Pharmacokinetics of Orally Administered GS-441524 in Dogs

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Abstract.

Despite being FDA-approved for COVID-19, the clinical efficacy of remdesivir (Veklury®) remains contentious. We previously pointed out pharmacokinetic, pharmacodynamic and toxicology reasons for why its parent nucleoside GS-441524, is better suited for COVID-19 treatment. Here, we assess the oral bioavailability of GS-441524 in beagle dogs and show that plasma concentrations ~24-fold higher than the EC_{50} against SARS-CoV-2 are easily and safely sustained. These data support translation of GS-441524 as an oral agent for COVID-19.

Remdesivir (RDV) is currently the only FDA-approved anti-viral drug for the treatment of COVID-19 despite exhibiting just modest efficacy in one double-blind, placebo-controlled randomized clinical trial (RCT) (1); other RCTs have thus far found no statistically significant improvement in mortality (2) or time to clinical improvement (3). As a phosphoramidate prodrug of the McGuigan class (4), RDV is structurally susceptible to conversion to its active nucleoside triphosphate (NTP; GS-443902) form by enzymes that are abundant in the liver (CES1/CTSA/HINT1) but minimally expressed in alveolar type 2 cells (AT2) (5), the cell type most susceptible to SARS-CoV-2 infection (6). Preferential liver metabolism of RDV results in on-target dose limiting toxicity (DLT) that precludes dose escalation despite its modest clinical performance (7, 8). Such shortcomings are exacerbated by the hydrophobic nature of RDV, which requires complex excipients that could implicate kidney function (9, 10). Another major drawback with RDV is its requisite intravenous (IV) administration (11), making outpatient therapy, early intervention, and prophylaxis impractical.

Cognizant of these limitations, we have asserted that the parent nucleoside of RDV, GS-441524, would be better suited for the treatment for COVID-19 (5). GS-441524 is the persistent, predominant metabolite in plasma following IV infusion of RDV in preclinical species (12–14) and in humans with a half-life ($T_{1/2}$) of >24 h (8, 15). The extent to which GS-441524 contributes to the overall anti-SARS-CoV-2 activity of RDV when administered to patients remains unclear. In contrast to RDV, which is preferentially bioconverted to GS-443902 by liver-abundant enzymes (16, 17), GS-441524 is bioconverted to GS-443902 by nucleoside kinases (likely adenosine kinase,

ADK) that are broadly expressed across all tissues. Due to its demonstrably better safety profile (7, 18), a significant advantage that GS-441524 possesses over RDV is the possibility for dose escalation without liver-related DLTs, as this would increase the concentration of bioactive GS-443902 in AT2 cells. Cell-based studies have shown that GS-441524 is a potent inhibitor of SARS-CoV-2-infected cells, with EC_{50} values on the same order of magnitude as that of RDV (EC_{50}= 0.47-1.09 µM) (19). Efficacy studies conducted in cats with natural presentations feline infectious peritonitis (FIP) as a result of infection by the closely related feline coronavirus (FCoV) have demonstrated up to 96% cure rate with subcutaneously (SC) administered GS-441524 (20–22). Efficacy studies conducted in mice infected with either SARS-CoV-2 or murine hepatitis virus (MHV, a closely related coronavirus) have shown that GS-441524 is capable of reducing viral loads in pathologically relevant organs without obvious adverse events (23). Given the scarcity of simple outpatient treatment options for COVID-19 (24), these especially encouraging data warrant translation of GS-441524 to the clinic. Here, we provide pharmacokinetic (PK) evidence in dogs supporting the rationale for GS-441524 to be investigated as an oral agent for COVID-19.

To validate our assertion that McGuigan prodrugs such as RDV are heavily subject to first-pass metabolism, we first conducted a single-dose, equimolar comparison between orally (PO) administered GS-441524 (6.5 mg/kg) and RDV (13 mg/kg) in dogs. Male beagles (N=1 per group) were administered excipient-less capsule formulations of either GS-441524 or RDV and plasma concentrations of GS-441524 were evaluated at predetermined timepoints (**Figure 1a**). No adverse events were observed in either

dosing group. As expected, plasma concentrations of GS-441524 following administration of RDV were poor, with C_{max} values roughly 25-fold lower than that observed when GS-441524 was administered directly (172 vs. 4580 ng/mL, respectively; **Figure 1a, c, Supplementary Data File 1**). Interestingly, there was an observable difference in T_{max} values, with plasma concentrations of GS-441524 peaking at approximately 3 h following RDV administration versus 1 h following direct dosing of GS-441524. This shift in T_{max} values with RDV PO administration suggests a mechanism of systemic release similar to that observed for the McGuigan prodrug sofosbuvir, wherein rapid hepatic extraction of intact prodrug forms a reservoir of active NTP and hydrolyzed nucleoside—the latter of which is then slowly released into systemic circulation (25). Given that plasma concentrations of GS-441524 following RDV administration are below the range of reported anti-SARS-CoV-2 EC_{50} values and that long-term PO dosing of RDV at 13 mg/kg is almost certainly therapeutically prohibitive in humans due to hepatotoxicity concerns (8), these data allude to the infeasibility of administering RDV PO for COVID-19. At the same time, we find that direct PO administration of GS-441524 results in plasma concentration exceeding the range of reported anti-SARS-CoV-2 EC_{50} values for at least 8 h (**Figure 1a, Supplementary Data File 1**). Peak concentrations of GS-441524 reached 15.45 µM and were obtained at approximately 1 h (**Figure 1a, c**), indicating high oral absorption of GS-441524 in dog even in the absence of excipients.

Prior studies assessing the oral bioavailability (F%) of GS-441524 in dogs following both IV (2 mg/kg) and PO (5 mg/kg) administration have found that the drug is efficiently

absorbed, with an F% of 85% (NCATS OpenData Portal). Such studies examined PO absorption of GS-441524 using a solution formulation prepared at a final concentration of 2.5 mg/mL. We sought to determine whether similarly favorable F% could be achieved using an excipient-less capsule formulation, which would greatly ease outpatient administration. The wide range of F% observed in other preclinical species (**Table 1**; NCATS OpenData Portal) and the unusual solubility properties of GS-441524 are reminiscent of that observed with the FDA-approved nucleoside analogue acyclovir (**Table 1**), which was ultimately formulated as an excipient-less tablet (26). Direct comparison of PK parameters between solution and capsule formulations of GS-441524 indicates a similar pattern of high drug absorption, with T_{max} values of 0.5 and 1 h, respectively (**Figure 1b**). Between solution and capsule formulations, PK parameters were generally similar; it should be noted that the capsule dose was slightly higher than the solution dose (5 mg/kg vs. 6.5 mg/kg). Adjusting for sampling timeframes, these data indicate that the C_{max} value was higher with the solution formulation (5060 vs. 4580 ng/mL) but the AUC value were somewhat higher with the capsule formulation (17,916 vs.19,151 ng*h/mL; **Figure 1c**). Such observations appear consistent with the general observation that liquid formulations tend to be more readily absorbed than their pill counterparts (27). Nevertheless, the estimated F% using this capsule formulation remains high at about 76% (**Figure 1b**). Pharmacodynamic comparison GS-441524 and RDV corroborates the ability for GS-441524 but not RDV to be administered orally. Quantification of NTP formation in whole blood using a highly sensitive dried blood spot assay found that plasma concentrations of GS-441524 following direct administration was able to generate levels of GS-443902 in whole blood; in contrast, GS-441524

produced as a metabolite of orally administered remdesivir was not able to form levels of NTP above the lower limit of quantification (**Figure 1a, dashed line**). These data hint at the feasibility of using an excipient-less pill formulation for GS-441524 for outpatient treatment.

There are some limitations associated with this study. First, the sample size in the capsule study is small, which may not capture possible variability associated with this formulation. Second, nucleoside analogues generally tend to exhibit higher F% in dogs than in other preclinical species perhaps due to the presence of a paracellular nucleoside transporter that is absent in humans and non-human primates (28). As a result, the F% of nucleoside analogues in dogs tends to overestimate that observed in humans (**Supplementary Data File 2**). While not specifically explored in this study, it should be noted that—at the other end of the F% spectrum—F% of nucleoside analogues in non-human primates tend to under-predict that observed in humans (**Supplementary Data File 3**). Nevertheless, these data suggest the feasibility of using a simple, excipient-less capsule formulation of GS-441524. As a prodrug inhibitor of the SARS-CoV-2 RNA-dependent RNA polymerase (RdRp), GS-441524 is aptly poised to demonstrate consistent efficacy among new mutations of SARS-CoV-2, as RdRp is much less susceptible to efficacy-altering mutations than is the spike protein (29, 30). Thus, clinical translation of GS-441524 is imperative to human health.

Drug formulation.

For capsule studies, GS-441524 and RDV were purchased at the highest commercially available quality from MedKoo Biosciences; purity was verified by ultra-performance liquid chromatography mass spectrometry (UPLC-MS) and nuclear magnetic resonance (NMR) spectroscopy (^{1}H, ^{13}C) in-house. For capsule studies, gelatin capsules (size 5, XPRS Nutra) were tightly packed with either GS-441524 (65 mg) or RDV (136.74 mg) without additional excipients. For solution studies, GS-441524 was purchased at the highest commercially available from AK Scientific and characterized by NCATS. Formulations for PO and IV studies conducted by NCATS are described on the NCATS OpenData Portal. Briefly, GS-441524 was dissolved in a solution containing 5% ethanol, 30% propylene glycol, 45% PEG-400, 20% water with 1 equivalent HCl for a final concentration of 2.5 mg/mL.

Single dose GS-441524 and RDV in dogs via capsule formulation.

All capsule form studies were performed at Charles River Laboratories (Wilmington, MA) with IACUC approval (#20236536). Fasted male adult beagles (10 kg; N=1 per compound) were administered either GS-441524 (6.5 mg/kg) or RDV (13 mg/kg). Plasma samples were taken for PK analysis at the following timepoints (h): -0.5, 0.5, 1, 3, 6, 8, 24. Animals were monitored continuously by veterinarians for any clinically relevant abnormalities during dosing and sample collection. PO solution and IV studies were performed by NCATS as described on the NCATS OpenData Portal with relevant committee and regulatory approval. Data from all studies were (re-)analyzed using PKSolver 2.0 and graphs were generated using GraphPad Prism 8.

Plasma pharmacokinetics

For capsule studies, plasma levels of GS-441524 were analyzed at Covance, Inc (Princeton, NJ) on a fee-for-service basis using a liquid chromatography mass spectrometry (LC-MS) assay previously described for quantification of GS-441524 following IV administration of RDV in NHP (12).

Quantification of GS-443902 in dried blood spots.

Analyses were performed at CU Anschutz adapted from previously published procedures (31–34). The assay range was 0.1 – 100 pmol/sample, with a lower limit of quantitation of 0.1 pmol/sample. A 7mm disc was punched from the DBS and extracted with two mL of 50:50 methanol:water of which, 1.6 mL was assayed (this was the sample). Results were then normalized to pmol per 7mm punch.

Author Contributions.

V.C.Y. and F.L.M. conceived of the study. V.C.Y. analyzed data and wrote the manuscript. C.D.P. prepared capsule formulations of GS-441524 and RDV. L.R.B., L.E.R., and P.L.A. performed pharmacodynamic analyses on dried blood spots. M.J.Y., A.J.Y., S.K., K.A., D.K.G., and J.J.A. provided technical assistance. V.C.Y., F.L.M. and C.L. oversaw the study.

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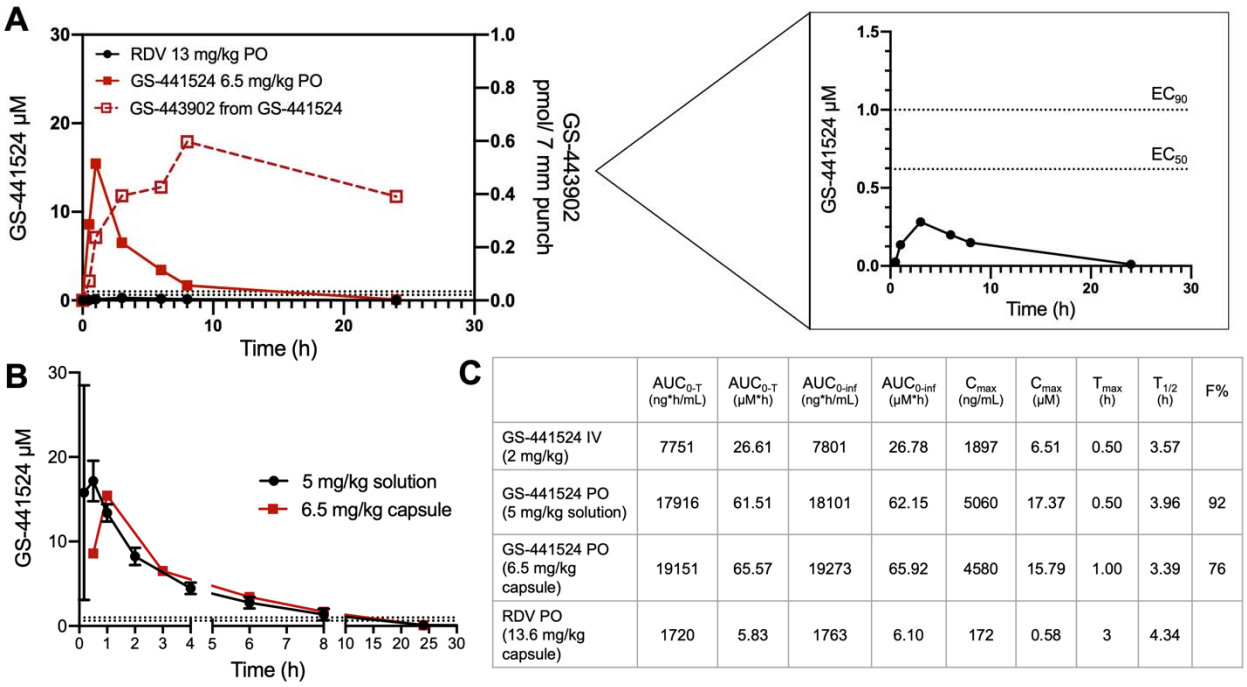
Conflicts of Interest

V.C.Y. is the CEO of Copycat Sciences, a company developing antiviral nucleoside analogues.



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	AUC_{0-T} (ng*h/mL)	AUC_{0-T} (µM*h)	AUC_{0-inf} (ng*h/mL)	AUC_{0-inf} (µM*h)	C_{max} (ng/mL)	C_{max} (µM)	T_{max} (h)	$T_{1/2}$ (h)	F%
GS-441524 IV (2 mg/kg)	7751	26.61	7801	26.78	1897	6.51	0.50	3.57	
GS-441524 PO (5 mg/kg solution)	17916	61.51	18101	62.15	5060	17.37	0.50	3.96	92
GS-441524 PO (6.5 mg/kg capsule)	19151	65.57	19273	65.92	4580	15.79	1.00	3.39	76
RDV PO (13.6 mg/kg capsule)	1720	5.83	1763	6.10	172	0.58	3	4.34	

Figure 1. Plasma concentrations of GS-441524 following a single oral dose of remdesivir or GS-441524 in dogs. (A) Head-to-head PK comparison following a single equimolar dose of remdesivir (black, 13.6 mg/kg) and GS-441524 (orange, 6.5 mg/kg) in male beagle dogs (N=1 per compound). Both compounds were administered in capsule form. Plasma concentrations of GS-441524 following compound administration are plotted for the following timepoints (h): 0.5, 1, 3, 6, 8, 24. A focused view of GS-441524 concentrations following oral administration of remdesivir is shown on the left. Dashed red line corresponds to the levels of GS-443902 (NTP) formed in whole blood

following oral administration of GS-441524 as quantified using a highly sensitive dried blood spot assay. Oral administration of RDV did not produce detectable levels of GS-443902 in whole blood. **(B)** Comparison of plasma concentrations of GS-441524 following oral administration as a solution (black, 5 mg/kg; N=3) or as a capsule (orange, 6.5 mg/kg; N=1). **(C)** Mean PK parameters following various routes of administration of GS-441524 and RDV. Raw values for GS-441524 dosed IV and PO dosed as a solution are adapted from NCATS OpenData Portal and have been re-calculated to match the sampling timeframe of the capsule studies (T=0.5-24 h). All PK parameters were calculated using PKSolver 2.0. In panels A and B, dotted lines correspond to EC_{50} (bottom) and EC_{90} (top) values reported for GS-441524 in SARS-CoV-2-infected Calu3 cells (19).

Species, F%	Acyclovir	GS-441524
Human	10-20	15-30*
Monkey	3.7	8.3
Dog	54-90	85-93

Table 1. Oral bioavailabilities of acyclovir and GS-441524 in preclinical species are similar. GS-441524 exhibits a similar pattern of F% as acyclovir across preclinical species. F% for GS-441524 were obtained from the NCATS OpenData Portal and F% for acyclovir were obtained from the FDA fact sheet on Zovirax (human), Laskin et al.

Clin. Pharm. (1983, monkey) (35) and Krasny et al. *J. Pharm. Exp. Ther.* (1981, dog) (36). *Anticipated F% of GS-441524 in humans.

References.

1. Beigel JH, Tomashek KM, Dodd LE, Mehta AK, Zingman BS, Kalil AC, Hohmann E, Chu HY, Luetkemeyer A, Kline S, Lopez de Castilla D, Finberg RW, Dierberg K, Tapson V, Hsieh L, Patterson TF, Paredes R, Sweeney DA, Short WR, Touloumi G, Lye DC, Ohmagari N, Oh M, Ruiz-Palacios GM, Benfield T, Fätkenheuer G, Kortepeter MG, Atmar RL, Creech CB, Lundgren J, Babiker AG, Pett S, Neaton JD, Burgess TH, Bonnett T, Green M, Makowski M, Osinusi A, Nayak S, Lane HC. 2020. Remdesivir for the Treatment of Covid-19 — Final Report. N Engl J Med https://doi.org/10.1056/NEJMoa2007764.
2. WHO Solidarity Trial Consortium. 2020. Repurposed Antiviral Drugs for Covid-19 - Interim WHO Solidarity Trial Results. N Engl J Med 1–15.
3. Wang Y, Zhang D, Du G, Du R, Zhao J, Jin Y, Fu S, Gao L, Cheng Z, Lu Q, Hu Y, Luo G, Wang K, Lu Y, Li H, Wang S, Ruan S, Yang C, Mei C, Wang Y, Ding D, Wu F, Tang X, Ye X, Ye Y, Liu B, Yang J, Yin W, Wang A, Fan G, Zhou F, Liu Z, Gu X, Xu J, Shang L, Zhang Y, Cao L, Guo T, Wan Y, Qin H, Jiang Y, Jaki T, Hayden FG, Horby PW, Cao B, Wang C. 2020. Remdesivir in adults with severe COVID-19: a randomised, double-blind, placebo-controlled, multicentre trial. Lancet 395:1569–1578.

4. Mcguigan C, Devine KG, O'oonnor TJ, Galpin SA, Jeffries DJ, Kinchington D. 1990. Synthesis and evaluation of some novel phosphoramidate derivatives of 3'-azido-3'-deoxythymidine (AZT) as anti-HIV compoundsAntiviral Chemistry & Chemotherapy.

5. Yan VC, Muller FL. 2020. Advantages of the Parent Nucleoside GS-441524 over Remdesivir for Covid-19 Treatment. ACS Med Chem Lett 11:1361–1366.

6. Schaefer I-M, Padera RF, Solomon IH, Kanjilal S, Hammer MM, Hornick JL, Sholl LM. 2020. In situ detection of SARS-CoV-2 in lungs and airways of patients with COVID-19. Mod Pathol 33:2104–2114.

7. Xu Y, Barauskas O, Kim C, Babusis D, Murakami E, Kornyeyev D, Lee G, Stepan G, Perron M, Bannister R, Schultz BE, Sakowicz R, Porter D, Cihlar T, Feng JY. 2020. Off-target In Vitro Profiling Demonstrates that Remdesivir Is a Highly Selective Antiviral Agent. Antimicrob Agents Chemother https://doi.org/10.1128/aac.02237-20.

8. Humeniuk R, Mathias A, Cao H, Osinusi A, Shen G, Chng E, Ling J, Vu A, German P. 2020. Safety, Tolerability, and Pharmacokinetics of Remdesivir, An Antiviral for Treatment of COVID-19, in Healthy Subjects. Clin Transl Sci cts.12840.

9. Yan VC, Muller FL. 2020. Captisol and GS-704277, but not GS-441524, are credible mediators of remdesivir's nephrotoxicity. Antimicrob Agents Chemother 64:01920–1920.

10. European Medicines Agency. 2020. Remdesivir: Summary on compassionate use EMA/178637/2020 - Rev. 1.

11. Food and Drug Administration. 2020. VEKLURY® (remdesivir).

12. Warren TK, Jordan R, Lo MK, Ray AS, Mackman RL, Soloveva V, Siegel D, Perron M, Bannister R, Hui HC, Larson N, Strickley R, Wells J, Stuthman KS, Van Tongeren SA, Garza NL, Donnelly G, Shurtleff AC, Retterer CJ, Gharaibeh D, Zamani R, Kenny T, Eaton BP, Grimes E, Welch LS, Gomba L, Wilhelmsen CL, Nichols DK, Nuss JE, Nagle ER, Kugelman JR, Palacios G, Doerffler E, Neville S, Carra E, Clarke MO, Zhang L, Lew W, Ross B, Wang Q, Chun K, Wolfe L, Babusis D, Park Y, Stray KM, Trancheva I, Feng JY, Barauskas O, Xu Y, Wong P, Braun MR, Flint M, McMullan LK, Chen S-S, Fearns R, Swaminathan S, Mayers DL, Spiropoulou CF, Lee WA, Nichol ST, Cihlar T, Bavari S. 2016. Therapeutic efficacy of the small molecule GS-5734 against Ebola virus in rhesus monkeys. Nature 531:381–385.

13. Sheahan TP, Sims AC, Graham RL, Menachery VD, Gralinski LE, Case JB, Leist SR, Pyrc K, Feng JY, Trantcheva I, Bannister R, Park Y, Babusis D, Clarke MO, Mackman RL, Spahn JE, Palmiotti CA, Siegel D, Ray AS, Cihlar T, Jordan R, Denison MR, Baric RS. 2017. Broad-spectrum antiviral GS-5734 inhibits both epidemic and zoonotic coronaviruses. Sci Transl Med 9:eaal3653.

14. Williamson BN, Feldmann F, Schwarz B, Meade-White K, Porter DP, Schulz J, Van Doremalen N, Leighton I, Yinda CK, Pérez-Pérez L, Okumura A, Lovaglio J, Hanley PW, Saturday G, Bosio CM, Anzick S, Barbian K, Cihlar T, Martens C, Scott DP, Munster VJ, De Wit E. 2020. Clinical benefit of remdesivir in rhesus macaques infected with SARS-CoV-2. Nature 585:273–276.

15. Tempestilli M, Caputi P, Avataneo V, Notari S, Forini O, Scorzolini L, Marchioni L,

312 Bartoli TA, Castilletti C, Lalle E, Capobianchi MR, Nicastri E, D'Avolio A, Ippolito
313 G, Agrati C. 2020. Pharmacokinetics of remdesivir and GS-441524 in two critically
314 ill patients who recovered from COVID-19. J Antimicrob Chemother dkaa239:1–4.
315 16. Murakami E, Wang T, Babusis D, Lepist E-I, Sauer D, Park Y, Vela JE, Shih R,
316 Birkus G, Stefanidis D, Kim CU, Cho A, Ray AS. 2014. Metabolism and
317 Pharmacokinetics of the Anti-Hepatitis C Virus Nucleotide Prodrug GS-6620.
318 Antimicrob Agents Chemother 58:1943–1951.
319 17. Lawitz E, Hill J, Marbury T, Hazan D, Gruener D, Webster L, Majauskas R,
320 Morrison R, DeMicco M, German P, Stefanidis D, Svaroskaia E, Arterburn S, Ray
321 A, Rossi S, McHutchinson J, Rodriguez-Torres M. 2012. GS-6620, A Liver-
322 Targeted Nucleotide Prodrug, Exhibits Antiviral Activity and Favorable Safety
323 Profile Over 5 Days in Treatment Naïve Chronic HCV Genotype 1 SubjectsEASL
324 47th Annual Meeting. EASL 47th Annual Meeting, Barcelona.
325 18. Lo MK, Jordan R, Arvey A, Sudhamsu J, Shrivastava-Ranjan P, Hotard AL, Flint
326 M, McMullan LK, Siegel D, Clarke MO, Mackman RL, Hui HC, Perron M, Ray AS,
327 Cihlar T, Nichol ST, Spiropoulou CF. 2017. GS-5734 and its parent nucleoside
328 analog inhibit Filo-, Pneumo-, and Paramyxoviruses. Sci Rep 7:43395.
329 19. Pruijssers AJ, George AS, Schä A, Baric RS, Denison MR, Sheahan TP. 2020.
330 Remdesivir Inhibits SARS-CoV-2 in Human Lung Cells and Chimeric SARS-CoV
331 Expressing the SARS-CoV-2 RNA Polymerase in Mice. Cell Rep 107940.
332 20. Pedersen NC, Perron M, Bannasch M, Montgomery E, Murakami E, Liepnieks M,
333 Liu H. 2019. Efficacy and safety of the nucleoside analog GS-441524 for
334 treatment of cats with naturally occurring feline infectious peritoniti. J Feline Med

Surg 21:271–281.

21. Murphy BG, Perron M, Murakami E, Bauer K, Park Y, Eckstrand C, Liepnieks M, Pedersen NC. 2018. The nucleoside analog GS-441524 strongly inhibits feline infectiousperitonitis (FIP) virus in tissue culture and experimental cat infection studies. Vet Microbiol 219:226–233.

22. Dickinson PJ, Bannasch M, Thomasy SM, Murthy VD, Vernau KM, Liepnieks M, Montgomery E, Knickelbein KE, Murphy B, Pedersen NC. 2020. Antiviral treatment using the adenosine nucleoside analogue GS-441524 in cats with clinically diagnosed neurological feline infectious peritonitis. J Vet Intern Med jvim.15780.

23. Li Y, Cao L, Li G, Cong F, Li Y, Sun J, Luo Y, Chen G. 2021. Remdesivir Metabolite GS-441524 Effectively Inhibits SARS-CoV□2 Infection in Mouse Models. J Med Chem https://doi.org/10.1021/acs.jmedchem.0c01929.

24. Lenze EJ, Mattar C, Zorumski CF, Stevens A, Schweiger J, Nicol GE, Miller JP, Yang L, Yingling M, Avidan MS, Reiersen AM. 2020. Fluvoxamine vs Placebo and Clinical Deterioration in Outpatients with Symptomatic COVID-19: A Randomized Clinical Trial. JAMA 324:2292–2300.

25. Wang T, Babusis D, Park Y, Niu C, Kim C, Zhao X, Lu B, Ma B, Muench RC, Sperger D, Ray AS, Murakami E. 2020. Species differences in liver accumulation and metabolism of nucleotide prodrug sofosbuvir. Drug Metab Pharmacokinet 35:334–340.

26. Food and Drug Administration. 2002. ZOVIRAX® (acyclovir).

27. Levene DL. 1973. The absorption of potassium chloride: Liquid vs. tablet. Can

Med Assoc J 108:1480–1483.

28. Hammond JR, Stolk M, Archer RGE, McConnell K. 2004. Pharmacological analysis and molecular cloning of the canine equilibrative nucleoside transporter 1. Eur J Pharmacol 491:9–19.

29. Agostini ML, Andres EL, Sims AC, Graham RL, Sheahan TP, Lu X, Clinton Smith E, Brett Case J, Feng JY, Jordan R, Ray AS, Cihlar T, Siegel D, Mackman RL, Clarke MO, Baric RS, Denison MR, Agostini CM, Gallagher T. 2018. Coronavirus Susceptibility to the Antiviral Remdesivir (GS-5734) Is Mediated by the Viral Polymerase and the Proofreading Exoribonuclease Downloaded from. MBio 9:ee00221-18.

30. Mahase E. 2021. Covid-19□: Novavax vaccine efficacy is 86 % against UK variant and 60 % against South African variant. BMJ 2021.

31. Grant RM, Lama JR, Anderson PL, McMahan V, Liu AY, Vargas L, Goicochea P, Casapía M, Guanira-Carranza JV, Ramirez-Cardich ME, Montoya-Herrera O, Fernández T, Veloso VG, Buchbinder SP, Chariyalertsak S, Schechter M, Bekker L-G, Mayer KH, Kallás EG, Amico KR, Mulligan K, Bushman LR, Hance RJ, Ganoza C, Defechereux P, Postle B, Wang F, McConnell JJ, Zheng J-H, Lee J, Rooney JF, Jaffe HS, Martinez AI, Burns DN, Glidden D V. 2010. Preexposure Chemoprophylaxis for HIV Prevention in Men Who Have Sex with Men. N Engl J Med 363:2587–2599.

32. Anderson PL, Glidden D V, Liu A, Buchbinder S, Lama JR, Guanira JV, Mcmahan V, Bushman LR, Casapía M, Montoya-Herrera O, Veloso VG, Mayer KH, Chariyalertsak S, Schechter M, Bekker L-G, Kallás EG, Grant RM. 2012.

Emtricitabine-Tenofovir Concentrations and Pre-Exposure Prophylaxis Efficacy in Men Who Have Sex with Men. Sci Transl Med 4:151ra125.

33. Zheng JH, Rower C, McAllister K, Castillo-Mancilla J, Klein B, Meditz A, Guida LA, Kiser JJ, Bushman LR, Anderson PL. 2016. Application of an intracellular assay for determination of tenofovir-diphosphate and emtricitabine-triphosphate from erythrocytes using dried blood spots. J Pharm Biomed Anal 122:16–20.

34. Bushman LR, Kiser JJ, Rower JE, Klein B, Zheng JH, Ray ML, Anderson PL. 2011. Determination of nucleoside analog mono-, di-, and tri-phosphates in cellular matrix by solid phase extraction and ultra-sensitive LC-MS/MS detection. J Pharm Biomed Anal 56:390–401.

35. Laskin OL. 1983. Clinical Pharmacokinetics of Acyclovir. Clin Pharmacokinet 8:187–201.

36. Krasny HC, De Miranda P, Blum MR, Elion GB. 1981. Pharmacokinetics and bioavailability of acyclovir in the dog. J Pharmacol Exp Ther 216:281–288.