SECTION 2.4—NONCLINICAL OVERVIEW

REMDESIVIR (GS-5734™)

Gilead Sciences

[redacted] **2020**

CONFIDENTIAL AND PROPRIETARY INFORMATION

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GLOSSARY OF ABBREVIATIONS AND DEFINITION OF TERMS

ADME	absorption, distribution, metabolism, and excretion
ALP	alkaline phosphatase
ALT	alanine aminotransferase
API	active pharmaceutical ingredient
ATP	adenosine triphosphate
AUC	area under the concentration versus time curve
AUC_{tau}	area under the concentration versus time curve over the dosing interval
$AUC_{x\text{-}xx}$	partial area under the concentration versus time curve from time "x" to time "xx"
BCRP	breast cancer resistance protein
BLQ	below the limit of quantitation
BSEP	bile salt export pump
BUN	blood urea nitrogen
CC_{50}	half-maximal cytotoxic concentration
CHMP	Committee for Medicinal Products for Human Use
C_{max}	maximum observed concentration of drug
CNS	central nervous system
CoV	Coronavirus
CYP	cytochrome P450 enzyme
DART	developmental and reproductive toxicity
DILI	Drug-induced liver injury
DNA	deoxyribonucleic acid
DNA pol	DNA polymerase
EBOV	Ebola virus
EC_{50}	half-maximal effective concentration
EMA	European Medicines Agency
EVD	Ebola virus disease
FDA	Food and Drug Administration
GI	gastrointestinal
GLP	Good Laboratory Practice
GMP	Good Manufacturing Practice
HAE	human airway epithelial
hERG	human ether-a-go-go-related gene
HIV	human immunodeficiency virus
HMVEC	human microvascular endothelial cells
IC_{50}	half-maximal inhibitory concentration
ICH	International Council for Harmonisation (of Technical Requirements for Pharmaceuticals for Human Use)
IFNb	interferon beta
IM	intramuscular

IV	Intravenous
LC-MS/MS	liquid chromatography-tandem mass spectrometry
LE	Long Evans
LPV	lopinavir
MARV	Marburg virus
MERS	Middle East respiratory syndrome
MHV	murine hepatitis virus
mRNA	messenger RNA
MRP	multidrug resistance-associated protein
MVD	Marburg viral disease
NA	not applicable
NHBE	normal human bronchial epithelial
NiV	Nipah virus
NOAEL	no observed adverse effect level
NOEL	no observed effect level
NTCP	sodium-taurocholate co-transporting polypeptide
OAT	organic anion transporter
OATP	organic anion transporting polypeptide
OECD	Organization for Economic Cooperation and Development
PBMC	peripheral blood mononuclear cell
PD	pharmacodynamic(s)
P-gp	P-glycoprotein
PK	pharmacokinetic(s)
RDV	remdesivir
RNA	ribonucleic acid
ROS	reactive oxydative species
RSV	Respiratory syncytial virus
RTV	ritonavir
SARS	Severe acute respiratory syndrome
SBECD	sulfobutylether-β-cyclodextrin
SC	subcutaneous
SD	Sprague-Dawley
$t_{1/2}$	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)
TK	Toxicokinetic(s)
UGT1A1	uridine diphosphate glucuronosyltransferase 1A1
ULN	upper limit of normal
USP	United States Pharmacopeia

NOTE TO THE REVIEWER

Remdesivir (RDV; GS-5734™) is a single diastereomer monophosphoramidate prodrug of a nucleoside analog, GS-441524. Early studies relevant to RDV have been conducted with the diastereomeric mixture GS-466547 (approximately 1:1 mixture of GS-5734 and its diastereomer at phosphorus). Based on antiviral activity, as well as in vitro and in vivo pharmacokinetic profiles, a single diastereoisomer (GS-5734) has been selected for further development. In these studies, the isomer GS-5734 performed similarly to the mixture GS-466547, and results generated with both GS-5734 and GS-466547 are presented in this document as they are considered relevant. 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. Descriptions of GS-5734 and its Diastereomers and Metabolites Referenced in the Text

Gilead No.	Description	Conversion Factors
Remdesivir (GS-5734, GS-643134)	Nucleotide prodrug	1 µM = 0.603 µg/mL
GS-466547	Diastereomeric mixture at phosphorus containing GS-5734	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

1. OVERVIEW OF THE NONCLINICAL TESTING STRATEGY

Remdesivir (RDV; GS-5734) is being developed by Gilead Sciences, Inc. (Gilead) and is formulated for intravenous (IV) administration. This document provides an overview of the nonclinical information that is relevant to the assessment of RDV. The overview is structured as a logical summary of the studies in the various disciplines, including primary pharmacodynamics (PD), secondary PD, safety pharmacology, pharmacokinetics (PK), and toxicology. A critical assessment of the completeness and relevance of the nonclinical testing program and the key findings are included. Specific cross-disciplinary topics and proposals for the inclusion of nonclinical items in the product labeling are discussed throughout the text, as appropriate, and summarized at the end of the document.

All nonclinical studies required to support the proposed use of RDV in coronavirus disease 2019 (COVID-19) patients have been completed. These studies included primary and secondary pharmacodynamics studies; complete core safety pharmacology studies; PK evaluation including absorption, distribution, metabolism, excretion, and drug interaction potential; repeat dose toxicity studies in rats and cynomolgus monkeys up to 4-weeks duration; in vitro and in vivo genotoxicity studies; assessment of fertility, early embryonic development, and pre- and postnatal development toxicity in rats; embryo-fetal development in rats and rabbits; and a 2-week toxicity study in monkeys for qualification of impurities. The nonclinical GLP safety program is consistent with the ICH M3(R2) guideline for a product with a proposed dosing regimen of less than 28 days.

The definitive safety pharmacology, toxicology, and toxicokinetic studies reported in this summary for RDV were conducted in accordance with guidelines issued by the International Council for Harmonisation (ICH) and with Good Laboratory Practice (GLP) or other applicable regulations promulgated by international health authorities. Pilot, exploratory, and mechanistic studies were either conducted in full compliance with GLP procedures or were conducted using appropriate protocols and documentation to assure data integrity.

Remdesivir is a nucleotide prodrug that efficiently distributes into cells and is intracellularly metabolized into an analog of adenosine triphosphate, GS-443902, that inhibits viral RNA polymerases. Remdesivir has potent activity against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the causative agent of COVID-19, as well as other coronaviruses (eg, severe acute respiratory syndrome [SARS] coronavirus [CoV], Middle East respiratory syndrome [MERS] CoV). Remdesivir exhibits in vivo therapeutic efficacy against SARS-CoV-2 in rhesus monkeys and prophylactic and therapeutic efficacy against MERS-CoV infection in rhesus monkeys as well as SARS-CoV and MERS-CoV infection in mice. In these animal studies, RDV treatment resulted in a significant reduction in clinical scores, signs of respiratory disease, and viral RNA levels compared to vehicle-treated animals. In addition, RDV has broad spectrum activity against members of the filoviruses (eg, Ebola virus [EBOV] and Marburg virus [MARV]) and paramyxoviruses (eg, respiratory syncytial virus [RSV], Nipah virus [NiV], and Hendra virus).

Tissue distribution studies following IV administration with [^{14}C]RDV showed RDV-derived material present in many tissues, including lungs. Subsequent studies confirmed that following IV administration, PK assessment shows that the rapid decline in plasma levels of RDV is accompanied by a sequential appearance of the GS-704277 and GS-441524 in plasma. Remdesivir also showed broad distribution and efficient activation of RDV to GS-443902 in respiratory tissues of marmosets and African green monkey at levels anticipated to match or exceed those targeted for efficacy. Similarly, high intracellular levels of GS-441524 and its phosphorylated metabolites were also observed in surrogate cells (peripheral blood mononuclear cells [PBMC]) from studies in African green, marmoset, cynomolgus, and rhesus monkeys. A half-life of 22 and > 24 hours was observed for GS-443902 in lung and PBMC, respectively, following IV administration to marmosets. These data support once-daily administration.

Systemic drug exposures including C_{max} and $AUC_{0\text{-}tau}$ values following RDV administration via IV infusion in healthy rhesus monkeys and humans, and in vivo efficacy studies in rhesus monkeys, support the proposed 200/100 mg RDV dose for clinical efficacy.

Remdesivir has a low potential for drug-drug interactions. Using the current FDA regulatory Guidelines, RDV at an initial intravenous dose of 200 mg and subsequent daily intravenous dose of 100 mg, is predicted not to cause drug interactions by induction and not to inhibit CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, UGT1A1, P-gp or BCRP. Inhibitory interactions with CYP3A, OATP1B1 and OATP1B3 are predicted. However, inhibition of these activities would be expected to be weak and transient due to the short clinical half-life (< 1 hour) of RDV.

Absorption, distribution, and metabolism studies support the selection of the Wistar-Han rat and cynomolgus monkey for the assessment of RDV toxicology. Both rat and monkey formed the intermediate metabolite GS-704277 and the nucleoside metabolite GS-441524. GS-441524 is the predominant metabolite observed in all nonclinical studies. Based on a similar in vitro stability profile in plasma and extracts, the monkey more closely mimics the behavior of RDV in humans. While forming the same major metabolites, rats had markedly reduced levels of intact RDV and correspondingly elevated and more persistent exposure to the intermediate metabolite GS-704277 and the nucleoside metabolite GS-441524.

Exposure margins in the single dose safety pharmacology studies were calculated from the C_{max} values in healthy adults following 30-minute IV infusion of RDV 200 mg on Day 1; exposure margins in the repeat-dose toxicology and reproduction studies were calculated from the $AUC_{0\text{-}tau}$ values in healthy adults following 30-minute IV infusion of RDV 100 mg on Day 5. As expected due to high levels of plasma esterase activity, RDV exposures in rodents were generally below the limit of quantitation (BLQ) and margins in rats were calculated for the major circulating metabolites, GS-441524 and GS-704277.

In safety pharmacology studies, RDV had no clinically relevant effect on the central nervous, cardiovascular, or respiratory systems. Following dosing in rats, and cynomolgus and rhesus monkeys for up to 4 weeks, the kidney was identified as the only target organ of toxicity (generally reversible, proximal convoluted tubule epithelial degeneration/regeneration). In all species, clinical chemistry, urinalysis, and/or urinary biomarkers were early predictors of the

observed kidney changes. At higher doses, microscopic kidney changes were generally reversible, and predictable based on changes in several validated urinary biomarkers. Remdesivir and GS-441524 exposures (AUC) at the no observed adverse effect level (NOAELs) are below the predicted steady-state exposure in humans at 200/100 mg.

Remdesivir is nongenotoxic. In the reproductive and development toxicity studies, the only notable finding was a decrease in corpora lutea, a consequent decrease in implantation sites and viable embryos, and lower ovary and uterus/cervix/oviduct weights in the rat fertility study; these changes were observed at a systemically toxic dose. There were no remarkable findings in male rats in the fertility study, no adverse findings in the developmental toxicity studies in rats and rabbits, and no adverse changes in the pre- and postnatal study in rats.

The nonclinical data discussed within this document support the favorable benefit/risk profile for the proposed use of RDV 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.

2. PHARMACOLOGY

2.1. Primary Pharmacodynamics

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). Remdesivir shows potent in vitro activity against SARS-CoV-2 and multiple genetically diverse CoVs. Importantly, RDV inhibits the human pathogenic CoVs SARS-CoV-2, SARS-CoV, and MERS-CoV in human cell types relevant for viral infection and in preclinical animal models.

2.1.1. In Vitro Studies

Remdesivir inhibited the in vitro replication of a clinical isolate of SARS-CoV-2 in primary human airway epithelial (HAE) cells with an average half-maximal effective concentration (EC_{50}) value of 0.0099 µM (m2.6.3, Section 2.1; PC-540-2003). 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 (m2.6.3, Section 2.1; PC-540-2002). 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 (m2.6.2, Section 3.1.1).

Biochemical studies have demonstrated that RDV is a selective inhibitor of viral RNA-dependent RNA polymerases. The nucleoside triphosphate GS-443902 acts as an analog of adenosine triphosphate (ATP) and competes with the natural ATP substrate to become incorporated into nascent RNA chains by the viral RNA-dependent RNA polymerase. This process results in delayed RNA chain termination during replication of the viral RNA. Delayed chain termination has been shown to be the mechanism of action of RDV inhibition of the SARS-CoV-2, SARS-CoV, and MERS-CoV polymerases (m2.6.3, Section 2.1; PC-540-2005) {Gordon 2020}. In contrast, host RNA and DNA polymerases, including mitochondrial polymerases, are not inhibited by GS-443902 at concentrations as high as 200 µM (m2.6.3, Section 3.1; PC-399-2017).

In vitro resistance profiling of RDV using the rodent CoV murine hepatitis virus (MHV) 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) reduced susceptibility to RDV. The mutant viruses showed reduced viral fitness in the absence of RDV in vitro suggesting that RDV-resistance mutations are unlikely to persist in the absence of treatment. Introduction of the corresponding mutations into SARS-CoV resulted in the same in vitro susceptibility changes seen with MHV suggesting that the conserved residues across divergent CoVs reflect conserved functions impaired by RDV, potentially implying common pathways to resistance across CoVs. Furthermore, SARS-CoV containing both mutations was attenuated in its ability to cause disease and replicated less efficiently than wild-type virus in a mouse model of SARS-CoV pathogenesis {Agostini 2018}.

2.1.2. In Vivo Studies

Remdesivir exhibits prophylactic and/or therapeutic efficacy in multiple animal models of coronavirus infection. Remdesivir demonstrated therapeutic efficacy in a study of RDV treatment in rhesus macaques inoculated with a clinical isolate of SARS-CoV-2 (m2.6.3, Section 2.2; PC-540-2004). Remdesivir was administered at 10 mg/kg via IV bolus injection 12 hours post-inoculation followed by additional once daily doses at 5 mg/kg through 6 days post-inoculation. Animals treated with RDV showed significantly reduced clinical signs of SARS-CoV-2 infection and reduced severity of pulmonary infiltrates as assessed by radiography compared to the vehicle control group. Upon scheduled necropsy at 7 days post-inoculation, RDV-treated animals had significantly reduced gross lung lesions and lower viral RNA levels in lung tissue compared to vehicle-treated animals.

Remdesivir also demonstrated prophylactic and therapeutic efficacy in a mouse model of SARS-CoV pathogenesis. Administration of 25 mg/kg RDV subcutaneously (SC) twice daily beginning 1 day before or 1 day after SARS-CoV inoculation through 4 (prophylactic) or 5 (therapeutic) days post-inoculation resulted in significantly reduced lung viral load and improved clinical signs of disease as well as lung function {Sheahan 2017}.

Similarly, in a mouse model of MERS-CoV pathogenesis, both prophylactic and therapeutic administration of 25 mg/kg RDV SC twice daily through 5 days post-inoculation improved pulmonary function and reduced lung viral loads and severe lung pathology. In contrast, prophylactic lopinavir/ritonavir and interferon beta (LPV/RTV-IFNb) slightly reduced viral loads without impacting other disease parameters. Therapeutic LPV/RTV-IFNb improved pulmonary function but did not reduce virus replication or severe lung pathology {Sheahan 2020}.

Remdesivir showed prophylactic and therapeutic efficacy in MERS-CoV-infected rhesus monkeys. Administration of RDV at 10 mg/kg (m2.6.3, Section 2.2; PC-399-2038) or 5 mg/kg {De Wit 2020} 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 to vehicle-treated animals (m2.6.3, Section 2.2, PC-399-2038). Therapeutic RDV treatment of 5 mg/kg once daily using IV bolus injection initiated 12 hours post-inoculation through 5 days post-inoculation also resulted in reduced clinical signs, reduced virus replication in the lungs, and decreased presence and severity of lung lesions {De Wit 2020}.

2.2. Secondary Pharmacodynamics

Remdesivir exhibits broad in vitro antiviral activity against filoviruses, including EBOV and MARV, as well as other RNA viruses such as pathogenic paramyxoviruses (Nipah and Hendra) with EC_{50} values ranging from 0.018 to 0.79 µM (m2.6.2, Section 4.2).

Remdesivir and the nucleoside analog GS-441524 were profiled for in vitro cytotoxicity and mitochondrial toxicity in multiple relevant cell types (m2.6.2, Sections 4.2 and 4.3). Remdesivir exhibited selectivity values > 170 (ie, ratio of half-maximal 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 susceptible to RDV-mediated toxicity, likely due to high cellular permeability and effective intracellular metabolism of the drug. While GS-704277 and GS-441524 are 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, due to their low C_{max} and minimal in vitro effects on hepatocytes. A relatively narrow in vitro cytotoxicity margin (ie, ratio of CC_{50} and clinical C_{max}), for a 200-mg IV infusion, of approximately 1-fold was determined for RDV in primary human hepatocytes following 5-days incubation. It should be noted in this context that systemic clinical exposures to RDV concentrations within the range of the hepatocyte cytotoxicity margin are transient, lasting only for the duration of drug administration by IV infusion due to the fast systemic clearance of RDV.

Molecular target screening studies with GS-441524 and GS-466547 (diastereomeric mixture) showed no significant binding (> 50%) at 10 µM (m2.6.3, Section 3.1; PC-399-2002 and PC-399-2001).

2.3. Safety Pharmacology

Safety pharmacology studies were conducted to examine the potential effects of RDV on the respiratory, CNS, and cardiovascular systems after IV administration (m2.6.3, Section 4.2; PC-399-2004, PC-399-2003, and PC-399-2005, respectively). In a 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 and returned to control levels by 24 hours postdose, resulting in a NOEL for respiratory function in male rats of 5 mg/kg, at exposures approximately 2.2-fold above the GS-441524 C_{max} at the 200 mg clinical dose. Remdesivir had no effect on the CNS of rats and no effect on cardiovascular parameters in monkeys. At the CNS NOEL of 50 mg/kg, exposures in rats were approximately 19-fold above the GS-441524 C_{max} at the 200-mg clinical dose. At the cardiovascular NOEL of 10 mg/kg, exposures in monkeys were approximately 0.3-fold and 2.7-fold for RDV and GS-441524, respectively, compared to the respective C_{max} values at the 200-mg clinical dose. The lack of in vivo cardiovascular effect is consistent with the weak in vitro inhibition of the hERG channel by RDV (IC_{50} 28.9 µM) and GS-441524 and GS-704277 (IC_{50}'s > 30 µM). Taken together, the risk for CNS, respiratory, or cardiovascular effects in the clinic is considered low.

2.4. Summary of Pharmacology

In summary, RDV shows potent in vitro and in vivo activity against SARS-CoV-2 and multiple genetically diverse coronaviruses. Importantly, RDV inhibits the human pathogenic coronaviruses SARS-CoV-2, SARS-CoV, and MERS-CoV in relevant human cell types for viral infection and in animal models. Remdesivir also exhibits broad in vitro antiviral activity against filoviruses, including EBOV and MARV, as well as other RNA viruses such as pathogenic paramyxoviruses (eg, NiV and Hendra).

The primary mechanism of inhibition by RDV is the incorporation of the triphosphate form of RDV into nascent RNA chains by the viral RNA-dependent RNA polymerase, causing delayed RNA chain termination during viral replication. Delayed chain termination has been shown to be the mechanism of action of RDV inhibition of the SARS-CoV-2, SARS-CoV, and MERS-CoV polymerases.

In vitro resistance profiling of RDV using the rodent coronavirus murine hepatitis virus demonstrated a high barrier to resistance development and identified two mutations in the viral polymerase at residues conserved across coronaviruses that conferred low-level (5.6-fold) reduced susceptibility to RDV. The mutant viruses showed reduced viral fitness in vitro and introduction of the analogous mutations into SARS-CoV resulted in attenuated SARS-CoV pathogenesis in a mouse model.

Importantly, RDV exhibits therapeutic efficacy against SARS-CoV-2 in rhesus monkeys, and prophylactic and therapeutic efficacy against MERS-CoV infection in rhesus monkeys as well as SARS-CoV and MERS-CoV infection in mice. In these animal studies, RDV treatment resulted in a significant reduction in clinical scores, signs of respiratory disease, and viral RNA levels compared to vehicle-treated animals.

In safety pharmacology studies, the only finding of potential clinical relevance was a transient increase in respiration rate in rats that was without an effect on tidal volume or minute volume. The risk for CNS, respiratory, or cardiovascular effects in the clinic is considered low.

3. PHARMACOKINETICS

3.1. Brief Overview

Remdesivir was selected for its ability to distribute into cells, including those within tissues, where it is metabolized to form the pharmacologically active nucleoside triphosphate metabolite, GS-443902. In cells and tissues, RDV is converted by hydrolase and phosphoramidase cleavage to the nucleoside analog monophosphate. Further phosphorylation by nucleotide kinases results in formation of GS-443902. Dephosphorylation of nucleotide metabolites results in conversion to the nucleoside analog, GS-441524, that is poorly re-phosphorylated. Efficient formation of the pharmacologically active triphosphate, GS-443902, has also been observed in several human lung cell types in vitro including NHBE and Calu-3 as well as in PBMC, macrophages, monocytes, and human microvascular endothelial cells (HMVEC). Once formed, GS-443902 has been observed to have a half-life in excess of 15 hours in the various cell types mentioned, following incubation with RDV in vitro.

In vitro hepatic stability of RDV in rat, dog, monkey, and human S9 fractions showed that across species, RDV was highly unstable in rat and increasingly more stable in the other species. Remdesivir was metabolized primarily via hydrolysis. In all species tested, RDV hydrolysis was associated predominantly with the formation of GS-704277 and to a lesser degree, formation of GS-441524.

Following IV administration, RDV generates sufficient levels of drug-related material in nonclinical species chosen for the assessment of toxicology. GS-704277 and GS-441524 are the predominant metabolites observed in plasma in rats and monkeys. While exposure to RDV is low in the rat due to high levels of plasma esterase activity, correspondingly high levels of the metabolites GS-704277 and GS-441524 are observed reflecting ester cleavage of the prodrug. Since all target cells relevant for SARS-CoV-2 infection are not fully understood and may not be easily monitored for drug levels, PBMC were initially used as a surrogate to assess intracellular activation following RDV administration.

Remdesivir also showed broad distribution and efficient activation to GS-443902, the pharmacologically active metabolite, in respiratory tissues of marmosets and African green monkeys at levels anticipated to match or exceed those targeted for efficacy. Similarly, high intracellular levels of GS-441524 and its phosphorylated metabolites were also observed in surrogate cells (PBMC) from studies in African green, marmoset, cynomolgus, and rhesus monkeys. A half-life of 22 and > 24 hours was observed for GS-443902 in lung and PBMC, respectively, following IV administration to marmosets. This data supports once-daily administration.

C_{max} and $AUC_{0\text{-}tau}$ values following RDV administration in healthy rhesus monkeys and humans, and in vivo efficacy studies in rhesus monkeys, support the proposed 200/100 mg RDV dose for clinical efficacy.

Tissue distribution following a single IV dose of [^{14}C]RDV to male non-pigmented and pigmented rats showed rapid and wide distribution to most tissues and radioactivity was eliminated from the majority of tissues by 96 hours postdose, with no preference for melanin binding. In rats, tissues showing the highest maximum concentrations included kidney cortex, kidney medulla, liver, arterial wall, nonpigmented skin, cecum, urinary bladder, and esophagus. The distribution of RDV was also determined following IV administration of [^{14}C]RDV to male cynomolgus monkeys. Tissues showing the highest mean radioactivity included gall bladder, kidneys, liver, prostate gland, salivary gland (mandibular), pancreas, and seminal vesicle(s). Moderate levels of radioactivity were also found in lung tissue. At one-week postdose in monkeys, tissues still showed low levels of retained dose, mostly in liver and muscle. Most of the radioactivity in select samples was associated with GS-441524, indicating metabolism of RDV. Renal and biliary excretion of RDV-related material were the major routes of elimination in both rats and monkeys.

Remdesivir has a low potential for drug-drug interactions. While a substrate for cytochrome P450 (CYP) 2C8, 2D6, and 3A4 in vitro, coadministration with inhibitors of these CYP isoforms is unlikely to markedly increase RDV levels as its metabolism is predominantly mediated by hydrolase activity. Remdesivir is a substrate for OATP1B1 and P-gp; however, the impact of these transporters on RDV disposition is likely minimized by the parenteral route of administration. Remdesivir is an inhibitor of CYP3A, and OATP1B1- and OATP1B3-mediated transport in vitro, however its potential to be the perpetrator of clinically significant drug-drug interactions is limited by its transient exposure at clinically relevant concentrations and its rapid clearance. In human microsomes, RDV was a weak inhibitor of CYP1A2, 2B6, 2C8, 2C9, 2C19, and 2D6. The most potent effects of RDV were upon CYP3A activities, with an IC_{50} of 11.0 μM determined with testosterone 6β-hydroxylase activity, and an IC_{50} of 1.6 μM determined with midazolam 1'-hydroxylase. Further analysis showed that there was no evidence for RDV to be a mechanism-based inhibitor of CYP3A. Assessment of RDV, GS-704277 and GS-441524 in human hepatocytes indicated they were unlikely to pose any induction risk.

In summary, RDV administered IV in multiple species exhibits a favorable and consistent PK profile including efficient delivery of high levels of the pharmacologically active nucleoside triphosphate metabolite into tissues and cells relevant for SARS-CoV-2 replication, supporting its consideration as a novel agent for the treatment of COVID-19.

3.2. Analytical Methods

Analyses of RDV and its metabolites in plasma and PBMC were performed during PK studies following single administration in the Wistar Han rat, and in African green, marmoset, cynomolgus, and rhesus monkeys. Single or repeat-dose non- GLP toxicokinetic (TK) studies in the Ces1c$^{-/-}$ mouse, rat, rabbit, cynomolgus, and rhesus monkey used high performance liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS). Methods used to analyze intracellular levels of the pharmacologically active GS-443902 in PBMC and lung tissue in PK/TK studies were also applied to analysis of GS-441524 and its phosphorylated metabolites in PBMC, macrophages, monocytes, human cervical carcinoma cell line, and HMVEC cells incubated in vitro and select respiratory and non-respiratory tissues in marmoset and African green monkeys. These non-GLP methods did not strictly conform to regulatory guidelines but

were evaluated for appropriate selectivity, sensitivity, and linearity, as well as intra-assay accuracy and precision.

Plasma concentrations of RDV and its metabolites GS-704277 and GS-441524 were quantified in toxicology studies using fully validated LC-MS/MS methods (m2.6.5, Section 2).

3.3. Absorption and Pharmacokinetics

Plasma pharmacokinetics following a single dose of RDV have been assessed after IV administration to the Wistar Han rat (m2.6.5, Section 3.2; AD-399-2003) and cynomolgus monkey (m2.6.5, Section 3.3; AD-399-2001, and Section 3.6; AD-399-2033), or intramuscular (IM) injection to rhesus monkeys (m2.6.5, Section 3.1; AD-399-2016). Intracellular PK in PBMC were also assessed following a single IV administration in rhesus monkeys (m2.6.5, Section 3.4; AD-399-2002, Section 3.5; AD-399-2022, and Section 5.7; AD-399-2028). The clearance of RDV exceeded liver blood flow in all species. Disappearance of RDV was followed by transient exposure to the intermediate metabolite GS-704277 and more persistent exposure to the nucleoside metabolite GS-441524. Dose-normalized exposure to RDV and its metabolites were similar in cynomolgus and rhesus monkeys. In rats, RDV had the shortest half-life and relatively high systemic levels of the metabolites GS-704277 and GS-441524. The different PK profile in the rat likely reflects high levels of plasma esterase activity and extracellular prodrug clearance by hydrolysis.

Maximal concentrations (C_{max}) and areas under the concentration-time curves over the dosing interval ($AUC_{0\text{-}tau}$) following RDV administration as a 30-minute IV infusion in healthy rhesus monkeys were compared to respective parameters in humans to support the 200/100 mg RDV dose for clinical efficacy. Administration of repeated doses of 5 mg/kg RDV in rhesus monkeys produced a similar $AUC_{0\text{-}24}$ to repeated doses of 100 mg RDV in humans (1430 ng•hr/mL [m2.6.5, Section 5.8; AD-399-2030] and 1585 ng•hr/mL [Study GS-US-399-5505], respectively). C_{max} values from repeated doses of 5 mg/kg RDV in rhesus monkeys were also similar to repeated doses of 100 mg RDV in humans (3350 ng/mL [m2.6.5, Section 5.8; AD-399-2030] and 2230 ng/mL [Study GS-US-399-5505], respectively). Given that full pharmacokinetic characterization of the 30-minute infusion in monkey was only assessed at 5 mg/kg, data from IV bolus administration showing dose proportional increases in RDV exposure from 3 to 10 mg/kg (m2.6.5, Section 3.4; AD-399-2002 and Section 3.5; AD-399-2022), support projection of the rhesus monkey efficacious dose of 10/5 mg/kg to the proposed 200/100 mg RDV clinical dose.

3.4. Repeat Dose Pharmacokinetics

Repeat-dose PK was assessed from TK data, which were obtained to support the respective toxicology studies. Toxicokinetic data from the toxicology studies in mice, rats, rabbits, and monkeys are presented in the Toxicology Tabulated Summary (m2.6.7, Section 3). The TK data after repeat-dose in nonclinical species generally showed no evidence for accumulation for RDV, GS-704277, or GS-441524. Accumulation was observed in Indian-origin rhesus monkeys for GS-441524 at higher doses. Sex-based differences were generally less than 2-fold for GS-441524 and generally greater than 2-fold for GS-704277, with males showing higher C_{max}

and AUC_{0-24} values for rats. Gender differences in monkeys were less than 2-fold for RDV, GS-704277, and GS-441524.

3.5. Distribution

Remdesivir had moderate protein binding in all species with a free fraction ranging from 8.0% in the rat to 14.2% in the cynomolgus monkey. The free fraction in humans was 12.1% (m2.6.5, Section 5.1, AD-399-2013). GS-704277 and GS-441524 exhibited very low protein binding in plasma from Wistar Han rats, cynomolgus monkeys, rhesus monkeys, and humans (m2.6.5, Section 5.1, AD-399-2031). After incubation of compounds with blood, RDV was found to be somewhat excluded from the blood cellular fraction, with mean whole blood/plasma concentration ratios of 0.71 and 0.76 for monkeys and humans, respectively (m2.6.5, Section 5.2; AD-540-2007). GS-441524 showed some association with the cellular fraction, with respective mean blood/plasma ratios of 1.36 and 1.19 for monkeys and humans.

Single-dose PK studies with RDV were conducted in male marmosets (m2.6.5, Section 5.6; AD-399-2023) and African green monkeys (m2.6.5, Section 5.9; AD-540-2003) following IV administration of RDV at 10 mg/kg. As seen in previous monkey studies, RDV was rapidly eliminated followed by the sequential appearance of GS-704277 and GS-441524 as well as efficient formation of the pharmacologically active triphosphate, GS-443902, observed in PBMCs and lungs. Once formed, GS-443902 appeared to persist with an approximate half-life of > 24 hours and 22 hours in PBMC and lung of marmosets, respectively. In African green monkeys, profiles for GS-441524 and its phosphorylated metabolites in PBMC and respiratory tissues and for total GS-441524 phosphorylated metabolites in liver, gastrointestinal (GI) tract, kidney, testis, and eye were obtained. At 24 hours postdose, levels of the pharmacologically active metabolite, GS-443902 persisted in respiratory tissues, suggestive of broad and efficient loading within these tissues following IV administration. Consistent with tissue distribution results in cynomolgus monkeys, but reflective of specific intracellular activation, high levels of GS-441524 phosphorylated metabolites were observed in the kidney and liver, moderate levels in the GI tract and more limited distribution observed in the testis and eye.

Tissue distribution following a single IV dose of [^{14}C]RDV at 10 mg/kg to male Sprague Dawley (SD; non-pigmented) and Long Evans (LE; pigmented) rats was determined by quantitative whole body autoradiography (m2.6.5, Section 5.4; AD-399-2017). [^{14}C]RDV-derived radioactivity was widely distributed to most tissues by the first collection time point (0.167 hours postdose) in both SD and LE male rats. Distribution of radioactivity was similar in both SD and LE rats. Most of the tissues reached maximum concentration (C_{max}) by the first collection time point for both SD and LE rats. Radioactivity was eliminated from majority of the tissues by 96 hours postdose in both SD and LE rats. Tissues showing the highest maximum concentrations of radioactivity included kidney cortex, kidney medulla, liver, arterial wall, nonpigmented skin, cecum, urinary bladder, and esophagus. At 168 hours postdose, radioactivity was still quantifiable in kidney, kidney cortex, and pigmented skin. No melanin binding was observed.

In monkeys administered a single IV dose of [^{14}C]RDV at 10 mg/kg (m2.6.5, Section 5.5; AD-399-2019), tissues showing the highest mean concentrations of radioactivity at 4 hours postdose, excluding the GI tract, were gall bladder, kidneys, liver, prostate gland, salivary gland

(mandibular), pancreas, and seminal vesicle(s). Notably, moderate levels of radioactivity were also found in lung tissue, while some of the lowest levels were seen in bone, brain, eye, and testis(es). Total radioactivity declined over 168 hours; elimination of radioactivity was not complete and radioactivity was still quantifiable in most tissues with a mean of 8.26% of the administered dose retained in the tissues, mostly in liver and muscle.

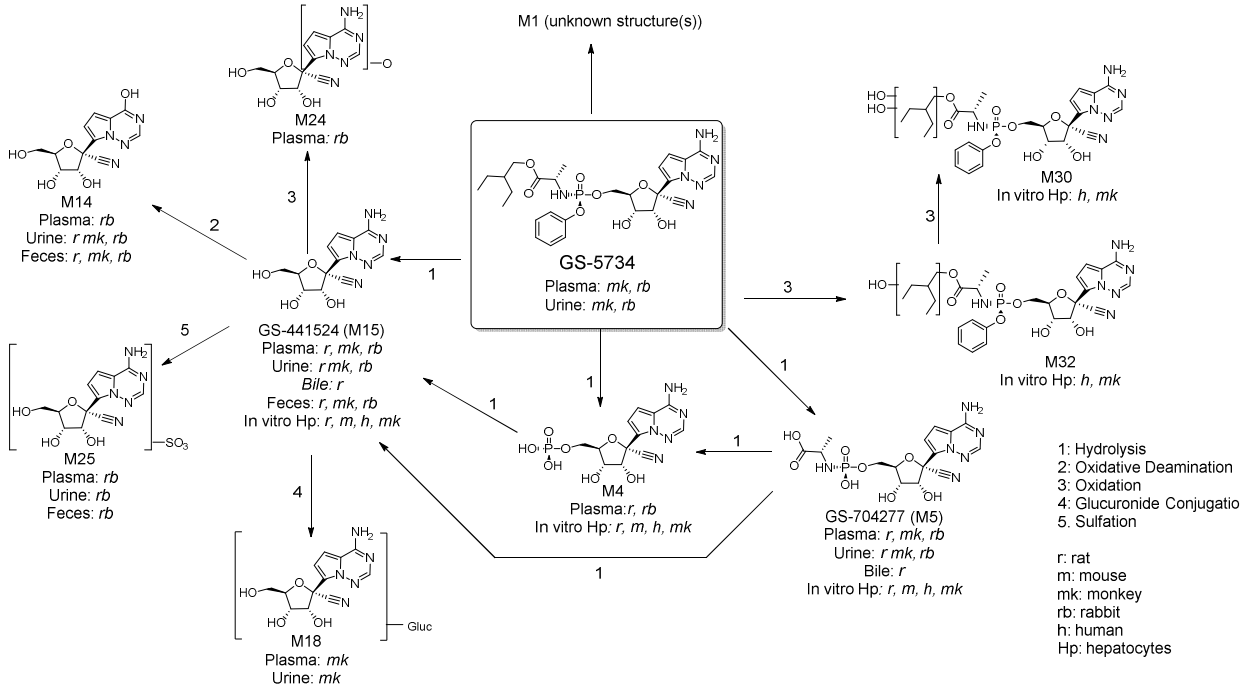
3.6. Metabolism

The stability of RDV in plasma from the SD rat, beagle dog, cynomolgus monkey, rhesus monkey, and humans was determined (m2.6.5, Section 7.1; AD-399-2012). Consistent with the presence of high esterase activity in plasma in many rodent species, RDV was unstable in rat plasma ($t_{1/2} \leq 0.9$ min). Remdesivir was substantially more stable in plasma from non-rodent species with $t_{1/2}$ ranging from 68.5 min in humans to 630 min in the dog.

The metabolism of [^{14}C]RDV was assessed in vitro using mouse, rat, monkey, and human cryopreserved hepatocytes (m2.6.5, Section 7.6; AD-399-2024). [^{14}C]RDV was metabolized by mouse, rat, monkey, and human hepatocytes, primarily via hydrolysis. The rate of biotransformation in the mouse and rat was faster relative to the monkey and humans. In all four species, most of the [^{14}C]RDV-derived radioactivity was associated with three major metabolites; GS-704277, GS-441524, and GS-441524-phosphate generated via hydrolysis.

In vivo metabolism of RDV was studied in the rat, rabbit and monkey (m2.6.5, Section 5.3 and Section 5.4; AD-399-2017, Section 5.5; AD-399-2019, and Section 10.4; AD-399-2025). Proposed biotransformation pathways described for the select species are also represented in Figure 1.

Figure 1. Proposed Biotransformation Pathways of Remdesivir Across Species



One of the main target tissues for SARS-CoV-2 is the lung, which is largely inaccessible for routine sampling. Therefore, a sampling surrogate could potentially be used to assess target distribution and metabolism. Circulating PBMCs may be appropriate as they are currently collected to assess active metabolite exposure and antiviral potency of nucleos(t)-ide analogs, or respective prodrugs, in the HIV population. To support nonclinical evaluations, and to assess potential differences in metabolism between monkey and human, the metabolism of RDV was compared in human and rhesus PBMCs and monocytes (m2.6.5, Section 7.3; AD-399-2015). Intracellular triphosphate concentrations were higher in human PBMCs (3.64-fold) and monocytes (4.24-fold) compared to respective monkey cell types in vitro. Additionally, as NHBE are representative target cells for SARS-CoV-2 infection, the kinetics of loading of NHBE by RDV and subsequent metabolism to GS-441524 and its phosphorylated metabolites, followed by their elimination, were studied following incubation with RDV, in vitro (m2.6.5, Section 7.8; AD-540-2001). GS-443902 was efficiently formed in NHBE cells and persisted with a $t_{1/2}$ of approximately 15 hours.

3.7. Excretion

The excretion profile of [^{14}C]RDV was characterized in rat, rabbit and monkey (m2.6.5, Section 10.1; AD-399-2017, Section 10.3; AD-399-2019, and Section 10.4; AD-399-2025). In all species, the majority of the radioactive dose was excreted by 48 hours postdose. In the rat, by 168 hours postdose, means of 63.0% and 27.8% of the administered radioactivity were recovered in urine and feces, respectively, while 22.7% of the administered radioactivity was excreted in bile of bile-duct-cannulated rats. Similarly, in rabbits by 168 hours postdose, means of 67.0 and 11.9% of the administered radioactivity were recovered in urine and feces, respectively. In the monkey by 168 hours postdose, means of 33.6% and 25.6% of the administered radioactivity were recovered in urine and feces, respectively. Significant radioactivity was also recovered in cage rinses, accounting for a mean of 16.9% of the dose. This indicated that renal and biliary excretion were the major routes of elimination in monkeys.

3.8. Pharmacokinetic Drug Interactions

3.8.1. Cytochrome P450/UGT Inhibition

RDV is unlikely to cause clinical drug interactions through inhibition of human CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19 or CYP2D6. Effects upon CYP3A activity would be limited based on the transient exposure to low concentrations of intact, unbound RDV following a 30-minute IV infusion. The potential for RDV to inhibit the catalytic activity of human uridine diphosphate glucuronosyltransferase 1A1 (UGT1A1) was also assessed (m2.6.5, Section 9.3; AD-540-2005). The rate of enzyme-specific metabolite formation from estradiol, the probe substrate, was quantified in the presence and absence of test compound and the IC_{50} value was determined. Remdesivir inhibited UGT1A1 activity with an IC_{50} of 9.78 µM, which is predicted to not be clinically relevant using the mechanistic static model of inhibition assessment.

3.8.2. Induction Liability

The potential of induction of CYP enzymes (CYP1A2, CYP2B6, and CYP3A4) following exposure of human hepatocytes to RDV, and its major systemic metabolites, GS-704277 and the nucleoside analog GS-441524, was assessed by quantitating CYP enzyme activities (m2.6.5, Section 9.4; AD-399-2027). Drug-drug interaction assessment using even the most sensitive donor in this assay predicts no induction liability for RDV, GS-704277, or GS-441524.

3.8.3. Interactions with Transporters

Studies were completed in transfected cell lines to determine if RDV is a substrate for hepatic uptake transporters OATP1B1 and OATP1B3 (m2.6.5, Section 11.3; AD-399-2008). Remdesivir was found to be a substrate for the OATP1B1 but not for OATP1B3. The rates of uptake of RDV increased in OATP1B1 transfected Chinese hamster ovary cells and its uptake was inhibited by the addition of rifampicin.

Remdesivir was assessed as a substrate for the efflux transporters P-gp and breast cancer resistance protein (BCRP) (m2.6.5, Section 11.2; AD-399-2007). Remdesivir was found to be a substrate for P-gp but not BCRP based on the changes in its permeability observed in P-gp but not BCRP over-expressing Madin-Darby canine kidney strain II cells. Consistent with P-gp dependent transport, the efflux ratio of RDV decreased in the presence of the P-gp inhibitor cyclosporin A in P-gp over-expressing cells.

In cells overexpressing human organic anion transporters (OAT) 1 and 3, no evidence for transport of RDV and its major systemic metabolites, GS-704277 and the nucleoside analog GS-441524, was observed (m2.6.3, Section 3.1; PC-399-2020). Similarly, rat OAT1 did not transport RDV or its metabolites, and rat OAT3 did not transport RDV or GS-441524. In contrast to human OAT3, cells overexpressing rat OAT3 were observed to transport GS-704277 and showed increased cytotoxicity compared to mock-transfected control cells.

The potential for RDV to inhibit drug transporters has been assessed in vitro in transfected cell lines expressing OATP1B1, OATP1B3, P-gp, and BCRP (m2.6.5, Section 11.4; AD-399-2005). Remdesivir did not inhibit P-gp transport at the highest concentration tested (40 μM). Remdesivir inhibited OATP1B1- and OATP1B3-dependent transport, with IC_{50} values of 2.8 and 2.1 μM, respectively. The potential for clinically significant drug-drug interactions caused by inhibition of OATP1B1 and OATP1B3 is limited by the transient exposure to low concentration of intact, unbound RDV following IV infusion.

Studies were also performed to investigate whether RDV, GS-704277 or GS-441524 are inhibitors of human bile salt export pump (BSEP), multidrug resistance-associated protein (MRP)2, MRP4, and sodium-taurocholate co-transporting polypeptide (NTCP) transporters using model substrates and transfected cell lines or membrane vesicles (m2.6.5, Section 11.6; AD-399-2029). Remdesivir inhibited BSEP-, MRP4- and NTCP-mediated probe substrate transport, with calculated IC_{50} values of 22, 5.1, and 72 μM, respectively. No interaction of RDV with MRP2 was observed at up to 100 μM. GS-704277 showed 25% and 44% inhibition of MRP2- and NTCP-mediated transport, respectively, at the 100 μM test concentration. No interaction of GS-704277 with BSEP or MRP4 was observed up to 100 μM.

GS-441524 showed 24% inhibition of NTCP-mediated transport at the 100 μM test concentration. No interaction of GS-441524 with BSEP, MRP2, or MRP4 was observed up to 100 μM.

Remdesivir metabolites GS-441524, GS-719700 (GS-441524-MP), GS-719699 (GS-441524-DP), GS-443902, and GS-704277 were investigated for interaction with the human BCRP, BSEP, MRP2, and MRP3 efflux transporters in the vesicular transporter inhibition assay (m2.6.5, Section 11.5; AD-399-2035). Transporter inhibition was only observed in the case of GS-719699 and GS-443902. GS-719699 inhibited BCRP (26%), while GS-443902 had an inhibitory effect on MRP2 (24%), both at 200 μM, thus no IC_{50} values were calculated. The remaining transporters were not affected by these test articles. GS-719700, GS-704277, and GS-441524 did not have an effect on the investigated transporters.

3.9. Drug-Drug Interaction Liability Assessment

The liability for RDV to cause PK drug interactions was assessed using current Food and Drug Administration (FDA) Guidelines (m2.6.5, Section 11.7; AD-540-2006) and representative clinical PK data. The only drug interaction liabilities identified for RDV are inhibition of CYP3A, OATP1B1, and OATP1B3. The inhibitory effects are weak and, due to the short half-life of RDV, the effects would only be manifest briefly. Further evaluation of GS-704277 and GS-441524 for possible interactions with drug metabolizing enzymes and transporters is ongoing.

3.10. Summary of Pharmacokinetics

Absorption, distribution, and metabolism studies support the selection of the Wistar Han rat and cynomolgus monkey for the assessment of the toxicology of RDV. Both rat and monkey formed the intermediate metabolite GS-704277 and the nucleoside metabolite GS-441524. GS-441524 is the predominant metabolite in plasma observed in all nonclinical studies. Based on a similar in vitro stability profile in plasma, hepatocytes and subcellular fractions, the monkey more closely mimics humans with respect to the behavior of RDV. While forming the same major metabolites, rats had markedly reduced levels of intact RDV in plasma and correspondingly elevated plasma exposure to the metabolites GS-704277 and GS-441524, consistent with higher levels of plasma esterase activity in rats.

Intracellular metabolism studies conducted in vitro illustrated effective activation of RDV in NHBE and Calu-3 (relevant cell types for SARS-CoV-2 infection). Additionally, high plasma stability observed in vitro in non-rodent species was confirmed in vivo where RDV demonstrated sufficient systemic exposure to load target tissues and cells supporting SARS-CoV-2 replication. In vivo studies performed in the cynomolgus monkey also showed distribution of [^{14}C]RDV-equivalents to the lungs, which are a target tissue. Additional distribution studies confirmed rapid delivery and efficient formation of the active triphosphate metabolite in marmoset and African green monkey lungs following IV administration of RDV. Finally, extensive studies in nonhuman primates demonstrated the utility of monitoring the active triphosphate in PBMC as an accessible surrogate for exposure in target tissues. The half-life of the pharmacologically active triphosphate GS-443902 observed in vitro in NHBE (15 hours), and

confirmed in vivo in marmoset lung and PBMC following IV administration (at least 22 hours), supports once-daily administration of RDV.

Remdesivir has a low potential for drug-drug interactions. Using the current FDA regulatory Guidelines, RDV at an initial intravenous dose of 200 mg and subsequent daily IV dose of 100 mg, would be predicted not to cause drug interactions by induction and not to inhibit CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, UGT1A1, P-gp, or BCRP. Inhibitory interactions with CYP3A, OATP1B1, and OATP1B3 are predicted. However, inhibition of these activities would be expected to be weak and transient due to the short clinical half-life (< 1 hour) of RDV. The once daily administration and short duration of RDV treatment may allow for temporary dose modification of other drugs as needed.

C_{max} and $AUC_{0\text{-}tau}$ values following RDV administration in healthy rhesus monkeys and humans, and in vivo efficacy studies in rhesus monkeys, support the proposed 200/100 mg RDV dose for clinical efficacy.

In summary, RDV administered IV exhibits a favorable and consistent PK profile as well as efficient delivery of high levels of the pharmacologically active nucleoside triphosphate metabolite into tissues and cells relevant for SARS-CoV-2 replication, supporting its consideration as a novel agent for the treatment of COVID-19.

4. TOXICOLOGY

4.1. Brief Overview

The nonclinical safety profile of RDV has been characterized in repeat-dose IV administration studies in rats and cynomolgus monkeys, in in vitro and in vivo genotoxicity studies, in a battery of developmental and reproductive toxicity (DART) studies in rats and rabbits, and in a hemolytic potential/plasma compatibility study. Additional studies included in vitro investigations to assess hepatotoxicity, non-GLP 7-day studies in carboxylesterase-knockout mice (Ces1c$^{-/-}$), cynomolgus monkeys, rhesus monkeys, and rabbits, and impurity qualification studies. Study designs and parameters evaluated were consistent with accepted principles and practices as outlined in ICH, Organization for Economic Co-Operation and Development (OECD), and national regulations (United States FDA and European Community Directives). All pivotal studies were conducted in accordance with US FDA or OECD GLP regulations. The extent of the nonclinical GLP safety program is consistent with the ICH M3(R2) guidelines for a product with a proposed dosing regimen of less than 28 days. The completed studies are described in detail in m2.6.6 and are listed in Table 2 below. For exposure margin calculations, AUC plasma values in the toxicology studies were divided by the AUC_{tau} values from PK analysis in healthy adults presented in m2.7.2, Study GS-US-399-5505.

Table 2. Overview of Remdesivir Toxicology Program

Study Type	Study Number	Concentration or Dose (mg/kg/day)	GLP Status	Tabulated Summary
Repeat-Dose Toxicity				
Non-Pivotal Studies				
1-week IV study in Rhesus Monkeys	TX-399-2021	0, 5, 10, 20	Non-GLP	2.6.7.6
Pivotal Studies				
2-Week IV study in Rats	TX-399-2003	0, 0, 5, 20, 50	GLP	2.6.7.7.1
4-week IV study in Rats	TX-399-2016	0, 1, 3, 10	GLP	2.6.7.7.2
2-Week IV study in Cynomolgus Monkeys	TX-399-2004	0, 0, 1, 3, 10	GLP	2.6.7.7.3
4-week IV study in Cynomolgus Monkeys	TX-399-2017	0, 1, 3, 10	GLP	2.6.7.7.4
Genotoxicity				
In Vitro Studies				
Bacterial reverse mutation assay	TX-399-2005	5 − 5000 µg/plate	GLP	2.6.7.8.1
In Vitro Cytogenetics	TX-399-2006	Without S9: 3 h: 117 - 171 µg/mL 24 h: 41.2 - 84.0 µg/mL With S9: 3 h: 120 - 245 µg/mL	GLP	2.6.7.8.2
In Vivo Studies				
Rat bone marrow micronucleus	TX-399-2003	0, 0, 5, 20, 50	GLP	2.6.7.9.1

Study Type	Study Number	Concentration or Dose (mg/kg/day)	GLP Status	Tabulated Summary
Reproduction and Developmental Toxicity				
IV fertility and early embryonic development to implantation study in rats	TX-399-2012	0, 1, 3, 10	GLP	2.6.7.12
IV study for effects on embryo-fetal development in rats	TX-399-2013	0, 2.5, 5, 10, 20	GLP	2.6.7.13.1
IV study of the effects on embryo-fetal development in rabbits	TX-399-2018	0, 2.5, 5, 10, 20	GLP	2.6.7.13.2
IV study of the effects on pre- and postnatal development, including maternal function, in rats	TX-399-2014	0, 1, 3, 10	GLP	2.6.7.14
Local Tolerance				
Bovine Corneal Opacity and Permeability (BCOP) Assay	TX-399-2025	750 µL of 20% w/v suspension	GLP	2.6.7.15
Skin Irritation/EpiDerm™	TX-399-2023	10 mg	GLP	2.6.7.15
Other Toxicity Studies				
Mechanistic Studies				
Human 3D Liver Tissue (GS-5734 and Metabolites GS-704277, and GS-441524)	TX-399-3022	GS-5734: 0.3 - 7.5 µM; GS-704277: 0.3 - 3 µM; GS-441524: 0.3 - 3 µM	Non-GLP	2.6.7.16
Rat, Monkey, and Human Hepatocytes (GS-5734 and Metabolites GS-704277, and GS-441524)	PC-399-2027	GS-5734: 30-0.12 µM GS-704277: 100-0.4 µM GS-441524: 300-1.2 µM	Non-GLP	2.6.7.16
Impurities				
2-Week impurity qualification IV study in Cynomolgus Monkeys	TX-399-2015	0, 0, 5, 5, 10, 10, 10	GLP	2.6.7.16
Bacterial reverse mutation assay with [redacted]	TX-399-2020	5 − 5000 µg/plate	GLP	2.6.7.16
Other Studies				2.6.7.16
1-Week IV study in Rats	TX-399-2009	0, 0, 20, 50	Non-GLP	2.6.7.16
1-Week IV study in Rabbits	TX-399-2010	0, 5, 15, 50	Non-GLP	2.6.7.16
1-Week SC study in Mice	TX-399-2019	10, 50	Non-GLP	2.6.7.16
1-Week IM study in Cynomolgus monkeys with GS-466547	TX-399-2001	0, 2.5, 7.5, 15	Non-GLP	2.6.7.16
Hemolytic Potential and Plasma Compatibility (Rat, Monkey and Human whole blood and plasma)	TX-399-2008	0, 1, 3, 10 mg/mL	GLP	2.6.7.16

4.2. Single Dose Toxicity

No single-dose toxicity studies with RDV have been conducted. In single-dose PK studies, RDV was well tolerated after IV doses up to 50 mg/kg in rats, 10 mg/kg in cynomolgus monkeys, and 10 mg/kg in rhesus monkeys (m2.6.4, Section 4.2).

4.3. Repeat Dose Toxicity

All GLP repeat-dose toxicity studies with RDV utilized IV injection (slow bolus over 1-2 minutes); the clinical route of administration is IV infusion over at least 30 minutes. Remdesivir was administered to rats or cynomolgus monkeys in a vehicle of 12% (w/v) sulfobutylether-β-cyclodextrin (SBECD) in Sterile Water for Injection, USP, pH 3.5 ± 0.1. The 2-week GLP repeat dose studies incorporated a saline and vehicle control group. Protocols for the 2- and 4-week studies included a comprehensive battery of kidney function/injury biomarkers; the 2-week studies also included 4-week recovery periods to assess reversibility from any observed effects. In all studies, blood was collected for plasma analysis of RDV and the nucleoside metabolite, GS-441524; the intermediate metabolite, GS-704277 was also analyzed in the 4-week studies and the reproduction studies. As expected, RDV exposures in rats were generally BLQ and margins were calculated for the major circulating metabolites, GS-441524 and GS-704277, in this species.

4.3.1. Target Organ Toxicity - Kidney

In the repeat dose studies with RDV, toxicity findings were consistent with dose-dependent and reversible kidney injury and dysfunction at doses greater than 3 mg/kg/day in rats and 5 mg/kg/day in rhesus monkeys. There were no observable kidney changes in cynomolgus monkeys administered IV RDV at 10 mg/kg/day. In clinical studies with healthy volunteers, no evidence of nephrotoxicity was observed after a single dose of 200 mg RDV followed by once-daily doses of 100 mg RDV for 10 days (Study GS-US-399-5505), or after multiple once daily doses of 150 mg RDV for up to 14 days (Study GS-US-399-1954). The clinical significance of the nephrotoxicity noted in animal species is unknown.

In rats, clinical chemistry and urinalysis findings, including increases in blood urea nitrogen and serum creatinine, and increases in urinary biomarkers of kidney injury, eg, total protein, n-acetyl-glucosaminidase, cystatin C, beta-2-microglobulin, and kidney injury molecule-1, were predictive of the microscopic changes observed in the kidney. Microscopic findings included a spectrum of degenerative, necrotic and regenerative changes to the renal tubular epithelium in the cortex. In the 2-week study (m2.6.7, Section 7.1; TX-399-2003), the changes in the kidney were reversible after a 4-week recovery period and correlated with the reversibility of the clinical chemistry, urinalysis and urinary biomarker findings. In the 4-week toxicity study (m2.6.7, Section 7.2; TX-399-2016), the NOAEL was 3 mg/kg/day, based on the nature and severity of the kidney changes at the 10 mg/kg/day dose level.

At the 3 mg/kg/day NOAEL in the 4-week rat study, AUC values (sexes combined) for GS-441524 and GS-704277 were 748 and 301 ng•h/mL, respectively, providing exposure margins of 0.3 and 0.7, respectively, versus clinical exposures at the proposed dose of 200/100 mg RDV. At the 10 mg/kg/day NOAEL in the 4-week cynomolgus monkey study, AUC values (sexes combined) for RDV, GS-441524 and GS-704277 were 1330, 2070 and 849 ng•h/mL, respectively, providing exposure margins of 0.8, 0.9 and 1.8, respectively.

In non-GLP exploratory studies, microscopic changes in the proximal tubules of the kidney were similar to those noted in rats after daily IM injections of 15 mg/kg/day GS-466547 (diastereomeric mixture) to cynomolgus monkeys for 7 days, and clinical pathology changes

correlated with the renal changes at the 15 mg/kg/day IM dose. In a 7-day IV study in (Indian-origin) rhesus monkeys, adverse kidney changes were observed at ≥ 5 mg/kg/day, with mortality noted in 1 animal administered 20 mg/kg/day. The reason for the possible increased sensitivity of rhesus monkeys administered IV RDV compared to cynomolgus monkeys is unknown

4.4. Genotoxicity

Remdesivir is nongenotoxic. Remdesivir and the nucleoside metabolite, GS-441524, were non-mutagenic in the in vitro Ames mutagenicity assay (m2.6.7, Section 8.1; TX-399-2005, and m2.6.7, Section 16; TX-195-2006, respectively), and RDV was negative in the rat micronucleus assay (m2.6.7, Section 9.1; TX-399-2003). In the in vitro chromosome aberrations assay with human lymphocytes, RDV was negative without metabolic activation, and equivocal in the 3-hour treatment with metabolic activation (m2.6.7, Section 8.2; TX-399-2006).

At the 50 mg/kg/day high dose in the rat micronucleus assay, AUC_{0-24} exposures on Day 15 were 284 ng•h/mL for RDV and 20,300 ng•h/mL for GS-441524 at exposure margins 0.2- and 9-fold versus clinical exposures at the 200/100 mg RDV dose (Study GS-US-399-5505).

4.5. Carcinogenicity

Current regulatory guidance does not require carcinogenicity studies with RDV for the proposed COVID-19 indication with a dosing duration of less than 3 months.

4.6. Reproductive Toxicity

A complete reproductive and development toxicity program has been completed with RDV. There were no effects on embryofetal development in rats and rabbits, and the NOAELs were 20 mg/kg/day in both species. There were no adverse effects in the pre- and postnatal toxicity study in rats, and the NOAEL was 10 mg/kg/day. There were no effects on male reproductive performance and spermatogenesis, and the NOAEL for male reproductive toxicity was 10 mg/kg/day. For females the NOAEL for reproductive toxicity and embryonic toxicity was 3 mg/kg/day, based on decreases in corpora lutea, numbers of implantation sites and viable embryos at the 10 mg/kg/day dose associated with systemic maternal toxicity. Exposure margins at the NOAELs in each study are provided in Table 3.

Table 3. Exposure Margins at NOAELs in Reproduction and Developmental Toxicity Studies

Study	NOAEL (mg/kg/day)	Exposure Margins[1]		
		Remdesivir	GS-441524	GS-704277
Rat Fertility (TX-399-2012)				
Males	10	NA	1.9	4.7
Females	3	NA	0.2	0.4
Embryofetal Development				
Rat (TX-399-2013)	20	NA	3.9	7.4
Rabbit (TX-399-2018)	20	1.8	4.0	25
Rat pre-/postnatal development (TX-399-2014)	10	NA	1.0	2.6

NA = Not applicable (RDV exposures were BLQ)
1 Exposure margins were calculated based on clinical AUC_{tau} values of 1585 ng•h/mL for RDV, 2230 ng•h/mL for GS-441524, and 461 ng•h/mL for GS-704277 (Study GS-US-399-5505).

4.7. Local Tolerance

Remdesivir is intended for IV administration. In the repeat dose studies, injection site reactions, such as red discoloration, were observed in rats. There were no similar reactions in monkeys. Remdesivir is not an irritant to skin, was classified as non-irritant to eyes, and is unlikely to be phototoxic based on the absence of binding to melanin-containing tissues (m2.6.4, Section 5.2; AD-399-2017), and its photochemical properties.

4.8. Other Toxicity Studies

4.8.1. Mechanistic Studies

In addition to studies using monoculture of human hepatocytes (Section 2.2), studies were conducted to investigate the potential of RDV and its major systemic metabolites, GS-441524 and GS-704277, to affect primary human and animal hepatocytes using several in vitro models (m2.6.7, Section 16; PC-399-2027 and TX-399-2022). Data from these in vitro studies demonstrated that human hepatocytes are susceptible to RDV-mediated toxicity, likely due to the high cellular permeability and effective intracellular metabolism of the drug. GS-441524 and GS-704277 are unlikely to contribute significantly to changes in liver enzymes observed in humans treated with repeated doses of RDV due to their low systemic exposure and minimal effects on hepatocytes demonstrated in several independent studies.

To understand the apparent increased sensitivity of rats to RDV-related nephrotoxicity, RDV, GS-441524 and GS-704277 were tested for their interaction with human and rat renal OATs (m2.6.3, Section 3.1; PC-399-2020). The data indicated that the intermediate metabolite GS-704277, but not RDV or the nucleoside metabolite GS-441524, was an effective substrate of rat OAT3 and exhibits rat OAT3-dependent cytotoxicity. In contrast, GS-704277 was not a substrate for human OATs, suggesting a reduced potential for renal adverse effects in human compared with rat due to lower renal accumulation.

4.8.2. Studies on Impurities/Degradation Products

The impurities or degradation products related to RDV have been identified in batches of the active pharmaceutical ingredient (API, drug substance) or drug product. The impurity profiles for batches of API or drug product used in nonclinical toxicology studies are provided in m2.6.7 Section 4.

One dedicated repeat-dose study was conducted in monkeys to determine if there were unexpected adverse effects from RDV-related process impurities and degradation products (m2.6.7, Section 16; TX-399-2015). No adverse treatment-related effects were observed and there were no differences in findings in animals treated with lots containing RDV-related process impurities and RDV-related degradants relative to a comparator lot. A reverse mutation assay with a potential impurity, [redacted], showed the material to be negative for mutagenicity (m2.6.7, Section 16; TX-399-2020).

Based on their impurity profiles, the multiple batches of RDV tested in the GLP toxicology program, in composite, are considered to be representative of the Good Manufacturing Process (GMP) material and support the specified limits of impurities proposed for commercial production (m3.2.S.4.5, Justification of Specification, and the specified limits of degradation products proposed, m3.2.P.5.6, Justification of Specifications).

4.8.3. Other Toxicity Studies

In a series of non-GLP studies, 7-day IV studies were conducted in rats and rabbits, an IM study was conducted in cynomolgus monkeys and a SC study was completed in mice (m2.6.7, Section 16; TX-399-2009, TX-399-2010, TX-399-2001, and TX-399-2019, respectively). In rats, the severity of renal changes and injection site reactions were greater in animals administered RDV in a 5% ethanol/5% polysorbate 80 vehicle versus the 12% [w/v] SBECD vehicle that was selected for further nonclinical and clinical development. In rabbits, dose limiting toxicity, observed at the high dose of 50 mg/kg/day, supported selection of the high dose of 20 mg/kg/day in the embryofetal development study (m2.6.7, Section 13.2; TX-399-2018). The SC study in Ces1c$^{-/-}$ mice supported the design and interpretation of the efficacy studies against SARS-CoV and MERS-CoV. Finally, early in the nonclinical development program for RDV, an IM study was conducted with GS-466547 (diastereomeric prodrug mixture containing RDV; m2.6.7, Section 16; TX-399-2001). Although the IM route was considered not optimal for parenteral delivery due to slow and variable release of RDV from muscle tissue, this study identified the kidney as a target organ of toxicity; clinical pathology changes in blood and urine were compatible with renal dysfunction and microscopic findings of proximal tubular epithelial cell degeneration/necrosis.

4.9. Target Organ Effects

4.9.1. Effects on Kidney

In the nonclinical studies, toxicity findings were consistent with dose-dependent and reversible kidney injury and dysfunction at doses greater than 3 mg/kg/day in rats and 5 mg/kg/day in rhesus monkeys. There were no observable kidney changes in cynomolgus monkeys administered RDV at 10 mg/kg/day. In all cases, clinical chemistry and urinalysis findings were predictive of the microscopic changes observed in the kidney. Exposure margins at the NOAELs in each study are provided in Table 4.

Table 4. Exposure Margins at the NOAELs in Repeat Dose IV Toxicity Studies with Remdesivir

Study	NOAEL (mg/kg/day)	Exposure Margins[1]		
		Remdesivir	GS-441524	GS-704277
4-week IV Rat (GLP) (TX-399-2016)	3	NA	0.3	0.7
4-week IV Cynomolgus Monkey (GLP) (TX-399-2017)	10	0.8	0.9	1.8
7-Day IV Rhesus Monkey (non-GLP) (TX-399-2021)	< 5	< 0.4	< 0.1	< 1.4

NA = Not applicable (RDV exposures were BLQ)
1 Exposure margins were calculated based on clinical AUC_{tau} values of 1585 ng•h/mL for RDV, 2230 ng•h/mL for GS-441524, and 461 ng•h/mL for GS-704277 (Study GS-US-399-5505)

In clinical studies, no evidence of nephrotoxicity has been observed with single doses of RDV up to 225 mg or multiple once-daily doses of RDV 150 mg for up to 14 days (Study GS-US-399-1954), or after a single dose of 200 mg followed by daily doses of 100 mg for 10 days (Study GS-US-399-5505). The increased sensitivity of rats to the renal effects of RDV may be related to the active tubular transport of RDV metabolites by rat renal OAT3; this interaction has not been detected with human renal OAT3 (m2.6.3, Section 3.1, PC-399-2020).

In summary, nonclinical findings were consistent with dose-dependent and reversible kidney injury and dysfunction. Remdesivir and GS-441524 exposures at the NOAELs are below the predicted steady-state exposure in humans at 200 mg. However, as no evidence of nephrotoxicity has been observed in clinical studies with RDV, the clinical significance of the nephrotoxicity noted in animal species is unclear.

4.9.2. Effects on Liver

In clinical studies with RDV, transient elevations in alanine aminotransferase (ALT) and aspartate aminotransferase have been observed. In the nonclinical program, there were no changes in the liver in rats or monkeys based on clinical chemistry parameters, liver weight, or microscopic observations. To better understand this specific effect associated with RDV

treatment in clinical studies, a series of in vitro mechanistic studies were completed to assess effects of RDV and its two systemically circulating metabolites, GS-441524 and GS-704277, on various parameters related to hepatotoxicity as described in Table 5.

Data from the studies in Table 5 demonstrate that human hepatocytes may be susceptible to RDV-mediated toxicity while the effects of the metabolites GS-704277 and GS-441524 on hepatocytes are minimal. An exact molecular mechanism for the toxicity was not identified. Key conclusions from these studies are as follows:

- Remdesivir and its major systemic metabolites, GS-704277 and GS-441524, exhibited low potential to adversely affect the function of the critical liver-specific human efflux transporters BSEP, MRP2, and NTCP at concentrations above the peak of systemic exposure observed in humans. Remdesivir, but not its metabolites, showed inhibition of MRP4-mediated transport at a concentration at least 5-fold higher than the unbound Cmax in humans. A contribution of MRP4 inhibition to ALT elevation is unlikely and would be limited to the short duration of infusion (m2.6.5, Section 11.6; AD-399-2029).

- Human, monkey, and rat hepatocytes in the HμRELTox™ co-culture model (consisting of primary hepatocytes of respective species co-cultured with cells of a non-parenchymal stromal type) exhibited signs of toxicity when exposed to RDV at concentrations below the peak of its systemic exposure observed in humans. Hepatocytes from all three species, human hepatocytes in particular, were substantially less sensitive to the systemic metabolites, GS-704277 and GS-441524, relative to the intact prodrug RDV (m2.6.7, Section 16; PC-399-2027).

- The Organovo™ exVive 3D liver tissue culture model (consisting of primary human hepatocytes, hepatic stellate cells, and endothelial cells) did not show conclusive evidence of toxicity for RDV or its metabolites (m2.6.7, Section 16; TX-399-2022).

- Remdesivir reduced both the mitochondrial spare respiration and viability of the primary human hepatocytes in two-dimensional mono-cultures of fresh primary human hepatocytes while the systemic metabolites GS-704277 and GS-441524 showed no effects at concentrations that exceed systemic exposures in humans. The RDV effect on cellular respiration was not selective and was rather a consequence of its direct cytotoxic effect (m2.6.3, Section 3.1; PC-399-2028).

- Remdesivir showed no to minimal effects on cellular reactive oxidative species (ROS) levels in the HepG2 human liver cell line at concentrations higher than the systemic drug exposures projected to be clinically efficacious in humans (m2.6.3, Section 3.1; PC-399-2050).

Table 5. Overview of In Vitro Mechanistic Studies

Study Number	Study Title
AD-399-2029	In Vitro Inhibition Assessment of GS-5734 and its Metabolites with Human BSEP, MRP2, MRP4, and NTCP Transporters
PC-399-2027	Hepatotoxic Profile of GS-5734 and its Metabolites in Rat, Monkey, and Human Hepatocytes Using the HμRELTox™ in vitro Platform
TX-399-2022	Evaluating the Toxicity of GS-5734, GS-441524, and GS-704277 in exVive3D™ Liver Tissues
PC-399-2028	Effect of GS-5734 on Cellular Respiration of Human Primary Hepatocytes
PC-399-2050	GS-5734 Effect on ROS level in HepG2 cells

BSEP = bile salt export pump; MRP2 = multidrug resistance-associated protein 2; MRP4 = multidrug resistance-associated protein 4; NTCP = sodium-taurocholate cotransporting polypeptide; ROS = reactive oxidative species

Data from the in vitro studies described above demonstrate that human hepatocytes may be susceptible to RDV-mediated toxicity, likely due to high cellular permeability and metabolic capacity to activate RDV. While GS-704277 and GS-441524 can be readily detected in plasma, these circulating metabolites are unlikely to contribute significantly to changes in liver enzymes observed in humans treated with repeated doses of RDV due to their minimal effects on hepatocytes demonstrated in several independent studies. Instead, the transaminase elevations observed following the administration of multiple doses of RDV to healthy human subjects may be related to direct liver exposure to the intact prodrug RDV. However, a specific mechanism of RDV-mediated transaminase elevations has not been identified. It should be noted that systemic clinical exposures to unbound RDV appear to be above the range of concentrations associated with in vitro hepatocyte cytotoxicity and are transient, lasting only for the duration of IV drug administration due to the rapid systemic clearance of RDV.

4.9.2.1. Human PK/Toxicity Modeling Using the DILIsym® Modeling Platform

Gilead engaged with DILIsym® Services, Inc. (DSSI) to assess the liver toxicity potential of RDV using the DILIsym® software platform {Yang 2018}. Combining mechanistic drug-induced liver injury (DILI) assay results (bile acid transporter inhibition, oxidative stress and mitochondrial toxicity) with physiologically based pharmacokinetic modeling-predicted liver concentrations for RDV and its metabolites, DILIsym (human SimPops v4A-1, n=285) predicted no ALT elevations for an RDV multiple-dose clinical protocol (1 hour IV infusion of 150 mg RDV once daily for 2 weeks). Dose escalation simulations showed that a 10-fold higher dose was required to elicit ALT elevations via inhibition of mitochondrial electron transport chain and bile acid transporters. At 10 × the clinical 150 mg dose, 11.2% (32 out of 285) and 1.4% (4 out of 285) of the simulated individuals showed peak serum ALT > 1 × the upper limit of normal (ULN) and ALT > 3 × ULN, respectively. These results suggest that clinically observed ALT increases in subjects administered RDV are unlikely to be due to mitochondrial electron transport chain inhibition and bile acid transporter inhibition, despite the in vitro assay signals. No elevations in liver biomarkers were predicted at the clinical dose of RDV in simulated populations (SimPops). Mechanisms other than mitochondrial toxicity, bile acid toxicity, or

oxidative stress might have contributed to the clinical elevation of liver transaminases following repeated dosing of RDV.

4.10. Formulation Excipient – SBECD

The vehicle used in the IV repeat-dose toxicity and DART studies and the hemolytic potential and plasma compatibility study contained 12% [w/v] SBECD in water, similar to the clinical formulation. The toxicity of SBECD has been well characterized in the RDV toxicology studies, and in several peer-reviewed publications {Hafner 2010, Luke 2010, Stella 2008}. There was no notable exacerbation of the SBECD-related effects when administered with RDV. Sulfobutylether β-cyclodextrin sodium is an approved excipient and used in several parenteral formulations, including 3 IV products (VFEND [voriconazole], Kyprolis [carfilzomib], and Nexterone [amiodarone]) and 2 intramuscular (IM) products (Geodon [ziprasidone mesylate] and Abilify [aripiprazole]) {Hanumegowda 2014}. The European Medicines Agency (EMA) has published a review summarizing the safety of cyclodextrins as excipients {Committee for Medicinal Products for Human Use (CHMP) 2014}, which indicates approximately 250 mg/kg/day of SBECD (~15 g/day based on a 60 kg human) for 6 months is safe in humans older than 2 years, although it was noted that SBECD is not indicated in Europe for newborn babies, infants under 2 years old, and patients with renal impairment. In addition, SBECD is listed in the European Pharmacopoeia {European Directorate for the Quality of Medicines & HealthCare (EDQM) 2015}, cited in the US FDA list of Inactive Pharmaceutic Ingredients {U. S. Food and Drug Administration 2015}, and generally considered safe.

5. INTEGRATED OVERVIEW AND CONCLUSIONS

5.1. Justification for Text in Labeling

The proposed Prescribing Information for RDV includes all relevant nonclinical safety findings. Based on findings in the nonclinical studies, the key safety points for consideration related to RDV include: use by women of childbearing potential during pregnancy and during lactation. In regard to these possible concerns, the following should be considered:

1) Animal data indicate that RDV does not cause reproductive or fetal toxicity.

2) Animal data indicate that RDV is nongenotoxic.

3) Long term carcinogenicity studies have not been conducted.

4) In animal studies, RDV and/or its metabolites were detected in the plasma of nursing rat pups likely due to the presence of RDV and/or its metabolites in milk, without effects on nursing pups.

5.2. Overall Conclusion

The pharmacologic basis for RDV for use in the treatment of COVID-19 is scientifically sound given the nonclinical in vitro and in vivo data. The absorption, distribution, metabolism, and excretion (ADME) and toxicologic profiles of RDV, are well characterized in multiple animal species. The overall nonclinical program supports the efficacy and safety of RDV based on the following considerations.

Remdesivir is a phosphoramidate prodrug of a novel adenine nucleoside analog that has broad spectrum antiviral activity against SARS-CoV-2 and the human pathogenic coronaviruses, MERS-CoV and SARS-CoV. The primary mechanism of inhibition by RDV is the incorporation of the triphosphate form of RDV into nascent RNA chains by the viral RNA-dependent RNA polymerase, causing delayed RNA chain termination during viral replication. Delayed chain termination has been shown to be the mechanism of action of RDV inhibition of the SARS-CoV-2, SARS-CoV, and MERS-CoV polymerases. In vitro resistance profiling of RDV in a representative coronavirus demonstrated a high barrier to resistance and identified two mutations in the viral polymerase at residues conserved across coronaviruses that conferred low-level (5.6-fold) reduced susceptibility to RDV. Remdesivir exhibits in vivo therapeutic efficacy against SARS-CoV-2 in rhesus monkeys and prophylactic and therapeutic efficacy MERS-CoV infection in rhesus monkeys as well as SARS-CoV and MERS-CoV infection in mice. In these animal studies, RDV treatment resulted in a significant reduction in clinical scores, signs of respiratory disease, and viral RNA levels compared to vehicle-treated animals.

Intracellular metabolism studies conducted in vitro illustrated effective activation of RDV in NHBE and Calu-3 (relevant cell types for SARS-CoV-2 infection). Additionally, high plasma stability observed in vitro in non-rodent species was confirmed in vivo where RDV demonstrated sufficient systemic exposure to load target tissues and cells supporting SARS-CoV-2 replication.

In vivo studies performed in the cynomolgus monkey also showed distribution of [^{14}C]RDV-equivalents to the lungs, which are a site of viral replication. Distribution studies confirmed rapid delivery and efficient formation of the active triphosphate metabolite in marmoset and African green monkey lungs following IV administration of RDV. Finally, extensive studies in nonhuman primates demonstrated the utility of monitoring the active triphosphate in PBMC as an accessible surrogate for exposure in target tissues. The half-life of the pharmacologically active triphosphate GS-443902 observed in vitro in NHBE (15 hours) and confirmed in vivo in marmoset lung and PBMC following IV administration (at least 22 hours) supports once-daily administration of RDV. Collectively, these results corroborate utility of RDV as a suitable prodrug for parenteral delivery.

Maximal concentrations and $AUC_{0\text{-}tau}$ values following RDV administration as a 30-minute IV infusion in healthy rhesus monkeys were compared to respective parameters in healthy humans to support the 200/100 mg RDV dose for clinical efficacy. Administration of repeated doses of 5 mg/kg RDV in rhesus monkeys produced a similar $AUC_{0\text{-}24}$ to repeated doses of 100 mg RDV in humans (1430 ng•hr/mL and 1585 ng•hr/mL, respectively). C_{max} values from repeated doses of 5 mg/kg RDV in rhesus monkeys were also similar to repeated doses of 100 mg RDV in humans (3350 ng/mL and 2230 ng/mL, respectively). Given that full PK characterization of the 30-minute infusion in monkey was only assessed at 5 mg/kg, data from IV bolus administration showing dose proportional increases in RDV exposure from 3 to 10 mg/kg support projection of the rhesus monkey efficacious dose of 10/5 mg/kg to the proposed 200/100 mg RDV clinical dose.

Remdesivir has a low potential for drug-drug interactions. Using the current FDA regulatory Guidelines, RDV at an initial IV dose of 200 mg and subsequent daily IV doses of 100 mg, would be predicted not to cause drug interactions by induction and not to inhibit CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, UGT1A1, P-gp, or BCRP. Inhibitory interactions with CYP3A, OATP1B1, and OATP1B3 are predicted. However, inhibition of these activities would be expected to be weak and transient due to the short clinical half-life (< 1 hour) of RDV. The once-daily administration and short duration of RDV treatment may allow for temporary dose modification of other drugs as needed.

In safety pharmacology studies, RDV had no clinically relevant effect on the central nervous, cardiovascular, or respiratory systems. Following dosing in rats, and cynomolgus and rhesus monkeys for up to 4 weeks, the kidney was identified as the only target organ of toxicity. Clinical chemistry, urinalysis, and/or urinary biomarkers were early predictors of the observed kidney changes. At higher doses, microscopic kidney changes were generally reversible. Remdesivir and GS-441524 exposures (AUC) at the NOAELs are below the predicted steady-state exposure in humans at 200/100 mg.

Remdesivir is nongenotoxic. In the reproductive and development toxicity studies, the only notable finding was a decrease in corpora lutea, a consequent decrease in implantation sites and viable embryos, and lower ovary and uterus/cervix/oviduct weights in the rat fertility study; these changes were only observed at a systemically toxic dose. There were no remarkable findings in male rats in the fertility study, no adverse findings in the developmental toxicity studies in rats and rabbits, and no adverse changes in the pre- and postnatal study in rats.

The nonclinical data support the favorable benefit/risk profile for the proposed use of RDV for the treatment of COVID-19.

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