SECTION 2.6.1—INTRODUCTION

REMDESIVIR (GS-5734™)

Gilead Sciences

[redacted] **2020**

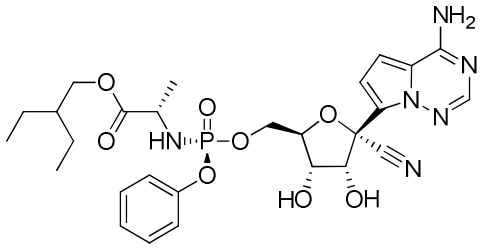
CONFIDENTIAL AND PROPRIETARY INFORMATION

1. NONCLINICAL SUMMARY

Remdesivir (GS-5734™) is a single diastereomer monophosphoramidate prodrug of a nucleoside analog, GS-441524, that is intracellularly metabolized into an analog of adenosine triphosphate that inhibits viral RNA polymerases and has broad-spectrum activity against members of the coronaviruses (eg, severe acute respiratory syndrome coronavirus [SARS-CoV]; SARS-CoV-2, the causative agent of coronavirus disease 2019 [COVID-19]; and Middle East respiratory syndrome coronavirus [MERS-CoV]), filoviruses (eg, Ebola virus [EBOV] and Marburg virus [MARV]), and paramyxoviruses (eg, respiratory syncytial virus [RSV], Nipah virus [NiV], and Hendra virus). Remdesivir is being developed for the treatment of patients with COVID-19.

Remdesivir has the chemical name 2-Ethylbutyl (2*S*)-2-{[(*S*)-{[(2*R*,3*S*,4*R*,5*R*)-5-(4-aminopyrrolo[2,1-f] [1,2,4]triazin-7-yl)-5-cyano-3,4-dihydroxytetrahydrofuran-2-yl] methoxy}(phenoxy)phosphoryl]amino}propanoate. The molecular formula is $C_{27}H_{35}N_6O_8P$ and the molecular weight is 602.6 g/mol (1 µM = 0.603 µg/mL). Remdesivir has the following structural formula (Figure 1).

Figure 1. Chemical Structure



Early studies relevant to remdesivir were conducted with the diastereomeric mixture, GS-466547 (approximately 1:1 mixture of remdesivir and its diastereomer at phosphorus). Based on antiviral activity, as well as in vitro and in vivo pharmacokinetic (PK) profiles, a single diastereomer (remdesivir; GS-5734) was selected for further development. In these studies, the isomer remdesivir performed similarly to the diastereomeric mixture, GS-466547.

Remdesivir has been selected for 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.

A comprehensive program of nonclinical studies has been carried out for remdesivir; summaries of the nonclinical data are provided in m2.6. The completed nonclinical study reports are provided in m4 and m5.

The nonclinical data presented in this dossier support the favorable benefit/risk profile for the proposed use of remdesivir for the treatment of COVID-19. All information from nonclinical

studies that is relevant to the prescriber and patient has been included in the proposed prescribing information and patient labeling.

SECTION 2.6.2—PHARMACOLOGY WRITTEN SUMMARY

REMDESIVIR (GS-5734™)

Gilead Sciences

[redacted] **2020**

CONFIDENTIAL AND PROPRIETARY INFORMATION

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LIST OF ABBREVIATIONS

5-FU	5-fluorouracil
ANOVA	analysis of variance
ATP	adenosine triphosphate
BAL	bronchoalveolar lavage
CC_{50}	50% cytotoxic concentration
CDC	Centers for Disease Control and Prevention
Ces1c	carboxylesterase 1c
C_{max}	maximum observed concentration of drug
CNS	central nervous system
CoV	coronavirus
COVID-19	coronavirus disease 2019
COX-1	cytochrome oxidase subunit 1
ddC	dideoxycytidine
DMSO	dimethylsulfoxide
DNA	deoxyribonucleic acid
dpi	days post-infection
DPP4	dipeptidyl peptidase-4
dTTP	deoxythymidine triphosphate
EBOV	Ebola virus
EC_{50}	50% effective concentration
EC_{90}	90% effective concentration
ECG	electrocardiogram
EVD	Ebola virus disease
FDA	Food and Drug Administration
Gilead	Gilead Sciences
GS-441524	nucleoside analog of remdesivir
GS-443902	pharmacologically active nucleoside triphosphate of remdesivir
GS-466547	diastereomeric mixture that contains remdesivir
GS-704277	metabolite of remdesivir
HAE	human airway epithelial
HBV	hepatitis B virus
HCV	hepatitis C virus
hDPP4	humanized dipeptidyl peptidase-4
hERG	human ether-a-go-go-related gene
HIV-1	human immunodeficiency virus type 1
HMVECs	human microvascular endothelial cells
IC_{50}	50% inhibitory concentration
ICH	International Conference on Harmonisation
IFNb	interferon beta

IV	intravenous
LPV/RTV-IFNb	lopinavir/ritonavir-interferon beta
MARV	Marburg virus
MERS	Middle East respiratory syndrome
MHV	murine hepatitis virus
mtDNA	mitochondrial DNA
mtRNA	mitochondrial RNA
NA	not applicable
[redacted]	[redacted]
NiV	Nipah virus
NOEL	no observed effect level
n.s.	not significant
OAT	organic anion transporter
PBMC	peripheral blood mononuclear cell
PCR	polymerase chain reaction
pfu	plaque-forming units
PHH	primary human hepatocytes
pol	polymerase
RdRp	RNA-dependent RNA polymerase
RDV	remdesivir
RML	Rocky Mountain Laboratories
RNA	ribonucleic acid
ROS	reactive oxygen species
RPTEC	renal proximal tubule cell
RSV	respiratory syncytial virus
SARS	severe acute respiratory syndrome
SBECD	sulfobutylether β-cyclodextrin sodium
SD	standard deviation
SDH-A	succinate dehydrogenase A
$t_{1/2}$	estimate of the terminal elimination half-life of the drug, calculated by dividing the natural log of 2 by the terminal elimination rate constant (λ_z)
$TCID_{50}$	50% Median Tissue Culture Infectious Dose
[redacted]	[redacted]
US	United States
[redacted]	[redacted]
USP	United States Pharmacopeia
w/v	weight-to-volume ratio
WHO	World Health Organization
wt	wild type

1. NOTE TO REVIEWER

Remdesivir (RDV; GS-5734™) is a single diastereomer monophosphoroamidate prodrug of a nucleoside analog GS-441524. Early studies relevant for RDV were conducted with the diastereomeric mixture GS-466547 (approximately 1:1 mixture of RDV and its diastereomer at phosphorous). Based on antiviral activity, as well as in vitro and in vivo pharmacokinetic profile, a single diastereoisomer (RDV) was selected for further development. In these early studies, the isomer RDV performed similarly to the mixture GS-466547. The majority of the reported studies have been performed with RDV and are the focus of this document as they are considered most relevant. To aid the reviewer, Table 1 lists the current nomenclature for RDV, the diastereomeric mixture, and the related metabolites referred to within the text of this document.

Table 1. Description of Remdesivir and its Diastereomers and Metabolites Referenced in the Text

Gilead No.	Description	Conversion Factors
Remdesivir (GS-5734)	Nucleotide prodrug	1 µM = 0.603 µg/mL
GS-466547	Diastereomeric mixture that contains RDV	1 µM = 0.603 µg/mL
GS-704277	Metabolite	1 µM = 0.442 µg/mL
GS-441524	Nucleoside analog	1 µM = 0.291 µg/mL
GS-719700	Nucleoside analog monophosphate	1 µM = 0.369 µg/mL
GS-719699	Nucleoside analog diphosphate	1 µM = 0.448 µg/mL
GS-443902	Pharmacologically active nucleoside triphosphate	1 µM = 0.527 µg/mL

2. BRIEF SUMMARY

Coronaviruses (CoVs) are positive-sense, single-stranded, enveloped RNA viruses, many of which are commonly found in humans and cause mild symptoms. However, over the past two decades, emerging pathogenic CoVs that can cause life-threatening disease in humans and animals have been identified, namely severe acute respiratory syndrome coronavirus (SARS-CoV; {Corman 2015, Hui 2016}), Middle Eastern respiratory syndrome coronavirus (MERS-CoV; {Assiri 2013, Choi 2016, Who Mers-Cov Research Group 2013}), and SARS-CoV-2 {Zhu 2020}). SARS-CoV-2 was identified as the cause of an outbreak of respiratory illness (COVID-19) that was first detected in Wuhan, China, in December 2019. The virus causes respiratory illness of varying severity in people and can spread from person to person {Center for Disease Control (CDC) 2020, Center for Disease Control and Prevention (CDC) 2020}. In severe cases, SARS-CoV-2 can cause pneumonia, severe acute respiratory distress syndrome, kidney failure, and death {World Health Organization (WHO) 2020}.

Remdesivir (GS-5734) is a single diastereomer monophosphoramidate prodrug of a nucleoside analog that is intracellularly metabolized into an analog of adenosine triphosphate acting as a potent and selective inhibitor of multiple viral RNA polymerases. Remdesivir has broad-spectrum activity against members of the coronaviruses (eg, SARS-CoV-2, SARS-CoV and MERS-CoV), filoviruses (eg, Ebola virus [EBOV] and Marburg virus [MARV]), and paramyxoviruses (eg, respiratory syncytial virus [RSV], Nipah virus [NiV], and Hendra virus). Remdesivir is being developed for the treatment of patients with COVID-19. The availability of an effective antiviral agent with a favorable benefit/risk profile would address a serious unmet medical need for the treatment of patients with COVID-19.

Remdesivir, the phosphoramidate prodrug of adenine nucleoside analog GS-441524, is being developed by Gilead Sciences, Inc. as an intravenous (IV) product for the treatment of COVID-19. This section provides a detailed overview of in vitro and in vivo pharmacology, pharmacodynamics, and safety pharmacology properties of the compound with the following highlights:

- Remdesivir showed potent in vitro activity against a clinical isolate of SARS-CoV-2 in primary human airway epithelial (HAE) cells (50% effective concentration [EC_{50}] = 0.0099 μM).
- Remdesivir potently inhibited a recombinant chimeric virus expressing the polymerase (nsp12) gene of SARS-CoV-2 in a backbone of SARS-CoV with a luciferase reporter in Huh7 cells (EC_{50} = 0.0035 μM).
- Remdesivir also showed potent in vitro activity against the human pathogenic coronaviruses MERS-CoV and SARS-CoV in multiple relevant human cell types.

- Remdesivir showed therapeutic efficacy in SARS-CoV-2-infected rhesus monkeys. Administration of 10/5 mg/kg (10 mg/kg first dose, followed by 5 mg/kg once daily thereafter) RDV using IV bolus injection initiated 12 hours post-inoculation with SARS-CoV-2 resulted in a significant reduction in clinical signs of respiratory disease, lung pathology and gross lung lesions, and viral RNA levels compared with vehicle-treated animals.

- Biochemical studies demonstrated that the active triphosphate metabolite of RDV, GS-443902, acts as an analog of adenosine triphosphate (ATP) and competes with the natural ATP substrate to selectively inhibit viral RNA-dependent RNA polymerases (RdRp). The primary mechanism of inhibition is incorporation of the nucleoside triphosphate GS-443902 into nascent RNA chains by RdRp, causing delayed RNA chain termination during the process of viral replication. The coronavirus RdRp of SARS-CoV-2, SARS-CoV, and MERS-CoV were shown to incorporate GS-443902 more efficiently than ATP, and also more efficiently than other viral RdRp such as those from EBOV or RSV.

- In vitro resistance selection experiments using the nucleoside analog of RDV and murine hepatitis virus (MHV), a related animal CoV, demonstrated a high barrier to resistance development and identified two mutations in the viral polymerase at residues conserved across CoVs that conferred low-level (5.6-fold) resistance to RDV. The mutant viruses showed reduced viral fitness in vitro, and introduction of the corresponding mutations into SARS-CoV resulted in attenuated SARS-CoV pathogenesis in a mouse model.

- In mouse models of SARS-CoV and MERS-CoV, administration of 25 mg/kg RDV subcutaneously twice daily beginning 1 day before or 1 day after virus inoculation resulted in significantly reduced lung viral load and improved clinical signs of disease as well as lung function.

- Remdesivir also showed prophylactic and therapeutic efficacy in MERS-CoV-infected rhesus monkeys. Administration of 10 mg/kg or 5 mg/kg RDV once daily for 7 days using IV bolus injection beginning 1 day prior to MERS-CoV inoculation resulted in a significant reduction of clinical scores, clinical signs of respiratory disease, and viral RNA levels compared with vehicle-treated animals. Therapeutic RDV treatment of 5 mg/kg once daily using IV bolus injection initiated 12 hours post-inoculation also resulted in reduced clinical signs, reduced virus replication in the lungs, and decreased presence and severity of lung lesions.

- Remdesivir is active against filoviruses (eg, EBOV and MARV), as well as other RNA viruses such as paramyxoviruses (eg, RSV and NiV).

- Remdesivir and its nucleoside GS-441524 were profiled for in vitro cytotoxicity and mitochondrial toxicity in multiple relevant cell types. Remdesivir exhibited selectivity values > 170 (ie, ratio of 50% cytotoxic concentration [CC_{50}]/EC_{50} against SARS-CoV-2 in HAE cells) in in vitro toxicity assays. Data from in vitro studies with liver cell culture systems demonstrated that human hepatocytes are the cell type most susceptible to RDV-mediated toxicity, likely due to rapid intracellular metabolism of prodrug.

- Molecular target screening studies with GS-441524 and GS-466547 (diastereomeric mixture) showed no significant binding inhibition (> 50%) at 10 μM.

- Safety pharmacology studies were conducted to examine the potential effects of RDV on the cardiovascular, respiratory, and central nervous system (CNS) systems after IV administration. Remdesivir had no effects on the cardiovascular system of monkeys (highest dose tested: 10 mg/kg IV) that correlated with the weak activity at the human ether-a-go-go-related gene (hERG) channel. In the respiratory safety study in rats, RDV had no effect on tidal volume or minute volume; however, respiration rates were increased from 0.75 to 6 hours postdose in animals administered ≥ 20 mg/kg IV. Remdesivir had no effects on the CNS of rats (highest dose tested: 50 mg/kg IV). Taken together, the risk for respiratory, CNS, or cardiovascular effects in the clinic is considered negligible.

In conclusion, RDV is a novel, small molecule inhibitor of CoV replication, with potent in vitro and in vivo activity against multiple genetically diverse CoVs. Importantly, RDV exhibits potent in vitro and in vivo antiviral activity against SARS-CoV-2. The overall nonclinical pharmacology profile of RDV supports its consideration as a novel agent for the treatment of COVID-19.

3. PRIMARY PHARMACODYNAMICS

3.1. In Vitro Pharmacodynamics

3.1.1. Antiviral Activity Against Coronaviruses

Remdesivir has been tested at [redacted] and independently in the laboratory of [redacted], as well as at [redacted], to establish the activity against human pathogenic CoVs, specifically SARS-CoV-2, SARS-CoV, and MERS-CoV, (m2.6.3, Section 2.1, PC-399-2008, PC-399-2019, PC-540-2001, PC-540-2002, and PC-540-2003, {Sheahan 2017}).

HAE cell cultures represent highly differentiated human airway epithelium containing ciliated and non-ciliated epithelial cells as well as goblet cells. They are among the most biologically relevant in vitro models of the lung, recapitulating the cellular complexity and physiology of the human conducting airway {Scobey 2013, Sims 2005}. Remdesivir inhibited the in vitro replication of SARS-CoV-2 (clinical isolate) in HAE cells with an EC_{50} value of 0.0099 µM after 48 hours of treatment (m2.6.3, Section 2.1, PC-540-2003, Table 2). The CC_{50} of RDV was previously established in HAE cells. No cytotoxicity was observed across the dose range measured in the assay (CC_{50} > 10 µM) {Sheahan 2017}. This suggests selective inhibition of the virus replication, with a selectivity index (CC_{50}/EC_{50}) > 1000.

Remdesivir also inhibited the in vitro replication of SARS-CoV-2 (clinical isolate) in African green monkey kidney Vero cells with EC_{50} values of 0.137 µM and 0.750 µM after 24 and 48 hours of treatment, respectively (Table 2). No cytotoxicity was observed with RDV up to 100 µM (CC_{50} > 100 µM), the highest concentration tested for both treatment durations, suggesting selective inhibition of virus replication.

Remdesivir inhibited the in vitro replication of a recombinant chimeric virus expressing the polymerase (nsp12) gene of SARS-CoV-2 in a backbone of SARS-CoV with a luciferase reporter (SARS/SARS-CoV-2 nsp12 nLUC) in human hepatoma Huh7 cells with a mean EC_{50} value of 0.0035 µM after 48 hours of treatment (m2.6.3, Section 2.1, PC-540-2002, Table 2). No cytotoxicity was previously reported for RDV in Huh7 cells across the dose range measured in the assay (CC_{50} > 10 µM) {Brown 2019}. This suggests selective inhibition of virus replication, with a selectivity index > 1000.

Table 2. In Vitro Activity of Remdesivir Against SARS-CoV-2 in HAE, Vero, and Huh7 Cells

Virus	Cell Type	EC_{50} (µM)	CC_{50} (µM)	Selectivity Index (CC_{50}/EC_{50})
SARS-CoV-2 clinical isolate	HAE	0.0099	> 10	> 1000
	Vero	0.137 after 24 hours 0.750 after 48 hours	> 100	> 730 > 133
SARS/SARS-CoV-2 nsp12 nLUC	Huh7	0.0035	> 10	> 1000

CC_{50} = 50% cytotoxic concentration; EC_{50} = 50% effective concentration
Source: m2.6.3, Section 2.1, PC-540-2003, PC-540-2001, PC-540-2002, {Brown 2019, Sheahan 2017}

The nsp12 viral polymerase of SARS-CoV-2 exhibits high sequence homology to the polymerases of SARS-CoV and MERS-CoV, with 96% and 71% amino acid sequence identity, respectively (m2.6.3, Section 2.1, PC-540-2005). Given the high sequence homology of the viral polymerases, data from in vitro and in vivo studies of RDV against SARS-CoV and MERS-CoV further substantiate its activity against SARS-CoV-2.

The activity of RDV against SARS-CoV and MERS-CoV was assessed using recombinant viruses expressing a fluorescent reporter protein in HAE cells (MERS-CoV and SARS-CoV) and a continuous human lung epithelial cell line 2B4 (Calu-3; MERS-CoV only). In HAE cells, RDV efficiently inhibited both SARS-CoV and MERS-CoV replication with EC_{50} values of 0.069 and 0.074 µM, respectively, (Table 3) {Sheahan 2017}. An independent experiment confirmed the antiviral activity of RDV against SARS-CoV with an EC_{50} of 0.0066 µM in a different donor of HAE cells. Remdesivir inhibited MERS-CoV replication in Calu-3 cells, with an average EC_{50} of 0.025 µM. In both HAE and Calu-3 cells, no cytotoxicity was observed at 10 µM RDV, the highest concentration tested, demonstrating that RDV has a favorable in vitro selectivity index (Table 3) {Sheahan 2017}.

Remdesivir inhibited the in vitro replication of SARS-CoV expressing luciferase (SARS-CoV nLUC) in human hepatoma Huh7 cells with a mean EC_{50} value of 0.0071 µM (m2.6.3, Section 2.1, PC-540-2002, Table 3). No cytotoxicity was previously reported for RDV in Huh7 cells across the dose range measured in the assay (CC_{50} > 10 µM) {Brown 2019}. This suggests selective inhibition of virus replication, with a selectivity index > 1000.

Remdesivir inhibited the in vitro replication of MERS-CoV in Vero E6 cells, with a mean EC_{50} value of 0.52 µM (Table 3; m2.6.3, Section 2.1, PC-399-2008, PC-399-2019). No cytotoxicity was observed at 10 µM, the highest concentration tested, indicating selective inhibition of virus replication with a selectivity index (CC_{50}/EC_{50}) > 19.

Table 3. In Vitro Activity of Remdesivir Against MERS-CoV and SARS-CoV in HAE, Calu-3, and Vero E6 Cells

Virus	Cell Type	Mean EC_{50} (µM)	CC_{50} (µM)	Selectivity Index (CC_{50}/EC_{50})
SARS-CoV	HAE	0.069	> 10	> 100
	HAE	0.0066	> 10	> 1000
	Huh7	0.0071	> 10	> 1000
MERS-CoV	HAE	0.074	> 10	> 100
	Calu-3	0.025	> 10	> 400
	Vero E6	0.52	> 10	> 19

CC_{50} = 50% cytotoxic concentration; EC_{50} = 50% effective concentration
Source: {Brown 2019, Sheahan 2017}, m2.6.3, Section 2.1, PC-540-2003, PC-540-2002, PC-399-2008, PC-399-2019

Remdesivir showed potent antiviral activity against endemic human CoVs OC43 and 229E, as well as the animal CoVs MHV and the genetically divergent porcine deltacoronavirus, with submicromolar EC_{50} values ranging from 0.02 to 0.15 µM in various cell types {Agostini 2018, Brown 2019}.

3.1.2. Mechanism of Action

Remdesivir has been designed to broadly and rapidly distribute into cells, including those within tissues, where it is efficiently metabolized to form the pharmacologically active nucleoside triphosphate metabolite, GS-443902. Efficient metabolism of RDV to GS-443902 has been demonstrated in multiple cell types, including normal human bronchial epithelial cells and the human lung adenocarcinoma cell line Calu-3 (m2.6.5, Section 7, AD-540-2001, AD-540-2002, AD-399-2004, AD-399-2006).

Biochemical studies demonstrate that the nucleoside triphosphate GS-443902 acts as an analog of ATP and competes with the natural ATP substrate to selectively inhibit viral RdRps. The primary mechanism of inhibition is the incorporation of the nucleoside triphosphate GS-443902 into nascent RNA chains by RdRp, which results in delayed RNA chain termination during replication of the viral RNA {Gordon 2020}. Delayed chain termination has been shown to be the mechanism of action of RDV inhibition of the SARS-CoV-2 (m2.6.3, Section 2.1, PC-540-2005), SARS-CoV (m2.6.3, Section 2.1, PC-540-2005), MERS-CoV {Gordon 2020}, EBOV {Tchesnokov 2019}, RSV {Warren 2016}, and NiV {Jordan 2018} polymerases. The ratio of Michaelis-Menten steady-state kinetic parameters for single nucleotide incorporation (V_{max}/K_m) of a natural nucleotide substrate over a nucleotide analogue defines its selectivity. Based on the steady-state kinetic parameters for single nucleotide incorporations in comparison with ATP, the coronaviruses SARS-CoV-2, SARS-CoV, and MERS-CoV were shown to incorporate GS-443902 more efficiently than ATP. GS-443902 selectivity values were 0.26, 0.32, and 0.35, respectively, compared with ATP {Gordon 2020} (m2.6.3, Section 2.1, PC-540-2005). GS-443902 inhibited MERS-CoV RNA polymerase with a 50% inhibitory concentration (IC_{50}) value of 0.032 µM {Gordon 2020}.

3.1.3. Viral Resistance

The in vitro development of resistance to RDV in CoVs has been assessed by cell culture passaging of MHV, a related animal CoV, in the presence of the RDV nucleoside analog GS-441524 {Agostini 2018}. After 23 passages, two mutations were selected in the nsp12 polymerase at residues conserved across sequenced α-, β-, and γ-CoVs: F476L and V553L, which reside within the predicted fingers domain of the conserved right-hand structure of the RdRp. Recombinant MHV containing the F476L and V553L RdRp mutations were generated to determine whether the mutations were necessary and sufficient for the observed resistance phenotype of the passage 23 virus population. Compared with wild-type virus, recombinant MHV containing the F476L mutation showed 2.4-fold reduced susceptibility to RDV, and MHV containing V553L demonstrated 5-fold reduced susceptibility; the double mutant conferred 5.6-fold reduced susceptibility to RDV in vitro, similar to passage 23 virus population. Neither the passage 23 virus population nor any of the recombinant viruses were completely resistant to RDV; all mutant viruses remained sensitive to higher but nontoxic concentrations of RDV.

Remdesivir decreases viral RNA levels, thus the RdRp resistance mutations partially restored RNA levels in the presence of RDV; the degree of restoration of RNA levels correlated with their fold resistance to RDV {Agostini 2018}. These results are consistent with the mechanism of action of RDV primarily targeting RdRp-mediated RNA synthesis (Section 3.1.2).

Although the replication capacity of recombinant MHV carrying the single and double mutants replicated similarly to wild-type MHV, both with respect to replication kinetics and observed peak titer, the mutant viruses were unable to compete with wild-type virus in coinfection experiments in the absence of RDV, demonstrating a viral fitness cost associated with these mutations {Agostini 2018}. By passage 2, the double mutant MHV was outcompeted by wild-type MHV in the population at every input ratio (1:1, 1:9, or 9:1), demonstrating a competitive fitness cost of the double mutations in the absence of RDV. This competitive fitness cost suggests that RDV resistance mutations are unlikely to persist in the absence of treatment.

Introduction of the MHV resistance mutations into the corresponding residues of SARS-CoV polymerase (F480L and V557L) resulted in the same in vitro susceptibility changes (6-fold reduced susceptibility to RDV), suggesting that the conserved residues across divergent CoVs reflect conserved functions impaired by RDV, potentially implying common pathways to resistance across CoVs {Agostini 2018}. In a mouse model of SARS-CoV infection, animals infected with the double mutant SARS-CoV showed attenuated disease pathogenesis versus wildtype SARS-CoV {Agostini 2018}. At 2 days post-infection, mouse lung viral titers were similar between wild-type and double mutant SARS-CoV. However, by 4 days post-infection, lung viral titers were significantly reduced ($P < 0.05$) in mice infected with the double mutant SARS-CoV. These data demonstrate that double mutant SARS-CoV is attenuated in its ability to cause disease and replicates less efficiently than wild type virus in a mouse model of SARS-CoV pathogenesis.

3.2. In Vivo Pharmacodynamics

Remdesivir was tested in SARS-CoV-2-infected rhesus monkeys at [redacted] (m2.6.3, Section 2.2, PC-540-2004).

Remdesivir has also been tested in murine models of SARS-CoV and MERS-CoV infection at [redacted] and in MERS-CoV-infected rhesus monkeys at [redacted] (m2.6.3, Section 2.2, PC-399-2038, {de Wit 2020, Sheahan 2017, Sheahan 2020}).

3.2.1. Therapeutic Efficacy of Remdesivir Against SARS-CoV-2 in Rhesus Monkeys

The therapeutic efficacy of RDV was determined in SARS-CoV-2-infected rhesus monkeys (m2.6.3, Section 2.2, PC-540-2004). Vehicle or RDV (10 mg/kg first dose, followed by 5 mg/kg thereafter) was administered once daily using IV bolus injection beginning 12 hours after SARS-CoV-2 inoculation through Day 6 post-inoculation (Table 4). Animals were inoculated on Day 0 with a total target dose of 2.6×10^6 tissue culture infectious dose 50 ($TCID_{50}$) of SARS-CoV-2 via the intranasal, ocular, oral, and intratracheal routes.

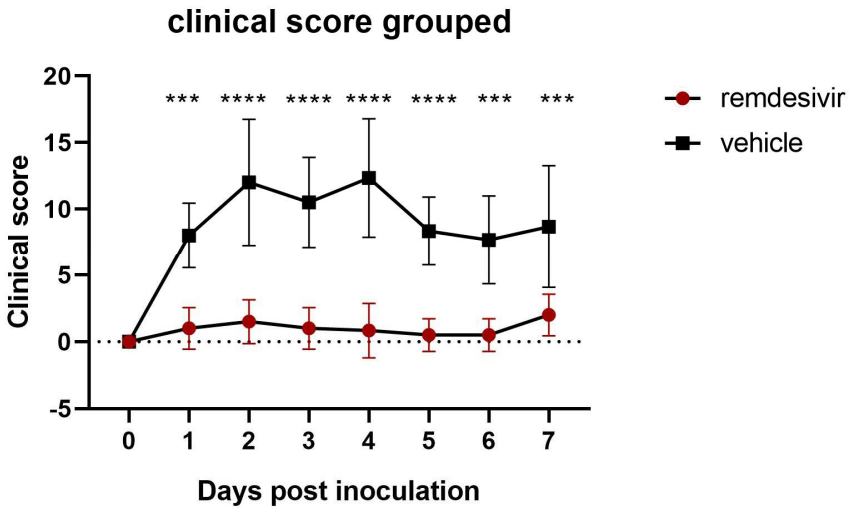
Table 4. Design of Therapeutic Efficacy Studies of 10/5 mg/kg Intravenous Remdesivir Against SARS-CoV-2 in Rhesus Monkeys

Group No.	No. of Animals (N)	Treatment (IV, Once Daily)
1	N = 6	12 hours post-inoculation: 10 mg/kg RDV Days 1-6 dpi: 5 mg/kg RDV
2	N = 6	12 hours post-inoculation: 2 mL/kg vehicle solution Days 1-6 dpi: 1 mL/kg vehicle solution

IV = intravenous; RDV = remdesivir

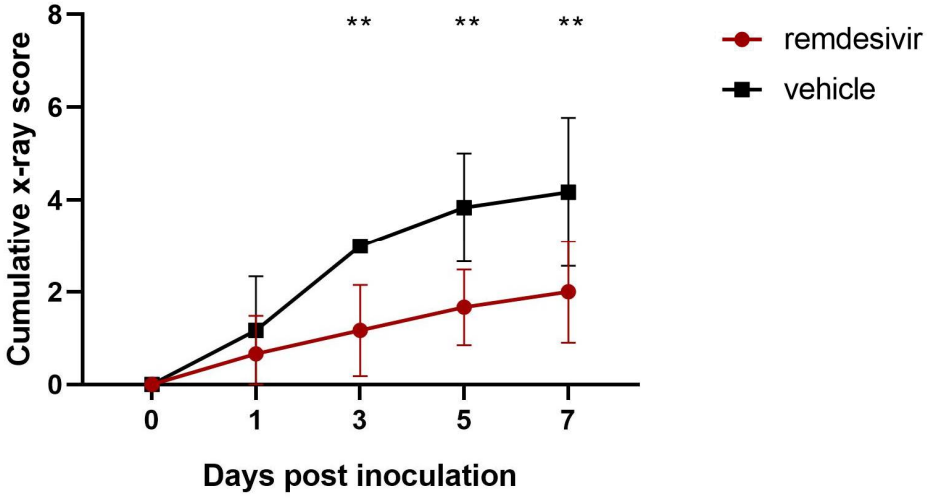
Remdesivir treatment significantly reduced SARS-CoV-2-induced clinical signs (Figure 1). Consistent with these observations, lung pathology assessed by radiography showed reduced severity of pulmonary infiltrates in the lungs of RDV-treated animals compared with vehicle-treated animals (Figure 2). At necropsy (7 days post-inoculation), RDV-treated animals had significantly reduced gross lung lesions (Figure 3) and an average 2.2 logs lower viral RNA levels in lung tissue compared with vehicle-treated animals (Figure 4).

Figure 1. Effect of 10/5 mg/kg Intravenous Remdesivir Once-Daily Therapeutic Administration on Clinical Score in SARS-CoV-2-Infected Rhesus Monkeys



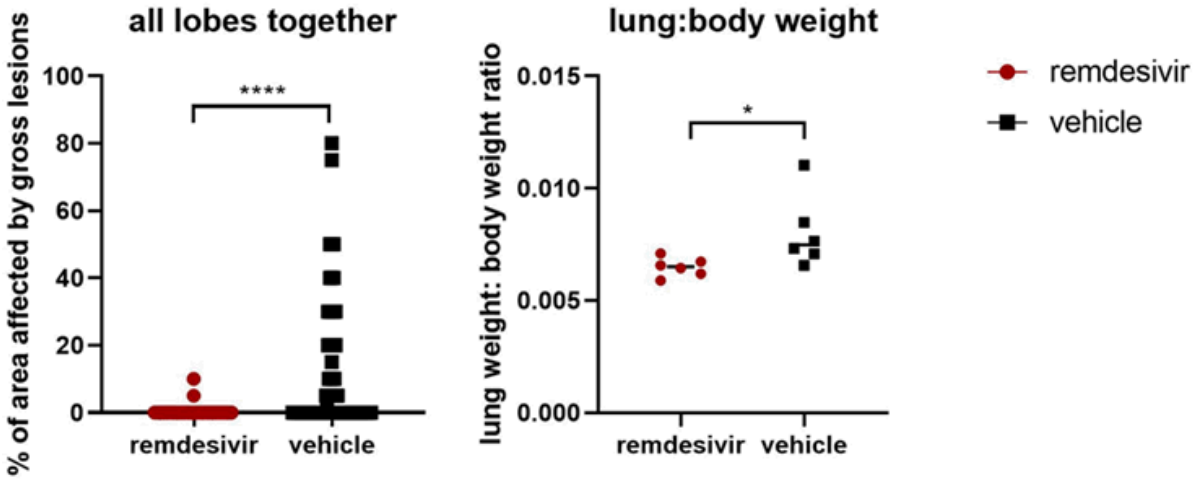
ANOVA = analysis of variance
Values represent averages from 6 animals per group. Asterisks indicate statistically significant differences in a two-way ANOVA with Dunnett's multiple comparisons. ***P < 0.001; ****P < 0.0001.
Source: m2.6.3, Section 2.2, PC-540-2004

Figure 2. Effect of 10/5 mg/kg Intravenous Remdesivir Once-Daily Therapeutic Administration on Lung Radiographic Findings in SARS-CoV-2-Infected Rhesus Monkeys



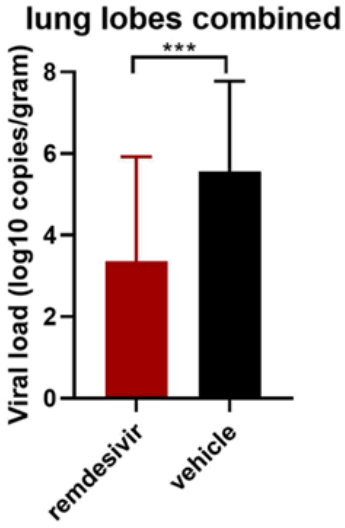
ANOVA = analysis of variance
Values represent averages from 6 animals per group. Asterisks indicate statistically significant differences in a one-way ANOVA with Dunnett's multiple comparisons. **P < 0.01.
Source: m2.6.3, Section 2.2, PC-540-2004

Figure 3. Effect of 10/5 mg/kg Intravenous Remdesivir Once-Daily Therapeutic Administration on Lung Pathologic Findings in SARS-CoV-2-Infected Rhesus Monkeys



ANOVA = analysis of variance
Left panel: The dots represent the individual scores from all lung lobes per animal. Right panel: 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 < 0.0001.
Source: m2.6.3, Section 2.2, PC-540-2004

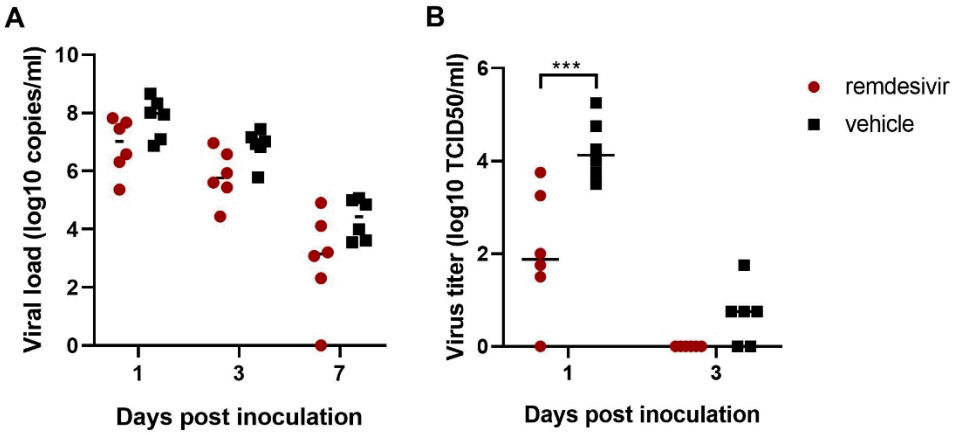
Figure 4. Effect of 10/5 mg/kg Intravenous Remdesivir Once-Daily Therapeutic Administration on Lung Viral Load in SARS-CoV-2-Infected Rhesus Monkeys



ANOVA = analysis of variance; SD = standard deviation
At necropsy, one sample from each lung lobe was collected per animal (6 samples per animal), RNA was extracted, and viral load was determined. Averages and SDs per group are indicated. Asterisks indicate statistically significant differences in a two-way ANOVA with Dunnett's multiple comparisons. ***P < 0.001.
Source: m2.6.3, Section 2.2, PC-540-2004

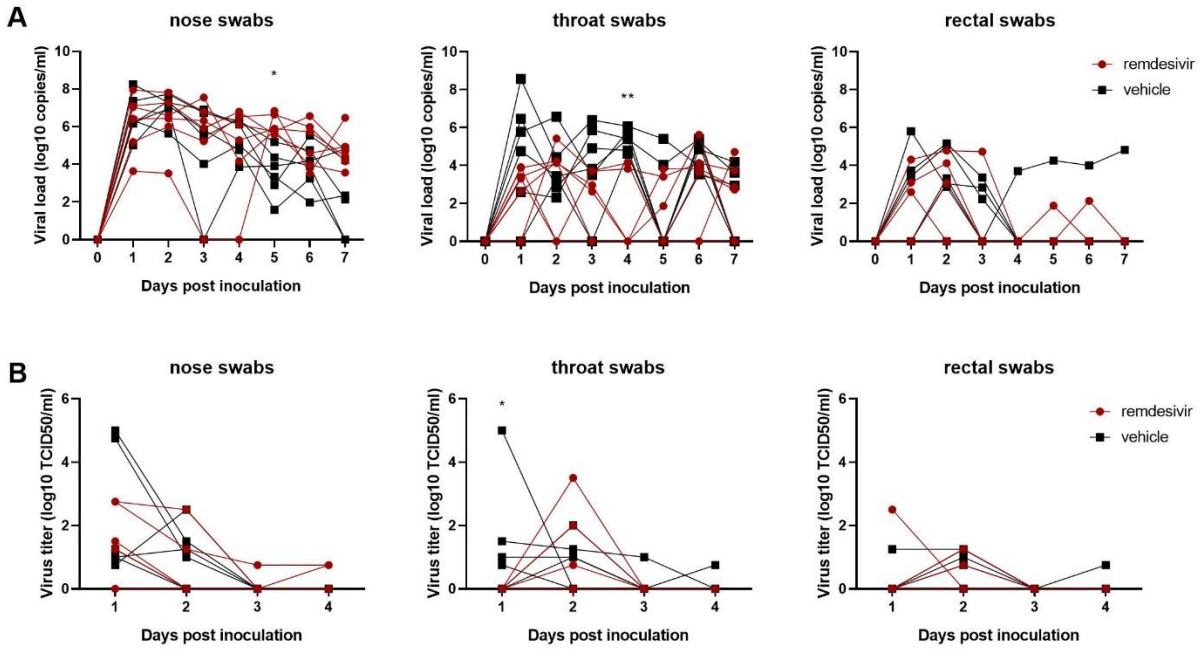
In bronchoalveolar lavage (BAL) fluid, viral loads were reduced in RDV-treated animals, although this difference was not statistically significant from vehicle-treated animals (Figure 5A). However, 12 hours after the first dose of RDV, the infectious virus titer in BAL was approximately 100-fold lower in RDV-treated animals compared to vehicle-treated animals (Figure 5B). By 3 days post-inoculation, infectious virus could no longer be detected in BAL from RDV-treated animals, whereas virus was still detected in BAL from 5 of 6 vehicle-treated animals (Figure 5B). Despite this reduction in virus replication in the lower respiratory tract, neither viral loads nor infectious virus titers were reduced in nose, throat, or rectal swabs collected from RDV-treated animals, with the exception of in virus titer in throat swabs collected on 1 day post-inoculation and in viral loads in throat swabs collected on 4 days post-inoculation, which were significantly different (Figure 6) {Williamson 2020}.

Figure 5. Effect of 10/5 mg/kg Intravenous Remdesivir Once-Daily Therapeutic Administration on Viral Loads and Virus Titers in Bronchoalveolar Lavage (BAL) in SARS-CoV-2-Infected Rhesus Monkeys



ANOVA = analysis of variance
Statistical analysis was performed using a 2-way ANOVA with Sidak's multiple comparisons test. *** $p < 0.001$
Source: {Williamson 2020}

Figure 6. Effect of 10/5 mg/kg Intravenous Remdesivir Once-Daily Therapeutic Administration on Viral Loads and Virus Titers in Swabs in SARS-CoV-2-Infected Rhesus Monkeys



ANOVA = analysis of variance
Statistical analysis was performed using a 2-way ANOVA with Sidak's multiple comparisons test. * $p < 0.05$; ** $p < 0.01$
Source: {Williamson 2020}

3.2.2. Efficacy of Remdesivir Against SARS-CoV in Mice

Compared with humans, the plasma stability of RDV is reduced in mice ($t_{1/2}$ = < 5 minutes) due to the expression of rodent-specific, secreted carboxylesterase 1c (Ces1c) {Li 2005}. To overcome this, C57BL/6 mice with a genetic deletion in Ces1c ($Ces1c^{-/-}$), in which plasma stability of RDV is increased, were used to evaluate the prophylactic and therapeutic efficacy of RDV in a murine model of SARS-CoV infection {Sheahan 2017}.

In a prophylactic and therapeutic study, RDV (25 mg/kg, twice daily) was administered subcutaneously starting 1 day prior to or 1 day after intranasal infection on Day 0 in mice with 10^4 plaque-forming units (pfu)/50 µL (prophylactic) or 10^3 pfu/50 µL (therapeutic) SARS-CoV (Table 5). Infected mice were subjected to whole body plethysmography and assigned a Penh score as a surrogate measure of bronchoconstriction or airway obstruction. Prophylactic administration of 25 mg/kg RDV subcutaneously twice daily, initiated 1 day prior to virus inoculation, substantially reduced SARS-CoV-induced weight loss (Figure 7), reduced virus titers in the lung (Figure 8), and improved pulmonary function (ie, reduced Penh scores) (Figure 9) compared with control vehicle-treated animals. Similarly, therapeutic administration of the same RDV dosing regimen initiated 1 day post-infection improved SARS-CoV-induced weight loss (Figure 7), reduced viral load in lung (Figure 8), and improved pulmonary function (ie, reduced Penh scores) (Figure 9), albeit to a lesser extent than the prophylactic regimen. In

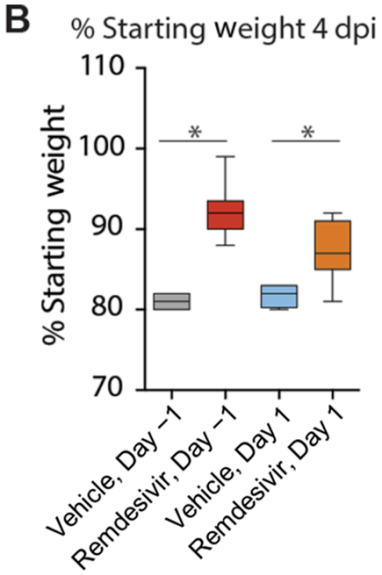
summary, subcutaneous administration of RDV, 25 mg/kg twice daily at 1-day post-infection, or earlier, in this murine model of SARS-CoV infection, suppressed viral replication in the lung and reduced disease severity.

Table 5. Design of Efficacy Study of Remdesivir Administered Subcutaneously Against SARS-CoV in Mice

Group, No. of Animals (N)	Treatment (Subcutaneous, Twice Daily)
Prophylactic Administration	
Group 1, N = 5	Vehicle, Days –1 to 4
Group 2, N = 10	RDV 25 mg/kg, Days –1 to 4
Therapeutic Administration	
Group 3, N = 4	Vehicle, Days 1 to 4
Group 4, N = 11	RDV 25 mg/kg, Days 1 to 4

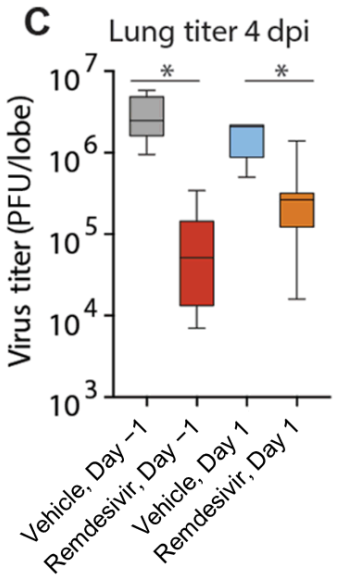
RDV = remdesivir

Figure 7. Effect of Remdesivir Twice-Daily Subcutaneous Prophylactic and Therapeutic Administration on Weight Loss in SARS-CoV-Infected Mice



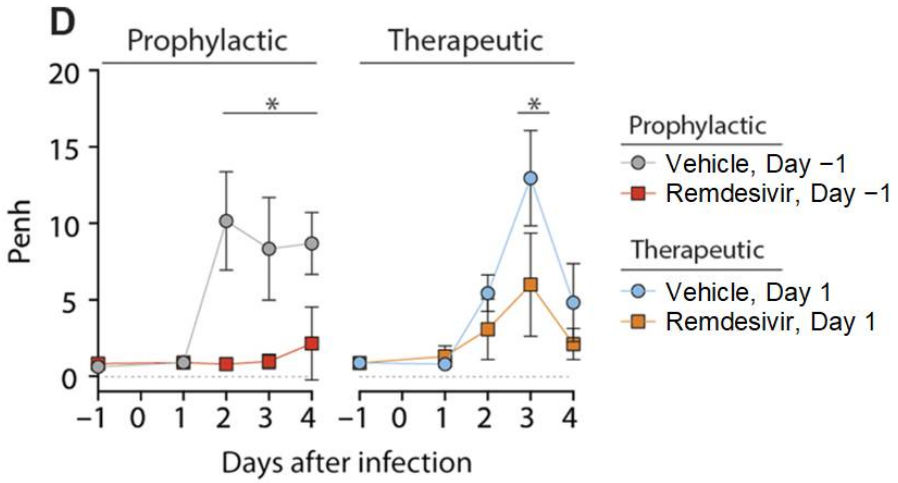
ANOVA = analysis of variance; dpi = days post-infection
*P = 0.05; calculated using the Mann-Whitney test comparing RDV with vehicle.
Source: {Sheahan 2017}

Figure 8. Effect of Remdesivir Twice-Daily Subcutaneous Prophylactic and Therapeutic Administration on Lung Viral Load in SARS-CoV-Infected Mice



dpi = days post-infection; PFU = plaque-forming units; RDV = remdesivir
*P = 0.05; calculated using the Mann-Whitney test comparing RDV with vehicle.
Source: {Sheahan 2017}

Figure 9. Effect of Remdesivir Twice-Daily Subcutaneous Prophylactic and Therapeutic Administration on Lung Function in SARS-CoV-Infected Mice



ANOVA = analysis of variance
Whole-body plethysmography was used to measure pulmonary function in SARS-CoV-infected mice treated with RDV, either 1 day prior to infection (prophylactic) or 1 day post-infection (therapeutic). Penh is a surrogate measure of bronchoconstriction or airway obstruction.
*P = 0.05; calculated using 2-way ANOVA with Šidák's multiple comparison test.
Source: {Sheahan 2017}

3.2.3. Efficacy of Remdesivir Against MERS-CoV in Mice

Standard laboratory mice are not susceptible to MERS-CoV infection due to differences in human and mouse dipeptidyl peptidase 4 (DPP4), the entry receptor for MERS-CoV. To enable testing of RDV in mice, Ces1c$^{-/-}$ mice (described in Section 3.2.1) were bred with mice harboring a modified humanized DPP4 (hDPP4) and the resulting Ces1c$^{-/-}$ hDPP4 mice were used to evaluate the prophylactic and therapeutic efficacy of RDV in a murine model of MERS-CoV infection {Sheahan 2020}.

3.2.3.1. Prophylactic Efficacy of Remdesivir Against MERS-CoV Infection in Mice

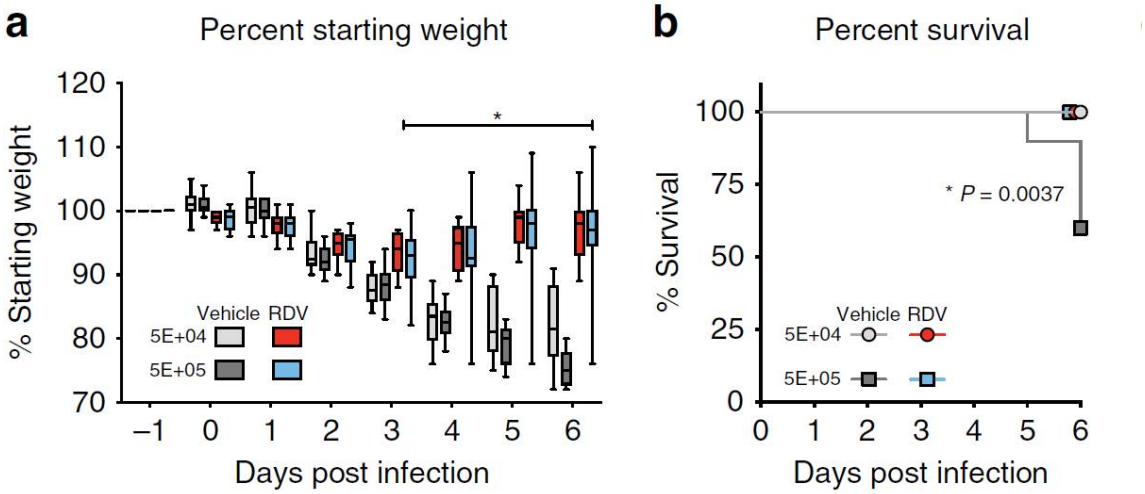
In a prophylactic study, RDV (25 mg/kg, twice daily) was administered subcutaneously 1 day prior to intranasal infection on Day 0 in mice with 5×10^4 pfu or 5×10^5 pfu of MERS-CoV (Table 6). In this model, RDV significantly diminished MERS-CoV-induced weight loss compared with control vehicle-treated animals and also prevented mortality in mice administered a lethal dose (ie, 5×10^5 pfu) of MERS-CoV (Figure 10). Remdesivir also significantly reduced virus lung titers on Days 4 and 6 post-infection (Figure 11), decreased lung hemorrhage scores, and diminished the pathological features of acute lung injury compared with control vehicle-treated animals. In contrast, a similarly designed study conducted in the same mouse model demonstrated that prophylactic lopinavir/ritonavir-interferon beta (LPV/RTV-IFNb) slightly reduced viral loads but did not impact other disease parameters {Sheahan 2020}.

Table 6. Design of Prophylactic Efficacy Study of Twice-Daily Subcutaneous Remdesivir Against MERS-CoV in Mice

Group, No. of Animals (N)	Treatment, Day of Treatment Initiation	Viral Inoculum (pfu)
Group 1, N = 14	Vehicle, Day –1	5×10^4
Group 2, N = 14	RDV 25 mg/kg, Day –1	5×10^4
Group 3, N = 14	Vehicle, Day –1	5×10^5
Group 4, N = 15	RDV 25 mg/kg, Day –1	5×10^5

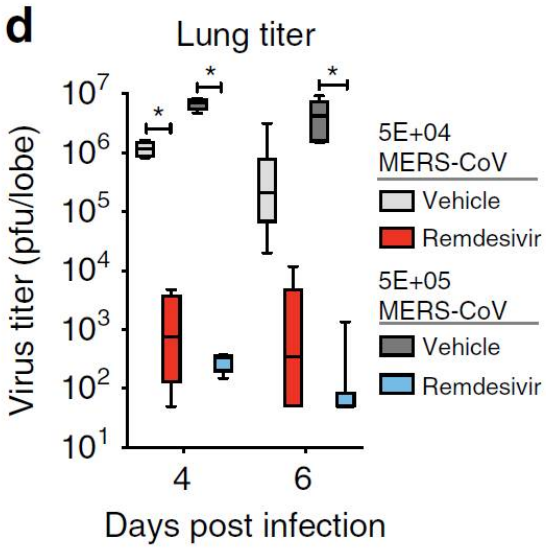
pfu = plaque-forming units; RDV = remdesivir
Treatment with RDV continued until scheduled euthanasia on Day 4 (4 animals per group) or Day 6 (all remaining animals).

Figure 10. Effect of Prophylactic Twice-Daily Subcutaneous Remdesivir on Weight Loss and Mortality in MERS-CoV-Infected Mice



Panel a: Asterisks indicate statistically significant differences ($P < 0.05$) as determined by 2-way ANOVA with Tukey's multiple comparison test.
Panel b: Survival analysis by Mantel–Cox test ($P < 0.05$).
Source: {Sheahan 2020}

Figure 11. Effect of Prophylactic Twice-Daily Subcutaneous Remdesivir on Lung Viral Load in MERS-CoV-Infected Mice



ANOVA = analysis of variance; pfu = plaque-forming unit
Day 4, n = 4 per group; Day 6, all remaining animals.
Asterisks indicate statistically significant differences ($P < 0.05$) as determined by 2-way ANOVA and Šidák's multiple comparison test.
Source: {Sheahan 2020}

3.2.3.2. Therapeutic Efficacy of Remdesivir Against MERS-CoV Infection in Mice

In a therapeutic study, RDV (25 mg/kg, twice daily) was administered subcutaneously 1 day after intranasal infection on Day 0 in mice with 5×10^4 pfu of MERS-CoV {Sheahan 2020}. The effect of therapeutic treatment with lopinavir/ritonavir (LPV/RTV) in combination with 2 different doses of interferon beta (IFNb) was also assessed as part of the same study (Table 7).

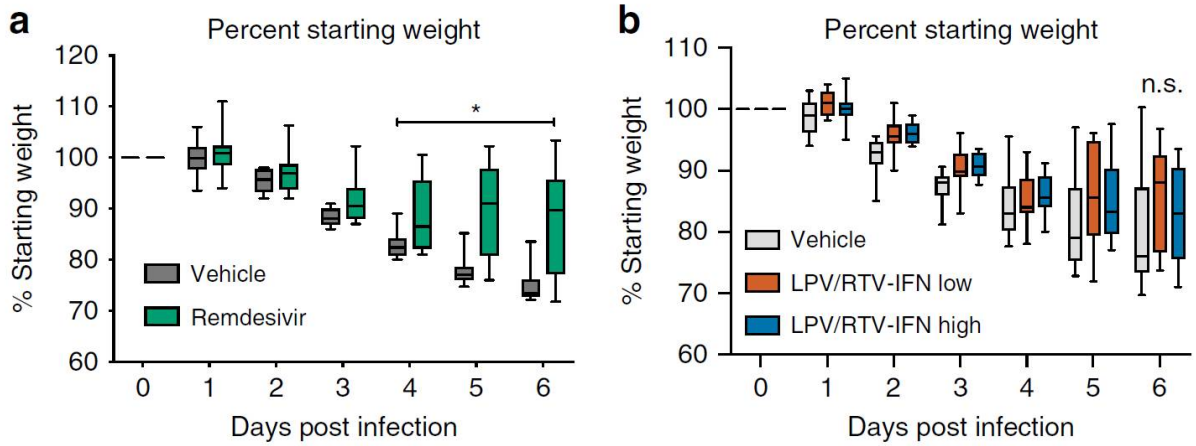
Table 7. Design of Therapeutic Efficacy Study of Twice-Daily Subcutaneous Remdesivir Compared With Once-Daily Oral Lopinavir/Ritonavir Plus Interferon-Beta Against MERS-CoV in Mice

Group, No. of Animals (N)	Treatment	Administration
Group 1, N = 13	RDV Vehicle, Days 1 to 5	Subcutaneous, Twice Daily
Group 2, N = 14	RDV 25 mg/kg, Days 1 to 5	Subcutaneous, Twice Daily
Group 3, N = 15	LPV/RTV-IFNb Vehicle, Days 1 to 5	Oral, Once Daily
Group 4, N = 16	LPV/RTV-IFNb Low[a], Days 1 to 5	Oral, Once Daily
Group 5, N = 16	LPV/RTV-IFNb High[b], Days 1 to 5	Oral, Once Daily

IFNb = interferon beta; LPV = lopinavir; RDV = remdesivir; RTV = ritonavir
a IFNb low is 1 × human equivalent dose of 1.6 million international units (MIU)/kg.
b IFNb low is 25 × human equivalent dose of 40 MIU/kg.

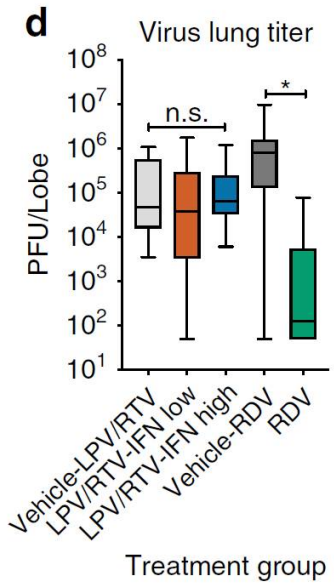
Only RDV substantially reduced MERS-CoV-induced body weight loss (Figure 12) and lung hemorrhage scores on Day 6 post-infection compared with control vehicle-treated animals. Similarly, only RDV treatment significantly reduced virus lung titers on Day 6 pos-infection compared with control vehicle-treated animals (Figure 13). Both RDV treatment and LPV/RTV-IFNb low treatment improved lung function as measured by several indicators of pulmonary function. However, only RDV reduced histologic features of acute lung injury compared with control vehicle-treated animals.

Figure 12. Effect of Therapeutic Twice-Daily Subcutaneous Remdesivir or Therapeutic Once-Daily Oral Lopinavir/Ritonavir Plus Interferon Beta on Weight Loss in MERS-CoV-Infected Mice



ANOVA = analysis of variance; IFN = interferon; LPV = lopinavir; n.s. = not significant; RTV = ritonavir
Asterisks indicate statistical differences ($P < 0.05$) by 2-way ANOVA with Tukey's multiple comparison test.
Source: {Sheahan 2020}

Figure 13. Effect of Therapeutic Twice-Daily Subcutaneous Remdesivir or Therapeutic Once-Daily Oral Lopinavir/Ritonavir Plus Interferon Beta on Lung Viral Load in MERS-CoV-Infected Mice



ANOVA = analysis of variance; IFN = interferon; LPV = lopinavir; n.s. = not significant; PFU = plaque-forming units; RDV = remdesivir; RTV = ritonavir
Asterisks indicate statistical significance ($P < 0.05$) by 1-way ANOVA with Kruskal-Wallis test.
Source: {Sheahan 2020}

3.2.4. Efficacy of Remdesivir Against MERS-CoV in Rhesus Monkeys

3.2.4.1. Prophylactic and Therapeutic Efficacy of 5 mg/kg Remdesivir Against MERS-CoV in Rhesus Monkeys

The prophylactic and therapeutic efficacy of a 5 mg/kg daily dose of RDV was determined in MERS-CoV-infected rhesus monkeys {de Wit 2020}. Vehicle or RDV 5 mg/kg was administered once daily using IV bolus injection beginning 24 hours prior to (prophylactic) or 12 hours after (therapeutic) MERS-CoV inoculation until Day 6 post-inoculation (Table 8). Animals were inoculated on Day 0 with a target dose of 7×10^6 $TCID_{50}$ of MERS-CoV via the intranasal, ocular, oral, and intratracheal routes.

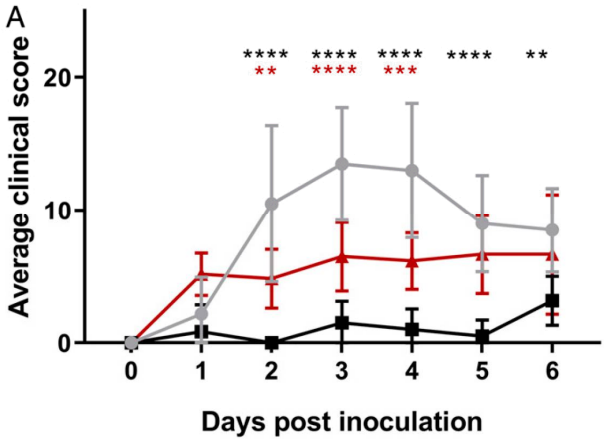
Table 8. Design of Prophylactic and Therapeutic Efficacy Studies of 5 mg/kg Intravenous Remdesivir Against MERS-CoV in Rhesus Monkeys

Group No.	No. of Animals (N)	Treatment (IV, Once Daily)
1	N = 6	RDV 5 mg/kg, Days –1 to 5
2	N = 3	Vehicle, Days –1 to 5
3	N = 6	RDV 5 mg/kg, 12 hrs to Day 5
4	N = 3	Vehicle, 12 hrs to Day 5

IV = intravenous; RDV = remdesivir

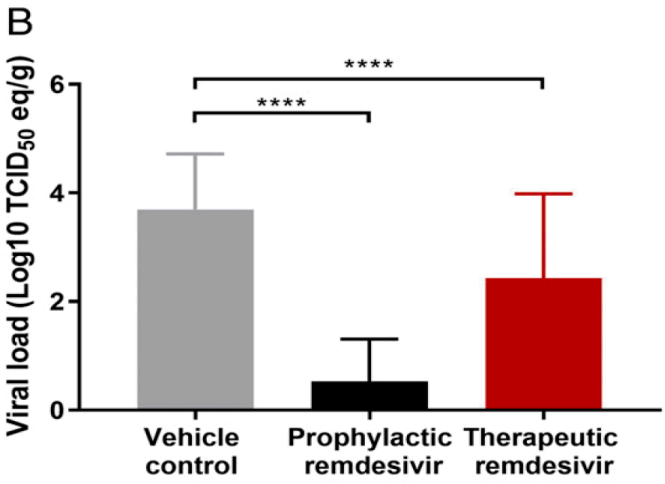
Both prophylactic and therapeutic RDV treatment significantly reduced MERS-CoV-induced clinical signs (Figure 14) and virus replication in respiratory tissues (Figure 15), and decreased presence and severity of lung lesions compared with vehicle-treated animals. These effects were more pronounced in the animals treated prophylactically.

Figure 14. Effect of 5 mg/kg Intravenous Remdesivir Once-Daily Prophylactic or Therapeutic Administration on Clinical Score in MERS-CoV-Infected Rhesus Monkeys



ANOVA = analysis of variance; RDV = remdesivir
Key: gray circles = vehicle control; black squares = prophylactic RDV; red triangles = therapeutic RDV
Two-way ANOVA with Dunnett's multiple comparisons; black asterisks indicate statistical significance between the vehicle control and prophylactic RDV groups and red asterisks indicate statistical significance between the vehicle control and therapeutic RDV groups.
*P < 0.05; **P < 0.01; ***P < 0.001; ****P < 0.0001. Vehicle control animals from the prophylactic and treatment groups were analyzed together.
Source: {de Wit 2020}

Figure 15. Effect of 5 mg/kg Intravenous Remdesivir Once-Daily Prophylactic or Therapeutic Administration on Lung Viral Load in MERS-CoV-Infected Rhesus Monkeys



ANOVA = analysis of variance
Asterisks indicate statistically significant differences in a 2-way ANOVA with Dunnett's multiple comparisons. *P < 0.05; **P < 0.01; ***P < 0.001; ****P < 0.0001.
Source: {de Wit 2020}

3.2.4.2. Prophylactic Efficacy of 10 mg/kg Remdesivir Against MERS-CoV Infection in Rhesus Monkeys

The prophylactic efficacy of a 10 mg/kg daily dose of RDV was determined in MERS-CoV-infected rhesus monkeys of Indian origin (m2.6.3, Section 2.2, PC-399-2038). Vehicle or RDV at 10 mg/kg was administered once daily for 7 days using IV bolus injection beginning 1 day prior to MERS-CoV inoculation (Table 9). Animals were inoculated on Day 0 with a target dose of 7×10^6 $TCID_{50}$ of MERS-CoV via the intranasal, ocular, oral, and intratracheal routes.

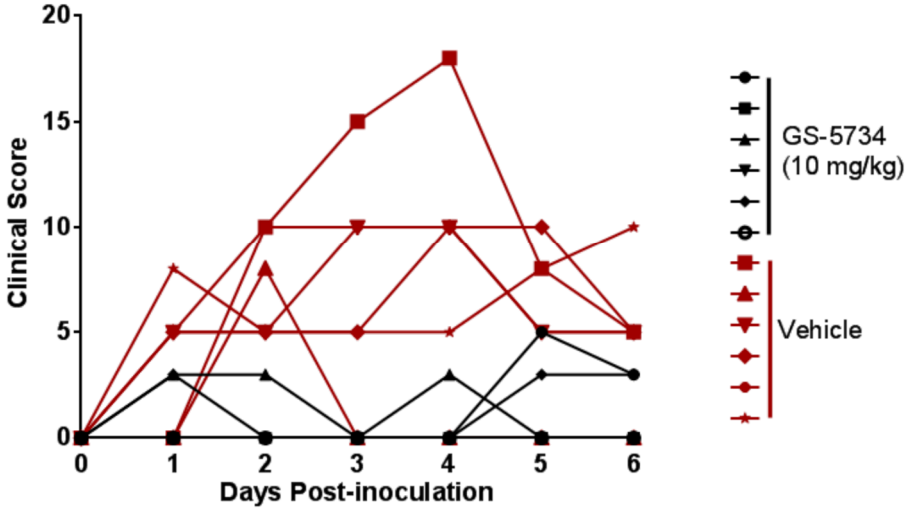
Table 9. Design of Prophylactic Study of 10 mg/kg Intravenous Remdesivir Against MERS-CoV in Rhesus Monkeys

Group No.	No. of Animals (N)	Treatment (IV, Once Daily)
1	N = 6	RDV 10 mg/kg, Days −1 to 5
2	N = 6	Vehicle, Days −1 to 5

IV = intravenous; RDV = remdesivir

Time-weighted average clinical scores were significantly higher in control vehicle-treated animals than in RDV-treated animals (P = 0.006; Figure 16). Clinical signs of respiratory disease, such as hunched posture and increased respiration rates observed in control vehicle-treated animals, were not observed in RDV-treated animals. At necropsy on Day 6 post-infection, viral RNA levels in lungs of RDV-treated animals were significantly reduced compared with vehicle-treated controls (Table 10). Animals treated with a 10 mg/kg dose of RDV displayed changes in serum creatinine and blood urea nitrogen suggestive of altered renal function (m2.6.3, Section 2.2, PC-399-2038), consistent with findings from the 7-day toxicity study in rhesus monkeys (m2.6.6, Section 4.2.1, TX-399-2021).

Figure 16. Effect of 10 mg/kg Intravenous Remdesivir Once-Daily Prophylactic Administration on Clinical Score in MERS-CoV-Infected Rhesus Monkeys



GS-5734 = remdesivir
Source: m2.6.3, Section 2.2, PC-399-2038

Table 10. Effect of 10 mg/kg Intravenous Remdesivir Once-Daily Prophylactic Administration on Lung Viral Load in MERS-CoV-Infected Rhesus Monkeys

Group, No. of Animals (N)	Treatment (IV, Once Daily, Days −1 to 5)	Day 6 Average Lung Viral Load (Log_{10} $TCID_{50}$ Eq/g) (SD)
Group 1, N = 6	RDV 10 mg/kg	0.26 (0.66)[a]
Group 2, N = 6	Vehicle	3.58 (0.89)

IV = intravenous; RDV = remdesivir; SD = standard deviation; $TCID_{50}$ = tissue culture infectious dose 50
a P = 0.0022; calculated from unpaired t-test comparing RDV-treated animals to control vehicle-treated animals.
Source: m2.6.3, Section 2.2, PC-399-2038

Taken together, these data suggest that RDV treatment is efficacious at reducing viral titers in the lung and alleviating clinical disease signs in a nonhuman primate model of MERS-CoV infection.

4. SECONDARY PHARMACODYNAMICS

4.1. In Vitro Activity Against Other Viruses

4.1.1. Antiviral Activity Against Filoviruses

The initial studies performed at [redacted] tested RDV and the nucleoside analog GS-441524 for in vitro anti-EBOV activity (m2.6.3, Section 3.1, PC-399-2007). Remdesivir exhibited potent anti-EBOV activity with EC_{50} values of 0.06 to 0.07 µM (Table 11). The nucleoside analog GS-441524 was approximately 10- to 20-fold less potent than RDV. In contrast, brincidofovir (CMX001), which was being considered for the treatment of Ebola virus disease (EVD), did not show any significant activity either in human microvascular endothelial cells (HMVECs) or in human hepatoma Huh7 cells.

Table 11. In Vitro Antiviral Activity of Remdesivir, the Nucleoside Analog GS-441524, and Control Compounds Against Ebola Virus

	EC_{50}/EC_{90}, EBOV-GFP [µM]	
Compound	**HMVEC**	**Huh7**
RDV	0.06/0.22	0.07/0.22
GS-441524 (Nucleoside Analog)	0.77/3.1	1.5/6.0
Brincidofovir (CMX001)	> 10/> 10	> 10/> 10

EBOV-GFP = genetically modified reporter Ebola virus strain expressing green fluorescent protein; EC_{50} = 50% effective concentration; EC_{90} = 90% effective concentration; HMVEC = human microvascular endothelial cells; Huh7 = human hepatoma cell line; RDV = remdesivir
Source: m2.6.3, Section 3.1, PC-399-2007

A separate test assessed the activity of the diastereomeric mixture GS-466547 specifically against wild-type Makona variant of EBOV that was isolated in 2014 during the outbreak in West Africa {Baize 2014}. Following a 3-day treatment of infected Huh7 cells, GS-466547 inhibited the yield of infectious virus and reduced the levels of viral RNA in cell culture supernatants with EC_{50} values of 0.01 and 0.001 µM, respectively (m2.6.3, Section 3.1, PC-399-2007).

In parallel, independent studies performed at [redacted], RDV showed antiviral activity against EBOV strain Kikwit (Zaire EBOV) with EC_{50} values of 0.086 and 0.14 µM in primary human macrophages and human cervical carcinoma cell line (HeLa) cell, respectively (Table 12; m2.6.3, Section 3.1, PC-399-2008). The nucleoside analog GS-441524 and several other compounds, including brincidofovir, favipiravir, and ribavirin, were inactive against EBOV. Brincidofovir has shown some antiviral activity in human foreskin fibroblasts (HFF-1) with EC_{50} of 1.8 µM. In contrast with RDV, compounds proposed to have activity against EBOV remained largely inactive in parallel assays under the same conditions. While this could be due in part to high stringency of the anti-EBOV assays that were optimized for a short, 48-hour incubation using a relatively high multiplicity of infection, it underscores superior in vitro activity of RDV compared with other small molecule inhibitors previously considered for the clinical treatment of EVD.

Table 12. In Vitro Antiviral Activity of Remdesivir, the Nucleoside Analog GS-441524, and Other Compounds Against Ebola Virus

Compound	EC_{50}/EC_{90} [µM]		
	HeLa	HFF-1	Human Macrophages
RDV	0.14/0.41	ND	0.086/0.18
GS-441524 (Nucleoside Analog)	> 20/> 20	> 20/> 20	> 20/> 20
Brincidofovir (CMX-001)	> 20/> 20	1.8/4.6	ND
Favipiravir (T-705)	> 20/> 20	ND	ND
Ribavirin	> 20/> 20	ND	ND

EC_{50} = 50% effective concentration; EC_{90} = 90% effective concentration; HeLa = human cervical carcinoma cell line; HFF-1 = human foreskin fibroblasts; ND = not determined; RDV = remdesivir
Source: m2.6.3, Section 3.1, PC-399-2008

Additional in vitro antiviral testing has shown that RDV exhibits consistent broad-spectrum antiviral activity against multiple EBOV-related filoviruses. In addition to inhibiting EBOV Kikwit and Makona, RDV has shown potent in vitro antiviral activity against the Sudan, Bundibugyo, and MARV, with EC_{50} values of 0.06 to 0.24 µM (Table 13; m2.6.3, Section 3.1, PC-399-2044) {Lo 2017, Warren 2016}.

Table 13. In Vitro Antiviral Activity of Remdesivir Against Filoviruses

Virus	Remdesivir EC_{50} (µM)
EBOV (Kikwit)	0.14
EBOV (Makona)	0.19
Bundibugyo	0.19
Sudan	0.24
MARV	0.06

EC_{50} = 50% effective concentration
Source: m2.6.3, Section 3.1, PC-399-2044, {Lo 2017, Warren 2016}

4.1.2. Antiviral Activity Against Other RNA and DNA Viruses

In addition to CoVs and filoviruses, the antiviral activity of RDV has been tested against paramyxoviruses and other RNA viruses representing significant emerging human pathogens (m2.6.3, Section 3.1, PC-399-2008) {Lo 2017, Warren 2016}.

Strong antiviral activity was observed across paramyxoviruses. Remdesivir and the nucleoside analog GS-441524 were tested in vitro against the highly pathogenic NiV Malaysia/1999 and NiV Bangladesh/2004 {Lo 2017}. Remdesivir demonstrated potent antiviral activity against both variants of NiV (Table 14). The nucleoside analog GS-441524 exhibited 10- to 26-fold reduced antiviral activity compared with RDV {Lo 2017}. Remdesivir also showed potent antiviral activity against RSV (Strain A2), as well as Hendra virus for which there are currently no antiviral therapeutics available (Table 14) (m2.6.3, Section 3.1, PC-399-2008) {Lo 2017}.

Weak in vitro antiviral activity of RDV against arenaviruses (Junin virus and Lassa fever virus) was observed with no measurable activity against togaviruses, rhabdoviruses, or bunyaviruses (Table 14) {Lo 2017, Warren 2016}.

Assessment of activity against flaviviruses demonstrated that RDV is effective against hepatitis C virus (HCV) genotypes 1b and 2a, with EC_{50} values of 0.097 and 0.084 µM, respectively (m2.6.3, Section 3.1, PC-399-2014). In contrast, weak activity of RDV was observed against tick-borne flaviviruses (Table 14) {Lo 2017}.

Remdesivir exhibited only weak antiviral activity against human rhinovirus serotype-10, with an EC_{50} value of 2.5 µM. Remdesivir was inactive against human immunodeficiency virus type-1 (HIV-1) and hepatitis B virus (HBV) (Table 14).

In general, the nucleoside analog GS-441524 exhibited 6- to 100-fold reduced antiviral activity compared with RDV (m2.6.3, Section 3.1, PC-399-2014) {Lo 2017}.

Table 14. In Vitro Activity of Remdesivir Against RNA and DNA Viruses

Virus Family	Virus	Strain	Assay Type	Remdesivir EC_{50}/EC_{90} (µM)
Paramyxovirus	NiV	wt MY 1999	VTR	0.047/0.083
		wt BD 2004	VTR	0.032/0.106
	HeV	wt HeV 1996	VTR	0.055/0.117
	RSV	A2-GFP	REP	0.021/0.059
	hPIV3	JS-GFP	REP	0.018/0.35
	MeV	EZ-GFP	REP	0.037/0.073
	MuV	wt IA 2006	AG	0.79/3.4
Arenavirus	LASV	wt Josiah	AG	4.5/5.1
	JUNV	Romero	AG	0.47/1.26
Bunyavirus	RVFV	ZH501-GFP	REP	No inhibition
	CCHFV	rec wt IbAr 10200	AG	No inhibition
	ANDV	Chile 9717869	AG	7.0/10.1
Rhabdovirus	VSV	New Jersey	CPE	No inhibition
Togavirus	CHIV	AF 15561	AG	> 20
	VEEV	SH3	AG	> 20
Flavivirus	AHFV	200300001	CPE	4.2/17.6
	KFDV	P9605	CPE	1.8/3.4
	TBEV	Hypr	CPE	2.1/3.5
	OHFV	Bogoluvovska	CPE	1.2/3.9
	HCV-1b	ND	REP	0.097
	HCV-2a	ND	REP	0.084
Lentivirus	HIV-1	IIIb	CPE	> 10
Hepadnavirus	HBV	ND	VTR	3.9[a]
Picornavirus	HRV-10	ND	CPE	2.5

AG = antigen reduction assay; AHFV = Alkhurma hemorrhagic fever virus; ANDV = Andes virus; CC_{50} = 50% cytotoxic concentration; CCHFV = Crimean Congo hemorrhagic fever virus; CHIV = Chikungunya virus; CPE = cytopathic effect assay; EC_{50} = 50% effective concentration; EC_{90} = 90% effective concentration; HBV = hepatitis B virus; HCV 1b = hepatitis C virus genotype 1b; HCV 2a = hepatitis C virus genotype 2a; HeV = Hendra virus; HIV-1 = human immunodeficiency virus type-1; hPIV3 = human parainfluenza virus type 3; HRV-10 = human rhinovirus serotype-10; JUNV = Junin virus; KFDV = Kyasanur Forest disease virus; LASV = Lassa fever virus; MeV = measles virus; MuV = mumps virus; ND = not determined; OHFV = Omsk hemorrhagic fever virus; REP = reporter assay; RSV = respiratory syncytial virus; RVFV = Rift Valley fever virus; TBEV = Tick-borne encephalitis virus; VEEV = Venezuelan equine encephalitis virus; VSV = vesicular stomatitis virus; VTR = virus titer reduction; wt = wild type

a Nonselective inhibition of HBV is a result of cytotoxic effect (CC_{50} = 7.4 µM).

Source: m2.6.3, Section 3.1, PC-399-2008 and PC-399-2014; {Lo 2017, Warren 2016}

4.2. In Vitro Cytotoxicity

4.2.1. Cytotoxicity in Human Cell Lines

The cytotoxicity of RDV and the nucleoside analog GS-441524 was tested in several immortalized human cell lines, including laryngeal, hepatoma, prostate, and lymphoblastoid transformed cell lines (Table 15; m2.6.3, Section 3.1, PC-399-2013). The CC_{50} values of RDV ranged from 1.7 to 8.9 µM, yielding a selectivity index of ~170 to 890 for all cell lines tested based upon the antiviral EC_{50} value of 0.0099 µM against SARS-CoV-2 in HAE cells (Table 2).

GS-441524 showed no cytotoxicity up to 100 µM in the tested cell lines, with the exception of the MT-4 T cell line, a model with the shortest doubling time among all cell types tested; MT-4 T cells were also the most sensitive cell type to the cytotoxicity of RDV. Control compound puromycin showed cytotoxic effects consistent with historical data, with CC_{50} values of 0.12 to 0.73 µM. MT-4 T cells were also the most sensitive cell type to the cytotoxicity of puromycin; the CC_{50} value for puromycin is approximately 10-fold lower than for RDV.

Table 15. In Vitro Cytotoxicity of Remdesivir and GS-441524 in Human Cell Lines

	CC_{50} (µM)[a]			
	HEp-2	HepG2	PC-3	MT-4
Compound	Laryngeal Carcinoma Cells	Hepatoma Cells	Prostate Cancer Cells	Lymphoblast T Cells
RDV	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

CC_{50} = 50% cytotoxic concentration; RDV = remdesivir; SD = standard deviation
a All CC_{50} values represent the mean ± SD of at least two independent experiments.
Source: m2.6.3, Section 3.1, PC-399-2013

4.2.2. Cytotoxicity in Primary Human Cells

The cytotoxicity of RDV and the nucleoside analog GS-441524 was tested against primary human hepatocytes, renal proximal tubule cells (RPTECs), and quiescent as well as stimulated peripheral blood mononuclear cells (PBMCs) (Table 16; m2.6.3, Section 3.1, PC-399-2013). The CC_{50} values of RDV ranged from 2.5 to > 20 µM with primary hepatocytes being the most sensitive cell type. These data yielded a selectivity index of ~250 to > 2000 for the primary cell types tested based upon the antiviral EC_{50} value of 0.0099 µM against SARS-CoV-2 in HAE cells (Table 2).

GS-441524 showed no cytotoxicity up to the highest concentration tested (100 µM) in all primary cells (Table 16).

Table 16. In Vitro Cytotoxicity of Remdesivir and GS-441524 in Primary Human Cells

Compound	CC_{50} (µM)[a]			
	Primary Hepatocytes	RPTECs	Quiescent PBMCs	Stimulated PBMCs
RDV	2.5 ± 0.6	12.9 ± 6.2	> 20	14.8 ± 5.8
GS-441524	> 100	> 100	> 100	> 100

CC_{50} = 50% cytotoxic concentration; PBMC = peripheral blood mononuclear cell; RDV = remdesivir; RPTEC = renal proximal tubule cell; SD = standard deviation
a All CC_{50} values represent the mean ± SD of at least two independent experiments.
Source: m2.6.3, Section 3.1, PC-399-2013

4.2.3. In Vitro Effect on Human Hematopoietic Progenitor Cells

Remdesivir, GS-441524, and control compound 5-fluorouracil (5-FU) were tested in vitro for their effects on the proliferation of erythroid, myeloid, and megakaryoid progenitors from three independent donors (m2.6.3, Section 3.1, PC-399-2018). The study was performed at [redacted], Canada). Following 11- to 14-day incubation, RDV exhibited CC_{50} values ranging from 2.3 to 10.5 µM across the three progenitor cell types from the three tested donors (Table 17). GS-441524 exhibited slightly weaker antiproliferative effects with CC_{50} values of 5.9 to 22.7 µM, and the control 5-FU was the most potent inhibitor of progenitor cell proliferation among the three compounds tested. No major differences in the sensitivity to RDV were observed among the different progenitor cell types and across the three tested donors. The sensitivity of progenitor cells to RDV is consistent with the results from general cytotoxicity screening against other primary human cells (Section 4.2.2), indicating that the human hematopoietic progenitors are not selectively sensitive to RDV.

Table 17. In Vitro Effect of Remdesivir and GS-441524 on Human Hematopoietic Progenitor Cells

Compound	Mean CC_{50} (Range), [µM][a]		
	Erythroid Progenitors	Myeloid Progenitors	Megakaryoid Progenitors
RDV	8.5 (6.7 – 10.5)	5.1 (3.4 – 7.7)	4.9 (2.3 – 7.2)
GS-441524	13.9 (12.4 – 15.0)	11.7 (5.9 – 22.7)	9.6 (7.2 – 12.0)
5-Fluorouracil	3.2 (1.7 – 4.8)	2.2 (1.6 – 2.8)	2.3 (0.7 – 4.1)

CC_{50} = 50% cytotoxic concentration; RDV = remdesivir
a The CC_{50} values represent a mean and a range from testing of human progenitor cells isolated from three independent donors.
Source: m2.6.3, Section 3.1, PC-399-2018

4.2.4. Effect on Production of Reactive Oxygen Species

Excessive production of reactive oxygen species (ROS) can be induced by drugs and may result in cellular damage and cell death {Shuhendler 2014}. The potential effect of RDV on ROS generation in HepG2 cells was assessed (m2.6.3, Section 3.1, PC-399-2050). After a 24-hour incubation, RDV-treated cells showed no detectable increase in ROS levels at concentrations up to 12.5 µM, and a 60% increase at 100 µM (P = 0.042 compared with DMSO control), the highest concentration tested. In comparison, the control compound menadione showed > 3-fold increase in ROS levels after a 30-minute incubation.

4.2.5. Effect on Renal Organic Anion Transporters

The active transport of RDV and its two major systemic metabolites, GS-441524 (parent nucleoside) and GS-704277 (nucleotide-alanine conjugate), was studied in cells over-expressing human or rat renal organic anion transporter (OAT)1/OAT3 renal transporters to determine their potential for OAT-mediated intracellular uptake and cytotoxicity (m2.6.3, Section 3.1, PC-399-2020).

Rat OAT3 expression increased the cytotoxicity of GS-704277 by 14.6-fold compared with mock-transfected control cells. In addition, intracellular accumulation of the triphosphate metabolite increased > 5-fold in rat OAT3-expressing cells compared with human OAT3-expressing cells when incubated with GS-704277. Consistent with active OAT-dependent GS-704277 uptake, the cytotoxicity and intracellular triphosphate formation were both substantially reduced upon simultaneous treatment with GS-704277 and the OAT inhibitor probenecid. In contrast, GS-704277 showed only marginal increases (< 2-fold) in its cytotoxicity in cells expressing rat OAT1, human OAT1, or human OAT3 transporters. Importantly, the intracellular accumulation of para-aminohippuric acid, a control OAT substrate, was similar in human and rat OAT-expressing cells, indicating bioequivalent transporter expression. The expression of neither rat nor human OATs significantly changed the cytotoxicity or intracellular triphosphate accumulation upon incubation with RDV or GS-441524 compared with mock-transfected control cells. These data indicate that GS-704277, but not RDV or GS-441524, is an effective substrate of rat OAT3 and exhibits rat OAT3-dependent cytotoxicity.

The control compound tenofovir displayed increased cytotoxicity in human and rat OAT-expressing cells in agreement with previous reports {Bam 2014}.

4.3. In Vitro Mitochondrial Toxicity

4.3.1. Cytotoxicity Under Aerobic Metabolic Conditions

Some nucleoside analogs have the potential to affect mitochondrial functions via diverse mechanisms. One of the generic approaches to assess effects of compounds of interest on mitochondrial functions is a comparison of their effect on cell viability in the presence of glucose-favoring glycolysis (ie, anaerobic metabolism) and galactose-favoring oxidative phosphorylation (ie, aerobic metabolism). The latter condition may sensitize cells to compounds affecting mitochondrial functions {Marroquin 2007}.

Using intracellular ATP quantification, the effects of glucose and galactose as a source of energy on the cytotoxicity of RDV and GS-441524 were assessed in the HepG2 hepatoma cell line that has previously been identified as a suitable model for testing compounds under aerobic conditions (m2.6.3, Section 3.1, PC-399-2013) {Marroquin 2007}. In addition, the same conditions were tested in the PC-3 prostate-derived cell line as a model of quickly proliferating cells. The CC_{50} values of RDV in HepG2 cells were 3.7-and 11.1 µM in the presence of glucose and galactose, respectively (Table 18), indicating that aerobic conditions do not enhance the cytotoxicity of RDV in this in vitro model. In comparison, PC-3 cells were more sensitive to RDV when cultured in the presence of galactose compared with glucose (CC_{50} = 1.4 vs 8.9 µM). The divergent results from HepG2 and PC-3 cells suggest the observed effects are cell line-dependent.

GS-441524 did not show any cytotoxicity in either PC-3 or HepG2 cells at the highest concentrations tested (100 µM), irrespective of the metabolic conditions (Table 18). Puromycin, used as general cytotoxic control, exhibited similar cytotoxicity in both cell types in the presence of glucose or galactose, matching historical data from these assays.

Table 18. In Vitro Cytotoxicity of Remdesivir and GS-441524 under Anaerobic and Aerobic Metabolic Conditions

	CC_{50} (µM)[a]			
	HepG2 Cells		PC-3 Cells	
Compound	Anaerobic Conditions (Glucose)	Aerobic Conditions (Galactose)	Anaerobic Conditions (Glucose)	Aerobic Conditions (Galactose)
RDV	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

CC_{50} = 50% cytotoxic concentration; RDV = remdesivir; SD = standard deviation
a All CC_{50} values represent the mean ± SD of at least two independent experiments.
Source: m2.6.3, Section 3.1, PC-399-2013

4.3.2. Effect on Mitochondrial DNA

The in vitro potential of RDV to affect mitochondrial DNA (mtDNA) was assessed by quantitative real-time polymerase chain reaction (PCR) analysis following continual treatment of HepG2 cells for 10 days (m2.6.3, Section 3.1, PC-399-2015). Dideoxycytidine (ddC), a known inhibitor of mtDNA replication, was used as a positive control. Treatment with 0.2 to 20 µM ddC resulted in a significant dose-dependent decrease in cellular mtDNA content relative to dimethylsulfoxide (DMSO) control (Table 19). In comparison, HepG2 cells treated with RDV up to 1.0 µM for 10 days showed no significant changes in their mtDNA content. Treatment with 2.0 µM RDV resulted in a 26% decrease in mtDNA content relative to DMSO. General cytotoxicity, but no apparent reduction in mtDNA, was detected at 10 µM RDV, the highest concentration tested. No changes in mtDNA levels were observed in cells treated with up to 10 µM of the nucleoside analog GS-441524. A minor reduction of 17% was detected at 100 µM GS-441524 (data not shown; m2.6.3, Section 3.1, PC-399-2015).

In addition, the active triphosphate metabolite of RDV GS-443902 did not inhibit the activity of mitochondrial RNA or DNA polymerase γ at concentrations up to 200 µM (Section 4.4; m2.6.3, Section 3.1, PC-399-2017). Together, these data suggest an overall low potential of RDV and the nucleoside analog GS-441524 to significantly affect mtDNA levels at therapeutically relevant drug concentrations.

Table 19. In Vitro Effects of Remdesivir on mtDNA Levels in HepG2 Cells

Exp.	Compound	Concentration (µM)	Relative Amount of mtDNA (% Control)[a]	*p*-value Compared to Control[b]
1	DMSO (control)	—	100.0 ± 22.2	—
	RDV	0.04	88.9 ± 30.4	0.215
		0.2	98.1 ± 30.1	0.702
		1.0	93.4 ± 27.1	0.436
	ddC	0.2	76.9 ± 12.8	0.005
		2.0	10.0 ± 3.8	< 0.001
		20.0	0.7 ± 0.2	< 0.001
2	DMSO (control)	—	100.0 ± 20.3	—
	RDV	0.4	122.8 ± 23.9	0.067
		2.0	74.2 ± 11.7	0.008
		10.0	148.0 ± 25.6	0.001
	ddC	0.2	94.7 ± 3.4	0.005
		2.0	56.5 ± 10.5	< 0.001
		20.0	12.2 ± 2.8	< 0.001

DMSO = dimethyl sulfoxide; ddC = dideoxycytidine; mtDNA = mitochondrial DNA; RDV = remdesivir
The data represent the mean ± SD of three independent experiments performed in triplicate.
Paired, two-tailed Student's t-test
Source: m2.6.3, Section 3.1, PC-399-2015

4.3.3. Effect on Mitochondrial Proteosynthesis

The effect of RDV and GS-441524 on mitochondrial protein synthesis was assessed following a 5-day incubation with the human cell line PC-3 (m2.6.3, Section 3.1, PC-399-2016). This particular cell model was chosen because of a high rate of cell division and protein synthesis. The selective effect of tested compounds on mitochondrial protein synthesis was determined by parallel quantification of the level of cytochrome oxidase subunit 1 (COX-1; encoded by mtDNA) and succinate dehydrogenase A (SDH-A; encoded by nuclear DNA). Remdesivir affected the levels of COX-1 and SDH-A to a similar extent, with CC_{50} values of 8.9 and 8.6 µM, respectively (Table 20). These effects manifested in the same range of concentrations as the cytotoxicity measured by cellular ATP levels, indicating a lack of any selective effect of RDV on mitochondrial proteosynthesis. GS-441524 showed no effect on proteosynthesis up to the highest concentration tested (100 µM); chloramphenicol was used as a positive control and its specific effect on mitochondrial proteosynthesis was consistent with historical data.

Table 20. In Vitro Effect of Remdesivir and GS-441524 on Mitochondrial Proteosynthesis

Compounds	Mitochondrial and Cellular Protein Synthesis 5-day CC_{50} (µM)[a]		
	COX-1	SDH-A	ATP
RDV	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

ATP = adenosine triphosphate; CC_{50} = 50% cytotoxic concentration; COX-1 = cytochrome oxidase subunit 1; RVD = remdesivir; SD = standard deviation; SDH-A = succinate dehydrogenase A
a CC_{50} values were reported as average of two or more independent experiments ± SD.
Source: m2.6.3, Section 3.1, PC-399-2016

4.3.4. Effect on Mitochondrial Respiration

Remdesivir and GS-441524 were further evaluated for their effects on mitochondrial respiration in multiple human cell types including PC-3 (prostate cancer) cells, HepG2 cells, and primary RPTECs by measuring the rate of oxygen consumption using a Seahorse Extracellular Flux Analyzer (m2.6.3, Section 3.1, PC-399-2016). The effect of RDV on spare mitochondrial respiration was dependent on cell type (Table 21). Whereas RDV inhibited mitochondrial spare respiration with a lower CC_{50} value (CC_{50} = 2.5 µM) than those observed for the inhibition of DNA and ATP levels (CC_{50} = 12.5 and 24.0 µM, respectively) in PC-3 cells, this differential effect on the spare respiration was only marginal in RPTECs and completely absent in HepG2 cells. GS-441524 showed no effect on mitochondrial protein synthesis or respiration at the highest concentration tested (100 µM).

Together, these data suggest that PC-3 cells might be uniquely sensitive to the effect of RDV on spare cellular respiration relative to either liver or kidney cells. This may be due to the fast cellular growth and vigorous metabolism in PC-3 cells.

Table 21. Effect of Remdesivir and GS-441524 on Mitochondrial Respiration

Cell Model	CC_{50} (µM)[a]					
	RDV			GS-441524		
	Spare Respiration	Total DNA Content	Cellular ATP	Spare Respiration	Total DNA Content	Cellular ATP
PC-3 Cells	2.5 ± 0.1	12.5 ± 0.7	24.0 ± 1.4	> 100	> 100	> 100
HepG2 Cells	10.6 ± 0.1	6.3 ± 0.9	7.9 ± 0.1	> 100	> 100	> 100
RPTECs	7.3 ± 2.7	14.3 ± 3.3	16.9 ± 4.1	> 100	> 100	> 100

CC_{50} = 50% cytotoxic concentration; RDV = remdesivir; SD = standard deviation
a CC_{50} values represent mean ± SD from at least two independent experiments following a 3-day incubation with the tested compounds.
Source: m2.6.3, Section 3.1, PC-399-2016

To assess the risk of mitochondrial toxicity in liver, RDV and its major systemic metabolites, GS-704277 and GS-441524, were studied for their effect on mitochondrial spare respiration in primary human hepatocytes (PHH) after a 4-hour or 3-day incubation (m2.6.3, Section 3.1, PC-399-2028). Remdesivir did not show any effect on total DNA level after a 4-hour treatment at the highest concentration tested (30 µM). Only mild (21% to 27%) changes in mitochondrial spare respiration and 17% to 31% changes in cellular ATP levels were detected in PHH after 4 hours treatment with 15 to 30 µM RDV, at concentrations that exceed the systemic levels of RDV observed in humans. After a 3-day treatment with RDV, PHH showed decreases in mitochondrial spare respiration, cellular ATP levels, and total DNA levels, with CC_{50} values of 7.6, 7.8, and 13.4 µM, respectively, suggesting that RDV-related cellular toxicity was not specific to mitochondria. Neither GS-704277 nor GS-441524, the 2 major systemic metabolites of RDV, exhibited any effects on mitochondrial spare respiration, cellular ATP level, or total DNA level at the highest concentration tested (100 µM) after either a 4-hour or a 3-day treatment. In conclusion, cellular toxicity of RDV in PHH was not observed after a 4-hour treatment at the highest concentration tested (30 µM), and the toxicity observed after a 3-day treatment was not specific to mitochondria. In addition, neither GS-704277 nor GS-441524 exhibited any degree of toxicity in PHH at concentrations substantially exceeding observed systemic levels in humans.

In conclusion, the anticipated potential of RDV to adversely affect mitochondrial function is low based on the transient exposure to RDV levels that occurs only during administration.

4.4. Interaction with Host RNA and DNA Polymerases

The active triphosphate metabolite of RDV GS-443902 has been tested in multiple in vitro biochemical assays to assess its interaction with important host DNA and RNA polymerases (m2.6.3, Section 3.1, PC-399-2017). The enzymatic activities of human DNA polymerases α and β, as well as that of RNA polymerase II, were unaffected by GS-443902 up to 200 µM, the highest concentration tested (Table 22). GS-443902 is a potent inhibitor of MERS-CoV RNA polymerase with an IC_{50} value of 0.032 µM{Gordon 2020}, suggesting a potential inhibitory selectivity of > 6000-fold for the target viral RNA polymerase over the host DNA and RNA polymerases.

Table 22. Inhibition of Host DNA and RNA Polymerases by the Active Triphosphate Metabolite GS-443902

	IC_{50} (µM)[a]				
Compound	**DNA Pol α**	**DNA Pol β**	**DNA Pol γ**	**RNA Pol II**	**mt RNA Pol**
GS-443902	> 200	> 200	> 200	> 200	> 200
Positive Control	Aphidicolin 4.7 ± 3.3	3′dTTP 1.9 ± 0.8	3′dTTP 1.2 ± 0.6	α-amanitin 0.0035 ± 0.0015	3′deoxy GTP 4.2 ± 1.4

dGTP = deoxyguanosine triphosphate; dTTP = deoxythymidine triphosphate; IC_{50} = 50% inhibitory concentration; Pol = polymerase; SD = standard deviation
a IC_{50} values represent mean ± SD from at least two independent experiments.
Source: m2.6.3, Section 3.1, PC-399-2017

In addition to direct inhibition of DNA and RNA polymerases, GS-443902 was tested for its incorporation into nucleic acids by host mitochondrial DNA and RNA polymerases using a single nucleotide incorporation assay (m2.6.3, Section 3.1, PC-399-2017). GS-443902 was not incorporated into DNA by mtDNA polymerase γ and was a poor substrate for mtRNA polymerase, with a rate of incorporation equal to 5.8% relative to ATP (Table 23). This result contrasts with the significantly higher incorporation rates observed with the triphosphates of BMS-986094 and balapiravir, two anti-HCV nucleosides associated with clinical toxicity. Together, these data further support the low potential of RDV to be associated with selective mitochondrial toxicity.

Table 23. Relative Rate of Incorporation of the Active Triphosphate Metabolite GS-443902 by Human Mitochondrial DNA and RNA Polymerases

	Rate of Incorporation (% of Natural dNTP or NTP)[a]	
Nucleotide Triphosphate	**DNA Polymerase γ**	**mtRNA Polymerase**
GS-443902	0%	5.8% ± 1.4%
Decitabine-TP	79% ± 8%	ND
BMS-986094-TP	ND	92% ± 33%
Balapiravir/RG1626-TP	ND	112% ± 10%

dNTP = deoxyribonucleotide triphosphate; ND = not determined; NTP = nucleotide triphosphate
a The rate of single nucleotide incorporation was measured in the presence of 500 μM nucleotide analog and expressed as % of the natural NTP incorporation at the same concentration. Data are presented as mean ± SD from at least two independent experiments.
Source: m2.6.3, Section 3.1, PC-399-2017

4.5. Molecular Target Screen of the Diastereomeric Mixture GS-466547 and the Nucleoside Analog GS-441524

The potential of the diastereomeric mixture GS-466547 and the nucleoside analog GS-441524 for off-target activity was evaluated against a panel of up to 87 targets consisting of receptors, ion channels, and transporters (m2.6.3, Section 3.1, PC-399-2002 and PC-399-2001). There were no responses (> 50% inhibition of ligand binding) considered related to either test article at a concentration of 10 μM.

5. SAFETY PHARMACOLOGY

The nonclinical safety profile of RDV was characterized in studies evaluating its potential pharmacologic effects on specific organ systems. Study designs and evaluated parameters were consistent with accepted principles and practices as outlined in International Conference on Harmonisation (ICH) and US FDA guidelines. All pivotal studies were conducted in accordance with US FDA Good Laboratory Practice regulations.

The rat and monkey were selected for in vivo investigations based on the formation of the same metabolites as expected in humans. As no gender-specific pharmacokinetic differences have been observed in rat and monkey toxicity studies (m2.6.6, Section 4), the use of males only in the cardiovascular, respiratory, and CNS safety pharmacology studies was acceptable. Consistent with its intended route of administration in humans, studies were conducted by the IV (slow bolus) injection route of administration. Formulations of the test material were prepared in 12% (w/v) SBECD in Sterile Water for Injection, USP, pH 3.5 ± 0.1 for all in vivo studies.

5.1. Cardiovascular System

5.1.1. In Vitro

5.1.1.1. Effect of Remdesivir on Cloned hERG Potassium Channels Expressed in Human Embryonic Kidney Cells

The in vitro effects of RDV on the hERG channel current (a surrogate for I_{Kr}, the rapidly activating delayed rectifier cardiac potassium current) was assessed at near-physiological temperature (m2.6.3, Section 4.1, PC-399-2006). The concentration that resulted in 20% inhibition (IC_{20}) and IC_{50} values for the inhibitory effect of RDV on hERG potassium current were 7.5 µM and 28.9 µM, respectively.

5.1.1.2. Effect of Remdesivir Metabolites on Cloned hERG Potassium Channels Expressed in CHO-hERG DUO Cells

In non-GLP studies, hERG IC_{50} values were > 30 µM for both GS-441524 and GS-704277 (m2.6.3, Section 4.1, PC-399-2025 and PC-399-2026).

5.1.2. In Vivo

5.1.2.1. Effect of Remdesivir on Telemetry-Instrumented Conscious Cynomolgus Monkeys

Four conscious, telemetered male cynomolgus monkeys were administered a single IV (slow bolus) injection of 0 (vehicle), 1, 3, or 10 mg/kg RDV in a Latin square dosing design (m2.6.3, Section 4.2, PC-399-2005), with a 3-day washout period between each of the dosing days. Cardiovascular parameters were continuously recorded for 2.5 hours prior to dosing and through a minimum of 19 hours after each dose. Cardiovascular parameters evaluated included heart rate, PR, QRS, RR, QT, and QTc intervals, as well as systolic, diastolic and mean arterial

pressures, and body temperature. Other parameters evaluated during the study included viability, clinical observations, food consumption, and body weight. In addition, blood samples were collected from all animals predose and at 6 hours postdose for each dosing interval to evaluate exposure to RDV and metabolite GS-441524.

No RDV-related mortality, morbidity, clinical observations, or effects on body weight, food consumption, or body temperature occurred. No RDV-related effects in echocardiogram (ECG; PR, QT, and QTc intervals and QRS duration) or hemodynamic (heart rate, pulse pressure, and systolic, diastolic, and mean arterial pressures) parameters through 19 hours postdose occurred at any dose level. No qualitative ECG abnormalities were attributed to the administration of RDV. Mean plasma concentrations of RDV approximately 6 hours postdose were 55 ng/mL at 10 mg/kg. Mean plasma concentrations for GS-441524 approximately 6 hours postdose were 13, 39, and 198 ng/mL, at 1, 3, and 10 mg/kg, respectively.

In summary, the no observed effect level (NOEL) for cardiovascular effects in male monkeys after IV administration is 10 mg/kg RDV. Exposures to RDV and GS-441524 at the NOEL were approximately 1180 and 381 ng/mL, respectively, based on Day 1 maximum observed concentration of drug (C_{max}) values in male monkeys administered 10 mg/kg in the 2-week repeat-dose toxicity study (m2.6.7, Section 7.3, TX-399-2004).

5.2. Respiratory System

5.2.1. Effect of Remdesivir on the Respiratory System of Wistar-Han Rats Using Head-Out Plethysmography

Crl:WI(Han) rats (8 males/group) were administered a single IV (slow bolus) injection of 0 (vehicle), 5, 20, or 50 mg/kg RDV (m2.6.3, Section 4.2, PC-399-2004). Assessment of respiratory function was based on analysis of tidal volume (mL), respiration rate (breaths/minute), and minute volume (mL/minute). Plethysmography data were collected continuously for approximately 2.5 hours 1 or 3 days prior to dosing (baseline), on Day 1 postdose from the time each animal was placed into the chamber through approximately 6 hours after dosing, and for 2.5 hours beginning approximately 22.5 hours after dosing (24 hours postdose time point; Day 2). Assessment of overall toxicity was based on mortality and clinical observations.

Remdesivir had no effect on tidal volume or minute volume through 24 hours postdose. Relative to control animals, administration of 20 or 50 mg/kg RDV was associated with an increased respiration rate from 0.75 through 6 hours postdose, although the increases were not clearly dose-dependent at all time points. Respiration rates were increased by up to 26 breaths per minute (21%) in animals administered 20 mg/kg RDV and by up to 33 breaths per minute (27%) in animals administered 50 mg/kg. Changes in respiration rate reached statistical significance from 3 to 3.5 hours and 4.5 to 6 hours postdose at 20 mg/kg, and at 3 and 5 hours postdose at 50 mg/kg. Respiration rates returned to control levels by 24 hours postdose.

In conclusion, the NOEL for respiratory effects in male rats was 5 mg/kg. GS-441524 exposure at the NOEL was approximately 315 ng/mL, based on the Day 1 C_{max} in male rats administered 5 mg/kg in the 2-week repeat-dose toxicity study (m2.6.7, Section 7.1, TX-399-2003).

5.3. Central Nervous System

5.3.1. Effect of Remdesivir on the Central Nervous System of Male Wistar-Han Rats

Crl:WI(Han) rats (8 males/group) were administered a single IV (slow bolus) injection of 0 (vehicle), 5, 20, or 50 mg/kg RDV (m2.6.3, Section 4.2, PC-399-2003). Assessment of potential neurological effects was based on observations collected approximately 0.25, 1.75, 3.25, 6, and 24 hours postdose using a modified Irwin battery of neurological assessments, including home cage, hand-held, open-field, and elicited response observations. General measures of toxicity consisting of mortality, clinical observations, and body temperature were also recorded.

No RDV-related effects on neurological function were observed in male rats through 24 hours post-dose. The NOEL for neurological effects for male rats was 50 mg/kg. GS-441524 exposure at the NOEL was approximately 2750 ng/mL, based on the Day 1 C_{max} in male rats administered 50 mg/kg in the 2-week repeat-dose toxicity study (m2.6.7, Section 7.1, TX-399-2003)

5.4. Pharmacologic Profiles of Metabolites, Stereoisomers, and Impurities

Diastereomeric mixture GS-466547 containing RDV and the opposite diastereomer at phosphorous was tested for in vitro antiviral activity against EBOV (Section 4.1).

The nucleoside analog GS-441524 was tested for in vitro antiviral activity against EBOV (Section 4.1.1) and other viruses (Section 4.1.2). In vitro barrier for the emergence of resistance to GS-441524 was tested in cell cultures infected with MHV (Section 3.1.3). In vitro cytotoxicity (Section 4.2) and mitochondrial toxicity (Section 4.3) of GS-441524 was assessed in multiple human cell lines and primary cells.

The active triphosphate metabolite GS-443902 was tested for in vitro inhibition of mitochondrial DNA (Section 4.3.2) as well as host DNA and RNA polymerases (Section 4.4). Mechanism of action studies evaluated the inhibition of SARS-CoV-2, SARS-CoV, MERS-CoV, EBOV, RSV, and NiV RNA-dependent RNA polymerases by GS-443902 (Section 3.1.2).

6. PHARMACODYNAMIC DRUG INTERACTIONS

No studies of pharmacodynamic drug interactions have been conducted.

7. DISCUSSION AND CONCLUSIONS

Remdesivir is a diastereomerically pure monophosphoramidate prodrug of a modified adenine nucleoside analog GS-441524. In multiple cell types relevant for CoV replication, RDV undergoes efficient conversion to the pharmacologically active triphosphate GS-443902 (m2.6.4, Section 6.1.3). The primary mechanism of inhibition is the incorporation of GS-443902 into nascent RNA chains by RdRp, causing delayed RNA chain termination during viral replication. The coronaviruses SARS-CoV-2, SARS-CoV, and MERS-CoV were shown to incorporate GS-443902 more efficiently than ATP, with selectivity values of 0.26, 0.32, and 0.35, respectively, compared with ATP. In contrast, GS-443902 does not inhibit host RNA and DNA polymerases, including mitochondrial polymerases, at concentrations as high as 200 μM.

Remdesivir shows potent in vitro activity against SARS-CoV-2 and multiple genetically diverse CoVs. Remdesivir inhibited the in vitro replication of a clinical isolate of SARS-CoV-2 in primary HAE cells with an average EC_{50} value of 0.0099 μM. Similarly, RDV potently inhibited a recombinant chimeric virus expressing the polymerase (nsp12) gene of SARS-CoV-2 in a backbone of SARS-CoV with a luciferase reporter in Huh7 cells with an EC_{50} of 0.0035 μM. Importantly, RDV also inhibits the human pathogenic CoVs SARS-CoV, and MERS-CoV in multiple human cell types relevant for viral infection with EC_{50} values ranging from 0.0066 to 0.52 μM. In addition, RDV exhibits potent in vitro antiviral activity against filoviruses (eg, EBOV, MARV), and paramyxoviruses (eg, RSV, NiV, and Hendra virus).

In vitro resistance selection experiments using the nucleoside analog of RDV and MHV, a related animal CoV, demonstrated a high barrier to resistance development and identified two mutations in the viral polymerase at residues conserved across CoVs that conferred low-level (5.6-fold) resistance to RDV. The mutant viruses showed reduced viral fitness in vitro, and introduction of the corresponding mutations into SARS-CoV resulted in attenuated SARS-CoV pathogenesis in a mouse model.

Importantly, RDV exhibited in vivo therapeutic efficacy against SARS-CoV-2 infection in rhesus monkeys, and prophylactic and therapeutic efficacy against SARS-CoV and MERS-CoV infection in mice as well as MERS-CoV infection in rhesus monkeys. In SARS-CoV-2-infected rhesus monkeys, administration of a 10/5 mg/kg regimen of RDV once daily via IV bolus injection initiated 12 hours post-inoculation with SARS-CoV-2, resulted in a significant reduction in clinical signs of respiratory disease, lung pathology and gross lung lesions, and viral RNA levels compared with vehicle-treated animals. In mouse models of SARS-CoV and MERS-CoV, administration of 25 mg/kg RDV subcutaneously twice daily beginning 1 day before or 1 day after virus inoculation resulted in significantly reduced lung viral load and improved clinical signs of disease as well as lung function. Remdesivir also showed prophylactic and therapeutic efficacy in MERS-CoV-infected rhesus monkeys. Administration of either 10 mg/kg or 5 mg/kg RDV once daily for 7 days via IV bolus injection beginning 1 day prior to MERS-CoV inoculation, resulted in a significant reduction of clinical scores, clinical signs of respiratory disease, and viral RNA levels compared with vehicle-treated animals. Therapeutic RDV treatment of 5 mg/kg once daily using IV bolus injection initiated 12 hours post-inoculation also resulted in reduced clinical signs, reduced virus replication in the lungs, and decreased presence and severity of lung lesions.

Remdesivir and the nucleoside analog GS-441524 were profiled for in vitro cytotoxicity and mitochondrial toxicity in multiple relevant cell types. Remdesivir exhibited selectivity values > 170 (CC_{50}/EC_{50} against SARS-CoV-2 in HAE cells) in in vitro toxicity assays. Data from in vitro studies with liver cell culture systems demonstrated that human hepatocytes are susceptible to RDV-mediated toxicity, likely due to high cellular permeability and efficient intracellular metabolism. While GS-704277 and GS-441524 are the main in vivo metabolites, and can be readily detected in plasma, these metabolites are unlikely to contribute significantly to changes in liver enzymes observed in humans treated with repeated doses of RDV, likely due to their low permeability and observed systemic exposures, as also evidenced in vitro by their lack of effect observed in hepatocytes.

Molecular target screening studies with GS-441524 and GS-466547 (diastereomeric mixture) showed no significant binding (> 50%) at 10 μM.

In the cardiovascular safety pharmacology study, RDV had no effects on the cardiovascular system of monkeys (highest dose tested: 10 mg/kg IV) that correlated with the weak activity of RDV, GS-441524, and GS-704277 at the hERG channel. In the respiratory safety study in rats, RDV had no effect on tidal volume or minute volume; however, respiration rates were transiently increased in animals administered ≥ 20 mg/kg IV. Remdesivir had no effects on the CNS of rats (highest dose tested: 50 mg/kg IV). Taken together, the risk for respiratory, CNS, or cardiovascular effects in the clinic is considered negligible.

In conclusion, RDV is a novel, small molecule inhibitor of CoV replication, with potent in vitro and in vivo activity against multiple genetically diverse CoVs. Importantly, RDV exhibits potent in vitro and in vivo antiviral activity against SARS-CoV-2. The overall nonclinical pharmacology profile of RDV supports its use as a novel agent for the treatment of COVID-19.

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SECTION 2.6.3—PHARMACOLOGY TABULATED SUMMARY

REMDESIVIR (GS-5734™)

Gilead Sciences

[redacted] **2020**

CONFIDENTIAL AND PROPRIETARY INFORMATION

TABLE OF CONTENTS

1. PHARMACOLOGY OVERVIEW

Test Article: remdesivir

Type of Study/Description	GLP[a]	Test System	Method of Administration	Testing Facility	Gilead Study No. (CRO Study No.)
Primary Pharmacodynamics					
In Vitro Antiviral Activity of Remdesivir against SARS-CoV-2 and SARS-CoV in Primary Human Airway Epithelial (HAE) Cells	No	Primary HAE cell cultures infected with SARS-CoV-2 or SARS-CoV	In vitro	[redacted]	PC-540-2003
In Vitro Antiviral Activity of Remdesivir against SARS-CoV-2 in Vero Cells	No	African green monkey kidney cell line (Vero) cultures infected with SARS-CoV-2	In vitro	[redacted], China	PC-540-2001
In Vitro Antiviral Activity of Remdesivir against SARS-CoV and SARS-CoV-2 in Huh7 Cells	No	Human transformed hepatocyte cell line (Huh-7) cultures infected with SARS-CoV-2 or SARS-CoV	In vitro	[redacted]	PC-540-2002
In Vitro Antiviral Activity of Remdesivir against Ebola Virus and Other RNA Viruses (USAMRIID Panel)	No	Human cervical carcinoma cells (HeLa), human foreskin fibroblasts (HFF-1), human macrophage cultures (HMP), monkey kidney epithelial cells cultures infected with Ebola virus or other RNA viruses	In vitro	[redacted]	PC-399-2008
In Vitro Antiviral Activity of Remdesivir and GS-466547 against Coronaviruses	No	Vero E6, HAE cell cultures	In vitro	[redacted]	PC-399-2019
In Vitro Antiviral Activity of Remdesivir against MERS-CoV and SARS-CoV	No	Calu-3, HAE cell cultures	In vitro	[redacted]	Sci Transl Med. 2017:1-20 {Sheahan 2017}

Test Article: remdesivir

Type of Study/Description	GLP[a]	Test System	Method of Administration	Testing Facility	Gilead Study No. (CRO Study No.)
In Vitro Antiviral Activity of Remdesivir against Murine Hepatitis Virus (MHV), MERS-CoV and SARS-CoV and Viral Resistance	No	Delayed brain tumor (DBT), HAE cultures	In vitro	[redacted] University of North Carolina (UNC) Chapel Hill, NC	mBio. 2018;9:e00221-00218 {Agostini 2018}
In Vitro Antiviral Activity of Remdesivir against Human CoVs and Porcine Deltacoronavirus	No	Huh7, porcine kidney (LLC-PK1) cell cultures	In vitro	[redacted]	Antiviral Res. 2019;169:104541 {Brown 2019}
In Vitro Activity of Remdesivir against SARS-CoV-2 RNA-dependent RNA polymerase	No	In vitro biochemical enzyme activity assays	In vitro	[redacted], Canada	PC-540-2005
In Vitro Activity of Remdesivir against MERS-CoV RNA-dependent RNA polymerase	No	In vitro biochemical enzyme activity assays	In vitro	[redacted], Canada	J Biol Chem. 2020 {Gordon 2020}
Efficacy of Remdesivir Treatment in the Rhesus Macaque Model of SARS-CoV-2	No	SARS-CoV-2-infected rhesus macaques	Intravenous (IV)	[redacted]	PC-540-2004
Prophylactic and Therapeutic Efficacy of Remdesivir in a Mouse Model of SARS-CoV Infection	No	SARS-CoV-infected Ces1c$^{-/-}$ mice	Subcutaneous (SC)	[redacted]	Sci Transl Med. 2017:1-20 {Sheahan 2017}
Prophylactic and Therapeutic Efficacy of Remdesivir in a Mouse Model of MERS-CoV Infection	No	MERS-CoV-infected Ces1c$^{-/-}$ hDPP4 mice	SC	[redacted]	Nat Commun. 2020;11:222 {Sheahan 2020}
Prophylactic and Therapeutic Efficacy of 5 mg/kg Remdesivir (GS-5734) Treatment in the Rhesus Macaque Model of MERS-CoV Infection	No	MERS-CoV-infected rhesus macaques	IV	[redacted]	Proc Natl Acad Sci USA. 2020;117:6771-6776 {de Wit 2020}

Test Article: remdesivir

Type of Study/Description	GLP[a]	Test System	Method of Administration	Testing Facility	Gilead Study No. (CRO Study No.)
Prophylactic Efficacy of 10 mg/kg Remdesivir (GS-5734) Treatment in the Rhesus Macaque Model of MERS-CoV Infection	No	MERS-CoV-infected rhesus macaques	IV	[redacted]	PC-399-2038
Secondary Pharmacodynamics					
In Vitro Antiviral Activity of Remdesivir against Ebola Virus in Huh-7 and HMVEC Cells (CDC Panel)	No	Human microvascular endothelial cells immortalized with the telomerase catalytic protein (HMVEC-TERT), Huh-7	In vitro	[redacted]	PC-399-2007
In Vitro Antiviral Activity of Remdesivir against Ebola Virus and Other RNA Viruses (USAMRIID Panel)	No	HeLa, HFF-1, HMP, monkey kidney epithelial cell cultures infected with Ebola virus or other RNA viruses	In vitro	[redacted]	PC-399-2008
In Vitro Antiviral Activity of GS-5734 against Filoviruses	No	HeLa cell cultures infected with filoviruses	In vitro	[redacted]	PC-399-2044
In Vitro Antiviral Activity of Remdesivir against Nipah Virus and Other RNA Viruses	No	HeLa cell cultures infected with RNA viruses	In vitro	[redacted]	Sci Rep. 2017;7:1-7 {Lo 2017}
Antiviral Activity of GS-441524 and Remdesivir against a Panel of Viruses Unrelated to Ebola Virus	No	HEp-2, Huh-7, lymphoblastoid T-cells (MT-4), HeLa, hepatoma cells (HepG2) infected with RNA and DNA viruses	In vitro	Gilead Sciences, Foster City, CA	PC-399-2014

Test Article: remdesivir

Type of Study/Description	GLP[a]	Test System	Method of Administration	Testing Facility	Gilead Study No. (CRO Study No.)
Cytotoxicity of Remdesivir in Human Cell Lines and Primary Cells	No	HEp-2, prostate cancer cells (PC-3), MT-4, primary human hepatocytes (PHH), renal proximal tubule epithelial cells (RPTEC), and quiescent as well as stimulated peripheral blood mononuclear cells (PBMC)	In vitro	Gilead Sciences, Foster City, CA	PC-399-2013
In Vitro Effect of Remdesivir on Human Hematopoietic Precursor Cells	No	Cultures of primary human myeloid, erythroid, and megakaryoid hematopoietic progenitor cells	In vitro	[redacted], Canada	PC-399-2018
Effect of Remdesivir on Levels of Reactive Oxygen Species	No	HepG2	In vitro	Gilead Sciences, Foster City, CA	PC-399-2050
In Vitro Evaluation of Remdesivir and Metabolites on Renal Organic Anion Transporters (OATs)	No	HEK	In vitro	Gilead Sciences, Foster City, CA	PC-399-2020
In Vitro Evaluation of Remdesivir Effects on Mitochondrial DNA Content	No	HepG2	In vitro	Gilead Sciences, Foster City, CA	PC-399-2015
Effect of Remdesivir on Mitochondrial Protein Synthesis and Respiration	No	PC-3, HepG2, RPTEC	In vitro	Gilead Sciences, Foster City, CA	PC-399-2016
Effect of Remdesivir on Mitochondrial Respiration	No	PHH	In vitro	Gilead Sciences, Foster City, CA	PC-399-2028
Interaction of GS-443902, the Active Nucleoside Triphosphate Metabolite of Remdesivir, with Host RNA and DNA Polymerase Enzymes	No	In vitro biochemical enzyme activity assays	In vitro	Gilead Sciences, Foster City, CA	PC-399-2017
Molecular Target Screen of GS-441524	No	Human, rabbit, rat, hamster, guinea pig, pig, and mouse receptors	In vitro	[redacted], Taiwan,	PC-399-2001 [redacted]
Molecular Target Screen of GS-466547	No	Human, rabbit, rat, hamster, guinea pig, pig, and mouse receptors	In vitro	[redacted], Taiwan,	PC-399-2002 [redacted]
Safety Pharmacology					
Effect of Remdesivir on the hERG Channel	Yes	Human embryonic kidney (HEK293) cell line	In vitro	[redacted], USA	PC-399-2006 [redacted]

Test Article: remdesivir

Type of Study/Description	GLP[a]	Test System	Method of Administration	Testing Facility	Gilead Study No. (CRO Study No.)
Effect of GS-441524 on the hERG Channel	No	CHO-hERG DUO cells	In vitro	Gilead Sciences, Foster City, CA	PC-399-2025
Effect of GS-704277 on the hERG Channel	No	CHO-hERG DUO cells	In vitro	Gilead Sciences, Foster City, CA	PC-399-2026
Effect of Remdesivir on the Cardiovascular System of the Monkey	Yes	Monkey/Cynomolgus	IV (slow bolus) injection	[redacted], USA	PC-399-2005 [redacted]
Effect of Remdesivir on the Respiratory System of Rats	Yes	Rat/Crl:WI(Han)	IV (slow bolus) injection	[redacted], USA	PC-399-2004 [redacted]
Effect of Remdesivir on the Central Nervous System of Rats	Yes	Rat/Crl:WI(Han)	IV (slow bolus) injection	[redacted], USA	PC-399-2003 [redacted]
Pharmacodynamic Drug Interactions					
No applicable studies	—	—	—	—	—

a An entry of "Yes" indicates that the study includes a GLP compliance statement.

2. PRIMARY PHARMACODYNAMICS

2.1. In Vitro Studies

Test Article: remdesivir

Organ Systems Evaluated	Species/Strain	Method of Administration	Concentration (µM)	Cell Line / Assay Format	Noteworthy Findings	GLP[a]	Gilead Study Number
In vitro cell-based antiviral assays	Primary human airway epithelial (HAE) cells	In vitro DMSO stock solution diluted in cell culture media	Dose response 4-fold serial dilutions starting at 2 µM	HAE cell culture 12-well format	SARS-CoV-2 EC_{50} = 0.0099 µM SARS-CoV EC_{50} = 0.0066 µM	No	PC-540-2003
In vitro cell-based antiviral assays	Vero cells (African Green monkey kidney epithelial cells) infected with SARS-CoV-2 isolate (C-Tan-nCoV Wuhan strain 01)	In vitro Stock solution diluted in cell culture media	24 hour dose response: 10-fold serial dilutions starting at 100 µM 48 hour dose response: 5-fold serial dilutions starting at 100 µM	Vero cell culture 96-well format	SARS-CoV-2 EC_{50} = 0.137 µM (24 hours), 0.750 (48 hours)	No	PC-540-2001
In vitro cell-based antiviral assays	Human hepatoma (Huh7) cells infected with SARS/SARS-CoV-2 nsp12 nLUC or SARS-CoV nLUC	In vitro Stock solution diluted in cell culture media	Dose response 3-fold serial dilutions starting at 10 µM	Huh7 cell culture 96-well format	SARS/SARS-CoV-2 nsp12 nLUC EC_{50} = 0.0035 µM SARS-CoV nLUC EC_{50} = 0.0071 µM	No	PC-540-2002

Test Article: remdesivir

Organ Systems Evaluated	Species/Strain	Method of Administration	Concentration (µM)	Cell Line / Assay Format	Noteworthy Findings	GLP[a]	Gilead Study Number
In vitro cell-based antiviral assays	Human and African Green monkey cell lines infected with Ebola virus (EBOV), Marburg virus (MARV), Junin virus (JUNV), Lassa fever virus (LASV), Middle East respiratory syndrome (MERS) virus, Chikungunya virus (CHIV), and Venezuelan equine encephalitis virus (VEEV)	In vitro DMSO stock solution diluted in cell culture media	Dose response 3-fold serial dilutions starting at 10 µM	HeLa, osteosarcoma cells (U2OS) and Vero E6 cell lines, HMP cultures 96-well format	MERS EC_{50} = 0.52 µM (Vero E6), EBOV EC_{50} = 0.14 µM (HeLa), 0.086 µM (HMP), MARV EC_{50} = 0.052 µM (HeLa), JUNV EC_{50} = 0.47 µM (HeLa), LASV EC_{50} = 1.65 µM (HeLa), CHIV EC_{50} > 20 µM (U2OS), VEEV EC_{50} > 20 µM (HeLa)	No	PC-399-2008
In vitro cell-based antiviral assays	African green monkey cell lines and human primary cell cultures infected with MERS-CoV, SARS-CoV	In vitro DMSO stock solution diluted in cell culture media	Dose response 3-fold serial dilutions starting at 10 µM	Vero E6, HAE cultures 384-well and 12-well format	MERS-CoV (Vero E6) EC_{50} = 0.52 µM Approximately 4-log reduction in infectious virus and viral RNA at 1 µM	No	PC-399-2019
Biochemical RNA polymerase assay	SARS-CoV SARS-CoV-2 LASV	In vitro	GS-443902 serial dilutions starting at 10 µM	Gel-based assay	Selectivity values for remdesivir triphosphate incorporation with viral polymerases: SARS-CoV = 0.32 SARS-CoV-2 = 0.26 MERS-CoV = 0.35 EBOV = 4.0 RSV = 2.7 LASV = 23	No	PC-540-2005
In vitro cell-based antiviral assays	Human primary cells and cell lines infected with MERS-CoV SARS-CoV	In vitro	Dose response serial dilutions starting at 10 µM	Calu-3, HAE cultures 96-well and 12-well format	MERS-CoV (Calu-3) IC_{50} = 0.025 µM MERS-CoV (HAE) IC_{50} = 0.074 µM SARS-CoV (HAE) IC_{50} = 0.069 µM	No	Sci Transl Med. 2017:1-20 {Sheahan 2017}

Test Article: remdesivir

Organ Systems Evaluated	Species/Strain	Method of Administration	Concentration (µM)	Cell Line / Assay Format	Noteworthy Findings	GLP[a]	Gilead Study Number
In vitro cell-based antiviral assays and viral resistance	Human primary cells and cell lines infected with murine hepatitis virus (MHV), MERS-CoV, and SARS-CoV	In vitro	Dose response serial dilutions starting at 10 µM	DBT, HAE cultures 96-well format	MHV (DBT) EC_{50} = 0.03 µM MERS-CoV (HAE) EC_{50} = 0.074 µM SARS-CoV (HAE) EC_{50} = 0.069 µM 2 mutations in nsp12 polymerase (F476L and V553L) conferred 5.6-fold reduced susceptibility to remdesivir; double mutant MHV outcompeted by wild-type MHV	No	mBio. 2018;9:e00221-00218 {Agostini 2018}
In vitro cell-based antiviral assays	Human and porcine cell lines infected with human CoVs OC43 and 229E and porcine deltacoronavirus	In vitro	Dose response 3-fold serial dilutions starting at 10 µM	LLC-PK1 cell cultures 96-well format	CoV OC43 (Huh7) EC_{50} = 0.15 µM CoV 229E (Huh7) EC_{50} = 0.024 µM deltacoronavirus (Huh7) EC_{50} = 0.02 µM deltacoronavirus (LLC-PK1) EC_{50} not determinable	No	Antiviral Res. 2019;169:104541 {Brown 2019}
Biochemical RNA polymerase assay	MERS-CoV	In vitro	GS-443902 serial dilutions starting at 200 µM	Gel-based assay	MERS-CoV IC_{50} = 0.032 µM Selectivity value for incoporation = 0.35	No	J Biol Chem. 2020 {Gordon 2020}

a An entry of "Yes" indicates that the study includes a GLP compliance statement.

2.2. In Vivo Studies

Test Article: remdesivir

Organ Systems Evaluated	Species / Strain	Method of Administration	Dose (mg/kg)	Gender and N per Group	Noteworthy Findings	GLP[a]	Gilead Study No. (CRO Study No.)
Systemic effect	Rhesus macaques infected with SARS-CoV-2	IV bolus	10 mg/kg once daily Day 0.5, then 5 mg/kg once daily Days 1-6	M/F N = 6 per group 12 total	Therapeutic remdesivir reduced clinical signs and virus replication in respiratory tissues, and decreased presence and severity of lung lesions.	No	PC-540-2004
Systemic effect	Esterase-deficient (*Ces1c*$^{-/-}$) C57BL/6 mice infected with SARS-CoV	Subcutaneous	25 mg/kg twice daily Days −1 to 4 (prophylactic) or Days 1 to 4 (therapeutic)	F N = 4-5 per group N = 10-11 per group (remdesivir) 30 total	Prophylactic remdesivir reduced virus titers in the lung and suppressed symptoms of disease. Therapeutic remdesivir had a similar effect, albeit to a lesser extent than the prophylactic regimen.	No	Sci Transl Med. 2017:1-20 {Sheahan 2017}
Systemic effect	Esterase-deficient (*Ces1c*$^{-/-}$) C57BL/6 mice infected with MERS-CoV	Subcutaneous	25 mg/kg twice daily Days −1 to scheduled termination (Day 2 or 6) (prophylactic) or Days 1 to 5 (therapeutic)	F N = 14-15 per group (prophylactic) N = 13-14 per group (therapeutic) 57 total	Prophylactic remdesivir prevented mortality in mice administered a lethal dose of MERS-CoV. Prophylactic remdesivir reduced virus titers in the lung and suppressed symptoms of disease. Therapeutic remdesivir reduced virus titers in the lung and suppressed symptoms of disease	No	Nat Commun. 2020;11:222 {Sheahan 2020}
Systemic effect	Rhesus macaques infected with MERS-CoV	IV bolus	5 mg/kg once daily Days −1 to 5 (prophylactic) or Days 0.5 to 5 (therapeutic)	M N = 3 per group (6 total across two vehicle groups) N = 6 per group (remdesivir) 18 total	Both prophylactic and therapeutic remdesivir reduced clinical signs and virus replication in respiratory tissues, and decreased presence and severity of lung lesions. Effects were more pronounced in the animals treated prophylactically.	No	Proc Natl Acad Sci USA. 2020;117:6771-6776 {de Wit 2020}

Test Article: remdesivir

Organ Systems Evaluated	Species / Strain	Method of Administration	Dose (mg/kg)	Gender and N per Group	Noteworthy Findings	GLP[a]	Gilead Study No. (CRO Study No.)
Systemic effect	Rhesus macaques infected with MERS-CoV	IV bolus	10 mg/kg once daily Days −1 to 5	M/F N = 6 per group 12 total	Prophylactic remdesivir reduced clinical signs and virus replication in respiratory tissues, and decreased presence and severity of lung lesions.	No	PC-399-2038

IV = intravenous
a An entry of "Yes" indicates that the study includes a GLP compliance statement.

3. SECONDARY PHARMACODYNAMICS

3.1. In Vitro Studies

Test Article: remdesivir

Organ Systems Evaluated	Species/Strain	Method of Administration	Concentration (µM)	Cell Line / Assay Format	Noteworthy Findings	GLP[a]	Gilead Study No. (CRO Study No.)
In vitro cell-based antiviral assays	Human cell lines infected with EBOV-GFP and Huh-7 cells infected with WT EBOV Makona 2014	In vitro DMSO stock solution diluted in cell culture media	Dose response 3-fold serial dilutions starting at 10 µM	HMVEC and Huh-7 96-well format	EBOV GFP EC_{50} = 0.07 µM (Huh-7) EC_{50} = 0.06 µM (HMVEC-TERT) EBOV Makona EC_{50} = 0.01 µM (Huh-7) Virus yield assay	No	PC-399-2007
In vitro cell-based antiviral assays	Human and African Green Monkey cell lines infected with EBOV, MARV, JUNV, LASV, MERS virus, CHIV, and VEEV	In vitro DMSO stock solution diluted in cell culture media	Dose response 3-fold serial dilutions starting at 10 µM	HeLa, osteosarcoma cells (U2OS), and Vero E6 cell lines, HMP cultures 96-well format	EBOV EC_{50} = 0.14 µM (HeLa), 0.086 µM (HMP), MARV EC_{50} = 0.052 µM (HeLa), JUNV EC_{50} = 0.47 µM (HeLa), LASV EC_{50} = 1.65 µM (HeLa), MERS EC_{50} = 0.52 µM (Vero E6), CHIV EC_{50} > 20 µM (U2OS), VEEV EC_{50} > 20 µM (HeLa)	No	PC-399-2008
In vitro cell-based antiviral assays	Human cell lines infected with EBOV, Bundibugyo, Sudan, and MARV	In vitro DMSO stock solution diluted in cell culture media	Dose response 2-fold serial dilutions starting at 10 µM	HeLa 384-well format	EBOV (Kikwit) EC_{50} = 0.12 µM EBOV (Makona) EC_{50} = 0.19 µM Bundibugyo EC_{50} = 0.19 µM Sudan EC_{50} = 0.24 µM MARV EC_{50} = 0.06 µM	No	PC-399-2044

Test Article: remdesivir

Organ Systems Evaluated	Species/Strain	Method of Administration	Concentration (µM)	Cell Line / Assay Format	Noteworthy Findings	GLP[a]	Gilead Study No. (CRO Study No.)
In vitro cell-based antiviral assays	Human cell line infected with Nipah virus (NiV) strains Malaysia/1999 and Bangladesh/2004	In vitro DMSO stock solution diluted in cell culture media	Dose response 3-fold serial dilutions starting at 10 µM	HeLa 96-well format	NiV MY 1999 EC_{50} = 0.047 µM NiV BD 2004 EC_{50} = 0.032 µM HeV EC_{50} = 0.055 µM RSV EC_{50} = 0.021 µM hPIV3 EC_{50} = 0.018 µM MeV EC_{50} = 0.037 µM MuV EC_{50} = 0.79 µM LASV EC_{50} = 4.5 µM RVFV = no inhibition CCHFV = no inhibition ANDV EC_{50} = 7.0 µM VSV = no inhibition AHFV EC_{50} = 4.2 µM KFDV EC_{50} = 1.8 µM TBEV EC_{50} = 2.1 µM OHFV EC_{50} = 1.2 µM	No	Sci Rep. 2017;7:1-7 {Lo 2017}
In vitro cell-based antiviral assays	Human cell lines infected with EBOV, respiratory syncytial virus (RSV), human rhinovirus (HRV), human immunodeficiency virus 1 (HIV-1), hepatitis B virus (HBV), hepatitis C virus (HCV) replicon cell lines	In vitro DMSO stock solution diluted in cell culture media	Dose response 3-fold serial dilutions starting at 10 to 50 µM	HEp-2, HeLa, MT-4, HepG2-NTCP, Huh-7 96-well format	RSV EC_{50} = 0.017 µM (HEp-2), HRV EC_{50} = 2.54 µM (HeLa), HIV-1 EC_{50} > 50 µM (MT-4), HBV EC_{50} = 3.92 µM* (HepG2-NTCP), HCV 1b EC_{50} = 0.097 µM (Huh-7), HCV 2a EC_{50} = 0.084 µM (Huh-7) *effect of non-specific cytotoxicity	No	PC-399-2014
In vitro cell-based cytotoxicity assays	Human primary cells and cell lines	In vitro DMSO stock solution diluted in cell culture media	Dose response 3-fold serial dilutions starting at 20 µM	HEp-2, HepG2, Gal HepG2, PC-3, Gal PC-3, MT-4, PHH, PBMC, PRPTEC 96-well format	HEp-2 CC_{50} = 6.0 µM, HepG2 CC_{50} = 3.7 µM, Gal HepG2 CC_{50} = 11.1 µM, PC-3 CC_{50} = 8.9 µM, Gal PC-3 CC_{50} = 1.4 µM, MT-4 CC_{50} = 1.7 µM, PHH CC_{50} = 2.5 µM, quiescent PBMC CC_{50} >20 µM, stim. PBMC CC_{50} = 14.8 µM, PRPTEC CC_{50} = 12.9 µM	No	PC-399-2013

Test Article: remdesivir

Organ Systems Evaluated	Species/Strain	Method of Administration	Concentration (µM)	Cell Line / Assay Format	Noteworthy Findings	GLP[a]	Gilead Study No. (CRO Study No.)
Human hematopoietic precursor cells	Human primary cells	In vitro DMSO stock solution diluted in cell culture media	Dose response 3-fold serial dilutions starting at 30 µM	Erythroid, myeloid, and megakaryoid cultures Colony formation in methyl cellulose	IC_{50} values for myeloid, erythroid, and megakaryoid progenitor cell colony formation were 8.5, 5.1, and 4.9 µM, respectively	No	PC-399-2018
In vitro cell-based reactive oxygen species (ROS) assay	Human cell lines	In vitro DMSO stock solution diluted in cell culture media	Dose response 2-fold serial dilutions starting at 100 µM	HepG2 96-well format	No increase in ROS up to 12.5 µM; 60% increase in ROS at 100 µM	No	PC-399-2050
In vitro cell-based OAT transporter and cytotoxicity assays	Human cell line transiently over-expressing human or rat OATs	In vitro DMSO (remdesivir, GS-441524) or water (GS-704277) stock solution diluted in cell culture media	Dose response Remdesivir: 3-fold serial dilutions starting at 200 µM GS-704277 and GS-441524: 3-fold serial dilutions starting at 2000 µM	HEK293 cell line 12-well and 96-well format	Remdesivir and GS441524 are not subject to rat or human OAT1 or OAT3-specific cellular uptake. GS-704277 is a substrate of rat OAT3 and exhibits rat OAT3-dependent cytotoxicity (14.6-fold vs mock-transfected control cells), but is not a substrate of rat or human OAT1 or human OAT3	No	PC-399-2020
In vitro cell-based mitochondrial cytotoxicity assays	Human cell lines	In vitro DMSO stock solution diluted in cell culture media	Dose response Remdesivir: 0.04 to 10 µM GS-441524: 1.0 to 100 µM	HepG2 12-well format	Remdesivir: No mitochondrial DNA (mtDNA) depletion at 1.0 and 10 µM; 26% mtDNA reduction at 2.0 µM GS-441524: 17% mtDNA reduction at 100 µM	No	PC-399-2015

Test Article: remdesivir

Organ Systems Evaluated	Species/Strain	Method of Administration	Concentration (µM)	Cell Line / Assay Format	Noteworthy Findings	GLP[a]	Gilead Study No. (CRO Study No.)
In vitro cell-based mitochondrial biogenesis and respiration assay	Human cell lines	In vitro DMSO stock solution diluted in cell culture media	Dose response 3-fold serial dilutions starting at 50 µM	PC-3, HepG2, and RPTEC 96-well format	Mitochondrial protein synthesis CC_{50} = 8.9 to 11.3 µM Spare respiration CC_{50} = 2.5 to 10.6 µM Total DNA CC_{50} = 6.3 to 14.3 µM ATP CC_{50} = 7.9 to 24.0 µM	No	PC-399-2016
In vitro cell-based mitochondrial respiration assay	Human primary cells	In vitro	Dose response Remdesivir: 2-fold serial dilutions starting at 30 µM GS-704277 and GS-441524: 2-fold serial dilutions starting at 100 µM	PHH 96 well format	4-h incubation: Only mild changes 3-day incubation: Spare respiration CC_{50} = 7.6 µM ATP CC_{50} = 7.8 µM Total DNA CC_{50} = 13.4 µM	No	PC-399-2028
Human DNA and RNA polymerase assays	DNA polymerase α, β, and γ; RNA polymerase II; mitochondrial RNA polymerase	In vitro	Dose response 3-fold serial dilutions starting at 200 µM	96 well format	No inhibition (IC_{50} > 200 µM) of DNA and RNA polymerases. No incorporation by DNA γ and 5.8% incorporation by mitochondrial RNA polymerase	No	PC-399-2017
Molecular target screen with GS-441524	Human receptors, transporters and ion channels	In vitro	10 µM	96 well format	No significant interaction at 10 µM	No	PC-399-2001 [redacted]
Molecular target screen with GS-466547	Human receptors, transporters and ion channels	In vitro	10 µM	96 well format	No significant interaction at 10 µM	No	PC-399-2002 [redacted]

GS-466547 is a diastereomeric mixture that contains remdesivir.
a An entry of "Yes" indicates that the study includes a GLP compliance statement.

4. SAFETY PHARMACOLOGY

4.1. In Vitro Studies

Test Article: remdesivir

Organ Systems Evaluated	Species/Strain	Method of Administration	Concentration (µM)[a]	Cells/Concentration	Noteworthy Findings	GLP[b]	Gilead Study No. (CRO Study No.)
Cardiovascular (hERG Inhibition)	Human recombinant hERG	In vitro	1, 3, 10, 30	Human HEK293 cell line	IC_{20} = 7.5 µM IC_{50} = 28.9 µM	Yes	PC-399-2006 [redacted]
Cardiovascular (hERG Inhibition)	Human recombinant hERG	In vitro	30 (GS-441524)	CHO-hERG DUO cells (Chinese Hamster Ovary cells stably expressing human ERG channel)	IC_{50} > 30 µM	No	PC-399-2025
Cardiovascular (hERG Inhibition)	Human recombinant hERG	In vitro	30 (GS-704277)	CHO-hERG DUO cells (Chinese Hamster Ovary cells stably expressing human ERG channel)	IC_{50} > 30 µM	No	PC-399-2026

CRO = clinical research organization; GLP= Good Laboratory Practice; hERG = human ether-a-go-go
a Free concentration
b An entry of "Yes" indicates that the study includes a GLP compliance statement.

4.2. In Vivo Studies

Test Article: remdesivir

Organ Systems Evaluated	Species/Strain	Method of Administration	Dose[a] (mg/kg)	Sex and No. per Group	Noteworthy Findings	GLP[b]	Gilead Study No. (CRO Study No.)
Cardiovascular System	Monkey/Cynomolgus	IV (slow bolus) injection	0[c], 1, 3, 10	4M (Latin Square Design)	None NOEL = 10 mg/kg	Yes	PC-399-2005 [redacted]
Respiratory System	Rat/Crl:WI(Han)	IV (slow bolus) injection	0[c], 5, 20, 50	8M/Group	Respiration rates increased 0.75 to 6 hours postdose in animals administered ≥ 20 mg/kg. No effect on tidal volume or minute volume at any dose level. NOEL = 5 mg/kg	Yes	PC-399-2004 [redacted]
Central Nervous System	Rat/Crl:WI(Han)	IV (slow bolus) injection	0[c], 5, 20, 50	8M/Group	None NOEL = 50 mg/kg	Yes	PC-399-2003 [redacted]

CRO = clinical research organization; GLP= Good Laboratory Practice; IV = intravenous; M = males; NOEL = no observed effect level

a Single dose

b An entry of "Yes" indicates that the study includes a GLP compliance statement.

c Vehicle control article was 12% (w/v) sulfobutylether-β-cyclodextrin in sterile Water for Injection, USP, pH 3.5 ± 0.1.

5. PHARMACODYNAMIC DRUG INTERACTIONS

Not applicable.

6. REFERENCES

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