EU RISK MANAGEMENT PLAN FOR ZOKOVEA

(ensitrelvir, S-217622)

RMP Version to be Assessed as Part of this Application:
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QPPV Name: Valentina Mancini

QPPV Signature:

The content of this RMP has been reviewed and approved by the MAH's QPPV. The final version of this document has been electronically signed, and electronic signature certificate is held on file.

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PART I: PRODUCT(S) OVERVIEW

Active substance(s) (INN or common name)	Ensitrelvir
Pharmacotherapeutic group(s) (ATC Code)	J05AE16
Marketing Authorisation Applicant	Shionogi B.V Herengracht 464 1017 CA, Amsterdam the Netherlands
Medicinal products to which this RMP refers	ZOKOVEA 125 mg Tablets
Invented name(s) in the European Economic Area (EEA)	ZOKOVEA
Marketing authorisation procedure	Centralised
Brief description of the product	Chemical class Oral antiviral drug
	Summary of mode of action: Ensitrelvir (S-217622) exerts the antiviral effect against the SARS-CoV-2 by inhibiting 3C-Like protease that is essential for the processing of the polyprotein encoded by the SARS-CoV-2 gene as well as the replication of the virus
	Important information about its composition: None
Hyperlink to the Product Information	EU SmPC
Indication(s) in the EEA	Current: The post-exposure prophylaxis of coronavirus disease 2019 (COVID-19) in adults and adolescents aged 12 years and older.
	Proposed (if applicable): Not applicable
Dosage in the EEA	Current: 375 mg orally once daily on day 1 and 125 mg from days 2 to 5 for a total of 5 days
	Proposed (if applicable): Not applicable

Pharmaceutical form(s) and strengths	Current (if applicable): Approximately 9 mm round, white to light yellow white, tablets debossed on one side with the company logo and “711”, and “125” on the other side.
	Proposed (if applicable): not applicable
Is/will the product be subject to additional monitoring in the EU?	Yes

LIST OF ABBREVIATIONS

Abbreviation	Description
ACE2	angiotensin-converting enzyme 2
APTT	activated partial thromboplastin time
AST	aspartate aminotransferase
AUC	area under the plasma concentration-time curve
$AUC_{0\text{-}24\ (48,\ 144)\ hr}$	area under the plasma concentration-time curve from time 0 to 24 (48, 144) hours post-dose
$AUC_{0\text{-}inf}$	area under the plasma concentration-time curve from time 0 to infinity
$AUC_{0\text{-}last}$	area under the plasma concentration-time curve from time zero to the time of the last quantifiable concentration after dosing
$AUC_{0\text{-}\tau}$	area under the concentration-time curve over the dosing interval τ (24 hours)
AUC_{all}	area under the mean concentration curve until 48 hours after first administration
BA	Bioavailability
$C_{24\ (48)\ hr}$	plasma concentration at 24 (48) hours after first administration
CC_{50}	concentration achieving 50% of cytotoxicity
CI	confidence interval
CL/F	apparent total clearance
CL_R	renal clearance
C_{max}	maximum plasma concentration
COVID-19	coronavirus disease 2019
CYP	cytochrome P450
D.Bil	direct bilirubin
DDI	drug-drug interaction
DMSO	dimethyl sulfoxide
EC_{50}	half maximal (50%) effective concentration
ECG	Electrocardiogram
EMA	European Medicines Agency
Fbg	Fibrinogen
FDA	Food and Drug Administration
GVP	Good Pharmacovigilance Practice
hAEC	human airway epithelial cells
HDL	high-density lipoprotein
hERG	human ether-a-go-go related gene
I.Bil	indirect bilirubin
LDH	lactate dehydrogenase
LDL	low-density lipoprotein
MERS-CoV	middle east respiratory syndrome coronavirus
mRNA	messenger RNA
NOAEL	no-observed adverse effect level
OAT	organic anion transporter
OATP	organic anion transporter polypeptide
OCT	organic cation transporter
$PA\text{-}EC_{50\ (90)}$	protein-adjusted $EC_{50\ (90)}$
PK	pharmacokinetic(s)
PND	postnatal day

Abbreviation	Description
QTc	correct QT interval
SARS-CoV	severe acute respiratory syndrome coronavirus
SARS-CoV-2	severe acute respiratory syndrome coronavirus 2
SD	standard deviation
$t_{1/2,z}$	terminal elimination half-life
T.Bil	total bilirubin
TG	Triglyceride
$Time_{High}$	total time above the target plasma concentration
T_{max}	time to maximum plasma concentration
VLDL	very low-density lipoprotein

PART II: SAFETY SPECIFICATION

PART II: MODULE SI - EPIDEMIOLOGY OF THE INDICATION AND TARGET POPULATION

Indication

Post-exposure prophylaxis of COVID-19 in adults and adolescents aged 12 years and older.

Overview of Target Disease, Incidence and Prevalence

On 30 Jan 2020, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection that causes coronavirus disease 2019 (COVID-19) was declared a public health emergency of international concern (PHEIC) by the World Health Organization (WHO). On 11 Mar 2020, the WHO determined that COVID-19 could be characterized as a pandemic (global epidemic) based on its worldwide spread and its severity [1]. The earliest cases reported of COVID-19 were in several areas in Wuhan City, Hubei Province, People's Republic of China in Dec 2019 following outbreaks of pneumonia of an unknown cause. Epidemiological studies confirmed the pneumonia was caused by the RNA virus, SARS CoV-2 [2].

The mechanisms of SARS-CoV-2 entry into host cells involves a multistep process including the binding of the SARS-CoV-2 viral envelope spike (S) protein to the angiotensin-converting enzyme 2 (ACE2) receptor expressed on the target host cell membrane with subsequent membrane fusion [3]. Angiotensin-converting enzyme 2 is expressed in nasal mucosal epithelial cells, alveolar epithelial cells, and small intestinal epithelial cells [2-4]. The ability of SARS-CoV-2 to infect a broad range of tissues explains the multiple systemic manifestations of the disease beyond respiratory tract involvement, and also the potential for inducing complications such as neurological manifestations and thromboses. The expression of ACE2 may be upregulated in some disease states, particularly if associated with endothelial dysfunction, which may explain why some comorbidities carry a higher risk of severe disease [5].

Symptoms of COVID-19 are induced by an inflammatory reaction or immune response to viral infection and include fever, cough, stuffy and runny nose, sore throat, muscle ache, diarrhoea, which are mostly similar to symptoms of the influenza with additional taste and smell disorders often reported [5]. The proportion of patients with severe disease or mortality is declining interdependently along with an increasing rate of vaccination and increasing acquired immunity due to frequent exposure. Based on the current status, the WHO terminated the PHEIC declaration on 06 May 2023 [7]; however, the threat due to SARS-CoV-2 infection has not been completely cleared since the upcoming epidemic situation remains unknown.

The incubation period of COVID-19 ranges from 2 to 14 days, with typical onset noted at approximately 5 days after exposure to SARS-CoV-2. The majority of infections present as mild to moderate in disease severity with recovery generally observed 7 days after onset of symptoms [8].

SARS-CoV-2 may be highly transmissible until 7 to 10 days after onset and this infectious period is associated with community transmission [9]. The known risk

factors for severe COVID-19 include age (65 years or older), hypertension, obesity, and smoking [10], and the risk of mortality increases with increasing age. A certain proportion of infected individuals may remain asymptomatic. This proportion in adult population can be up to 30% and the disease may be more likely to remain asymptomatic or mildly symptomatic in children [11,12]. It is known that various symptoms of COVID-19 may persist or recur for varied durations in some patients even after recovery, a condition typically referred to as 'long COVID', affecting patients' health in both the short and long term (long COVID) [13,14].

As recently stated by the WHO expert committee for COVID-19 vaccine strain selection, SARS-CoV-2 continues to circulate globally, causing severe disease, long COVID-19, and death. In the EU, the mortality due to COVID-19 was 12,593 deaths per million persons at a maximum with regional variation based on socioeconomic factors. Morbidity and mortality continue, particularly in vulnerable populations in the EU [15]. The majority of COVID-19 deaths continue to occur in individuals aged 65 years and older and those with coexisting conditions. Some countries have reported an increase in incidence of COVID-19-related hospitalizations and deaths among children under 1 year of age, as compared to young adults, although this group still accounts for a small proportion of total COVID-19 hospitalizations and deaths [1616].

Demographics of the population in the proposed indication (age, gender, racial and ethnic origin), and risk factors for the disease

Whilst early studies suggested that the risk for acute COVID-19 is modulated by age, with lower frequencies of infection in children and adolescents, relative to adults [17], more recent studies have shown that individuals of any age including children are all at similar risk of SARS-CoV-2 infection [18]. However, the risk of severe COVID-19 increases with age.

Patients with COVID-19 can experience a wide range of symptoms from mild to critical illness [19]. Mild symptoms include fever or chills, cough, shortness of breath or difficulty breathing, fatigue, muscle or body ache, headache, new loss of taste or smell, sore throat, congestion or runny nose, nausea or vomiting, diarrhoea [19].

Older adults and persons with medical conditions, including cardiovascular disease, chronic kidney disease, chronic liver disease, cancer, obesity, diabetes, pre-existing hypertension, pulmonary disease, immunosuppression, and sickle cell disease, are at increased risk of severe or critical disease [20].

Studies suggest that the number of confirmed COVID-19 cases is comparable among men and women; however, men may have more severe illness and higher mortality from COVID-19 than women [21,22]. Non-white ethnicity has been recognized as a predictor for more severe disease, and/or risk of hospitalization. However, the role of socio-economic status and comorbidities in confounding these associations remains to be clarified [23].

Current Treatment of Target Disease

Prophylaxis

Despite good efficacy of COVID-19 vaccines, breakthrough infections of fully vaccinated individuals are emerging in the general population, probably because of emergence of new variants [24]. In addition, there are some populations at high risk of infection after vaccination because of immunosuppression, either because of concomitant use of immunosuppressive drugs or diseases associated with immunosuppression [25,26,27]. Data have shown that approximately 3% of the population are unable to mount an optimal immune response to COVID-19 vaccines and are not receiving the full benefit of vaccination [28].

A number of antibody therapies eg, RONAPREVE (casirivimab and imdevimab) as pre-exposure prophylaxis and/or post- exposure prophylaxis have been approved in Europe. However, their activity is subject to molecular variations in the virus.

Post-exposure prophylaxis (PEP) of COVID-19

The following list provides an overview of those drugs used in pre-or post-exposure prophylaxis of COVID-19. Please refer to local product information for the latest detailed information. (Drugs solely indicated for the treatment of COVID-19 are not listed.)

- Casirivimab and imdevimab (genetic recombination) (anti-SARS-CoV-2 neutralizing antibody drug)
  Indication: the treatment of COVID-19 in adults and adolescents aged 12 years and older weighing at least 40 kg who do not require supplemental oxygen and who are at increased risk of progressing to severe COVID-19 and prevention of COVID-19 in adults and adolescents aged 12 years and older weighing at least 40 kg. (EU MAA withdrawn Dec 2025)
- Sipavibart
  Indication: the pre-exposure prophylaxis of COVID-19 in adults and adolescents 12 years of age and older weighing at least 40 kg and who are immunocompromised due to a medical condition or receipt of immunosuppressive treatments.
- Tixagevimab and cilgavimab (anti-SARS-CoV-2 neutralizing antibody drug)
  Indication: the treatment and pre-exposure prophylaxis of COVID-19 in adults and adolescents aged 12 years and older weighing at least 40 kg. (EU MAA withdrawn Sep 2025)

Natural history of COVID-19 in the untreated population, including mortality and morbidity

COVID-19 can be classified as asymptomatic or pre-symptomatic infection, or mild, moderate, severe symptomatic disease, and critical illness. Transmission of SARS-CoV-2 may occur from pre-symptomatic, through to critically ill patients [29]. Asymptomatic infection is estimated to occur in 40% to 45% of individuals [30].

The majority of symptomatic patients have mild to moderate disease. A meta-analysis including more than 280,000 persons from 11 countries/regions estimated the proportion of individuals with severe (and critical) disease as 22.9% [31], but currently this number is believed to be significantly lower.

The prognosis of COVID-19 is largely dependent on various factors that include the patient's age, the severity of illness at presentation, pre-existing conditions, how quickly treatment can be implemented, and response to treatment. WHO estimated the global case fatality rate for COVID-19 to be 2.2%. However, the case fatality rate is affected by factors such as age, underlying pre-existing conditions, and severity of illness and current values in Europe are significantly lower.

Complications associated with COVID-19

COVID-19 can be regarded as a systemic viral illness based on its involvement in multiple major organ systems [29].

The most common complication of severe COVID-19 illness is progressive or sudden clinical deterioration leading to acute respiratory failure and ARDS and/or multiorgan failure leading to death.

Other complications include cardiac injury including myocardial infarction, thrombotic complications including pulmonary embolism and acute stroke, probably as a result of hypercoagulability developing in severe COVID-19. Various gastrointestinal pathologies including ischaemia can occur as can renal impairment including renal failure [29].

A multisystem inflammatory syndrome with clinical features similar to those of Kawasaki disease and toxic shock syndrome has been described in children with COVID-19 [32,33]. A similar syndrome has also been reported in adults following COVID-19 [34].

Long-term complications of COVID-19 following recovery from the acute illness of any severity (so-called Long COVID), can affect up to 1 in 5 people (data representative of 2021 - now they are believed to be lower). Although sequalae are chronic and often debilitating, Long COVID remains poorly characterized in current COVID-19 prevention and treatment strategies [35,36]. Multiple organ systems can be affected, including respiratory, cardiovascular, nervous system, musculoskeletal, cutaneous and neuropsychiatric manifestations [35,36].

Important comorbidities

There are no known comorbidities coexisting within the target population that are deemed to be clinically relevant or have an impact on the administration of ensitrelvir.

PART II: MODULE SII - NON-CLINICAL PART OF THE SAFETY SPECIFICATION

Toxicology

Single Dose Toxicity

Although independent single-dose toxicity studies to evaluate the acute toxicity of S-217622 fumaric acid were not conducted, acute toxicity was evaluated based on the results of exploratory single-dose TK studies in rats and monkeys, and the results on the first day of dosing in 2-week repeated oral administration toxicity studies in rats and monkeys, and in vivo micronucleus study in rats. In rat 2-week repeated oral administration toxicity study and micronucleus study, no death or moribund occurred on the first day of dosing up to the highest doses of 1000 and 2000 mg/kg, respectively. In a 2-week repeated oral administration toxicity study in monkeys, no death or moribund occurred on the first day of dosing up to the highest dose of 1000 mg/kg/day (male) or 300 mg/kg/day (female), but vomiting and a decrease of food consumption were observed in males. The approximate lethal doses for rats and monkeys were determined to be greater than 2000 and 1000 mg/kg, respectively.

When S-217622 fumaric acid was administered to rats at a dose of 1000 mg/kg/day, C_{max} and AUC024hr values (294 to 375 µg/mL and 5400 to 6720 µg·hr/mL) of S-217622 in plasma on the first day of dosing were both 10 to 13 times higher than C_{max} and AUC0-24hr values (28.1 µg/mL and 518.3 µg·hr/mL) in the clinical dosing regimen of S-217622 at 375/125 mg (375 mg for loading dose on Day 1, 125 mg for maintenance dose from Days 2 to 5 once daily), respectively.

In addition, when S-217622 fumaric acid was administered to male monkeys at a dose of 1000 mg/kg/day, and to female at a dose of 300 mg/kg/day, C_{max} and $AUC_{0\text{-}24hr}$ values (433 to 520 µg/mL and 8920 to 10700 µg·hr/mL) of S-217622 in plasma on the first day of dosing were 15 to 19 times and 17 to 21 times higher than C_{max} and $AUC_{0\text{-}24hr}$ values in the clinical dosing regimen of S-217622 at 375/125 mg, respectively.

Repeated Dose Toxicity

A summary of the findings observed in repeated oral toxicity studies in rats and monkeys are shown in Table SII.1. The plasma exposure at the non observed adverse effect level (NOAEL) (C_{max} and $AUC_{0\text{-}24hr}$) in each study and ratio to the exposure in the clinical dosing regimen of S-217622 at 375/125 mg in Table SII.2.

In the 2-week (dose levels: 20, 100, and 1000 mg/kg/day) and 4-week (dose levels: 20, 50, and 1000 mg/kg/day) repeated oral toxicity studies in rats, no adverse effects were observed up to the dose of 1000 mg/kg/day, and NOAEL was judged to be 1000 mg/kg/day in any of studies (Table SII.1). Plasma exposure (C_{max} and $AUC_{0\text{-}24hr}$) of S-217622 at the NOAEL (1000 mg/kg/day) in 4week repeated oral toxicity study in rats have sufficient difference to C_{max} and $AUC_{0\text{-}24hr}$ values (28.1 µg/mL and 518.3 µg·hr/mL) in the clinical dosing regimen of S217622 at 375/125 mg, which were 9 to 13 times and 8 to 10 times higher, respectively (Table SII.2).

In the 2-week oral toxicity study in monkeys (doses levels for male: 10, 50, or 1000/300/100 mg/kg/day [dose level reduction from 1000 to 300 mg/kg/day on Day 3, and from 300 to 100 mg/kg/day on Day 8 of dosing], dose levels for female: 10, 50, or 300/100 mg/kg/day [dose level reduction from 300 to 100 mg/kg/day on Day 9 of dosing]), and in the 4-week oral toxicity study in monkeys (dose levels for male: 3, 10, or 30 mg/kg/day), adverse effects attributable to S-217622 fumaric acid included the following: death/moribund, vomiting, decrease in body weight and food consumption, and decreased erythrocytes parameters and platelet counts, and

inflammatory changes in multiple organs accompanied by changes in blood inflammatory markers (Table SII.1). Plasma exposures (C_{max} and AUC_{0-24hr}) of S217622 at the NOAEL (10 mg/kg/day) in the 4-week oral toxicity study in monkeys were 2.7 to 3.4 times and 2.3 to 3.6 times higher than plasma exposures (C_{max}: 28.1 µg/mL, AUC_{0-24hr} 518.3 µg·hr/mL) in the clinical dosing regimen of S-217622 at 375/125 mg, respectively (Table SII.2).

In the 2-week repeated oral toxicity study in monkeys, death or moribund occurred in males administered at a dose of 1000 mg/kg/day, and at a reduced dose of 1000 mg/kg/day to 300 mg/kg/day, and in a female at a dose of 300 mg/kg/day. In addition, there were animals required veterinary care due to continuous reduction of food consumption at doses of 1000/300/100 mg/kg/day (male) and 300/100 mg/kg/day (female) in the 2-week repeated oral toxicity study in monkeys, and 30 mg/day of the 4-week repeated oral toxicity study in monkey. These individuals presented with hypoalbuminemia (2.2 to 3.0 g/dL [333 to 455 µmol/L]), which was speculated to be due to decreased food consumption and inflammatory changes. The major serum binding protein of the S-217622 is albumin. In general, when the molar concentration of the drug in plasma exceeds the molar concentration of albumin in plasma, it is known that the unbound drug concentration in plasma increases rapidly due to the saturation of albumin binding [37]. The C_{max} of individuals with severe toxicity was 211 µg/mL (397 µmol/L) or higher, which was equal to or exceeded the molar concentration of albumin in plasma. It is possible that the toxicity might be more severe as the unbound plasma concentration of S-217622 increased.

As other major findings derived from S-217622 fumaric acid, increased total bilirubin (T.Bil), mainly increased indirect bilirubin (I.Bil), and decreased HDL- and low density lipoprotein (LDL)- cholesterol in monkeys were observed. However, none of the changes was associated with histopathological changes and therefore they are not adverse. The adverse effects observed in monkeys (death/moribund, vomiting, decreased food consumption, decreased erythrocyte parameters, decreased platelet count and inflammatory changes in multiple organs), and increased bilirubin are discussed below.

- Causes of death/moribund

In the 2-week repeated oral toxicity studies in monkeys, death or moribund was observed after vomiting or decreased food consumptions in 2 males with repeated doses of 1000 mg/kg/day and 300 mg/kg/day and 1 female with repeated doses of 300 mg/kg/day.

The cause of death/moribundity in the 2 males was considered to be decreased food consumption and opportunistic infection, caused by the stress-related immunosuppression. The cause of moribundity in the 1 female was considered to be decreased food consumption and anaemia.

C_{max} and AUC_{0-24hr} values (378 to 404 µg/mL and 7390 to 7810 µg·hr/mL) on Day 14 of dosing at 50 mg/kg/day with no mortality/moribund in 2-week repeated oral toxicity study in monkeys were 13 to 14 times and 14 to 15 times higher than C_{max} and AUC_{0-24hr} values (28.1 µg/mL and 518.3 µg·hr/mL) in the clinical dosing regimen of S-217622 at 375/125 mg. On the other hand, the C_{max} was 409 µg/mL (769 µmol/L) or higher when administered at a dose of 300 mg/kg/day or higher, in

which moribund or dead cases were observed. The C_{max} of S-217622 exceeded the plasma albumin concentration during the acclimatization period (3.5 to 4.5 g/dL [530 to 682 µmol/L]). Furthermore, the moribund individual presented with hypoalbuminemia (plasma albumin concentration: 2.8 or 3.0 g/dL [424 or 455 µmol/L]) due to decreased food consumption and inflammatory changes. In individuals with death and moribund, the toxicity was considered to be more severe as the concentration of unbound plasma S-217622 increased as described above. On the other hand, the C_{max} value in the clinical dosing regimen of S-217622 at 375/125 mg was 28.1 µg/mL (52.8 µmol/L), which is substantially lower than the human albumin concentration (4.1 to 5.1 g/dL [621 to 773 µmol/L] [38]). It is unlikely that unbound plasma S-217622 concentration will increase due to saturation of albumin binding in humans.

The reduced food consumption and vomiting observed in death/moribund individuals were also observed in other surviving cases, and the exposure in the clinical dosing regimen of S-217622 at 375/125 mg was lower than the exposure level at the NOAEL in the repeated oral administration for 2 weeks and 4 weeks in monkeys, as shown in Table SII.2.

Based on the above, it is considered that the possibility of serious adverse events leading to death/moribund is extremely low in the clinical dosing regimen of S-217622 at 375/125 mg.

- Vomiting

Vomiting was observed in males in the 1000/300/100 mg/kg/day group and females in the 300/100 mg/kg/day group in the 2-week repeated oral toxicity study in monkeys. The frequency of vomiting was higher in both males and females in the first half of the administration period, and the frequency of vomiting tended to decrease as the dose decreased. No vomiting was observed during the 2-week washout period.

Vomiting is roughly divided into central and peripheral depending on the cause that stimulates the vomiting center, and as one of the central causes, action by nonselective PDE4 inhibition has been reported in multiple animal species including monkeys [39-42]. In secondary pharmacological evaluation, S-217622 showed the inhibitory effects on PDE4A1A, PDE4B1, PDE4C1 and PDE4D2 (IC_{50} = 63.2 to 75.7 µmol/L). Unbound C_{max} value of males in the 1000/300/100 mg/kg/day group and females in the 300/100 mg/kg/day group with vomiting in 2-week repeated oral toxicity studies in monkeys was 14 to 17 µmol/L (calculated based on total S-217622 concentration [433 to520 µg/mL], molecular weight [531.88] and monkey plasma protein binding ratio [98.3%]). The ratio between unbound C_{max} value in individual with vomiting and the IC_{50} value for various PDE4 inhibition was 3.7 to 5.4 times. As mentioned above, it is possible that albumin binding was saturated and unbound plasma S-217622 concentration increased in the high-dose monkey group. The vomiting observed in the high-dose group of the 2-week repeated oral toxicity study in monkeys may be due to nonspecific PDE4 inhibition. In humans, the IC_{50} values of PDE4s (63.2 to 75.7 µmol/L) are 53 to 63 times higher than unbound C_{max} (1.2 µmol/L; calculated from total S-217622 concentration [28.1 µg/mL], molecular weight [531.88] and the protein binding rate [97.7%]) in the clinical dosing regimen of S-217622 at 375/125 mg. It was considered that there is little concern about vomiting by the nonspecific PDE4 inhibition in human.

The exposure level in the clinical dosing regimen of S-217622 at 375/125 mg was lower than those at the NOAEL (C_{max}: 2.7 to 3.4 times and AUC: 2.3 to 3.6 times) in the 4-week repeated oral toxicity study in monkeys (Table 2). There is little concern for vomiting and nausea in the clinical dosing regimen of S-217622 at 375/125 mg.

- Decreased food consumption

Decreased food consumption was observed at 50 mg/kg/day and above in the 2-week repeat-dose oral toxicity study in monkeys and at 30 mg/kg/day in the 4-week repeat-dose oral toxicity study in monkeys. No irritative or degenerative findings in the upper gastrointestinal tract were observed in any of the studies. Decreased food consumption at 30 mg/kg/day group in the 4-week repeat-dose oral toxicity study in monkeys was observed after Day 10 of dosing, while the clinical dosing regimen of S-217622 is as short as 5 days. The decreased food consumption showed reversibility by the drug withdrawal.

The exposure levels in the clinical dosing regimen of S-217622 at 375/125 mg are lower than those at the NOAEL (C_{max}: 2.7 to 3.4 times and $AUC_{0\text{-}24hr}$: 2.3 to 3.6 times) in the 4-week repeated oral toxicity study in monkeys (Table 2). There is little concern for decreased appetite and related adverse events in the clinical dosing regimen of S-217622 at 375/125 mg.

- Decreased erythrocyte parameters

Decreased erythrocyte parameters (erythrocyte count, haemoglobin concentration, and haematocrit value) were observed at 50 mg/kg/day or higher in the 2-week repeat-dose oral toxicity study in monkeys and at 30 mg/kg/day in the 4-week repeat-dose oral toxicity study in monkeys. Some animals showed increased reticulocyte counts, erythrophagia in Kupffer cells, or hematopoietic reactions in the bone marrow, liver and spleen associated with decreased erythrocyte parameters.

The decrease in erythrocyte parameters was considered to be due to extravascular haemolysis based on the reasons as follows: (1) no obvious change in mean corpuscular volume or mean corpuscular haemoglobin concentration, (2) no haematological and histopathological changes indicating intravascular haemolysis such as increased blood aspartate transaminase and lactate dehydrogenase or haemoglobinuria-suspected abnormal urine colour were noted in these animals, and (3) congestion in the spleen with increased spleen weights were noted in these animals. Decreased erythrocyte parameters showed reversibility by withdrawal of S-217622.

Although the decreased erythrocyte parameter (anaemia) observed in the 30 mg/kg/day group in the monkey 4-week repeated oral toxicity study occurred between Days 11 and 27 of dosing, the clinical dosing regimen of S-217622 is as short as 5 days. In addition, as mentioned above, the exposure levels in the clinical dosing regimen of S-217622 at 375/125 mg is lower than those at the NOAEL in the 4-week repeated oral toxicity study in monkeys (C_{max}: 2.7 to 3.4 times and $AUC_{0\text{-}24hr}$: 2.3 to 3.6 times) (Table SII.2). There is little concern for anaemia and related adverse events in the clinical dosing regimen of S-217622 at 375/125 mg.

- Decreased platelet count

Decreased platelet count was observed in 1 female at 50 mg/kg/day and 1 male at 1000/300/100 mg/kg/day in the 2-week repeat-dose oral toxicity study in monkeys. In males at 1000/300/100 mg/kg/day group, an increased cellularity of megakaryocytes was observed in the bone marrow, suggesting decreased platelet count was not mediated by myelosuppression. Decreased in platelet count was not observed in the recovery period. In the 4-week repeated oral toxicity study in monkeys, no effect on platelet count was observed at doses up to 30 mg/kg/day.

The exposure levels in the clinical dosing regimen of S-217622 at 375/125 mg are lower than those at the NOAEL (C_{max}: 2.7 to 3.4 times and $AUC_{0\text{-}24hr}$: 2.3 to 3.6 times) in the 4-week repeated oral toxicity study in monkeys (Table SII.2). There is little concern for decreased platelet count and related adverse events in the clinical dosing regimen of S-217622 at 375/125 mg.

- Inflammatory changes in multiple organs

Increased histiocytes and plasma cells in the spleen and lymph nodes, and infiltration by inflammatory cells, mainly mononuclear cells, was noted in the perivascular area in the lung, epididymis, and the oesophagus, lacrimal gland, eyeballs (bulbar conjunctiva), and lamina propria of the gallbladder were observed in the administration group of 50 mg/kg/day or higher in the 2-week repeated oral toxicity study in monkeys. In addition, in the 4-week toxicity study in monkeys, inflammatory cell infiltration was observed in the muscular layer of gallbladder and the uterus in one female at a dose of 30 mg/kg/day. Parenchymal cell damage or vascular wall degeneration were not observed in any of the organs in the surviving individuals. These results suggested that the inflammatory cell infiltration observed during repeated administration in monkeys was not due to the response to cytotoxicity or vascular wall degeneration. Inflammatory lesions derived from mainly mononuclear inflammatory cells such as histiocytes and plasma cells were found in organs known to have inflammatory lesions as background lesions [43,44] Since these are considered to be organs where inflammation is likely to occur due to foreign substances, it is possible that the above inflammatory changes were induced by the enhancement of immune function against foreign substances by S-217622 fumaric acid. In addition, in males in the 50 mg/k/day administration group, males in the 1000/300/100 mg/kg/day administration group, and females in the 300/100 mg/kg/day administration group in the 2-week repeated oral toxicity study in monkeys, increased fibrinogen concentration was observed. These changes were considered to be related to the above-mentioned inflammatory lesions derived from mainly mononuclear inflammatory cells.

All inflammatory changes and inflammatory marker changes observed in the repeated oral toxicity study in monkeys were recovered by withdrawal.

The exposure levels in the clinical dosing regimen of S-217622 at 375/125 mg are lower than those in the NOAEL range (C_{max}: 2.7 to 3.4 times and $AUC_{0\text{-}24hr}$: 2.3 to 3.6 times) in the 4-week repeated oral toxicity study in monkeys (Table 2). There is little concern for inflammation and related adverse events in the clinical dosing regimen of S-217622 at 375/125 mg.

- Increased bilirubin

Increased T.Bil and direct bilirubin (D.Bil) were observed in the administration groups of 50 mg/kg/day or higher in the 2-week repeated oral toxicity study in monkeys and the 30 mg/kg/day group of the 4-week repeated oral toxicity study in monkeys. The increase in D.Bil was smaller than the increase in T.Bil, and the increase in serum bilirubin was mainly due to the increase in I.Bil. However, no other changes suggestive of cholestasis and hepatobiliary disorders were observed on blood chemistry or histopathological examinations in any of the individuals. S-217622 fumaric acid has inhibitory effects on OATP1B1 and OATP1B3. I.Bil produced in the blood is taken up into the liver via OATP1B1 and OATP1B3, conjugated with glucuronic acid, and then excreted [45]. The unbound C_{max} value of S-217622 was greater than 7 µmol/L (calculated based on total S-217622 concentration [211 µg/mL], molecular weight [531.88] and monkey plasma protein binding rate [98.3%]) in monkeys with increased I.Bil when administered at doses of 30 mg/kg/day or higher in repeated oral toxicity studies. The unbound C_{max} value of S-217622 above was close to the IC_{50} value (13.2 µmol/L) for the inhibitory effect of OATP1B1, and higher than the IC_{50} value (3.51 µmol/L) for the inhibitory effect of OATP1B3. These results suggest that the increase in serum bilirubin level observed in the repeated oral toxicity study in monkeys is mainly related to OATP inhibition. As mentioned above, extravascular haemolysis is suggested at doses of 50 mg/kg/day or higher in 2-week repeated oral toxicity study and 30 mg/kg/day in 4-week repeated oral toxicity study. It is possible that extravascular haemolysis also contributes to the increase in serum bilirubin in individuals with decreased erythrocyte parameters.

The unbound C_{max} (1.2 µmol/L) value at the clinical dosing regimen of 375/125 mg was lower than the IC_{50} value (3.51 and 13.2 µmol/L) for OATP inhibition, and it is unlikely the bilirubin level will increase due to OATP inhibition.

Table SII.1 Main Adverse Effect in Repeated Oral Administration Toxicity Study of S-217622 Fumaric Acid

Animal Species	Dosing Period	Dose [a] (mg/kg/day)	Main Adverse Effect
Rat	2 weeks	20, 100, <u>1000</u>	No adverse effect
	4 weeks	20, 50, <u>1000</u>	No adverse effect
Monkey	2 weeks	<u>10</u>, 50, [male] 1000/300/100[b], [female] 300/100[c]	[males] 1000/300/100 mg/kg/day, [females] 300/100 mg/kg/day: Death/moribund and vomiting ≥ 50 mg/kg/day: Decreased body weight with food consumptions, decreased erythrocyte parameters and platelet count, inflammatory changes in multiple organs with changes in blood inflammatory markers
	4 weeks	3, <u>10</u>, 30	30 mg/kg/day: Decreased erythrocyte parameters, decreased body weights with food consumptions, inflammatory changes in the muscular layer/fibrous membrane of gallbladder and in the muscular layer of uterus with changes in blood inflammatory markers

a The underline shows the NOAEL.

b Since death occurred before the administration on Day 3 of dosing, the dose was reduced from 1000 mg/kg/day to 300 mg/kg/day from Day 3 of dosing. Furthermore, since death occurred before the administration on day 8 of dosing, the dose was reduced to 100 mg/kg/day from Day 8 of dosing.

c Since moribund occurred on Day 8 of dosing, the dose was reduced from 300 mg/kg/day to 100 mg/kg/day from Day 9 of dosing.

Table SII.2 TK Parameters of S-217622 at NOAEL in Repeated Oral Administration Toxicity Study of S-217622 Fumaric Acid, and Ratio to Exposure in Human

Animal Species (Dosing Period)	Dose (mg/kg/day)	Gender	C_{max} (μg/mL)	AUC_{0-24hr} (μg·hr/mL)	Exposure Ratio	
					C_{max}[a]	AUC_{0-24hr}[b]
Rat (2 weeks)	1000	Male	269	3850	9.6	7.4
		Female	319	3940	11	7.6
Rat (4 weeks)	1000	Male	263	4270	9.4	8.2
		Female	359	5380	13	10
Monkey (2 weeks)	10	Male	77.6	1220	2.8	2.4
		Female	75.2	1170	2.7	2.3
Monkey (4 weeks)	10	Male	76.8	1170	2.7	2.3
		Female	95.9	1850	3.4	3.6
Human (5 days)	375/125 mg	Female	28.1	518.3	N/A	N/A

a The ratio of C_{max} value on final day of dosing in the toxicity study in animals, and C_{max} value (28.1 μg/mL) on Day 5 in clinical dosing regimen of 375/125 mg (375 mg on Day 1 of dosing, 125 mg once daily on Days 2 to 5 of dosing) in healthy adult Phase 1 study (Study T1211)

b The ratio of AUC0-24hr value on final day of dosing in the toxicity study in animals, and AUC0-24hr value (518.3 μg·hr/mL) on Day 5 in clinical dosing regimen of 375/125 mg (375 mg on Day 1 of dosing, 125 mg once daily on Days 2 to 5 of dosing) in healthy adult Phase 1 study (Study T1211)

Genotoxicity

S-217622 fumaric acid has no genotoxic potential based on negative results the bacterial reverse micronucleus test with the human lymphoblast cell line, or the in vivo micronucleus test in rat.

Carcinogenicity

Carcinogenicity study was not conducted with S-217622 fumaric acid since S-217622 fumaric acid is considered to have low carcinogenic potential based for the following reasons: the intended clinical use of S-217622 fumaric acid is 5 day-administration; S-217622 fumaric acid has no concern for genotoxicity

Reproductive and Developmental Toxicity

The main adverse effects and the NOAELs observed in the reproductive and developmental toxicity studies are shown in Table 3, and the plasma exposures (C_{max} and AUC0-24hr) in each study and the ratio to the plasma exposures in the clinical dosing regimen of S-217622 at 375/125 mg are shown in Table SII.4.

In the study on fertility and early embryonic development in rats (dose levels: 20, 60, and 1000 mg/kg/day), no adverse effects were observed. The NOAEL for general toxicity and reproductive function in parental animals and early embryonic development in this study was 1000 mg/kg. In the study on embryo/fetal development in rats (dose levels: 20, 60, and 1000 mg/kg/day), no embryo/fetal morphological abnormalities or lethality were observed at doses up to 1000 mg/kg/day, and there were no effects on reproductive function of the maternal animal. Decreased food consumption and body weight gain were noted in dams at 1000 mg/kg/day, and slight

fetal growth retardation (low fetal body weight and decreased number of ossified sternebrae) were noted in fetuses at the dose with maternal toxicity. High frequency of short supernumerary rib was observed at 1000 mg/kg/day, but this change is known to disappear with growth after birth [46,47]. The NOAEL was 60 mg/kg/day for embryo-fetal development and maternal general toxicity and 1000 mg/kg/day for maternal reproductive toxicity.

Plasma exposure was not measured in the study on fertility and early embryonic development, but C_{max} and $AUC_{0\text{-}24hr}$ values at 1000 mg/kg/day in 4-week repeated oral dose toxicity study in rats were 9 to 13 times and 8 to 10 times higher than those in the clinical dosing regimen of S217622 at 375/125 mg, respectively (Table SII.2).

In the study on embryo/fetal development in rabbits (dose: 30, 100 and 300 mg/kg/day), body weight loss accompanied by decreased food consumption was observed in the maternal animal and skeletal abnormalities (abnormalities in axial skeleton morphology such as vertebrae, ribs, and sternum segments) and short tails in the fetus at doses of 100 mg/kg/day or higher. In addition, a low embryo/fetal survival rate was observed at a dose of 300 mg/kg/day. Furthermore, abortion was observed in one individual showing a marked decrease in food consumption at a dose of 100 mg/kg/day. Abortion and premature birth due to marked decreased food consumption have been reported in pregnant rabbits [48], suggesting that the abortion observed in this study was due to decreased food consumption by S-217622 fumaric acid administration. From these results, it was judged that the NOAEL for the general toxicity of the maternal animal, for the reproductive toxicity of the maternal animal, and for the embryo/fetal development in this study were all 30 mg/kg/day. Plasma exposures of S-217622 (C_{max} and $AUC_{0\text{-}24hr}$) in pregnant rabbits were 68.8 µg/mL and 1260 µg·hr/mL, respectively, which were 2.4 times higher than the exposure in the clinical dosing regimen of S-217622 at 375/125 mg (Table SII.4).

In order to investigate the effects of maternal toxicity and short-term administration (3 to 4 days) of 300 mg/kg/day during the divided organogenesis period, additional embryo-fetal development study in rabbits were conducted. S-217622 fumaric acid induced axial skeletal malformations and embryo-fetal lethality, the effects of which were marked at early phase of the organogenesis period.

In the study for pre- and postnatal developmental and maternal function in rats (dose: 20, 60 and 1000 mg/kg/day), maternal animals at a dose of 1000 mg/kg/day showed the tendency for decreased body weight gain and low body weight, and showed decreased food consumption. Total litter loss was observed in 5 from 20 animals in the early stage of lactation. In addition, the pup numbers, 0-day and 4-day-old survival rates, low birth weight throughout the lactation period, eyelids opening and delayed sexual maturation of males and females considered to be due to maternal toxicity were observed in F1 animals at a dose of 1000 mg/kg/day. Based on the above results, the NOAEL for maternal reproductive function and general toxicity and pre- and postnatal development in this study was determined to be 60 mg/kg/day. Although plasma exposure was not measured in this study, based on the exposures of the study on embryo and fetal development in rats, the exposures (C_{max} and $AUC_{0\text{-}24hr}$) of pregnant female rats at the NOAEL in this study are 134 µg/mL and 1990 µg·hr/mL, respectively, which were 4.8 times and 3.8 times higher than the plasma exposures in the clinical dosing regimen of S-217622 at 375/125 mg, respectively (Table SII.4).

As described above, in the reproductive developmental toxicity studies conducted so far, abnormal axial skeleton morphology such as fetal vertebrae, ribs, and sternum segments and short tails were observed in rabbits. The administration of S-217622 fumaric acid is contraindicated in pregnant women or women who may be pregnant.

In pre- and postnatal development and maternal function studies, administration of S-217622 fumaric acid during late pregnancy and lactation periods affected on the growth and development of infants at the doses which is adverse to maternal animals in rats (equivalent to 6.6 times the clinical exposure). In addition, S-217622 fumaric acid transfers to the fetus through the placenta in rats and to the milk of nursing rats, but it is unclear for the transfer to the milk in human. Considering the safety margin of the general toxicity studies, the safety risk potentially exists for infant via breast milk. For lactating women, breastfeeding is not recommended during the treatment of S-217622 fumaric acid and for a certain period after the end of administration.

Table SII.3 Main Adverse Effect and NOAEL in Reproductive Toxicity Study of S-217622 Fumaric Acid

Animal Species	Study	Dosing Route	Dose (mg/kg/day)	Main Adverse Effect and NOAEL
Rat	Fertility and early embryonic development	Oral	20, 60, 1000	No adverse effect NOAEL Male general toxicity, male reproductive function, female general toxicity, female reproductive function and early embryonic development: 1000 mg/kg/day
	Pre- and postnatal developmental and maternal function	Oral	20, 60, 1000	Dam • 1000 mg/kg/day: Decreased body weight gain, low trend of body weight, decreased food consumption, and total litter loss F1 animals • 1000 mg/kg/day: Low birth numbers, reduced survival rate at 0 and 4 days after birth, low body weight, delayed eyelid opening and delayed sexual maturation NOAEL Maternal toxicity, maternal reproductive function, pre- and postnatal development: 60 mg/kg/day
	Embryo/fetal development	Oral	20, 60, 1000	Maternal animal • 1000 mg/kg/day: Decreased body weight gain and decreased food consumption Embryo/fetus • 1000 mg/kg/day: Low body weight in fetus, delayed ossification, and increased frequency of short and small excess ribs NOAEL Maternal toxicity, embryo/fetal development: 60 mg/kg/day Maternal reproductive function: 1000 mg/kg/day

Animal Species	Study	Dosing Route	Dose (mg/kg/day)	Main Adverse Effect and NOAEL
Rabbit	Embryo/fetal development	Oral	30, 100, 300	Maternal animal • 100 mg/kg/day: Abortion • ≥ 100 mg/kg/day: Low body weight and decreased food consumption Embryo/fetus • ≥ 100 mg/kg/day: Skeletal morphological abnormalities (sternum segment / rib fusion, rib abnormalities, vertebrae misalignment, etc.) • 300 mg/kg/day: Low survival rate of embryo/fetus • NOAEL Maternal toxicity, maternal reproductive function embryo/ fetal development: 30 mg/kg/day

Table SII.4 Plasma Exposure Ratio of S-217622 between Reproductive Developmental Toxicity Studies and Clinical Dosing Regimen

Animal Species (Dosing Period)	Dose (mg/kg/day)	C_{max} (µg/mL)	AUC_{0-24hr} (µg·hr/mL)	Exposure Ratio	
				C_{max} [a]	AUC_{0-24hr} [b]
Pregnant Rat	20	46.1	618	1.6	1.2
	60 (NOAEL)	134	1990	4.8	3.8
	1000	240	3400	8.5	6.6
Pregnant Rabbit	30 (NOAEL)	68.8	1260	2.4	2.4
	100	167	2580	5.9	5.0
	300	220	3840	7.8	7.4
Human (5 days)	375/125 mg	28.1	518.3	N/A	N/A

a The ratio of C_{max} value on final day of dosing in the toxicity study in animals, and C_{max} value (28.1 µg/mL) on Day 5 in clinical dosing regimen of 375/125 mg (375 mg on Day 1 of dosing, 125 mg once daily on Days 2 to 5 of dosing) in healthy adult Phase 1 study (Study T1211)

b The ratio of AUC0-24hr value on final day of dosing in the toxicity study in animals, and area under the concentration time curve over the dosing interval τ (AUC0-τ) value (518.3 µg·hr/mL) on Day 5 in clinical dosing regimen of 375/125 mg (375 mg on Day 1 of dosing, 125 mg once daily on Days 2 to 5 of dosing) in healthy adult Phase 1 study (Study T1211)

Lactation

S-217622 fumaric acid transfers to the milk of nursing rats but there are no data from humans. Considering the safety margin of the general toxicity studies, a risk potentially exists for infants via breast milk and therefore breastfeeding is not recommended during treatment with S-217622 fumaric acid.

Safety Pharmacology

As a safety pharmacological core battery study, the effects of S-217622 fumaric acid on the central nervous system, cardiovascular system, and respiratory system were investigated. The results of the safety pharmacological core battery study, the plasma exposure, and the ratio of plasma exposure to that of clinical dosing regimen are shown in Table SII.5.

Table SII.5 Results of Safety Pharmacology Core Battery Study of S-217622 Fumaric Acid and Ratio to Exposure in Human

Study	Dose or concentration of S-217622	Notable findings	Plasma exposure at NOEL (C_{max}) (Ratio to exposure in human)
Central nervous system in rats	0 (Control), 20, 100, 1000 mg/kg	No effects on central nervous system (NOEL 1000 mg/kg)	294 µg/mL (× 10.5)[a]
hERG assay	0 (Control), 10, 30, 100 µmol/L (0, 5.32, 16.0, 53.2 µg/mL in each)	IC_{50} > 100 µmol/L (53.2 µg/mL)	–
Cardiovascular system in monkey	0 (Control), 10, 50, 150 mg/kg	Mild increase in heart rate (≥ 50 mg/kg) (NOEL 10 mg/kg)	43.8 µg/mL (× 1.6)[b]
Respiratory system in monkey	0 (Control), 50, 150 mg/kg	No effects (NOEL 150 mg/kg)	326 µg/mL (× 11.6)[b]

NOEL = non observed effect level; hERG = human ether-à-go-go related gene

a The ratio of C_{max} value on Day 1 of administration in the male 1000 mg/kg/day administration group in 2-week repeated oral administration toxicity study in rats, and C_{max} value (28.1 µg/mL) on day 5 in clinical dosing regimen of 375/125 mg (375 mg on Day 1 of dosing, 125 mg once daily on Days 2 to 5 of dosing) in healthy adult Phase 1 study (Study T1211)

b The ratio of C_{max} value in the NOEL and Cmax value (28.1 µg/mL) on Day 5 in clinical dosing regimen of 375/125 mg (Study T1211)

In the study to evaluate the effects on central nervous system in rats, there was no change suggesting an effect on the central nervous system up to the 1000 mg/kg administration group, and the non-observed effect level (NOEL) was judged to be 1000 mg/kg. The ratio of C_{max} between the NOEL in rats for central nervous system after single oral administration and clinical dosing regimen of S-217622 at 375 mg/125 mg (28.1 µg/mL) was 10.5-fold (Table SII.5).

In a study on the effect on potassium current in human ether-à-go-go related gene (hERG)-introduced cells, IC_{50} value on hERG current was estimated to exceed 100 µmol/L (53.2 µg/mL). In addition, in a study evaluating the effects on the monkey cardiovascular system and respiratory system, a slight increase in heart rate was observed at doses of 50 and 150 mg/kg, but no effects were observed on blood pressure, electrocardiogram, respiratory rate or blood gas parameters up to doses of 150 mg/kg. The ratio of C_{max} values in the NOELs for cardiovascular system (10 mg/kg), and for respiratory system (150 mg/kg) in monkeys after single oral administration, to C_{max} value in the clinical dosing regimen of S-217622 at 375 mg/125 mg on Day 5 in Study T1211 (28.1 µg/mL) were 1.6 or 11.6 times, respectively (Table SII.5).

Based on the results of the above safety pharmacological studies, there is no or negligible concern for the central nervous system, cardiovascular system, and respiratory system in the clinical dosing regimen of S-217622 fumaric acid at 375/125 mg.

Other Toxicity

- **Phototoxicity Study**

The neutral red uptake phototoxicity study in 3T3 mouse fibroblasts was negative, and it was judged the concern for S-217622 fumaric acid to cause phototoxicity in humans be very low.

- **Mechanistic Studies for HDL- Cholesterol Reduction**

Mechanistic study was conducted for HDL-cholesterol reduction observed in the Phase 1 study of S-217622 fumaric acid. Lipoprotein of HDL, which contains Apolipoprotein A1 (Apo-A1) as the main protein, recovers excess cholesterol in peripheral tissues from peripheral tissue cells and macrophages. The recovered cholesterol is esterified by LCAT and incorporated into HDL. In the process of cholesterol recovery and esterification, HDL2/3 cholesterol with an increased particle size is formed, and then cholesterol is taken up by the liver and excreted from the body [49-53].

Each lipoprotein (HDL, LDL, very low density lipoprotein [VLDL]) and chylomicron [CM] after administration of 750/250 mg in the Phase 1 study of S-217622 fumaric acid was fractionated in more detail according to the particle size, and lipid analysis in each fraction was performed, which found the initial lipid fluctuation was a decrease in cholesterol in medium-sized HDL. Medium-sized HDL is considered to be a fraction in which HDL recovers free cholesterol from peripheral tissues, resulting in an increased size [54]. In addition, as a result of an in vitro study on the effect of HDL on cholesterol recovery, it was considered unlikely that S-217622 directly inhibits cholesterol recovery ability by HDL and lecithin cholesterol acyltransferase (LCAT). From the above, it was speculated that the decrease in HDL cholesterol due to S-217622 administration was caused by the maturation process or excretion of HDL cholesterol after cholesterol recovery by HDL, but the mechanism was unknown.

As a result of lipid analysis after administration of 750/250 mg in the Phase 1 study of S-217622 fumaric acid, the major changes observed in addition to the decrease in HDL cholesterol were the decrease in cholesterol in small LDL and the increase in triglyceride in large LDL and small VLDL. Cholesterol in HDL is esterified by LCAT and then exchanged for triglycerides in the LDL and VLDL fractions by cholesteryl ester transfer protein (CETP) [55]. It is considered that administration of S-217622 causes a decrease in cholesterol in medium-sized HDL, and as a secondary change, the exchange of HDL cholesterol and triglycerides of LDL and VLDL by CETP is reduced, followed by the decrease of cholesterol in small LDL, and triglyceride in small VLDL and large LDL.

In a 4-week repeated oral toxicity study of S-217622 fumaric acid in monkey, HDL and LDL cholesterol decreased at doses of 10 mg/kg/day or more on Days 10 and 27 of dosing. Decreased total cholesterol at a dose of 10 mg/kg/day and increased total cholesterol and triglyceride at a dose of 30 mg/kg/day were observed.

Histopathological examination revealed hepatocyte hypertrophy around the portal vein. The only histopathological changes associated with lipid fluctuations observed in monkeys were hepatocyte hypertrophy around the portal vein, which is presumed to be due to increased synthesis of lipoprotein or cholesterol in the liver associated with decreased blood cholesterol. It was judged that there is little concern that the effects on lipids will lead to further adverse events.

Table SII.6 Key Safety Findings from non-clinical studies

Key Safety findings from non-clinical Studies	Relevance to Human Usage
Toxicology In monkeys [males] 1000/300/100 mg/kg/day, [females] 300/100 mg/kg/day: Death/moribund and vomiting ≥ 50 mg/kg/day: Decreased body weight with food consumptions, decreased erythrocyte parameters and platelet count, inflammatory changes in multiple organs with changes in blood inflammatory markers 30 mg/kg/day: Decreased erythrocyte parameters, increased bilirubin, decreased body weights and food consumption, inflammatory changes in the muscular layer/fibrous membrane of gallbladder and in the muscular layer of uterus with changes in blood inflammatory markers	To date, there have been no reports of clinically significant treatment-emergent changes in clinical pathology parameters related to red cells, platelets inflammation or liver function. Increases in bilirubin, without evidence of liver pathology or haemolysis have been reported in individual patients in clinical studies
Reproductive/developmental toxicity There was maternal toxicity and embryo/foetal development. In rats there was maternal decreased food consumption and toxicity with litter loss and pre- and postnatal development in pups with a NOEL of 60 mg/kg/day In rabbits there was maternal with decreased food consumption with low body weights and associated abortions. In kittens there were skeletal morphological abnormalities (sternum segment / rib fusion, rib abnormalities, vertebrae misalignment, etc.) and low survival rates with a NOEL of 30 mg/kg/day. There was delayed development of kittens with delayed eyelid opening and sexual maturation with a NOEL of 60 mg/kg/day	The estimated plasma levels at which effects on foetuses were observed are around 5 times higher than those measured using the proposed dosage regimen in humans. However, given these findings ensitrelvir should not be used in pregnancy.

PART II: MODULE SIII - CLINICAL TRIAL EXPOSURE

The below tabulations present exposure to ensitrelvir in pivotal clinical studies in patients with COVID-19 or in PEP, ie, study 2108T1221(SCORPIO-SR), study 2117T1224 (SCORPIO-HR) and study 2206T1331 (SCORPIO-PEP).

In addition to these patients, 76 healthy participants were exposed to a single dose of S-217622 across 4 single-dose studies (2135T1216; 2127T1213; 2128T1214; 2130T1215), and a further 189 participants were exposed to multiple doses of S-217622: 161 in multiple-dose cohorts in Study 2102T1211 and 28 in Study 2305T1218.

Table SIII-1 Duration of exposure for Ensitrelvir Studies (Adults and Adolescents)

Duration of Exposure	Treatment		PEP	All indications
	S-217622 375/125 mg N = 2003	S-217622 750/250 mg N = 964	S-217622 375/125 mg N = 1190	S-217622 [a] N = 4157
1 day	15	9	2	26
2 days	7	4	3	14
3 days	7	8	2	17
4 days	12	3	8	23
5 days	1962	940	1175	4077
Total person-time (days) [b]	9908	4753	5921	20582

Study: SCORPIO-SR (2108T1221), SCORPIO-HR (2117T1224; ACTIV-2d/A5407), SCORPIO-PEP (2206T1331)

Treatment: SCORPIO-SR + SCORPIO-HR, PEP: SCORPIO-PEP, All indications: pooling the three studies

[a] All doses (125 mg and 250 mg) are included.

[b] Person-time is defined as the sum of actual exposure time for every participant.

Table SIII- 2 Age group and gender Exposure for Ensitrelvir Studies (Adults and Adolescents)

Age / Sex	Treatment				PEP		All indications		All indications	
	S-217622 375/125 mg N = 2003		S-217622 750/250 mg N = 964		S-217622 375/125 mg N = 1190		S-217622 [a] N = 4157		Person time (days) [b]	
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
Adolescents (≥ 12 to ≤ 17)	7	8	14	5	38	34	59	47	295	234
Adults (≥ 18 to ≤ 65)	938	998	516	417	426	586	1880	2001	9322	9888
Elderly People 1 (≥ 66 to ≤ 75)	18	25	2	10	33	38	53	73	265	360
Elderly People 2 (> 75)	4	5	0	0	8	27	12	32	58	160
Total	967	1036	532	432	505	685	2004	2153	9940	10642

Study: SCORPIO-SR (2108T1221), SCORPIO-HR (2117T1224; ACTIV-2d/A5407), SCORPIO-PEP (2206T1331)

Treatment: SCORPIO-SR + SCORPIO-HR, PEP: SCORPIO-PEP, All indications: pooling the three studies

[a] All doses (125 mg and 250 mg) are included.

[b] Person-time is defined as the sum of actual exposure time for every participant.

Table SIII- 3 Dose of exposure for Ensitrelvir Studies (Adults and Adolescents)

Total Dose of Exposure	Treatment		PEP	All indications	All indications
	S-217622 375/125 mg N = 2003	S-217622 750/250 mg N = 964	S-217622 375/125 mg N = 1190	S-217622 [a] N = 4157	Person Time (days) [b]
375 mg	15	0	2	17	17
500 mg	7	0	3	10	20
625 mg	7	0	2	9	27
750 mg	11	9	8	28	85
812.5 mg	1	0	0	1	5
875 mg	1962	0	1175	3137	15684
1000 mg	0	4	0	4	8
1250 mg	0	8	0	8	24
1500 mg	0	3	0	3	12
1625 mg	0	3	0	3	15
1750 mg	0	937	0	937	4685
Total	2003	964	1190	4157	20582

Study: SCORPIO-SR (2108T1221), SCORPIO-HR (2117T1224; ACTIV-2d/A5407), SCORPIO-PEP (2206T1331)

Treatment: SCORPIO-SR + SCORPIO-HR, PEP: SCORPIO-PEP, All indications: pooling the three studies

[a] All doses (125 mg and 250 mg) are included.

[b] Person-time is defined as the sum of actual exposure time for every participant.

Table SIII-4 Demographic characteristics (Race) for Ensitrelvir Studies (Adults and Adolescents)

Race	Treatment		PEP	All indications	All indications
	S-217622 375/125 mg N = 2003	S-217622 750/250 mg N = 964	S-217622 375/125 mg N = 1190	S-217622 [a] N = 4157	Person Time (days) [b]
Asian	1370	961	376	2707	13395
American Indian or Alaska Native	13	0	3	16	80
Black or African American	87	1	60	148	739
Native Hawaiian or Other Pacific Islander	0	1	0	1	5
White	456	0	728	1184	5861
Multiple	7	0	0	7	35
Not Reported	24	0	0	24	120
Other	44	1	23	68	337
Unknown	2	0	0	2	10
Total	2003	964	1190	4157	20582

Study: SCORPIO-SR (2108T1221), SCORPIO-HR (2117T1224; ACTIV-2d/A5407), SCORPIO-PEP (2206T1331)

Treatment: SCORPIO-SR + SCORPIO-HR, PEP: SCORPIO-PEP, All indications: pooling the three studies

[a] All doses (125 mg and 250 mg) are included.

[b] Person-time is defined as the sum of actual exposure time for every participant.

PART II: MODULE SIV - POPULATIONS NOT STUDIED IN CLINICAL TRIALS

SIV.1 Exclusion criteria in pivotal clinical studies within the development programme

Important exclusion criteria in the pivotal clinical studies (2108T1221; 2117T1224; 2206T1331) are listed below

Exclusion criteria	Reason for exclusion	Missing information (Yes/No)	Rationale for not including as missing information
Pregnancy	Reason for exclusion: Women who were pregnant were excluded from the clinical studies to avoid potential harm to the unborn foetus	No	Due to the findings in nonclinical studies, use of ensitrelvir in pregnant women will be contraindicated.
Patients < 12 years of age	This population was excluded from clinical studies based on the general principle that young children are not exposed to an investigational product where the benefit-risk profile for an older population has not yet been established, rather than due to any specific safety concern.	No	Paediatric subjects < 12 years of age are not relevant for the proposed indication. Safety in the paediatric population will be assessed as part of the paediatric investigation plan
Patients who have used approved or unapproved drugs for the treatment of SARS-CoV-2 infection within 7 days prior to randomization	Patients were excluded in order to avoid factors that may confound a complete understanding of the efficacy data of ensitrelvir and ensure interpretability of data	No	This population was not excluded on the basis of safety and there is no scientific rationale to suspect that the safety profile of ensitrelvir observed in the general population would be impacted by COVID-19 vaccination or prior treatment with a mAb/biologic indicated for the prevention of SARS-CoV-2.
Patients who have used strong cytochrome P450 3A inhibitors or inducers or products containing St John's Wort	Patients were excluded in order to avoid factors that may confound a complete understanding of the efficacy data of ensitrelvir and ensure interpretability of data.	No	As ensitrelvir is known to affect plasma levels of medicines dependent of P450 3A for clearance and ensitrelvir levels are affected by inducers of P450 3A concomitant use of medicines highly dependent on P450 3A for clearance are contraindicated in the SmPC.

Exclusion criteria	Reason for exclusion	Missing information (Yes/No)	Rationale for not including as missing information
Current or chronic history of severe liver disease or known hepatic or biliary disorders	Pharmacokinetics of ensitrelvir was not yet determined in subjects with hepatic impairment at the time the pivotal studies started	No	Information collected with study 2127T1213 (Phase 1 study of pharmacokinetics, safety and tolerability of ensitrelvir in subjects with mild, moderate hepatic impairment).
Current or chronic history of moderate or severe kidney disease	Pharmacokinetics of ensitrelvir was not yet determined in subjects with renal impairment at the time the pivotal studies started	No	Information collected with study 2128T1214 Phase 1 study of pharmacokinetics, safety and tolerability of ensitrelvir in subjects with mild, moderate and severe renal impairment

SIV.2 Limitations to detect adverse reactions in clinical trial development programmes

The clinical development programme is unlikely to detect certain types of adverse reactions such as rare adverse reactions and/or adverse reactions with a long latency.

Ability to Detect Adverse Reactions	Limitation of Trial Programme	Discussion of Implications for Target Population
Which are uncommon and associated only with ensitrelvir	4157 subjects treated with at least 1 dose of ensitrelvir, in pivotal studies	Only ADRs with a frequency of 1 in 1385 associated with a single dose could be detected with a 95% probability or more.
Which have a long latency	Long latency effects are unlikely to be detected	Long latency effects are unlikely to be associated with a 5-day course of treatment especially given the short half-life.

SIV.3 Limitations in respect to populations typically under-represented in clinical trial development programmes

Table SIV.3-1 Exposure of special populations included or not in clinical trial development programmes

Type of special population	Exposure
Pregnant women	not included in the clinical development programme
Breastfeeding women	
Patients with relevant comorbidities: Patients with hepatic impairment Patients with renal impairment Patients with cardiovascular impairment Immunocompromised patients Patients with a disease severity different from inclusion criteria in clinical trials	not included in the clinical development programme (patients with hepatic and renal impairment studied in phase 1 single dose studies, 2127T1213 and 2128T1214, respectively)

Type of special population	Exposure
Population with relevant different ethnic origin	White patients: 1184 Asian patients: 2707 Refer to Table SIII-4 for further details
Subpopulations carrying relevant genetic polymorphisms	not included in the clinical development programme
Paediatric population	Refer to Table SIII- 2 for information on paediatric patients' exposure. Children younger than 12 years old were not included in pivotal clinical studies
Elderly population	Refer to Table SIII- 2 for information on elderly patients' exposure

PART II: MODULE SV - POST-AUTHORISATION EXPERIENCE

SV.1 Post-authorisation exposure

Ensitrelvir obtained an emergency regulatory approval in Japan on 22 Nov 2022 and full approval on 5 Mar 2024. Data on exposure are available from Japan only, for the indication of "Treatment of adults and adolescents (aged 12 years and older) with COVID-19 (as monotherapy)". Of note, the clinical dosing regimen in Japan is the same as the proposed in Europe.

SV.1.1 Method used to calculate exposure

The data on Japanese patient exposure was based on the total number of patients estimated from the number of patients registered through the registration centre and the delivered quantity of product, shipped to wholesaler and medical institutions, considering 5-day treatment.

SV.1.2 Exposure

	Estimated patients who received ensitrelvir tablets 125 mg
	From the International Birth Date (22 Nov 2022) to 02 Mar 2025
Post-marketing (Japan)	2,034,907

PART II: MODULE SVI - ADDITIONAL EU REQUIREMENTS FOR THE SAFETY SPECIFICATION

Potential for misuse for illegal purposes.

In view of the mechanism of action of ensitrelvir, no potential for misuse for illegal purposes exists.

PART II: MODULE SVII - IDENTIFIED AND POTENTIAL RISKS

SVII.1 Identification of safety concerns in the initial RMP submission

SVII.1.1. Risks not considered important for inclusion in the list of safety concerns in the RMP

Risks with minimal clinical impact on patients (in relation to the severity of the indication treated):

Headache, diarrhoea, nausea, vomiting, abdominal discomfort, dyspepsia, dyslipidaemia, hypertriglyceridemia, hyperbilirubinaemia

The above events reported as adverse reactions were generally mild or moderate in severity and did not cause withdrawal of treatment. They therefore had minimal impact on patients, particularly given that ensitrelvir is indicated only for a short treatment period. In addition, these events are generally transient or reversible after ceasing the treatment.

Known risks that require no further characterisation and are followed up via routine pharmacovigilance namely through signal detection and adverse reaction reporting, and for which the risk minimisation messages in the product information are adhered by prescribers (eg, actions being part of standard clinical practice in each EU Member state where the product is authorised):

Anaphylaxis, rash, drug eruption, eczema, urticaria, pruritus

All the above are indicative of cutaneous manifestations of hypersensitivity to ensitrelvir. Clinical manifestation can occasionally be severe (rare cases of anaphylactic shock have been reported during post-marketing experience in Japan), but Healthcare professionals are familiar with risk of hypersensitivity with use of medicines and its management is part of routine clinical practice.

Non important missing information:

Safety in patients with severe hepatic impairment

As ensitrelvir is principally metabolised by the liver, characterisation of the effects of liver impairment on the pharmacokinetic profile and safety profile of the drug in patients with mild or moderate liver disease have been conducted, with no clinically meaningful differences in PK in participants with mild or moderate hepatic impairment, compared with participants with normal hepatic function. No clinical studies have been conducted in patients with severe hepatic impairment. However, since from PK studies, hepatic impairment tends to decrease (not to increase) the exposures of ensitrelvir with increased severity of hepatic function, no safety concerns due to an increase exposure are expected if the product is administered in patient with severe hepatic impairment.

Safety in pregnancy / embryo-foetal toxicity

The use of ZOKOVEA is contraindicated during pregnancy and pregnancy must be excluded prior to start of ensitrelvir in women of childbearing potential. Therefore the use in pregnant women is not expected (and otherwise will be very limited). Reproductive and developmental toxicity findings observed in non-clinical studies are adequately reflected in the SmPC and routine pharmacovigilance is considered sufficient to monitor any emerging information.

Use during breastfeeding

It is unknown whether ensitrelvir is excreted in human milk. A non-clinical study in rats showed that ensitrelvir is excreted in milk and a risk to the suckling child cannot be excluded. Breastfeeding is not recommended during treatment with ensitrelvir and therefore as above the use is expected to be very limited. Information regarding use during lactation is adequately addressed in the SmPC and routine pharmacovigilance is considered sufficient to further monitor any emerging information.

SVII.1.2. Risks considered important for inclusion in the list of safety concerns in the RMP

Important Identified Risk

There are no important risks identified for ensitrelvir.

Important Potential Risk

There are no important potential risks for ensitrelvir.

Missing information:

There is no important missing information for ensitrelvir.

SVII.2 New safety concerns and reclassification with a submission of an updated RMP

Not applicable

SVII.3 Details of important identified risks, important potential risks, and missing information

SVII.3.1. Presentation of important identified risks and important potential risks

Important Identified Risk: None

Important Potential Risk: None

SVII.3.2. Presentation of the missing information

Missing information: None

PART II: MODULE SVIII - SUMMARY OF THE SAFETY CONCERNS

Table SVIII- 1 Summary of safety concerns

Summary of safety concerns	
Important identified risks	None
Important potential risks	None
Missing information	None

PART III: PHARMACOVIGILANCE PLAN (INCLUDING POST-AUTHORISATION SAFETY STUDIES)

III.1 Routine pharmacovigilance activities

Routine pharmacovigilance undertaken by the MAH include individual review of AE reports; periodic review of aggregate AE reports; weekly review of published literature; review of Eudravigilance database, to the extent the MAH has access to it. Discussion of risks (embryofetal-toxicity) and missing information (use during breastfeeding) identified, but not included in the RMP because of contraindication, as well as any new signals will be included in the Periodic Safety Update Reports (PSURs). The MAH will promptly inform the Pharmacovigilance Risk Assessment Committee (PRAC) of new signals that are considered to meet the definition of an emerging safety issue, as defined in the Guideline Good Pharmacovigilance Practices (GVP) Module VI Management of Adverse Reactions to Medicinal Medical Products and GVP Module IX Signal Management.

III.1.1 Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection

Specific adverse reaction and pregnancy follow-up questionnaires:

None

III.2 Additional pharmacovigilance activities

No additional pharmacovigilance activities are proposed.

III.3 Summary Table of additional Pharmacovigilance activities

None

PART IV: PLANS FOR POST-AUTHORISATION EFFICACY STUDIES

All efficacy studies have been completed, with final study report currently in preparation. There are no plans for any additional efficacy studies following marketing authorization.

Table Part IV- 1 Planned and on-going post-authorisation efficacy studies that are conditions of the marketing authorisation or that are specific obligations.

Study Status	Summary of objectives	Efficacy uncertainties addressed	Milestones	Due Date
Efficacy studies which are conditions of the marketing authorisation.				
none				
Efficacy studies which are Specific Obligations in the context of a conditional marketing authorisation or a marketing authorisation under exceptional circumstances				
none				

PART V: RISK MINIMISATION MEASURES (INCLUDING EVALUATION OF THE EFFECTIVENESS OF RISK MINIMISATION ACTIVITIES)

Risk Minimisation Plan

V.1. Routine Risk Minimisation Measures

Routine Risk Minimization measures are sufficient to manage use of Ensitrelvir.

The routine risk minimization measures for ZOKOVEA in the EU comprise the SmPC, the package leaflet (PL), and the legal status of the product. There are no individual safety concerns for ZOKOVEA.

V.2. Additional Risk Minimisation Measures

Routine risk minimisation activities as described in Part V.1 and are sufficient to manage the safety concerns of the medicinal product. No additional risk minimization measures are warranted, as there are no safety concerns for the medicinal product.

V.3 Summary of risk minimisation measures

Not applicable

PART VI: SUMMARY OF THE RISK MANAGEMENT PLAN

Summary of risk management plan for ZOKOVEA (ensitrelvir)

This is a summary of the risk management plan (RMP) for ZOKOVEA. The RMP details important risks of ZOKOVEA. how these risks can be minimised, and how more information will be obtained about ZOKOVEA 's risks and uncertainties (missing information).

ZOKOVEA 's summary of product characteristics (SmPC) and its package leaflet give essential information to healthcare professionals and patients on how ZOKOVEA should be used.

This summary of the RMP for ZOKOVEA should be read in the context of all this information including the assessment report of the evaluation and its plain-language summary, all which is part of the European Public Assessment Report (EPAR).

Important new concerns or changes to the current ones will be included in updates of ZOKOVEA's RMP.

I. The medicine and what it is used for

Zokovea contains the active substance ensitrelvir. This is a medicine that helps to fight viral infections throughout the whole body.

ZOKOVEA is used forpost exposure prophylaxis of coronavirus disease 2019 (COVID-19) in adults and adolescents aged 12 years and older. (see SmPC for the full product information).

Pharmaceutical form and strength: Approximately 9 mm round, white to light yellow white, tablets debossed on one side with the company logo and “711”, and “125” on the other side.

Further information about the evaluation of ensitrelvir’s benefits can be found in ZOKOVEA’s EPAR, including in its plain-language summary, available on the EMA website, under the medicine’s webpage <link to the EPAR summary landing page>.

II. Risks associated with the medicine and activities to minimise or further characterise the risks

Important risks of ZOKOVEA together with measures to minimise such risks and the proposed studies for learning more about ZOKOVEA's risks, are outlined below.

Measures to minimise the risks identified for medicinal products can be:

- Specific information, such as warnings, precautions, and advice on correct use, in the package leaflet and SmPC addressed to patients and healthcare professionals.
- Important advice on the medicine’s packaging.
- The authorised pack size — the amount of medicine in a pack is chosen so to ensure that the medicine is used correctly.

- The medicine's legal status — the way a medicine is supplied to the patient (eg, with or without prescription) can help to minimise its risks.

Together, these measures constitute *routine risk minimisation* measures.

In addition to these measures, information about adverse reactions is collected continuously and regularly analysed, including PSUR assessment so that immediate action can be taken as necessary. These measures constitute routine pharmacovigilance activities.

If important information that may affect the safe use of ZOKOVEA is not yet available, it is listed under 'missing information' below.

II.A List of important risks and missing information

Important risks of ZOKOVEA are risks that need special risk management activities to further investigate or minimise the risk, so that the medicinal product can be safely taken. Important risks can be regarded as identified or potential. Identified risks are concerns for which there is sufficient proof of a link with the use of ZOKOVEA. Potential risks are concerns for which an association with the use of this medicine is possible based on available data, but this association has not been established yet and needs further evaluation. Missing information refers to information on the safety of the medicinal product that is currently missing and needs to be collected (eg, on the long-term use of the medicine);

List of important risks and missing information	
Important identified risks	None
Important potential risks	None
Missing information	None

II.B Summary of important risks

None

II.C Post-authorisation development plan

II.C.1 Studies which are conditions of the marketing authorisation

There are no studies required for ZOKOVEA.

PART VII: ANNEXES

Annex 1 Eudravigilance interface

Not applicable

[Annex 1 of the RMP is not required to be submitted in eCTD; the electronic file is submitted in accordance to GVP Module V Section V.C.2 and the guidance on the website[1]. Therefore Annex 1 is left empty].

1 http://www.ema.europa.eu/ema/index.jsp?curl=pages/regulation/general/general_content_000683.jsp&mid=WC0b01ac058067a113

Annex 2 Tabulated summary of planned, ongoing, and completed pharmacovigilance study programme

Not applicable

Annex 3 Protocols for proposed, on-going and completed studies in the pharmacovigilance plan

Not applicable

Annex 4 Specific adverse drug reaction follow-up forms

Not applicable

Annex 5 Protocols for proposed and on-going studies in RMP part IV

Not applicable

Annex 6 Details of proposed additional risk minimisation activities (if applicable)

Not applicable

Annex 7 Other supporting data (including referenced material)

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Annex 8 Summary of changes to the risk management plan over time

Version	Approval date Procedure	Change
1.0	<At the time of authorisation> <procedure number> dd/mm/yyyy	Not applicable