Xocova - Mechanism of Action

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.**

SUMMARY

- SARS-CoV-2 is the virus that causes COVID-19. 3CL protease is a key enzyme in the SARS-CoV-2 viral replication cycle[1]
- Xocova (ensitrelvir) is a noncovalent, nonpeptidic, orally bioavailable, SARS-CoV-2 main protease (also referred to as M^{pro}, $3CL^{pro}$ or nsp5 protease) inhibitor that prevents the formation of enzymes essential for viral replication, thereby preventing the SARS-CoV-2 virus from replicating[1,2]

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SARS-COV-2 VIRAL REPLICATION CYCLE

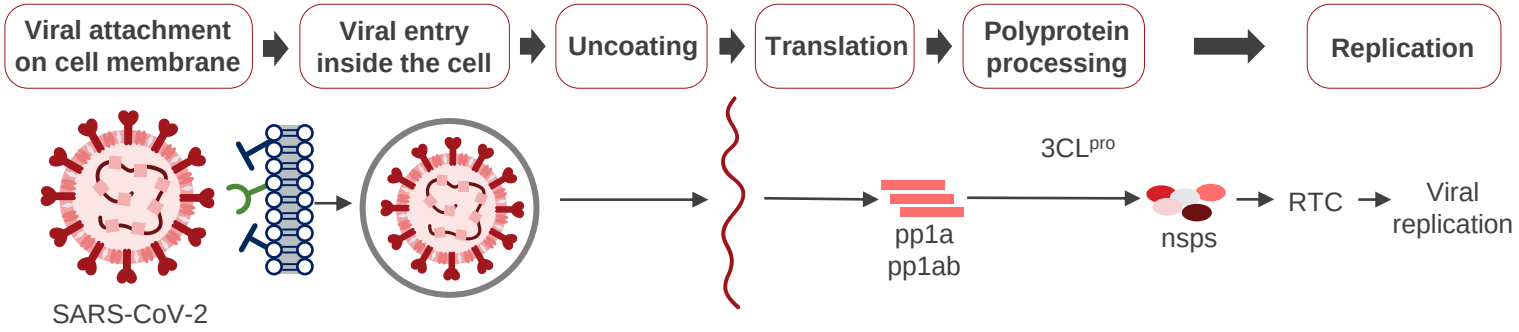
SARS-CoV-2 is an enveloped positive-sense single-stranded ribonucleic acid (RNA) virus that belongs to the genus *Betacoronavirus* and causes COVID-19.[1]

SARS-CoV-2 attaches to the cell membrane through binding of its spike glycoprotein to angiotensin-converting enzyme 2 (ACE2) on the host cell (Figure 1). Fusion of SARS-CoV-2 with the host cell membrane is facilitated by transmembrane serine protease 2 (TMPRSS2), followed by uncoating of the virus and release of viral RNA genome into the cell.[1,3]

SARS-CoV-2 viral RNA is translated by the host cell into 2 polyproteins, pp1a and pp1ab, which are subsequently autoproteolytically processed into 16 individual nonstructural proteins (nsp1-16), by main protease (M^{pro}; also known as 3CL protease or nsp5) and papain-like protease (PL^{pro}). 3CL protease is a key enzyme in the SARS-CoV-2 viral replication cycle that cleaves the polyproteins at 11 distinct sites into replication-essential enzymes.[1,3]

Proteases with substrate specificity similar to 3CL protease are not produced in humans, reducing the likelihood of off-target effects and making inhibition of 3CL protease a potential therapeutic strategy for treating SARS-CoV-2 infection.[3]

Figure 1. Overview of SARS-CoV-2 Replication Cycle[1,3]



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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.

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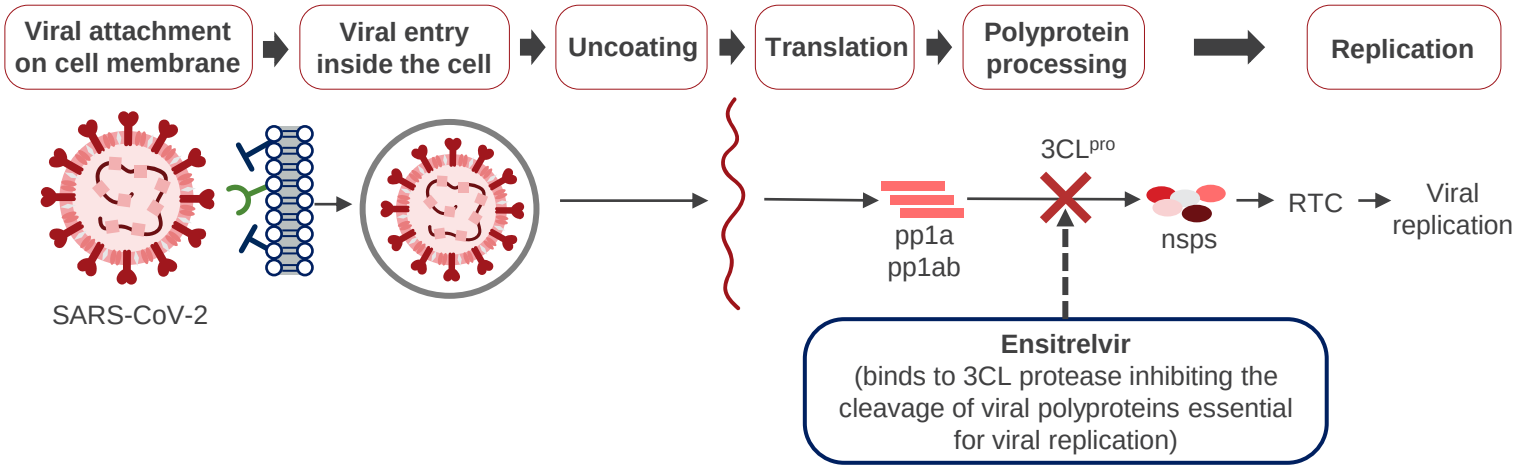
ENSITRELVIR MECHANISM OF ACTION

Ensitrelvir is a noncovalent, nonpeptidic, orally bioavailable SARS-CoV-2 3CL protease inhibitor indicated for post-exposure prophylaxis of COVID-19 in adults and adolescents 12 years of age and older following contact with an individual who has COVID-19.[1,2,4-6] By inhibiting the activity of 3CL protease, ensitrelvir prevents the formation of enzymes essential for viral replication, thereby preventing the SARS-CoV-2 virus from replicating (Figure 2).[1,3,4]

Ensitrelvir was identified using a structure-based drug design strategy via docking-based virtual screening, followed by biological screening of an in-house compound library. Optimization of the resulting hit yielded S-217622, designated ensitrelvir.[1]

Ensitrelvir was shown to be complexed with 3CL protease in X-ray crystallography studies. Ensitrelvir inhibited SARS-CoV-2 3CL protease activity in vitro with a half-maximal (50%) inhibitory concentration (IC_{50}) value of 0.0132 µM, and in antiviral assays with a half-maximal (50%) effective concentration (EC_{50}) value of 0.37 µM.[1,7]

Figure 2. Overview of SARS-CoV-2 Replication Cycle and Mechanism of Action of Ensitrelvir[1,6,7]



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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.

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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.[2]

CONTRAINDICATIONS

Xocova is contraindicated in patients with a history of clinically significant hypersensitivity reactions to ensitrelvir or any other components of the product.[2]

Xocova is contraindicated in patients taking drugs that are:

- 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.[2]

As stated in the Xocova Full Prescribing Information:

Section 12.4 – Microbiology

Mechanism of Action

Ensitrelvir is an inhibitor of the SARS-CoV-2 main protease (M^{pro}), also referred to as 3C-like protease ($3CL^{pro}$) or nonstructural protein 5 (nsp5) protease. Inhibition of SARS-CoV-2 M^{pro} renders it incapable of processing the viral polyproteins pp1a and pp1ab, preventing viral replication. Ensitrelvir inhibited the activity of recombinant SARS-CoV-2 M^{pro} in a biochemical assay with an IC_{50} value of 13.2 nM. Ensitrelvir was found to bind directly to the SARS-CoV-2 M^{pro} active site by X-ray crystallography.[2]

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.

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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 information on ensitrelvir's mechanism of action. For your review, these references are listed in the bibliography that follows:

Lin C, Jiang H, Li W, et al. Structural basis for the inhibition of coronaviral main proteases by ensitrelvir. *Structure*. 2023;31(9):1016-1024.e3. doi:10.1016/j.str.2023.06.010.

Lin M, Zeng X, Duan Y, et al. Molecular mechanism of ensitrelvir inhibiting SARS-CoV-2 main protease and its variants. *Commun Biol*. 2023;6(1):694. doi:10.1038/s42003-023-05071-y.

Hongyu H, Wu T, He F, et al. The binding mechanism of failed, in processing and succeed inhibitors target SARS-CoV-2 main protease. *J Biomol Struct Dyn*. Published online September 21, 2023. doi:10.1080/07391102.2023.2257800.

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 it should not be considered all-inclusive.

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Abbreviations

3CL	3-chymotrypsin-like
ACE2	angiotensin-converting enzyme 2
COVID-19	coronavirus disease 19
EC_{50}	half-maximal (50%) effective concentration
IC_{50}	half-maximal (50%) inhibitory concentration
MHLW	Ministry of Health, Labour and Welfare
M^{pro}	main protease
nsp	nonstructural protein
PL^{pro}	papain-like protease
pp1a/pp1ab	polyproteins 1a/1ab
RNA	ribonucleic acid
RTC	replicase-transcriptase complex
SARS-CoV-2	severe acute respiratory syndrome coronavirus 2
TMPRSS2	transmembrane serine protease 2

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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. Xocova® (ensitrelvir) tablets, for oral use [Prescribing Information]. Shionogi Inc.
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4. Mukae H, Yotsuyanagi H, Ohmagari N, et al. A randomized phase 2/3 study of ensitrelvir, a novel oral SARS-CoV-2 3C-Like protease inhibitor, in Japanese patients with mild-to-moderate COVID-19 or asymptomatic SARS-CoV-2 infection: results of the phase 2a part. [Published online ahead of print 13 September 2022]. Antimicrob Agents Chemother. 2022:e0069722. doi:10.1128/aac.00697-22.
5. Mukae H, Yotsuyanagi H, Ohmagari N, et al. Efficacy and Safety of Ensitrelvir in Patients With Mild-to-Moderate Coronavirus Disease 2019: The Phase 2b Part of a Randomized, Placebo-Controlled, Phase 2/3 Study. Clin Infect Dis. 2023;76(8):1403-1411. doi:10.1093/cid/ciac933.
6. 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.
7. 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)(Supplement):S1-S39. doi:10.1021/acs.jmedchem.2c00117.

ENCLOSURE

Xocova® (ensitrelvir) tablets, for oral use [Prescribing Information]. Shionogi Inc.