The combined treatment of Molnupiravir and Favipiravir results in a marked potentiation of efficacy in a SARS-CoV2 hamster infection model through an increased frequency of mutations in the viral genome.

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Author Contributions

R.A., C.S.F. and J.N. designed the studies; R.A., C.S.F., S.J.F.K., X.Z., J.B., J.P. and L.L. performed the studies ; R.A., C.S.F., J.B, J.P. and B.W. analyzed data; D.J. and J.N. provided advice on the interpretation of data; R.A., C.S.F. and J.N. wrote the paper with input from co-authors; A.K.C. and S.D.J provided essential reagents; V.G. and E.H. provided and facilitated access to essential infrastructure; R.A., C.S.F., R.W. and J.N. supervised the study; L.V., L.C., P.L., J.N. and K.D. acquired funding.

Abstract

The experimental anti-influenza drug Molnupiravir, an orally bioavailable nucleoside analogue, has earlier been reported to exert antiviral activity against SARS-CoV2. We here report on the antiviral activity of Molnupiravir in a robust Syrian hamster SARS-CoV-2 infection model. Oral treatment of infected hamsters for four consecutive days, starting from the day of infection, markedly reduced (in a dose-dependent manner) viral loads in the lungs and improved the lung histopathology score. When onset of treatment with a high dose was delayed until 24h post-infection, a modest but still significant antiviral effect was observed at endpoint (96hr post-infection). When animals were treated with suboptimal doses of Molnupiravir and Favipiravir, another influenza drug with anti-coronavirus activity, a marked combined potency was observed with complete reduction (~5 $\log_{10}$) of infectious virus titers in the lungs of most of the combo-treated animals. Both drugs resulted in an increased mutation frequency of the viral RNA recovered from the lungs. In the combo-treated hamsters an increased frequency of C-to-T and G-to-A mutations as compared to the single treatment groups (including the highest dose of Molnupiravir) was observed which explains the pronounced antiviral potency of the combination. The combined all oral treatment and/or prophylaxis of SARS-CoV-2 infections with the combination of Molnupiravir and Favipiravir should be explored in clinical studies.

Keywords

SARS-CoV-2; Antivirals; Molnupiravir; Favipiravir, hamsters, coronavirus

Introduction

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a β-coronavirus that was first identified in Wuhan, China in December 2019 (1). Since then, the virus rapidly spread around the globe with more than 109 million cases and 2.4 million deaths reported until February 2021 [www.covid19.who.int]. Infection with SARS-CoV-2 results in coronavirus-induced disease (COVID-19) which is characterized by a wide range of symptoms including fever, dry cough, muscle and/or joint pain, headache, decreased sense of taste and smell and diarrhea. The disease can progress into severe complications such as acute respiratory distress syndrome (ARDS), respiratory failure, septic shock as well as multi-organ failure, which are mainly attributed to a massive cytokine storm and exaggerated immune response (2).

To date, there are no approved, selective coronavirus antivirals to treat or prevent infections. The use of potent antivirals against SARS-CoV-2 will reduce viral loads and may hence reduce the chance to progress to a severe disease. In addition, such antiviral drugs may be useful to protect for example health care workers and high-risk groups in a prophylactic setting. Since the *de novo* development and approval of (a) specific, highly potent antiviral(s) for SARS-CoV-2 may require years, the main focus for COVID-19 treatment in the current pandemic is to repurpose drugs that have been approved or in clinical trials for other diseases (3).

The ribonucleoside analogue, N4-hydroxycytidine (NHC, EIDD-1931), was initially developed as an influenza inhibitor, but exerts also broader-spectrum antiviral activity against multiple viruses belonging to different families of RNA viruses. Activity against SARS-CoV and SARS-CoV-2 has been reported in cell lines and primary human airway epithelial cell cultures (4). Acting through lethal mutagenesis, its incorporation into viral RNA results in the accumulation of deleterious transition mutations beyond a permissible error threshold to sustain the virus population, leading to error catastrophe (5). The orally bioavailable, pro-drug counterpart of NHC (6), Molnupiravir (EIDD-2801, MK-4482) is currently being assessed for its potential as an antiviral treatment of SARS-CoV-2 infection

in Phase 2 clinical trials of infected patients (NCT04405570, NCT04405739). To our knowledge, three recent studies reported on the activity of orally dosed EIDD-2801 in SARS-CoV-2-infected animals. Oral treatment of SARS-CoV-2 infected Syrian hamsters with high doses of Molnupiravir (EIDD-2801) was reported to result in marked reduction (1 to 2 log_{10}) of viral loads when administered either in a pre-exposure (12h before infection) or post-exposure (start of treatment 12h post-infection, pi) settings (7). In a ferret model infection model, Molnupiravir (EIDD-2801) was reported to significantly reduce virus loads in the lungs when start of treatment was delayed until 12 or 36h pi and to block also SARS-CoV-2 contact transmission (8). In a humanized mouse model i.e. implanted with human lung tissues, Molnupiravir (EIDD-2801) prevented SARS-CoV-2 infection in a pre-exposure prophylaxis setting (9).

We recently demonstrated that the influenza drug Favipiravir results in a pronounced antiviral activity in SARS-CoV-2-infected hamsters, whereas hydroxychloroquine lacks antiviral activity in this model (10). Here, we use the same hamster model to obtain further information on the antiviral activity of Molnupiravir (EIDD-2801) either when used alone or in combination with Favipiravir and on the mechanism of the combined treatment.

Results

In vivo efficacy of Molnupiravir against SARS-CoV-2 at the time of infection

First, we evaluated the (dose-response) effect of Molnupiravir (EIDD-2801) in SARS-CoV-2-infected hamsters. Briefly, 6-8 weeks female SG hamsters were treated orally with Molnupiravir (either 75, 150, 200 or 500 mg/kg, BID) or the vehicle (i.e. the control group) for four consecutive days starting one hour before intranasal infection with SARS-CoV-2 [BetaCov/Belgium/GHB-03021/2020 (EPI ISL 109 407976|2020-02-03)]. At day four post-infection (pi), the animals were euthanized and lungs were collected for quantification of viral RNA, infectious virus titers and lung histopathology as described previously (10) (Fig. 1A). Molnupiravir treatment resulted in a dose-dependent reduction in the viral RNA copies per mg of lung tissue with 1.3 (P=0.002), 1.9 (P<0.0001), 3.3 (P<0.0001) and 2.8 (P=0.01) log_{10} reduction was noted in the groups that had been treated BID with 75, 150, 200 and 500 mg/kg,

respectively (Fig. 1B). A similar pattern was observed for the infectious virus load in the lungs whereas the high doses, but not the 75 mg/kg dose BID, significantly reduced infectious virus lung titers (Fig. 1C). The reduction in infectious virus titers ($TCID_{50}$ / mg tissue) in the lungs of hamsters treated BID with 150, 200 and 500 mg/kg was 1.3 (P=0.0002), 3.5 (P<0.0001) and 1 (P=0.0002) log_{10}, respectively (Fig. 1C). However, some variations in viral loads reduction was observed in the group treated with the highest dose.

Treatment with 75, 150 and 200 mg/kg Molnupiravir BID significantly reduced the histological lung disease score (P=0.0025, P=0.005, P<0.0001, respectively), because of a large variation of the individual data points surprisingly no significant protective activity was noted at the highest dose used (Fig. 1D). All the doses studied were well tolerated without significant weight loss or any obvious adverse effects (Fig. 1E).

In vivo efficacy of Molnupiravir against SARS-CoV-2 in a post-exposure setting

We next explored whether delayed Molnupiravir (EIDD-2801) treatment [started at 24 h after infection] (Fig. 2A) has an impact on the infection. Delaying the start of treatment with Molnupiravir (200 mg/kg or 500 mg/kg BID) by 1 day resulted in 0.4 (P=0.03) and 1 (P=0.05, ns) log_{10} reduction of viral RNA copies/mg lung, respectively (Fig. 2B). Likewise no substantial reduction of infectious virus load in the 200 mg/kg Day 1 group was noted, whereas a modest but significant reduction [1 log_{10} reduction in TCID50/mg lung tissue (P=0.0003)] in the 500 mg/kg Day 1 group was observed (Fig. 2C). A modest reduction of the histological lung disease score was observed in the 200 mg/kg (P=0.0007) and 500 mg/kg (P=0.27, ns) Day 1 treatment groups (Fig. 2D). Thus even though the delayed start of treatment with EIDD-2801 is not sufficient to efficiently stop viral replication as assessed 72h later, the drug may still able to delay to some extend disease progression.

The combined treatment of Molnupiravir and Favipiravir results in a marked potentiation of efficacy.

We earlier demonstrated that a high dose of Favipiravir (i.e. 500 mg/kg, BID) can reduce infectious virus loads of SARS-CoV2 in the lungs of hamsters to undetectable levels. We here explored whether a

similar potency can be achieved by combined treatment of infected hamsters with suboptimal doses of Favipiravir and Molnupiravir (EIDD-2801) (Fig. 3A). A single treatment with Favipiravir (300 mg/kg, BID, intraperitoneal injection) reduced viral RNA and infectious virus loads in the lungs of infected hamsters by 0.7 (P=0.0009) and 1.2 (P=0.0002) log_{10}/mg tissue, respectively (Fig. 3B/C). The combination resulted in enhanced antiviral efficacy with a reduction of 2.7 log_{10} viral RNA per mg lung tissue (Fig. 3B), but interestingly, in a markedly enhanced reduction in infectious virus titers (4.5 log_{10} $TCID_{50}$ per mg lung, P=0.02 as compared to Molnupiravir alone) (Fig. 3C). Notably, six out of ten hamsters in the combined treatment group had no detectable infectious virus in their lungs (Fig. 3C). A marked improvement in the histological lung pathology scores was also observed in the combined treatment group (Fig. 3D). No significant weight loss or toxicity signs were observed in the combined treatment group (Supplementary Fig. S1).

Molnupiravir is known to increase the mutation frequency of MERS-CoV viral RNA in infected mice (4). To test whether this is also the case in SARS-CoV-2-infected hamsters, we used Illumina deep sequencing to determine the SARS-CoV-2 mutations rate in remaining viral RNA in lung samples of hamsters after treatment. A dose-dependent increase in the mutation count (in particular, C-to-T and G-to-A transitions) in samples from EIDD-2801 treated hamsters was observed as compared to the vehicle control group (Supplementary Fig. S2). The Molnupiravir (150mg/kg) + Favipiravir (300 mg/kg) combination resulted in a markedly higher number of C-to-T and G-to-A mutations (68 and 50, respectively), as compared to the single dose groups [150 mg/kg Molnupiravir group (33 and 31, respectively) and 300 mg/kg Favipiravir (14 and 21, respectively)] (Fig. 3E). The C-to-T and G-to-A mutation count in the combination group was also markedly higher than in the highest dose group of Molnupiravir (47 and 32, respectively) (Supplementary Fig. S2). These results may at least partially explain the markedly enhanced reduction in infectious viral loads observed in the combination treatment group.

Discussion

We demonstrate in the robust SARS-CoV2 hamster infection model (10, 11) that Molnupiravir markedly reduces, albeit at a relatively high dose, SARS-CoV-2 infection and virus induced pathology. In another study (7) a somewhat higher dose (250 mg/kg) was less effective (1 $\log_{10}$ reduction in viral RNA and 2 $\log_{10}$ reduction in infectious virus titers) despite the fact that a much lower inoculum was used for challenge than is the case in our study. At high doses, the drug was also reported to be effective in SARS-CoV-infected C57/BL6 mice either when administered prophylactically or when treatment was delayed until 48 hr after infection (4). In a humanized mouse model of SARS-CoV-2 infection, high dose prophylaxis resulted in a ~6 $\log_{10}$ reduction in viral lung titres (9). The drug proved also effective at relatively low doses (15 mg/kg, BID) to inhibit viral replication in the upper respiratory tract of ferrets even when start of treatment was delayed until 36 hpi. It also prevented contact transmission when only index animals were treated (8). Our data on the monotherapy of infected hamsters lend further support to these studies.

The antiviral drug, Remdesivir (Veklury), is the first drug to receive FDA approval for use in hospitalised COVID19 patients, although the World Health Organisation has recently recommended against its use. Remdesivir needs to be administrated intravenously which precludes its use in the early stages of the infection/disease or even prophylactic use. On the other hand, Molnupiravir can be dosed via the oral route. Both drugs have a high barrier to resistance and resistant variants have a loss in fitness (6, 11). Favipiravir is another broad-spectrum antiviral drug that can be dosed orally. It is currently being studied in clinical trials against SARS-CoV-2 in several countries (12). Akin to Molnupiravir (5, 11) also Favipiravir induces lethal viral mutagenesis (13). We previously showed that treatment of SARS-CoV-2-infected hamsters with a high doses of Favipiravir can reduce infectious SARS-CoV2 titers in the lungs of infected hamsters to undetectable levels and results as a consequence in a markedly improved lung pathology (10).

We here demonstrate that the combination of suboptimal doses of Molnupiravir (150 mg/kg, BID) and Favipiravir (300 mg/kg, BID) results in a marked antiviral in the hamster model. Infectious virus titers were reduced to undectable in 6 out of 10 treated animals. A median reduction of 4.5 $\log_{10}$ TCID50/mg lung tissue was achieved, which is much more pronounced than what could be expected from an additive activity of either Molnupiravir (1.3 $\log_{10}$) or Favipiravir (1.1 $\log_{10}$) when dosed alone. This pronounced efficacy of the combination Favipiravir may at least to some extend be explained by the markedly increased total mutation count in the viral RNA collected from the lungs of combo-treated hamsters as compared to the single treatment groups.

In conclusion our data lend further support for the development of Molnupiravir as an antiviral drug for the prophylaxis/treatment of SARS-CoV2 infections. The more than additive activity of the combination of Molnupiravir and Favipiravir is also reflected in an enhanced accumulation of mutations in the viral genome in animals that received both drugs. Our findings lay the basis for the design of clinical studies to test the efficacy of the combination of Molnupiravir and Favipiravir, both of which can be dosed orally.

Materials and methods

SARS-CoV-2

The SARS-CoV-2 strain used in this study, BetaCov/Belgium/GHB-03021/2020 (EPI ISL 109 407976|2020-02-03), was recovered from a nasopharyngeal swab taken from an RT-qPCR confirmed asymptomatic patient who returned from Wuhan, China in the beginning of February 2020. A close relation with the prototypic Wuhan-Hu-1 2019-nCoV (GenBank accession 112 number MN908947.3) strain was confirmed by phylogenetic analysis. Infectious virus was isolated by serial passaging on HuH7 and Vero E6 cells (10); passage 6 virus was used for the study described here. The titer of the virus stock was determined by end-point dilution on Vero E6 cells by the Reed and Muench method (14). Live virus-related work was conducted in the high-containment A3 and BSL3+ facilities of the KU Leuven Rega Institute (3CAPS) under licenses AMV 30112018 SBB 219 2018 0892 and AMV 23102017 SBB 219 20170589 according to institutional guidelines.

Cells

Vero E6 cells (African green monkey kidney, ATCC CRL-1586) were cultured in minimal essential medium (Gibco) supplemented with 10% fetal bovine serum (Integro), 1% L- glutamine (Gibco) and 1% bicarbonate (Gibco). End-point titrations were performed with medium containing 2% fetal bovine serum instead of 10%.

Compounds

For the first pilot experiment, EIDD-2801 was kindly provided by Calibr at Scripps Research (USA). For further studies, Molnupiravir (EIDD-2801) was purchased from Excenen Pharmatech Co., Ltd (China) and was formulated as 50 or 100 mg/ml (for groups with the highest dose) stocks in a vehicle containing 10%PEG400 and 2.5% Kolliphor-EL in water. Favipiravir was purchased from BOC Sciences (USA) and was formulated as a 50 mg/mL stock in 3% sodium bicarbonate.

SARS-CoV-2 infection model in hamsters

The hamster infection model of SARS-CoV-2 has been described before (10, 15). In brief, wild-type Syrian Golden hamsters (Mesocricetus auratus) were purchased from Janvier Laboratories and were housed per two in ventilated isolator cages (IsoCage N Biocontainment System, Tecniplast) with ad libitum access to food and water and cage enrichment (wood block). The animals were acclimated for 4 days prior to study start. Housing conditions and experimental procedures were approved by the ethics committee of animal experimentation of KU Leuven (license P065-2020). Female hamsters of 6-8 weeks old were anesthetized with ketamine/xylazine/atropine and inoculated intranasally with 50 µL containing 2×10^6 TCID50 SARS-CoV-2 (day 0).

Treatment regimen

For D0 treatment, animals were treated twice daily with 75, 150, 200 or 500 mg/kg of EIDD-2801 by oral gavage just before infection with SARS-CoV-2. For delayed treatment groups, animals were treated with either, 200 or 500 mg/kg of EIDD-2801 starting from day1 post-infection (pi) by oral gavage. For combination therapy, hamsters were treated from day0 with 150 mg/kg EIDD-2801 (oral gavage) and 300 mg/kg Favipiravir (intraperitoneal, i.p.) twice daily. All the treatments continued until day 3 pi. Hamsters were monitored for appearance, behavior and weight. At day 4 pi, hamsters were euthanized by i.p. injection of 500 µL Dolethal (200mg/mL sodium pentobarbital, Vétoquinol SA). Lungs were collected and viral RNA and infectious virus were quantified by RT-qPCR and end-point virus titration, respectively.

SARS-CoV-2 RT-qPCR

Hamster lung tissues were collected after sacrifice and were homogenized using bead disruption (Precellys) in 350 µL TRK lysis buffer (E.Z.N.A.® Total RNA Kit, Omega Bio-tek) and centrifuged (10.000 rpm, 5 min) to pellet the cell debris. RNA was extracted according to the manufacturer's instructions. Of 50 µL eluate, 4 µL was used as a template in RT-qPCR reactions. RT-qPCR was performed on a LightCycler96 platform (Roche) using the iTaq Universal Probes One-Step RT-qPCR kit (BioRad) with N2

primers and probes targeting the nucleocapsid (10). Standards of SARS-CoV-2 cDNA (IDT) were used to express viral genome copies per mg tissue.

End-point virus titrations

Lung tissues were homogenized using bead disruption (Precellys) in 350 µL minimal essential medium and centrifuged (10,000 rpm, 5min, 4°C) to pellet the cell debris. To quantify infectious SARS-CoV-2 particles, endpoint titrations were performed on confluent Vero E6 cells in 96- well plates. Viral titers were calculated by the Reed and Muench method (14) using the Lindenbach calculator and were expressed as 50% tissue culture infectious dose (TCID50) per mg tissue.

Histology

For histological examination, the lungs were fixed overnight in 4% formaldehyde and embedded in paraffin. Tissue sections (5 µm) were analyzed after staining with hematoxylin and eosin and scored blindly for lung damage by an expert pathologist. The scored parameters, to which a cumulative score of 1 to 3 was attributed, were the following: congestion, intra-alveolar hemorrhagic, apoptotic bodies in bronchus wall, necrotizing bronchiolitis, perivascular edema, bronchopneumonia, perivascular inflammation, peribronchial inflammation and vasculitis.

Deep sequencing and analysis of whole genome sequences

Genomic sequences from all samples were obtained using SureSelect^{XT} target enrichment and Illumina sequencing. Reads generated were trimmed with Trim Galore (https://github.com/FelixKrueger/TrimGalore). Duplicated reads were removed using Picard (http://broadinstitute.github.io/picard). Reads from the inoculation sample were mapped to the SARS-CoV-2 reference genome (NC_045512) from GenBank using BWA-MEM (16). The mapping quality was checked using Qualimap and the consensus whole genome sequence was generated using QUASR (17, 18). Reads from the lung samples were mapped to this unique reference sequence. Genomes with less than less than a 100 read depth were excluded. Variants above 1% and with a minimum of 2 supporting reads per strand were identified at sites with a read depth of ≥ 10 using VarScan (19).

255 **Statistics**

256 GraphPad Prism (GraphPad Software, Inc.) was used to perform statistical analysis. Statistical
257 significance was determined using the non-parametric Mann Whitney U-test. P-values of ≤0.05 were
258 considered significant.

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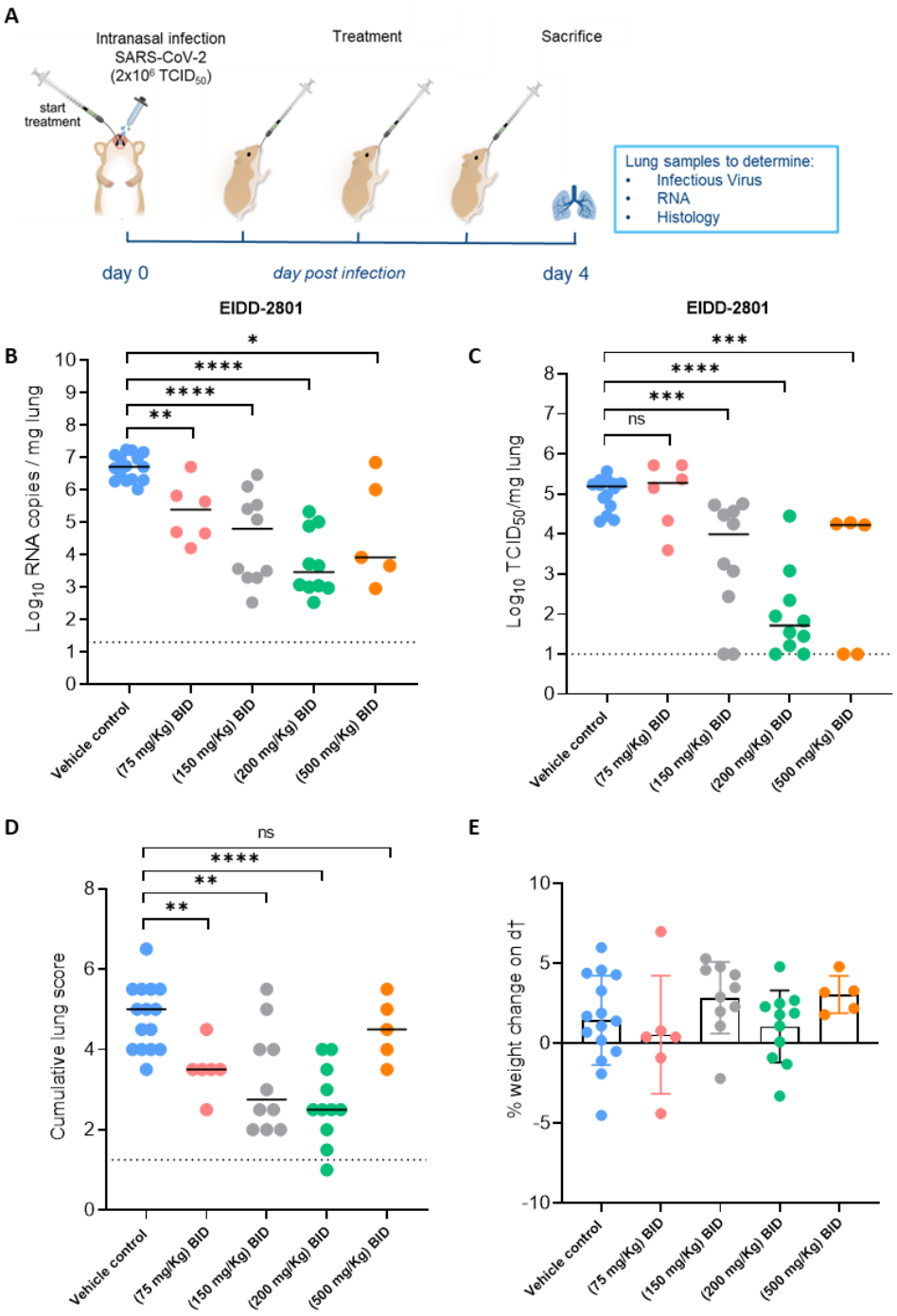


Fig.1. Efficacy of Molnupiravir (EIDD-2801) against SARS-CoV-2 in a hamster infection model. (A) Set-up of the study. (B) Viral RNA levels in the lungs of control (vehicle-treated) and EIDD-2801-treated (75, 150, 200 or 500 mg/kg, BID) SARS-CoV-2–infected hamsters at day 4 post-infection (pi) are expressed as log_{10} SARS-CoV-2 RNA copies per mg lung tissue. Individual data and median values are presented. (C) Infectious viral loads in the lungs of control (vehicle-treated) and EIDD-2801-treated SARS-CoV-2–infected hamsters at day 4 pi are expressed as log_{10} $TCID_{50}$ per mg lung tissue Individual data and median values are presented. (D) Cumulative severity score from H&E stained slides of lungs from control (vehicle-treated) and EIDD-2801-treated SARS-CoV-2–infected hamsters. Individual data and median values are presented and the dotted line represents the median score of untreated non-infected hamsters. (E) Weight change at day 4 pi in percentage, normalized to the body weight at the time of infection. Bars represent means ± SD. Data were analyzed with the Mann–Whitney U test. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001, ns=non-significant. All data (panels B, C, D, E) are from two independent experiments except for the 75 and 500 mg/kg groups. The number of animals were 15, 6, 10, 10, 5 for respectively the vehicle 75, 150, 200 and 500 mg/kg condition.

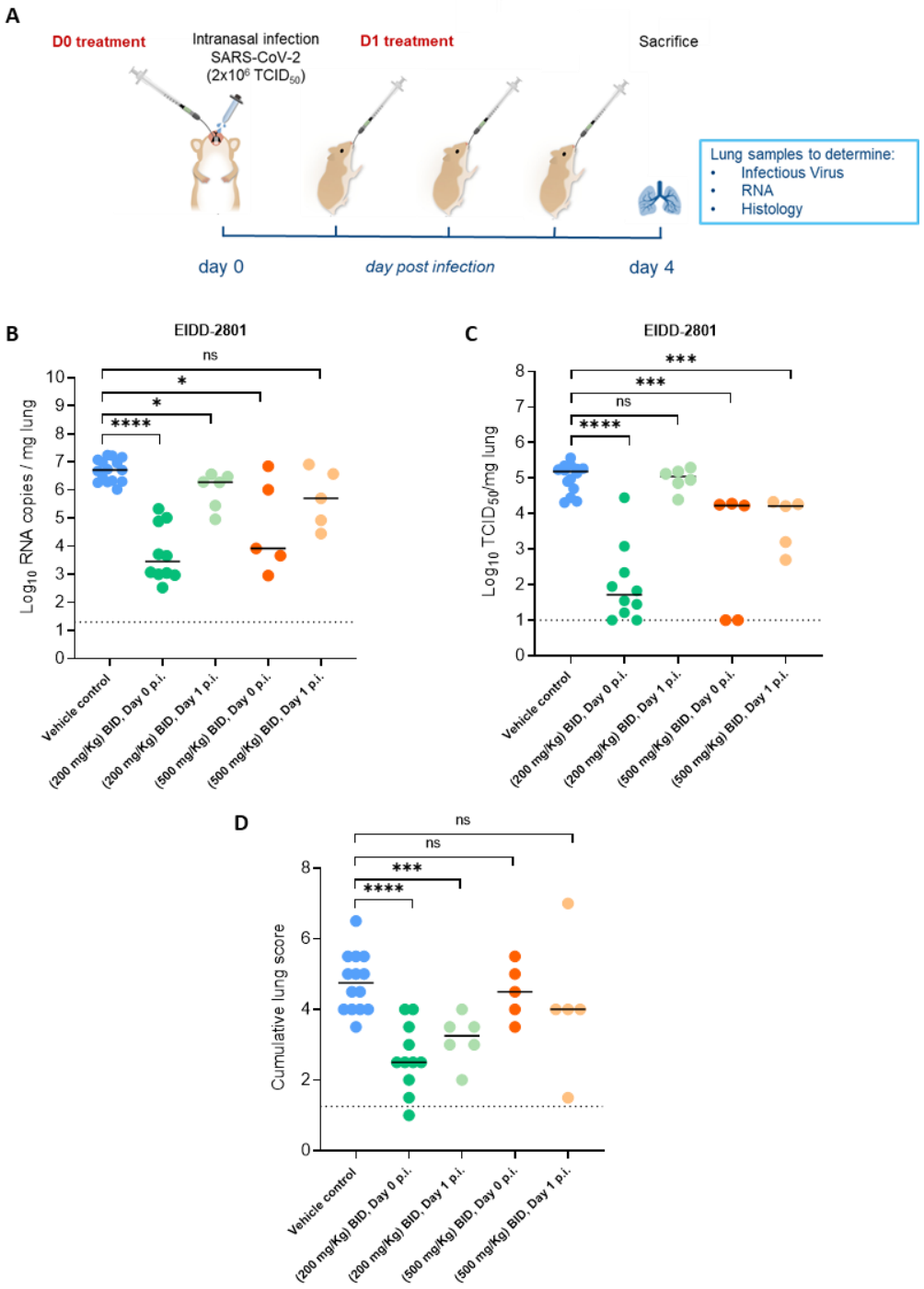


Fig.2. Effect of Molnupiravir (EIDD-2801) against SARS-CoV-2 in a hamster infection model in a post-exposure setting. (A) Set-up of the study. (B) Viral RNA levels in the lungs of control (vehicle-treated) and EIDD-2801-treated (200 or 500 mg/kg, BID starting from day 0 or day 1 post-infection, p.i.) SARS-CoV-2–infected hamsters at day 4 post-infection (pi) are expressed as log10 SARS-CoV-2 RNA copies per mg lung tissue. Individual data and median values are presented. (C) Infectious viral loads in the lungs of control (vehicle-treated) and EIDD-2801-treated SARS-CoV-2–infected hamsters at day 4 pi are expressed as log10 $TCID_{50}$ per mg lung tissue. Individual data and median values are presented. (D) Cumulative severity score from H&E stained slides of lungs from control (vehicle-treated) and EIDD-2801-treated SARS-CoV-2–infected hamsters. Individual data and median values are presented and the dotted line represents the median score of untreated non-infected hamsters. Data were analyzed with the Mann–Whitney U test. *P < 0.05, ***P < 0.001, ****P < 0.0001, ns=non-significant. Data for the vehicle and 200 mg/kg day 0 p.i. groups are from respectively 15 and 10 animals and two independent experiments. Data from the 200 mg/kg day 1 p.i, 500 mg/kg day 0 p.i and 500 mg/kg day1 p.i. condition are from a single experiment with respectively 6, 5 and 5 animals.

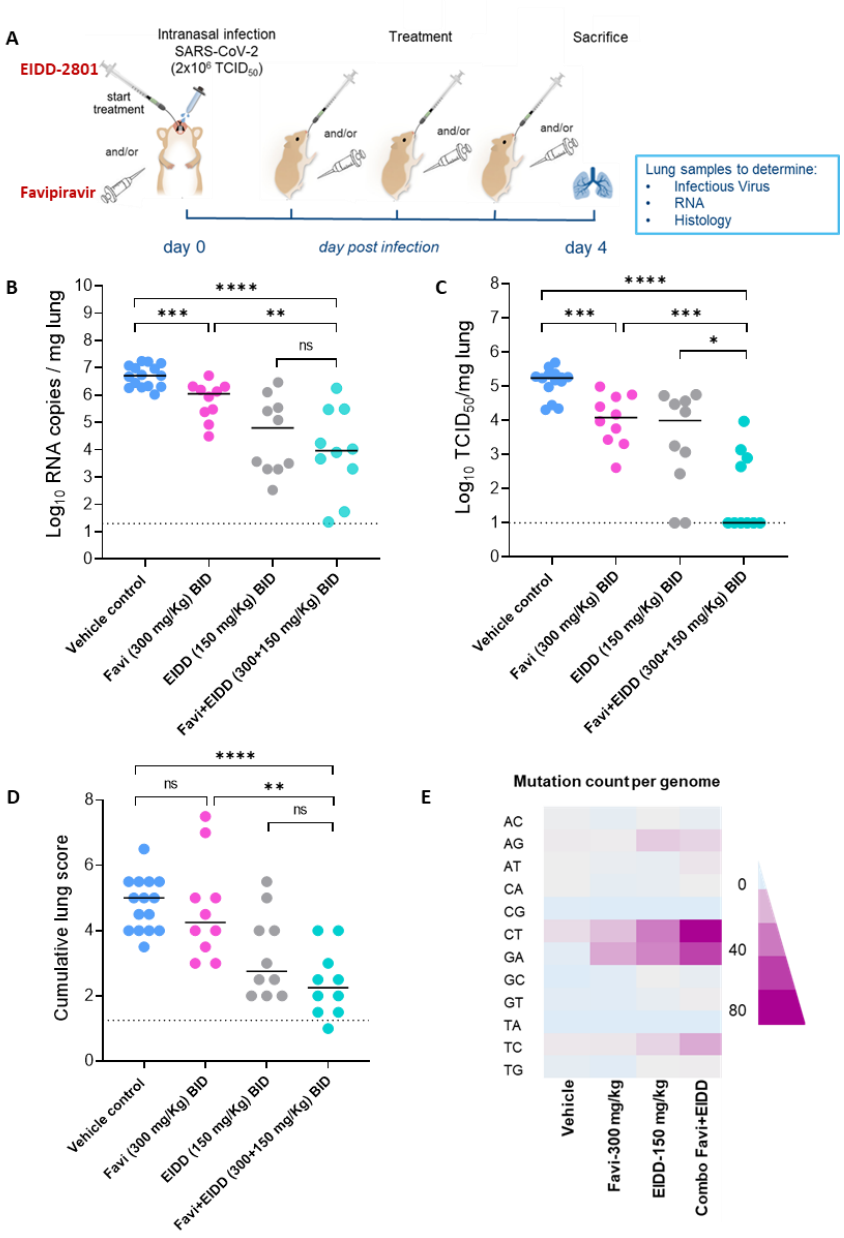
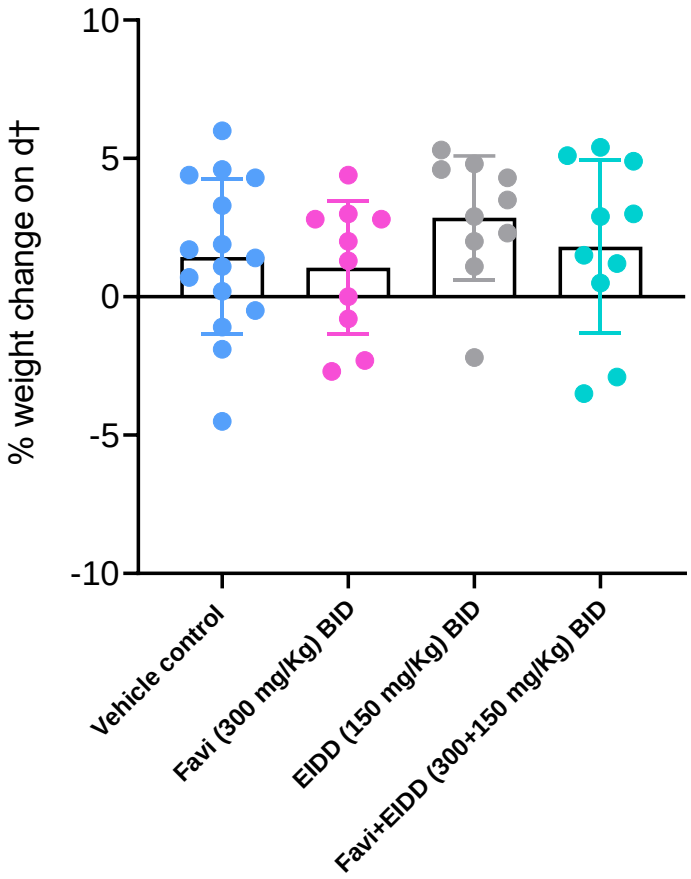


Fig.3. Combined efficacy of Favipiravir and Molnupiravir (EIDD-2801) against SARS-CoV-2 in a hamster infection model. (A) Set-up of the study. (B) Viral RNA levels in the lungs of control (vehicle-treated), Favipiravir-treated (300 mg/kg, BID), EIDD-2801-treated (150 mg/kg, BID) and combination-treated (Favipiravir+EIDD-2801 at 300+150 mg/kg, BID, respectively) SARS-CoV-2−infected hamsters at day 4 post-infection (pi) are expressed as log_{10} SARS-CoV-2 RNA copies per mg lung tissue. Individual data and median values are presented. (C) Infectious viral loads in the lungs of control (vehicle-treated), Favipiravir-treated, EIDD-2801-treated and combination-treated (Favipiravir+EIDD-2801) SARS-CoV-2−infected hamsters at day 4 pi are expressed as log_{10} $TCID_{50}$ per mg lung tissue. Individual data and median values are presented. (D) Cumulative severity score from H&E stained slides of lungs from control (vehicle-treated), Favipiravir-treated, EIDD-2801-treated and combination-treated (Favipiravir+EIDD-2801) SARS-CoV-2−infected hamsters. Individual data and median values are presented and the dotted line represents the median score of untreated non-infected hamsters. (E) Mean mutation count (per the whole genome) in the viral RNA isolated from the lungs of control (vehicle-treated), Favipiravir-treated (300 mg/kg, BID), EIDD-2801-treated (150 mg/kg, BID) and combination-treated (Favipiravir+EIDD-2801 at 300+150 mg/kg, BID, respectively) SARS-CoV-2−infected hamsters at day 4 post-infection (pi). Data were analyzed with the Mann−Whitney U test. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001, ns=non-significant. Favi=Favipiravir, EIDD=EIDD-2801. All data (panels B, C, D) are from two independent experiments with 15, 10, 10, 10 animals for respectively the vehicle, Favipiravir 300 mg/kg, EIDD-2801 150 mg/kg and Favipiravir+EIDD-2801 condition.



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367 **Fig. S1. Tolerability of combined treatment with Favipiravir and EIDD-2801 in SARS-CoV-2-infected hamsters.**
368 Weight change at day 4 post-infection in percentage, normalized to the body weight at the time of infection.
369 Bars represent means ± SD.

370

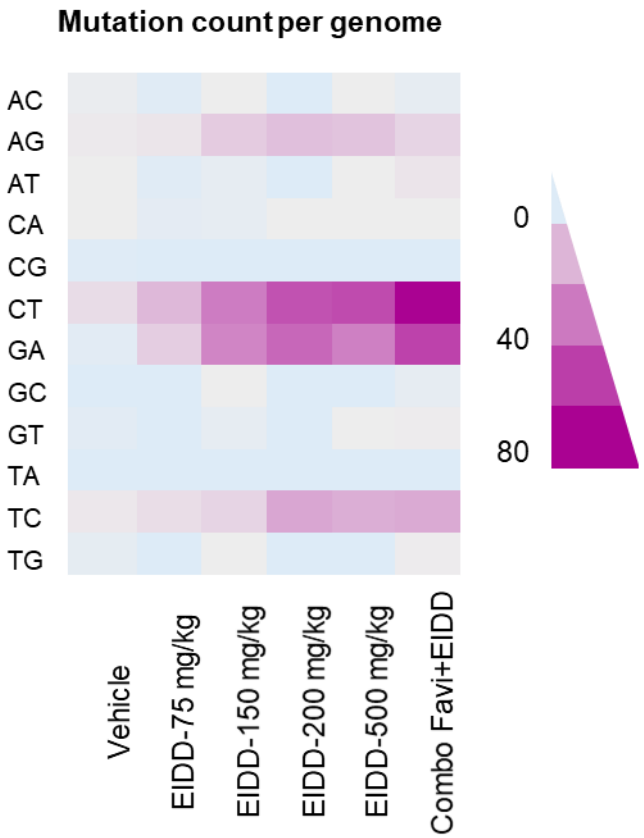

Fig. S2. Dose-dependent increase in mutation count in SARS-CoV-2 viral RNA by Molnupiravir (EIDD-2801). Mean mutation count (per the whole genome) in the viral RNA isolated from the lungs of control (vehicle-treated), EIDD-2801-treated (75, 150, 200 or 500 mg/kg, BID) and combination-treated (Favipiravir+EIDD-2801 at 300+150 mg/kg, BID, respectively) SARS-CoV-2–infected hamsters at day 4 post-infection (pi).