First-in-Woman Safety, Tolerability, and Pharmacokinetics of Orally Administered GS-441524: A Broad-Spectrum Antiviral Treatment for COVID-19

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ABSTRACT

GS-441524 is a nucleoside analogue with broad-spectrum antiviral activity against RNA viruses, including severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), severe acute respiratory syndrome coronavirus (SARS-CoV), Middle East respiratory syndrome coronavirus (MERS-CoV), and feline coronavirus (FCoV). GS-441524 is the main circulating metabolite following intravenous administration of remdesivir (Veklury®), with a plasma half-life of approximately 24 hours. The safety, tolerability, and pharmacokinetics of GS-441524 was evaluated in a healthy human volunteer (N=1) when administered directly as an oral solution (750 mg) once daily for 7 days (Part 1) and 3 times daily for 3 days (Part 2). In Part 1 of the study, the effect of food on the absorption of GS-441524 was also evaluated. GS-441524 appeared rapidly in plasma, with an average time of maximum concentration of 0.5 hours during once-per-day dosing and exhibited an initial half-life phase of approximately 3.3 hours in the fasted state. Negligible accumulation was observed during part 1 of the multiday study. In Part 2 of the study, GS-441524 was administered 3 times daily, every 3 hours. A 2-4-fold accumulation of GS-441524 was observed approximately 3 hours after the third dose was administered, with a time of maximum concentration of 9 hours and a maximum concentration of 12.01

μM, exceeding the concentration reported to eradicate SARS-CoV-2 *in vitro*. For the duration of the study, GS-441524 was well-tolerated. There were no treatment-related adverse events and no clinically significant findings in clinical laboratory, vital signs, or electrocardiography. Taken together, these results demonstrate the safety and viability of orally administered GS-441524 for the treatment of COVID-19 and emerging viral infections.

INTRODUCTION

In December 2019, a novel coronavirus (SARS-CoV-2) was identified in Wuhan, China and is the causative agent of the COVID-19 pandemic (1). A concerted global effort to develop safe and effective antiviral therapies, especially with the emergence of SARS-CoV-2 variants (2), has thus been exigent. Currently, remdesivir (RDV; Veklury®) is the only FDA-approved treatment for COVID-19 (3) on the basis of a single placebo-controlled randomized clinical trial (RCT) demonstrating accelerated time-to-recovery in treated adults hospitalized with severe COVID-19 (4). However, the efficacy of RDV remains contentious, with other reports demonstrating no statistically significant benefit in patients treated with RDV (5, 6). As a phosphoramidate prodrug, RDV delivers the active nucleotide triphosphate (NTP; GS-443902) that is incorporated into nascent viral RNA strands by the SARS-CoV-2 RNA-dependent polymerase (RdRp), which leads to chain termination (7, 8). The active NTP exhibits high specificity for the SARS-CoV-2 RdRp, with minimal specificity for human DNA and RNA polymerases ($IC_{50} > 200 \mu M$) (9). As a result, treatment with RDV would be expected to yield reductions in viral titers; however, the largest study to-date did not find significant differences in viral titer reduction

between placebo control and RDV-treated patients (5). Modifications to the RDV dosing regimen to increase concentrations of active NTP in the pneumocytes, the main site of SARS-CoV-2 replication (10), are constrained due to dose-limiting toxicities to the liver and kidney (9, 11, 12) as a consequence of RDV's identity as a hydrophobic, esterase-labile McGuigan prodrug (13, 14). RDV is also susceptible to premature hydrolysis by serum esterases, releasing a renally-cleared anionic metabolite (GS-704277), which can be further hydrolyzed to its parent nucleoside, GS-441524 (**Figure 1**) (12, 15).

GS-441524 is the main metabolite of RDV following intravenous (IV) administration (12, 15), with a half-life ($T_{1/2}$) of over 24 hours. Patients treated with RDV have been exposed to maximum concentrations (C_{max}) of GS-441524 of 0.5-0.8 μM (12, 15), while patients with compromised renal function have been exposed to C_{max} concentrations of GS-441524 of up to 5 μM (16). As an adenosine analogue, GS-441524 is phosphorylated to the active NTP through cellular nucleoside and nucleotide kinases that are broadly expressed across all tissues (13). In contrast to the bioactivation pathway for RDV, which relies on enzymes concentrated in the liver, bioactivation of GS-441524 is more even across organs (13). While both GS-441524 and RDV deliver the same, potent NTP inhibitor, differences in prodrug bioactivation pathways result in dramatically different safety profiles. Unlike RDV, which is mechanistically dose-limited by liver toxicities, GS-441524 is a remarkably safe drug, with the CC_{50} values exceeding 100 μM in most cell lines (versus < 10 μM for RDV) (9). Head-to-head comparison between GS-441524 and RDV in SARS-CoV-2-infected cells *in vitro* in Vero E6 and Calu-3 cells demonstrates

similar potency, with the EC₅₀ value for GS-441524 being 0.47 and 0.62 µM, respectively (17).

The long-term safety and efficacy of GS-441524 has been evaluated in cats infected with feline coronavirus (FCoV), which causes feline infectious peritonitis (FIP), a uniformly fatal disease. FIP-afflicted cats treated with GS-441524 (5-12 mg/kg) for up to 12 weeks demonstrated 75-96% cure rates without any treatment-related adverse events (AEs) (18–20). GS-441524 has also demonstrated robust antiviral activity in mouse models of COVID-19 and murine hepatitis virus (MHV), a closely related coronavirus (CoV), without any treatment-related AEs (21, 22). In total, GS-441524 has shown to be exceptionally safe in mice, rats, dogs, cats, and non-human primates. Further studies to reinforce the long-term safety of GS-441524 across preclinical species are ongoing (NCATS OpenData Portal). The genotoxic potential of the active NTP has been evaluated in a standard battery of *in vitro* and *in vivo* genotoxicity evaluations conducted for RDV and was found to be non-genotoxic in these studies (11).

There is an urgent need to expeditiously translate safe and effective anti-SARS-CoV-2 outpatient therapies to the clinic. The most clinically advanced, orally bioavailable direct-acting-antivirals are molnupiravir (23) (NCT04575597) and AT-527 (NCT04709835); however, their shortcomings—mutagenicity (24) and limited anti-SARS-CoV-2 efficacy (25, 26), respectively—warrants continued efforts to develop safe and potent antivirals to the clinic. The exceptional safety profile (9), strong anti-SARS-CoV-2 potency (17, 21, 25), and indirect clinical experience with GS-41524 (12, 15, 16) compels its rapid clinical

translation and investigation as an orally administered antiviral treatment for COVID-19. Here, a two-part open-label study on the safety, tolerability, and pharmacokinetic (PK) parameters of GS-441524 is described in a healthy human volunteer (author V.C.Y.). In Part 1 of the study, the participant was administered 750 mg GS-441524 once per day (QD) for 7 days. An evaluation of fasted (D1-5) and fed (D6-7) states on the pharmacokinetics of the drug was conducted. In Part 2 of the study, the participant was administered 750 mg GS-441524 3 times per day (TID), 3 hours apart, for 3 days. The primary objective of this study was to assess the safety and characterize the pharmacokinetics of orally administered GS-441524 in woman.

METHODS

Ethics

Written informed consent was obtained from the participant (author V.C.Y) enrolled in the study. This study is compliant with Article 5 of the Nuremberg Code (27). Requirements outlined in the Declaration of Helsinki are not applicable to this study, as Article 30 presumes that the investigator and the study participant(s) are different parties. In the present study, there is no delineation between the investigator and the participant and the Declaration of Helsinki does not address such situations (28).

Participant characteristics

The participant (author V.C.Y.) is a healthy, nonpregnant 23-year-old Asian female with a body mass index of 23 kg/m² and no history of illness. Prior to study initiation, the participant received blood analysis (comprehensive metabolic panel (CMP) and complete

blood count (CBC)). Test results were unremarkable (**Tables 1-2; Supplementary File 1**). The participant was screened for and tested negative for HIV, hepatitis B virus, and hepatitis C virus. The participant had not donated plasma or blood within 7 and 56 days prior to study commencement, respectively, and has no history of taking prescription drugs.

Study design

For the duration of the study, GS-441524 (750 mg) was formulated as an oral solution containing 0.4% DMSO and 3% raw, unfiltered honey in water (500 mL, 80°C, pH=6). For reference, this approximates a 16 Oz cup of tea. The solution was consumed over 10 minutes at approximately 55°C. This formulation was selected to maximize the aqueous solubility of GS-441524.

Part 1: 7-day QD study. The participant was administered 750 mg GS-441524 for 7 days. To probe the effects of food on drug absorption, the participant was in the fasted state prior to administration of GS-441524 during D1-D5 and in the fed state during D6-D7 of the study (**Figure 2**). The participant was evaluated for changes in blood chemistry (CMP, CBC) on D4 and on D7 after study completion. Commencement of the 3-day TID study was not initiated until the safety, tolerability, and plasma concentrations of GS-441524 were thoroughly evaluated to ensure that the TID study would proceed safely.

Part 2: 3-day TID study. Initiation of the TID study began 40 days after the QD study for continual assessment of the participant's health. The participant was administered 750

mg GS-441524 TID with a 3 h interval between doses, for 3 days. The initial daily dose was consumed in the fasted state (**Figure 3**). The participant was evaluated for changes in blood chemistry (CMP, CBC) on D4 after study completion.

Dose selection

The 750 mg dose was selected based on the aqueous solubility properties of GS-441524 and safety data observed in preclinical species. Repeated oral administration of GS-441524 has been well-tolerated at 150 mg/kg in mice (unpublished data) and 20 mg/kg in non-human primates (Gilead Sciences, personal communication). The selected 750 mg dose corresponds to a dose of 13 mg/kg.

Blood chemistry

All CBC and CMP measurements were performed Clinical Pathology Laboratories, Inc. (Austin, TX) on a fee-for-service basis.

Pharmacokinetic evaluation

The participant was administered GS-441524 solution orally over a 10-minute period. Post-dose blood samples were collected as early as 5 minutes and up to 24 hours; an additional sample was collected at 48 hours at the conclusion of the 7-day QD study. For D1 of the 3-day TID study, blood samples were collected 1 and 3 hours after each the dose was consumed.

Bioanalytical procedures

Plasma concentrations of GS-441524 were determined using a validated ultra-performance liquid chromatography tandem mass spectrometry (UPLC-MS/MS) method. The validated range for GS-441524 in plasma was 1-3000 ng/mL. Pharmacokinetic parameters were calculated using GraphPad Prism 8.

Safety analyses

For the duration of the study, safety was assessed through reporting of treatment-emergent AEs, clinical laboratory tests (hematology profile, chemistry profile) according to the Division of Microbiology and Infectious Diseases (DMID) toxicity grading (29). Physical examinations and vital signs were also assessed at various points throughout the study. During the multidose study, the participant was periodically assessed for shifts in 6-lead ECGs (AliveCor KardiaMobile 6L).

Sample size and statistical analyses

No formal sample size calculation was performed, as this was an N=1 study. Where applicable, data are presented using descriptive statistics.

RESULTS

Participant demographics and baseline characteristics.

The participant (author V.C.Y.) is a 23-year-old healthy Asian female with unremarkable baseline characteristics (**Table 1-2, Supplementary File 1**).

Safety and tolerability.

GS-441524 was well-tolerated in both parts of the study. No treatment-related AEs or laboratory abnormalities were observed in either part of the study (**Tables 1-2, Supplementary File 1**). For the 3-day TID study, no pattern of clinically relevant changes in vital signs or shifts in 6-lead ECGs were observed during the study.

Pharmacokinetics

Part 1: 7-day single dose study

The plasma pharmacokinetics of GS-441524 following 750 mg QD dosing is summarized in **Figure 2**. Overall, the 7-day pharmacokinetics of GS-441524 appeared consistent. Drug absorption appeared to be improved when fasted. Following administration, GS-441524 appeared rapidly in plasma, with an average time of maximum concentration (T_{max}) value for D1-5 of approximately 0.5 hours in the fasted state and a corresponding average C_{max} value of 888 ng/mL (3.05 μ M). In the fed state (D6-7) a food effect could be observed, with shifts in T_{max} (4 hours) and C_{max} (704 ng/mL, 2.42 μ M) values. In both fasted and fed states, GS-441524 exhibited a long $T_{1/2}$ of approximately 3.3 and 3.7 hours, respectively.

Part 2: 3-day triple dose study

The plasma pharmacokinetics of GS-441524 following 750 mg TID dosing on D1 is summarized in **Figure 3**. Following administration, GS-441524 appeared rapidly in plasma, with a concentration of 1288 ng/mL (4.42 μ M) 1 hour post dose. As anticipated with 3-hour intervals between dosing, each successive dose resulted in accumulation of GS-441524 in plasma. At 1 hour post second dose, the concentration of GS-441524 was

found to be 2030 ng/mL (6.97 μ M), indicating approximately 1.5-fold accumulation. After administration of the third dose, the $C_{\max}$ value (3498 ng/mL; 12.01 μ M) was achieved at 9 hours (3 hours post third dose), indicating a 2.7-4-fold accumulation in plasma GS-441524 compared to single dosing. At the 26-hour timepoint, GS-441524 remained in plasma at a concentration of 291 ng/mL (1 μ M), indicating the likelihood of multi-day bioaccumulation. The area-under-the-curve (AUC_{0-T}) for D1 of the TID study was found to be 48444 ng*h /mL (164.1 μ M*h).

DISCUSSION

The highly transmissible nature of COVID-19 coupled with the risk of severe illness prompts urgent translation of safe and effective anti-SARS-CoV-2 outpatient therapies. GS-441524 is the parent nucleoside and main metabolite of RDV (12, 15) and has exhibited an exceptional safety profile *in vitro* (9, 30) and *in vivo* (19–21). Against SARS-CoV-2 and related CoV models, GS-441524 has demonstrated strong antiviral activity *in vitro* (9, 30) and *in vivo* (19–21). In contrast to the structurally labile nature of RDV that precludes oral administration for COVID-19 (31), GS-441524 possesses no such structural vulnerabilities. On account of its remarkable safety profile and prompted by the growing urgency to develop the safe and effective outpatient therapies (2), the present study was undertaken to evaluate the safety, tolerability, and pharmacokinetics of orally administered GS-441524 in woman.

This paper reports the results of an N=1, first-in-woman study, which comprised of a 7-day QD dosing phase and a 3-day TID dosing phase. Supportive of preclinical findings,

no treatment-related AEs or laboratory abnormalities were observed throughout the entirety of the study. The demonstrated safety profile in woman refutes the initial claim by Le and colleagues that GS-441524 is involved in acute kidney injury (AKI), as plasma $C_{\max}$ concentrations of GS-441524 were approximately 6-fold higher during the 3-day TID study than the trough concentrations of GS-441524 reported in patients with AKI treated with RDV (12.01 vs. 1.93 μM , respectively) (14, 32, 33). In addition to establishing the safety of GS-441524 in human, this report also indicates the viability of orally administered GS-441524 using the described solution formulation. Single-dose preclinical investigations into the oral bioavailability ($F\%$) of GS-441524 have yielded a wide range of $F\%$ from 85% in dog to 8.3% in cynomolgus macaques (NCATS OpenData Portal). The results of this study point to the feasibility of an orally administered GS-441524 solution and indicate that effective anti-SARS-CoV-2 activity may be achieved with BID or TID dosing. Drug absorption appeared to be influenced by a food effect, which has been observed for other orally administered nucleoside analogues (23); further studies should be conducted to verify this preliminary finding, as PK parameters in the fed state are presented as an average of 2 days in a single volunteer. For D1-5, which were conducted in the fasted state, GS-441524 appeared rapidly in plasma, with a $T_{\max}$ of 0.5 hours and an AUC_{0-24} of 9803 $\text{ng}\cdot\text{h}/\text{mL}$ (33.96 $\mu\text{M}\cdot\text{h}$). Following QD dosing, GS-441524 appeared to demonstrate two-phase decay kinetics, with an initial $T_{1/2}$ of approximately 3.3 hours and an estimated $T_{1/2}$ for the second phase of approximately 5 hours. The discrepancy between the prolonged $T_{1/2}$ when GS-441524 is produced as a metabolite of RDV and when administered directly can likely be explained by the fact that RDV permeates red blood cells and forms a reservoir of GS-441524 upon hydrolysis of

its L-alanyl intermediate metabolite. This would enable slow, delayed release of GS-441524 into the bloodstream and explains the prolonged $T_{1/2}$ of GS-441524 as a metabolite of RDV. It should also be noted that the $T_{1/2}$ of GS-441524 would likely be longer in the older patients or in individuals with compromised renal function, as kidney filtration rate tends to decrease with age and given that GS-441524 is renally eliminated (11). While not specifically explored in this study, the consistent pharmacokinetics of GS-441524 in the fasted state over 5 days suggests that BID dosing regimens would result in bioaccumulation, as observed during the TID phase of this study.

PK parameters for D1 of the TID study shows the C_{max} value (3498 ng/mL; 12.01 μ M) of GS-441524 was achieved 3 hours after the third dose was administered (9 hours post initial dose). Under the described TID conditions, the C_{max} value of 12.01 μ M and large drug exposure (164.1 μ M*h) easily surpasses the reported EC_{50} (0.47-0.62 μ M) and EC_{90} (0.7-1 μ M) values for GS-441524 against SARS-CoV-2 and exceeds the concentration required to completely eradicate the SARS-CoV-2 *in vitro* (10 μ M to drive levels of SARS-CoV-2 below the limit of detection in Calu-3 cells (17)). These results sharply contrast those observed for RDV, which achieves transient C_{max} concentrations of 7.34 μ M and 3.78 μ M for IV doses of 225 mg and 150 mg, respectively (12), and falls short of the concentration required to eradicate SARS-CoV-2 *in vitro* (20 μ M for RDV in Calu-3 cells (17)) (**Figure 4**). PK parameters for the 200 mg loading dose of RDV could not be found as this dose was not investigated in the phase 1 study and as subsequent studies have reported PK parameters observed during the cumulative RDV dose (200 mg loading, 100 mg maintenance for 5-10 days) (15). Evaluated in the context of its transient C_{max} ,

low AUC, short drug exposure, and preferential hepatic extraction (12), it is unlikely that the current dose of RDV is sufficient to deliver levels of active NTP required to achieve robust reductions in viral titers in the lungs. Indeed, the largest study to-date did not find significant differences in viral titers in the upper and lower respiratory tracts between RDV- and placebo-treated patients (5), despite the low nanomolar affinity ($K_m \sim 9$ nM) of the active NTP for the SARS-CoV-2 RdRp (7). The present study demonstrates that GS-441524, which forms the same active NTP through a distinct bioactivation pathway (13), achieves sustained plasma exposures reported to inhibit and eradicate SARS-CoV-2 *in vitro* (17). With TID dosing, the AUC_{0-24} of 164.1 $\mu M \cdot h$ surpasses those observed at the highest investigated concentrations of molnupiravir (AUC_{0-last} at 1600 mg = 63 $\mu M \cdot h$; AUC_{0-last} at 800 mg BID = 25 $\mu M \cdot h$) (23), which is has a similar EC_{50} as GS-441524 in culture and is currently being investigated as an oral therapy for COVID-19 in phase 3 trials (NCT04575584, NCT04575597) (**Figure 5**). While these results were most clearly achieved with TID dosing, it is possible that a BID regimen at similar or at a higher dose may likewise achieve similarly favorable results. Due to the N=1 nature of this study, assessment of other dosing regimens was not conducted and the given limitations of an N=1 study inevitably apply. Here, a TID regimen was selected over a BID regimen because the primary goal of this study was to evaluate the multi-day safety of GS-441524 at the highest achievable plasma concentrations. The absence of any hints of saturability upon drug absorption during the TID study coupled with the dose-dependent increases in plasma GS-441524 concentrations, strongly suggests that even higher plasma concentrations could have been attained with higher or additional doses. More comprehensive studies should be conducted to assess the PK and safety of GS-441524

across a range of doses and schedules. Nevertheless, the most resounding finding of this study was demonstration of the excellent safety profile of GS-441524 when directly administered to a human, which has been repeatedly emphasized in multitude preclinical studies (9, 11, 17–20, 22, 30, 34, 35). This indicates that there is sufficient leeway to safely modify the dose to achieve a more pronounced antiviral effect, when necessary. Taken together, this study is the first to report the multi-day safety, tolerability, and pharmacokinetics of orally administered GS-441524 in woman following QD and TID dosing regimens. These results add to the growing list of species (mice, rats, dog, cat, non-human primates, and now human) demonstrating the safety and well-tolerated nature of GS-441524. Because of the highly conserved nature of RdRp across RNA viruses and SARS-CoV-2, GS-441524 is much less susceptible to efficacy-altering mutations, which reinforces the necessity of its development with the emergence of SARS-CoV-2 variants (2, 22). Given the excellent safety profile and sustained plasma concentrations exceeding reported *in vitro* values necessary to eradicate SARS-CoV-2, expedient clinical advancement of GS-441524 is thus imperative.

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Author Contributions

V.C.Y. conceived, designed, and oversaw the study. V.C.Y. analyzed data and wrote the manuscript

Competing Interests

V.C.Y. is the CEO of Copycat Sciences.

Figure 1. Mechanisms of bioactivation for GS-441524 and RDV. GS-441524 and RDV are prodrugs that are intracellularly converted to the same active NTP, GS-443902, through distinct bioactivation pathways. Promoieties and corresponding enzymes indicated in blue for RDV indicate those that are highly expressed in the liver (13). No formal studies have identified the precise enzyme responsible for the initial phosphorylation of GS-441524, but as an adenosine analogue it is likely phosphorylated by adenosine kinase (ADK). K_m values for GS-443902 against the SARS-CoV-2 RdRp were obtained from Gordon et al. *J. Biol. Chem.* (2020) (7).

Part 1: 7-day QD study

Key blood measurements	D0 (baseline)	D4	D8	Ref
Hemoglobin, g/L	13.2	12.8	12.6	11.5-15.5
Hematocrit	40.80	40.50	39.5	34.0-45.0
Creatinine, mg/dL	0.79	0.81	0.81	0.60-1.30
Potassium, mmol/L	4.4	5	5	3.5-5.4
Calcium, mg/dL	9.7	9.7	10	8.5-10.5
eGFR, mL/min	106	102	102	>60
Alanine aminotransferase, U/L	21	20	31	5-40
Aspartate aminotransferase, U/L	32	23	30	9-40

Table 1. Blood chemistry remained normal throughout the 7-day QD study.

Participant was evaluated for changes in baseline blood chemistry midway through the study (D4) and 1 day after study completion (D8) no abnormalities were observed for the duration of the study. Full CBC and CMP results can be found in **Supplementary File 1**.

Part 2: 3-day TID study

Key blood measurements	D0 (baseline)	D4	Ref
Hemoglobin, g/L	12.5	12.6	11.5-15.5
Hematocrit	38.8	39.2	34.0-45.0
Creatinine, mg/dL	0.71	0.71	0.60-1.30
Potassium, mmol/L	4	4.4	3.5-5.4
Calcium, mg/dL	9.5	9.5	8.5-10.5
eGFR, mL/min	120	120	>60
Alanine aminotransferase, U/L	28	24	5-40
Aspartate aminotransferase, U/L	27	18	9-40

Table 2. Blood chemistry remained normal throughout the 3-day TID study.

Participant was evaluated for changes in baseline blood chemistry 1 day after study completion (D4). No abnormalities were observed between baseline and D4. Full CBC and CMP results can be found in **Supplementary File 1**.

Figure 2. Study design and pharmacokinetics of GS-441524 during the 7-day QD study. **(A)** Study schematic. Blood chemistry values were monitored on day 0, 4, and 8 (1 day post final dose). Participant was in the fasted state during D1-5 of the study and in the fed state during D6-7 of the study. **(B)** Plasma PK curves of GS-441524 throughout the 7-day experiment. Blue arrows indicate dosing times. **(C)** Pharmacokinetics of GS-441524 when administered in the fasted state (red, mean $\pm$ SD of 5 repeats) and in the fed state (black, mean $\pm$ SD of 2 repeats) and corresponding summary of PK parameters. Full report on plasma concentrations of GS-441524 can be found in **Supplementary File 2**.

Figure 3. Study design and pharmacokinetics of GS-441524 on D1 of the 3-day TID study. (A) Dosing schematic. Doses were administered 3 hours apart. The initial daily dose was administered in the fasted state. After the first dose was given, the participant was unfasted. The second and third daily doses were administered in the fed state. Blood chemistry values were monitored on day 0 and day 4 (1 day post final dose). **(B)** Plasma PK curve of GS-441524 administered TID. The C_{max} concentration (3489 ng/mL, 12.01 μM) was obtained 3 hours after the third dose (9 hours). At 26 hours post initial dose, the plasma concentration of GS-441524 was 291 ng/mL (1 μM). Data on EC₅₀, EC₉₀, and SARS-CoV-2 eradication concentrations were obtained from Puijssers et al. *Cell Rep.* (2020) (17). **(C)** Summary of key PK parameters. Full report on plasma concentrations of GS-441524 can be found in **Supplementary File 2**.

Figure 4. GS-441524 but not RDV can safely achieve plasma concentrations required to eradicate SARS-CoV-2 *in vitro*. **(A)** Plasma PK of PO 750 mg GS-441524 administered QD (red, dashed line), TID (dark red, solid line), IV RDV 150 mg (light grey, dashed line) and 225 mg (dark grey, solid line) plotted in log scale. Red traces correspond to plasma concentrations of GS-441524. Grey traces correspond to plasma concentrations of RDV. **(B)** Plasma PK data represented in linear scale. While 750 mg TID GS-441524 achieves plasma concentrations capable of eradicating SARS-CoV-2 *in vitro* ($C_{max} = 12.01 \mu\text{M}$), the highest tested concentration of RDV does not ($C_{max} = 7.34 \mu\text{M}$) (17). **(C)** Summary of key PK parameters. Values for RDV were obtained from Humeniuk et al. *Clin. Transl. Sci.* (2020) (12).

Figure 5. Pharmacokinetics of orally administered GS-441524 in the context of oral antivirals in late-stage clinical trials. (A) Plasma PK curves of GS-441524 QD (red dashed line) and TID (red solid line) compared to molnupiravir administered at 1600 mg QD (light grey dashed line) and 800 mg BD (dark grey solid line) (23). **(B)** Plasma PK curves of GS-441524 QD (red dashed line) and TID (red solid line) compared to 600 mg AT-527 QD (black line) (36). **(C)** Summary of PK values.

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