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APPLICATION NUMBER:

214787Orig1s000

CLINICAL MICROBIOLOGY/VIROLOGY
REVIEW(S)

DIVISION OF ANTIVIRAL PRODUCTS (HFD-530)
VIROLOGY REVIEW
NDA: 214787 SDN: 000 DATE REVIEWED: 08/10/2020
Virology Reviewer: Eric F. Donaldson, Ph.D.

Reviewer: Eric F. Donaldson, Ph.D.

Dates Submitted: 04/08/2020 **Dates Assigned:** 04/09/2020

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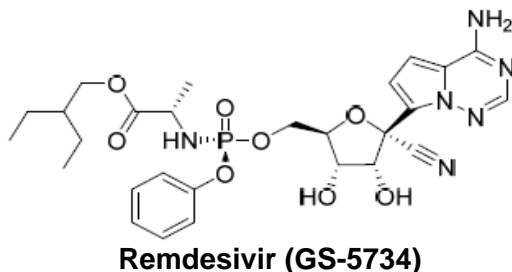
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011	06/25/2020	06/25/2020	07/16/2020
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014	07/15/2020	07/15/2020	07/16/2020
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024	08/13/2020	08/13/2020	08/14/2020
029	08/21/2020	08/21/2020	08/24/2020
033	08/28/2020	08/28/2020	08/31/2020
035	09/01/2020	09/02/2020	09/02/2020
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039	09/08/2020	09/08/2020	09/09/2020
040	09/09/2020	09/09/2020	09/10/2020
044	09/16/2020	09/16/2020	09/17/2020

Product Names: remdesivir (GS-5734)

Chemical Names: 2-Ethylbutyl (2S)-2-[[[(S)-[[[(2R,3S,4R,5R)-5-(4-aminopyrrolo[2,1-f][1,2,4]triazin-7-yl)-5-cyano-3,4-dihydroxytetrahydrofuran-2-yl]methoxy}(phenoxy)phosphoryl]amino}propanoate

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Structure:



Molecular Formula: C₂₇H₃₅N₆O₈P

Molecular Weight: 602.6

Drug Category: antiviral

Indication: Treatment of hospitalized patients with SARS-CoV-2 (aka 2019 nCoV) infection and coronavirus infectious disease 2019 (COVID-19)

Dosage Form/Route of administration: 100 mg powder for aqueous reconstitution/intravenous

Supporting documents: Pre-IND 125566 submissions; IND147753; IND14771

Abbreviations: ACE2, Angiotensin-1 Converting Enzyme-2; ALT, alanine aminotransferase; ARDS, acute respiratory distress syndrome; AST, aspartate aminotransferase; ATP, adenosine triphosphate; BAL, bronchioalveolar lavage; CI, confidence interval; CoV, coronavirus; COVID-19, coronavirus infectious disease originating in 2019; COX-1, cytochrome c oxidase subunit 1; CPE, cytopathic effect; CQ, chloroquine phosphate; CU, compassionate use; DAA, direct-acting antiviral; dpi, days post infection or inoculation; EC₅₀ value; effective concentration 50%; ECMO, extracorporeal membrane oxygenation; EUA, emergency use authorization; ExoN, exoribonuclease; HAE, human airway epithelial; HCoV, human coronavirus; HCV, hepatitis C virus; HCQ, hydroxychloroquine sulfate; HMVEC, human microvascular endothelial cells; IC₅₀ value, inhibitory concentration 50%; IM, intramuscular; IN, intranasal; IQR, interquartile range; IV, intravenous; MERS, Middle East respiratory syndrome; MOI, multiplicity of infection; MS, mass spectrometry; NDA, new drug application; NHBE, normal human bronchial epithelial; nsp, nonstructural protein; NTP, nucleoside triphosphate; OP, oropharyngeal; PBMC, peripheral blood mononuclear cells; PBS, phosphate buffered saline; PEP, postexposure prophylaxis; PFU, plaque forming units; POLRMT, human mitochondrial RNA polymerase (POLRMT); PP, polyprotein; PrEP, pre-exposure prophylaxis; RCT, randomized controlled clinical trial; RdRp, RNA dependent RNA polymerase; RDV, remdesivir; RSV, respiratory syncytial virus; RT-PCR, reverse transcriptase polymerase chain reaction; SAE, serious adverse event; SARS, severe acute respiratory syndrome; SARS-CoV-2, severe acute respiratory syndrome coronavirus type 2; SDH-A, succinate dehydrogenase complex, subunit A; SOC, standard of care; TCID₅₀, tissue culture infectious dose 50%; TP, triphosphate; TRS, transcriptional regulatory sequences;

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EXECUTIVE SUMMARY

Remdesivir is a severe acute respiratory virus type 2 (SARS-CoV-2) nucleotide analog RNA polymerase inhibitor indicated for the treatment of adults and pediatric patients 12 years of age and older, weighing at least 40 kg, and hospitalized with coronavirus infectious disease 2019 (COVID-19). Remdesivir is an adenosine nucleotide prodrug that distributes into cells where it is metabolized to a nucleoside intermediate by carboxyesterase 1 and/or cathepsin A to form a pharmacologically active nucleoside that is phosphorylated by cellular kinases to form a triphosphate analog of ATP, which functions as an inhibitor of the SARS-CoV-2 RNA-dependent RNA polymerase (RdRp) to disrupt viral mRNA and progeny genome synthesis.

SARS-CoV-2 was identified as the cause of an outbreak of respiratory illness (COVID-19) that was first detected in Wuhan, China, in late November 2019 and its genome is ~79% similar to SARS-CoV, but 96% similar to bat CoV RaTG13. SARS-CoV-2 has caused a human pandemic ([Guo et al., 2020](#)), and as of September 15, 2020, there have been over 6,595,000 confirmed cases and more than 195,500 deaths in the US ([Johns Hopkins CSSE](#)).

Remdesivir (RDV) was an early investigational drug candidate considered for the treatment of SARS-CoV-2 infection and COVID-19 disease because it was known to exhibit antiviral activity against SARS-CoV (type 1) and Middle East Respiratory Syndrome CoV (MERS-CoV) in cell culture and in animal models ([Brown et al., 2019](#); [Agostini et al., 2018](#); [Sheahan et al., 2017](#)), two betacoronaviruses closely related to SARS-CoV-2 and with high amino acid sequence identity in the viral encoded RNA-dependent RNA polymerase (nsp12). Safety and pharmacokinetic data from Phase 1 studies helped inform the selection of the dose and durations that were evaluated in the Phase 3 clinical trials.

Remdesivir received Emergency Use Authorization (EUA) on May 1, 2020. Clinical Virology was supportive of the EUA for remdesivir based on the nonclinical virology data for remdesivir against SARS-CoV-2 and topline results from ACTT-1 (CO-US-540-5776; [NCT04280705](#)) which showed that the median time to recovery was 11 days in the remdesivir group compared to 15 days in the placebo group (hazard ratio 1.31; 95% CI 1.12 to 1.54, $p < 0.001$). Clinical Virology was in agreement with authorizing the EUA for remdesivir because based on the totality of the scientific evidence available at the time, it was reasonable to believe that remdesivir may be effective for the treatment of COVID-19 and that the known and potential benefits outweighed the known and potential risks.

Given the rapidly emergent nature of the 2019-2020 pandemic and the urgent need for medical countermeasures against SARS-CoV-2, the development of remdesivir as an antiviral drug for the treatment of SARS-CoV-2 infection and severe COVID-19 disease has occurred rapidly, but each step of the process has been guided by robust scientific data that provided evidence that remdesivir exhibited antiviral activity against SARS-CoV-2 in cell culture and in animal models and provided a clinical benefit for hospitalized patients with severe COVID-19. Importantly, the approval process for remdesivir was not compromised in any manner due to the expedited review timeline.

Clinical Virology recommended that the Applicant assess cell culture combination activity relationships of drugs that may be used in combination with remdesivir. Because hydroxychloroquine sulfate (HCQ) and chloroquine phosphate (CQ) received emergency use authorization on March 28, 2020 (revoked on June 15, 2020), the Applicant performed combination activity assessments between remdesivir and CQ in HEp-2 cells infected with respiratory syncytial virus (RSV). The antiviral activity of RDV against RSV in HEp-2 cells was antagonized in a CQ-dose-dependent manner when CQ was co-incubated in the cultures at clinically relevant concentrations ranging from 10 to 2,560 nM resulting in increases in

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the remdesivir EC₅₀ value ranging from 1.19- to 4.43-fold. Based on these data, RDV and CQ/HCQ should not be co-administered.

No Clinical Virology data were provided as part of the NDA for remdesivir, although the following recommendations were made and the protocols contained language to collect these data when feasible: quantify SARS-CoV-2 RNA from oropharyngeal (OP), plasma, stool, and urine samples; monitor subjects for as long as they continue to shed SARS-CoV-2 RNA in oropharyngeal and stool samples; and collect samples to assess for the development of resistance to remdesivir. The use of a central laboratory was recommended, but was not feasible due to SARS-CoV-2 shipping constraints. The Applicant noted that there were feasibility issues that limited the number of samples that could be collected and the amount of data that could be processed by the time of the NDA submission. Additional data will be required by post-marketing actions.

The nonclinical virology package for remdesivir against SARS-CoV-2 was sufficient to initiate clinical trials. The data from ACTT-1 indicate that there is a clinical benefit for hospitalized patients infected with SARS-CoV-2 with COVID-19 who are treated with remdesivir compared to placebo. No clinical virology data were provided to support efficacy, but based on the demonstrated clinical benefit in the Phase 3 trials and the Applicant's agreement to provide additional clinical virology data post-marketing, this application is approvable from the Clinical Virology perspective.

1 Recommendations

1.1 Recommendation and Conclusion on Approvability

This original NDA for remdesivir is approvable from the Clinical Virology perspective for the treatment of infection caused by SARS-CoV-2 leading to COVID-19.

1.2 Recommendation on Phase 4 Commitments

Clinical Virology reached agreement on the following post-marketing actions with the Applicant:

1. **PMR 4:** Conduct a study to select for remdesivir resistant SARS-CoV-2 variants in cell culture and characterize several independent isolates phenotypically and genotypically.
2. **PMR 5:** Submit all SARS-CoV-2 viral shedding and viral load data from ACTT-1, GS-540-5773, and GS-US-5774 assessing remdesivir including quantitation of viral shedding and viral load for any subject samples that have not been completed to date.
3. **PMC 1:** Submit all sequencing data from ACTT-1, GS-540-5773, and GS-US-540-5774 assessing remdesivir including sequencing of any subject samples that have not been completed to date.
4. **PMC 2:** Submit a comprehensive resistance study reports from ACTT-1, GS-US-540-5773, and GS-54-5774 describing all resistance assessments performed for remdesivir.
5. **PMC 3:** Submit a complete study report for the assessment of the antagonistic effect of chloroquine/hydroxychloroquine on the antiviral activity of RDV against SARS-CoV-2 in human lung cells.

2 Summary of Virology Assessments

2.1 Nonclinical Virology

Mechanism of Action

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- Remdesivir (RDV; GS-5734™) is a single diastereomer monophosphoramidate prodrug of an adenosine nucleoside analog, GS-441524. Once inside cells, remdesivir undergoes rapid and efficient conversion to the pharmacologically active nucleoside triphosphate form GS-443902. Remdesivir is activated to the pharmacologically active nucleoside analog triphosphate, GS-443902, by a sequential metabolic activation pathway. In cells and tissues, remdesivir is converted by hydrolase and phosphoramidase cleavage to the nucleoside analog monophosphate. Further phosphorylation by nucleotide kinases results in formation of GS-443902. Efficient formation of the pharmacologically active triphosphate, GS-443902, has also been observed in a number of human lung cell types in cell culture including normal human bronchial epithelial (NHBE) and Calu-3 cells as well as in peripheral blood mononuclear (PBMC)s, macrophages, monocytes, and human microvascular endothelial cells. Once formed, GS-443902 has been observed to have a half-life in excess of 15 hours in the various cell types mentioned, following incubation with remdesivir in cell culture.
- Remdesivir interacts with the SARS-CoV-2 RdRp and is preferentially incorporated, with a selectivity value of 0.26 (3.65-fold), into the nascent RNA strand in place of native ATP, which results in a delayed chain termination at position i+3.
- In a biological assay assessing RDV triphosphate (RDV-TP) incorporation by the MERS-CoV RdRp complex, increasing concentrations of RDV-TP caused a reduction in 14-mer RNA synthesis products due to RNA synthesis arrest at position i+3 with an IC₅₀ value of 0.032 μM.
- Mechanism of action studies indicated that RDV is a direct-acting inhibitor of SARS-CoV-2 RdRp (nsp12).

Cell Culture Antiviral Activity

- Remdesivir exhibited cell culture antiviral activity against a clinical isolate of SARS-CoV-2 in primary human airway epithelial (HAE) cells with an EC₅₀ value of 9.9 nM after 48 hours of treatment. The selectivity index for RDV against SARS-CoV-2 in primary HAE cells is >1,000.
- Remdesivir inhibited the replication of SARS-CoV-2 in Vero cells with an EC₅₀ value of 137 nM and 750 nM after 24- and 48-hours of treatment, respectively. The CC₅₀ value for remdesivir was >100 μM and selectivity index >730 for the 24-hour and >133 for the 48-hour treatment. No cytotoxicity was observed for remdesivir in primary HAE cells at concentrations up to 10 μM (CC₅₀ value >10 μM).
- Two different EC₅₀ values were reported for remdesivir against SARS-CoV in HAE cultures derived from different donors but assayed under the same experimental conditions:
- An EC₅₀ value of 6.6 nM was reported in Study [PC-540-2003](#), which was >10-fold lower than an EC₅₀ value of 69 nM reported for RDV against SARS-CoV in HAE cultures derived from a different donor but assayed under the same experimental conditions ([Sheahan et al., 2017](#)).
- This comparison highlights a 10-fold difference in remdesivir antiviral activity associated with host genotype that predicts the potential for highly variable results between individuals treated with this drug.
- These studies indicate that remdesivir is a direct-acting antiviral that inhibits replication of SARS-CoV-2 by interacting with the RdRp (nsp12) to terminate chain elongation.
- To estimate the plasma protein-adjusted antiviral potency of remdesivir (GS-5734), the ratio of free drug fraction between cell culture media and human plasma was taken into account. Competitive equilibrium dialysis followed by LC-MS analysis detected a 4.1-fold higher free drug

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fraction in cell culture media compared to human plasma. Based on these analyses, there would be a 4.1-fold increase in the EC₅₀ value for remdesivir in the presence of human plasma.

Cytotoxicity

- Remdesivir (GS-5734) was generally cytotoxic at low micromolar concentrations against a variety of cell lines and primary cells representing different tissues.
- Remdesivir (GS-5734) also had low micromolar cytotoxicity for human myeloid, erythroid and megakaryoid bone marrow progenitor cells in colony formation assays.
- In general, the remdesivir (GS-5734) monophosphate prodrug was substantially more cytotoxic than the non-phosphorylated parent nucleoside, GS-441524, although both compounds had bone marrow progenitor cytotoxicity.
- GS-443902 (active triphosphate of GS-5734) showed no inhibition of human DNA polymerases α , β , or γ , human RNA polymerase II.

Mitochondrial toxicity

- Remdesivir (GS-5734) did not have a consistent signal of specific mitochondrial toxicity based on multiple different cell-based assays, including assessments of cytotoxicity in cells grown in glucose- versus galactose-containing medium (“crabtree effect”), mitochondrial-specific protein synthesis, mitochondrial respiration capacity, and mitochondrial DNA levels.
- GS-443902 also was not incorporated into DNA by the human mitochondrial DNA polymerase γ , and was a relatively poor substrate (~6% relative to ATP) for incorporation into RNA by human mitochondrial RNA polymerase (Study Report [PC-399-2017](#)).

Cell Culture Combination Activity Relationships

- Because HCQ and CQ received emergency use authorization on March 28, 2020, combination antiviral activity assessments were performed with remdesivir and CQ in HEp-2 cells infected with respiratory syncytial virus (RSV). The antiviral activity of RDV against RSV in HEp-2 cells was antagonized in a CQ-dose-dependent manner when CQ was co-incubated in the cultures at clinically relevant concentrations ranging from 10 to 2,560 nM with EC₅₀ value increases ranging from 1.19- to 4.43-fold ([PC-540-2009](#)).
- Co-incubation of RDV and CQ resulted in a CQ dose-dependent reduction in formation of intracellular RDV-TP, indicating an antagonistic effect of CQ on RDV metabolism to its active triphosphate. Significant CQ antagonism of RDV-TP formation was observed at 8 h post treatment with 1 and 10 μ M CQ concentrations resulting in >2-fold reductions of RDV-TP, respectively. RDV-TP reductions persisted for the 48-hour duration of the assay. The antagonism of remdesivir by CQ was addressed in labeling.

Animal Studies of RDV

- None of the animal model studies assessed RDV (GS-5734) in a robust treatment paradigm where treatment was initiated 3 or 4 (or more) days after CoV infection.
- Only one study to date has been conducted in rhesus macaques infected with SARS-CoV-2. All six rhesus macaques treated with remdesivir had significantly reduced clinical signs of SARS-CoV-2 infection compared to the vehicle-treated animals (P <0.001). Consistent with these observations, lung pathology assessed by radiography showed reduced pulmonary infiltrates in the lungs of remdesivir-treated animals compared to the vehicle control (P <0.01). Upon

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necropsy at 7 days post-infection (dpi), remdesivir-treated animals had significantly reduced gross lung lesions ($P < 0.0001$) and on an average $2.2 \log_{10}$ lower viral RNA levels in lung tissue compared to vehicle-treated animals ($P < 0.001$). Minimal differences in viral RNA levels were observed in nose and throat swabs, while no statistically significant differences were observed in rectal swab and bronchoalveolar lavage (BAL) between the groups. SARS-CoV-2 was not detected in BAL at 3 dpi and in lung tissue at 7 dpi in remdesivir-treated animals but could still be isolated from the vehicle-treated animals.

- These data support early remdesivir treatment in COVID-19 patients to prevent progression to severe pneumonia. Dose initiation at twelve hours would seem to be too early for disease signs to have developed so this study may be more representative of post-exposure prophylaxis. Analysis of viral shedding and viral load data appears to be consistent with this.
- RDV (GS-5734) exhibited antiviral activity in the rhesus macaque SARS-CoV-2 challenge model, indicating that RDV (GS-5734) treatment early in the SARS-CoV-2 infection may be effective. It is not clear if RDV (GS-5734) treatment later in the infection cycle will be beneficial.

Resistance assessments

- SARS-CoV-2 RdRp (nsp12) substitutions leading to reduced susceptibility to RDV (GS-5734) will likely occur when the drug is in widespread use.
- There are currently no known polymorphisms among clinical isolates in the SARS-CoV-2 active site or within 10 Å of the active site; however, the potential for SARS-CoV-2 resistance to RDV (GS-5734) has not been adequately described.

2.2 Clinical Virology

Days Symptomatic Prior to RDV (GS-5734) Treatment

Clinical Virology was interested in determining if the clinical benefit observed for patients treated with remdesivir was greater among patients who were treated earlier in the SARS-CoV-2 infection cycle. In general, hospitalized patients infected with SARS-CoV-2 were treated 8-10 days (based on median values) after the onset of symptoms.

NIAID ACTT-1 Trial ([NCT04280705](#)). The median time from symptom onset prior to enrollment was 9 days for all subjects. Subgroup analysis compared efficacy among patients who were randomized within 10 days of symptom onset to patients who were enrolled 10 days or later after the onset of symptoms. Both subgroups experienced a greater clinical benefit than the placebo group with rate ratios for recovery of >1.0 , but a significant difference was not seen between these subgroups. Patients who were randomized within 10 days of symptom onset had a rate ratio for recovery of 1.28 (95% CI, 1.05 to 1.57; 664 patients), whereas patients who underwent randomization more than 10 days after the onset of symptoms had a rate ratio for recovery of 1.38 (95% CI, 1.05 to 1.81; 380 patients) ([Beigel et al., 2020](#)).

Study GS-US-540-5773 ([NCT04292899](#)). Median duration of symptoms prior to first dose of RDV was 8 days for patients in the 5-Days RDV Treatment Group and 9 days for patients in the 10-Days RDV Treatment Group. The median duration of hospitalization prior to the first dose of RDV (2 days) were similar across treatment groups ([Goldman et al., 2020](#)).

Study GS-US-540-5774 ([NCT04292730](#)). The median duration of symptoms prior to treatment in the standard of care group was 9 days (IQR, 6-11 days) compared with 8 days (IQR, 5-11 days) for both

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groups receiving remdesivir. The median duration of hospitalization prior to the first dose of RDV (2 days) were similar across treatment groups ([Spinner et al., 2020](#)).

SARS-CoV-2 RNA Shedding

No Clinical Virology data were provided as part of the NDA for remdesivir, although the following recommendations were made and the protocols contained language to collect these data when feasible: quantify SARS-CoV-2 RNA from oropharyngeal, plasma, stool, and urine samples; monitor subjects for as long as they continue to shed SARS-CoV-2 RNA in oropharyngeal and stool samples; and collect samples to assess for the development of resistance to remdesivir. The use of a central laboratory was recommended, but was not possible due to SARS-CoV-2 shipping constraints. The Applicant noted that there were feasibility issues that limited the number of samples that could be collected and the amount of data that could be processed by the time of the NDA submission. Additional data will be required by post-marketing actions.

SARS-CoV-2 Resistance to RDV (GS-5734)

No data were submitted from the clinical trials.

Baseline RDV (GS-5734) Resistance Analysis

No data were submitted from the clinical trials.

3 Administrative Signatures

3.1 Reviewer's Signature

Eric F. Donaldson, Ph.D.
Clinical Virology Reviewer, Division of Antivirals

3.2 Concurrence

Julian J. O'Rear, Ph.D.
Clinical Virology Team Leader, Division of Antivirals

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OND VIROLOGY REVIEW

1 Introduction and Background

1.1 Important Milestones in Product Development

Compassionate Use

Remdesivir was an early investigational drug candidate considered for the treatment of SARS-CoV-2 infection and COVID-19 disease because it was known to exhibit antiviral activity against SARS-CoV and MERS-CoV in cell culture and in animal models ([Brown et al., 2019](#); [Agostini et al., 2018](#); [Sheahan et al., 2017](#)), two *betacoronaviruses* related to SARS-CoV-2.

Emergency Use Authorization

Remdesivir received Emergency Use Authorization (EUA) on May 1, 2020. The sponsor submitted a Pre-EUA request for remdesivir on April 8, 2020. On April 17, 2020 the sponsor submitted a formal EUA request under [EUA-000046](#). From the initial pre-EUA request through the approval of the EUA on May 1, 2020, there were several rounds of revisions to the EUA template, the Health Care Professionals Fact Sheet, and the Patient/Caregiver Fact Sheet. Clinical Virology was supportive of the EUA for remdesivir based on the topline results from CO-US-540-5776 ([NCT04280705](#)) which showed that the median time to recovery was 11 days in the remdesivir group compared to 15 days in the placebo group (hazard ratio 1.31; 95% CI 1.12 to 1.54, $p < 0.001$). Mortality was 8.0% for the remdesivir group versus 11.6% for the placebo group ($p = 0.059$). Clinical Virology was in agreement with authorizing the EUA for remdesivir because based on the totality of the scientific evidence available at the time, it was reasonable to believe that remdesivir may be effective for the treatment of COVID-19 and that the known and potential benefits outweighed the known and potential risks. Of note, no clinical virology data have been submitted from the NIAID clinical trial.

1.2 Methodology

Sampling for Clinical Trials

Clinical Virology made several recommendations to the Applicant during clinical trial protocol development:

1. Use a standardized method for collection of oropharyngeal samples for virologic assessments and have analyses performed by a central laboratory.
2. Provide a detailed description of the methodology and performance characteristics of the quantitative RT-PCR assay in the final clinical study report.
3. Quantify viral RNA in stool and plasma in addition to oropharyngeal samples.
4. Monitor subjects for as long as they continue to shed viral RNA in oropharyngeal and stool samples.
5. If possible, collect samples from subjects who show a lack of response or rebound for resistance analysis.

RT-PCR analysis of viral shedding

At the time of protocol development, an RT-PCR assay specific to SARS-CoV-2 had not been developed by the Applicant, and it was not clear which assays would be used to confirm SARS-CoV-2 infection at the time of treatment initiation at the various clinical trial study sites. The protocols allowed some flexibility as different study sites could use different assays. The Applicant agreed to collect

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information on the quantitative RT-PCR assays used at each site and provide assay details in the final clinical study report.

Resistance Assessments

The Applicant planned to work with a subset of clinical trial study sites that had the capability to perform viral sequencing from patient samples to generate data for resistance analysis.

1.3 Prior FDA Virology Reviews

A total of 27 Clinical Virology reviews were written for submissions to the investigational new drug development program for remdesivir used in the treatment of SARS-CoV-2, which spanned from 2/25/2020 to 8/03/2020.

1.4 Major Virology Issues that Arose during Product Development

Therapeutic Window

Early in the SARS-CoV-2 pandemic, it appeared that SARS-CoV-2 replication primarily occurred during the first week of infection with severe cases of COVID-19 developing in the second week driven by the development of a cytokine storm, which contributed to acute respiratory distress and severe pneumonia ([Huang et al., 2020](#)). It was not clear at the time if an antiviral drug would have much impact after the first week of infection when viral replication was starting to decline, indicating that antiviral drug treatment should be initiated as early after infection as possible. However, remdesivir is administered by IV, which limited the patient population to those who were hospitalized with more severe COVID-19 cases who were often beyond the first week of SARS-CoV-2 infection (median range of 8-10 days symptomatic before treatment initiation in clinical trials described in this application). As the pandemic progressed and additional information about SARS-CoV-2 viral kinetics became available, the scientific data indicated that SARS-CoV-2 viral kinetics are highly variable and may be impacted to some degree by age, host genetics, and the disease state of the patient (asymptomatic, mild, moderate, severe, and critical), with more severe cases shedding SARS-CoV-2 RNA (as detected by RT-PCR) from respiratory samples for longer durations ([Chang et al., 2020](#); [He et al., 2020](#); [Li et al., 2020](#); [Xiao et al., 2020](#); [Zhou et al., 2020](#)).

Only a few studies have compared the detection of SARS-CoV-2 viral RNA measured by RT-PCR to replication-competent SARS-CoV-2 as measured by plaque assay ([Bullard et al., 2020](#); [Wölfel et al., 2020](#)). One study that looked at SARS-CoV-2 RNA measured by RT-PCR, replicating SARS-CoV-2 measured by plaque assay, and seroconversion concluded that virus elimination does not directly correlate with seroconversion, but results in a slow but steady decline of viral load in sputum ([Wölfel et al., 2020](#)). These observations indicate that the therapeutic window for some patients with SARS-CoV-2 may extend beyond 1-2 weeks, and that direct-acting antivirals may be effective in reducing the disease course. However, treatment as early as possible in the disease course may provide the greatest clinical benefit.

CQ/HCQ Antagonism

In response to the original IND submission on February 28, 2020, Clinical Virology recommended that the Applicant assess cell culture combination activity relationships of drugs that may be used in combination with remdesivir. The Applicant performed combination activity assessments between remdesivir and CQ in HEp-2 cells infected with RSV. The antiviral activity of RDV against RSV in HEp-2 cells was antagonized in a CQ-dose-dependent manner when CQ was co-incubated in the cultures at clinically relevant concentrations ranging from 10 to 2,560 nM with increases in the RDV EC₅₀ value ranging from 1.19- to 4.43-fold ([PC-540-2009](#)).

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Co-incubation of RDV and CQ resulted in a CQ dose-dependent reduction in formation of intracellular RDV-TP, indicating an antagonistic effect of CQ on RDV metabolism to its active triphosphate. Significant CQ antagonism of RDV-TP formation was observed at 8 hours post treatment with 1 and 10 μM CQ concentrations resulting in >2-fold reductions of RDV-TP, respectively. RDV-TP reductions persisted for the 48-hour duration of the assay. The antagonism of remdesivir by CQ was addressed in labeling.

1.5 State of Antivirals Used for the Indication Sought

There are no approved antivirals for the treatment of SARS-CoV-2 to date.

2 Nonclinical Virology

Coronaviruses (CoVs) are enveloped, positive-sense, single-stranded, RNA viruses, with a genome size of approximately 30 kilobases, the largest of all RNA viruses. Coronaviruses belong to the Family *Coronaviridae*, and are divided into four genera (*Alpha-*, *Beta-*, *Gamma-* and *Deltacoronavirus*). There are 7 known strains of CoVs that infect humans (HCoVs) and cause respiratory diseases, and these can be loosely categorized as the *common cold coronaviruses* (two from the *Alphacoronavirus* genus: HCoV-NL63 and -229E and two from the *Betacoronavirus* genus: -HKU1 and -OC43) that are responsible for ~30% of common colds each year, and the *severe respiratory syndrome coronaviruses* that cause severe lung disease resulting in acute respiratory distress syndrome (ARDS) and higher mortality rates, including SARS-CoV, SARS-CoV-2, and MERS-CoV. SARS-CoV and SARS-CoV-2 belong to lineage B of the *Betacoronavirus* genus, while MERS-CoV belongs to lineage C of the genus.

SARS-CoV-2 was identified as the cause of an outbreak of respiratory illness (COVID-19) that was first detected in Wuhan, China, in late November 2019 and its genome is ~79% similar to SARS-CoV, but 96% similar to bat CoV RaTG13. SARS-CoV-2 has caused a human pandemic ([Guo et al., 2020](#)), and as of September 15, 2020, there have been nearly 6,595,000 confirmed cases and more than 195,500 deaths in the US ([Johns Hopkins CSSE](#)).

Coronaviruses are spherical or pleomorphic, with a diameter of 80–120 nm. Under the electron microscope, the virion surface is decorated with club-like projections constituted by the trimeric S, which gives the virus a crown-like appearance ([Fung and Liu, 2019](#)). The CoV transmembrane S protein trimers promote attachment and fusion of the viral and cellular membranes, are essential for virus infectivity, and are the main target of the humoral immune response. For SARS-CoV-2 (and SARS-CoV) the primary cellular receptor used for attachment to cells is Angiotensin-1 Converting Enzyme-2 (ACE2) ([Wan et al., 2020](#); [Shang et al., 2020](#)).

The SARS-CoV-2 positive-sense genomic RNA has a 5'-cap and a 3'-poly(A) tail and acts as an mRNA for immediate translation of the viral polyproteins. In addition, both 5'- and 3'-ends of the genomic RNA present a highly structured untranslated region (UTR) that plays an important role in the regulation of RNA replication and transcription. The SARS-CoV-2 genome contains 14 open reading frames (ORFs), preceded by transcriptional regulatory sequences (TRSs). The two main transcriptional units, ORF1a and ORF1ab, encode replicase polyprotein 1a (PP1a) and polyprotein 1ab (PP1ab), respectively (Figure 2-1). The largest polyprotein PP1ab contains non-structural proteins (Nsp1-16), which form the complex replicase machinery. At the 3' end, the viral genome encodes four structural proteins (spike, envelope, membrane, nucleocapsid), which are components of the mature virus and play a crucial role in viral structure. Interspersed among the structural genes, the 3' end of the genome also contains nine putative ORFs for accessory factors (Figure 2-1). The structural and accessory proteins are translated from a nested set of sub-genomic mRNAs whose synthesis require the TRSs.

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TRSs are present in the leader sequence at the 5' end of the genome and precede each viral structural and accessory protein gene. During antisense RNA synthesis, the viral RNA polymerase pauses at each TRS element, and if antisense strand synthesis is not halted, it continues synthesis through the TRS into the adjacent gene. If termination of the antisense strand synthesis occurs at one of the TRSs, the viral RNA polymerase switches to the nascent antisense strand and uses it as a template to generate the subgenomic mRNA (reviewed in [Romano et al., 2020](#) and references therein). Transcription of the antisense strand continues to the 5' end of the antisense template such that all subgenomic mRNAs have a 3' end equivalent to the genomic RNA ([Sola et al., 2015](#)). This RNA synthesis process results in an enrichment of nonstructural subgenomic mRNAs and an enrichment of nonstructural proteins based on their position in the genome (Nucleocapsid >Membrane >Envelope >Spike) ([Romano et al., 2020](#)).

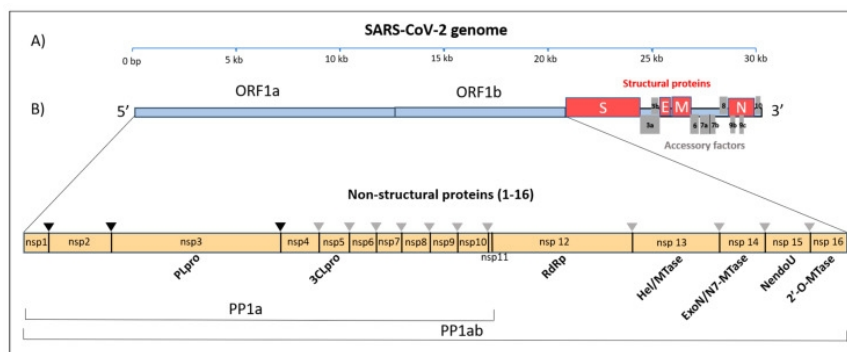


Figure 2-1. SARS-CoV-2 polycistronic genome (Figure 1 and legend copied from [Romano et al., 2020](#)). (A) Genome of SARS-CoV-2 organized in individual ORFs. (B) Polyprotein 1ab (PP1ab) embeds 16 non-structural proteins (nsps); the black and grey triangles indicate the cleavage sites of the protease PLpro and 3CLpro, respectively. Names of confirmed and putative functional domains in the nsps are also indicated.

The viral RNA replication machinery of SARS-CoV-2 involves an array of functional proteins from the N- to C-termini of the polyprotein, PP1ab (Figure 2.1). These include the essential RNA-dependent RNA polymerase (RdRp, nsp12), the zinc-binding helicase (HEL, nsp13) and a number of other enzymatic functions related to viral RNA modification, such as mRNA capping (nsp14, nsp16), RNA proofreading (nsp14), and uridylate-specific endoribonuclease activity (NendoU, nsp15), which has been shown to counteract double-strand RNA sensing. The activity of these enzymes is further regulated by the association with other non-structural proteins (nsp7–nsp10) that are likely necessary to achieve all of the replication and transcription processes. As observed for other nidoviruses, all of these protein subunits likely associate in a replication transcription enzyme complex anchored to membranes derived from the host cell ER, which drives the synthesis of new genome molecules and also sub-genomic mRNAs.

2.1 Mechanism of Action

Metabolism to active triphosphate

Remdesivir (RDV; GS-5734™) is a single diastereomer monophosphoramidate prodrug of an adenosine nucleoside analog, GS-441524. Early studies relevant to RDV were conducted with the diastereomeric mixture GS-466547 (approximately 1:1 mixture of remdesivir [GS-5734] and its diastereomer at phosphorus). Based on antiviral activity, as well as cell culture and in vivo pharmacokinetic profiles, a single diastereoisomer (remdesivir, GS-5734) was selected for further development. In nonclinical studies, the isomer remdesivir (GS-5734) performed similarly to the mixture GS-466547, and results generated with both remdesivir (GS-5734) and GS-466547 were considered relevant and included in various study reports reviewed in this section. Table 2.1-1 lists the current

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nomenclature for RDV, the diastereomeric mixture, and the related metabolites referred to throughout this review.

Table 2.1-1. Remdesivir (GS-5734) and its diastereomers and metabolites, with molarity-mass/volume conversion factors (Table 1, page 6, Nonclinical Overview).

Gilead No.	Description	Conversion Factors
Remdesivir (GS-5734, GS-643134)	Nucleotide prodrug	1 μ M = 0.603 μ g/mL
GS-466547	Diastereomeric mixture at phosphorus containing GS-5734	1 μ M = 0.603 μ g/mL
GS-704277	Metabolite	1 μ M = 0.442 μ g/mL
GS-441524	Nucleoside analog	1 μ M = 0.291 μ g/mL
GS-719700	Nucleoside analog monophosphate	1 μ M = 0.369 μ g/mL
GS-719699	Nucleoside analog diphosphate	1 μ M = 0.448 μ g/mL
GS-443902	Pharmacologically active nucleoside triphosphate	1 μ M = 0.527 μ g/mL

Remdesivir was selected for further development based on its ability to widely distribute into tissues and their cells. Once inside cells, remdesivir undergoes rapid and efficient conversion to the pharmacologically active nucleoside triphosphate form GS-443902. Efficient distribution of remdesivir and/or the diastereomeric mixture GS-466547 and subsequent metabolism to the nucleoside triphosphate GS-443902 has been demonstrated in multiple cell types (discussed below).

Combined results from nonclinical pharmacology and PK studies led to the proposed intracellular metabolic/activation pathway of RDV (Figure 2.1-1).

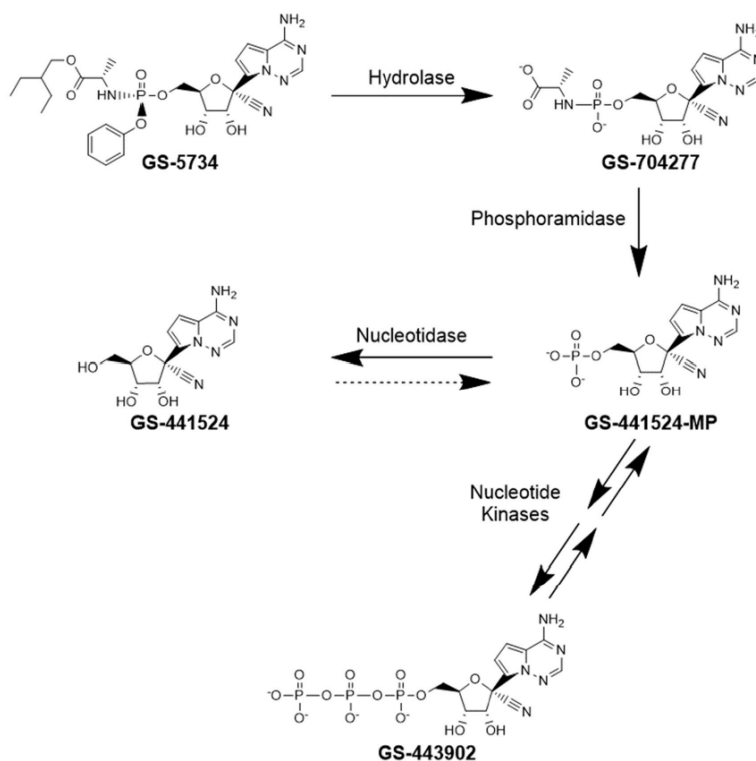


Figure 2.1-1. Proposed Intracellular Metabolic Pathway of RDV (Figure 2, page 25, Summary Clinical Pharmacology).

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Remdesivir is activated to the pharmacologically active nucleoside analog triphosphate, GS-443902, by a sequential metabolic activation pathway: (i) hydrolase activity removes the ester, resulting in the release of 2-ethyl-butanol; (ii) a chemical step that releases phenol results in formation of the intermediate metabolite, GS-704277; (iii) phosphoramidase activity cleaves the phosphoramidate bond, liberating the nucleoside analog monophosphate and alanine; and (iv) nucleotide kinases likely catalyze the conversion to the active triphosphate, GS-443902.

Remdesivir was selected for its ability to distribute into cells, including those within tissues, where it is metabolized to form the pharmacologically active nucleoside triphosphate metabolite, GS-443902. In cells and tissues, remdesivir is converted by hydrolase and phosphoramidase cleavage to the nucleoside analog monophosphate. Further phosphorylation by nucleotide kinases results in formation of GS-443902. Efficient formation of the pharmacologically active triphosphate, GS-443902, has also been observed in a number of human lung cell types in cell culture including normal human bronchial epithelial (NHBE) and Calu-3 as well as in PBMCs, macrophages, monocytes, and human microvascular endothelial cells (HMVEC). Once formed, GS-443902 has been observed to have a half-life in excess of 15 h in the various cell types mentioned, following incubation with remdesivir in cell culture.

One of the main target tissues for SARS-CoV-2 is the lung, which is largely inaccessible for routine sampling. Therefore, a sampling surrogate was used to assess target distribution and metabolism of RDV. Circulating PBMCs are frequently collected to assess active metabolite exposure and antiviral potency of nucleos(t)-ide analogs, or respective prodrugs, in human patients. To support nonclinical evaluations, and to assess potential differences in metabolism between monkey and human, the metabolism of remdesivir was compared in human and rhesus PBMC and monocytes. Intracellular triphosphate concentrations were higher in human PBMC (3.64-fold) and monocytes (4.24-fold) compared to respective monkey cell types in these cells (Figure 2.1-2).

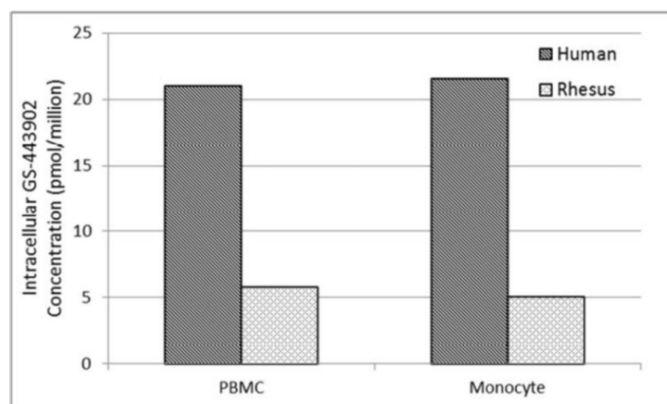


Figure 2.1-2. Comparison of intracellular GS-443902 concentrations formed in peripheral blood mononuclear cells and monocytes from human and rhesus monkey during incubation with remdesivir (Figure 17, page 43, Pharmacokinetics Written Summary). Concentrations measured following a 2 h incubation with 1 μ M remdesivir. Values are the average of n = 2 donors with duplicate measurements in the cells from each donor.

NHBE cells are representative target cells for SARS-CoV-2 infection. Therefore, the kinetics of loading of NHBE by remdesivir and subsequent metabolism to GS-441524 and its phosphorylated metabolites, followed by their elimination, were studied following a 2 h pulse incubation with 1 μ M remdesivir, in NHBE cell culture. The pulse incubation is designed to mimic the transient exposure to prodrug following IV administration. The pharmacologically active triphosphate analog, GS-443902, was

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efficiently formed in NHBE cells from three different donors (mean GS-443902 C_{max} of 14.5 ± 12.7 pmol/million cells and persisted with a $t_{1/2}$ of approximately 15 h (Figure 2.1-3).

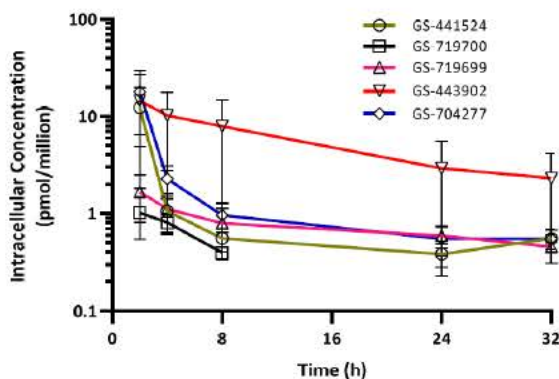


Figure 2.1-3. Mean intracellular concentrations of GS-441524 and metabolites in NHBE cells following a 2-hour continuous incubation with remdesivir (1 μM) and for up to 30 hours following removal from extracellular media (mean, $n=3$ experiments) (Figure 18, page 44, Pharmacokinetics Written Summary).

Calu-3 is a lung cell line which may be used to assess antiviral activity against SARS-CoV-2. An experiment was carried out to characterize GS-443902 concentrations following continuous incubation for 48 h with either 1 μM remdesivir or its nucleoside metabolite, GS-441524 at 10 μM in Calu-3 cells. GS-443902 was again efficiently formed intracellularly, with almost 3-fold higher levels following incubation with remdesivir compared to GS-441524, on a dose-normalized basis.

Antiviral mechanism of action

The Applicant provided one study report to support the mechanism of action for remdesivir against SARS-CoV-2.

Study Title: In Vitro Antiviral Activity of Remdesivir against SARS-CoV-2 RNA-Dependent RNA Polymerase

Study Report: [PC-540-2005](#)

Objective: The purpose of this study was to characterize the inhibitory activity and mechanism of action of remdesivir against several coronavirus RdRps including SARS-CoV-2 RdRp in biochemical assays.

Methods: The active SARS-CoV-2 RdRp complex comprised of recombinant non-structural proteins nsp8 and nsp12 was expressed and purified and used to biochemically assess the inhibitory activity of remdesivir. The pFastBac-1 ^{(b) (4)} plasmid with the codon-optimized synthetic DNA sequences ^{(b) (4)} coding for a portion of the SARS-CoV (NCBI: AAP33696.1) and SARS-CoV-2 (NCBI: QHD43415.1) PP1ab polyproteins containing nsp5 (Mpro), nsp7 (replicase co-factor), nsp8 (replicase co-factor), and nsp12 (RdRp) proteins were used as starting material for protein expression in Sf9 insect cells from a recombinant baculovirus. The SARS-CoV and SARS-CoV-2 protein complexes were purified using Ni-NTA affinity chromatography of the nsp8 N-terminal eight-histidine tag. Modified SARS-CoV and SARS-CoV-2 nsp12 proteins with amino acid substitutions in the conserved motif C (SDD to SNN) to inactivate the catalytic site were also expressed and used as inactive controls.

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For comparison, the Lassa virus L protein was also expressed in insect cells in the baculovirus expression system and *Zaire ebolavirus* RdRp complexes were generated as previously described ([Tchesnokov et al., 2018](#)). The identities of the purified LASV, SARS-CoV, and SARS-CoV-2 proteins were confirmed by mass spectrometry (MS) analysis.

To study whether RDV-TP was able to compete with its natural counterpart ATP, RNA synthesis was monitored at a fixed concentration of NTPs (0.02 μM) and increasing concentrations of RDV-TP. The RNA synthesis assay consisted of mixing (final concentrations) Tris-HCl (pH 8, 25 mM), RNA primer (200 μM), RNA template (2 μM), [α - ^{32}P]-NTP (0.1 μM), various concentrations and combinations (as indicated) of NTP and NTP analogues, and MERS-, SARS- or SARS-CoV-2 RdRp complex ($\sim$ 0.1 μM) on ice. Reactions with LASV L protein contained $\sim$ 0.02 μM of enzyme. Reaction mixtures (10 μL) were incubated for 10 min at 30°C followed by the addition of 5 μL of MgCl_2 (5 mM). Reactions were stopped after 30 min by the addition of 15 μL of formamide/EDTA (50 mM) mixture and incubated at 95°C for 10 min. Data was collected and analyzed using GraphPad Prism 7.0. The assay measured incorporation of [α - ^{32}P]GTP into the primer. With this assay, it was not possible to quantify the fraction of the extended primer; however, the reaction products were clearly defined by the correct incorporation of [α - ^{32}P]GTP.

Structural models of the SARS-CoV-2 replication complex in its elongating state were generated based on a recent cryo-EM structure of the apo SARS-CoV nsp12/nsp7/nsp8 complex (PDB: 6NUR). With high amino acid sequence identity (nsp12 = 96%, nsp7 = 98%, nsp8 = 97%), a homology model was generated for SARS-CoV-2 using [Prime](#). An x-ray structure of hepatitis C virus (HCV) NS5B (RdRp) in its elongating state (PDB: 4WTD) was aligned to the SARS-CoV-2 model. Key residues that interact with the RNA in the HCV NS5B or SARS-CoV-2 nsp12 RNA binding channel were aligned. These included A97, F193, G283, and S367 in NS5B and S501, G590, G683, and S814 in nsp12. The two Mn^{++} ions and the ADP substrate from the NS5B structure were changed to Mg^{++} and ATP, and these, as well as the aligned RNA, were added to the SARS-CoV-2 model. Several active site residues were manually adjusted to accommodate the incoming metals and substrate following the NS5B structure. These SARS-CoV-2 nsp12 residues included K545, R555, D618, S682, G683, D760 and D761. A series of constrained optimizations was performed and each time a similar model resulted. Remdesivir triphosphate and other inhibitors were modeled into the nsp12 active site.

Results: Wild type and mutant SARS-CoV and SARS-CoV-2 nsp12 enzymes were expressed and tested for RNA synthesis on short model primer/templates mimicking a random elongation complex (Figure 2.1-4A). Incorporation of a radio-labeled nucleotide allowed gel-based detection of reaction products. While wild type SARS-CoV and SARS-CoV-2 RdRp complexes were able to synthesize RNA from a 4-mer primer and a 14-mer template, the corresponding active site mutant RdRps showed no template-base specific nucleotide incorporation (Figure 2.1-4B). These data indicate that RNA synthesis activity is mediated by SARS-CoV-2 and SARS-CoV nsp12.

The ratio of Michaelis-Menten steady-state kinetic parameters V_{max}/K_m for a single incorporation of a natural nucleotide over a nucleotide analogue defines selectivity. With the limitations of a steady-state approach, a selectivity value lower than 1 indicates the analogue is incorporated more efficiently than the natural NTP. Conversely, a selectivity value higher than 1 indicates the analogue is incorporated less efficiently than the natural NTP.

To measure selectivity for RDV-TP incorporation by SARS-CoV-2 RdRp, the steady-state kinetic parameters for single nucleotide incorporations were determined in comparison with ATP (Table 2.1-2). Previously, a selectivity value of 0.35 for RDV-TP incorporation with MERS-CoV RdRp was reported ([Gordon et al., 2020](#)).

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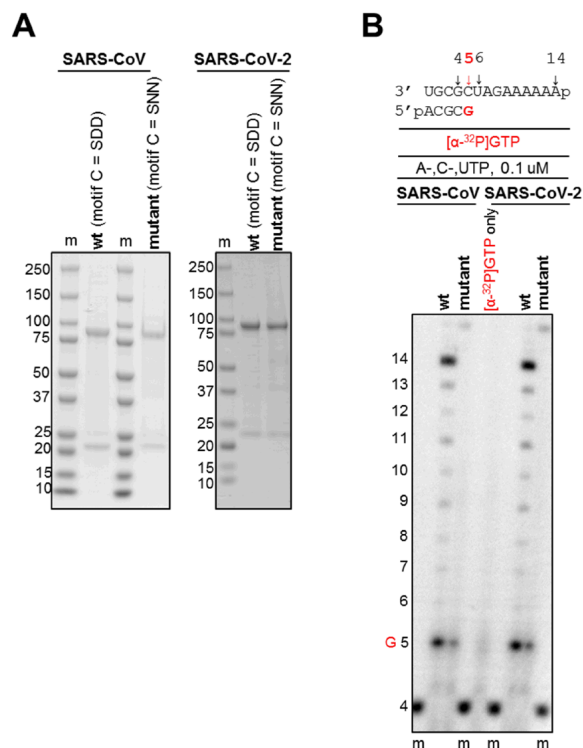


Figure 2.1-4. Expression, purification and characterization of the SARS-CoV and SARS-CoV-2 RdRp complexes (Figure 1, page 10, Study Report PC-540-2005). (A) SDS PAGE migration pattern of the purified enzyme preparations stained with Coomassie Brilliant Blue G-250 dye. Bands migrating at ~100 kDa and ~25 kDa contain nsp12 and nsp8, respectively. (B) RNA synthesis on a short model primer/template substrate. Template and primer were both phosphorylated (p) at their 5'-ends. G indicates incorporation of the radiolabeled nucleotide opposite template position 5. RNA synthesis was monitored with the purified RdRp complexes representing wild type (wt, motif C = SDD) and the active site mutant (motif C = SNN).

SARS-CoV-2 and SARS-CoV showed selectivity values of 0.26 and 0.32, respectively. For EBOV, RSV, and LASV enzymes, higher selectivity values of 4.0, 2.7, and 23, respectively were measured. The combined results indicate that the ability of RDV-TP to be incorporated in place of the natural substrate ATP is highest with the SARS-CoV-2 and other coronavirus RdRp complexes.

Table 2.1-2. Selectivity values of RDV-TP with SARS-CoV-2 RdRp (modified from Table 1, page 12, Study Report PC-540-2005).

	ATP			RDV-TP			Selectivity ^c (fold)
	V _{max} ^a (product fraction)	K _m ^b (μM)	V _{max} /K _m	V _{max} (product fraction)	K _m (μM)	V _{max} /K _m	
SARS-CoV-2	n = 4			n = 3			0.26
	0.77	0.03	23	0.74	0.009	84	
±	0.013	0.0028		0.020	0.0009		
% error	7	19		4	18		
SARS-CoV	n = 4 ^d			n = 3			0.32
	0.73	0.03	24	0.70	0.010	68	
±	0.016	0.003		0.011	0.0005		
% error	6	25		11	8		

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- ^a Vmax is a Michaelis–Menten parameter reflecting the maximal velocity of nucleotide incorporation.
- ^b Km is a Michaelis–Menten parameter reflecting the concentration of the nucleotide substrate at which the velocity of nucleotide incorporation is half of Vmax.
- ^c Selectivity of a viral RNA polymerase for a nucleotide substrate analogue is calculated as the ratio of the Vmax/Km values for NTP and NTP analogue, respectively.
- ^d All reported values have been calculated on the basis of a 9-data point experiment repeated indicated number of times (n).

Several other nucleotide analogues (dATP, favipiravir, sofosbuvir, ribavirin, etc.) were evaluated using the same selectivity protocol with a focus on SARS-CoV-2. The results indicated that the remdesivir triphosphate selectivity was more favorable for incorporation than native ATP (0.26) and ATP was more favorably incorporated than any of the other compounds tested with fold increases in selectivity that ranged from 25-1225 (data reviewed but not shown).

The active site of the betacoronavirus RdRp (nsp12) is conserved despite sequence variations with SARS-CoV and SARS-CoV-2 sharing 96% identity compared to 71% for MERS-CoV-2 compared to SARS-CoV-2 (Figure 2.1-5).

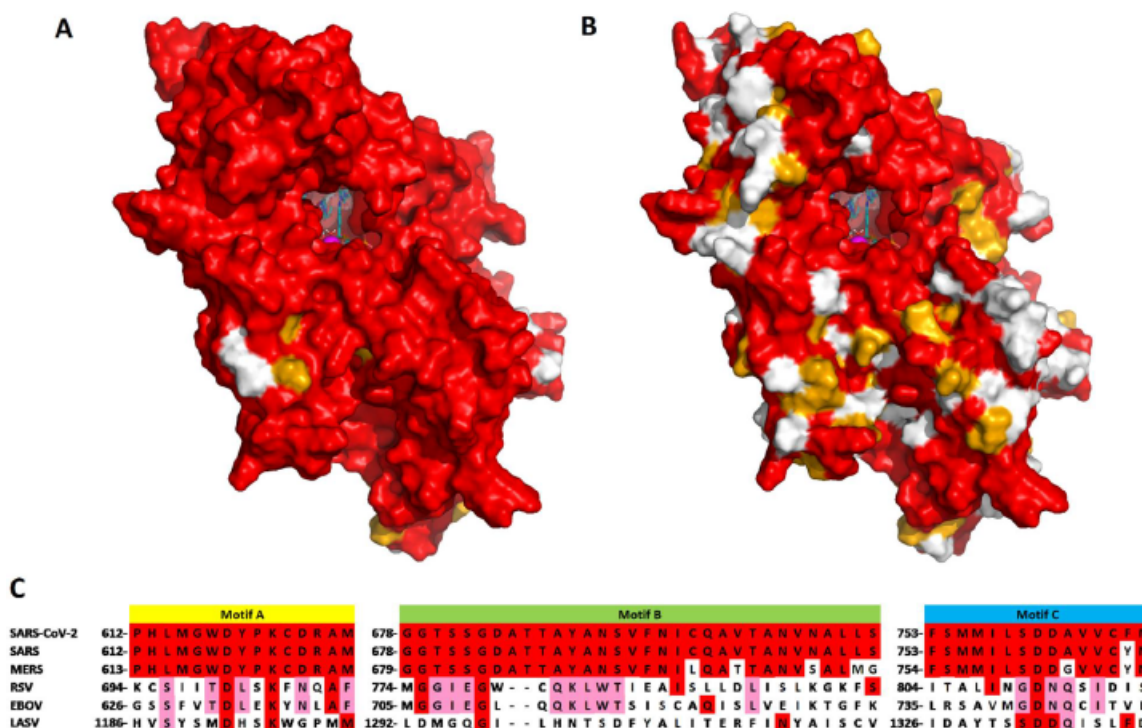


Figure 2.1-5. Structural and amino acid sequence comparison of the active site of viral polymerases (Figure 3, page 14, Study Report PC-540-2005). The nsp12 active site is well conserved between SARS-CoV-2 and (A) SARS-CoV and (B) MERS. Residues shown in red are identical, yellow similar and white dissimilar. The active site is visible near the center of each image. (C) The active site of other polymerases, while maintaining the same basic structure defined by motifs A, B and C, is only conserved at key residues (highlighted in red). RSV and EBOV are similar in the nature of their active sites (highlighted in pink), while LASV is different from both CoV and RSV-like structures in key aspects.

A computational model of an elongating SARS-CoV-2 RdRp complex based on the cryo-electron microscopy structure of the apo SARS-CoV RdRp complex composed of nsp7, nsp8, nsp12 indicated that the catalytic metal ions in SARS-CoV-2 nsp12 are coordinated by D618, D760, and D761, and the substrate β -phosphate is stabilized by R555. Of particular note, amino acid residues D623, S682, and N691, involved in 2'OH recognition of the incoming nucleotide, are conserved (Figure 2.1-6).

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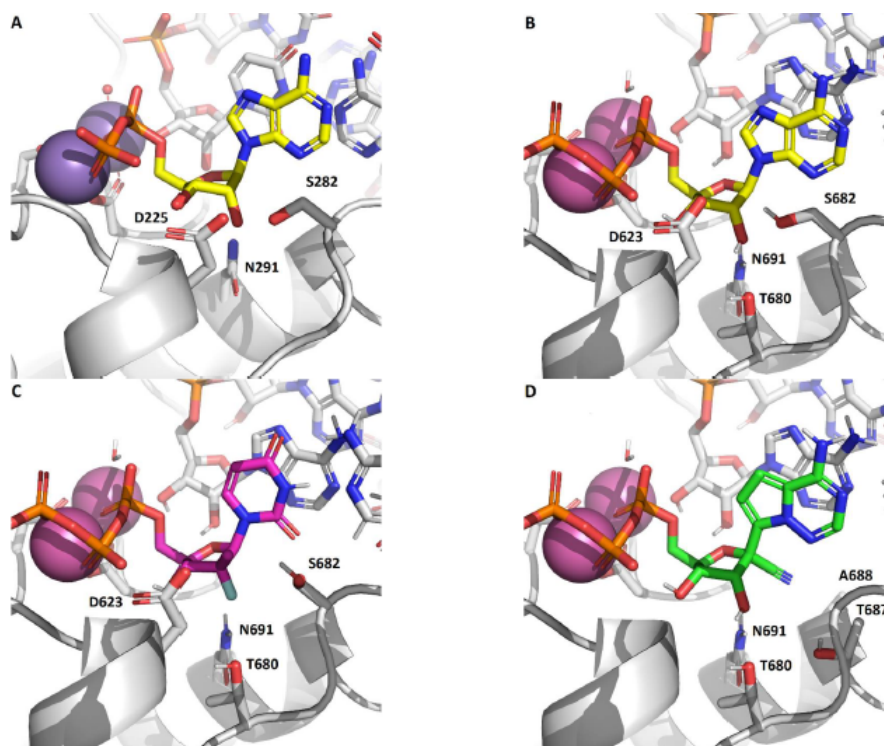


Figure 2.1-6. Structural models of RDV-TP, ATP, and ADP interacting with the HCV and SARS-CoV-2 RdRps (Figure 5, page 19, Study Report PC-540-2005). (A) X-ray structure of HCV NS5B with an incoming non-hydrolysable ADP substrate (PDB: 4WTD). The 2'OH of the substrate is recognized by the trio of residues, D225, S282 and N291, with hydrogen bonds formed to S282 and N291 in this pre-incorporation state. (B) Model of SARS-CoV-2 nsp12 with incoming ATP. In addition to the analogous ASP/SER/ASN residues, T680 is positioned to alter the hydrogen bonding network and effectively pull the substrate lower into the pocket relative to NS5B. (C) Model of SARS-CoV-2 with SOF-TP. The greater occlusion of the 2' position due to D623 and S682 makes 2' β -methyl substitution less effective than with NS5B. (D) Model of SARS-CoV-2 with remdesivir-TP. The remdesivir 1'CN sits in a pocket formed by residues T687 and A688. Residues D623 and S682 (not shown) adopt the same conformations as with ATP.

However, while HCV, norovirus, and poliovirus RdRp's use these serine and asparagine residues to coordinate the 2'OH during incorporation, the coronavirus nsp12 appears to rely on an additional threonine residue (T680) not present in the other enzymes (Figure 2.1-6B). This interaction has the effect of pulling the substrate deeper into the pocket. The lower positioning of the NTP in the active site is also seen in the interaction of the 3'OH with the protein.

In HCV RdRp, 3'OH forms a hydrogen bond to the D225 backbone NH, while in the coronaviruses, the model instead suggests that the β -phosphate coordinates to the analogous D623 NH. As a consequence of this repositioning of the substrate, the activity of various inhibitors across the coronaviruses is expected to diverge from that seen with other viral RNA polymerases. The preference for 2'OH > 2'd2'F >> 2'deoxy is evident from the nature of the polar residues coordinating that position and is similar to the other enzymes. In contrast, SOF-TP and 2'CM_e-ATP have increased clashes between the 2' β -Me and D623 and S682 (Figure 2.1-6C). This is partially relieved by a conformational change in S682, but overall, the poorer incorporation efficiencies of the 2' β -Me substituted inhibitors is likely due to these putative steric clashes. In contrast, RDV-TP (Figure 2.1-6D) is recognized at 2'OH in a manner similar to ATP, and the 1'CN modification is well positioned in a pocket formed between T687 and A688. The high efficiency of incorporation reported here is consistent with this model.

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RNA primer/template substrates (Figure 2.1-7A) were used to compare the inhibitory effects of RDV-TP against MERS-CoV, SARS-CoV, SARS-CoV-2 and LASV RdRps.

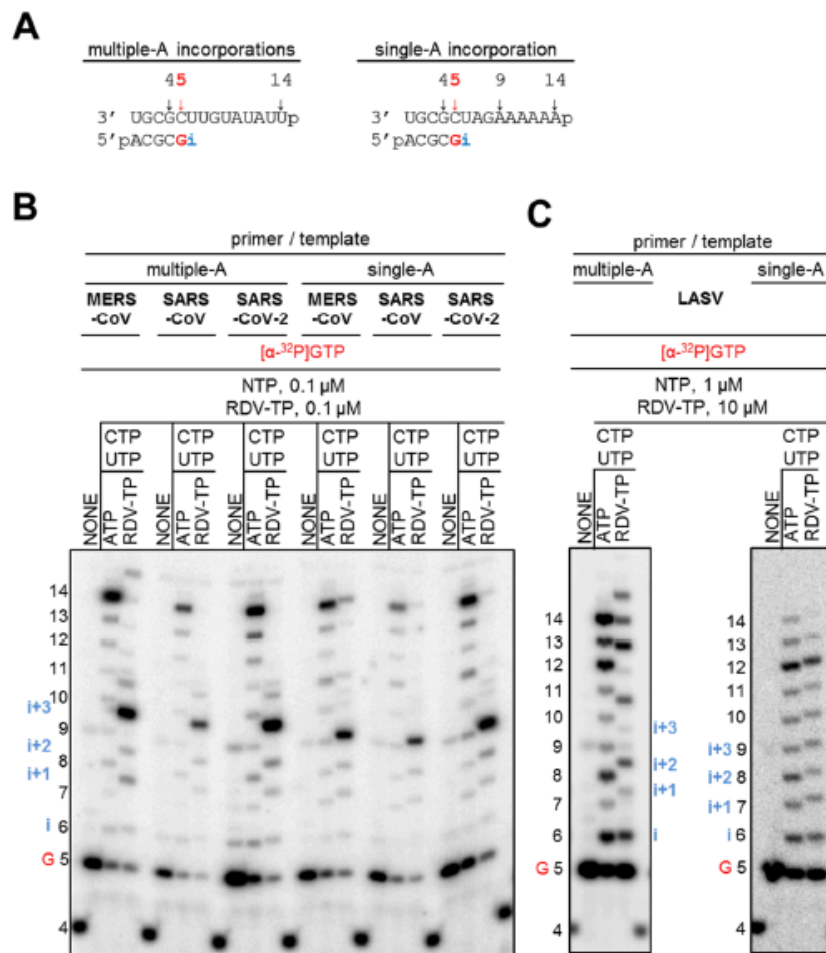


Figure 2.1-7. Patterns of inhibition of RNA synthesis with RDV-TP (Figure 6, page 20, Study Report PC-540-2005). (A) RNA primer/template substrates used to test multiple (left) or single (right) incorporations of RDV-TP. G indicates incorporation of the radiolabeled nucleotide opposite template position 5. RDV-TP incorporation is indicated by “i”. RDV-TP incorporation was monitored with purified CoV RdRp complexes (B) and LASV L protein (C) in the presence of indicated combinations of NTPs and RDV-TP.

CoV RdRp complexed with templates allowing multiple incorporations, and termination of RNA synthesis at positions i+3 and i+4 was observed. A faint band representing the full-length product suggests low levels of read-through and high potency of RDV to terminate RNA synthesis (Figure 2.1-7B). On a template that allowed only a single RDV-TP incorporation, termination is seen solely at position i+3. Full-length product formation is generally more pronounced under these conditions, following the order MERS-CoV RdRp > SARS-CoV RdRp > SARS-CoV-2 RdRp, indicating that SARS-CoV-2 RdRp is most efficiently inhibited by RDV among the tested coronaviruses RdRps.

In the presence of increasing concentrations of UTP the signal at i+3 decreases concomitantly with an increase in the full-length product. UTP concentrations that are ~100-fold higher than RDV-TP can cause significant reductions in delayed chain-termination.

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The biochemical mechanism described in this study report characterizes RDV as a direct-acting antiviral (DAA) and provided a model for the mechanism of action for RDV (Figure 2.1-8).

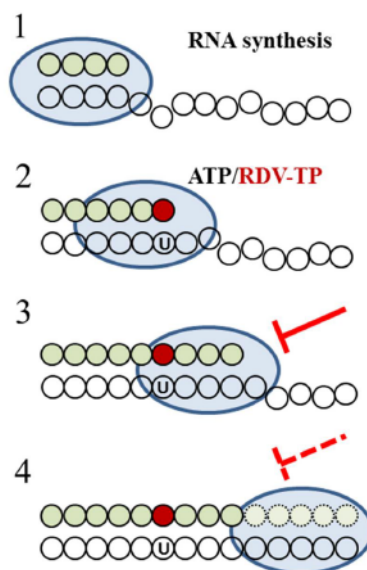


Figure 2.1-8. Mechanism of inhibition of CoV RdRp by RDV-TP (Figure 9, page 25, Study Report PC-540-2004). 1. RNA synthesis is shown schematically with green circles representing residues of the priming strand, colorless circles representing residues of the template and the blue oval represents the active CoV RdRp complex. 2. Competition of RDV-TP with its natural counterpart ATP opposite template uridine (U). The incorporated nucleotide analogue is illustrated by the red circle. 3. RNA synthesis is terminated after the addition of three more nucleotides, which is referred to as delayed chain-termination. 4. Delayed chain-termination can be overcome by high ratios of NTP/RDV-TP.

Conclusions

- GS-443902, the active triphosphate form of RDV (RDV-TP), is efficiently incorporated into the nascent RNA strand, and termination of RNA synthesis occurs at position $i+3$. RDV-TP has a unique property resulting in its high selectivity over incorporation of its natural nucleotide counterpart adenosine triphosphate.
- In the presence of increasing concentrations of UTP the signal at $i+3$ decreases concomitantly with an increase in the full-length product. UTP concentrations that are ~100-fold higher than RDV-TP can cause significant reductions in delayed chain-termination.
- The ratio of Michaelis-Menten steady-state kinetic parameters V_{max}/K_m for a single incorporation of a natural nucleotide over a nucleotide analogue, where a selectivity value lower than 1 indicates the analogue is incorporated more efficiently than the natural NTP and greater than 1 indicates less efficient incorporation than a natural NTP, was used to assess selectivity for RDV-TP incorporation by recombinant SARS-CoV-2 RdRp for single nucleotide incorporations in comparison with ATP. Steady-state kinetic parameters for single nucleotide incorporations of RDV-TP were performed in comparison with ATP. Previously, a selectivity value of 0.35 for RDV-TP incorporation with MERS-CoV RdRp was reported ([Gordon et al., 2020](#)). SARS-CoV-2 and SARS-CoV showed selectivity values of 0.26 and 0.32, respectively, indicating that RDV-TP is incorporated more selectively than ATP.
- The active site of the betacoronavirus RdRp (nsp12) is conserved despite sequence variations with SARS-CoV and SARS-CoV-2 sharing 96% identity compared to 71% for MERS-CoV-2 compared to SARS-CoV-2.

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- The catalytic metal ions in SARS-CoV-2 nsp12 are coordinated by a trio of aspartates, D618, D760, and D761, and the substrate β -phosphate is stabilized by R555. Amino acid residues D623, S682, and N691 are involved in 2'OH recognition of the incoming nucleotide, and these amino acids are conserved across betacoronavirus RdRps.
- The collective data in this study report provide a unifying, refined mechanism of inhibition of RDV against coronaviruses and define the nucleotide analogue as a direct-acting antiviral.

Post Marketing considerations: none.

Additional Mechanism of Action Information

Additional information about the activity of remdesivir was reported in a journal article focused on remdesivir against MERS-CoV ([Gordon et al., 2020](#)). In this report, which included authors from Gilead Sciences, Inc., a study was performed to assess whether RDV-TP was able to compete with its natural counterpart ATP during RNA synthesis with purified MERS RdRp complex in the presence of 0.02 μ M ATP, CTP and UTP mix and increasing concentrations of RDV-TP (Figure 2.1-9A, upper panel).

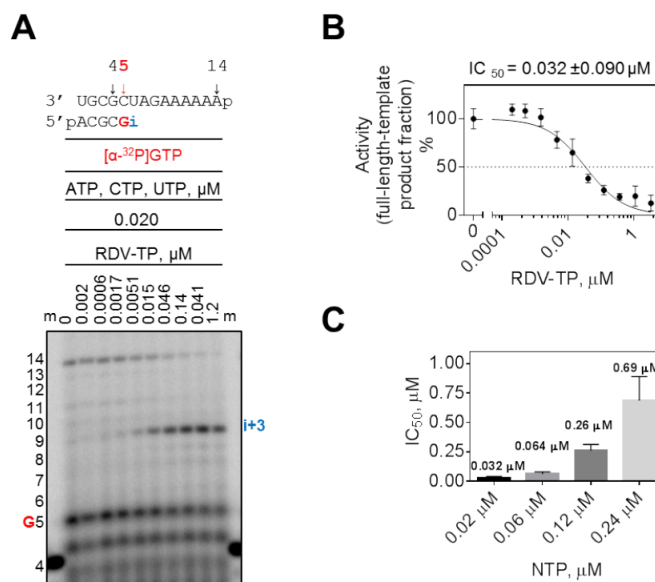


Figure 2.1-9. Competition between RDV-TP and ATP (copied from [Gordon et al., 2020](#)). (A) The RNA primer/template substrate used in this assay is shown above the gel. G indicates incorporation of the radiolabeled nucleotide opposite template position 5. Position i allows incorporation of ATP or RDV-TP. RNA synthesis was monitored with purified MERS RdRp complex in the presence of 0.02 μ M ATP, CTP and UTP mix and increasing concentrations of RDV-TP as indicated. (B) Graphic representation and IC_{50} determination fitting of quantified data from panel A. Error bars represent standard deviation of the data within four independent experiments. (C) Graphic representation of the relationship between IC_{50} values for RDV-TP measured at different NTP concentrations. Average IC_{50} values for RDV-TP are shown above the corresponding bars. Error bars represent standard deviation of the data within at least three independent experiments.

The paper reported that increasing concentrations of RDV-TP caused a reduction of the 14-mer product due to increases in RNA synthesis arrest at position i+3. Of note, the formation of the full-length 14-mer product was not evident at RDV-TP concentrations $>0.041 \mu$ M. An IC_{50} value of 0.032μ M was determined for RDV-TP under these conditions (Figure 2.1-9B). This IC_{50} value was only 1.5-fold higher than the ATP concentration, which indicated efficient use of RDV-TP by the MERS-CoV RdRp complex.

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Increasing concentrations of ATP caused a corresponding increase in IC₅₀ values for RDV-TP (Figure 2.1-9C), which indicated that RDV-TP is a competitive inhibitor.

Conclusions of Mechanism of Action Assessments:

- Remdesivir (RDV; GS-5734™) is a single diastereomer monophosphoramidate prodrug of an adenosine nucleoside analog, GS-441524. Once inside cells, remdesivir undergoes rapid and efficient conversion to the pharmacologically active nucleoside triphosphate form GS-443902. Remdesivir is activated to the pharmacologically active nucleoside analog triphosphate, GS-443902, by a sequential metabolic activation pathway. In cells and tissues, remdesivir is converted by hydrolase and phosphoramidase cleavage to the nucleoside analog monophosphate. Further phosphorylation by nucleotide kinases results in formation of GS-443902. Efficient formation of the pharmacologically active triphosphate, GS-443902, has also been observed in a number of human lung cell types in cell culture including normal human bronchial epithelial and Calu-3 cells as well as in PBMCs, macrophages, monocytes, and human microvascular endothelial cells. Once formed, GS-443902 has been observed to have a half-life in excess of 15 h in the various cell types mentioned, following incubation with remdesivir in cell culture.
- Remdesivir interacts with the SARS-CoV-2 RdRp and is preferentially incorporated, with a selectivity value of 0.26, into the nascent RNA strand in place of native ATP, which results in a delayed chain termination at position i+3.
- In a biological assay assessing RDV-TP incorporation by the MERS-CoV RdRp complex, increasing concentrations of RDV-TP caused a reduction in 14-mer RNA synthesis products due to RNA synthesis arrest at position i+3 with an IC₅₀ value of 0.032 μM.
- Mechanism of action studies indicated that RDV is a direct-acting inhibitor of SARS-CoV-2 RdRp (nsp12).

2.2 Cell Culture Studies

Several study reports were submitted to show that remdesivir has antiviral activity in cell culture against SARS-CoV-2 and several other viruses with an RNA genome. The study reports supporting antiviral activity against SARS-CoV-2 and other coronaviruses are reviewed here, ordered by relevance to SARS-CoV-2 antiviral activity. Summaries from other cell culture activity studies are also presented.

Study Title: In Vitro Antiviral Activity of Remdesivir against SARS-CoV-2 and SARS-CoV in Primary Human Airway Epithelial Cells

Study Report: [PC-540-2003](#)

Objective: The purpose of this study was to characterize the antiviral activity of remdesivir against SARS-CoV-2 in primary HAE cells.

Methods: The cell culture antiviral activity of remdesivir against SARS-CoV-2 was assessed in primary human airway epithelial (HAE) cells using virus isolated from clinical specimens of a patient in Washington, USA ([Holshue et al., 2020](#)). The nucleotide sequence for this isolate of SARS-CoV-2 is available through GenBank (accession number [MN985325](#)). After differentiation of primary human airway epithelial cells at the air-liquid interface, cultures showed a density of approximately 1,000,000 cells per well. Before infection, HAE cells were washed with phosphate-buffered saline and moved into air-liquid interface medium containing various doses of remdesivir ranging from 10 to 0.00260 μM (final

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DMSO, <0.05%). HAE cultures were then infected at a MOI of 0.5 for 2 hours at 37°C, after which the virus was removed, and cultures were washed three times with PBS, and then incubated at 37°C for 48 hours. *Reviewer's note:* The study report methods section stated that an RDV concentration range of 10 to 0.00260 mM was used, but based on the EC₅₀ and CC₅₀ values, presumably the correct units were μM.

Results: Remdesivir inhibited replication of SARS-CoV-2 in HAE cells with a mean EC₅₀ value of 9.9 nM after 48 hours of treatment (Table 2.2-1, Figure 2.2-1). A comparable activity was observed for remdesivir against SARS-CoV in HAE cells (EC₅₀ value = 6.6 nM), similar to previous independent studies of SARS-CoV ([Sheahan et al., 2017](#)).

Table 2.2-1. Antiviral activity of remdesivir against SARS-CoV-2 and SARS-CoV in primary HAE cells after 48-hour treatment (Table 1, page 8, PC-540-2003 Study Report).

Construct	Remdesivir EC ₅₀ , μM		
	1 st experiment	2 nd experiment	Average (±st.dev)
SARS-CoV-2	0.0104	0.0094	0.0099 (±0.0007)
SARS-CoV	0.0066	ND	0.0066 (ND)

The report stated that the CC₅₀ value of remdesivir in HAE cells was previously determined to be >10 μM, resulting in a selectivity index (CC₅₀/EC₅₀) >1000.

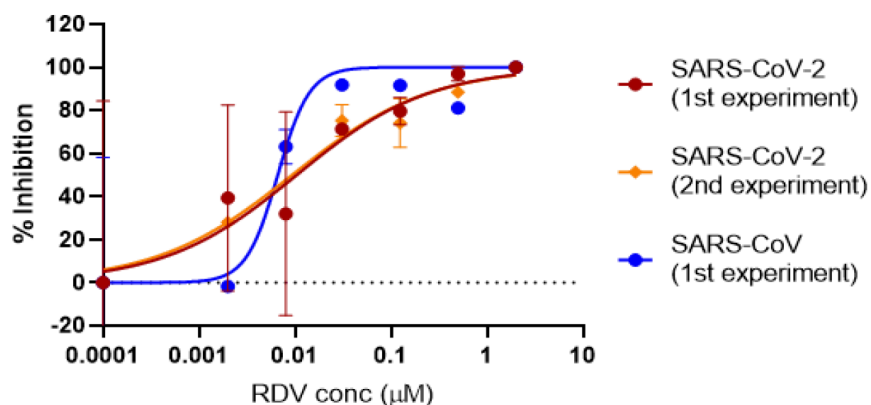


Figure 2.2-1. Antiviral activity of remdesivir at different concentrations against SARS-CoV-2 and SARS-CoV in HAE cells following 48-hour incubation (Figure 1, page 8, PC-540-2003 Study Report).

Reviewer's Note: The dose response curve of RDV against SARS-CoV-2 exhibited a shallow dose-dependent increase in inhibition compared to the response against SARS-CoV, indicating that RDV may be more active against SARS-CoV in HAE cells. Alternatively, there could be a lag in formation of the active diphosphate partially overcome by higher concentrations due to slow uptake by the cells, slow metabolism of the prodrug to the monophosphate, or a slow phosphorylation step.

Reviewer's Note #2: The EC₅₀ value reported in this assay for remdesivir against SARS-CoV was 6.6 nM, which is >10-fold lower than the EC₅₀ value of 69 nM reported for RDV against SARS-CoV in HAE cultures derived from a different donor but assayed under the same experimental conditions described in this report ([Sheahan et al., 2017](#)). This comparison highlights a 10-fold difference in remdesivir antiviral activity associated with host genotype that indicates the potential for highly variable results between individuals treated with this drug.

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Conclusions

- Remdesivir exhibited cell culture antiviral activity against a clinical isolate of SARS-CoV-2 in primary HAE cells with an EC₅₀ value of 9.9 nM after 48 hours of treatment.
- Remdesivir exhibited cell culture antiviral activity against a clinical isolate of SARS-CoV in primary HAE cells with an EC₅₀ value of 6.6 nM after 48 hours of treatment.
- No cytotoxicity was observed for remdesivir in primary HAE cells at concentrations up to 10 µM (CC₅₀ value >10 µM).
- The selectivity index for RDV against SARS-CoV-2 in primary HAE cells is >1,000.
- The EC₅₀ value reported in this assay for remdesivir against SARS-CoV was 6.6 nM, which was >10-fold lower than the EC₅₀ value of 69 nM reported for RDV against SARS-CoV in HAE cultures derived from a different donor but assayed under the same experimental conditions described in this report ([Sheahan et al., 2017](#)). This comparison highlights a 10-fold difference in remdesivir antiviral activity associated with host genotype that predicts the potential for highly variable results between individuals treated with this drug.

Post-Marketing Considerations: None.

Study Title: In Vitro Antiviral Activity of Remdesivir against SARS-CoV-2 in Vero Cells

Study Report: [PC-540-2001](#)

Objective: The purpose of this study was to characterize the antiviral activity of remdesivir against SARS-CoV-2 in Vero cells.

Methods: The cell culture antiviral activity of remdesivir against SARS-CoV-2 in Vero cells was evaluated by quantification of viral nucleic acid in the supernatant by reverse transcription, quantitative RT-PCR. Vero cells were seeded in a 96 well plate using 10,000 Vero cells/well in 100 µL cell culture media. After 24 hours, the cells were infected with SARS-CoV-2 at a multiplicity of infection (MOI) of 0.01 plaque forming units (PFU) per cell (100 PFU per well). The cells were washed with DMEM and remdesivir was added to the cells 2 hours post infection in a 10-fold serial dilution: 0.001 µM, 0.01 µM, 0.1 µM, 1 µM, 10 µM, and 100 µM. The experiment was performed once and the remdesivir treatment was done in triplicates for each dilution. The infected cell cultures were incubated with remdesivir for 24 hours.

In addition, a second experiment was performed using similar conditions as described above but with the following exceptions: 20,000 Vero cells were seeded per well and the cells were infected with SARS-CoV-2 using MOI of 0.01 PFU per cell (200 PFU per well). The following remdesivir concentrations were used: 0.032 µM, 0.16 µM, 0.8 µM, 4 µM, 20 µM, 100 µM. The infected cell cultures were incubated with remdesivir for 48 hours.

Viral RNA was extracted from 100 µL supernatant per well using the automated nucleic acid extraction system (b) (4) according to the manufacturer's recommendations. SARS-CoV-2 RNA was measured using qRT-PCR with primers and a probe designed for ORF 1a (nsp3) using One Step PrimeScript RT-PCR kit (TaKaRa, Japan) on the LightCycler 480 Real-Time PCR system (Roche, Rotkreuz, Switzerland). The RNA copy number of SARS-CoV-2 in the remdesivir treated cells were

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compared to the untreated SARS-CoV-2 infected control. An inhibition curve was generated by plotting the remdesivir concentration against the percent inhibition of viral replication.

Results: Remdesivir inhibited the replication of SARS-CoV-2 in Vero cells with an EC₅₀ value of 137 nM and 750 nM after 24- and 48-hours of treatment, respectively. The CC₅₀ value for remdesivir was >100 μM and selectivity index >730 for the 24-hour and >133 for the 48-hour treatment (Figure 2.2-2). Similar activity has been observed in independent studies for MERS-CoV and SARS-CoV in multiple relevant human cell types.

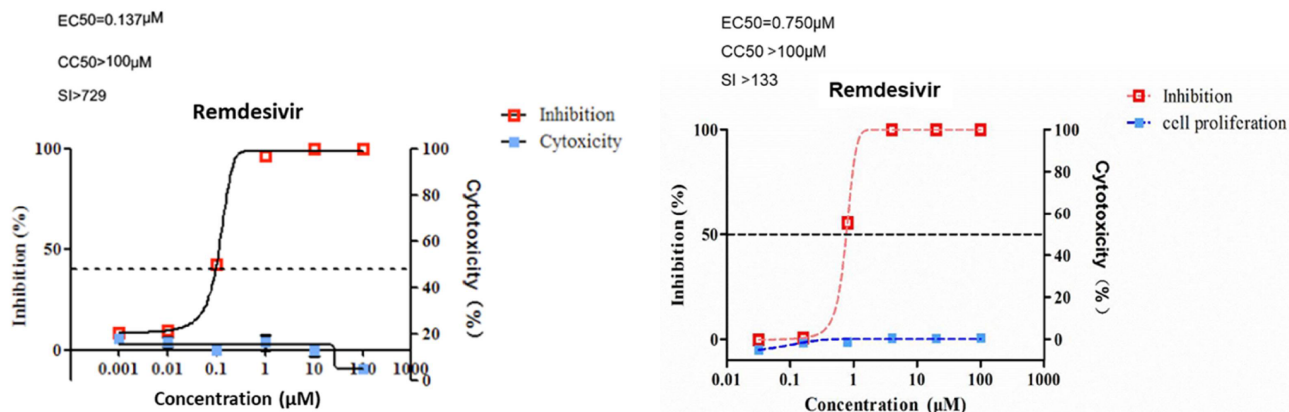


Figure 2.2-2. Cell culture activity of remdesivir for 24- and 48-hours at different concentrations against SARS-CoV-2 in Vero cells (Figures 1 and 2, pages 8 and 9, Study Report PC-540-2001). Values represent averages from three replicates. Left panel, 24-hours of treatment; Right panel, 48-hours of treatment.

Conclusions

- The EC₅₀ values of remdesivir against SARS-CoV-2 in Vero cells was 137 nM at 24 hours and 750 nM at 48 hours post-treatment ([PC-540-2001](#)). An EC₅₀ value of 770 nM in Vero cells has been reported for RDV against SARS-CoV-2 by the Wuhan Institute of Virology ([Wang et al., 2020](#)).

Post-Marketing Considerations: None.

Study Title: In Vitro Antiviral Activity of Remdesivir against SARS-CoV and SARS-CoV-2 in Huh7 Cells

Study Report: [PC-540-2002](#)

Objective: The purpose of this study was to characterize the antiviral activity of remdesivir against SARS/SARS-CoV-2 nsp12 nLUC in Huh7 cells.

Methods: The cell culture antiviral activity of remdesivir was assessed against SARS-CoV and SARS-CoV-2 using a recombinant chimeric SARS-CoV expressing the RdRp gene (nsp12) of SARS-CoV-2 in the backbone of SARS-CoV with a luciferase reporter (inserted in place of ORF7) in Huh7 cells. Cells were plated at a density of 8×10⁴ cells per well. Twenty-four hours later, fresh medium was added. In triplicate, cells were infected for 1 hour with SARS/SARS-CoV-2 nsp12 nLUC or SARS-CoV nLUC diluted in growth medium at a 1:100 dilution for 1 hour at 37°C. After an hour dilution, virus was removed, and cultures were rinsed once with media, and fresh media containing dilutions of remdesivir

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(GS-5734) or vehicle was added. At 48 hours post-infection, virus replication was measured by nLUC assay (Promega) and read on a SpectraMax plate reader (Molecular Devices).

Results: Remdesivir inhibited replication of SARS/SARS-CoV-2 nsp12 nLUC with a mean EC₅₀ values of 3.5 nM in Huh7 cells after 48 hours of treatment (Figure 2.2-3).

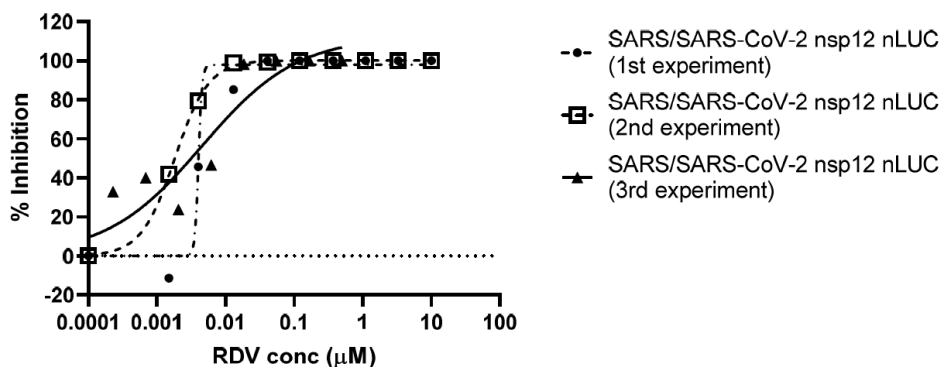


Figure 2.2-3. Cell culture activity of remdesivir for 48-hours at different concentrations against SARS/SARS-CoV-2 nsp12 nLUC in Huh7 cells (Figure 1, page 8, Study Report PC-540-2002).

Remdesivir inhibited replication of SARS-CoV nLUC with a mean EC₅₀ value of 7.1 nM in Huh7 cells after 48 hours of treatment (Figure 2.2-4). No cytotoxicity was previously reported for remdesivir in Huh7 cells across the dose range measured in the assay (CC₅₀ value >10 µM), resulting in a presumed selectivity index >1000.

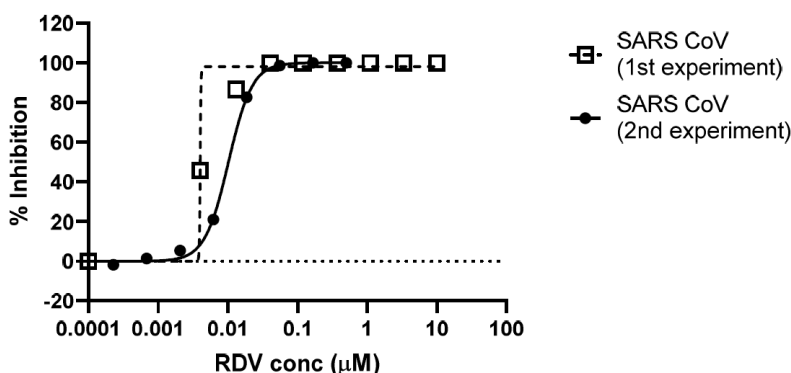


Figure 2.2-4. Cell culture activity of remdesivir for 48-hours at different concentrations against SARS-CoV nLUC in Huh7 cells (Figure 2, page 9, Study Report PC-540-2002). Values represent averages from at least two replicates in each experiment.

Conclusions

- Remdesivir inhibited a recombinant chimeric virus expressing the RdRp gene (nsp12) of SARS-CoV-2 in a backbone of SARS-CoV with a fluorescent reporter protein in Huh7 cells with an EC₅₀ value = 3.5 nM after 48 hours of treatment.
- Remdesivir inhibited replication of SARS-CoV nLUC with a mean EC₅₀ value of 7.1 nM in Huh7 cells after 48 hours of treatment.
- No cytotoxicity was previously reported for remdesivir in Huh7 cells across the dose range measured in the assay (CC₅₀ value >10 µM), resulting in a presumed selectivity index >1000.

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Post-Marketing Considerations: None

Study Title: In Vitro Antiviral Activity of GS-5734 and GS-466547 against Coronaviruses

Study Report: [PC-399-2019](#)

Objective: The purpose of this study was to characterize the cell culture antiviral activity and selectivity of remdesivir (GS-5734) and GS-466547 against MERS-CoV and SARS-CoV in transformed cell lines and physiologically relevant human airway epithelial cell cultures.

Methods: Cell culture antiviral assessments were performed by two independent research organizations, one at USAMRIID and one at University of North Carolina (UNC).

For experiments conducted by USAMRIID, Vero E6 cells were treated with serial dilutions of remdesivir (GS-5734) or GS-466547 added to the assay plates by direct titration and cultures were infected with MERS-CoV (Strain Jordan N3) at a multiplicity of infection (MOI) of 0.5 plaque forming unit (pfu) per cell. The infected cultures were incubated for 48 hours. The level of virus replication in compound-treated and control vehicle-treated cultures was determined by quantifying the level of virus-specific antigen following immuno-staining with antibody against the MERS-CoV spike (S) protein.

At UNC, HAE cell cultures isolated from human donor lung tissue were cultured for 6 weeks at the air liquid interface to promote differentiation. The apical surfaces of the HAE cultures were washed at 24 h and 1 h prior to infection with 1x PBS for 1 hour at 37°C. Recombinant MERS-CoV expressing red fluorescent protein (MERS-CoV RFP) and SARS-CoV expressing green fluorescent protein (SARS-CoV GFP) were used to apically infect the differentiated HAE cultures at a multiplicity of infection of 0.1 pfu per cell. Five 3-fold serial dilutions of GS-466547 starting at 10 µM were prepared in triplicate and added to HAE ALI media on the basolateral side of the culture approximately 30 minutes prior to infection. Virus replication was assessed by fluorescence imaging of cell cultures following 48-hours of incubation in a tissue culture incubator. In addition, virus replication was quantified by measuring the production of infectious virus in HAE apical washes by plaque assay on Vero cell monolayers and by quantifying viral RNA production from total cell RNA by real-time RT-PCR assay.

Results: Remdesivir and GS-466547 were tested in cell culture antiviral activity assessments against human pathogenic coronaviruses, specifically MERS-CoV and SARS-CoV. Remdesivir and GS-466547 inhibited replication of MERS-CoV in Vero E6 cells, with mean EC₅₀ values of 520 ± 90 and 420 ± 60 nM, respectively. No cytotoxicity was observed at 10 µM, the highest concentration tested, indicating selective inhibition of virus replication with selectivity indices of >19 and >24, respectively.

Table 2.2-2. Cell culture antiviral activity of remdesivir (GS-5734) and GS-466547 against MERS-CoV (Table 1, page 9, PC-399-2019 Study Report).

Compound	EC ₅₀ , µM Replicate #1 ^a	EC ₅₀ , µM Replicate #2 ^a	Mean ± S.D.	CC ₅₀ , µM
GS-5734	0.58	0.45	0.52 ± 0.09	> 10
GS-466547	0.46	0.38	0.42 ± 0.06	> 10

^a Antiviral assay conducted in Vero E6 cells at USMARIID (Fort Detrick, MD).

In addition, GS-466547 inhibited both MERS-CoV and SARS-CoV replication in HAE cultures as observed by the reduction in the expression of the RFP or GFP reporter genes compound

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concentrations ranging from at 0.1 to 1.1 μM (Figure 2.2-5). The treatment of MERS-CoV and SARS-CoV infected HAE cultures with 1.1 μM GS-466547 resulted in the reduction of virus titers by approximately 4 $\log_{10}$ PFU/mL (Figure 2.2-5). GS-466547 also inhibited the production of MERS-CoV and SARS-CoV RNAs in HAE cell culture supernatants. The compound activity manifested in a dose-dependent manner with approximately 4 $\log_{10}$ and 3.5 $\log_{10}$ reductions in the levels of MERS-CoV and SARS-CoV RNAs, respectively, following the treatment with 1.1 μM compound.

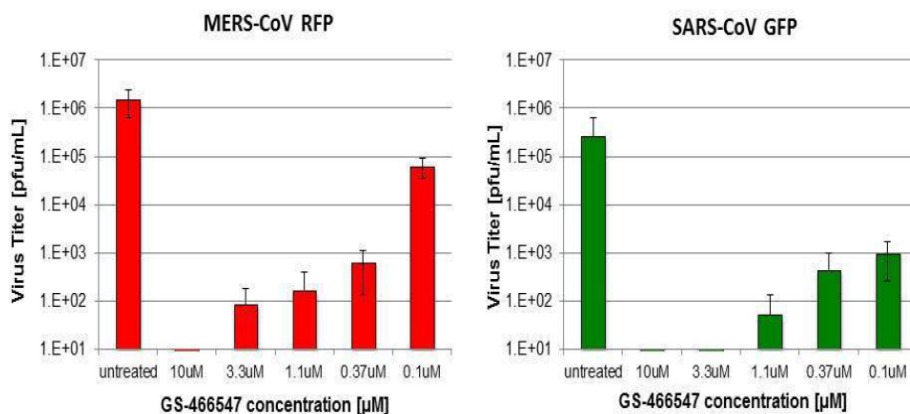


Figure 2.2-5. GS-466547 inhibits production of infectious MERS-CoV and SARS-CoV in HAE cultures (Figure 3, page 10, PC-399-2019 Study Report).

Conclusions

- Remdesivir and GS-466547 (an opposite diastereomer) were tested in cell culture antiviral activity assessments against SARS-CoV and MERS-CoV. Remdesivir and GS-466547 inhibited replication of MERS-CoV in Vero E6 cells with mean EC_{50} values of 520 ± 90 and 420 ± 60 nM, respectively. No cytotoxicity was observed at 10 μM , the highest concentration tested, indicating selective inhibition of virus replication with selectivity indices of >19 and >24 , respectively.
- GS-466547 inhibited SARS-CoV and MERS-CoV replication in HAE cultures as measured by the reduction in the expression of a fluorescent reporter protein at compound concentrations ranging from 0.1 to 1.1 μM .

Additional Information for RDV Activity against Coronaviruses

The sponsor provided references that described additional cell culture antiviral assessments of RDV against coronaviruses, and these are summarized below:

- The activity of remdesivir against SARS-CoV and MERS-CoV was assessed using recombinant viruses expressing a fluorescent reporter protein in a continuous human lung epithelial cell line, 2B4 (Calu-3; MERS-CoV only) and primary HAE cells (SARS-CoV and MERS-CoV). Remdesivir inhibited MERS-CoV replication in Calu-3 cells, with a mean EC_{50} value of 25 nM ([Sheahan et al., 2017](#)).
- In HAE cells, remdesivir inhibited both SARS-CoV and MERS-CoV replication with EC_{50} values of 690 and 740 nM, respectively. In both HAE and Calu-3 cells, no cytotoxicity was observed at 10 μM of remdesivir, the highest concentration tested, indicating that remdesivir has selectivity indices >100 in these cell culture systems ([Sheahan et al., 2017](#)).
- Remdesivir showed cell culture antiviral activity against human betacoronavirus OC43 and alphacoronavirus 229E as well as the animal betacoronavirus murine hepatitis virus and the

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genetically divergent porcine deltacoronavirus with submicromolar EC₅₀ values ranging from 20 to 250 nM in various cell types ([Agostini et al., 2018](#); [Brown et al., 2019](#); [Sheahan et al., 2017](#)).

Post-Marketing Considerations: None.

Antiviral Activity Against Other Viruses

The sponsor performed a variety of cell culture antiviral activity assessments with other RNA viruses to determine the specificity of remdesivir antiviral activity, which are summarized below.

Remdesivir (GS-5734) had nanomolar activity against RSV in HEp-2 cells and HCV in Huh-7 cells (Table 2.2-3). GS-5734 had modest activity against HRV serotype 10 in HeLa cells, but no measurable activity at non-cytotoxic concentrations against HIV-1 in MT-4 cells or HBV in HepG2 NTCP cells. The lack of antiviral activity against HIV-1 or HBV is consistent with GS-443902 having activity specifically against viral RNA-dependent RNA polymerases, as HIV-1 and HBV do not utilize RNA-dependent RNA polymerases for replication ([PC-399-2014](#)).

Table 2.2-3. Cell culture antiviral activity of remdesivir (GS-5734) against panel of other viruses (Table 1, page 10, Study Report PC-399-2014).

Virus	Positive Control ^a		GS-5734 ^a			GS-441524 ^a	
	Compound	EC ₅₀ (nM)	EC ₅₀ (nM)	CC ₅₀ (nM)	SI	EC ₅₀ (nM)	CC ₅₀ (nM)
RSV A2	YM-53403	370 ± 165	17.3 ± 2.6	5,675 ± 1,156	328	447 ± 228	> 100,000
HCV 1b replicon	ITMN-191	4.4 ± 2.6	97.1 ± 54.6	42,217 ± 16,308	435	8,774 ± 82	> 88,000
HCV 2a replicon	ITMN-191	24.2 ± 12.3	84.3 ± 20.4	28,597 ± 4,186	339	9,659 ± 487	> 88,000
HRV-10	Rupintrivir	51.3 ± 18.0	2,543 ± 447	> 50,000	19.7	15,533 ± 4,581	> 100,000
HIV-1	Zidovudine	38.9 ± 30.3	> 50,000	1,808 ± 648	< 1.0	> 100,000	69,298 ± 25,651
HBV	Lamivudine	42.3 ± 22.7	3,915 ± 2,807	7,430 ± 3,635	1.9	> 50,000	> 50,000

^a All values represent the mean ± S.D. of at least two independent experiments.
SI: Selectivity index calculated as the ratio of CC₅₀/EC₅₀

Remdesivir (GS-5734) exhibited cell culture antiviral activity against multiple, unrelated human RNA viruses including, EBOV, Marburg virus (MARV), Junin virus (JUNV), Lassa fever virus (LASV), and Middle East MERS-CoV (Table 2.2-3)([PC-399-2008](#)). Remdesivir did not have antiviral activity against Chikungunya virus (CHIK), and Venezuelan equine encephalitis virus (VEEV) (EC₅₀ values >20 μM).

Table 2.2-4. Antiviral activity of remdesivir across multiple RNA viruses (data derived from PC-399-2008). GS-5734, remdesivir. EBOV-HeLa, assessments conducted with EBOV in HeLa cells; EBOV-HMP, assessments conducted with EBOV in human macrophages.

Compound	Antiviral Activity; EC ₅₀ value / EC ₉₀ value							
	MERS-CoV	EBOV-HeLa	EBOV-HMP	MARV	JUNV	LASV	CHIV	VEEV
GS-5734	0.52 / 1.29	0.14 / 0.41	0.086 / 0.18	0.052 / 0.13	0.47 / 1.26	1.65 / 3.31	>20 / >20	>20 / >20
GS-466547	0.42 / 1.18	0.18 / 0.59	0.10 / 0.31	0.04 / 0.13	0.49 / 1.25	2.0 / 4.21	>20 / >20	>20 / >20

n.d. – Not determined

CC₅₀ values for all compounds and cell types were greater than the highest concentration tested (>20 μM).

To estimate the plasma protein-adjusted antiviral potency of remdesivir (GS-5734), the ratio of free drug fraction between cell culture media and human plasma was taken into account. Competitive equilibrium dialysis followed by LC-MS analysis detected a 4.1-fold higher free drug fraction in cell culture media compared to human plasma. Based on this result, the estimated protein-adjusted EC₅₀ and EC₉₀ values of remdesivir (GS-5734) against EBOV in primary human macrophages were 350 and

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740 nM, respectively. The estimated protein-adjusted EC₅₀ and EC₉₀ values of remdesivir (GS-5734) in HeLa cells was 574 nM and 1.68 μM, respectively.

Conclusions

- Remdesivir (GS-5734) demonstrated cell culture antiviral activity against RSV A2, HCV-1b, and HCV-2a with EC₅₀ values of 17.3 ± 2.6 nM, 97.1 ± 54.6 nM, and 84.3 ± 20.4 nM, respectively.
- Remdesivir (GS-5734) had weak activity against HRV-10 with an EC₅₀ value = 2,543 ± 447 nM.
- GS-5734 was inactive against HIV-1 and HBV. The lack of antiviral activity against HIV-1 or HBV is consistent with GS-443902 having activity specifically against viral RNA-dependent RNA polymerases, as HIV-1 and HBV do not utilize RNA-dependent RNA polymerases for replication.
- The antiviral activity of remdesivir (GS-5734), GS-466547, and GS-441524 were evaluated against other human RNA viral pathogens including Marburg virus (MARV), Junin virus (JUNV), Lassa fever virus (LASV), Middle East respiratory syndrome virus (MERS), Chikungunya virus (CHIK), and Venezuelan equine encephalitis virus (VEEV). Both remdesivir (GS-5734) and GS-466547 were active against all viruses tested with the exception of the alphaviruses, CHIK and VEEV, with EC₅₀ values ranging from 20 nM to 2.0 μM.

Post-Marketing Considerations: None.

Conclusions for Cell Culture Antiviral Activity Assessments:

Table 2.2-5 summarized the cell culture antiviral activity for RDV against SARS-CoV-2 and other betacoronaviruses. Against SARS-CoV-2, remdesivir had the following cell culture antiviral activity:

- Remdesivir exhibited cell culture antiviral activity against a clinical isolate of SARS-CoV-2 in primary HAE cells with an EC₅₀ value of 9.9 nM after 48 hours of treatment. The selectivity index for RDV against SARS-CoV-2 in primary HAE cells is >1,000.
- Remdesivir inhibited the replication of SARS-CoV-2 in Vero cells with an EC₅₀ value of 137 nM and 750 nM after 24- and 48-hours of treatment, respectively. The CC₅₀ value for remdesivir was >100 μM and selectivity index >730 for the 24-hour and >133 for the 48-hour treatment. No cytotoxicity was observed for remdesivir in primary HAE cells at concentrations up to 10 μM (CC₅₀ value >10 μM).

Table 2.2-5. Summary of cell culture antiviral activity for RDV against SARS-CoV-2 and other betacoronaviruses (compiled by DAV).

Virus	Cells	EC ₅₀ value (nM)	CC ₅₀ value (nM)	SI	Source
Sars-CoV-2	HAE	9.9 ± 0.7 (48 h)	>10,000	>1,000	PC-540-2003
	Vero	137 (24 h)	>100,000	>730	PC-540-2001
	Vero	750 (48 h)	>100,000	>133	PC-540-2001
	Vero E6	770 (48 h)	>100,000	>129.87	Wang et al., 2020
SARS-CoV/SARS-CoV-2 nsp12 nLUC	Huh7	3.5 (48 h)	>10,000	>1,000	PC-540-2002
SARS-CoV	HAE	6.6 (48 h)	>10,000	>1,500	PC-540-2003
	HAE	69 (48 h)	>10,000	>130	Sheahan et al., 2017
SARS-CoV nLUC	Huh7	7.1 (48 h)	>10,000	>1,000	PC-540-2002
MERS-CoV	Vero E6	520 ± 90 (48 h)	>10,000	>19	PC-399-2019
	HAE	74 (48 h)	>10,000	>130	Sheahan et al., 2017
	Calu-3 2B4	25 (48 h)	>10,000	>400	Sheahan et al., 2017

- Two different EC₅₀ values were reported for remdesivir against SARS-CoV in HAE cultures

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derived from different donors but assayed under the same experimental conditions:

- An EC₅₀ value of 6.6 nM was reported in Study [PC-540-2003](#), which was >10-fold lower than an EC₅₀ value of 69 nM reported for RDV against SARS-CoV in HAE cultures derived from a different donor but assayed under the same experimental conditions ([Sheahan et al., 2017](#)).
- This comparison highlights a 10-fold difference in remdesivir antiviral activity associated with host genotype that predicts the potential for highly variable results between individuals treated with this drug.
- These studies indicate that remdesivir is a direct-acting antiviral that inhibits replication of SARS-CoV-2 by interacting with the RdRp (nsp12) to terminate chain elongation.

2.3 Antiviral Activity in Cell Culture in the Presence of Serum and Serum Proteins

Remdesivir (GS-5734) had moderate plasma protein binding across multiple species, with a free fraction ranging from 8-14%, with 12.1% for humans. The distribution of remdesivir and GS-441524 between the cellular and soluble fractions of blood from rhesus monkey and human was assessed. After incubation of blood with the compounds (initial concentration 0.5 µM) remdesivir was found to be somewhat excluded from the blood cellular fraction in both species, with mean whole blood/plasma concentration ratios of 0.71 and 0.76 for monkey and human, respectively, while GS-441524 showed some association with the cellular fraction with respective mean blood/plasma ratios of 1.36 and 1.19 for monkey and human.

The sponsor did not evaluate directly the impact of human serum/plasma on remdesivir (GS-5734) antiviral activity. According to the sponsor's response to a 4/10/2015 virology comment, the assays used for the assessment of GS-5734 antiviral activity have not been optimized for performance in the presence of human serum. In study [PC-399-2008](#), the Applicant estimated the plasma protein-adjusted antiviral potency of remdesivir (GS-5734), the ratio of free drug fraction between cell culture media and human plasma was taken into account. Competitive equilibrium dialysis followed by liquid chromatography–mass spectrometry analysis detected a 4.1-fold higher free drug fraction in cell culture media compared to human plasma. Based on these analyses, there would be a 4.1-fold increase in the EC₅₀ value for remdesivir in the presence of human plasma.

Presumably, the low stability of remdesivir (GS-5734) in human plasma is a key reason why the Applicant did not evaluate the impact of human plasma protein on the antiviral activity of remdesivir (GS-5734). The mean half-life of remdesivir (GS-5734) was 68.5 minutes in human plasma compared to 467 minutes (7-fold more stable) in rhesus macaque plasma (Report AD-399-2012). Variable plasma stability was also observed in other animal species: ≤0.9 minutes for Sprague-Dawley rat, 630 minutes for beagle dog, and 385 minutes cynomolgus monkey.

2.4 Cytotoxicity Assessments

Cytotoxicity

Cytotoxicity was assessed by reviewing two study reports listed below:

1. Study Report [PC-399-2013](#): Cytotoxicity of GS-5734 in Human Cell Lines and Primary Cells. This study was conducted to determine the cell culture cytotoxicity of the remdesivir prodrug (GS-5734) and its parent nucleoside GS-441524 in a broad panel of human cell lines and primary cell cultures.
2. Study Report [PC-399-2018](#): In Vitro Effect of GS-5734 on Human Hematopoietic Precursor

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Cells. The aim of this study was to evaluate the potential effects of a continuous incubation with test articles remdesivir (GS-5734) and GS-441524 and control article 5-Fluorouracil (5-FU) at 8 concentrations on human bone marrow derived erythroid and myeloid progenitor proliferation in MethoCult™ and megakaryoid progenitor proliferation in MegaCult-C™ media using three different pre-qualified frozen bone marrow lots.

Overview of Cytotoxicity Results

Remdesivir (GS-5734) had low micromolar cytotoxicity (CC₅₀ values of 1.7 µM to 14.8 µM) against a variety of cell lines and primary cells cultured in the presence of test compound for 4 to 5 days (Table 2.4-1), illustrating a modest therapeutic window for remdesivir (GS-5734) anti-SARS-CoV-2 activity (Study Report PC-399-2013). Lack of measurable remdesivir (GS-5734) cytotoxicity (CC₅₀ value >20 µM) was only observed in quiescent PBMCs in these assessments (Table 2.4-1). The GS-441524 parent nucleoside analog had reduced cytotoxicity relative to remdesivir (GS-5734) for all cell types evaluated, indicating that intracellular levels of the active nucleotide triphosphate metabolite (or mono- or di-phosphate) may be contributing to the cytotoxicity. The study report did not clearly describe the proliferation status of the cells in these assays, with the exception of stimulated versus quiescent PBMCs, although the cell line assessments included puromycin as a positive control for cytotoxicity. Cell viability was assessed in these studies using Cell-Titer Glo® luminescence reagent (Promega, Madison, WI), which quantifies ATP levels as an indicator of metabolically active cells.

Reviewer’s note: A recommendation was sent to the Applicant requesting that they assess if GS-443902 (ATP analog) interferes with the Cell-Titer Glo® assay. In response, the Applicant stated that they would assess the potential of GS-443902 to interfere with the Cell-Titer Glo® cytotoxicity readout and if any significant interference was detected, they agreed to conduct additional cytotoxicity studies using alternative ATP-independent readout(s) for cell viability.

Table 2.4-1. Cytotoxicity of GS-5734 and GS-441524 against human cell lines (top) and primary cells (bottom) (Tables 2 and 3, page 10, Study Report PC-399-2013). RPTECs, primary renal proximal tubule epithelial cells; PBMCs, peripheral blood mononuclear cells. GS-5734, remdesivir.

Compound	CC ₅₀ (µM) ^a			
	HEp-2	HepG2	PC-3	MT-4
	Laryngeal Carcinoma Cells	Hepatoma Cells	Prostate Cancer Cells	Lymphoblast T-Cells
GS-5734	6.0 ± 1.5	3.7 ± 0.2	8.9 ± 1.6	1.7 ± 0.4
GS-441524	> 100	> 100	> 100	69.3 ± 25.7
Puromycin	0.53 ± 0.10	0.73 ± 0.01	0.52 ± 0.11	0.12 ± 0.03
Compound	Primary Hepatocytes	RPTECs	Quiescent PBMCs	Stimulated PBMCs
GS-5734	2.5 ± 0.6	12.9 ± 6.2	> 20	14.8 ± 5.8
GS-441524	> 100	> 100	> 100	> 100

Remdesivir (GS-5734) GS-5734 also had low micromolar cytotoxicity for human myeloid, erythroid and megakaryoid bone marrow progenitor cells in colony formation assays after 11 to 14 days of drug exposure, with mean CC₅₀ values of 4.9-8.5 µM. The parent nucleoside, GS-441524, also had low µM cytotoxicity in these assays with mean CC₅₀ values of 9.6-13.9 µM. Remdesivir (GS-5734) and GS-441524 were only slightly less cytotoxic in these assays compared to the positive control, 5-fluorouracil, which had mean CC₅₀ values of 2.2-3.2 µM (Study Report PC-399-2018).

Conclusions

- Remdesivir (GS-5734) was generally cytotoxic at low micromolar concentrations against a variety of cell lines and primary cells representing different tissues.

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- Remdesivir (GS-5734) also had low micromolar cytotoxicity for human myeloid, erythroid and megakaryoid bone marrow progenitor cells in colony formation assays.
- In general, the remdesivir (GS-5734) monophosphate prodrug was substantially more cytotoxic than the non-phosphorylated parent nucleoside, GS-441524, although both compounds had bone marrow progenitor cytotoxicity.
- GS-443902 (active triphosphate of GS-5734) showed no inhibition of human DNA polymerases α , β , or γ , human RNA polymerase II

Mitochondrial Toxicity

Mitochondrial toxicity was assessed in these study reports:

1. Study Report [PC-399-2013](#): Cytotoxicity of GS-5734 in Human Cell Lines and Primary Cells. In this study report, the cytotoxicity of remdesivir prodrug (GS-5734) and its parent nucleoside GS-441524 in HepG2 and PC-3 cultures (quickly proliferating cells) was compared in the presence of glucose- and galactose-containing culture media to assess for mitochondrial toxicity.
2. Study Report [PC-399-2015](#): In Vitro Evaluation of GS-5734 Effects on Mitochondrial DNA Content. The purpose of this study was to assess the potential of remdesivir (GS-5734) and GS-441524 to affect the mitochondrial DNA content in HepG2 cells.
3. Study Report [PC-399-2016](#): Effect of GS-5734 on Mitochondrial Protein Synthesis and Respiration. This study was conducted to determine the in vitro effect of remdesivir (GS-5734) and GS-441524 on mitochondrial protein synthesis in PC-3 cells and mitochondrial spare respiration in human PC-3 cells, hepatoblastoma HepG2 cells, and primary human renal proximal tubule epithelial cells (RPTEC).
4. Study Report [PC-399-2017](#): Interaction of GS-443902, the Active Nucleoside Triphosphate Metabolite of GS-5734, with Host RNA and DNA Polymerase Enzymes. This study was conducted to determine the potential of GS-443902, the active triphosphate metabolite of GS-5734, to inhibit the human DNA polymerases α , β , and γ and the RNA polymerases II (POLII) and POLRMT. In addition, the potential for GS-443902 to be incorporated into DNA and RNA by DNA POL γ and POLRMT, respectively, was evaluated.
5. Study Report [PC-399-2028](#): Effect of GS-5734 on Cellular Respiration of Human Primary Hepatocytes. This study was conducted to determine the cell culture effect of remdesivir (GS-5734) and its two systemic metabolites GS-704277 and GS-441524 on mitochondrial spare respiration, cellular ATP levels, and DNA levels in primary human hepatocytes.

Mitochondrial toxicity was characterized by assessing cytotoxicity in cell lines maintained in glucose-versus galactose-containing medium (Study Report [PC-399-2013](#)). Adapting cell lines to grow in the presence of galactose circumvents the “crabtree effect” and increases the cells reliance on mitochondrial respiration for the generation of ATP; thus, enhanced cytotoxicity of a compound for cells that use galactose as a source of energy may indicate potential mitochondrial dysfunction ([Marroquin et al., 2007](#)). The Applicant evaluated the cytotoxicity of remdesivir (GS-5734) and GS-441524 in this manner for two human different cell lines, HepG2 (hepatoma cells) and PC-3 (prostate cancer cells) (Table 2.4-2). Again, cell viability was assessed in these studies using Cell-Titer Glo[®] luminescence reagent (Promega, Madison, WI). The impact of cell maintenance in glucose- versus galactose-containing medium on GS-5734 cytotoxicity was inconsistent across both cell lines, with enhanced cytotoxicity observed in PC-3 cells grown in galactose-containing medium, but the converse observed for HepG2 cells. Note that an appropriate positive control with specific mitochondrial toxicity was not included in these assessments. Nevertheless, GS-5734 had low micromolar toxicity for both cell lines

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in both growing conditions, indicating the compound is relatively cytotoxic regardless of any specific effect on mitochondria function.

Table 2.4-2. Cytotoxicity of remdesivir (GS-5734) and GS-441524 for cells grown in presence of glucose versus galactose (Table 3, page 11, Study Report PC-399-2013).

Compound	CC ₅₀ (μM) ^a			
	HepG2 Cells		PC-3 Cells	
	Anaerobic Conditions (Glucose)	Aerobic Conditions (Galactose)	Anaerobic Conditions (Glucose)	Aerobic Conditions (Galactose)
GS-5734	3.7 ± 0.2	11.1 ± 1.2	8.9 ± 1.6	1.4 ± 0.1
GS-441524	> 100	> 100	> 100	> 100
Puromycin	0.73 ± 0.01	0.96 ± 0.13	0.52 ± 0.11	0.48 ± 0.01

^a All CC₅₀ values represent the mean ± s.d. of at least 2 independent experiments.

Remdesivir (GS-5734) did not specifically inhibit mitochondrial protein synthesis in PC-3 cells after 5 days of treatment based on a study using the MitoTox™ MitoBiogenesis™ In-Cell ELISA Kit (MitoSciences/Abcam, Eugene, OR) (Study Report [PC-399-2016](#)) (Table 2.4-3). This assay compared levels of two cellular enzymes, cytochrome c oxidase subunit 1 (COX-1) encoded by mitochondrial DNA, and succinate dehydrogenase A (SDH-A) encoded by nuclear DNA. A skewed reduction in COX-1 levels relative to succinate dehydrogenase complex, subunit A (SDH-A) levels would indicate specific inhibition of mitochondrial protein synthesis as shown for chloramphenicol.

Table 2.4-3. Assessment of remdesivir (GS-5734) and GS-441524 impact on mitochondrial protein synthesis (Table 1, page 9, Study Report PC-399-2016).

Compounds	CC ₅₀ (μM) ^a		
	COX-1 (mtDNA-encoded Protein)	SDH-A (nucDNA-encoded Protein)	ATP
GS-5734	8.9 ± 1.1	8.6 ± 1.3	11.3 ± 3.3
GS-441524	> 100	> 100	> 100
Chloramphenicol (positive control)	2.6 ± 0.6	> 25	14.1 ± 3.6

^a CC₅₀ values were reported as average of two or more independent experiments ± standard deviation.

Remdesivir (GS-5734) had cell-type-dependent effects on mitochondrial respiration capacity at low micromolar levels after 3 days of exposure based on the [XF Cell Mito Stress Test Kit](#) (Seahorse Biosciences, North Billerica, MA) (Study Report [PC-399-2016](#)) (Table 2.4-4). This assay measures the impact of test compounds on the oxygen consumption rate of cells exposed to various modulators of cellular respiration targeting components of the electron transport chain in mitochondria. A specific inhibition of spare mitochondrial respiration was observed in PC-3 (prostate cancer) cells. GS-441524 (parent nucleoside analog) had no measurable cytotoxicity by any of the three measurements for any of the three cell lines (CC₅₀ values >100 μM). The sponsor concluded that PC-3 cells might be uniquely sensitive to the effects of GS-5734 on spare cellular respiration relative to either liver (i.e., HepG2) or kidney (i.e., RPTEC) cells, possibly due to the fast cellular proliferation and metabolism of PC-3 cells.

Table 2.4-4. Assessment of GS-5734 impact on mitochondrial respiration (Table 2, page 10, Study Report PC-399-2016).

Cell Models	CC ₅₀ (μM) ^a		
	Spare Respiration Capacity	DNA	ATP
PC-3 Cells	2.5 ± 0.1	12.5 ± 0.7	24.0 ± 1.4
HepG2 Cells	10.6 ± 0.1	6.3 ± 0.9	7.9 ± 0.1
RPTEC	7.3 ± 2.7	14.3 ± 3.3	16.9 ± 4.1

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Remdesivir (GS-5734) did not cause a specific depletion of mitochondrial DNA levels in HepG2 (hepatoma) cells after 10 days (presumably stationary) of drug exposure at concentrations up to 1 μM compared to ddC, which was used as a positive control (Study Report [PC-399-2015](#)) (Table 2.4-5). In a second experiment, at 2 μM GS-5734 mitochondrial DNA levels were reduced by 26%, but at 10 μM GS-5734 mitochondrial DNA levels were not specifically reduced, but rather total cellular DNA levels were reduced by 75%, which the sponsor attributed to the cytotoxicity of GS-5734 over the 10-day incubation presumably in stationary cells. Again, these results are consistent with the low micromolar cytotoxicity of GS-5734 for multiple cell types.

Table 2.4-5. Impact of remdesivir (GS-5734) on mitochondrial DNA levels (Tables 3 and 4, pages 8 and 9, Study Report [PC-399-2015](#)). Shown are results from two experiments. ddC, 2',3'-dideoxycytidine is a known inhibitor of mitochondrial DNA polymerase γ .

Compound	Compound Concentration (μM)	DNA Yield (% Control)	Relative Amount of mtDNA (% Control) ^a	p-value Compared to DMSO (vs Control) ^b
DMSO (control)	—	100.0 $\pm$ 26.8	100.0 $\pm$ 22.2	—
GS-5734	0.04	114.6 $\pm$ 39.0	88.9 $\pm$ 30.4	0.215
	0.2	86.0 $\pm$ 31.5	98.1 $\pm$ 30.1	0.702
	1.0	100.3 $\pm$ 32.1	93.4 $\pm$ 27.1	0.436
ddC	0.2	93.1 $\pm$ 26.3	76.9 $\pm$ 12.8	0.005
	2.0	91.4 $\pm$ 32.1	10.0 $\pm$ 3.8	< 0.001
	20.0	86.8 $\pm$ 25.7	0.7 $\pm$ 0.2	< 0.001
Compound	Compound Concentration (μM)	DNA Yield (% Control)	Relative Amount of mtDNA (% Control) ^a	p-value compared to DMSO (vs Control) ^b
DMSO (control)	—	100.0 $\pm$ 16.7	100.0 $\pm$ 20.3	—
GS-5734	0.4	104.1 $\pm$ 30.0	122.8 $\pm$ 23.9	0.067
	2.0	106.4 $\pm$ 36.2	74.2 $\pm$ 11.7	0.008
	10.0	25.2 $\pm$ 14.1	148.0 $\pm$ 25.6	0.001
ddC	0.2	125.1 $\pm$ 20.3	94.7 $\pm$ 3.4	0.419
	2.0	128.6 $\pm$ 34.4	56.5 $\pm$ 10.5	< 0.001
	20.0	129.4 $\pm$ 19.3	12.2 $\pm$ 2.8	< 0.001

In biochemical assays, GS-443902 (active triphosphate) showed no inhibition of human DNA polymerases α , β , or γ , human RNA polymerase II, or human mitochondrial RNA polymerase (POLRMT) at concentrations up to 200 μM (Table 2.4-6) (Study Report [PC-399-2017](#)).

Table 2.4-6. Inhibition of human DNA and RNA polymerases by GS-443902 (Table 3, page 10, Study Report [PC-399-2017](#)). POLRMT, human mitochondrial RNA polymerase. In the POLRMT assay, natural NTPs were included at 2.5 μM .

Compounds	IC ₅₀ (μM) ^a				
	DNA Pol α ^b	DNA Pol β ^b	DNA Pol γ ^b	RNA Pol II ^c	POLRMT
GS-443902	> 200	> 200	> 200	> 200	> 200
Positive Controls (IC ₅₀)	Aphidicolin (4.7 $\pm$ 3.3)	3'dTTP (1.9 $\pm$ 0.8)	3'dTTP (1.2 $\pm$ 0.6)	α -amanitin (0.0035 $\pm$ 0.0015)	3'deoxy GTP ^d (4.2 $\pm$ 1.4)

- a IC₅₀ values were reported as arithmetic means of two to three independent experiments. All nucleoside/tide analogs were tested using their corresponding 5'-triphosphate active form. For simplification, the active form of each compound was denoted as NI-TP where NI refers to the prodrug or nucleoside used in clinical trial or pre-clinical studies wherever applicable.
- b The natural dNTP concentrations were kept at 2 μM .
- c The measured K_m values for natural NTP were 25 μM for ATP and 5 μM for GTP, CTP, and UTP.
- d IC₅₀ values for 3'deoxy ATP, 3'-deoxy GTP, 3'deoxy CTP, and 3'deoxy UTP were 4.6, 1.9, 1.4, and 4.7 μM , respectively.

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GS-443902 was not incorporated into DNA by the human mitochondrial DNA polymerase γ , and was a relatively poor substrate (~6% relative to ATP) for incorporation into RNA by human mitochondrial RNA polymerase (Table 2.4-7) (Study Report [PC-399-2017](#)). It is possible that this level of incorporation by mitochondrial RNA polymerase could be significant if intracellular GS-443902 levels surpass natural ATP levels and incorporation inhibits further polymerase activity. However, as shown in Table 2.4-6 high levels of GS-443902 did not appear to inhibit mitochondrial RNA polymerase activity. Furthermore, as shown in Table 2.4-7, GS-5734 did not have a specific impact on mitochondrial protein synthesis in PC-3 cells.

Table 2.4-7. Incorporation of GS-443902 by human mitochondrial DNA polymerase γ or human mitochondrial RNA polymerase (Table 4, page 10, Study Report PC-399-2017). POLRMT, human mitochondrial RNA polymerase

Nucleotide Triphosphates	Rate of Incorporation (% of Rate for Natural dNTP or NTP)	
	DNA POL γ	POLRMT
GS-443902	0%	5.8 ± 1.4%
Decitabine-TP	79 ± 8%	ND ^b
BMS-986094-TP	ND	92 ± 33%
Balapiravir/RG1626-TP	ND	112 ± 10%

- a Values present the average of at least two independent experiments. All compounds were tested using the 5'-triphosphate active form.
b ND = not determined

Additional Data

A recent study looked at the genetic pathways involved in cellular remdesivir metabolism and cytotoxicity and reported that remdesivir treatment leads to a repression of mitochondrial respiratory activity that is associated with five human genes, the loss of which significantly reduces remdesivir cytotoxicity ([Akinci et al., 2020](#) – preprint).

Conclusions

- Remdesivir (GS-5734) did not have a consistent signal of specific mitochondrial toxicity based on multiple different cell-based assays, including assessments of cytotoxicity in cells grown in glucose-versus galactose-containing medium (“crabtree effect”), mitochondrial-specific protein synthesis, mitochondrial respiration capacity, and mitochondrial DNA levels.
- GS-443902 also was not incorporated into DNA by the human mitochondrial DNA polymerase γ , and was a relatively poor substrate (~6% relative to ATP) for incorporation into RNA by human mitochondrial RNA polymerase (Study Report [PC-399-2017](#)).

Considerations for Post-Marketing Actions: None.

2.5 Cell Culture Combination Antiviral Activity Relationship Studies

Remdesivir was issued Emergency Use Authorization for the treatment of SARS-CoV-2 infection on May 1, 2020. An Emergency Use Authorization was issued for chloroquine phosphate (CQ) and hydroxychloroquine sulfate (HCQ) on March 28, 2020, although, the EUA for CQ and HCQ was later revoked on June 15, 2020 when results from randomized controlled clinical trials did not support its

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use. Given that the two drugs were in wide use through EUAs, the Applicant performed a cell culture combination antiviral activity relationship assessment of the two drugs to determine if one antagonized the other ([Bobrowski et al., 2020](#) – preprint).

Study Title: Chloroquine antagonizes the antiviral activity of remdesivir by reducing the formation of its active triphosphate metabolite

Study Report: [PC-540-2009](#)

Objective: The purpose of this study is to evaluate the in vitro effects of clinically relevant concentrations of chloroquine phosphate on the intracellular metabolism and antiviral activity of RDV.

Methods

A surrogate RSV inhibition assay was used to assess the impact of CQ doses recommended under the EUA guidance for COVID-19. Here are a few details regarding the surrogate RSV inhibition assay:

1. The experiments were performed in the HEp-2 human epithelial carcinoma cell line infected with RSV strain A2 at a multiplicity of infection (MOI) of 4.
2. RDV exhibits cell culture antiviral activity against RSV with an EC₅₀ value of 19 nM ([Warren et al., 2016](#)).
3. CQ did not inhibit RSV replication at concentrations up to 5 µM (internal data not shown).

Assaying for CQ antagonism against RDV. To determine the antagonistic effect of CQ on RDV antiviral activity, the RDV dilution series was additionally spiked with DMSO or 4X increasing concentrations of CQ (final concentrations: 10, 40, 160, 640, or 2,560 nM). The cells were then infected with RSV A2 virus diluted in DMEM + GlutaMAX at a MOI=4 and incubated for 4 days at 37°C and 5% CO₂. Uninfected and untreated wells were included as controls for 100% cell viability. Virus-induced cytopathic effect was then assessed by measuring luminescence signal using an Envision plate reader.

Results

The sponsor noted that RDV, CQ and HCQ have been granted EUA by the FDA for the treatment of COVID-19 in the ongoing SARS-CoV-2 pandemic. Because there might be interest in combining these drugs for potentially added clinical benefit, the sponsor examined the effect of clinically relevant concentrations of CQ on the cell culture antiviral activity of RDV.

According to the sponsor, at CQ doses recommended under the EUA guidance for COVID-19, the average systemic CQ exposures ranged from 610-760 nM and could exceed 20 µM in the lung. This range of concentration was used to assess the impact of CQ on RDV antiviral activity. Because the sponsor has limited immediate access to BSL-3 facilities, they did their initial experiment using RSV instead of SARS-CoV-2 in a surrogate RSV inhibition assay in HEp-2 human epithelial carcinoma cell line. The sponsor reported that similar experiments using SARS-CoV-2 are currently ongoing.

The antiviral activities of RDV and CQ against RSV were evaluated in HEp-2 epithelial carcinoma cells. RDV inhibited RSV with a mean EC₅₀ value of 49.5 nM in this assay (Table 2.5-1).

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Table 2.5-1. Cell culture antiviral activity of RDV against RSV in HEp-2 cells treated with increasing CQ concentrations (Table 1, page 9, Study Report PC-540-2009).

Chloroquine (nM)	RDV RSV EC ₅₀ (nM) ^a	RDV RSV EC ₅₀ Fold-change ^b	P value ^c
0	49.5 ± 13.1	-	-
10	58.4 ± 12.8	1.19 ± 0.10	0.075
40	72.1 ± 24.8	1.45 ± 0.19	0.052
160	99.6 ± 30.3	2.02 ± 0.17	0.009
640	138.3 ± 33.8	2.86 ± 0.50	0.023
2560	209.0 ± 18.2	4.43 ± 1.13	0.034

a Values are mean ± standard deviation of two independent replicates in HEp-2 cells
b Fold-change represents the mean RDV RSV EC₅₀ at each chloroquine concentration divided by the mean RDV EC₅₀ in the absence of chloroquine
c p value is determined using paired t test compared to the DMSO control in GraphPad Prism

The antiviral activity of RDV against RSV decreased when combined with CQ, in a CQ-dose-dependent manner (Table 2.5-1 and Figure 2.5-1). Co-incubation of cultures with 160 and 640 nM CQ decreased RDV antiviral activity against RSV, as determined by increases in EC₅₀ values, by approximately 2-fold (p = 0.009) and 3-fold (p = 0.023), respectively (Table 2.5-1). The sponsor noted that these concentrations are within the range of plasma concentrations of CQ following administration of a dose of 500 mg CQ (Zhao et al., 2014). A greater reduction in RDV antiviral activity against RSV (4.43-fold) was observed in the presence of 2,560 nM CQ (Table 2.5-1).

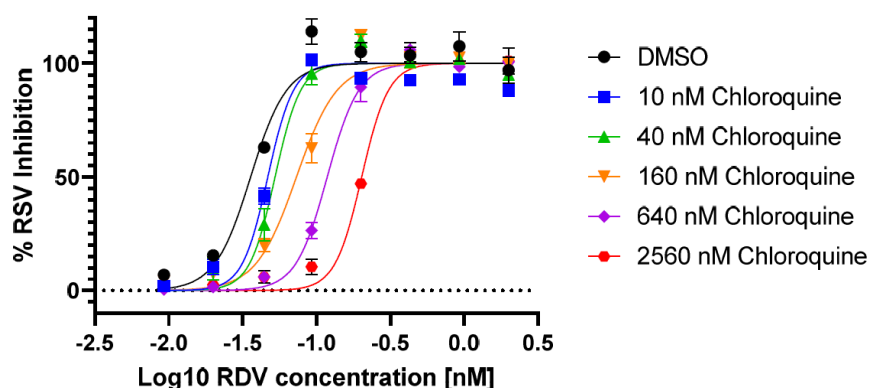


Figure 2.5-1. Chloroquine antagonizes RDV RSV antiviral activity in a dose-dependent manner (Figure 1, page 10, Study Report PC-540-2009).

- The antiviral activity of RDV against RSV in HEp-2 cells was antagonized in a CQ-dose-dependent manner when CQ was co-incubated in the cultures at clinically relevant concentrations ranging from 10 to 2,560 nM with EC₅₀ value increases ranging from 1.19- to 4.43-fold.

RDV triphosphate metabolism analysis in NHBE cells. Normal human bronchial epithelial (NHBE) cells were incubated at 37°C with medium containing 1 μM RDV alone, or in combination with 1 or 10 μM CQ. At 8, 24, and 48h post drug addition, cells were prepared for analysis by Liquid chromatography–mass spectrometry (LC-MS)/MS, using a multi-stage linear Gradient. Detection was performed on a Qtrap 6500+ (AB Sciex) mass spectrometer operating in positive ion and multiple reaction monitoring

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modes. Analytes were quantified using a 7-point standard curve ranging from 0.624 to 160 pM per million cells prepared in extracts from untreated cells.

Table 2.5-2. Co-Incubation with CQ reduces RDV-TP formation in a dose-dependent manner (Table 2, page 10, Study Report PC-540-2009).

Treatment time (h)	Mean RDV-TP (pmol/million cells) ^a		
	1 μ M RDV	1 μ M RDV + 1 μ M CQ	1 μ M RDV + 10 μ M CQ
8	11.1 $\pm$ 0.8	5.1 $\pm$ 0.7	2.7 $\pm$ 1.5
24	10.5 $\pm$ 1.3	8.9 $\pm$ 0.7	4.9 $\pm$ 0.4
48	12.6 $\pm$ 6.6	10.5 $\pm$ 2.6	6.1 $\pm$ 0.3
Average concentration	10.3 $\pm$ 1.2	7.6 $\pm$ 0.6	4.2 $\pm$ 0.5
P value ^b	NA	0.006	0.005

a Values are mean $\pm$ standard deviation of duplicate samples collected at each timepoint from two separate experiments
b P value is determined using 2-tails, paired t-test compared to RDV alone; P value refers to comparison of average RDV-TP concentration.

Co-incubation of RDV and CQ resulted in a CQ dose-dependent reduction in formation of intracellular RDV-TP, indicating an antagonistic effect of CQ on RDV metabolism to its active triphosphate (Table 2.5-2 and Figure 2.5-2). Significant CQ antagonism of RDV-TP formation was observed at 8 h post treatment with 1 and 10 μ M CQ concentrations resulting in >2-fold reductions of RDV-TP, respectively. RDV-TP reductions persisted for the 48-hour duration of the assay, with a 2-fold reduction maintained for the 10 μ M CQ concentration (Table 2.5-2).

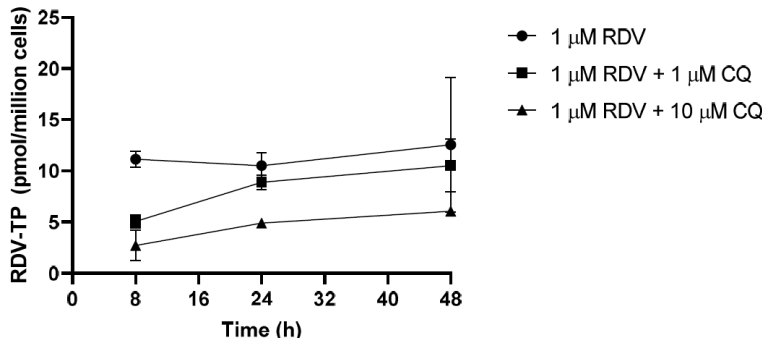


Figure 2.5-2. Co-Incubation of CQ with 1 μ M RDV results in dose-dependent inhibition of RDV-TP formation (Figure 2, page 11, Study Report PC-540-2009).

- Co-incubation of RDV and CQ resulted in a CQ dose-dependent reduction in formation of intracellular RDV-TP, indicating an antagonistic effect of CQ on RDV metabolism to its active triphosphate. Significant CQ antagonism of RDV-TP formation was observed at 8 h post treatment with 1 and 10 μ M CQ concentrations resulting in >2-fold reductions of RDV-TP, respectively. RDV-TP reductions persisted for the 48-hour duration of the assay.

CQ pharmacokinetics. The sponsor used simulated PK profiles to predict accumulation of CQ after multiple daily doses and noted that after stopping dosing, it also takes approximately 7 days for CQ plasma concentrations to drop to below 0.1 μ M. Because plasma levels of CQ and its active metabolite persist with estimated terminal elimination half-lives of 14-60 days ([Smit et al., 2020](#), [Wong et al., 2020](#); [Yao et al., 2020](#)), patients who have recently been on chloroquine therapy may also experience decreased efficacy of RDV treatment. Of note, concentrations of HCQ are significantly higher in the human lung than in blood ([Yao et al., 2020](#)). In a population PK model ([Carmichael et al., 2003](#)), the

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average steady-state blood concentrations were 974 µg/L (2.9 µM) for the 310-mg doses of HCQ base (equivalent to 400 mg HCQ). In animals, chloroquine and HCQ showed similar tissue distribution patterns, with high concentrations in the liver, spleen, kidney, and lung reaching levels of 200 - 700 times higher than those in the plasma ([Popert, 1976](#)). Interestingly, a recent report indicates that CQ does not block infection in TMPRSS2-expressing human Calu-3 lung cells because chloroquine targets a pathway for viral activation that is not active in lung cells ([Hoffmann et al., 2020](#)). It is unknown if or to what extent HCQ/CQ antagonizes RDV in human lung cells.

- Based on the evidence that CQ antagonizes the metabolism of RDV to its active RDV-TP, RDV should not be co-administered with CQ or hydroxychloroquine sulfate (nearly identical chemical structures and mechanisms of action). In addition, subjects who received CQ/HCQ in a prophylaxis clinical trial who later become infected with SARS-CoV-2 may experience reduced efficacy if treatment with RDV is initiated within 60 days of the end of CQ/HCQ prophylaxis.

Conclusions

- This study assessed for antagonism between remdesivir and chloroquine phosphate in cell culture, and described the mechanism of chloroquine phosphate antagonism of the remdesivir active triphosphate metabolite. The Applicant did not describe the specific mechanism as CQ could impact uptake of the prodrug, processing of the prodrug to the monophosphate and/or subsequent phosphorylation steps, all of which could lead to a reduction in the active form of remdesivir.
- The antiviral activity of RDV against RSV in HEp-2 cells was antagonized in a CQ-dose-dependent manner when CQ was co-incubated in the cultures at clinically relevant concentrations ranging from 10 to 2,560 nM with EC₅₀ value increases ranging from 1.19- to 4.43-fold.
- Co-incubation of RDV and CQ resulted in a CQ dose-dependent reduction in formation of intracellular RDV-TP, indicating an antagonistic effect of CQ on RDV metabolism to its active triphosphate. Significant CQ antagonism of RDV-TP formation was observed at 8 h post treatment with 1 and 10 µM CQ concentrations resulting in >2-fold reductions of RDV-TP, respectively. RDV-TP reductions persisted for the 48-hour duration of the assay.
- Simulated PK profiles were used to predict accumulation of CQ after multiple daily doses and indicated that after stopping dosing, it could take approximately 7 days for CQ plasma concentrations to drop to below 0.1 µM. Because plasma levels of CQ and its active metabolite persist with estimated terminal elimination half-lives of 14-60 days, patients who have recently been on chloroquine therapy may also experience decreased efficacy of RDV treatment.
- Based on the evidence that CQ antagonizes the metabolism of RDV to its active RDV-TP, RDV should not be co-administered with CQ or hydroxychloroquine sulfate (nearly identical chemical structures and mechanisms of action). In addition, subjects who received CQ or HCQ in a prophylaxis clinical trial who later become infected with SARS-CoV-2 may experience reduced efficacy if treatment with RDV is initiated within 60 days of the end of CQ/HCQ prophylaxis.
- Language describing the antagonism of CQ on RDV was added to the RDV EUA and will be included in the labeling for this drug.
- The Applicant indicated experiments are underway to investigate the effect of CQ on the antiviral activity of RDV against SARS-CoV-2. This study report will be requested as a post-marketing commitment.

Considerations for Post Marketing Actions

1. **Antagonism PMC #3:** Submit a complete study report for the assessment of the antagonistic

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effect of chloroquine/hydroxychloroquine on the antiviral activity of RDV against SARS-CoV-2 in cell culture when it is available.

2.6 Animal Studies

Study Title: Topline report: Evaluation of the Efficacy (sic) of Remdesivir against SARS-CoV-2 in a Rhesus Macaque Model

Study Report: [PC-540-2004](#)

Objectives: The purpose of this study was to characterize the in vivo antiviral activity of remdesivir against SARS-CoV-2 in rhesus macaque model ([Munster et al., 2020](#)). Preliminary results of the study are presented in this topline report.

Challenge virus: SARS-CoV-2 isolate nCoV-WA1-2020 ([MN985325.1](#)) (passage 4) was used. The isolate originated from CDC and was propagated once in Vero E6 cells. Next-generation sequencing (NGS) of virus stock confirmed 100% identity with sequence in Genbank. No data were provided regarding the outcome of the patient from whom this isolate was collected.

Rhesus macaque model of SARS-CoV-2 infection: This nonlethal model reportedly experiences mild to moderate, transient lower respiratory tract disease with pulmonary infiltrates visible on radiographs, and a shedding pattern similar to that observed in COVID-19 patients ([Munster et al., 2020](#)).

Experimental Design

Two groups of six rhesus macaques (3 males and 3 females each; 3.6-5.7 kg) were infected with SARS-CoV-2 and treated with intravenous remdesivir or an equal volume of vehicle solution. Clinical, virological, and histological parameters were assessed to determine treatment effect. It's unclear if administration of remdesivir was done in a blinded fashion; however, the person conducting daily assessments of animals after treatment, the veterinarian, and the pathologist were blinded to the group assignments of the animals.

- Animals were randomly assigned to groups and inoculated with a total dose of 2.6×10^6 TCID₅₀ of SARS-CoV-2 strain nCoV-WA1-2020 via a combination of intranasal, oral, ocular and intratracheal routes
- RDV treatment was initiated at 12 hours after inoculation with SARS-CoV-2 and continued once daily through 6 days post inoculation (dpi)
 - The treatment group received a loading dose of 10 mg/kg remdesivir, followed by a 5 mg/kg dose 12 hours after the loading dose, and then a daily maintenance dose of 5 mg/kg for 5 additional days delivered as a slow intravenous bolus injection
 - The infected controls group was treated with an equal volume (i.e., 2 mL/kg loading dose and 1 mL/kg thereafter) of vehicle solution (12% sulfobutylether- β -cyclodextrin in water and hydrochloric acid, pH^{(b)(4)}) according to the same treatment schedule
- Animals were monitored at least twice daily throughout the experiment for clinical signs of disease using a standardized scoring sheet
- The predetermined endpoint for this experiment was 7 dpi
- Nose, throat and rectal swabs were collected daily during treatment administration

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- On 1, 3, and 7 dpi a bronchoalveolar lavage was performed using 10 mL of sterile saline. After euthanasia on 7 dpi, necropsies were performed

Reviewer's note: Dosing was started 12 hours after inoculation and so we consider this a post-exposure prophylaxis study and not a treatment study.

Virology Assessments

- SARS-CoV-2 Virus Isolation and Titration Assay.** Virus titrations were performed by end-point titration in Vero E6 cells. Tissue was homogenized in 1 mL DMEM using a TissueLyzer (Qiagen). Vero E6 cells were inoculated with 10-fold serial dilutions of BAL samples. One hour after inoculation of cells, the inoculum was removed and replaced with 100 μ L DMEM (b) (4) supplemented with 2% fetal bovine serum, 1 mM L-glutamine, 50 U/mL penicillin and 50 μ g/mL streptomycin. Six days after inoculation cytopathic effect (CPE) was scored and the TCID₅₀ was calculated. Virus isolation was performed on lung tissues by homogenizing the tissue in 1mL DMEM and inoculating Vero E6 cells in a 24 well plate with 250 μ L of cleared homogenate and a 1:10 dilution thereof. One hour after inoculation of cells, the inoculum was removed and replaced with 500 μ L DMEM (b) (4) supplemented with 2% fetal bovine serum, 1 mM L-glutamine, 50 U/mL penicillin and 50 μ g/mL streptomycin. Six days after inoculation, CPE was scored.
- SARS-CoV-2 Viral Load Assay.** RNA was extracted from swabs and BAL using the QiaAmp Viral RNA kit (Qiagen) according to the manufacturer's instructions. Tissues (30 mg) were homogenized in RLT buffer (Qiagen), and RNA was extracted using the RNeasy kit (Qiagen) according to the manufacturer's instructions. For detection of viral RNA, 5 μ L of RNA was used in a one-step real-time RT-PCR assay targeting the SARS-CoV-2 Envelope gene as previously described (Corman et al., 2020). In each run, dilutions of RNA standard were run in parallel, to calculate copy numbers in the samples.

Results

Clinical signs. Remdesivir reduced clinical disease signs in SARS-CoV-2-infected rhesus macaques. The vehicle-treated animals displayed signs of disease, such as increased respiration and shortness of breath after inoculation, starting as early as 1 dpi. Clinical scores were assessed in blinded fashion and were statistically significantly lower in remdesivir-treated animals than in vehicle-treated control animals at 1 to 7 dpi (Figure 2.6-1).

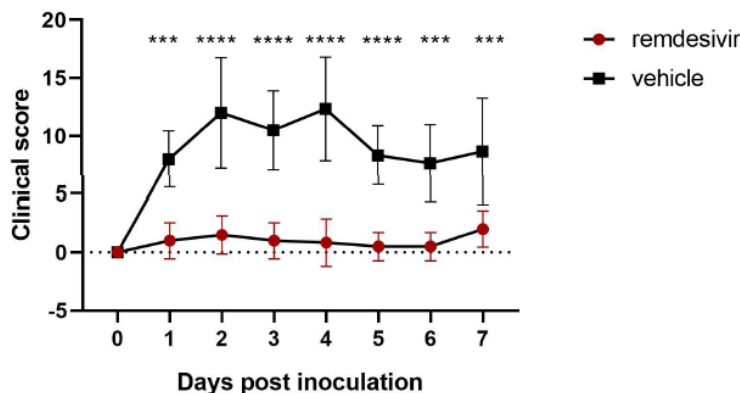


Figure 2.6-1. Clinical signs in rhesus macaques inoculated with SARS-CoV-2 and treated with remdesivir or vehicle (Figure 1, page 9, Study Report PC-540-2004). Values represent averages from 6 animals per group. Asterisks indicate statistically significant differences in a two-way ANOVA with Dunnett's multiple comparisons. ***P < 0.001; ****P < 0.0001.

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Gross lung lesions. Upon necropsy at 7 dpi, the area of each lung lobe affected by gross lesions was estimated by a board-certified veterinary pathologist who was blinded to the group assignments of the animals. Among animals treated with remdesivir, 1 of 6 animals had >10% of area affected by gross lesions in the upper and lower parts of the right lung lobe. In contrast, gross lung lesions were present in several lung lobes of all the vehicle-treated control animals (Figure 2.6-2A). The total area of the lungs affected by gross lesions was statistically significantly smaller in the remdesivir treated animals than in vehicle-treated control animals (Figure 2.6-2B). In addition, the vehicle-treated control animals had an increased lung weight:body weight ratio indicative of pulmonary edema. The ratio between lung and body weight was significantly lower in remdesivir-treated animals than in vehicle-treated control animals (Figure 2.6-2C).

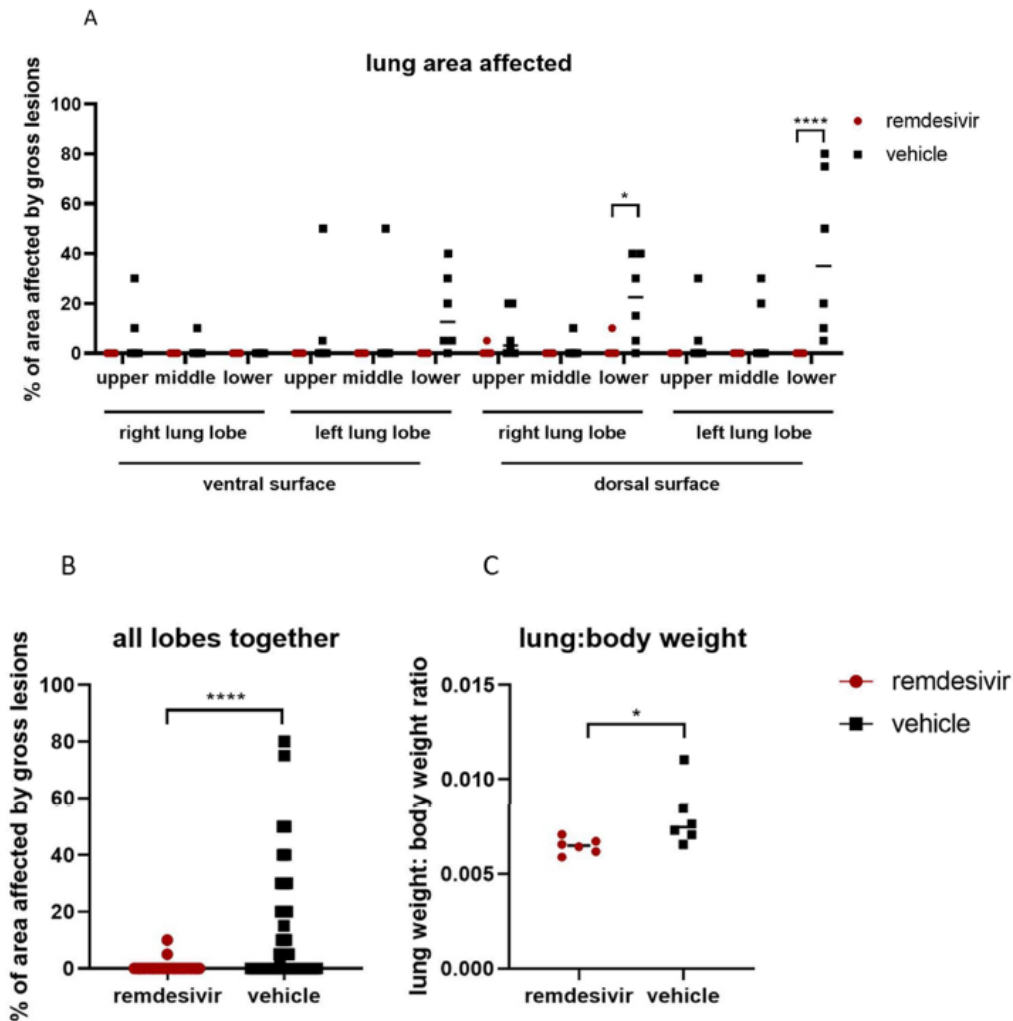


Figure 2.6-2. Pathological findings in the lung of rhesus macaques inoculated with SARS-CoV-2 and treated with remdesivir or vehicle (Figure 2, page 10, Study Report PC-540-2004). Each dot represents results from one animal (A and C). Each dot represents the individual scores from all lung lobes and animals (B). Asterisks indicate statistically significant differences in a two-way ANOVA with Dunnett's multiple comparisons. *P < 0.05; ****P < 0.0001.

Pulmonary infiltrates. Radiographs were used to score individual lung lobes for severity of pulmonary infiltrates by a clinical veterinarian (blinded to the group assignment of the animals) according to a

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standard scoring system. At 3 to 7 dpi, there were statistically significantly fewer infiltrates in the lungs of animals treated with remdesivir as compared to vehicle-treated control animals (Figure 2.6-3).

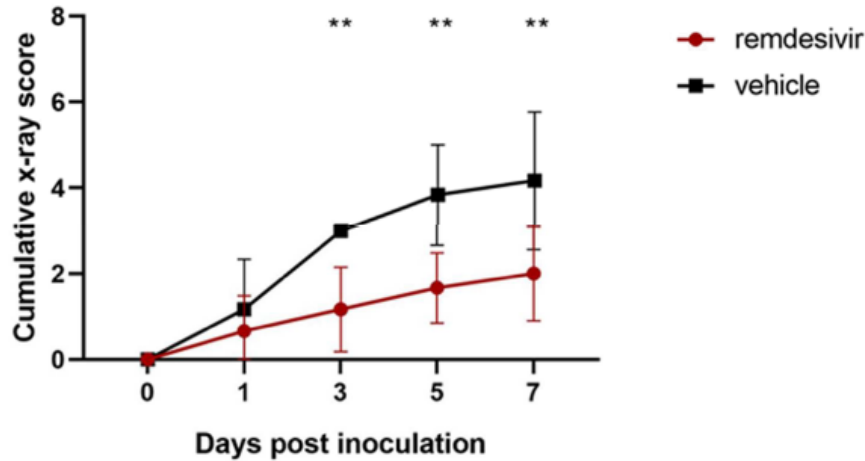


Figure 2.6-3. Lung radiographs of NHPs inoculated with SARS-CoV-2 and treated with remdesivir or vehicle (Figure 3, page 11, Study Report PC-540-2004).

Lung viral RNA loads. At 7 dpi, all animals were euthanized, and respiratory tissues were collected for quantitative analysis of the levels of viral RNA by quantitative RT-PCR. Compared to vehicle-treated control animals, remdesivir treatment resulted in significantly lower levels of SARS-CoV-2 replication in the lungs, with the average lung viral RNA 2.2 log₁₀ lower in the combined lung lobes (Figure 2.6-4). At necropsy, one sample per lung lobe was collected per animal (6 samples per animal) and viral load was determined. Averages and standard deviations per group are indicated. Asterisks indicate statistically significant differences in a two-way ANOVA with Dunnett’s multiple comparisons. ***P < 0.001.

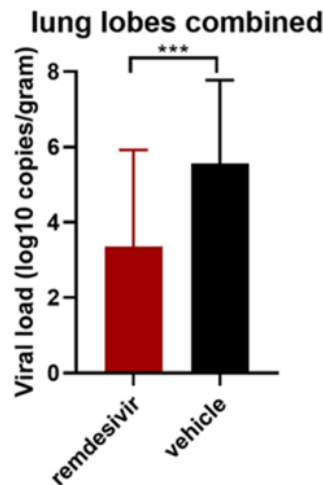


Figure 2.6-4. Viral RNA load in lung lobes of NHPs (Figure 4, page 12, Study Report PC-540-2004).

Additionally, remdesivir-treated animals had significantly lower viral shedding as measured by viral RNA copies in throat swabs on 4 dpi compared to vehicle-treated control animals (Figure 2.6-5). No difference was observed in bronchoalveolar lavage, nose swabs and rectal swabs, except for the nose swabs at 5 dpi where viral shedding was lower in vehicle control compared to remdesivir-treated animals.

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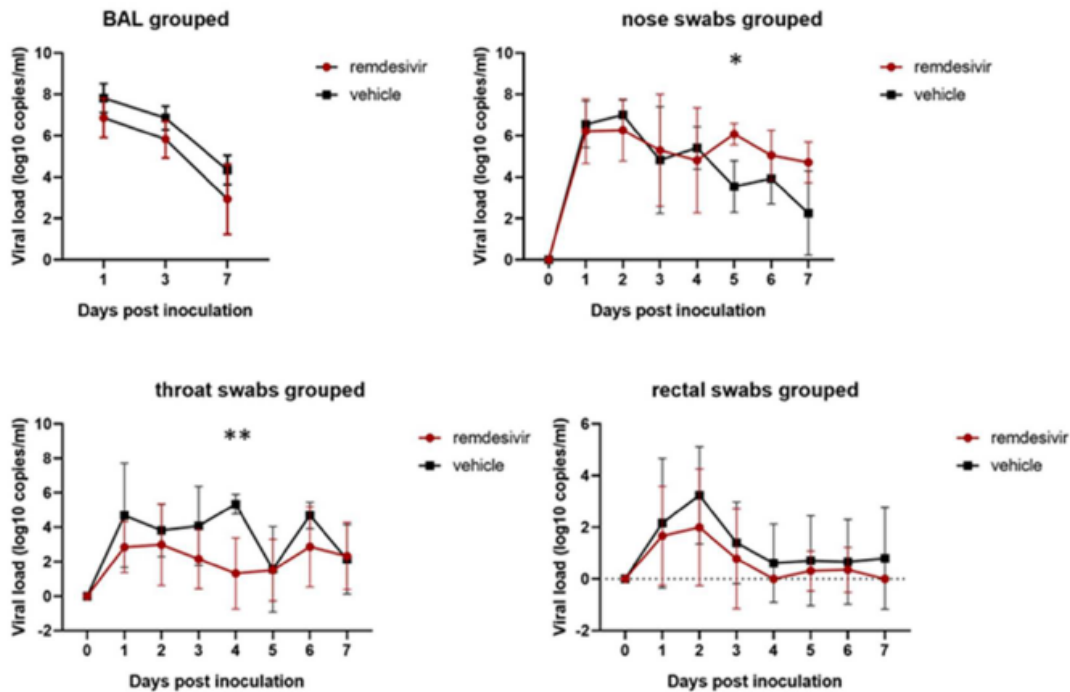


Figure 2.6-5. Viral shedding in swabs from NHPs inoculated with SARS-CoV-2 and treated with remdesivir or vehicle (Figure 5, page 13, Study Report PC-540-2004). Values represent averages from 6 animals per group. Asterisks indicate statistically significant differences in a two-way ANOVA with Dunnett's multiple comparisons. *P < 0.05; **P < 0.01. BAL, Bronchoalveolar lavage.

Reduced SARS-CoV-2 viral titers. Replication-competent virus was isolated from BAL fluid at 1 and 3 dpi. In BAL collected on 1 dpi, the viral titer was significantly lower in remdesivir-treated animals compared to vehicle-treated control animals. At 3 dpi virus was no longer detected in BAL from remdesivir-treated animals but could still be isolated from the vehicle-treated animals (Figure 2.6-6). At 7 dpi, all animals were euthanized, and respiratory tissues from 1 to 2 lung lobes per animal were collected for virus isolation. Virus was isolated from 5 of 6 vehicle-treated control animals but was not detectable in any of the remdesivir-treated animals.

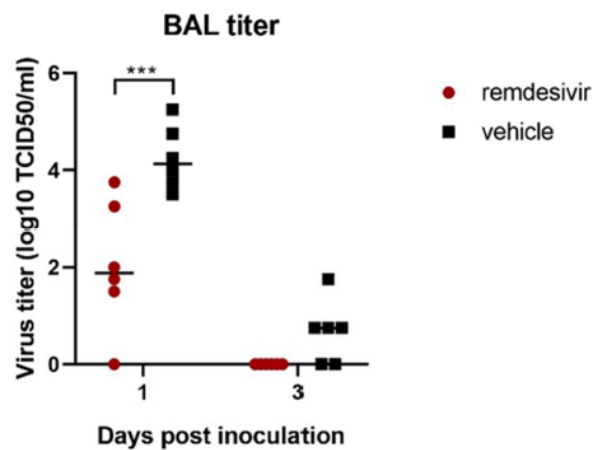


Figure 2.6-6. Viral titers in swabs from NHPs inoculated with SARS-CoV-2 and treated with remdesivir or vehicle (Figure 6, page 14, Study Report PC-540-2004). Each dot represents results from one animal. Asterisks indicate statistically significant differences in a two-way ANOVA with Dunnett's multiple comparisons. *P < 0.05; ***P < 0.001. BAL, Bronchoalveolar lavage.

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Conclusions

- All six rhesus macaques treated with remdesivir had significantly reduced clinical signs of SARS-CoV-2 infection compared to the vehicle-treated animals ($P < 0.001$). Consistent with these observations, lung pathology assessed by radiography showed reduced severity of pulmonary infiltrates in the lungs of remdesivir-treated animals compared to the vehicle control ($P < 0.01$). Upon necropsy at 7 dpi, remdesivir-treated animals had significantly reduced gross lung lesions ($P < 0.0001$) and on an average 2.2 $\log_{10}$ lower viral RNA levels in lung tissue compared to vehicle-treated animals ($P < 0.001$).
- Minimal differences in viral RNA levels were observed in nose and throat swabs, while no statistically significant differences were observed in rectal swab and bronchoalveolar lavage between the groups. SARS-CoV-2 was not detectable in BAL at 3 dpi and in lung tissue at 7 dpi in remdesivir-treated animals but could still be isolated from the vehicle-treated animals.
- These data support early remdesivir treatment in COVID-19 patients to prevent progression to severe pneumonia. The relevance of these data to treatment in humans is unclear. Dose initiation at twelve hours would seem to be too early for disease signs to have developed so this study may be more representative of post-exposure prophylaxis. Analysis of viral shedding and viral load data appears to be consistent with this.
- The relationship to viral shedding and symptoms is unclear for SARS-CoV-2 but in other human respiratory viral infections, viral shedding is typically declining around the time symptoms appear.
- Quantification of viral RNA in nose and throat swabs from some of the untreated animals appears to show peaks around Days 3-4 (excluding Day 1 which is probably residual inoculum), which seems more likely to represent the replicating viral population. Consistent with this interpretation is the occurrence of peak clinical scores on Days 3-4 for some animals.
- Remdesivir showed antiviral activity in SARS-CoV-2-infected rhesus monkeys. Administration of remdesivir at 10/5 mg/kg (10 mg/kg first dose, followed by 5 mg/kg once daily thereafter) using IV bolus injection initiated 12 hours post-inoculation with SARS-CoV-2 resulted in a reduction in clinical signs of respiratory disease, lung pathology and gross lung lesions, and lung viral RNA levels compared with vehicle-treated animals ([Williamson et al., 2020 -- preprint](#)).

Additional information for RDV activity against SARS-CoV-2 in animal models.

An engineered chimeric mouse-adapted SARS-CoV variant encoding the RNA-dependent RNA polymerase (nsp12) of SARS-CoV-2 was used in a study to assess the impact of RDV (GS-5734) in mice. To determine if the chimeric SARS/SARS2-RdRp virus had similar activity against RDV (GS-5734), EC_{50} values of the chimera and wildtype SARS-CoV were determined in Huh7 cells and were comparable (SARS-CoV mean EC_{50} value = 7 nM, SARS/SARS2-RdRp mean EC_{50} value = 3 nM) ([Pruijssers et al., 2020](#)). The study authors noted that mice produce a serum esterase absent in humans, known as carboxyl esterase 1c (Ces1c), which dramatically reduces the half-life of RDV (GS-5734). To circumvent this variable, this mouse study using RDV (GS-5734) was performed in transgenic C57BL/6 *Ces1c*^{-/-} mice. C57BL/6 *Ces1c*^{-/-} mice were challenged intranasally with 10^3 PFU in 50 μ L of SARS/SARS2-RdRp and RDV (GS-5734) was administered subcutaneously with 25 mg/kg BID starting at 1-day post-infection (dpi).

Weight loss and lung hemorrhage did not differ significantly between vehicle- and RDV (GS-5734)-treated animals, but differences were noted in pulmonary function, as measured by whole-body plethysmography between RDV (GS-5734) - and vehicle-treated animals. RDV (GS-5734) treatment

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reduced viral shedding in the lung at 5 dpi as measured by plaque assay ([Pruijssers et al., 2020](#)) (Figure 2.6-7).

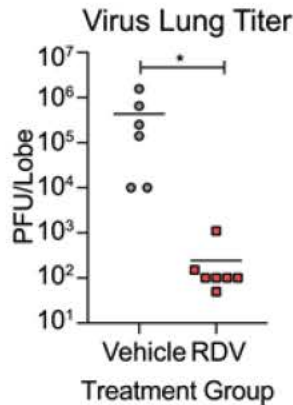


Figure 2.6-7. RDV (GS-5734) reduces SARS/SARS2-RdRp shedding in the lungs of C57BL/6 *Ces1c*^{-/-} mice (Figure 5 and legend copied and modified from [Pruijssers et al., 2020](#)).

Study Title: A Blinded, Randomized, Vehicle-Controlled Evaluation of the Efficacy (sic) of Intravenous GS-5734 Against Middle East Respiratory Syndrome Coronavirus (MERS-COV) in Rhesus Monkeys

Study Report: [RML 2016-018-E.1](#)

Objectives: The objective of this study was to characterize the antiviral activity of GS-5734 in a non-human primate (rhesus macaque) MERS-CoV disease model. In this study, the prophylactic antiviral activity of GS-5734 administered as an IV by slow bolus once-daily for seven days beginning one day before virus exposure was tested. The primary objectives of the study were to assess the effect of GS-5734 treatment on MERS-CoV viral shedding in lung tissue, disease-related clinical signs, radiographic lung assessments, and lung pathology and histopathology at Day 6 post-infection.

Challenge virus: MERS-CoV isolate HCoV-EMC/2012 was provided by (b) (4) and propagated in VeroE6 cells in DMEM (b) (4) supplemented with 2% (vol/vol) FCS (b) (4), 1 mM L-glutamine (b) (4), 50 U/mL penicillin, and 50 µg/mL streptomycin (b) (4).

Animal Model: Experimentally naive, Indian-origin, male rhesus macaques (3-5 kg; 2-3 years of age) were used in this study. Animals were sourced from the (b) (4) facility (b) (4) and were kept in quarantine for 30 days prior to the study initiation. Animals were transferred to the BLS-4 laboratory and acclimatized for at least 7 days.

Experimental Design

Experimentally naive rhesus macaques were randomly assigned to treatment groups and balanced by body weight (Table 2.6-1). MERS-CoV isolate HCoV-EMC/2012 was used as the challenge virus. All animals were exposed to a target dose of 7 x 10⁶ tissue culture infectious dose 50% (TCID₅₀) of MERS-CoV virus diluted in DMEM for inoculation. Animals were inoculated under anesthesia (10 mg/kg ketamine IM) through a combination of intratracheal (4 mL), intranasal (0.5 mL each nostril), oral (1 mL) and ocular (1 mL) with MERS-CoV (1x10⁶ TCID₅₀/mL). The day on which animals were challenged was designated as Day 0.

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Table 2.6-1. Experimental design (Table 1, page 7, Study Report RML 2016-018-E.1).

Group No.	No. males/No. females	Treatment	GS-5734 Dose (mg/kg)	Timing and Duration	Treatment Route	Challenge
1	6/0	GS-5734 in vehicle	10	Once Daily, D-1 to D5	IV bolus	MERS-CoV
2	6/0	Vehicle	0	Once Daily, D-1 to D5		

Methods to control bias included experimental blinding. Specifically, study personnel who administered GS-5734 or vehicle treatments or routinely evaluated animal health were experimentally blinded to the group assignment of all animals for the duration of the in-life phase. In addition, the veterinary pathologist was also blinded to the study group assignments during necropsy (lung lesion evaluation) and histopathology evaluations.

Virology Assessments

The primary endpoint for the study was viral load in lung tissue at Day 6 post-infection. Animal health was monitored at least twice daily for the duration of the in-life phase and clinical disease signs were recorded. On Days -7, 0, 1, 3, 5 and 6, clinical exams were performed on all animals to determine bodyweight, body temperature, respirations/minute (under anesthesia), and to collect x-rays, nose and throat swabs. Whole blood and serum were collected for hematology and biochemistry. On Day 6, a scheduled necropsy was performed on all animals; gross lung pathology was scored (as % of lung lobe affected by gross lesions) by a board-certified veterinary pathologist. Nineteen tissues were collected for histopathological and virological analysis conjunctiva; nasal mucosa; tonsil; oronasopharynx; trachea; right bronchus; left bronchus; lung (upper right lobe, middle right lobe, lower right lobe, upper left lobe, middle left lobe and lower left lobe); liver; spleen; kidney; bladder; mediastinal lymph node; and mandibular lymph node.

Results

All six animals in Group 1, treated with a 10 mg/kg dose of GS-5734 beginning on Day -1, had reduced clinical signs of MERS-Co V infection compared to the vehicle control animals (Figure 2.6-8).

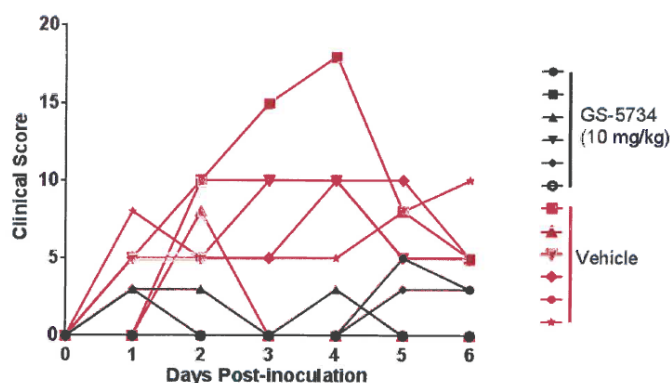


Figure 2.6-8. Clinical sign scores in MERS-CoV-infected rhesus monkeys treated with vehicle or 10 mg/kg RDV (GS-5734) (Figure 1, page 10, Study Report RML 2016-018-E.1). Clinical scoring is based upon a selected set of parameters designed to assess general health of the animals and distress due to MERS-Co V infection. GS-5734, remdesivir.

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At Day 6 post-infection, RDV (GS-5734)-treated animals had viral RNA levels in lung tissue that were 10- to 100-fold lower than viral RNA levels in vehicle-treated animals (Figure 2.6-9). Consistent with these observations, lung pathology assessed by radiography and histopathology showed clear improvement in the GS-5734-treated animals relative to animals in the vehicle control arm (data provided and reviewed but not shown).

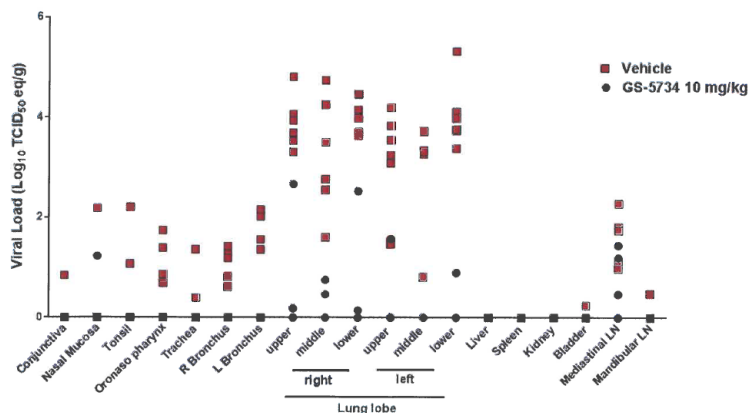


Figure 2.6-9. Tissue viral RNA concentrations by treatment group (Figure 3, page 12, Study Report RML 2016-018-E.1). Viral load was measured by qRT-PCR on Day 6. TCID₅₀ equivalents were extrapolated from standard curves generated by 10-fold dilutions of a MERS-CoV stock with known virus titer in parallel with each analysis. GS-5734, remdesivir.

While there were no statistically significant differences between GS-5734 and vehicle treatment on viral load in the nose and throat, the duration of shedding was notably reduced in RDV (GS-5734)-treated animals (5/6 animals with negative nasal swab) compared to the vehicle controls (5/6 animals with positive nasal swab) (data provided and reviewed but not shown).

Conclusions

Data from this pre-exposure prophylaxis study indicated that a 7-day treatment regimen of 10 mg/kg RDV (GS-5734) administered once daily by IV bolus injection beginning 1 day prior to virus exposure reduced viral shedding in the lung and clinical disease signs in MERS-CoV-infected rhesus macaques. However, RDV (GS-5734) was given prior to the onset of peak viremia, and so this study did not provide evidence of a treatment effect. In several of the MERS-CoV infected animals, the treatment with RDV (GS-5734) adversely affected renal function.

Additional information for RDV activity against coronaviruses in rhesus macaques

A PEP/PrEP study in a nonlethal model was published in 2020 by the NIH Rocky Mountain Labs wherein rhesus macaques were challenged with MERS-CoV (HCoV-EMC/2012) and treated with RDV (GS-5734) at 12 hours post challenge or prophylactically 24 hour prior to challenge [de Wit et al., 2020](#). In this blinded study, 18 rhesus macaques were randomly assigned to three groups of six animals. Three animals in the control group received 1 mL/kg vehicle solution 24 h before MERS-CoV inoculation, and three animals received the same vehicle solution at 12 h after MERS-CoV inoculation. Another group of six rhesus macaques was administered 5 mg/kg RDV (GS-5734) prophylactically 24 h before MERS-CoV inoculation, and one group of six animals was treated with 5 mg/kg RDV (GS-5734) at 12 h post-inoculation with MERS-CoV. RDV (GS-5734) was administered once daily until 6 dpi, when animals were euthanized and necropsied.

At 6 dpi, all animals were euthanized, and respiratory tissues were collected for quantitative analysis of the levels of viral RNA by qRT-PCR. Compared to vehicle-treated control animals, prophylactic

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administration of RDV (GS-5734) resulted in lower levels of MERS-CoV replication in the lungs, with lung viral loads 2.5 to 4 log₁₀ lower in each lung lobe (Figure 2.6-10A). Lung viral loads were, on average, lower in individual lung lobes after therapeutic treatment; however, there was larger variation between animals in the therapeutically treated group (Figure 2.6-10A). However, when all lung lobes were combined, the lung viral load in therapeutically treated animals was clearly lower than in vehicle-treated animals (Figure 2.6-10B). Additionally, viral loads were significantly lower in trachea, bronchi, tonsils, and mediastinal lymph nodes of animals treated prophylactically and therapeutically with remdesivir than in vehicle-treated control animals (Figure 2.6-10C); viral RNA was not detected in kidney tissue samples.

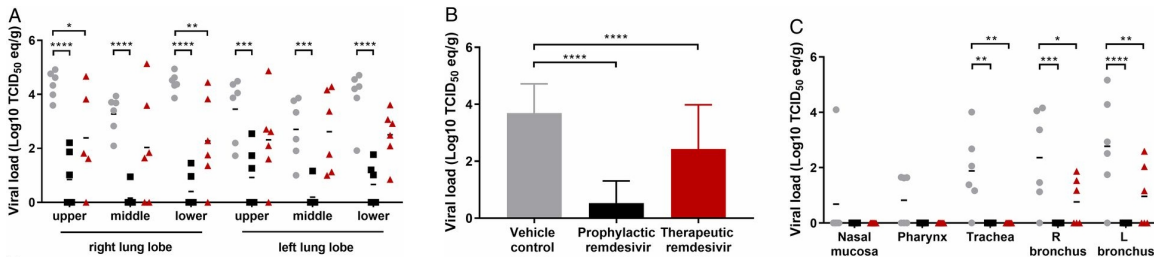


Figure 2.6-10. Viral loads in respiratory tract tissues of rhesus macaques inoculated with MERS-CoV and treated with RDV (GS-5734) (Figure 3 figure and legend copied from [de Wit et al., 2020](#)). Three groups of six rhesus macaques were inoculated with MERS-CoV strain HCoV-EMC/2012; one group was i.v.-administered 1 mL/kg vehicle solution (vehicle control; gray circles), one group was administered 5 mg/kg remdesivir starting at 24 h before inoculation (prophylactic remdesivir; black squares), and one group was administered 5 mg/kg remdesivir starting at 12 h after inoculation (therapeutic remdesivir; red triangles). Treatment was continued once daily until 6 dpi, when all animals were euthanized and necropsies were performed. At necropsy, tissue samples were collected from all six lung lobes, RNA was extracted, and viral load was determined as TCID₅₀ equivalents per gram tissue. Individual animals and lung lobes are indicated (A), and averages and SDs per group (B). Similarly, viral loads were determined in additional tissues from the respiratory tract of each animal (C). R: right; L: left. Asterisks indicate statistically significant differences in a two-way ANOVA with Dunnett's multiple comparisons. **P* < 0.05; ***P* < 0.01; ****P* < 0.001; *****P* < 0.0001.

Conclusions on Animal Studies of RDV

- None of the animal model studies assessed RDV (GS-5734) in a robust treatment paradigm where treatment was initiated 3 or 4 (or more) days after CoV infection.
- Only one study to date has been conducted in rhesus macaques infected with SARS-CoV-2. All six rhesus macaques treated with remdesivir had significantly reduced clinical signs of SARS-CoV-2 infection compared to the vehicle-treated animals (*P* < 0.001). Consistent with these observations, lung pathology assessed by radiography showed reduced pulmonary infiltrates in the lungs of remdesivir-treated animals compared to the vehicle control (*P* < 0.01). Upon necropsy at 7 dpi, remdesivir-treated animals had significantly reduced gross lung lesions (*P* < 0.0001) and on an average 2.2 log₁₀ lower viral RNA levels in lung tissue compared to vehicle-treated animals (*P* < 0.001). Minimal differences in viral RNA levels were observed in nose and throat swabs, while no statistically significant differences were observed in rectal swab and BAL between the groups. SARS-CoV-2 was not detectable in BAL at 3 dpi and in lung tissue at 7 dpi in remdesivir-treated animals but could still be isolated from the vehicle-treated animals.
- These data support early remdesivir treatment in COVID-19 patients to prevent progression to severe pneumonia. Dose initiation at twelve hours would seem to be too early for disease signs to have developed so this study may be more representative of post-exposure prophylaxis. Analysis of viral shedding and viral load data appears to be consistent with this.

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- RDV (GS-5734) exhibited antiviral activity in the rhesus macaque SARS-CoV-2 challenge model, indicating that RDV (GS-5734) treatment early in the SARS-CoV-2 infection may be effective. It is not clear if RDV (GS-5734) treatment later in the infection cycle will be beneficial.

2.7 Resistance Studies

The Applicant has not conducted any cell culture selection experiments to assess the development of SARS-CoV-2 resistance to RDV (GS-5734) in infected cells. During the expedited development program, this was requested of the Applicant, and these experiments are underway. This will be addressed as a post-marketing requirement.

Resistance PMR: Conduct a study to select for remdesivir resistant SARS-CoV-2 variants in cell culture and characterize several independent isolates phenotypically and genotypically.

Characterization of remdesivir resistant murine hepatitis virus (MHV) variants

The development of resistance to RDV (GS-5734) in coronaviruses has been assessed by cell culture passaging of MHV, a coronavirus, in the presence of the remdesivir parent nucleoside, GS-441524. After 23 passages, two substitutions were selected in the nsp12 polymerase at residues conserved across coronaviruses: F476L and V553L (Agostini et al., 2018) (Figure 2.7-1).

Conserved resistance motifs

	F476L	V553L
PDCoV	DKYLEPY	ARTVAGV
229E	ARYFDCY	ARTVGGV
NL63	YKYFEIY	ARTVAGV
OC43	YKYFEIY	ARTVAGV
MHV	NKYFEIY	ARTVAGV
HKU1	YKYFEIY	ARTVAGV
MERS	NKYFEIY	ARTVAGV
HKU3-HKU1	DKYFDCY	ARTVAGV
SARS-CoV-2	DKYFDCY	ARTVAGV
HKU3	DKYFDCY	ARTVAGV
SARS-CoV	DKYFDCY	ARTVAGV
SHC014	DKYFDCY	ARTVAGV

Figure 2.7-1. Conservation of motifs that contain RDV resistance-associated substitutions across *Coronaviridae* (Figure 3C copied from Sheahan et al., 2020). Amino acid sequence of CoVs at known resistance motifs to RDV (GS-5734). For SARS-CoV, the positions in this figure are F480L and V557L.

Compared to wildtype MHV, the F476L substitution conferred a 2.4-fold increase in EC₅₀ value against RDV (GS-5734), and the V553L substitution conferred a 5-fold increase in EC₅₀ value against RDV (GS-5734). The two substitutions combined mediated a 5.6-fold increase in EC₅₀ value against RDV (GS-5734) (Table 2.7-1).

Table 2.7-1. MHV nsp12 F476L and V553L substitutions confer resistance to RDV (GS-5734) (Table 2 modified from Agostini et al., 2018). Mean EC₅₀ values ± SD and fold resistance of RDV (GS-5734)-resistant viruses were calculated using viral titer data following infection of DBT cells with the indicated virus at a MOI of 0.01 PFU/cell and treatment with increasing concentrations of RDV (GS-5734). Fold resistance was calculated as EC₅₀ of mutant/EC₅₀ of WT. The data represent the results from 3 independent experiments, each with 3 replicates.

Virus	EC ₅₀ (µM)	Fold shift in EC ₅₀ value
Wildtype MHV	0.024 ± 0.011	1
F476L	0.057 ± 0.040	2.4
V553L	0.12 ± 0.06	5.0
F476L + V553L	0.13 ± 0.06	5.6

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The replication capacity of recombinant MHV bearing the F476L, V553L, and F476L + V553L substitutions was assessed, and each of the viruses replicated similarly to wildtype MHV, both in replication kinetics and in observed peak titer, although a competitive fitness cost was observed for the MHV F476L + V553L variant in the absence of GS-5734 ([Agostini et al., 2018](#)).

The F476L, V553L, and F476L + V553L substitutions were also engineered into the analogous positions of the recombinant SARS-CoV genome (F480L, V557L, and F480L + V557L) and these variants were assessed for reduced susceptibility to RDV (GS-5734) in Calu-3 2B4 cells. RDV (GS-5734) had an EC₅₀ value of 10 nM against wildtype SARS-CoV and an EC₅₀ value of 60 nM against the SARS-CoV F480L + V557L recombinant virus, representing a 6-fold shift in susceptibility to RDV (GS-5734), nearly identical to the reduction in RDV (GS-5734) susceptibility to the MHV F476L + V553L variant; however, the SARS-CoV F480L + V557L recombinant virus was attenuated in mice compared to wildtype SARS-CoV ([Agostini et al., 2018](#)).

Of note, sequencing the MHV virus that grew in the presence of RDV (GS-5734) after 23 passages revealed a total 6 amino acid substitutions in four viral protein-coding regions, including the nsp13 helicase (A335V), the nsp2 2',5' phosphodiesterase (Q67H), the spike glycoprotein (A34V and I924T), and the nsp12 RdRp (F476L and V553L). The other 4 substitutions associated with reduced RDV (GS-5734) susceptibility were not characterized ([Agostini et al., 2018](#)).

Conclusions

- The nsp12 F476L, V553L, and F476L + V553L substitutions identified in MHV and assessed in SARS-CoV are likely resistance-associated substitutions that will confer reduced susceptibility to SARS-CoV-2.
- Compared to wild-type virus, recombinant MHV containing the F476L substitution showed 2.4-fold reduced susceptibility to RDV, and MHV containing the V553L substitution demonstrated 5-fold reduced susceptibility, while the double mutant conferred 5.6-fold reduced susceptibility to RDV in cell culture ([Agostini et al., 2018](#)). The potential relevance of this finding to SARS-CoV-2 is unknown.
- This information will be included in labeling for RDV (GS-5734).

Assessment of Conservation of the SARS-CoV-2 RdRp Among Clinical Isolates

Study Title: Genetic Conservation of RNA-Dependent-RNA Polymerase of SARS-CoV-2 in Patient Isolates

Study Report: [PC-540-2006](#)

Objectives: The purpose of this study was to evaluate the prevalence of the amino acid substitutions in nsp12 that may be associated with reduced susceptibility to RDV (GS-5734) in patient isolates of SARS-CoV-2 from December 2019 to March of 2020.

Methods

SARS-CoV-2 sequences were obtained from GISAID EpiCov database (GISAID, [Elbe and Buckland-Merett, 2018](#)) on March 27th, 2020. These sequences were submitted to the database from 12/26/2019 through 3/27/2020. Sequences were aligned to Wuhan-Hu-1 viral isolate ([NC_045512](#)) using the Mafft Multiple Sequence Aligner ([Katoh et al., 2005](#)). The nsp12 sequences were adjusted for the ribosomal slippage at nucleotide position 13468 (pp1a/pp1ab interface). Amino acid changes from reference

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Wuhan-Hu-1 viral isolate ([NC_045512](#)) were tabulated. Amino acid residues F476 and V553 of MHV nsp12 were identified to correspond to F480 and V557 SARS-CoV-2 nsp12, respectively. Sequences with ambiguous bases (N) were excluded or trimmed.

Results

A total of n=1993 sequences with full or near-full genomes from 52 countries were selected and aligned to the reference strain Wuhan-Hu-1 viral isolate. No amino acid substitutions were observed at any of the conserved amino acid residues in nsp12 that directly interact with the metal ions or RDV-TP or may contribute to the inhibitor's delayed chain termination. Furthermore, a larger set of residues were also considered that are located within 5Å and 10Å from the active site and residues that are located within 7Å of the primer RNA C1' positions. Only one substitution (A547V) within 10 Å of the active site was observed in one sequence of 1993 clinical isolates investigated ([PC-540-2006](#)).

Conclusions

- No polymorphisms in SARS-CoV-2 clinical isolates sequences were detected in or near the nsp12 active site where RDV (GS-5734) interacts with the SARS-CoV-2 RdRp for incorporation into the nascent RNA leading to delayed chain termination.

Resistance Conclusions

- SARS-CoV-2 RdRp (nsp12) substitutions leading to reduced susceptibility to RDV (GS-5734) will likely occur when the drug is in widespread use.
- There are currently no known polymorphisms among clinical isolates in the SARS-CoV-2 active site or within 10 Å of the active site; however, the potential for SARS-CoV-2 resistance to RDV (GS-5734) has not been adequately described.

Post Marketing Considerations

Several post-marketing actions will be required to further address RDV resistance:

1. Conduct a study to select for remdesivir resistant SARS-CoV-2 variants in cell culture and characterize several independent isolates phenotypically and genotypically.
2. Submit all SARS-CoV-2 viral shedding and viral load data from clinical trials assessing remdesivir including quantitation of viral shedding and viral load for any subject samples that have not been completed to date.
3. Submit all sequencing data from clinical trials assessing remdesivir including sequencing or any subject samples that have not been completed to date.
4. Submit a comprehensive resistance study report describing all resistance assessments performed for remdesivir.

3 Relevant Findings from Other Disciplines

3.1 Summary of Efficacy in Phase 3 Trials

Study Report: [Adaptive COVID-19 Treatment Trial \(ACTT\)](#)

Protocol Numbers:

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- **DMID Protocol Number:** 20-0006 ([NCT04280705](#))
- **INSIGHT Protocol Number:** 010
- **Gilead Protocol Number:** CO-US-540-5776

Title: A Multicenter, Adaptive, Randomized Blinded Controlled Trial of the Safety and Efficacy of Investigational Therapeutics for the Treatment of COVID-19 in Hospitalized Adults

Summary of NIAID ACTT-1 Trial in Subjects with Mild/Moderate and Severe COVID-19

A randomized, double-blind, placebo-controlled clinical trial evaluated Veklury 200 mg once daily for 1 day followed by Veklury 100 mg once daily for 9 days (for a total of up to 10 days of intravenously administered therapy) in hospitalized adult subjects with COVID-19 with evidence of lower respiratory tract involvement. Treatment with Veklury was stopped in subjects who were discharged from the hospital prior to the completion of 10 days of treatment. The trial enrolled 1,062 subjects: 105 (9.9%) subjects with mild/moderate disease and 957 (90.1%) subjects with severe disease. A total of 285 subjects (26.8%) (n=131 received Veklury) were on invasive mechanical ventilation/Extracorporeal membrane oxygenation (ECMO). Subjects were randomized in a 1:1 manner, stratified by disease severity at enrollment, to receive Veklury (n=541) or placebo (n=521), plus standard of care.

The primary clinical endpoint was time to recovery within 29 days after randomization, defined as either discharged from the hospital or hospitalized but not requiring supplemental oxygen and no longer requiring ongoing medical care. The median time to recovery was 10 days in the Veklury group compared to 15 days in the placebo group (recovery rate ratio, 1.29; [95% CI 1.12 to 1.49]; p<0.001). Overall, the odds of improvement in the ordinal scale were higher in the Veklury group at Day 15 when compared to the placebo group (odds ratio, 1.6; [95% CI, 1.3 to 1.9]; p<0.001). 14-day mortality based on 607 recoveries was 7.1% for the Veklury group versus 11.9% for the placebo group (hazard ratio 0.70 [95% CI 0.47, 1.04], p=0.07). Overall, 29-day mortality was 11% in the RDV group vs. 15% in the PBO group (hazard ratio, HR 0.73 [95% CI 0.52, 1.02], p=0.066).

Among subjects with severe disease at enrollment (n=957), the median time to recovery was 11 days in the Veklury group compared to 18 days in the placebo group (recovery rate ratio, 1.31; [95% CI, 1.12 to 1.52]; p<0.001); the odds of improvement in the ordinal scale in the Veklury group at Day 15 when compared to the placebo group were as follows: (odds ratio, 1.56; [95% CI, 1.24 to 1.95].

Study Report: [Study GS-US-540-5773 in Subjects with Severe COVID-19](#)

Protocol Number: GS-US-540-5773 ([NCT04292899](#))

Title: A Phase 3 Randomized Study to Evaluate the Safety and Antiviral Activity of Remdesivir (GS-5734™) in Participants with Severe COVID-19

Summary of Study GS-US-540-5773 in Subjects with Severe COVID-19

A randomized, open-label multi-center clinical trial (Study GS-US-540-5773) of hospitalized subjects at least 12 years of age with confirmed SARS-CoV-2 infection, oxygen saturation of $\leq 94\%$ on room air, and radiological evidence of pneumonia compared 200 subjects who received IV Veklury (remdesivir) for 5 days with 197 subjects who received IV Veklury for 10 days. Patients on mechanical ventilation at screening were excluded. All subjects received 200 mg of Veklury on Day 1 and 100 mg once daily on subsequent days, plus standard of care. Treatment with VEKLURY was stopped in subjects who were discharged from the hospital prior to completion of their protocol-defined duration of treatment. The

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primary endpoint was clinical status on Day 14 assessed on a 7-point ordinal scale consisting of the following categories: 1, death; 2, hospitalized, receiving ventilation or high-flow oxygen devices; 4, hospitalized, requiring low-flow supplemental oxygen; 5, hospitalized, not requiring supplemental oxygen but receiving ongoing medical care (related or not related to COVID-19); 6, hospitalized, requiring neither supplemental oxygen nor ongoing medical care (other than that specified in the protocol for remdesivir administration); and 7, not hospitalized. After adjusting for between-group differences at baseline, patients receiving a 10-day course of Veklury had similar clinical status at Day 14 as those receiving a 5-day course (odds ratio for improvement, 0.75; [95% CI 0.51 to 1.12]). 28-day mortality was 11.5% and 14.2% in the 5- and 10-day treatment groups, respectively.

Study Report: [Study GS-US-540-5774 in Subjects with Moderate COVID-19](#)

Protocol Number: [GS-US-540-5774 \(NCT04292730\)](#)

Title: A Phase 3 Randomized Study to Evaluate the Safety and Antiviral Activity of Remdesivir (GS-5734™) in Participants with Moderate COVID-19 Compared to Standard of Care Treatment

Summary of Study GS-US-540-5774 in Subjects with Moderate COVID-19

A randomized, open-label multi-center clinical trial (Study GS-US-540-5774) of hospitalized subjects at least 12 years of age with confirmed SARS-CoV-2 infection and radiological evidence of pneumonia without an oxygen requirement during screening compared treatment with Veklury for 5 days (n=191) and treatment with Veklury for 10 days (n=193) with standard of care (SOC) (n=200). Subjects treated with Veklury received 200 mg on Day 1 and 100 mg once daily on subsequent days. Treatment with VEKLURY was stopped in subjects who were discharged from the hospital prior to completion of their protocol-defined duration of treatment.

The primary endpoint was clinical status on Day 11 assessed on a 7-point ordinal scale consisting of the following categories: 1, death; 2, hospitalized, receiving invasive mechanical ventilation or ECMO; 3, hospitalized, receiving noninvasive ventilation or high-flow oxygen devices; 4, hospitalized, requiring low-flow supplemental oxygen; 5, hospitalized, not requiring supplemental oxygen but receiving ongoing medical care (related or not related to COVID-19); 6, hospitalized, requiring neither supplemental oxygen nor ongoing medical care (other than that specified in the protocol for remdesivir administration); and 7, not hospitalized. Baseline clinical status, oxygen support status, and median duration of symptoms and hospitalization prior to first dose of Veklury were similar across treatment groups. Overall, the odds of improvement in clinical status were higher in the 5-day Veklury group at Day 11 when compared to those receiving only SOC (odds ratio, 1.65; [95% CI, 1.09 to 2.48]; p=0.017). The odds of improvement in clinical status with the 10-day treatment group when compared to those receiving only SOC were not statistically significantly different (odds ratio, 1.31; [95% CI 0.88 to 1.95]; p=0.183). At Day 28, mortality was ≤ 2% in all treatment groups.

3.2 Summary of Safety in Phase 3 Trials

For safety information, please see the Clinical review written by Dr. Kirk Chan-Tack, M.D.

4 Clinical Virology

During the clinical trial protocol development, Clinical Virology recommended that the Applicant:

1. Quantify SARS-CoV-2 RNA in oropharyngeal, plasma, stool, and urine samples during the

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course of treatment

2. Use a standardized method for collection of oropharyngeal samples for virologic assessments and use a central laboratory for sample processing.
3. Provide detailed descriptions of the assays used to assess Clinical Virology markers and performance characteristics of the quantitative RT-PCR assay in their final study reports.
4. Monitoring subjects for as long as they continue to shed SARS-CoV-2 RNA in oropharyngeal and stool samples.
5. Collect samples to assess for the development of resistance to remdesivir.

In response, the Applicant noted that there were feasibility issues that would limit the amount of data that could be collected, and they explained that the use of a central laboratory was not possible due to SARS-CoV-2 shipping constraints.

One PMR and two PMCs will address Clinical Virology deficiencies:

1. **Clinical Virology SARS-CoV-2 RNA Shedding PMR:** Submit all SARS-CoV-2 viral shedding and viral load data from ACTT-1, GS-540-5773, and GS-US-5774 assessing remdesivir including quantitation of viral shedding and viral load for any subject samples that have not been completed to date.
2. **Clinical Virology SARS-CoV-2 Sequencing PMC:** Submit all sequencing data from ACTT-1, GS-540-5773, and GS-US-540-5774 assessing remdesivir including sequencing of any subject samples that have not been completed to date.
3. **Clinical Virology SARS-CoV-2 Resistance PMC:** Submit a comprehensive resistance study reports from ACTT-1, GS-US-540-5773, and GS-54-5774 describing all resistance assessments performed for remdesivir.

4.1 SARS-CoV-2 RNA shedding

NIAID ACTT-1 Trial ([NCT04280705](#))

No PK/PD assessments were performed for this clinical trial.

The following exploratory endpoints were part of the protocol, but assessments were not provided in the study report:

- Qualitative and quantitative RT-PCR for SARS-CoV-2 in OP swab on Days 1; 3, 5, 8, 11 (while hospitalized); and Day 15 and 29 (if able to return to clinic or still hospitalized).
- Qualitative and quantitative PCR for SARS-CoV-2 in blood on Days 1; 3, 5, 8, 11 (while hospitalized).

Study GS-US-540-5773 ([NCT04292899](#))

The following “other endpoint” was listed as part of the protocol, but assessments were not provided in the study report

- Time to first negative SARS-CoV-2 RT-PCR

The study report noted that the endpoint of *time to first negative SARS-CoV-2 RT-PCR* was updated to the *number of participants with negative RT-PCR on Days 5 and 10* because RT-PCR was not collected on a routine basis.

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The protocol also stated that:

SARS-CoV-2 testing may include quantitative RT-PCR to detect or quantify SARS-CoV-2 or virus sequencing results. If feasible, oropharyngeal, saliva, sputum, stool, and/or blood samples may be collected and assayed using RT-PCR to quantify SARS-CoV-2 viral load. Pretreatment and posttreatment samples with detectable SARS-CoV-2 may be sequenced for resistance monitoring of the viral polymerase gene if possible. No data were provided.

Results for detection of SARS-CoV-2 RNA by quantitative RT-PCR on Days 5 and 10 were provided in the Clinical Study Report presented by treatment group for the overall study population but no meaningful interpretation could be made from the limited data provided (data were available for $\leq 10\%$ of patients who received RDV for 5 days and $< 14\%$ of patients treated with RDV for 10 days).

Study GS-US-540-5774 (NCT04292730)

The following “other endpoint” was listed as part of the protocol, but assessments were not provided in the study report

- Time to first negative SARS-CoV-2 RT-PCR

The protocol also stated that:

SARS-CoV-2 testing may include quantitative RT-PCR to detect or quantify SARS-CoV-2 or virus sequencing results. If feasible, oropharyngeal, saliva, sputum, stool, and/or blood samples may be collected and assayed using RT-PCR to quantify SARS-CoV-2 viral load. Pretreatment and posttreatment samples with detectable SARS-CoV-2 may be sequenced for resistance monitoring of the viral polymerase gene if possible. No data were provided.

Results for detection of SARS-CoV-2 RNA by quantitative RT-PCR on Days 5 and 10 were provided in the Clinical Study Report presented by treatment group for the overall study population but no meaningful interpretation could be made from the limited data provided (data were available for $< 20\%$ of patients).

4.2 SARS-CoV-2 Resistance to RDV (GS-5734)

NIAID ACTT-1 Trial (NCT04280705)

No resistance assessments were performed for this clinical trial.

The protocol indicated that OP samples would be taken on Day 3, 5, 8, 11, 15, and 29 to monitor for the development of SARS-CoV-2 resistance to remdesivir. No data were provided in the Clinical Study Report.

Study GS-US-540-5773 (NCT04292899)

No resistance assessments were performed for this clinical trial.

The protocol also stated that:

SARS-CoV-2 testing may include quantitative RT-PCR to detect or quantify SARS-CoV-2 or virus sequencing results. If feasible, oropharyngeal, saliva, sputum, stool, and/or blood samples may be collected and assayed using RT-PCR to quantify SARS-CoV-2 viral load. Pretreatment and posttreatment samples with detectable SARS-CoV-2 may be sequenced for resistance monitoring of the viral polymerase gene if possible. No data were provided.

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Study GS-US-540-5774 (NCT04292730)

No resistance assessments were performed for this clinical trial.

The protocol also stated that:

SARS-CoV-2 testing may include quantitative RT-PCR to detect or quantify SARS-CoV-2 or virus sequencing results. If feasible, oropharyngeal, saliva, sputum, stool, and/or blood samples may be collected and assayed using RT-PCR to quantify SARS-CoV-2 viral load. Pretreatment and posttreatment samples with detectable SARS-CoV-2 may be sequenced for resistance monitoring of the viral polymerase gene if possible. No data were provided.

4.3 Days Symptomatic Prior to RDV (GS-5734) Treatment

NIAID ACTT-1 Trial (NCT04280705)

Clinical Virology was interested in determining if the clinical benefit observed for patients treated with remdesivir was greater among patients who were treated earlier in the SARS-CoV-2 infection cycle. The median time from symptom onset prior to enrollment was 9 days for all subjects. Subgroup analysis compared efficacy among patients who were randomization within 10 days of symptom onset to patients who were enrolled 10 days or later after the onset of symptoms. Both subgroups experienced a greater clinical benefit than the placebo group with rate ratios for recovery of >1.0. Patients who were randomization within 10 days of symptom onset had a rate ratio for recovery of 1.28 (95% CI, 1.05 to 1.57; 664 patients), whereas patients who underwent randomization more than 10 days after the onset of symptoms had a rate ratio for recovery of 1.38 (95% CI, 1.05 to 1.81; 380 patients) ([Beigel et al., 2020](#)).

Study GS-US-540-5773 (NCT04292899)

Median duration of symptoms prior to first dose of RDV was 8 days for patients in the 5-Days RDV Treatment Group and 9 days for patients in the 10-Days RDV Treatment Group. The median duration of hospitalization prior to the first dose of RDV (2 days) were similar across treatment groups ([Goldman et al., 2020](#)).

Study GS-US-540-5774 (NCT04292730)

The median duration of symptoms prior to treatment in the standard of care group was 9 days (IQR, 6-11 days) compared with 8 days (IQR, 5-11 days) for both groups receiving remdesivir. The median duration of hospitalization prior to the first dose of RDV (2 days) were similar across treatment groups ([Spinner et al., 2020](#)).

4.4 Baseline RDV (GS-5734) Resistance Analysis

NIAID ACTT-1 Trial (NCT04280705)

No baseline resistance assessments were performed to identify polymorphisms with reduced susceptibility to remdesivir.

Study GS-US-540-5773 (NCT04292899)

No baseline resistance assessments were performed to identify polymorphisms with reduced susceptibility to remdesivir.

Study GS-US-540-5774 (NCT04292730)

No baseline resistance assessments were performed to identify polymorphisms with reduced susceptibility to remdesivir.

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5 Conclusion

The nonclinical virology package for remdesivir against SARS-CoV-2 was sufficient to initiate clinical trials. The clinical trial data from ACCT-1 indicate that there is a clinical benefit for hospitalized patients infected with SARS-CoV-2 with COVID-19 who are treated with remdesivir compared to placebo. No clinical virology data were provided to support efficacy, but based on the demonstrated clinical benefit in the Phase 3 trials and the Applicant's agreement to provide additional clinical virology data post-marketing, this application is approvable from the Clinical Virology perspective.

6 Package Insert

6.1 Proposed Package Insert (with Reviewer-recommended changes)

Revisions to sections of the label that were reviewed by Clinical Virology. **Red font** indicates changes from the originally proposed label to a near complete label as of September 16, 2020.

-----INDICATIONS AND USAGE-----

[REDACTED] (b) (4)

5 WARNINGS AND PRECAUTIONS

5.3 Risk of Reduced Antiviral Activity When Coadministered with Chloroquine or Hydroxychloroquine

Coadministration of VEKLURY and chloroquine phosphate or hydroxychloroquine sulfate is not recommended based on cell culture data demonstrating an antagonistic effect of chloroquine on the intracellular metabolic activation and antiviral activity of VEKLURY [see Drug Interactions (7) and Microbiology (12.4)].

7 DRUG INTERACTIONS

Due to antagonism observed in (b) (4) cell culture, concomitant use of VEKLURY with chloroquine phosphate or hydroxychloroquine sulfate is not recommended [see Warnings and Precautions (5.3) and Microbiology (12.4)].

12.1 Mechanism of Action

Remdesivir is an antiviral drug with activity against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) [see Microbiology (12.4)]

12.4 Microbiology

Mechanism of Action

Remdesivir is an inhibitor of the SARS-CoV-2 RNA-dependent RNA polymerase (RdRp), which is essential for viral replication. Remdesivir is an adenosine nucleotide prodrug that distributes into cells

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where it is metabolized to a nucleoside monophosphate intermediate by carboxyesterase 1 and/or cathepsin A, depending upon the cell type. The nucleoside monophosphate is subsequently phosphorylated by cellular kinases to form the pharmacologically active nucleoside triphosphate metabolite. (b) (4)

Remdesivir triphosphate (RDV TP) acts as an analog of adenosine triphosphate (ATP) and competes with high selectivity (3.65-fold) over the natural ATP substrate for incorporation into nascent RNA chains by the SARS-CoV-2 RNA-dependent RNA polymerase, which results in delayed chain termination (position i+3) during replication of the viral RNA. In a biochemical assay assessing RDV-TP incorporation by the MERS-CoV RdRp complex, RDV-TP inhibited RNA synthesis with an IC50 value of 0.032 μM. Remdesivir triphosphate is a weak inhibitor of mammalian DNA and RNA polymerases (b) (4) including the human mitochondrial (b) (4) RNA polymerase.

Antiviral Activity

Remdesivir exhibited cell culture antiviral activity against a clinical isolate of SARS-CoV-2 in primary human airway epithelial (HAE) cells with a 50% effective concentration (EC₅₀) of 9.9 nM after 48 hours of treatment (b) (4)

The antiviral activity of remdesivir was antagonized by chloroquine phosphate in a dose-dependent manner when the two drugs were co-incubated at clinically relevant concentrations in HEp-2 cells infected with respiratory syncytial virus (RSV). Higher remdesivir EC₅₀ values were observed with increasing concentrations of chloroquine phosphate. Increasing concentrations of chloroquine phosphate reduced formation of remdesivir triphosphate in normal human bronchial epithelial cells.

Resistance

No clinical data are available on the development of SARS-CoV-2 resistance to remdesivir. The cell culture development of SARS-CoV-2 resistance to remdesivir has not been assessed to date.

Cell culture resistance profiling of remdesivir using the rodent CoV murine hepatitis virus identified 2 substitutions (F476L and V553L) in the viral RNA-dependent RNA polymerase at residues conserved across CoVs that. The combination of these two substitutions conferred a 5.6-fold reduced reduction in susceptibility to remdesivir. The mutant viruses showed reduced viral fitness in cell culture and introduction of the corresponding substitutions (F480L and V557L) into SARS-CoV resulted in 6-fold (b) (4) reduction in susceptibility to remdesivir in cell culture and attenuated SARS-CoV pathogenesis in a mouse model.

6.2 Final Approved Package Insert

The label was not finalized at the time this review was completed.

7 Recommendations

Clinical Virology recommends approval of remdesivir for the treatment of adults and pediatric patients 12 years of age and older hospitalized with COVID-19, resulting from SARS-CoV-2 infection. Given the mechanism of action data submitted in this NDA, it is expected that remdesivir will likely have a greater impact on reducing viral replication when administered as early as possible after SARS-CoV-2 infection is detected; however, there was a clear benefit in hospitalized COVID-19 patients who started remdesivir treatment more than 9 days after symptom onset indicating that the therapeutic window for RDV (GS-5734) may be wider than expected.

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8 References

1. Guo YR, Cao QD, Hong ZS, et al. The origin, transmission and clinical therapies on coronavirus disease 2019 (COVID-19) outbreak - an update on the status. *Mil Med Res.* 2020;7(1):11. Published 2020 Mar 13. doi:10.1186/s40779-020-00240-0
2. Johns Hopkins CSSE: <https://coronavirus.jhu.edu/map.html>
3. Brown AJ, Won JJ, Graham RL, et al. Broad spectrum antiviral remdesivir inhibits human endemic and zoonotic deltacoronaviruses with a highly divergent RNA dependent RNA polymerase. *Antiviral Res.* 2019;169:104541. doi:10.1016/j.antiviral.2019.104541
4. Agostini ML, Andres EL, Sims AC, et al. Coronavirus Susceptibility to the Antiviral Remdesivir (GS-5734) Is Mediated by the Viral Polymerase and the Proofreading Exoribonuclease. *mBio.* 2018;9(2):e00221-18. Published 2018 Mar 6. doi:10.1128/mBio.00221-18
5. Sheahan TP, Sims AC, Graham RL, et al. Broad-spectrum antiviral GS-5734 inhibits both epidemic and zoonotic coronaviruses. *Sci Transl Med.* 2017;9(396):eaal3653. doi:10.1126/scitranslmed.aal3653
6. Tchesnokov EP, Feng JY, Porter DP, Götte M. Mechanism of Inhibition of Ebola Virus RNA-Dependent RNA Polymerase by Remdesivir. *Viruses.* 2019;11(4):326. Published 2019 Apr 4. doi:10.3390/v11040326
7. Beigel JH, Tomashek KM, Dodd LE, et al. Remdesivir for the Treatment of Covid-19 - Preliminary Report [published online ahead of print, 2020 May 22]. *N Engl J Med.* 2020;NEJMoa2007764. doi:10.1056/NEJMoa2007764
8. Goldman JD, Lye DCB, Hui DS, et al. Remdesivir for 5 or 10 Days in Patients with Severe Covid-19 [published online ahead of print, 2020 May 27]. *N Engl J Med.* 2020;NEJMoa2015301. doi:10.1056/NEJMoa2015301
9. Spinner CD, Gottlieb RL, Criner GJ, et al. Effect of Remdesivir vs Standard Care on Clinical Status at 11 Days in Patients with Moderate COVID-19: A Randomized Clinical Trial. *JAMA.* 2020;324(11):1048–1057. doi:10.1001/jama.2020.16349
10. Huang C, Wang Y, Li X, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China [published correction appears in *Lancet.* 2020 Jan 30;:]. *Lancet.* 2020;395(10223):497-506. doi:10.1016/S0140-6736(20)30183-5
11. Chang D, Mo G, Yuan X, et al. Time Kinetics of Viral Clearance and Resolution of Symptoms in Novel Coronavirus Infection. *Am J Respir Crit Care Med.* 2020;201(9):1150-1152. doi:10.1164/rccm.202003-0524LE
12. He, X., Lau, E.H.Y., Wu, P. *et al.* Temporal dynamics in viral shedding and transmissibility of COVID-19. *Nat Med* **26**, 672–675 (2020). <https://doi.org/10.1038/s41591-020-0869-5>
13. Li W, Su YY, Zhi SS, et al. Virus shedding dynamics in asymptomatic and mildly symptomatic patients infected with SARS-CoV-2 [published online ahead of print, 2020 Jul 9]. *Clin Microbiol Infect.* 2020;S1198-743X(20)30410-9. doi:10.1016/j.cmi.2020.07.008
14. Xiao AT, Tong YX, Zhang S. Profile of RT-PCR for SARS-CoV-2: a preliminary study from 56 COVID-19 patients [published online ahead of print, 2020 Apr 19]. *Clin Infect Dis.* 2020;ciaa460. doi:10.1093/cid/ciaa460
15. Zhou F, Yu T, Du R, et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study [published correction appears in *Lancet.* 2020 Mar 28;395(10229):1038] [published correction appears in *Lancet.* 2020 Mar 28;395(10229):1038]. *Lancet.* 2020;395(10229):1054-1062. doi:10.1016/S0140-6736(20)30566-3
16. Bullard J, Dust K, Funk D, et al. Predicting infectious SARS-CoV-2 from diagnostic samples [published online ahead of print, 2020 May 22]. *Clin Infect Dis.* 2020;ciaa638. doi:10.1093/cid/ciaa638
17. Wölfel, R., Corman, V.M., Guggemos, W. et al. Virological assessment of hospitalized patients with COVID-2019. *Nature* 581, 465–469 (2020). <https://doi.org/10.1038/s41586-020-2196-x>

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18. Fung TS, Liu DX. Human Coronavirus: Host-Pathogen Interaction. *Annu Rev Microbiol.* 2019;73:529-557. doi:10.1146/annurev-micro-020518-115759
19. Wan Y, Shang J, Graham R, Baric RS, Li F. Receptor Recognition by the Novel Coronavirus from Wuhan: an Analysis Based on Decade-Long Structural Studies of SARS Coronavirus. *J Virol.* 2020;94(7):e00127-20. Published 2020 Mar 17. doi:10.1128/JVI.00127-20
20. Shang J, Wan Y, Luo C, et al. Cell entry mechanisms of SARS-CoV-2. *Proc Natl Acad Sci U S A.* 2020;117(21):11727-11734. doi:10.1073/pnas.2003138117
21. Romano M, Ruggiero A, Squeglia F, Maga G, Berisio R. A Structural View of SARS-CoV-2 RNA Replication Machinery: RNA Synthesis, Proofreading and Final Capping. *Cells.* 2020;9(5):1267. Published 2020 May 20. doi:10.3390/cells9051267
22. Sola I, Almazán F, Zúñiga S, Enjuanes L. Continuous and Discontinuous RNA Synthesis in Coronaviruses. *Annu Rev Virol.* 2015;2(1):265-288. doi:10.1146/annurev-virology-100114-055218
23. Tchesnokov, E.P., Raesismakiani, P., Ngure, M. et al. Recombinant RNA-Dependent RNA Polymerase Complex of Ebola Virus. *Sci Rep* 8, 3970 (2018). <https://doi.org/10.1038/s41598-018-22328-3>
24. Prime: <https://www.schrodinger.com/prime>
25. Gordon CJ, Tchesnokov EP, Feng JY, Porter DP, Götte M. The antiviral compound remdesivir potently inhibits RNA-dependent RNA polymerase from Middle East respiratory syndrome coronavirus. *J Biol Chem.* 2020;295(15):4773-4779. doi:10.1074/jbc.AC120.013056
26. Holshue ML, DeBolt C, Lindquist S, et al. First Case of 2019 Novel Coronavirus in the United States. *N Engl J Med.* 2020;382(10):929-936. doi:10.1056/NEJMoa2001191
27. Wang, M., Cao, R., Zhang, L. et al. Remdesivir and chloroquine effectively inhibit the recently emerged novel coronavirus (2019-nCoV) in vitro. *Cell Res* 30, 269–271 (2020). <https://doi.org/10.1038/s41422-020-0282-0>
28. Marroquin LD, Hynes J, Dykens JA, Jamieson JD, Will Y. Circumventing the Crabtree effect: replacing media glucose with galactose increases susceptibility of HepG2 cells to mitochondrial toxicants. *Toxicol Sci.* 2007;97(2):539-547. doi:10.1093/toxsci/kfm052
29. Akinci E, Cha M, Lin L, et al. Elucidation of remdesivir cytotoxicity pathways through genome-wide CRISPR-Cas9 screening and transcriptomics. Preprint. *bioRxiv.* 2020;2020.08.27.270819. Published 2020 Aug 28. doi:10.1101/2020.08.27.270819
30. Bobrowski T, Chen L, Eastman RT, et al. Discovery of Synergistic and Antagonistic Drug Combinations against SARS-CoV-2 In Vitro. Preprint. *bioRxiv.* 2020;2020.06.29.178889. Published 2020 Jun 30. doi:10.1101/2020.06.29.178889
31. Warren, T., Jordan, R., Lo, M. et al. Therapeutic efficacy of the small molecule GS-5734 against Ebola virus in rhesus monkeys. *Nature* 531, 381–385 (2016). <https://doi.org/10.1038/nature17180>
32. Zhao Q, Tensfeldt TG, Chandra R, Mould DR. Population pharmacokinetics of azithromycin and chloroquine in healthy adults and paediatric malaria subjects following oral administration of fixed-dose azithromycin and chloroquine combination tablets. *Malar J.* 2014;13:36. Published 2014 Jan 29. doi:10.1186/1475-2875-13-36
33. Smit C, Peeters MYM, van den Anker JN, Knibbe CAJ. Chloroquine for SARS-CoV-2: Implications of Its Unique Pharmacokinetic and Safety Properties. *Clin Pharmacokinet.* 2020;59(6):659-669. doi:10.1007/s40262-020-00891-1
34. Wong YK, Yang J, He Y. Caution and clarity required in the use of chloroquine for COVID-19. *Lancet Rheumatol.* 2020;2(5):e255. doi:10.1016/S2665-9913(20)30093-X
35. Yao X, Ye F, Zhang M, et al. In Vitro Antiviral Activity and Projection of Optimized Dosing Design of Hydroxychloroquine for the Treatment of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). *Clin Infect Dis.* 2020;71(15):732-739. doi:10.1093/cid/ciaa237

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36. Carmichael SJ, Charles B, Tett SE. Population pharmacokinetics of hydroxychloroquine in patients with rheumatoid arthritis. *Ther Drug Monit.* 2003;25(6):671-681. doi:10.1097/00007691-200312000-00005
37. Popert AJ. Chloroquine: a review. *Rheumatol Rehabil.* 1976;15(3):235-238. doi:10.1093/rheumatology/15.3.235
38. Hoffmann, M., Mösbauer, K., Hofmann-Winkler, H. et al. Chloroquine does not inhibit infection of human lung cells with SARS-CoV-2. *Nature* (2020).
39. Munster, V.J., Feldmann, F., Williamson, B.N. et al. Respiratory disease in rhesus macaques inoculated with SARS-CoV-2. *Nature* 585, 268–272 (2020). <https://doi.org/10.1038/s41586-020-2324-7>
40. Corman VM, Landt O, Kaiser M, et al. Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. *Euro Surveill.* 2020;25(3):2000045. doi:10.2807/1560-7917.ES.2020.25.3.2000045
41. Williamson BN, Feldmann F, Schwarz B, et al. Clinical benefit of remdesivir in rhesus macaques infected with SARS-CoV-2. Preprint. *bioRxiv.* 2020;2020.04.15.043166. Published 2020 Apr 22. doi:10.1101/2020.04.15.043166
42. Pruijssers 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. doi:10.1016/j.celrep.2020.107940
43. de Wit E, Feldmann F, Cronin J, et al. Prophylactic and therapeutic remdesivir (GS-5734) treatment in the rhesus macaque model of MERS-CoV infection. *Proc Natl Acad Sci U S A.* 2020;117(12):6771-6776. doi:10.1073/pnas.1922083117
44. Sheahan TP, Sims AC, Zhou S, et al. An orally bioavailable broad-spectrum antiviral inhibits SARS-CoV-2 in human airway epithelial cell cultures and multiple coronaviruses in mice. *Sci Transl Med.* 2020;12(541):eabb5883. doi:10.1126/scitranslmed.abb5883

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