Report on the Deliberation Results

November 22, 2022

Pharmaceutical Evaluation Division, Pharmaceutical Safety and Environmental Health Bureau
Ministry of Health, Labour and Welfare

Brand Name	Xocova Tablets 125 mg
Non-proprietary Name	Ensitrelvir Fumaric Acid (JAN*)
Applicant	Shionogi & Co., Ltd.
Date of Application	February 25, 2022

Results of Deliberation

The applicant submitted an application for the approval of the product on February 25, 2022. The Act on Securing Quality, Efficacy and Safety of Products Including Pharmaceuticals and Medical Devices (Act No. 145 of 1960) was amended on May 20, 2022. In response to the amendment, the applicant requested that the Emergency Approval system based on Article 14-2-2, Paragraph 1 of the Act be applied to the application, in the current pandemic of COVID-19.

The Second Committee on New Drugs held a meeting on June 22, 2022. Then, the Committee and the Pharmaceutical Affairs Department of the Pharmaceutical Affairs and Food Sanitation Council held a joint meeting on July 20, 2022. As a result of deliberation in the meetings, the Committee and the Department concluded that the efficacy of the product cannot be presumed from the results of phase IIb part of the global phase II/III study (Study T1221) and other data, and decided that they would deliberate again after the submission of data from the then-ongoing phase III part of the study.

In a joint meeting held on November 22, 2022, the Committee and the Department deliberated again based on data submitted from phase III part of the study, and concluded that the product may be granted Emergency Approval with an effective period of 1 year.

The product is not classified as a biological product or a specified biological product. Both the drug product and its drug substance are classified as powerful drugs.

Approval Conditions

1. The applicant is required to develop and appropriately implement a risk management plan.
2. The applicant is required to request that physicians administer the product only to patients considered eligible for treatment with the product who, or whose legally acceptable representatives, have been provided with the efficacy and safety information of the product in written form, and have provided written informed consent before the treatment.
3. The applicant is required to promptly compile and submit data showing the efficacy of the product in phase III part of the global phase II/III study (Study T1221).

**Japanese Accepted Name (modified INN)*

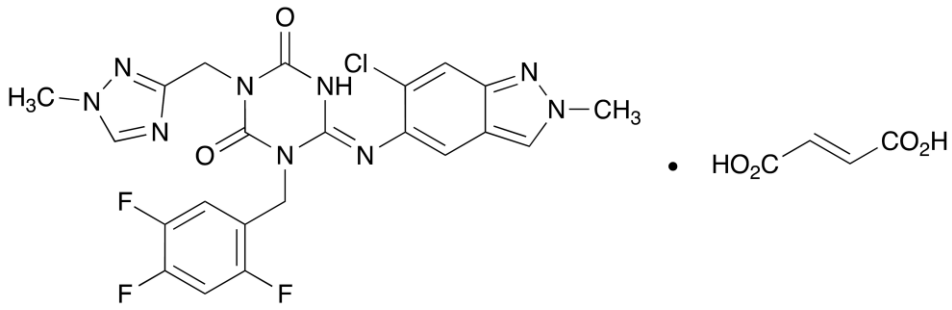
Review Report

June 17, 2022
Pharmaceuticals and Medical Devices Agency

The following are the results of the review of the following pharmaceutical product submitted for marketing approval conducted by the Pharmaceuticals and Medical Devices Agency (PMDA).

Brand Name	Xocova Tablets 125 mg
Non-proprietary Name	Ensitrelvir Fumaric Acid
Applicant	Shionogi & Co., Ltd.
Date of Application	February 25, 2022
Dosage Form/Strength	Each tablet contains 152.3 mg of ensitrelvir fumaric acid (125 mg of ensitrelvir).
Application Classification	Prescription drug, (1) Drug with a new active ingredient

Chemical Structure



Molecular formula:	$C_{22}H_{17}ClF_3N_9O_2$ $C_4H_4O_4$
Molecular weight:	647.95
Chemical name:	(6*E*)-6-[(6-Chloro-2-methyl-2*H*-indazol-5-yl)imino]-3-[(1-methyl-1*H*-1,2,4-triazol-3-yl)methyl]-1-[(2,4,5-trifluorophenyl)methyl]-1,3,5-triazinane-2,4-dione monofumaric acid]

Items Warranting Special Mention	The product was handled as a product expected to be fall under the framework of approval from the Minister of Health, Labour and Welfare prescribed in Article 14 of the Pharmaceuticals and Medical Devices Act, pursuant to the provisions of Article 14-2, Paragraph 2 of the Act. ("Handling of a drug whose applicant is requesting a change of the application category to Emergency Approval (request)" (PSEHB/PED Notification No. 0526-16, dated May 26, 2022). Priority Review based on "Policy on regulatory review of drugs, etc. against coronavirus disease (COVID-19) (No. 2)" (PSEHB/PED Notification No. 0617-9 and PSEHB/MDED Notification No. 0617-1, dated June 17, 2021)
Reviewing Office	Office of New Drug IV

Results of Review

On the basis of the data submitted (see attachment), PMDA does not deny that the product tended to reduce viral load, as shown by results from phase IIa and IIb parts of the global phase II/III study (Study T1221), but cannot conclude that the product is presumed to have efficacy for the proposed indication. Therefore, further investigation is needed based on data including results from phase III part of the study. This is PMDA's conclusion regarding the efficacy of the product against COVID-19. Nevertheless, from a medical and social point of view, it is also acceptable to consider making the product available early. If the product is approved based on the data currently available, its efficacy should be re-evaluated after the approval based on results from phase III part of the global phase II/III study (Study T1221) and, depending on the results, appropriate actions should be taken including considering the withdrawal of marketing approval.

Results of phase IIa and IIb parts of the global phase II/III study (Study T1221) do not show any significant safety concerns with the product, showing a certain level of tolerability of the product. However, because the use experience with the product in patients with COVID-19 is limited, new safety concerns may arise when the product is used in many patients after the market launch. If the product is approved based on the currently available data, the package insert should include appropriate precautionary statements regarding potential teratogenic risks, drug interactions, and others. The safety of the product should be further investigated using data including results to be obtained from the ongoing phase IIb/III part and phase III part of the global phase II/III study (Study T1221), and new findings should be provided appropriately to healthcare professionals.

Indication

Disease caused by SARS-CoV-2 infection (COVID-19)

Dosage and Administration

The usual dose in ≥12-year-old pediatric patients and adults is ensitrelvir 375 mg on Day 1 and ensitrelvir 125 mg from Days 2 to 5, administered orally once daily.

Approval Conditions

1. The applicant is required to develop and appropriately implement a risk management plan.
2. The applicant is required to request that physicians administer the product only to patients considered eligible for treatment with the product who, or whose legally acceptable representatives, have been provided with the efficacy and safety information of the product in written form, and have provided written informed consent before the start of treatment.
3. The applicant is required to submit data from phase III part of the global phase II/III study (Study T1221) promptly after the study completion.

Attachment

Review Report (1)

June 9, 2022

The following is an outline of the data submitted by the applicant and content of the review conducted by the Pharmaceuticals and Medical Devices Agency (PMDA).

Product Submitted for Approval

Brand Name	Xocova Tablets 125 mg
Non-proprietary Name	Ensitrelvir Fumaric Acid
Applicant	Shionogi & Co., Ltd.
Date of Application	February 25, 2022
Dosage Form/Strength	Each tablet contains 152.3 mg of ensitrelvir fumaric acid (125 mg of ensitrelvir).

Proposed Indication

Disease caused by SARS-CoV-2 infection (COVID-19)

Proposed Dosage and Administration

The usual dose in ≥12-year-old patients is ensitrelvir 375 mg on Day 1 and ensitrelvir 125 mg from Days 2 to 5, administered orally once daily, for a total of 5 days.

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List of Abbreviations

See Appendix.

1. Origin or History of Discovery, Use in Foreign Countries, and Other Information

COVID-19 is a disease caused by SARS-CoV-2 infection. Symptoms at the onset of the disease include, in the order of frequency, pyrexia, respiratory symptoms, malaise, headache, gastrointestinal symptoms, runny nose, taste abnormality, dysosmia, arthralgia, and myalgia. In patients infected with the Omicron variant, the frequency of common cold-like symptoms (e.g., runny nose, headache, malaise, and sore throat) increases, while the frequency of dysosmia and taste disorder decreases, compared with the frequency observed with the original strain (Guidelines for Diagnosis and Treatment of COVID-19, ver. 7.2, dated May 9, 2022). As of May 30, 2022, the total number of people infected with SARS-CoV-2 (those testing positive by polymerase chain reaction [PCR]) in Japan is 8,830,300. Among them, 254,935 required hospitalization or other treatment (including 93 with severe disease). In total, 8,530,400 were discharged or released from medical treatment, and 30,582 died.[1)]

In Japan, the following drugs are approved for marketing for the treatment of COVID-19: remdesivir (brand name: Veklury for Intravenous Injection 100 mg), casirivimab/imdevimab (brand name: Ronapreve for Intravenous Infusion Set 300, etc.), sotrovimab (genetical recombination) (brand name: Xevudy for Intravenous Infusion 500 mg), molnupiravir (brand name: Lagevrio Capsules 200 mg), and nirmatrelvir/ritonavir (brand name: Paxlovid PACK). Also, baricitinib (brand name: Olumiant Tablets 4 mg, etc.) and tocilizumab (genetical recombination) (brand name: Actemra 80 mg, etc. for Intravenous Infusion) are approved for marketing for the treatment of pneumonia caused by COVID-19. In addition, multiple vaccines against COVID-19 are approved for marketing.

Ensitrelvir fumaric acid (hereafter, ensitrelvir) is an inhibitor of 3C-like (3CL) protease of SARS-CoV-2, a compound discovered by the joint study of Hokkaido University and Shionogi & Co., Ltd. Ensitrelvir suppresses viral replication by inhibiting the cleavage of the polyprotein.

The applicant has submitted an application for the approval of ensitrelvir based on the results of phase IIa and phase IIb parts of the global phase II/III study (Study T1221) involving patients with COVID-19. Subsequently, the Pharmaceuticals and Medical Devices Act was revised on May 20, 2022. In response to this, the applicant requested that the Emergency Approval system be applied to the application of ensitrelvir, claiming that ensitrelvir is qualified for approval from the Minister of Health, Labour and Welfare prescribed in Article 14 of the Pharmaceuticals and Medical Devices Act, pursuant to the provisions of Article 14-2, Paragraph 2 of the Act.

As of May 2022, ensitrelvir has not been approved in any country or region.

This report contains the results of review conducted by PMDA based on the “Principles for approval review within the Emergency Approval system” (PSEHB/PED Notification No. 0520-1, dated May 20, 2022) and “Handling of a drug whose applicant is requesting a change of the application category to Emergency Approval (request) (request)” (PSEHB/PED Notification No. 0526-16, dated May 26, 2022).

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1) Ministry of Health, Labour and Welfare: https://www.mhlw.go.jp/stf/covid-19/kokunainohasseijoukyou.html (last accessed on May 31, 2022)

2. Quality and Outline of the Review Conducted by PMDA

2.1 Drug substance

2.1.1 Characterization

The drug substance is a white powder. The determined general properties include description, solubility, hygroscopicity, thermal analysis, pH, acid dissociation constant, distribution coefficient, and polymorphism. The drug substance is present in at least 2 types of fumaric acid cocrystals ([a] [redacted], [b] [redacted], [redacted], [redacted]). Only [a] [redacted] shown to be formed in the commercial-scale manufacturing.

The chemical structure of the drug substance was elucidated by elemental analysis, mass spectrometry, infrared absorption spectroscopy, nuclear magnetic resonance spectrometry (^{1}H-NMR and ^{13}C-NMR), powder X-ray diffraction, and single crystal X-ray diffraction.

2.1.2 Manufacturing process

The drug substance is synthesized using the following starting materials: Starting Material A, Starting Material B, and Starting Material C.

The quality control strategy was designed based on the following investigations (Table 1):

- Identification of critical quality attributes (CQAs)
- Identification of critical process parameters based on the quality risk assessment

Table 1. Summary of quality control strategy

CQA[a)]	Control method
Content	Specifications
Description	Manufacturing process, specifications
Identity	Manufacturing process, specifications
Related substances	Manufacturing process, specifications
[redacted]	Manufacturing process, specifications
Residual solvents	Manufacturing process, specifications
[redacted]	Specifications
Residue on ignition	Specifications
Water content	Specifications

a) The risk assessment of the drug substance confirmed that there was no possibility of [redacted] remaining in excess of [redacted]. Therefore, [redacted] not included in CQA.

The synthetic process of Intermediate D,[2)] the synthetic process of Intermediate E, and [redacted] were identified as the critical steps. Intermediates D and E are controlled as critical intermediates.

2.1.3 Control of drug substance

The proposed specifications for the drug substance include content (ensitrelvir and fumaric acid), description, identification (infrared absorption spectroscopy and liquid chromatography), purity (related substances [liquid chromatography], Related Substance F[3)] [redacted], residual solvents [gas chromatography]), water content, residue on ignition, and assay (ensitrelvir [liquid chromatography], fumaric acid [liquid chromatography]).

2) Intermediate D

3) Controlled as a mutagenic impurity (Class 2) by the specifications for the drug substance.

During the review process, the test for fumaric acid was changed from purity test to assay, and was included in content test.

2.1.4 Stability of drug substance

Table 2 shows the main stability studies performed on the drug substance. Results demonstrated the stability of the drug substance. A photostability testing showed that the drug substance was photostable.

Table 2. Stability studies of drug substance

Study	Primary batch	Temperature	Humidity	Storage condition	Storage period
Long-term	3 pilot-scale batches	25°C	60% RH	Low-density polyethylene bag (double-layered) + fiber drum	3 months [a)]
Accelerated	3 pilot-scale batches	40°C	75% RH		3 months [a)]

a) The applicant submitted data on 2 batches up to 6 months and data on 1 batch up to 3 months (3 pilot-scale batches in total).

Based on the above, a retest period of 6 months was proposed for the drug substance stored in a double-layered polyethylene bag placed in a fiber drum at room temperature. The long-term stability study will be continued for [redacted] months.

2.2 Drug product

2.2.1 Description and composition of drug product and formulation development

The drug product is uncoated tablets. Each tablet contains 152.3 mg of the drug substance (125 mg of ensitrelvir). Excipients contained in the drug product are D-mannitol, croscarmellose sodium, hydroxypropylcellulose, light anhydrous silicic acid, crystalline cellulose, and magnesium stearate.

2.2.2 Manufacturing process

The drug product is manufactured through [redacted], tableting, filling, packaging/labeling, testing, and storage. [redacted] was identified as the critical step, and in-process control parameters and action limits have been established for [redacted].

The quality control strategy was designed based on the following investigations (Table 3).

- Identification of CQAs
- Identification of critical process parameters based on quality risk assessment

Table 3. Summary of control strategy for drug product

CQA	Control method
Strength	Manufacturing process, specifications
Description	Manufacturing process, specifications
Identity	Specifications
Uniformity of dosage unit	Manufacturing process, specifications
Dissolution	Manufacturing process, specifications
Related substances	Specifications

2.2.3 Control of drug product

The proposed specifications for the drug product include strength, description, identification (liquid chromatography and ultraviolet-visible spectroscopy), purity (related substances [liquid

chromatography]), uniformity of dosage units (mass variation), dissolution (liquid chromatography), and assay (liquid chromatography).

2.2.4 Stability of drug product

Table 4 shows the main stability studies performed on the drug product. Results demonstrated the stability of the drug product. A photostability test showed that the drug product was photostable.

Table 4. Stability studies of drug product

Study	Primary batches	Temperature	Humidity	Storage form	Storage period
Long-term	1 pilot scale batch 2 small scale batches	25°C	60% RH	Blister package ([redacted])	6 months
Accelerated	1 pilot scale batch 2 small scale batches	40°C	75% RH		6 months

Based on the above, a shelf life of 12 months has been proposed for the drug product stored in a blister package ([redacted]) at room temperature, based on the ICH Q1E Guideline. Long-term stability study will be continued for [redacted] months.

2.R Outline of the review conducted by PMDA

Based on the submitted data and the following review, PMDA has concluded that the quality of the drug substance and the drug product is controlled in an appropriate manner.

2.R.1 Strategy for controlling cocrystallized drug substance

The applicant explained the reason why the cocrystal of ensitrelvir and fumaric acid (hereinafter, "ensitrelvir-fumaric acid cocrystal") was selected as the drug substance and the strategy for controlling the ensitrelvir-fumaric acid cocrystal.

The applicant's explanation:

Ensitrelvir in free form is poorly absorbed orally due to its low solubility. Therefore, in order to select drug substance candidates, salts and cocrystals were screened based on their solubility, hygroscopicity, and preliminary evaluation of solid stability. Among the selected candidates, the ensitrelvir-fumaric acid cocrystal was selected considering the control of crystal polymorphism and other factors.

As for the control strategy of the ensitrelvir-fumaric acid cocrystal, the assessment of the effect of the manufacturing process parameters on the crystalline form showed that none of the parameters affected the crystalline form, and that [redacted] of the ensitrelvir-fumaric acid cocrystal were produced constantly. Accordingly, the crystalline form is not included in the COAs of the drug substance, obviating the need for controlling the crystalline form by in- process tests or by the specifications for the drug substance. The unnecessariness of controlling the crystalline form is also supported by the following facts:

(a) [redacted] ensitrelvir ([redacted] with the ensitrelvir-fumaric acid cocrystal) was not observed in any batches of the drug substance either during the development of manufacturing process or at the commercial-scale manufacturing.

(b) Since the specifications for the drug substance include [redacted], the drug substance ([redacted] of the ensitrelvir-fumaric acid cocrystal) can be distinguished from [redacted] ensitrelvir.

PMDA's view regarding the appropriateness of the proposed control strategy of the ensitrelvir-fumaric acid cocrystal:
The applicant explained that the crystalline form of the ensitrelvir-fumaric acid cocrystal is ensured by the manufacturing process and therefore it need not be controlled by in-process tests or by the specifications for the drug substance. However, the following facts indicate that potential risks remain in the proposed control strategy:

- The acceptable range of the effect of the manufacturing process parameters on the crystalline form was assessed by a test on a laboratory scale. At the current moment, no sufficient information is available on the effect of the parameters when the ensitrelvir-fumaric acid cocrystal (the drug substance) is manufactured at commercial scale.
- As for the control of the crystalline form, the applicant has not explained in detail the comparability of the analytical sensitivity, etc., between [redacted] used in the specifications for the ensitrelvir-fumaric acid cocrystal (the drug substance) and [redacted] used in the characterization analysis etc., employed during the development process.

Thus, the crystalline form should be controlled by [redacted] in commercial manufacturing.

The applicant's response:
[redacted] was used in [redacted] of [redacted]. It will also be used for controlling the crystalline form in commercial manufacturing as a condition of marketing authorization.

PMDA accepted the applicant's explanation.

2.R.2 Strategy for controlling residual Solvent A in the drug substance and the drug product

The applicant's explanation about the results of batch analysis constantly showing residual Solvent A of [redacted] ppm in the drug substance:
Based on the following results, the drug substance is considered to be [redacted]. Therefore, Solvent A should be controlled as a residual solvent, not as an adduct (solvated adduct).

- The amount of residual Solvent A in the drug substance obtained between the early development stage and process validation was [redacted] ppm ([redacted] in terms of ensitrelvir), showing that the residual amount varied significantly among batches. Further, even the maximum amount of residual Solvent A is too little to form solvation.
- Single-crystal X-ray diffraction showed that there was no space for solvent in crystals and there was no peak corresponding to solvent-derived residue.

As a strategy for controlling the residual Solvent A, the limit for the residual Solvent A in the specifications for the drug substance should not be ≤5,000 ppm (Option 1 limit for Class 3 solvents in the ICH Q3C Guideline) but should be ≤[redacted] ppm (Option 2 limit), for the following reasons:

- The effect of the process parameters in the synthetic process of Intermediate E (in which Solvent A is used as the solvent and the final crystallization is performed) on the amount of residual Solvent A was investigated during the development process. Results showed that none of the manufacturing process parameters affected the amount of residual Solvent A. Further, the batch analysis at commercial scale showed a similar amount of residual Solvent A to that obtained between the early development stage and process validation. Furthermore, Solvent A was selected as the solvent to ensure the robustness of controlling the crystalline form. Therefore, it is difficult, from the aspect of control strategy of the drug substance, to reduce the residual amount of Solvent A from the present level by changing the manufacturing process.
- In the control of the drug product based on Option 2 for Class 3 solvents in the ICH Q3C Guideline, the drug substance at the maximum daily dose (375 mg) results in approximately [redacted]mg daily intake of Solvent A (derived from the drug substance) even if the drug substance contains [redacted] ppm of Solvent A. Thus, Solvent A intake can be controlled at a level ≤50 mg/day, the permitted daily exposure stipulated by the ICH Q3C Guideline. Further, the drug product does not contain Solvent A as an excipient.

PMDA's view:
It is acceptable to control Solvent A as a residual solvent, not as the adduct (solvated adduct). The proposed specification limit for the residual Solvent A is also acceptable. Results of the safety studies so far available do not show any effect of Solvent A on the container/closure system. However, PMDA instructed the applicant to follow the behavior of residual Solvent A in the drug substance and the drug product continuously in the ongoing stability studies, and to evaluate its effects on the container/closure system. The applicant accepted the PMDA's instruction.

2.R.3 Retest period of drug substance and shelf life of drug product

Since ensitrelvir is a drug with a new active ingredient, the drug substance and the drug product are required to undergo formal stability tests, to confirm the stability of at least 12 months for 3 batches in the long-term stability study and at least 6 months for 3 batches in the accelerated study, according to the ICH Q1A Guideline. The guideline also requires that 2 of 3 primary batches of the drug product in the stability studies should be manufactured at the pilot or larger scale. In the present application of ensitrelvir, the applicant proposed the retest period of 6 months for the drug substance and the shelf life of 12 months for the drug product (i.e., twice the stable period confirmed in the long-term storage studies on 3 batches), according to the ICH Q1E Guideline. However, these stability studies did not meet the above conditions for batches or the minimum storage period [see Sections 2.1.4 and 2.2.4, etc.]. The applicant plans to submit additional data from the 12-month stability study and the 6-month accelerated study to PMDA when they become available.

PMDA concluded that the proposed retest period of the drug substance and the shelf life of the drug product were acceptable, for the following reasons:

- Ensitrelvir has been developed as a drug against COVID-19, a newly emerging infectious disease, within an extremely short period compared with usual drug development. It is unavoidable that only limited data of stability tests are available.
- The stability of the drug substance has been confirmed by the results of stability studies so far obtained [see Section 2.1.4].
- The stability of the drug product has been confirmed by the results of stability studies so far obtained [see Section 2.2.4]. [redacted] is not included in the specifications for the drug product, but results of the stress test (40°C/75% RH, in the dark, in a glass bottle without a cap) showed that [redacted] increased to [redacted]% after 1 month but remained unchanged at [redacted]% after 2 months. This suggests that [redacted] does not exceed approximately [redacted]% even after long-term storage. In addition, 2-month data from the stress test showed no change from baseline in any test parameters other than [redacted].
- The following data are available on 3 process-validation batches (commercial scale) of the drug product. Results showed no batch size-dependent effect on the quality attributes or stability of the drug product.
  - Batch analysis results for 3 commercial-scale batches were similar to those for 1 pilot scale batch and 2 small scale batches.
  - One-month data from the accelerated study (40°C/75% RH, protected from light) of 2 commercial-scale batches showed no clear changes in the quality attributes from baseline, except for [redacted]. The increasing trend of [redacted] was similar to that observed with 1 pilot scale batch and with 2 small scale batches.

3. Non-clinical Pharmacology and Outline of the Review Conducted by PMDA

The applicant submitted data on pharmacodynamic studies, secondary pharmacology studies, and safety pharmacology studies. In this section, data are expressed in means unless specified otherwise

3.1 Primary pharmacodynamics

3.1.1 Inhibition of SARS-CoV-2 3CL protease (CTD 4.2.1.1-01, 20)

The inhibitory activity of ensitrelvir against SARS-CoV-2 3CL protease was evaluated based on the reaction between SARS-CoV-2 3CL proteases containing various amino acid substitutions and their substrates. Table 5 shows the results.

Table 5. Inhibitory activity of ensitrelvir against SARS-CoV-2 3CL protease

Amino acid substitution of SARS-CoV-2 3CL protease	IC_{50} (nmol/L)
No amino acid substitution [a)]	13.2
G15S [b)]	8.0
T21I	14.3
L89F	15.0
K90R [c)]	9.7
P108S	13.2
P132H [d)]	14.4

a) Amino acid sequence observed in the original strain, the Alpha, Gamma, Delta variants, etc.
b) Amino acid substitution observed in the Lambda variant
c) Amino acid substitution observed in the Beta variant and a recombinant strain (XA)
d) Amino acid substitution observed in the Omicron variant (BA.1, BA.2, BA.3, BA4, BA5 and their sub-lineages) and in recombinant strains (XD and XE)

3.1.2 *In vitro* antiviral activity

3.1.2.1 *In vitro* antiviral activity (CTD 4.2.1.1-02 to 07, 21)

Using various cells infected with clinical isolates of SARS-CoV-2 or with a mouse-adapted strain, the antiviral activity of ensitrelvir was evaluated based on cell degeneration. Table 6 shows results.

Table 6. *In vitro* antiviral activity of ensitrelvir against SARS-CoV-2

Cells	Viral isolate	Viral strain	EC_{50} (μmol/L)	
			Ensitrelvir	Remdesivir
VeroE6/TMPRSS2	hCoV-19/Japan/TY/WK-521/2020	A (original strain)	0.37	1.9
	hCoV-19/Japan/QK002/2020	B.1.1.7 (Alpha variant)	0.33	0.87
	hCoV-19/Japan/QHN001/2020		0.31	0.97
	hCoV-19/Japan/QHN002/2020		0.46	0.99
	hCoV-19/Japan/TY8-612/2021	B.1.351 (Beta variant)	0.40	1.2
	hCoV-19/Japan/TY7-501/2021	P.1 (Gamma variant)	0.50	2.1
	hCoV-19/Japan/TY7-503/2021		0.43	1.0
	hCoV-19/Japan/TY11-927-P1/2021	B.1.617.2 (Delta variant)	0.41	1.6
	hCoV-19/Japan/TY38-873/2021	B.1.1.529/BA.1 (Omicron variant)	0.29	1.1
	hCoV-19/Japan/TY38-871/2021	B.1.1.529/BA.1.1 (Omicron variant)	0.36	1.0
	hCoV-19/Japan/TY40-385/2022	B.1.1.529/BA.2 (Omicron variant)	0.52	1.0
	SARS-CoV-2 MA-P10[a)]	A (original strain)	0.12	1.6
HEK293T/ACE2-TMPRSS2	hCoV-19/Japan/TY/WK-521/2020	A (original strain)	0.027	0.012
	hCoV-19/Japan/QK002/2020	B.1.1.7 (Alpha variant)	0.044	0.011
	hCoV-19/Japan/TY8-612/2021	B.1.351 (Beta variant)	0.038	0.010
	hCoV-19/Japan/TY7-501/2021	P.1 (Gamma variant)	0.026	0.0061
	hCoV-19/Japan/TY11-927-P1/2021	B.1.617.2 (Delta variant)	0.058	0.0094
	hCoV-19/Japan/TY38-873/2021	B.1.1.529/BA.1 (Omicron variant)	0.064	0.015
3D organ culture model of human tracheal epithelium prepared from primary human nasal cavity-derived cells	hCoV-19/Japan/TY11-927-P1/2021	B.1.617.2 (Delta variant)	0.0570 [b)]	-

-: Not measured
a) Mice-adapted strain of hCoV-19/Japan/TY/WK-521/2020
b) EC_{90} was 0.117 μmol/ L (0.0623 μg/mL).

The 50% cytotoxic concentration (CC_{50}) of ensitrelvir against VeroE6/TMPRSS2 cells and HEK293T/ACE2-TMPRSS2 cells was >100 μmol/L and 55 μmol/L, respectively. Also, using the cytotoxic activity (CC_{50}) and the antiviral activity (50% effective concentration [EC_{50}]) of ensitrelvir, its selectivity ratio (CC_{50}/EC_{50}) was calculated to be >192.[4)]

3.1.2.2 Antiviral activity in the presence of serum (CTD 4.2.1.1-08)

Antiviral activity of ensitrelvir was investigated in the presence of serum, using VeroE6/TMPRSS2 cells infected with SARS-CoV-2 (isolate hCoV-19/Japan/TY7-501/2021, strain P.1 [Gamma variant]). Linear regression was used to estimate EC_{50} in 100% serum from EC_{50} in the presence of human

4) Calculated using EC_{50} (0.52 μmol/L) of isolate hCoV-19/Japan/TY40-385/2022, which showed the highest EC_{50} when assayed using VeroE6/TMPRSS2 cells.

serum (0-25%) or mouse serum (0-12.5%). EC_{50} in the presence of 100% human or mouse serum was estimated to be 3.02 and 3.93 μmol/L, respectively. EC_{50} in the presence of 100% serum (both human and mouse serum) was approximately 6 times higher than that in its absence.

3.1.3 Resistance profile

3.1.3.1 *In vitro* resistance acquisition (CTD 4.2.1.1-09 to 11)

VeroE6/TMPRSS2 cells infected with a clinical isolate of SARS-CoV-2 were cultured in the presence of ensitrelvir (0.041 to 10 μmol/L). The supernatant was collected and, after 4 subcultures, the virus in the supernatant was subjected to sequencing of SARS-CoV-2 3CL protease region. Results showed amino acid substitutions of D48G, M49L, P52S, S144A, and M49(M/L)/S144A. Table 7 shows the antiviral activity of ensitrelvir and remdesivir against these strains with amino acid substitutions.

Table 7. Antiviral activity of ensitrelvir and remdesivir against mutant strains

Parent isolate (strain)	Amino acid substitution in 3CL protease	Ensitrelvir		Remdesivir	
		EC_{50} (μmol/L)	Sensitivity change [a)]	EC_{50} (μmol/L)	Sensitivity change [a)]
hCoV-19/Japan/TY/WK-521/2020 (A, original strain)	M49L	5.9	12	1.1	0.40
hCoV-19/Japan/QHN001/2020 (B.1.1.7, Alpha variant)	M49L	12	26	1.6	0.58
	S144A	4.0	8.5	1.4	0.49
	D48G	1.8	3.7	2.0	0.71
hCoV-19/Japan/TY8-612/2021 (B.1.351, Beta variant)	M49L	10	21	1.1	0.66
	P52S	2.6	5.5	1.4	0.86
hCoV-19/Japan/TY7-501/2021 (P.1, Gamma variant)	M49L	13	27	3.1	1.8
	M49 (M/L)/S144A[b)]	49	100	1.6	0.97
	S144A	5.4	11	3.0	1.8
hCoV-19/Japan/TY11-927-P1/2021 (B.1.617.2, Delta variant)	M49L	13	41	1.7	1.1
	D48G	2.0	6.5	2.7	1.7

VeroE6/TMPRSS2 cells were used.
a) "EC_{50} against variant" / "EC_{50} against parent strain"
b) Mixture of 2 variants with different amino acid substitutions (S144A and M49L/S144A)

3.1.3.2 Antiviral activity against recombinant SARS-CoV-2 viruses with amino acid substitutions and the replication capacity of the recombinant viruses (CTD 4.2.1.1-12 to 13)

Amino acid substitutions (D48G, M49L, P52S, S144A, M49L/S144A) of SARS-CoV-2 3CL protease were observed in the *in vitro* resistance acquisition study [see Section 3.1.3.1]. Recombinant viruses were generated by introducing these amino acid substitutions into SARS-CoV-2 (rgSARS-CoV-2/Hu/DP/Kng/19-020, lineage A) by reverse genetics technique. Antiviral activity of the following drugs against the recombinant viruses was investigated: ensitrelvir, remdesivir, nirmatrelvir, and the combination of casirivimab and imdevimab.[5)] Table 8 shows the results. Also, VeroE6/TMPRSS2 cells were infected with the recombinant viruses, and then the replication capacity of the viruses was evaluated based on viral titer (50% tissue culture infectious dose [$TCID_{50}$]). The changes over time in the viral titer were similar in the parent strains and the recombinant viruses.

5) Clone REGN10933 and Clone REGN10987(Cell Science)

Table 8. Antiviral activity against recombinant viruses with amino acid substitutions

Amino acid substitution	Ensitrelvir		Remdesivir		Nirmatrelvir		Casirivimab + Imdevimab[a)]	
	EC_{50} (μmol/L)	Change in sensitivity[b)]	EC_{50} (μmol/L)	Change in sensitivity[b)]	EC_{50} (μmol/L)	Change in sensitivity[b)]	EC_{50} (μmol/L)	Change in sensitivity[b)]
D48G	0.49	4.3	1.7	1.0	0.038	1.0	0.19	0.91
M49L	1.7	17	0.85	0.52	0.029	0.68	0.21	0.93
P52S	0.44	3.7	1.8	1.0	0.021	0.54	0.19	0.91
S144A	0.92	9.2	1.5	0.90	0.057	1.4	0.22	1.0
M49L/S144A	11	100	0.86	0.53	0.055	1.3	0.21	0.95

VeroE6/TMPRSS2 cells were used.

a) Clone REGN10933 and Clone REGN10987 (Cell Science)

b) "EC_{50} against recombinant virus with amino acid substitution" / "EC_{50} against parent strain"

3.1.4 Effect of coadministration of ensitrelvir with other anti-SARS-CoV-2 drugs (CTD 4.2.1.1-14 to 15)

The effect of ensitrelvir in combination with other anti-SARS-CoV-2 drugs was investigated. Table 9 shows the results.

Table 9. Effect of coadministration of ensitrelvir with other anti-SARS-CoV-2 drugs

Viral isolate (strain)	Concomitant drug	Combination Index[a)]	Assessment[b)]
hCoV-19/Japan/TY11-927-P1/2021 (B.1.617.2, Delta variant)	Remdesivir	1.11	Additive
		1.06	Additive
		1.03	Additive
	Casirivimab + Imdevimab[c)]	0.913	Additive
		0.742	Synergistic
		0.730	Synergistic
rgSARS-CoV-2/Hu/DP/Kng/19-020 (A, original strain)[e)]	*N*-Hydroxycytidine[d)]	1.07	Additive
		1.19	Additive
		1.12	Additive
	Nirmatrelvir	1.08	Additive
		1.12	Additive
		1.15	Additive

VeroE6/TMPRSS2 cells were used.

a) Drugs for coadministration were mixed at a ratio approximate to EC_{50} of each drug according to Chou-Talalay method (*Adv Enzyme Regul*. 1984;22:27-55), and the combination index was calculated under the conditions where the two drugs contribute almost equally to the inhibitory effect.

b) CI ≤0.8 was considered as synergistic effect, 0.8<CI<1.2 as additive effect, and 1.2≤CI as competitive effect.

c) Clone REGN10933 and Clone REGN10987 (Cell Science)

d) The main metabolite of molnupiravir

e) Recombinant viral strain transfected with reporter gene [redacted]

3.1.5 *In vitro* antiviral activity

3.1.5.1 Antiviral activity of ensitrelvir in SARS-CoV-2-infected mice and PK/PD analysis (CTD 4.2.1.1-16)

BALB/c mice (10 females in vehicle group, 5 females in each ensitrelvir group) were intranasally inoculated with SARS-CoV-2 (1.00×10^4 $TCID_{50}$/animal, isolate hCoV-19/Japan/TY7-501/2021 [Gamma variant]). Immediately after that, they started to receive oral administration of (a) vehicle[6)] or ensitrelvir 1 to 32 mg/kg twice daily, (b) ensitrelvir 0.5 to 16 mg/kg 4 times daily, or (c) ensitrelvir 2 to 64 mg/kg as a single dose. Viral titer ($TCID_{50}$) in lung tissue at 24 hours after the start of administration was evaluated. All dosage regimens resulted in a dose-dependent decrease in the viral titer.

SARS-CoV-2 (1.00×10^4 $TCID_{50}$/animal, isolate hCoV-19/Japan/TY7-501/2021 [Gamma variant]) was inoculated intranasally into BALB/c mice (4 females per group). Immediately after that,

6) 0.5% (w/v) methylcellulose

ensitrelvir 2 to 64 mg/kg was administered orally in a single dose. The applicant investigated the correlation between pharmacokinetic (PK) parameters (C_{max}/EC_{50}, $AUC_{0\text{-}24\ h}/EC_{50}$, $C_{24\ h}/EC_{50}$, total time above the target plasma concentration [$Time_{High}$][7])[8] and the decrease in viral titer ($TCID_{50}$) in lung tissue at 24 hours after the start of administration. C_{max}/EC_{50} and $AUC_{0\text{-}24\ h}/EC_{50}$ showed a higher correlation with decreased $TCID_{50}$ than other PK parameters.

3.1.5.2 Antiviral activity of delayed ensitrelvir administration in SARS-CoV-2-infected mice and PK/PD analysis (CTD 4.2.1.1-17)

SARS-CoV-2 (1.00×10^4 $TCID_{50}$/animal, isolate hCoV-19/Japan/TY7-501/2021, [Gamma variant]) was inoculated intranasally into BALB/c mice (5 females per group). At 24 hours after inoculation, the animals received a single oral dose of ensitrelvir 32 to 64 mg/kg, or started to receive a 2-day oral administration of (a) vehicle[6] or ensitrelvir 8 to 64 mg/kg twice daily, (b) ensitrelvir 8 to 64 mg/kg 3 times daily, or (c) ensitrelvir 16 to 64 mg once daily. Viral titer ($TCID_{50}$) in lung tissue was measured at 24 and 48 hours after the start of administration. A dose-dependent decrease in viral titer was observed both at 24 and 48 hours after the start of administration, except in the single-dose group.

The applicant investigated the correlation between PK parameters (C_{max}/EC_{50}, $AUC_{0\text{-}48\ h}/EC_{50}$, $C_{48\ h}/EC_{50}$, $Time_{High}$ [$1 \times EC_{50}$, $3 \times EC_{50}$, $5 \times EC_{50}$, $10 \times EC_{50}$][9])[8] and the decrease in viral titer ($TCID_{50}$) in lung tissue at 48 hours after the start of administration. $C_{48\ h}/EC_{50}$, $Time_{High}$ ($5 \times EC_{50}$ and $10 \times EC_{50}$), and $AUC_{0\text{-}48\ h}/EC_{50}$ were more correlated with decreased $TCID_{50}$ than other PK parameters. The applicant explained that the results suggested the importance of maintaining plasma ensitrelvir concentration throughout the treatment period.

$C_{48\ h}/EC_{50}$ of ensitrelvir when viral titer in lung tissue ($TCID_{50}$) decreased by 1 log_{10}, 2 log_{10}, and 3 log_{10} at 48 hours after the start of administration was 0.769, 3.78, and 14.7, respectively. $C_{48\ h}$ required to reduce viral titer in lung tissue was calculated from these values and EC_{50} in the presence of 100% human or mouse serum (3.02 and 3.93 μmol/L, respectively [estimates]) (see Section 3.1.2.2). $C_{48\ h}$ required to reduce the viral titer by 1 log_{10}, 2 log_{10}, and 3 log_{10} was 1.24, 6.09, and 23.7 μg/mL respectively in humans and 1.61, 7.90, and 30.7 μg/mL respectively in mice.

3.1.5.3 Therapeutic effect of delayed ensitrelvir administration in SARS-CoV-2-infected mice (CTD 4.2.1.1-18)

SARS-CoV-2 (1.00×10^3 $TCID_{50}$/animal, SARS-CoV-2 MA-P10[10] strain) was inoculated intranasally into BALB/c mice (8 females in vehicle group, 12 females in each ensitrelvir group). At 24 hours after the inoculation, the animals started to receive oral administration of vehicle[6] or ensitrelvir 4 to 32 mg/kg twice daily for 5 days. The body weight change rate, survival rate, and survival period were evaluated for 14 days after viral inoculation. Ensitrelvir groups showed dose-dependent suppression of body weight loss compared with the vehicle group. Body weight at 14 days after viral inoculation was 86.4% (vehicle group) and 94.6% to 100.1% (ensitrelvir groups) of the baseline. All ensitrelvir groups showed a higher survival rate and a longer survival period than the vehicle group. The survival rate at

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7) The period during which plasma ensitrelvir concentration exceeded EC_{50}.
8) EC_{50}: EC_{50} of ensitrelvir in the presence of 100% mouse serum (3.93 μmol/L, estimated value) was used (see Section 3.1.2.2).
9) The period during which plasma ensitrelvir concentration exceeds $1 \times EC_{50}$, $3 \times EC_{50}$, $5 \times EC_{50}$, and $10 \times EC_{50}$, respectively.
10) hCoV-19/Japan/TY/WK-521/2020 strain adapted to mice

14 days after viral inoculation was 37.5% (vehicle group), 91.7% (ensitrelvir 4 mg/kg group), and 100% (ensitrelvir 8, 16, and 32 mg/kg groups).

3.2 Secondary pharmacodynamics

3.2.1 Effect on receptors, ion channels, transporters, and enzymes (CTD 4.2.1.2-01)

In vitro effect of ensitrelvir (100 μmol/L) on 108 types of receptors, ion channels, transporters, and enzymes was investigated. The following were inhibited by ≥50%: phosphodiesterase (PDE)4A1A, PDE4B1, PDE4C1, PDE4D2, PDE7A, PDE7B, and cholecystokinin $(CCK)_2$ receptors, nicotinic acetylcholine (Ach) receptor subunit α1, and adenosine transporter.

The applicant also investigated the effects of ensitrelvir (0.03 to 100 μmol/L) on the above 9 types of receptors or transporters and on 10 types of peptidases (angiotensin-converting enzyme, caspase 2, chymotrypsin, cathepsin B, cathepsin D, cathepsin G, cathepsin L, neutrophil elastase, HIV-1 protease, and thrombin). The following were inhibited by ≥50%: PDE4A1A, PDE4B1, PDE4C1, PDE4D2, and adenosine uptake, with 50% inhibitory concentration (IC_{50}) of 63.2, 69.1, 75.7, 73.4, and 1.43 μmol/L, respectively. The other receptors etc., were not inhibited by ≥50% by ensitrelvir at ≤100 μmol/L.

3.2.2 Toxicity to human primary cells (CTD 4.2.1.2-02)

Cytotoxicity of ensitrelvir (0.55 to 135 μmol/L) against human primary cells (human liver cells, human normal renal mixed epithelial cells, and human peripheral $CD4^+$ T cells) was evaluated during the cell growth and non-growth phases, based on intracellular adenosine triphosphate (ATP) level. CC_{50} was >135 μmol/L (72 μg/mL) in all cells tested.

3.2.3 Cytotoxicity against human cell lines of various tissue origin (CTD 4.2.1.2-03)

Cytotoxicity of ensitrelvir (0.55 to 135 μmol/L) against the following human cell lines of various tissue origin was evaluated based on intracellular ATP level: HepG2 cells (human hepatoma cells), HK-2 cells (human renal proximal tubule cells), HEK293 cells (human embryonic kidney cells), SH-SY-5Y cells (human neuroblastoma cells), SK-N-SH cells (human neuroblastoma cells), Jurkat cells (human T cell leukemia cells), E006AA-hT cells (human prostatic cancer cells), HUVEC cells (human vascular endothelial cells), and MRC-5 cells (human pulmonary fibroblasts). CC_{50} was >135 μmol/L (>72 μg/mL) against all of the cells tested.

3.2.4 Mitochondrial toxicity in human cells (CTD 4.2.1.2-04)

HepG2 cells were cultured in a glucose- or galactose-containing medium in the presence of ensitrelvir (0.18 to 564 μmol/mL), and CC_{50} was calculated based on the amount of intracellular ATP, to evaluate mitochondrial toxicity.[11] CC_{50} was similar in both mediums, showing no mitochondrial toxicity.

[11] Cells in a glucose-containing medium can produce intracellular ATP by glycolytic pathway, showing resistance to mitochondrial toxicity of a test compound. On the other hand, cells in a galactose-containing medium cannot use glycolytic pathway for ATP production, showing a sensitivity to mitochondrial toxicity. Based on this mechanism, mitochondrial toxicity was evaluated by comparing cytotoxicity observed in cells cultured in each medium.

3.3 Safety pharmacology

3.3.1 Effect on central nervous system (CTD 4.2.1.3-01)

A single dose of ensitrelvir (20, 100, or 1,000 mg/kg) was administered orally to Sprague Dawley (SD) rats (6 males per group). The effect of ensitrelvir on clinical symptoms and neurobehavioral function was investigated by functional observation battery (FOB). The 1,000 mg/kg group showed a decrease in feces and urine volume, but had no events related to neurobehavioral function; the applicant therefore concluded that ensitrelvir does not affect the central nervous system.

3.3.2 Effect on cardiovascular system

3.3.2.1 *In vitro* study

3.3.2.1.1 Effect on hERG potassium current (CTD 4.2.1.3-02)

Using CHO cells engineered to express human ether-a-go-go-related gene (hERG) channel, the effect of ensitrelvir on hERG current was investigated by whole-cell patch-clamping. Ensitrelvir 10, 30, 100 μmol/L suppressed hERG potassium current by 9%, 16%, and 39%, respectively, with IC_{50} of >100 μmol/L against hERG current.

3.3.2.2 *In vivo* study (CTD 4.2.1.3-03)

A single oral dose of ensitrelvir 10, 50, and 150 mg/kg was sequentially administered to unanesthetized cynomolgus monkeys (4 males per group[12)]). The effects on blood pressure, heart rate, and electrocardiogram (PR interval, QRS interval, QT interval, and corrected QT interval [QTc]) were investigated by telemetry. A mild increase in heart rate (≤28%) was observed following the administration of ensitrelvir at 50 and 150 mg/kg. The no-observed-effect level (NOEL) in cynomolgus monkeys was determined to be 10 mg/kg/dose. The plasma ensitrelvir exposure (C_{max}: 43.8 μg/mL) at 10 mg/kg/dose (NOEL) was approximately 1.6 times the plasma exposure in humans receiving ensitrelvir (C_{max}: 28.1 μg/mL).[13)] The applicant explained that ensitrelvir is unlikely to cause safety problem in clinical use, for the following reasons: (a) Ensitrelvir has no risk of QT interval prolongation in humans [see Section 6.2.3], (b) palpitation observed in 1 subject in the ensitrelvir 375/125 mg group was the only cardiovascular risk-related adverse event observed in clinical studies, and (c) there is thus no evidence suggestive of any significant effect on humans.

3.3.3 Effect on respiratory system (CTD 4.2.1.3-03)

A single oral dose of ensitrelvir 50 and 150 mg/kg was sequentially administered to cynomolgus monkeys (4 males per group). The effects on respiratory rate and blood gas parameters (arterial blood pH, arterial partial pressure of oxygen, arterial carbon dioxide pressure, and hemoglobin oxygen saturation) were investigated. Ensitrelvir had no effects on these parameters.

3.R Outline of the review conducted by PMDA

3.R.1 Antiviral activity of ensitrelvir against SARS-CoV-2

The applicant's explanation about the antiviral activity of ensitrelvir against SARS-CoV-2:

When SARS-CoV-2 enters cells, the viral ribonucleic acid (RNA) is translated into proteins. Non-structural proteins are first produced as polyproteins, which are then processed by SARS-CoV-2

12) Blood pressure after 10 mg/kg administration was measured in 3 animals.

13) Plasma ensitrelvir exposure on Day 5 of administration in healthy adult women who received 375 mg ensitrelvir once on Day 1 and 125 mg ensitrelvir once daily from Days 2 to 5 in a phase I study (Study T1211) [see Section 6.2.1.2].

3CL protease, etc., and are eventually converted into non-structural proteins essential for viral replication, etc. (*Nat Commun.* 2021;12:6055). Ensitrelvir inhibits SARS-CoV-2 3CL protease [see Section 3.1.1], thereby inhibiting polyprotein processing, resulting in suppression of SARS-CoV-2 replication. The binding site of ensitrelvir with SARS-CoV-2 3CL protease[14] and their binding mode were investigated by X-ray diffraction for cocrystal structural analysis. Table 10 shows the results.

Table 10. Binding site of ensitrelvir to 3CL protease and their binding mode

Binding site of ensitrelvir [a]	Binding mode of ensitrelvir and the binding site
T25	Hydrogen bond with NH donor of the main chain
H41	π-π interaction with the side chain
M49	CH-π interaction with ε carbon of the side chain
G143	Hydrogen bond with NH donor of the main chain
C145	Hydrogen bond with NH donor of the main chain
H163	Hydrogen bond with NH donor of the side chain
M165	CH-π interaction with β carbon of the side chain
E166	Hydrogen bond with NH donor of the main chain, CH-π interaction with β carbon of the side chain

a) Amino acid residues of SARS-CoV-2 3CL protease located within 5Å from ensitrelvir

In vitro studies showed that ensitrelvir had a similar level of antiviral activity against the original strain, the Alpha, Beta, Gamma, and Delta variants and the Omicron variant (subvariants BA.1, BA.1.1, and BA.2) [see Section 3.1.2.1]. *In vitro* antiviral activity of ensitrelvir against the Lambda variant, the Omicron variant (BA.1, BA.2, BA.3, BA.4, BA.5, and their sub-lineages), and recombinant strains (XA, XD, and XE strains), has not been evaluated. Nevertheless, ensitrelvir is presumed to have similar antiviral activity against these variants and recombinant strains as against the original strain, for the following reasons:

(a) Ensitrelvir has inhibitory activity against SARS-CoV-2 3CL protease containing amino acid substitutions observed in these variants and recombinant strains (G15S[15] in the Lambda variant, P132H[16] in the Omicron variant, K90R[16] in recombinant strain XA, P132H[16] in recombinant strains XD and XE) [see Section 3.1.1].

(b) These substituted amino acid residues are not the binding sites of ensitrelvir (Table 10).

Furthermore, the following findings suggest that ensitrelvir has similar antiviral activity against the Omicron variant (BA.1.1.2 strain) and the Mu variant as against the original strain.

(a) The Omicron variant (BA.1.1.2 strain) has amino acid substitutions (P132H and T169S) in SARS-CoV-2 3CL protease, and ensitrelvir inhibits SARS-CoV-2 3CL protease containing P132H substitution.

(b) Neither of the substituted amino acid residues (P132H or T169S) is the binding site of ensitrelvir (Table 10).

(c) The Mu variant has no amino acid substitution in SARS-CoV-2 3CL protease.[17]

PMDA's view:

The applicant's explanation about the antiviral activity of ensitrelvir against SARS-CoV-2 is acceptable. Since antiviral activity of ensitrelvir against novel variants is critical information regarding the efficacy, relevant information should be collected continuously. In particular, the effect of amino

14) The amino acid sequence of SARS-CoV-2 3CL protease from the original strain (isolate hCoV-19/Japan/TY/WK-521/2020, strain A) was used.

15) Refer to the gene sequence of isolate hCoV-19/Japan/TY33-456/2021 registered in GISAID (https://www.gisaid.org/)

16) Refer to the gene sequences of the Omicron sublineages and recombinant strains registered in GISAID (https://www.gisaid.org/) as of May 23, 2022.

17) Refer to the gene sequence of isolate hCoV-19/Japan/TY26-717/2021 registered in GISAID (https://www.gisaid.org/).

acid substitutions on the antiviral activity of ensitrelvir should be evaluated whenever necessary, and appropriate actions should be taken when new information is obtained. SARS-CoV-2 strains that infected the subjects enrolled in clinical studies are described in Section 7.1.

3.R.2 Resistance profile of SARS-CoV-2 against ensitrelvir

The applicant's explanation about the resistance profile of SARS-CoV-2 against ensitrelvir:

In vitro studies on resistance acquisition showed variants with amino acid substitutions (D48G, M49L, P52S, S144A, and M49 [M/L]/S144A) located near the active center of SARS-CoV-2 3CL protease. These variants were found to be 3.7 to 100 times less sensitive to ensitrelvir than the parent strain [see Sections 3.1.3.1 and 3.1.3.2]. Also, recombinant SARS-CoV-2 viruses with these amino acid substitutions showed a similar time-course of viral titer as the parent strain did, with no significant effects on the replication capacity [see Section 3.1.3.2]. In the database of the Global Initiative on Sharing Avian Influenza Data (GISAID)[18] as of May 27, 2022, the percentage of strains containing a single mutation of these amino acids is ≤0.001%, and amino acid substitution of M49L/S144A has not been reported.

Before and after administration of ensitrelvir, samples were obtained from 34 subjects[19] who received ensitrelvir in the phase IIa part of the global phase II/III study (Study T1221). Amino acid sequence of SARS-CoV-2 3CL protease in the samples was analyzed.[20] Amino acid substitutions were detected in 10 subjects after ensitrelvir administration (Table 11). However, neither viral titer nor viral RNA level increased again after the detection of these mutations. These amino acid substitutions were not detected in samples subsequently obtained from 2 subjects (No. 7 and 9) that contained a sufficient amount of RNA for base sequence analysis. The amino acid substitutions were not located at the active center of SARS-CoV-2 3CL protease or at the binding sites of ensitrelvir [Section 3.R.1, Table 10]. Further, these substitutions were different from those detected in the *in vitro* resistance acquisition study [see Section 3.1.3.1]. A similar analysis is planned in the phase IIb part but has not been initiated. When the results of the analysis become available is therefore unclear at present.

Table 11. Amino acid substitutions in SARS-CoV-2 3CL protease detected in subjects who received ensitrelvir in phase IIa part of global phase II/III study (Study T1221)

Subject	Treatment	Amino acid substitution [a]
1	750/250 mg	A234S
2	375/125 mg	L87F
3	375/125 mg	H246Y
4	750/250 mg	T198I
5	375/125 mg	A94A/V
6	750/250 mg	L272L/P
7	750/250 mg	T45T/S
8	750/250 mg	M130M/V
9	750/250 mg	K100K/Stop M130M/I
10	375/125 mg	D263D/E

Stop: Stop codon

a) Subjects No. 5 to 10 had both a strain with amino acid substitution and a strain without.

18) https://www.gisaid.org/

19) Thirty subjects (ITT population) who were randomized and tested positive for RT-PCR performed on the nasal swab obtained before the start of study treatment, and 4 subjects who were not RT-PCR-positive but had RNA in an amount sufficient for base sequence analysis.

20) The analysis used all samples obtained at baseline, on Day 6, and on Day 9 as well as samples on Day 14 and 21 that tested positive for qualitative PCR.

PMDA's view:

In vitro studies detected amino acid substitutions (D48G, M49L, P52S, S144A, and M49L/S144A) in SARS-CoV-2 3CL protease in the presence of ensitrelvir, showing that SARS-CoV-2 with these mutations become less sensitive to ensitrelvir. Neither viral titer nor viral RNA increased again in subjects who showed amino acid substitutions in the phase IIa part of the global phase II/III study (Study T1221). However, there is extremely limited information on the resistant mutations against ensitrelvir in clinical studies, including the information on the relationship between amino acid substitutions and the efficacy of ensitrelvir. Further, i*n vitro* studies detected variants with decreased sensitivity to ensitrelvir. This suggests that use of ensitrelvir in clinical setting may result in emergence of resistant variants with amino acid substitutions that affect the efficacy of ensitrelvir. Therefore, as soon as results from the phase IIb part of the global phase II/III study (Study T1221) become available, they should be evaluated, and appropriate actions should be taken based on newly obtained information. The emergence of resistant mutations is critical information regarding the efficacy of ensitrelvir. New information, including that available from published reports, should be collected continuously and appropriate actions should be taken based on the new information.

3.R.3 Inhibition of adenosine uptake

The applicant's explanation about adenosine uptake inhibition by ensitrelvir observed in the secondary pharmacodynamics study:

The following effects of adenosine have been reported: Central nervous system (sedation and decreased locomotor activity), cardiovascular system (decreased heart rate, decreased blood pressure due to dilatation of coronary and peripheral arteries), renal function (renal artery contraction, decreased glomerular filtration rate), cellular metabolic system (suppression of lipid degradation, suppression of insulin secretion, glucagon release), and anti-inflammatory effect or immune suppression in intense physical exercises and in inflammatory diseases (*Nat Rev Drug Discov*. 2013;12:265-86). IC_{50} (1.43 μmol/L) of ensitrelvir against adenosine uptake is close to the clinical exposure (unbound form: C_{max}: 1.2 μmol/L[21)]), but the half-life of adenosine in blood in the body is only ≤30 seconds (*Eur J Pharmacol.* 2004;495:1-16). This suggests that there is a certain discrepancy between IC_{50} of ensitrelvir against adenosine uptake and the ensitrelvir concentration that increases adenosine concentration in blood. Also, the clinical studies have reported no serious adverse events of immediate safety concerns in terms of adenosine uptake inhibition [see Section 7.R.3]. Thus, adenosine-related safety problems are unlikely to occur in clinical use of ensitrelvir.

PMDA's view:

The applicant's explanation is acceptable at the current moment. However, given the small difference between IC_{50} and the clinical exposure together with the limited clinical study results, attention should be paid continuously to the occurrence of adverse events.

[21)] Calculated from the plasma ensitrelvir exposure (C_{max}: 28.1 μg/mL) following ensitrelvir administration in humans [see Section 6.2.1.2] and from the protein binding rate in human serum (97.7%) [see Section 4.2.2].

4. Non-clinical Pharmacokinetics and Outline of the Review Conducted by PMDA

PK was investigated in rats, rabbits, and monkeys that received unlabeled or ^{14}C-labeled ensitrelvir. Also, serum protein binding, drug-metabolizing enzymes, drug transporters, etc., were investigated using biological samples of humans and animals.

Ensitrelvir concentration in plasma was measured by liquid chromatography-tandem mass spectrometry (LC-MS/MS) (lower quantitation limit: 1.00 or 100 ng/mL in rats, 100 ng/mL in rabbits, 10.0 or 100 ng/mL in monkeys). Radioactivity concentration in biological samples was measured by a liquid scintillation counter (lower quantitation limit: background level or twice the background level) or by quantitative whole-body autoradiography (lower quantitation limit: 0.015 µg Eq/g).

In this section, the dose and concentration of ensitrelvir fumaric acid are expressed as those of ensitrelvir. PK parameters are expressed as mean ± standard deviation, unless specified otherwise.

4.1 Absorption

4.1.1 Single dose (CTD 4.2.2.2-01 to 02)

Table 12 shows plasma PK parameters (unlabeled or ^{14}C-labeled ensitrelvir) in rats or monkeys that received a single dose (intravenous or oral) of unlabeled or ^{14}C-labeled ensitrelvir. Absolute bioavailability (BA) following a single oral dose of ensitrelvir in rats was 85.5% ± 7.1%.

Table 12. PK parameters of ensitrelvir or total radioactivity in plasma following a single dose (intravenous or oral) of unlabeled or ^{14}C-labeled ensitrelvir

Animal species	Analyte	Route of administration	Dose (mg/kg)	Sex	N	C_{max} (µg/mL)	t_{max} (h)	$t_{1/2}$ (h)	AUC_{inf} (µg·h/mL)
Rat	Ensitrelvir	i.v.	1	Male	4	7.11 ± 1.85 a)	-	2.84 ± 0.27	10.4 ± 2.70
		p.o.	2 b)	Male	4	2.22 ± 0.59	2.00 [1.00, 2.00]	3.61 ± 0.56	17.8 ± 1.50
	Total radio activity	p.o.	2 b)	Male	4	2.32 ± 0.45 c)	2.00 [2.00, 2.00]	5.24 ± 0.18	21.1 ± 0.60 d)
Monkey	Ensitrelvir	p.o.	2 b)	Male	3	3.68 ± 0.30	8.00 [8.00, 8.00]	11.6 ± 1.5	98.4 ± 11.5
	Total radio activity	p.o.	2 b)	Male	3	4.12 ± 0.36 c)	8.00 [8.00, 8.00]	14.1 ± 1.5	119 ± 17.0 d)

Mean ± SD; t_{max} is expressed as median [range]; -, not calculated.
a) $C_{0\,h}$ (calculated by non-compartment analysis); b) ^{14}C-labeled ensitrelvir; c) unit, µg Eq/mL; d) unit, µg Eq·h/mL

4.1.2 Repeated doses (CTD 4.2.3.2-02 to 03, 4.2.3.5-02 to 03)

Table 13 shows plasma PK parameters of ensitrelvir in rats, rabbits, and monkeys that received repeated doses of ensitrelvir once daily.

Table 13. Plasma PK parameters of ensitrelvir administered orally once daily

Animal species	Dose (mg/kg/day)	N	Day of measurement	C_{max} (μg/mL)		t_{max} (h)		$AUC_{0\text{-}24\ h}$ (μg·h/mL)	
				Male	Female	Male	Female	Male	Female
Rat	20	6 males and 6 females	Day 1	26.0 ± 5.25	44.1 ± 6.52	1.00 [1.00, 2.00]	1.00 [1.00, 2.00]	214 ± 62.1	327 ± 44.5
			Day 28	39.6 ± 7.25	51.6 ± 5.90	2.00 [2.00, 2.00]	1.50 [1.00, 2.00]	388 ± 93.1	500 ± 66.4
	50	6 males and 6 females	Day 1	68.4 ± 8.92	114 ± 12.6	2.00 [2.00, 2.00]	2.00 [1.00, 2.00]	758 ± 187	1,110 ± 139
			Day 28	114 ± 14.7	142 ± 17.7	2.00 [2.00, 8.00]	1.00 [1.00, 2.00]	1,700 ± 331	1,780 ± 245
	1,000	6 males and 6 females	Day 1	272 ± 33.2	381 ± 65.7	5.00 [1.00, 8.00]	2.00 [1.00, 24.0]	4,420 ± 445	6,060 ± 476
			Day 28	263 ± 28.5	359 ± 28.1	6.00 [2.00, 8.00]	2.00 [1.00, 8.00]	4,270 ± 571	5,380 ± 692
Pregnant rat	20 [a)]	5 females	Day 1	-	36.9 ± 4.75	-	1.00 [1.00, 2.00]	-	368 ± 89.8
			Day 12	-	46.1 ± 7.70	-	1.00 [1.00, 1.00]	-	618 ± 187
	60 [a)]	5 females	Day 1	-	125 ± 14.7	-	1.00 [1.00, 2.00]	-	1,590 ± 274
			Day 12	-	134 ± 12.7	-	1.00 [1.00, 1.00]	-	1,990 ± 335
	1,000 [a)]	5 females	Day 1	-	266 ± 24.7	-	1.00 [1.00, 4.00]	-	4,070 ± 294
			Day 12	-	240 ± 29.9	-	1.00 [1.00, 2.00]	-	3,400 ± 918
Pregnant rabbit	30 [b)]	5 females	Day 1	-	36.6 ± 9.75	-	2.00 [1.00, 4.00]	-	450 ± 122
			Day 14	-	68.8 ± 26.6	-	1.00 [1.00, 24.0]	-	1,260 ± 872
	100 [b)]	4 females	Day 1	-	123 ± 27.1	-	4.00 [2.00, 8.00]	-	2,010 ± 559
			Day 14	-	167 ± 53.4	-	4.00 [1.00, 8.00]	-	2,580 ± 1,300
	300 [b)]	3 females	Day 1	-	307 ± 52.0	-	2.00 [1.00, 24.0]	-	6,010 ± 676
			Day 14	-	220 ± 87.9	-	4.00 [4.00, 8.00]	-	3,840 ± 1,660
Monkey	10	3 males and 3 females	Day 1	37.1 ± 2.85	36.1 ± 0.721	2.00 [1.00, 2.00]	1.00 [1.00, 1.00]	550 ± 65.0	503 ± 83.2
			Day 14	77.6 ± 23.3	75.2 ± 4.50	4.00 [4.00, 4.00]	1.00 [1.00, 1.00]	1,220 ± 426	1,170 ± 124
	50	3 males and 3 females	Day 1	142 ± 4.04	174 ± 37.7	8.00 [4.00, 8.00]	4.00 [4.00, 4.00]	2,940 ± 130	2,980 ± 414
			Day 14	404 ± 90.6	378 ± 77.5	4.00 [4.00, 4.00]	1.00 [1.00, 4.00]	7,810 ± 1,750	7,390 ± 1,970
	Male: 1,000/300/100 [c)] Female: 300/100 [d)]	3 males and 3 females	Day 1	520 ± 112	433 ± 87.5	8.00 [8.00, 24.0]	8.00 [4.00, 8.00]	10,700 ± 2,220	8,920 ± 1,900
			Day 14	409 ± 206 [e)]	430 ± 72.3 [f)]	4.00 [1.00, 4.00] [e)]	2.00 [1.00, 4.00] [f)]	7,840 ± 4,000 [e)]	8,170 ± 1,590 [f)]

Mean ± SD; t_{max} is expressed as median [range]; -, not applicable

a) Repeated administration for 12 days from gestation day 6 to 17
b) Repeated administration for 14 days from gestation day 6 to 19
c) The starting dose was 1,000 mg/kg/day. With the worsening of systemic conditions, the dose was reduced to 300 mg/kg/day from Day 3 and to 100 mg/kg/day from Day 8.
d) The starting dose was 300 mg/kg/day. With the worsening of systemic conditions, the dose was reduced to 100 mg/kg/day from Day 9.
e) Three animals
f) Four animals

4.2 Distribution

4.2.1 Tissue distribution (CTD 4.2.2.3-01)

Following a single oral dose of ^{14}C-labeled ensitrelvir (2 mg/kg) to pigmented rats (9 males, 1 animal per time point), tissue distribution[22)] of radioactivity was investigated up to 840 hours post-dose. Most of the radioactivity was detected in the content of the digestive tract, and the remaining radioactivity was widely distributed in tissues. In most of the tissues studied, radioactivity concentration peaked at approximately 4 hours post-dose. Tissues (other than contents in the digestive tract) showing a radioactivity concentration higher than that in plasma (1.92 μg Eq/g) were large intestinal mucosa (3.69 μg Eq/g), the liver (3.33 μg Eq/g), and small intestinal mucosa (2.51 μg Eq/g). Then, radioactivity concentrations in each tissue decreased over time and, in most of the tissues, decreased below the lower quantitation limit at 72 hours post-dose. Radioactivity in the nasal mucosa, the liver, and uvea was detectable up to 168 hours post-dose, but decreased to an undetectable level by 336 hours post-dose. The change over time in the radioactivity concentration in melanin-containing tissues such as uvea and pigmented skin was not significantly different from the change in other tissues, suggesting the low affinity of ensitrelvir and its metabolites to melanin.

4.2.2 Serum protein binding (CTD 5.3.2.1-01 to 02)

Following the addition of ^{14}C-labeled ensitrelvir (0.5, 5, 50 μg/mL) to rat, rabbit, monkey, and human serums, serum protein-binding rate (ultrafiltration method) was 98.3% to 98.8%, 97.4% to >98.9%, 98.3% to 98.9%, and 97.7% to 98.7%, respectively.

Following the addition of ^{14}C-labeled ensitrelvir (0.5, 5, 50 μg/mL) to serum albumin, α1-acid glycoprotein, and γ-globulin, protein binding rate (equilibrium dialysis method) was 94.7% to 94.8%, 3.7% to 9.4%, and 6.8% to 14.7%, respectively, showing that ensitrelvir bound mainly to albumin in human serum.

4.2.3 Distribution in blood cells (CTD 4.2.2.2-01 to 02, 5.3.2.1-02)

Following the addition of ^{14}C-labeled ensitrelvir (0.5, 5, 50 μg/mL) to rat, monkey, and human blood samples, the distribution rate in blood cells was 5.6% to 12.9%, 9.0% to 10.4%, and 5.2% to 8.0%, respectively.

Following a single oral dose of ^{14}C-labeled ensitrelvir (2 mg/kg) to rats and monkeys, the distribution rate of radioactivity in blood cells was 10.3% to 25.7% and 1.1% to 10.2%, respectively.

4.2.4 Placental transfer (CTD 4.2.2.3-01)

Following a single oral dose of ^{14}C-labeled ensitrelvir (2 mg/kg) to rats (gestation day 17) (6 rats, 1 animal per time point), tissue concentrations of radioactivity in maternal animals and fetuses were measured up to 48 hours post-dose. In fetuses, radioactivity was distributed in the brain, blood

22) The following tissues were investigated for radioactivity: Cecal content, cecal mucosa, large intestinal content, large intestinal mucosa, esophagus, rectal mucosa, small intestinal content, small intestinal mucosa, gastric content, forestomach mucosa, glandular stomach mucosa, cerebellum, cerebrum, choroid plexus, meninges, spinal cord, bone surface, periosteum, pigmented skin, non-pigmented skin, adrenal cortex, adrenal medulla, pineal body, pituitary gland, thyroid, liver, kidney (cortex), kidney (medulla), kidney (whole), bladder content, bladder wall, extraorbital lacrimal gland, intraorbital lacrimal gland, Harderian gland, pancreas, salivary gland, brown fat, white fat, eye (ciliary body and iris), eye (lens), eye (uvea), eye (whole), bulbourethral gland, epididymis, preputial gland, prostate gland, vesicular gland, testis, lung, nasal mucosa, trachea, aortic wall, skeletal muscle, cardiac muscle, tongue, caval wall, plasma, blood (intracardiac), bone marrow, mandibular lymph node, lymph nodes, spleen, thymus, and dental pulp

(intracardiac), the liver, and the lung among the tissues investigated.[23)] Radioactivity concentration in these tissues peaked at 4 hours post-dose, as with plasma radioactivity concentration in maternal animals, then decreased below the lower quantitation limit at 24 hours post-dose. The ratio of radioactivity concentration in fetal tissue to that in maternal plasma was 0.01 for the brain, 0.04 for blood (intracardiac), 0.05 for the liver, and 0.03 for the lung. Results suggested that ensitrelvir and its metabolites were transferred to fetuses through the placenta.

4.3 Metabolism

4.3.1 Postulated metabolic pathways

Based on the results of the investigations in Sections 4.3.2 and 4.3.3, the metabolic pathway of ensitrelvir was postulated as shown in Figure 1.

[23)] Radioactivity concentrations in the following tissues were investigated: Brain, blood (intracardiac), eyeballs, liver, and lung

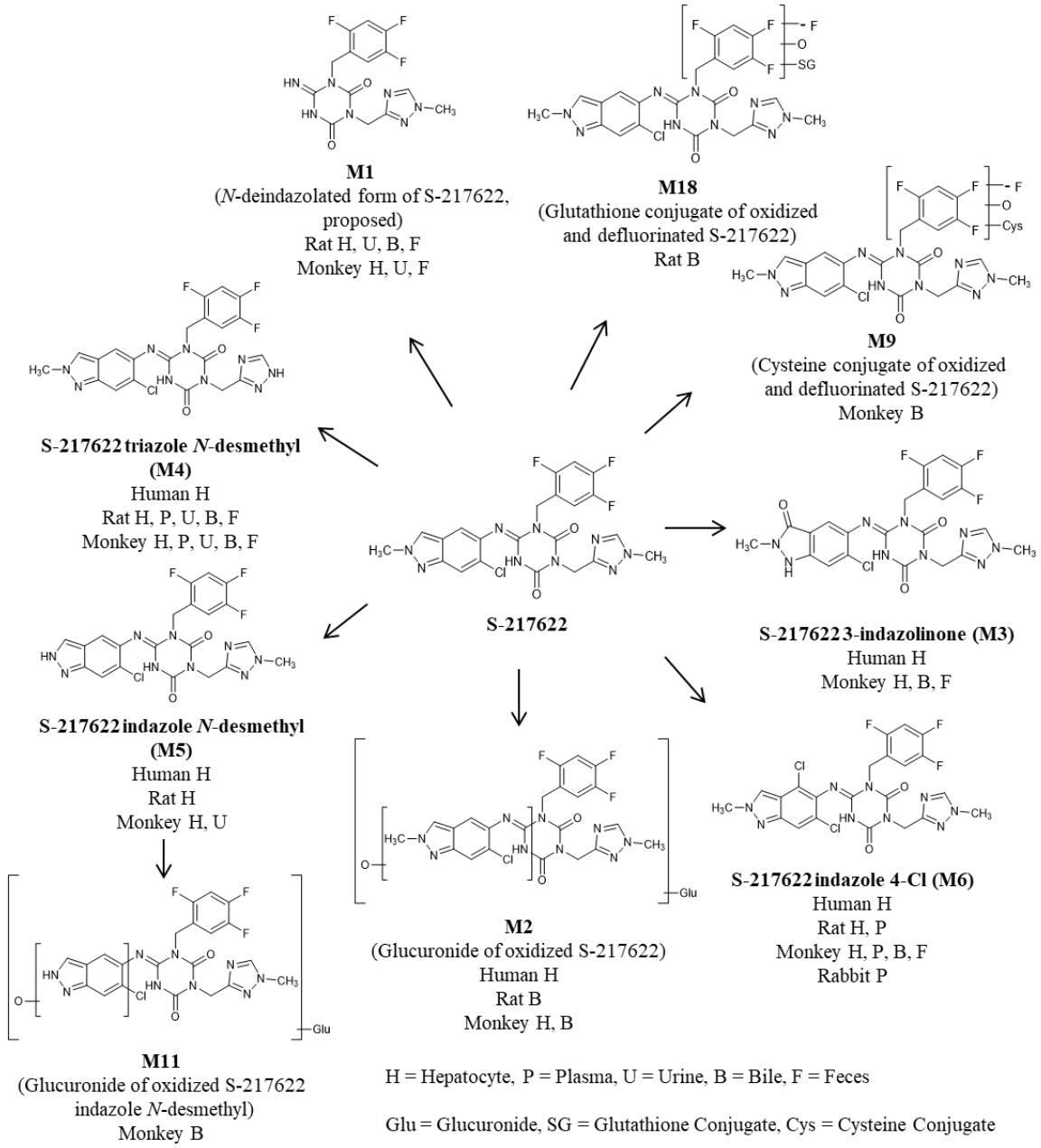

Figure 1. Postulated metabolic pathways of ensitrelvir (cited from CTD 2.6.4, Figure 2.6.4.5-1)

4.3.2 *In vitro* metabolism (CTD 5.3.2.2-01 to 02)

^{14}C-labeled ensitrelvir (5 μmol/L) was added to liver cells of rats, monkeys, and humans, and the mixtures were incubated for 4 hours. The major radioactive compound detected was the unchanged compound in all animal species (percentage to the total radioactivity: 72.3% in rats, 79.8% in monkeys, and 75.1% to 79.2% in humans). The following metabolites were detected: M1 in rats and monkeys, M2 and M3 in monkeys and humans, and M4, M5, and M6 in rats, monkeys, and humans. The percentage of each metabolite to the total radioactivity was ≤3.5%.

^{14}C-labeled ensitrelvir (50 μmol/L) was added to expression systems of human cytochrome P450 (CYP) isoforms (CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP3A4, and CYP3A5), and the mixtures were incubated for 90 minutes. The percentage of the unchanged

compound to the total radioactivity was 94.9% to 97.7%,[24] with only very small amounts of metabolites formed: M3 (0.11% to 0.15%)[25], M4 (0.10% to 0.54%)[25] and M5 (0.03% to 1.08%).[25]

^{14}C-labeled ensitrelvir (50 µmol/L) was added to human liver microsomes, and the mixture was incubated for 60 minutes in the presence or absence of an inhibitor[26] of each CYP isoform (CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, or CYP3A4/5), to investigate the inhibition rate of M3, M4, and M5 formation. M3 formation was inhibited by 6.3% by the inhibitor of CYP3A4/5. M4 formation was inhibited by 38.1% by the inhibitor of CYP3A4/5. M5 formation was inhibited by 50.0% by the inhibitor of CYP3A4/5, 10.2% by the inhibitor of CYP2B6, 14.1% by the inhibitor of CYP2C8, 7.7% by the inhibitor of CYP2C9, and 2.6% by the inhibitor of CYP2D6. The other CYP inhibitors did not inhibit M3, M4, or M5 formation. Formation of ensitrelvir metabolites (M3, M4, M5) was inhibited only partially by the inhibitors of CYP isoforms, suggesting the contribution of multiple CYP isoforms in the metabolism.

4.3.3 *In vivo* metabolism (CTD 4.2.2.4-01 to 03)

In vivo metabolism of ensitrelvir was investigated following a single oral dose of ^{14}C-labeled ensitrelvir (2 mg/kg) to bile duct-cannulated or non-cannulated rats, rabbits, and monkeys. Table 14 shows the percentage of the radioactivity of ensitrelvir and metabolites to the total radioactivity in plasma. Table 15 shows the percentage of the radioactivity of ensitrelvir and metabolites to the total radioactivity in urine, feces, and bile. Table 16 shows metabolites in plasma, urine, feces, and bile.

Table 14. Percentage of radioactivity of ensitrelvir and metabolites to the total radioactivity in plasma following a single oral dose of ^{14}C-labeled ensitrelvir (2 mg/kg)

Animal species	Bile duct cannulation	Sex	No. of animals/ time point	Sample	Measurement time point (h)	Percentage to total radioactivity in plasma (%)	
						Ensitrelvir	Metabolites
Rats	No	Male	2	Plasma	2	85.1	1.3
					8	83.2	3.9
					24	43.6	3.7
Rabbits	No	Female	1	Plasma	4	86.0	-
					24	74.2	1.5
Monkeys	No	Male	3	Plasma	4	91.0	1.7
					24	81.5	5.4
					48	54.6	9.7

Mean, -: Undetectable

[24] Following the incubation of the control expression system and ^{14}C-labeled ensitrelvir, the percentage of radioactivity of ensitrelvir and metabolites to total radioactivity was as follows: 97.0% for ensitrelvir (unchanged compound), 0.10% for M3, 0.07% for M4, and 0.02% for M5.

[25] Percentage of the radioactivity of each metabolite to total radioactivity

[26] Inhibitors against CYP isoforms: furafylline (CYP1A2), thio-TEPA (CYP2B6), montelukast (CYP2C8), sulfaphenazole (CYP2C9), S-(+)-N-3-benzylnirvanol (CYP2C19), quinidine (CYP2D6), and ketoconazole (CYP3A4/5). The dose of the inhibitors was 5 µmol/L, except for Thio-TEPA (10 µmol/L) and ketoconazole (1 µmol/L).

Table 15. Percentage of radioactivity of ensitrelvir and metabolites to total radioactivity in urine, feces, and bile following a single oral dose of ^{14}C-labeled ensitrelvir (2 mg/kg)

Animal species	Bile duct cannulation	Sex	No. of animals	Sample	Measurement Time point (h)	Percentage to total radioactivity (%)	
						Ensitrelvir	Metabolites
Rats	No	Male	4	Urine	0 - 24	0.3	1.4
				Feces	0 - 48	36.7	13.1
	Yes	Male	4	Bile	0 - 48	4.5	28.1
Monkeys	No	Male	3	Urine	0 - 72	0.8	6.7
				Feces	0 - 96	24.5	16.2
	Yes	Male	2	Bile	0 - 72	4.9	26

Table 16. Metabolite profile in plasma, urine, feces, and bile following a single oral dose of ^{14}C-labeled ensitrelvir (2 mg/kg)

Animal species	Bile duct cannulation	Sex	Sample			
			Plasma	Urine	Feces	Bile
Rats	No	Male	Unchanged compound, M4, M6	Unchanged compound, M1, M4, M8, M10, M13, M14, M15, M17	Unchanged compound, M1, M4, M8, M20	-
	Yes	Male	-	-	-	Unchanged compound, M1, M2, M4, M8, M15, M16, M17, M18, M19, M20
Rabbits	No	Female	Unchanged compound, M6	-	-	-
Monkeys	No	Male	Unchanged compound, M4, M6	Unchanged compound, M1, M4, M5, M8, M10	Unchanged compound, M1, M3, M4	-
	Yes	Male	-	-	-	Unchanged compound, M2, M3, M4, M9, M11, M12

-: Not investigated.

4.4 Excretion

4.4.1 Urinary, fecal, and biliary excretion (CTD 4.2.2.2-01 to 02)

Following a single oral dose of ^{14}C-labeled ensitrelvir (2 mg/kg) to bile duct-cannulated or non-cannulated rats and monkeys, urinary, fecal, and biliary excretion rates were investigated. The following results were obtained:

- In bile duct-non-cannulated rats (4 males), the mean percentage of radioactivity in urine, feces and cage wash collected up to 168 hours post-dose to the administered radioactivity was 2.9%, 94.1%, and 0.5%, respectively.
- In bile duct-cannulated rats (4 males), the mean percentage of radioactivity in urine, feces, bile, and cage wash collected up to 72 hours post-dose to the administered radioactivity was 3.8%, 33.6%, 60.1%, and 0.3%, respectively.
- In bile duct-non-cannulated monkeys (3 males), the mean percentage of radioactivity in urine, feces and cage wash collected up to 168 hours post-dose to the administered radioactivity was 11.2%, 84.9%, and 1.5%, respectively.
- In bile duct-cannulated monkeys (2 males), the mean percentage of radioactivity in urine, feces, bile, and cage wash collected up to 72 hours post-dose to the administered radioactivity was 14.4%, 27.2%, 57.5%, and 1.6%, respectively.

4.4.2 Excretion in milk (CTD 4.2.2.5-01)

Lactating rats (n = 5) on postpartum day 11 received a single oral dose of ^{14}C-labeled ensitrelvir (2 mg/kg) and radioactivity distribution in milk was investigated up to 48 hours post-dose. Radioactivity concentration in milk peaked at 4 hours post-dose (1.75 ± 0.25 μg Eq/mL, 1.25 ± 0.21 times the plasma radioactivity concentration), then decreased over time ($t_{1/2}$ of radioactivity in maternal plasma and milk was 5.71 ± 0.84 and 5.95 ± 0.68 hours, respectively).

4.5 Pharmacokinetic interactions

4.5.1 Inhibition of drug-metabolizing enzymes (CTD 5.3.2.2-04 to 05)

Using human liver microsomes, the inhibitory effect of ensitrelvir against the metabolic activity of substrates[27)] of each CYP isoform (CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, and CYP3A4/5) was investigated as follows:

- Human liver microsomes and ensitrelvir (0.1 to 100 μmol/L) were incubated in the presence of the substrate of each CYP isoform and NADPH, and the inhibitory effect of ensitrelvir against the substrate-metabolizing activity was investigated. Ensitrelvir inhibited the metabolic activity of CYP2C8 (IC_{50}: 35 μmol/L) but did not inhibit the metabolism of substrates of other CYP isoforms tested (IC_{50}: >100 μmol/L).
- Human liver microsomes and ensitrelvir (0.1 to 100 μmol/L) were incubated in the presence of NADPH, followed by incubation with substrates of CYP isoforms. Then, time-dependent inhibitory effect of ensitrelvir against the metabolism of substrates was investigated. Ensitrelvir inhibited the metabolic activity of CYP3A4/5 in a time-dependent manner. k_{inact} and half-maximal inactivation concentration (K_I) were 0.046 min^{-1} (k_{inact}) and 84 μmol/L (K_I) against the metabolism of midazolam and 0.055 min^{-1} (k_{inact}) and 86 μmol/L (K_I) against metabolism of testosterone. Ensitrelvir did not show any clear time-dependent inhibitory effect against the metabolism of substrates of other CYP isoforms.

Human liver microsomes and ensitrelvir were incubated in the presence of substrates of UDP glucuronosyltransferase (UGT) isoforms (UGT1A1, 1A3, 1A4, 1A6, 1A9, 2B7, or 2B15) and UDPGA, to investigate inhibitory effect of ensitrelvir (0.1 to 100 μmol/L) against the metabolism of substrates.[28)] Ensitrelvir did not show clear inhibitory effect in any UGT isoforms tested (IC_{50}: >100 μmol/L).

4.5.2 Induction of drug-metabolizing enzymes (CTD 5.3.2.2-03)

Using primary human liver cells, activity of ensitrelvir to induce each CYP isoform (CYP1A2, 2B6, 2C8, 2C9, 2C19, and 3A4) was investigated. Following the incubation of primary human liver cells with ensitrelvir (1 to 100 μmol/L) for 72 hours, mRNA expression of CYP2B6, CYP2C8, CYP2C9, and CYP3A4 increased up to ≥2-fold relative to that in the vehicle control (0.1% dimethyl sulfoxide [DMSO]). The mRNA expression of CYP2C19 increased to a level similar to that observed with

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27) Substrates (concentration) used:
CYP1A2, phenacetin (90 μmol/L); CYP2B6, efavirenz (5 μmol/L); CYP2C8, amodiaquine (2 μmol/L); CYP2C9, diclofenac (12 μmol/L); CYP2C19, *S*-mephenytoin (60 μmol/L); CYP2D6, dextromethorphan (10 μmol/L); CYP3A, testosterone (60 μmol/L) and midazolam (3 μmol/L).

28) Substrates (concentration) used: UGT1A1, 17β-estradiol (12 μmol/L); UGT1A3, chenodeoxycholic acid (160 μmol/L); UGT1A4, trifluoperazine (20 μmol/L); UGT1A6, 1-naphthol (2 μmol/L); UGT1A9, propofol (16 μmol/L); UGT2B7, morphine (400 μmol/L); UGT2B15, oxazepam (100 μmol/L).

rifampicin, the positive control[29] (up to 1.70-fold with rifampicin, up to 1.80-fold with ensitrelvir).[30] Also, enzyme activity of CYP1A2 increased in the presence of ensitrelvir up to more than twice the level observed in the presence of the vehicle control (0.1%DMSO).

These results suggest that ensitrelvir induce all CYP isoforms tested (CYP1A2, 2B6, 2C8, 2C9, 2C19, and 3A4).

4.5.3 Assessment of ensitrelvir as a substrate for drug transporters (CTD 5.3.2.2-06)

Using Caco-2 cells, the efflux ratio (basal-to-apical apparent permeation coefficient/apical-to-basal apparent permeation coefficient) of ^{14}C-ensitrelvir (1, 10 μmol/L) was investigated in the presence or absence of P-glycoprotein (P-gp) or breast cancer resistance protein (BCRP) inhibitor.[31]

The efflux ratio at 1 and 10 μmol/L ^{14}C-ensitrelvir, respectively, was as follows: 5.5 and 4.7 in the absence of the inhibitor, 2.3 and 2.0 in the presence of the P-gp inhibitor, and 3.5 and 3.1 in the presence of the BCRP inhibitor. These results suggest that ensitrelvir is a substrate of both P-gp and BCRP.

Transport of ^{14}C-ensitrelvir (1, 10 μmol/L) was investigated using HEK293 cells engineered to express human organic anion transporting polypeptide (OATP)1B1, OATP1B3, organic cation transporter (OCT)1, OCT2, organic anion transporter (OAT)1, OAT3, multidrug and toxin extrusion protein (MATE)1, or MATE2-K. The extent of uptake of ^{14}C-ensitrelvir into transporter-expressing HEK293 cells was similar to that observed in the control cells regardless of the type of the transporter, and the uptake was not reduced by the inhibitor of each transporter.[32] These results suggested that ensitrelvir was not a substrate for OATP1B1, OATP1B3, OCT1, OCT2, OAT1, OAT3, MATE1, or MATE2-K.

4.5.4 Inhibition of drug transporters (CTD 5.3.2.2-06)

Using Caco-2 cells, the inhibitory effect of ensitrelvir (0.3 to 250 μmol/L) against the uptake of substrates[33] of P-gp and BCRP was investigated. Also, using HEK293 cells engineered to express human OATP1B1, OATP1B3, OCT1, OCT2, OAT1, OAT3, MATE1, or MATE2-K, inhibitory effect of ensitrelvir (0.3 to 250 μmol/L) against uptake of the substrates[34] of these transporters was investigated. IC_{50} of ensitrelvir against P-gp, BCRP, OATP1B1, OATP1B3, OCT1, OCT2, OAT1, OAT3, and MATE1 was 11.5, 8.71, 13.2, 3.51, 7.24, 202, 47.7, 8.37, and 82.3 μmol/L, respectively, while IC_{50} against MATE2-K was >250 μmol/L.

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29) Positive controls (concentration) used: CYP1A2, omeprazole (50 μmol/L); CYP2B6, phenobarbital (1,000 μmol/L); CYP2C8, CYP2C9, CYP2C19, and CYP3A4, rifampicin (20 μmol/L)

30) Even the positive control rifampicin showed a less than 2-fold increase in CYP2C19 mRNA over the vehicle control (0.1% DMSO). The applicant provided the following explanation about this finding:
The fold increase of pregnane X receptor-mediated CYP2C19 mRNA is known to be smaller than that of CYP2C8, CYP2C9, and CYP3A4 mRNA (*Drug Metab Dispos.* 2001;29:242-51); similar tendencies were observed in this study as well. Further, ensitrelvir showed a more than 2-fold increase in CYP2C8, CYP2C9, and CYP3A4 mRNA over the vehicle control (0.1% DMSO). Therefore, the CYP2C19 induction by ensitrelvir can be evaluated based on the results of this study.

31) Inhibitors (concentration) used: P-gp, verapamil (30 μmol/L); BCRP, Ko143(1 μmol/L).

32) Inhibitors (concentration) used: OATP1B1 and OATP1B3, rifampicin (10 μmol/L); OAT1 and OAT3, probenecid (100 μmol/L); OCT1, quinidine (100 μmol/L); OCT2, quinidine (300 μmol/L); MATE1, cimetidine (10 μmol/L); MATE2-K, cimetidine (100 μmol/L).

33) Substrates (concentration) used: P-gp, ^{3}H-labeled digoxin (1 μmol/L); BCRP, ^{3}H-labeled estrone sulfate (0.1 μmol/L).

34) Substrates (concentration) used: OATP1B1 and OATP1B3, ^{3}H-labeled estradiol 17 β-D-glucuronide (0.05 μmol/L); OAT3, ^{3}H-labeled estrone sulfate (0.05 μmol/L); OCT1, OCT2, MATE1, and MATE2-K, ^{14}C-labeled metformin (10 μmol/L); OAT1, ^{3}H-labeled 4-aminohippuric acid (1 μmol/L).

4.R Outline of the review conducted by PMDA

PMDA considers that the nonclinical pharmacokinetics have been well characterized. Based on the results of the *in vitro* drug interaction studies, the following interactions are discussed further in Section 6.R.6 in accordance with "Guideline on drug interaction for drug development and appropriate provision of information" (PSEHB/PED Notification No. 0723-4 dated July 23, 2018, by the Pharmaceutical Evaluation Division, Pharmaceutical Safety and Environmental Health Bureau, Ministry of Health, Labour and Welfare):

(a) Drug interaction between ensitrelvir and CYP3A, P-gp or BCRP inhibitors or CYP3A inducers (because these drug interaction may occur in clinical use of ensitrelvir).
(b) Drug interaction mediated by the inhibitory effect of ensitrelvir against CYP3A, P-gp, BCRP, OATP1B1, OATP1B3, OCT1, and OAT3.

5. Toxicity and Outline of the Review Conducted by PMDA

The applicant submitted the data on a repeated-dose toxicity study, a genotoxicity study, a reproductive and developmental toxicity study, and other toxicity studies (phototoxicity study, study on impurities, and study on the mechanism of decrease in high density lipoprotein [HDL] cholesterol).

In this section, the dose and concentration of ensitrelvir fumaric acid are expressed as those of ensitrelvir. Aqueous 0.5%(w/v) methylcellulose solution was used as the vehicle unless specified otherwise.

5.1 Single-dose toxicity

No single-dose toxicity study was conducted. Instead, acute toxicity was evaluated in exploratory single-dose TK studies in rats and monkeys (CTD 4.2.3.1-01 and 02), repeated-dose toxicity studies in rats and monkeys [see Section 5.2], and a micronucleus assay in rats [see Section 5.3]. No death occurred after the first dose in any of the studies, and the approximate lethal dose was estimated to be >2,000 mg/kg in rats and >1,000 mg/kg in monkeys. The main acute symptom observed was vomiting in male monkeys receiving 1,000 mg/kg. The applicant explained that PDE4 inhibition by ensitrelvir may be involved in the mechanism of inducing vomiting, because vomit-inducing effect of PDE4 inhibitors in monkeys was reported (*Toxicol Pathol*. 2004;32:295-308) and PDE inhibition by ensitrelvir was observed in the secondary pharmacodynamics study [see Section 3.2.1].

5.2 Repeated-dose toxicity

Two- and four-week repeated dose toxicity studies were conducted in rats (Table 17). The main abnormal finding observed was decreased level of direct and indirect bilirubin concentrations in blood. Since the finding was not accompanied by related histopathological changes, the applicant does not consider the event as toxicity. Other laboratory values that showed significant changes compared with the vehicle group were not accompanied by related histopathological changes or were within the range of the laboratory historical data. Accordingly, the applicant considers that these changes are of low toxicological significance. The no-observed-adverse-effect level (NOAEL) in the 4-week repeated-dose toxicity study in rats was determined to be 1,000 mg/kg/day. The plasma ensitrelvir exposure at the NOAEL ($AUC_{0\text{-}24\,h}$: 4,270 μg•h/mL [male], 5,380 μg•h/mL [female]) was

approximately 8.2 (male) or 10 (female) times the plasma ensitrelvir exposure in humans receiving ensitrelvir[35] (AUC_{tau}: 518.3 μg•h/mL).

Table 17. Summary of repeated-dose toxicity studies in rats

Test system	Route of administration	Administration period	Dose (mg/kg/day)	Main findings	NOAEL (mg/kg/day)	Attached document CTD
Male and female rats (SD)	Forced p.o.	2 weeks (once daily) + 2-week recovery period	0, 20, 100, 1,000	1000: Decreased food consumption and decreased blood total bilirubin (males and females); decreased blood direct bilirubin, decreased body weight and weight gain, and decreased blood glucose (males) Recovery period: None	1,000	4.2.3.2-01
Male and female rats (SD)	Forced p.o.	4 weeks (once daily) + 4-week recovery period	0, 20, 50, 1,000	1,000: Decreased blood direct and indirect bilirubin (males and females) and increased lymphocyte count (males) Recovery period: None	1,000	4.2.3.2-02

Two- and four-week repeated dose toxicity studies were conducted in monkeys (Table 18). Main toxicities and abnormal findings were as follows:

- Dehydration, no food consumption, decreased food consumption, and decreased body weight, followed by death due to deterioration of clinical symptoms
- Increased direct and total bilirubin concentrations in blood
- Decreased blood cholesterol
- Decreased erythrocyte parameters and platelet count
- Findings suggesting immune suppression such as decreased blood lymphocyte count and decreased lymphocyte density in lymphatic tissues (stress-associated secondary changes)
- Abnormalities in coagulation system
- Hemorrhagic changes in organs and tissues in the whole body
- QT/QTc interval prolongation associated with hypocalcaemia/hypokalaemia

The following events were observed only in animals that died:

- Opportunistic infection (*Toxicol Pathol.* 2021;49:397-407, *J Immunotoxicol.* 2010;7:128-37) related to immune suppression associated with decreased lymphatic parameters due to stress caused by deterioration of clinical symptoms (*Toxicol Pathol.* 2013; 41: 560-614),
- Secondary changes related to infection-associated inflammatory reactions
- Decreased hematopoietic cell count in bone marrow

The following events were observed in surviving animals:

- Increased histiocyte and plasma cell counts in the spleen and lymph nodes not related to inflammatory reaction caused by bacterial infection
- Infiltration of inflammatory cells (mainly mononuclear cells) in multiple organs and tissues
- Fluctuations in blood inflammatory markers
- Secondary changes related to inflammatory changes.

[35] The plasma ensitrelvir exposure on Day 5 in healthy adult women who received 375 mg ensitrelvir once on Day 1 and 125 mg ensitrelvir once daily from Day 2 through 5 in the phase I study (Study T1211) [see Section 6.2.1.2].

The following significant changes were observed in the ensitrelvir group compared with the vehicle group: Decreases in urine analysis and blood electrolytes, decreased $CD3^+CD8^+$cells/$CD3^+CD4^+$cells ratio in blood, an increased activity of alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) in blood, high spleen weight, hepatocyte hypertrophy, adipose tissue atrophy in heart/hypodermal tissue, decreased lipid droplets in adrenal fasciculata, and periportal monocyte infiltration. The applicant considers that these changes are of low toxicological significance because they are not accompanied by related changes or are within the range of the laboratory historical data. The NOAEL in the 4-week repeated dose toxicity study in cynomolgus monkeys was estimated to be 10 mg/kg/day. The plasma ensitrelvir exposure at the NOAEL ($AUC_{0-24\,h}$: 1,170 µg• h/mL in males, 1,850 µg•h/mL in females) was approximately 2.3 (male) or 3.6 (female) times the plasma ensitrelvir exposure in humans receiving ensitrelvir[35] (AUC_{tau}: 518.3 µg•h/mL).

Table 18. Summary of repeated dose toxicity studies in cynomolgus monkeys

Test system	Route of administration	Administration period	Dose (mg/kg/day)	Main findings	NOAEL (mg/kg/day)	Attached data CTD
Male and female cynomolgus monkeys	Forced p.o.	2 weeks (once daily) + 2-week recovery period	Male: 0, 10, 50, 1,000/300/100[a)] Female: 0, 10, 50, 300/100[b)]	<u>Dead animals [c)]</u> 1,000/300/100[a)]: 2 of 5 (male) 300/100[b)]: 1 of 5 (female) Vomiting, lateral position, decreased body temperature, decreased contact behavior, decreased spontaneous locomotion, sitting position, sign of dehydration, decreased food consumption, no food consumption, decreased erythrocyte count/hemoglobin/hematocrit/lymphocyte count, increased PT/APTT/fibrinogen, increased or decreased blood glucose, increased blood LDH/CK/total bilirubin/direct bilirubin/triglycerides/urea nitrogen/creatinine/potassium, decreased total blood protein/albumin/"A/G" ratio, decreased calcium/sodium/chloride, adrenal enlargement, accumulation of white foamy solution in trachea and bronchi, red foci in heart, enlarged cecum/colon, pulmonary edema, induration of tissues around submandibular lymph nodes, white foci in both submandibular glands, edema in submandibular gland/submandibular lymph nodes, pulmonary edema, alveolar exudate, increased adrenal/lung/liver/kidney weight, adrenocortical hemorrhage/diffuse hypertrophy, bacterial-like particles in bone marrow/lung/submandibular lymph nodes/macrophages/neutrophils, inflammatory cell infiltration in bone marrow/heart (in blood vessels)/ hepatic sinusoid/lung (in blood vessels)/ submandibular lymph nodes/pituitary gland/submandibular gland, decreased bone marrow hematopoietic cells, increased megakaryocytes, atrophy of corneal epithelium, degeneration of arterial walls of colon/gall bladder/heart/liver, swelling of hepatocytes (including cells around portal vein)/localized necrosis of hepatocytes, dilatation of distal renal tubule, fibrin-like exudates, decreased lymphocytes in mesentery/submandibular lymph nodes/Peyer's patch/white pulp of spleen, increased histiocytes in submandibular lymph nodes, bleeding from adrenal cortex/cystic arterial wall/cardiac adipose tissue/endocardium/epicardium/ submandibular lymph nodes/alveoli (multifocal)/pancreatic islet/posterior pituitary gland/duodenal serosa/splenic white pulp/thymus/endometrium, necrosis of colic arterial wall (localized), decreased secretion from pancreatic acinar cells, atrophy of skeletal muscle fibers, atrophy of adipose tissue of aorta/heart/hypodermis, atrophy of duodenal Brunner's gland, splenic congestion, edema of submandibular gland stroma, thymic atrophy/increased tangible body macrophages/increased cortical single cell necrosis, and atrophy of lingual mucosal epithelium <u>Surviving animals</u> ≥50: Increased blood total/direct bilirubin, mononuclear cell infiltration around pulmonary/bronchial blood vessels, increased histiocytes/plasma cells in submandibular lymph nodes, splenic congestion (males and females), decreased body weight/food consumption, decreased urinary pH/chloride, increased urinary urobilinogen/protein, decreased red blood cell count/hemoglobin/hematocrit, increased monocyte count/fibrinogen, increased blood triglycerides, decreased blood calcium/sodium, darkening of adrenals, increased liver weight, enhanced myelopoiesis/ serous atrophy of adipose tissue, mononuclear cell infiltration around epididymal blood vessels/in esophageal mucosal epithelium/in lacrimal gland, atrophy of cardiac adipose tissue, swollen perihepatic portal vein cells, decreased lymphocyte count in submandibular lymph nodes/Peyer's patch/splenic white pulp, decreased secretion from pancreatic acinar cells, atrophy of duodenal Brunner's gland, thymic atrophy (males), increased blood total cholesterol, increased splenic weight, and increased histiocyte count in splenic white pulp (females). 1,000/300/100[a)]: Dehydration, decreased contact behavior, vomiting, diarrhea, decreased urinary potassium, decreased platelet count, increased reticulocyte count/ratio, decreased	10	4.2.3.2-03

Test system	Route of administration	Administration period	Dose (mg/kg/day)	Main findings	NOAEL (mg/kg/day)	Attached data CTD
				blood total protein/albumin/glucose/inorganic phosphate, turbid pale red ascites, red foci in heart, multifocal red foci in gastric/large intestinal mucosa, pale lung, dark spleen, increased splenic weight, decreased thymic weight, diffuse hypertrophy of adrenal cortex, bleeding from arterial adipose tissue/proper lamina of gallbladder mucosa/cardiac adipose tissue/"endo/exo"-cardiac wall/renal papillary fat/submucosal tissue/"gastric, small intestinal, and large intestinal proper lamina"/lung/bronchus/testis, increased bone marrow megakaryocytes, atrophy of esophageal mucosa, infiltration of mononuclear cells into bulbar conjunctiva/proper lamina of gallbladder mucosa/skin/ hypodermal tissue/oral mucosa/sublingual gland/ submandibular gland, ulcer/mucosa-localized erosion of large intestine, degeneration of hepatic arterial wall, infiltration of inflammatory cells in hepatic portal vein, decreased lymphocytes in mesenteric lymph nodes, neutrophil infiltration into submandibular lymph node sinusoid, infiltration of plasma cells in posterior pituitary gland, atrophy of skeletal muscle fibers, enhanced extramedullary hematopoiesis in spleen, swelling of mucilage cells in sublingual gland, atrophy of lingual mucosal epithelium (male) 300/100[b)]: Dehydration, decreased contact behavior, decreased food consumption, vomiting, decreased red blood cell count/hemoglobin/hematocrit, increased reticulocyte count and ratio/neutrophil count/monocyte count/fibrinogen, increased blood triglycerides, decreased blood calcium, pale lung, diffuse hypertrophy of adrenal cortex, enhanced myelopoiesis, atrophy and inflammatory cell infiltration in cardiac adipose tissue, increased histiocytes and plasma cells in mesenteric lymph node, neutrophil infiltration in submandibular lymph node sinusoid, decreased secretion from pancreatic acinar cells, increased plasma cells in Peyer's patch and splenic white pulp, bleeding from gastric lamina propria, thymic atrophy (females). 50: Swollen spleen, decreased lipid droplets in adrenal fasciculata (males and females), increased weight of submandibular gland/spleen/lung, swollen hepatocytes around hepatic portal vein, atrophy of subcutaneous adipose tissue, increased histiocytes and plasma cells in splenic white pulp, decreased lymphocytes in submandibular lymph nodes/splenic white pulp, bleeding from uterine submucosal tissue (females) Recovered.		
Male and female cynomolgus monkeys	Forced p.o.	4 weeks (once daily) + 4-week recovery period	0, 3, 10, 30	<u>Systemic toxicity</u> ≥10: Decreased blood HDL/LDL/total cholesterol (males and females) 30: Increased blood total/direct bilirubin, increased liver weight, decreased lipid droplets in adrenal fasciculata (males and females), decreased body weight/food consumption, QT/QTc interval prolongation, decreased urine pH, increased urinary ketone body/bilirubin/urobilinogen/ protein/glucose/white blood cell count, decreased red blood cell count/hemoglobin/hematocrit, increased reticulocyte ratio and count/lymphocyte count/neutrophil count/ monocyte count/basophil count/fibrinogen, increased blood ALP/LDH/triglycerides/ferritin/C-reactive protein, decreased blood albumin/"A/G" ratio/glucose/calcium/ sodium/chloride, accumulated cardiac effusion, increased spleen weight, increased bone marrow hematopoietic cells, inflammatory cell infiltration into muscular layer of gallbladder, necrosis/granulomatous inflammation of pericardium/large intestine/skin and subcutaneous tissue/adipose tissue of urinary bladder, erythrophagocytosis by hepatic Kupffer cells/enhanced extramedullary hematopoiesis/hepatocyte hypertrophy, mononuclear cell infiltration in portal vein, vacuolated hepatocytes around portal vein, decreased	10	4.2.3.2-04

Test system	Route of administration	Administration period	Dose (mg/kg/ day)	Main findings	NOAEL (mg/kg/ day)	Attached data CTD
				lymphocyte count in submandibular lymph nodes, enlarged pancreatic acinus and decreased secretion, decreased secretion from parotid gland, splenic congestion/enhanced extramedullary hematopoiesis, monocyte infiltration around vessels in myometrial layer (females) <u>Immunotoxicity</u> 30: Decreased CD3+CD8+ cell ratio (males and females), decreased blood CD3+CD8+ cell count (males), decreases in blood CD3+ cell ratio, CD3+CD4+ cell ratio, and CD3−CD20+ cell ratio (females) Recovered.		

a) The starting dose was 1,000 mg/kg/day but, with the worsening of systemic conditions, the dose was decreased to 300 mg/kg/day from Day 3 and to 100 mg/kg/day from Day 8.
b) The starting dose was 300 mg/kg/day but, with the worsening of systemic conditions, the dose was decreased to 100 mg/kg/ from Day 9.
c) Only main systemic toxicities are presented.

5.3 Genotoxicity

Genotoxicity studies consisted of a bacterial reverse mutation assay (Ames test), an *in vitro* micronucleus assay using human lymphoblastoma-derived TK6 cells, and a rat micronucleus assay (Table 19). No genotoxicity was observed in any of the assays. Accordingly, the applicant concluded that ensitrelvir had no genotoxicity.

Table 19. Summary of genotoxicity studies

Study		Test system	Metabolic activation (duration)	Concentration or dose	Results	Attached data CTD
in vitro	Ames test	*Salmonella Typhimurium:* TA98, TA100, TA1535 *Escherichia coli:* WP2*uvrA*	S9−/+	0[a], 156, 313, 625, 1250, 2500, 5000 μg/plate	Negative	4.2.3.3-01
		Salmonella Typhimurium: TA1537		0[a], 39.1, 78.1, 156, 313, 625, 1250, 2500, 5000 μg/plate		
	Micronucleus test	Human lymphoblastoma-derived TK6 cells	S9−/+ (3 hours)	0[a], 150, 200, 250 μg/mL	Negative	4.2.3.3-03
			S9− (24 hours)	0[a], 75, 100, 125 μg/mL		
in vivo	Rat micronucleus test	Male rat (SD) bone marrow cells		0, 500, 1,000, 2000 mg/kg/day (forced p.o., 3 days)	Negative	4.2.3.3-05

a) Vehicle: DMSO

5.4 Carcinogenicity

No carcinogenicity test was conducted because ensitrelvir is administered for 5 days usually and has no genotoxicity [see Section 5.3].

5.5 Reproductive and developmental toxicity

The following reproductive and developmental toxicity studies were conducted: (a) A study of fertility and early embryonic development to implantation in rats, (b) studies of embryofetal development in rats and rabbits, and (c) a study of effects on pre- and postnatal development, including maternal function in rats (Table 20). Main toxicity findings were (a) skeletal anomalies in rat fetuses and (b) embryo/fetal death, malformation/variation of fetal axial skeleton, and brachyury and spina bifida as external anomalies in rabbits. The following findings were observed in the rat offspring: death of all pups, decreased survival rate/birth rate, and changes related to growth retardation such as delayed sexual maturation after weaning. In male rats, decreased sperm count in the epididymis and decreased sperm activity and motility were observed but the applicant does not consider these observations as

toxicities because they did not affect fertility. The NOAEL was estimated to be 60 mg/kg/day in rat embryos/fetuses and offspring and 30 mg/kg/day in rabbit embryos/fetuses. The plasma ensitrelvir exposure at the NOAEL ($AUC_{0\text{-}24\ h}$: 1,990 µg•h/mL in rats, 1,260 µg•h/mL in rabbits) was approximately 3.8 (rats) and 2.4 (rabbits) times the plasma exposure[35)] in humans receiving ensitrelvir (AUC_{tau}: 518.3 µg•h/mL).

Table 20. Summary of reproductive and developmental toxicity studies

Study type	Test system	Route of administration	Administration period	Dose (mg/kg/day)	Main findings	NOAEL (mg/kg/day)	Attached data CTD
Fertility and early embryonic development to implantation	Male and female rats (SD)	Forced p.o.	Male: 28 days before mating to 1 day before autopsy Female: 14 days before mating to gestation day 7 (once daily)	0, 20, 60, 1,000	Parental animals *Male* 1,000: Decreased cauda epididymis weight/ sperm motility/percentage of motile sperms/total sperm count in epididymis *Female* None Fertility None Early embryonic development None	Parental animals (general toxicity, fertility): 1,000 Early embryonic development: 1,000	4.2.3.5-01
Embryofetal development	Female rats (SD)	Forced p.o.	Gestation day 6 to 17 (once daily) Caesarean section: Gestation day 20	0, 20, 60, 1,000	Maternal animals 1,000: Decreased weight gain/food consumption Embryofetal development 1,000: Decreased body weight, increased rate of maternal animals carrying fetuses with skeletal anomalies, increased rate of fetuses with skeletal anomalies, decreased number of ossified sternebrae [b)]/short extra ribs [b)]	Maternal animals (general toxicity): 60 Maternal animals (fertility): 1,000 Embryofetal development: 60	4.2.3.5-02
	Female rabbits (NZW)	Forced p.o.	Gestation day 6 to 19 (once daily) Caesarean section: Gestation day 28	0, 30, 100, 300	Maternal animals ≥100: Decreased feces, decreased body weight/weight gain/food consumption Embryofetal development ≥100: Increased placental weight, short tail,[a)] increased rate of maternal animals carrying fetuses with skeletal anomalies, increased rate of fetuses with skeletal anomalies, fusion of sternebrae, [a)] branching ribs, [a)] fusion of coccygeal vertebrae,[a)] complete extra ribs [b)] 300: Decreased fetal survival rate/number of live fetuses, increased post-implantation embryonic mortality/fetal deaths, increased rate of fetuses with skeletal anomalies, fused ribs, [a)] missing ribs, [a)] fused cervical vertebral bodies, [a)] supernumerary thoracic vertebrae,[a)] fused[a)] or small[a)] thoracic vertebral arch, fusion [a)] or abnormal alignment [a)] of thoracic vertebral body, abnormal alignment of lumbar vertebrae, abnormal alignment of coccygeal vertebrae,[a)] abnormal alignment of sacral spine/caudal vertebra, [a)] extra lumbar vertebrae [b)]	Maternal animals (general toxicity, fertility): 30 Embryofetal development: 30	4.2.3.5-03

a) deformity, b) variation

Table 20. Summary of reproductive and developmental toxicity studies (continued)

Study type	Test system	Route of administration	Administration period	Dose (mg/kg/day)	Main findings	NOAEL (mg/kg/day)	Attached data CTD
Embryofetal development	Female rabbits (NZW)	Forced p.o.	Gestation day 6-9, 10 to 12, 13 to 15, or 16 to 19 (once daily) Caesarean section: Gestation day 28	0, 300	**Administration from gestation day 6 to 9** Maternal animals 300: Decreased feces, decreased body weight/weight gain/food consumption, decreased monocyte count/lymphocyte count, increased blood triglycerides/creatinine Embryofetal development 300: increased postimplantation loss, decreased fetal survival rate/fetal body weight, short tail, [a] spina bifida, [a] increased rate of fetuses with skeletal anomalies/increased rate of maternal animals carrying fetuses with skeletal anomalies, abnormal alignment of sternebrae, [a] missing ribs, [a] bifurcated ribs, [a] fused ribs, [a] abnormal alignment of cervical vertebrae,[a] fused thoracic vertebral arch, [a] missing/fused vertebral body,[a] dumbbell-shaped ossification,[b] thoracic hemivertebrae,[a] thoracic hemivertebrae,[a] supernumerary thoracic vertebrae,[a] fused sacral vertebral body,[a] sacral hemivertebrae,[a] fused coccygeal vertebrae,[a] abnormal alignment of sacral spine/caudal vertebra [a]		4.2.3.5-08
					Administration from gestation day 10 to 12 Maternal animals 300: Decreased feces, decreased body weight/weight gain/food consumption, increased platelet count, decreased white blood cell count/neutrophil count/lymphocyte count/eosinophil count/monocyte count/basophil count/APTT, increased blood creatinine/triglycerides Embryofetal development 300: fusion of sternebrae,[a] malposition of sternebrae, missing ribs,[a] abnormal alignment of sacral spine/caudal vertebra [a]		
					Administration from gestation day 13 to 15 Maternal animals 300: Decreased feces, abortion, decreased body weight/weight gain/food consumption, decreased white blood cell count/lymphocyte count, increased blood creatinine Embryofetal development 300: fusion of sternebrae,[a] bifurcated ribs,[a] thoracic hemivertebrae, [a] missing sacral and caudal vertebral arch, [a] abnormal alignment of sacral spine/caudal vertebra [a]		
					Administration from gestation day 16 to 19 Maternal animals 300: Decreased feces, abortion, decreased weight gain/food consumption, decreased reticulocyte ratio/white blood cell count/lymphocyte count/ monocyte count/basophil count, increased blood triglycerides/creatinine Embryofetal development 300: Decreased fetal weight, decreased ossified sternebrae [b]		
Effects on pre- and postnatal development, including maternal function	Female rats (SD)	Forced p.o.	Gestation day 6 to lactation day 20 (once daily)	0, 20, 60, 1,000	Maternal animals 1,000: Decreased body weight/weight gain/food consumption, death of all pups F_1 offspring 1,000: Decreases in the number of liter mates, number of live births, birth rate, 4-day survival rate after birth, body weight of pups, rate of open eyelids, prepuce separation rate, and vaginal opening rate	Maternal animals (general toxicity, fertility): 60 F1 offspring development: 60	4.2.3.5-05

-: Not counted
a) deformity, b) variation

5.6 Other studies

5.6.1 Phototoxicity

Ensitrelvir absorbed light within the wave range of 290 to 700 nm (CTD 4.2.3.7-01). An *in vitro* phototoxicity study was conducted using mouse fibroblast cells (Table 21). Based on the results, the applicant concluded that ensitrelvir was unlikely to be phototoxic.

Table 21. Summary of phototoxicity test

Test system	Testing method	Main findings	Attached data CTD
Mouse fibroblasts (Balb/c 3T3)	0[a], 0.781, 1.56, 3.13, 6.25, 12.5, 25, 50, 100 μg/mL UVA (5 J/cm^2) and UVB (68.7 mJ/cm^2) were irradiated for 50 minutes.	PIF, D[b]; MPE, 0.022 No phototoxicity	4.2.3.7-02

a) Vehicle: DMSO
b) Not calculated because of the absence of cytotoxicity

5.6.2 Safety assessment of impurities (CTD 4.2.3.7-05 to 16)

According to the ICH-M7 Guideline, Ames test was conducted on the following impurities that are present in the drug substance or the drug product or may emerge during the manufacturing process or storage: Impurities A, B, C, D, E, F, G, H, I, J, K, L, and M. Based on the test results, Impurities G, H, I, J, K, and L were considered to be mutagenic. The applicant explained that the residual amount of the mutagenic impurities in the drug substance will be controlled at a level to ensure that human exposure to the impurities remains below the acceptable threshold recommended by the Threshold of Toxicological Concern (TTC) [see Sections 2.1.2 and 2.1.3].

5.6.3 Mechanism of decrease in HDL cholesterol

In the Japanese phase I study (Study T1211), a decrease in blood HDL cholesterol was observed in subjects receiving ensitrelvir. The following studies were conducted in order to elucidate the mechanism of decreased HDL cholesterol (Table 22).

(a) Using rat plasma, the effect of ensitrelvir on the activity of plasma LCAT (lecithin: cholesterol acyltransferase) was investigated (LCAT is an enzyme involved in the HDL-mediated uptake of cholesterol). No inhibitory effect was observed.
(b) Using macrophage-like cells, the effect of ensitrelvir on HDL-mediated cholesterol retrieval was investigated. No inhibitory effect was observed up to the maximum concentration (200 μg/mL).
(c) Plasma samples obtained from some subjects in the Japanese phase I study (Study T1211) were subjected to lipid analysis. A decrease in intermediate HDL cholesterol was observed.

Based on the above, the applicant suspected that the ensitrelvir-induced decrease in HDL cholesterol was due to the effect on process(es) after the retrieval of cholesterol from peripheral tissues by HDL, but explained that the exact mechanism was unclear.

Table 22. Summary of studies on the mechanism of decrease in HDL cholesterol

Study	Test system	Testing method	Results	Attached data CTD
Effect on LCAT activity in rat plasma	Rat plasma	Ensitrelvir was added to rat plasma to achieve a final concentration of 0[a], 0.206, 0.617, 1.85, 5.56, 16.7, 50.0, 150, and 450 µg/mL, and LCAT activity was measured.	Ensitrelvir did not inhibit LCAT activity. IC_{50}: >450 µg/mL	4.2.3.7-03
Effect on removal of cholesterol from macrophage-like cells	THP-1 cells	THP-1 cells were treated with an inducer of cholesterol efflux transporter (ABCA1)[b] and a macrophage-like cell differentiation inducer.[c] Then, ensitrelvir was added to these cells to achieve a final concentration of 0,[a] 6.25, 12.5, 25.0, 50.0, 100, and 200 µg/mL, and cholesterol concentration in the cells and in the culture supernatant was measured.	Ensitrelvir had no effect on cholesterol retrieval by HDL. IC_{50}: >200 µg/mL	5.3.2.3-01
Analysis of human plasma lipids	Human plasma	Using plasma samples collected from subjects in Cohort G (oral administration of 750 mg once on Day 1 and 250 mg once daily from Days 2 to 5, or placebo once daily for 5 days) in the phase I study (Study T1211), lipoprotein fractions were measured (4 main fractions [CM, VLDL, LDL, and HDL], 20 detailed fractions [2 CM fractions, 5 VLDL fractions, 6 LDL fractions, 7 HDL fractions]).	Decreased medium HDL and small LDL cholesterol and increased large LDL and small VLDL triglycerides were observed in the ensitrelvir group (n = 4) compared with the placebo group (n = 2).	5.3.2.3-02

a) Vehicle: DMSO
b) 9-*cis*-retinoic acid
c) phorbol 12-myristate 13-acetate

5.R Outline of the review conducted by PMDA

5.R.1 Effect on the decrease in blood cholesterol

Decreases in blood total cholesterol and HDL and low-density lipoprotein (LDL) cholesterol were observed in the repeated-dose toxicity study in cynomolgus monkeys.

PMDA's view:

Findings suggestive of tissue injuries related to decreased blood cholesterol or physiological dysfunctions were not observed in repeated dose toxicity studies, but lipid-related adverse events were observed in clinical studies [see Sections 7.2.1, 7.2.2]. Tolerability in humans should be evaluated based on the occurrences of lipid-related adverse events in clinical studies [see Section 7.R.3].

5.R.2 Effect on erythrocyte parameters

The applicant's explanation about the decrease in erythrocyte parameters observed in the repeated-dose toxicity studies in cynomolgus monkeys:

The observed decrease in erythrocyte parameters may have been caused by extravascular hemolysis, for the following reasons: (a) No changes were observed either in red cell volume or in hemoglobin concentration in red blood cells; (b) splenic congestion was observed; (c) neither blood chemistry test nor urinalysis showed findings suggestive of intravascular hemolysis.

Further, safety problems are unlikely to arise in clinical use because no related adverse events such as anemia were observed in clinical studies [see Sections 7.1.1, 7.1.2].

PMDA's view:

Whether these toxicity findings are relevant to the safety in humans should be investigated based on the occurrence of adverse events such as anemia in clinical studies [see Section 7.R.3].

5.R.3 Increase in blood bilirubin

The applicant explained that the increased blood bilirubin observed in repeated-dose toxicity studies in cynomolgus monkeys may be due to extravascular hemolysis and/or to inhibition of OATP1B1- and OATP1B3-mediated indirect bilirubin uptake into the liver due to inhibition of these transporters by ensitrelvir.

PMDA's view:

No adverse events related to increased blood bilirubin were observed in nonclinical studies, but the safety margin is narrow [see Section 5.2]. In clinical studies, an increase in blood bilirubin was observed at the proposed dosage [see Section 7.1.1, 7.1.2], and the mechanism of increased blood bilirubin may be relevant to humans. Thus, tolerability in humans should be investigated, also taking account of the incidences of adverse events related to blood bilirubin increase [see Section 7.R.3].

5.R.4 Effect on immune system

The following findings were observed in the repeated-dose toxicity study in cynomolgus monkeys: (a) the increased histiocytes and plasma cells in the spleen and lymph nodes and (b) mononuclear cell-dominant inflammatory cell infiltration in multiple organs and tissues.

The applicant's explanation:

No degenerative histopathological lesions were found in parenchymal cells or vascular walls of organs/tissues where inflammatory changes were observed in surviving animals. Inflammatory changes were observed in organs/tissues prone to inflammation due to foreign matters. This suggests that the inflammatory changes were induced not by reactions to cellular damages but by enhanced immune function.

PMDA's view on the toxicity findings observed in the repeated-dose toxicity study in cynomolgus monkeys:

The above toxicity findings do not immediately pose any safety concern at the current moment, for the following reasons:

(a) There is a certain safety margin between the plasma ensitrelvir exposure in cynomolgus monkeys receiving ensitrelvir at the NOAEL and that in humans receiving ensitrelvir [see Section 5.2].
(b) No changes were observed in inflammation-related laboratory markers in clinical studies.

However, the relevance of these ensitrelvir-induced toxicity findings to humans cannot be evaluated accurately because the mechanism of these findings is unknown and, in usual clinical studies, it is impossible to conduct histological tests on organs/tissues of the whole body. If ensitrelvir is approved, the information on the toxicity findings should be provided to healthcare professionals through the package insert.

5.R.5 Effect on blood coagulation and easy bleeding

The following findings were observed in the repeated-dose toxicity studies in cynomolgus monkeys: prolongation of prothrombin time (PT) and activated partial thromboplastin time (APTT), decreased platelet count, and hemorrhagic lesions in organs/tissues of the whole body.

The applicant's explanation about the relevance of these findings to the safety in humans:
PT and APTT prolongations were observed in animals that died. This suggests that they were caused by (a) vitamin K deficiency due to decreased food consumption caused by worsening of systemic conditions and/or by (b) impaired production of blood coagulation factors in the liver. No PT- or APTT prolongation-related changes were observed in the secondary pharmacodynamic studies [see Section 3.2.1]. Decreased platelet count is probably a toxicity finding related to ensitrelvir, but the mechanism of its development is unknown. In clinical studies, on the other hand, neither ensitrelvir-related PT or APTT prolongation nor decreased platelet count was observed, and no adverse events suggestive of systemic multiple hemorrhage were observed [see Sections 7.1.1 and 7.1.2]. Accordingly, clinical use of ensitrelvir is unlikely to cause the above safety problems.

PMDA's view:
The applicant's explanation about the mechanism of PT and APTT prolongation is understandable. Decreased platelet count and hemorrhagic lesion were observed in animals without worsening of systemic conditions as well and the mechanism of these disorders is unknown. Therefore, the relevance of these toxicity findings to the safety in humans should be investigated based on the occurrences of hemorrhage-related adverse events in clinical studies [see Section 7.R.3].

5.R.6 Effect on embryos/fetuses

The applicant's explanation about the effect on embryos/fetuses:
In the embryofetal development studies, malformation of fetal axial skeleton was observed in rabbits but not in rats. Further, there is no information that suggests the effect of 3CL protease inhibition. Thus, the relevance of this finding to humans is unclear. According to the foreign guideline,[36)] this finding corresponds to the case "where it is desirable not to administer the product to pregnant or possibly pregnant women." This will be mentioned in the package insert to raise caution.

PMDA's view:
The study of embryofetal development in rabbits showed findings suggestive of malformation of axial skeleton and external anomalies in fetuses. This means that ensitrelvir is potentially teratogenic. The ratio of "the plasma ensitrelvir exposure in rabbits receiving ensitrelvir at the NOAEL for the above toxicity in rabbit embryos/fetuses" to "the plasma ensitrelvir exposure in humans receiving ensitrelvir" was approximately 2.4, which is not a sufficiently wide safety margin [see Section 5.5]. Also, since pregnant or possibly pregnant women were excluded from the clinical studies, there is no information available on the safety of ensitrelvir in pregnant women. Thus, the safety in human fetuses has not been established. Since there are anti-COVID-19 drugs that are approved for use in pregnant women

36) GUIDELINE ON RISK ASSESSMENT OF MEDICINAL PRODUCTS ON HUMAN REPRODUCTION AND LACTATION: FROM DATA TO LABELLING:
https://www.ema.europa.eu/en/documents/scientific-guideline/guideline-risk-assessment-medicinalproducts-human-reproduction-lactation-data-labelling_en.pdf (last accessed on June 7, 2022)

in Japan, ensitrelvir should be contraindicated in pregnant or possibly pregnant women, should the product be approved.

5.R.7 Effect on development of offspring

The total offspring death, decreased live birth rate, decreased survival rate, delayed post-weaning development, and delayed sexual maturation were observed in the study of effects on pre- and postnatal development, including maternal function in rats.

The applicant's explanation about these findings:

Ensitrelvir is transferred to fetuses through the placenta [see Section 4.2.4] and is distributed in milk [see Section 4.4.2]. This suggests that the toxic findings in offspring may possibly be due to exposure to ensitrelvir through placenta or mother's milk, but probably the main cause was the toxic effects on maternal animals, for the following reasons:

- The above study showed the following findings:
  (a) In maternal animals that showed a decrease in weight gain from lactation day 0 to 4, the survival rate of the offspring tended to be lower on Day 4 after birth.
  (b) The maternal animals with total offspring death showed an extensive body weight loss during the lactation period.

  Decreased food consumption in maternal animals adversely affects the fetal nutrition, causing decreased survival rate (*Reproductive Toxicology*. 2009;28:489-94). This suggests that the decreased survival rate of the offspring was due to the toxicity in maternal animals.
- In the above study, food consumption in maternal animals was correlated with a body weight increase in the offspring. Decreased food consumption in maternal animals leads to decreased body weight of the offspring (*Journal of Japanese Society of Nutrition and Food Science*. 2011;64:011-17 [in Japanese]). Thus, the decreased body weight in the offspring is likely to be due to the toxicity in the maternal animals.
- The low body weight in the offspring is known to be correlated with delayed development and sexual maturation (*Juntendo Medical Journal.* 2009;55:27-3, *Biology of Reproduction.* 2003;68:390-400). In the above study, individuals with low body weight tended to show delayed development and sexual maturation. This suggests that the toxic findings were related to low body weight of the offspring caused by the toxicity in the maternal animals such as decreased food consumption.

PMDA's view:

The applicant's explanation about the relationship between the toxicity of ensitrelvir in the maternal animals and the effect on the development of the offspring is understandable. However, since the risk of ensitrelvir in lactating women is currently unclear, information on the toxicity findings observed in nonclinical studies should be disseminated through the package insert, if ensitrelvir is approved.

6. Summary of Biopharmaceutic Studies and Associated Analytical Methods, Clinical Pharmacology, and Outline of the Review Conducted by PMDA

6.1 Summary of biopharmaceutic studies and associated analytical methods

In clinical studies of ensitrelvir, both ensitrelvir suspension and tablets (125, 250 mg) were used. The 125 mg-tablets are the proposed commercial formulation. Table 23 shows formulations used in each clinical study.

Table 23. Formulations used in each clinical study

Formulation		Clinical study
Suspension		Japanese phase I study (Study T1211, Cohorts A to H and J)
Tablets	125-mg tablets [a)]	Japanese phase I study (Study T1211, Cohort N) Global phase II/III study (Study T1221)
	250-mg tablets [b)]	Japanese phase I study (Study T1211, Cohorts L, M, and O) Global phase II/III study (Study T1221)

a) Proposed commercial formulation
b) The 250-mg tablet was shown to be comparable to the 125-mg tablet (the proposed commercial formulation) in the dissolution test conducted according to "Guideline for Bioequivalence Studies for Different Strengths of Oral Solid Dosage Forms" (PMSB/ELD Notification No. 64 dated February 14, 2000, partially revised by PSEHB/PED Notification No. 0319-1 dated March 19, 2020).

Ensitrelvir and M6 (main metabolite) in plasma and urine were measured by LC-MS/MS (lower quantitation limit: ensitrelvir, 10.0 ng/mL in plasma and urine; M6, 5.00 ng/mL in plasma).

In this section, the dose and concentration of ensitrelvir fumaric acid are expressed as those of ensitrelvir. PK parameters are expressed in geometric means (coefficient of variation [CV]%).

6.1.1 Food effect (CTD 5.3.3.1-01: Study T1211 [ongoing since July 2021; data cut-off on February 2022] Cohort C)

Following a single oral dose of ensitrelvir 250 mg (suspension) to Japanese healthy adults (8 PK-evaluable subjects) under fasting conditions or after intake of high-fat diet (863 kcal, lipid 58.1%), PK of ensitrelvir was investigated by a 2-group 2-period crossover study.

The ratio of the least squares geometric means of C_{max} and AUC_{last} of plasma ensitrelvir under fed conditions to those under fasted conditions (fed/fasted) [90% CI] was 0.8508 [0.7507, 0.9644] (C_{max}) and 1.0470 [1.0061, 1.0895] (AUC_{last}).

6.2 Clinical pharmacology

In the present application, results of PK analysis, population pharmacokinetic (PPK) analysis, exposure-response analysis, etc., in healthy subjects were submitted. Results of *in vitro* studies using human biological samples are presented in Sections 4.2.2, 4.2.3 and 4.3.2.

6.2.1 Japanese phase I study (CTD 5.3.3.1-01: Study T1211 [ongoing since July 2021; data cut-off in February 2022])

6.2.1.1 Single dose in Japanese healthy adults (Cohorts A, B, C, D, E, J)

PK parameters were measured following a single oral dose of ensitrelvir suspension in Japanese healthy adult men under fasting conditions. Table 24 shows the results.

Table 24. PK parameters of ensitrelvir following a single oral dose of ensitrelvir (suspension) in Japanese healthy adult men under fasted conditions

Dose (mg)	N	C_{max} (μg/mL)	t_{max} (h)	AUC_{last} (μg·h/mL)	AUC_{inf} (μg·h/mL)	$t_{1/2}$ (h)	CL/F (L/h)	V/F (L)	$Feu_{0\text{-}144\ h}$ (%)
20	6	1.70 (15.0)	2.50 [1.00, 4.00]	82.00 (19.5)	91.44 (24.3)	42.6 (18.6)	0.219 (24.3)	13.5 (10.2)	12.9 (14.6)
70	6	5.20 (18.5)	1.50 [1.00, 4.00]	289.1 (15.4)	291.0 (15.7)	45.7 (11.9)	0.241 (15.7)	15.9 (9.0)	14.7 (27.4)
250	8	15.2 (23.6)	2.50 [1.00, 12.0]	906.8 (15.8)	913.7 (16.2)	43.1 (20.2)	0.274 (16.2)	17.0 (8.8)	16.0 (16.6)
500	6	32.6 (19.0)	2.00 [1.00, 4.00]	1,975 (15.9)	1,987 (16.1)	42.2 (14.6)	0.252 (16.1)	15.3 (13.5)	21.8 (15.4)
1,000	6	63.8 (39.1)	2.75 [1.00, 6.00]	3,341 (35.2)	3,370 (35.5)	48.1 (11.3)	0.297 (35.5)	20.6 (26.2)	19.4 (29.5)
2,000	6	96.9 (16.5)	4.00 [1.50, 8.00]	6,311 (22.0)	6,346 (22.2)	43.1 (15.6)	0.315 (22.2)	19.6 (21.7)	-

Geometric mean (geometric CV%), t_{max} in median [range], -: not tested
$Feu_{0\text{-}144\ h}$: Urinary excretion rate of unchanged compound in urine relative to the administered dose from 0 to 144 hours post-dose.

Metabolites in plasma were investigated following a single oral dose of ensitrelvir suspension 70 mg in Japanese healthy adult men.[37)] The unchanged compound was the main ensitrelvir-derived component in plasma, and M6 was the main metabolite detected.

6.2.1.2 Multiple doses in Japanese and non-Japanese healthy adults (Cohorts F, G, H, L, N, and O)

Ensitrelvir (suspension or tablets) was administered orally to Japanese and non-Japanese (Caucasian) healthy adults at the dose of (a) 375 mg once on Day 1 and 125 mg once daily from Days 2 to 5 (375/125 mg group) or (b) 750 mg once on Day 1 and 250 mg once daily from days 2 to 5 (750/250 mg group). PK parameters of ensitrelvir were measured. Table 25 shows the results.

The plasma ensitrelvir exposure (C_{max} and AUC_{tau}) in Japanese healthy adults was slightly higher than that in non-Japanese healthy adults. The mean body weight in the Japanese and non-Japanese subjects was 66.58 kg and 74.68 kg respectively. The applicant explained that the difference in body weight may be the cause of the difference in plasma ensitrelvir exposure.

Table 25. PK parameters of ensitrelvir following multiple oral doses of ensitrelvir (suspension or tablets) in Japanese and non-Japanese healthy adults

Formulation	Dose (mg)	Race	Sex	N	Day of measurement	C_{max} (μg/mL)	t_{max} (h)	$C_{24\ h}$ (μg/mL)	AUC_{tau} (μg·h/mL)
Suspension	375/125 a)	Japanese	Male	8	Day 1	29.5 (18.6)	1.50 [1.50, 4.00]	17.1 (15.3)	484.5 (13.6)
				8	Day 5	30.4 (8.0)	2.00 [1.00, 6.00]	21.3 (10.6)	597.4 (10.2)
		Caucasians		8	Day 1	22.7 (10.9)	1.75 [0.50, 4.00]	12.7 (15.3)	350.8 (10.2)
				8	Day 5	26.3 (15.3)	1.50 [0.50, 4.00]	19.0 (19.2)	516.5 (14.6)
	750/250 b)	Japanese		8	Day 1	44.8 (21.4)	3.00 [2.00, 3.00]	29.9 (19.6)	818.4 (20.8)
				7	Day 5	66.3 (16.0)	2.50 [0.50, 6.00]	48.9 (13.8)	1,337 (15.0)
Tablets	375/125 a)	Japanese	Female	8	Day 1	22.3 (14.8)	2.50 [1.50, 8.00]	14.0 (11.3)	372.9 (12.0)
				7	Day 5	28.1 (15.6)	2.00 [1.00, 8.00]	17.7 (10.7)	518.3 (13.0)
	750/250 b)		Male	14	Day 1	32.4 (20.0)	2.50 [1.00, 8.00]	-	545.2 (16.3)
				14	Day 5	43.9 (14.7)	4.00 [1.00, 8.00]	-	852.8 (16.6)
			Female	8	Day 1	39.9 (18.3)	3.50 [2.00, 8.00]	23.6 (23.1)	644.4 (21.0)
				8	Day 5	55.8 (15.2)	2.25 [1.50, 4.00]	36.1 (17.3)	1,019 (16.3)

Geometric mean (geometric CV%), t_{max} in median [range], -: not calculated
a) Oral administration of 375 mg once on Day 1 and 125 mg once daily from Days 2 to 5
b) Oral administration of 750 mg once on Day 1 and 250 mg once daily from Days 2 to 5

37) The chromatogram obtained by liquid chromatography of plasma samples was used to identify metabolites.

Following the administration of ensitrelvir tablets to Japanese healthy adult men at the dose of 750 mg once on Day 1 and 250 mg once daily from Days 2 to 5, the geometric mean ratio of AUC (M6/unchanged compound) was 0.00397 (AUC_{tau}) on Day 1, and 0.00866 (AUC_{tau}) and 0.0448 (AUC_{inf}) on Day 5. The applicant provided the following explanation:

> There are no metabolites with >10% exposure relative to the plasma ensitrelvir exposure, as the main metabolite in plasma following a single oral dose was M6 [see Section 6.2.1.1].

6.2.2 Drug interactions (CTD 5.3.3.1-01: Study T1211 [ongoing since July 2021; data cut-off on February 2022] Cohorts G, L, and M)

Effect of ensitrelvir on PK of concomitant drugs was investigated. Table 26 shows the ratio of the least squares geometric means of plasma PK parameters of drugs coadministered with ensitrelvir to that of the drugs administered without ensitrelvir (with ensitrelvir/without ensitrelvir) [90% confidence interval (CI)].

Table 26. Effect of ensitrelvir on PK parameters of concomitant drugs

Dosage regimen of ensitrelvir	Concomitant drug			Number of subjects [a]	Ratio of the least squares geometric means [90% CI] (with ensitrelvir/without ensitrelvir)	
	Drug	Dosage regimen	Day of administration		C_{max}	AUC_{inf}
750/250 mg [b] (suspension)	Midazolam (p.o.)	2 mg once	Day 6	7 [c]/8	2.78 [2.33, 3.30]	8.80 [6.71, 11.5]
750/250 mg [d] (tablets)	Dexamethasone (p.o.)	1 mg once	Day 5	14 [e]/14 [f]	1.47 [1.30, 1.67]	3.47 [3.23, 3.72]
			Day 9	14/14 [f]	1.24 [1.09, 1.40]	2.38 [2.23, 2.54]
			Day 14	14 [e]/14 [f]	1.17 [1.04, 1.33]	1.58 [1.47, 1.70]
750/250 mg [d] (tablets)	Prednisolone (p.o.)	10 mg once	Day 5	14/14	1.11 [1.00, 1.24]	1.25 [1.22, 1.28]
			Day 9	14/14	1.10 [0.99, 1.22]	1.12 [1.10, 1.15]
			Day 14	14/14	0.99 [0.89, 1.10]	1.04 [1.01, 1.07]

a) Number of subjects with/without ensitrelvir coadministration
b) Oral administration of ensitrelvir at 750 mg once on Day 1 and at 250 mg once daily from Days 2 to 6
c) AUC_{inf} was measured in 5 subjects
d) Oral administration of ensitrelvir at 750 mg once on Day 1 and at 250 mg once daily from Days 2 to 5
e) AUC_{inf} was measured in 11 subjects
f) AUC_{inf} was measured in 13 subjects

6.2.3 Evaluation of QT/QTc interval prolongation based on the plasma drug concentration-response analysis (CTD 5.3.4.1-01)

The relationship between plasma ensitrelvir concentration and the change from baseline in Fridericia-adjusted QTc interval (hereinafter referred to as ΔQTcF) was investigated using the linear mixed effect model, based on the plasma ensitrelvir concentration [see Section 6.2.1.1] and electrocardiogram data following a single oral dose of ensitrelvir (20, 70, 250, 500, 1000, or 2000 mg) in Japanese healthy adults in the Japanese phase I study (Study T1211).

The least squares mean of the difference [90% CI] of ΔQTcF between a single dose of ensitrelvir 2,000 mg and placebo (hereinafter referred to as "ΔΔQTcF") was predicted to be 1.79 [−1.97, 5.54] ms, and the upper limit of 90% CI was below 10 ms. ΔΔQTcF was close to 0 ms within the range of the plasma concentration [C_{max} (geometric mean) 1.70 to 96.9 μg/mL]) studied, showing no risk of QT/QTc interval prolongation.

6.2.4 Pharmacokinetic analysis

6.2.4.1 PPK analysis (CTD 5.3.3.5-01)

PPK analysis was conducted (software: NONMEM version 7.4 and up) using plasma ensitrelvir concentration data obtained at 3663 time points from 443 subjects (sex [283 males and 160 females], formulation [suspension in 62 subjects, tablets in 381 subjects], health conditions [120 healthy subjects, 323 subjects infected with SARS-CoV-2], race [435 Asians, 8 Caucasians]) in 2 clinical studies that used ensitrelvir suspension and tablets (Japanese phase I study [Study T1211] and phase IIa and IIb parts of the global phase II/III study [Study T1221]).[38)]

In this analysis, the following parameters were investigated as candidate covariates:

- Effect of food (fasted or fed) and effect of formulation (suspension or tablets) for the first order absorption rate constant (Ka) of ensitrelvir
- Effect of food (fasted or fed), effect of formulation (suspension or tablets), and health conditions (healthy subjects or subjects infected with SARS-CoV-2) for relative BA (F_1)
- Body weight, body mass index (BMI), age, sex, race (Asians or Caucasians), aspartate aminotransferase (AST), alanine aminotransferase (ALT), albumin, total bilirubin, creatinine clearance (CrCL),[39)] serum creatinine concentration (Scr), estimated glomerular filtration rate (eGFR),[40)] eGFR not adjusted for body surface area, and health conditions (healthy subjects or subjects infected with SARS-CoV-2) for apparent total clearance (CL/F)
- Body weight, BMI, age, sex, race (Asians or Caucasians), albumin, and health conditions (healthy subjects or subjects infected with SARS-CoV-2) for apparent volume of distribution in central compartment (Vc/F)

As a result, the final model was described by a 2-compartment model with the first order absorption process using exponential error model for inter-individual variations and a proportional error model for intra-individual variations, and the following parameters were selected as covariates: body weight and health conditions (healthy adults or those infected with SARS-CoV-2) for CL/F; body weight for Vc/F, and effect of food (fasted or fed) and effect of formulation (suspension or tablets) for Ka.

The effects of these covariates on PK parameters (CL/F, Vc/F, and Ka) were estimated based on the final model (see Figure 2).

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38) Characteristics (median [range]) of subjects included in PPK analysis were as follows:
Body weight, 63.0 [35.0, 115.0] kg; BMI, 22.0 [14.2, 37.3] kg/m^2; age, 32 [12, 69] years; AST, 21 [12, 215] U/L; ALT, 19 [5, 287] U/L; albumin, 4.5 [3.2, 5.5] g/dL; total bilirubin, 0.5 [0.1, 1.8] mg/dL; CrCL, 118.0 [50.3, 322.3] mL/min; Scr, 0.76 [0.37, 1.26] mg/dL; eGFR, 90.9 [49.9, 156.8] mL/min/1.73 m^2; eGFR not adjusted for body surface area, 87.9 [47.9, 184.6] mL/min.

39) Calculated by the following equation:
CrCL (mL/min) = (body weight) × (140 − age)/(72 × Scr) × (0.85 [in women])

40) Calculated by the following equation:
Asians: eGFR (mL/min/1.73 m^2) = 194 × (age)$^{-0.287}$ × (Scr)$^{-1.094}$ × (0.739 [in women])
Non-Asians: eGFR (mL/min/1.73 m^2) = 175 × (age)$^{-0.203}$ × (Scr)$^{-1.154}$ × (0.742 [in women]) × (1.212 [in African Americans])

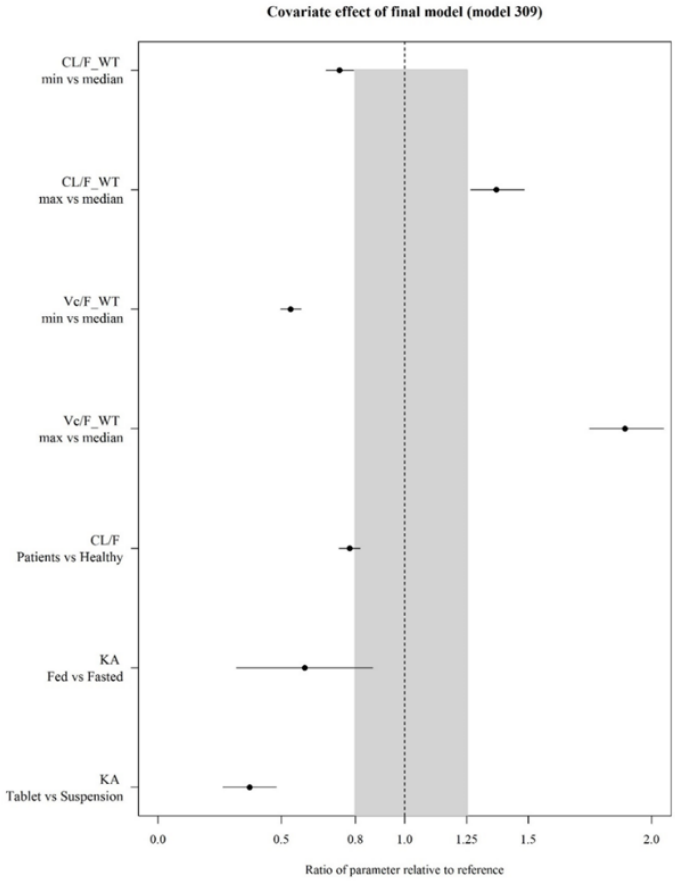

Figure 2. Effects of covariates on PK parameters of ensitrelvir, estimated by the final model
Black point: Median ratio of parameter in the subgroup of the covariate to that in the reference population.
Black solid line: 95% CI of the ratio of parameter in the subgroup of the covariate to that in the reference population.
Dotted center line: Point where the ratio of parameter in the subgroup of the covariate to that in the reference population is 1.
Grey shaded area: Area where the ratio of parameter in the subgroup of the covariate to that in the reference population is 0.8 to 1.25.

Using the final model, PK parameters of ensitrelvir in subjects of phase IIa and IIb parts of the global phase II/III study (Study T1221) were estimated. Table 27 shows the results.

Table 27. PK parameters of ensitrelvir in subjects of phase IIa and IIb parts of global phase II/III study (Study T1221) (estimates)

Dose (mg)	N	Day of measurement	C_{max} (μg/mL)	$C_{24\,h}$ (μg/mL)	AUC_{tau} (μg·h/mL)
375/125 [a)]	161	Day 1	19.6 ± 4.07	16.5 ± 3.17	384.6 ± 89.63
	154	Day 5	25.7 ± 4.51	20.8 ± 3.44	697.7 ± 207.5
750/250 [b)]	162	Day 1	40.4 ± 7.76	34.4 ± 6.36	783.6 ± 170.2
	161	Day 5	54.3 ± 9.30	44.2 ± 7.20	1,489 ± 499.8

Mean ± SD
a) Oral administration of 375 mg once on Day 1 and 125 mg once daily from Days 2 to 5
b) Oral administration of 750 mg once on Day 1 and 250 mg once daily from Days 2 to 5

6.R Outline of the review conducted by PMDA

6.R.1 Food effect of proposed commercial formulation

The applicant explained the food effect of the proposed commercial formulation based on the results of PK analysis of Cohort P in the ongoing Japanese phase I study (Study T1211).

The applicant's explanation:

A single oral dose of ensitrelvir 375 mg (three 125-mg tablets of proposed commercial formulation) was administered to Japanese healthy adults (14 PK-evaluable subjects) under fasting conditions or after high-fat meal (863 kcal, fat 58.1%). Then, PK of ensitrelvir was investigated by a 2-group, 2-period crossover method. The ratio of the least squares geometric mean [90% CI] (fed/fasted) of C_{max} and AUC_{inf} of plasma ensitrelvir were 0.9320 [0.8134, 1.0679] and 1.2447 [1.1396, 1.3596], respectively, with no clinically significant difference. This suggests that instructions for dose timing in relation to meals are not needed. The report of the study will be submitted later.

PMDA's view:

The applicant's explanation is acceptable. If ensitrelvir is approved, information on the results of the study should be provided appropriately to healthcare professionals as soon as the clinical study report of Study T1211 is prepared.

6.R.2 Rationale for the dosage regimen in adult patients

The applicant's explanation about the rationale for the dosage regimen in adult patients in the phase IIa and IIb parts of the global phase II/III study (Study T1221):

The results of changes in plasma ensitrelvir concentration and the elimination half-life following a single dose of ensitrelvir in the Japanese phase I study (Study T1211) [see Section 6.2.1.1] showed that a loading dose and a maintenance dose should be used in combination to rapidly achieve a blood ensitrelvir concentration necessary for exhibiting its effectiveness. Thus, if a 3-times higher dose than the maintenance dose is administered on Day 1, the plasma ensitrelvir concentration would reach an almost steady state on Day 1.

In the Japanese phase I study (Study T1211), ensitrelvir suspension was administered orally at the following dosage regimens:

(a) 375 mg once on Day 1 and 125 mg once daily from Days 2 to 5 (375/125 mg regimen); or
(b) 750 mg once on Day 1 and 250 mg once daily from Days 2 to 5 (750/250 mg regimen)

In both dosage regimens, the trough concentration ($C_{24\,h}$) [see Section 6.2.1.2] exceeded both of the following levels from Day 1 and throughout the administration period:

(a) 90% effective concentration (EC_{90}) of ensitrelvir against SARS-CoV-2 in the presence of 100% human serum *in vitro* (0.374 μg/mL)[41)]; and
(b) Plasma ensitrelvir exposure necessary for reducing the viral titer in lung tissue by 2 log_{10}, estimated from the study in SARS-CoV-2-infected mice ($C_{48\,h}$: 6.09 μg/mL) [see Section 3.1.5.2].

41) EC_{50} of ensitrelvir in the presence of 100% human serum was approximately 6 times higher than that in the absence of human serum [see Section 3.1.2.2]. Accordingly, this EC_{90} value is 6 times the EC_{90} (0.117 μmol/L [0.0623 μg/mL]) of ensitrelvir against SARS-CoV-2 (isolate hCoV-19/Japan/TY11-927-P1/2021 [B.1.617.2 strain, Delta variant]) in the 3-D organ culture model of human tracheal epithelium derived from primary human nasal cavity cells [see Section 3.1.2.1].

Based on this, the dosage regimens in the phase IIa and IIb parts of the global phase II/III study (Study T1221) were determined to be 375/125 mg and 750/250 mg.

As a result, the estimated trough concentration ($C_{24\,h}$, mean ± SD) of ensitrelvir administration in the subjects of phase IIa and IIb parts of the global phase II/III study (Study T1221) was 16.5 ± 3.17 μg/mL (Day 1) and 20.8 ± 3.44 μg/mL (Day 5) in the 375/125 mg group and 34.4 ± 6.36 μg/mL (Day 1) and 44.2 ± 7.20 μg/mL (Day 5) in the 750/250 mg group [see Section 6.2.4.1]. The concentrations exceeded the target exposure level from Day 1 in both dosage regimens.

PMDA's view:
The applicant's explanation is acceptable from the point of view of clinical pharmacology. The appropriateness of the proposed dosage and administration is discussed further in Sections 7.R.2, 7.R.3, and 7.R.5, taking account of the efficacy and safety in clinical studies.

6.R.3 Rationale for the dosage regimen in pediatric patients ≥12 years old

The applicant proposed the following dosage and administration for pediatric patients ≥12 years old. This is the same dosage proposed for adults:

> Oral administration of ensitrelvir 375 mg on Day 1 and ensitrelvir 125 mg from Days 2 to 5, administered orally once daily (375/125 mg regimen)

The applicant's explanation:
Pediatric patients ≥10 years old who receive the same dosage as adults are expected to achieve a plasma ensitrelvir exposure similar to that in adults, for the following reasons:

(a) Ensitrelvir inhibits viral replication through inhibition of 3CL protease of SARS-CoV-2. Accordingly, there is no difference in the plasma ensitrelvir exposure required for the efficacy between adults and pediatric individuals, and;
(b) In general, the expression levels of main drug-metabolizing enzymes and transporters and renal functions do not significantly differ between pediatric individuals ≥10 years old and adults (*Pharmaceutics*. 2011;3:53-72).

In the phase IIa and IIb parts of the global phase II/III study (Study T1221), pediatric patients ≥12 years old received the same dosage as adults, showing no safety concerns unique to pediatric patients [see Section 7.R.3].

Pediatric patients ≥12 years old could be enrolled in Study T1221 only if they weighed ≥40 kg. However, restriction on body weight are considered unnecessary for the proposed dosage and administration in pediatric patients ≥12 years old, because the following findings show that safety concerns unique to patients weighing <40 kg are unlikely to arise:

- Using the final model of PPK analysis [see Section 6.2.4.1], PK of ensitrelvir after administration of ensitrelvir at 375/125 mg was predicted in patients weighing 30 kg, which was below the mean body weight (41.5 to 43.4 kg)[42)] in boys and girls 12 years old. Results showed

[42)] Handbook of Health and Welfare Statistics, Ministry of Health, Labour and Welfare (2020) (https://www.mhlw.go.jp/toukei/youran/indexyk_2_1.html)

that the ratios of C_{max} and AUC_{tau} of ensitrelvir in patients weighing 30 kg to those of patients weighing 63 kg (median body weight in the general population in PPK analysis) were 1.64 and 1.59, respectively, falling below 2.

- In the global phase II/III study (Study T1221), the safety profile of ensitrelvir was confirmed to be tolerable in subjects receiving ensitrelvir 750/250 mg, twice the proposed dosage [see Section 7.R.3].
- In the phase IIa and IIb parts of the global phase II/III study (Study T1221), ensitrelvir was administered to 2 adult subjects weighing <40 kg (1 in 375/125 mg group [35 kg], 1 in 750/250 mg group [38.5 kg]). No adverse events were observed in these subjects. The ratios of C_{max} and AUC_{tau} on Day 1 in the 2 subjects to the mean ratios in the general population were estimated by the final model of PPK analysis [see Section 6.2.4.1]. The ratios were 1.45 (C_{max}) and 1.53 (AUC_{tau}) in the subject weighing 35 kg and 1.45 (C_{max}) and 1.14 (AUC_{tau}) in the subject weighing 38.5 kg. Thus, ensitrelvir exposures in both subjects were less than twice the exposure in the general population.

Based on the above, it is appropriate to use the same dosage regimen in pediatric patients ≥12 years old as in adults.

PMDA's view:

The final model of PPK analysis (see Section 6.2.4.1) was used to estimate PK in patients weighing <40 kg, but the final model was constructed mainly from PK data of adults. PK data of subjects weighing <40 kg are from only 2 subjects. Further, PK parameters in patients weighing 30 kg was predicted using the allometric coefficient[43)] (0.524 [for CL/F] and 1.06 [for Vc/F]) estimated from the PK data. (The allometric coefficient expresses the relationship between CL/F or Vc/F and body weight.) These facts mean that there is uncertainty about applying the predicted PK parameters in patients weighing 30 kg to pediatric patients. PMDA therefore requested that the applicant predict PK parameters using the allometric coefficient of body weight to CL/F and Vc/F empirically used to investigate PK in children (i.e., 0.75 for CL/F and 1 for Vc/F), as a conservative prediction. The predicted exposure did not exceed the exposure in the 750/250 mg group (ratios[44)] of C_{max} and AUC_{tau} was 0.69 and 0.56, respectively, on Day 1 and 0.86 and 0.74, respectively, on Day 5).

On the basis of the applicant's explanation including the additional investigation, PMDA concluded that the proposed dosage in pediatric patients ≥12 years old including those weighing <40 kg (i.e., oral administration of ensitrelvir at 375 mg once on Day 1, then at 125 mg once daily from Days 2 to 5) was acceptable, from the point of view of clinical pharmacology. If ensitrelvir is approved, safety data should be collected after the market launch and new findings should be provided appropriately to healthcare professionals, because experience with ensitrelvir in pediatric patients ≥12 years old is limited, and particularly because ensitrelvir has never been administered to pediatric patients weighing <40 kg.

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43) Effects of body weight on CL/F and Vc/F are expressed in the following equations:
CL/F = a × (body weight/63 kg [population mean body weight])^b
Vc/F = c × (body weight /63 kg [population mean body weight])^d
a or c, fixed effect of CL/F or Vc/F; b or d, allometric coefficient expressing the relationship between body weight and the fixed effect of CL/F or Vc/F.

44) "Predicted value in SARS-CoV-2-infected people weighing 30 kg"/"median value in the ensitrelvir 750/250 mg group (post hoc estimate)"

Appropriateness of the proposed dosage and administration is discussed in Sections 7.R.2, 7.R.3, and 7.R.5, based on the efficacy and safety in clinical studies.

6.R.4 Ensitrelvir administration in patients with hepatic impairment

The applicant's explanation about ensitrelvir administration in patients with hepatic impairment:
No clinical pharmacology study was conducted in subjects with hepatic impairment. Ensitrelvir administration in this patient group is unlikely to pose any particular safety concerns, for the following reasons:

- In the final model of PPK analysis, hepatic function-related laboratory values such as AST, ALT, albumin, and bilirubin were not selected as significant covariates for CL/F of ensitrelvir [see Section 6.2.4.1].
- In the global phase II/III study (Study T1221), the incidence of adverse events observed in subjects with mild hepatic impairment[45] in the ensitrelvir group was as follows:

  Phase IIa part: 0 of 1 subject in the ensitrelvir 375/125 mg group and 1 of 2 subjects (high density lipoprotein decreased) in the ensitrelvir 750/250 mg group

  Phase IIb part: 1 of 4 in the ensitrelvir 375/125 mg group (AST increased, ALT increased, blood lactate dehydrogenase increased, and high-density lipoprotein decreased in 1 each [this subject had 4 events]) and 1 of 2 in the ensitrelvir 750/250 mg group (high density lipoprotein decreased)

  All adverse events were nonserious and resolved. The incidence of adverse events in this patient group was not different from that in the entire population.

A clinical pharmacology study involving subjects with mild (Child-Pugh class A) and moderate (Child-Pugh class B) hepatic impairment will be started in July 2022 and its clinical study report will be completed in June 2023.

PMDA's view:
Taking account of the explanation of the applicant, ensitrelvir administration in patients with mild hepatic impairment is unlikely to cause any particular safety concerns.

In patients with moderate hepatic impairment as well, plasma ensitrelvir exposure is unlikely to increase to a level that causes clinically serious problems, because hepatic function is preserved to a certain extent in such patients, and in view of the following information available on ensitrelvir.

- The main elimination pathway of ensitrelvir is metabolism in the liver [see Section 4.3.3], but the urinary excretion rate of the unchanged compound following a single dose is 12.9% to 21.8% [see Section 6.2.1.1], indicating that ensitrelvir is partly eliminated by renal excretion as the unchanged compound.
- In the global phase II/III study (Study T1221), the safety profile of ensitrelvir was confirmed to be tolerable in subjects receiving ensitrelvir 750/250 mg, which is twice the proposed dosage [see Section 7.R.3].

[45] Subjects with complications classified as "hepatobiliary disorders" in MedDRA SOC. Since the study excluded subjects with hepatic impairment of Grade ≥2 according to CTCAE, all of the subjects had Grade 1 hepatic impairment.

Nevertheless, because ensitrelvir has never been used in patients with moderate hepatic impairment, the package insert should include a precautionary statement to raise caution against the possible increase in ensitrelvir exposure in such patients, if ensitrelvir is approved.

As for patients with severe hepatic impairment, the package insert should state that ensitrelvir administration is not recommended in this patient group, if ensitrelvir is approved, for the following reasons: (a) Due to the severe reduction of hepatic function, plasma ensitrelvir exposure may exceed the level with confirmed tolerability, and (b) ensitrelvir has never been administered to patients with severe hepatic impairment.

If ensitrelvir is approved, information on the safety of patients with hepatic impairment, including the results of the clinical pharmacology study to be conducted in subjects with hepatic impairment, should be collected continuously after marketing, and new information should be promptly provided to healthcare professionals.

6.R.5 Ensitrelvir administration in patients with renal impairment

The main elimination pathway of ensitrelvir is metabolism in the liver [see Section 4.3.3]. Ensitrelvir is a substrate for CYP3A and at the same time inhibits CYP3A in a time-dependent manner. This raise the possibility that repeated administration of ensitrelvir may inhibit metabolism of ensitrelvir metabolism by CYP3A, leading to increased renal excretion rate of ensitrelvir relative to the overall excretion. Based on the above concern, PMDA asked the applicant to explain ensitrelvir administration in patients with renal impairment:

The applicant's explanation:
Although no clinical pharmacology study was conducted in subjects with renal impairment, there are no particular concerns in administering ensitrelvir to patients with renal impairment, for the following reasons:

- In the final model of PPK analysis, laboratory values related to renal functions (CrCL, Scr, eGFR, and eGFR not adjusted for body surface) were not selected as significant covariates for CL/F [see Sections 6.2.4.1].
- Subjects in the phase IIa and IIb parts of the global phase II/III study (Study T1221) were evaluated for distribution of the post-hoc estimates of C_{max} and AUC_{tau} of ensitrelvir in plasma by renal function level (eGFR [mL/min/1.73 m^2]: normal [≥90], mild impairment [≥60 to <90], moderate impairment [≥30 to <60]). No clear difference was observed between populations with different renal function levels (Figure 3).

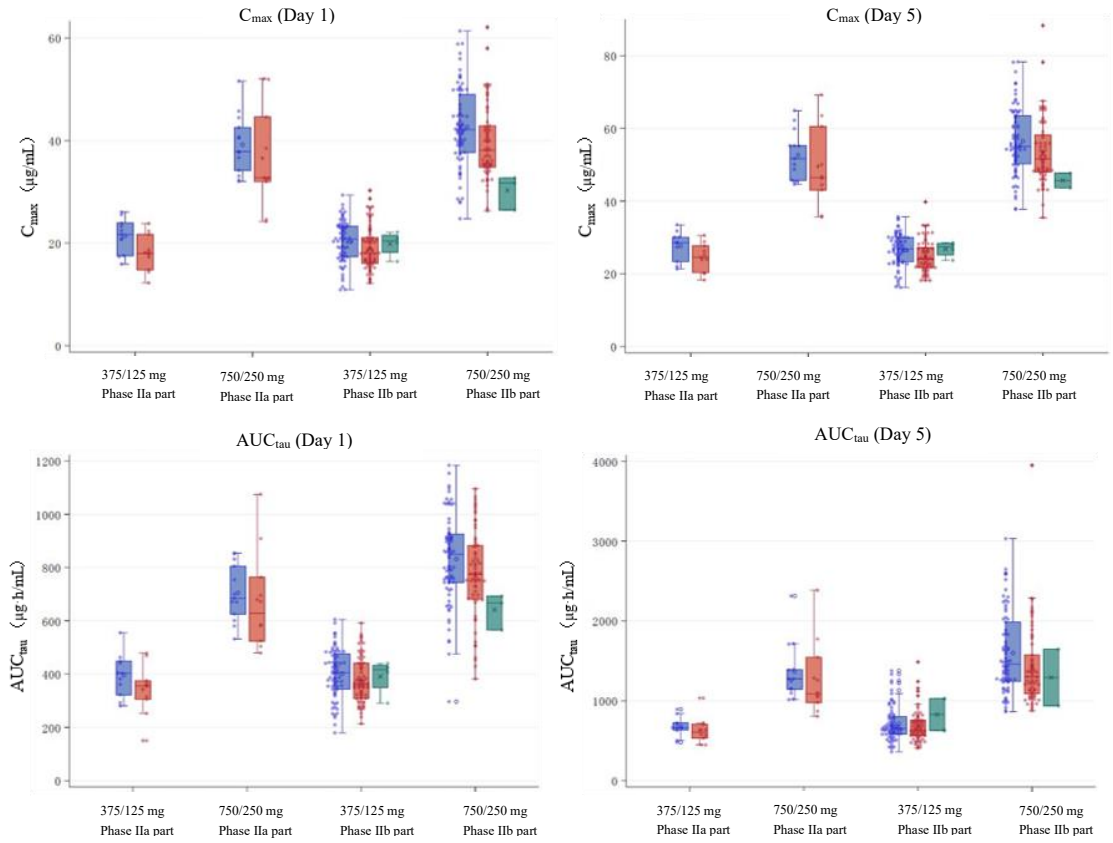

Figure 3. Exposure by the level of renal function in subjects of phase IIa and IIb parts of global phase II/III study (Study T1221) (distribution of C_{max} and AUC_{tau})

Blue: subjects with normal renal function (phase IIa part [11 subjects in the ensitrelvir 375/125 mg group; 13 subjects in the ensitrelvir 750/250 mg group], phase IIb part [69 in the ensitrelvir 375/125 mg group; 74 in the ensitrelvir 750/250 mg group])
Red: subjects with mild renal impairment (phase IIa part [10 in the ensitrelvir 375/125 mg group; 10 in the ensitrelvir 750/250 mg group], phase IIb part [67 in the ensitrelvir 375/125 mg group; 62 in the ensitrelvir 750/250 mg group])
Green: subjects with moderate renal impairment (phase IIa part [0 in the ensitrelvir 375/125 mg group; 0 in the ensitrelvir 750/250 mg group], phase IIb part [4 in the ensitrelvir 375/125 mg group; 3 in the ensitrelvir 750/250 mg group])
The bottom, middle, and top lines of each box indicate 25 percentile, median, and 75 percentile, respectively.
The top and bottom ends of each whisker indicate the lower limit and upper limit, respectively, excluding values beyond 1.5 times the quartile from the end of the box.

- In the ensitrelvir groups of the global phase II/III study (Study T1221), the number of subjects with renal impairment enrolled and evaluated for safety was as follows [see Section 7.R.3]:
  Subjects with mild renal impairment
  Phase IIa part: 10 in the ensitrelvir 375/125 mg group; 10 in the ensitrelvir 750/250 mg group
  Phase IIb part: 67 in the ensitrelvir 375/125 mg group; 63 in the ensitrelvir 750/250 mg group
  Subjects with moderate renal impairment
  Phase IIa part: 0 in the ensitrelvir 375/125 mg group; 0 in the ensitrelvir 750/250 mg group
  Phase IIb part: 4 in the ensitrelvir 375/125 mg group; 3 in the ensitrelvir 750/250 mg group

A clinical pharmacology study involving subjects with mild, moderate, and severe renal impairment will be started in June 2022 and its clinical study report will be completed in [redacted].

PMDA's view:
The safety profile of ensitrelvir was tolerable in subjects receiving ensitrelvir 750/250 mg, twice the proposed dosage in the global phase II/III study (Study T1221) [see Section 7.R.3]. On the basis of

this finding and the applicant's explanation, there is no evidence suggesting any particular safety concern regarding the use of ensitrelvir in patients with renal impairment. However, if ensitrelvir is approved, the applicant should continue to collect safety data in patients with renal impairment after the market launch (including the results of the clinical pharmacology study to be conducted in subjects with renal impairment) and should provide new information promptly to healthcare professionals.

6.R.6 Drug interactions

6.R.6.1 Effect of concomitant drug on PK of ensitrelvir

In vitro studies suggested that ensitrelvir was metabolized mainly by CYP3A and served as a substrate of P-gp and BCRP [see Section 4.3.2 and 4.5.3].

The applicant provided the following explanation about the effect of concomitant drugs on the PK of ensitrelvir:

Coadministration with CYP3A inhibitor

- Ensitrelvir is metabolized mainly by CYP3A and, at the same time, inhibits CYP3A in a time-dependent manner [see Section 4.5.1]. Coadministration of ensitrelvir 750/250 mg[46)] with midazolam (typical CYP3A substrate) caused an 8.80-fold increase in AUC_{inf} of midazolam on Day 6 of ensitrelvir administration, showing a potent CYP3A-inhibitory effect of ensitrelvir [see Section 6.2.2].
- The accumulation rate under the steady state was estimated from the elimination half-life following a single dose of ensitrelvir [see Section 6.2.1.1] without regard to the time-dependent inhibitory effect of ensitrelvir against CYP3A. Results predicted that AUC_{tau} on Day 1 would be similar to that on Day 5 with the dosage regimen of "750 mg ensitrelvir once on Day 1, followed by 250 mg ensitrelvir once daily from Days 2 to 5 (750/250 mg)."
- However, in the Japanese phase I study (Study T1211), the actual AUC_{tau} on Day 5 was 1.56 to 1.80 times that on Day 1 in subjects receiving 750/250 mg [see Section 6.2.1.2]. This suggests that the observed ratio of the exposure on Day 5 to that on Day 1 (1.56- to 1.80-fold) was probably due to the time-dependent CYP3A inhibitory effect associated with the repeated administration of ensitrelvir.
- Taking account of the above estimate, even if ensitrelvir is coadministered with a potent CYP3A inhibitor on Day 1 of treatment (when the time-dependent CYP3A-inhibitory effect of ensitrelvir is not fully expressed yet), the increase in the exposure will be similar to the above-observed exposure ratio (1.50 to 1.80-fold). On Day 5, ensitrelvir itself fully expresses a potent time-dependent inhibitory effect of against CYP3A, and therefore the inhibitory effect is unlikely to be affected by coadministration of another potent CYP3A inhibitor. This suggests that, on Day 5, the ratio of "plasma ensitrelvir exposure following administration of ensitrelvir 375/125 mg (the proposed dosage) + a CYP3A inhibitor" to "plasma exposure following administration ensitrelvir 375/125 mg alone" will not exceed 1.80.
- In the global phase II/III study (Study T1221), the safety profile of ensitrelvir was shown to be well tolerated in the group receiving ensitrelvir 750/250 mg, the twice the proposed dosage [see Section 7.R.3]. Coadministration of ensitrelvir with a CYP3A inhibitor is therefore unlikely to cause any

[46)] Oral administration of ensitrelvir at 750 mg once on Day 1, then at 250 mg once daily from Days 2 to 6.

clinically significant drug interactions. Accordingly, it is unnecessary to provide cautions in the package insert.

Coadministration with P-gp or BCRP inhibitors

The following study results suggest that BA of ensitrelvir is high and that ensitrelvir absorption from the digestive tract is unlikely to be affected by P-gp or BCRP inhibition.

- Ensitrelvir showed a high BA in rats [see Section 4.1.1].
- A study with Caco-2 cells showed that the apical-to-basal apparent permeation coefficient of ensitrelvir ($P_{app\ A \rightarrow B}$) was 4.24×10^{-6} cm/ms. Drugs with $P_{app\ A \rightarrow B}$ of $\geq 1 \times 10^{-6}$ cm/ms have good oral absorption (*Adv Drug Deliv Rev*. 2001;46:27-43).

In the phase IIa and IIb parts of the global phase II/III study (Study T1221), ensitrelvir was coadministered with drugs[47)] with P-gp-inhibitory effects to 4 subjects. Adverse events were observed in 2 of them (high density lipoprotein decreased, hyperbilirubinaemia, and myalgia in 1 each [1 subject had >1 event]), but there were no adverse events unique to subjects receiving ensitrelvir in combination with a drug with P-gp-inhibitory effect.

Based on the above, it is unnecessary to raise caution against coadministration with drugs with P-gp- or BCRP-inhibitory activity because such coadministration is unlikely to cause clinically significant drug interactions.

Coadministration with CYP3A inducers

Since ensitrelvir is metabolized mainly by CYP3A, there is a concern that coadministration of ensitrelvir with a CYP3A inducer may cause a decrease in plasma ensitrelvir concentration, which may result in decreased efficacy of ensitrelvir. Taking account of the significance of the effect, coadministration of potent CYP3A inducers will be prohibited, and the package insert will include a precautionary statement regarding coadministration of moderate CYP3A inducers.

PMDA's view:

The applicant's explanation is acceptable. However, because of the limited information available on the interactions between ensitrelvir and CYP3A, P-gp, or BCRP inhibitors or CYP3A inducers, the applicant should continue to collect information on drug interactions in ensitrelvir administration, including the information in published reports, after the market launch, and should promptly provide new information to healthcare professionals, if ensitrelvir is approved.

6.R.6.2 Effect of ensitrelvir on PK of concomitant drugs

6.R.6.2.1 Coadministration of ensitrelvir with CYP3A substrate

In the study of the drug interaction between ensitrelvir and midazolam, AUC_{inf} of midazolam coadministered with ensitrelvir was 8.80 times that of midazolam administered without ensitrelvir; this indicates a potent CYP3A-inhibitory effect of ensitrelvir [see Section 6.2.2]. In view of this, the applicant explained the CYP3A-inhibitory effect of ensitrelvir administered orally at the proposed dosage (375 mg once on Day 1, followed by 125 mg once daily from Days 2 to 5 [375/125 mg]).

47) Clarithromycin in 2 subjects, azithromycin in 1 subject, and cyclosporine in 1 subject.

The applicant's explanation:

The dosage regimen used in this study (750 mg once on Day 1, followed by 250 mg once daily from Days 2 to 5 [750/250 mg]) was higher than the proposed dosage (375/125 mg). Therefore, the drug interaction with midazolam at the proposed dosage (375/125 mg) of ensitrelvir was investigated using the physiologically-based pharmacokinetic (PBPK) model.[48] AUC_{inf} of midazolam coadministered with ensitrelvir 375/125 mg was estimated to be 3.83 times that of midazolam administered without ensitrelvir. This suggests that ensitrelvir is a moderate CYP3A inhibitor.

PMDA's view:

PBPK model analysis used for the investigation of drug interaction between ensitrelvir and a concomitant drug requires a high degree of predictability, for the following reasons:

- In the drug interaction study, AUC_{inf} of midazolam coadministered with ensitrelvir 750/250 mg increased was 8.80 times that of midazolam administered without ensitrelvir, demonstrating the potent CYP3A-inhibitory effect of ensitrelvir [see Section 6.2.2].
- The drug interaction between a CYP3A substrate and ensitrelvir at the proposed dosage (375 mg/125 mg) was investigated only by the PBPK model analysis.
- This PBPK model is used in making a very high-risk decision, i.e., deciding which CYP3A substrates should be prohibited from being coadministered with ensitrelvir.

However, the parameters and the hypothesis used in the above analysis raise concerns listed in Table 28. Accordingly, the extent of CYP3A inhibition by ensitrelvir at the proposed dosage cannot be explained based only on the predictions by the PBPK model.

[48] SimCYP version 20 was employed in the analysis using PBPK model. First-order model was selected as the model of ensitrelvir absorption, and minimal PBPK model as the distribution model. fa was determined to be 1 for the suspension and 0.7 for the tablets, based on the results of administration of ensitrelvir suspension and ensitrelvir tablet in the Japanese phase I study (Study T1211) [see Section 6.2.1.2]. The fraction of unbound drug in gut cells ($f_{u,\,gut}$) was determined to be 0.013 (predicted by SimCYP). The contribution rate of CYP3A4 to elimination was determined to be 40% based on the investigation using the accumulation rate after single- and multiple-dose administration of ensitrelvir [see Section 6.R.6.1]. CLr of ensitrelvir was determined to be 0.0498 L/h based on the results of single-dose administration of ensitrelvir in the Japanese phase I study (Study T1211) [see Section 6.2.1.1]. K_I and k_{inact} of ensitrelvir against CYP3A4/5 were determined to be 84 μmol/L and 2.76 1/h, respectively, based on the results of the *in vitro* study [see Section 4.5.1]. The fraction of unbound drug in microsomes ($f_{u,mic}$) was determined to be 0.5 based on the rate of increase in AUC_{inf} (8.80-fold) in coadministration of ensitrelvir suspension 750/250 mg and midazolam [see Section 6.2.2]. The model in coadministration of ensitrelvir and a CYP3A substrate was validated based on the results of the clinical drug interaction study of ensitrelvir and dexamethasone (Cohort L in the Japanese phase I study [Study T1211]). PBPK model in ensitrelvir monotherapy was validated based on the results of Cohorts F, G, H, L, M, N, and O of the Japanese phase I study (Study T1211). The model in midazolam or dexamethasone monotherapy was validated based on the results of Cohorts G and L in the Japanese phase I study (Study T1211).

Table 28. Major concerns about PBPK model analysis used in the investigation of drug interactions between ensitrelvir and CYP3A substrates

- Since results of a mass-balance study of ensitrelvir in humans are not available, only limited information is available on the oral absorption rate of ensitrelvir and on the contribution rate of metabolism to the elimination of ensitrelvir.
- Ensitrelvir suspension was used in the clinical drug interaction study between ensitrelvir and midazolam, whereas the proposed formulation is tablet. In the PBPK model, taking account of the difference in the formulation, fa of ensitrelvir was assumed to be 1 for suspension and 0.7 for tablet, based on the results of administration of ensitrelvir suspension and tablets [see Section 6.2.1.2]. However, in the final model of PPK analysis [see Section 6.2.4.1], it was deemed unnecessary to consider the effect of formulation on fa of ensitrelvir.
- The contribution rate of CYP3A4 to the elimination of ensitrelvir was estimated from the accumulation rate in the repeated administration [see Section 6.R.6.1], without conducting a clinical drug interaction study with a potent CYP3A inhibitor. Thus only limited data are available for investigating the contribution rate of CYP3A4 to the elimination of ensitrelvir. Also, in the PPK analysis, a model without regard to time-dependent inhibitory effect was employed as the final model, and the applicant explained that the time-course of plasma concentration in repeated ensitrelvir administration could be described by a model that did not consider the time-dependent inhibitory effect. As a result, the above parameters are fraught with uncertainties.
- K_I and k_{inact} of ensitrelvir against CYP3A4/5 were determined based on data from the *in vitro* study [see Section 4.5.1]. Ensitrelvir was shown to induce CYP3A4 in the *in vitro* study [see Section 4.5.2], but the CYP3A4/5-inducting effect of ensitrelvir was not taken into account in the PBPK model.
- Validation of the PBPK model of coadministration of ensitrelvir and a CYP3A substrate was conducted based on data from the clinical drug interaction study using 750/250 mg, a higher dose than the proposed dosage (375/125 mg). This means that the model of CYP3A-inhibitory effect of ensitrelvir within the range of plasma exposure to ensitrelvir administered at the proposed dosage, has not been fully validated.
- When the fraction of unbound drug in microsomes ($f_{u,mic}$)[49)] was changed over the range from 0.4 to 1.0 in a sensitivity analysis, the predicted increase rate of AUC_{inf} of midazolam changed over a wide range by 5.12- to 10.5-fold, albeit based on the assessment in the ensitrelvir 750/250 mg administration.
- The predicted changes in plasma concentration of midazolam or dexamethasone coadministered with ensitrelvir 750/250 mg tended to differ from the actually measured changes.[50)] The applicant has not sufficiently investigated the effect of this difference on the prediction of PK of midazolam coadministered with ensitrelvir tablets at the proposed dosage (375/125 mg).
- $f_{u,gut}$ of ensitrelvir was assumed to be 0.013, but the appropriateness of this parameter value has not been fully explained. Also, the applicant has not investigated the effect of this parameter value on (a) setting of other parameters or (b) the estimation of PK of midazolam coadministered with ensitrelvir at the proposed dosage (375/125 mg).

The applicant's explanation:

Taking account of the PMDA's view, a new clinical drug interaction study will be conducted to investigate the interaction between ensitrelvir at the proposed dosage and midazolam. The precautions for coadministration of ensitrelvir and a CYP3A substrate will be advised based on the maximum conceivable risks at the current moment. Thus, after the results of this study become available, the content of contraindications and precautions for concomitant use will be decided based on the increase

49) $f_{u,mic}$ was estimated to be 0.5 based on the rate of increase in AUC_{inf} of midazolam (8.80-fold, see Section 6.2.2) when coadministered with ensitrelvir (750/250 mg).

50) According to the applicant's explanation, the observed difference was due to the difference between (a) the formulations of midazolam and dexamethasone used in the clinical drug interaction study (midazolam, syrup; dexamethasone, tablets [marketed in Japan]) and (b) those used in the construction of PBPK model (midazolam, tablets [*Clin Pharmacol Ther*. 1981;30:653-61]; dexamethasone, tablets [marketed in foreign countries] [*Eur Clin Respir J*. 2017;4:1353395]).

in AUC_{inf} of midazolam (8.80-fold) in the clinical drug interaction study of ensitrelvir 750/250 mg and midazolam (see Section 6.2.2), and based on the package inserts for other potent CYP3A inhibitors.

PMDA accepted the explanation of the applicant.

6.R.6.2.2 Coadministration of ensitrelvir with substrates of P-gp, BCRP, OATP1B1, OATP1B3, OCT1, and OAT3

In *in vitro* studies, the extent of inhibition of P-gp, BCRP, OATP1B1, OATP1B3, OCT1, and OAT3 by ensitrelvir exceeded the cut-off value that may require conducting a clinical drug interaction study, as stipulated in "Guideline on Drug Interaction for Drug Development and Appropriate Provision of Information" (PSEHB/PED Notification No. 0723-4 dated July 23, 2018, by the Pharmaceutical Evaluation Division, Pharmaceutical Safety and Environmental Health Bureau, Ministry of Health, Labour and Welfare). The applicant explained the drug interactions between ensitrelvir and the substrates of these transporters, taking account of the above guidelines.

The applicant's explanation:
A clinical drug interaction study (T1215) was conducted using cocktail substrates to investigate drug interactions between ensitrelvir and substrates of P-gp, BCRP, OATP1B1, OATP1B3, and OCT1. Although the study report is yet to be completed, results of PK analysis are available. Table 29 shows the ratio of the least squares geometric mean [90% CI] of plasma PK parameters of concomitant drugs (digoxin, rosuvastatin, metformin) in the presence versus absence of ensitrelvir.

The exposure to digoxin and rosuvastatin increased after coadministration with ensitrelvir. Therefore, the following information will be included in the package insert, taking into account the safety margin of the concomitant drugs and the seriousness of possible adverse reactions:

(a) Precautions for concomitant use: digoxin and dabigatran etexilate methanesulfonate (substrates of P-gp) and rosuvastatin calcium (substrate of BCRP, OATP1B1 and OATP1B3)
(b) Ensitrelvir may increase plasma concentration of these drugs.

Table 29. Effect of ensitrelvir on PK parameters of concomitant drugs

Dosage regimen of ensitrelvir	Concomitant drug		Number of subjects [a]	Ratio of least squares geometric mean [90% CI] (with ensitrelvir/without ensitrelvir)	
	Drug	Dosage regimen [a]		C_{max}	AUC_{inf}
500 mg single dose [b]	Digoxin (p.o.)	0.25 mg, single dose	14 [c]/14 [d]	2.17 [1.72, 2.73]	1.31 [1.13, 1.52]
	Rosuvastatin (p.o.)	2.5 mg, single dose	14/14	1.97 [1.73, 2.25]	1.65 [1.47, 1.84]
	Metformin (p.o.)	500 mg, single dose	14/14	1.03 [0.91, 1.16]	1.02 [0.94, 1.11]

a) "Number of subjects receiving concomitant ensitrelvir" / "Number of subjects not receiving concomitant ensitrelvir"
b) Tablets
c) AUC_{inf} was calculated in 11 subjects.
d) AUC_{inf} was calculated in 13 subjects.

No clinical drug interaction study of ensitrelvir and OAT3 substrates was conducted, leaving open the possibility that coadministration with ensitrelvir may increase the plasma concentration of OAT3 substrates. Methotrexate, an OAT3 substrate with a narrow safety margin, will be listed under "Precautions for concomitant use" and a precautionary statement (i.e., "coadministration of ensitrelvir

and methotrexate may increase plasma methotrexate concentration") will be included in the package insert.

PMDA's view:
The applicant's explanation is acceptable. However, since no information is available on the extent of the effect of ensitrelvir on plasma concentration of OAT3 substrates, the applicant should continue to collect information on the coadministration of ensitrelvir with OAT3 substrates, including the information in published reports, after the market launch.

7. Clinical Efficacy and Safety and Outline of the Review Conducted by PMDA

In the present application, the applicant submitted the results of clinical studies shown in Table 30 as the main efficacy and safety data.

Table 30. Summary of main clinical study

Category	Region	Study code	Phase	Population	No. of subjects enrolled	Outline of dosage regimen	Main endpoints
Evaluation	Global	T1221	II/III[a)]	Phase IIa part Asymptomatic SARS-CoV-2 carriers and patients with COVID-19 Phase IIb part Patients with COVID-19	Phase IIa part (a) 22 (b) 23 (c) 24 Phase IIb part (a) 142 (b) 143 (c) 143	Both parts (a) Oral administration of ensitrelvir 375 mg once on Day 1 and ensitrelvir 125 mg once daily from Days 2 to 5 (b) Oral administration of ensitrelvir 750 mg once on Day 1 and ensitrelvir 250 mg once daily from Days 2 to 5 (c) Oral administration of placebo once daily for 5 days	Efficacy Safety

a) The study also includes phase III part and IIb/III part. Data from these parts are yet to be submitted.

7.1 Global phase II/III study (CTD 5.3.5.1-01: Study T1221 [ongoing since September 9, 2021])

7.1.1 Phase IIa part [September 2021 to January 2022]

A randomized, double-blind, placebo-controlled, parallel-group study was conducted at 56 study sites in Japan to investigate the antiviral activity and safety of ensitrelvir in asymptomatic SARS-CoV-2 carriers and patients with COVID-19 (target sample size, 69 [23 in each group]). Table 31 shows the main inclusion/exclusion criteria of the study.

Table 31. Main inclusion/exclusion criteria

Inclusion criteria	1. Subjects ≥12 to <20 years old weighing ≥40 kg, and those ≥20 to <70 years old [a)] 2. SARS-CoV-2 positive (confirmed by PCR etc. on samples collected within 120 hours before randomization) 3. Subjects who meet either of the following regarding the symptoms of COVID-19: (1) Symptomatic (patients with COVID-19): Subjects who meet both of the following. • Onset of at least 1 of 14 symptoms [b)] of COVID-19 within 120 hours before randomization • At least 1 moderate (score 2) [c)] or severe symptom among 12 symptoms [d)] of COVID-19 at the time of randomization. Subjects with symptoms that have been present since before the onset of COVID-19 are eligible only if they consider that the symptoms have worsened after SARS-CoV-2 infection.[e)] (2) Asymptomatic (SARS-CoV-2 carriers without symptoms) • Subjects who have none of the 14 symptoms [b)] of COVID-19 within 2 weeks before randomization. Subjects with symptoms that have been present since before SARS-CoV-2 infection are eligible only if none of the symptoms have worsened at baseline. 4. Subjects who can take contraceptive measures from the start of study treatment through 10 days after the end of study treatment. 5. Women who are not pregnant or possibly pregnant
Exclusion criteria [f)]	1. SpO_2 ≤93% (room air) [g)] 2. Subjects who need oxygen therapy 3. Subjects who need mechanical ventilation 4. Current or chronic history of moderate or severe liver disease (Grade ≥2 in CTCAE, ver. 5.0) 5. Current or chronic history of moderate or severe kidney disease (Grade ≥2 in CTCAE, ver. 5.0)

a) In the protocol ver. 5 (October 19, 2021), the age criterion was changed from "≥20 to <70 years" to "≥12 to <70 years" (those <20 years old are limited to those weighing ≥40 kg).
b) (1) Malaise or tiredness, (2) muscle or body aches, (3) headache, (4) chills or sweating, (5) feverish or pyrexia, (6) stuffy or runny nose, (7) sore throat, (8) cough, (9) shortness of breath (dyspnea), (10) nausea, (11) vomiting, (12) diarrhea, (13) taste abnormality, and (14) dysosmia
c) Symptoms were scored by the subject on a 4-point scale (0, none; 1, mild; 2, moderate; 3, severe).
d) (1) Malaise or tiredness, (2) muscle or body aches, (3) headache, (4) chills or sweating, (5) feverish or pyrexia, (6) stuffy or runny nose, (7) sore throat, (8) cough, (9) shortness of breath (dyspnea), (10) nausea, (11) vomiting, (12) diarrhea
e) Changed from "except symptoms that have been present since before the onset of COVID-19" in the protocol ver. 5 (October 19, 2021).
f) "Subjects strongly suspected of having pneumonia" was deleted in the protocol ver. 5 (October 19, 2021).
g) SpO_2 was changed from ≤96% (room air) to ≤93% (room air) in the protocol ver. 5 (October 19, 2021).

The study drug was administered orally at the following dosage: (a) ensitrelvir at 375 mg once on Day 1 and at 125 mg once daily from Days 2 to 5 (ensitrelvir 375/125 mg group), (b) ensitrelvir at 750 mg once on Day 1 and at 250 mg once daily from Days 2 to 5 (ensitrelvir 750/250 mg group), or (c) placebo once daily for 5 days.

Of 69 randomized subjects (22 in the ensitrelvir 375/125 mg group, 23 in the ensitrelvir 750/250 mg group, 24 in the placebo group), 68 (21 in the ensitrelvir 375/125 mg group, 23 in the ensitrelvir 750/250 mg group, 24 in the placebo group) who received at least 1 dose of the study drug were included in the safety analysis population. Among the 69 randomized subjects, 43 (15 in the ensitrelvir 375/125 mg group, 14 in the ensitrelvir 750/250 mg group, 14 in the placebo group) tested positive for both (a) SARS-CoV-2 by reverse transcription PCR (RT-PCR) on nasal swab sample[51)] on the first day of study treatment (before administration) and (b) SARS-CoV-2 virus titer in nasal swab sample at baseline. The 43 subjects were included in the mITT population and in the population for antiviral activity analysis. Of the subjects of the mITT population, 2 subjects each in each group met the criterion of "asymptomatic" in the inclusion criteria (see Table 31).

A total of 4 subjects (1 in the ensitrelvir 375/125 mg group, 3 in the placebo group) discontinued the study due to disease progression (1 subject in the placebo group) and at their own will (3 subjects [1 in the ensitrelvir 375/125 mg group, 2 in the placebo group]).

51) The last sample obtained before the start of study treatment.

Table 32 and Figure 4 show the antiviral activity evaluated by the primary endpoint "change in SARS-CoV-2 virus titer from baseline to each time point (nasal swab samples)."

Table 32. Change in SARS-CoV-2 virus titer (nasal swab sample) at each time point from baseline (Phase IIa part: mITT population)

Evaluation time point	Ensitrelvir 375/125 mg			Ensitrelvir 750/250 mg			Placebo		
	N	Viral titer	Change from baseline	N	Viral titer	Change from baseline	N	Viral titer	Change from baseline
Baseline	15	3.36 ± 1.35	-	14	3.66 ± 1.18	-	14	3.29 ± 1.19	-
Day 2	15	2.31 ± 1.24	−1.05 ± 1.17	14	1.64 ± 0.88	−2.03 ± 1.21	14	2.42 ± 1.52	−0.86 ± 0.93
Day 4	15	0.94 ± 0.29	−2.42 ± 1.42	14	0.85 ± 0.19	−2.81 ± 1.21	14	1.74 ± 1.17	−1.54 ± 0.74
Day 6	15	0.80 ± 0.00	−2.56 ± 1.35	14	0.90 ± 0.25	−2.76 ± 1.19	13	1.04 ± 0.57	−2.08 ± 0.91
Day 9	14	0.85 ± 0.19	−2.69 ± 1.27	13	0.80 ± 0.00	−2.78 ± 1.18	13	0.91 ± 0.26	−2.21 ± 1.11

Mean ± SD
Viral titer: $\log_{10}$ $TCID_{50}$/mL
Titer below the lower detection limit (0.8 $\log_{10}$ $TCID_{50}$/mL) was assumed to be 0.8 $\log_{10}$ $TCID_{50}$/mL.
Missing values were not imputed.

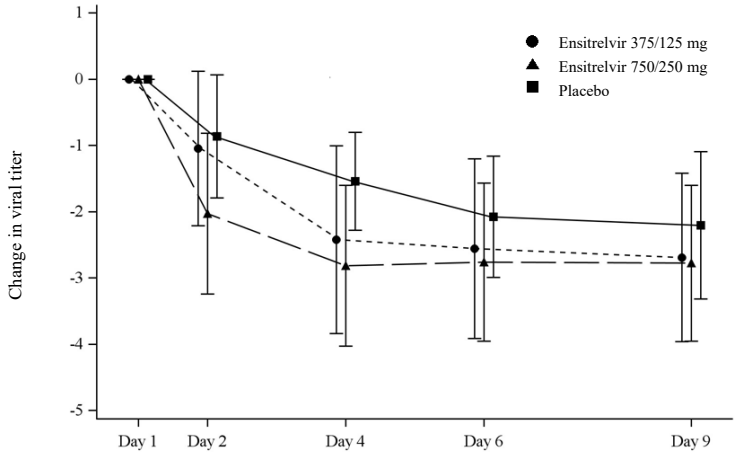

Figure 4. Change in SARS-CoV-2 virus titer ($\log_{10}$ $TCID_{50}$/mL) (nasal swab sample) from baseline to each time point (mean ± SD)

SARS-CoV-2 strains infecting the subjects (ITT population[52)]) were the Delta variant (81.3% [13 of 16 subjects] in the ensitrelvir 375/125 mg group; 92.9% [13 of 14] in the ensitrelvir 750/250 mg group; 94.1% [16 of 17] in the placebo group) and the Omicron variant (18.8% [3 of 16] in the ensitrelvir 375/125 mg group; 7.1% [1 of 14] in the ensitrelvir 750/250 mg group; 5.9% [1 of 17] in the placebo group).

As for safety,[53)] adverse events and adverse reactions[54)] were observed in 52.4% (11 of 21) and 23.8% (5 of 21) of subjects, respectively, in the ensitrelvir 375/125 mg group; 69.6% (16 of 23) and 43.5% (10 of 23) of subjects, respectively, in the ensitrelvir 750/250 mg group; and 37.5% (9 of 24) and 0% (0 of 24) of subjects, respectively, in the placebo group. Table 33 shows the incidences of adverse events and adverse events observed in ≥1 subject in any group.

52) Randomized subjects who tested positive by RT-PCR on a nasal swab sample obtained before the start of study treatment.
53) Adverse events and adverse reactions observed until 23 days after the completion of study treatment.
54) Adverse events considered by the investigator, etc. to be related to the study drug.

Table 33. Adverse events and adverse reactions observed in ≥1 subject in any group (phase IIa part: safety analysis population)

Event	Adverse events			Adverse reactions		
	Ensitrelvir 375/125 mg (N = 21)	Ensitrelvir 750/250 mg (N = 23)	Placebo (N = 24)	Ensitrelvir 375/125 mg (N = 21)	Ensitrelvir 750/250 mg (N = 23)	Placebo (N = 24)
All events	11 (52.4)	16 (69.6)	9 (37.5)	5 (23.8)	10 (43.5)	0
High density lipoprotein decreased	3 (14.3)	12 (52.2)	2 (8.3)	3 (14.3)	8 (34.8)	0
Nasopharyngitis	2 (9.5)	0	0	0	0	0
Rhinalgia	2 (9.5)	0	0	0	0	0
Headache	1 (4.8)	3 (13.0)	0	0	0	0
AST increased	1 (4.8)	1 (4.3)	2 (8.3)	0	0	0
Nausea	1 (4.8)	1 (4.3)	0	1 (4.8)	0	0
ALT increased	1 (4.8)	0	2 (8.3)	0	0	0
γ-GTP increased	1 (4.8)	0	1 (4.2)	0	0	0
Neutropenia	1 (4.8)	0	0	1 (4.8)	0	0
Abdominal discomfort	1 (4.8)	0	0	1 (4.8)	0	0
Abdominal pain upper	1 (4.8)	0	0	1 (4.8)	0	0
Insomnia	1 (4.8)	0	0	0	0	0
Photophobia	1 (4.8)	0	0	0	0	0
Oropharyngeal pain	1 (4.8)	0	0	0	0	0
Fatigue	1 (4.8)	0	0	0	0	0
Pyrexia	1 (4.8)	0	0	0	0	0
Urine ketone body present	1 (4.8)	0	0	0	0	0
Blood triglycerides increased	0	3 (13.0)	0	0	2 (8.7)	0
Blood bilirubin increased	0	2 (8.7)	0	0	1 (4.3)	0
White blood cell count increased	0	1 (4.3)	1 (4.2)	0	0	0
Hyperbilirubinaemia	0	1 (4.3)	0	0	1 (4.3)	0
Blood iron increased	0	1 (4.3)	0	0	1 (4.3)	0
Gout	0	1 (4.3)	0	0	0	0
Hypoaesthesia	0	1 (4.3)	0	0	0	0
Conjunctival hyperaemia	0	1 (4.3)	0	0	0	0
Diarrhoea	0	1 (4.3)	0	0	0	0
Vomiting	0	1 (4.3)	0	0	0	0
Rash	0	1 (4.3)	0	0	0	0
Arthralgia	0	1 (4.3)	0	0	0	0
Blood cholesterol decreased	0	1 (4.3)	0	0	0	0
Blood creatine phosphokinase increased	0	1 (4.3)	0	0	0	0
Blood immunoglobulin G decreased	0	1 (4.3)	0	0	0	0
Presyncope	0	0	1 (4.2)	0	0	0
Abdominal distension	0	0	1 (4.2)	0	0	0
Proctalgia	0	0	1 (4.2)	0	0	0
Eczema	0	0	1 (4.2)	0	0	0
Back pain	0	0	1 (4.2)	0	0	0
Dysmenorrhoea	0	0	1 (4.2)	0	0	0
Blood calcium increased	0	0	1 (4.2)	0	0	0
Blood uric acid increased	0	0	1 (4.2)	0	0	0
Protein urine present	0	0	1 (4.2)	0	0	0

n (%), MedDRA ver.24.0

There were no adverse events that resulted in death, were serious, or led to study discontinuation.

7.1.2 Phase IIb part [January to March 2022]

A randomized, double-blind, placebo-controlled, parallel-group study was conducted at 88 study sites in Japan and Korea to investigate the efficacy and safety of ensitrelvir in patients with COVID-19

(target sample size 435 [145 per group][55]). Table 34 shows the main inclusion/exclusion criteria in this study.

Table 34. Main inclusion/exclusion criteria

Inclusion criteria	1. Subjects ≥12 to <20 years old weighing ≥40 kg, and those ≥20 to <70 years old 2. SARS-CoV-2 positive (confirmed by PCR etc. on a sample collected within 120 hours before randomization) 3. Onset of at least 1 of 14 symptoms [a] of SARS-CoV-2 within 120 hours before randomization 4. At least 1 moderate (score 2)[b] or severe symptom among 12 symptoms [c] of COVID-19 at the time of randomization. Subjects with symptoms that have been present since before the onset of COVID-19 are eligible only if they consider that the symptoms have worsened after SARS-CoV-2 infection. 5. Subjects who can take contraceptive measures from the start of the study treatment until 10 days after the end of the study treatment 6. Women who are not pregnant or possibly pregnant
Exclusion criteria	1. SpO_2 ≤93% (room air) 2. Subjects who need oxygen therapy 3. Subjects who need a mechanical ventilation 4. Current or chronic history of moderate or severe liver disease (Grade ≥2 in CTCAE, ver. 5.0) 5. Current or chronic history of moderate or severe kidney disease (Grade ≥2 in CTCAE, ver. 5.0)

a) (1) Malaise or tiredness, (2) muscle or body aches, (3) headache, (4) chills or sweating, (5) feverish or pyrexia, (6) stuffy or runny nose, (7) sore throat, (8) cough, (9) shortness of breath (dyspnea), (10) nausea, (11) vomiting, (12) diarrhea, (13) taste abnormality, and (14) dysosmia
b) Symptoms were scored by the subject on a 4-point scale (0, none; 1, mild; 2, moderate; 3, severe).
c) (1) Malaise (tiredness), (2) muscle or body aches, (3) headache, (4) chills or sweating, (5) feverish or pyrexia, (6) stuffy or runny nose, (7) sore throat, (8) cough, (9) shortness of breath (dyspnea), (10) nausea, (11) vomiting, (12) diarrhea

The study drug was administered orally at the following dosage regimens: (a) ensitrelvir at 375 mg once on Day 1 and at 125 mg once daily from Days 2 to 5 (375/125 mg group), (b) ensitrelvir at 750 mg once on Day 1 and at 250 mg once daily from Days 2 to 5 (750/250 mg group), or (c) placebo once daily for 5 days.

Of 428 randomized subjects (142 in the ensitrelvir 375/125 mg group, 143 in the ensitrelvir 750/250 mg group, 143 in the placebo group), 421 (140 in the ensitrelvir 375/125 mg group, 140 in the ensitrelvir 750/250 mg group, 141 in the placebo group) received at least 1 dose of the study drug. The 421 subjects were included in the safety analysis population.

Among the 428 randomized subjects, 341 (114 in the ensitrelvir 375/125 mg group, 116 in the ensitrelvir 750/250 mg group, 111 in the placebo group) tested positive for SARS-CoV-2 virus titer with nasal swab sample,[51] and were included in the ITT1 population. The ITT1 population was handled as the efficacy analysis population.

A total of 19 subjects (8 in the ensitrelvir 375/125 mg group, 7 in the ensitrelvir 750/250 mg group, 4 in the placebo group) discontinued the study. The reasons for the discontinuation were subject's request in 11 subjects (4 in the ensitrelvir 375/125 mg group, 4 in the ensitrelvir 750/250 mg group, 3 in the placebo group), lost to follow up in 4 subjects (2 in the ensitrelvir 375/125 mg group, 1 in the

[55] One of the primary endpoints was "change per unit time in the total score of 12 COVID-19 symptoms from the start of study treatment (Day 1) to 120 hours (Day 6)." For this primary endpoint, the expected difference between the placebo group and each ensitrelvir group was assumed to be -1, and the standard deviation in each treatment group was assumed to be 2.6. With these assumptions, the required sample size was calculated to be 108 subjects per group in order to detect the difference with 80% statistical power at 1-sided significance level of 2.5%. By assuming the drop-out rate due to a negative viral titer to be 25%, the target sample size was determined to be 435 (145 per group). The other primary endpoint was "change in SARS-CoV-2 viral titer from baseline to Day 4." For this primary endpoint, the expected difference between the placebo group and each ensitrelvir group was assumed to be −0.5 $\log_{10}$ $TCID_{50}$/mL, and the standard deviation in each treatment group was assumed to be 0.7. With these assumptions, 99.9% statistical power is obtained from 435 subjects (145 in each group) with a 1-sided significance level of 2.5%.

ensitrelvir 750/250 mg group, 1 in the placebo group), adverse event in 1 subject (ensitrelvir 375/125 mg group), SARS-CoV-2 negative in 1 subject (ensitrelvir 750/250 mg group), disease progression in 1 subject (ensitrelvir 750/250 mg group), and other[56)] in 1 subject (ensitrelvir 375/125 mg group).

For the assessment of efficacy, the following co-primary endpoints were employed in this study:

- Change per unit time in the total score of 12 COVID-19 symptoms from the start of study treatment (Day 1) to 120 hours (Day 6)[57)]; and
- Change from baseline to Day 4 in SARS-CoV-2 viral titer

Table 35 shows the results, falling short of fulfilling the pre-defined success criteria of the study.

Table 35. Results of the primary endpoints (phase IIb part: ITT1 population)

	Ensitrelvir 375/125 mg (N = 114)	Ensitrelvir 750/250 mg (N = 116)	Placebo (N = 111)
Change per unit time in the total score of 12 COVID-19 symptoms from the start of study treatment (Day 1) to 120 hours (Day 6)	−5.95 ± 4.02 (n =109)[a)]	−5.42 ± 3.70 (n = 113)[a)]	−4.92 ± 3.25 (n = 110)[a)]
Difference from placebo [95% CI] [b)] *P* value [b)c)]	−0.24 [−0.83, 0.34] 0.4171	−0.04 [−0.62, 0.53] -	-
Change from baseline to Day 4 in SARS-CoV-2 viral titer (log_{10} $TCID_{50}$/mL) [d)]	−1.69 ± 0.84 (n = 106)[a)]	−1.43 ± 0.83 (n = 112)[a)]	−1.06 ± 0.99 (n = 107)[a)]
Difference from placebo [95% CI] [e)] *P* value [c)e)]	−0.41 [−0.51, −0.31] <0.0001	−0.41 [−0.51, −0.31] -	-

Mean ± SD

a) Subjects without scores at baseline or after the start of study and those without Day 4 virus titer were excluded from analysis.
b) ANCOVA (Analysis of Covariance) model with covariates of (a) baseline total score of 12 symptoms, (b) time from onset of COVID-19 to randomization (<72 h or ≥72 h), and (c) prior vaccination against SARS-CoV-2 (yes, no).
c) The significance level of the entire study was 5% (2-sided). In order to adjust the multiplicity of hypothesis testing, the ensitrelvir 750/250 mg group was to be compared with the placebo group only if statistical significance was observed in comparison between the ensitrelvir 375/125 mg and placebo groups in both of the 2 primary endpoints.
d) Titer below the lower detection level (1.1 log_{10} $TCID_{50}$/mL) was assumed to be 1.1 log_{10} $TCID_{50}$/mL.
e) ANCOVA model with covariates of (a) baseline SARS-CoV-2 virus titer, (b) time from onset of COVID-19 to randomization (<72 h or ≥72 h), and (c) prior vaccination against SARS-CoV-2 (yes, no).

Figure 5 shows time course of the total score of 12 symptoms and its changes from baseline. Figure 6 shows time course of the scores of each of 12 symptoms.

56) In order to treat coexisting disease.
57) The value calculated according to the following procedures:
(a) Each of the following 12 COVID-19 symptoms are scored by the subject on a 4-point scale (0, none; 1, mild; 2, moderate; 3, severe), and then the scores are totaled: (1) malaise or tiredness, (2) muscle or body aches, (3) headache, (4) chills or sweating, (5) feverish or pyrexia, (6) stuffy or runny nose, (7) sore throat, (8) cough, (9) shortness of breath (dyspnea), (10) nausea, (11) vomiting, (12) diarrhea.
(b) AUC of the "change in the total of the 12 scores at each measuring time point from baseline" was calculated by trapezoidal method from the start of study treatment (Day 1) to 120 hours (Day 6), and divided by the length of the evaluation period (unit: hour)

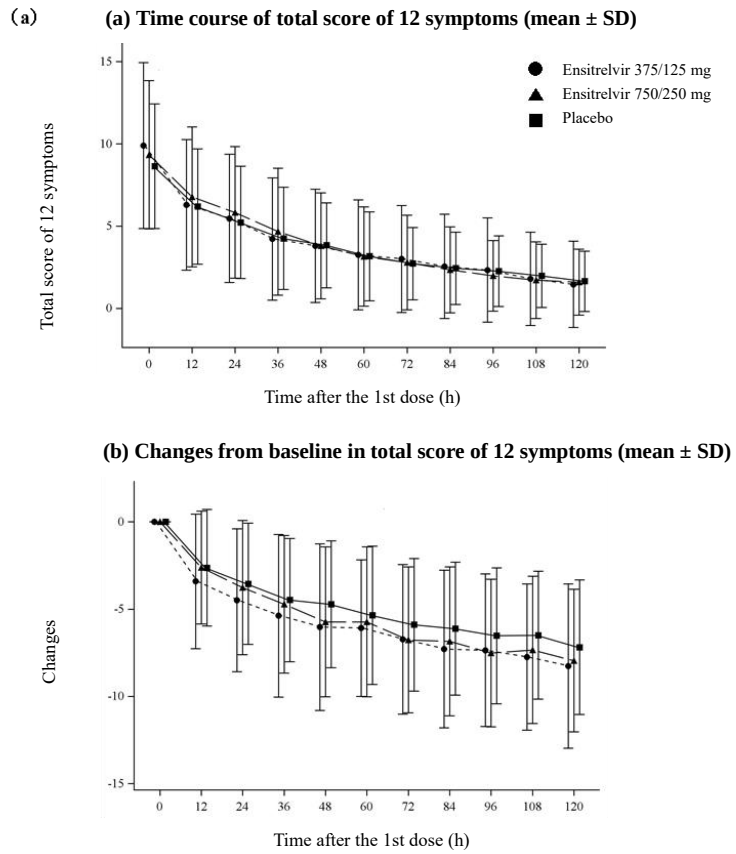

Figure 5. Time course of total score of 12 symptoms and its changes from baseline (phase IIb part: ITT1 population)

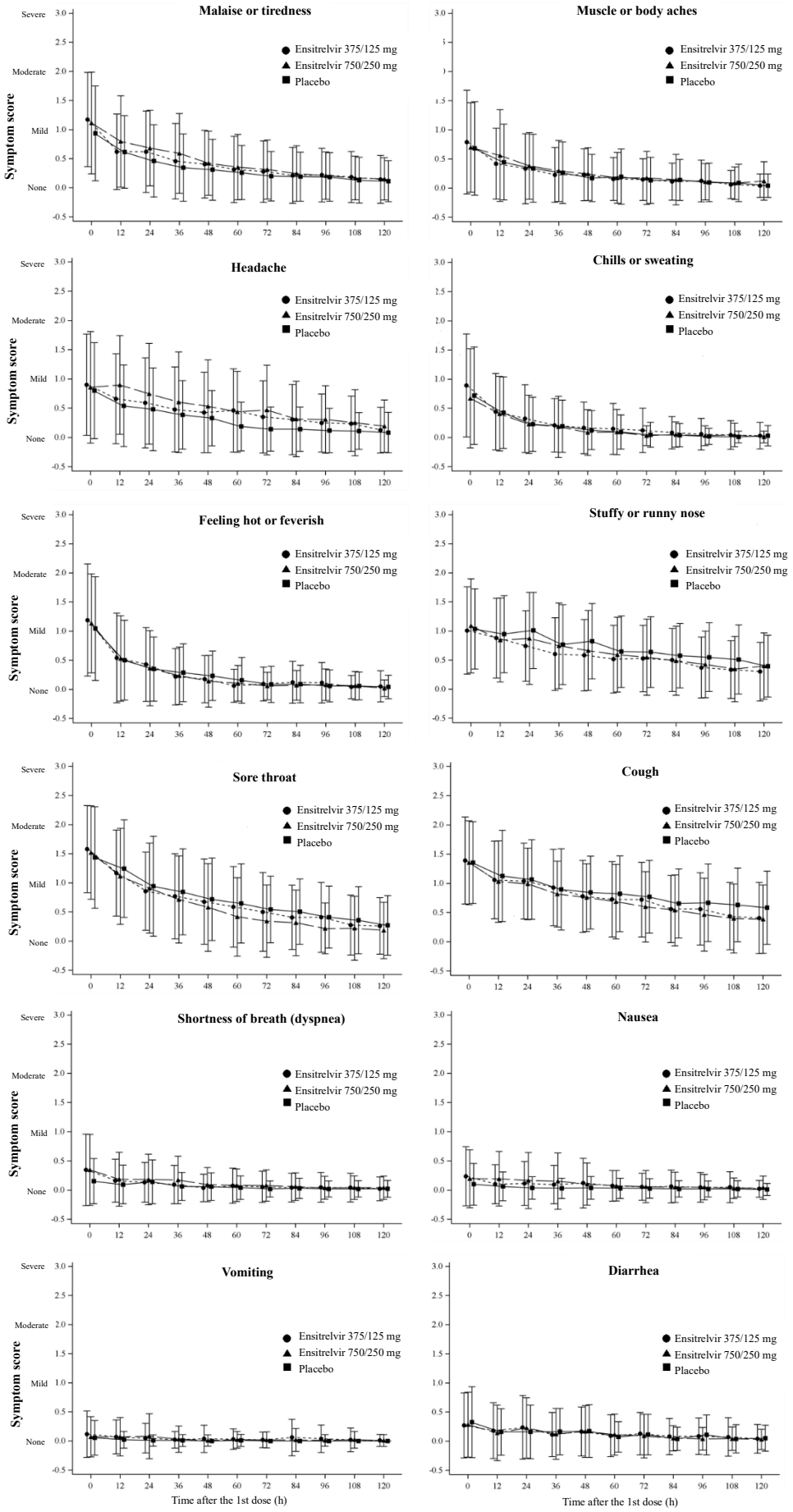

Figure 6. Change over time in score of each of 12 symptoms of COVID-19 (phase IIb part: ITT1 population)

Of 428 randomized subjects, 419 were enrolled in the study in Japan. During the period of enrollment of these subjects, approximately 94% of SARS-CoV-2 strains identified in Japan was the Omicron variant.[58)]

As for safety, adverse events and adverse reactions[54)] were observed in 34.3% (48 of 140) and 13.6% (19 of 140) of subjects, respectively, in the ensitrelvir 375/125 mg group, in 42.9% (60 of 140) and 22.1% (31 of 140) of subjects in the ensitrelvir 750/250 mg group, and in 31.2% (44 of 141) and 5.0% (7 of 141) of subjects in the placebo group. Table 36 shows the incidences of adverse events and adverse reactions observed in at least 1 subject in the ensitrelvir groups.

58) National Institute of Infectious Diseases: SARS-CoV-2 variant-specific detection by genome surveillance in Japan https://www.mhlw.go.jp/stf/seisakunitsuite/newpage_00061.html (last accessed on May 30, 2022)

Table 36. Adverse events and adverse reactions observed in ≥1 subject in the ensitrelvir groups (phase IIb part: Safety analysis population)

Event	Adverse events			Adverse reactions		
	Ensitrelvir 375/125 mg (N = 140)	Ensitrelvir 750/250 mg (N = 140)	Placebo (N = 141)	Ensitrelvir 375/125 mg (N = 140)	Ensitrelvir 750/250 mg (N = 140)	Placebo (N = 141)
All events	48 (34.3)	60 (42.9)	44 (31.2)	19 (13.6)	31 (22.1)	7 (5.0)
High density lipoprotein decreased	31 (22.1)	40 (28.6)	5 (3.5)	13 (9.3)	22 (15.7)	0
Headache	3 (2.1)	3 (2.1)	0	1 (0.7)	1 (0.7)	0
Diarrhoea	2 (1.4)	3 (2.1)	1 (0.7)	1 (0.7)	2 (1.4)	0
Rash	2 (1.4)	1 (0.7)	3 (2.1)	1 (0.7)	1 (0.7)	0
ALT increased	2 (1.4)	1 (0.7)	1 (0.7)	1 (0.7)	0	1 (0.7)
AST increased	2 (1.4)	0	0	1 (0.7)	0	0
Blood triglycerides increased	1 (0.7)	9 (6.4)	1 (0.7)	1 (0.7)	1 (0.7)	0
Back pain	1 (0.7)	3 (2.1)	1 (0.7)	0	0	0
Nausea	1 (0.7)	2 (1.4)	1 (0.7)	1 (0.7)	2 (1.4)	1 (0.7)
Blood bilirubin increased	1 (0.7)	2 (1.4)	1 (0.7)	1 (0.7)	1 (0.7)	1 (0.7)
Blood iron increased	1 (0.7)	1 (0.7)	0	1 (0.7)	1 (0.7)	0
Insomnia	1 (0.7)	1 (0.7)	0	0	0	0
Vomiting	1 (0.7)	1 (0.7)	0	0	0	0
Palpitations	1 (0.7)	0	1 (0.7)	1 (0.7)	0	0
Eczema	1 (0.7)	0	1 (0.7)	1 (0.7)	0	0
Sinusitis	1 (0.7)	0	1 (0.7)	0	0	0
Cystitis	1 (0.7)	0	0	0	0	0
Haemoptysis	1 (0.7)	0	0	0	0	0
Abdominal discomfort	1 (0.7)	0	0	0	0	0
Constipation	1 (0.7)	0	0	0	0	0
Arthralgia	1 (0.7)	0	0	0	0	0
Pain in extremity	1 (0.7)	0	0	0	0	0
Chest pain	1 (0.7)	0	0	0	0	0
Blood lactate dehydrogenase increased	1 (0.7)	0	0	0	0	0
Glucose urine present	1 (0.7)	0	0	0	0	0
Low density lipoprotein increased	1 (0.7)	0	0	0	0	0
Jaw fracture	1 (0.7)	0	0	0	0	0
Skin abrasion	1 (0.7)	0	0	0	0	0
Dyslipidaemia	0	3 (2.1)	0	0	3 (2.1)	0
Oral herpes	0	2 (1.4)	0	0	0	0
Seasonal allergy	0	2 (1.4)	0	0	0	0
Hypoaesthesia	0	1 (0.7)	2 (1.4)	0	1 (0.7)	0
Nasopharyngitis	0	1 (0.7)	1 (0.7)	0	0	0
Epistaxis	0	1 (0.7)	1 (0.7)	0	0	0
Gastrointestinal disorder	0	1 (0.7)	1 (0.7)	0	0	0
Dry skin	0	1 (0.7)	1 (0.7)	0	0	0
Intervertebral disc protrusion	0	1 (0.7)	1 (0.7)	0	0	0
Pruritus	0	1 (0.7)	0	0	1 (0.7)	0
Blood uric acid increased	0	1 (0.7)	0	0	1 (0.7)	0
Hepatic enzyme increased	0	1 (0.7)	0	0	1 (0.7)	0
Streptococcal infection	0	1 (0.7)	0	0	0	0
Gastrooesophageal reflux disease	0	1 (0.7)	0	0	0	0
Myalgia	0	1 (0.7)	0	0	0	0
Bilirubin conjugated increased	0	1 (0.7)	0	0	0	0
Blood cholesterol decreased	0	1 (0.7)	0	0	0	0

n (%), MedDRA ver.24.0

There was no adverse event resulting in death.

Serious adverse events were observed in 2 subjects in the placebo group (thoracic vertebral fracture and facial paralysis in 1 subject each). Causal relationship to the study drug was ruled out for both of them. The outcome was “recovering” or “recovered.”

Adverse events leading to study discontinuation were observed in 2 subjects in the ensitrelvir 375/125 mg group (eczema, nausea, and headache in 1 subject each [1 subject had 2 events]). All of these events were considered to be related to the study drug, with the outcome of "recovered."

7.R Outline of the Review Conducted by PMDA

7.R.1 Plan for global phase II/III study (Study T1221)

The applicant's explanation about the ongoing global phase II/III study (Study T1221), the main clinical study on the efficacy and safety of ensitrelvir:

As shown in Table 37, the study consists of 4 parts. Phase III part and phase IIb/III part are currently ongoing. For the present application, the efficacy and safety of ensitrelvir were evaluated based on the results of phase IIa and IIb parts.

Table 37. Structure of global phase II/III study (Study T1221)

Part	Main objective
Phase IIa part	To confirm the antiviral activity of ensitrelvir in patients with mild to moderate COVID-19 and in asymptomatic SARS-CoV-2 carriers.
Phase IIb part	To evaluate clinical symptom improvement by ensitrelvir and the antiviral activity of ensitrelvir in patients with mild to moderate COVID-19.
Phase III part	To evaluate the efficacy of ensitrelvir in patients with mild to moderate COVID-19
Phase IIb/III part	To evaluate the efficacy of ensitrelvir in asymptomatic SARS-CoV-2 carriers and in patients with only mild symptoms of COVID-19.

Up to the protocol ver. 6 (dated December 28, 2021) of the global phase II/III study (Study T1221), a phase IIb/III part in patients with mild to moderate COVID-19 had been planned (former phase IIb/III part). However, during the conduct of the former phase IIb/III part, the protocol was amended to ver. 7 (dated February 7, 2022) to introduce a phase IIb part and to reclassify the former phase IIb/III part as a phase III part.

The study was conducted as a global study because intrinsic and extrinsic ethnic factors were considered unlikely to affect the evaluation of ensitrelvir, for the following reasons:

(a) There is no significant difference in the definition of COVID-19, diagnostic methods, and treatment methods in Japan and in other countries.
(b) Patients with mild to moderate symptoms, especially those with risk factors for severe COVID-19, are treated with antiviral drugs and neutralizing antibodies.
(c) There was no clear difference in PK of ensitrelvir between Japanese and non-Japanese subjects [see Section 6.2.1.2].
(d) In nonclinical studies, ensitrelvir was shown to have antiviral activity against multiple SARS-CoV-2 variants, suggesting that the antiviral efficacy of ensitrelvir is unlikely to be affected by the type of the prevalent strain [see Section 3.1.2.1].

The phase IIb part used the following co-primary endpoints:

- Change per unit time in the total score of 12 COVID-19 symptoms from the start of study treatment (Day 1) to 120 hours (Day 6)
- Change in SARS-CoV-2 viral titer from baseline to Day 4

These co-primary endpoints were selected based on the results of the phase IIa part and the following conditions:

- Clinical symptoms were evaluated based on the value obtained by the following calculation: AUC of "change from baseline in the total score" was calculated by trapezoidal method from the start of study treatment (Day 1) to 120 hours (Day 6) and divided by the length of the evaluation period (unit: hour), for the following reasons:
  (a) The change from baseline to Day 6 (the next day of the end of study treatment) only provides indirect information on treatment effect at time points before Day 6.
  (b) AUC of changes in total symptom scores was used as the secondary endpoint in a clinical study of another drug against COVID-19.[59)]
  (c) Having multiple symptoms is a burden for patients, and reducing the burden is considered to be of clinical significance.
  (d) Reducing the total score of 12 symptoms at an early stage is considered to contribute to reducing the time to recovery from symptoms.
- In most of the subjects with COVID-19 symptoms in the placebo group of phase IIa part, viral titer on Day 6 was below the lower detection limit. Accordingly, Day 4 was selected as the measurement point of viral titer. Viral RNA load was not used as the parameter for SARS-CoV-2 infection but instead viral titer was used, because viral RNA assessment also measures viral genome fragments without infective capacity, whereas viral titration quantitatively measures only viruses with infective capacity and therefore provides a higher clinical significance. Further, "changes in viral titer" was used because it can evaluate the antiviral effect quantitatively at a higher sensitivity than "the presence or absence of viral titer."
- Both the pharmacological endpoint and the clinical endpoint are included in the co-primary endpoints in order to evaluate the efficacy of ensitrelvir based on its antiviral effect and to ensure that antiviral effect is reflected in clinical efficacy.

PMDA's view:

The applicant's explanation about conducting the study as a global study is acceptable. Appropriateness of the primary endpoints is discussed in Section 7.R.2. The phase IIb part was added after the start of study; making such a major change in the midst of conducting the confirmatory study was inappropriate. However, given the changes in the epidemic situation of COVID-19, the change of the study protocol is acceptable to a certain extent. Also, since the change was done under the double-blind conditions, it is acceptable to evaluate the study results after the change.

7.R.2 Efficacy

The applicant's explanation about the efficacy of ensitrelvir:

In the phase IIa part of the global phase II/III study (Study T1221), regarding the primary endpoint "change in SARS-CoV-2 viral titer from baseline to each time point (nasal swab sample, mITT population[60)])," the mean change on Day 4 was 0.88 $\log_{10}$ $TCID_{50}$/mL in the ensitrelvir 375/125 mg group and 1.27 $\log_{10}$ $TCID_{50}$/mL in the ensitrelvir 750/250 group. The ensitrelvir groups showed

[59)] *JAMA*. 2022;327:1236-46

[60)] Randomized subjects who met both of the following criteria: (a) Tested positive by RT-PCR on the nasal swab sample on the first day of study treatment (before administration) and (b) SARS-CoV-2 virus titer was detected in the baseline nasal swab sample.

greater changes in the viral titer than the placebo group, demonstrating the tendency of decrease in the viral titer [see Section 7.1.1].

In the phase IIb part of the global phase II/III study (Study T1221), the following co-primary endpoints were used:

- Change per unit time in the total score of 12 COVID-19 symptoms from the start of study treatment (Day 1) to 120 hours (Day 6)
- Change from baseline to Day 4 in SARS-CoV-2 viral titer (ITT1 population[61])

Results showed a statistically significant difference in the viral titer between the ensitrelvir 375/125 mg group and the placebo group, and between the ensitrelvir 750/250 mg group and the placebo group, albeit not adjusted for the multiplicity of hypothesis testing. In both ensitrelvir groups, the change (least squares mean) was greater than the change in the placebo group by 0.41 $\log_{10}$ $TCID_{50}$/mL, showing a tendency of decrease in viral titer. On the other hand, the change in the total score of 12 COVID-19 symptoms showed no significant difference between the placebo group and either of the ensitrelvir groups, failing to achieve the primary objective of the phase IIb part.

The "change per unit time in scores of 12 COVID-19 symptoms by symptom category[62] from the start of study treatment (Day 1) to 120 hours (Day 6)" is a secondary endpoint of the phase IIb part of the global phase II/III study (Study T1221). Table 38 shows results of a symptom category, namely the respiratory symptoms unique to the Omicron variant (stuffy or runny nose, sore throat, cough, and shortness of breath [dyspnea]). Both the ensitrelvir 375/125 mg and 750/250 mg groups showed a statistically significant difference from the placebo group in the change in the respiratory symptoms, albeit not adjusted for multiplicity of hypothesis testing (P = 0.0153 and 0.0033, respectively, for the ensitrelvir 375/125 mg and 750/250 mg groups [2-sided]).

Table 38. Change per unit time in respiratory symptom scores from the start of study treatment (Day 1) to 120 hours (Day 6) (phase IIb part: ITT1 population)

	Ensitrelvir 375/125 mg (n = 114)	Ensitrelvir 750/250 mg (n = 116)	Placebo (n = 111)
Change per unit time in respiratory symptom scores (stuffy or runny nose, sore throat, cough, shortness of breath [dyspnea]) from the start of study treatment (Day 1) to 120 hours (Day 6)	−2.28 ± 1.54 (n = 106)[b]	−2.33 ± 1.58 (n = 111)[b]	−1.67 ± 1.44 (n = 109)[b]
Difference from placebo [95% CI] [a]	−0.37 [−0.67, −0.07]	−0.44 [−0.74, −0.15]	-

Mean ± SD

a) ANCOVA model with covariates of (a) total score of 12 symptoms at baseline, (b) time from the onset of COVID-19 until randomization (<72 hours, ≥72 hours), (c) and history of vaccination against SARS-CoV-2 (yes, no).

b) Subjects without scores at baseline or after the start of treatment were excluded from analysis.

In order to assess the effect of ensitrelvir to improve clinical symptoms characteristic to the Omicron variant, a post-hoc analysis was conducted on the change per unit time (mean ± SD) in the following 5 symptoms from the start of the study treatment (Day 1) to 120 hours (Day 6):

61) Randomized subjects with a baseline nasal swab sample in which SARS-CoV-2 virus titer was detected.

62) Symptoms were classified into the following symptom categories:
- Acute symptoms: Sore throat, cough, feverish or pyrexia
- Main clinical symptoms: Stuffy or runny nose, sore throat, cough, chills or sweating, feverish or pyrexia
- Respiratory symptoms: Stuffy or runny nose, sore throat, cough, shortness of breath (dyspnea)
- Systemic symptoms: Malaise (tiredness), muscle or body aches, headache, chills or sweating, feverish or pyrexia
- Gastrointestinal symptoms: Nausea, vomiting, diarrhea

- Among 12 symptoms of COVID-19, those with a mean baseline score of ≥1 (stuffy or runny nose, sore throat, cough, feverish or pyrexia).
- Shortness of breath (dyspnea), which is one of the parameters for severity of COVID-19.

The change was −3.17 ± 1.79 in the ensitrelvir 375/125 mg group, −3.26 ± 1.81 in the ensitrelvir 750/250 mg group, and −2.49 ± 1.66 in the placebo group. The least squares mean [95% CI] of the difference between the ensitrelvir 375/125 mg group and the placebo group was −0.40 [−0.73, -0.07], and that between the ensitrelvir 750/250 mg group and the placebo group was −0.48 [−0.80, −0.15]. These results showed a statistically significant difference between the placebo group and each of the ensitrelvir groups (P = 0.0164 and 0.0039, respectively, for the ensitrelvir 375/125 mg and 750/250 mg groups, 2-sided), although not adjusted for the multiplicity of hypothesis testing.

In addition, in both the phase IIa and phase IIb parts, the mean decrease in the level of viral RNA (RT-PCR) from baseline to Day 4 tended to be greater by approximately 1 $\log_{10}$ (copies/mL) in the ensitrelvir group than in the placebo group.

Based on the collective assessment of the above findings, the applicant considers that ensitrelvir has demonstrated its antiviral effect against SARS-CoV-2 and the effect to improve clinical symptoms.

PMDA's view on the results of phase IIa and phase IIb parts of the global phase II/III study (Study T1221):

- The results of the phase IIb part did not meet the success criteria for the prespecified primary efficacy endpoints of the study. In the phase IIb part, co-primary endpoints consisting of 2 primary endpoints were used but multiplicity adjustment for hypothesis testing of the 2 endpoints was not planned. Accordingly, since the statistically significant difference was not demonstrated in "change per unit time in the total score of 12 COVID-19 symptoms from the start of study treatment (Day 1) to 120 hours (Day 6)," it is impossible to evaluate the statistically significant difference in "change from baseline to Day 4 in SARS-CoV-2 viral titer."
- As for the ensitrelvir's effect to improve clinical symptoms, "change per unit time in the total score of 12 COVID-19 symptoms[57)] (AUC divided by 120 hours)" was used as one of the primary endpoints of the phase IIb part. However, this may lead to inconsistency between the evaluation results and the outcome at the last evaluation time point. The significance of evaluating the time-course of symptoms scores by AUC is therefore unclear. The time-course of the total score of 12 symptoms was generally similar between the ensitrelvir groups and the placebo group [see Figure 5 in Section 7.1.2].
- The applicant explained that the change per unit time in the following scores was greater in the ensitrelvir groups than in the placebo group:
    (a) Total score of respiratory symptoms (stuffy or runny nose, sore throat, cough, shortness of breath (dyspnea), which are considered to be characteristic to the Omicron variant among 12 symptoms of COVID-19; and
    (b) Total score of 5 symptoms consisting of symptoms with a mean baseline score of ≥1 (stuffy or runny nose, sore throat, cough, feverish or pyrexia) and shortness of breath (dyspnea), which is one of the parameters for severity of COVID-19.

However, PMDA cannot conclude that ensitrelvir showed the clinical symptom-improving effect, for the following reasons.

- Since COVID-19 causes a variety of symptoms, there are limitations to interpreting the clinical symptom-improving effect from results of only a part of symptom scores.
- The change per unit time tended to be greater in the ensitrelvir groups than in the placebo group. However, since the estimated between-group differences were less than 1, the minimal unit for each symptom score, the results cannot be interpreted as showing a significant between-group difference in these symptoms.

- No clear difference was observed between the ensitrelvir groups and the placebo group in any of the secondary endpoints related to clinical symptoms.[63)]
- The applicant explained that, in both phase IIa and phase IIb parts, the mean decrease in viral titer from baseline to Day 4 was greater in the ensitrelvir groups than in the placebo group. It is important to confirm in clinical studies that the viral RNA level and viral titer in the ensitrelvir groups decrease to lower levels (or decrease sooner) than those in the placebo group. However, evaluating the clinical significance of the viral titer decrease is difficult, for the following reasons:
  - In COVID-19, the viral titer usually decreases within a relatively short period even under the natural course.
  - A relative decrease in viral titer compared with placebo may be interpreted as the significant clinical effect of a drug, but the assessment of viral titer depends on multiple conditions: (a) viral strain (variant), (b) sampling site and method and the analytical method, (c) timing of measurement, (d) the extent of difference in viral titer between the drug group and the placebo group. Nevertheless, no sufficient data have been collected to identify the optimal conditions under which a relative decrease in viral titer compared with placebo can be interpreted as the significant clinical effect of the drug. For similar reasons, there are limitations to a simple comparison of a virus decrease between ensitrelvir and other drugs because data on other drugs are from clinical studies conducted at a different timing under different conditions for viral measurement.
  - As described above, no clinically significant changes in clinical symptoms associated with decreased viral titer was confirmed. According to the discussions by the regulatory agencies of other countries, evaluation of an anti-COVID-19 drug should focus on whether the drug has demonstrated clinically meaningful effects in survival or patient function.[64)]

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63) Secondary endpoints related to clinical symptoms in phase IIb part of the global phase II/III study (Study T1221):
- Percentage of subjects who had a score of ≥1, ≥2, ≥3, ≥4, ≥5, ≥6, or 7 on an 8-Point Ordinal Scale at each time point and during the follow-up evaluation period.
- Time to achieve a score of ≥1, ≥2, ≥3, ≥4, ≥5, ≥6, or 7 on an 8-Point Ordinal Scale.
- SpO_2 at each time point
- Change in EQ-5D-5L from baseline
- Time to recovery from COVID-19 symptoms
- Time to recovery from COVID-19 symptoms (duration of recovered state ≥72 h)
- Time to recovery from COVID-19 symptoms (duration of recovered state ≥120 h)
- Time to recovery from each COVID-19 symptom
- Change in total score of COVID-19 symptoms from baseline to each time point
- Percentage of subjects who recovered from symptoms and each symptom of COVID-19 at each time point
- Percentage of subjects with taste abnormality or dysosmia at each time point
- Time to recovery to normal temperature

64) ICMRA COVID-19 Treatments and Clinical Trials Workshop: http://www.icmra.info/drupal/news/20july2020/summary (last accessed on June 2, 2022)

PMDA's conclusion based on the above view:
PMDA does not deny that the data from phase IIa and phase IIb parts of the global phase II/III study (Study T1221) show a decreasing trend in the viral load in subjects treated with ensitrelvir, but cannot conclude that ensitrelvir is presumed to have efficacy for the proposed indication. The efficacy should be further evaluated based on the results of the phase III part of Study T1221 and other data. This is PMDA's efficacy assessment, but it is also acceptable to discuss whether ensitrelvir be authorized for expedited use from medical and social standpoints. If ensitrelvir is approved based on the currently available data etc., its efficacy should be re-evaluated based on the phase III part of the global phase II/III study (Study T1221). Depending on the results of the re-evaluation, appropriate actions should be taken, including considering the withdrawal of marketing approval.

This conclusion by PMDA will be discussed at the Expert Discussion.

7.R.3 Safety

The applicant's explanation about the safety profiles of ensitrelvir:
Table 39 shows the summary of safety data from the phase IIa and IIb parts of the global phase II/III study (Study T1221).

Table 39. Summary of safety in global phase II/III study (Study T1221) (safety analysis population)

	Phase IIa part			Phase IIb part		
	Ensitrelvir 375/215 mg (N = 21)	Ensitrelvir 750/250 mg (N = 23)	Placebo (N = 24)	Ensitrelvir 375/215 mg (N = 140)	Ensitrelvir 750/250 mg (N = 140)	Placebo (N = 141)
Adverse events	11 (52.4)	16 (69.6)	9 (37.5)	48 (34.3)	60 (42.9)	44 (31.2)
Adverse reactions	5 (23.8)	10 (43.5)	0	19 (13.6)	31 (22.1)	7 (5.0)
Serious adverse events	0	0	0	0	0	2 (1.4)
Adverse events resulting in death	0	0	0	0	0	0
Adverse events leading to treatment discontinuation	0	0	0	2 (1.4)	0	0

n (%)

The incidences of adverse events and adverse reactions tended to be higher in the ensitrelvir groups than in the placebo group, and in the ensitrelvir 750/250 mg group than in the ensitrelvir 375/125 mg group. Neither serious adverse events nor adverse events resulting in death were observed in the ensitrelvir groups. In the ensitrelvir 375/125 mg group of the phase IIb part, adverse events leading to treatment discontinuation occurred in 2 subjects (eczema, nausea, and headache in 1 subject each [1 subject had 2 events]). All of the events were considered related to the study drug, with the outcome of "recovered."

The safety analysis population included the following number of subjects <20 or ≥65 years old:

<20 years old: Phase IIa part: 1 in the ensitrelvir 375/125 mg group, none in the ensitrelvir 750/250 mg group, and 1 in the placebo group
Phase IIb part: 7 in the ensitrelvir 375/125 mg group, 7 in the ensitrelvir 750/250 mg group, and 4 in the placebo group

≥65 years old: Phase IIa part: none
Phase IIb part: 1 in the ensitrelvir 375/125 mg group, 2 in the ensitrelvir 750/250 mg group, and 1 in the placebo group

There were no safety concerns unique to pediatric or elderly subjects.

Although the safety data on ensitrelvir in patients are limited, no particular safety concerns have been identified. The applicant thus considers that the safety profile of ensitrelvir is tolerable.

PMDA's view:
PMDA confirmed that the ensitrelvir groups had no serious adverse events or adverse events resulting in death in the phase IIa and IIb parts of the global phase II/III study (Study T1221). However, the incidences of adverse events and adverse reactions tended to be higher in the ensitrelvir groups than in the placebo group, and especially high density lipoprotein decreased (a decrease in HDL cholesterol) occurred frequently in the ensitrelvir groups (Table 40). Also, the incidence of other lipid-related events in the ensitrelvir 750/250 mg group tended to be higher than that in the placebo group. All lipid-related events were nonserious. The outcome of these events was "recovered, " except in the following subjects:

- Five subjects with "not recovered" due to failure to retrieve Day-14 and Day-28 samples, consisting of 3 in the ensitrelvir 750/250 mg group (high density lipoprotein decreased in 3, blood triglycerides increased in 1 [1 subject had 2 events]) and 2 in the placebo group (high density lipoprotein decreased in 2)
- One subject with "recovering" (blood triglycerides increased in 1 subject in the ensitrelvir 750/250 mg group).

Accordingly, these lipid-related events are unlikely to pose any significant clinical concerns at the current moment. However, if ensitrelvir is approved, the package insert should include a precautionary statement regarding "high density lipoprotein decreased," which occurred more frequently in the 375/125 mg group (the proposed dosage) than in the placebo group.

Table 40. Lipid-related adverse events observed in ≥1 subject in the ensitrelvir group in global phase II/III study (Study T1221) (safety analysis population)

	Event	Adverse events			Adverse reactions		
		Ensitrelvir 375/125 mg (N = 21)	Ensitrelvir 750/250 mg (N = 23)	Placebo (N = 24)	Ensitrelvir 375/125 mg (N = 21)	Ensitrelvir 750/250 mg (N = 23)	Placebo (N = 24)
Phase IIa part	All events [a)]	11 (52.4)	16 (69.6)	9 (37.5)	5 (23.8)	10 (43.5)	0
	High density lipoprotein decreased	3 (14.3)	12 (52.2)	2 (8.3)	3 (14.3)	8 (34.8)	0
	Blood triglycerides increased	0	3 (13.0)	0	0	2 (8.7)	0
	Blood cholesterol decreased	0	1 (4.3)	0	0	0	0
	Event	Adverse events			Adverse reactions		
		Ensitrelvir 375/125 mg (N = 140)	Ensitrelvir 750/250 mg (N = 140)	Placebo (N = 141)	Ensitrelvir 375/125 mg (N = 140)	Ensitrelvir 750/250 mg (N = 140)	Placebo (N = 141)
Phase IIb part	All events [a)]	48 (34.3)	60 (42.9)	44 (31.2)	19 (13.6)	31 (22.1)	7 (5.0)
	High density lipoprotein decreased	31 (22.1)	40 (28.6)	5 (3.5)	13 (9.3)	22 (15.7)	0
	Blood triglycerides increased	1 (0.7)	9 (6.4)	1 (0.7)	1 (0.7)	1 (0.7)	0
	Low density lipoprotein increased	1 (0.7)	0	0	0	0	0
	Dyslipidaemia	0	3 (2.1)	0	0	3 (2.1)	0
	Blood cholesterol decreased	0	1 (0.7)	0	0	0	0

n (%), MedDRA ver.24.0
a) All events regardless of relationship to lipids

The following findings and pharmacological effects were observed in nonclinical studies: decreased blood cholesterol, decreased erythrocyte parameters, increased blood bilirubin, prolonged blood coagulation time and easy bleeding, inhibition of adenosine uptake [see Sections 5.R.1 to 3 and 5, and 3.R.3]. There have been no serious adverse events related to these findings or pharmacological effects that raise immediate safety concerns to date. However, because of the limited use experience with ensitrelvir in patients, attentions should be paid to the incidences of related events.

Results of phase IIa and IIb parts of the global phase II/III study (Study T1221) do not show any significant safety concerns with ensitrelvir, showing a certain level of tolerability of ensitrelvir. However, because the use experience with ensitrelvir in patients with COVID-19 is limited, new safety concerns may arise when ensitrelvir is used in many patients after the market launch. Safety of ensitrelvir should be further investigated, including the assessment of data to be obtained from the ongoing phase IIb/III part and phase III part of the global phase II/III study (Study T1221).

If ensitrelvir is approved, the following actions should be taken based on the currently available data:

- Findings suggestive of fetal malformation were observed in nonclinical studies, indicating potential teratogenic risks with ensitrelvir. Therefore, ensitrelvir should be contraindicated in pregnant or possibly pregnant women [see Section 5.R.6].
- Ensitrelvir may possibly interact with other drugs, as demonstrated by CYP3A-inhibitory effect of ensitrelvir. Appropriate precaution should be given [see Section 6.R.6].
- Only 324 patients received ensitrelvir in the phase IIa and IIb parts of the global phase II/III study (Study T1221). A system should be established to ensure collection of safety data [see Section 7.R.6].

This conclusion by PMDA will be discussed at the Expert Discussion.

7.R.4 Clinical positioning and indication

The applicant's explanation about the clinical positioning of ensitrelvir:

The global phase II/III study (Study T1221) was conducted in asymptomatic SARS-CoV-2 carriers and patients with COVID-19 not requiring oxygen therapy. According to the results of phase IIa and IIb parts of Study T1221, ensitrelvir had antiviral effect against SARS-CoV-2 and improved symptoms of COVID-19 [see Section 7.R.2]. No particular safety concerns were observed in the treatment with ensitrelvir [see Section 7.R.3]. These results suggest that starting treatment with ensitrelvir at an early stage after SARS-CoV-2 infection will rapidly suppress viral replication, improve clinical symptoms by inhibiting excessive inflammation and immune reaction caused by viral infection, and reduce the quarantine period of hospitalization or recuperation, thereby relieving the burden to patients and to already tight healthcare resources. In the phase IIa and phase IIb parts of the study, approximately 80% of subjects had received at least 1 dose of an anti-SARS-CoV-2 vaccine and, in the phase IIb part, approximately 30% of subjects had a risk factor(s) for severe COVID-19.[65)] These facts suggest that ensitrelvir offers a novel treatment option to be used widely in asymptomatic SARS-CoV-2 carriers and patients with COVID-19 not requiring oxygen therapy, regardless of history of vaccination or risk factors for severe COVID-19.

The proposed indication is COVID-19 (disease caused by SARS-CoV-2 infection).

PMDA's view:

PMDA cannot conclude that ensitrelvir is presumed to have efficacy based on the phase IIb part of the global phase II/III study (Study T1221) [see Section 7.R.2]. Accordingly, at the current moment, PMDA does not consider that ensitrelvir offers a treatment option for patients with COVID-19.

If ensitrelvir is approved based on the currently available information, as with the other approved oral drugs, ensitrelvir should be indicated only for patients with COVID-19 or at risk of severe COVID-19 who require pharmacotherapy but cannot use other therapeutic agents.

This conclusion by PMDA will be discussed at the Expert Discussion.

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65) Main risk factors for severe COVID-19 in the subjects (ITT1 population):
- Smoking (23.7% [27 of 114] in the ensitrelvir 375/125 mg group, 17.2% [20 of 116] in the ensitrelvir 750/250 mg group, 27.0% [30 of 111] in the placebo group)
- Dyslipidaemia (8.8% [10 of 114] in the ensitrelvir 375/125 mg group, 6.9% [8 of 116] in the ensitrelvir 750/250 mg group, 11.7% [13 of 111] in the placebo group)
- Hypertension (7.9% [9 of 114] in the ensitrelvir 375/125 mg group, 7.8% [9 of 116] in the ensitrelvir 750/250 mg group, 11.7% [13 of 111] in the placebo group)
- BMI ≥30 kg/m^2 (6.1% [7 of 114] in the ensitrelvir 375/125 mg group, 6.0% [7 of 116] in the ensitrelvir 750/250 mg group, 8.1% [9 of 111] in the placebo group)
- Diabetes mellitus (4.4% [5 of 114] in the ensitrelvir 375/125 mg group, 0.9% [1 of 116] in the ensitrelvir 750/250 mg group, 5.4% (6 of 111] in the placebo group)

7.R.5 Dosage and administration

The applicant's rationale for the proposed dosage and administration of ensitrelvir:

Based on the evaluation of the results of nonclinical studies and the phase I study (Study T1211), the following dosage regimens were selected for the phase IIa and IIb parts of the global phase II/III study (Study T1221) [see Section 6.R.2]:

(a) Oral administration of ensitrelvir 375 mg once on Day 1 and ensitrelvir 125 mg once daily from Days 2 to 5.
(b) Oral administration of ensitrelvir 750 mg once on Day 1 and ensitrelvir 250 mg once daily from Days 2 to 5.

Both dosage regimens showed antiviral activity against SARS-CoV-2 and improved clinical symptoms, without any particular safety concerns [see Sections 7.R.2 and 7.R.3].

Since ensitrelvir 750/250 mg has a potent inhibitory effect against CYP3A [see Section 6.R.6.2.1], the applicant has proposed ensitrelvir 375/125 mg for the dosage and administration because it may make risk control easier.

Based on the investigation of clinical pharmacology [see Section 6.R.3], the same dosage should be used in pediatric patients ≥12 years old as in adults.

PMDA's view:

If ensitrelvir is approved, the dosage and administration in pediatric patients ≥12 years old and adult patients should be as follows: Oral administration of ensitrelvir 375 mg once on Day 1 and ensitrelvir 125 mg once daily from Days 2 to 5.

This conclusion by PMDA will be discussed at the Expert Discussion.

7.R.6 Post-marketing investigations

The applicant plans to conduct, after the market launch, a specified use-results survey (target sample size, 300) to confirm the safety of ensitrelvir in elderly patients.

PMDA's view:

Because of the limited use experience with ensitrelvir in patients with COVID-19, a use-results survey should be conducted in patients not limited to the elderly after the approval of ensitrelvir.

This conclusion by PMDA will be discussed at the Expert Discussion.

8. Results of Compliance Assessment Concerning the New Drug Application Data and Conclusion Reached by PMDA

8.1 PMDA's conclusion concerning the results of document-based GLP/GCP inspections and data integrity assessment

The inspection is currently ongoing. Results and the conclusion of PMDA are reported in Review Report (2).

8.2 PMDA's conclusion concerning the results of the on-site GCP inspection

The inspection is currently ongoing. Results and the conclusion of PMDA are reported in Review Report (2).

9. Overall Evaluation during Preparation of the Review Report (1)

PMDA does not deny that ensitrelvir tended to reduce viral load, as shown by results from phase IIa and IIb parts of the global phase II/III study (Study T1221), but cannot conclude that ensitrelvir is presumed to have efficacy for the proposed indication. Therefore, further investigation is needed based on data including results from phase III part of the study. This is PMDA's conclusion regarding the efficacy of ensitrelvir. Nevertheless, from a medical and social point of view, it is also acceptable to consider making ensitrelvir available early. If ensitrelvir is approved based on the data currently available, its efficacy should be re-evaluated after the approval based on results from phase III part of the global phase II/III study (Study T1221) and, depending on the results, appropriate actions should be taken including considering the withdrawal of marketing approval.

As for safety, if ensitrelvir is approved, the package insert should include appropriate precautionary statements regarding potential teratogenic risks, drug interactions, and others. Use experience with ensitrelvir in patients with COVID-19 is limited, and therefore new safety concerns may arise when ensitrelvir is used in many patients after the market launch. Safety data should therefore be collected after the market launch and new findings should be provided appropriately to healthcare professionals.

The applicant submitted their opinions on the Review Report (1) (see Addendum).

Addendum

The applicant's opinions on the review report (1).

Matters related to Section "7.R.2 Efficacy"

In addition to the description in Section "7.R.2 Efficacy," the applicant considers the efficacy of ensitrelvir as follows.

In the first place, the failure to demonstrate a significant difference in the change in the total score of 12 COVID-19 symptoms may be due to the change of prevalent strains during the study period. This endpoint was selected based on the results of the phase IIa part, which was conducted when the Delta variant was dominant, but the Omicron variant became dominant when the phase IIb part was conducted.

Of the 12 symptoms of COVID-19, those with a mean baseline score of ≥1 (mild) were stuffy or runny nose, sore throat, cough, and feverish or pyrexia, which were consistent with symptoms unique to the Omicron variant.[66)]

On the other hand, other systemic symptoms and gastrointestinal symptoms were barely detectable or very mild with a mean baseline score of <1 (mild); therefore detecting changes in these clinical symptoms in a sensitive and appropriate manner was difficult. Since the pre-defined 12 symptoms of COVID-19 included these symptoms, it was difficult to detect the symptom-improving effect of ensitrelvir and to confirm the statistically significant difference in the 12 symptoms.

In the development of therapeutic agents against COVID-19, drug developers are required to confirm and demonstrate the efficacy of an antiviral agent in the midst of various changes, including alteration of prevalent strains due to frequent viral mutations, acquisition of herd immunity through vaccination, and resulting changes in the characteristics of the population evaluated in clinical studies and in the healthcare delivery system and public health system. For these reasons, the applicant considered that the following were more important than showing the superiority of ensitrelvir to placebo in pre-determined endpoints: (a) confirming the virologically meaningful antiviral effect against the strain prevalent during the study period, and (b) evaluating the symptom-improving effect in a manner tailored to the characteristics of the prevalent strain. In particular, the characteristics of the Omicron variant are very different from those of the original strain, making it difficult to use the same endpoints.

A foreign regulatory agency recommends that time to disappearance of symptoms be evaluated,[67)] [redacted]. In response, the applicant defined "time to resolution of COVID-19

66) National Institute of Infectious Diseases: Active epidemiological investigation on SARS-CoV-2 infection caused by the Omicron variant (lineage B.1.1.529) (Report No. 5): Epidemiological and clinical characteristics (February 18, 2022): https://www.niid.go.jp/niid/ja/2019-ncov/2484-idsc/10969-covid19-72.html

67) FDA: COVID-19: Developing Drugs and Biological Products for Treatment or Prevention. Guidance for Industry. February 2021

symptoms," which expresses the time required for the scores of all 12 symptoms[68] to return to the level before the disease onset or to null,[69] and conducted additional post-hoc analysis of data from the phase IIb part. Results showed that "median time to resolution of COVID-19 symptoms" in the ITT1 population of phase IIb part was shortened by 74 hours (approximately 3 days) in both the ensitrelvir 375/125 mg and 750/250 mg groups, compared with the placebo group (P = 0.0939 in the ensitrelvir 375/125 mg group, P = 0.0406 in the ensitrelvir 750/250 mg group by stratified log-rank test). The applicant thus confirmed that ensitrelvir clearly reduced the time to disappearance of 12 symptoms of COVID-19, but a large-scale clinical study is necessary for statistically confirming this result. The applicant is currently conducting the phase III part with the primary endpoint of "time to resolution of COVID-19 symptoms."

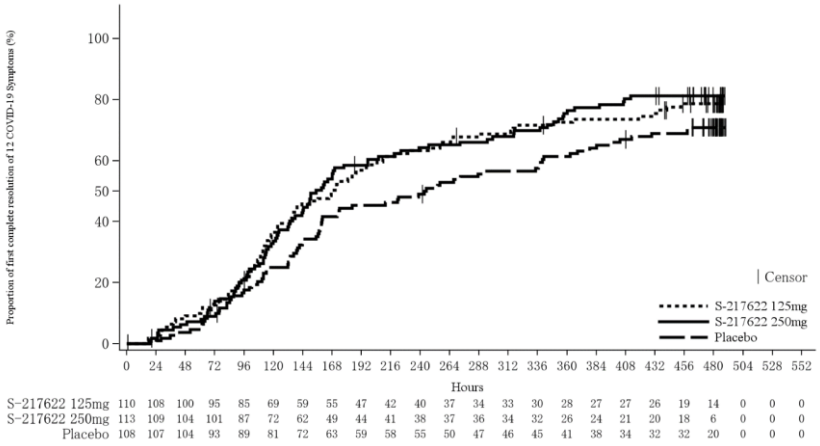

Figure. Kaplan-Meier curves of time to resolution of 12 symptoms of COVID-19 (phase IIb part: ITT1 population)

In foreign countries, a re-increase of viral RNA load and relapse of symptoms 10 to 14 days after the start of treatment with an anti-COVID-19 drug has become problems.[70][71] In contrast, in the phase IIa part of the global phase II/III study, the re-increase of viral titer or viral RNA load was not observed in subjects receiving ensitrelvir who had SARS-CoV-2 with amino acid substitutions. In the phase IIb part of the study as well, neither a clear re-increase in viral titer or viral RNA load nor a relapse of symptoms was observed in the ensitrelvir groups compared to the placebo group. Based on these findings and taking account of the PMDA's view in Section "7.R.3 Efficacy," the applicant's view is as follows:

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68) Each of the following 12 symptoms of COVID-19 was scored by the subject on a 4-point scale (0, none; 1, mild; 2, moderate; 3, severe): (1) malaise or tiredness, (2) muscle or body aches, (3) headache, (4) chills or sweating, (5) feverish or pyrexia, (6) stuffy or runny nose, (7) sore throat, (8) cough, (9) shortness of breath (dyspnea), (10) nausea, (11) vomiting, (12) diarrhea.

69) "Resolution of COVID-19 symptoms" refers to the time point when all 12 symptoms of COVID-19 have disappeared, improved, or remained as shown below from the start of study treatment. When such conditions continued for at least 24 hours, it is concluded that the subject has achieved the endpoint.
- Symptoms that had been present before the onset of COVID-19 and were considered by the subject to have worsened at baseline (at test before administration) must improve from, or persist at, the severity at the baseline.
- Symptoms that had been present before the onset of COVID-19 and were NOT considered by the subject to have worsened at baseline (at test before administration) must persist at, or improve from, the severity at the baseline.
- Other symptoms (i.e., symptoms that were not present before the onset of COVID-19 and developed after baseline [test before administration]) must disappear completely.

70) FDA: Emergency Use Authorization for Paxlovid Center for Drug Evaluation and Research Review. 22 December 2021

71) Preprint doi: https://doi.org/10.21203/rs.3.rs-1588371/v3

The phase IIa part of the global phase II/III study (Study T1221) was conducted when the Delta variant was dominant, whereas the phase IIb part was conducted when the Omicron variant was dominant. In the phase IIa part of the study conducted during the dominance of the Delta variant, the applicant confirmed a statistically significant decrease in both viral titer and viral RNA load, and also confirmed that the total score of 12 symptoms (major characteristic symptoms of COVID-19 before the prevalence of the Omicron variant) decreased rapidly during the 120 hours after the start of ensitrelvir therapy, compared with the decrease after placebo administration. On the other hand, it was difficult in this study to statistically assess the efficacy of ensitrelvir using the endpoints of "hospitalization rate and mortality rate in patients with risk factors for severe COVID-19" (a criterion for emergency use authorization employed in foreign countries), because phase IIa and IIb parts enrolled patients regardless of risk factors for severe COVID-19 or history of vaccination. Under the situations where most of the people with risk factors have completed vaccination, it is difficult not only in Japan but also in other counties to demonstrate a decrease in hospitalization rate and mortality rate in a statistically significant manner in unvaccinated patients with risk factors for severe COVID-19. These situations necessitated use of surrogate endpoints. Since a quick recovery from moderate or severe symptoms was considered important for reducing the patient's burden, the phase IIb part used the following primary clinical endpoint to evaluate the symptom-improving effect associated with early virus reduction, in view of data from phase IIa part, which was conducted when the Delta variant was dominant:

> "Change per unit time in the total score of 12 COVID-19 symptoms from the start of administration to 120 hours."

In the phase IIb part, as it turned out, the dominant strain changed to the Omicron variant with characteristics substantially different from the original strain. This resulted in the failure to demonstrate the efficacy based on the 12 symptoms, the endpoint established based on the data from the phase IIa part. On the other hand, a statistically significant difference was observed between the ensitrelvir groups and the placebo group in 4 respiratory symptoms (which are reported to be characteristic to the Omicron variant) and 5 symptoms (consisting of the 4 respiratory symptoms and pyrexia), when expressed as change per unit time in the total score of 12 COVID-19 symptoms from the start of administration to 120 hours. By the way, AUC (change per unit time multiplied by 120 hours), which is also a parameter expressing the change per unit time of the total score of 12 symptoms, is an endpoint used in other companies' clinical studies regarding COVID-19 and RSV infection. This endpoint is now being used increasingly for evaluating the treatment effect of acute infections.[72)][73)] The phase IIb part used the primary endpoint of "change per unit time in the total score of 12 COVID-19 symptoms from the start of administration to 120 hours," in order to mainly evaluate rapid improvement in symptoms early after infection; the results suggested a certain degree of efficacy. Particularly, in patients infected with the Omicron variant, mild symptoms may persist and it may take time to achieve complete recovery. It is therefore important to evaluate the efficacy based on a clinical endpoint related to the disappearance of symptoms.

Accordingly, the applicant analyzed the time to disappearance of symptoms (a parameter recommended by foreign regulatory agencies) as the primary endpoint in global phase III study.

[72)] EDP-938, a Respiratory Syncytial Virus Inhibitor, in a Human Virus Challenge (abstract in Japanese) (*The New England Journal of Medicine: Japanese version*) https://www.nejm.jp/abstract/vol386.p655)

[73)] *Antivir Ther.* 2000;5:205-13

Results confirmed reduction in time to symptom disappearance, suggesting the clinical effect compared with placebo. In addition, Kaplan-Meier curves confirmed the difference in the disease duration between the ensitrelvir groups and the placebo group, with the difference becoming obvious at around 120 hours, and the difference trend persisted after 120 hours as well, suggesting that it is appropriate to evaluate the symptom scores at 120 hours post-dose.

In conclusion, evaluating the efficacy based on hospitalization rate or mortality rate is difficult, because the dominant Omicron variant has characteristics substantially different from those of the original strain,[66)] with a lower risk of causing severe COVID-19, and because the vaccination program resulted in the decreased rate of severe COVID-19 among those infected with SARS-CoV-2. Despite these difficulties, a tendency of improvement of symptoms was observed in patients infected with the Omicron variant, which was the dominant strain during the phase IIb part study. This means that ensitrelvir is presumed to have efficacy.

Matters related to Section "7.R.4 Clinical positioning and indication"

The applicant's opinions in response to the PMDA's view presented in Section "7.R.4 in Clinical positioning and indication":

It is difficult to evaluate the efficacy of a drug against COVID-19 based on the hospitalization rate and mortality rate because the rate of severe disease is low in patients infected with the Omicron variant and has been reduced by the vaccination program. There are substantial differences in clinical characteristics between the Omicron variant and the original strain.[66)] Therefore, evaluating symptoms characteristic to the Omicron variant is more important than evaluating the 12 symptoms selected based on the characteristics of the original strain.

The phase IIb part was conducted in patients regardless of vaccination status or risk factors for severe COVID-19, and the results showed a tendency of improvement in symptoms characteristics to infection by the Omicron variant. This finding is very clinically meaningful.

Also, the potent antiviral activity of ensitrelvir is expected to reduce the risk of long COVID, prevent severe COVID-19, suppress disease transmission, and thereby serve as a very useful drug from the public health standpoint, as detailed below:

- There are a certain number of patients who are suffering from long COVID despite disappearance of main symptoms of COVID-19. Even a single symptom of long COVID reduces QOL, causing social problems. The mechanism of long COVID is largely unknown but residual viruses, direct damage to virus-infected tissues, progression of inflammation due to insufficient immunoregulation after viral infection, etc., are supposed to be possible contributing factors.[74)] In the phase IIb part, the ensitrelvir groups had significantly lower scores of taste abnormality and dysosmia (known symptoms of long COVID) than the placebo group at multiple time points tested after the start of treatment; the percentage of subjects with taste abnormality or dysosmia was also lower in the ensitrelvir groups than in the placebo group. These results suggest that ensitrelvir not only

[74)] Guidelines for Diagnosis and Treatment of COVID 19; supplementary volume, Management of Long COVID (tentative edition) dated December 1, 2021 (in Japanese) by the Ministry of Health, Labour and Welfare.

improves symptoms occurring immediately after infection but also may reduce the risk of long COVID.

- The pathogenesis of COVID-19 during the early stage is inflammatory reaction due to viral replication.[75] For the treatment of COVID-19, it is beneficial to start treatment with an anti-viral drug at an early stage of SARS-CoV-2 infection, thereby promptly inhibiting viral replication and suppressing excessive inflammatory and immune reactions due to viral infection. It has been suggested that virus elimination at an early stage may lead to recovery from symptoms and prevention of severe COVID-19.[76] Results of the phase IIa and IIb parts of the global phase II/III study (Study T1221) demonstrated the superior antiviral effect of ensitrelvir. Therefore, viral elimination by ensitrelvir during the early stage is expected to lead to prevention of severe COVID-19.
- The Omicron variant is reported to be more contagious than the original strain,[77] and "breakthrough infection" after vaccination is now causing problems. Using a time-course model of viral load in infected people (index cases), viral load in index cases at the time of household contact that led to transmission, was predicted. The transmission rate increased with the increase in viral load in the index case at the time of contact.[78] This simulation shows that promptly reducing the viral load is expected to suppress viral transmission; this suggests that the antiviral effect of ensitrelvir is also expected to suppress viral transmission. Suppressing viral transmission is effective in reducing the risk of secondary infection in close contacts and of household transmission, thereby preventing the collapse of the healthcare system due to a growing number of infected patients. In addition, loss of labor power due to quarantine of infected people for a certain period is causing problems, and suppression of viral transmission is expected to reduce the quarantine period and to contribute to the infection control.

As expressed by the ICMRA statement, development of therapeutic agents for patients with COVID-19 of varying severity including initially mild to moderate disease, is expected to offer novel benefits.[79] On the other hand, despite the fact that most patients with COVID-19 have mild to moderate I symptoms (translator's note: In Japan, the severity of COVID-19 is classified into "mild," "moderate I," "moderate II," and "severe."), drugs and treatment options for these patients are limited. Some of the approved drugs are used in patients with mild to moderate I symptoms, but as a general rule they are used only in patients with risk factors for severe COVID-19. Moreover, some drugs are not recommended for patients infected with the Omicron variant because of their reduced efficacy against the variant. Thus, there are drugs for patients with mild to moderate I symptoms, which is the majority group requiring treatment in clinical practice, but these drugs are insufficient for the treatment of COVID-19 because their target patient populations are limited. In addition, the existing drugs were approved based on the results of clinical studies conducted in unvaccinated patients or during the epidemic of strains preceding the Omicron variant; whereas clinical evaluation of ensitrelvir was conducted not only during the Delta-dominant period (phase IIa part) but also during the Omicron-dominant period (phase IIb part). Ensitrelvir is thus the only drug with evidence that the efficacy was evaluated against the currently dominant strains.

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75) The Japanese Association for Infectious Diseases: Drug treatment for COVID-19, ver. 13.1
76) *Int J Infect Dis*. 2021;105:532-9
77) *Nature*. 2022;doi:10.1038/d41586-022-00632-3.
78) *Elife*. 2021;10:e69302
79) ICMRA COVID-19 Treatments and Clinical Trials Workshop: http://www.icmra.info/drupal/news/20july2020/summary

Many people have been vaccinated in Japan,[80] but there are limitations to the prevention of COVID-19 by vaccination for the following reasons:

(a) Breakthrough infection occurs among vaccinated people. [81][82]

(b) The vaccination rate appears to decrease with repeated booster vaccinations.

(c) A certain percentage of people avoid vaccination.

In addition to the above problems of vaccination, there are problems that the existing drugs cannot be used in some groups of patients and that even patients with mild symptoms may suffer from long COVID. In the face of the current medical needs and the next and future outbreaks of COVID-19, the applicant considers that providing more treatment options is important from the public health standpoint, in order to avoid extensive adverse impacts on people's lives and on national economy so that people can live with a sense of security.

Based on the above, the applicant considers that ensitrelvir can be used widely in asymptomatic SARS-CoV-2 carriers and in patients with COVID-19 not requiring oxygen therapy, regardless of history of vaccination or risk factors for severe COVID-19. After the Emergency Approval, all patients prescribed ensitrelvir will be controlled by the patient registration center. Through the center, the applicant will promptly collect and evaluate information on adverse reactions and other detailed information from all patients prescribed ensitrelvir during the early post-marketing phase vigilance, in order to ensure the safety of patients. The applicant will collect adverse reaction data through the early post-marketing phase vigilance, provide information every week to the Japanese regulatory agencies and to healthcare professionals. Preparations have been made to take safe measures if a severe adverse reaction occurs. Immediately after the completion of the phase III part ongoing mainly in Japan, the applicant will submit an application for the official approval of ensitrelvir. The applicant also conducts a global phase III study in the United States and other countries to confirm the efficacy against everchanging SARS-CoV-2 variants spreading worldwide. The applicant will continue to make efforts to analyze the efficacy and safety data, and will publish the data as soon as they become available. The applicant will discuss more appropriate clinical positioning of ensitrelvir with the Japanese regulatory agencies based on new data to be obtained and thereby contribute to healthcare policy against COVID-19 through developing and fostering of ensitrelvir.

80) Prime Minister's Office in Japan: COVID-19 Vaccines: https://www.kantei.go.jp/jp/headline/kansensho/vaccine.html

81) National Institute of Infectious Diseases: Findings obtained from COVID-19 cluster surveillance in medical and welfare institutions including breakthrough infection cases (abbreviated version), dated December 8, 2021 https://www.niid.go.jp/niid/ja/2019-ncov/10834-covid19-22.html

82) *Lancet*. 2022;399:625-6

Review Report (2)

June 17, 2022

Product Submitted for Approval

Brand Name	Xocova Tablets 125 mg
Non-proprietary Name	Ensitrelvir Fumaric Acid
Applicant	Shionogi & Co., Ltd.
Date of Application	February 25, 2022

List of Abbreviations
See Appendix.

1. Content of the Review

Comments made during the Expert Discussion and the subsequent review conducted by the Pharmaceuticals and Medical Devices Agency (PMDA) are summarized below. The expert advisors present during the Expert Discussion were nominated based on their declarations, etc., concerning the product submitted for marketing approval, in accordance with the provisions of the Rules for Convening Expert Discussions, etc., by Pharmaceuticals and Medical Devices Agency (PMDA Administrative Rule No. 8/2008 dated December 25, 2008).

At the Expert Discussion, the expert advisors supported PMDA's conclusion on issues described in Sections "7.R.3 Safety," "7.R.5 Dosage and Administration," and "7.R.6 Post-marketing investigations (use-result surveys)" in the Review Report (1), based on the Review Report (1) and its addendum.

PMDA conducted further reviews on the following points:

1.1 Efficacy

At the expert discussion, PMDA presented the following conclusion regarding Sections "7.R.2 Safety" and "7.R.4 Clinical positioning and indication" in the Review Report (1):

> PMDA cannot conclude that ensitrelvir is presumed to have the efficacy but, from a medical and social point of view, it is acceptable to consider making ensitrelvir available early.

Based on the Review Report (1) and its addendum, the Expert Advisors generally supported the PMDA's conclusion. However, an Expert Advisor commented that it cannot be completely denied that ensitrelvir is presumed to have efficacy. The following are main comments made by the Expert Advisors:

• There are limitations to the interpretation of the submitted data:

(a) It is difficult to evaluate symptom improvement based on the scores of some symptoms selected from numerous symptoms.

(b) The estimated between-group differences were less than 1, the minimal unit for each symptom score. This makes it difficult to evaluating the significance of the results.

However, it cannot be completely denied that ensitrelvir is presumed to have efficacy for the proposed indication, considering the following:

(a) The dominant SARS-CoV-2 strain has changed from the Delta variant to the Omicron variant, accompanied by changes in clinical symptoms.
(b) In the development of a drug against COVID-19, it is difficult to demonstrate the improvement of clinical symptoms caused by ever-changing SARS-CoV-2 strains. This should be taken into account.

- I do not deny the importance of making a drug available early to meet the medical and social needs. However, approving a drug without any evidence suggesting the clinical efficacy has a potential risk of not only providing an unnecessary treatment but also (a) robbing patients of a chance to receive other effective treatments or (b) making widely available a drug with minimal benefits compared with the risks. Approving such a drug might be a gross violation of the medical and social expectation. This should be taken into account.
- If ensitrelvir is approved with no evidence of efficacy, its use should be limited to patients requiring a therapeutic agent (e.g., those with risk factors for severe COVID-19) who cannot use other drugs because of contraindications or unavailability due to the limited supply, etc.

PMDA's view:

Under the Emergency Approval system, the efficacy of a drug can be evaluated based on the results of an exploratory clinical study without data from a confirming study. The presumption of the efficacy of a drug based on an exploratory clinical study means that the efficacy is evaluated using uncertain data in the exploratory phase. It is therefore important to obtain results that meet, or closely meet, the success criteria in an exploratory clinical study that has been appropriately planned and conducted. For example, the following conditions should be satisfied:

(a) The exploratory study should use the same primary endpoint employed in the confirmatory study.
(b) The efficacy should be suggested consistently by results of the primary and other endpoints consistently, or should be demonstrated by results of a parameter clearly positioned as a surrogate endpoint of the primary endpoint of the confirmatory study.

PMDA does not deny that ensitrelvir tended to reduce viral load, as shown by results from phase IIa and IIb parts of the global phase II/III study (Study T1221), but cannot conclude that ensitrelvir is presumed to have efficacy for the proposed indication. Therefore, further investigation is needed based on data including results from phase III part of the study. This is PMDA's conclusion regarding the efficacy of ensitrelvir. Based on the deliberations of the Expert Discussion, however, it is also acceptable to consider making ensitrelvir available early from a medical and social point of view (e.g., to ensure stable supply of drugs). If ensitrelvir is approved based on the data currently available, its efficacy should be re-evaluated after the approval based on results from phase III part of the global phase II/III study (Study T1221) and, depending on the results, appropriate actions should be taken including considering the withdrawal of marketing approval.

1.2 Risk management plan (draft)

Based on its review presented in the Review Report (1) and on the comments raised by the expert advisors at the Expert Discussion, PMDA has concluded that the following are needed, in addition to collection of data through usual safety vigilance activities:

- The risk management plan (draft) for ensitrelvir should include the safety and efficacy specifications presented in Table 41.
- The applicant should conduct additional pharmacovigilance activities, efficacy survey and studies, and additional risk minimization activities presented in Tables 42, 43, and 44.

Table 41. Safety and efficacy specifications in the risk management plan (draft)

Safety specification		
Important identified risks	Important potential risks	Important missing information
None	Teratogenicity	Safety in patients with moderate or severe hepatic impairment
Efficacy specification		
Efficacy in phase III part of global phase II/III study (Study T1221)		

Table 42. Summary of additional pharmacovigilance activities, efficacy survey and studies, and additional risk minimization activities included under the risk management plan (draft)

Additional pharmacovigilance activities	Efficacy survey and studies	Additional risk minimization activities
• Early post-marketing phase vigilance • Use-results survey • Post-marketing clinical study (clinical pharmacology study in subjects with hepatic impairment)	• Post-marketing clinical study (global phase II/III study [T1221])	• Provide information to patients and enhance their understanding before starting treatment (through an informed consent form and a brochure for patients) • Organize and disseminate information for healthcare professionals (regarding teratogenicity) • Disseminate data gathered during early post-marketing phase vigilance

Table 43. Summary of phase III part of global phase II/III study (Study T1221)

Study design	Randomized, double-blind, placebo-controlled, parallel-group study
Population	Patients with COVID-19
Dosage and administration	(a) Oral administration of ensitrelvir 375 mg once on Day 1 and ensitrelvir 125 mg once daily from Days 2 to 5 (b) Oral administration of ensitrelvir 750 mg once on Day 1 and ensitrelvir 250 mg once daily from Days 2 to 5 (c) Oral administration of placebo once daily for 5 days
Target sample size	1,785 (595 per group)
Primary endpoint	Time [b)] to resolution of symptoms [a)] of COVID-19

a) The following 12 symptoms of COVID-19 are evaluated by the subject on a 4-point scale (0, none; 1, mild; 2, moderate; 3, severe): (1) malaise or tiredness, (2) muscle or body aches, (3) headache, (4) chills or sweating, (5) feverish or pyrexia, (6) stuffy or runny nose, (7) sore throat, (8) cough, (9) shortness of breath (dyspnea), (10) nausea, (11) vomiting, and (12) diarrhea.

b) Resolution of COVID-19 is expressed by the period between the start of study treatment and the point when all symptoms meet the following criteria:
- Symptoms that had been present before the onset of COVID-19 and were considered by the subject to have worsened at baseline (at test before administration): Improved severity from baseline or the same severity as baseline (i.e., from severe to moderate or better, from moderate to mild or better, or from mild to mild or better) is maintained for 24 hours.
- Symptoms that had been present before the onset of COVID-19 and were NOT considered by the subject to have worsened at baseline (at test before administration): Improved severity from baseline or the same severity as baseline (i.e., from severe to severe or better, from moderate to moderate or better, or from mild to mild or better) is maintained for 24 hours.
- Other symptoms (i.e., symptoms that were not present before the onset of COVID-19 and developed after baseline [test before administration]): An asymptomatic condition is maintained for 24 hours.

Table 44. Outline of use-results survey (draft)

Objective	To evaluate the safety of ensitrelvir in routine clinical practice
Survey method	Survey including consecutive patients
Population	Patients treated with ensitrelvir
Observation period	14 days from the start of administration
Planned sample size	3,000

2. Results of Compliance Assessment Concerning the New Drug Application Data and Conclusion Reached by PMDA

2.1 PMDA's conclusion concerning the results of document-based GLP/GCP inspections and data integrity assessment

The inspection is currently ongoing. Results and the conclusion of PMDA will be reported separately.

2.2 PMDA's conclusion concerning the results of the on-site GCP inspection

The inspection is currently ongoing. Results and the conclusion of PMDA will be reported separately.

3. Overall Evaluation

As a result of the above review, PMDA does not deny that ensitrelvir tended to reduce viral load, as shown by results from phase IIa and IIb parts of the global phase II/III study (Study T1221), but cannot conclude that ensitrelvir is presumed to have efficacy for the proposed indication. Therefore, further investigation is needed based on data including results from phase III part of the study. This is PMDA's conclusion regarding the efficacy of ensitrelvir. Nevertheless, from a medical and social point of view, it is also acceptable to consider making ensitrelvir available early. If ensitrelvir is approved based on the data currently available, its efficacy should be re-evaluated after the approval based on results from phase III part of the global phase II/III study (Study T1221) and, depending on the results, appropriate actions should be taken including considering the withdrawal of marketing approval. The applicant plans to submit the clinical study report of the phase III part of the global phase II/III study (Study T1221) in November 2022. The effective period of the Emergency Approval should be 1 year in accordance with Article 14-2-2, Paragraph 1 of the Pharmaceuticals and Medical Devices Act (Act No. 145, 1960)."

If the product is approved, the proposed indication and dosage and administration should be as follows, with the following approval conditions. The product is not classified as a biological product or a specified biological product. Both the drug product and its drug substance are classified as powerful drugs.

Indication

Disease caused by SARS-CoV-2 infection (COVID-19)

(No change)

Dosage and Administration

The usual dose in ≥12-year-old pediatric patients and adults is ensitrelvir 375 mg on Day 1 and ensitrelvir 125 mg from Days 2 to 5, administered orally once daily~~, for a total of 5 days~~.

(The underlined words were added to the proposed dosage, and strike-through denotes deleted text.)

Approval Conditions

1. The applicant is required to develop and appropriately implement a risk management plan.
2. The applicant is required to request that physicians administer the product only to patients considered eligible for treatment with the product who, or whose legally acceptable representatives, have been provided with the efficacy and safety information of the product in written form, and have provided written informed consent before the start of treatment.
3. The applicant is required to submit data from phase III part of the global phase II/III study (Study T1221) promptly after the study completion.

Appendix

List of Abbreviations

3CL	3C-like
A/G	Albumin/Globulin ratio
ACE	Angiotensin-converting enzyme
ACh	Acetylcholine
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
APTT	Activated partial thromboplastin time
AST	Aspartate aminotransferase
ATP	Adenosine tri-phosphate
AUC	Area under the concentration versus time curve
$AUC_{0\text{-}t\ h}$	Area under the concentration-time curve from time 0 to t hours post-dose
AUC_{inf}	Area under the concentration-time curve from time 0 to infinity
AUC_{last}	Area under the concentration-time curve from time 0 to the last observed concentration
AUC_{tau}	Area under the concentration-time curve over the dosing interval
BA	Bioavailability
BCRP	Breast cancer resistance protein
BMI	Body mass index
Casirivimab	Casirivimab (genetical recombination)
CC_{50}	Concentration achieving 50% of cytotoxicity
CCK	Cholecystokinin
CD	Cluster of differentiation
CI	Confidence interval
CK	Creatine kinase
CL/F	Apparent total clearance
CLr	Renal clearance
C_{max}	Maximum concentration
CQA	Critical quality attribute
CrCL	Creatinine clearance
$C_{t\ h}$	The concentration at t hours post dose
CV	Coefficient of variation
CYP	Cytochrome P450
DMSO	Dimethyl sulfoxide
EC_{50}	50% effective concentration
EC_{90}	90% effective concentration
eGFR	Estimated glomerular filtration rate
GISAID	Global Initiative on Sharing Avian Influenza Data
HDL	High density lipoprotein
hERG	Human ether-a-go-go-related gene
HPLC	High performance liquid chromatography
IC_{50}	50% inhibitory concentration
ICH Q1A Guideline	Revision of the Guideline on Stability Testing of New Drug Substances and Products (PFSB/ELD Notification No. 0603001 dated June 3, 2003)
ICH Q1E Guideline	Guideline on Evaluation of Stability Data (PFSB/ELD Notification No. 0603004 dated June 3, 2003)
ICH Q3C Guideline	Guideline for Residual Solvents (PMSB/ELD Notification No.307 dated March 30, 1998)
Imdevimab	Imdevimab (genetical recombination)
Ka	First order absorption rate constant
K_I	Half-maximal inactivation concentration
k_{intact}	Maximal inactivation rate constant
LCAT	Lecithin:cholesterol acyltransferase
LC-MS/MS	Liquid chromatography-tandem mass spectrometry
LDH	Lactate dehydrogenase
LDL	Low density lipoprotein
MATE	Multidrug and toxin extrusion protein
MedDRA	Medical Dictionary for Regulatory Activities
MPE	Mean photo effect
NZW	New Zealand White
OAT	Organic anion transporter
OATP	Organic anion transporting polypeptide
OCT	Organic cation transporter
$P_{app\ A\rightarrow B}$	Apparent permeability in apical to basolateral direction
PBPK	Physiologically-based pharmacokinetics

PCR	Polymerase chain reaction
PD	Pharmacodynamics
PDE	Phosphodiesterase
P-gp	P-glycoprotein
Pharmaceuticals and Medical Devices Act.	The Act on Securing Quality, Efficacy and Safety of Products Including Pharmaceuticals and Medical Devices (Act No. 145, 1960)
PIF	Photo irritation factor
PK	Pharmacokinetics
PMDA	Pharmaceuticals and Medical Devices Agency
PPK	Population pharmacokinetics
PT	Prothrombin time
QTc	Corrected QT interval
RNA	Ribonucleic acid
RT-PCR	Reverse transcription PCR
SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2
Scr	Serum creatinine concentration
SD	Sprague Dawley
Study T1211	Study 2102T1211
Study T1215	Study 2130T1215
Study T1221	Study 2108T1221
$t_{1/2}$	Terminal elimination half-life
$TCID_{50}$	50% tissue culture infectious dose
$Time_{High}$	Total time above the target plasma concentration
t_{max}	Time to maximum concentration
TMPRSS2	Transmembrane protease, serine 2
UGT	UDP glucuronosyltransferase
UV	Ultraviolet
V/F	Apparent volume of distribution
Vc/F	Apparent volume of distribution in central compartment
VLDL	Very low-density lipoprotein
γ-GTP	Gamma-glutamyl transferase

Second Review Report

July 12, 2022
Pharmaceuticals and Medical Devices Agency

Brand Name	Xocova Tablets 125 mg
Non-proprietary Name	Ensitrelvir Fumaric Acid
Applicant	Shionogi & Co., Ltd.
Date of Application	February, 25, 2022

Results of Review

After the finalization of the Review Report, the applicant submitted new study data on the clinical drug interaction between midazolam and ensitrelvir at the proposed dosage.

1. Clinical Pharmacology and Outline of the Review Conducted by PMDA

1.1 Drug interaction (CTD 5.3.3.1-01: Study T1211 [July 2021 to June 2022] Cohort T)

In total, 14 healthy Japanese adults received oral ensitrelvir at the proposed dosage (375 mg on Day 1, followed by 125 mg once daily from Days 2 to 5 [hereinafter referred to as "375/125 mg"]). They also received midazolam 2 mg, a typical CYP3A substrate, at 2 days before and 5 days after the start of ensitrelvir administration. PK parameters of midazolam were investigated. The ratio of the least squares geometric means [90% confidence interval] of C_{max} and AUC_{inf} of midazolam coadministered with ensitrelvir to that of midazolam administered without ensitrelvir (with ensitrelvir/without ensitrelvir) was 2.80 [2.38, 3.30] and 6.77 [6.16, 7.44], respectively.

1.R Coadministration of ensitrelvir with CYP3A substrate drugs

The applicant's explanation:

The investigation of clinical drug interaction between midazolam and ensitrelvir 375/125 mg (the proposed dosage) showed that AUC_{inf} of midazolam coadministered with ensitrelvir was 6.77 times higher than that of midazolam administered without ensitrelvir [see Section 1.1], demonstrating a potent inhibition of CYP3A by ensitrelvir. AUC and C_{max} of midazolam coadministered with clarithromycin, a potent CYP3A inhibitor, are reported to increase approximately 6.42- and 2.76-fold,[1)] respectively. Contraindications and precautions for concomitant use in the package insert for ensitrelvir will be determined based on the package insert for clarithromycin.

[1)] Mean ratio of increase in AUC (4.85, 5.48, 6.32, 6.50, 7.00, and 8.39-fold) and in C_{max} (2.17, 2.39, 2.69, 2.75, 2.77, and 3.80-fold) of midazolam coadministered with clarithromycin, reported in literature (*Clin Pharmacol Ther.* 2017; 101: 519-30, *Clin Pharmacol Ther*. 2008; 83: 61-9, *Br J Clin Pharmacol.* 2008; 65: 98-109, *Drug Metab Pharmacokinet.* 2021; 36: 100374, *Clin Pharmacol Ther*. 1998; 64: 133-43, and *J Clin Pharmacol.* 2006; 46: 201-13, respectively)

PMDA's view:

It cannot be concluded that the drug interaction risk of ensitrelvir is clearly different from that of potent CYP3A inhibitors other than clarithromycin. Therefore, contraindications and precautions for concomitant use for ensitrelvir should be determined based on those for not only clarithromycin but also other potent CYP3A inhibitors. When a CYP3A substrate drug is administered to a patient who has completed treatment with ensitrelvir, attention should be paid to the possibility of residual CYP3A-inhibitory effect of ensitrelvir because ensitrelvir has a time-dependent inhibitory effect against CYP3A [see Sections 4.5.1 and 6.2.2 of Review Report (1)].

As described above and in Section 6.R.6.2 of Review Report (1), ensitrelvir may interact with other drugs. Healthcare professionals should be appropriately advised to take precautions and should be provided with information, including about the time-dependent inhibition of CYP3A by ensitrelvir.

2. Results of Compliance Assessment Concerning the New Drug Application Data and Conclusion Reached by PMDA

3.1 PMDA's conclusion concerning the results of document-based GLP/GCP inspections and data integrity assessment

The new drug application data were subjected to a document-based compliance inspection and a data integrity assessment in accordance with the provisions of the Act on Securing Quality, Efficacy and Safety of Products Including Pharmaceuticals and Medical Devices. On the basis of the inspection and assessment, PMDA concluded that there were no obstacles to conducting its review based on the application documents submitted.

3.2 PMDA's conclusion concerning the results of the on-site GCP inspection

The new drug application data (CTD 5.3.5.1-01) were subjected to an on-site GCP inspection, in accordance with the provisions of the Act on Securing Quality, Efficacy and Safety of Products Including Pharmaceuticals and Medical Devices. On the basis of the inspection, PMDA concluded that there were no obstacles to conducting its review based on the application documents submitted.

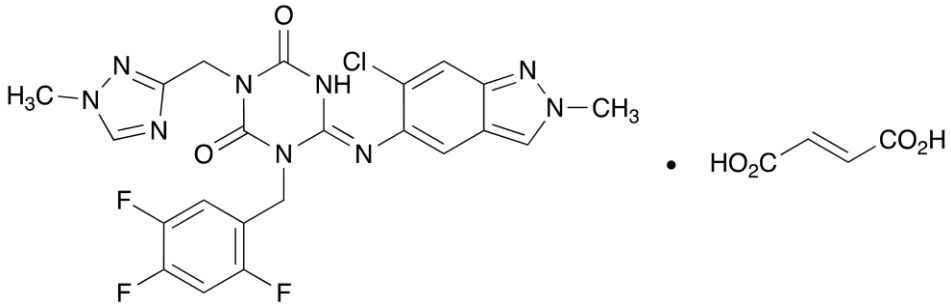
Third Review Report

November 15, 2022
Pharmaceuticals and Medical Devices Agency

The following are the results of the review of the following pharmaceutical product submitted for marketing approval conducted by the Pharmaceuticals and Medical Devices Agency (PMDA).

Brand Name	Xocova Tablets 125 mg
Non-proprietary Name	Ensitrelvir Fumaric Acid
Applicant	Shionogi & Co., Ltd.
Date of Application	February 25, 2022
Dosage Form/Strength	Each tablet contains 152.3 mg of ensitrelvir fumaric acid (125 mg of ensitrelvir).
Application classification	Prescription drug (1) Drug with a new active ingredient

Chemical structure



Molecular formula: $C_{22}H_{17}ClF_3N_9O2 \cdot C_4H_4O_4$
Molecular weight: 647.95
Chemical name: (6*E*)-6-[(6-Chloro-2-methyl-2*H*-indazol-5-yl)imino]-3-[(1-methyl-1*H*-1,2,4-triazol-3-yl)methyl]-1-[(2,4,5-trifluorophenyl)methyl]-1,3,5-triazinane-2,4-dione monofumaric acid

Items Warranting Special Mention	The product was handled as a product expected to be fall under the framework of approval from the Minister of Health, Labour and Welfare prescribed in Article 14 of the Pharmaceuticals and Medical Devices Act, pursuant to the provisions of Article 14-2, Paragraph 2 of the Act. ("Handling of a drug whose applicant is requesting a change of the application category to Emergency Approval (request)" (PSEHB/PED Notification No. 0526-16, dated May 26, 2022). Priority Review based on "Policy on regulatory review of drugs, etc. against coronavirus disease (COVID-19) (No. 2)" (PSEHB/PED Notification No. 0617-9 and PSEHB/MDED Notification No. 0617-1, dated June 17, 2021)
Reviewing Office	Office of New Drug IV

Results of review

On the basis of the data submitted (see Attachment), PMDA has concluded that currently available data are enough to presume that the product has efficacy against COVID-19, taking account of the following finding:

> In phase III part of the global phase II/III study (Study T1221), the ensitrelvir 375/125 mg group had a shorter time to resolution of 5 symptoms and tended to have a shorter time to resolution of 12 symptoms than the placebo group, when the population was limited to patients with COVID-19 who were randomized <72 hours after the onset of symptoms.

Note, however, that this is the conclusion at this moment that has been reached based on limited data for review. The appropriateness of the presumed efficacy of the product should be re-evaluated by additional analyses on efficacy and, based on the results, appropriate actions should be taken, including considering the withdrawal of marketing approval.

As for the safety of the product, PMDA considers that the safety risk of the product can be controlled by issuing appropriate alerts regarding the risk of teratogenicity, drug interactions, and others. Safety data should be collected after the market launch and new findings should be provided appropriately to healthcare professionals, because experience with treatment with the product in pediatric patients is limited, and particularly because the product has never been administered to pediatric patients weighing <40 kg.

Indication

Disease caused by SARS-CoV-2 infection (COVID-19)

Dosage and Administration

The usual dose in ≥12-year-old pediatric patients and adults is ensitrelvir 375 mg on Day 1 and ensitrelvir 125 mg from Days 2 to 5, administered orally once daily.

Approval Conditions

1. The applicant is required to develop and appropriately implement a risk management plan.
2. The applicant is required to request that physicians administer the product only to patients considered eligible for treatment with the product who, or whose legally acceptable representatives, have been provided with the efficacy and safety information of the product in written form, and have provided written informed consent before the treatment.
3. The applicant is required to promptly compile and submit data showing the efficacy of the product in phase III part of the global phase II/III study (Study T1221).

Attachment

Review Report (3)

October 25, 2022
Pharmaceuticals and Medical Devices Agency

Brand Name	Xocova Tablets 125 mg
Non-proprietary Name	Ensitrelvir Fumaric Acid
Applicant	Shionogi & Co., Ltd.
Date of Application	February, 25, 2022

List of Abbreviations
See Appendix.

1. History of review

Based on the data of phase IIa and phase IIb parts of the global phase II/III study (Study T1221), the appropriateness of Emergency Approval of Xocova was deliberated in the meeting of the Second Committee on New Drugs on June 22, 2022, and in the joint meeting of the Committee and the Pharmaceutical Affairs Department of the Pharmaceutical Affairs and Food Sanitation Council on July 20, 2022. As a result of deliberation in the meetings, the Committee and the Department concluded that the efficacy of Xocova cannot be presumed from the available data, and that the appropriateness of Emergency Approval of Xocova should be deliberated again based on results from phase III part of the global phase II/III study (Study T1221).

The applicant recently submitted preliminary results of the primary endpoint in phase III part of the global phase II/III study (Study T1221) involving patients with COVID-19. PMDA reviewed the data. The applicant also submitted, around the same time, additional study data on quality and nonclinical pharmacology. PMDA also reviewed the data.

2. Data Relating to Quality and Outline of the Review Conducted by PMDA

2.1 Drug product

2.1.1 Stability of drug product

Table 1 shows the main stability studies of the drug product. Results demonstrated the stability of the drug product.

Table 1. Stability studies of drug product

Study	Primary batch	Temperature	Humidity	Storage package	Storage period
Long-term	1 pilot scale batch 2 small scale batches	25˚C	60% RH	Blister package ([redacted])	12 months [a)]
Accelerated	1 pilot scale batch 2 small scale batches	40˚C	75% RH		6 months

a) The storage period was 6 months at the time of Review Report (1). Data on 9- and 12-month storage were submitted additionally.

Based on the above, a shelf life of 24 months has been proposed for the drug product stored in a blister package ([redacted]) at room temperature, based on the ICH Q1E Guideline "Evaluation for Stability Data" (PFSB/ELD Notification No. 0603004 dated June 3, 2003).

PMDA confirmed that the quality of the drug product was adequately controlled, based on the data submitted.

3. Non-clinical Pharmacology and Outline of the Review Conducted by PMDA

3.1 Primary pharmacodynamics

3.1.1 *In vitro* antiviral activity (CTD 4.2.1.1-22 to 24)

Using VeroE6/TMPRSS2 cells infected with clinical isolates of SARS-CoV-2, the antiviral activities of ensitrelvir and remdesivir were investigated based on cytopathic effect. Table 2 shows the results. The mean 50% effective concentration (EC_{50}) of ensitrelvir against each clinical isolate was similar to that against the original strain.[2)] PMDA confirmed that ensitrelvir had antiviral activity against each clinical isolate.

Table 2. *In vitro* antiviral activity of ensitrelvir and remdesivir against SARS-CoV-2

Viral strain	Lineage	Mean EC_{50} (μmol/L)	
		Ensitrelvir	Remdesivir
hCoV-19/Japan/TY28-444/2021	P.3 (Theta)	0.29	0.98
hCoV-19/Japan/TY33-456/2021	C.37 (Lambda)	0.27	3.2
hCoV-19/Japan/TY26-717/2021	B.1.621 (Mu)	0.43	3.9
hCoV-19/Japan/TY41-716/2022	B.1.1.529/BA.2.75 (Omicron)	0.30	0.91
hCoV-19/Japan/TY41-703/2022	B.1.1.529/BA.4 (Omicron)	0.22	0.65
hCoV-19/Japan/TY41-702/2022	B.1.1.529/BA.5 (Omicron)	0.40	1.3
hCoV-19/Japan/TY41-686/2022	B.1.1.529/XE [a)] (Omicron)	0.44	1.1

a) A recombinant of B.1.1.529/BA.1 and B.1.1.529/BA.2

4. Clinical Efficacy and Safety and Outline of the Review Conducted by PMDA

4.1 Global phase II/III study (CTD 5.3.5.1-01: Study T1221, jRCT2031210350 [ongoing since September 2021])

4.1.1 Phase III part [data cut off in August 2022; preliminary results]

A randomized, double-blind, placebo-controlled, parallel-group study was conducted in 3 countries (Japan, Vietnam, and South Korea) to investigate the efficacy and safety of ensitrelvir in patients with COVID-19 (target sample size, 780 subjects [260 per group]). Table 3 shows the main inclusion and exclusion criteria. Main changes made to the study protocol during the conduct of phase III part are presented in Section 6.1.

[2)] EC_{50}: 0.37 μmol/L (see Table 6 in Section 3.1.2.1 of Review Report (1)).

Table 3. Main inclusion/exclusion criteria

Inclusion criteria	1. Subjects ≥12 to <18 years old weighing ≥40 kg, and those ≥18 to <70 years old[a)] 2. SARS-CoV-2 positive (confirmed by PCR etc. on a sample collected within 120 hours before randomization) 3. Onset of at least 1 of 14 symptoms[b)] of COVID-19 within 120 hours before randomization 4. At least 1 moderate (score 2)[c)] or severe symptom among 12 symptoms[d)] of COVID-19 at the time of randomization. Subjects with symptoms that have been present since before the onset of COVID-19 are eligible only if they consider that the symptoms have worsened after SARS-CoV-2 infection. 5. Subjects who can take contraceptive measures from the start of study treatment through 10 days after the end of study treatment. 6. Women who are not pregnant or possibly pregnant
Exclusion criteria	1. SpO_2 ≤93% (room air) 2. Subjects who need oxygen therapy 3. Subjects who need a mechanical ventilation 4. Current or chronic history of moderate or severe liver disease (Grade ≥2 in CTCAE, ver. 5.0) 5. Current or chronic history of moderate or severe kidney disease (Grade ≥2 in CTCAE, ver. 5.0)

a) Age limitation for subjects weighing ≥40 kg was changed from "≥12 to <20 years" to "≥12 to <18 years" in the protocol ver. 9 (dated July 8, 2022).
b) (1) Malaise or tiredness, (2) muscle or body aches, (3) headache, (4) chills or sweating, (5) feverish or pyrexia, (6) stuffy or runny nose, (7) sore throat, (8) cough, (9) shortness of breath (dyspnea), (10) nausea, (11) vomiting, (12) diarrhea, (13) taste abnormality, and (14) dysosmia
c) Symptoms were scored by the subject on a 4-point scale (0, none; 1, mild; 2, moderate; 3, severe).
d) (1) Malaise or tiredness, (2) muscle or body aches, (3) headache, (4) chills or sweating, (5) feverish or pyrexia, (6) stuffy or runny nose, (7) sore throat, (8) cough, (9) shortness of breath (dyspnea), (10) nausea, (11) vomiting, (12) diarrhea

The study drug was administered orally at the following dosage: (a) ensitrelvir at 375 mg on Day 1 and at 125 mg once daily from Days 2 to 5 (ensitrelvir 375/125 mg group), (b) ensitrelvir at 750 mg on Day 1 and at 250 mg once daily from Days 2 to 5 (ensitrelvir 750/250 mg group), or (c) placebo once daily for 5 days.

The phase III part was planned to start after the completion of enrollment of subjects in phase IIb part, and actually started in February 10, 2022. The observation period (follow-up period: Day 1 (the first day of study treatment] through Day 28) in all subjects was completed on August 8, 2022. The protocol was amended to ver. 10 on September 20, 2022 (last amendment before unblinding on September 23, 2022), in order to change the dose for efficacy evaluation, the primary endpoint, main efficacy analysis population, sample size required, method for the primary analysis, etc. (Table 4). After the amendment, data from phase III part were analyzed.

Table 4. Main changes made to the study protocol (underlined parts: differences between the versions. See Section 6.1 for main changes made to the study protocol during the conduct of the study)

	Before amendment Ver. 9 (amended on July 8, 2022)	After amendment Ver. 10 (amended on September 20, 2022)
Dose evaluated for efficacy	・Ensitrelvir 375/125 mg ・Ensitrelvir 750/250 mg	・Ensitrelvir 375/125 mg
Primary endpoint	Time from the start of study treatment to resolution of 12 symptoms of COVID-19 12 symptoms: (1) Malaise or tiredness, (2) muscle or body aches, (3) headache, (4) chills or sweating, (5) feverish or pyrexia, (6) stuffy or runny nose, (7) sore throat, (8) cough, (9) shortness of breath (dyspnea), (10) nausea, (11) vomiting, and (12) diarrhea	Time from the start of study treatment to resolution of 5 symptoms of COVID-19 5 symptoms: (1) Malaise or tiredness, (2) feverish or pyrexia, (3) stuffy or runny nose, (4) sore throat, and (5) cough
Primary efficacy analysis population	ITT population	Subjects who experienced the onset of COVID-19 symptoms within <72 hours before randomization in ITT population
Sample size required	1,590 subjects (530 per group)	780 subjects (260 per group)
Method for primary analysis	Stratified log-rank test	Stratified Peto-Prentice's generalized Wilcoxon test

Of 1,821 randomized subjects (607 in the ensitrelvir 375/125 mg group, 606 in the ensitrelvir 750/250 mg group, 608 in the placebo group), 1,808 who received at least 1 dose of the study drug (604 in the ensitrelvir 375/125 mg group, 599 in the ensitrelvir 750/250 mg group, 605 in the placebo group) were included in the safety analysis population. Of the 1,821 randomized subjects, 1,798 were included in the ITT population (603 in the ensitrelvir 375/125 mg group, 595 in the ensitrelvir 750/250 mg group, 600 in the placebo group) because they tested positive for SARS-CoV-2 by RT-PCR on baseline nasopharyngeal swab.[3)] Of the subjects in the ITT population, 1,030 were included in the primary efficacy population (347 in the ensitrelvir 375/125 mg group, 340 in the ensitrelvir 750/250 mg group, 343 in the placebo group) because they experienced the onset of COVID-19 symptoms within <72 hours before randomization.

In total, 52 subjects discontinued the study (11 in the ensitrelvir 375/125 mg group, 21 in the ensitrelvir 750/250 mg group, 20 in the placebo group). The reasons for discontinuation were subject's request in 24 subjects (3 in the ensitrelvir 375/125 mg group, 11 in the ensitrelvir 750/250 mg group, 10 in the placebo group), protocol violation in 5 subjects (3 in the ensitrelvir 375/125 mg group, 1 in the ensitrelvir 750/250 mg group, 1 in the placebo group), adverse events in 4 subjects (2 in the ensitrelvir 750/250 mg group, 2 in the placebo group), disease progression in 1 subject (placebo group), and other causes in 18 subjects (5 in the ensitrelvir 375/125 mg group, 7 in the ensitrelvir 750/250 mg group, 6 in the placebo group).

The primary endpoint for efficacy was defined as "the time from the start of study treatment to resolution of the 5 symptoms of COVID-19." "Resolution" was defined as the condition in which all 5 symptoms met the following criteria:

- Symptoms that had been present before the onset of COVID-19 and were considered by the subject to have worsened at baseline (at test before administration): Improved severity[4)] from baseline or the same severity as baseline (i.e., from severe to moderate or better, from moderate to mild or

[3)] Samples were collected the day before or on the day of starting the study treatment.
[4)] For severity assessment, symptoms were scored by the subject on a 4-point scale (0, none; 1, mild; 2, moderate; 3, severe).

better, or from mild to mild or better) is maintained for at least 24 hours.

- Symptoms that had been present before the onset of COVID-19 and were NOT considered by the subject to have worsened at baseline (at test before administration): Improved severity[4)] from baseline or the same severity as baseline (i.e., from severe to severe or better, from moderate to moderate or better, or from mild to mild or better) is maintained for at least 24 hours.
- Other symptoms (i.e., symptoms that were not present before the onset of COVID-19 and developed after baseline [test before administration]): An asymptomatic condition is maintained for at least 24 hours.

Table 5 and Figure 1 show the results of the primary endpoint, demonstrating a statistically significant difference between the ensitrelvir 375/125 mg and the placebo groups. No death or hospitalization occurred as of Day 28 in either group in the ITT population.

SARS-CoV-2 variants detected in the participating countries during the subject enrollment period were mainly BA.1, BA.2, and BA.5.[5)]

Table 5. Time to resolution of 5 symptoms of COVID-19 (primary endpoint)
(only in subjects who experienced the onset of COVID-19 symptoms within <72 hours before randomization in the ITT population of phase III part)

		Ensitrelvir 375/125 mg	Ensitrelvir 750/250 mg	Placebo
Entire population	Number of subjects [a)]	336	329	321
	Number of subjects who achieved resolution	254	262	233
	Median time (h) to resolution of 5 symptoms of COVID-19	167.9	171.2	192.2
	p value [b)]	0.0407	-	
	Hazard ratio [95% confidence interval] [c)]	1.14 [0.95, 1.36]	1.22 [1.03, 1.46]	
Japanese subpopulation	Number of subjects [a)]	179	154	163
	Number of subjects who achieved resolution	134	123	120
	Median time (h) to resolution of 5 symptoms of COVID-19	165.8	151.6	172.1
	p value [b)]	1.04 [0.81, 1.33]	1.27 [0.98, 1.63]	

a) Subjects were excluded from the analysis if all scores of baseline 5 symptoms were 0 or if a baseline score(s) were missing.
b) Peto-Prentice's generalized Wilcoxon test stratified by vaccination status against COVID-19 (yes, no), at a 2-sided significance level of 5%
c) Cox proportional hazard model stratified by vaccination status against COVID-19 (yes, no)

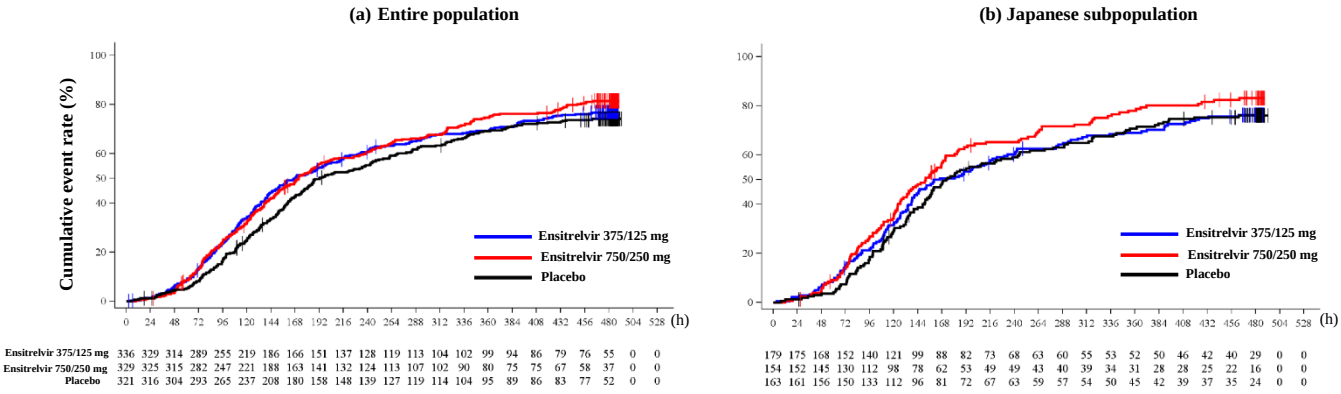


Figure 1. Cumulative rate of "time to resolution of 5 symptoms of COVID-19" (primary endpoint)
(only in subjects who experienced the onset of COVID-19 symptoms within <72 hours before randomization in the ITT population of phase III part)

[5)]CoVariants. Overview of Variants in Countries: https://covariants.org/

As for safety,[6)] the following are the incidences of adverse events and adverse reactions,[7)] respectively:

The ensitrelvir 375/125 mg group: 44.2% (267 of 604) and 24.5% (148 of 604) of subjects

The ensitrelvir 750/250 group: 53.6% (321 of 599) and 36.2% (217 of 599) of subjects

The placebo group: 24.8% (150 of 605) and 9.9% (60 of 605) of subjects

Table 6 shows the incidences of adverse events and adverse reactions observed in ≥2 subjects in at least 1 group.

[6)] Adverse events and adverse reactions observed on or before Day 28

[7)] Adverse events considered to be related to the study drug by the investigator, etc.

Table 6. Adverse events and adverse reactions observed in ≥2 subjects in at least 1 group (phase III part: safety analysis population)

Event terms	Adverse events			Adverse reactions		
	Ensitrelvir 375/125 mg (N = 604)	Ensitrelvir 750/250 mg (N = 599)	Placebo (N = 605)	Ensitrelvir 375/125 mg (N = 604)	Ensitrelvir 750/250 mg (N = 599)	Placebo (N = 605)
All events	267 (44.2)	321 (53.6)	150 (24.8)	148 (24.5)	217 (36.2)	60 (9.9)
High density lipoprotein decreased	188 (31.1)	231 (38.6)	23 (3.8)	111 (18.4)	157 (26.2)	9 (1.5)
Blood triglycerides increased	49 (8.1)	74 (12.4)	32 (5.3)	16 (2.6)	37 (6.2)	17 (2.8)
Blood bilirubin increased	36 (6.0)	56 (9.3)	6 (1.0)	17 (2.8)	35 (5.8)	3 (0.5)
Blood cholesterol decreased	20 (3.3)	28 (4.7)	3 (0.5)	8 (1.3)	12 (2.0)	1 (0.2)
Bilirubin conjugated increased	15 (2.5)	20 (3.3)	3 (0.5)	1 (0.2)	2 (0.3)	1 (0.2)
Blood creatine phosphokinase increased	14 (2.3)	8 (1.3)	11 (1.8)	4 (0.7)	1 (0.2)	1 (0.2)
Headache	13 (2.2)	20 (3.3)	14 (2.3)	4 (0.7)	13 (2.2)	2 (0.3)
Blood lactate dehydrogenase increased	6 (1.0)	15 (2.5)	6 (1.0)	4 (0.7)	8 (1.3)	4 (0.7)
ALT increased	6 (1.0)	11 (1.8)	11 (1.8)	3 (0.5)	6 (1.0)	7 (1.2)
Diarrhoea	6 (1.0)	9 (1.5)	12 (2.0)	5 (0.8)	8 (1.3)	7 (1.2)
Dizziness	6 (1.0)	1 (0.2)	4 (0.7)	4 (0.7)	1 (0.2)	2 (0.3)
Blood potassium decreased	6 (1.0)	0	1 (0.2)	1 (0.2)	0	1 (0.2)
Low density lipoprotein increased	5 (0.8)	3 (0.5)	4 (0.7)	2 (0.3)	1 (0.2)	3 (0.5)
Nausea	4 (0.7)	11 (1.8)	1 (0.2)	2 (0.3)	5 (0.8)	0
AST increased	4 (0.7)	9 (1.5)	12 (2.0)	1 (0.2)	5 (0.8)	7 (1.2)
Vomiting	4 (0.7)	8 (1.3)	0	2 (0.3)	4 (0.7)	0
Blood uric acid increased	4 (0.7)	6 (1.0)	4 (0.7)	2 (0.3)	3 (0.5)	1 (0.2)
Blood phosphorus increased	3 (0.5)	2 (0.3)	3 (0.5)	1 (0.2)	2 (0.3)	2 (0.3)
Somnolence	3 (0.5)	2 (0.3)	0	3 (0.5)	1 (0.2)	0
Seasonal allergy	3 (0.5)	1 (0.2)	1 (0.2)	0	0	0
Paraesthesia	3 (0.5)	0	1 (0.2)	2 (0.3)	0	0
Rash	2 (0.3)	7 (1.2)	5 (0.8)	0	5 (0.8)	1 (0.2)
Urticaria	2 (0.3)	3 (0.5)	1 (0.2)	0	2 (0.3)	0
Blood cholesterol increased	2 (0.3)	2 (0.3)	3 (0.5)	0	1 (0.2)	0
Back pain	2 (0.3)	2 (0.3)	3 (0.5)	0	0	0
Nasopharyngitis	2 (0.3)	2 (0.3)	2 (0.3)	0	0	0
Hypertriglyceridaemia	2 (0.3)	2 (0.3)	1 (0.2)	1 (0.2)	2 (0.3)	1 (0.2)
Dyspepsia	2 (0.3)	2 (0.3)	0	1 (0.2)	1 (0.2)	0
Blood iron increased	2 (0.3)	2 (0.3)	0	0	0	0
Constipation	2 (0.3)	1 (0.2)	3 (0.5)	0	0	0
Blood alkaline phosphatase increased	2 (0.3)	1 (0.2)	0	0	1 (0.2)	0
Hypertension	2 (0.3)	1 (0.2)	0	0	0	0
Chest pain	2 (0.3)	1 (0.2)	0	0	0	0
Dyslipidaemia	2 (0.3)	0	1 (0.2)	2 (0.3)	0	0
Blood bilirubin unconjugated increased	2 (0.3)	0	0	2 (0.3)	0	0
Eosinophil count increased	2 (0.3)	0	0	0	0	0
White blood cell count decreased	2 (0.3)	0	0	0	0	0
Tonsillitis	2 (0.3)	0	0	0	0	0
Anaemia	2 (0.3)	0	0	0	0	0
Insomnia	1 (0.2)	3 (0.5)	2 (0.3)	0	2 (0.3)	1 (0.2)
Abdominal discomfort	1 (0.2)	3 (0.5)	0	0	1 (0.2)	0
Eczema	1 (0.2)	2 (0.3)	1 (0.2)	1 (0.2)	0	1 (0.2)
Blood pressure increased	1 (0.2)	1 (0.2)	4 (0.7)	0	0	0
γ-GTP increased	1 (0.2)	1 (0.2)	3 (0.5)	1 (0.2)	0	2 (0.3)
C-reactive protein increased	1 (0.2)	1 (0.2)	2 (0.3)	0	0	2 (0.3)
Heavy menstrual bleeding	1 (0.2)	1 (0.2)	2 (0.3)	0	0	0
White blood cell count increased	1 (0.2)	0	2 (0.3)	0	0	0
Tension headache	1 (0.2)	0	2 (0.3)	0	0	0
Pruritus	1 (0.2)	0	2 (0.3)	0	0	0
Pyrexia	1 (0.2)	0	2 (0.3)	0	0	0
Transaminases increased	0	3 (0.5)	0	0	1 (0.2)	0
Blood cholinesterase increased	0	2 (0.3)	0	0	0	0
Blood urea increased	0	2 (0.3)	0	0	0	0
Toothache	0	2 (0.3)	0	0	0	0
Low density lipoprotein decreased	0	1 (0.2)	2 (0.3)	0	1 (0.2)	2 (0.3)
Neutrophil count decreased	0	0	2 (0.3)	0	0	1 (0.2)
Pain in extremity	0	0	2 (0.3)	0	0	0

n (%), MedDRA ver.24.0

There were no adverse events resulting in death.

Serious adverse events were observed in 1 subject (heavy menstrual bleeding) in the ensitrelvir 375/125 mg group and in 1 subject (cholecystitis acute) in the placebo group. Both events were unrelated to the study drug, with the outcome of "recovered."

Adverse events leading to treatment discontinuation were observed in 4 subjects (eczema, vomiting, rash, and hypertension in 1 subject each) in the ensitrelvir 375/125 mg group, 6 subjects (rash in 2 subjects; nausea, headache, abdominal pain, presyncope, and vomiting in 1 subject each [some subjects had more than 1 event]) in the ensitrelvir 750/250 mg group, and 2 subjects (hypoaesthesia, muscular weakness, headache in 1 subject each [1 subject had 2 events]) in the placebo group. The events in the following subjects were related to the study drug: 2 subjects (eczema and vomiting in 1 subject each) in the ensitrelvir 375/125 mg group, 2 subjects (rash in 2 subjects) in the ensitrelvir 750/250 mg group, and 1 subject (hypoaesthesia and muscular weakness in the same subject) in the placebo group; the outcome of these events was "recovered" or "recovering."

The following are the incidences of adverse events and adverse reactions, respectively, in the Japanese subpopulation:

The ensitrelvir 375/125 mg group: 52.8% (179 of 339) and 23.9% (81 of 339) of subjects

The ensitrelvir 750/250 mg group: 64.6% (201 of 311) and 38.6% (120 of 311) of subjects

The placebo group: 25.3% (81 of 320) and 5.0% (16 of 320) of subjects

Table 7 shows adverse events and adverse reactions observed in ≥2 subjects in at least 1 group.

Table 7. Adverse events and adverse reactions observed in ≥2 subjects in at least 1 group in the Japanese subpopulation (phase III part: safety analysis population)

Event terms	Adverse events			Adverse reactions		
	Ensitrelvir 375/125 mg (N = 339)	Ensitrelvir 750/250 mg (N = 311)	Placebo (N = 320)	Ensitrelvir 375/125 mg (N = 339)	Ensitrelvir 750/250 mg (N = 311)	Placebo (N = 320)
All events	179 (52.8)	201 (64.6)	81 (25.3)	81 (23.9)	120 (38.6)	16 (5.0)
High density lipoprotein decreased	142 (41.9)	171 (55.0)	17 (5.3)	68 (20.1)	104 (33.4)	4 (1.3)
Blood triglycerides increased	29 (8.6)	36 (11.6)	15 (4.7)	1 (0.3)	7 (2.3)	4 (1.3)
Blood bilirubin increased	18 (5.3)	25 (8.0)	2 (0.6)	0	6 (1.9)	1 (0.3)
Bilirubin conjugated increased	14 (4.1)	16 (5.1)	1 (0.3)	0	0	0
Blood cholesterol decreased	13 (3.8)	16 (5.1)	3 (0.9)	1 (0.3)	1 (0.3)	1 (0.3)
Headache	10 (2.9)	10 (3.2)	12 (3.8)	1 (0.3)	3 (1.0)	0
Blood creatine phosphokinase increased	7 (2.1)	5 (1.6)	7 (2.2)	0	0	1 (0.3)
Nausea	4 (1.2)	10 (3.2)	1 (0.3)	2 (0.6)	4 (1.3)	0
Diarrhoea	4 (1.2)	5 (1.6)	7 (2.2)	3 (0.9)	4 (1.3)	3 (0.9)
Vomiting	4 (1.2)	4 (1.3)	0	2 (0.6)	1 (0.3)	0
ALT increased	3 (0.9)	5 (1.6)	4 (1.3)	1 (0.3)	0	2 (0.6)
AST increased	3 (0.9)	3 (1.0)	3 (0.9)	1 (0.3)	0	2 (0.6)
Seasonal allergy	3 (0.9)	1 (0.3)	1 (0.3)	0	0	0
Rash	2 (0.6)	4 (1.3)	3 (0.9)	0	2 (0.6)	1 (0.3)
Urticaria	2 (0.6)	3 (1.0)	1 (0.3)	0	2 (0.6)	0
Back pain	2 (0.6)	2 (0.6)	3 (0.9)	0	0	0
Nasopharyngitis	2 (0.6)	2 (0.6)	2 (0.6)	0	0	0
Blood iron increased	2 (0.6)	2 (0.6)	0	0	0	0
Constipation	2 (0.6)	1 (0.3)	3 (0.9)	0	0	0
Chest pain	2 (0.6)	1 (0.3)	0	0	0	0
Dyslipidaemia	2 (0.6)	0	0	2 (0.6)	0	0
White blood cell count decreased	2 (0.6)	0	0	0	0	0
Tonsillitis	2 (0.6)	0	0	0	0	0
Eczema	1 (0.3)	2 (0.6)	1 (0.3)	1 (0.3)	0	1 (0.3)
γ-GTP increased	1 (0.3)	1 (0.3)	2 (0.6)	1 (0.3)	0	1 (0.3)
Tension headache	1 (0.3)	0	2 (0.6)	0	0	0
Transaminases increased	0	3 (1.0)	0	0	1 (0.3)	0
Dyspepsia	0	2 (0.6)	0	0	1 (0.3)	0
Blood lactate dehydrogenase increased	0	2 (0.6)	0	0	0	0
Toothache	0	2 (0.6)	0	0	0	0
Low density lipoprotein decreased	0	0	2 (0.6)	0	0	2 (0.6)
Pyrexia	0	0	2 (0.6)	0	0	0

n (%), MedDRA ver.24.0

There were no adverse events resulting in death.

A serious adverse event occurred in 1 subject (heavy menstrual bleeding) in the ensitrelvir 375/125 mg group. The event was unrelated to the study drug, with the outcome of "recovered."

Adverse events leading to treatment discontinuation were observed in 3 subjects (eczema, vomiting, and rash in 1 subject each) in the ensitrelvir 375/125 mg group, 5 subjects (rash, nausea, headache, abdominal pain, presyncope, and vomiting in 1 subject each [some subjects had more than 1 event]) in the ensitrelvir 750/250 mg group, and 1 subject (headache) in the placebo group. The events in the following subjects were related to the study drug: 2 subjects (eczema and vomiting in 1 subject each) in the ensitrelvir 375/125 mg group and in 1 subject (rash) in the ensitrelvir 750/250 mg group; the outcome of these events was "recovered."

4.R Outline of the review conducted by PMDA

4.R.1 Protocol of the global phase II/III study (Study T1221)

In phase III part of the global phase II/III study (Study T1221), changes were made to the primary endpoint, the primary efficacy analysis population, etc., under blinded conditions immediately before unblinding [see Section 4.1.1, Table 4].

The applicant's explanation for the reason of these changes:
Based on the characteristics of the viral strain prevalent during the period of subject accrual to the phase III part, the applicant had a discussion with medical experts and investigated the following points (see below). As a result, the applicant concluded that the primary efficacy endpoint and the primary analysis population should be changed and, based on them, the efficacy should be evaluated.

The applicant's investigation:

- Phase IIb and III parts of the global phase II/III study (Study T1221) were conducted when the Omicron variant was dominant. In Figure 2, five symptoms are circled in a red frame because only a small proportion of subjects rated the severity of them as "none" or "as usual." These symptoms overlapped with symptoms (respiratory symptoms, pyrexia, general malaise) occurring frequently in patients hospitalized around the same time, according to data in Japan.[8)] Accordingly, evaluating the time to resolution of the 5 symptoms was considered clinically meaningful.

[8)] Data from Hiroshima prefecture for the 94th meeting of COVID-19 Advisory Board (August 10, 2022): Findings from J-SPEED data of COVID-19 in Hiroshima prefecture: https://www.mhlw.go.jp/content/10900000/000975398.pdf (last accessed on October 25, 2022)

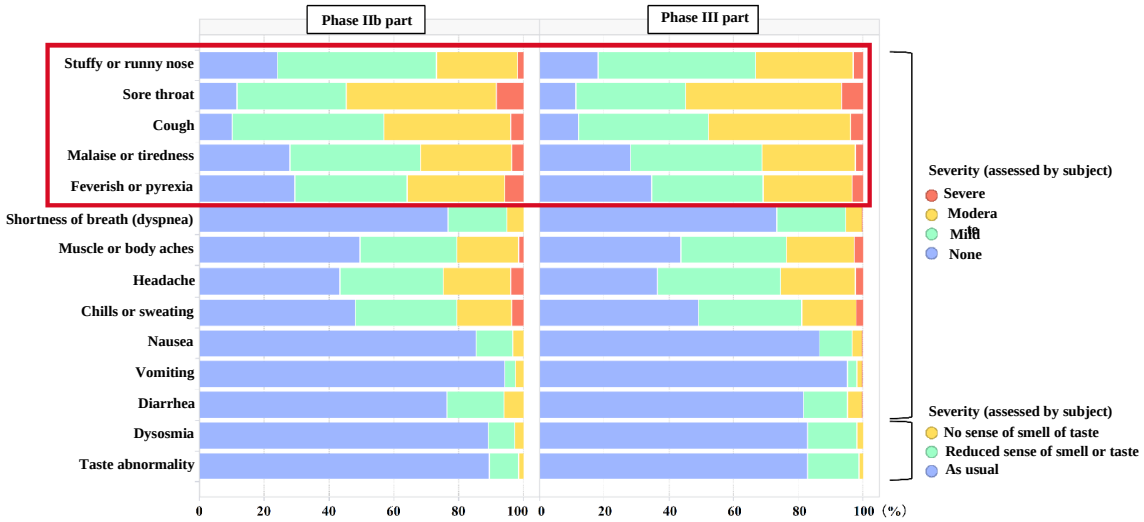


Figure 2. Baseline symptom scores in phase IIb part [a)] and phase III part [b)] of global phase II/III study (Study T1221)

a) Of the subjects in the ITT1 population (randomized subjects with a positive SARS-CoV-2 viral titer in baseline nasopharyngeal swab), 333 subjects with baseline symptom scores recorded are included (110 in the ensitrelvir 375/125 mg group, 113 in the ensitrelvir 750/250 mg group, 110 in the placebo group).

b) A total of 1768 randomized subjects with baseline symptom scores recorded are included (cut-off data as of August 15, 2022. Breakdown of treatment groups is unknown because the data were yet to be unblinded)

- Ensitrelvir achieves maximum treatment effect when administered before symptoms become aggravated. According to the data in Japan,[8)] approximately 60% of hospitalized patients experienced symptom aggravation within 3 days after the onset of symptoms. These findings suggested that the primary efficacy analysis population should consist of subjects who experienced the onset of COVID-19 symptoms within <72 hours before randomization.[9)]

The required sample size was changed in response to the change of the primary efficacy endpoint and the analysis population [see Section 4.1.1]. Also, the following changes were made taking account of the results of phase IIb part:

- Results of phase IIb part showed that there was no clear difference in efficacy between the 2 dose groups, with sufficient antiviral effect achieved even at the lower dose. Also, in order to increase the margin against the risks of teratogenicity and drug interactions, only data on ensitrelvir 375/125 mg were subjected to efficacy assessment.
- In phase IIb part, the between-group difference (the ensitrelvir 375/125 mg group vs. the placebo group) in the percentage of subjects who achieved resolution of 5 symptoms[10)] of COVID-19 was large at the early stage after the start of study treatment, but became smaller at the end of the observation period. These findings suggest that it is more appropriate to use stratified Peto-Prentice's generalized Wilcoxon test (which places more weight on events at early time points) than using the stratified long-rank test (which places equal weight at all time points). The statistical method was changed accordingly.

PMDA's view:

The applicant changed the primary endpoint, the primary efficacy population, etc., immediately before unblinding [see Section 4.11, Table 4]. Although this change was made under blinded conditions, this

[9)] Subjects were randomized on Day 1 (acceptable range: −1 day), and the study treatment was started on Day 1.

[10)] The applicant explained that, in phase III part, the efficacy was evaluated using the following 5 symptoms of COVID-19: (1) Malaise or tiredness, (2) feverish or pyrexia, (3) stuffy or runny nose, (4) sore throat, and (5) cough.

action was not appropriate because it damaged the credibility of the study results. Accordingly, PMDA evaluated the efficacy of ensitrelvir based on not only the results obtained after the change but also the analysis results obtained under the pre-change conditions [see Section 4.R.2].

That being said, the applicant has developed ensitrelvir in the midst of repeated spread of SARS-CoV-2 infection and the growing attention in Japan to the speed of development of therapeutic agents. Taking account of this situation and the following points, the change of the primary efficacy endpoint and the efficacy analysis population should not be denied:

- As for the symptoms included in the primary endpoint, the post-change 5 symptoms were extracted from the pre-change 12 symptoms,[11)] and are thus not unique to the new SARS-CoV-2 variant. Evaluating ensitrelvir based on the 12 symptoms is clinically reasonable to a certain extent because COVID-19 causes a variety of symptoms. Nevertheless, PMDA does not deny that there is a certain significance in evaluating ensitrelvir based on the 5 symptoms, which occur frequently in patients with COVID-19.
- Ensitrelvir had to be developed under the circumstance where the proportion of patients who experience severe COVID-19 was reduced due to the progress of vaccination program and mutations of epidemic strains. In this circumstance, there is a certain logic in using "symptom alleviation in patients in the early phase of COVID-19" as an efficacy endpoint in the development of ensitrelvir, which mainly acts by its antiviral effect.

4.R.2 Efficacy

The applicant's explanation about the efficacy of ensitrelvir in patients with COVID-19:

In phase III part of the global phase II/III study (Study T1221), a statistically significant difference was observed between the ensitrelvir 375/125 mg and placebo groups in the primary endpoint "time from the start of study treatment to resolution of the 5 symptoms[10)] of COVID-19" (only in subjects who experienced the onset of COVID-19 symptoms within <72 hours before randomization in the ITT population) [see Section 4.1.1, Table 5]. The median time in the ensitrelvir 375/125 mg group was shorter by 24.3 hours than that in the placebo group (95% CI of difference from placebo: −78.7, 11.7 hours). In subjects who experienced the onset of COVID-19 symptoms within <72 hours before randomization in the ITT population, the median time from the start of study treatment to resolution of 12 symptoms[11)] of COVID-19 tended to be shorter by 34.0 hours (95% CI of the difference from placebo: −85.9, 8.3 hours) in the ensitrelvir 375/125 mg group (179.2 hours) than in the placebo group (213.2 hours) [see Table 8 and Figure 3]. In subjects who experienced the onset of COVID-19 symptoms within <72 hours before randomization in the ITT population, the change in SARS-CoV-2 RNA level from baseline to Day 4 differed between the ensitrelvir 375/125 mg and placebo groups (the difference, 1.47 $\log_{10}$ copies/mL), showing a greater decrease in the RNA level in the ensitrelvir 375/125 mg group than in the placebo group (Table 9). These results demonstrate the efficacy of ensitrelvir 375/125 mg against COVID-19.

[11)] The 12 symptoms of COVID-19: (1) Malaise or tiredness, (2) muscle or body aches, (3) headache, (4) chills or sweating, (5) feverish or pyrexia, (6) stuffy or runny nose, (7) sore throat, (8) cough, (9) shortness of breath (dyspnea), (10) nausea, (11) vomiting, and (12) diarrhea.

Table 8. Time from the start of study treatment to resolution of COVID-19 symptoms (only in subjects who experienced the onset of COVID-19 symptoms within <72 hours before randomization in the ITT population of phase III part)

		Ensitrelvir 375/125 mg	Ensitrelvir 750/250 mg	Placebo
5 symptoms	Number of subjects [a)]	336	329	321
	Number of subjects who achieved resolution	254	262	233
	Median time (h) to resolution of COVID-19 symptoms	167.9	171.2	192.2
	p value [b)]	0.0407	-	
	Hazard ratio [95% confidence interval] [c)]	1.14 [0.95, 1.36]	1.22 [1.03, 1.46]	
12 symptoms	Number of subjects [a)]	336	330	321
	Number of subjects who achieved resolution	244	258	227
	Median time (h) to resolution of COVID-19 symptoms	179.2	184.9	213.2
	Hazard ratio [95% confidence interval] [c)]	1.11 [0.93, 1.33]	1.19 [1.00, 1.42]	

a) Subjects were excluded if all of baseline symptom scores were 0 or a baseline score(s) were missing.
b) Peto-Prentice's generalized Wilcoxon test stratified by vaccination status against COVID-19 (yes, no), at a 2-sided significance level of 5%
c) Cox proportional hazard model stratified by vaccination status against COVID-19 (yes, no)

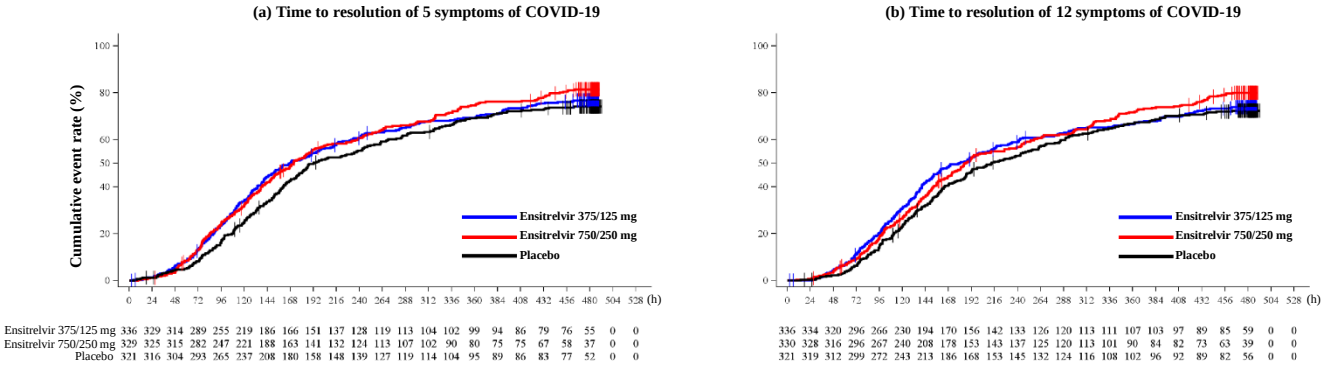


Figure 3. Cumulative rate of "time from the start of study treatment to resolution of COVID-19 symptoms" (only in subjects who experienced the onset of COVID-19 symptoms within <72 hours before randomization in the ITT population of phase III part)

Table 9. Change in SARS-CoV-2 RNA level (log_{10} copies/mL) from baseline to Day 4 (phase III part)

	Subjects experiencing the onset of COVID-19 symptoms within <72 hours before randomization in the ITT population			Subjects experiencing the onset of COVID-19 symptoms within 120 hours before randomization (ITT population) [a)]		
	Ensitrelvir 375/125 mg (N = 340)	Ensitrelvir 750/250 mg (N = 333)	Placebo (N = 337)	Ensitrelvir 375/125 mg (N = 592)	Ensitrelvir 750/250 mg (N = 579)	Placebo (N = 589)
Change in SARS-CoV-2 RNA level (log_{10} copies/mL) from baseline to Day 4	−2.737 ± 1.085	−2.690 ± 0.974	−1.235 ± 1.528	−2.646 ± 1.097	−2.594 ± 1.010	−1.419 ± 1.423
Difference from placebo [95% confidence interval]	−1.47 [b)] [−1.63, −1.31]	−1.48 [b)] [−1.64, −1.32]	–	−1.20 [c)] [−1.32, −1.08]	−1.20 [c)] [−1.32, −1.08]	–

Mean ± standard deviation
When a qualitative PCR result was positive, the result was used. When a qualitative PCR result was negative, RNA level was regarded as 2.27 log_{10} copies/mL, the lower limit of positivity. When an RNA level in quantitative PCR was below the lower quantitation limit (2.08 log_{10} copies/mL), it was regarded as 2.08 log_{10} copies/mL.
a) Subjects without viral RNA data available at baseline or Day 4 were excluded from analysis.
b) Analysis of covariance with covariates of (a) baseline SARS-CoV-2 virus RNA level and (b) vaccination status against SARS-CoV-2 (yes, no).
c) Analysis of covariance with covariates of (a) time from the onset of COVID-19 symptoms to randomization (<72 hours, ≥72 hours), (b) baseline SARS-CoV-2 virus RNA level, and (c) vaccination status against SARS-CoV-2 (yes, no).

PMDA's view:

The following are the main results obtained from phase III part of the global phase II/III study (Study T1221):

- A statistically significant difference was observed between the ensitrelvir 375/125 mg and placebo groups in the primary endpoint "time from the start of study treatment to resolution of 5 symptoms of COVID-19 (only in subjects who experienced the onset of COVID-19 symptoms within <72 hours before randomization in the ITT population) [see Section 4.1.1, Table 5]. In the Japanese subpopulation, the difference between the ensitrelvir 375/125 mg and placebo groups tended to be smaller, compared with the difference in the entire population [see Section 4.1.1, Table 5].
- Results of the analysis under the conditions before the protocol amendment [see Section 4.1.1, Table 4] are shown in Table 10 and Figure 4. There was no statistically significant difference between the ensitrelvir 375/125 mg and placebo groups in the former primary endpoint "time from the start of study treatment to resolution of 12 symptoms of COVID-19 in the ITT population." In the ITT population, no clear difference was observed between the ensitrelvir 375/125 mg and placebo groups in the time to resolution of symptoms, even when 5 symptoms were used as the endpoint (Table 10).

Table 10. Time from the start of study treatment to resolution of COVID-19 symptoms (phase III part: ITT population)

		Ensitrelvir 375/125 mg	Ensitrelvir 750/250 mg	Placebo
12 symptoms	Number of subjects [a)]	582	577	572
	Number of subjects who achieved resolution	401	423	403
	Median time to resolution of COVID-19 symptoms (h)	200.0	192.1	221.5
	p values [b)c)]	0.7830	0.2903	
	Hazard ratio [95% confidence interval] [d)]	0.98 [0.85, 1.12]	1.08 [0.94, 1.23]	
5 symptoms	Number of subjects [a)]	582	575	572
	Number of subjects who achieved resolution	425	433	412
	Median time to resolution of COVID-19 symptoms (h)	189.7	177.3	200.3
	Hazard ratio [95% confidence interval] [d)]	1.03 [0.90, 1.18]	1.11 [0.97, 1.27]	

a) Subjects were excluded from analysis if all of baseline symptom scores were 0 or a baseline score(s) were missing.
b) Log-rank test stratified by time from the onset of COVID-19 symptoms to randomization (<72 hours, ≥72 hours) and vaccination status against SARS-CoV-2 (yes, no).
c) The 2-sided significance level of the entire study was 5%. Multiplicity of the hypothesis testing was adjusted by Bonferroni' s method. Comparison between ensitrelvir 375/125 mg and placebo and between ensitrelvir 750/250 mg and placebo was done at a 2-sided significance level of 2.5%.
d) Cox proportional hazard model stratified by time from the onset of COVID-19 symptoms to randomization (<72 hours, ≥72 hours) and vaccination status against SARS-CoV-2 (yes, no).

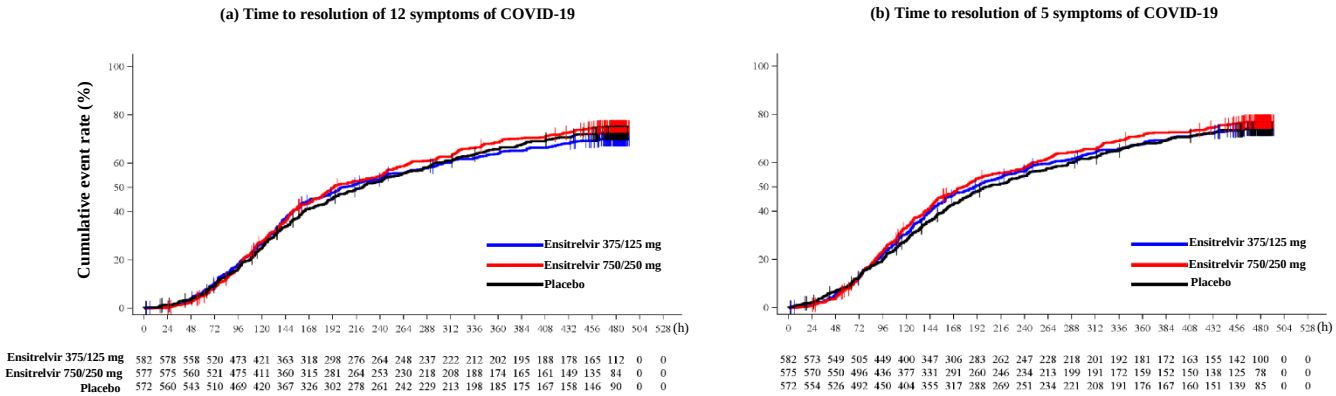


Figure 4. Cumulative rate of "time from the start of study treatment to resolution of COVID-19 symptoms" (phase III part: ITT population)

The difference in the primary endpoint between the ensitrelvir 375/125 mg and placebo groups tended to be smaller in the Japanese subpopulation than in the entire population. Further, there was no statistically significant difference in the former primary endpoint between the ensitrelvir 375/125 mg and placebo groups. The causes of these results can be discussed to a certain extent even at the current moment, but information available for assessment and discussion is limited. The results should be further evaluated based on data from the clinical study report to be prepared.

The ensitrelvir 375/125 mg group had a shorter time to resolution of 5 symptoms and tended to have a shorter time to resolution of 12 symptoms than the placebo group, when the population was limited to patients with COVID-19 who experienced the onset of symptoms within <72 hours before randomization. Based on this finding, PMDA has concluded that currently available data are enough to presume that ensitrelvir has efficacy against COVID-19. Note, however, that this is the conclusion at this moment that has been reached based on limited data for review. The presumed efficacy of ensitrelvir should be re-evaluated using additional efficacy data from the clinical study report of Study T1221. Depending on the evaluation results, appropriate actions should be taken, including considering the withdrawal of marketing approval.

This conclusion by PMDA will be discussed at the Expert Discussion.

4.R.3 Safety

The applicant's explanation about the safety profile of ensitrelvir:

Table 11 shows the summary of safety in the global phase II/III study (Study T1221).

Table 11. Summary of safety in global phase II/III study (Study T1221) (safety analysis population)

		Phase IIa part [a)]			Phase IIb part			Phase III part		
Entire population		Ensitrelvir 375/125 mg (N = 21)	Ensitrelvir 750/250 mg (N = 23)	Placebo (N = 24)	Ensitrelvir 375/125 mg (N = 140)	Ensitrelvir 750/250 mg (N = 140)	Placebo (N = 141)	Ensitrelvir 375/125 mg (N = 604)	Ensitrelvir 750/250 mg (N = 599)	Placebo (N = 605)
	Adverse events	11 (52.4)	16 (69.6)	9 (37.5)	48 (34.3)	60 (42.9)	44 (31.2)	267 (44.2)	321 (53.6)	150 (24.8)
	Adverse reactions	5 (23.8)	10 (43.5)	0	19 (13.6)	31 (22.1)	7 (5.0)	148 (24.5)	217 (36.2)	60 (9.9)
	Serious adverse reactions	0	0	0	0	0	2 (1.4)	1 (0.2)	0	1 (0.2)
	Adverse reactions resulting in death	0	0	0	0	0	0	0	0	0
	Adverse reactions leading to treatment discontinuation	0	0	0	2 (1.4)	0	0	4 (0.7)	6 (1.0)	2 (0.3)
Japanese subpopulation		Ensitrelvir 375/125 mg (N = 21)	Ensitrelvir 750/250 mg (N = 23)	Placebo (N = 24)	Ensitrelvir 375/125 mg (N = 138)	Ensitrelvir 750/250 mg (N = 137)	Placebo (N = 138)	Ensitrelvir 375/125 mg (N = 339)	Ensitrelvir 750/250 mg (N = 311)	Placebo (N = 320)
	Adverse events	11 (52.4)	16 (69.6)	9 (37.5)	48 (34.8)	59 (43.1)	44 (31.9)	179 (52.8)	201 (64.6)	81 (25.3)
	Adverse reactions	5 (23.8)	10 (43.5)	0	19 (13.8)	30 (21.9)	7 (5.1)	81 (23.9)	120 (38.6)	16 (5.0)
	Serious adverse reactions	0	0	0	0	0	2 (1.4)	1 (0.3)	0	0
	Adverse reactions resulting in death	0	0	0	0	0	0	0	0	0
	Adverse reactions leading to treatment discontinuation	0	0	0	2 (1.4)	0	0	3 (0.9)	5 (1.6)	1 (0.3)

n (%)

a) Since phase IIa part was conducted in Japan, the data of the entire population and the Japanese subpopulations in this part are the same.

In all parts, the incidences of adverse events and adverse reactions tended to be higher in the ensitrelvir groups than in the placebo group, and were higher in the ensitrelvir 750/250 mg group than in the ensitrelvir 375/125 mg group.

A serious adverse event was observed in 1 subject (heavy menstrual bleeding) in the ensitrelvir 375/125 mg group of phase III part; this event was unrelated to the study drug, with the outcome of "recovered." Adverse events resulting in death were not observed in any part. Adverse events leading to treatment discontinuation were observed in 2 subjects in the ensitrelvir 375/125 mg group (eczema, nausea, and headache [1 subject had 2 events]) of phase IIb part, and in 4 subjects in the ensitrelvir 375/125 mg group (eczema, vomiting, rash, and hypertension in 1 subject each) and in 6 subjects in the ensitrelvir 750/250 mg group (rash in 2 subjects, nausea, headache, abdominal pain, presyncope, and vomiting in 1 subject each [some subjects had more than 1 event]) of the phase III part. The events in the following subjects were related to the study drug: 2 subjects in the ensitrelvir 375/125 mg group (eczema, nausea, and headache in 1 subject each [1 subject had 2 events]) of the phase IIb part and in 2 subjects in the ensitrelvir 375/125 mg group (eczema and vomiting in 1 subject each) and in the ensitrelvir 750/250 mg group (rash in 2 subjects) of the phase III part; the outcome of these events was "recovered" or "recovering."

Subjects <18 years old included in the safety analysis population were as follows: 11 in the ensitrelvir 375/125 mg group, 16 in the ensitrelvir 750/250 mg group, and 22 in the placebo group. There were no adverse events, serious adverse events, adverse events resulting in death, or adverse events leading to treatment discontinuation that were observed only in subjects <18 years old in the ensitrelvir groups.

All subjects in phase IIa part and 98.1% (413 of 421) of subjects in phase IIb part were Japanese. In order to evaluate the safety in Japanese subjects, the applicant compared data in the Japanese

subpopulation and the entire population in phase III part. The incidence of adverse events in the ensitrelvir group tended to be higher in the Japanese subpopulation than in the entire population, but the incidences of adverse reactions, serious adverse events, and adverse events leading to treatment discontinuation did not differ significantly between the entire population and the Japanese subpopulation.

After the database lock of phase IIb part, the applicant found that data from the following subjects had not been entered into EDC: 1 subject (neck pain) in the ensitrelvir 750/250 mg group and 2 subjects (blood creatine phosphokinase increased and chest discomfort in 1 subject each) in the placebo group. Since all events were nonserious and unrelated to the study drug, the applicant concluded that this had no significant effect on the safety evaluation of ensitrelvir.

Thus, the safety profile of ensitrelvir is considered to be tolerable, with no particular safety concerns.

PMDA's view:
In the global phase II/III study (Study T1221), the incidences of adverse events and adverse reactions tended to be higher in the ensitrelvir groups than in the placebo group. In particular, high density lipoprotein decreased (HDL cholesterol decreased) was observed frequently in the ensitrelvir groups (Table 12). Incidences of lipid-related events, including high density lipoprotein decreased, are shown in Table 12. All of them are nonserious and unlikely to cause significant clinical concerns. Nevertheless, the package insert should include a precautionary statement regarding high density lipoprotein decreased because lipid-related events occurred in the clinical study.

Table 12. Lipid-related adverse events observed in ≥1 subject receiving ensitrelvir in global phase II/III study (Study T1221) [a)]

Event terms	Adverse events			Adverse reactions		
	Ensitrelvir 375/125 mg (N = 763)	Ensitrelvir 750/250 mg (N = 759)	Placebo (N = 766)	Ensitrelvir 375/125 mg (N = 763)	Ensitrelvir 750/250 mg (N = 759)	Placebo (N = 766)
All events [b)]	325 (42.6)	395 (52.0)	205 (26.8)	172 (22.5)	255 (33.6)	67 (8.7)
High density lipoprotein decreased	222 (29.1)	281 (37.0)	30 (3.9)	127 (16.6)	185 (24.4)	9 (1.2)
Blood triglycerides increased	50 (6.6)	85 (11.2)	33 (4.3)	17 (2.2)	39 (5.1)	17 (2.2)
Blood cholesterol decreased	20 (2.6)	30 (4.0)	3 (0.4)	8 (1.0)	12 (1.6)	1 (0.1)
Low density lipoprotein increased	6 (0.8)	3 (0.4)	4 (0.5)	2 (0.3)	1 (0.1)	3 (0.4)
Dyslipidaemia	2 (0.3)	3 (0.4)	1 (0.1)	2 (0.3)	3 (0.4)	0
Blood cholesterol increased	2 (0.3)	2 (0.3)	3 (0.4)	0	1 (0.1)	0
Hypertriglyceridaemia	2 (0.3)	2 (0.3)	2 (0.3)	1 (0.1)	2 (0.3)	1 (0.1)
Low density lipoprotein decreased	0	1 (0.1)	2 (0.3)	0	1 (0.1)	2 (0.3)
Blood triglycerides decreased	0	1 (0.1)	0	0	1 (0.1)	0

n (%), MedDRA ver.24.0

a) Combined data from phase IIa, IIb, and III parts. Of the subjects in the safety analysis population of phase IIa part, 9 subjects without COVID-19 symptoms (2 in the ensitrelvir 375/125 mg group, 3 in the ensitrelvir 750/250 mg group, and 4 in the placebo group) were excluded from the analysis. Among the subjects excluded, 2 in the ensitrelvir 750/250 mg group had lipid-related adverse events (high density lipoprotein decreased in 2 subjects, blood triglycerides increased in 1 subject [1 subject had 2 events]).

b) All events regardless of relation to lipid

The following findings and pharmacological effects were observed in the nonclinical studies: decreased blood cholesterol, decreased erythrocyte parameters, increased blood bilirubin, prolonged blood clotting and bleeding tendency, adenosine uptake-inhibitory activity [see Sections 5.R.1 to 3, Section 5, and

Section 3.R.3 of Review Report (1)]. PMDA confirmed that there were no serious adverse events of immediate safety concerns related to these findings and pharmacological effects.

The above review and the occurrences of adverse events in the global phase II/III study (Study T1221) show that ensitrelvir has not indicated any significant safety concerns in patients with COVID-19, and that the main adverse event occurring frequently in both the entire population and the Japanese subpopulation was high density lipoprotein decreased. Accordingly, PMDA considers that ensitrelvir is well-tolerated. PMDA also considers that the safety risk of ensitrelvir is controllable by including an appropriate precautionary statement in the package insert based on the information obtained from Study T1221 and by taking the actions listed below. Experience with ensitrelvir therapy in pediatric patients is limited, and particularly ensitrelvir has never been administered to pediatric patients weighing <40 kg; therefore, safety data should be collected after the market launch and new findings should be provided appropriately to healthcare professionals.

- The nonclinical studies showed findings suggestive of teratogenicity in fetuses, indicating a potential teratogenic risk of ensitrelvir. Therefore, ensitrelvir should be contraindicated in pregnant or possibly pregnant women [see Section 5.R.6 of Review Report (1)].
- As ensitrelvir inhibits CYP3A, it may interact with other drugs. Appropriate caution should be raised [see Section 6.R.6 of Review Report (1) and Section 1.R of Second Review Report].

This conclusion by PMDA will be discussed at the Expert Discussion.

4.R.4 Clinical positioning and indication

The applicant's explanation about the clinical positioning of ensitrelvir.

Phase III part of the global phase II/III study (Study T1221) enrolled patients with COVID-19 regardless of risk factors for severe COVID-19. It turned out that approximately 30%[12)] of the subjects enrolled had risk factors for severe COVID-19. Results in this part demonstrated the efficacy of ensitrelvir 375/125 mg against COVID-19 [see Section 4.R.2]. Thus ensitrelvir serves as an oral antiviral drug that can be administered to patients with COVID-19 regardless of vaccination status[13)] or risk factors for severe COVID-19. The proposed indication is COVID-19.

PMDA's view:

Patients who have mild to moderate I[14)] symptoms of COVID-19 were eligible for enrollment in phase III part of the global phase II/III study (Study T1221). Accordingly, ensitrelvir offers a treatment option for this patient population. Also, ensitrelvir can be administered regardless of vaccination status or risk

[12)] The following are main risk factors for severe COVID-19 identified in the subjects (i.e., those who experienced the onset of COVID-19 symptoms within <72 hours before randomization in the ITT population):
Smoking (ensitrelvir 375/125 mg, 15.9% [55 of 347 subjects]; ensitrelvir 750/250 mg, 16.5% [56 of 340 subjects]; and placebo, 14.0% [48 of 343 subjects])
Dyslipidaemia (ensitrelvir 375/125 mg, 8.4% [29 of 347 subjects]; ensitrelvir 750/250 mg, 5.0% [17 of 340 subjects]; and placebo, 7.6% [26 of 343 subjects])
BMI ≥30 kg/m^2 (ensitrelvir 375/125 mg, 6.6% [23 of 347 subjects]; ensitrelvir 750/250 mg, 4.4% [15 of 340 subjects]; and placebo, 3.5% [12 of 343 subjects])
Hypertension (ensitrelvir 375/125 mg, 6.3% [22 of 347 subjects]; ensitrelvir 750/250 mg, 2.9% [10 of 340 subjects]; and placebo, 4.1% [14 of 343 subjects])

[13)] In the ITT population, approximately 90% of subjects who experienced the onset of COVID-19 within <72 hours before randomization had received a vaccine against COVID-19.

[14)] Guidelines for Diagnosis and Treatment of COVID-19, ver. 8.1, by the Ministry of Health, Labour and Welfare

factors for severe COVID-19. The package insert should state that ensitrelvir has been evaluated for its effect on the symptoms of COVID-19. On the basis of the review presented in Section 4.R.2, the package insert should include a precautionary statement that the efficacy of ensitrelvir against COVID-19 was demonstrated in patients who experienced the onset of symptoms within <72 hours before the start of treatment. The proposed indication of ensitrelvir (i.e., COVID-19) is acceptable.

This conclusion by PMDA will be discussed at the Expert Discussion.

4.R.5 Dosage and administration

On the basis of the review presented in Sections 6.R.2 and 6.R.3 of Review Report (1) and the discussion on the efficacy and safety based on results from phase III part of the global phase II/III study (Study T1221) [Sections 4.R.2 and 4.R.3], PMDA has concluded that the following dosage and administration is acceptable:

Ensitrelvir 375 mg on Day 1 and ensitrelvir 125 mg from Days 2 to 5, administered orally once daily in ≥12-year-old pediatric patients and adults.

This conclusion by PMDA will be discussed at the Expert Discussion.

5. Overall Evaluation during Preparation of the Review Report (3)

On the basis of the data submitted, PMDA has concluded that currently available data are enough to presume that ensitrelvir has efficacy against COVID-19, taking account of the following finding:

In phase III part of the global phase II/III study (Study T1221), the ensitrelvir 375/125 mg group had a shorter time to resolution of 5 symptoms and tended to have a shorter time to resolution of 12 symptoms than the placebo group, when the population was limited to patients with COVID-19 who experienced the onset of symptoms within <72 hours before randomization.

Note, however, that this is the conclusion at this moment that has been reached based on limited data for review. The presumed efficacy of ensitrelvir should be re-evaluated using additional efficacy data from the clinical study report of Study T1221. Depending on the evaluation results, appropriate actions should be taken, including considering the withdrawal of marketing approval.

As for the safety of ensitrelvir, PMDA considers that the safety risk of ensitrelvir can be controlled by issuing appropriate alerts regarding the risk of teratogenicity, drug interactions, and others. Experience with ensitrelvir therapy in pediatric patients is limited, and particularly ensitrelvir has never been administered to pediatric patients weighing <40 kg; therefore, safety data should be collected after the market launch and new findings should be provided appropriately to healthcare professionals.

6. Other

6.1 Main changes made to the study protocol during the conduct of phase III part of global phase II/III study (Study T1221)

Main changes made to the study protocol during the conduct of phase III part of Study T1221:

Protocol ver. 7 (amended on February 7, 2022)	
Doses for efficacy evaluation	Ensitrelvir 375/125 mg and ensitrelvir 750/250 mg
Primary endpoint	Time from the start of study treatment to recovery [a)] from 12 symptoms [b)] of COVID-19
Primary efficacy population	Subjects with ≥3 moderate symptoms in the ITT2 population (ITT2 population consisted of randomized subjects who tested positive by RT-PCR on nasopharyngeal swab either at Visit 1 [before the start of treatment] or Visit 2)
Sample size required	1,260 subjects (420 per group)
Justification for sample size	The median time to recovery from symptoms was assumed to be 7 days in the placebo group and 5.5 days in both ensitrelvir groups (hazard ratio to the placebo group assumed to be 1.27). The drop-out rate due to a negative RT-PCR test performed immediately before enrollment was assumed to be approximately 10%. With these assumptions, the sample size required to ensure 80% statistical power by log-rank test with a 2-sided significance level of 2.5% was calculated to be 1,128 patients with COVID-19 who have ≥3 moderate symptoms in the 3 groups combined (376 patients per group).
Analytical method for the primary endpoint	Stratified log-rank test
Method for adjusting multiplicity of hypothesis testing	Bonferroni's method was used. The 2-sided significance level was 2.5% for the comparison between each ensitrelvir group and the placebo group in the analysis of the primary endpoint.
Interim analysis	An interim analysis is conducted for early termination for efficacy when follow-up is completed in 50% of the target number of subjects. Multiplicity of hypothesis testing in the interim analysis is adjusted by O'Brien–Fleming type α-spending function.
Protocol ver. 8 (amended on April 5, 2022)	
Doses for efficacy evaluation	Ensitrelvir 375/125 mg and ensitrelvir 750/250 mg
Primary endpoint	Time from the start of study treatment to resolution [c)] of 12 symptoms [b)] of COVID-19
Primary efficacy population	ITT1 population (randomized subjects with a positive SARS-CoV-2 viral titer in baseline nasopharyngeal swab)
Sample size required	1,785 subjects (595 per group)
Justification for sample size	The median time to resolution of symptoms was assumed to be 10 days in the placebo group and 8 days in both ensitrelvir groups (hazard ratio to the placebo group assumed to be 1.25). The drop-out rate due to a negative SARS-CoV-2 viral titer at Visit 1 (before administration) was assumed to be 20%. With these assumptions, the sample size required to ensure 80% statistical power by log-rank test at a 2-sided significance level of 2.5% was calculated to be 1,785 patients with COVID-19 in the 3 groups combined (595 patients per group).
Analytical method for the primary endpoint	Stratified log-rank test
Method for adjusting multiplicity of hypothesis testing	Bonferroni's method was used. The 2-sided significance level was 2.5% for the comparison between each ensitrelvir group and the placebo group in the analysis of the primary endpoint.
Interim analysis	Description on the interim analysis was deleted because, based on the progress of enrollment, the target sample size (1,785) was expected to be reached before the time of interim analysis.
Protocol ver. 9 (amended on July 8, 2022)	
Doses for efficacy evaluation	Ensitrelvir 375/125 mg and ensitrelvir 750/250 mg
Primary endpoint	Time from the start of study treatment to resolution [c)] of 12 symptoms [b)] of COVID-19
Primary efficacy population	ITT population
Sample size required	1,590 subjects (530 per group)
Justification for sample size	The median time to resolution of symptoms was assumed to be 10 days in the placebo group and 8 days in both ensitrelvir groups (hazard ratio to the placebo group assumed to be 1.25). The drop-out rate due to a negative RT-PCR test performed immediately before enrollment was assumed to be 10%. With these assumptions, the sample size required to ensure 80% statistical power by log-rank test at a 2-sided significance level of 2.5% was calculated to be 1,590 patients with COVID-19 in the 3 groups combined (530 patients per group).
Analytical method for the primary endpoint	Stratified log-rank test
Method for adjusting multiplicity of hypothesis testing	Bonferroni's method was used. The 2-sided significance level was 2.5% for the comparison between each ensitrelvir group and the placebo group in the analysis of the primary endpoint.

Protocol ver. 10 (amended on September 20, 2022)	
Dose for efficacy evaluation	Ensitrelvir 375/125 mg
Primary endpoint	Time from the start of study treatment to resolution [c)] of 5 symptoms [d)] of COVID-19
Primary efficacy population	Only in subjects who experienced the onset of COVID-19 symptoms within <72 hours before randomization in the ITT population
Sample size required	780 subjects (260 per group)
Justification for sample size	Weibull distribution was assumed (median Weibull distribution assumed to be 8.3 days in the ensitrelvir 375/125 mg group and 11.1 days in the placebo group), based on the Kaplan-Meier curve of the time to resolution of symptoms in subjects who experienced the onset of COVID-19 symptoms within <72 hours before randomization in phase IIb part. The drop-out rate due to a negative RT-PCR test performed immediately before enrollment was assumed to be 10%. With these assumptions, the number of subjects required to ensure 80% statistical power by Peto-Prentice's generalized Wilcoxon test at a 2-sided significance level of 5%, was calculated to be 780 patients with COVID-19 who experienced the onset of symptoms within <72 hours before randomization in the 3 groups combined (230 patients per group).
Analytical method for the primary endpoint	Stratified Peto-Prentice's generalized Wilcoxon test
Method for adjusting multiplicity of hypothesis testing	Since only the 375/125 mg dose is used, adjustment for the multiplicity of hypothesis testing is not required.

a) "Recovery" was defined as the condition in which all 12 symptoms met the following criteria:
- Symptoms that had been present before the onset of COVID-19 and were considered by the subject to have worsened at baseline (at test before administration): Improved severity from baseline (i.e., from severe to moderate or better, from moderate to mild or better) is maintained for at least 24 hours.
- Symptoms that had been present before the onset of COVID-19 and were NOT considered by the subject to have worsened at baseline (at test before administration): Improved severity from baseline or the same severity as baseline (i.e., from severe to severe or better, from moderate to moderate or better) is maintained for at least 24 hours.
- Other symptoms (i.e., symptoms that were not present before the onset of COVID-19 and developed after baseline [test before administration]): A mild or asymptomatic condition is maintained for at least 24 hours.

b) The 12 symptoms caused by SARS-CoV-2: (1) Malaise or tiredness, (2) muscle or body aches, (3) headache, (4) chills or sweating, (5) feverish or pyrexia, (6) stuffy or runny nose, (7) sore throat, (8) cough, (9) shortness of breath (dyspnea), (10) nausea, (11) vomiting, and (12) diarrhea

c) See Section 4.1.1 for the definition of "resolution."

d) The 5 symptoms caused by SARS-CoV-2: (1) Malaise or tiredness, (2) feverish or pyrexia, (3) stuffy or runny nose, (4) sore throat, and (5) cough

Review Report (4)

November 15, 2022

Product Submitted for Approval

Brand Name	Xocova Tablets 125 mg
Non-proprietary Name	Ensitrelvir Fumaric Acid
Applicant	Shionogi & Co., Ltd.
Date of Application	February 25, 2022

List of Abbreviations
See Appendix.

1. Content of the Review

Comments made during the Expert Discussion and the subsequent review conducted by the Pharmaceuticals and Medical Devices Agency (PMDA) are summarized below. The expert advisors present during the Expert Discussion were nominated based on their declarations etc., concerning the product submitted for marketing approval, in accordance with the provisions of the Rules for Convening Expert Discussions etc. by Pharmaceuticals and Medical Devices Agency (PMDA Administrative Rule No. 8/2008 dated December 25, 2008).

At the Expert Discussion, the expert advisors supported PMDA's conclusions presented in Sections "4.R.2 Efficacy," "4.R.3 Safety," and "4.R.5 Dosage and Administration" of Review Report (3).

1.1 Clinical positioning

At the Expert Discussion, the expert advisors supported PMDA's conclusions presented in Section "4.R.4 Clinical positioning and indication" of Review Report (3), and made the following additional comments:

- The package insert will include a precautionary statement on the timing of starting treatment. The package insert should clearly state that the reason for issuing the statement is not due to safety concerns, but due to the fact that the efficacy was presumed in patients who received the treatment on or before Day 3 after the onset of symptoms (Day 0).
- Ensitrelvir is expected to be used in a wide range of patients with COVID-19, in view of the population treated (those with mild to moderate I symptoms regardless of risk factors of severe COVID-19) in phase III part of the global phase II/III study (Study T1221). At present, there is no particular concern for resistance acquisition or adverse events, but these concerns may materialize when ensitrelvir is used widely. Physicians should be advised to carefully examine the necessity of using ensitrelvir, based on a full understanding of the primary endpoint and results in phase III part of the global phase II/III study (Study T1221).
- Data on severe COVID-19 (i.e., death and hospitalization) in patients using ensitrelvir in routine clinical practice should be collected through the use results survey.

Based on the comments raised in the Expert Discussion, PMDA instructed the applicant to add the following precautionary statements in the package insert and to collect data on severe COVID-19 in the use-results survey. The applicant agreed.

Precautions Concerning Indication

The necessity of ensitrelvir therapy should be carefully examined by a physician with a full understanding of the information presented in Section "17. Clinical Studies" and of the efficacy and safety of ensitrelvir.

Precautions Concerning Dosage and Administration

Ensitrelvir should be administered promptly after the onset of COVID-19 symptoms. Ensitrelvir is presumed to have efficacy when administered within 3 days after the onset of symptoms.

1.2 Risk management plan (draft)

In view of the discussions presented in Review Report, Second Review Report, and Review Report (3), and comments from the expert advisors at the Expert Discussion, PMDA has concluded that, in addition to collecting information through usual pharmacovigilance activities, the risk management plan should include the safety and efficacy specifications presented in Table 13, and that the applicant should conduct additional pharmacovigilance activities, efficacy survey and studies, and additional risk minimization activities presented in Tables 14 and 15.

Table 13. Safety and efficacy specifications in the risk management plan (draft)

Safety specification		
Important identified risks	Important potential risks	Important missing information
None	Teratogenicity	Safety in patients with moderate or severe hepatic impairment
Efficacy specification		
Efficacy in phase III part of global phase II/III study (Study T1221)		

Table 14. Summary of additional pharmacovigilance activities, efficacy survey and studies, and additional risk minimization activities included under the risk management plan (draft)

Additional pharmacovigilance activities	Efficacy survey and studies	Additional risk minimization activities
• Early post-marketing phase vigilance • Use-results survey • Post-marketing clinical study (A clinical pharmacological study in patients with hepatic impairment)	• Post-marketing clinical study (global phase II/III study [Study T1221])	• Provide information to patients and enhance their understanding before starting treatment (through an informed consent form) • Information materials for healthcare professionals (teratogenicity) • Disseminate data gathered during early post-marketing phase vigilance

Table 15. Outline of use-results survey (draft)

Objective	To evaluate the safety of ensitrelvir in routine clinical practice
Survey method	Consecutive patients
Population	Patients treated with ensitrelvir
Observation period	28 days after the start of treatment [a)]
Planned sample size	3,000
Key survey items	Patient characteristics, exposure to ensitrelvir, concomitant drugs, symptoms before and after treatment, adverse events, hospitalization or death after the start of treatment[a)]

a) Based on the comments made by the expert advisors at the Expert Discussion, the observation period was changed from 14 days to 28 days, and "hospitalization or death after the start of treatment" was added to survey items.

2. Overall evaluation

On the basis of the above review, PMDA has concluded that currently available data are enough to presume that the product has efficacy against COVID-19, taking account of the following finding:

> In phase III part of the global phase II/III study (Study T1221), the ensitrelvir 375/125 mg group had a shorter time to resolution of 5 symptoms and tended to have a shorter time to resolution of 12 symptoms than the placebo group, when the population was limited to patients with COVID-19 who experienced the onset of symptoms within <72 hours before randomization.

Note, however, that this is the conclusion at this moment that has been reached based on limited data for review. The appropriateness of the presumed efficacy of the product should be re-evaluated by additional analyses on efficacy and, based on the results, appropriate actions should be taken, including considering the withdrawal of marketing approval.

As for the safety of the product, PMDA considers that the safety risk of the product can be controlled by issuing appropriate alerts regarding the risk of teratogenicity, drug interactions, etc. Experience with treatment with the product in pediatric patients is limited, and particularly the product has never been administered to pediatric patients weighing <40 kg; therefore, safety data should be collected after the market launch and new findings should be provided appropriately to healthcare professionals.

The applicant should reassess the appropriateness of the presumed efficacy of the product based on new data in the clinical study report of phase III part of the global phase II/III study (T1221) and, according to the results, should comprehensively assess the benefits and risks of the product. In order to ensure sufficient time for the applicant to conduct the assessments, the effective period of the Emergency Approval (based on Article 14-2-2, Paragraph 1 of the Pharmaceuticals and Medical Devices Act) of the product should be 1 year.

If the product is approved, the proposed indication and dosage and administration should be modified as follows and the following approval conditions should apply. The product is not classified as a biological product or a specified biological product. Both the drug product and its drug substance are classified as powerful drugs.

Indication

Disease caused by SARS-CoV-2 infection (COVID-19)

(No change)

Dosage and Administration

The usual dose in ≥12-year-old pediatric patients and adults is ensitrelvir 375 mg on Day 1 and ensitrelvir 125 mg from Days 2 to 5, administered orally once daily~~, for a total of 5 days~~.

(The underlined words are added to, and the strikethrough words are deleted from, the proposed text.)

Approval Conditions

1. The applicant is required to develop and appropriately implement a risk management plan.
2. The applicant is required to request that physicians administer the product only to patients considered eligible for treatment with the product who, or whose legally acceptable representatives, have been provided with the efficacy and safety information of the product in written form, and have provided written informed consent before the treatment.
3. The applicant is required to promptly compile and submit data showing the efficacy of the product in phase III part of the global phase II/III study (Study T1221).

Appendix

List of Abbreviations

ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
BMI	Body mass index
EC_{50}	50% effective concentration
HDL	High density lipoprotein
MedDRA	Medical Dictionary for Regulatory Activities
PCR	Polymerase chain reaction
Pharmaceuticals and Medical Devices Act	Act on Securing Quality, Efficacy and Safety of Products Including Pharmaceuticals and Medical Devices (Act No. 145 of 1960)
PMDA	Pharmaceuticals and Medical Devices Agency
RNA	Ribonucleic acid
RT-PCR	Reverse transcription PCR
SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2
Study T1211	Study 2102T1211
Study T1221	Study 2108T1221
TMPRSS2	Transmembrane protease, serine 2
γ-GTP	Gamma-glutamyltransferase