Molnupiravir (EIDD-2801) inhibits SARS-CoV2 replication in Syrian hamsters model

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Author Contributions

R.A., C.S.F. and J.N. designed research; R.A., C.S.F., S.J.F.K., X.Z. and L.L. performed research; R.A., C.S.F. and B.W. analyzed data; 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.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. and J.N. supervised the study; L.V., J.N. and K.D. acquired funding.

Abstract

Since its emergence in Wuhan, China in December 2019, the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has spread worldwide resulting in a global pandemic with >1.5 million deaths until now. In the search for small molecule inhibitors of SARS-CoV-2, drug repurposing is being extensively explored. Molnupiravir (EIDD-2801) is an orally bioavailable nucleoside analog that possesses a relatively broad-spectrum antiviral activity including against coronaviruses. We here studied the effect of EIDD-2801 in a well-established Syrian hamster SARS-CoV2 infection model. Treatment of SARS-CoV-2-infected hamsters with 200 mg/kg BID of EIDD-2801 for four consecutive days, starting from the day of infection, significantly reduced infectious virus titers and viral RNA loads in the lungs and markedly improved lung histopathology. When onset of treatment was delayed until 1 or 2 days after infection, a very modest antiviral effect was observed. The potential of EIDD-2801 for the treatment and or prevention of SARS-CoV2 deserves further attention.

Keywords

SARS-CoV-2; Antivirals; EIDD-2801; MK-4482; Molnupiravir; preclinical model.

34 **Main Text**

35 The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a β-coronavirus that was first
36 identified in Wuhan, China in December 2019 (1). Since then, the virus rapidly spread around the globe
37 with more than 66 million cases reported by the end of 2020, including >1.5 million deaths
38 [https://covid19.who.int/]. Infection with SARS-CoV-2 results in COVID-19 which is characterized by a
39 wide range of symptoms including fever, dry cough, muscle and/or joint pain, headache, decreased
40 sense of taste and smell and diarrhea. The disease can also progress into severe complications such as
41 acute respiratory distress syndrome (ARDS), respiratory failure, septic shock and multi-organ failure,
42 which are mainly attributed to a massive cytokine storm and exaggerated immune response (2).

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

50 The ribonucleoside analogue, N4-hydroxycytidine (NHC, EIDD-1931), has broad-spectrum antiviral
51 activity against multiple viruses belonging to different families of RNA viruses. Activity against SARS-
52 CoV and SARS-CoV-2 has been reported in cell lines and primary human airway epithelial cell cultures
53 (4). Acting through lethal mutagenesis, its incorporation into viral RNA results in the accumulation of
54 deleterious transition mutations beyond a permissible error threshold to sustain the virus population,
55 leading to error catastrophe (5). The orally bioavailable, pro-drug counterpart of NHC (6), Molnupiravir
56 (EIDD-2801, MK-4482) is currently being assessed for its potential as an antiviral treatment of SARS-
57 CoV-2 infection in Phase 2 clinical trials of infected patients (NCT04405570, NCT04405739). To our
58 knowledge, three very recent studies reported on activity of orally dosed EIDD-2801 in SARS-CoV-2

59 infected animals. Oral treatment of SARS-CoV2 infected Syrian hamsters with EIDD-2801 resulted in
60 marked reduction of viral loads when administered either in a pre- or post-exposure settings (7). In a
61 ferret model, EIDD-2801 significantly reduced virus load in a therapeutic setting and blocked SARS-
62 CoV-2 contact transmission (8), while in a humanized mouse model, EIDD-2801 prevented SARS-CoV-
63 2 infection in a pre-exposure prophylaxis setting (9).

64 Recently, we have established and characterized a Syrian Gold (SG) hamster model for the evaluation
65 of antiviral drugs against SARS-CoV-2 (10, 11). Using this model, we have shown that a high dose of the
66 influenza drug Favipiravir results in a pronounced antiviral activity in SARS-CoV-2 infected hamsters,
67 whereas hydroxychloroquine lacks antiviral activity in this model. Here, we use the same hamster
68 model to assess the anti-SARS-COV2 of EIDD-2801 against SARS-CoV-2 in infected hamsters.

69 First, we evaluated the effect when the drug was administered at a dose of either 75 or 200 mg/kg BID.
70 Briefly, 6-8 weeks female SG hamsters were treated with the intended dose of the compound or the
71 vehicle (i.e. the control group) for four consecutive days starting one hour before intranasal infection
72 with 50 µL containing 2×10^6 $TCID_{50}$ SARS-CoV-2 [BetaCov/Belgium/GHB-03021/2020 (EPI ISL 109
73 407976|2020-02-03)]. At day four post-infection (pi), the animals were euthanized and lungs were
74 collected for quantification of viral RNA, infectious virus titers and lung histopathology as described
75 previously (10) (Fig. 1A). Treatment of hamsters with 75 mg/kg BID EIDD-2801 resulted in 1.2 $\log_{10}$
76 reduction in the viral RNA copies per mg of lung tissue (P=0.01), compared to the vehicle-treated
77 infected hamsters (Fig. 1B). On the other hand, in the lungs of hamsters that had been treated with
78 200 mg/kg BID EIDD-2801 a $3\log_{10}$ reduction in the viral RNA copies/mg of lung tissue was noted (P
79 <0.0001) (Fig. 1B). A similar pattern was observed for the infectious virus load in the lungs, the high
80 dose of 200 mg/kg, but not the 75 mg/kg dose, reduced infectious virus lung titers by 3.3 $\log_{10}$
81 (P<0.0001) $TCID_{50}$ per mg (Fig. 1C).

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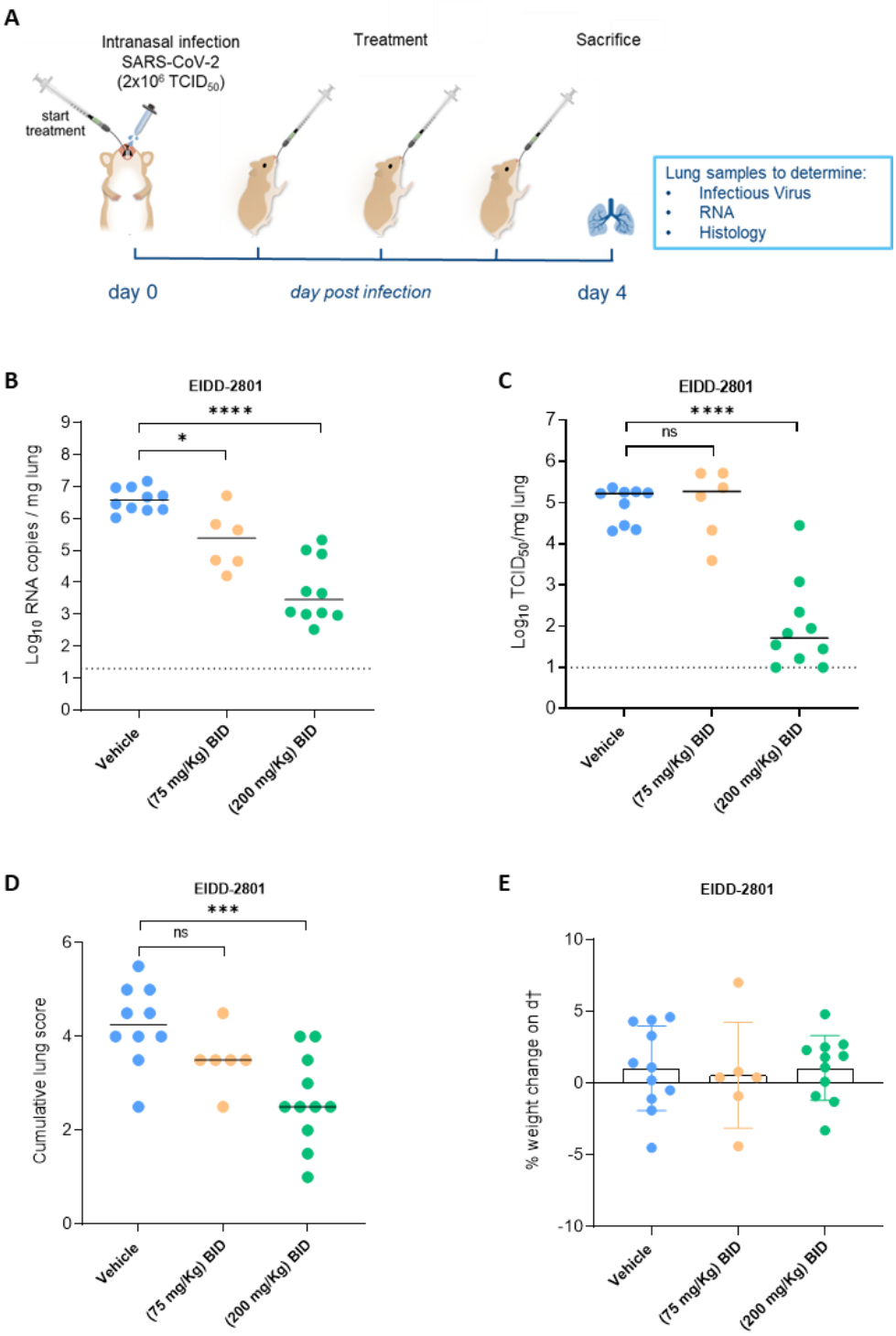

Fig.1. *In vivo* efficacy of EIDD-2801 against SARS-CoV2 at the time of infection. (A) Set-up of the study. (B) Viral RNA levels in the lungs of control (vehicle-treated) and EIDD-2801-treated SARS-CoV-2–infected hamsters at day 4 post-infection (pi) are expressed as log_{10} SARS-CoV2 RNA copies per mg lung tissue. The bars represent median values. (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. The bars represent median values. (D) Cumulative severity score from H&E stained slides of lungs from control (vehicle-treated) and EIDD-2801-treated hamsters. The bars represent median values. (E) Weight change at day 4 pi in percentage, normalized to the body weight at the day of infection. Bars represent means ± SD. Data were analyzed with the Mann–Whitney U test. *P < 0.05; ***P < 0.001, ****P < 0.0001.

95 Importantly, treatment with 200 mg/kg EIDD-2801 BID significantly reduced the histological lung
96 disease score (P=0.001), whereas the lower dose resulted only in slight reduction in lung pathology
97 (P=0.05, ns, Fig. 1D). Both doses were well-tolerated without significant weight loss or any obvious
98 adverse effects (Fig. 1E).

99 We next explored whether the high dose can also be used in a post-exposure setting, i.e. when
100 treatment is started at 24 or 48 h after infection (Fig. 2A). Delaying the start of treatment with EIDD-
101 2801 (200 mg/kg, BID) by 1 or 2 days resulted in a loss of potency with only a rather slight reduction
102 of viral RNA copies/mg lung [0.3 to 0.4 $\log_{10}$ respectively] (Fig. 2B). Similarly, virus titrations of the lung
103 tissues also revealed no significant reduction of infectious virus load in both groups with delayed
104 treatment compared with the vehicle-treated group (Fig. 2C). However, a modest but significant
105 reduction of the histological lung disease score was observed in both the day 1 (p=0.016) and day 2
106 (p=0.01) delayed treatment groups (Fig. 2D). These results suggest that even if the delayed treatment
107 with the 200 mg/kg BID dose was not sufficient to efficiently stop the viral replication, it may still able
108 to delay the disease progression in the lung of infected hamsters.

109 In summary, EIDD-2801 is able to markedly reduce, albeit at a relatively high dose, SARS-CoV2 infection
110 and virus induced pathology in hamsters, in particular when given at the time of Infection. EIDD-2801
111 has been previously demonstrated to exert antiviral activity against MERS-CoV and SARS-CoV in cell
112 culture and mouse models. Very recently activity was also reported against SARS-CoV-2 (4, 8, 9). In
113 another SARS-CoV2 Syrian hamster model, 250 mg/kg of EIDD-2801 was given orally every 12 hours
114 starting either 12 hours pre-infection or 12 hours post-infection (7). Although a slightly higher
115 compound dose (250 mg/kg, BID) and a much lower virus inoculum (5×10^2 $TCID_{50}$) was used in their
116 hamster study, the observed antiviral effect was less pronounced (1 $\log_{10}$ reduction in viral RNA and 2
117 $\log_{10}$ reduction in infectious virus titers) than in our study (7).

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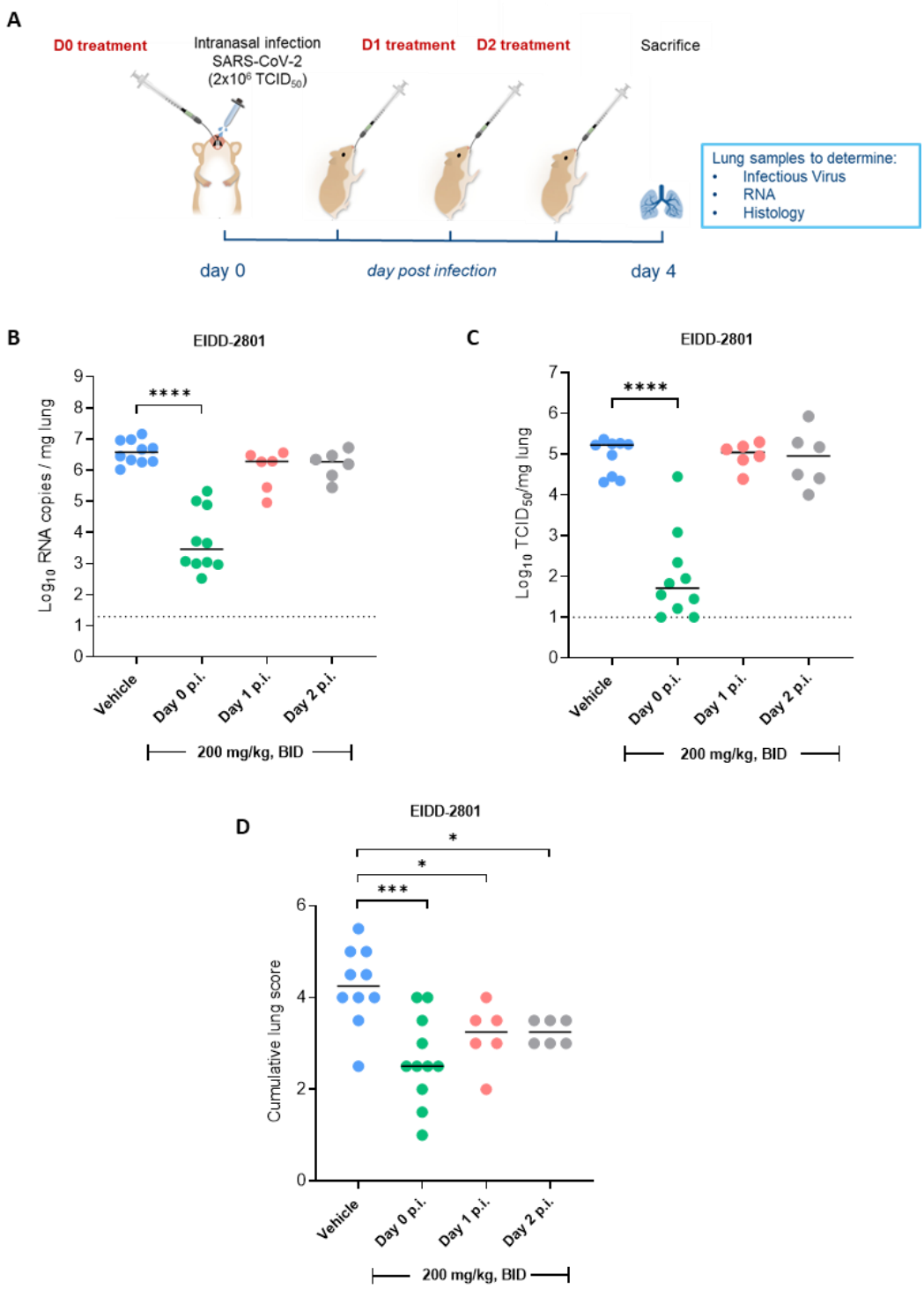

Fig.2. *In vivo* efficacy of EIDD-2801 against SARS-CoV2 in a post-exposure setting. (A) Set-up of the study. (B) Viral RNA levels in the lungs of control (vehicle-treated) and EIDD-2801-treated SARS-CoV-2–infected hamsters at day 4 post-infection (pi) are expressed as log10 SARS-CoV2 RNA copies per mg lung tissue. The bars represent median values. (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. The bars represent median values. (D) Cumulative severity score from H&E stained slides of lungs from control (vehicle-treated) and EIDD-2801-treated hamsters. The bars represent median values. Data were analyzed with the Mann–Whitney U test. *P < 0.05, ***P < 0.001, ****P < 0.0001.

130 EIDD-2801 has also been reported to be effective in SARS-CoV infected C57/BL6 mice either when
131 administered prophylactically (start of treatment 2 h before infection) or therapeutically (start of
132 treatment delayed until 48 hpi) at a dose of 500 mg/kg twice daily (Sheahan et al., 2020). In a
133 humanized mouse model of SARS-CoV-2 infection, pre-exposure (prophylaxis) (12 h before infection)
134 with 500 mg/kg of EIDD-2801 twice daily was efficacious in preventing SARS-CoV-2 infection, with a ~6
135 log_{10} reduction in virus lung titers (9). In a very recent report, EIDD-2801 was found to markedly reduce
136 SARS-CoV-2 viral titers in in the upper respiratory tract of ferrets when start of treatment was delayed
137 until 12 hpi to 36 hpi. Moreover, treating SARS-CoV2 infected ferrets with EIDD-2801 starting at 12 hpi
138 prevented contact transmission when they were co-housed with non-infected untreated contact
139 ferrets (8).

140 Our results are consistent with other recent studies (in hamster, mouse and ferret models) showing
141 that pre-emptive and early intervention with oral EIDD-2801 results in antiviral activity. However, at a
142 dose of 200 mg/kg we did not observe a reduction of virus in the lung when treatment was initiated
143 24 to 48 hpi in our Syrian hamster model (although some improvement in lung pathology was observed
144 in these groups). Further studies are ongoing to explore therapeutic (delayed start of treatment) effect
145 at higher doses.

146 The antiviral drug, Remdesivir (Veklury), is the first treatment for COVID-19 to receive FDA approval
147 for use in hospitalised patients, although the World Health Organisation has recently recommended
148 against its use (https://www.fda.gov/news-events/press-announcements/fda-approves-first-
149 treatment-covid-19; https://www.who.int/news-room/feature-stories/detail/who-recommends-
150 against-the-use-of-remdesivir-in-covid-19-patients). Both Remdesivir and EIDD-2801 are nucleoside
151 analogues acting on the viral RNA replication pathway, with Remdesivir resulting in chain termination
152 and EIDD-2801 in lethal mutagenesis (5, 12). Additionally, both have a high resistance barrier, with
153 resistant viral mutants having a loss in fitness (6, 13). However, Remdesivir needs to be administrated
154 intravenously, whereas EIDD-2801 can be dosed via the oral route. This is a significant advantage in

155 terms of the practicalities of treatment management, where we can envision that asymptomatic
156 patients who have had high-risk contact or patients presenting mild symptoms are prescribed EIDD-
157 2801 pills in a non-hospitalised setting.

158 By demonstrating the antiviral efficacy of the orally-bioavailable EIDD-2801 against SARS-CoV-2 in the
159 Syrian hamster model, we contribute to the pre-clinical profiling of EIDD-2801, and provide further
160 evidence in support of the ongoing Phase II clinical trials. The outcomes of these trials could play a
161 pivotal role in the control and management of this devastating pandemic.

Acknowledgments

We thank Carolien De Keyzer, Lindsey Bervoets, Thibault Francken, Elke Maas, Jasper Rymenants, Birgit Voeten, Dagmar Buyst, Niels Cremers, Bo Corbeels and Kathleen Van den Eynde for excellent technical assistance. We are grateful to Piet Maes for kindly providing the SARS-CoV-2 strain used in this study. We thank Jef Arnout and Annelies Sterckx (KU Leuven Faculty of Medicine, Biomedical Sciences Group Management) and Animalia and Biosafety Departments of KU Leuven for facilitating the animal studies. This project has received funding from the Covid-19-Fund KU Leuven/UZ Leuven and the COVID-19 call of FWO (G0G4820N), the European Union's Horizon 2020 research and innovation program under grant agreements No 101003627 (SCORE project) and Bill & Melinda Gates Foundation (BGMF) under grant agreement INV-00636. R.A., C.S.F. and L.L. were supported by a KU Leuven internal project fund. X.Z. received funding of the China Scholarship Council (grant No.201906170033).

Competing Interest Statement: None to declare.

175 **References**

176 1. N. Zhu, *et al.*, A Novel Coronavirus from Patients with Pneumonia in China, 2019. *N. Engl. J.*
177 *Med.* **382**, 727–733 (2020).

178 2. M. Z. Tay, C. M. Poh, L. Rénia, P. A. MacAry, L. F. P. Ng, The trinity of COVID-19: immunity,
179 inflammation and intervention. *Nat. Rev. Immunol.* **20**, 363–374 (2020).

180 3. L. Delang, J. Neyts, Medical treatment options for COVID-19. *Eur. Hear. J. Acute Cardiovasc.*
181 *Care*, 204887262092279 (2020).

182 4. T. P. Sheahan, *et al.*, An orally bioavailable broad-spectrum antiviral inhibits SARS-CoV-2 in
183 human airway epithelial cell cultures and multiple coronaviruses in mice. *Sci. Transl. Med.* **12**
184 (2020).

185 5. N. Urakova, *et al.*, β-D- N 4 -Hydroxycytidine Is a Potent Anti-alphavirus Compound That
186 Induces a High Level of Mutations in the Viral Genome . *J. Virol.* **92** (2017).

187 6. M. Toots, *et al.*, Characterization of orally efficacious influenza drug with high resistance
188 barrier in ferrets and human airway epithelia. *Sci. Transl. Med.* **11** (2019).

189 7. K. Rosenke, *et al.*, Orally delivered MK-4482 inhibits SARS-CoV-2 replication in the Syrian
190 hamster model. *Res. Sq.* (2020).

191 8. R. M. Cox, J. D. Wolf, R. K. Plemper, Therapeutically administered ribonucleoside analogue
192 MK-4482/EIDD-2801 blocks SARS-CoV-2 transmission in ferrets. *Nat. Microbiol.* (2020).

193 9. A. Wahl, *et al.*, Acute SARS-CoV-2 Infection is Highly Cytopathic, Elicits a Robust Innate
194 Immune Response and is Efficiently Prevented by EIDD-2801. *Res. Sq.* (2020).

195 10. S. J. F. Kaptein, *et al.*, Favipiravir at high doses has potent antiviral activity in SARS-CoV-
196 2−infected hamsters, whereas hydroxychloroquine lacks activity. *Proc. Natl. Acad. Sci. U. S. A.*
197 **117**, 26955–26965 (2020).

198 11. R. Boudewijns, *et al.*, STAT2 signaling restricts viral dissemination but drives severe
199 pneumonia in SARS-CoV-2 infected hamsters. *Nat. Commun.* **11**, 5838 (2020).

200 12. T. P. Sheahan, *et al.*, Broad-spectrum antiviral GS-5734 inhibits both epidemic and zoonotic
201 coronaviruses. *Sci. Transl. Med.* **9** (2017).

202 13. M. L. Agostini, *et al.*, Coronavirus susceptibility to the antiviral remdesivir (GS-5734) is
203 mediated by the viral polymerase and the proofreading exoribonuclease. *MBio* **9** (2018).

204