Oral prodrug of remdesivir parent GS-441524 is efficacious against SARS-CoV-2 and a variant of concern in ferrets

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Oral prodrug of remdesivir parent GS-441524 is efficacious against SARS-CoV-2 and a variant of concern in ferrets

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Short title: Oral remdesivir analog blocks SARS-CoV-2

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24 **Abstract**

25 Remdesivir is the only small-molecule antiviral approved to date for COVID-19 treatment, but its
26 wider use is limited by intravenous delivery. An orally bioavailable remdesivir analog may boost
27 therapeutic benefit by facilitating early administration to non-hospitalized patients. This study
28 characterized the anti-SARS-CoV-2 efficacy of GS-621763, an oral prodrug of remdesivir parent
29 nucleoside GS-441524. Both GS-621763 and GS-441524 inhibited SARS-CoV-2, including variants of
30 concern (VoC) in cell culture. Oral GS-621763 was efficiently converted to plasma metabolite GS-
31 441524, and in lungs to the triphosphate metabolite identical to that generated by remdesivir,
32 demonstrating a consistent mechanism of activity. Twice-daily oral administration of 10 mg/kg GS-
33 621763 reduced SARS-CoV-2 burden to near-undetectable levels. When dosed therapeutically against
34 VoC P.1 gamma (γ), oral GS-621763 blocked virus replication and prevented transmission to untreated
35 contact animals. These results demonstrate therapeutic efficacy of a much-needed orally bioavailable
36 analog of remdesivir in a relevant animal model of SARS-CoV-2 infection.

37 **Introduction**

38 The global COVID-19 pandemic has resulted in far more than 150 million cases and an estimated
39 3.5 million deaths. The socioeconomic and geopolitical impact of the pandemic is unprecedented and
40 will most likely become fully evident only in post-pandemic years. To date, the ribonucleoside analog
41 remdesivir is the only small molecule antiviral therapeutic approved by the FDA for the treatment of
42 COVID-19¹. Originally discovered for respiratory syncytial virus², remdesivir is a mono-
43 phosphoramidate prodrug that must be administered intravenously³. The obligatory parenteral delivery
44 limits access of non-hospitalized COVID-19 patients to remdesivir treatment in early disease stages,
45 thereby significantly narrowing the therapeutic window in which a direct-acting antiviral targeting an
46 acute viral infection is expected to have its greatest effect⁴.

47 Orally active COVID-19 therapeutics have potential to maximize patient benefit and ideally prevent
48 progression to severe disease. Several oral therapeutic candidates such as EIDD-2801/molnupiravir⁵⁻⁷
49 and AT-527⁸ are in advanced stages of clinical trials, but currently only remdesivir has shown confirmed
50 efficacy for COVID-19 treatment in humans⁹. Following intravenous administration and despite its short
51 plasma half-life, remdesivir is sufficiently stable in non-rodent species to distribute to tissues such as the
52 lung, where it is rapidly converted to its monophosphate metabolite and then efficiently anabolized to
53 the bioactive triphosphate GS-443902 (Fig. 1a)². An alternative strategy to generate GS-443902 could
54 be to directly administer the remdesivir parent nucleoside GS-441524, but due to less efficient
55 metabolism of GS-441524 to its monophosphate, higher daily systemic exposures of GS-441524 than
56 those obtained from intravenous remdesivir are required to generate equivalent concentrations of GS-
57 443902 in lung tissue. In order to obtain high exposures after oral delivery, a prodrug strategy to
58 improve oral absorption was employed leading to the identification of GS-621763, which demonstrated
59 high oral bioavailability in several species².

60 SARS-CoV-2 efficiently infects mustelids such as ferrets and mink, and both direct and reverse
61 zoonotic transmission between mink and humans have been reported^{10,11}. Infected ferrets show only
62 mild clinical signs, but the virus readily replicates in the upper respiratory tract and shed virus load in
63 nasal lavages is high, supporting efficient animal-to-animal transmission⁶. Consequently, ferrets
64 recapitulate the presentation of SARS-CoV-2 in the majority of human cases, especially in children and
65 younger adults^{12,13}.

66 In this study, we used the ferret model to test oral anti-SARS-CoV-2 efficacy of GS-621763. The
67 GS-621763 prodrug is pre-systemically hydrolyzed to afford high systemic exposures of GS-441524
68 (Fig. 1a). Having determined the plasma pharmacokinetic (PK) profile of GS-621763 in ferrets, we
69 examined the effect of oral GS-621763 administered therapeutically against the original SARS-CoV-2
70 USA-WA1/2020 (WA1/2020) strain and the recently emerged, highly prevalent VoC P.1 (γ) lineage^{14,15}.

71

72 **Results**

73 Prior to *in vivo* testing, we assessed antiviral potency of both GS-621763 and its metabolite GS-
74 441524 against lineage A isolate WA1/2020 and three recently emerged VoC, hCoV-
75 19/USA/CA_UCSD_5574/2020 ((α), lineage B.1.1.7; CA/2020), hCoV-19-South Africa/KRISP-
76 K005325/2020 ((β), lineage B.1.351; SA/2020), and hCoV-19/Japan/TY7-503/2021 ((γ), lineage P.1;
77 BZ/2021) in cultured cells.

78 **Antiviral Activity against VoCs**

79 Half-maximal effective concentrations (EC₅₀) were highly consistent, ranging from 0.11 to 0.73 μ M
80 for GS-621763 (Fig. 1b, extended data Table 1) and from 0.11 to 0.68 μ M for GS-441524 (Fig. 1c,
81 extended data Table 1). Analogous potency ranges were obtained when luciferase expressing WA1/2020
82 reporter viruses were examined in dose-response assays in A549 cells stably expressing human ACE2

83 (A549-hACE2) (extended data Table 1). Toxicity-testing of GS-621763, remdesivir, and GS-441524 in
84 different cell lines and primary human cells derived from different donors revealed half-maximal
85 cytotoxic concentrations (CC_{50}) of 40 to $>100\ \mu\text{M}$ (Fig. 1d, extended data Table 1), 36 to $>100\ \mu\text{M}$
86 (extended data Fig. 1, extended data Table 1) and $>100\ \mu\text{M}$ (Fig. 1e, extended data Table 1),
87 respectively, corresponding to selectivity indices ($SI = CC_{50}/EC_{50}$) of GS-621763 >137 in VeroE6 and
88 >51 in A549-ACE2 cells.

89 **Pharmacokinetics following oral administration**

90 Assessment of GS-621763 plasma PK parameters in the ferret revealed excellent oral bioavailability
91 (Fig. 2a), extensive cleavage pre-systemically to generate high exposures of GS-441524 in the blood
92 (extended data Table 2), efficient distribution to soft tissues of the respiratory system (lung), and
93 confirmed anabolism to bioactive GS-443902 (extended data Table 3). Following a single 30 mg/kg oral
94 dose of GS-621763 in ferrets, the daily systemic exposure (AUC_{0-24h}) of GS-441524 was $81\ \mu\text{M}\cdot\text{h}$, 4.5
95 fold higher than the exposure following IV remdesivir at 10 mg/kg and approximately 12-fold greater
96 than that observed following an 200 mg IV remdesivir dose in human¹⁶. Lower levels of bioactive GS-
97 443902 were formed from oral 30 mg/kg GS-621763 dosing compared to 10 mg/kg IV remdesivir
98 (extended data Table 3), illustrating the difference in intracellular activation efficiency of the
99 phosphoramidate prodrug remdesivir compared to systemic parent nucleoside GS-441524.

100 **Prophylactic efficacy in ferrets**

101 To test antiviral efficacy, we infected ferrets intranasally with 1×10^5 plaque forming units (pfu) of
102 WA1/2020, followed by twice daily (b.i.d.) oral treatment with GS-621763 at 20 mg/kg body weight for
103 four days (Fig. 2b). Treatment was initiated at the time of infection, nasal lavages collected in 12-hour
104 intervals, and respiratory tissues harvested 4 days after infection. Shed SARS-CoV-2 load in nasal
105 lavages of vehicle-treated animals reached plateau 1.5 days after infection at approximately 1×10^4

106 pfu/mL, whereas virus was transiently detectable in lavages of only one ferret of the GS-621763-
107 treatment group at 12 hours after infection (Fig. 2c). Clinical signs overall are minor in the ferret model⁶.
108 However, only animals of the vehicle group showed elevated body temperature (Fig. 2d) and reduced
109 weight gain (Fig. 2e). Virus was undetectable in the nasal turbinates extracted from treated animals 4
110 days after infection, compared to a robust load of approximately 5×10^4 pfu/g nasal turbinate of animals
111 of the vehicle group (Fig. 2f). Viral RNA copy numbers found in lavages (Fig. 2g) and turbinates (Fig.
112 2h) mirrored the infectious titer results, revealing a consistent, statistically significant difference
113 between vehicle and treatment groups of two and three orders of magnitude, respectively. Consistent
114 with prior studies⁶, no infectious virions or viral RNA were detectable in the lower respiratory tract (Fig.
115 2i,j).

116 **Therapeutic efficacy and lowest efficacious dose**

117 To determine the lowest efficacious dose in a clinically more relevant therapeutic setting, we
118 initiated oral treatment 12 hours after infection at the 10 mg/kg and 3 mg/kg body weight levels,
119 administered b.i.d. (Fig 3a). EIDD-2801/molnupiravir at 5 mg/kg b.i.d. was given as reference following
120 an identical therapeutic b.i.d. regimen⁶. Shed virus load was significantly lower in all treated animals
121 than in the vehicle group within 12 hours of treatment onset (Fig. 3b). Virus load in nasal lavages of
122 ferrets receiving GS-621763 at 3 mg/kg plateaued approximately one order of magnitude lower than in
123 those from vehicle animals, while treatment with GS-621763 at 10 mg/kg or EIDD-2801/molnupiravir
124 reduced shedding to near-detection level by day 3 after infection. Consistent with this inhibitory effect,
125 treatment with 3 mg/kg GS-621763 reduced burden in the turbinates by one order of magnitude (Fig.
126 3c), while virus burden approached the limit of detection in animals of the 10 mg/kg GS-621763 and
127 EIDD-2801/molnupiravir treatment groups. No significant differences in clinical signs were noted
128 between vehicle animals and any of the treatment groups (Fig. 3d,e).

129 Viral RNA was detectable in nasal lavages and turbinates of all animals, underscoring efficient
130 infection. However, RNA copies showed a statistically significant mean reduction in the 10 mg/kg GS-
131 621763 and EIDD-2801/molnupiravir groups compared to vehicle (Fig. 3f,g). These results confirm oral
132 efficacy of therapeutic GS-621763 against WA1/2020 in a relevant animal model of upper respiratory
133 infection.

134 **Inhibition of replication and transmission of a the BZ/2021 VoC**

135 To probe the anti-SARS-CoV-2 indication spectrum of GS-621763, we applied the efficacious
136 regimen, 10 mg/kg GS-621763 b.i.d. started 12 hours after infection, to recently emerged VoC BZ/2021
137 ¹⁷ in a combined efficacy and transmission study (Fig. 4a). After an initial replication delay, shed virus
138 became detectable in vehicle-treated animals 1.5 days after infection, then rapidly reached a robust
139 plateau of nearly 10⁴ pfu/mL nasal lavage on day 2 after infection (Fig. 4b). Quantitation of viral RNA
140 copies mimicked the profile of the infectious titers, although a low viral RNA load was present in
141 lavages already on the first day after infection (Fig. 4c). Viral titers and RNA copies in nasal turbinates
142 determined 4 days after infection were likewise high, ranging from 10⁴ to 10⁵ pfu/g tissue (Fig. 4d) and
143 10⁸ to 10¹⁰ RNA copies/g tissue (Fig. 4e), respectively. However, no infectious BZ/2021 virions or viral
144 RNA were detected in the lungs of any of these animals (Fig. 4f,g), and no clinical signs such as changes
145 in body weight or fever emerged (extended data Fig. 2a,b). This presentation mimicked our previous
146 experience with WA1/2020 ⁶, indicating that BZ/2021 does not invade the ferret host more aggressively
147 than WA1/2020. Treatment of BZ/2021 infection with oral GS-621763 was highly efficacious, reducing
148 both shed virus burden and tissue titers to undetectable levels (Fig. 4b,d) and lowering viral RNA copies
149 in nasal lavages and turbinates by over three orders of magnitude (Fig. 4c,e).

150 Whole genome sequencing of the virus inoculum and virus populations extracted from nasal
151 turbinates confirmed the presence of mutations characteristic for the P.1 VoC¹⁸ (Fig. 4h, supplementary

dataset 1). In addition, we noted an L260F substitution in nsp6 associated with SARS-CoV-2 adaptation to weasels¹⁰ that had a 60%-allele frequency in the BZ/2021 inoculum. Four days after infection of ferrets, this mutation had become fully dominant and a second characteristic weasel mutation, Y453F in the spike protein that was first noted in several clusters of SARS-CoV-2 outbreaks in mink farms¹⁰, had emerged in addition (Fig. 4h, supplementary dataset 1). We furthermore noted the presence of an F184V exchange in nsp6 of the BZ/2021 inoculum, which arose during amplification in VeroE6 cells¹⁸ and was rapidly counterselected against in the ferret host. In contrast, the WA1/2020 inoculum used for our ferret studies did not contain any unreported additional changes (Fig. 4h, supplementary dataset 2). WA1/2020 also acquired a weasel-characteristic mutation when passaged through ferrets, N501T in the receptor binding domain of the spike protein¹⁰, but no changes in nsp6 were detected and only one animal harbored a virus population with Y453F substitution in low (8%) allele frequency. Neither the GS-621763-experienced BZ/20201 nor WA1/2020 populations contained remdesivir resistance mutations previously selected in SARS-CoV-2 (i.e. E802D in nsp12¹⁹) or the related mouse hepatitis virus²⁰ (i.e. F476 L and V553L in nsp12) nor did any new variants arise in nsp12 at >5% allele frequency, when viruses were extracted from treated animals at the time of termination (Fig. 4h, supplementary datasets 1,2).

All vehicle-treated animals efficiently transmitted VoC BZ/2021 to untreated direct contact ferrets (Fig. 4b-e). Co-housing was started 54 hours after infection and continued until termination of the source animals. Shed BZ/2021 replicated in the contacts without delay, becoming first detectable in nasal lavages within 12 hours after initiation of co-housing. This altered replication profile corroborated BZ/2021 adaptation to the ferret host in the source animals, and virus populations recovered from contacts of vehicle-treated source animals indeed contained both the L260F exchange in nsp6 and the Y453F mutation in spike (Fig. 4h, supplementary dataset 1). Consistent with efficient inhibition of

175 BZ/2021 replication in the treated source animals by oral GS-621763, treatment completely blocked
176 virus transmission to untreated direct-contact animals. None of the contacts of treated source ferrets shed
177 infectious particles or viral RNA at any time (Fig. 4b,c), infectious viral particles were absent from nasal
178 turbinates 5.5 days after initiation of co-housing (Fig. 4d), and only a low level of viral RNA ($<10^5$
179 copies/g nasal turbinate) was detected in nasal turbinates of the contact animals (Fig. 4e).

180

181 **Discussion**

182 This study demonstrates oral efficacy of the remdesivir analog GS-621763 against the early clinical
183 SARS-CoV-2 isolate WA1/2020 and one of the recently emerged VoC in the ferret model. After oral
184 administration of GS-621763, only low systemic levels of intact prodrug were transiently observed. The
185 parent nucleoside GS-441524 was the major metabolite detected in blood, more than three orders of
186 magnitude higher than intact prodrug, indicating efficient conversion upon intestinal absorption. GS-
187 441524 metabolism inside cells to the same intracellular bioactive triphosphate GS-443902 in tissues
188 links GS-621763 to the well-understood mechanism of action of remdesivir, which blocks SARS-CoV-2
189 replication by triggering delayed chain termination of the nascent viral RNA chain²¹ and/or template-
190 dependent inhibition after incorporation into viral antigenomic RNA²². Systemic GS-441524 is less
191 efficient at forming the bioactive GS-443902 in the lungs of ferrets compared to systemic remdesivir,
192 but the exposure of GS-441524 achieved following oral dosing of GS-621763 is sufficient to overcome
193 this difference and generate substantial levels of bioactive GS-443902. The more persistent but lower
194 plasma levels of GS-441524 following intravenous remdesivir are a result of efficient formation of
195 triphosphate in tissues from remdesivir, followed by slow dephosphorylation to GS-441524 which then
196 appears in plasma, as evidenced by its similar half-life with that of the triphosphate GS-443902 in
197 PBMC¹⁶.

198 Although GS-443902 levels in ferret respiratory tissues after a single 30 mg/kg oral dose of GS-
199 621763 trailed those of intravenous 10 mg/kg remdesivir by approximately 4-fold at 24 hours, a minimal
200 daily oral dose of 20 mg/kg GS-621763 (10 mg/kg b.i.d.) provided full therapeutic benefit, rapidly
201 reducing shed virus titers to near-undetectable. As the GS-443902 active metabolite is quantitated from
202 gross tissue samples, we cannot compare GS-443902 levels resulting from either GS-621763 or
203 remdesivir dosing in distinct respiratory cell types. If human oral bioavailability of GS-621763 is
204 consistent with that observed in ferrets, and the model is predictive of antiviral potential, the dose levels
205 identified in the ferret study as fully efficacious in upper respiratory tract correspond to a feasible human
206 daily dose of approximately 250 mg oral GS-621763.

207 An observed difference of approximately two to three orders of magnitude between infectious titers
208 and viral RNA copy numbers in the ferrets closely resembled that described in earlier reports^{6,23,24},
209 demonstrating high reproducibility of the model. At present, the correlation between upper respiratory
210 tract SARS-CoV-2 load at early stages after human infection and the likelihood of progression to viral
211 pneumonia and severe COVID-19 has not been fully understood. However, clinical studies have linked
212 higher upper respiratory virus burden to a heightened patient risk of developing severe COVID-19,
213 requiring intubation or intensive care, and having an unfavorable outcome²⁵. Based on these clinical data
214 and the rapid reduction of upper respiratory virus burden seen in the ferret model, we expect that early
215 initiation of oral treatment, ideally immediately following a positive diagnostic test and before the onset
216 of clinical signs^{26,27}, holds high promise to block viral invasion of the lower respiratory tract and viral
217 pneumonia that necessitates hospitalization.

218 Recently emerged SARS-CoV-2 V such as BZ/2021 are a major concern because of their high
219 prevalence and increased frequency of transmission^{14,28}. Ferrets infected with BZ/2021 did not develop
220 more severe clinical signs than after infection with WA1/2020 and the virus did not advance to the lower

221 respiratory tract, indicating that pathogenicity of this VoC is not fundamentally different from that of
222 WA1/2020 in this model. Peak viral burden and shed virus loads of BZ/2021-infected ferrets were
223 equivalent to those of WA1/2020. Reflecting that analysis of circulating SARS-CoV-2 VoC worldwide
224 has yielded no evidence of widespread transmission of remdesivir-resistant strains^{19,20,29}, GS-621763
225 and its metabolite GS-441524 were comparably potent against CA/2020, SA/2020, and BZ/2021 as
226 against WA1/2020 in cell culture. Importantly, oral GS-621763 was highly efficacious against BZ/2021
227 *in vivo*, reducing infectious titers to near-undetectable levels rapidly after treatment initiation. Whole
228 genome sequencing of GS-621763-experienced viruses recovered from treated ferrets likewise revealed
229 no allele variations in candidate remdesivir resistance sites, indicating that SARS-CoV-2 does not
230 rapidly escape from inhibition by GS-621763 *in vivo*.

231 Based on the high transmissibility of BZ/2021 in the field and very efficient direct contact
232 transmission of SARS-CoV-2 between ferrets³⁰, we assessed the impact of oral GS-621763 on BZ/2021
233 spread among ferrets. To recapitulate what we consider a realistic scenario for exploring
234 pharmacological interference in community transmission, we therapeutically treated the infected source
235 animals but left their contacts untreated. In this relevant experimental setting, treatment with the same
236 GS-621763 dose of 10 mg/kg b.i.d. completely blocked transmission of BZ/2021, resembling the
237 performance of oral EIDD-2801/molnupiravir in the model⁶.

238 These results build confidence that a new generation of orally available broad-spectrum antivirals is
239 emerging that should allow initiation of treatment early after infection and promise to efficiently
240 interrupt community transmission chains. In addition to immediately contributing to ending the COVID-
241 19 pandemic and containing the continued evolution of increasingly contagious SARS-CoV-2 VoC³¹⁻³³,
242 an oral analog of the broad-spectrum antiviral drug remdesivir with confirmed *in vivo* efficacy such as
243 GS-621763 may become a cornerstone in the first-line defense against future pandemic threats.

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Fig. 1. Antiviral potency of remdesivir analog GS-621763. **a**, Schematic depicting metabolism of remdesivir (RDV) and GS-621763 after injection or oral uptake, respectively. Remdesivir distributes into tissues (e.g. lung) and is efficiently metabolized intracellularly to GS-443902. Conversely, GS-441524 is the dominant plasma metabolite after intestinal absorption of orally administered GS-621763 and is subsequently anabolized to GS-443902 in the tissues. **b-c**, Virus yield reduction of SARS-CoV-2 clinical isolates WA1/2020, CA/2020, SA/2020, and BZ/2021 representing the A, B.1.1.7 (α), B.1.351 (β) and P.1 (γ) lineages, respectively, by GS-621763 (b) and GS-441524 (c) on VeroE6 cells. EC₅₀ concentrations are specified. **d-e**, *In vitro* cytotoxicity profiles of GS-621763 (d) and GS-441524 (e) on

361 VeroE6, HEp-2, BHK-21, HCT-8 and a panel of primary HAE cells from independent donors (“F2”,
 362 “F3”, “M2”, “M6”, “DF2”). In (b-e), symbols represent individual biological repeats (n=3), error bars
 363 show standard deviations, lines depict non-linear regression models.
 364

365 **Fig. 2. Prophylactic efficacy of GS-621763.** a, Single-dose PK study in ferrets showing plasma
 366 concentrations of GS-441524, GS-621763, and remdesivir (RDV) as specified after dosing with GS-
 367 621763 (30 mg/kg; p.o.), remdesivir (10 mg/kg; i.v.), and GS-441524 (20 mg/kg; i.v.). Symbols
 368 represent individual biological repeats (n=3), error bars show standard deviations, lines depict sample
 369

370 means. **b**, Schematic of the prophylactic efficacy study design. Ferrets were infected intranasally with
371 1×10^5 pfu WA1/2020 (virus symbol). Groups (n=4) were gavaged b.i.d. (first aid symbol) with vehicle
372 or GS-621763 (20 mg/kg) starting at the time of infection. Nasal lavages (pipet symbol) were harvested
373 twice daily. All animals were terminated 4 days after infection. **c**, Virus titers from nasal lavages; LoD,
374 limit of detection. **d**, Temperature measurements collected once daily. **e**, Body weight measured once
375 daily. **f**, Infectious titers of SARS-CoV-2 in nasal turbinates harvested four days after infection. **g**,
376 SARS-CoV-2 RNA copies present in nasal lavages. **h**, SARS-CoV-2 RNA copies detected in nasal
377 turbinates. **i-j**, SARS-CoV-2 infectious particles (i) and SARS-CoV-2 RNA copies (j) in lungs four days
378 after infection. The number of independent biological repeats (individual animals) is shown in each
379 subpanel, symbols represent independent biological repeats, lines (c, d, e, g) and bar graphs (f, h-j)
380 connect or show samples mean, respectively, and P values are stated. 2-way ANOVA with Sidak's post-
381 hoc multiple comparison tests (c, d, e, g) or two tailed t-test (f, h).

Fig. 3. Therapeutic efficacy of GS-621763. **a**, Schematic of the therapeutic efficacy study design.

Ferrets were infected intranasally with 1×10⁵ pfu WA1/2020. Symbols as described for Fig. 2a. Starting 12 hours after infection, groups of ferrets (n=4) were gavaged b.i.d. with vehicle GS-621763 (3 mg/kg (purple first aid symbol) or 10 mg/kg (pink first aid symbol)), or treated with EIDD-2801 (5 mg/kg (green first aid symbol)). Nasal lavages were harvested twice daily. Animals were terminated 4 days after infection. **b**, Virus titers from nasal lavages. **c**, Infectious titers of SARS-CoV-2 in nasal turbinates harvested four days after infection. **d**, Temperature measurements collected once daily. **e**, Body weight measured once daily. **f**, SARS-CoV-2 RNA copies present in nasal lavages. **g**, SARS-CoV-2 RNA

391 copies detected in nasal turbinates. The number of independent biological repeats (individual animals) is
392 shown in each subpanel. Symbols represent independent biological repeats, lines (b, d, e, f) and bar
393 graphs (c, g) connect or show samples mean, respectively, and P values are stated. 1-way (c, g) or 2-way
394 (b, d, e, f) ANOVA with Dunnett's post-hoc multiple comparison tests.

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Fig. 4. GS-621763 blocks replication and transmission of SARS-CoV-2 VoC BZ/2021. **a**, Schematic of the efficacy and contact transmission study design. All ferrets were infected intranasally with 1×10^5 pfu BZ/2021 (virus symbol). Symbols as described for Fig. 2a. Groups of ferrets (n=4) were gavaged b.i.d. with vehicle or GS-621763 (10 mg/kg) starting at 12 hours after infection. Nasal lavages were harvested twice daily. Source ferrets were terminated 4 days after infection and all contact animals were terminated on study day 8. **b**, Virus titers from nasal lavages. **c**, SARS-CoV-2 RNA copies present in nasal lavages. **d**, Infectious titers of SARS-CoV-2 in nasal turbinates harvested four days after infection. **e**, SARS-CoV-2 RNA copies detected in nasal turbinates. **f**, Infectious titers of SARS-CoV-2 in lung tissue. **g**, SARS-CoV-2 RNA copies present in lung tissue. In (b-g), the number of independent biological repeats (individual animals) is shown in each subpanel. Symbols represent independent biological repeats, lines (b, c) and bar graphs (d, e, f, g) connect or show sample means, respectively, and P values are stated. 1-way (d, e) or 2-way (b, c) ANOVA with Tukey's (d, e) or Sidak's (b, c) post-hoc multiple comparison tests. **h**, Metagenome sequence analysis of inoculum WA1/2020 and BZ/2021 viruses, virus populations extracted from ferret nasal turbinates four days after infection, and BZ/2021 populations extracted from nasal lavages of contacts of vehicle-treated source animals. Relative allele frequencies of signature residues are shown. Symbols represent independent biological repeats (virus population of individual animals), columns show group means.

Methods

Study design

Ferrets (*Mustela putorius furo*) were used as an *in vivo* model to determine efficacy of the orally available remdesivir analog GS-621763 against SARS-CoV-2 infection and transmission to untreated and uninfected contact animals. Oral bioavailability and pharmacokinetic properties of GS-621763 in

419 ferrets were assessed prior to infecting any animals with SARS-CoV-2. For efficacy and transmission
420 studies, anesthetized ferrets were inoculated intranasally with SARS-CoV-2. Nasal lavages were
421 performed periodically at predefined time points to measure virus load. Temperature and body weight
422 were measured once daily for all animals. At four days after infection (study day 8 for contact ferrets),
423 animals were euthanized and nasal turbinates extracted to measure virus load in the upper respiratory
424 tract. SARS-CoV-2 titers were determined by plaque assay and viral RNA copies quantified by RT-
425 qPCR.

426 **Cells and viruses**

427 African green monkey kidney VeroE6 (ATCC®, cat# CRL-1586™), human lung adenocarcinoma
428 epithelial Calu-3 (ATCC® HTB-55™), human epithelial/HeLa contaminant HEP-2 (ATCC®, cat#
429 CCL-23™), and baby hamster kidney BHK-21 (ATCC®, cat# CCL-10™) cells were cultivated in a
430 humidified chamber at 37°C and 5% CO₂ in Dulbecco's Modified Eagle's medium (DMEM) (Corning,
431 cat# 10-013-CV, lot# 05721000) supplemented with 7.5% (10% for Calu-3) heat-inactivated fetal
432 bovine serum (FBS) (Corning, cat# 35-010-CV, lot# 14020001). Human epithelial colon
433 adenocarcinoma HCT-8 cells (ATCC® cat# CCL-244™ lot# 70036111) were cultivated at 37°C and 5%
434 CO₂ in Roswell Park Memorial Institute (RPMI) medium (Quality biological, cat# 112-024-101, lot#
435 723411) supplemented with 2 mM L-glutamine (Gibco, cat# 23030-081) and 10% heat-inactivated FBS.

436 A549-hACE2 cells that stably express human angiotensin-converting enzyme 2 (hACE2) were
437 grown in the culture medium supplemented with 10 µg/mL Blasticidin S. Primary human airway
438 epithelial (HAE) cells from multiple donors were cultivated at 37°C and 5% CO₂ in Bronchial Epithelial
439 Cell Growth Medium (BEGM) BulletKit following the provider's instructions (Lonza, cat# CC-3171
440 lot# 0000889952 with supplement cat# CC-4175 lot# 0000848033). Human Bronchial Tracheal
441 Epithelial cells (HBTEC) were derived from the following donors: "F2" from a 29-year old Caucasian

female (Lifeline, cat# FC-0035, lot# 5101); “F3” from a 42-year old Caucasian female (Lonza, cat# CC-2540S, lot# 0000519670); “M2” from a 40-year old Caucasian male (Lonza, cat# CC-2540S, lot# 0000667744); and “M6” from a 48-year old Caucasian male (Lonza, cat# CC-2540S, lot# 0000544414). Diseased (Asthma) Human Bronchial Epithelial (DHBE) cells “DF2” were from a 55-year old Caucasian female (Lonza, cat# 00194911S, lot# 0000534647). Primary HAE were used for cytotoxicity assays at passage ≤ 3 . Cell lines were routinely checked for mycoplasma and bacterial contamination.

SARS-CoV-2 strains were obtained from BEI and propagated using Calu-3 cells supplemented with 2% FBS in accordance with approved biosafety level 3 protocols. Virus stocks were stored at -80°C . Stock virus titers were determined by plaque assay and stocks authenticated through metagenomic sequencing.

Plaque assays

Vero E6 cells were seeded in 12-well plates at 3×10^5 cells per well. The following day, samples were serially diluted in DMEM containing Antibiotic-Antimycotic (Gibco) supplemented with 2% FBS. Dilutions were then added to cells and incubated for 1 hour at 37°C . Cells were subsequently overlaid with 1.2% Avicel 581-NF (FMC BioPolymer) in DMEM containing Antibiotic-Antimycotic (Gibco) and allowed to incubate for 3 days at 37°C with 5% CO_2 . After 3 days, the overlay was removed, cells were washed once with phosphate buffered saline (PBS) and fixed with neutral buffered formalin (10%) for 15 minutes. Plaques were then visualized using 1% crystal violet followed by washing with water.

Compound sources and chemical synthesis

Remdesivir was either purchased from MedChemExpress (cat# HY-104077, batch# 46182) or synthesized at Gilead Sciences, Inc. GS-441524 was either purchased from MedChemExpress (cat# HY-103586, batch# 62110) or synthesized at Gilead Sciences Inc. GS-621763 was synthesized at Gilead

464 Sciences Inc. All EIDD-2801/molnupiravir used in this study was provided by Gilead Sciences Inc.,
465 sourced from MedChemExpress.

466 **Cytotoxicity assays**

467 7,500 cells were seeded in each well of 96-well plates (Corning, cat# 3598). Cells were incubated
468 with 3-fold serial dilutions of compound from a 100 μ M maximum concentration. Each plate included 4
469 wells of positive (100 μ M cycloheximide (Millipore Sigma, cat# C7698-5G)) and negative (vehicle
470 (0.2% dimethyl sulfoxide (DMSO)) controls for normalization. Plates were incubated in a humidified
471 chamber at 37°C and 5% CO₂ for 72 hours. PrestoBlue™ Cell Viability Reagent (ThermoFisher
472 Scientific, cat# A13262) was added in each well (10 μ L/well) and fluorescence recorded on a Synergy
473 H1 multimode microplate reader (BioTek) after 1-hour incubation (excitation 560 nm, emission 590
474 nm). Raw data was normalized with the formula: % cell viability = $100 \times (\text{signal sample} - \text{signal positive control}) / (\text{signal negative control} - \text{signal positive control})$. 50% cytotoxic concentrations (CC₅₀) and 95
475 % confidence intervals after non-linear regression were determined using the inhibitor vs normalized
476 response equation in Prism 9.1.0 for MacOS (GraphPad). For cytotoxicity assays in A549-hACE2 cells,
477 compounds (200 nL) were spotted onto 384-well plates prior to seeding 5000 A549-hACE2 cells/well in
478 a volume of 40 μ L culture medium. The plates were incubated at 37°C for 48 hours with 5% CO₂. On
479 day 2, 40 μ L of CellTiter-Glo (Promega) was added and mixed 5 times. Plates were read for
480 luminescence on an Envision (PerkinElmer) and CC₅₀ values calculated using a nonlinear four
481 parameter regression model.
482

483 **Virus yield reduction**

484 2×10^5 VeroE6 cells were seeded per well in 12-well plates 16 hours before infection. Confluent
485 monolayers were then infected with the indicated virus at a multiplicity of infection (MOI) of 0.1
486 pfu/cell for 1 hour at 37°C with frequent rocking. Inoculum was removed and replaced with 1 mL of

487 DMEM with 2% FBS and the indicated concentration of compound. Cells were incubated at 37°C and
488 5% CO₂ for 48 hours. Supernatant were harvested, aliquoted and stored at -80°C before being analyzed
489 by plaque assay.

490 **Reporter virus assays**

491 A549-hACE2 cells (12,000 cells per well in medium containing 2% FBS) were plated into a white
492 clear-bottomed 96-well plate (Corning) at a volume of 50 µl. On the next day, compounds were added
493 directly to cultures as 3-fold serial dilutions with a Tecan D300e digital liquid dispenser, with DMSO
494 volumes normalized to that of the highest compound concentration (final DMSO concentration <0.1%).
495 The diluted compound solutions were mixed with 50 µl of SARS-CoV-2-Nluc (MOI 0.025 pfu/cell),
496 expressing a nano luciferase reporter protein (kind gift of Xuping Xie and Pei-Yong Shi (University of
497 Texas Medical Branch; Galveston, TX), were added. At 48 h post-infection, 75 µl Nano luciferase
498 substrate solution (Promega) was added to each well. Luciferase signals were measured using an
499 Envision microplate reader (Perkin Elmer). The relative luciferase signals were calculated by
500 normalizing the luciferase signals of the compound-treated groups to that of the DMSO-treated groups
501 (set as 100%). EC₅₀ values were calculated using a nonlinear four parameter variable slope regression
502 model.

503 **Pharmacokinetics**

504 Female ferrets were either intravenously administered 10 mg/kg remdesivir as a 30-minute infusion or
505 orally administered 30 mg/kg GS-621763, after which plasma was isolated at 7 to 9 timepoints post
506 administration. Plasma samples underwent methanol protein precipitation followed by centrifugation.
507 Resulting supernatants were isolated, evaporated to dryness under nitrogen and reconstituted with 5%
508 acetonitrile for injection onto an LC-MS/MS system. Concentrations of remdesivir, GS-621763 and GS-
509 441524 were determined using 9-point calibration curves spanning at least 3 orders of magnitude, with

quality control samples to ensure accuracy and precision, prepared in normal ferret plasma. Analytes were separated by a 50 × 3.0 mm, 2.55 µm Synergi Polar-RP 30A column (Phenomenex, Inc.) using a mobile phase A consisting of 10 mM ammonium formate with 0.1% formic acid and a mobile phase B consisting of 0.1% formic acid in acetonitrile. A multi-stage linear gradient from 5% to 95% mobile phase B at a flow rate of 1 mL/min was employed for analyte separation. Pharmacokinetic parameters were calculated using Phoenix WinNonlin (version 8.2, Certara) and concentration-time profiles generated using Prism (version 8, GraphPad).

Ferret efficacy studies

Female ferrets (6-10 months old, *Mustela putorius furo*) were purchased from Triple F Farms. Ferrets were rested for 7 days after arrival. Ferrets were then housed individually or in groups of 2 in ventilated negative-pressure cages in an ABSL-3 facility. Based on previous experiments⁶, ferrets were randomly assigned to groups (n=4) and used as an *in vivo* model to examine the efficacy of orally administered compounds against SARS-CoV-2 infection. No blinding of investigators was performed. Ferrets were anesthetized using dexmedetomidine/ketamine and infected intranasally with 1×10⁵ pfu 2019-nCoV/USA-WA1/2020 in 1 mL (0.5 mL per nare). Body weight and temperature were measured once daily. Nasal lavages were performed twice daily using 1 mL sterile PBS (containing Antibiotic-Antimycotic (Gibco)). Nasal lavage samples were stored at -80°C until virus titration could be performed by plaque assay. Treatment (once daily (q.d.) or twice daily (b.i.d.)) was initiated at either 0 or 12 hours after infection and continued until 4 days post infection with either vehicle (2.5% dimethyl sulfoxide; 10% Kolliphor HS-15; 10% Labrasol; 2.5% propylene glycol; 75% water) or compound. Four days after infection, ferrets were euthanized, and tissues and organs were harvested and stored at -80°C until processed.

Contact transmission in ferrets

533 Eight ferrets were anesthetized and inoculated intranasally with 1×10^5 pfu of hCoV-19/Japan/TY7-
534 503/2021. Twelve hours after infection, ferrets were split into two groups (n=4; 2 ferrets per cage) and
535 treated with vehicle or GS-621763 (10 mg kg⁻¹) twice daily (b.i.d.) via oral gavage. At 54 hours after
536 infection, uninfected and untreated contact ferrets (two contacts for GS-621763; three contacts for
537 vehicle) were co-housed with source ferrets. Co-housing was continued until 96 hours after infection
538 and source ferrets were euthanized. Contact ferrets were housed individually and monitored for an
539 additional 4 days after separation from source ferrets and subsequently euthanized. Nasal lavages were
540 performed on all source ferrets every 12 hours and all contact ferrets every 24 hours. For all ferrets,
541 nasal turbinates and lung tissues were harvested to determine viral titers and the detection of viral RNA.

542 **SARS-CoV-2 titration in tissue extracts**

543 Selected tissues were weighed and mechanically homogenized in sterile PBS. Homogenates were
544 clarified by centrifugation (2,000×g) for 5 minutes at 4°C. Clarified homogenates were then serially
545 diluted and used in plaque assays to determine virus titer as described above.

546 **Quantitation of SARS-CoV-2 RNA copy numbers**

547 To probe viral RNA in selected tissues, samples were harvested and stored in RNeasy lysis buffer at -80°C.
548 Total RNA from tissues was isolated using a RNeasy mini kit (Qiagen), in accordance with the
549 manufacturer's protocol. For nasal lavage samples, total RNA was extracted using a ZR viral RNA kit
550 (Zymo Research) in accordance with the manufacturer's protocol. SARS-CoV-2 RNA was detected as
551 previously described⁶ using the nCoV_IP2 primer-probe set (National Reference Center for Respiratory
552 Viruses, Pasteur Institute). An Applied Biosystems 7500 using the StepOnePlus real-time PCR system
553 was used to perform RT-qPCR reactions. The nCoV_IP2 primer-probe set was used in combination
554 with TaqMan fast virus 1-step master mix (Thermo Fisher Scientific) to detect viral RNA. SARS-CoV-2
555 RNA copy numbers were calculated using a standard curve created from serial dilutions of a PCR

556 fragment (12669-14146 nt of the SARS-CoV-2 genome), as previously described⁶. For RNA copies in
557 tissue samples, RNA copies were normalized to the weights of the tissues used.

558 **Next generation sequencing**

559 To authenticate virus stocks, metagenomic sequencing was performed as described^{34,35}, while to
560 sequence lower viral load *in vivo* samples the COVID-Seq (Illumina) amplicon tiling protocol was used.
561 For metagenomic sequencing, viral RNA was treated with Turbo DNase I (Thermo Fisher), converted to
562 cDNA using random hexamers and SuperScript IV reverse transcriptase, and double stranded cDNA
563 created using Sequenase v2.0. Sequencing libraries for both sets of libraries were generated using
564 Nextera Flex (illumina) and cleaned using 0.8×Ampure XP beads and pooled equimolarly before
565 sequencing on an Illumina 1×100 bp NextSeq2000 run. Raw fastq reads were adapter- and quality-
566 trimmed with Trimmomatic v0.39³⁶. To interrogate potential resistance alleles, reference-based
567 mapping to NC_045512.2 was carried out using our modified Longitudinal Analysis of Viral Alleles
568 (LAVA - <https://github.com/michellejlin/lava>)³⁷ pipeline. LAVA constructs a candidate reference
569 genome from early passage virus using bwa³⁸, removes PCR duplicates with Picard, calls variants with
570 VarScan^{39,40}, and converts these changes into amino acid changes with Annovar⁴¹. Consensus sequences
571 were called with TAYLOR³⁴ and deposited in NCBI GenBank. Accession numbers (supplementary
572 Table 4) are as follows: input strain WA1/2020, MZ433205; WA1/2020 recovered from ferrets,
573 MZ433206 - MZ433213; input strain BZ/2021, MZ433225; BZ/2021 recovered from source ferrets,
574 MZ433214 - MZ433221; BZ/2021 recovered from contacts of vehicle-treated source ferrets, MZ433222
575 - MZ433224. Raw reads for these sequences are publicly available on SRA (BioProject PRJNA740065).

576 **Ethics statement**

577 All *in vivo* efficacy studies were conducted at Georgia State University in compliance with the
578 Animal Welfare Act Code of Federal Regulations and the Guide for the Care and Use of Laboratory

579 Animals of the National Institutes of Health. All studies involving SARS-CoV-2 infected ferrets were
580 approved by the Georgia State Institutional Animal Care and Use Committee under protocol A20031.
581 Experiments at Georgia State University using infectious SARS-CoV-2 were performed in BSL-
582 3/ABSL-3 facilities at Georgia State University and approved by the Georgia State Institutional
583 Biosafety Committee under protocol B20016. Experiments at Gilead Sciences, Inc. using infectious
584 SARS-CoV-2 were performed in a BSL-3 facility and approved by an institutional biosafety committee.

585 **Statistics and reproducibility**

586 The Microsoft Excel (version 16.48), GraphPad Prism (version 9.1.0), and Numbers (version 10.1)
587 software packages were used for data collection and analysis. One-way or two-way ANOVA with
588 Dunnett's or Tukey's multiple comparisons post-hoc test were used to evaluate statistical significance
589 when comparing more than two groups or two independent variables. When comparing two variables, a
590 two-tailed unpaired t-test was performed to determine statistical significance. The specific statistical test
591 used to individual studies is specified in the figure legends. RT-qPCR data were collected and analyzed
592 using the StepOnePlus (version 2.1; Applied Biosystems) software package. Final figures were
593 assembled in Adobe Illustrator (version CS6). All numerical raw data and summaries of individual
594 statistical analyses are provided in supplementary datasets 3 and 4. Effect sizes between groups in the
595 ANOVAs were calculated as $\eta^2 = (SS_{\text{effect}}) / (SS_{\text{total}})$ for one-way ANOVA and $\omega^2 = (SS_{\text{effect}} -$
596 $(df_{\text{effect}})(MS_{\text{error}})) / MS_{\text{error}} + SS_{\text{total}}$ for two-way ANOVA (SS_{effect} , sum of squares for the effect; SS_{total} ,
597 sum of squares for total; df_{effect} , degrees of freedom for the effect; MS_{error} , mean squared error). The
598 statistical significance level α was set to <0.05 for all experiments. Exact P values are shown in the
599 individual graphs.

600

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606

607 **Author Contributions**

608 J. P. B. and R.K.P. coordinated the study. R.M.C. and J.S. performed virus-stock preparations and
609 virus titrations. R.M.C., J.D.W., C.M.L., and R.K.P. performed animal inoculations, nasal lavage
610 sampling, animal necropsies, and/or titration of virus from ferrets R.M.C. extracted RNA from all
611 animal samples and performed all RT-qPCR experiments and analyses. J.S. performed virus reduction
612 assays and cytotoxicity assessments. D.B. coordinated pharmacokinetics studies in ferrets. K.B.
613 prepared compound formulations. V.D.P., J.P.B., and J.C. performed reporter virus and cytotoxicity
614 assays in ACE2-A549 cells. R.K, K.C, and R.L.M. synthesized and sourced compounds. M.J.L. and
615 A.L.G. performed all next-generation sequencing and analyses. R.M.C. created all figure schematics.
616 R.M.C., J.S., J.P.B., and R.K.P. were responsible for experimental design, data analysis, and data
617 presentation. R.M.C., J.D.W., C.M.L., J.S., D.B., L.S.M., A.L.G., R.L.M., J.P.B., and T.C. edited the
618 manuscript. J.P.B. and R.K.P. conceived and designed the study. R.K.P. wrote the manuscript.

619

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Competing Interests Declaration

All authors affiliated with Gilead Sciences may hold stock or stock options in Gilead Sciences Inc. R.K.P. was the principal investigator of a Gilead-sponsored research agreement with Georgia State University. He has received funding from Gilead Sciences Inc. to support parts of this work. All other authors declare no competing interests.

Additional Information

Supplementary Information

Supplementary Information is available for this paper.

Correspondence

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Data Availability

All data generated and analyzed during this study are included in this published article (and its supplementary information files). Source data and statistical analyses for figures 1-4, extended data tables 1-3 and extended data figures 1-2 are provided with the paper in supplementary datasets 3-4.

Code Availability

This study does not use proprietary codes. All computer codes and algorithms used are specified in the Methods section.

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648 **Extended Data Table 1. Antiviral potency and cytotoxicity.**

virus	host cells	GS-621763		GS-441524		remdesivir	
		EC ₅₀ [μM]	CC ₅₀ [μM]	EC ₅₀ [μM]	CC ₅₀ [μM]	EC ₅₀ [μM]	CC ₅₀ [μM]
WA1/2020-nano luciferase	A549-ACE2	0.98 ^a	>50 ^b	1.4 ± 0.9 ^c	>50 ^b	0.075 ± 0.03 ^d	17.3 ± 4.9 ^e
WA1/2020 ^f	VeroE6	0.73	>100	0.68	>100	n.d.	>100
CA/2020 (α) ^f	VeroE6	0.21	>100	0.11	>100	n.d.	>100
SA/2020 (β) ^f	VeroE6	0.11	>100	0.34	>100	n.d.	>100
BZ/2021 (γ) ^f	VeroE6	0.22	>100	0.55	>100	n.d.	>100
n.a. ^f	HEp-2	n.a.	79.4	n.a.	>100	n.a.	45.12
n.a. ^f	VeroE6	n.a.	>100	n.a.	>100	n.a.	>100
n.a. ^f	BHK-21	n.a.	>100	n.a.	>100	n.a.	>100
n.a. ^f	HCT-8	n.a.	74.6	n.a.	>100	n.a.	36.4
n.a. ^f	"F2" HAE	n.a.	43.8	n.a.	>100	n.a.	85.5
n.a. ^f	"F3" HAE	n.a.	43.8	n.a.	>100	n.a.	104.4
n.a. ^f	"M2" HAE	n.a.	39.7	n.a.	>100	n.a.	101.9
n.a. ^f	"M6" HAE	n.a.	92.3	n.a.	>100	n.a.	>100
n.a. ^f	"DF2" HAE	n.a.	86	n.a.	>100	n.a.	>33

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650 ^amean (n=2); data represent mean of two independent experiments, each with technical duplicates.

651 ^bmean (n=2); data represent mean of two independent experiments, each with technical triplicates or
652 quadruplicates.

653 ^cmean ± SD (n=8); data represent mean of eight independent experiments, each with technical
654 duplicates.

655 ^dmean ± SD (n=12); data represent mean of twelve independent experiments, each with technical
656 duplicates.

657 ^emean ± SD (n=6); data represent mean of six independent experiments, each with technical triplicates
658 or quadruplicates.

659 ^fmean (n=3); all other data represent mean of three independent experiments.

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661 **Extended Data Table 2. Single dose pharmacokinetic parameters of GS-441524 following**
662 **administration of either intravenous GS-441524 or remdesivir or oral GS-621763 in ferrets.**

compound	route	dose [mg/kg]	t _{1/2} [hours]	IV – CL [L/hours/kg] PO – T _{max} [hours]	C _{max} [μM]	AUC _{last} [μM.h]	F [%]
GS-441524	i.v.	20	3.4	0.86	54.2	81.1	n/a
remdesivir	i.v.	10	6.09	n/a	2.81	18.2	n/a
GS-621763 ^a	p.o.	30	2.68 ± 0.15	4.0 ± 3.5	15.8 ± 4.7	80.8 ± 14.6	115 ± 21

^aapproximately 10 nM GS-621763 transiently observed in first two hours

Extended Data Table 3. GS-441524 and its metabolite concentrations in ferret lung tissue.

compound	route	dose	lung GS-443902 [nmol/g]	lung total nuc [nmol/g]
GS-441524	i.v.	20 mg kg ⁻¹	0.53 ± 0.10	0.66 ± 0.21
remdesivir	i.v.	10 mg kg ⁻¹	1.28 ^a	2.96
GS-621763	p.o.	30 mg kg ⁻¹	0.30 ± 0.19	0.88 ± 0.13

^a one lung from remdesivir i.v. dosing was BLQ for all metabolites

Extended Data Fig. 1. Remdesivir cytotoxicity. *In vitro* cytotoxicity profile of remdesivir on HEp-2, VeroE6, BHK-21, HCT-8 and the panel of primary HAE cells (“F2”, “F3”, “M2”, “M6”, “DF2”) cells examined in (Fig. 2b-e). Symbols represent individual biological repeats (n=3), error bars show standard deviations, lines depict non-linear regression models.

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Extended Data Fig. 2. Clinical signs in source and contact animals infected with BZ/2021. a,

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Temperature measurements collected once daily. b, Body weight measured once daily.

Supplementary Files

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- [Supplementarydataset2.html](#)