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Reproductive and developmental safety of nirmatrelvir (PF-07321332), an oral SARS-CoV-2 M^{pro} inhibitor in animal models

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ABSTRACT

Nirmatrelvir (PF-07321332; NMV) the antiviral component of PAXLOVID™ is a potent and selective inhibitor of the SARS-CoV-2 main protease (M^{pro}), which plays a critical role in viral replication. PAXLOVID, comprised of nirmatrelvir and ritonavir (used as a pharmacokinetic enhancer), is an oral therapy currently in development as a therapeutic option for those infected with SARS-CoV-2 to prevent progression to severe disease, hospitalization, and death. PAXLOVID has been shown to be efficacious against hospitalization and death in two Phase 2/3 clinical studies that evaluated non hospitalized patients both with and without high risk factors for progression to severe illness. Given that males and females of reproductive age are included in the intended patient population, we assessed the potential effects of NMV up to the limit dose of 1000 mg/kg/day in ICH guideline embryo-fetal development studies in rats and rabbits, and a fertility and early embryonic development study in rats. There were no effects on male and female fertility or early embryonic development in rats, and no severe manifestations of developmental toxicity in rats or rabbits. The lack of adverse findings reported here in nonclinical species is consistent with the intended therapeutic target of NMV (a virus specific protein not present in mammalian cells), the favorable off-target selectivity profile, and lack of genetic toxicity. The results of these nonclinical studies with NMV along with existing ritonavir safety information indicate that there are no clinically relevant risks associated with PAXLOVID administration during pregnancy and in males and females of reproductive age.

1. Introduction

The coronavirus disease 2019 (COVID-19) global pandemic caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has resulted in over 250 million confirmed COVID-19 cases and over 5 million deaths worldwide as of November 2021, prior to the emergency use authorization (EUA) of PAXLOVID. While vaccines have proven to be effective at preventing COVID-19, therapeutic options are still needed to treat COVID-19 in individuals that are immunocompromised and may not response to the vaccine and in those whose titers that have waned over time, which is particularly relevant as new variants of concern emerge. To address the need for therapeutic options for those infected with SARS-CoV-2, the oral small molecule nirmatrelvir (PF-07321332; NMV) is being developed to prevent progression to severe disease, hospitalization, and death. NMV is a potent and selective inhibitor of the SARS-CoV-2 main protease (M^{pro}), which plays a critical role in viral replication [1]. NMV is orally bioavailable, providing a valuable oral antiviral treatment option outside of the hospital setting. Other therapeutic options currently available, as of November 2021, through EUA by FDA for COVID-19 include SARS-CoV-2 targeting monoclonal antibodies (REGEN−COV [casirivimab/imdevimab], sotrovimab, bamlanivimab, and etesevimab), the antiviral drug remdesivir (approved in individuals 12 years of age and older and weighing at least 40 kg; EUA for hospitalized pediatrics weighing at least 3.5 kg), and immune modulators baricitinib and tocilizumab.

In a final analysis of data from a randomized, double blind Phase 2/3 clinical trial in non-hospitalized high-risk adults, PAXLOVID was found to significantly reduce hospitalization or death by 89 % compared to placebo, confirming results of an interim analysis [2,3]. Additionally, NMV has a favorable in vivo safety profile, as demonstrated by lack of adverse findings in 2-week general toxicology studies in rats and monkeys at doses up to 1000 mg/kg/day and 600 mg/kg/day, respectively,

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and lack of genetic toxicity [1]. The lack of adverse findings in nonclinical species is consistent with the high selectivity of NMV for the intended therapeutic target, which is a virus specific protein not present in mammalian cells [1]. Due to the predominant role of CYP3A4 in the metabolism of NMV, co-administration with ritonavir, a CYP3A4 inhibitor, is being used to enhance therapeutic concentrations of NMV, a clinically proven approach to enhancing pharmacokinetics of protease inhibitors [1,4].

Given that males and females of reproductive age are included in the intended patient population, low risk of effects on fertility and embryo-fetal development, which includes a lack of genetic toxicity, are desirable for widespread use of PAXLOVID. In addition, favorable developmental toxicity and genetic toxicity profiles are needed to support potential use in pregnant women, which may be of importance given the evidence of higher risk related to COVID-19 during pregnancy [5–15]. Here we report the results of the reproductive and developmental toxicity studies with NMV, the antiviral component of PAXLOVID.

2. Materials and methods

All studies were conducted in compliance with US Food and Drug Administration Good Laboratory Practice (GLP) regulations in a test facility that was accredited by the Association of Assessment and Accreditation of Laboratory Animal Care (AAALAC) with oversight by an Institutional Animal Care and Use Committee (IACUC).

2.1. Test article

For the embryo-fetal development (EFD) and fertility studies, NMV was manufactured as a 50 % spray dried dispersion (SDD; 50 % active PF-07321332, 50 % HPMCAS-MG) and supplied by Pfizer, Inc. (New York, NY, USA) and was formulated for each study in an aqueous vehicle consisting of a suspension of 1 % (w/v) Soluplus and 0.5 % (w/v) methylcellulose A4M in purified water. For the vehicle control, the MF grade of HPMCAS was used to more closely match the particle size that resulted from the SDD process and was formulated in the same manner as the test article. For each study, NMV concentrations were analyzed and confirmed in dosing formulations.

2.2. Animals and husbandry

Male and female Wistar Han (Crl:WI[Han]) rats (Charles River Laboratories, Inc., Raleigh, NC) and female [Hra:(NZW)SPF] New Zealand White rabbits (Envigo Global Services, Inc., Denver, PA) were used in these studies. Animals were group housed, except when pregnant or paired for mating. Rats were provided Certified Irradiated Rodent Diet 2916C (Envigo Teklad Global Diet) available ad libitum. Rabbits were provided 150 g/day of Certified Rabbit Diet 2030C (Envigo Teklad Global Diet). Municipal drinking water purified by reverse osmosis was provided ad libitum. Environmental conditions across studies were set to maintain relative humidity at 30 %–70 % and temperature of 68 °F–79 °F and 61 °F–72 °F for rats and rabbits, respectively. Room lighting was set to provide a 12-h light/dark cycle.

2.3. Study design and test article administration

2.3.1. Fertility and early embryonic development study in rats

Male (9–14 weeks old and 326–397 g at dose initiation) and female (12–14 weeks old and 196–261 g at dose initiation) rats were acclimated and randomly assigned to four groups (n = 20 per group/sex). NMV doses of 0, 60, 200, or 1000 mg/kg/day were orally (gavage) administered to both sexes 14 days prior to mating, throughout the mating phase, and until gestation day (GD) 6 for the females and for a total of 32 doses in the males. NMV was tested up to the limit dose of 1000 mg/kg/day in accordance with ICH guidelines [16]. Control animals were administered the vehicle by the same dosing route and regimen. The dosing volume was 10 mL/kg. Blood samples were taken from male and female rats at 0.5 h after dosing on day 10, and systemic exposure was consistent with previous studies (data not shown).

2.3.2. Embryo-fetal development studies in rats and rabbits

A total of 80 presumed pregnant rats (8–11 weeks old and 192−253 g at dose initiation) and 80 presumed pregnant rabbits (4–6 months old and 2.8–3.6 kg at dose initiation) were acclimated and randomly assigned to dose groups (n = 20 per group). Rats and rabbits were administered 0, 100, 300, or 1000 mg/kg/day NMV by oral gavage once daily from GD 6 through 17 and GD 7 through 19, respectively. NMV was tested up to the limit dose of 1000 mg/kg/day in accordance with ICH guidelines [16]. Control animals were administered the vehicle by the same dosing route and regimen. The dose volume for both species was 10 mL/kg. Blood samples were taken from maternal animals (n = 5 per group) at 0 (predose), 0.5, 1, 2, and 4 h after the GD 17 and GD 19 dose in rats and rabbits, respectively, to determine NMV systemic concentrations.

2.3.3. Observations and measurements

Clinical signs, body weight, and food consumption were monitored throughout all studies.

The study design, toxicokinetic analysis, and statistical analysis used for the fertility and rat and rabbit EFD studies have been previously described [17,18]. Briefly, in the fertility study estrous cycling was monitored in the female rats 14 days prior to dose initiation, during dosing, and continuing until positive evidence of mating was observed (sperm present in smear of vaginal contents or presence of copulatory plug). The day on which evidence of mating was found was designated as GD 0. Females were euthanized on GD 14 by gas anesthesia (isoflurane) followed by exsanguination, and underwent a macroscopic evaluation of the abdominal, thoracic, and pelvic viscera. The ovaries and uterine contents were examined for number and distribution of corpora lutea, implantation sites, placentae, and viable and nonviable embryos. Apparently non-gravid uteri were stained with 10 % ammonium sulfide to visualize potential implantation sites [19]. Male rats were euthanized after 32 days of dose administration, and also underwent macroscopic evaluation of the abdominal, thoracic, and pelvic viscera. The testes, epididymides, and accessory sex organs (prostate gland and seminal vesicles) were examined and retained for possible microscopic evaluation.

In the EFD studies, maternal rats and rabbits were euthanized on GD 21 and GD 29, respectively, via gas anesthesia (isoflurane) followed by exsanguination (rats) or intravenous injection of a barbiturate followed by exsanguination (rabbits). Following euthanasia, maternal animals underwent macroscopic examination of the abdominal, thoracic, and pelvic viscera, and examination of ovarian and uterine contents (number and distribution of corpora lutea, implantation sites, placentae [size, color, or shape], live and dead fetuses, and early and late resorptions). Fetuses were removed from the uterus, weighed individually, and examined for sex and external abnormalities. All fetal rats and rabbits were examined for visceral abnormalities using a modification of the microdissection technique of Staples [20] and following evisceration, cleared, and stained with alizarin red S [21], they were examined for skeletal abnormalities.

3. Results

3.1. Fertility and early embryonic development

NMV was well-tolerated, with no evidence of systemic toxicity in both males and females as demonstrated by lack of NMV-related clinical signs, food consumption or body weights (Table 1). Lower food consumption observed during the premating phase for the males at 1000 mg/kg/day was not considered NMV-related due to the low magnitude, lack of consistency, and there was no overall impact on body weight.

Table 1
Summary of body weight, food consumption, fertility, and mid-gestation uterine examination data from the fertility study in rats.

	Dose (mg/kg/day)			
	0	60	200	1000
Number of females/males	20/20	20/20	20/20	20/20
Male Terminal Body Weight (g)	408.9 ± 23.1[a]	409.9 ± 22.7	411.6 ± 22.2	408.9 ± 19.7
Male Food Consumption (g/day)				
Premating Day 1–14	22.4 ± 1.3	22.0 ± 1.1	23.0 ± 1.2	20.9 ± 0.9 *
Mating Day 15–19	18.4 ± 2.2	19.3 ± 2.8	18.0 ± 3.1	18.3 ± 3.2
Female Terminal Body Weight (g)	273.6 ± 15.8	277.0 ± 24.1	272.0 ± 13.3	277.8 ± 14.1
Female Food Consumption (g/day)				
Premating Day 1–14	14.7 ± 0.7	15.3 ± 1.2	14.8 ± 0.8	14.7 ± 1.0
Gestation Day 0–14	19.2 ± 2.0	19.2 ± 2.1	19.0 ± 2.2	19.0 ± 1.6
Fertility Data				
Mean no. of estrous cycles	2.8 ± 0.4	2.7 ± 0.5	2.7 ± 0.5	2.6 ± 0.5
Precoital interval (days)[b]	3.7 ± 3.5	3.6 ± 3.5	3.0 ± 2.2	3.2 ± 2.9
Mating index (%)[c]	20/20 (100 %)	20/20 (100 %)	20/20 (100 %)	20/20 (100 %)
Fecundity Index (%)[d]	20/20 (100 %)	19/20 (95 %)	18/20 (90 %)	20/20 (100 %)
Fertility Index (%)[e]	20/20 (100 %)	19/20 (95 %)	18/20 (90 %)	20/20 (100 %)
Number of pregnant females	20	19	18	20
Uterine Examination Data				
Corpora lutea	13.3 ± 1.5	13.8 ± 1.3	14.1 ± 1.7	12.7 ± 1.6
Implantation sites	10.3 ± 4.2	12.5 ± 1.4	12.6 ± 3.0	10.5 ± 3.1
Live embryos	8.9 ± 4.4	11.8 ± 1.4	11.5 ± 3.2	9.6 ± 3.2
Resorptions	1.4 ± 1.3	0.7 ± 0.7	1.1 ± 1.6	1.0 ± 0.8
Preimplantation loss (%)	23.8 ± 28.9	9.7 ± 8.1	11.0 ± 18.4	16.9 ± 22.8
Postimplantation loss (%)	18.1 ± 24.2	5.3 ± 5.7	9.5 ± 12.8	10.6 ± 11.9

* $p \leq 0.05$.
[a] Data presented as mean per group ± standard deviation.
[b] Precoital interval is the number of days cohabitated before confirmed copulation (sperm observed in vaginal smear or copulatory plug observed in vagina).
[c] Mating index is the number of mating pairs with confirmed mating/the number of mating pairs cohabitated.
[d] Fecundity index is the number of mating pairs with a pregnant female/the number of mating pairs with confirmed copulation.
[e] Fertility index is the number of mating pairs with a pregnant female/the number of mating pairs cohabitated.

There were no NMV-related effects on male or female fertility, as evidenced by lack of effects on estrous cyclicity, precoital interval, and mating, fecundity, and fertility indices (Table 1). In addition, there were no NMV-related effects on uterine examination endpoints such as the number of corpora lutea, implantation sites, and embryo viability, providing further evidence for lack of effects on male and female fertility as well as early embryonic development and a no observed adverse effect level (NOAEL) of 1000 mg/kg/day (the highest dose tested).

3.2. Rat embryo-fetal development

There was no NMV-related maternal toxicity, as demonstrated by lack of NMV-related maternal mortality or clinical signs (data not shown) as well as lack of effects on maternal body weight gain, body weight, or food consumption at any dose (Table 2). There were also no NMV-related effects on embryo-fetal viability, based on similar percentage of post-implantation loss across all dose groups, including control (Table 2). While the number of live fetuses is slightly lower in the

Table 2
Maternal data, cesarean section observations, and fetal weights from the rat embryo-fetal development study.

	Dose (mg/kg)				Historical Control Data[b]
	0	100	300	1000	
Number of Pregnant Dams	18	17	20	19	–
Maternal Body Weight (GD 21; g)	304.5 ± 26.7[a]	298.3 ± 11.3	303.6 ± 15.7	310.6 ± 21.7	–
Maternal Body Weight Gain (GD 6–21; g)	86.7 ± 20.1	85.6 ± 8.3	87.9 ± 14.5	92.1 ± 14.8	–
Maternal Food Consumption (GD 6–21; g/day)	320.4 ± 42.3	313.4 ± 32.0	322.3 ± 20.8	309.5 ± 43.1	–
Corpora lutea	12.1 ± 1.6	11.4 ± 1.1	11.5 ± 1.5	11.5 ± 1.5	11.9 (11.1–12.9)
Implantation sites	11.2 ± 1.7	9.9 ± 1.5	9.3 ± 2.4	9.7 ± 2.6	10.3 (8.9–11.5)
Pre-implantation loss (%)	7.1 ± 10.4	12.8 ± 11.1	18.7 ± 19.9	15.3 ± 21.0	13.0 (5.8–18.9)
Post-implantation loss (%)	9.7 ± 13.1	6.5 ± 15.4	6.8 ± 14.9	9.7 ± 14.1	7.3 (3.6–13.5)
Live fetuses/litter	10.2 ± 2.3	9.2 ± 1.9	8.7 ± 2.7	8.9 ± 3.0	9.6 (8.5–10.5)
Sex ratio (% male)	50.5 ± 16.0	49.3 ± 13.6	48.9 ± 18.1	50.9 ± 18.5	48.3 (41.4–56.5)
Fetal weight (g)/ Litter	5.2 ± 0.4	5.3 ± 0.3	5.5 ± 0.3	5.4 ± 0.3	5.2 (5.1–5.4)

(g) = grams; GD = Gestation Day.
[a] Data presented as mean per group ± standard deviation.
[b] Pfizer, Groton, CT; Wistar Han (Crl:WI[Han]) rats; 2017–2021; data from 10 studies presented as mean (minimum-maximum).

NMV dosed groups, this is not related to NMV administration, but rather to the higher percent pre-implantation loss and resultant lower number of implantation sites in these groups and because NMV administration was initiated after implantation already occurred. Additionally, the number of live fetuses across all dose groups up to 1000 mg/kg/day, were within the normal range of historical control (8.5–10.5 live fetuses/litter). There were no NMV-related effects on fetal body weight (Table 2), and no effects on fetal external, visceral, or skeletal morphological development (Supplemental Table 1). All fetal visceral and skeletal findings that were observed in the study were considered to be incidental and unrelated to NMV because the findings were not dose-dependent, limited to single incidences within a dose group, and/or occurred at an incidence within the normal range of historical control. Based on the lack of adverse effects in this study, the NOAEL was 1000 mg/kg/day (the highest dose tested). Toxicokinetic analysis revealed that NMV exposure increased with increasing dose on GD 17 (Table 3).

3.3. Rabbit embryo-fetal development

There was no NMV-related maternal mortality or clinical signs at any dose (data not shown). In the 1000 mg/kg/day group, mean maternal body weight gains and food consumption were slightly lower than control, with effects on maternal body weight gain and food consumption at ≤300 mg/kg/day (Table 4). There were no NMV-related effects on embryo-fetal viability. Lower fetal body weights (91 % of controls) were observed at 1000 mg/kg/day (Table 4), in the presence of the lower maternal body weight gain and food consumption at this dose. Fetal examinations showed no NMV-related effects on external, visceral, or skeletal morphological development (Supplemental Table 2). All fetal findings that were observed in the study were considered to be incidental and unrelated to NMV because the findings were not dose-dependent, limited to single incidences within a dose group, and/or

Table 3
Maternal mean total systemic exposure in the rat and rabbit.

	Sampling Day	Dose (mg/kg/day)	Mean Systemic Exposure		Exposure Margin[a]	
			C_{max} (μg/mL)	AUC_{24} (μgh*/mL)	C_{max}	AUC_{24}
Rat EFD Study	GD 17	100	29.0 ± 11.0	75.5 ± 12.4	7.0x	1.1x
		300	43.2 ± 14.4	346 ± 92.0	10x	5.0x
		1000	65.4 ± 18.7	535 ± 330	16x	7.8x
Rabbit EFD Study	GD 19	100	17.0 ± 6.1	98.7 ± 27.4	4.1x	1.4x
		300	42.9 ± 11.1	195 ± 56.8	10x	2.8x
		1000	99.6 ± 46.5	689 ± 206	24x	10x

[a] Calculated using animal total exposures relative to predicted human total PF-07321332 C_{max} of 4.14 μg/mL and AUC_{24} of 68.6 μg h/mL at a twice daily dose of 300/100 mg PF-07321332/ritonavir.

Table 4
Maternal data, cesarean section observations, and fetal weights from the rabbit embryo-fetal development study.

	Dose (mg/kg)				Historical Control Data[b]
	0	100	300	1000	
Number of Pregnant Does	20	20	19	19	–
Maternal Body Weight (GD 29; kg)	3.6 ± 0.2[a]	3.6 ± 0.2	3.6 ± 0.2	3.6 ± 0.2	–
Maternal Body Weight Gain (GD 7–29; kg)	0.5 ± 0.2	0.5 ± 0.1	0.4 ± 0.1	0.4 ± 0.1	–
Maternal Food Consumption (GD 7–29; g)	2979.6 ± 398.4	2983.9 ± 289.5	2908.9 ± 500.1	2703.0 ± 514.2	–
Corpora lutea	10.2 ± 1.5	9.7 ± 1.5	9.4 ± 2.1	9.5 ± 1.7	9.8 (9.3–10.2)
Implantation sites	9.0 ± 1.3	8.2 ± 1.7	8.6 ± 2.3	8.8 ± 1.6	9.0 (8.4–9.5)
Pre-implantation loss (%)	11.3 ± 10.0	14.4 ± 17.9	9.5 ± 10.2	6.4 ± 11.8	7.9 (6.1–10.1)
Post-implantation loss (%)	3.7 ± 6.7	3.3 ± 7.7	5.4 ± 7.4	3.2 ± 4.9	3.2 (0.4–7.1)
Live fetuses/litter	8.6 ± 1.3	7.9 ± 1.7	8.1 ± 2.1	8.5 ± 1.5	8.7 (7.9–9.2)
Sex ratio (% male)	46.9 ± 14.6	49.7 ± 19.2	48.0 ± 23.8	44.8 ± 16.4	49.1 (44.1–54.0)
Fetal weight (g)/Litter	41.6 ± 2.6	40.2 ± 3.0	40.3 ± 4.5	37.7 ± 4.4**	41.6 (40.3–43.0)

(g) = grams; GD = Gestation Day.
** $p \leq 0.01$.
[a] Data presented as mean per group ± standard deviation.
[b] Pfizer, Groton, CT; New Zealand White [Hra:(NZW)SPF] rabbits; 2017–2021; data from 10 studies presented as mean (minimum-maximum).

occurred at an incidence within the normal range of historical control. There was no effect on fetal viability or morphological development, however, the NOAEL for developmental toxicity was 300 mg/kg/day based on lower fetal weights at 1000 mg/kg/day. Toxicokinetic analysis revealed that NMV exposure increased with increasing dose on GD 19 (Table 3).

4. Discussion

NMV did not cause malformations or lower embryo-fetal survival when administered to pregnant rats and rabbits. The only finding was a slight reduction in rabbit fetal body weights in the presence of lower maternal gestational body weight gain. The lower fetal body weight is consistent with delayed development and occurred following exposure throughout the period of organogenesis (GD 7–19 in rabbits that corresponds with first trimester gestation in humans) which represents 43 % (13 days of dosing out of 30-day gestation period) of the total gestational period in rabbits. In comparison, the intended 5-day course of PAXLOVID treatment represents < 2% (5 days of treatment for 280 days of gestation) of the human gestational period. Additionally, the reduction in rabbit fetal weights occurred at 10-fold the highest anticipated exposure in humans (Table 3) and therefore the lower rabbit fetal weight is not considered a meaningful human risk. There were no effects on male or female fertility or pre-implantation embryonic development. Taken together, the studies presented here indicate that NMV has a favorable reproductive safety profile based on the lack of clinically meaningful effects on embryo-fetal development and male or female fertility in animals along with a lack of findings in a battery of genetic toxicology tests [1].

In addition, the intended therapeutic target of NMV further supports the favorable reproductive risk assessment. NMV inhibits SARS-CoV-2 3CL M^{pro}, a virus specific enzyme that is not present in mammalian cells and therefore would not be expected to interfere with pathways involved in mammalian development. Furthermore, NMV demonstrated a favorable off-target selectivity profile [1], further supporting the favorable reproductive risk assessment.

The lack of genotoxicity and off-target activity for NMV is an important consideration for the pregnancy risk assessment of antiviral compounds. Similar to NMV, remdesivir, a viral RNA-dependent RNA polymerase inhibitor approved for treatment of hospitalized COVID-19 patients, demonstrated no adverse effects on embryo-fetal development in rats or rabbits up the highest dose tested, 20 mg/kg/day [22]. In contrast, molnupiravir, an antiviral oral treatment that is authorized in the UK, resulted in severe manifestations of developmental toxicity (malformations and embryo-fetal lethality) as well as lower fetal body weight and developmental variations at the highest dose of molnupiravir in rats, 1000 mg/kg/day, with lower fetal body weights and delayed ossification observed in the presence of decreased maternal body weight gain at 500 mg/kg/day and no developmental toxicity at 250 mg/kg/day [23]. Developmental toxicity of molnupiravir in rabbits was limited to lower fetal body weights in the presence of slight maternal toxicity (lower food consumption, body weight gain, and abnormal fecal output) at 750 mg/kg/day with no developmental toxicity observed at 400 mg/kg/day [23]. Molnupiravir is a nucleoside analog that induces lethal mutagenesis after it is metabolized into a ribonucleoside analog that resembles cytidine, which is then incorporated into newly made RNA in place of cytidine. Continued replication of RNA containing deleterious mutations results in an accumulation of errors in the viral genome leading to inhibition of replication, an effect known as viral error catastrophe [23]. Other antiviral compounds that work by targeting incorporation into viral RNA include ribavirin (RBV) and favipiravir (FAV). Both of these compounds have shown to be teratogenic in multiple species. RBV has been shown to be embryocidal and/or teratogenic in rats, rabbits, and guinea pigs [24–26] and FAV for which teratogenesis has been reported in monkeys, mice, rats, and rabbits [27]. There is a concern that that the mutagenic mechanism that results in antiviral activity of these compounds could theoretically result in incorporation and subsequent mutagenesis of the host DNA [28,29]. Consistent with the mechanism of action, molnupiravir and its active metabolite were positive in the in vitro bacterial reverse mutation assay (Ames assay) and a mammalian cell hypoxanthine phosphoribosyltransferase (HPRT) gene mutation assay; however it was reported to be negative in the Big Blue® (cII Locus) transgenic rodent mutagenesis

assay [23,29,30]. Therefore, it seems unlikely that mutagenesis is the mechanism responsible for the embryo-fetal toxicity observed in animals with molnupiravir.

NMV is being administered in combination with ritonavir as a pharmacokinetic enhancer. Inhibition of CYP3A with ritonavir increases the bioavailability of NMV by inhibiting its metabolism, thereby increasing the duration of therapeutic levels. While no longer widely used as a sole antiviral agent, several antiviral regimens use ritonavir to reduce metabolism and prolong exposure, and as such there is a body of human literature on the use of ritonavir in a broad patient population that includes pediatrics, males and females of reproductive age, and pregnant women. Specifically in regards to use during pregnancy, in 3453 live births following exposure to ritonavir-containing regimens in the first-trimester there was no difference in the rate of overall birth defects for ritonavir (2.35 %) compared with the background birth defect rate of 2.72 % in the U.S. reference population of the Metropolitan Atlanta Congenital Defects Program (MACDP) [31]. The number of first trimester exposures to ritonavir is sufficient to detect at least a 1.5-fold increase in risk of overall birth defects and a 2-fold increase in risk of birth defects in the more common classes, cardiovascular and genitourinary system; no such increases have been observed [31]. In nonclinical studies with ritonavir, no evidence of fetal malformations was observed in rat and rabbit EFD studies at doses of 15, 35, and 75 mg/kg and 25, 50, and 110 mg/kg/day, respectively. Developmental toxicity associated with ritonavir administration in EFD studies in rats and/or rabbits (higher resorptions, lower fetal body weight, ossification delays, and increased skeletal variations) occurred only at the highest dose evaluated in each species, which were also maternally toxic doses. Most of these findings typically represent a developmental delay and are often associated with exposure throughout the entire period of organogenesis rather than to a specific sensitive window of gestation. Extending the treatment period through the end of gestation and through lactation (pre-and postnatal development study [PPND]) in rats did not appear to exacerbate these findings.

In addition to the findings mentioned above, cryptorchidism was reported in the mid (35 mg/kg/day) and high (75 mg/kg/day) ritonavir doses in the rat EFD study; however, there is limited biologic plausibility of identifying cryptorchidism in a rat EFD study as testicular descent does not occur until after weaning [32]. In addition, there were no reports of cryptorchidism in postnatal developing rats in the rat PPND study in which maternal rats are dosed from GD 6 through parturition and continuing out to postpartum day 20 and offspring are allowed to develop until adulthood. Mature male offspring in the PPND study showed normal reproductive function, supporting the lack of effects of ritonavir on the developing male reproductive system. Ritonavir was also evaluated in combination with paritapevir in embryo fetal toxicity studies in rats and mice. There were no effects on embryo-fetal development in either species at the highest combination dose, which contained 45 mg/kg/day and 30 mg/kg/day ritonavir in rats and mice, respectively [33], further indicating that the observation of cryptorchidism was spurious.

Women who are pregnant are at increased risk for severe illness with COVID-19 and pregnant women with COVID-19 are more likely to have premature birth and might be at increased risk for pregnancy complications or loss, highlighting the need for antiviral therapies and the importance of reproductive safety of these potential therapies [5–15]. The potential risks and benefits of PAXLOVID use during pregnancy should be discussed with the patient's physician. However, the available information indicates low concern for fetal harm with PAXLOVID, a potent and selective inhibitor of the SARS-CoV-2 main protease (NMV) given in combination with ritonavir, a pharmacokinetic enhancer. This is based on the intended pharmacological target and target selectivity of NMV, limited duration of clinical administration, human data with ritonavir, lack of clinically meaningful effects on embryo-fetal development in nonclinical studies with NMV, and lack of genetic toxicity with NMV and ritonavir. The results of these nonclinical studies with NMV along with existing ritonavir safety information indicate that there are no clinically relevant risks associated with PAXLOVID administration during pregnancy and in males and females of reproductive age.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.reprotox.2022.01.006.

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