]

Uehara et al. (2025) presented an analysis of the SCORPIO-SR study to assess the impact of SARS-Cov-2 *nsp5* TEAASs on virological and clinical outcomes in patients treated with ensitrelvir. Viral genome sequences of *nsp5* (encoding $3CL^{pro}$) were analyzed using sequencing from pre-/post-treatment samples in ensitrelvir 125 mg-treated patients. The impact of TEAASs on time to resolution of the 5 COVID-19 symptoms and the effect on viral RNA and viral titer were investigated.[3,12]

Study Population

Among the 1821 patients enrolled in the SCORPIO-SR study, 1769 completed the study and 1030 were randomized in less than 72 hours of disease onset (primary analysis population).[4] Baseline demographics and disease characteristics of patients, including mean viral RNA level (6.98 ± 1.01 vs 6.93 ± 0.99 $\log_{10}$ copies/mL, respectively), were similar between the ensitrelvir 125 mg and placebo groups among the primary analysis population.[3]

Distribution of TEAAS

The analysis included 345 patients in the ensitrelvir 125 mg group, 204 of whom had paired sequence data and 9.3% (19/204) had *nsp5* TEAASs. The most commonly observed TEAASs (≥2 patients) were M49L (n=12), M49I (n=3), and S144A (n=2) (**Table 2**), including one patient where both M49L and M49I were identified, on Day 6 and Day 9, respectively.[3,12]

Table 2. Distribution of *nsp5* TEAAS in Patients from the SCORPIO-SR Phase 3 Clinical Study[3,12]

Patients with *nsp5* TEAASs, n (%)	Ensitrelvir 125 mg		Placebo	
	Patients with paired sequence data[a] (n=204)[b]	ITT population (n=345)	Patients with paired sequence data[a] (n=137)[b]	ITT population (n=341)
Any	19 (9.3)	19 (5.5)	3 (2.2)	3 (0.9)
M49L	12 (5.9)[c]	12 (3.5)	0	0
M49I	3 (1.5)[c]	3 (0.9)	0	0
S144A	2 (1.0)[d]	2 (0.6)	0	0
D216H	1 (0.5)	1 (0.3)	0	0
D48Y	1 (0.5)	1 (0.3)	0	0
S123Y	1 (0.5)	1 (0.3)	1 (0.7)	1 (0.3)
T25I	1 (0.5)[d]	1 (0.3)	0	0
T25A	1 (0.5)[c]	1 (0.3)	0	0
P252L	0	0	1 (0.7)	1 (0.3)
V212I	0	0	1 (0.7)	1 (0.3)

Abbreviations: ITT, intent-to-treat; TEAAS, treatment-emergent amino acid substitutions.
[a]Pre- (baseline) and post-treatment data.
[b]Only participants with a variant frequency of ≥15% were included in this analysis. If a patient experienced a particular substitution at the same position more than once, they were counted only once for that substitution; however, if a patient experienced different substitutions (whether at the same position and/or the same time point), they were counted once for each substitution.
[c]Including 1 participant with *nsp5* TEAASs T25A and M49L on Day 6 and M49I on Day 9.
[d]Including 1 participant with *nsp5* TEAASs T25I and S144A on Day 14.

Impact of TEAASs

In patients with or without M49L, M49, or S144A, the median time to resolution of the 5 COVID-19 symptoms was comparable (158.8 hours [95%CI 112.1–281.9] vs 189.7 hours [95%CI151.4–234.4], respectively) (**Table 3**).[3,12]

Table 3. Impact of TEAASs (M49L, M49I or S144A) on Time to Resolution of 5 COVID-19 Symptoms in the SCORPIO-SR Clinical Trial (ITT population)[a3,12]

	Ensitrelvir 125 mg		Placebo	
	With TEAASs (n=16)[b]	Without TEAASs (n=188)	With TEAASs (n=0)	Without TEAASs (n=137)
Patients, no.[c]	15	182	0	130
Events, n (%)	11 (73.3)	138 (75.8)	-	86 (66.2)
Censored, n (%)	4 (26.7)	44 (24.2)	-	44 (33.8)
Kaplan-Meier estimate (hours)				
Median (95% CI)	158.8 (112.1 – 281.9)	189.7 (151.4 – 234.4)	-	236.9 (174.5 – 318.6)

Abbreviations: CI, confidence interval; ITT, intent-to-treat; TEAASs, treatment-emergent amino acid substitutions.
[a]Randomized <72 h from disease onset and treated.
[b]1 patient in whom both M49L and M49I were detected was counted just once, thus 14 patients had either M49I or M49L and 2 had S144A.
[c]Analyzed population, which excluded participants with a score of 0 for all 5 COVID-19 symptoms and those with missing data.

Viral RNA reduction was slower in patients with M49L, M49I or S144A when compared to those without the TEAASs, but reduction in viral titers were comparable (**Figure 3**).[3,12]

Figure 3. Effect of the Frequently Encountered TEAASs (M49L/M49I/S144A) on (A) Viral RNA (ITT population) and (B) Viral Titer (mITT population) in SCORPIO-SR[3]

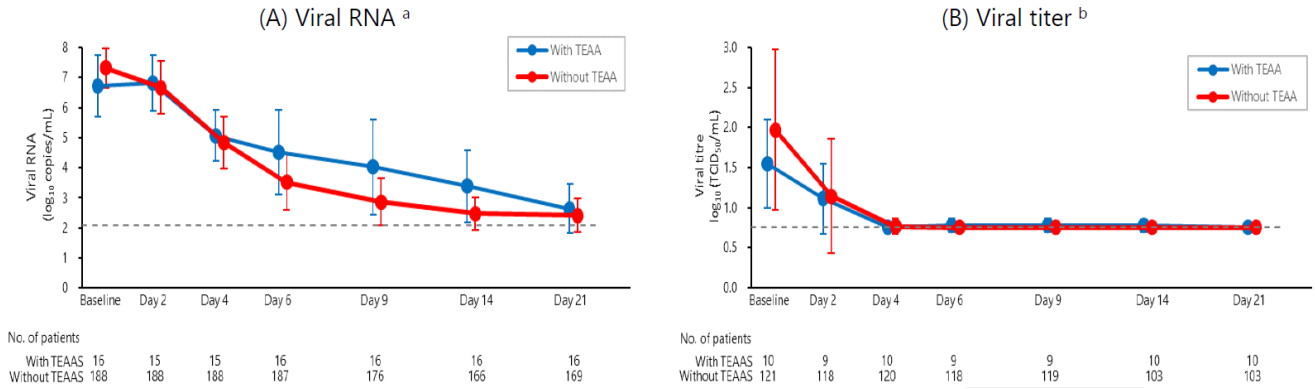


Figure adopted from Tsuge et al. OPTIONS XII. 2024.
Abbreviations: ITT, intent-to-treat; SD, standard deviation; TEAASs, treatment-emergent amino acid substitutions.
Data: mean ± SD
[a]ITT population: All patients randomly assigned to the study intervention and with RT-PCR–confirmed SARS-CoV-2 infection
[b]mITT (modified intention to treat) population: All patients randomly assigned to the study intervention with RT-PCR–confirmed SARS-CoV-2 infection on Visit 1 (pre-intervention) and with SARS-CoV-2 viral titre detected at baseline.

Uehara et al. (2025) also conducted *in vitro* studies to report the characteristics of ensitrelvir-resistant AASs in 3CL^{pro} in cultured cells, which were compared to those identified in SCORPIO-SR (see "*In Vitro* Data").[12]

4. SURVEILLANCE STUDIES

Genomic Analysis of SARS-CoV-2 Mutations Associated with Ensitrelvir Therapy

Inoue et al. (2025) conducted a retrospective descriptive study to identify gene mutations resulting in resistance to 3CL protease inhibitors in patients at Kansai Medical University General Medical Center in Osaka, Japan, between January 2022 and July 2024. The study analyses were performed in three parts:[13]

- **Part 1**: To test for drug resistance mutations in randomly selected samples. Samples were collected prior to treatment initiation, although some cases had received COVID-19 antivirals in other hospitals before study entry. Samples were subjected to genomic testing, and the frequency of amino acid substitutions was investigated. Ensitrelvir usage history was assessed based on the AAS identified.[13]
- **Part 2**: In patients treated with ensitrelvir for 5 days with no history of other antiviral use, genomic analysis was performed in those with a viral load ≥5000 copies/µL 10 days after ensitrelvir administration.[13]
- **Part 3**: In select patients with previously identified persistent COVID-19 after ensitrelvir treatment and confirmed *nsp5* drug resistance mutations, genomic analysis was performed to assess mutation rates and timing of emergence.[13]

Results

- **Part 1**: Viral genome analysis was performed on 1156 random samples. T25A, T25I, and T45N mutations were found in 1/1156 cases each, and M49L mutations were found in 6/1156 cases; four of these nine cases had not been previously treated with ensitrelvir. All five patients who had previously received ensitrelvir treatment had an underlying medical history of either malignant lymphoma or acute myeloid leukemia.[13]
- **Part 2**: Genomic analysis was performed on 16 patients with viral load ≥5000 copies/μL 10 days after ensitrelvir administration. T25A, T25N, M49I, and M49L mutations were found in 19% (3/16), 13% (2/16), 31% (5/16), and 63% (10/16) of cases, respectively. Among the 10 cases with M49L mutations, 7 exhibited amino acid substitutions of 90% or higher. All but two patients with drug resistance mutations did not receive immunomodulators, corticosteroids, and/or baricitinib.[13]
- **Part 3**: Genomic analysis was performed on 6 patients with persistent infection and drug resistance mutations, all of whom were considered immunocompromised with a history of B-cell lymphoma or adult T-cell leukemia/lymphoma. In case III-1, the M49I and D48G mutations appeared on Day 5 following the initiation of ensitrelvir therapy, while in case III-3, the M49L mutation emerged on Day 6. Over time, M49L mutations tended to become the predominant variant, whereas other mutations generally remained minor and eventually declined in frequency (**Figure 4**).[13]

Figure 4. De Novo Non-Synonymous Mutations in 6 Cases of Persistent Infection[13]

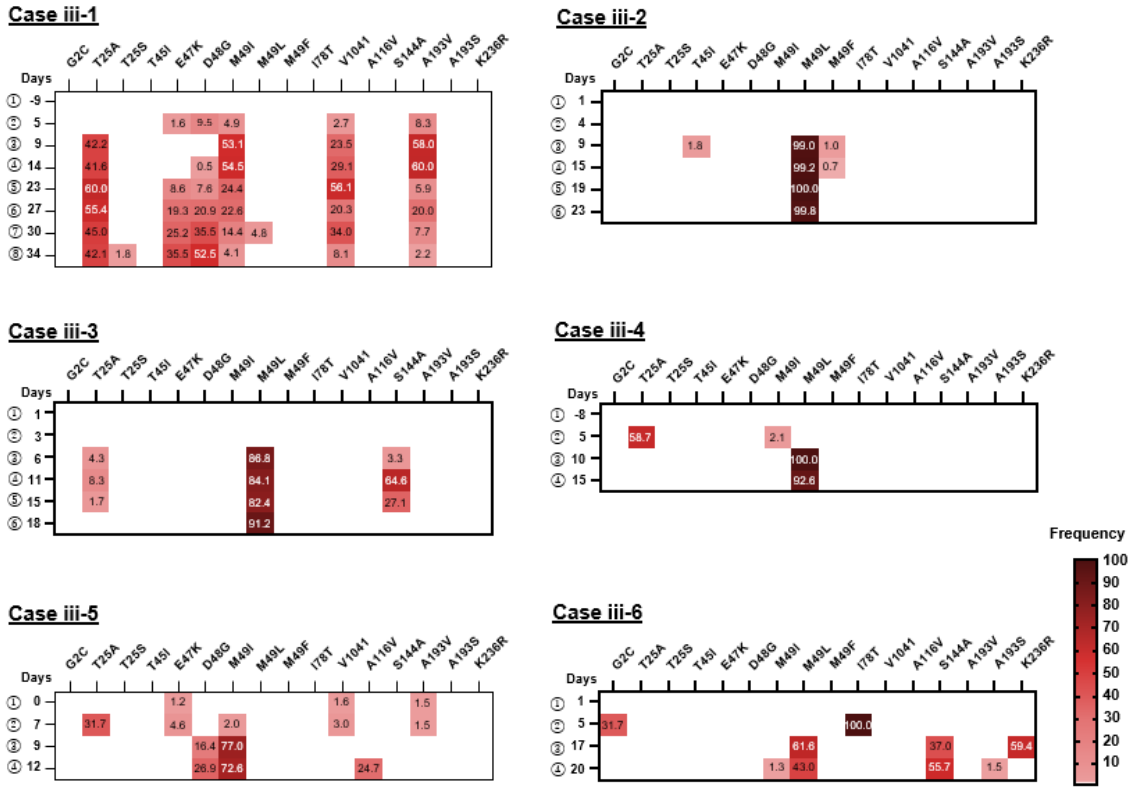


Figure adapted from Inoue et al. *Antiviral Res*. 2025.
The y-axis represents the days since the start of ensitrelvir administration, with the start date as 0. Heatmap is colored according to the frequency of mutations. Numbers indicate the frequency of mutation in percentages, and blanks indicate wild type at that residue. Heatmap was created using Prism 10 (Version 10.2.2).

Frequency and Distribution of *NSP5* Mutations Associated with 3CL Protease Inhibitor Resistance

Doi et al. (2024) conducted an analysis using data from the GISAID EpiCoV™ database to detect AAS mutations in *nsp5*, which is associated with conferred resistance to ensitrelvir, from March to December 2023. This period marks the first 9 months of ensitrelvir's full approval in Japan, while it remained investigational in the rest of the world. GISAID EpiCoV™ database provides virus genome sequencing of samples from COVID-19 patients globally.[14]

- Most cases of *nsp5* AAS substitutions were not associated with ensitrelvir treatment, although certain AASs were observed as having a regional bias (**Table 4**).[14]
  - M49L was reported in 277/845,796 (0.03%) of cases globally, 92.4% (256/277) of which were found in Japan.[14]
  - M49I, was reported in 90 cases, most of which were reported in May 2023 in the US.[14]

Table 4. Number of Mutations in *nsp5* Causing Ensitrelvir Resistance in Japan (March 31 – December 31, 2023)[a][14]

Amino Acid Substitutions[c]	No. cases from GISAID database[b]					
	Globally	Japan	United States	Europe	China	Others
T45I	25	0	4	15 (60.0)	1	5
D48Y	0	0	0	0	0	0
M49I	90	6	77 (85.6)	5	0	2
M49L	277	256 (92.4)	15	0	2	4
M49T	1	0	0	0	0	1
M49V	1	0	1	0	0	0
L50F	85	6	31	26	2	20
P52L	4	2	0	0	0	2
Y54C	0	0	0	0	0	0
S144A	4	2	0	1	0	1
E166A	1	1	0	0	0	0
E166V	23	0	4	10	1	8
L167F	0	0	0	0	0	0
P168del	4	0	0	0	0	4
A173T	23	0	6	11	1	5
A173V	23	2	6	3	0	12 (52.2)
Q192R	2	0	0	1	0	1

Table adapted from Doi et al. *Emerg Infect Dis*. 2024.
Abbreviations: del, deletion; GISAID, Global Initiative on Sharing All Influenza Data; *nsp5*, nonstructural protein 5.
[a]Numbers in parentheses are percentages of total global cases (if a particular mutation was found in >10 cases and was responsible for >50% of global cases).
[b]Sequences were extracted from the GISAID EpiCoV database (https://www.gisaid.org).
[c]Amino acid substitutions in *nsp5* that trigger emergence of ensitrelvir-resistant SARS-CoV-2.

5. *IN VITRO* DATA

In Vitro Evaluation of Ensitrelvir Susceptibility and Viral Fitness in Recombinant SARS-CoV-2 Variants

Baba et al. (2024) conducted 2 *in vitro* studies to report the characteristics of recombinant SARS-CoV-2 with ensitrelvir-resistant AASs in $3CL^{pro}$ in cultured cells.[4]

- Part 1 aimed to identify AASs by conducting viral passage in the presence of ensitrelvir (0.041 to 10 μmol/L) and sequencing the isolated viruses (**Figure 5**).[4]

- Part 2 aimed to analyze the inhibitory effects of ensitrelvir against recombinant viruses containing AASs (generated by CPER method) by measuring EC_{50} 3 days after virus inoculation. Virus fitness and competitive fitness were also analyzed.[4]

Figure 5. Diagram of Ensitrelvir *In Vitro* Virus Passage Study[4]

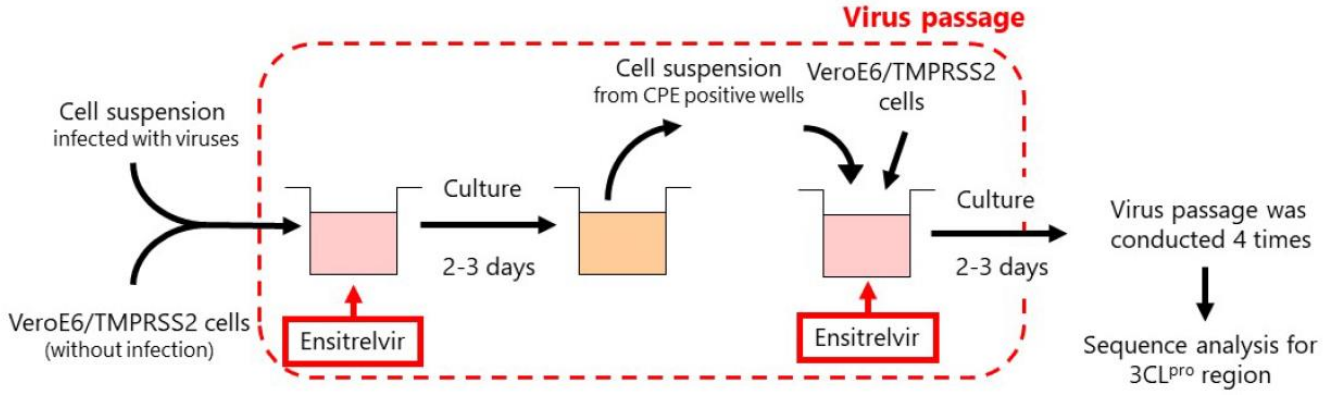


Figure adopted from Baba et al. OPTIONS XII. 2024.
Abbreviations: 3CL^{pro}, 3-chymotrypsin-like protease; CPE, cytopathic effects; VeroE6/TMPRSS2, Vero cell 6/Transmembrane serine protease 2.

Part 1: AAS Identification

From the virus passage study, AASs in the 3CL^{pro} region were identified as shown in **Table 5**. M49L and S144A are located within 5Å from the ensitrelvir binding site in 3CL^{pro} and were also observed after treatment in the ensitrelvir clinical study SCORPIO-SR.[4]

Table 5. AASs in 3CL^{pro} Detected in the Virus Passage Study[4]

Strains	Pango lineage	Detected AASs
hCoV-19/Japan/TY/WK-521/2020	A	M49L
hCoV-19/Japan/TY7-501/2021	P.1	M49L, S144A, M49M/L+S144A
hCoV-19/Japan/QHN001/2020	B.1.1.7	D48G, M49L, S144A, M49M/L+S144A
hCoV-19/Japan/TY8-612/2021	B.1.351	M49L, P52S
hCoV-19/Japan/TY11-927-P1/2021	B.1.617.2	D48G, M49L

Table adapted from Baba et al. OPTIONS XII 2024.
Abbreviations: 3CL^{pro}, 3-chymotrypsin-like protease; AAS, amino acid substitution.

Part 2: Susceptibility and Fitness

The total 9 AAS-containing recombinant virus strains each showed reduced susceptibility to ensitrelvir (range 2.2- to 100-fold) as shown in **Table 6**.[4]

Table 6. Susceptibility of Recombinant Viruses with *nsp5* AASs to Ensitrelvir and Remdesivir[4]

Strains[a]	Ensitrelvir		Remdesivir	
	EC_{50} (µmol/L)	FC	EC_{50} (µmol/L)	FC
Experiment 1				
Wild-type	0.11	–	1.6	–
nsp5/M49L	1.7	17	0.85	0.52
nsp5/S144A	0.92	9.2	1.5	0.90
nsp5/M49L/S144A	11	100	0.86	0.53
nsp5/D48G	0.49	4.3	1.7	1.0
nsp5/P52S	0.44	3.7	1.8	1.0
Experiment 2				
Wild-type	0.12	–	2.0	–
nsp5/T25A	1.7	14.4	2.6	1.3
nsp5/T25I	0.34	2.86	1.5	0.74
nsp5/D48Y	0.26	2.2	2.0	0.97
nsp5/M49I	0.63	5.25	1.5	0.73

Table adapted from Baba et al. OPTIONS XII 2024.
Abbreviations: $3CL^{pro}$, 3-chymotrypsin-like protease, AAS, amino acid substitution; EC_{50}, half maximal effective concentration; FC, fold change.
Inhibitory effects on cytopathic effect induced by virus infection (EC_{50}) was measured in VeroE6/TMPRSS2 cells. AASs of T25A, T25I, D48Y, M49I, M49L and S144A were detected after ensitrelvir treatment in the SCORPIO-SR clinical study.
[a]Strains were variations of rgSARS-CoV-2/Hu/DP/Kng/19-020.

Several of the AAs from the *in vitro* passage study were identified in the ensitrelvir 125 mg arm of the SCORPIO-SR clinical study as well. Combining the results of the SCORPIO-SR AAS strain susceptibility and the results of the *in vitro* passage study AAS strain susceptibility reveals the comparative fold change in EC_{50} for 9 different strains against ensitrelvir, remdesivir, and nirmatrelvir. No cross-resistance between ensitrelvir and other antiviral drugs was observed (**Table 7**).[12]

Fitness and competitive fitness analysis indicated that recombinant viruses with P52S or M49L+S144A had lower replicative fitness than wild-type, and the replicative capacity of recombinant viruses with D48G, M49L, or S144A was not higher compared with wild-type (**Figure 6** and **Figure 7**).[4]

Table 7. Comparison of Susceptibility to Antiviral Drugs of Strains Harboring *nsp5* AASs[12]

Reverse genetics-derived SARS-CoV-2 strains with *nsp5* AASs	Source	Fold change in EC_{50} (vs wild-type)[a]		
		Ensitrelvir	Remdesivir	Nirmatrelvir
T25A	SCORPIO-SR	14.4	1.3	1.1
T25I	SCORPIO-SR	2.9	0.7	0.6
D48G	*In vitro* passage study	4.9	1.5	1.5
D48Y	SCORPIO-SR	2.2	1.0	1.2
M49I[b,c]	SCORPIO-SR	5.3	0.7	0.2
M49L[b,c]	SCORPIO-SR; *In vitro* passage study	33.0	1.0	0.8
P52S	*In vitro* passage study	4.4	0.8	0.6
S144A[b,c]	SCORPIO-SR; *In vitro* passage study	8.2	1.5	1.4
M49L/S144A	*In vitro* passage study	124	1.4	1.6

Table adapted from Uehara et al. *Antiviral Res.* 2025.
Abbreviations: AASs, amino acid substitutions; EC_{50}, half maximal effective concentration; TEAASs, treatment-emergent amino acid substitutions.
[a]Calculated by dividing the mean EC_{50} of each tested virus by that of the cognate wild-type virus.
[b]Observed in ≥2 patients in the ensitrelvir 125mg arm of SCORPIO-SR.
[c]TEAASs with mutations at the amino acid position in the *in vitro* resistance isolation test.

Figure 6. Fitness of Recombinant Viruses in VeroE6/TMPRSS2 Cells[4]

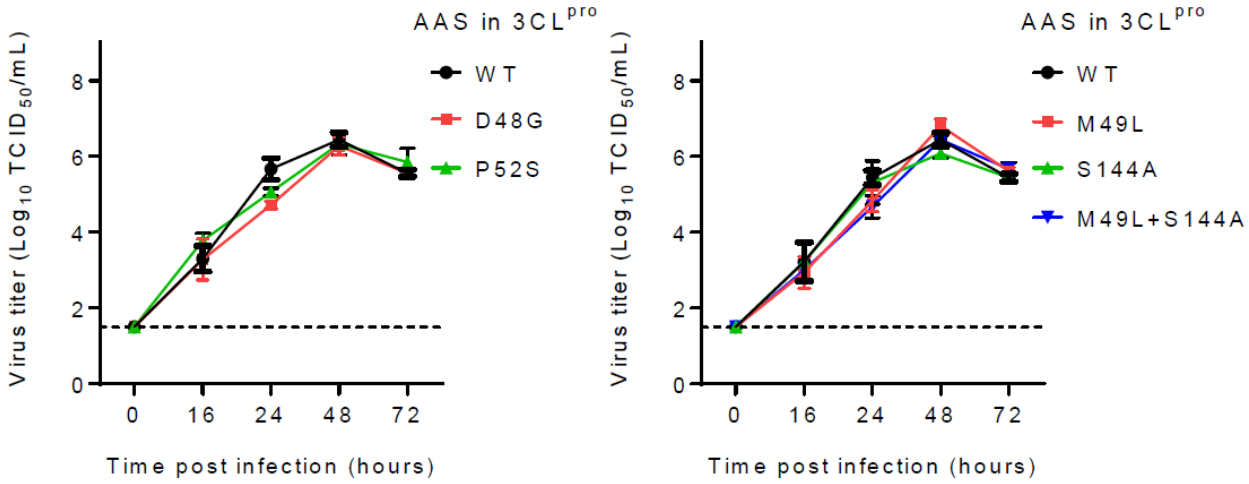


Figure adopted from Baba et al. OPTIONS XII 2024.
Abbreviations: AAS, amino acid substitution; $TCID_{50}$, median tissue culture infectious dose; WT, wild-type.
Virus fitness analyses were conducted in triplicate for each recombinant virus.

Figure 7. Competitive Fitness of Recombinant Viruses in MucilAir™[4]

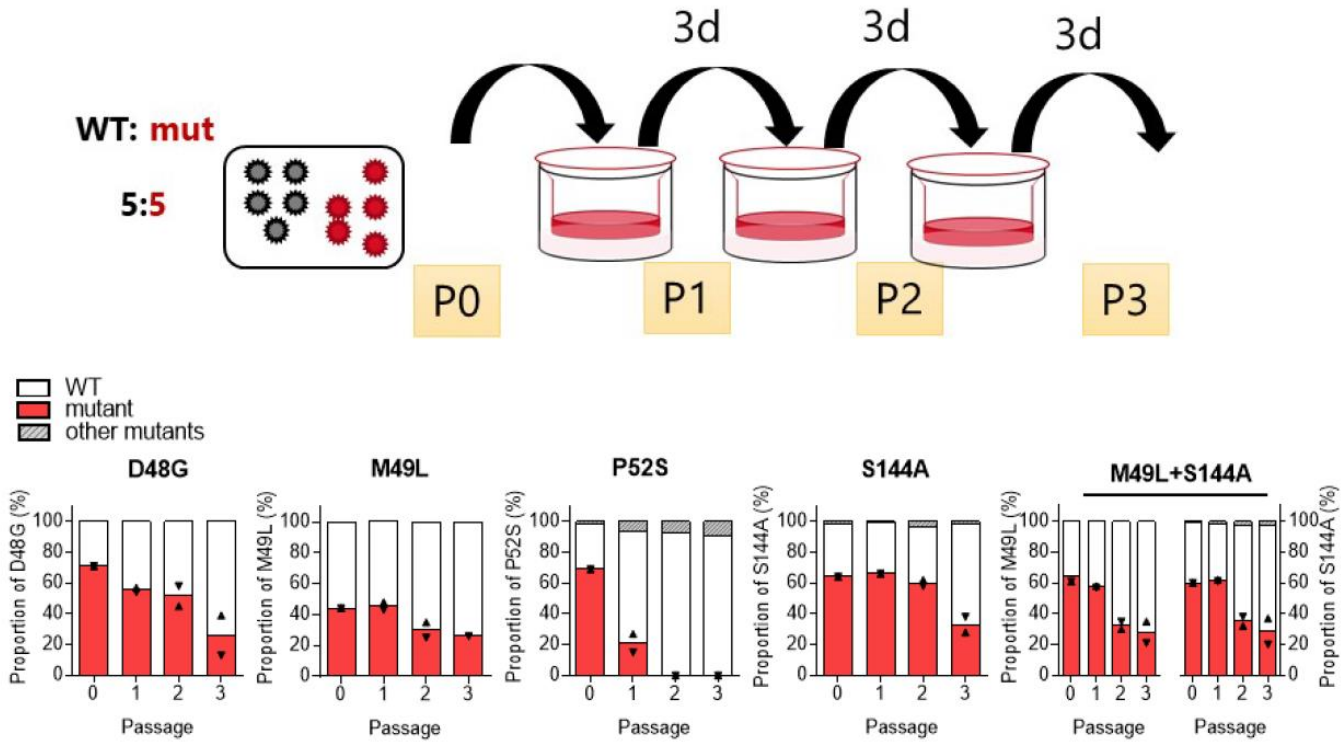


Figure adopted from Baba et al. OPTIONS XII 2024.
Abbreviations: AAS, amino acid substitution; d, days; mut, mutant; P, passage; $TCID_{50}$, median tissue culture infectious dose; WT, wild-type.
Virus passage was conducted 3 times in total in duplicate cultures. ≧1% variants were analyzed by next-generation sequencing. In some samples, some variants other than target AASs (other mutants) were detected but the proportion of these variants was <10%.

In Vitro Assessment of SARS-CoV-2 M^{pro} Mutations on Ensitrelvir and Nirmatrelvir Susceptibility

Rauch et al. (2024) conducted a study in a VSV-based system that analyzed a wide range of $3CL^{pro}$ mutations and found L89F, P96S, A129V, T225I, H164N, P184H, I78V, T243I, R279C, M276V and R279H to be AAS mutations that potentially preserved virally fitness, with L89F and H164N also frequently reported in the GISAID EpiCoV™ database. T25N, C44Y, P52H, L89F, H163Q, H164N, E166K, L167F, R188W and Q189L were also found to be close to the ensitrelvir binding site. Selected mutations were analyzed further and demonstrated varying degrees of reduced susceptibility against ensitrelvir and nirmatrelvir as shown in **Table 8**.[15]

Table 8. Ensitrelvir and Nirmatrelvir IC_{50} Fold-Changes of M^{pro} Mutations to Parental Strains[15]

M^{pro} mutation	TM_{50} fold-change	IC_{50} fold-change ensitrelvir	IC_{50} fold-change nirmatrelvir
Single mutations			
T25A	0.84	146.33[a]	20.93
O-T25A	0.95	23.48	2.19
T25N	0.90	41.16	0.55
O-T25N	0.95	48.04	0.89
C44Y	0.98	47.50	3.35
P52H	0.91	46.09	5.06
O-L89F	0.94	2.75	0.75
H163Q	1.26	9.50	3.79
H164N	1.08	2.43	1.76
E166K	1.14	69.46	16.00
O-R188W	1.03	4.31	4.38
Double mutations			
E166K-T25A	0.92	>1000[a]	169.98[a]
E166K-Q189L	1.18	193.11[a]	17.57
R188W-T25A	1.00	27.45	8.80
O-R188W-L89F	1.03	9.53	4.50

Table adapted from Rauch et al. *Antiviral Res*. 2024.
Abbreviations: IC_{50}, half maximal inhibitory concentration; M^{pro}, main protease; TM_{50}, time to reach the half-maximum signal.
[a]Strong phenotype with wide confidence interval.

6. RELEVANT PRESCRIBING INFORMATION

INDICATIONS AND USAGE

XOCOVA is indicated for post-exposure prophylaxis of coronavirus disease 2019 (COVID-19) in adults and adolescents 12 years of age and older following contact with an individual who has COVID-19.[22]

CONTRAINDICATIONS

XOCOVA is contraindicated in patients with a history of clinically significant hypersensitivity reactions to ensitrelvir or any other components of the product.[22]

XOCOVA is contraindicated in patients taking drugs that are:[22]

- Primarily metabolized by CYP3A for which elevated concentrations may be associated with serious and/or life-threatening reactions and
- Strong CYP3A inducers because they may significantly reduce ensitrelvir plasma concentrations, leading to the potential loss of virologic response.[22]

As stated in the XOCOVA **Full Prescribing Information**:[22]

Section 12.4 – Microbiology

Antiviral Resistance

SARS-CoV-2 M^{pro} residues associated with reduced susceptibility to ensitrelvir have been identified using a variety of methods, including SARS-CoV-2 resistance selection experiments, testing of recombinant SARS-CoV-2 viruses, replicons, or enzymes with laboratory-engineered M^{pro} substitutions in cell culture or biochemical assays, and evaluation of samples collected from subjects treated with XOCOVA in clinical trials.[22]

In Cell Culture and Biochemical Assays

In cell culture, ensitrelvir selected the SARS-CoV-2 M^{pro} D48G, M49L, P52S, S144A, E166A, and T169I substitutions, as well as combinations of these substitutions, all of which reduced ensitrelvir activity ≥3 fold in cell culture (**Table 9**).[22] M^{pro} cleavage site substitutions have not been associated with reduced ensitrelvir susceptibility in cell culture.[22]

Table 9. SARS-CoV-2 M^{pro} Amino Acid Substitutions Selected by Ensitrelvir in Cell Culture[22a]

Single Substitutions (EC_{50} value fold change in cell culture)	D48G (3.7-6.5), M49L (12-71), P52S (3.7-5.5), S144A (8.5-34), E166A (3.4-9.1), T169I (1.4-4.8)
≥2 Substitutions (EC_{50} value fold change in cell culture)	M49L+S144A (100-290), M49L+E166A (133-197), M49L+S144A+T169I (131-660)

[a]EC_{50} value fold change ranges are shown in instances where multiple data points have been reported.

In cell culture or biochemical assays using recombinant SARS-CoV-2 viruses, replicons, or enzymes with laboratory-engineered M^{pro} substitutions, the following M^{pro} substitutions and deletions reduced ensitrelvir activity ≥3-fold: T25E/V, C44Y, M49T, P52H, Y54A/C, L57F, N133H, F140A, N142S, G143S, H164N, M165V, E166C/G/H/I/L/Q/S/V, L167F, P168del, A173V, R188S, A191T/V, and Q192L/T, as well as combinations of these and other substitutions.[22] However, these M^{pro} substitutions and deletions were not observed in SARS-CoV-2 resistance selection experiments using ensitrelvir in cell culture or in XOCOVA-treated subjects in clinical trials, and the clinical significance of these substitutions is unknown.[22]

In Clinical Trials

In Trial SCORPIO-PEP, post-baseline sequencing data were provided for 29% (93/318) of XOCOVA-treated ITT subjects who had at least one SARS-CoV-2 RT-PCR-positive post-baseline sample. SARS-CoV-2 M^{pro} amino acid changes (relative to the Wuhan-Hu-1 reference sequence) were classified as XOCOVA resistance-associated substitutions (RAS) based on the number of subjects in each arm with the substitution, the frequency of the substitution within the samples, and the impact of the substitution on ensitrelvir susceptibility in cell culture (generally a ≥3-fold decrease).[22] The following M^{pro} amino acid changes were considered XOCOVA RAS (n: number of XOCOVA-treated subjects): T25A/I/N (n=9), D48G (n=2), M49I/L (n=29), P52S (n=5), S144A (n=1), and E166A (n=1). In some subjects, multiple M^{pro} substitutions were observed, either as single substitutions in different viruses or as combinations in the same virus, with T25A+E166A and M49I+S144A detected as combinations in the same virus. All of the single M^{pro} substitutions except T25I reduced ensitrelvir activity ≥3-fold in cell culture, whereas the combinations have not been tested (**Table 10**).[22] The proportion of XOCOVA-treated subjects with XOCOVA RAS was 3.4% (40/1,190) in the ITT, 1.9% (20/1,030) in the mITT, and 18% (20/114) in the

ITT baseline positive populations. There were insufficient numbers of subjects to determine the impact of XOCOVA RAS on viral RNA shedding and clinical outcomes.[22]

Table 10. SARS-CoV-2 M^{pro} Amino Acid Substitutions Observed in XOCOVA-Treated Subjects in Clinical Trials[22a]

Single Substitutions (EC_{50} value fold change in cell culture)	T25A (7.0-14), T25I (2.9), T25N (4.8-28), D48G (3.7-6.5), M49I (5.3), M49L (12-71), P52S (3.7-5.5), S144A (8.5-34), E166A (3.4-9.1), E166K (ND)
≥2 Substitutions (EC_{50} value fold change in cell culture)	T25A+E166A (ND), M49I+S144A (ND)

[a]EC_{50} value fold change ranges are shown in instances where multiple data points have been reported.
Abbreviation: ND=no data.

In other clinical trials, the following M^{pro} amino acid changes were considered XOCOVA RAS (n: number of XOCOVA-treated subjects): T25A/I/N (n=7), M49I/L (n=40), P52S (n=1), S144A (n=2), and E166A/K (n=2). Some of these M^{pro} substitutions were observed as combinations in the same subject. Most of these M^{pro} substitutions reduced ensitrelvir activity ≥3-fold in cell culture (**Table 10**).[22] In other trials, the proportion of XOCOVA-treated subjects with XOCOVA RAS was 2.1% (47/2,226).[22]

XOCOVA RAS have been detected at frequencies <1% among globally circulating viruses based on analysis of public sequence databases. In clinical trials, only a single subject had a XOCOVA RAS (T25I) identified in a pre-treatment sample.[22]

Cross-Resistance

Ensitrelvir and nirmatrelvir are both SARS-CoV-2 M^{pro} inhibitors and exhibit partial cross-resistance. Nirmatrelvir had activity against the most common ensitrelvir RAS T25A/I/N and M49I/L in cell culture (EC_{50} value fold changes: 0.2-1.8) and biochemical assays (IC_{50}/K_i value fold changes: 0.2-2.2). Conversely, ensitrelvir activity was either unchanged or reduced to variable extents by the nirmatrelvir RAS E166V in cell culture (EC_{50} value fold changes: 1.0-23) and biochemical assays (IC_{50}/K_i value fold changes: 1.1-599).[22] Cross-resistance is not expected between ensitrelvir and remdesivir or any other SARS-CoV-2 antivirals with different mechanisms of action (i.e., antivirals that are not M^{pro} inhibitors).[22]

Please read the attached XOCOVA **Full Prescribing Information**. The use of XOCOVA in any manner other than as described in the Full Prescribing Information approved by the U.S. Food and Drug Administration (FDA) is not recommended.

7. LITERATURE SEARCH

As of April 2026, a computerized search of the published medical literature using internal and external databases (ie, Medline® and Embase®) has identified additional relevant references regarding ensitrelvir and resistance. For your review, these references are listed in the bibliography that follows:

Abe S, Wannigama DL, Suzuki Y, et al. Real world effectiveness of early ensitrelvir treatment in patients with SARS-CoV-2, a retrospective case series. *New Microbes New Infect*. 2024;62:101522. [Web Link]

Bhandari D, Gerlits O, Keable S, et al. Characterization of an unusual SARS-CoV-2 main protease natural variant exhibiting resistance to nirmatrelvir and ensitrelvir. *Commun Biol*. 2025;8(1):1061. [Web Link]

Bouzidi HS, Driouich JS, Klitting R, et al. Generation and evaluation of protease inhibitor-resistant SARS-CoV-2 strains. *Antiviral Res*. 2024;222:105814. [Web Link]
Costacurta F, Dodaro A, Falch A, et al. A novel dual-chimeric surrogate virus to select, test, and characterize mutants of protease and glycoprotein inhibitors. *bioRxiv* [Preprint]. 2026. [Web Link]

Esler MA, Shi K, Rollie JA, et al. Structural basis for varying drug resistance of SARS-CoV-2 M^{pro} E166 variants. *mBio*. 2025;16(7):e0262424. [Web Link]

Kawashima S, Nobori H, Okane M, et al. *In vitro* resistance profiling of S-892216, a second generation SARS-CoV-2 $3CL^{pro}$ inhibitor for the treatment of COVID-19. *Open Forum Infect. Dis.*2025. [Web Link]

Kovalevsky A, Aniana A, Ghirlando R, et al. Effects of SARS-CoV-2 main protease mutations at positions L50, E166, and L167 rendering resistance to covalent and noncovalent inhibitors. *J Med Chem*. 2024;67(20):18478-18490. [Web Link]

Krismer L, Schöppe H, Rauch S, et al. Study of key residues in MERS-CoV and SARS-CoV-2 main proteases for resistance against clinically applied inhibitors nirmatrelvir and ensitrelvir. *Npj Viruses*. 2024;2(1):23. [Web Link]

Lo CW, Kariv O, Hao C, et al. Replication capacity and susceptibility of nirmatrelvir-resistant mutants to next-generation Mpro inhibitors in a SARS-CoV-2 replicon system. *Antiviral Res*. 2024;231:106022. [Web Link]

Ma Y, Ye C, Rahisuddin R, et al. Identification and characterization of a SARS-CoV-2 M^{pro} G23 deletion ensitrelvir-resistant mutant. *bioRxiv* [Preprint]. 2025. [Web Link]

Min SC, Seo J-J, Jeong JH, et al. A SARS-CoV-2 M^{pro} mutation conferring ensitrelvir resistance paradoxically increases nirmatrelvir susceptibility. *Nat Commun.*2025;16(1):10737. [Web Link]

Mizuno A, Nakayoshi T, Kato K, et al. Computational estimation of residues involving resistance to the SARS-CoV-2 main protease inhibitor ensitrelvir based on virtual alanine scan of the active site. *Biol Pharm Bull*. 2024;47(5):967-977. [Web Link]

Su H, Nie T, Chen G, et al. Structure-based development of ultra-broad-spectrum 3C-like protease inhibitors. *Adv Sci (Weinh)*. 2026;13(11):e12342. [Web Link]

Tan H, Chi X, Deng X, Wang J. Characterization of the cross-resistance of SARS-CoV-2 main protease inhibitiors, ibuzatrelvir, ensitrelvir, and nirmatrelvir. *ACS Pharmacol Transl Sci*. 2026;9(2):404-413. [Web Link]

Zhang L, Xie X, Luo H, et al. Resistance mechanisms of SARS-CoV-2 3CLpro to the non-covalent inhibitor WU-04. *Cell Discov*. 2024;10(1):40. [Web Link]

Flynn JM, Huang QYJ, Zvornicanin SN, et al. Systematic analyses of the resistance potential of drugs targeting SARS-CoV-2 main protease. *ACS Infect Dis*. 2023;9(7):1372-1386. [Web Link]

Ip JD, Wing-Ho Chu A, Chan WM, et al. Global prevalence of SARS-CoV-2 3CL protease mutations associated with nirmatrelvir or ensitrelvir resistance. *EBioMedicine*. 2023;91:104559. [Web Link]

Sayan M, Arikan A, Sanlidag E. Molecular epidemiology of SARS-CoV-2 omicron sub-lineages isolated from Turkish patients infected with COVID-19. *Viruses*. 2023;15(5):1066. [Web Link]

Unoh Y, Hirai K, Uehara S, et al. Discovery of the clinical candidate S-892216: a second-generation of SARS-CoV-2 3CL protease inhibitor for treating COVID-19. *J Med Chem*. 2025;68(20):21099-21119. [Web Link]

Xiong D, Zhao X, Luo S, et al. Molecular mechanism of the non-covalent orally targeted SARS-CoV-2 M^{pro} inhibitor S-217622 and computational assessment of its effectiveness against mainstream variants. *J Phys Chem Lett*. 2022;13(38):8893-8901. [Web Link]

Zhou Y, Gammeltoft KA, Tjørnelund-Sjursen HD, et al. SARS-CoV-2 M^{pro} inhibitor ensitrelvir:asymmetrical cross-resistance with nirmatrelvir and emerging resistance hotspots. *Emerg Microbes Infect*. 2025;14(1):2552716. [Web Link]

Please keep in mind that medical literature databases, while considered a major source of biomedical information, are selective in their sources, and the information derived from them should not be considered all-inclusive.

ABBREVIATIONS

3CL	3-chymotrypsin-like	nsps	nonstructural proteins
$3CL^{pro}$	3-chymotrypsin-like protease	O	Omicron variant
AAS	amino acid substitution	PK	pharmacokinetic
CI	confidence interval	pp1a/pp1ab	polyproteins 1a/1ab
COVID-19	coronavirus disease 2019	RAS	resistance-associated substitutions
CPE	cytopathic effects	RNA	ribonucleic acid
EC_{50}	half maximal effective concentration	RTC	replicase-transcriptase complex
FC	fold change	SARS-CoV-2	severe acute respiratory syndrome coronavirus 2
GISAID	Global Initiative on Sharing All Influenza Data	TEAAS	treatment-emergent amino acid substitution
IC_{50}	half maximal inhibitory concentration	TM_{50}	time to reach the half-maximum signal
ITT	intent-to-treat	$TCID_{50}$	median tissue culture infectious dose
MHLW	Ministry of Health, Labour, and Welfare	US	United States
mITT	modified intent-to-treat	VeroE6/ TMPRSS2	Vero cell 6/Transmembrane serine protease 2
M^{pro}	main protease	WT	wild-type

REFERENCES

1. Unoh Y, Uehara S, Nakahara K, et al. Discovery of S-217622, a noncovalent oral SARS-CoV-2 3CL protease inhibitor clinical candidate for treating COVID-19. *J Med Chem*. 2022;65(9):6499-6512. doi:10.1021/acs.jmedchem.2c00117.

2. Zhang L, Lin D, Sun X, et al. Crystal structure of SARS-CoV-2 main protease provides a basis for design of improved α-ketoamide inhibitors. *Science*. Apr 24 2020;368(6489):409-412. doi:10.1126/science.abb3405.
3. Tsuge Y, Yotsuyanagi H, Ohmagari N, et al. Analysis of the ensitrelvir treatment-emergent amino acid substitutions observed in the SCORPIO-SR phase 3 trial. Presentation presented at: OPTIONS XII; September 15 – October 2, 2024; Brisbane, Australia.
4. Baba K, Nobori H, Miyamoto T, et al. Characterization of reverse genetics-derived SARS-CoV-2 with amino acid substitutions observerd in ensitrelvir *in vitro* virus passage study. Poster presented at: OPTIONS XII; September 15 – October 2, 2024; Brisbane, Australia.
5. Noske GD, de Souza Silva E, de Godoy MO, et al. Structural basis of nirmatrelvir and ensitrelvir activity against naturally occurring polymorphisms of the SARS-CoV-2 main protease. *J Biol Chem*. 2023;299(3):103004. doi:10.1016/j.jbc.2023.103004
6. Heilmann E, Costacurta F, Moghadasi SA, et al. SARS-CoV-2 3CL(pro) mutations selected in a VSV-based system confer resistance to nirmatrelvir, ensitrelvir, and GC376. *Sci Transl Med*. 2023;15(678):eabq7360. doi:10.1126/scitranslmed.abq7360
7. Iketani S, Mohri H, Culbertson B, et al. Multiple pathways for SARS-CoV-2 resistance to nirmatrelvir. *Nature*. 2023;613(7944):558-564. doi:10.1038/s41586-022-05514-2
8. Moghadasi SA, Heilmann E, Khalil AM, et al. Transmissible SARS-CoV-2 variants with resistance to clinical protease inhibitors. *Sci Adv*. 2023;9(13):eade8778. doi:10.1126/sciadv.ade8778
9. Stanford University. Corona Antiviral and Resistance Database. SARS-CoV-2 Resistance Mutations – 3CLpro inhibitors. Last update August 21, 2024. Accessed on November 25, 2024. https://covdb.stanford.edu/drms/3clpro/#abstract
10. Hayden FG, Shinkai M, Clark TW, et al. Ensitrelvir for Covid-19 Postexposure Prophylaxis in Household Contacts. *New England Journal of Medicine*. 2026;394(19):1905-1915. doi:10.1056/NEJMoa2509306
11. Choudhary MC, Deo R, Luetkemeyer A, et al. SARS-CoV-2 ensitrelvir treatment-emergent resistance-associated mutations in Phase III randomized clinical trial. Poster presented at: CROI; March 9 - 12, 2025; San Francisco, CA.
12. Uehara T, Yotsuyanagi H, Ohmagari N, et al. Ensitrelvir treatment-emergent amino acid substitutions in SARS-CoV-2 3CL(pro) detected in the SCORPIO-SR phase 3 trial. *Antiviral research*. 2025;236:106097. doi:10.1016/j.antiviral.2025.106097.
13. Inoue A, Ichikawa T, Wada D, et al. M49L and other drug resistance mutations emerging in individuals after administration of ensitrelvir in Japanese clinical settings. *Antiviral research*. 2025;236:106118. doi:10.1016/j.antiviral.2025.106118
14. Doi A, Ota M, Saito M, Matsuyama S. Sporadic occurrence of ensitrelvir-resistant SARS-CoV-2, Japan. *Emerg Infect Dis*. 2024;30(6):1289-1291. doi:10.3201/eid3006.240023
15. Rauch S, Costacurta F, Schöppe H, et al. Highly specific SARS-CoV-2 main protease (M(pro)) mutations against the clinical antiviral ensitrelvir selected in a safe, VSV-based system. *Antiviral research*. 2024;231:105969. doi:10.1016/j.antiviral.2024.105969
16. Jeong GU, Song H, Yoon GY, Kim D, Kwon YC. Therapeutic strategies against COVID-19 and structural characterization of SARS-CoV-2: a review. *Front Microbiol*. 2020;11:1723. doi:10.3389/fmicb.2020.01723.
17. Phase 3 study of S-217622 in prevention of symptomatic SARS-CoV-2 infection (SCORPIO-PEP). NLM Identifier: NCT05897541. Last accessed June 18, 2025. https://clinicaltrials.gov/study/NCT05897541?term=scorpio-pep&rank=1.
18. Hayden FG, Shinkai M, Clark TW, et al. Ensitrelvir for Covid-19 postexposure prophylaxis in household contacts. *New England Journal of Medicine*. 2026;394(19)(Suppl):S1-S33. doi:10.1056/NEJMoa2509306
19. Luetkemeyer AF, Chew KW, Lacey S, et al. Ensitrelvir for the treatment of nonhospitalized adults with COVID-19: results from the SCORPIO-HR, phase 3, randomized, double-blind, placebo-controlled trial. *Clin Infect Dis*. 2025;doi:/10.1093/cid/ciaf029/8017725

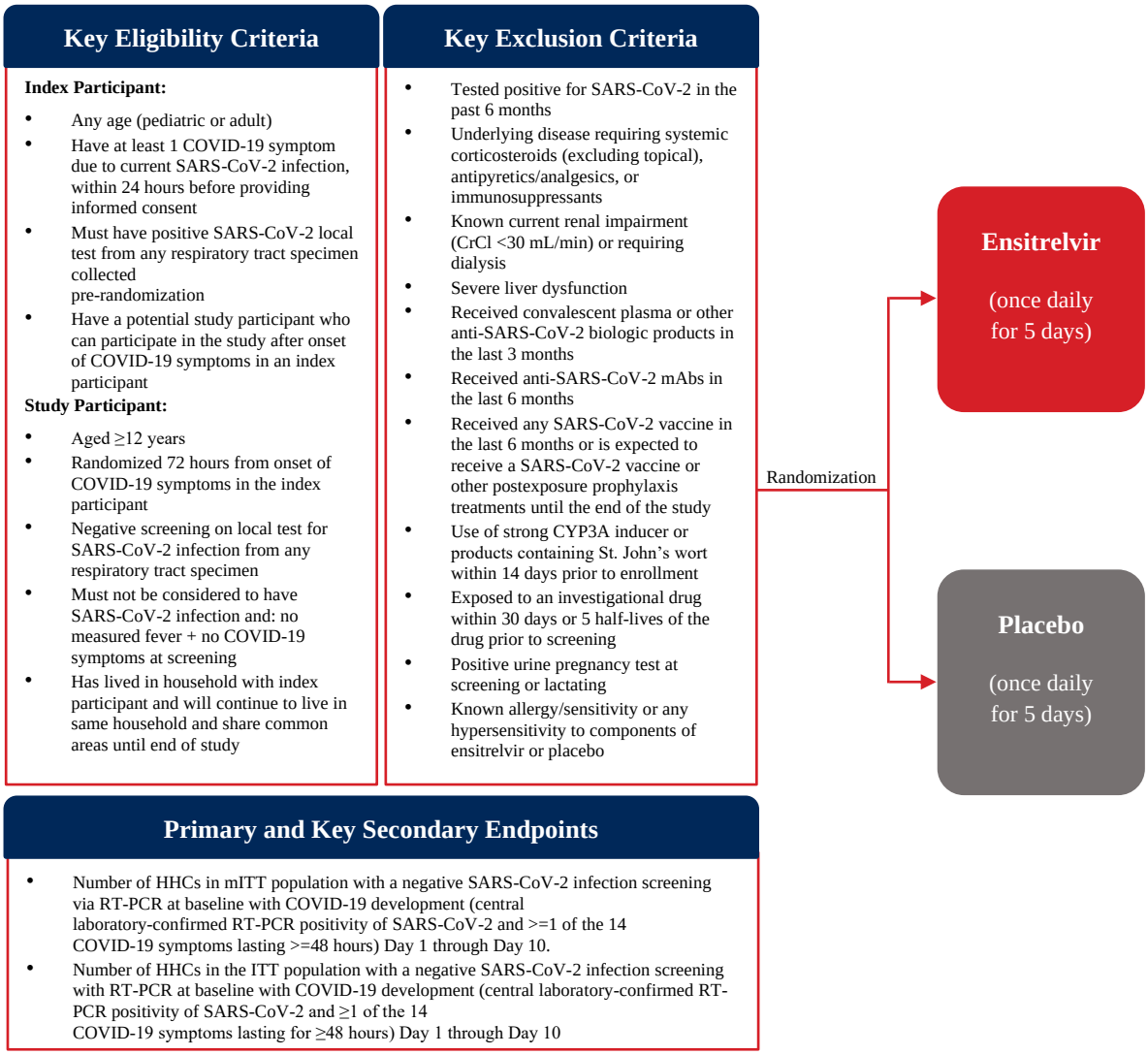
20. Luetkemeyer AF, Chew KW, Lacey S, et al. Ensitrelvir for the treatment of nonhospitalized adults with COVID-19: results from the SCORPIO-HR, phase 3, randomized, double-blind, placebo-controlled trial. *Clin Infect Dis*. Feb 17 2025;doi:10.1093/cid/ciaf029
21. Yotsuyanagi H, Ohmagari N, Doi Y, et al. Efficacy and safety of 5-day oral ensitrelvir for patients with mild to moderate COVID-19 the SCORPIO-SR randomized clinical trial. *JAMA Netw Open*. 2024;7(2):e2354991. doi:10.1001/jamanetworkopen.2023.54991.
22. Xocova® (ensitrelvir) tablets, for oral use [Prescribing Information]. Shionogi Inc.

8. APPENDIX

SCORPIO-PEP (Phase 3)

Study Design

Figure 8. SCORPIO-PEP Study Design[10,17]



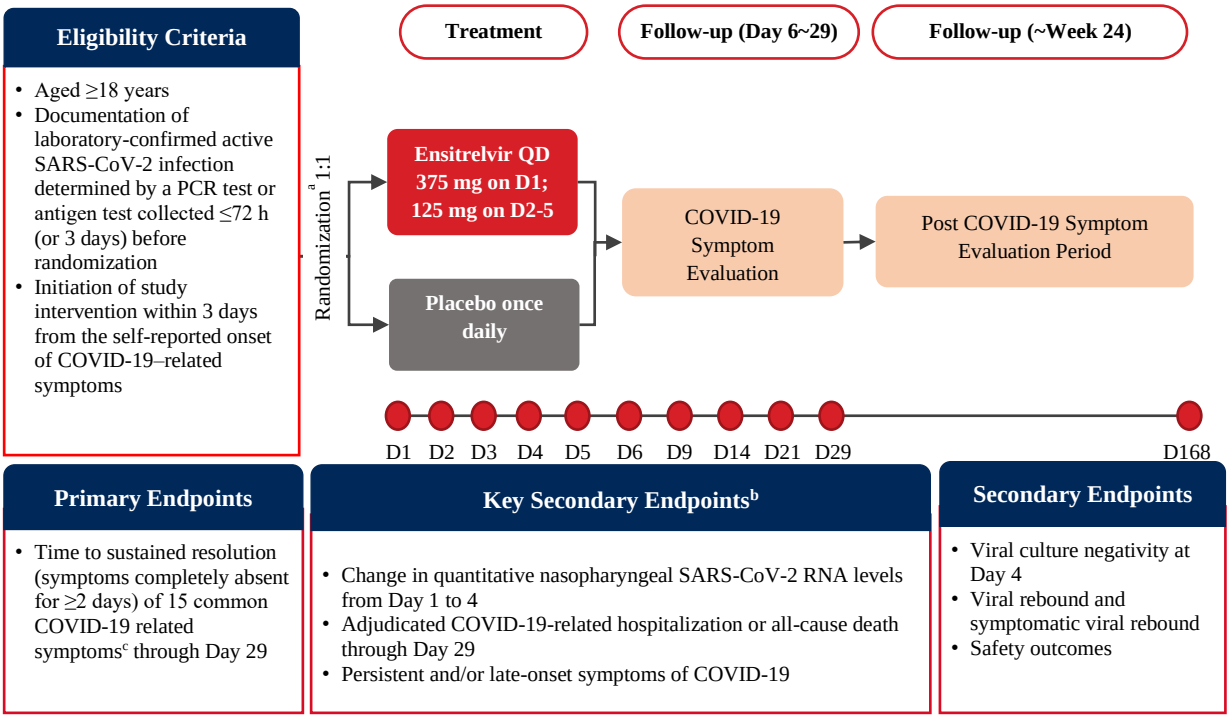
Results

- **Primary Endpoint**: 2.9% (30/1030) of HHCs in the ensitrelvir group developed symptomatic COVID-19 compared to 9.0% (91/1011) of HHC participants on placebo through Day 10, a 67% reduction in the risk of developing COVID-19 (risk ratio: 0.33; 95% CI: 0.22-0.49; P < 0.0001) in uninfected individuals (mITT population) treated after exposure.[10]
- TEAEs occurred in 15.1% (180/1190) of patients in the ensitrelvir group and 15.5% (184/1187) of patients in the placebo group, with 1 event in each group leading to discontinuation of treatment.[10]
- 2 serious TEAEs occurred in each group and they were considered to be unrelated to the study drug.[10]
- There were no AEs that led to hospitalization and no deaths were observed in either group.[10]

SCORPIO-HR (Phase 3)

Study Design

Figure 9. SCORPIO-HR Study Design[19]



Abbreviations: COVID-19, coronavirus disease 19; D, day; h, hours; n; RNA, ribonucleic acid; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.
[a]N=~2000; ~1000 patients in each treatment arm
[b]Primary and key secondary endpoints were tested using a fixed-sequence hierarchical approach at two-sided significance level of 0.05.
[c]15 COVID-19 symptoms: stuffy nose, runny nose, cough, sore throat, low energy or tiredness, feeling hot or feverish, shortness of breath or difficulty breathing, chills or shivering, muscle or body aches, diarrhea, nausea, vomiting, headache, loss of taste and loss of smell.

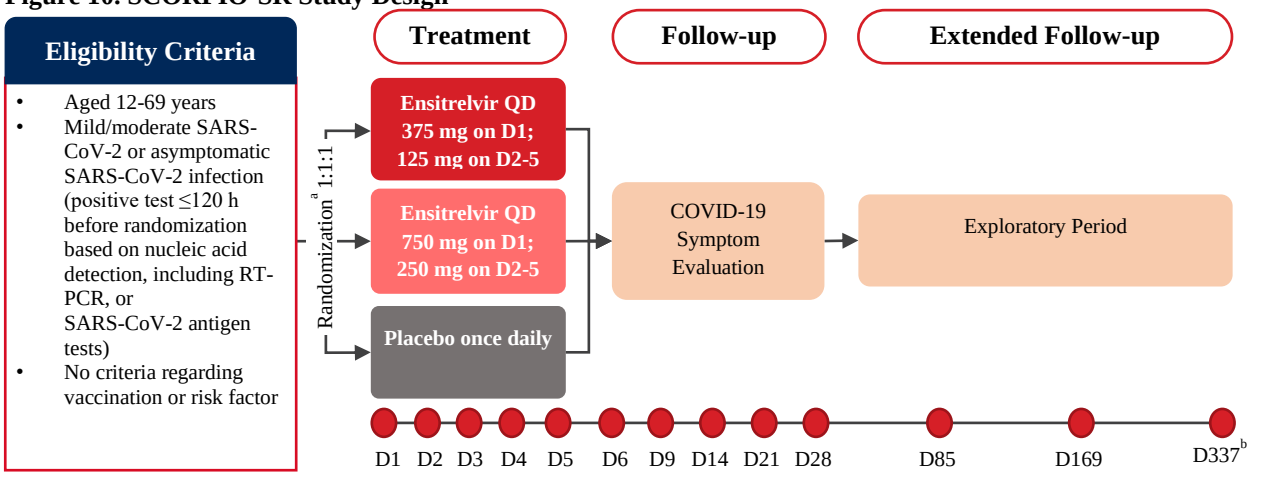
Results

- **Primary Endpoint**: In the modified intent-to-treat (mITT) population, the restricted mean time to sustained symptom resolution was not significantly different between the ensitrelvir (12.5 days) and placebo (13.1 days) groups (difference, 0.6 days; 95% CI: 1.38, 0.19; P=0.14).[19]
- Primary and key secondary endpoints were tested using a fixed-sequence hierarchical approach at two-sided significance level of 0.05. For SCORPIO-HR, the primary efficacy endpoint was not statistically significant, therefore secondary endpoint results are descriptive in nature; as such, secondary endpoints should be interpreted in an exploratory manner.[19]
- Adverse events occurred in 638 patients treated with ensitrelvir (61.5%; n=1037), and 635 patients treated with placebo (60.6%; n=1048). Treatment-related adverse events occurred in 86/1037 (8.3%) of patients treated with ensitrelvir and 74/1048 (7.1%) of patients that were given placebo. Serious treatment emergent adverse events (TEAE) occurred in 5/1037 (0.5%) of the ensitrelvir-treated participants and 6/1048 (0.6%) of the patients given placebo. No serious treatment-related adverse events occurred in either group.[19]
- COVID-19–related hospitalization was observed in 3/1037 (0.3%) ensitrelvir-treated patients and 1/1048 (0.1%) placebo-treated participant. No deaths were observed in either group.[19]

SCORPIO-SR (Phase 3)

Study Design

Figure 10. SCORPIO-SR Study Design[21]



Phase 3 Endpoints

Population: Mild/moderate

Primary:

- Time to resolution of 5 COVID-19 symptoms

Secondary outcomes included:

- Change from baseline in SARS-CoV-2 viral RNA on D4, time to first negative SARS-CoV-2 viral titer (key endpoints), and safety by D28

Abbreviations: COVID-19, coronavirus disease 19; D, Day; PCC, post-COVID-19 condition; RNA, ribonucleic acid; RT-PCR, reverse transcription polymerase chain reaction; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

Results

- **Primary Endpoint:** The time to resolution of 5 COVID-19 symptoms was significantly reduced with ensitrelvir 125 mg vs placebo in the primary analysis population (P = 0.04 with a Peto-Prentice generalized Wilcoxon test).[21]
- Most treatment-related adverse events (TRAEs) were mild in severity, and a decrease in high-density lipoprotein (HDL) was the most commonly reported TRAE. There were two serious adverse events (AEs) reported in the study (one in the ensitrelvir 125 mg arm and one in the placebo arm), both of which were judged as not treatment related.[21]