Review

Molnupiravir revisited – critical assessment of studies in animal models of Covid-19

Henrik Berg Rasmussen * and And Peter Riis Hansen

Posted Date: 6 September 2023

doi: 10.20944/preprints202309.0334.v1

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Review

Molnupiravir Revisited—Critical Assessment of Studies in Animal Models of COVID-19

Henrik Berg Rasmussen [1,2,*] and Peter Riis Hansen [3,4]

[1] Institute of Biological Psychiatry, Mental Health Centre Sct. Hans, 4000 Roskilde, Denmark; henrik.berg.rasmussen@regionh.dk
[2] Department of Science and Environment, Roskilde University, 4000, Roskilde, Denmark
[3] Department of Cardiology, Herlev and Gentofte Hospital, Copenhagen University Hospital, 2900 Hellerup, Denmark; peter.riis.hansen@regionh.dk
[4] Department of Clinical Medicine, Faculty of Health and Medical Sciences, University of Copenhagen, 2200 Copenhagen, Denmark;
* Correspondence: henrik.berg.rasmussen@regionh.dk

Abstract: Molnupiravir, a prodrug known for its broad antiviral activity, demonstrated efficacy in animal models of Covid-19, prompting clinical trials where initial results indicated a significant effect against the disease. However, subsequent clinical studies did not confirm these findings, leading to the rejection of molnupiravir for permanent market authorization in many countries. This report critically assessed 19 studies published in 17 reports that investigated the efficacy of molnupiravir in animal models of Covid-19 with the purpose of determining how well the design of these models informed human studies. We found that the administered doses of molnupiravir in most studies involving animal Covid-19 models were disproportionately higher than the dose recommended for human use. Specifically, when adjusted for body surface area, half of the doses of molnupiravir used in the animal studies were more than twice as high as the human dose. Additionally, the drug was frequently given prophylactically or shortly after SARS-CoV-2 inoculation in these models, in contrast to clinical trials where such timing is not consistently achieved. Furthermore, the recommended five-day treatment duration for humans was exceeded in several animal studies. Collectively, we suggest that these design elements in the reported animal studies contributed to a bias favoring molnupiravir, and thus inflated expectations for its efficacy against Covid-19. Addressing these elements may offer avenues to enhance the clinical efficacy of molnupiravir for treatment of Covid-19 that include dose increment, early treatment, and administration by inhalation along with use of molnupiravir in antiviral combination therapy.

Keywords: molnupiravir; COVID-19; antiviral efficacy; animal models; animal-to-human extrapolation

1. Background

Molnupiravir is the isopropylester derivative of the nucleoside analog β-D-N4-hydroxycytidine (NHC). This nucleoside analog possesses broad antiviral activity and initially garnered attention as agent for treatment of infections caused by the equine encephalitis viruses and other Alphavirus infections [1–4]. Due to low bioavailability in non-human primates, NHC was redesigned as the ester prodrug molnupiravir, which entered preclinical testing for treatment of seasonal influenza [2,5]. However, with the emergence of coronavirus disease 2019 (Covid-19), molnupiravir was repurposed to treat this new pandemic.

Findings of substantial in vitro anti-SARS-CoV-2 activity led to preclinical efficacy studies of molnupiravir in animal models of Covid-19 [6]. Studies in mice, Syrian hamsters, Roborovski dwarf hamsters, ferrets and nonhuman primates all indicated substantial in vivo anti-SARS-CoV-2 activity of molnupiravir as evidenced by effective viral clearance, improved clinical signs and reduced lung pathology [6]. Also, molnupiravir effectively blocked viral transmission in the ferret model of Covid-19, a model that is characterized by robust upper airway replication of SARS-CoV-2 while expressing

only mild symptoms, and therefore imitating infections with the virus in children and teenagers [7]. Assessment of the efficacy of molnupiravir in nonhuman primates was partially hampered by mild clinical disease after inoculation with SARS-CoV-2, but the agent reduced viral load in the airways of these animals [8,9].

During the Covid-19 pandemic molnupiravir entered human clinical trials to assess its efficacy against the disease, and on October 25, 2021, the European Medicines Agency initiated a rolling review of the agent based on results from preclinical studies and the MOVe-OUT trial, a randomized placebo-controlled clinical trial [10]. A few months later, on December 23, 2021, the United States Food and Drug Administration issued an emergency use authorization for molnupiravir. In several other countries use of the drug also became possible without formal market authorization. Subsequently, data from additional clinical trials in humans became available that did not confirm the substantial benefits reported by the MOVe-OUT trial [10–12] , and in February 2023 the European Medicines Agency recommended against marketing authorization of the agent [13].

Early treatment of SARS-CoV-2 infections with compounds that act by inhibiting viral replication is critical to achieve a significant reduction in the number of infectious viral particles and symptom improvement [14]. This reflects that an increasing number of cells undergo infection in the initial phase of a viral invasion, known as the proliferation phase, culminating in the viral load peak, where the majority of susceptible cells are infected, resulting in a diminished number of viral replications that can be targeted and inhibited by the antiviral agent [15]. Hence, knowledge about the trajectory of the viral load including the time of the peak is pivotal for timing of treatment with direct-acting antiviral agents such as molnupiravir. In fact, achieving a favorable treatment outcome may rely on initiating treatment with these agents before the viral load peak is reached [15–17].

Since the exact time at which infection with SARS-CoV-2 occurs in humans often is difficult to ascertain, viral load peak is commonly reported with reference to symptom onset. Symptoms most often appear four to five days after infection in humans, albeit with shorter asymptomatic incubation periods for some SARS-CoV-2 variants [18]. Viral load peak occurs from symptom onset to a couple of days later in the upper respiratory tract of patients with Covid-19, and generally within one week after onset of symptoms [19–22]. However, levels of SARS-CoV-2 in the upper respiratory tract have also been reported to peak before symptoms onset [23,24].

Generally, the disease course in animal models of Covid-19 is shorter and more compressed than that humans with symptoms appearing two to three days after or even as early as one day after inoculation in the Roborovski dwarf hamster model [25,26]. Likewise, viral load peaks early in the ferret, hamster, K18-hACE2 transgenic mouse and macaque models of Covid-19, i.e., from 2 to 4 days after inoculation [26–29].

This report critically reviews studies investigating the efficacy of molnupiravir in animal models of Covid-19, assessing whether their designs might have favored the efficacy of the agent, and thus contributed to inflated expectations about its anti-SARS-CoV-2 activity in humans. Based on this review, we suggest potential strategies to enhance the effectiveness of the agent in Covid-19.

2. Collection of data

We searched PUBMED for studies examining the efficacy of molnupiravir in animal models using the search terms “Molnupiravir” AND “Covid-19” AND “Animal”. In subsequent searches the term “Animal” was replaced with “Mice”, “Ferret”, “Hamster” and “Macaque”. Based on this search strategy, we identified 17 relevant reports. As two of these reports evaluated the efficacy of molnupiravir in two distinct animal species, a total of 19 animal studies were assessed. We retrieved information regarding the experimental design and molnupiravir efficacy (Table 1). The experimental design included details such as the tested viral isolates, viral inoculation doses, dose levels of molnupiravir (mg/kg), time of initiation of treatment relative to inoculation, and treatment duration. Time from inoculation to viral load peak was evaluated based on measurements in control animals, i.e., animals administered vehicle instead of molnupiravir or other agent. We normalized doses of molnupiravir in mg/kg according to body surface area in the animal species, which served as models of Covid-19, using previously reported conversion factors [30]. A standard human body area surface

 doi:10.20944/preprints202309.0334.v1

was calculated using the duBois formula [31], with a height of 175 cm and weight of 75 kg. This weight was used to account for the increased risk of severe illness in COVID-19 due to obesity [32], potentially resulting in higher body weights among Covid-19 patients eligible for antiviral treatment, while also factoring in a lower body weight of females and differences in body weight across geographical regions

3. Evaluation of data

We evaluated the experimental design in the reported animal studies of Covid-19, with emphasis on the dose levels of molnupiravir and the timing of treatment with the drug. Evaluation of efficacy was based on treatment outcomes that varied across studies and included load of SARS-CoV-2 in tissues, swabs and nasal and bronchal lavage, body weight loss, survival, lung pathology and prevention of contact transmission. Determination of the viral load included quantification of infectious viruses (viral titer), viral RNA and protein produced by the virus.

Table 1. Doses, treatment timing, and efficacy of molnupiravir in animal models of Covid-19.

Animal model[1]	SARS-CoV-2 isolate used for inoculation[2]	Viral dose[3]	Oral drug dose in mg/kg, bidaily	Oral drug dose in mg/m^2, bidaily[4]	Start of treatment relative to time of inoculation	Treatment duration[5]	Efficacy of molnupiravir[6]	Study
SCID mouse	Beta	10^5 $TCID_{50}$	200	600	At inoculation	3 days	Reduced viral titers and viral RNA in lungs; improved lung pathology	Abdelnabi et al. 2022 [33]
K18-hACE2 mouse	Original type	5 MLD_{50}	20	60	6 hours after	5 days	Modest weight loss protection; improved clinical score; decreased viral RNA; viral titers in lungs largely unchanged; increased survival	Jeong et al. 2022 [34]
Lung-only mice	Original type	1-3 x 10^5 PFU	500	1500	12 hours before	2 days and 12 hours	Markedly reduced lung viral titers with pre-inoculation treatment being most effective; lower viral antigen in lungs; improved lung pathology	Wahl et al. 2021 [35]
					24 hours after	2 days		
					48 hours after	2 days		
K18-hACE-2 mouse	Original type	300 FFU	50	150	2 hours before	3 days and 8 hours	Reduction in lung viral titers; lung pathology not improved	Stegmann et al. 2022 [36]
Syrian hamster	Original type, Alpha, Beta	10^5 $TCID_{50}$	200	1000	1 hours before	4 days	Reduced viral titers and viral RNA in lungs; improved lung pathology; major weight increase for original viral type and Beta variant	Abdelnabi et al. 2021 [37]
Syrian hamster	Original type	2 x 10^6 $TCID_{50}$	75	375	1 hour before	4 days	Lung virus titers not reduced by 75 mg/kg dose of molnupiravir but lowered by the higher doses; 150 mg/kg dose probably suboptimal for monotherapy	Abdelnabi et al. 2021 [38]
			150	750				
			200	1000				
Syrian hamster	Beta, Omicron	10^4 $TCID_{50}$	150	750	At inoculation	4 days	Reduction in viral titers and virus RNA in lungs; lung pathology not significantly improved; no effect on body weight	Abdelnabi et al. 2022 [39]
Syrian hamster	Original type	10^6 PFU	50	250	4 hours before	5 days and 4 hours	Full protection against weight loss with molnupiravir at 500 mg/kg and partial weight loss protection with the lower	Bakowski et al. 2021 [40]
			150	750				
			500	2,500				

doi:10.20944/preprints202309.0334.v1

Animal model[1]	SARS-CoV-2 isolate used for inoculation[2]	Viral dose[3]	Oral drug dose in mg/kg, bidaily	Oral drug dose in mg/m^2, bidaily[4]	Start of treatment relative to time of inoculation	Treatment duration[5]	Efficacy of molnupiravir[6]	Study
							doses; lung viral titers decreased below detection limit with molnupiravir at 150 and 500 mg/kg; lung pathology improved	
Syrian hamster	Beta	10^4 $TCID_{50}$	150	750	1 hours before	4 days	Reduced virus titers and virus RNA in lungs; improved lung pathology	Foo et al. 2022 [41]
Syrian hamster	Omicron	10^3 PFU	500	2,500	24 hours after	3 days	Viral titers reduced in lungs but not in nasal turbinates	Uraki et al. 2022 [42]
Syrian hamster	Omicron	10^3 PFU	500	2,500	24 hours after	3 days	Reduction in nasal turbinate viral titer on second day after inoculation; viable virus not detected in lungs during period treatment	Uraki et al. 2022 [43]
Syrian hamster	Original type	5×10^2 $TCID_{50}$	250	1,250	12 hours before	4 days and 12 hours	Reduced viral RNA, viral titers and viral antigen in lungs for treatments initiated both before and after inoculation; improved lung pathology; no effect on viral load in oral swabs	Rosenke et al. 2021 [44]
					2 hours before	4 days and 2 hours		
					12 hours after	3 days and 12 hours		
Syrian hamster	Alpha, Beta, Delta, Omicron	10^3 or 10^4 $TCID_{50}$	250	1,250	12 hours after	3 days and 12 hours	Reduced viral titers and viral antigen of all examined variants in lungs; reduced lung disease; no reduction of viral load in oral swabs except for Omicron variant	Rosenke et al. 2022 [45]
Syrian hamster	Original type	10^4 $TCID_{50}$	250	1,250	24 hours before	8 days	Small decrease in nasal viral titer and in weight loss; improved lung pathology	Stegmann et al. 2022 [36]
Roborovski dwarf hamster	Original type, Alpha, Beta, Gamma, Delta, Omicron	10^5 PFU or 3×10^4 PFU (Delta)	250	900	12 hours after	12 days and 12 hours	Prevented death by all SARS-CoV-2 types; reduced viral titers and viral RNA in lungs with larger reduction for Gamma variant; improved lung pathology for all virus variants	Lieber et al. 2022 [46]

doi:10.20944/preprints202309.0334.v1

Animal model[1]	SARS-CoV-2 isolate used for inoculation[2]	Viral dose[3]	Oral drug dose in mg/kg, bidaily	Oral drug dose in mg/m², bidaily[4]	Start of treatment relative to time of inoculation	Treatment duration[5]	Efficacy of molnupiravir[6]	Study
Ferret	Original type, Alpha, Beta, Gamma, Delta, Omicron	10^5 PFU	5	35	12 hours after	4 days and 12 hours	Titers in nasal lavages of all viral isolates below detection level 12 hours after treatment start; blocks contact transmission; not all virus variants established productive infection	Lieber et al. 2022 [46]
Ferret	Original type	10^5 PFU	5	35	12 hours after	4 days	Viral titers below detection limit in nasal lavages within 1 day and 1.5 days for treatment started 12 and 36 hours after inoculation; no contact transmission	Cox et al. 2021 [7]
			15	105	12 hours after	4 days		
			15	105	36 hours after	3 days		
Rhesus macaque	Original type	5.15 x 10^6 or 6.08 x 10^6 $TCID_{50}$	75	900	At inoculation	7 days	Reduced nasal swab viral titers and viral RNA in bronchoalveolar lavages by administration of 250 mg/kg molnupiravir compared to dose of 75 mg/kg and vehicle; no effect on virus titers in bronchoalveolar lavage; lung pathology difficult to evaluate	Johnson et al. 2023 [8]
			250	3,000				
Rhesus macaque	Delta	2 × 10^6 $TCID_{50}$	130[1]	1,560	12 hours after	4 days	Reduced viral titers but not viral RNA in nasal and oral swabs; largely unchanged viral titers and viral RNA in lower airways; slightly milder disease course; less severe lung pathology	Rosenke et al. 2023 [9]

[1] SCID mice: severe combined immunodeficiency mice; K18-hACE-2 mice: mice expressing human angiotensin converting enzyme 2 under the control of the human keratin 18 (K18) promoter. [2]Only WHO label is given. [3]TCID: 50% tissue culture infectious dose; PFU: plaque forming units; FFU: focus forming unit; *MLD_{50}:* median *lethal dose*. [4] Body surface area-based doses. Conversion factors were based on standard body weights and surfaces [30]. For the dwarf hamster a body weight of 25 g and a body surface of 0.007 m^2 were used. The recommended oral human dose of molnupiravir is 800 mg bidaily (10.7 mg/kg), which corresponds to 400 mg/m^2 bidaily for a standard person with a body weight of 75 kg, a height of 175 cm and a body surface area of 2.00 m^2. [5]In several studies, some animals were sacrificed before the end of treatment. These intermediate time points are not included in the table, which only provides the reported total duration of treatment. [6]In many studies the viral load was determined prior to end of treatment.

doi:10.20944/preprints202309.0334.v1

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3.1. Viral doses and variants

Different methods for quantification of viral inoculation doses were used in the reported animal studies including median tissue culture infectious dose ($TCID_{50}$), plaque forming units (PFUs), focus forming units (FFUs) and 50% mouse lethal dose (MLD_{50}). Viral quantification by $TCID_{50}$ and PFUs were mostly used, while determination of FFUs and MLD_{50} were only used in a single study each. The doses ranged from 5×10^2 to about 6×10^6 $TCID_{50}$ and from 10^3 to 10^6 PFUs, in addition to doses of 300 FFUs and 5 MLD_{50}. A few studies tested both the original types of SARS-CoV-2 and the Alpha, Beta, Delta and Omicron variants.

Assessment of antiviral treatment efficacy in animal models often involves use of viral doses of a magnitude sufficient to induce noticeable clinical signs and high viral loads that facilitate detection of treatment effects. However, this empirical and somewhat abitrary approach complicates comparisons of viral doses across studies. Moreover, the use of various methodologies for viral quantification and distinct susceptibilities to SARS-CoV-2 among the animal species further complicates meaningful comparison of viral doses in the reported studies. Likewise, pathogenicity was dependent on the SARS-CoV-2 variant, with the Delta variant being the most pathogenic of those tested in the dwarf hamster model [46]. Observations in the latter model also suggested that molnupiravir reduced lung titers of all the tested SARS-CoV-2 variants, with the efficacy being more pronounced for the Gamma variant compared to other variants [46]. Additionally, in the Syrian hamster model of COVID-19, the drug reduced lung titers of the Alpha, Beta, and Delta variants, apparently possessing highest activity against the latter, while the lung titer decrease observed for the Omicron variants did not reach statistical significance [45].

3.2. Dose levels of molnupiravir

The molnupiravir doses varied significantly across the examined studies. Specifically, the bidaily oral doses ranged from 5 mg/kg in ferrets to 500 mg/kg in Syrian hamsters and lung-only mice. When we normalized the doses according to body surface areas in the individual species, they ranged from 57 to 3,000 mg/m^2 bidaily. In four studies based on various mouse models of Covid-19 including K18-hACE-2 mice, SCID mice and lung-only mice, molnupiravir doses ranging from 20 to 500 mg/kg were used [33–36]. Two of these studies found significantly reduced pulmonary viral titers accompanied by improved lung pathology [33,35]. In one of these two studies, namely, the study based on the lung-only mouse model, molnupiravir at 500 mg/kg twice daily was chosen as this dose was assumed to provide intracellular 5′-triphosphate NHC levels similar to those in humans administered a dose of 1,600 mg per day [35]. However, in humans, a daily dose of 1,600 mg (800 mg twice daily) is equivalent to a body surface area-based dose of 400 mg/m^2 twice daily, which is more than three times lower than the dose of 1,500 mg/m^2 twice daily (500 mg/kg twice daily) in the lung-only mouse model. In the other study, the 20 mg/kg dose administered to K18-hACE-2 mice was selected based on a dose-optimization study and considered optimal for low-dose therapeutic efficacy [34].

Studies of the efficacy of molnupiravir in Syrian hamsters inoculated with SARS-CoV-2 were done with doses ranging from 50 to 500 mg/kg twice daily [36–45]. Specifically, a dose-response study suggested that a dose of molnupiravir of 150 mg/kg twice daily was suboptimal, and that a dose of at least 200 mg/kg twice daily was necessary to reduce pulmonary viral titers to levels approaching the detection limit [38].

In another study using the Syrian hamster model, the administration of molnupiravir at a dose of 150 mg/kg twice daily not only reduced pulmonary viral titers to below the detection limit, but also provided partial protection against weight loss, which was negligible with a higher dose of molnupiravir at 500 mg/kg twice daily. [40].

Several of the animal studies aimed to assess the effect of combining molnupiravir with another antiviral agent or a different type of agent. These studies used a suboptimal dose of molnupiravir to compare the effect of this agent in monotherapy with combination therapies, thereby facilitating detection of potential synergistic effects [9,34,36,38,39,41]. For example, the suboptimal molnupiravir

 doi:10.20944/preprints202309.0334.v1

dose of 150 mg/kg bidaily in Syrian hamsters was used for such studies [38,39,41]. Importantly, the combination of this dose of molnupiravir with favipiravir or GS-441524 (the nucleobase of remdesivir), exhibited potent antiviral activity in Syrian hamsters [38,39]. Combining doses of 250 mg/kg of molnupiravir and 10 mg/kg of teriflunomide twice daily in the Syrian hamster model also suggested superiority of the combination over both compounds in monotherapy, with molnupiravir alone reducing nasal lavage titers by only 1.5 $\log_{10}$ [36].

In some studies of molnupiravir in the Syrian hamster and dwarf hamster models, the doses were selected based on findings from other animal models of Covid-19 [43,46]. For example, the dose of molnupiravir of 500 mg/kg twice daily was administered to Syrian hamsters as this dose was previously demonstrated to be efficacious in the lung-only mouse model of Covid-19 [43]. Moreover, the choice of a bidaily dose of 250 mg/kg molnupiravir for dwarf hamsters was motivated by the heightened metabolic activity in these animals compared to ferrets, which were administered 5 mg/kg bidaily, and aligned with the doses employed in Syrian hamsters and lung-only mice of 250 and 500 mg/kg bidaily, respectively [46]. Ferrets received molnupiravir in bidaily doses of 5 and 15 mg/kg [7,46]. These doses were the lowest among those used in the reported animal models, both when expressed as mg/kg and mg/m^2. However, they effectively eliminated SARS-CoV-2 from nasal passages in the ferrets after 0.5 to 1.5 days of treatment and blocked contact transmission [7,46].

Rhesus macaque monkeys were administered molnupiravir at doses of 75, 130 and 250 mg/kg twice daily [8,9]. The 75 and 250 mg/kg twice daily dose levels were selected based on previous knowledge about plasma exposure of molnupiravir in macaque monkeys and the in vitro inhibitory activity against SARS-CoV-2 of the agent [8]. The 130 mg/kg twice daily dose of molnupiravir, which is equivalent to a body surface area-based bidaily dose of 1,950 mg/m^2, was reported to be allometrically derived from the 800 mg twice daily dose recommended for treatment of COVID-19, although the calculation underlying this dose conversion was not specified [9]. The molnupiravir doses of 75 and 130 mg/kg, administered twice daily, appeared to be therapeutically suboptimal, with varying efficacy across treatment outcomes [8,9]. Additionally, a dose of 250 mg/kg twice daily did not consistently reduce viral RNA and titers in macaques [8]. Generally, assessment of the efficacy of molnupiravir in nonhuman primates was hampered by mild clinical disease after inoculation with SARS-CoV-2, but the agent reduced the load of SARS-CoV-2 in the airways [8,9].

Most of the molnupiravir doses used in the reported animal models of Covid-19, expressed in both mg/kg and mg/m^2, exceeded the corresponding bidaily doses of 10.7 mg/kg and of 400 mg/m^2, respectively, that both were derived from the recommended human dose of 800 mg twice daily. Notably, in half of the animal studies, including the two studies performed in macaques, the calculated body surface area-based doses of molnupiravir exceeded the human equivalent dose of 400 mg/m^2 by a factor of two or more. Among the animal species used to assess the efficacy of molnupiravir against Covid-19, the macaque species is the one most closely related to humans and together with other nonhuman primates, macaques have played an instrumental role in developing and preclinical testing of novel therapeutics against Covid-19 [27].

Overall, the lack of clarity in the rationale behind selection of doses of molnupiravir in several of the reported animal studies is remarkable. Significantly, the body surface area-based scaling of molnupiravir enables the comparison of doses across species, under the assumption of absence of species differences in the pharmacokinetics. However, achieving more accurate inter-species knowledge of the relationships between drug dose, exposure, and efficacy may require sophisticated modeling founded on species-specific pharmacological parameters, as exemplified by studies of remdesivir and GS-441524 [47,48].

3.3. Time from inoculation to viral load peak

Due to lack of a sufficient number of consecutive samplings, it was only possible to determine the time of the viral load peak relative to that of inoculation in some of the animal studies. In the lung-only mouse model, the viral titer was reported to peak two days after inoculation with SARS-CoV-2 [35]. Viral load peaked in nasal turbinates on the second day after inoculation with SARS-CoV-2 and one to two days later in the lungs of Syrian hamsters, with these levels remaining high for

several days [43]. Likewise, the RNA levels of the Alpha, Beta, Delta and Omicron variants in oral swabs collected from Syrian hamsters appeared to plateau at a constantly high level from 2 to 3.5 days after inoculation [45]. Also, findings suggested that viral titers in nasal washings from Syrian hamsters decreased from two to four days after inoculation consistent with virus load peak occurring within the first couple of days of the inoculation [36]. By contrast, virus load peaked in the lungs of dwarf hamsters as early as 12 to 24 hours after inoculation [46]. Likewise, replication of SARS-CoV-2 appeared to reach an early maximum in the upper respiratory tract of ferrets, where the viral load has been found to peak in nasal washings at 24 to 36 hours after inoculation [7]. This is consistent with other findings in ferrets suggesting that viral load peak occured one to three days after inoculation, depending upon the virus variant [46]. Moreover, findings in macaques suggested that the viral load in nasal swabs peaked about two days after inoculation with SARS-CoV-2 [8,9].

3.4. Time to initiation of treatment

Treatment with molnupiravir was initiated at various time points in the animal studies, spanning from 24 hours before to 48 hours after SARS-CoV-2 inoculation, with most treatments being initiated before or concurrently with the viral inoculation. Several studies permitted comparison of the outcome of treatments initiated at different time points relative to the time of inoculation, thereby providing the basis for determination of a therapeutic time window, i.e., the time interval where the treatment is likely to be most effective. This included a study in the lung-only mouse model, where initiation of treatment with 500 mg/kg of molnupiravir twice daily 12 hours before inoculation led to lower lung viral titers and improved lung pathology compared to treatments using the same dose but initiated 24 and 48 hours after inoculation, with the lowest therapeutic effect observed in the animals initiating treatment 48 hours after inoculation [35]. Likewise, a study using the Syrian hamster model of Covid-19 reported that treatment with molnupiravir at 250 mg/kg twice daily initiated 12 hours prior to inoculation resulted in significantly lower levels of viral titer, RNA and antigen in the lungs than treatments initiated 2 hours prior to or 12 hours after inoculation [44]. In line with these findings, lower nasal titers of SARS-CoV-2 was reported in ferrets that initiated treatment with molnupiravir 12 hours after inoculation compared to those initiating molnupiravir treatment 36 hours after inoculation [7].

Although molnupiravir treatment was initiated before or simultaneously with inoculation in most studies, several studies exclusively initiated the treatment after inoculation. This included studies in mice, Syrian hamsters, dwarf hamsters, and macaques, which started treatment at 6, 12 and 24 hours after viral inoculation [7,9,34,42,43,45,46]. For example, treatment of K-18 hACE2 mice with molnupiravir at 20 mg/kg twice daily was initiated 6 hours after inoculation and provided only modest protection against weight loss, but improved the clinical score and decreased mortality significantly [34]. Additionally, Syrian hamsters initiating treatment with molnupiravir at doses of 250 or 500 mg/kg twice a day at 12 and 24 hours post-inoculation, exhibited significantly decreased lung viral titers for both the original and variant strains of SARS-CoV-2, while viral reductions in nasal turbinates and oral swabs were not consistent over the course of the experiments [42,43,45]. Finally, treatment of experimentally infected macaques with a 130 mg/kg twice daily dose of molnupiravir initiated 12 hours after inoculation, reduced lung viral antigen and viral titers in both the upper and lower parts of the respiratory system, but did not significantly reduce clinical signs or reduce viral RNA in the respiratory system [9].

In general, findings from the animal studies where treatment was exclusively initiated post-inoculation, indicated that molnupiravir was effective in eliminating SARS-CoV-2, although its efficacy differed across outcome measures. It is, however, noteworthy that these studies used molnupiravir doses at the upper end of those administered in the reported animal models, namely, doses of 130, 250 and 500 mg/kg twice daily, with the exception of the 20 mg/kg dose twice daily used in the K-18 hACE2 mouse model [34], and 5 and 15 mg/kg twice daily used for ferrets [7,46].

Since treatment was initiated within two days after infection with SARS-CoV-2 in most of the reported animal studies, exposure to the drug likely commenced before viral load peak, although in dwarf hamsters and ferrets, the viral load peak may occur earlier in the infection course and therefore

 doi:10.20944/preprints202309.0334.v1

lead to a shorter therapeutic time window. In the MOVe-OUT study, where molnupiravir was deemed clinically effective, about half of the patients initiated treatment within three days or less from symptom onset, while treatment was started four to five days after symptom onset in the remainder of the patients [10]. The median time from symptom onset to treatment initiation was three to five days in the subsequent PANORAMIC study, which failed to demonstrate reduced risk of death and hospitalization in patients treated with molnupiravir [12]. If the viral load often peaks within a few days after symptom onset or even earlier in humans infected with SARS-CoV-2, treatment initiation in the MOVe-OUT and PANORAMIC studies would have come belatedly, resulting in decreased overall efficacy of molnupiravir. Conversely, in the preclinical studies with animals the efficacy of molnupiravir against SARS-CoV-2 was likely augmented by treatments initiated early relative to the viral load peak, which may have contributed to high expectations for outcomes in human clinical trials with the agent. However, comparisons of the duration of the therapeutic windows of molnupiravir in Covid-19 and animal models of the disease is challenging due to the typically condensed disease course and earlier viral load peak in the animal models

3.5. Duration of treatment

The duration of treatment varied in the animal studies, ranging from 2 days in lung-only mice to over 12 days in Roborovski dwarf hamsters, with a median of 4 days, reflecting differences in study designs. The study with the treatment duration of only 2 or 2.5 days was performed in the lung-only mouse model and used the highest molnupiravir doses among all the reported animal studies, namely, 500 mg/kg [35]. The longest treatment durations, spanning 7, 8, and 12.5 days, were observed in studies using macaques [8], Syrian hamsters [36], and Roborovski dwarf hamsters [46]. These latter treatment durations exceeded the recommended five-days course for treatment of Covid-19. Overall, while the disease course in animal models of Covid-19 is generally more condensed than in humans, this difference did not necessarily translate into proportionately shorter treatment durations with molnupiravir in the reported studies. This again underscores the challenges associated with application of animal models of Covid-19 including extrapolation to humans, and raises the question of whether the choice of treatment length in these models offered appropriate guidance for determining the length of treatment required to achieve maximum efficacy in Covid-19.

4. Conclusion and future perspectives

Potentially significant limitations in the studies that assessed efficay of molnupiravir against Covid-19 based on animal models of the disease pertain to dose levels, treatment timing, and duration of treatment. We suggest that the dose levels of molnupiravir in several animal studies were excessively high compared to the recommended human dose, leading to significantly higher drug exposures and higher antiviral activities. Similarly, prophylactic treatment with molnupiravir, and treatment that was initiated shortly after viral inoculation, i.e., prior to viral load peak, in the animal models, may have favored molnupiravir and increased its treatment effect. By contrast, in the human clinical trials, a considerable number of patients may have initiated treatment after viral load peak was reached. Furthermore, in many animal studies, the treatment duration appeare4d to be disproportionately longer than the recommended duration of treatment in humans, given the typically short and condensed disease course in animal models of Covid-19, with several studies exceeding this treatment duration. Collectively, we posit that design elements in the animal studies assessing the efficacy of molnupiravir contributed to overestimation of the efficacy of the agent, and thus inflated the expectations for its application in Covid-19.

While molnupiravir did not exhibit high efficacy across all outcome measures in the reported animal models of Covid-19, the collective findings in these studies indicated a substantial efficacy of the agent against SARS-CoV-2. We speculate that addressing the limitations in the animal studies, which likely biased results in favor of molnupiravir, might enhance its efficacy in humans. Therefore, we propose achieving enhanced efficacy of molnupiravir in Covid-19 by using higher doses than the currently recommended oral dose of 800 mg twice daily. Also, administration of molnupiravir or NHC by inhalation could increase the intracellular concentration of 5′-triphosphate NHC and

enhance antiviral activity in the airways, while early identification of infected cases in the asymptomatic phase might widen the therapeutic time window of the agent. Concurrently, combining molnupiravir with other antivirals also holds promise for enhanced efficacy. Although these suggestions may raise hope for the use of molnupiravir in treating Covid-19, all new clinical developments of the agent should obviously be accompanied by rigorous safety data.

Author Contributions: Conceptualization, H.B.R.; writing—original draft preparation, H.B.R and P.R.H.; writing—review and editing, H.B.R. and P.R.H. Both authors have read and agreed to the published version of the manuscript.

Funding: This report received no external funding.

Data Availability Statement: The data presented in the report and used for calculations were derived from publicly available studies.

Conflicts of Interest: The authors declare no conflict of interest.

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