Nucleoside analog GS-441524
pharmacokinetics in different species, safety, and potential effectiveness against Covid-19
Rasmussen, Henrik Berg; Thomsen, Ragnar; Hansen, Peter Riis

Published in:
Pharmacology Research and Perspectives

DOI:
10.1002/prp2.945

Publication date:
2022

Document Version
Publisher's PDF, also known as Version of record

Citation for published version (APA):
Rasmussen, H. B., Thomsen, R., & Hansen, P. R. (2022). Nucleoside analog GS-441524: pharmacokinetics in different species, safety, and potential effectiveness against Covid-19. Pharmacology Research and Perspectives, 10(2), Article e00945. https://doi.org/10.1002/prp2.945

General rights
Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

• Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
• You may not further distribute the material or use it for any profit-making activity or commercial gain.
• You may freely distribute the URL identifying the publication in the public portal.

Take down policy
If you believe that this document breaches copyright please contact rucforsk@kb.dk providing details, and we will remove access to the work immediately and investigate your claim.
Nucleoside analog GS-441524: pharmacokinetics in different species, safety, and potential effectiveness against Covid-19

Henrik Berg Rasmussen\(^1\)\(^2\) | Ragnar Thomsen\(^3\) | Peter Riis Hansen\(^4\)

\(^1\)Institute of Biological Psychiatry, Mental Health Centre Sct. Hans, Roskilde, Denmark
\(^2\)Department of Science and Environment, Roskilde University Center, Roskilde, Denmark
\(^3\)Section of Forensic Chemistry, Department of Forensic Medicine, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark
\(^4\)Department of Cardiology, Herlev and Gentofte Hospital, Hellerup, Denmark

**Abstract**

GS-441524, the parent nucleoside of remdesivir, has been proposed to be effective against Covid-19 based on in vitro studies and studies in animals. However, randomized clinical trials of the agent to treat Covid-19 have not been conducted. Here, we evaluated GS-441524 for Covid-19 treatment based on studies reporting pharmacokinetic parameters of the agent in mice, rats, cats, dogs, monkeys, and the single individual in the first-in-human trial supplemented with information about its activity against severe acute respiratory syndrome coronavirus 2 and safety. A dosing interval of 8 h was considered clinically relevant and used to calculate steady-state plasma concentrations of GS-441524. These ranged from 0.27 to 234.41 μM, reflecting differences in species, doses, and administration routes. Fifty percent maximal inhibitory concentrations of GS-441524 against severe acute respiratory syndrome coronavirus 2 ranged from 0.08 μM to above 10 μM with a median of 0.87 μM whereas concentrations required to produce 90% of the maximal inhibition of the virus varied from 0.18 μM to more than 20 μM with a median of 1.42 μM in the collected data. Most of these concentrations were substantially lower than the calculated steady-state plasma concentrations of the agent. Plasma exposures to orally administered GS-441524, calculated after normalization of doses, were larger for dogs, mice, and rats than cynomolgus monkeys and humans, probably reflecting interspecies differences in oral uptake with reported oral bioavailabilities below 8.0% in cynomolgus monkeys and values as high as 92% in dogs. Reported oral bioavailabilities in rodents ranged from 12% to 57%. Using different presumptions, we estimated human oral bioavailability of GS-441524 at 13% and 20%. Importantly, doses of GS-441524 lower than the 13 mg/kg dose used in the first-in-human trial may be effective against Covid-19. Also, GS-441524 appears to be well-tolerated. In conclusion, GS-441524 has potential for oral treatment of Covid-19.

**KEYWORDS**
GS-441524, coronavirus disease 2019, in vitro–in vivo extrapolation, nucleoside analog, pharmacokinetics

---

**Abbreviations:** AUC\(_{0-24}\), area under the plasma drug concentration–time curve from time zero to 24 h; AUC\(_{0-\infty}\), area under the plasma drug concentration–time curve from time zero to infinity; CC\(_{50}\), 50 percent cytotoxic concentration; C\(_{av,ss}\), average plasma drug concentration at steady state; Covid-19, coronavirus disease 2019; FIP, feline infectious peritonitis; HED, human equivalent dose; IC\(_{50}\), 50 percent of maximal inhibitory concentration (half maximal inhibitory concentration); IC\(_{90}\), 90 percent of maximal inhibitory concentration; PK, pharmacokinetics; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

This is an open access article under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.

© 2022 The Authors. Pharmacology Research & Perspectives published by John Wiley & Sons Ltd, British Pharmacological Society and American Society for Pharmacology and Experimental Therapeutics.
1 | INTRODUCTION

There remains an unmet need for easily administrable therapeutic agents with high effectiveness in the treatment of coronavirus disease 2019 (Covid-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Drug repurposing has attracted interest to accelerate discovery and clinical development of new pharmacological Covid-19 treatments. Remdesivir is a repurposed antiviral drug, which was originally designed to treat Ebola and other viral infections with a pandemic potential. This drug received approval for treatment of Covid-19 in USA and Europe based on the first stage of the Adaptive COVID-19 Treatment Trial known as ACTT-1. However, subsequent results from the larger Solidarity trial questioned its effectiveness against Covid-19. Activity of GS-441524 against Covid-19 is supported by findings that it markedly inhibited SARS-CoV-2 in cell lines, and possessed anti-SARS-CoV-2 activity in mouse models of Covid-19. GS-441524 has been found to be highly effective for treatment of feline infectious peritonitis (FIP), a coronavirus disease in cats.

Since GS-441524 is a nucleoside analog lacking the Mcguigan moiety characteristic of ProTides, its pharmacokinetics (PK) differs markedly from that of remdesivir. Notably, cellular uptake of GS-441524 is believed to be dependent upon membrane-bound transporters since it is hydrophilic with limited ability to cross cell membranes by diffusion. By contrast, uptake of remdesivir appears to be mediated by diffusion, which is facilitated by its hydrophobic prodrug moieties, thus probably to a large extent being independent of membrane-bound transporters. Once inside the cells, the GS-441524 is phosphorylated to the active antiviral GS-441524 triphosphate metabolite, also known as GS-443902, with adenosine kinase probably being responsible for catalyzing the first and perceived rate-limiting step in the formation of this metabolite. Importantly, the relatively simple chemical structure of GS-441524 may permit fast manufacture of the agent in large amounts.

Randomized clinical trials of GS-441524 against Covid-19 have not been conducted. Further knowledge about the pharmacokinetics, toxicity, and effectiveness against Covid-19 of GS-441524 may pave the way for the agent to reach such trials. Based on publicly available in vitro and in vivo data including data from a variety of different species, we here review the PK, anti-SARS-CoV-2 activity and safety of GS-441524 supplemented with calculations to critically evaluate the potential of this nucleoside analog for treatment of Covid-19.

2 | DATA SOURCES AND CALCULATIONS

PubMed, medRxiv and bioRxiv were searched on August 19, 2021 and revisited on December 19, 2021 using ‘GS-441524’ as search term to identify publications and preprints reporting on the PK, in vitro and in vivo anti-SARS-CoV inhibitory activities in addition to toxicity of the agent. PK data were also collected from the National Center for Advancing Translational Sciences. Information about plasma protein binding of GS-441524 and PK parameters of GS-441524 triphosphate after administration of remdesivir in humans were obtained from recent publications supplemented with reports assessing remdesivir for treatment of Covid-19 prepared by the European Medicines Agency and the Australian Department of Health.

In the event that a study presented plots of time versus plasma concentration for GS-441524 but did not provide PK variables, we extracted data from the plots using WebplotDigitizer. Using the PKSolver, these data were used for calculation of PK parameters for a noncompartmental model including areas under the plasma drug concentration–time curve from time zero to 24 h (AUC₀–₂₄) and from time zero to infinity (AUC₀–inf). Conversions of animal doses to human equivalent doses (HEDs) and the yet only human dose to animal equivalent doses were based on allometric scaling with normalization to body surface area using the equation: HED = Animal dose x (Animal weight/Human weight)⁽¹⁻⁰·₆₇⁾, where HED and animal dose are in mg/kg, animal and human weights are in kg and 0.67 is the body surface-based scaling factor. Although this scaling factor is well-established for estimation of HEDs, 0.74 and 0.75 may perform better as factors for allometric-based interspecies drug dose conversion. Hence, both 0.67 and 0.75 were used as scaling factor for estimation of human oral bioavailability of GS-441524.

3 | PLASMA PHARMACOKINETICS OF GS-441524 IN DIFFERENT SPECIES

GS-441524 doses and exposures following oral, intragastric, subcutaneous, intramuscular, and intravenous administration in mice, rats, cats, dogs, cynomolgus monkeys and a single human are listed (Table 1). Since the AUC₀–₂₄ and AUC₀–inf values were almost identical, GS-441524 does not appear to accumulate in plasma over a dosing interval of 24 h (data not shown). Therefore, we calculated average plasma concentrations of the agent at steady state (Cav/st) using a dosing interval of 8 h and found that these concentrations ranged from 0.27 to 234.41 μM.

Assuming linear PK, the AUC₀–inf values in the animal species scaled to the only reported (n = 1) human dose of 13 mg/kg ranged from 20.23 μM · hour in cynomolgus monkey to 290.86 μM · hour in dogs (Table 2). Remarkably, the AUC₀–inf in cynomolgus monkey was closer to the value observed in the human of 31.08 μM · hour than it was to those reported in the other animal species.

Oral bioavailability of GS-441524 differed significantly between species, with cynomolgus monkeys displaying markedly lower ability
TABLE 1 Plasma pharmacokinetics of GS-441524 in various species

| Species                  | Single dose route of administration | Dose (mg/kg) | Human equivalent dose (mg/kg)a | \( \text{AUC}_{0-\text{inf}} \) (\( \mu \text{M·h} \)) | \( C_{\text{av,ss}} \) for three daily doses (\( \mu \text{M} \))b | Reference               |
|-------------------------|-------------------------------------|-------------|-------------------------------|-------------------------------------------------|-------------------------------------------------|--------------------------|
| Cat                     | Subcutaneous                        | 5           | NA                            | 41.26\(^c\)                                      | 5.16                                            | Murphy et al.\(^{35}\)   |
| Cat                     | Intravenous                         | 5           | 2.05                          | 42.42\(^c\)                                      | 5.30                                            | Murphy et al.\(^{35}\)   |
| Dog                     | Oral (capsule)                      | 6.5         | 3.61                          | 65.92                                            | 8.24                                            | Yan et al.\(^{24}\)      |
| Rat                     | Intravenous                         | 30          | 4.84                          | 1875.28                                          | 234.41                                          | Li et al.\(^{9}\)        |
| Rat                     | Intragastric                        | 30          | 4.84                          | 68.64                                            | 8.58                                            | Li et al.\(^{9}\)        |
| Rat                     | Intravenous                         | 5           | 0.81                          | 11.07                                            | 1.38                                            | NCATS\(^{14}\)           |
| Rat                     | Oral                                | 10          | 1.61                          | 7.47                                             | 0.93                                            | NCATS\(^{14}\)           |
| Mouse                   | Intravenous                         | 5           | 0.41                          | 11.08                                            | 1.38                                            | NCATS\(^{14}\)           |
| Mouse                   | Oral                                | 10          | 0.81                          | 8.71                                             | 1.09                                            | NCATS\(^{14}\)           |
| Dog                     | Intravenous                         | 2           | 1.11                          | 28.73                                            | 3.59                                            | NCATS\(^{14}\)           |
| Dog                     | Oral (solution)                     | 5           | 2.78                          | 65.50                                            | 8.19                                            | NCATS\(^{14}\)           |
| Cynomolgus monkey       | Intravenous                         | 2           | 0.65                          | 12.38                                            | 1.55                                            | NCATS\(^{14}\)           |
| Cynomolgus monkey       | Oral                                | 5           | 1.61                          | 2.51                                             | 0.31                                            | NCATS\(^{14}\)           |
| Rat                     | Intramuscular                       | 67          | NA                            | 634.88\(^c\)                                      | 79.36                                           | Shi et al.\(^{49}\)      |
| Mouse                   | Intramuscular                       | 67          | NA                            | 357.81\(^c\)                                      | 44.73                                           | Shi et al.\(^{49}\)      |
| Mouse                   | Intravenous                         | 10          | 0.81                          | 30.40                                            | 3.80                                            | Scherf-Clavel et al.\(^{21}\) |
| Mouse                   | Intravenous                         | 5           | 0.41                          | 14.80                                            | 1.85                                            | Xie and Wang\(^{32}\)    |
| Mouse                   | Oral                                | 10          | 0.81                          | 16.84                                            | 2.10                                            | Xie and Wang\(^{32}\)    |
| Rat                     | Oral                                | 10          | 1.61                          | 2.14\(^c\)                                       | 0.27                                            | Yin et al.\(^{50}\)      |
| Rat                     | Intravenous                         | 2           | 0.32                          | 2.49\(^c\)                                       | 0.31                                            | Yin et al.\(^{50}\)      |
| Human                   | Oral, fasted                        | 13          | 13                            | 31.08\(^d\)                                      | 3.89                                            | Yan\(^{54}\)             |

Note: NCATS: The National Center for Advancing Translational Sciences; \( \text{AUC}_{0-\text{inf}} \): area under the plasma drug concentration–time curve from time zero to infinity; \( C_{\text{av,ss}} \): average plasma drug concentration at steady state; NA: not applicable.

aAnimal doses were converted to human equivalent doses using the exponent 0.67 in body surface area-based allometric scaling. Since interspecies conversion of drug doses by allometric scaling is not supported for subcutaneous and intramuscular administration, only human equivalent doses for oral and intravenous administrations were calculated.

bCalculated as \( \text{AUC}_{0-\text{inf}} \tau \), where \( \tau \) is the doing interval (8 h).

cFor studies that presented plots of time versus drug concentrations without providing \( \text{AUC}_{0-\text{inf}} \) values, these values were calculated using PKSolver after extraction of data from the plots. Moreover, we recalculated areas under the plasma drug concentration–time curve from time zero to 12 or 24 h based on data extracted from plots and found that these did not deviate with more than 5% from the corresponding parameters of exposure reported by the studies in question (data not shown).

dCalculated based on the supplementary data appended the first-in-human study (\( n = 1 \)).

for oral uptake than mice and dogs (Table 3). Human oral bioavailability of GS-441524 was estimated at 13% and 20% using the scaling factors of 0.67 and 0.75, respectively. These levels of human bioavailability are consistent with previous estimates ranging from 15% to 30% obtained by comparison with acyclovir.\(^{24}\) Hence, the human oral bioavailabilities calculated using different approaches suggest that oral administration of GS-441524 is feasible in humans.

4 | IN VITRO ANTI-SARS COV-2 ACTIVITY OF GS-441524

Using different isolates of SARS-CoV-2, viral quantification methods and types of cells, studies have reported fifty percent maximal inhibitory concentration (IC\(_{50}\)) values from 0.08 to above 10 \( \mu \text{M} \) with a median of 0.87 \( \mu \text{M} \) and 90% maximal inhibitory concentration (IC\(_{90}\)) values from 0.18 to above 20 \( \mu \text{M} \) corresponding to a median of 1.42 \( \mu \text{M} \) for the activity of GS-441524 against the virus (Table 4). Most of the calculated \( C_{\text{av,ss}} \) values exceeded these medians (Table 1). A study showed that GS-441524 at 3.7 \( \mu \text{M} \) reduced the load of SARS-CoV-2 RNA in Vero cells by more than four log\(_{10}\) units to levels below lower limit of detection but did not determine the IC\(_{50}\) and IC\(_{90}\) values for its activity against the virus.\(^{25}\) Consistent with this, exposure to GS-441524 at a concentration of 3 \( \mu \text{M} \) reduced the load of SARS-CoV-2 with up to four log\(_{10}\) units in cultured human airway epithelial cells, which may represent a more appropriate model for studying the activity of anti-SARS-CoV-2 agents than cancer cell lines.\(^{26}\) Other findings, also based on human airway epithelial cell
ANTI-SARS-COV-2 ACTIVITY OF GS-441524

A single intravenous injection of mice with GS-441524 at a dose of 10 mg/kg has been reported to produce wet weight concentrations above 1 µmol/kg in most organs, except for lungs and nasal mucosa, leading to the suggestion that administration of the agent at 10 mg/kg twice daily in mice would produce concentrations above reported IC50 values for its anti-SARS-CoV-2 activity in most organs.31 In support of this suggestion, a plot of time versus concentration showed that administration of a single oral dose of 20 mg/kg GS-441524 to mice resulted in concentrations above 2 µmol/kg in the lungs and up to 10 µmol/kg in the liver at 1, 2 and 4 h post dosing.32 Also, a daily intraperitoneal GS-441524 dose of 25 mg/kg resulted in significant inhibition of SARS-CoV-2 in a mouse model of Covid-19 after 2 days of treatment.9 Given that the intracellular volume in many tissues is around 0.8 mL/g tissue,33 intracellular concentrations of GS-441524 in µM are likely to be 25% higher than the reported wet weight.

5 | TISSUE DISTRIBUTION AND IN VIVO

| Species                  | Administered dose (mg/kg) | Observed AUC0–inf (µM · h)a | Scaled AUC0–inf (µM · h)b | Referencec |
|--------------------------|---------------------------|-----------------------------|---------------------------|-------------|
| Mouse                    | 10                        | 8.71                        | 139.27                    | NCATS14     |
| Rat                      | 10                        | 7.47                        | 60.21                     | NCATS14     |
| Cynomolgus monkey        | 5                         | 2.51                        | 20.23                     | NCATS14     |
| Dog                      | 5                         | 65.50                       | 306.54                    | NCATS14     |
| Human                    | 13                        | 31.08                       | NA                        | Yan 202124  |

Note: NCATS, The National Center for Advancing Translational Sciences.
aOral administration formulation was a solution.
bValue estimated at 3.4%, which we rounded to 3%.
cCalculated using the equation: Oral bioavailability = 100 · AUCoral / AUCIV · DoseIV/Doseoral, where AUCoral and AUCIV are the areas under the plasma drug concentration–time curves after administration of Doseoral and DoseIV respectively. A human AUCIV of 31.08 µM-h measured after administration of an oral dose of 13 mg/kg was used for the calculation. Given that the AUCIV of GS-441524 has not been determined in humans, this plasma exposure in the equation was replaced with the AUCIV of 12.38 µM-h reported in cynomolgus monkeys after administration of 2 mg/kg. Furthermore, a human equivalent intravenous dose of 0.65 mg/kg, which we obtained by body surface-based allometric scaling of the monkey intravenous dose of 2 mg/kg (scaling factor = 0.67), served as intravenous dose, thus assuming that the scaled dose produces an AUCIV in humans similar to that observed in cynomolgus monkeys. Using a scaling factor of 0.75, human oral bioavailability of GS-441524 was estimated at 20%.

Importantly, plasma protein binding of GS-441524 is low with the unbound fraction being above 85%16 or even as high as 98% to 99%.17 Hence, a major fraction of GS-441524 in human plasma appears to be available for cellular uptake and subsequent intracellular phosphorylation to the active antiviral GS-441524 triphosphate metabolite.

Based on detection of fine changes in cellular morphology, studies have reported that GS-441524 potently rescued the phenotypic profile induced by SARS-CoV-2 and strongly suppressed alterations in this profiles, thus suggesting activity against the virus.28,29 By contrast, findings based on a cellular thermal shift assay coupled to mass spectrometry, did not show anti-SARS-CoV-2 activity of GS-441524 in HepG2 cells, possibly reflecting limited cellular uptake of the agent.30 However, these analytical procedures were designed for high-throughput screening and may therefore not provide accurate information about the anti-SARS-CoV activity of all agents.

TABLE 2 Area under the plasma drug concentration–time curve after oral administration of GS-441524 adjusted to human dose in different animal species

| Species                  | Oral bioavailability (%)a | Reference |
|--------------------------|---------------------------|-----------|
| Mouse                    | 39                        | NCATS14   |
| Mouse                    | 57                        | Xie and Wang32 |
| Rat                      | 12                        | Mackman et al.51 |
| Rat                      | 16                        | Yin et al.50 |
| Rat                      | 33                        | NCATS14   |
| Dog                      | 85                        | NCATS14   |
| Dog                      | 89                        | Mackman et al.51 |
| Dog                      | 92                        | Yan et al.24 |
| Cynomolgus monkey        | 3b                        | Mackman et al.51 |
| Cynomolgus monkey        | 8                         | NCATS14   |
| Human                    | 13c                       | Present review |

Note: NA, not applicable; NCATS, The National Center for Advancing Translational Sciences; AUC0–inf, area under the plasma drug concentration curve from time zero to infinity.
aDetermined using PKSolver.
bAnimal AUC0–inf values scaled to a human dose of 13 mg/kg under the assumption of dose-related pharmacokinetics. These AUC0–inf values were calculated by multiplying observed animal AUC0–inf values with the ratio between the animal equivalent dose and the administered animal dose, where the animal equivalent doses were derived by body surface-based allometric scaling of the human dose.
cTo increase comparability, animal data from NCATS only were used.
dFirst-in-human study (n = 1).

cultures, found that GS-441524 at a concentration of 2 µM resulted in complete elimination of SARS-CoV-2, whereas 1 µM of the agent conferred intermediate anti-SARS-CoV-2 activity.27
| SARS-CoV-2 strain or isolate | Cell line or primary cell culture | Virus quantification method | IC₅₀ (μM) | IC₉₀ (μM) | Reference |
|----------------------------|----------------------------------|-----------------------------|-----------|-----------|-----------|
| hCoV-19/CHN/SYSU-IHV/2020  | Vero E6                          | RT-qPCR                     | 0.70      | -         | Li et al.⁹ |
| hCoV-19/CHN/SYSU-IHV/2020  | Calu-3                           | RT-qPCR                     | 3.21      | -         | Li et al.⁹ |
| hCoV-19/CHN/SYSU-IHV/2020  | Caco-2                           | RT-qPCR                     | 3.62      | -         | Li et al.⁹ |
| hCoV-19/USA/WA1/2020       | Vero E6                          | Plaque forming assay        | 0.47      | 0.71      | Pruijssers et al.⁸ |
| hCoV-19/USA/WA1/2020       | Vero E6                          | RT-qPCR                     | 0.47      | 0.80      | Pruijssers et al.⁸ |
| hCoV-19/USA/WA1/2020       | Calu3 2B4                        | Plaque forming assay        | 0.62      | 1.34      | Pruijssers et al.⁸ |
| hCoV-19/USA/WA1/2020       | Calu3 2B4                        | RT-qPCR                     | 1.09      | 1.37      | Pruijssers et al.⁸ |
| hCoV-19/Harbin/HRB-26/2020 | Vero E6                          | Plaque forming assay        | 5.19      | -         | Shi et al.⁹ |
| hCoV-19/mouse/Harbin/HRB- 26m/2020 (mouse-adapted) | Vero E6                          | Plaque forming assay        | 5.05      | -         | Shi et al.⁹ |
| hCoV-19/USA/WA1/2020 with insertion of nanoluciferase gene | A549 expressing human angiotensin-converting enzyme 2 | Luciferase signal | 0.87      | -         | Xie et al.⁵² |
| hCoV-19/Wuhan/WIV04/2019   | Vero E6                          | RT-qPCR                     | 0.48      | -         | Yin et al.⁵⁰ |
| hCoV-19/USA/WA1/2020 and B.1.351 (beta) isolate | Calu-3                          | Counting infected cells using fluorescence microscopy | 0.10      | -         | Schultz et al.⁵³ |
| hCoV-19/Wuhan/Hu-1/2019 encoding firefly luciferase and green fluorescence fusion protein | 293T                          | Counting cells expressing reporter or measuring luciferase activity | 0.60      | -         | He et al.⁵⁴ |
| hCoV-19/Wuhan/Hu-1/2019 encoding firefly luciferase and green fluorescence fusion protein | Vero                          | Counting cells expressing reporter or measuring luciferase activity | 0.30      | -         | He et al.⁵⁴ |
| hCoV-19/Wuhan/Hu-1/2019 encoding firefly luciferase and green fluorescence fusion protein | Huh-7.5                        | Counting cells expressing reporter or measuring luciferase activity | 1.03      | -         | He et al.⁵⁴ |
| hCoV-19/Wuhan/Hu-1/2019 encoding firefly luciferase and green fluorescence fusion protein | Calu-1                         | Counting cells expressing reporter or measuring luciferase activity | 1.33      | -         | He et al.⁵⁴ |
| hCoV-19/Wuhan/Hu-1/2019 encoding firefly luciferase and green fluorescence fusion protein | A549                           | Counting cells expressing reporter or measuring luciferase activity | 1.47      | -         | He et al.⁵⁴ |
| hCoV-19/Belgium/GHB-03021/2020 | Vero E6 expressing enhanced green fluorescent protein | Fluorescence intensity measurement | 2.74b     | -         | Saul et al.⁵⁵ |
| hCoV-19/Belgium/GHB-03021/2020 | Vero E6 expressing green fluorescent protein | Fluorescence-based imaging | 0.78–0.89c | -         | Do et al.²⁷ |
| hCoV-19/Belgium/GHB-03021/2020 | Huh-7                           | Cytopathic effect           | 1.10–1.50c | -         | Do et al.²⁷ |
| hCoV-19/Belgium/GHB-03021/2020 and hCoV-19/Germany/ BY-ChVir-929/2020 | Human airway epithelial cells | RT-qPCR                     | 0.51      | -         | Do et al.²⁷ |
| hCoV-19/USA/WA1/2020       | Vero                             | RT-qPCR                     | 8.2       | 13.2      | Zandi et al.⁵⁶ |
| hCoV-19/USA/WA1/2020       | Huh-7                            | RT-qPCR                     | >10       | >20       | Zandi et al.⁵⁶ |
| hCoV-19/CHN/SYSU-IHV/2020  | Vero E6                          | RT-qPCR                     | 1.71      | -         | Cao et al.⁵⁷ |
| SARS_CoV-2_human_ CHN_20S18530_2020 | Vero E6                          | RT-qPCR                     | 1.35      | -         | Cao et al.⁵⁷ |
| B.1.617.2 isolate          | Vero E6                          | RT-qPCR                     | 0.96      | -         | Cao et al.⁵⁷ |
| hCoV-19/Wuhan/WIV04/2019   | Vero E6                          | RT-qPCR                     | 0.48      | -         | Wei et al.⁵⁸ |
| hCoV-19/USA/WA1/2020 expressing nanoluciferase | A549 expressing human angiotensin-converting enzyme 2 | Luminescence intensity measurement | 3.37      | -         | Schäfer et al.⁵⁹ |
| hCoV-19/USA/WA1/2020 expressing firefly luciferase | Normal human bronchial epithelial cells | Luminescence intensity measurement | 2.45      | -         | Schäfer et al.⁵⁹ |

(Continues)
6 | CELLULAR PHARMACOKINETICS OF GS-441524 TRIPHOSPHATE

A study reported intracellular GS-441525 triphosphate levels from 1 to 1.25 μM in Crandell-Rees feline kidney cells exposed to GS-441524 at 1 μM over a three-day period. Moreover, intracellular levels of GS-441524 triphosphate of 0.85 and 1.78 pmol/million cells were reported in Calu3 2B4 cells and Vero E6 cells, respectively, after incubation with GS-441524 for 24 h. Assuming an intracellular average volume of 1 pL in cultured mammalian cells, these levels translate into intracellular concentrations of 0.85 and 1.78 μM. Based on intracellular volumes determined specifically for Calu-3 cells of 2.7 pL/cell and Vero cells ranging from 0.59 to 0.74 pL/cell, the intracellular concentrations of GS-441524 triphosphate were estimated at 0.31 μM in Calu cells and from 2.41 to 3.02 μM in Vero E6 cells suggesting major differences between different cell types in uptake and ability to phosphorylate adenosine analogs.

Also, large variation in concentrations of GS-441524 triphosphate between organs after administration of GS-441524 have been reported. Notably, a plot of time versus concentration showed that administration of a single oral GS-441524 dose of 20 mg/kg to mice led to concentrations in the range from about 0.20 to 0.63 μmol/kg and from 0.04 to 0.10 μmol/kg in homogenized liver and lung, respectively, 1–4 h after dosing. The GS-441524 triphosphate concentrations observed in lung homogenate translate into intracellular concentrations in the range from 0.05 to 0.13 μM assuming an intracellular volume in the lung of 0.8 mL/g tissue. Since conversion of a human dose of GS-441524 at 13 mg/kg by

---

**TABLE 4 (Continued)**

| SARS-CoV-2 strain or isolate | Cell line or primary cell culture | Virus quantification method | IC₅₀ (μM) | IC₉₀ (μM) | Reference |
|-----------------------------|----------------------------------|-----------------------------|----------|----------|-----------|
| hCoV-19/USA/WA1/2020 expressing mNeonGreen protein | Vero E6 | Fluorescent-reporter imaging to quantitate focus-forming units | 0.42 | 0.60 | Lo et al.⁶⁰ |
| hCoV-19/USA/WA1/2020 expressing mNeonGreen protein | Huh-7 | Fluorescent-reporter imaging to quantitate focus-forming units | 0.69 | 1.50 | Lo et al.⁶⁰ |
| hCoV-19/USA/WA1/2020 | Vero E6 | RT-qPCR | 0.38 | 0.77 | Schooley et al.⁶¹ |
| hCoV-19/USA/WA1/2020 | Human pluripotent stem cell-derived lung cells | RT-qPCR | 0.74 | 2.62 | Schooley et al.⁶¹ |
| hCoV-19/USA/WA1/2020 | Calu-3 | RT-qPCR | 0.15 | 0.18 | Schooley et al.⁶¹ |
| hCoV-19/USA/WA1/2020 | Huh-7.5 | RT-qPCR | 0.32 | 0.73 | Schooley et al.⁶¹ |
| hCoV-19/USA/WA1/2020 | Caco-2 | RT-qPCR | 0.96 | 1.75 | Schooley et al.⁶¹ |
| hCoV-19/USA/WA1/2020 | Vero E6 | RT-qPCR | 1.10 | 3.90 | Tao et al.⁶² |
| hCoV-19/USA/WA1/2020 | Vero | RT-qPCR | 0.80 | 1.60 | Tao et al.⁶² |
| hCoV-19/USA/WA1/2020 | Calu-3 | RT-qPCR | 0.25 | 2.35 | Tao et al.⁶² |
| hCoV-19/USA/WA1/2020 | Caco-2 | RT-qPCR | 0.08 | 1.42 | Tao et al.⁶² |
| hCoV-19/USA/WA1/2020 | Calu-3 | Fluorescent-reporter imaging to quantitate focus-forming units | 1.30 | - | Tao et al.⁶² |

Note: Origin of cell lines: Vero and Vero E6, African green monkey kidney; Calu-1, Calu-3 and Calu3 2B4, human metastatic lung adenocarcinoma; Caco-2, human colorectal adenocarcinoma; A549, human epithelial lung carcinoma; 293T, human embryonic kidney; Huh-7 and Huh-7.5, human hepatocellular carcinoma; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; IC₅₀, 50 percent of maximal inhibitory concentration (half maximal inhibitory concentration); IC₉₀, 90 percent of maximal inhibitory concentration; RT-qPCR, reverse transcriptase quantitative polymerase chain reaction.

Isolate designation is based on GISAID (https://www.gisolad.org/) when possible. Isolate hCoV-19/USA/WA1/2020 is now designated hCoV-19/USA/WA-CDC-02982586-001/2020, and hCoV-19/CHN/SYSU-IHV/2020 is now known as hCoV-19/Guangdong/SYSU-IHV/2020.

Value estimated based on a plot.

References:

⁶¹ Interquartile range (Q1–Q3).

Concentrations. Assuming such ratio between volume and weight, we derived intracellular concentrations of GS-441524 from 1.25 to above 10 μM in mice after a single GS-441524 dose of 10 mg/kg intravenously or 20 mg/kg orally, i.e. equal to or higher than the median IC₅₀ and IC₉₀ values of 0.87 and 1.42 μM for the anti-SARS-CoV-2 activity of the agent with its concentration in the liver being more than 7-fold higher than the IC₅₀ median value (Table 4). Also, the intracellular concentrations of GS-441524 exceeded the IC₅₀ and IC₉₀ values by more than 4-fold in a large number of other organs.

We calculated HEDs of 1.63, 1.63 and 2.03 μM/kg/day by body surface-based scaling of the GS-441524 dose of 10 mg/kg suggested to be administered intravenously twice a day, the single peroral dose of 20 mg/kg and the daily intraperitoneal injection of 25 mg/kg, which all possessed potential for inhibition of SARS-CoV-2 in mice. These HEDs were considerably lower than the 13 mg/kg dose administered once or three times daily in the first-in-human study.
body surface-based interspecies scaling yields a mouse equivalent dose of 160 mg/kg, i.e. eight times higher than the 20 mg/kg dose.\textsuperscript{32} A single dose of 160 mg/kg is expected to produce intracellular lung concentrations of GS-441524 triphosphate from 0.40 to 1.00 μM in mice under the assumption of linear PK and validity of interspecies extrapolation.

Using data extracted from a plot of time versus concentration\textsuperscript{35} followed by calculation of AUC\textsubscript{0-24}, we derived intracellular GS-441524 triphosphate average concentrations of 8 and 17 μM over a period of 24 h in peripheral blood mononuclear cells from cats after a single intravenous or subcutaneous administration of GS-441524 at 5 mg/kg, respectively, corresponding to a HED of 2.16 mg/kg. For comparison, remdesivir administered intravenously to healthy human subjects at therapeutic doses, i.e. 100 mg daily after an initiation dose of 200 mg, has been found to produce a steady-state AUC of GS-441524 triphosphate of 240 h · μM in peripheral blood mononuclear cells.\textsuperscript{15} This translates into a \(C_{\text{ass}}\) of 10 μM over a 24-h dosing interval, which is almost equal to or lower than the estimated GS-441524 triphosphate concentrations of 9 and 17 μM in peripheral blood mononuclear cells from cats administered a single GS-441524 dose of 5 mg/kg.\textsuperscript{35} A lower average GS-441524 triphosphate concentration of 6.54 μM in peripheral blood mononuclear cells was calculated for the first 24 h following a remdesivir initiation dose of 200 mg/kg in humans.\textsuperscript{15}

We derived IC\textsubscript{50} values for the intracellular anti-SARS-CoV-2 activity of GS-441524 triphosphate by multiplication of the ratios between intracellular GS-441524 triphosphate and extracellular GS-441524 levels with the IC\textsubscript{50} values for the anti-SARS-CoV-2 activities of extracellular GS-441524 in Calu3 2B4 and Vero E6 cells, respectively, based on cell-specific intracellular volumes.\textsuperscript{8} Using this approach, we obtained an IC\textsubscript{50} value of approximately 0.27 μM in Calu3 2B4 cells and IC\textsubscript{50} values from 1.13 to 1.42 μM in Vero E6 cells. These intracellular IC\textsubscript{50} values are markedly higher than the intracellular concentrations of GS-441524 triphosphate ranging from 0.05 to 0.13 μM in mouse lungs but only slightly higher than the intracellular lung concentrations in mice estimated assuming administration of a mouse dose equivalent with the human dose of 13 mg/kg.\textsuperscript{32,34} Also, the IC\textsubscript{50} values for the intracellular anti-SARS-CoV-2 activity of GS-441524 triphosphate were comparable to the observed concentrations of this compound in mouse liver after conversion of wet weight concentrations to intracellular concentrations,\textsuperscript{32} but significantly lower than the intracellular GS-441524 triphosphate concentration in peripheral blood cells from cats administered GS-441524 and humans administered remdesivir.\textsuperscript{15,35} Notably, the IC\textsubscript{50} value of 0.27 μM was almost 63-fold lower than the GS-441524 triphosphate concentration of 17 μM in cat peripheral blood mononuclear cells.\textsuperscript{35} Therefore, GS-441524 triphosphate seems to be formed at sufficiently high intracellular levels after administration of GS-441524 and remdesivir in several types of cells to inhibit SARS-CoV-2 with the level of this active metabolite probably being lower in the lungs than in most other organs.

7 | SAFETY AND TOXICITY

Reported values for the 50% cytotoxic concentration (CC\textsubscript{50}) of GS-441524 ranged from 7 to above 1000 μM suggesting low general in vitro cytotoxicity of the agent (Table 5). In line with this, the recommendations on compassionate use of remdesivir by the European Medicines Agency concluded that high levels of GS-441524 did not induce in vitro cytotoxic effects, although CC\textsubscript{50} values in the range from 9.6 to 13.9 μM affected hematopoietic stem cell proliferation.\textsuperscript{16} However, these concentrations are 3 to 4.5-fold higher than the \(C_{\max}\) of 3.05 μM observed after administration of a single dose of GS-441524 at 13 mg/kg in the first-in-human study.\textsuperscript{34} Moreover, GS-441524 at a concentration as high as 50 μM was required to produce alteration in the in vitro antigen-induced memory T cell proliferation.\textsuperscript{38} Consistent with this, concentrations of GS-441524 in the range between 10 and 100 μM were necessary to inhibit proliferation of NRK-49F cells and HK-2 cells, derived from rat and human kidneys, after stimulation with transforming growth factor-β, whereas marked inhibition of the protein expression of fibrotic markers such as fibronectin was detected at a concentration of this nucleoside analog of 10 μM.\textsuperscript{39}

In line with the in vitro findings, preclinical animal studies have suggested that GS-441524 is devoid of adverse effects at high doses. This includes multiple oral doses of 150 mg/kg in mice and 20 mg/kg in non-human primates.\textsuperscript{34} Additionally, a dose range-finding study suggested that GS-441524 was well-tolerated at maximum feasible oral doses of 1,000, 1,500 and 2,000 mg/kg/day in cynomolgus monkeys, rats and dogs, respectively.\textsuperscript{34}

A clinical trial with 31 cats suffering from FIP found no systemic toxicity after treatment with doses of GS-441524 at 2 and 4 mg/kg for 12 to 30 weeks.\textsuperscript{10} Moreover, administration of GS-441524 at doses of 5, 8 and 10 mg/kg for up to 19 weeks did not produce major adverse reactions in four cats with FIP.\textsuperscript{40} The therapeutic doses of 2, 4, 5, and 10 mg/kg, which appeared to be well-tolerated in cats, correspond to HEDs in the range from 0.82 to 4.10 mg/kg per day. These HEDs are significantly lower than the GS-441524 dose of 13 mg/kg that was administered to a healthy human volunteer once daily for seven days and three times daily for three days, respectively, and reported to not be associated with major adverse reactions or significant alterations of key blood parameters.\textsuperscript{34} Hence, the current evidence suggests that GS-441524 is safe in a range of species, albeit with a scarcity of human data being available at present.

8 | EFFECTS OF EXTRACELLULAR AND INTRACELLULAR ADENOSINE LEVELS ON THERAPEUTIC EFFECTIVENESS

Previously, we suggested that endogenous adenosine competes with GS-441524 for cellular uptake by nucleoside transporters and the perceived rate-limiting first step of its phosphorylation to GS-441524 monophosphate eventually leading to the formation of GS-441524 triphosphate.\textsuperscript{11} Under physiological conditions,
### TABLE 5 Cytotoxicity of GS-441524

| Cell line or primary cell culture | CC50 (µM)a | Reference |
|----------------------------------|------------|-----------|
| Vero E6                          | >50        | Li et al.9 |
| Calu-3                           | >50        | Li et al.9 |
| Caco-2                           | >50        | Li et al.7 |
| Vero E6                          | >250       | Shi et al.49 |
| A549 expressing human angiotensin-converting enzyme 2 | >50 | Xie et al.52 |
| Vero E6                          | >100       | Yin et al.50 |
| 293T                             | >30b       | He et al.54 |
| VeroE6 tagged green fluorescent protein | 49–83c | Do et al.27 |
| Huh-7                            | 37–59c     | Do et al.27 |
| Calu-3                           | >50        | Schulz et al.53 |
| Human peripheral blood mononuclear cells | >100 | Zandi et al.56 |
| CEM                              | >100       | Zandi et al.56 |
| Vero                             | >100       | Zandi et al.56 |
| Huh-7                            | >100       | Zandi et al.56 |
| CRFK                             | >100       | Cook et al.63 |
| CRFK                             | >100       | Murphy et al.35 |
| NRK-49F stimulated with transforming growth factor-β | >100 | Xu et al.39 |
| Vero E6                          | >50        | Cao et al.57 |
| HEL                              | >100       | Stevaert et al.64 |
| Vero E6                          | >100       | Lo et al.60 |
| Huh-7                            | >100       | Lo et al.60 |
| HSAEC1-KT                        | >100       | Lo et al.60 |
| TIME                             | >100       | Lo et al.60 |
| Vero E6                          | >100       | Schooley et al.61 |
| Human pluripotent stem cell-derived lung cells | >100 | Schooley et al.61 |
| Calu-3                           | >100       | Schooley et al.61 |
| Huh-7.5                          | >100       | Schooley et al.61 |
| Caco-2                           | >100       | Schooley et al.61 |
| Vero CCL-81                      | >100       | Tao et al.52 |
| Calu-3                           | >100       | Tao et al.62 |
| Caco-2                           | >100       | Tao et al.62 |
| Huh-7                            | >100       | Tao et al.62 |

Note: Origin of cell lines: Vero and Vero E6, African green monkey kidney; Calu-3, human metastatic lung adenocarcinoma; Caco-2, human colorectal adenocarcinoma; A549, human epithelial lung carcinoma; 293T, human embryonic kidney; Huh-7 and Huh-7.5, human hepatocellular carcinoma; CEM, human acute lymphoblastic leukemia; CRFK, cat kidney cortex; NRK-49F, normal rat kidney (fibroblasts); HEL, human erythroleukemia; HSAEC1-KT, human small airway epithelial cells (human telomerase reverse transcriptase-immortalized); TIME, telomerase-immortalized human microvascular endothelium; CC50, 50 percent cytotoxic concentration.

aSeveral studies reported upper concentrations of serial dilutions of GS-441524 for which cell toxicity was not observed but did not determine CC50 values. In such instances, these upper tested concentrations were used as substitutes for CC50 values.
bDetermined based on a plot.
cInterquartile range (Q1–Q3).

eXtracellular and intracellular adenosine levels are in the submicro-molar range, but these levels are significantly elevated in hypoxia and critical illness, occasionally being increased by 5- to 10-fold or more, e.g. reaching a level of 8.4 µM in sepsis.41–44 Such high adeno-sine levels exceed most of the calculated Cav values of GS-441524 including that of 3.89 µM reported in the first-in-human study (Table 1). Importantly, high blood levels of uridine were recently suggested to competitively inhibit cellular uptake of EIDD-1931 (https://www.guidetopharmacology.org/GRAC/DatabaseSearchForward?searchString=EIDD-1931&searchCategories=all&species=none&type=all&comments=includeComments&order=rank), the main metabolite of the cytosine analog prodrug molnupiravir (https://www.guidetopharmacology.org/GRAC/DatabaseSearchForward?searchString=molnupiravir&searchCategories=all&species=none&type=all&comments=includeComments&order=rank&submit=Search+GtoPdb) with activity against SARS-CoV-2,45 thus supporting the notion that levels of endogenous nucleotides can affect therapeutic effectiveness of nucleoside analogs.

## 9 CONCLUSION

In aggregate, publicly available data suggest that GS-441524 has potential for oral treatment of Covid-19 although human oral bioavailability does not appear to be high. Of note, a lower oral dose than the 13 mg/kg dose administered in the so far only reported human PK and safety study (n=1) may be effective against Covid-19. However, GS-441524 may not be equally effective in eliminating SARS-CoV-2 from all tissues and organs, potentially having lower activity against the virus in the airways than in other organs. Also, cellular uptake and intracellular phosphorylation of GS-441524 necessary for antiviral activity could be reduced by competitive inhibition due to increased adenosine levels in subjects with severe Covid-19. GS-441524 appears to be well tolerated in animal species, and although to our knowledge, new clinical studies are not currently registered in major clinical trial registries, further clinical development of the agent appears to be justified.

### 9.1 Nomenclature of targets and ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY,46 and are permanently archived in the Concise Guide to PHARMACOLOGY 2021/22.47,48

**CONFLICT OF INTEREST**

The authors declare no conflict of interest.

**AUTHOR CONTRIBUTIONS**

Henrik Berg Rasmussen identified and collected relevant findings from publicly available sources, conducted calculations, and drafted
the article. Peter Riis Hansen co-wrote the article and provided critical revision. Ragnar Thomsen provided critical revision and suggestions. All three authors interpreted the results from the calculations and approved the final manuscript.

**DATA AVAILABILITY STATEMENT**

No data available in the study.

**ORCID**

Henrik Berg Rasmussen https://orcid.org/0000-0001-9979-7627

**REFERENCES**

1. Srivastava K, Singh MK. Drug repurposing in COVID-19: a review with past, present and future. Metab Open. 2021;12:100121.

2. Beigel JH, Tomashek KM, Dodd LE, et al. Remdesivir for the treatment of Covid-19—final report. N Engl J Med. 2020;383:1813-1826.

3. WHO Solidarity Trial Consortium. Repurposed Antiviral Drugs for Covid-19—Interim WHO Solidarity Trial Results. N Engl J Med. 2021;384(6):497-511.

4. Al-Abdouh A, Bizanti A, Barbarawi M, et al. Remdesivir for the treatment of COVID-19: a systematic review and meta-analysis of randomized controlled trials. Contemp Clin Trials. 2021;101:106272.

5. Eastman RT, Roth JS, Brimacombe KR, et al. Remdesivir: a review of its discovery and development leading to emergency use authorization for treatment of COVID-19. ACS Cent Sci. 2020;6(5):672-683.

6. Mehellou Y, Rattan HS, Balsarini J. The ProTide Prodrug Technology: from the concept to the clinic. J Med Chem. 2018;61(6):2211-2226.

7. Yan VC, Muller FL. Advantages of the parent nucleoside GS-441524 over remdesivir for Covid-19 treatment. ACS Med Chem Lett. 2020;11(7):1361-1366.

8. Prijssers AJ, George AS, Schäfer A, et al. Remdesivir inhibits SARS-CoV-2 in human lung cells and chimeric SARS-CoV expressing the SARS-CoV-2 RNA polymerase in mice. Cell Rep. 2020;32(3):107940.

9. Li Y, Cao L, Li GE, et al. Remdesivir metabolite GS-441524 effectively inhibits SARS-CoV-2 infection in mouse models. J Med Chem. 2022;65(4):2785-2793.

10. Pedersen NC, Perron M, Bannasch M, et al. Efficacy and safety of the nucleoside analog GS-441524 for treatment of cats with naturally occurring feline infectious peritonitis. J Feline Med Surg. 2019;21(4):271-281.

11. Rasmussen HB, Jürgens G, Thomsen R, et al. Cellular uptake and intracellular phosphorylation of GS-441524: implications for its effectiveness against COVID-19. Viruses. 2021;13(7):1369.

12. Nies AT, König J, Hofmann U, Kötz C, Fromm MF, Schwab M. Interaction of remdesivir with clinically relevant hepatic drug uptake transporters. Pharmaceutics. 2021;13(3):369.

13. Van Rompay A, Johansson M, Karlsson A. Phosphorylation of nucleosides and nucleoside analogs by mammalian nucleoside monophosphate kinases. Pharmacol Ther. 2000;87:189-198.

14. National Center for Advancing Translational Sciences. GS-441524 studies. Accessed August 26, 2021. https://opendata.ncats.nih.gov/covid19/GS-441524

15. Humeinik R, Mathias A, Kirby BJ, et al. Pharmacokinetic, pharmacodynamic, and drug-interaction profile of remdesivir, a SARS-CoV-2 replication inhibitor. Clin Pharmacokinet. 2021;60(5):569-583.

16. European Medicines Agency. Summary on compassionate use: Remdesivir Gilead. 03 April 2020; EMA/178637/2020 – Rev.2.

17. Australian Government, Department of Health. Therapeutic Goods Administration. Australian Public Assessment Report for Remdesivir. Published online July 2020.

18. Zhang Y, Huo M, Zhou J, Xie S. PKSolver: an add-in program for pharmacokinetic and pharmacodynamic data analysis in Microsoft Excel. Comput Methods Programs Biomed. 2010;99(3):306-314.

19. Janhavi P, Divyashree S, Sanjail KP, Muthukumar SP. DoseCal: a virtual calculator for dosage conversion between human and different animal species. Arch Physiol Biochem. 2019;1-5. Published online.

20. Nair AB, Jacob S. A simple practice guide for dose conversion between animals and human. J Basic Clin Pharm. 2016;7(2):27-31.

21. Saadh MJ, Haddad M, Dababneh MF, Bayan MF, Al-Jaidi BA. A guide for estimating the maximum safe starting dose and conversion it between animals and humans. Syst Rev Pharm. 2020;11(8):98-101.

22. Watanabe K, Bois FY, Zeise L. Interspecies extrapolation: a reexamination of acute toxicity data. Risk Anal Off Publ Soc Risk Anal. 1992;12(2):301-310.

23. Travis CC, White RK. Interspecific scaling of toxicity data. Risk Anal. 1998;8(1):119-130.

24. Yan VC, Khadka S, Arthur K, Ackroyd JJ, Georgiou DK, Muller FL. Pharmacokinetics of Orally Administered GS-441524 in Dogs. bioRxiv. Published online 2021 February 5:2021.02.04.429674.

25. Van Damme E, De Meyer S, Bojkova D, et al. Intravivo activity of itraconazole against SARS-CoV-2. J Med Virol. 2021;93(7):4454-4460.

26. Abdelnabi R, Foo CS, Jochmans D, et al. The oral protease inhibitor (PF-07321332) protects Syrian hamsters against infection with SARS-CoV-2 variants of concern. Nat Commun. 2022;13(1):719.

27. Do TND, Donckers K, Vangeel L, et al. A robust SARS-CoV-2 replication model in primary human epithelial cells at the air liquid interface to assess antiviral agents. Antiviral Res. 2021;192:105122.

28. Cuccarese MF, Earnshaw BA, Heiser K, et al. Functional immune mapping with deep-learning enabled phenomics applied to immunomodulatory and COVID-19 drug discovery. bioRxiv. Published online 2020 August 14:2020.08.02.233064.

29. Heiser K, McLean PF, Davis CT, et al. Identification of potential treatments for COVID-19 through artificial intelligence-enabled phenomic analysis of human cells infected with SARS-CoV-2. bioRxiv. Published online 23 April 2020:2020.04.21.054387.

30. Friman T, Chernobrovkina A, Martinez Molina D, Arnold L. CETSA MS profiling for a comparative assessment of FDA-approved antivirals repurposed for COVID-19 therapy identifies TRIP13 as a remdesivir off-target. SLAS Discov. 2021;26(3):336-344.

31. Scherf-Clavel O, Kaczmarek E, Kinzig M, et al. Tissue level profile of SARS-CoV-2 antivirals in mice to predict their effects in COVID-19 multiorgan failure. bioRxiv. Published online 24 September 2020.

32. Xie J, Wang Z. Can remdesivir and its parent nucleoside GS-441524 be potential oral drugs? An in vitro and in vivo DMPK assessment. Acta Pharm Sin B. 2021;11(6):1607-1616.

33. Traut TW. Physiological concentrations of purines and pyrimidines. Mol Cell Biochem. 1994;140(1):1-22.

34. Yan VC. First-in-human safety, tolerability, and pharmacokinetics of orally administered GS-441524: a broad-spectrum antiviral treatment for COVID-19. OSF Preprints. 2021 April 9:am5s8.

35. Murphy BG, Perron M, Murakami E, et al. The nucleoside analog GS-441524 strongly inhibits feline infectious peritonitis (FIP) virus in tissue culture and experimental cat infection studies. Vet Microbiol. 2018;219:226-233.

36. Min KA, Talattof A, Tsune Y, et al. The extracellular microenvironment explains variations in passive drug transport across different airway epithelial cell types. Pharm Res. 2013;30(8):2118-2132.

37. Noorafshan A, Motamedifar M, Karbalay-Doust S. Volume and surface changes in vero cell and its nucleus after infection with SARS-CoV-2 and SARS-CoV-2 variants of concern. Vet Microbiol. 2021;219:226-233.

38. Kleinman E, Sierra G, Mao B, MacIsaac D, George MV, Daftarian PM. Adenosine-related small molecules show utility of recall antigen assay to screen compounds for off-target effects on memory T cells. Sci Rep. 2021;11(1):9561.

39. Xu L, Tan B, Huang D, et al. Remdesivir inhibits tubulointerstitial fibrosis in obstructed kidneys. Front Pharmacol. 2021;12:626510.
40. Dickinson PJ, Bannasch M, Thomasy SM, et al. Antiviral treatment using the adenosine nucleoside analogue GS-441524 in cats with clinically diagnosed neurological feline infectious peritonitis. J Vet Intern Med. 2020;34(4):1587-1593.

41. Fredholm BB. Physiological and pathophysiological roles of adenosine. Sleep Biol Rhythms. 2011;9(Suppl 1):24-28.

42. Kumar V. Adenosine as an endogenous immunoregulator in cancer pathogenesis: where to go? Purinergic Signal. 2013;9(2):145-165.

43. Rasmussen HB, Thomsen R, Hansen PR. Nucleoside analog GS-441524: pharmacokinetics in different species, safety, and potential effectiveness against Covid-19. Pharmacol Res Perspect. 2022;10:e00945.

44. Martin C, Leone M, Viviani X, Ayem ML, Guieu R. High adenosine plasma concentration as a prognostic index for outcome in patients with septic shock. Crit Care Med. 2000;28(9):3198-3202.

45. Painter WP, Holman W, Bush JA, et al. Human safety, tolerability, and pharmacokinetics of molnupiravir, a novel broad-spectrum oral antiviral agent with activity against SARS-CoV-2. Antimicrob Agents Chemother. 2021;65(5):e02428-e02520.

46. Harding SD, Sharman JL, Faccenda E, et al. The IUPHAR/BPS Guide to PHARMACOLOGY in 2018: updates and expansion to encompass the new guide to IMMUNOPHARMACOLOGY. Nucleic Acids Res. 2018;46(D1):D1091-D1106.

47. Schooley RT, Carlin AF, Beadle JR, et al. Rethinking remdesivir: synthesis, antiviral activity, and pharmacokinetics of oral lipid produgs. Antimicrob Agents Chemother. 2021;65(10):e0115521.

48. Tao S, Zandi K, Bassit L, et al. Comparison of anti-SARS-CoV-2 activity and intracellular metabolism of remdesivir and its parent nucleoside. Curr Res Pharmacol Drug Discov. 2021;2:100045.

49. Schäfer A, Martinez DR, Won JJ, et al. Therapeutic efficacy of an oral nucleoside analogue of remdesivir against SARS-CoV-2 pathogenesis in mice. bioRxiv. Published online 2021 September 17:2021.09.13.460111.

50. Stevaert A, Krasniqi B, Van Loy B, et al. Betulonic acid derivatives interfering with human coronavirus 229E replication via the nsp15 endoribonuclease. J Med Chem. 2021;64(9):5632-5644.

How to cite this article: Rasmussen HB, Thomsen R, Hansen PR. Nucleoside analog GS-441524: pharmacokinetics in different species, safety, and potential effectiveness against Covid-19. Pharmacol Res Perspect. 2022;10:e00945. doi:10.1002/prp2.945