SECTION 2.6.6—TOXICOLOGY WRITTEN SUMMARY

REMDESIVIR (GS-5734™)

Gilead Sciences

[redacted] **2020**

CONFIDENTIAL AND PROPRIETARY INFORMATION

TABLE OF CONTENTS

LIST OF IN-TEXT TABLES

LIST OF ABBREVIATIONS

AUC_{0-24}	the area under the concentration versus time curve
AUC_{x-xx}	partial area under the concentration versus time curve from time "x" to time "xx"
β2M	beta-2-microglobulin
BUN	blood urea nitrogen
Ces1c	carboxylesterase 1c
C_{max}	the maximum observed concentration of drug
COVID-19	coronavirus disease 2019
DART	developmental and reproductive toxicity
DMSO	dimethylsulfoxide
EVD	Ebola Virus Disease
FIH	first-in-human
g	gram
GD	gestation day
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
IC_{50}	50% inhibitory concentration
ICH	International Conference on Harmonisation
IM	intramuscular
IV	intravenous
kg	kilogram
KIM-1	kidney injury molecule-1
µg	microgram
µl	microliter
µm	micrometer
µM	micromolar
mg	milligram
ml	milliliter
M	Molar
MERS	Middle East respiratory syndrome
mol	mole
NAG	n-acetyl-glucosaminidase
ng	nanogram
NOAEL	no-observable-adverse-effect level
po	oral
SARS	severe acute respiratory syndrome
SBECD	sulfobutylether-β-cyclodextrin sodium
SD	standard deviation

SE	standard error
TK	toxicokinetics

Abbreviations are in accordance with those accepted by *Toxicology and Applied Pharmacology*.

1. NOTE TO REVIEWER

Remdesivir (GS-5734) is a single diastereomer monophosphoroamidate prodrug of a nucleoside analog GS-441524. Early studies relevant for remdesivir have been conducted with the diastereomeric mixture GS-466547 (approximately 1:1 mixture of remdesivir and its diastereomer at phosphorous). Based on antiviral activity, as well as in vitro and in vivo pharmacokinetic profile, a single diastereoisomer (remdesivir) was selected for further development. In these studies, the isomer remdesivir performed similarly to the mixture GS-466547, and results generated with both remdesivir and GS-466547 are presented in this document as they are considered relevant. To aid the reviewer, Table 1 lists the current nomenclature for remdesivir, 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 at phosphorous containing remdesivir	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-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 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 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 that inhibits viral RNA polymerases and 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.

The nonclinical safety profile of remdesivir has been characterized in repeat dose intravenous (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 (Table 2). 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 International Conference on Harmonization (ICH), Organization for Economic Co-Operation and Development (OECD), and national regulations (United States Food and Drug Administration [US FDA], and European Community Directives). All pivotal studies were conducted in accordance with US FDA or OECD Good Laboratory Practice (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.

Table 2. Remdesivir Toxicology Program

Study Type and Duration	Route of Administration	GLP Status	Species
Repeat-Dose Toxicity			
7 day with 10 day recovery	IV (Slow Bolus)	non-GLP	Rhesus Monkey
2 weeks with 4 week recovery	IV (Slow Bolus)	GLP	Rat/Cynomolgus Monkey
4 weeks	IV (Slow Bolus)	GLP	Rat/Cynomolgus Monkey
Genotoxicity			
In vitro reverse mutation assay	In Vitro	GLP	Bacteria (Salmonella typhimurium, Escherichia coli)
In vitro chromosome aberration assay	In Vitro	GLP	Human lymphocytes
In vivo micronucleus assay	IV (Slow Bolus)	GLP	Rat
Reproductive and Developmental Toxicity			
Fertility and early embryonic development	IV (Slow Bolus)	GLP	Rat
Embryo-fetal development	IV (Slow Bolus)	GLP	Rat/Rabbit
Prenatal and postnatal development, including maternal function	IV (Slow Bolus)	GLP	Rat
Local Tolerance			
Dermal irritation	In Vitro	GLP	Human epidermis
Eye irritation	In Vitro	GLP	Bovine
Mechanistic Studies	In Vitro	non-GLP	Rat, Monkey, Human hepatocytes
Impurities			
2 weeks	IV (Slow Bolus)	GLP	Cynomolgus Monkey
In vitro reverse mutation assay	In Vitro	GLP	Bacteria (Salmonella typhimurium, Escherichia coli)
Other Studies			
1 week toxicity	IV (Slow Bolus)	non-GLP	Rats, Rabbits
1 week toxicity	SC	non-GLP	Mice
1 week toxicity	IM	non-GLP	Cynomolgus Monkey
Hemolytic Potential and Plasma Compatibility	In Vitro	GLP	Rat, Monkey and Human whole blood and plasma

IM = intramuscular; IV = intravenous

Absorption, distribution, and metabolism studies support the selection of Wistar-Han rat and cynomolgus monkey for the repeat dose toxicology assessment of remdesivir, and the rat and rabbit for the assessment of reproductive and developmental toxicity. Rat, monkey and rabbit all formed the intermediate metabolite GS-704277 and the nucleoside metabolite GS-441524. GS-441524 is the predominant metabolite observed in all nonclinical studies, and in humans. As expected for a monophosphoroamidate prodrug that is rapidly cleaved by esterases in the plasma of rodents, low levels of remdesivir were detected in the systemic circulation of rodents after dosing, and dose-response relationships were based on the toxicokinetics (TK) of the parent nucleoside, GS-441524. Consistent with its intended route of administration in humans, repeat-dose toxicity studies were conducted by the IV route of administration. The vehicle used in the toxicology program was 12% [w/v] sulfobutylether-β-cyclodextrin (SBECD) in sterile water for injection, pH 3.5 ± 0.1, similar to the vehicle used in the clinical studies.

Following daily IV 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. In rats and cynomolgus monkeys, microscopic kidney changes were reversible. There were no liver changes in rats or monkeys based on clinical chemistry parameters, liver weights, or microscopic observations. Remdesivir and GS-441524 exposures (AUC) at the no observed adverse effect levels (NOAELs) in the 4-week GLP studies are below the predicted steady-state exposure in humans at the recommended 200 mg dose. The sensitivity of the rat to the renal effects of remdesivir may be related to the active tubular transport of remdesivir metabolites by rat renal organic anion transporter 3 (OAT3); this interaction has not been detected with human renal OAT3. In clinical studies, no evidence of nephrotoxicity has been observed with single doses of remdesivir up to 225 mg or multiple once-daily doses of remdesivir 150 mg for up to 14 days.

Remdesivir is nongenotoxic. 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. In the rat fertility and early embryonic development study, there was a decrease in corpora lutea, a consequent decrease in implantation sites and viable embryos, and lower ovary and uterus/cervix/oviduct weights; these changes were observed at a systemically toxic dose level.

The remdesivir nonclinical toxicology studies provide an adequate basis to evaluate potential toxicity of remdesivir, and for comparing and interpreting results from clinical studies. All information from the nonclinical toxicology studies that is of relevance to the prescriber and patient has been included in the proposed prescribing information. The nonclinical toxicological assessment of remdesivir supports its use for the treatment of patients with COVID-19.

3. SINGLE DOSE TOXICITY

No single-dose toxicity studies with remdesivir have been conducted. In single-dose pharmacokinetic studies, remdesivir 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. REPEAT DOSE TOXICITY

All GLP repeat-dose toxicity studies with remdesivir utilized IV injection (slow bolus over 1-2 minutes), similar to the clinical route of administration (IV infusion for 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. Dose levels in the 2-week GLP studies were based on single-dose intravenous pharmacokinetic studies in rats and cynomolgus monkeys (m2.6.4, Section 4.2), and target organ toxicity observed in a non-GLP repeat-dose 7-day IM study in cynomolgus monkeys (m2.6.7, Section 16, TX-399-2001). 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 the all studies, blood was collected for plasma analysis of remdesivir, the nucleoside metabolite, GS-441524, and the intermediate metabolite, GS-704277.

4.1. Rat

4.1.1. 2-Week Intravenous (Slow Bolus) Toxicity and Toxicokinetic Study with Remdesivir in Wistar-Han Rats with a 4 Week Recovery Phase and Bone Marrow Micronucleus Assay

Male and female Crl:WI (Han) rats (15/sex/group) were administered remdesivir via IV injection (slow bolus) once daily for 15 days at dose levels of 0 (saline), 0 (vehicle), 5, 20, or 50 mg/kg/day at a dose volume of 5 mL/kg (m2.6.7, Section 7.1, TX-399-2003). After 15 days of dosing, 10 animals/sex/group were necropsied; the remaining 5 animals/sex in the vehicle control and high-dose groups were retained for an additional 4 weeks to assess recovery from any observed effects. At the 2-week necropsy, bone marrow was extracted from 5 animals/sex/group for micronucleus evaluation. An additional group of animals was administered cyclophosphamide (30 mg/kg, positive control article for micronucleus testing) via oral gavage on Day 15.

Assessment of toxicity was based on mortality, clinical observations, body weights, body weight change, food consumption, ophthalmic examinations, and clinical (hematology and clinical chemistry on Day 16; urine chemistry, urine biomarkers, and urinalysis on Days 4, 7, and 16; cardiac Troponin I on Days 4 and 16) and anatomic pathology. Blood samples were collected on Days 1 and 15 from similarly dosed animals for TK evaluation of remdesivir (prodrug) and GS-441524 (metabolite), and liver samples were collected for hepatic microsomal analysis.

No remdesivir-related mortality, ophthalmic findings, or macroscopic findings were observed. Remdesivir-related clinical observations included red discoloration of the tail skin (injection sites) of males administered ≥ 20 mg/kg/day and females administered ≥ 5 mg/kg/day. In the absence of any correlating microscopic findings, these clinical observations were not considered adverse. The only notable change in microsomal enzyme analysis was an approximate 2.0-fold increase in CYP3A activity in males administered 20 mg/kg/day.

Decreases in body weight were noted in saline, vehicle, and remdesivir-dosed animals between Days 1 and 4, with larger decreases seen in animals administered 20 and 50 mg/kg/day. After Day 4, all groups gained body weight. Over the entire dosing phase, statistically significant decreases in mean body weight gain versus vehicle controls were considered adverse in males administered ≥ 5 mg/kg/day (approximately 18 to 66% decreases) and females administered ≥ 20 mg/kg/day (approximately 71%). Decreases in body weight gain were more pronounced during the first week of dosing than the second. Decreases in food consumption correlated with decreases in body weight gain. During the recovery period, high dose animals showed complete recovery from the body weight and food consumption changes.

The SBECD vehicle had no toxicologically relevant effects on hematology, coagulation, clinical chemistry, urinalysis, urine chemistry or urine biomarker results.

Remdesivir-related clinical and anatomic pathology effects indicated kidney injury and/or dysfunction. Some clinical pathology changes were present at all dose levels and were generally dose-dependent (Table 3). Some effects on urine chemistry and urinalysis did not persist to Day 15, and others were more pronounced at early test intervals (Day 4 and Day 7). These clinical pathology changes included increases in BUN; creatinine; ratios of urinary total protein, NAG, cystatin C, β2M, and KIM-1 to urinary creatinine; urine sodium, potassium, and chloride excretion; urine protein, blood, white blood cells, and granular casts. At 5 mg/kg/day, effects on urine total protein:urine creatinine ratio and urine NAG:urine creatinine ratio were of minimal to mild severity for males and, when present, only minimal severity for females; urine biomarker effects were of minimal severity for females and only slightly greater severity for males. At 50 mg/kg/day, the magnitude of the effect on urine total protein:urine creatinine ratio was much greater on Day 4 than on Days 7 or 16, and effects on urine biomarkers were greater on Day 4 than on Day 16.

The only remdesivir-related urine chemistry effect at 50 mg/kg/day that did not exhibit clear evidence of reversibility at the end of the recovery phase was minimally higher urine chloride excretion (+42%) for females. Although higher urine total protein:urine creatinine ratio for males exhibited clear evidence of reversibility, urine total protein:urine creatinine ratio remained mildly higher (+188%) at the end of the recovery phase for males administered 50 mg/kg/day and was consistent with the finding of increased incidence of positive urine protein using the reagent strip method. All remdesivir-related effects on urine biomarkers were reversed at the end of the recovery phase.

Table 3. Selected Urine Chemistry and Biomarker Findings in Rats Administered Remdesivir for 2-Weeks with a 4-Week Recovery Period

	Males					Females				
	Saline	Vehicle				Saline	Vehicle			
Remdesivir Dose (mg/kg/day)	**0**	**0**	**5**	**20**	**50**	**0**	**0**	**5**	**20**	**50**
Day 4										
Total protein:creatinine ratio	0.53	0.69†	1.23*	7.14*	12.58*	0.42	0.41	0.46	1.51*	6.05*
% difference from control	-23	—	+78	+935	+1723	+2	—	+12	+268	+1376
NAG:creatinine ratio	0.7	0.6	0.7	1.3*	2.5*	0.5	0.5	0.6	0.8*	1.4*
% difference from control	+17	—	+17	+117	+317	0	—	+20	+60	+180
Cystatin C:creatinine ratio	2.3	2.4	5.3*	107.8*	725.9*	0.9	1.0	1.7*	8.4*	134.0*
multiple of control mean	0.96×	—	2.2×	44.9×	302.5×	0.9×	—	1.7×	8.4×	134.0×
β2M:creatinine ratio	51.16	77.19	205.25*	1253.53*	2524.52*	13.21	11.16†	15.82*	160.32*	1510.63*
multiple of control mean	0.66×	—	2.7×	16.2×	32.7×	1.2×	—	1.4×	14.4×	135.4×
KIM-1:creatinine ratio	0.015	0.014	0.035*	0.284*	0.869*	0.013	0.011	0.019*	0.218*	0.825*
multiple of control mean	1.1×	—	2.5×	20.3×	62.1×	1.2×	—	1.7×	19.8×	75.0×
Day 16										
Total protein:creatinine ratio	0.65	0.90	2.96*	3.41*	3.77*	0.35	0.32	0.44*	0.56*	0.78*
% difference from control	-28	—	+229	+279	+319	+9	—	+38	+75	+144
NAG:creatinine ratio	0.4	0.3	0.6*	0.9*	1.4*	0.4	0.3	0.4*	0.5*	0.7*
% difference from control	+33	—	+100	+200	+367	+33	—	+33	+67	+133
Cystatin C:creatinine ratio	1.7	1.6	21.2*	70.2*	263.5*	0.8	0.7	2.3*	10.9*	28.1*
multiple of control mean	1.1×	—	13.3×	43.9×	164.7×	1.1×	—	3.3×	15.6×	40.1×
β2M:creatinine ratio	52.89	61.49	565.40*	960.22*	1043.41*	11.25	10.69	22.69*	328.49*	446.29*
multiple of control mean	0.86×	—	9.2×	15.6×	17.0×	1.1×	—	2.1×	30.7×	41.7×
KIM-1:creatinine ratio	0.010	0.007†	0.013*	0.063*	0.138*	0.009	0.008	0.009*	0.034*	0.086*
multiple of control mean	1.4×	—	1.9×	9.0×	19.7×	1.1×	—	1.1×	4.3×	10.8×
Recovery Day 29										
Total protein:creatinine ratio	—	0.67	—	—	1.93	—	0.27	—	—	0.32

	Males					Females				
	Saline	Vehicle				Saline	Vehicle			
Remdesivir Dose (mg/kg/day)	**0**	**0**	**5**	**20**	**50**	**0**	**0**	**5**	**20**	**50**
% difference from control	—	—	—	—	+188	—	—	—	—	+19
NAG:creatinine ratio	—	0.3	—	—	0.3	—	0.3	—	—	0.3
% difference from control	—	—	—	—	0	—	—	—	—	0
Cystatin C:creatinine ratio	—	1.2	—	—	2.2	—	0.9	—	—	0.6
multiple of control mean	—	—	—	—	1.8×	—	—	—	—	0.67×
β2M:creatinine ratio	—	32.51	—	—	64.61	—	7.94	—	—	7.00
multiple of control mean	—	—	—	—	2.0×	—	—	—	—	0.88×
KIM-1:creatinine ratio	—	0.005	—	—	0.005	—	0.005	—	—	0.006
multiple of control mean	—	—	—	—	1.0×	—	—	—	—	1.2×

+/- = Higher/Lower; — = Not applicable
* = Statistically significant at $p \leq 0.05$ versus vehicle control group
† = Statistically significant at $p \leq 0.05$ versus saline control group
Note: Results presented are group mean values. Ratios are expressed as urine analyte:urine creatinine.

Remdesivir-related microscopic findings at the terminal sacrifice were limited to the kidney and included a spectrum of degenerative, necrotic, and regenerative changes of the renal tubular epithelium in the cortex (Table 4). Dose-dependent increases in the incidence and severity of minimal to marked regenerative changes (i.e., basophilic tubules and increased mitosis) occurred with increased incidence and severity in males compared with females at 5 mg/kg/day and correlated with increased kidney weights in animals administered 50 mg/kg/day. Minimal focal/multifocal tubular degeneration, affecting no more than 5% of renal cortical tubules, was seen in 3 males and 1 female administered 50 mg/kg/day. Basophilic tubules and increased mitosis likely represented a reparative response of the tubular epithelium. At the recovery sacrifice, remdesivir-related degenerative/necrotic and regenerative changes in the kidney were fully reversible with the exceptions of minimal basophilic (regenerative) tubules in males only and increased kidney weights in females that lacked microscopic correlates. The reversibility of these remdesivir-related changes correlated with clinical chemistry and urinalysis findings. At the recovery sacrifice, an increased incidence and severity of minimal to slight chronic progressive nephropathy was noted in males. This likely represented a remdesivir-related exacerbation or accelerated onset of this spontaneously occurring degenerative change commonly seen in male rats and is of limited relevance to humans. Remdesivir-related microscopic findings were not evident at the injection site.

Vehicle-related (SBECD) microscopic findings included diffuse tubule cell vacuolation and focal/multifocal tubule cell hypertrophy in the kidney (Table 4). These findings were present in vehicle-dosed controls and all remdesivir-dosed groups, and were partially to fully reversible after a 4-week recovery phase, with the exception of slightly increased severity of tubule cell hypertrophy in males that previously received 50 mg/kg/day remdesivir. These vehicle-related changes were not considered adverse.

Table 4. Summary Incidence and Severity of Selected Microscopic Findings in the Kidney of Rats Administered Remdesivir for 2-Weeks with a 4-Week Recovery Period

	Males					Females				
	Saline	Vehicle				Saline	Vehicle			
Remdesivir Dose (mg/kg/day)	**0**	**0**	**5**	**20**	**50**	**0**	**0**	**5**	**20**	**50**
Terminal Necropsy										
No. Examined	10	10	10	10	10	10	10	10	10	10
Basophilic tubules										
Total number affected	0	0	10	10	10	0	0	6	10	10
Minimal	0	0	0	0	0	0	0	5	0	0
Slight	0	0	6	0	0	0	0	1	2	0
Moderate	0	0	4	9	6	0	0	0	8	6
Marked	0	0	0	1	4	0	0	0	0	4
Mitosis, increased										
Total number affected	0	0	8	7	9	0	0	2	10	9
Minimal	0	0	8	7	7	0	0	2	10	8
Slight	0	0	0	0	2	0	0	0	0	1
Degeneration, tubule, focal/multifocal										
Total number affected	0	0	0	0	3	0	0	0	0	1
Minimal	0	0	0	0	3	0	0	0	0	1
Vacuolation, tubule cell, diffuse										
Total number affected	0	10	10	10	10	0	10	10	10	10
Minimal	0	2	2	3	4	0	3	2	3	4
Slight	0	8	8	6	6	0	7	8	7	6
Moderate	0	0	0	1	0	0	0	0	0	0
Hypertrophy, tubule cell, focal/multifocal										
Total number affected	0	2	0	3	5	0	2	3	0	5
Minimal	0	2	0	2	4	0	2	3	0	4
Slight	0	0	0	1	1	0	0	0	0	1

	Males					Females				
	Saline	Vehicle				Saline	Vehicle			
Remdesivir Dose (mg/kg/day)	**0**	**0**	**5**	**20**	**50**	**0**	**0**	**5**	**20**	**50**
Recovery Necropsy										
No. Examined	—	5	—	—	5	—	5	—	—	5
Basophilic tubules, diffuse	—		—	—		—		—	—	
Total number affected	—	0	—	—	2	—	0	—	—	0
Minimal	—	0	—	—	2	—	0	—	—	0
Nephropathy, chronic progressive	—		—	—		—		—	—	
Total number affected	—	3	—	—	5	—	2	—	—	3
Minimal	—	3	—	—	3	—	2	—	—	3
Slight	—	0	—	—	2	—	0	—	—	0
Vacuolation, tubule cell, diffuse	—		—	—		—		—	—	
Total number affected	—	2	—	—	0	—	4	—	—	0
Minimal	—	2	—	—	0	—	4	—	—	0
Hypertrophy, tubule cell, focal/multifocal	—		—	—		—		—	—	
Total number affected	—	2	—	—	5	—	1	—	—	4
Minimal	—	2	—	—	2	—	1	—	—	4
Slight	—	0	—	—	3	—	0	—	—	0

— = Not applicable

Remdesivir did not induce increases in micronucleated PCEs, and therefore, was considered negative in the rat bone marrow micronucleus assay.

Plasma concentrations of remdesivir were only measurable at 50 mg/kg/day on Days 1 and 15, and in females at 20 mg/kg/day on Day 15. No accumulation of remdesivir was observed after multiple doses of 50 mg/kg/day. Exposure to the nucleoside metabolite, GS-441524, increased with the increase in remdesivir dose from 5 to 50 mg/kg/day (Table 5). Sex-based differences were less than 2-fold in GS-441524 C_{max} and AUC_{0-24} values. In general, the increases in C_{max} and AUC_{0-24} were approximately dose proportional between the 5 to 50 mg/kg/day dose levels. Slight accumulation (approximately 1.3- to 3.8-fold) of GS-441524 was observed after multiple doses of remdesivir. As expected, due to the high esterase levels in rats, remdesivir was rapidly and extensively converted to GS-441524.

Table 5. Toxicokinetic Parameters for GS-441524 in Rats Administered Remdesivir for 2-Weeks

Remdesivir (mg/kg/day)	Sex	AUC_{0-24} (ng•h/mL)		C_{max} (ng/mL)	
		Day 1	Day 15	Day 1	Day 15
5	Male	1190	1880	315	371
	Female	942	1420	294	324
	Combined-sex	1080	1650	295	347
20	Male	4560	10000	1240	1760
	Female	3350	12700	879	1690
	Combined-sex	3950	11400	1040	1520
50	Male	12900	21700	2750	3560
	Female	14300	19000	2170	2900
	Combined-sex	13500	20300	2360	3230

In conclusion, once daily remdesivir administration via intravenous injection to male and female rats for 15 days at 5, 20, and 50 mg/kg/day resulted in decreases in body weight gain and food consumption, and clinical pathology and microscopic findings indicative of kidney injury and/or dysfunction in males administered ≥ 5 mg/kg/day and in females administered ≥ 20 mg/kg/day. Effects on body weight gain and food consumption, and clinical pathology and microscopic findings were reversible after a 4-week recovery period. The NOAEL for remdesivir was not identified for males, and was 5 mg/kg/day for females. In animals administered 5 mg/kg/day, GS-441524 exposures on Day 15 were: AUC_{0-24} 1880 ng•h/mL and C_{max} 371 ng/mL (males) and AUC_{0-24} 1420 ng•h/mL and C_{max} 324 ng/mL (females).

4.1.2. 4-Week Intravenous (Slow Bolus) Toxicity and Toxicokinetic Study with Remdesivir in Wistar-Han Rats

Male and female Crl:WI(Han) rats (10/sex/group) were administered remdesivir via IV (slow bolus) injection for at least 4 weeks at dose levels of 0 (vehicle), 1, 3, or 10 mg/kg/day at a dose volume of 5 mL/kg (m2.6.7, Section 7.2, TX-399-2016). A satellite group of animals (3/sex in the vehicle control group, 6/sex/treatment group) were included for toxicokinetic evaluation on Day 1 and during Week 4. Assessment of toxicity was based on mortality, clinical observations, body weight, body weight change, food consumption, ophthalmic examinations, and clinical (including urine biomarkers) and anatomic pathology. Blood samples were collected for toxicokinetic evaluations of remdesivir and its major metabolites, GS-441524 and GS-704277.

Two toxicity females administered 10 mg/kg/day died at an unscheduled interval. One female was found dead shortly following dosing on Day 14 and the other was sacrificed in moribund condition prior to dosing on Day 19. Clinical observations prior to death included one or more of the following: pale body and/or ears and eyes, labored respiration, piloerection, ataxia, tremors or convulsions. No prior remarkable clinical observations were noted for either animal, and both had been gaining weight up through the most proximate scheduled body weight collection. The animal sacrificed in a moribund condition had no remdesivir-related hematology or clinical chemistry changes, and none of the test results indicated a reason for its poor condition. At necropsy, no macroscopic findings were noted for either animal. However, both animals exhibited remdesivir-related microscopic findings in the renal cortex, which were noted in other animals at the terminal sacrifice. Cause of death or moribund condition for these animals could not be determined and the deaths were considered of uncertain relationship to remdesivir.

In animals that survived to the terminal sacrifice there were no remdesivir-related clinical, ophthalmic, or macroscopic observations.

Remdesivir-related adverse effects on body weight and food consumption were observed in animals administered 10 mg/kg/day. On Day 29, mean body weights were decreased 12% and 9% and mean body weight changes from Days 1 through 29 were decreased 33% and 35% compared to controls for males and females, respectively. Decreased mean food consumption corresponded with decreased mean body weight and body weight changes.

Remdesivir administration at a dose of 1 mg/kg/day had no effect on clinical pathology test results. Remdesivir-related clinical pathology effects at a dose of ≥ 3 mg/kg/day indicated kidney injury and/or dysfunction (Table 6). These effects included increases in the following urine creatinine ratios: total protein, urine n-acetyl-beta-glucosaminidase (NAG), urine cystatin C, urine beta-2-microglobulin, and urine kidney injury marker-1 (KIM-1) in males administered ≥ 3 mg/kg/day and females administered 10 mg/kg/day. Additional changes included increased serum creatinine in animals administered 10 mg/kg/day; higher urine sodium and chloride excretion and increased incidence and/or severity of positive urine protein and glucose (reagent strip methodology) in males administered 10 mg/kg/day. The effects appeared dose dependent, were larger in males and usually most pronounced on Day 30, and were generally consistent with the microscopic appearance of the kidney at the end of the dosing phase.

Table 6. Urine Chemistry Findings in Rats Administered Remdesivir for 4-Weeks

	Males				Females			
Remdesivir Dose (mg/kg/day)	**0**	**1**	**3**	**10**	**0**	**1**	**3**	**10**
Day 4								
Total protein:creatinine ratio	0.53	0.54	0.72	0.93*	0.41	0.50	0.39	0.48
% difference from control	—	+2	+36	+75	—	+22	-5	+17
NAG:creatinine ratio	0.5	0.6	0.7*	0.8*	0.6	0.6	0.5	0.6
% difference from control	—	+20	+40	+60	—	0	-17	0
Sodium excretion (mmol)	0.56	0.67	0.68	0.82*	0.57	0.48	0.46	0.48
% difference from control	—	+20	+21	+46	—	-16	-19	-16
Chloride excretion (mmol)	0.36	0.42	0.42	0.45	0.32	0.28	0.25	0.24
% difference from control	—	+17	+17	+25	—	-13	-22	-25
Day 30								
Total protein:creatinine ratio	0.98	1.06	1.95*	3.08*	0.36	0.30	0.47	0.63*
% difference from control	—	+8	+99	+214	—	-17	+31	+75
NAG:creatinine ratio	0.3	0.3	0.5*	0.7*	0.3	0.4	0.3	0.4
% difference from control	—	0	+67	+133	—	+33	0	+33
Sodium excretion (mmol)	0.47	0.43	0.45	0.74*	0.35	0.38	0.30	0.32
% difference from control	—	-9	-4	+57	—	+9	-14	-9
Chloride excretion (mmol)	0.25	0.26	0.27	0.39	0.24	0.19	0.17	0.15
% difference from control	—	+4	+8	+56	—	-21	-29	-38

+/- = Higher/Lower; — = Not applicable
* = Statistically significant at $p \leq 0.05$ versus control group
Note: Results presented are group mean values. Ratios are expressed as urine analyte:urine creatinine.

Remdesivir-related microscopic findings at the terminal euthanasia were limited to the cortex of the kidney in males administered ≥ 3 mg/kg/day and females administered 10 mg/kg/day and were consistent with a regenerative process secondary to a sustained, low-level injury to the cortical tubules; findings included basophilic tubules and karyomegaly (Table 7). These findings were associated with increased absolute and/or relative kidney weights for males. Based on the nature of the change and the severity of the findings, the changes in the kidney were considered not adverse.

Vehicle control article-related microscopic findings occurred in the urinary tract, spleen, lymph nodes, adrenal cortex, liver, and stifle joint of animals administered vehicle control article alone or with remdesivir.

Table 7. Summary Incidence and Severity of Remdesivir-Related Microscopic Findings in the Kidney of Rats Administered Remdesivir for 4-Weeks

Remdesivir Dose (mg/kg/day)	Males				Females			
	0	1	3	10	0	1	3	10
Kidney								
No. Examined	10	10	10	10	10	10	10	8
Basophilic tubules								
Total number affected	0	0	10	10	0	0	0	8
Minimal	0	0	10	1	0	0	0	1
Slight	0	0	0	9	0	0	0	7
Karyomegaly								
Total number affected	0	0	10	10	0	0	0	8
Minimal	0	0	10	6	0	0	0	8
Slight	0	0	0	4	0	0	0	0

Remdesivir was rapidly cleared and extensively metabolized to GS-441524 and GS-704277 in rats following intravenous administration. Exposure to GS-441524 increased with the increase in remdesivir dose level from 1 to 10 mg/kg/day (Table 8). The increases in GS-441524 C_{max} and AUC_{0-24} values were generally greater than dose proportional on Days 1 and 28, with differences generally within 2-fold. Sex-based differences were generally less than 2-fold in GS-441524 C_{max} and AUC_{0-24} values. No accumulation of GS-441524 was observed after multiple doses of remdesivir in rats. Exposure to GS-704277 increased with the increase in remdesivir dose level from 1 to 10 mg/kg/day (Table 9).The increases in GS-704277 C_{max} and AUC_{0-24} values were, in general, approximately dose proportional between the 3 and 10 mg/kg/day remdesivir dose levels on Day 1, and slightly greater than dose proportional between 1 and 10 mg/kg/day on Day 28. Males had higher GS-704277 C_{max} and AUC_{0-24} values than females, with sex-based differences generally greater than 2-fold for AUC_{0-24} values. No accumulation of GS-704277 was observed after multiple doses of remdesivir in rats.

Table 8. Toxicokinetic Parameters for GS-441524 in Rats Administered Remdesivir for 4-Weeks

Remdesivir (mg/kg/day)	Sex	AUC_{0-24} (ng•h/mL)		C_{max} (ng/mL)	
		Day 1	Day 28	Day 1	Day 28
1	Male	151	209	44.1	50.1
	Female	121	140	36.0	38.8
	Combined-sex	136	190	40.0	44.5
3	Male	473	1000	124	208
	Female	419	493	115	127
	Combined-sex	446	748	119	156
10	Male	1880	4210	448	754
	Female	1700	2940	397	521
	Combined-sex	1790	3570	423	615

Table 9. Toxicokinetic Parameters for GS-704277 in Rats Administered Remdesivir for 4-Weeks

Remdesivir (mg/kg/day)	Sex	AUC_{0-24} (ng•h/mL)		C_{max} (ng/mL)	
		Day 1	Day 28	Day 1	Day 28
1	Male	NR	82.3	160	213
	Female	NR	NR	139	130
	Combined-sex	NR	72.0	150	171
3	Male	292	424	526	1020
	Female	141	167	388	288
	Combined-sex	209	301	457	654
10	Male	782	2150	2190	4210
	Female	676	904	1550	2040
	Combined-sex	729	1530	1870	3130

NR= Not reported

In conclusion, once daily remdesivir administration via IV injection to male and female rats for 29 days resulted in clinical pathology and microscopic findings indicative of kidney injury and/or dysfunction in animals administered ≥ 3 mg/kg/day, and unscheduled deaths of 2 females and decreased body weight gain and food consumption at 10 mg/kg/day. Based on the nature and severity of the kidney changes the NOAEL for remdesivir is 3 mg/kg/day (Day 28 GS-441524 C_{max} and AUC_{0-24}: 208 and 127 ng/mL and 1000 and 493 ng•h/mL in males and females, respectively; GS-704277 C_{max} and AUC_{0-24}: 1020 and 288 ng/mL and 424 and 167 ng•h/mL in males and females, respectively).

4.2. Monkey

4.2.1. 7-Day Intravenous (Slow Bolus) Toxicity Study of Remdesivir in Male Rhesus Monkeys with a 10-Day Recovery Period (non-GLP)

Male Indian-origin rhesus monkeys (6/group in the vehicle control and high-dose groups and 3/group in the low- and mid-dose groups) were administered remdesivir via IV injection (slow bolus) at dose levels of 0 (vehicle), 5, 10, and 20 mg/kg/day at a dose volume of 2 mL/kg (m2.6.7, Section 6, TX-399-2021). [Note: Indian-origin Rhesus monkeys were specifically used in this study to support MERS-CoV efficacy studies (m2.6.2, Section 3.2.4.2.)] Parameters evaluated included mortality, clinical observations, body weight, qualitative food consumption, clinical pathology (hematology, coagulation, serum chemistry, and urinalysis), anatomic pathology (macroscopic and microscopic examination), including organ weights. Blood samples were collected on Days 0 and 6 for toxicokinetic evaluation of remdesivir and metabolites GS-441524 and GS-704277.

Remdesivir-related morbidity and subsequent early euthanasia was noted in a single 20 mg/kg/day group male on Day 6. The cause of morbidity was attributed to remdesivir-related kidney findings (marked tubular atrophy with slight tubular basophilia and casts in the kidney). Clinical observations noted prior to early euthanasia included hypoactivity, labored respiration, partial closure of the eye(s), dermal atonia, red mucoid feces, decreased body temperature and red or brown material around the anogenital area. Microscopically, this animal also had ulcerations or necrosis in the stomach, jejunum, colon, and rectum, and decreased cellularity of the lymphoid follicles in the spleen.

No remdesivir-related clinical findings were noted for the surviving animals or effects on food consumption. Nonadverse, remdesivir-related slight body weight losses and lower body weight gains were noted at ≥ 10 mg/kg/day groups during the dosing period. These slight body weight changes showed improvement during the recovery period.

The most notable clinical pathology changes indicated kidney injury and/or dysfunction. These effects were dose dependent, and correlated with histopathology findings of renal tubular atrophy and basophilia and casts. Increased mean urea nitrogen was noted at 20 mg/kg/day at Day 4 and at ≥ 5 mg/kg/day at Day 6, and increased mean creatinine was noted at 20 mg/kg/day at Day 2, ≥ 10 mg/kg/day at Day 4 and ≥ 5 mg/kg/day at Day 6. Decreased mean albumin concentrations, potentially related to urinary loss of albumin, with consequently decreased total protein was noted at 20 mg/kg/day at Day 6. Clinical pathology alterations related to acid base imbalance or altered renal ability to maintain electrolyte balance included increased mean chloride at 20 mg/kg/day at Days 4 and 6, and decreased pH at 20 mg/kg/day at Day 6. Inadequately concentrated urine was noted in a few or all animals at 20 mg/kg/day at Days 2 and ≥ 5 mg/kg/day at Day 6, with increased urine volume noted in a few animals at 10 mg/kg/day at Day 6 and 20 mg/kg/day at Days 2 and 6. Positive protein reaction was noted at ≥ 10 mg/kg/day at Days 2 and/or 6. Inadequately concentrated urine and increased urine volume were still noted at 20 mg/kg/day at Day 16, indicating lack of recovery.

Alterations potentially related to stress included increased mean absolute neutrophil and monocyte counts at 20 mg/kg/day at Day 6 and decreased absolute lymphocyte count at 20 mg/kg/day at Days 4 and 6.

Clinical pathology alterations of uncertain mechanisms included increased mean PT at 10 mg/kg/day at Days 4 and 6 and increased mean PT and APTT and decreased mean fibrinogen at 20 mg/kg/day at Days 2, 4, and 6; decreased mean cholesterol at ≥ 5 mg/kg/day at Days 2, 4, and 6; and decreased mean phosphorus at 20 mg/kg/day at Days 4 and 6 and at 10 mg/kg/day at Day 6.

Remdesivir-related clinical pathology changes noted at 20 mg/kg/day during the recovery phase (Day 9 and/or 16) included decreased mean absolute lymphocyte count, fibrinogen, cholesterol, albumin, total protein, and phosphorus and increased PT, APTT, urea nitrogen, and creatinine with inadequately concentrated urine and increased urine volume.

Remdesivir-related macroscopic findings at the primary necropsy included discoloration or pale kidneys in a single 20 mg/kg/day group animal that correlated microscopically to tubular atrophy and basophilia, and a small thymus in a single 10 mg/kg/day group animal which correlated microscopically to mild decreased lymphocytes in the cortex of the thymus. Pale kidneys in one 20 mg/kg recovery animal correlated microscopically to moderate interstitial fibrosis. There were no remdesivir-related macroscopic findings noted at the recovery necropsy.

Remdesivir-related organ weight changes were limited to the primary necropsy and included increased kidney and spleen weights at ≥ 5 mg/kg/day.

Remdesivir-related microscopic findings at the primary necropsy included increased severity of tubular atrophy and basophilic tubules with casts in the kidneys at ≥ 5 mg/kg/day and decreased cellularity in the lymphoid follicles of the lymph nodes, spleen, and thymus at 20 mg/kg/day. At the recovery necropsy, remdesivir-related microscopic findings persisted in the kidneys and the thymus. The kidney findings indicating altered kidney function were considered adverse at all dose levels, as there was a loss of tubules and resulting interstitial fibrosis in one recovery animal suggestive of the finding progressing to chronicity.

Exposure to remdesivir increased with the increase in dosage level from 5 to 20 mg/kg/day (Table 10). The increases in C_{max} and AUC_{0-24} were generally greater than dose proportional between the 5 and 20 mg/kg/day. No accumulation of remdesivir was observed after multiple doses.

Exposure to GS-441524 and GS-704277 increased with the increase in remdesivir dose from 5 to 20 mg/kg/day (Table 10). The increases in C_{max} and AUC_{0-24} were approximately dose proportional between the 5 and 20 mg/kg/day on Day 0 for GS-441524 and GS-704277 and between the 5 and 10 mg/kg/day on Day 6 for GS-704277. The increases in C_{max} and AUC_{0-24} were greater than dose proportional between the 5 and 20 mg/kg/day on Day 6 for GS-441524 and between the 10 and 20 mg/kg/day on Day 6 for GS-704277. No accumulation of GS-441524 was observed after multiple doses of remdesivir in monkeys at 5 mg/kg/day; however, accumulation was observed at the 10 and 20 mg/kg/day. No accumulation of GS-704277 was observed after multiple doses of remdesivir in monkeys. The mean C_{max} and AUC_{0-24} metabolite

to parent ratios indicate that remdesivir was rapidly and extensively metabolized to GS-441524 and GS-704277 in monkeys following intravenous (slow bolus) injection administration of remdesivir.

Table 10. Toxicokinetic Parameters for Remdesivir, GS-441524, and GS-704277 in Male Rhesus Monkeys Administered Remdesivir for 7 Days

Remdesivir Dose (mg/kg/day)	AUC_{0-24} (ng•h/mL)[a]		C_{max} (ng/mL)[a]	
	Day 0	Day 6	Day 0	Day 6
Remdesivir				
5	361	653	389	537
10	815	2120	755	1380
20	2700	5600	2210	3960
GS-441524				
5	121	173	934	1390
10	282	512	2080	6050
20	600	1620	4550	21,000
GS-704277				
5	696	637	528	602
10	1090	1300	911	1500
20	2610	4910	2310	7890

In summary, IV administration of remdesivir to male rhesus monkeys at dosage levels of 5, 10, and 20 mg/kg/day for 7 days resulted in one 20 mg/kg/day animal euthanized early on Day 6. Findings considered adverse at all dose levels consisted of increased mean urea nitrogen and increased mean creatinine indicating altered kidney function, with correlating histopathology findings of renal tubular atrophy and basophilia and casts. The kidney findings were considered adverse at all dose levels as there was a loss of tubules and resulting interstitial fibrosis in one recovery animal suggestive of the finding progressing to chronicity. Based on these findings, a NOAEL cannot be assigned to any of the dose levels under the conditions of this study.

4.2.2. 2-Week Intravenous (Slow Bolus) Toxicity and Toxicokinetic Study with Remdesivir in Cynomolgus Monkeys with a 4 Week Recovery

Male and female cynomolgus monkeys (4-6 animals/sex/group) were administered remdesivir via IV (slow bolus) injection once daily for 15 days at dose levels of 0 (saline), 0 (vehicle), 1, 3, or 10 mg/kg/day at a dose volume of 2 mL/kg (m2.6.7, Section 7.3, TX-399-2004). On Day 16, 4 animals/sex/group were necropsied, and 2 animals/sex in the vehicle control article and 10 mg/kg/day remdesivir groups underwent 4 weeks of recovery.

Assessment of toxicity was based on mortality; clinical observations; body weights; body weight change; food consumption; ophthalmic examinations; electrocardiogram (ECG) examinations; hematology and coagulation on Days 7 and 13; D-dimer on Days 7 and 16; clinical chemistry, cardiac Troponin I, urine chemistry, urine biomarkers, and urinalysis on Days 4, 7, and 13; and anatomic pathology. Blood samples were collected on Days 1 and 15 from similarly dosed animals for TK evaluation of remdesivir and GS-441524 (metabolite), and liver samples were collected for hepatic microsomal analysis.

No remdesivir-related mortality; clinical observations; ophthalmic observations; or effects on body weights, qualitative food consumption, ECG parameters, clinical pathology parameters, organ weights, macroscopic findings, or microscopic findings were observed at any dose level. With the exception of increased CYP1A activity (approximately 2.3-fold) in females administered 10 mg/kg/day, no notable changes were observed in hepatic microsomal enzyme activities.

The only clinical chemistry finding considered remdesivir-related was mildly decreased cholesterol concentration on Days 4, 7, and 13 of the dosing phase for animals administered 10 mg/kg/day (-18 to -26% relative to the respective baseline mean closest to initiation of dosing). Although a mechanism for this minor effect was not apparent, it exhibited reversibility at the end of the recovery phase, and in the absence of correlative findings, was not considered adverse.

There were no remdesivir-related changes in serum chemistry (BUN and creatinine), urinalysis, urine chemistry or urinary biomarkers (total protein, creatinine, NAG, cystatin C, B2M, microalbumin, and KIM-1) to suggest an effect on kidney function or kidney injury. Of uncertain relationship to remdesivir was minimally decreased urine pH on Day 13 in animals administered 10 mg/kg/day (-6 to -9% relative to the respective baseline mean closest to initiation of dosing). At the end of the recovery phase, 2/4 recovery animals administered 10 mg/kg/day continued to have minimally decreased urine pH relative to baseline, but 3/4 recovery control animals also showed decreased urine pH, and no clear difference was noted between groups.

Vehicle-related (SBECD) microscopic findings included diffuse tubule cell vacuolation and focal/multifocal tubule cell hypertrophy in the kidney. These findings were present in all remdesivir-dosed groups and vehicle-dosed controls, and were partially to fully reversible after a 4-week recovery phase, with the exception of slightly increased severity of tubule cell hypertrophy in males that previously received 10 mg/kg/day remdesivir. These vehicle-related changes were not considered adverse.

Sex based differences were less than 2-fold in remdesivir and GS-441524 mean C_{max} and AUC_{0-24} values. Exposure to remdesivir and GS-441524 increased with the increase in remdesivir dose level from 1 to 10 mg/kg/day. The increases in remdesivir C_{max} and AUC_{0-24} were generally dose proportional between 1 and 3 mg/kg/day, and greater than dose proportional between 3 and 10 mg/kg/day (Table 11). The increases in GS-441524 C_{max} and AUC_{0-24} were generally dose proportional between the 1 to 10 mg/kg/day dose levels (Table 12). No accumulation of remdesivir or GS-441524 was observed after multiple doses of remdesivir in monkeys. Remdesivir was extensively converted to GS-441524 in monkeys following intravenous injection of remdesivir.

Table 11. Toxicokinetic Parameters for Remdesivir in Monkeys Administered Remdesivir for 2-Weeks

Remdesivir (mg/kg/day)	Sex	AUC_{0-24} (ng•h/mL)		C_{max} (ng/mL)	
		Day 1	Day 15	Day 1	Day 15
1	Male	76.7	88.7	82.1	81.7
	Female	92.7	111	100	96
	Combined-sex	84.7	99.8	91.2	88.9
3	Male	215	363	243	316
	Female	297	342	318	295
	Combined-sex	256	352	281	305
10	Male	1320	1720	1180	1490
	Female	1710	1780	1490	1510
	Combined-sex	1510	1750	1340	1500

Table 12. Toxicokinetic Parameters for GS-441524 in Monkeys Administered Remdesivir for 2-Weeks

Remdesivir (mg/kg/day)	Sex	AUC_{0-24} (ng•h/mL)		C_{max} (ng/mL)	
		Day 1	Day 15	Day 1	Day 15
1	Male	152	192	27.5	26.5
	Female	172	214	30.8	27.5
	Combined-sex	162	203	29.2	27
3	Male	730	804	107	100
	Female	547	577	90	81.6
	Combined-sex	638	690	98.4	90.8
10	Male	2310	2450	381	363
	Female	2350	2330	390	335
	Combined-sex	2330	2390	385	349

In conclusion, remdesivir administration once daily via IV injection to male and female cynomolgus monkeys for 15 days at 1, 3, and 10 mg/kg/day did not result in any adverse findings. Therefore, the NOAEL for remdesivir in monkeys is 10 mg/kg/day (Day 15 mean remdesivir AUC_{0-24}:1750 ng•h/mL and C_{max}: 1500 ng/mL; GS-441524 AUC_{0-24}: 2390 ng•h/mL and C_{max}: 349 ng/mL; combined sexes).

4.2.3. 4-Week Intravenous (Slow Bolus) Toxicity and Toxicokinetic Study with Remdesivir in Cynomolgus Monkeys

Male and female cynomolgus monkeys were assigned to 4 groups (4 animals/sex/group) and administered remdesivir via slow bolus IV injection once daily for 4 weeks at dose levels of 0 (vehicle), 1, 3, or 10 mg/kg/day at a dose volume of 2 mL/kg (m2.6.7, Section 7.4, TX-399-2017). On Day 29, all animals were necropsied. Assessment of toxicity was based on mortality, clinical observations, body weights, body weight change, food consumption, ophthalmic observations, electrocardiographic (ECG) measurements, and clinical and anatomic pathology. Blood samples were collected for toxicokinetic evaluations of remdesivir and its metabolites, GS-441524 and GS-704277.

All animals survived to their scheduled sacrifice. No remdesivir-related clinical observations or effects on body weight, food consumption, ophthalmoscopy, or ECGs were noted. The only clinical pathology findings considered remdesivir-related were mildly decreased cholesterol concentrations on Days 4 and 29 in females administered 10 mg/kg/day; the effect was not associated with correlative findings and was considered non-adverse. With one possible exception, hematology, coagulation, urinalysis, urine chemistry, and urine biomarker test results were unaffected by remdesivir administration. Minimally increased urine beta-2-microglobulin:creatinine ratio on Day 29 in females administered 10 mg/kg/day was considered of uncertain relationship to remdesivir administration because of its small magnitude of change and lack of correlative findings for other markers of renal integrity and function.

No remdesivir-related macroscopic findings, organ weight differences, or microscopic findings were noted in terminal sacrifice animals. A vehicle control article-related microscopic finding of vacuolation was observed in organs of the urinary tract, lymph nodes, and liver of animals in all groups, including control. The vehicle control article-related findings and the affected organs included tubule cell and transitional cell vacuolation in the kidney; transitional cell vacuolation in the urinary bladder, ureter, and urethra; infiltrates of vacuolated macrophages in the inguinal, mandibular, mesenteric, and popliteal lymph nodes; and Kupffer cell vacuolation in the liver.

All concentration values of remdesivir, GS-441524, and GS-704277 in the vehicle control group were below the lower limit of quantitation. Exposure to remdesivir, GS-441524, and GS-704277 increased with the increase in remdesivir dose level from 1 to 10 mg/kg/day (Table 13). The increases in remdesivir, GS-441524, and GS-704277 C_{max} and AUC_{0-24} were approximately dose proportional between the 1 and 10 mg/kg/day remdesivir dose levels. Sex-based differences were less than 2-fold in remdesivir, GS-441524, and GS-704277 C_{max} and AUC_{0-24} values. No accumulation of remdesivir, GS-441524, and GS-704277 was observed after multiple doses of remdesivir in monkeys. The mean AUC_{0-24} metabolite to parent ratios indicate that remdesivir was extensively metabolized to GS-441524 and GS-704277 in monkeys.

Table 13. Toxicokinetic Parameters for Remdesivir, GS-441524, and GS-704277 in Cynomolgus Monkeys Administered Remdesivir for 4-Weeks

Remdesivir Dose (mg/kg/day)	AUC_{0-24} (ng•h/mL)[a]		C_{max} (ng/mL)[a]	
	Day 1	Day 28	Day 1	Day 28
Remdesivir				
1	73.1	102	79.5	100
3	205	265	229	243
10	1050	1330	1020	1160
GS-441524				
1	167	190	27.4	28.9
3	424	443	65.6	66.9
10	1810	2070	292	288
GS-704277				
1	62.4	67.1	71.0	64.2
3	193	185	221	168
10	789	849	818	680

[a] Male and female, combined

In conclusion, administration of remdesivir to male and female cynomolgus monkeys was well tolerated as a daily IV slow bolus injection at a dose level up to 10 mg/kg/day for 4 weeks. No adverse test article-related findings were noted. The NOAEL is 10 mg/kg/day (Day 28 mean remdesivir combined sexes AUC_{0-24} of 1330 ng•h/mL and C_{max} of 1160 ng/mL; GS-441524 combined sexes AUC_{0-24} of 2070 ng•h/mL and C_{max} of 288 ng/mL; GS-704277 combined sexes AUC_{0-24} of 849 ng•h/mL and C_{max} of 680 ng/mL).

5. GENOTOXICITY

5.1. In Vitro

5.1.1. Bacterial Reverse Mutation Assay Plate Incorporation Method with Remdesivir

Remdesivir mutagenic potential was evaluated in a bacterial reverse mutation assay using histidine-dependent mutant strains of *Salmonella typhimurium* (*Salmonella*; TA98, TA100, TA1535, and TA1537), and the tryptophan-dependent *Escherichia coli* (*E. coli*) strain, WP2*uvr*A, in the presence or absence of an exogenous mammalian metabolic activation system (S9) using the plate incorporation methodology (m2.6.7, Section 8.1, TX-399-2005). Remdesivir was formulated in dimethylsulfoxide (DMSO), and evaluated in all five tester strains at doses up to 5000 µg/plate with and without S9 metabolic activation. Bacteria were also incubated with standard positive control agents, and the response of the various bacterial strains to these agents confirmed the sensitivity of the test system and the activity of the S9 mix.

No toxicity was observed at any tester strain at any tested dose level in the presence or absence of S9. There were no positive increases in the mean number of revertant colonies observed at any remdesivir dose level with any tester strain in the presence or absence of S9. Precipitate was observed at 5000 µg/plate in all the tester strains in the presence and absence of S9. Under the conditions of this study, remdesivir was considered negative in the bacterial reverse mutation assay.

5.1.2. Chromosomal Aberrations in Cultured Human Peripheral Blood Lymphocytes with Remdesivir

The objective of this *in vitro* assay was to evaluate the ability of remdesivir to cause structural chromosomal aberrations in cultured human lymphocytes with and without metabolic activation (m2.6.7, Section 8.2, TX-399-2006). In the initial test, DMSO vehicle or remdesivir (3.39 - 500 µg/mL) was evaluated for 3-hour treatment periods with and without metabolic activation and for an approximate 24 hour continuous treatment without metabolic activation. In the 3-hour treatment assay without metabolic activation, chromosomal aberrations were analyzed from the cultures treated with 117, 146, and 171 µg/mL. In the 24-hour treatment assay without metabolic activation, chromosomal aberrations were analyzed from the cultures treated with 41.2, 58.8, and 84.0 µg/mL. In the 3-hour treatment assay with metabolic activation, chromosomal aberrations were analyzed from the cultures treated with 120, 172, and 245 µg/mL.

There were no statistically significant or biologically relevant increases in the number of cells with chromosomal aberrations, polyploidy, or endoreduplication observed in the cultures analyzed under the 3 or 24-hour treatment without metabolic activation. In the 3-hour treatment with metabolic activation, there was a slight but statistically significant increase in cells with chromosome aberrations which was observed only at the high dose level of 245 µg/mL and inducing 48% toxicity. This increase was small, was within the historical control range and only observed at a high level of cytotoxicity. Hence, the response observed is considered equivocal and of questionable biological significance.

Treatment of cultures with the vehicle and positive control articles produced the expected response meeting acceptance criteria and validating test sensitivity.

In summary, remdesivir was considered negative for inducing chromosomal aberrations in cultured human lymphocytes when tested up to the limits of cytotoxicity in 3 and 24-hour test conditions without metabolic activation and equivocal for inducing chromosome aberrations in cultured human lymphocytes when tested up to the limits of cytotoxicity in the 3-hour treatment with metabolic activation.

5.2. In Vivo

5.2.1. 2-Week Intravenous (Slow Bolus) Toxicity and Toxicokinetic Study with Remdesivir in Wistar-Han Rats with a 4 Week Recovery Phase and Bone Marrow Micronucleus Assay

The objective of the micronucleus portion of the 2-week rat toxicity study was to evaluate remdesivir for *in vivo* clastogenic activity and/or disruption of the mitotic apparatus by counting micronuclei in polychromatic erythrocytes in male and female rat bone marrow following 2 weeks of intravenous (slow bolus) injection dosing of remdesivir at 0 (saline), 0 (vehicle), 5, 20 and 50 mg/kg/day (m2.6.7, Section 9.1, TX-399-2003). Femoral bone marrow was extracted and at least 2000 polychromatic erythrocytes (PCEs) per animal (5/sex/group) were analyzed for the frequency of micronuclei. Cytotoxicity was assessed by scoring the number of PCEs and normochromatic erythrocytes (NCEs) in at least 1000 total erythrocytes for each animal. The positive control, 30 mg/kg cyclophosphamide administered 24 hours prior to bone marrow harvest, induced increases in micronucleated PCEs confirming appropriate sensitivity of the test system.

Remdesivir was evaluated as negative in the male and female rat bone marrow micronucleus assay when administered intravenously at daily doses up to 50 mg/kg/day for 15 days. At 50 mg/kg/day, $AUC_{0\text{-}24}$ exposures on Day 15 were 284 ng•h/mL for remdesivir, and 20,300 ng•h/mL for GS-441524 (sexes combined).

6. CARCINOGENICITY

Carcinogenicity studies with remdesivir have not been conducted and are not considered necessary for the proposed indication with a dosing duration of less than 3 months (ICH S1A).

7. REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

7.1. Fertility and Early Embryonic Development

7.1.1. Intravenous (Slow Push) Study of Fertility and Early Embryonic Development to Implantation of Remdesivir in Sprague Dawley Rats

Male and female Crl:CD(SD) rats were randomized into 4 groups (25/sex/group) and administered the 0 (vehicle) or remdesivir at 1, 3, and 10 mg/kg/day via once-daily IV injection (slow push over 1-2 minutes) in a lateral caudal vein at a dose volume of 5 mL/kg (m2.6.7, Section 12, TX-399-2012). Males received 28 daily doses prior to mating and were dosed throughout the mating period through 1 day prior to euthanasia for a total of 63-64 doses. Females received 14 daily doses prior to cohabitation and were dosed through gestation day (GD) 7 for a total of 22-36 doses. Female necropsy and cesarean section were performed on GD 15. Male necropsy was performed following the necropsy of the last female.

Assessment of toxicity was based on mortality, clinical observations, body weights, food consumption, reproductive performance, estrous cyclicity (females), macroscopic observations, organ weights, laparohysterectomy findings (females: numbers of corpora lutea, implantation sites, resorptions, and live embryos), and sperm analysis (males: sperm motility, morphology, and counts).

All animals survived to the scheduled necropsies. There were no remdesivir-related macroscopic findings noted at any dosage level. No remdesivir-related clinical observations were noted in the 1 and 3 mg/kg/day group males and females or the 10 mg/kg/day group females. Remdesivir-related, adverse lower mean body weights, body weight gains, and food consumption were noted in the 10 mg/kg/day group males and females, with a corresponding slight increase in the incidence of decreased defecation noted in the 10 mg/kg/day group males. Mean body weights were 21.9% lower in males on study day 63 and 9.5% lower in females on GD 7 compared to the vehicle control group. Remdesivir-related slightly lower mean body weights, body weight gains, and food consumption were noted in the 3 mg/kg/day group males; these findings were considered non-adverse due to the sporadic nature and the magnitude of the effects on mean body weight. No remdesivir-related effects on mean body weights, body weight gains, or food consumption were noted in the 1 mg/kg/day group males and females and the 3 mg/kg/day group females.

No remdesivir-related effects on reproductive performance (male and female mating, fertility, and copulation/conception indices, estrous cyclicity, and the number of days between pairing and mating) or male spermatogenesis parameters were noted at any dosage level. Lower mean male reproductive organ weights (excluding testes) in the 10 mg/kg/day group were considered secondary to the effects on mean body weights. Lower mean number of corpora lutea and consequently lower mean numbers of implantation sites and viable embryos were noted in the 10 mg/kg/day group females, accompanied by lower mean ovary and uterus/cervix/oviduct weights. These findings were considered remdesivir-related and adverse.

Based on the lower mean body weights, body weight gains, and food consumption at 10 mg/kg/day, the NOAEL for male and female systemic toxicity of remdesivir when administered via once-daily intravenous injection to Crl:CD(SD) rats was 3 mg/kg/day. Based on the lack of effects on male reproductive performance and spermatogenesis data, the NOAEL for male reproductive toxicity was considered to be 10 mg/kg/day. For females at 10 mg/kg/day, a statistically significantly lower mean number of corpora lutea, and consequently lower mean numbers of implantation sites and viable embryos and lower mean ovary and uterus/cervix/oviduct weights were noted. Therefore, the NOAEL for female reproductive toxicity and embryonic toxicity was considered to be 3 mg/kg/day.

7.2. Embryo-Fetal Development

7.2.1. Intravenous (Slow Push) Study of the Effects of Remdesivir on Embryo-Fetal Development with a Toxicokinetic Phase in Sprague Dawley Rats

The objectives of the study were to determine the maternal and in utero toxicity and toxicokinetics of remdesivir when administered at doses of 0 (vehicle), 2.5, 5, 10, and 20 mg/kg/day via once daily IV injection (slow-push over 1-2 minutes) in the lateral caudal vein to pregnant Crl:CD(SD) rats (25/group) during organogenesis (GD 6 to 17) at a dose volume of 5 mL/kg (m2.6.7, Section 13.1, TX-399-2013). Assessment of toxicity was based on mortality, clinical observations, body weight, food consumption, necropsy findings, laparohysterectomy data, and fetal external, visceral, and skeletal evaluations. Additional mated females were used for toxicokinetic analysis on GD 6 and 17.

All females survived to the scheduled laparohysterectomy on GD 21. No test article-related clinical findings were noted at the daily examinations or approximately 1 hour following dose administration. No test article macroscopic findings were noted at the scheduled necropsy.

Mean body weight losses were noted for females in the 10 and 20 mg/kg/day groups during GD 6-9, with corresponding lower mean food consumption during GD 6-12 (10 mg/kg/day) or throughout the treatment period (20 mg/kg/day). As a result, mean body weights in the 10 and 20 mg/kg/day groups were up to 5.6% and 6.3%, respectively, lower than the vehicle control group. Absolute mean maternal body weights were comparable across all groups at the end of the treatment period. Due to the small magnitude of the effect on mean body weights (<10% relative to the vehicle control group) and the lack of any effects on gravid uterine weights, mean net body weight, and body weight change, the initial lower body weights noted at 10 and 20 mg/kg/day were not considered adverse.

Intrauterine growth and survival and fetal morphology were unaffected by maternal test article administration at all dosage levels.

All concentration values of remdesivir were below the lower limit of quantitation (< 2.00 ng/mL) on GD 6 and 17 therefore no toxicokinetic parameters were calculated. These results indicated that remdesivir was rapidly cleared and extensively metabolized in pregnant rats following IV administration of remdesivir. Exposure to GS-441524 and GS-704277 increased with the increase in remdesivir dose level from 2.5 to 20 mg/kg/day (Table 14). For GS-441524, the increase in C_{max} and AUC_{0-24} values were approximately dose proportional between the 2.5 to

20 mg/kg/day remdesivir dose levels. For GS-704277, the increase in C_{max} and AUC_{0-24} values were, in general, approximately dose proportional between the 2.5 to 20 mg/kg/day remdesivir dose levels, with the exception of GD 17 where the increases were greater than dose proportional from 5 to 20 mg/kg/day. AUC_{0-24} values were slightly higher on GD 17 than on GD 6 for GS-441524 (1.3- to 2.5-fold) and GS-704277 (1.2- to 2.4-fold) after multiple doses of remdesivir in pregnant rats.

Table 14. Toxicokinetic Parameters for GS-441524 and GS-704277 in Pregnant Sprague Dawley Rats Administered Remdesivir From Gestational Day 6 to Gestational Day 17

Remdesivir Dose (mg/kg/day)	AUC_{0-24} (ng•h/mL)		C_{max} (ng/mL)	
	GD 6	GD 17	GD 6	GD 17
GS-441524				
2.5	453	642	126	137
5	961	1210	249	272
10	1800	3680	462	676
20	3510	8740	799	1580
GS-704277				
2.5	158	333	415	458
5	328	391	882	1020
10	812	1180	1910	2850
20	1420	3420	3810	8620

In conclusion, based on the absence of adverse effects at any dosage level, a dosage level of 20 mg/kg/day, the highest dosage level evaluated, was considered to be the NOAEL for maternal toxicity and embryo/fetal development when remdesivir was administered via once daily intravenous injection to mated Crl:CD(SD) rats. This corresponded to GS-441524 AUC_{0-24} and C_{max} values of 8740 ng•h/mL and 1580 ng/mL, respectively, and GS-704277 AUC_{0-24} and C_{max} values of 3420 ng•h/mL and 8620 ng/mL, respectively, on GD 17.

7.2.2. Intravenous (Slow Push) Study of the Effects of Remdesivir on Embryo/Fetal Development with a Toxicokinetic Phase in Rabbits

The objectives of the study were to determine the maternal and in utero toxicity and toxicokinetics of remdesivir when administered at doses of 0 (vehicle), 2.5, 5, 10, and 20 mg/kg/day via once daily IV injection (slow-push over approximately 3 minutes) in the left marginal ear vein to time-mated New Zealand White rabbits (22/group) during organogenesis (GD 7 to 20) at a dose volume of 5 mL/kg (m2.6.7, Section 13.2, TX-399-2018). Assessment of toxicity was based on mortality, clinical observations, body weight, food consumption, necropsy findings, laparohysterectomy data, and fetal external, visceral, and skeletal evaluations. Additional time-mated females (4/group) were used for toxicokinetic analysis on GD 7 and 20.

No test article-related mortality or moribundity was noted in the test article-treated groups. Clinical observations were limited to decreased defecation in the 20 mg/kg/day group at the daily examinations. No other test article-related clinical or macroscopic findings were noted.

Mean body weight losses, with corresponding effects on mean food consumption, were noted for females in the 20 mg/kg/day group during GD 7-13. A mean body weight loss was noted for females in the 10 mg/kg/day group during GD 7-10, with corresponding effects on mean food consumption during GD 7-13. These results were considered test article-related and resulted in lower mean body weights in these groups (up to 5.1% and 8.3%, respectively). Throughout the remainder of the treatment and post-treatment periods, mean body weights gains in these groups were generally higher than the vehicle control group and mean food consumption was comparable. Because the initial mean body weight loss for females in the 10 mg/kg/day group was transient and the resulting effects on mean body weights were slight, this was not considered adverse. Mean body weights, body weight gains, and food consumption in the 2.5 and 5 mg/kg/day groups were unaffected by test article administration. Mean net body weights, net body weight gains, and gravid uterine weights at all dosage levels were unaffected by test article administration.

Intrauterine growth and survival and fetal morphology were unaffected by maternal test article administration at all dosage levels.

Exposure to remdesivir increased with dose level from 2.5 to 20 mg/kg/day (Table 15). The increases in C_{max} and AUC_{0-24} were generally greater than dose proportional between 2.5 and 20 mg/kg/day. Values for mean C_{max} and AUC_{0-24} were approximately 5- to 13-fold higher on GD 20 than on GD 7; however, concentration values were generally below the lower limit of quantitation by 12 hours postdose, indicating no accumulation of remdesivir after multiple doses. Exposure to metabolites GS-441524 and GS-704277 increased with the increase in remdesivir dose level from 2.5 to 20 mg/kg/day. The increases in C_{max} and AUC_{0-24} were approximately dose proportional between the 2.5 and 10 mg/kg/day dose levels and greater than dose proportional between the 10 and 20 mg/kg/day dose levels. No accumulation of GS-441524 and GS-704277 was observed after multiple doses of remdesivir. Mean T_{max} values and C_{max} and AUC_{0-24} metabolite to parent ratios indicate that remdesivir was rapidly and extensively metabolized to GS-441524 and GS-704277 in rabbits.

Table 15. Toxicokinetic Parameters for Remdesivir and its Metabolites, GS-441524 and GS-704277, in Pregnant Rabbits Administered Remdesivir

Remdesivir (mg/kg/day)	AUC_{0-24} (ng•h/mL)		C_{max} (ng/mL)	
	GD 7	GD 20	GD 7	GD 20
Remdesivir				
2.5	28.2	196	18.1	204
5	72.7	643	81.6	558
10	214	2110	226	1600
20	586	2830	604	2950
GS-441524				
2.5	462	429	150	119
5	1030	1090	308	247
10	1710	1790	526	380
20	4960	8930	1330	1680
GS-704277				
2.5	946	657	1560	1140
5	1870	1280	3060	2050
10	3180	2860	5270	3630
20	9860	11700	14800	13200

In conclusion, based on the body weight losses, with corresponding reduced food consumption and body weights at 20 mg/kg/day, the NOAEL for maternal toxicity was 10 mg/kg/day, and the NOAEL for embryo/fetal development was the high dose of 20 mg/kg/day when remdesivir was administered via once daily IV injection to time-mated New Zealand White rabbits. Exposures on GD 20 at the 20 mg/kg/day dose level were: remdesivir AUC_{0-24} and C_{max} values of 2830 ng•h/mL and 2950 ng/mL, respectively; GS-441524 AUC_{0-24} and C_{max} values of 8930 ng•h/mL and 1680 ng/mL, respectively, and GS-704277 AUC_{0-24} and C_{max} values of 11,700 ng•h/mL and 13,200 ng/mL, respectively, on GD 17.

7.3. Prenatal and Postnatal Development, Including Maternal Function

7.3.1. Intravenous (Slow Push) Study of the Effects of Remdesivir on Pre- and Postnatal Development, Including Maternal Function in Sprague Dawley Rats

Four groups of mated female Crl:CD(SD) rats (25/group) were administered remdesivir at 0 (vehicle), 1, 3, and 10 mg/kg/day at a dose volume of 5 mL/kg once daily via IV injection (slow-push over 1-2 minutes) in a lateral tail vein from GD 6 through lactation day (LD) 20

(m2.6.7, Section 14, TX-399-2014). One or 2 weanlings/sex/litter were randomly selected to form the F1 generation (behavioral/reproductive phase; 25 animals/sex/group, when possible) and were not directly administered remdesivir. Satellite maternal animals (4 females in Group 1 and 10 females/group in Groups 2-4) were dosed from GD 6 through LD 10 and blood samples were collected for toxicokinetic evaluation on GD 6 and LD 10 at selected time points. Blood samples were also collected on PND 10 from 1 pup/sex/litter from litters whose dams were used for the same blood collection time points on LD 10.

Toxicity assessments for the F_0 females were based on mortality, clinical signs, body weight, body weight changes, food consumption, and findings at necropsy. Rats were also evaluated for clinical observations during parturition, the length of gestation, live litter size, and maternal behavior. F_1 pup viability, body weights, and clinical signs were recorded for each litter. For the F_1 generation, mortality, clinical signs, body weight, body weight changes, physical signs of sexual maturation (vaginal opening from postnatal day [PND] 25; balanopreputial separation from PND 35) were conducted for all animals. Subsets of F1 animals were evaluated for behavioral performance (auditory startle response on PND 20 and 60, motor activity on PND 21 and 61, and Biel water maze on PND 22 and 62) and reproductive function assessment. The stage of the estrous cycle of each F_1 female assigned to the breeding phase was assessed for 10 consecutive days prior to cohabitation. At approximately 85 days of age, a subset of the F_1 generation was assigned to a 15-day cohabitation period. All F_1 females were allowed to deliver and rear their pups until PND 4. For the F_2 generation, clinical observations, body weights, and sexes were recorded at appropriate intervals. All surviving F_1 females were necropsied on LD 4, post-mating day 25, or post-cohabitation day 25. F_1 males were euthanized following the last female necropsy. Gross necropsies were performed on all F_2 pups found dead; all remaining F_2 pups were euthanized and discarded without examination on PND 4.

All F_0 females survived to the scheduled necropsy. No test article-related clinical findings were noted at the daily examination or approximately 1 hour following dose administration at any dosage level.

A test article-related initial mean body weight loss (GD 6-9) and sporadically lower mean body weight gains throughout the remainder of the gestation treatment period (GD 9-20), with corresponding effects on mean food consumption, were noted for females in the 10 mg/kg/day group. As a result, mean body weights for these females were up to 6.8% lower than the vehicle control group during GD 9 through LD 7. Mean body weight gains and food consumption for females in the 10 mg/kg/day group during lactation were comparable to the vehicle control group. Based on the small magnitude of change and the lack of any effects on the F1 offspring and because maternal body weights were comparable to the vehicle control group at the end of lactation, these decrements in maternal body weights during gestation, and the first week of the lactation period, were considered test article-related, but not adverse. Mean body weights, body weight gains, and food consumption for the 1 and 3 mg/kg/day groups were comparable to the vehicle control group throughout the study.

There were no test article-related effects on mean gestation lengths or the process of parturition for F_0 females, and no test article-related macroscopic findings or effects on the mean number of former implantation sites and unaccounted-for sites.

There were no test article-related effects on the mean number of F_1 pups born, live litter size, percentage of males at birth, postnatal survival, clinical observations, developmental landmarks (balanopreputial separation and vaginal patency), neurobehavior, and reproductive performance or necropsy findings, or birth weights at any dosage level. Mean F_1 pup body weights and body weight gains were unaffected by maternal test article administration.

No test article-related effects on survival, clinical condition, or mean body weights and body weight gains were noted for F_1 males and females at any dosage level. F_1 reproductive endpoints (pre-coital intervals, estrous cycle lengths, and mating, fertility, and copulation/conception indices) were unaffected by F_0 maternal test article administration. There were no test article-related effects on the F_1 gestation lengths or the process of parturition at any dosage level. There were no test article-related macroscopic findings in the F_1 males and females in any group or effects on the mean numbers of former implantation sites, unaccounted-for sites, and corpora lutea at any dosage level.

There were no test article-related effects of F_0 maternal test article administration on the mean number of F_2 pups born, viability on PND 0, postnatal survival, physical condition, mean body weights, body weight gains, or necropsy findings of F_2 pups that were found dead.

All concentration values of remdesivir, GS-441524, and GS-704277 for maternal and pup rats in the vehicle control group were below the lower limit of quantitation (Table 16). All concentration values of remdesivir on GD 6 and LD 10 for maternal rats and the majority of concentration values of remdesivir on PND 10 for pups were below the lower limit of quantitation (< 2.00 ng/mL); therefore, no toxicokinetic parameters were calculated. Exposure to GS-441524 and GS-704277 increased with the increase in maternal remdesivir dose level from 1 to 10 mg/kg/day for maternal rats and exposure to GS-441524 slightly increased with the increase in maternal remdesivir dose level from 3 to 10 mg/kg/day for pups. The increase in C_{max} and AUC_{0-24} values were approximately dose proportional between the 1 and 10 mg/kg/day remdesivir dose levels for maternal rats on GD 6 and LD 10, and were slightly greater than dose proportional for GS-704277 on LD 10. No accumulation of GS-441524 and GS-704277 was observed after multiple doses of remdesivir in maternal rats. Due to limited data in pups for GS-441524, assessment of dose proportionality and gender were not possible. Exposure to GS-441524, in regards to C_{max}, was higher in maternal rats than in pups, with maternal:pup C_{max} ratios of 42.6 for females at the 3 mg/kg/day remdesivir dose level and 143 and 114 for males and females, respectively, at the 10 mg/kg/day remdesivir dose level. Concentrations of GS-704277 were not measurable in pups.

Table 16. Toxicokinetic Parameters for GS-441524 and GS-704277 in Maternal Rats Administered Remdesivir

Remdesivir (mg/kg/day)	AUC_{0-24} (ng•h/mL)		C_{max} (ng/mL)	
	Gestation Day 6	Lactation Day 10	Gestation Day 6	Lactation Day 10
GS-441524				
1	137	349	49.5	60.3
3	415	538	142	149
10	1610	2310	477	572
GS-704277				
1	71.2	70.7	190	196
3	213	343	576	570
10	775	1190	2320	2680

In conclusion, due to the lack of adverse effects at any dosage level, a dosage level of 10 mg/kg/day (the highest dosage level evaluated) was considered to be the NOAEL for F_0 maternal systemic toxicity of remdesivir when administered via IV injection (slow-push over 1-2 minutes) to Crl:CD(SD) rats. This dosage level corresponded to maternal GS-441524 and GS-704277 C_{max} values of 572 and 2680 ng/mL, respectively, and AUC_{0-24} values of 2310 and 1190 ng•h/mL, respectively, on LD 10. Based on lack of effects at any dosage level, a dosage level of 10 mg/kg/day (the highest dosage level evaluated) was considered to be the NOAEL for F_1 developmental/neonatal, F_1 parental systemic, F_1 reproductive, and F_2 neonatal/early postnatal toxicity. This dosage level corresponded to a F_1 pup (combined sex) GS-441524 C_{max} value of 4.51 ng/mL on PND 10.

8. LOCAL TOLERANCE

8.1. Intravenous, Perivenous, and Intra-Arterial Tolerance Studies

Remdesivir is intended for IV administration. Dedicated local tolerance studies with remdesivir have not been conducted; however, evaluation of local tolerance at the site of intravenous injection was conducted during the repeat-dose GLP studies. In the 2-week rat study, remdesivir-related injection site observations included red discoloration of tail skin observed in males administered ≥ 20 mg/kg/day and females administered ≥ 5 mg/kg/day. In the absence of any correlating microscopic findings, these observations were not considered adverse (m2.6.7, Section 7.1, TX-399-2003). There were no treatment-related injection site changes observed in monkeys administered remdesivir up to 10 mg/kg/day for 2 weeks (m2.6.7, Section 7.3, TX-399-2004).

The injection site reactions and associated inflammatory responses observed after repeated daily IM injections in cynomolgus monkeys are not considered clinically relevant due to the IM route of administration and the non-optimized formulation (5% ethanol / 95% propylene glycol) that was used in the study.

8.2. Dermal Irritation Studies

The dermal irritation potential of remdesivir has been evaluated in the Episkin® reconstructed human epidermis model (m2.6.7, Section 15, TX-399-2023). Remdesivir was not an irritant in this assay.

8.3. Ocular Irritation Studies

The potential ocular irritation of remdesivir was evaluated in the bovine corneal opacity (BCOP) assay (m2.6.7, Section 15, TX-399-2025). The BCOP assay concluded remdesivir as ‘non-irritant’.

8.4. Phototoxicity

The ultraviolet-visible absorption spectrum of remdesivir reference standard was recorded in methanol. The spectrum exhibits absorbance maxima at 209, 246 and 274 nm, and has no absorbance above 350 nm. The molar extinction coefficients were 1.7 x 10^4 M^{-1} cm^{-1} at 209 nm, 3.8 x 10^4 M^{-1} cm^{-1} at 246 nm, and 0.6 x 10^4 M^{-1} cm^{-1} at 274 nm (m3.2.S.3.1). Based on these photochemical properties, remdesivir is not considered to be sufficiently photoreactive to result in direct phototoxicity, as per ICH S10.

9. OTHER TOXICITY STUDIES

9.1. Antigenicity

Nonclinical studies of the antigenicity of remdesivir have not been performed. Remdesivir is a small molecular weight compound and unlikely to be antigenic.

9.2. Immunotoxicity

No specific immunotoxicity studies were conducted with remdesivir. There were no findings in the repeat dose toxicity studies with remdesivir to indicate an immunological concern.

9.3. Mechanistic Studies

9.3.1. In Vitro Effect on Human and Animal Hepatocytes

In addition to a monoculture of human hepatocytes (m2.6.2, Section 4.2), studies have been conducted to investigate the potential of remdesivir and its major systemic metabolites, GS-704277 and the nucleoside analog GS-441524, to affect primary human and animal hepatocytes using several in vitro models. Results of these studies are briefly summarized below.

The in vitro cross-species hepatotoxicity profiles of remdesivir, GS-704277, and GS-441524 were assessed using the HμRELTox™ (co-culture system consisting of primary hepatocytes of respective species co-cultured with cells of a nonparenchymal stromal type in human, monkey, and rat primary hepatocyte co-cultures; m2.6.7, Section 16, PC-399-2027). Albumin secretion, cell culture integrity, and metabolic activity were quantified as indicators of in vitro hepatotoxicity. Remdesivir was more toxic in primary human hepatocytes (PHH) than in rat or monkey hepatocytes. Of the 3 cytotoxicity readouts, albumin was the most sensitive followed closely by the culture integrity assessed by impedance. After 14 days of continuous exposure in culture, remdesivir IC_{50} values, based on the decrease in albumin secretion, were < 0.12, 0.96, and 2.1 μM in human, rat, and monkey hepatocytes, respectively. Metabolic activity measured by intracellular ATP was the least sensitive marker of remdesivir-induced cytotoxicity. The 14-day IC_{50} values of remdesivir in human hepatocytes measured by albumin production, culture integrity, and ATP levels were < 0.12, < 0.12, and 0.68 μM, respectively. Remdesivir was substantially more toxic than its metabolites GS-704277 and GS-441524 across the 3 tested species, and particularly in human hepatocytes, indicating that remdesivir itself, but not its systemic metabolites, is likely the drug species associated with the low-grade changes in liver enzymes observed in humans treated with multiple doses of remdesivir. While a significant inhibition of albumin production by remdesivir was detected in these in vitro experiments, no effects on albumin levels were observed in healthy human subjects following 7- to 14-day dosing of remdesivir, indicating a likely increased sensitivity of this in vitro model to continual remdesivir exposure.

The cytotoxicity profiles of remdesivir, GS-704277, and GS-441524 were evaluated in the Organovo™ exVive3D Liver system (bioprinted 3D tissues composed of PHH, hepatic stellate cells, and endothelial cells; m2.6.7, Section 16, TX-399-2022). Treatment groups included

3 concentrations each of remdesivir, GS-704277, and GS-441524, along with a 0.1% dimethylsulfoxide (DMSO) vehicle control dosed daily for 14 days. In order to mimic the clinical pharmacokinetics of remdesivir and its metabolites, tissues treated with remdesivir (0.3, 1.5, and 7.5 μM) and GS-704277 (0.3, 1.0, and 3.0 μM) were exposed to the compounds for 2 hours daily, followed by 22 hours of exposure to vehicle alone; tissues were continuously exposed to GS-441524 (0.3, 1.0, and 3.0 μM). Spent media samples were analyzed for albumin, lactate dehydrogenase (LDH), and ALT production to monitor tissue health. Tissues were assessed histologically to determine morphology. Under the conditions run, there was little to no conclusive evidence of toxicity in the exVive3D human liver tissues and the design of the study does not support drawing conclusions regarding mechanism.

In conclusion, data from these in vitro studies demonstrate that human hepatocytes are susceptible to remdesivir-mediated toxicity, likely due to the high cellular permeability and effective intracellular metabolism of the drug. While GS-704277 and GS-441524 are products of the in vivo systemic metabolism of remdesivir 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 remdesivir due to their low systemic exposure and minimal effects on hepatocytes demonstrated in several independent studies.

9.3.2. Assessment of Potential Etiology of Remdesivir Nephrotoxicity in Rats: Interaction with Renal Organic Anion Transporters

To explore the potential etiology of remdesivir nephrotoxicity observed in rat repeat-dose toxicity studies (Section 4.1), remdesivir and its major systemic metabolites GS-441524 and GS-704277 were tested for their interaction with human and rat renal OATs (m2.6.3, Section 3, PC-399-2020). Rat OAT3 expression increased the cytotoxicity of the intermediate metabolite GS-704277 by approximately 15-fold. In addition, intracellular accumulation of the active triphosphate metabolite GS-443902 increased in rat OAT3-expressing cells following exposure to GS-704277. In contrast, the cytotoxicity of GS-704277 changed minimally (< 2-fold) in cells expressing rat OAT1, human OAT1, or human OAT3 transporters compared with control cells. Neither rat nor human OATs expression significantly changed cytotoxicity or intracellular triphosphate accumulation upon incubation with remdesivir or the nucleoside metabolite GS-441524. These data indicate that the intermediate metabolite GS-704277, but not remdesivir or the nucleoside metabolite GS-441524, is an effective substrate of rat OAT3 and exhibits rat OAT3-dependent cytotoxicity. In contrast, GS-704277 is not a substrate for human OATs, suggesting a reduced potential for renal adverse effects in human compared with rat due to lower renal accumulation.

9.4. Dependence

No specific studies on dependency of remdesivir were conducted. Direct acting antiviral agents have no known properties that would suggest development of dependence. There was no evidence of development of dependence in nonclinical studies with remdesivir. Tissue distribution studies using radiolabeled remdesivir (m2.6.4, Section 4.2) indicated that relatively low concentrations of radioactivity at C_{max} were observed in the central nervous system. Consequently, dependency studies with remdesivir are not considered warranted.

9.5. Studies on Metabolites

No specific repeat dose toxicology studies with remdesivir metabolites were conducted as there were no unique human metabolites; all major human metabolites were also found in the nonclinical species (m2.6.4, Section 6.1). Metabolite levels in nonclinical species used for toxicology studies exceed those observed clinically.

One non-GLP reverse mutation assay was conducted with GS-441524 in four *S. typhimurium* strains (TA97a, TA98, TA100, and TA1535) and E. coli WP2*uvr*A with and without S9 (m2.6.7, Section 16, TX-195-2006). The assay met acceptance criteria and GS-441524 was negative for mutagenicity.

9.6. Impurities

9.6.1. A 14-Day Intravenous (Slow Bolus) Impurity Qualification Toxicity Study with Remdesivir in Male Cynomolgus Monkeys

The purpose of this study was to evaluate the toxicity and determine the toxicokinetics of different lots of remdesivir when administered daily via IV (slow bolus) injection to male cynomolgus monkeys for at least 14 days (m2.6.7, Section 16, TX-399-2015).

Male cynomolgus monkeys were assigned to 6 groups, and were administered the vehicle control, Test Article 1 (remdesivir) at 10 mg/kg/day, Test Article 2 (remdesivir+Impurities; remdesivir-I) at 5 or 10 mg/kg/day, or Test Article 3 (remdesivir+Degradants; remdesivir-D) at 5 or 10 mg/kg/day. Animals were dosed via slow bolus injection through the saphenous vein over 1 to 2 minutes once a day for 14 days. Assessment of toxicity was based on mortality, clinical observations, food consumption, body weights, ophthalmic observations, electrocardiographic measurements, and clinical and anatomic pathology. Blood samples were collected for toxicokinetic evaluations of remdesivir and metabolites on Day 1 and Day 14.

All animals survived to the terminal sacrifice. There were no test article-related clinical observations or effects on body weight, food consumption ophthalmoscopy, or electrocardiography. Remdesivir administration had no effect on clinical pathology test results, except for a mild, non-dose-related decrease in cholesterol in animals administered remdesivir, and remdesivir-I and remdesivir-D at both dose levels. Although a mechanism for this minor decrease in cholesterol concentration was not apparent, it was not considered adverse because of its small magnitude and absence of correlative findings.

No test article-related organ weight differences, or macroscopic or microscopic findings were noted.

Exposure to remdesivir and metabolites, GS-441524 and GS-704277, increased with the increase in remdesivir-I and remdesivir-D dose level from 5 to 10 mg/kg/day. Increases in C_{max} and AUC_{0-24} were approximately dose proportional between the 5 to 10 mg/kg/day dose levels. No accumulation of remdesivir, GS-441524 or GS-704277 was observed after multiple doses of remdesivir, remdesivir-I, or remdesivir-D. Exposures, based on AUC_{0-24}, to remdesivir, GS-441524 and GS-704277 were generally similar after administration of remdesivir,

remdesivir-I and remdesivir-D with differences less than 2-fold. The mean C_{max} and AUC_{0-24} metabolite to parent ratios indicate that remdesivir was extensively metabolized to GS-441524 and GS-704277 following administration of all test articles. Metabolite to parent ratios were slightly higher after administration of remdesivir-D than with remdesivir or remdesivir-I; however, differences were generally less than 2-fold.

In conclusion, administration of three lots of remdesivir (remdesivir, remdesivir-I, and remdesivir-D) to male cynomolgus monkeys was well tolerated as an IV slow bolus injection at dose levels up to 10 mg/kg/day for 14 days. No adverse test article-related findings were noted. The NOAEL for each lot of remdesivir is 10 mg/kg/day. This dose level corresponded to Day 14 mean C_{max} values of 1130, 1660, and 1110 ng/mL and AUC_{0-24} values of 1400, 1880, and 1130 ng·h/mL for remdesivir, remdesivir-I, and remdesivir-D, respectively.

9.6.2. In Vitro Genotoxicity Studies

One GLP reverse mutation assay was conducted in four *S. typhimurium* strains (TA98, TA100, TA1535 and TA1537) and E. coli WP2*uvr*A with and without S9 for [redacted] (m2.6.7, Section 16, TX-399-2020). The assay met acceptance criteria and [redacted] was negative for mutagenciity.

9.7. Other Toxicity Studies

9.7.1. A 7-Day Subcutaneous Tolerability Study of Remdesivir in Ces1c$^{-/-}$ Mice

The purposes of this study (m2.6.7, Section 16, TX-399-2019) were to evaluate the toxicity and toxicokinetics of remdesivir in Ces1c$^{-/-}$ (carboxylesterase 1c knockout) mice administered daily subcutaneous doses for at least 7 days, and to support the designs of the efficacy studies against SARS-CoV and MERS-CoV in Ces1c$^{-/-}$ mice (m2.6.2, Sections 3.2.2 and 3.2.3). Male and female Ces1c$^{-/-}$ mice were assigned to two toxicity groups (5/sex/group) and two toxicokinetic groups (9/sex/group) and administered 10 or 50 mg/kg remdesivir via subcutaneous injection once daily for 7 days at a volume of 10 mL/kg. Assessment of toxicity was based on mortality, clinical observations, food consumption, body weights, and clinical pathology. Blood and lung samples were collected for toxicokinetic evaluations on Day 7. Tails were collected for genotyping analysis from all toxicity animals and from the 24 hour-toxicokinetic animals.

Following subcutaneous injection in animals administered 10 mg/kg or 50 mg/kg, remdesivir was rapidly cleared from the systemic circulation. The intermediate metabolite, GS-704277, was also rapidly formed and cleared from plasma. Concomitant with the decline of remdesivir and GS-704277, the nucleoside metabolite GS-441524 was observed in plasma and persisted over the 24 hour period. Efficient formation of the pharmacologically active triphosphate, GS-443902, was observed in the lung and increased proportionally with dose. Lung levels of triphosphate were substantially lower at 24 hours, suggesting a short half-life (≤10 hours). No significant sex difference in lung GS-443902 levels was observed.

All animals survived until their terminal sacrifice on Day 8. No changes in clinical observations occurred. Decreases in body weight occurred in animals administered 50 mg/kg over the course of the study (-6.5% for males and -1.0% for females). These decreases correlated to a decrease in

food consumption from Days 1 to 4. Remdesivir administration for 7 days had no obvious or adverse effect on clinical chemistry test results, and macroscopic findings were considered not test article related. Genotyping data showed that one toxicity male administered 10 mg/kg as well as one toxicokinetic male and one toxicokinetic female administered 10 mg/kg were Ces1c wild type.

In conclusion, male and female Ces1c$^{-/-}$ mice were administered 10 or 50 mg/kg remdesivir via subcutaneous injection once daily for 7 days. No changes in clinical condition occurred. Decreases in body weight and food consumption occurred in animals administered 50 mg/kg. No changes occurred in clinical chemistry parameters and no test article-related macroscopic findings were noted.

9.7.2. 7-Day Intravenous (Slow Bolus) Investigative Toxicity and Toxicokinetic Study with Remdesivir Formulated in Two Vehicles, in Male Wistar-Han Rats

The purpose of this study was to evaluate the toxicity and determine the toxicokinetics of remdesivir formulated in two different vehicles and administered once daily via IV (slow bolus) injection to male rats for at least 7 days (m2.6.7, Section 16, TX-399-2009). Male Crl:WI(Han) rats were administered remdesivir via IV injection (slow bolus) once daily for at least 7 days at a dose level of 0 (vehicle 1; 12% [w/v] SBECD in water; pH 3.0 to 3.5), 0 (vehicle 2; 90% normal saline/5% ethanol/5% polysorbate 80 [v/v/v] and hydrochloric acid; pH 3.0), or 20 or 50 mg/kg/day in each vehicle at a dose volume of 5 mL/kg. Assessment of toxicity was based on mortality, clinical observations, body weights, body weight change, food consumption, and clinical (hematology, coagulation, and clinical chemistry on Day 8; urine chemistry and urinalysis on Days 4 and 8) and anatomic pathology (kidney evaluation). Blood samples were collected on Days 1 and 7 from similarly dosed animals for toxicokinetic evaluation of remdesivir and metabolites, and kidney samples were collected for possible test article bioanalysis.

Remdesivir-related mortality was observed on Day 7 for two animals administered 50 mg/kg/day in vehicle 2. One toxicity animal was sacrificed in moribund condition, and one toxicokinetic animal was found dead. Clinical observations for these animals were limited to the tail and included swelling, red discharge, and purple and red discoloration of skin. Clinical pathology and microscopic findings in the toxicity animal were similar to those for members of the group surviving to scheduled sacrifice.

Remdesivir-related clinical observations in surviving animals were limited to the tail and included red or purple discoloration for animals administered ≥ 20 mg/kg/day in both vehicles and swelling, sores, and scabs on tails of animals administered 50 mg/kg/day in vehicle 2.

Decreases in body weight were noted in all groups between Days 1 and 4, with larger decreases seen in animals administered 20 or 50 mg/kg/day in either vehicle. After Day 4, all groups gained body weight. Over the entire dosing phase, significant decreases in mean body weight gain were considered adverse in all groups administered remdesivir. Decreases in food consumption correlated with decreases in body weight gain.

Remdesivir-related clinical pathology effects indicative of kidney injury and/or dysfunction were present in animals administered remdesivir in either vehicle and were associated with microscopic renal findings. Most changes were present at both dose levels, appeared dose-dependent, and were of similar magnitude in either vehicle. Clinical pathology changes included increases in serum urea nitrogen; creatinine; inorganic phosphorus; increases in ratios of urine total protein and urine n-acetyl-beta-glucosaminidase to urine creatinine; urine sodium excretion; fractional clearance of sodium, potassium, and chloride; urine protein; blood; ketones; epithelial cells; and granular casts. Some clinical pathology changes (urine sodium excretion, fractional clearance of sodium and potassium) were present only with remdesivir in vehicle 1. Effects on urine chemistry and urinalysis in either vehicle were generally more pronounced on Day 4 than Day 8.

Remdesivir-related increases in absolute kidney weights were observed in animals administered ≥20 mg/kg/day in either vehicle. Macroscopic findings at or near the injection site were recorded under skin/subcutis, tail or intravenous injection site and occurred more frequently with remdesivir in vehicle 2. One male administered 50 mg/kg/day remdesivir in vehicle 2 had tan discolored kidneys that were considered remdesivir-related.

At the terminal sacrifice, remdesivir-related microscopic findings in the kidney were observed in animals administered ≥ 20 mg/kg/day in either vehicle and included multifocal moderate to marked basophilic tubules, increased mitosis, proteinaceous casts, and vacuolation in renal tubular epithelial cells. In addition, remdesivir in vehicle 2 also resulted in minimal to slight focal/multifocal degeneration of renal tubules. In general, the severity of renal changes was greater in animals administered remdesivir in vehicle 2 versus vehicle 1.

Plasma concentrations of remdesivir were generally below the lower limit of quantitation by 6 hours postdose indicating that remdesivir was rapidly and extensively converted to GS-441524 and GS-704277. Exposure to remdesivir generally increased with the increase in dose level from 20 to 50 mg/kg/day in both formulations. Exposure to GS-441524 and GS-704277 generally increased with the increase in remdesivir dose level from 20 to 50 mg/kg/day. The increases in GS-441524 C_{max} and AUC values were generally greater-than-dose proportional between 20 and 50 mg/kg/day, while increases in GS-704277 C_{max} and AUC values were greater-than-dose proportional between 20 and 50 mg/kg/day on Day 1 and approximately dose-proportional between 20 and 50 mg/kg/day on Day 7 for either formulation. Potential accumulation of GS-441524 was observed after multiple doses of remdesivir, while no accumulation of GS-704227 was observed. Exposures to remdesivir and metabolites, GS-441524 and GS-704277, were similar between formulations.

In conclusion, once daily intravenous administration of 20 or 50 mg/kg/day remdesivir in 2 different vehicles to male rats for 7 days resulted in adverse decreases in body weight gain and food consumption and clinical pathology and microscopic findings indicative of kidney injury and/or dysfunction in males administered ≥ 20 mg/kg/day in either vehicle. In general, the severity of renal changes and injection site reactions were greater in animals administered remdesivir in vehicle 2 versus vehicle 1.

9.7.3. 7-Day Intramuscular Injection Toxicity and Toxicokinetic Study with GS-466547 in Male Cynomolgus Monkeys

Male monkeys were assigned to 4 groups (3 animals/group) and administered vehicle [5% ethanol, 95% propylene glycol (v/v)] or GS-466547 (diastereomeric prodrug mixture containing remdesivir) at a dose level of 0 (vehicle: 95% propylene glycol, 5% ethanol), 2.5, 7.5, or 15 mg/kg/day (m2.6.7, Section 16, TX-399-2001). Doses were administered once daily via IM injection at a dose volume of 0.1 mL/kg. Assessment of toxicity was based on mortality, clinical observations, body weights, food consumption, and clinical and anatomic pathology. Plasma samples were collected on Day 1 and Day 7 and peripheral blood mononuclear cells (PBMC) samples were collected on Day 8 for toxicokinetic analysis of GS-466547 and its two major metabolites GS-441524 and GS-704277.

All animals survived to the study termination. No GS-466547-related changes in clinical condition occurred. A non-adverse decrease in mean body weights was noted for all groups, including controls, from Days 1 to 7. GS-466547-related decreases in food consumption occurred in the group administered 15 mg/kg/day beginning on Days 4 or 5 for all animals in that group.

GS-466547-related clinical pathology findings were compatible with renal injury in animals administered 15 mg/kg/day, and inflammation correlated with local injection site reactions for animals administered ≥ 2.5 mg/kg/day. Increased serum creatinine concentration and the lack of adequate urine concentration and 1 or 2+ urine protein was compatible with renal dysfunction. Changes compatible with inflammation included increased neutrophil and monocyte counts in animals administered ≥ 7.5 mg/kg/day and increased fibrinogen concentrations and platelet counts in animals administered ≥ 2.5 mg/kg/day. Decreases in serum albumin concentration and albumin:globulin ratio in animals administered ≥ 2.5 mg/kg/day may have been due, at least in part, to inflammation (negative acute phase response), but some increased renal loss in the urine may have also been contributory. Animals administered 15 mg/kg/day did not have the expected regenerative erythroid response after repeated blood sampling (e.g., lower reticulocyte counts, compared with controls with increased reticulocyte counts). Slightly decreased inorganic phosphorus in animals administered ≥ 2.5 mg/kg/day, and decreased serum triglyceride in animals administered 15 mg/kg/day were of uncertain toxicological significance in the absence of microscopic correlates.

Animals administered 15 mg/kg/day had adverse kidney changes (proximal tubular epithelial cell degeneration/necrosis) that was represented macroscopically as pale kidney cortices, and correlated with increased kidney weights in animals administered ≥ 7.5 mg/kg/day. Clinical pathology changes in blood and urine were compatible with renal dysfunction. Adverse injection site reactions in the muscle of the caudal thigh occurred in a high percentage of animals administered ≥ 2.5 mg/kg/day and consisted of induration (firmness) at necropsy; this finding correlated microscopically with myofiber necrosis and extensive neutrophilic or mixed-cell inflammation. The popliteal and/or inguinal lymph nodes from some GS-466547-dosed animals had correlative secondary inflammatory changes. Animals administered 15 mg/kg/day had decreased thymus weights, and one animal had the microscopic correlate of slightly decreased cortical lymphocytes in the thymus, which was consistent with a secondary stress response.

Following intramuscular injection, GS-466547 and its metabolites, GS-441524 and GS-704277, were observed in plasma in all dose groups except the vehicle control (Table 17). On Day 1, exposure to GS-466547 and its metabolites increased proportionally with dose between 2.5 and 7.5 mg/kg/day but the increase was less than proportional between 7.5 mg/kg and 15 mg/kg/day. On Day 7, exposure to GS-466547 was similar to that observed on Day 1. GS-441524 and GS-704277 exposure levels on Day 1 and Day7 were within 2-fold at 2.5 mg/kg/day and accumulated approximately 2.5-fold between Day 1 and Day 7 at 7.5 mg/kg/day. Compared to the lower dose groups, higher accumulation was observed at 15 mg/kg/day for GS-441524 and GS-704277 (9.4- and 5.7-fold, respectively). Total metabolite levels in PBMCs assessed 24 hours after dosing on Day 7 increased with dose.

Table 17. Toxicokinetic Parameters for GS-466547, GS-441524 and GS-704277 in Male Monkeys Administered GS-466547 for 7 Days

GS-466547 (mg/kg/day)	AUC_{0-24} (ng•h/mL)		C_{max} (ng/mL)	
	Day 1	Day 7	Day 1	Day 7
GS-466547				
2.5	900	956	292	282
7.5	2530	4350	312	1580
15	3840	2850	295	708
GS-441524				
2.5	437	771	49.5	58.7
7.5	1350	3370	83	239
15	1930	18200	105	923
GS-704277				
2.5	232	340	69.9	63.4
7.5	1340	2980	104	353
15	2080	11800	129	694

In conclusion, male monkeys were administered vehicle or GS-466547 at dose levels of 2.5, 7.5, or 15 mg/kg/day via IM injection once daily for 7 days. Minor decreases in body weight were observed for all treatment groups including controls with decreases in food consumption observed at 15 mg/kg/day. Findings compatible with renal injury included clinical and anatomic pathology changes at 15 mg/kg/day. Inflammation correlated with local injection site reactions for animals administered ≥ 2.5 mg/kg/day. Due to the adverse injection site reactions seen at all dose levels, a NOAEL for local tolerance was not identified. The NOAEL for systemic toxicity was considered 7.5 mg/kg/day (Day 7 AUC_{0-24}: GS-466547 4350 ng•h/mL; GS-441524 3370 ng•h/mL; GS-704277 2980 ng•h/mL).

9.7.4. A 7-Day Intravenous (Slow Push) Tolerability Study of Remdesivir in Nonpregnant New Zealand White Rabbits

The objective of this study was to determine the tolerability and toxicokinetics of remdesivir in nonpregnant female rabbits following IV (slow bolus) administration for up to 7 days (m2.6.7, Section 16, TX-399-2010). The results of this study allowed for dose selection for a subsequent embryo/fetal development toxicity study in rabbits (Section 7.2.2). Nonpregnant female New Zealand White rabbits were randomized into 4 groups (3/group) and administered the vehicle or remdesivir at 5, 15, and 50 mg/kg/day by once-daily IV injection (slow-push over 1-2 minutes) in the left marginal ear vein on study days 0 through 6 at a dose volume of 5 mL/kg. Assessment of toxicity was based on mortality, clinical observations, body weight, food consumption, and gross necropsy observations. In addition, a toxicokinetic assessment of plasma levels of remdesivir and metabolites was performed on Days 0 and 6.

One female in the 50 mg/kg/day group was found dead on Day 6 following clinical findings of impaired equilibrium, ataxia, constricted pupils, exophthalmos, partial closure of the eyes, and decreased defecation. Clinical observations noted for surviving females in the 50 mg/kg/day group included ataxia, impaired use of the hindlimbs, decreased or shallow respiration rate, constricted pupils, clear or white discharge of the eyes, and/or exophthalmos generally throughout the treatment period. The aforementioned clinical observations were generally noted 1 minute to approximately 15 minutes following dose administration. Decreased defecation (Days 5-7) was noted at the daily examinations. No remarkable clinical observations were noted in the 5 and 15 mg/kg/day groups.

Severe body weight losses with corresponding minimal food consumption were noted for all 3 females in the 50 mg/kg/day group generally throughout the treatment period, resulting in individual body weight losses of 5.2% to 16.6% from study day 0 to the day of death or scheduled euthanasia. Mean body weight gains, body weights, and food consumption in the 5 and 15 mg/kg/day groups were comparable to the vehicle control group throughout the study.

Exposure to remdesivir, GS-441524, and GS-704277 increased with the increase in remdesivir dose from 5 to 50 mg/kg/day. The increases in remdesivir, GS-441524, and GS-704277 C_{max} and AUC_{0-24} values were generally greater than dose proportional between the 5 to 50 mg/kg/day dose levels. No accumulation of remdesivir or GS-704277 was observed while accumulation of GS-441524 was generally observed after multiple doses of remdesivir. Remdesivir was extensively converted to GS-441524 and GS-704277 in rabbits.

In conclusion, based on mortality, clinical findings, severe body weight losses, and reduced food consumption noted for females at 50 mg/kg/day group, dosage levels of 2.5, 5, 10, and 20 mg/kg/day were selected to be evaluated in the rabbit embryo/fetal development study.

9.7.5. Hemolytic Potential and Plasma Compatibility Study of Remdesivir in Human, Cynomolgus Monkey, and Rat Blood and Plasma

This study assessed the hemolytic potential and plasma compatibility of the vehicle [12% (w/v) (SBECD) in Sterile Water for Injection, pH 3.5 ± 0.1] and remdesivir in the vehicle at concentrations of 1, 3, and 10 mg/mL in cynomologus monkey, Han Wistar rat, and human whole blood and plasma (m2.6.7, Section 16, TX-399-2008).

No hemolysis was observed in monkey, rat, or human whole blood when combined with the vehicle alone or with the 1, 3, or 10 mg/mL remdesivir formulations. Additionally, no macroscopic changes were noted in monkey, rat, or human plasma when combined with the vehicle alone or with the 1, 3, and 10 mg/mL remdesivir formulations. In conclusion, the remdesivir SBECD-based formulations were compatible with monkey, rat, and human whole blood and plasma.

10. DISCUSSION AND CONCLUSIONS

Remdesivir is a nucleotide prodrug of an adenosine nucleoside analog GS-441524 that is being developed for IV administration for the treatment COVID-19. The nonclinical toxicology profile of remdesivir has been characterized through the conduct of repeat-dose studies in rats and cynomolgus monkeys with once-daily IV dosing up to 4 weeks in duration, studies to evaluate the genotoxic potential of the compound, and a battery of reproduction and developmental studies (fertility in rats, embryofetal development in rats and rabbits, and a pre- and postnatal developmental study in rats). Additional studies include a series of in vitro investigations to assess kidney and liver toxicity, impurity qualification studies, and worker safety studies. Following repeated dosing in rats and monkeys, the kidney was identified as the only target organ of toxicity. In both species, clinical chemistry, urinalysis, and/or urinary biomarkers were early predictors of the observed kidney changes. There were no changes in the liver in rats or monkeys based on clinical chemistry parameters, liver weight, or microscopic observations. Remdesivir is considered nongenotoxic. Remdesivir is not considered phototoxic and is not a skin or eye irritant. No major concerns were identified in the reproductive and developmental toxicity studies. Overall, the nonclinical studies have adequately characterized the nonclinical safety profile of remdesivir.

10.1. Target Organ Toxicity - 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 remdesivir at 10 mg/kg/day. In clinical studies, no evidence of nephrotoxicity has been observed with single doses of remdesivir up to 225 mg or multiple once-daily doses of remdesivir 150 mg for up to 14 days.

In rats administered remdesivir via daily IV (slow bolus) injection for up to 4 weeks, decreases in body weight gain and food consumption, and clinical pathology and microscopic findings indicative of kidney injury and/or dysfunction were observed in animals administered ≥ 3 mg/kg/day, with unscheduled deaths and decreased body weight gain and food consumption at 10 mg/kg/day. Clinical chemistry and urinalysis findings, including increases in BUN and serum creatinine, and increases in urinary biomarkers of kidney injury, e.g., total protein, NAG, cystatin C, β2M, and KIM-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, 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, the NOAEL was 3 mg/kg/day, based on the nature and severity of the kidney changes at this dose level. The sensitivity of rats to renal effects of remdesivir may be related to the active tubular transport of remdesivir metabolites by rat renal OAT3; this interaction has not been detected with human renal OAT3.

In cynomolgus monkeys administered remdesivir via daily IV (slow bolus) injection for up to 4 weeks, there were no changes indicative of an effect in the kidney, and the NOAEL was the high dose of 10 mg/kg/day. After daily IM injections of 15 mg/kg/day GS-466547 (diastereomeric mixture) to cynomolgus monkeys, similar microscopic changes were observed in the proximal tubules of the kidney to those noted in rats; clinical pathology changes correlated with the renal changes at the 15 mg/kg/day IM dose. Exposures at the NOAEL in the 7-day IM-study were slightly higher than at the NOAEL in the 4-week IV study. 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 compared to cynomolgus monkeys is unknown.

In summary, nonclinical findings were consistent with dose-dependent and reversible kidney injury and dysfunction. The clinical significance of the nephrotoxicity noted in animal species is unknown as no evidence of nephrotoxicity has been observed in clinical studies with remdesivir.

10.2. Hepatotoxicity

In clinical studies with remdesivir, transient elevations in alanine aminotransferase (ALT) and aspartate aminotransferase (AST) have been observed with single doses of remdesivir up to 225 mg and multiple once-daily doses of remdesivir 150 mg for up to 14 days, with mild, reversible prothrombin time (PT) prolongation in some subjects but without any clinically relevant change in international normalized ratio (INR) or other evidence of hepatic effects. The mechanism of these elevations is currently unknown.

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. Remdesivir and the nucleoside analog GS-441524 have been profiled for in vitro cytotoxicity and mitochondrial toxicity in multiple relevant cell types (m2.6.2, Sections 4.2 and 4.3). Data from in vitro studies with liver cell culture systems (Section 9.3.1) demonstrated that human hepatocytes are more susceptible to remdesivir-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 administered repeated doses of remdesivir due to their low toxicity on hepatocytes observed in vitro.

10.3. 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 remdesivir toxicology studies, and in several peer-reviewed publications {Hafner 2010, Luke 2010, Stella 2008}. Sulfobutylether β-cyclodextrin-related microscopic findings were not considered adverse, nor associated with any clinical pathology effects indicative of changes in kidney function, and have been previously described {Luke 2010, Stella 2008}. There was no notable exacerbation of the SBECD-related effects when administered with remdesivir.

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}. Kidney vacuolation and hypertrophy are both previously described changes associated with SBECD administration in nonclinical species {Luke 2010, Stella 2008}. 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 Food and Drug Administration (FDA) list of Inactive Pharmaceutic Ingredients {U. S. Food and Drug Administration 2015}, and generally considered safe. Because SBECD is renally cleared, and accumulates in patients with decreased renal function, administration of drugs formulated with SBECD is not recommended in patients with moderate to severe renal impairment unless the benefit of therapy outweighs the risk {Roerig (Division of Pfizer) Inc 2015}.

10.4. Genotoxicity

Remdesivir was non-mutagenic in the *in vitro* Ames mutagenicity assay and negative in the rat micronucleus assay. In the *in vitro* chromosome aberrations assay with human lymphocytes, remdesivir was negative without metabolic activation, and equivocal in the 3-hour treatment with metabolic activation. In the 3-hour treatment with metabolic activation, a slight but statistically significant increase in cells with chromosome aberrations was observed only at the high dose level of 245 µg/mL. This increase was small, was within the historical control range and only observed at a high level of cytotoxicity. Hence, the response observed is considered equivocal, of questionable biological significance, and remdesivir is considered nongenotoxic.

10.5. Reproductive and Developmental Toxicity

A complete reproductive and development toxicity program has been completed with remdesivir. 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 at 10 mg/kg/day, adverse effects on body weight and food consumption were noted, along with a lower mean number of corpora lutea, and consequently lower mean numbers of implantation sites and viable embryos, and lower mean ovary and uterus/cervix/oviduct weights, and the NOAEL for female reproductive toxicity and embryonic toxicity was 3 mg/kg/day.

10.6. Conclusions

The nonclinical safety program completed for remdesivir supports its use for the treatment of COVID-19. All information from the nonclinical toxicology studies that is of relevance to the prescriber and patient has been included in the proposed prescribing information.

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SECTION 2.6.7—TOXICOLOGY TABULATED SUMMARY

REMDESIVIR (GS-5734™)

Gilead Sciences

[redacted] **2020**

CONFIDENTIAL AND PROPRIETARY INFORMATION

TABLE OF CONTENTS

1. TOXICOLOGY: OVERVIEW

Test Article: Remdesivir

Type of Study/Species/Strain	Method of Administration	Duration of Dosing	Dose (mg/kg)[a]	GLP[b]	Testing Facility	Gilead Study No. (CRO Study No.)
Repeat-Dose Toxicity						
Rat/Crl:WI(Han)	Intravenous (slow bolus) Injection	2 weeks with 4-week recovery	0 (saline), 0 (vehicle[c]), 5[d], 20, 50	Yes	[redacted] USA	TX-399-2003 [redacted]
Rat/Crl:WI(Han)	Intravenous (slow bolus) Injection	4 weeks	0 (vehicle[c]), 1, 3, 10	Yes	[redacted] USA	TX-399-2016 [redacted]
Monkey/Cynomolgus	Intravenous (slow bolus) Injection	2 weeks with 4-week recovery	0 (saline), 0 (vehicle[c]), 1, 3, 10	Yes	[redacted] USA	TX-399-2004 [redacted]
Monkey/Cynomolgus	Intravenous (slow bolus) Injection	4 weeks	0 (vehicle[c]), 1, 3, 10	Yes	[redacted] USA	TX-399-2017 [redacted]
Monkey/ Rhesus	Intravenous (slow bolus) Injection	1 week with 10-day recovery	0 (vehicle[c]), 5, 10, 20[e]	No	[redacted] USA	TX-399-2021 [redacted]
Genotoxicity						
Bacterial mutation study/*Salmonella typhimurium* and *Escherichia coli*	*In Vitro*	52 ± 4 hours	5 − 5000 µg/plate	Yes	[redacted] USA	TX-399-2005 [redacted]

Test Article: Remdesivir

Type of Study/Species/Strain	Method of Administration	Duration of Dosing	Dose (mg/kg)[a]	GLP[b]	Testing Facility	Gilead Study No. (CRO Study No.)
Chromosome aberration / Cultured human peripheral blood lymphocytes	*In Vitro*	3 hours (± S9) or 24 hours (-S9 only)	*Without S9:* 3 h: 117, 147, and 171 µg/mL 24 h: 41.2, 58.8, and 84.0 µg/mL *With S9:* 3 h: 120, 172, and 245 µg/mL	Yes	[redacted] USA	TX-399-2006 [redacted]
In Vivo Micronucleus / Rat/Crl:WI(Han)	Intravenous (slow bolus) Injection	2 weeks	0 (saline), 0 (vehicle[c]), 5, 20, 50	Yes	[redacted] USA	TX-399-2003 [redacted]
Reproductive and Developmental Toxicity						
Fertility/Rat/Crl:CD(SD)	Intravenous (slow push) Injection	Males: 28 days prior to mating through day prior to necropsy Females: 14 days prior to mating through GD 7	0 (vehicle[c]), 1, 3[f], 10[f]	Yes	[redacted] USA	TX-399-2012 [redacted]
Embryo-fetal development/ Rat/Crl:CD(SD)	Intravenous (slow push) Injection	GD 6 to GD 17	0 (vehicle[c]), 2.5, 5, 10, 20	Yes	[redacted] USA	TX-399-2013 [redacted]
Embryo-fetal development/ Rabbit/Hra:(NZW)SPF	Intravenous (slow push) Injection	GD 7 to GD 20	0 (vehicle[c]), 2.5, 5, 10[g], 20[g]	Yes	[redacted] USA	TX-399-2018 [redacted]

Test Article: Remdesivir

Type of Study/Species/Strain	Method of Administration	Duration of Dosing	Dose (mg/kg)[a]	GLP[b]	Testing Facility	Gilead Study No. (CRO Study No.)
Prenatal and postnataldevelopmental toxicity/Rat/ Crl:CD(SD)	Intravenous (slow push) Injection	F_0 dosed from GD 6 to LD 20	0 (vehicle[c]), 1, 3, 10	Yes	[redacted] USA	TX-399-2014 [redacted]
Local Tolerance						
Bovine Corneal Opacity and Permeability (BCOP) Assay	In Vitro	Opacity assessment (4 hours) followed by permeability assessment (90 minutes)	750 µL of 20% w/v suspension	Yes	[redacted] UK	TX-399-2025 [redacted]
Skin Irritation/EpiDerm™	In Vitro	15 minute treatment	10 mg	Yes	[redacted] UK	TX-399-2023 [redacted]
Other Toxicity Studies						
Hemolytic Potential and Plasma Compatibility in Human, Cynomolgus Monkey, and Rat Blood and Plasma	*In Vitro*	41 minutes	0 (vehicle[c]), 1, 3, 10 mg/mL	Yes	[redacted] USA	TX-399-2008 [redacted]
Mouse/Ces1c$^{-/-}$	Subcutaneous	1 week	10, 50	No	[redacted] USA	TX-399-2019 [redacted]
Rat/Crl:WI(Han)	Intravenous (slow bolus) Injection	1 week	0 (vehicle[c]), 0 (vehicle[l]), 20, 50	No	[redacted] USA	TX-399-2009 [redacted]

Test Article: Remdesivir

Type of Study/Species/Strain	Method of Administration	Duration of Dosing	Dose (mg/kg)[a]	GLP[b]	Testing Facility	Gilead Study No. (CRO Study No.)
Rabbit/Hra:(NZW)SPF	Intravenous (slow bolus) Injection	1 week	0[c], 5, 15[h], 50	No	[redacted] USA	TX-399-2010 [redacted]
Monkey/Cynomolgus (GS-466547[k])	Intramuscular (bolus) Injection	1 week	0[i], 2.5, 7.5[j], 15	No	[redacted] USA	TX-399-2001 [redacted]
Impurity Qualification/ Monkey/Cynomolgus	Intravenous (slow bolus) Injection	2 weeks	0 (saline), 0 (vehicle[c]), 5[n], 5[o], 10[m], 10[n], 10[o]	Yes	[redacted] USA	TX-399-2015 [redacted]
Impurity Bacterial Mutation /*Salmonella typhimurium* and *E.coli* ([redacted][p])	*In Vitro*	52 ± 4 hours	5 − 5000 µg/plate	Yes	[redacted] USA	TX-399-2020 [redacted]
Metabolite Bacterial Mutation /*Salmonella typhimurium* and *E.coli* (*GS-441524*[q])	*In Vitro*	72 hours	5 − 250 µg/well	Yes	[redacted]	TX-195-2006 [redacted]
Human 3D Liver Tissue (GS-5734 and Metabolites GS-704277, and GS-441524)	*In Vitro*	2 weeks	GS-5734: 7.5, 1.5, 0.3 µM GS-704277: 3, 1, 0.3 µM GS-441524: 3, 1, 0.3 µM	No	[redacted] USA	TX-399-2022 [redacted]
Rat, Monkey, and Human Hepatocytes (GS-5734 and Metabolites GS-704277, and GS-441524)	*In Vitro*	2 weeks	GS-5734: 30-0.12 µM GS-704277: 100-0.4 µM GS-441524: 300-1.2 µM	No	[redacted] USA	PC-399-2027 [redacted]

CRO = contract research organization; F = female; GD = gestation day; GLP = Good Laboratory Practice; LD = lactation day; Remdesivir = GS-5734

a Unless otherwise specified for Repeat-Dose Toxicity, the highest No-Observed-Adverse-Effect Level (NOAEL) is underlined.

b 'Yes', indicates 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 (pH range may vary between 3.0-3.5).

d Female NOAEL identified no NOAEL established for males

e NOAEL or NOEL not established

f NOAEL for male and female systemic toxicity 3mg/kg and 10 mg/kg for male reproductive toxicity
g NOAEL for maternal toxicity 10 mg/kg and 20 mg/kg for embryo fetal development
h NOAEL/NOEL was not identified
i Vehicle control article was 5% (v/v) ethanol (EtOH) in 95% propylene glycol (PG).
j NOAEL identified for systemic toxicity and no NOAEL established for local tolerance
k GS-466547 (diastereomeric prodrug mixture containing Remdesivir)
l Vehicle control article was 90% normal saline, 5% ethanol (EtOH), 5% polysorbate 80 (v/v/v) and hydrochloric acid pH 3.0
m Remdesivir
n Remdesivir-I (with impurities)
o Remdesivir-D (with degradants and impurities)
p [redacted] is an impurity of Remdesivir
q GS-441524 is a metabolite of Remdesivir

2. TOXICOKINETICS: OVERVIEW OF TOXICOKINETICS STUDIES

Test Article: Remdesivir

Type of Study	Test System	Method of Administration	Dose (mg/kg)	GLP[a]	Gilead Study No. (CRO Study No.)
1-week Toxicity	Rat	Intravenous (slow bolus) Injection	0 (vehicle[b]), 0 (vehicle[c]), 20, 50	No	TX-399-2009 [redacted]
2-week Toxicity	Rat	Intravenous (slow bolus) Injection	0 (saline), 0 (vehicle), 5, 20, 50	Yes	TX-399-2003 [redacted]
4-week Toxicity	Rat	Intravenous (slow bolus) Injection	0 (vehicle), 1, 3, 10	Yes	TX-399-2016 [redacted]
1-week Toxicity[d]	Cynomolgus Monkey	Intramuscular (bolus) Injection	0, 2.5, 7.5, 15	No	TX-399-2001 [redacted]
2-week Toxicity	Cynomolgus Monkey	Intravenous (slow bolus) Injection	0 (saline), 0 (vehicle), 1, 3, 10	Yes	TX-399-2004 [redacted]
4-week Toxicity	Cynomolgus Monkey	Intravenous (slow bolus) Injection	0 (vehicle), 1, 3, 10	No	TX-399-2017 [redacted]
1-week Toxicity	Rhesus Monkey	Intravenous (slow bolus) Injection	0 (vehicle), 5, 10, 20	No	TX-399-2021 [redacted]
Embryo-Fetal Development	Rat	Intravenous (slow bolus) Injection	0 (vehicle), 2.5, 5, 10, 20	Yes	TX-399-2013 [redacted]
Pre- and Postnatal Development	Rat	Intravenous (slow bolus) Injection	0 (vehicle), 1, 3, 10	Yes	TX-399-2014 [redacted]
7-Day Tolerability Study	Rabbit	Intravenous (slow bolus) Injection	0, 5, 15, 50	No	TX-399-2010 [redacted]
Embryo-Fetal Development	Rabbit	Intravenous (slow bolus) Injection	0 (vehicle), 2.5, 5, 10, 20	Yes	TX-399-2018 [redacted]
2-Week Repeat Dose Impurity Qualification	Cynomolgus Monkey	Intravenous (slow bolus) Injection	0 (saline), 0 (vehicle), 5, 5, 10, 10, 10	Yes	TX-399-2015 [redacted]

CRO = contract research organization; GLP = Good Laboratory Practice
a 'Yes', indicates study includes a GLP Compliance statement.
b Vehicle control article was 12% (w/v) sulfobutylether-β-cyclodextrin in Sterile Water for Injection, USP, pH 3.5 ± 0.1 (pH range varies 3.0-3.5).
c Vehicle control article was 90% normal saline, 5% ethanol (EtOH), 5% polysorbate 80 (v/v/v) and hydrochloric acid, pH 3.0
d Test article was GS-466547 (diastereomeric prodrug mixture containing Remdesivir)

3. TOXICOKINETICS: OVERVIEW OF TOXICOKINETICS DATA

3.1. Remdesivir

Test Article: Remdesivir

	Steady State GS-5734 AUC_{0-24} (ng•h/mL)						
Species	**Rat**		**Rabbit**	**Cynomolgus Monkey**		**Cynomolgus Monkey**	**Rhesus**
Method of Administration	**IV (Slow Bolus) Injection**		**IV (Slow Bolus) Injection**	**IV (Slow Bolus) Injection**		**IM Injection**	**IV (Slow Bolus) Injection**
Sex (M/F)	**M**	**F**	**F**	**M**	**F**	**M**	**M**
Dose (mg/kg)							
1	NR[c]	NR[c], NR[h]	—	88.7[b], 101[d]	111[b], 103[d]	—	—
2.5	—	NR[g]	196[i]	—	—	956[j]	—
3	NR[c]	NR[c], NR[h]	—	363[b], 265[d]	342[b], 265[d]	—	—
5	NR[a]	NR[a], NR[g]	298[f], 643[i]	—	—	—	653[e]
7.5	—	—	—	—	—	4350[j]	—
10	NR[c]	NR[c], NR[g], NR[h]	2110[i]	1720[b], 1260[d]	1780[b], 1400[d]	—	2120[e]
15	—	—	3480[f]	—	—	2850[j]	—
20	NR[a], NR[k1], 13[k2]	NR[a], NR[g],	2830[i]	—	—	—	5600[e]
50	NR[a], 32[k1], 121[k2]	NR[a],	46,400[f]	—	—	—	—

AUC_{0-24} = area under the plasma concentration-time curve from 0 to 24 hours postdose; F= female; IV = intravenous; IM = intramuscular; M = male
— = species and/or sex was not dosed at this level; NR = not reported due to limited plasma concentrations below the GS-5734 limit of quantitation in rat (< 2.00 ng/mL)
a TX-399-2003 Day 15
b TX-399-2004 Day 15
c TX-399-2016 Day 28
d TX-399-2017 Day 28
e TX-399-2021 Day 6
f TX-399-2010 Day 6

g TX-399-2013 Day 17
h TX-399-2014 LD 10
i TX-399-2018 GD 20
j TX-399-2001 Day 7 Test article was GS-466547 (diastereomeric prodrug mixture)
k TX-399-2009 Day 7 in vehicle 1[1](12% (w/v) sulfobutylether-β-cyclodextrin in Sterile Water for Injection, USP, pH 3.5 ± 0.1 (pH range varies 3.0-3.5) and vehicle 2[2] (Vehicle control article was 90% normal saline, 5% ethanol (EtOH), 5% polysorbate 80 (v/v/v) and hydrochloric acid, pH 3.0).

3.2. GS-441524

Test Article: Remdesivir

	Steady State GS-441524 AUC_{0-24} (ng•h/mL)						
Species	**Rat**		**Rabbit**	**Cynomolgus Monkey**		**Cynomolgus Monkey**	**Rhesus**
Method of Administration	**IV (Slow Bolus) Injection**		**IV (Slow Bolus) Injection**	**IV (Slow Bolus) Injection**		**IM Injection**	**IV (Slow Bolus) Injection**
Sex (M/F)	**M**	**F**	**F**	**M**	**F**	**M**	**M**
Dose (mg/kg)							
1	209[c]	140[c], 349[h]	—	192[b], 167[d]	214[b], 213[d]	—	—
2.5	—	642[g]	429[i]	—	—	771[j]	—
3	1000[c]	493[c], 538[h]	—	804[b], 404[d]	577[b], 482[d]	—	—
5	1880[a]	1420[a], 1210[g]	844[f], 1090[i]	—	—	—	1390[e]
7.5	—	—	—	—	—	3370[j]	—
10	4210[c]	2940[c], 3680[g], 2310[h]	1790[i]	2450[b], 1890[d]	2330[b], 2240[d]	—	6050[e]
15	—	—	5030[f]	—	—	18,200[j]	—
20	10,000[a], 10,000[k1], 13,400[k2]	12,700[a], 8740[g]	8930[i]	—	—	—	21,000[e]
50	21,700[a], 30,900[k1], 44,400[k2]	19,000[a]	50,700[f]	—	—	—	—

AUC_{0-24} = area under the plasma concentration-time curve from 0 to 24 hours postdose; F= female; IV = intravenous; IM = intramuscular; M = male
— = species and/or sex was not dosed at this level
a TX-399-2003 Day 15
b TX-399-2004 Day 15
c TX-399-2016 Day 28
d TX-399-2017 Day 28
e TX-399-2021 Day 6

f TX-399-2010 Day 6
g TX-399-2013 Day 17
h TX-399-2014 LD 10
i TX-399-2018 GD 20
j TX-399-2001 Day 7 Test article was GS-466547 (diastereomeric prodrug mixture)
k TX-399-2009 Day 7 in vehicle 1[1](12% (w/v) sulfobutylether-β-cyclodextrin in Sterile Water for Injection, USP, pH 3.5 ± 0.1 (pH range varies 3.0-3.5) and vehicle 2[2] (Vehicle control article was 90% normal saline, 5% ethanol (EtOH), 5% polysorbate 80 (v/v/v) and hydrochloric acid, pH 3.0).

3.3. GS-704277

Test Article: Remdesivir

	Steady State GS-704277 AUC_{0-24} (ng•h/mL)						
Species	**Rat**		**Rabbit**	**Cynomolgus Monkey**		**Cynomolgus Monkey**	**Rhesus**
Method of Administration	**IV (Slow Bolus) Injection**		**IV (Slow Bolus) Injection**	**IV (Slow Bolus) Injection**		**IM Injection**	**IV (Slow Bolus) Injection**
Sex (M/F)	**M**	**F**	**F**	**M**	**F**	**M**	**M**
Dose (mg/kg)							
1	82.3[c]	NR[c], 70.7[h]	—	65.7[d]	68.6[d]	—	—
2.5	—	333[g]	657[i]	—	—	340[j]	—
3	424[c]	167[c], 343[h]		160[d]	209[d]	—	—
5	—	391[g]	1280[i], 953f	—	—	—	602[e]
7.5	—	—	—	—	—	2980[j]	—
10	2150[c]	904[c], 1180[g], 1190[h]	2860[i]	794[d]	903[d]	—	1500[e]
15	—	—	6150[f]	—	—	11,800[j]	—
20	12,000[k1], 19,700[k2]	3420[g]	11,700[i]	—	—	—	7890[e]
50	39,600[k1], 46,700[k2]	—	80,900[f]	—	—	—	—

AUC_{0-24} = area under the plasma concentration-time curve from 0 to 24 hours postdose; F= female; IV = intravenous; IM = intramuscular; M = male
— = species and/or sex was not dosed at this level; NR = not reported due to limited plasma concentrations below the GS-704277 limit of quantitation in rat (< 5.00 ng/mL)
a TX-399-2003 Day 15
b TX-399-2004 Day 15
c TX-399-2016 Day 28
d TX-399-2017 Day 28
e TX-399-2021 Day 6
f TX-399-2010 Day 6

g TX-399-2013 Day 17
h TX-399-2014 LD10
i TX-399-2018 GD20
j TX-399-2001 Day 7 Test article was GS-466547 (diastereomeric prodrug mixture)
k TX-399-2009 Day 7 in vehicle 1[1](12% (w/v) sulfobutylether-β-cyclodextrin in Sterile Water for Injection, USP, pH 3.5 ± 0.1 (pH range varies 3.0-3.5) and vehicle 2[2] (Vehicle control article was 90% normal saline, 5% ethanol (EtOH), 5% polysorbate 80 (v/v/v) and hydrochloric acid, pH 3.0).

4. TOXICOLOGY: DRUG SUBSTANCE

Test Article: Remdesivir

Batch No.	Purity (%)[a]	Observed Impurities (%)																Type of Study
		不純物1*	不純物2*	[redacted]	[redacted]	不純物5*	[redacted]	[redacted]	不純物3*	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	不純物4*	不純物8*	
Proposed Specification	[redacted]	[redacted]	[redacted]	[redacted]		[redacted]	[redacted]	[redacted]										
[redacted]	84.1	6.25	0.05 (Trace)	0.18	—	0.73	0.24	0.29	—	—	—	—	—	—	—	—	—	c
[redacted]	98.6	0.60	0.04 (Trace)	—	—	0.21	0.03 (Trace)	0.04 (Trace)	—	—	—	0.03 (Trace)	—	—	—	0.08	0.08	d
[redacted]	97.9	1.12	—	—	—	0.06	—	—	—	—	—	0.09	0.07	—	—	—	—	e, f
[redacted]	77.7	0.52	0.17	—	—	2.52	0.98	0.62	—	0.72	—	0.77	0.30	0.50	4.51	1.44	1.00	f
[redacted]	96.2	1.07	0.33	—	—	—	—	—	5.56	0.24	0.57	0.07 (Trace)	0.06 (Trace)	—	—	—	—	f
[redacted]	95.0	3.87	0.05	0.02	0.04	0.13	—	—	—	—	—	—	—	—	—	—	—	g
[redacted]	98.3	0.76	—	—	—	0.06	—	—	—	—	—	0.06	0.08	—	—	—	—	h

All batches in the table are reprocessed for re-labeling RRT names of identified impurities to GS-codes and applying relative response factors per analytical test method validation results. The batch analysis data for the toxicology batches that were used in impurity qualification studies are also included in Module 3 Sections 3.2.S.4.4 and 3.2.P.5.4 for Remdesivir Injection.

a Purity is determined by external standard.
b Unidentified impurities below 0.10% are not included.
c Used for study numbers: TX-399-2003, TX-399-2004, TX-399-2005, TX-399-2006, TX-399-2008, PC-399-2003, PC-399-2004, PC-399-2005, PC-399-2006 in repeat dose, safety pharmacology and gene tox.
d Used for study numbers: TX-399-2016, TX-399-2017, TX-399-2012, TX-399-2014, TX-399-2021, TX-399-2023, TX-399-2025 in repeat dose, repro tox., and worker safety studies
e Used for study numbers: TX-399-2013, TX-399-2015, TX-399-2018 in repro tox, and impurity qualification studies
f Used for study number: TX-399-2015 in impurity qualification
g Used for study number: TX-399-2009 in investigative repeat dose
h Used for study number: TX-399-2010 in rabbit tolerability

*情報提供時に置き換え

Test Article: Remdesivir

Batch No.	Observed Impurities (%)																	Type of Study
	不純物6*	不純物7*	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	
Proposed Specification								[redacted]	[redacted]			[redacted]	[redacted]					
[redacted]	—	—	—	—	—	0.09	—	0.36	0.15	0.15		0.33	0.14	0.02 (Trace)	0.32	0.14	0.03 (Trace)	c
[redacted]	0.11	0.08	—	—	—	0.05	0.05 (Trace)	—	—	—	—	—	—	—	—	—	—	d
[redacted]	—	0.36	—	—	—	0.32	—	—	—	—	—	—	—	—	—	—	—	e, f
[redacted]	1.58	1.41	1.98	0.72	—	0.05	—	0.10	—	—	—	—	—	0.24	—	—	—	f
[redacted]	—	0.31	—	—	0.52	0.30	—	—	—	—	0.11	—	—	—	—	—	—	f
[redacted]	—	—	—	—	—	—	—	—	—	—	—	—	—	0.03	—	0.07	0.14	g
[redacted]	—	0.31	—	—	—	0.36	—	—	—	0.05	—	—	—	—	—	—	—	h

All batches in the table are reprocessed for re-labeling RRT names of identified impurities to GS-codes and applying relative response factors per analytical test method validation results. The batch analysis data for the toxicology batches that were used in impurity qualification studies are also included in Module 3 Sections 3.2.S.4.4 and 3.2.P.5.4 for Remdesivir Injection.

a Purity is determined by external standard.
b Unidentified impurities below 0.10% are not included.
c Used for study numbers: TX-399-2003, TX-399-2004, TX-399-2005, TX-399-2006, TX-399-2008, PC-399-2003, PC-399-2004, PC-399-2005, PC-399-2006 in repeat dose, safety pharmacology and gene tox.
d Used for study numbers: TX-399-2016, TX-399-2017, TX-399-2012, TX-399-2014, TX-399-2021, TX-399-2023, TX-399-2025 in repeat dose, repro tox., and worker safety studies
e Used for study numbers: TX-399-2013, TX-399-2015, TX-399-2018 in repro tox, and impurity qualification studies
f Used for study number: TX-399-2015 in impurity qualification
g Used for study number: TX-399-2009 in investigative repeat dose
h Used for study number: TX-399-2010 in rabbit tolerability study

*情報提供時に置き換え

Test Article: Remdesivir

Batch No.	Observed Impurities (%)																				Type of Study
	[redacted]																				
Proposed Specification	[redacted]			[redacted]				[redacted]							[redacted]		[redacted]			[redacted]	
[redacted]	0.18	0.18	0.17	0.17	0.29		0.10	0.29	0.10	0.10	0.24	0.19	0.07	0.50	0.10	0.10	0.24	0.11	—	0.11	c
[redacted]	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	d
[redacted]	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	e, f
[redacted]	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0.13	—	f
[redacted]	—	—	—	—	—	0.49	—	—	—	—	—	—	—	—	—	—	—	—	—	—	f
[redacted]	—	—	—	—	—	0.06	—	—	0.06	0.04	—	0.09	0.13	—	—	—	—	—	—	—	g
[redacted]	—	—	—	—	—		—	—	—	—	—	—	—	—	—	—	—	—	—	—	h

All batches in the table are reprocessed for re-labeling RRT names of identified impurities to GS-codes and applying relative response factors per analytical test method validation results. The batch analysis data for the toxicology batches that were used in impurity qualification studies are also included in Module 3 Sections 3.2.S.4.4 and 3.2.P.5.4 for Remdesivir Injection.

a Purity is determined by external standard.
b Unidentified impurities below 0.10% are not included.
c Used for study numbers: TX-399-2003, TX-399-2004, TX-399-2005, TX-399-2006, TX-399-2008, PC-399-2003, PC-399-2004, PC-399-2005, PC-399-2006 in repeat dose, safety pharmacology and gene tox.
d Used for study numbers: TX-399-2016, TX-399-2017, TX-399-2012, TX-399-2014, TX-399-2021, TX-399-2023, TX-399-2025 in repeat dose, repro tox., and worker safety studies
e Used for study numbers: TX-399-2013, TX-399-2015, TX-399-2018 in repro tox, and impurity qualification studies
f Used for study number: TX-399-2015 in impurity qualification
g Used for study number: TX-399-2009 in investigative repeat dose study
h Used for study number: TX-399-2010 in rabbit tolerability study

5. TOXICOLOGY: SINGLE-DOSE TOXICITY

Not applicable.

6. REPEAT-DOSE TOXICITY (NONPIVOTAL STUDIES)

Test Article: Remdesivir

Species/Strain	Method of Administration (Vehicle/Formulation)	Duration of Dosing	Dose (mg/kg/day)	Sex and No. per Group	NOAEL (mg/kg/day)	Noteworthy Findings	Gilead Study No. (CRO Study No.)
Monkey / Rhesus	IV (slow bolus) Injection	7 days with 10-day recovery period	0[a], 5, 10, 20	3M (main study); 3M (recovery) in the vehicle control and 20 mg/kg/day groups	NOAEL could not be determined	One animal at 20 mg/kg/day euthanized early on Day 6 due to remdesivir-related kidney findings. Adverse findings at ≥ 5 mg/kg/day: ↑ urea nitrogen and creatinine indicating altered kidney function, with correlating histopathology findings of renal tubular atrophy and basophilia and casts. Kidney findings considered adverse at all dose levels due to loss of tubules and resulting interstitial fibrosis in one recovery animal suggestive of the finding progressing to chronicity. A NOAEL could not be determined.	TX-399-2021 [redacted]

NOAEL = No-Observed-Adverse-Effect Level; ↑ = increased from vehicle control; M = male
a Vehicle control article was 12% (w/v) SBECD in Sterile Water for Injection, USP, pH 3.5 ± 0.1

7. TOXICOLOGY: REPEAT-DOSE TOXICITY (PIVOTAL STUDIES)

7.1. 2-Week Rat

Test Article: Remdesivir

Report Title: 2-Week Intravenous (Slow Bolus) Toxicity and Toxicokinetic Study with GS-5734 in Wistar-Han Rats with a 4 Week Recovery Phase and Bone Marrow Micronucleus Assay		**Gilead Study No.:** TX-399-2003 [redacted]
Species/Strain: Crl:WI(Han) Rats	**Duration of Dosing:** 2 Weeks	**Location in CTD:** 4.2.3.2
Initial Age: Six to seven weeks	**Duration of Postdose:** 4 Weeks	**GLP Compliance:** Yes
Date of First Dose: [redacted] 20[redacted]	**Method of Administration:** Intravenous (Slow Bolus) Injection	**Lot Number:** [redacted]
Vehicle: 12% (w/v) sulfobutylether-β-cyclodextrin (SBECD) in Sterile water for Injection, USP, pH 3.5 ± 0.1		
Special Features: Urine biomarker analysis, bone marrow micronucleus assay, hepatic microsomal fractions		
No Observed Adverse Effect Level: Not achieved for male rats, 5 mg/kg/day for female rats		

Daily Dose (mg/kg/day)	**0 (Saline Control)**		**0 (Vehicle Control)**		**5**		**20**		**50**	
Gender: Number of Animals (TK)	**M: 3**	**F: 3**	**M: 3**	**F: 3**	**M: 9**	**F: 9**	**M: 9**	**F: 9**	**M: 9**	**F: 9**
GS-5734 Toxicokinetics: AUC_{0-24} (ng•h/mL)										
Day 1	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
Day 15	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	385	BLQ
GS-5734 Toxicokinetics: C_{max} (ng/mL)										
Day 1	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	79.5	157
Day 15	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	8.46	520	233
GS-441524 Toxicokinetics: AUC_{0-24} (ng•h/mL)										
Day 1	BLQ	BLQ	BLQ	BLQ	1190	942	4560	3350	12900	14300
Day 15	BLQ	BLQ	BLQ	BLQ	1880	1420	10000	12700	21700	19000
GS-441524 Toxicokinetics: C_{max} (ng/mL)										
Day 1	BLQ	BLQ	BLQ	BLQ	315	294	1240	879	2750	2170
Day 15	BLQ	BLQ	BLQ	BLQ	371	324	1760	1690	3560	2900

Test Article: Remdesivir

Daily Dose (mg/kg/day)	**0 (Saline Control)**		**0 (Vehicle Control)**		**5**		**20**		**50**	
Gender: Number of Toxicity Animals	**M: 10**	**F: 10**	**M: 15**	**F: 15**	**M: 10**	**F: 10**	**M: 10**	**F: 10**	**M: 15**	**F: 15**
Noteworthy Findings										
Died or sacrificed moribund	0	0	0	0	0	0	0	0	0	0
Body Weight (%)[a]										
Days 1 to 15	56 g	26 g	56 g	24 g	-17.9*	-8.3	-58.9*	-70.8*	-66.1*	-70.8*
Food Consumption (%)[a]										
Days 1 to 4	15 g	11 g	13 g	11 g	0.0	0.0	-7.7*	-18.2	-15.4*	-18.2
Days 4 to 7	18 g	15 g	17 g	14 g	0.0	14.3	-11.8	-14.3*	-23.5*	-21.4*
Days 7 to 11	21 g	15 g	20 g	18 g	-5.0	-16.7	-20.0*	-27.8	-20.0*	-27.8
Days 11 to 15	22 g	16 g	21 g	15 g	-9.5*	0.0	-14.3*	-20.0*	-19.0*	-13.3*
Clinical Observations										
Discolored Skin, Tail Distal, Red	0	0	0	0	0	2	2	1	2	0
Discolored Skin, Tail Mid, Red	0	0	0	0	0	2	2	3	5	6
Ophthalmoscopy	No test article related findings									
Hematology										
Red Blood Cell Count (E6/uL)	—	8.28	—	8.45	—	8.51	—	8.67	—	7.98*
Hemoglobin (g/dL)	16.2	15.9	16.0	15.8	16.2	16.0	16.2	16.3	15.5	14.7*
Hematocrit (%)	50.4	49.0	49.5	48.0	50.0	49.0	50.0	49.5	47.6*	45.2*
Mean Corpuscular Volume (fL)	59.3	—	59.0	—	58.7	—	58.0	—	57.1*	—
Reticulocyte Count (E3/uL)	307.6	272.6	332.0	260.5	264.4*	235.4	217.9*	157.0*	244.5*	219.4*
Coagulation										
Activated Partial Thromboplastin (sec)	12.7	13.9	13.5	13.1	12.6	12.5	14.5	9.8*	10.9	10.5*
Clinical Chemistry										
Urea Nitrogen (mg/dL)	11	—	11	—	14*	—	13*	—	12	—
Creatinine (mg/dL)	0.5	0.6	0.5	0.6	0.6*	0.6	0.7*	0.8*	0.7*	0.7*
Inorganic Phosphorus (mg/dL)	—	8.3	—	7.6*	—	7.7	—	8.2*	—	8.6*
Chloride (mg/dL)	101	102	101	102	100	100	101	103	103*	105*
Urinalysis										
Day 4										
Urine Volume (mL)	29.5	15.3	18.7*	14.5	16.4	15.3	23.8	23.7*	35.9*	28.0*

Test Article: Remdesivir

Daily Dose (mg/kg/day)	**0 (Saline Control)**		**0 (Vehicle Control)**		**5**		**20**		**50**	
Gender: Number of Toxicity Animals	**M: 10**	**F: 10**	**M: 15**	**F: 15**	**M: 10**	**F: 10**	**M: 10**	**F: 10**	**M: 15**	**F: 15**
Urine pH	7.2	—	7.2	—	7.1	—	7.0	—	6.3*	—
White Blood Cells in Urine	0	1 (1)	1 (1)	1 (2) QNS (1)	1 (1)	1 (2)	1 (2)	1 (3)	0	1 (5) 2 (1)
Granular Casts in Urine	0	0	0	QNS (1)	1 (1)	0	1 (1)	0	3 (1)	1 (4)
Urine Protein	Trace (1)	—	Trace (4) 1+ (1)	Trace (4) QNS (1)	Trace (3) 1+ (4)	1+ (1)	1+ (2) 2+ (8)	Trace (3) 1+ (3)	2+ (11) 3+ (4)	1+ (2) 2+ (12)
Urine Blood	Trace (2) 1+ (2)	Trace (1)	Trace (10) 1+ (3)	Trace (4) 1+ (2) QNS (1)	Trace (5) 1+ (1)	Trace (1) 1+ (1)	Trace (6) 1+ (2) 3+ (1)	Trace (5) 1+ (2) 2+ (1)	Trace (5) 1+ (9) 2+ (1)	Trace (5) 1+ (8) 2+ (2)
Urine Glucose	—	—	—	QNS (1)	—	—	Trace (1)	—	Trace (5) 1+ (5)	—
Day 16										
Urine Protein	Trace (3) 1+ (2)	Trace (1)	Trace (4) 1+ (5) 2+ (1)	Trace (1)	Trace (1) 1+ (7) 2+ (2)	Trace (1) 1+ (1)	Trace (2) 1+ (7) 2+ (1)	Trace (1) 1+ (3)	Trace (1) 1+ (12) 2+ (2)	Trace (5) 1+ (7)
Urine Blood	Trace (1) 1+ (1)	—	Trace (2)	Trace (1)	Trace (3)	Trace (1)	Trace (2)	Trace (3) 1+ (1)	Trace (5) 1+ (6)	Trace (1) 1+ (1)
Urine Glucose	—	—	—	—	—	—	Trace (2) 1+ (1)	—	Trace (5) 1+ (2) 2+ (1)	Trace (1)
Urine Chemistry										
Day 4										
Urine Total Protein:Urine Creatinine	0.53	0.42	0.69*	0.41	1.23*	0.46	7.14*	1.51*	12.58*	6.05*
Urine NAG:Urine Creatinine	0.7	0.5	0.6	0.5	0.7	0.6	1.3*	0.8*	2.5*	1.4*
Urine Sodium Excretion (mmol/L)	0.66	0.39	0.56	0.39	0.64	0.42	0.93*	0.82*	0.81*	0.63*
Urine Potassium Excretion (mmol/L)	0.76	0.50	0.66	0.43	0.61	0.46	0.87*	0.64*	1.07*	0.78*
Urine Chloride Excretion (mmol/L)	0.52	0.29	0.38*	0.23	0.36	0.25	0.38	0.38*	0.44	0.33*
Day 16										
Urine Total Protein:Urine Creatinine	0.65	0.35	0.90	0.32	2.96*	0.44*	3.41*	0.56*	3.77*	0.78*
Urine NAG:Urine Creatinine	0.4	0.4	0.3	0.3	0.6*	0.4*	0.9*	0.5*	1.4*	0.7*
Urine Sodium Excretion (mmol/L)	0.62	0.41	0.55	0.42	0.61	0.38	0.70	0.50	1.12*	0.54*

Test Article: Remdesivir

Daily Dose (mg/kg/day)	**0 (Saline Control)**		**0 (Vehicle Control)**		**5**		**20**		**50**	
Gender: Number of Toxicity Animals	**M: 10**	**F: 10**	**M: 15**	**F: 15**	**M: 10**	**F: 10**	**M: 10**	**F: 10**	**M: 15**	**F: 15**
Urine Potassium Excretion (mmol/L)	0.89	0.55	0.69	0.49	0.78	0.45	0.70	0.54	0.82	0.53
Urine Chloride Excretion (mmol/L)	0.46	0.33	0.38	0.27	0.33	0.23	0.43	0.28	0.59*	0.41*
Urine Biomarker										
Day 4										
Urine Cystatin C:Urine Creatinine Ratio	2.3	0.9	2.4	1.0	5.3*	1.7*	107.8*	8.4*	725.9*	134.0*
Urine Beta-2-Microglobulin: Urine Creatinine Ratio	51.16	13.21	77.19	11.16*	205.25*	15.82*	1253.53*	160.32*	2524.52*	1510.63*
Urine KIM-1:Urine Creatinine Ratio	0.015	0.013	0.014	0.011	0.035*	0.019*	0.284*	0.218*	0.869*	0.825*
Day 16										
Urine Cystatin C:Urine Creatinine Ratio	1.7	0.8	1.6	0.7	21.2*	2.3*	70.2*	10.9*	263.5*	28.1*
Urine Beta-2-Microglobulin: Urine Creatinine Ratio	52.89	11.25	61.49	10.69	565.40*	22.69*	960.22*	328.49*	1043.41*	446.29*
Urine KIM-1:Urine Creatinine Ratio	0.010	0.009	0.007*	0.008	0.013*	0.009*	0.063*	0.034*	0.138*	0.086*
Organ Weights[a]										
Kidney										
Absolute Organ Weight (%)	1.6108 g	1.2573 g	1.6555 g	1.2530 g	-7	+1	0	+5*	+13*	+24*
Organ to Body Weight (%)	0.7196	0.7599	0.7412	0.7502	0	+2	+18*	+17*	+40*	+39*
Organ to Brain Weight (%)	83.2251	67.9963	88.1147	69.2117	-10*	-1	+1	+3	+13*	+23*
Gross Pathology	No test article related findings									
Histopathology										
Number Evaluated	M: 10	F: 10	M: 10	F: 10	M: 10	F: 10	M: 10	F: 10	M: 10	F: 10
Kidney, Basophilic Tubules										
Minimal	0	0	0	0	0	5	0	0	0	0
Slight	0	0	0	0	6	1	0	2	0	0
Moderate	0	0	0	0	4	0	9	8	6	6
Marked	0	0	0	0	0	0	1	0	4	4
Kidney, Mitosis, Increased										
Minimal	0	0	0	0	8	2	7	10	7	8

Test Article: Remdesivir

Daily Dose (mg/kg/day)	**0 (Saline Control)**		**0 (Vehicle Control)**		**5**		**20**		**50**	
Gender: Number of Toxicity Animals	**M: 10**	**F: 10**	**M: 15**	**F: 15**	**M: 10**	**F: 10**	**M: 10**	**F: 10**	**M: 15**	**F: 15**
Slight	0	0	0	0	0	0	0	0	2	1
Kidney, Degeneration, Tubule, Focal/Multifocal										
Minimal	0	0	0	0	0	0	0	0	3	1
Kidney, Vacuolation, Tubule Cell, Diffuse										
Minimal	0	0	2	3	2	2	3	3	4	4
Slight	0	0	8	7	8	8	6	7	6	6
Moderate	0	0	0	0	0	0	1	0	0	0
Kidney, Hypertrophy, Tubule Cell, Focal/Multifocal										
Minimal	0	0	2	2	0	3	2	0	4	4
Slight	0	0	0	0	0	0	1	0	1	1
Hepatic Microsomal Enzyme	No test article related findings									
Bone Marrow Micronucleus	No test article related findings									
Number of Toxicity Animals in Recovery	**0**	**0**	**M: 5**	**F: 5**	**0**	**0**	**0**	**0**	**M: 5**	**F: 5**
Recovery Phase										
Body Weight (%)	No test article related findings									
Food Consumption (%)	No test article related findings									
Clinical Observations	No test article related findings									
Hematology	No test article related findings									
Clinical Chemistry										
Cholesterol (mg/dL)	NA	NA	—	49	NA	NA	NA	NA	—	5
Urinalysis										
Urine Protein	NA	NA	Trace (1) 1+ (2)	-	NA	NA	NA	NA	Trace (1) 1+ (3) 2+ (1)	-
Urine Chemistry										
Urine Chloride (mmol/L)	NA	NA	—	49	NA	NA	NA	NA	—	58
Total Protein:Urine Creatinine	NA	NA	0.67	—	NA	NA	NA	NA	1.93	—
Organ Weights[a]										
Kidney										

Test Article: Remdesivir

Daily Dose (mg/kg/day)	**0 (Saline Control)**		**0 (Vehicle Control)**		**5**		**20**		**50**	
Gender: Number of Toxicity Animals	**M: 10**	**F: 10**	**M: 15**	**F: 15**	**M: 10**	**F: 10**	**M: 10**	**F: 10**	**M: 15**	**F: 15**
Absolute Organ Weight (%)	NA	NA	—	1.2351 g	NA	NA	NA	NA	—	+31
Organ to Body Weight (%)	NA	NA	—	0.6170	NA	NA	NA	NA	—	+31
Organ to Brain Weight (%)	NA	NA	—	67.7520	NA	NA	NA	NA	—	+31
Histopathology										
Number Evaluated	0	0	M: 5	F: 5	0	0	0	0	M: 5	F: 5
Kidney, Basophilic Tubules, Diffuse										
Minimal	NA	NA	0	0	NA	NA	NA	NA	2	0
Kidney, Nephropathy, Chronic, Progressive										
Minimal	NA	NA	3	2	NA	NA	NA	NA	3	3
Slight	NA	NA	0	0	NA	NA	NA	NA	2	0
Kidney, Vacuolation, Tubule Cell, Diffuse										
Minimal	NA	NA	2	4	NA	NA	NA	NA	0	0
Kidney, Hypertrophy, Tubule Cell, Focal/Multifocal										
Minimal	NA	NA	2	1	NA	NA	NA	NA	2	4
Slight	NA	NA	0	0	NA	NA	NA	NA	3	0

AUC_{0-24}= Area under the concentration-time curve from hour 0 to hour 24, estimated by the linear trapezoidal rule; BLQ = below the lower limit of quantitation (< 2.00 ng/mL for GS-5734 and GS-441524 and < 5.00 ng/mL for GS-704277); C_{max}= Maximum observed concentration; F = Female; GLP = Good Laboratory Practice; M = Male;
NA = NotApplicable; NR= Not reported; NC = Not calculable; TK = toxicokinetic; − Comparable to control animals or no test article-related findings; * = $p \leq 0.05$

a For controls, group means are shown. For treated groups, percent differences from vehicle controls (Group 2) are shown. Statistical significance is based on actual data not on the percent.

7.2. 4-Week Rat

Test Article: Remdesivir

Report Title: A 4-Week Intravenous Slow Bolus Injection Toxicity and Toxicokinetic Study with GS-5734 in Wistar Han Rats		**Gilead Study No.** TX-399-2016 [redacted]
Species / Strain: Crl:WI(Han) rats	**Duration of Dosing:** 4 weeks	**Location in** CTD**:**
Initial Age: 6 to 7 weeks old	**Duration of Postdose:** N/A	**GLP Compliance:** Yes
Date of First Dose: [redacted]20[redacted]	**Method of Administration:** Slow Bolus Injection	**Lot Number:** [redacted]
Vehicle: 12% (w/v) sulfobutylether-b-cyclodextrin (Dexolve-7™ [SBE-b-CD]) in Sterile Water for Injection, USP, pH 3.5 ± 0.1		
Special Features: kidney urine biomarker analysis		
No Observed Adverse Effect Level: 3 mg/kg/day		

Daily Dose (mg/kg/day)	**0 (Control)**		**1 (Low)**		**3 (Mid)**		**10 (High)**	
Sex: Number of Animals (TK)	M: 3	F: 3	M: 6	F: 6	M: 6	F: 6	M: 6	F: 6
GS-5734 Toxicokinetics: AUC_{0-24} (ng·h/mL)								
Day 1	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
Day 28	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
GS-5734 Toxicokinetics: Cmax (ng/mL)								
Day 1	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
Day 28	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
GS-441524 Toxicokinetics: AUC_{0-24} (ng·h/mL)								
Day 1	BLQ	BLQ	151	121	473	419	1880	1700
Day 28	BLQ	BLQ	209	140	1000	493	4210	2940
GS-441524 Toxicokinetics: Cmax (ng/mL)								
Day 1	BLQ	BLQ	44.1	36.0	124	115	448	397
Day 28	BLQ	BLQ	50.1	38.8	208	127	754	521
GS-704277 Toxicokinetics: AUC_{0-24} (ng·h/mL)								
Day 1	BLQ	BLQ	NR[a]	NR[a]	292	141	782	676
Day 28	BLQ	BLQ	82.3	NR[a]	424	167	2150	904
GS-704277 Toxicokinetics: Cmax (ng/mL)								
Day 1	BLQ	BLQ	160	139	526	388	2190	1550
Day 28	BLQ	BLQ	213	130	1020	288	4210	2040

Test Article: Remdesivir

Daily Dose (mg/kg/day)	**0 (Control)**		**1 (Low)**		**3 (Mid)**		**10 (High)**	
Sex: Number of Animals (Main)	**M: 10**	**F: 10**	**M: 10**	**F: 10**	**M: 10**	**F: 10**	**M: 10**	**F: 10**
Noteworthy Findings								
Died or sacrificed moribund	0	0	0	0	0	0	0	2[b]
Clinical Observations	No test article-related findings[b]							
Ophthalmoscopy	No test-article related findings.							
Body Weight (%)[c]								
Day 22	261	188	-	-	-	-	-9.96*	-6.38
Day 29	282	199	-	-	-	-	-12.4*	-8.54
Body Weight Change (g)[c]								
Day 1-29	108g	7g	-	-	-	-	-33	-35
Food Consumption (g)								
Days 1-8	17	13	-	-	-	-	15*	11*
Days 8-15	23	17	-	-	-	-	21	15*
Days 15-22	20	15	-	-	-	-	18	13*
Days 22-29	20	15	-	-	-	-	17*	13*
Hematology								
Day 30								
hemoglobin concentration (g/dL)	15.9	-	16.0	-	15.7	-	15.3*	-
hematocrit (%)	48.6	-	48.9	-	48.0	-	46.9	-
mean corpuscular volume (fL)	57.8	-	58.3	-	57.3	-	56.5	-
absolute reticulocyte count (E3/uL)	194.6	-	214.4	-	192.8	-	136.1*	-
Coagulation	No test article-related findings.							
Clinical Chemistry								
Day 30								
creatinine (mg/dL)	0.6	0.6	0.6	0.7	0.6	0.7	0.7	0.7
Urinalysis								

Test Article: Remdesivir

Daily Dose (mg/kg/day)	**0 (Control)**		**1 (Low)**		**3 (Mid)**		**10 (High)**	
Sex: Number of Animals (Main)	**M: 10**	**F: 10**	**M: 10**	**F: 10**	**M: 10**	**F: 10**	**M: 10**	**F: 10**
Day 30								
Urine qualitative protein	2 (trace) 3 (1+)	-	3 (trace) 4 (1+)	-	6 (trace) 4 (1+)	-	10 (1+)	-
Urine qualitative glucose	0	-	0	-	0	-	1 (trace) 4 (1+) 1 (2+) 1 (3+)	-
Urine Chemistry								
Day 4								
Total protein:creatinine ratio	0.53	0.41	0.54	0.50	0.72	0.39	0.93*	0.48
NAG:creatinine ratio	0.5	0.6	0.6	0.6	0.7*	0.5	0.8*	0.6
Sodium excretion (mmol)	0.56	-	0.67	-	0.68	-	0.82*	-
Chloride excretion (mmol)	0.36	-	0.42	-	0.42	-	0.45	-
Day 30								
Total protein:creatinine ratio	0.98	0.36	1.06	0.30	1.95*	0.47	3.08*	0.63*
NAG:creatinine ratio	0.3	0.3	0.3	0.4	0.5*	0.3	0.7*	0.4
Sodium excretion (mmol)	0.47	0.35	0.43	0.38	0.45	0.30	0.74*	0.32
Chloride excretion (mmol)	0.25	0.24	0.26	0.19	0.27	0.17	0.39	0.15
Urine Biomarker								
Day 4								
Cystatin C:creatinine ratio	2.1	1.0	2.2	1.0	3.8*	1.2	8.2*	1.7*
Beta-2- microglobulin: creatinine ratio	30.01	NC	20.84	NC	56.20	6.99	168.22*	8.81
KIM-1:creatinine ratio	0.007	0.009	0.008	0.011	0.011*	0.009	0.087*	0.036*
Day 30								
Cystatin C:creatinine ratio	1.5	0.9	2.5	1.1	12.5*	1.2	42.9*	6.0*
Beta-2- microglobulin: creatinine ratio	25.51	6.48	47.32	14.75	299.35*	6.49	575.93*	130.19*

Test Article: Remdesivir

Daily Dose (mg/kg/day)	**0 (Control)**		**1 (Low)**		**3 (Mid)**		**10 (High)**	
Sex: Number of Animals (Main)	**M: 10**	**F: 10**	**M: 10**	**F: 10**	**M: 10**	**F: 10**	**M: 10**	**F: 10**
KIM-1:creatinine ratio	0.003	0.004	0.003	0.005	0.005*	0.005	0.012*	0.007*
Organ Weights								
Kidney								
Absolute weight[c] (%)	1.804 g	1.4464 g	+3	-2	+11	-6	+5	-14
Relative to body weight (%)	0.6934	0.7990	0.7268	0.8060	0.7603*	0.7681	0.8335*	0.7379
Relative to brain weight (%)	90.6404	76.1143	91.8626	74.2282	100.4357*	71.9715	94.6153	65.2225
Macroscopic Observations	No test article-related findings.							
Microscopic Observations								
Number Evaluated	10	10	10	10	10	10	10	8
Kidney								
Basophilic tubules								
Minimal	0	0	0	0	10	0	1	1
Slight	0	0	0	0	0	0	9	7
Karyomegaly								
Minimal	0	0	0	0	10	0	6	8
Slight	0	0	0	0	0	0	4	0
Additional Microscopic Observations	Vehicle control article (SBE-b-CD)-related microscopic findings occurred in the urinary tract, spleen, lymph nodes, adrenal cortex, liver, and stifle joint of animals administered vehicle control article alone or with remdesivir							

$AUC_{0\text{-}24}$= Area under the concentration-time curve from hour 0 to hour 24, estimated by the linear trapezoidal rule; BLQ = below the lower limit of quantitation (< 2.00 ng/mL for GS-5734 and GS-441524 and < 5.00 ng/mL for GS-704277); C_{max}= Maximum observed concentration; M = Male; F = Female; GLP = Good Laboratory Practice; M = Male; NA = Not Applicable; NR= Not reported; NC = Not calculable; TK = toxicokinetic; − Comparable to control animals or no test article-related findings; * = $p \leq 0.05$

a Value not reported due to less than three measurable concentrations in the profile.

b One 10 mg/kg/day female died on Day 14 soon after removal from the dosing restraint device, at which time it was noted with pale body and eyes, labored respiration, and a clonic convulsion greater than 1 minute. Another 10 /mg/kg/day female was euthanized prior to dosing on Day 19 following clinical observations of pale eyes and ears, piloerection, ataxia, and continuous body tremors. No prior remarkable clinical observations or body weight loss were noted for either animal.

c For controls, group means are shown. For treated groups, percent differences from controls are shown. Statistical significance is based on actual data not on the percent.

7.3. 2-Week Monkey

Test Article: Remdesivir

Report Title: A 2-Week Intravenous (Slow Bolus) Toxicity and Toxicokinetic Study with GS-5734 in Cynomolgus Monkeys with a 4 Week Recovery Phase		**Gilead Study No.:** TX-399-2004 [redacted]
Species/Strain: Cynomolgus monkeys (*Macaca fascicularis*)	**Duration of Dosing:** 2 Weeks	**Location in CTD:** 4.2.3.2
Initial Age: Two to four years	**Duration of Postdose:** 4 Weeks	**GLP Compliance:** Yes
Date of First Dose: [redacted]20[redacted]	**Method of Administration:** Intravenous (Slow Bolus) Injection	**Lot Number:** [redacted]
Vehicle: 12% (w/v) sulfobutylether-β-cyclodextrin (SBECD) in Sterile water for Injection, USP, pH 3.5 ± 0.1		
Special Features: D-Dimer analysis, urine biomarker analysis, hepatic microsomal fractions		
No Observed Adverse Effect Level: 10 mg/kg/day		

Daily Dose (mg/kg/day)	**0 (Saline Control)**		**0 (Vehicle Control)**		**1**		**3**		**10**	
Gender: Number of Animals	**M: 4**	**F: 4**	**M: 6**	**F: 6**	**M: 4**	**F: 4**	**M: 4**	**F: 4**	**M: 6**	**F: 6**
GS-5734 Toxicokinetics: AUC_{0-24} (ng•h/mL)										
Day 1	BLQ	BLQ	BLQ	BLQ	76.7	92.7	215	297	1320	1710
Day 15	BLQ	BLQ	BLQ	BLQ	88.7	111	363	342	1720	1780
GS-5734 Toxicokinetics: C_{max} (ng/mL)										
Day 1	BLQ	BLQ	BLQ	BLQ	82.1	100	243	318	1180	1490
Day 15	BLQ	BLQ	BLQ	BLQ	81.7	96.0	316	295	1490	1510
GS-441524 Toxicokinetics: AUC_{0-24} (ng•h/mL)										
Day 1	BLQ	BLQ	BLQ	BLQ	152	172	730	547	2310	2350
Day 15	BLQ	BLQ	BLQ	BLQ	192	214	804	577	2450	2330
GS-441524 Toxicokinetics: C_{max} (ng/mL)										
Day 1	BLQ	BLQ	BLQ	BLQ	27.5	30.8	107	90.0	381	390
Day 15	BLQ	BLQ	BLQ	BLQ	26.5	27.5	100	81.6	363	335

Test Article: Remdesivir

Daily Dose (mg/kg/day)	**0 (Saline Control)**		**0 (Vehicle Control)**		**1**		**3**		**10**	
Gender: Number of Animals	**M: 4**	**F: 4**	**M: 6**	**F: 6**	**M: 4**	**F: 4**	**M: 4**	**F: 4**	**M: 6**	**F: 6**
Noteworthy Findings										
Died or sacrificed moribund	0	0	0	0	0	0	0	0	0	0
Body Weight (%)	No test article-related findings									
Clinical Observations	No test article-related findings									
Ophthalmoscopy	No test article-related findings									
Electrocardiogram Examinations	No test article-related findings									
Hematology and Coagulation	No test article-related findings									
Clinical Chemistry										
Cholesterol										
Day 4 (mg/dL)	149	138	158	133	105*	134	127*	136	110*	119
Day 7 (mg/dL)	147	133	159	132	103*	136	120*	137	112*	120
Day 13 (mg/dL)	147	121	154	136	111*	138	126*	126	113*	118
Urinalysis	No test article-related findings									
Urine Chemistry	No test article-related findings									
Biomarker Urinalysis	No test article-related findings									
Organ Weights	No test article-related findings									
Gross Pathology	No test article-related findings									
Histopathology										
Number of Animals Examined	M: 4	F: 4	M: 4	F: 4	M: 4	F: 4	M: 4	F: 4	M: 4	F: 4
Kidney, Vacuolation, Tubule Cell, Minimal	0	0	1	1	1	1	2	0	1	1

Daily Dose (mg/kg/day)	0 (Saline Control)		0 (Vehicle Control)		1		3		10	
Gender: Number of Animals	**M: 0**	**F: 0**	**M: 2**	**F: 2**	**M: 0**	**F: 0**	**M: 0**	**F: 0**	**M: 2**	**F: 2**
Recovery Phase										
Body Weight (%)	No test article-related findings									
Clinical Observations	No test article-related findings									
Hematology and Coagulation	No test article-related findings									
Clinical Chemistry	No test article-related findings									
Biomarker Urinalysis	No test article-related findings									
Organ Weights	No test article-related findings									
Gross Pathology	No test article-related findings									
Histopathology										
Number of Animals Examined	0	0	M: 2	F: 2	0	0	0	0	M: 2	F: 2
Kidney, Vacuolation, Tubule Cell, Minimal	NA	NA	1	1	NA	NA	NA	NA	2	1

$AUC_{0\text{-}24}$ = Area under concentration-time curve from time 0 to 24 hours postdose; C_{max} = Maximum (peak) observed drug concentration; GLP = Good Laboratory Practices; M = Male; F= Female; NA = Not applicable; TK = toxicokinetic; BLQ = Values below the lower limit of quantitation for monkey (< 4.00 ng/mL for GS-5734 and < 2.00 ng/mL for GS-441524); * $p \leq 0.05$

7.4. 4-Week Monkey

Test Article: Remdesivir

Report Title: A 4-Week Intravenous Slow Bolus Injection Toxicity and Toxicokinetic Study with GS-5734 in Cynomolgus Monkeys		**Gilead Study No**. TX-399-2017 [redacted]
Species / Strain: Cynomolgus Monkeys	**Duration of Dosing:** 4 weeks	**Location in CTD**: 4.2.3.2
Initial Age: 2-3 years	**Duration of Postdose:** Not Applicable	**GLP Compliance**: yes
Date of First Dose: [redacted]20[redacted]	**Method of Administration:** intravenous slow bolus injection	**Lot Number:** [redacted]
Vehicle Control Article: 12% (w/v) sulfobutylether-b-cyclodextrin (Dexolve-7™ [SBE-b-CD]) in Sterile water for Injection, USP (SWFI), pH 3.5 ± 0.1		
Special Features: urine biomarkers		
No Observed Adverse Effect Level: 10 mg/kg/day		

Daily Dose (mg/kg/day)	**0 (Control)**		**1 (Low)**		**3 (Mid)**		**10 (High)**	
Sex: Number of Animals	**M: 4**	**F: 4**	**M: 4**	**F: 4**	**M: 4**	**F: 4**	**M: 4**	**F: 4**
Toxicokinetics: $AUC_{0\text{-}24}$ (ng·h/mL)								
Day 1								
GS-5734	BLQ	BLQ	79.2	67.0	206	203	1100	999
GS-441524	BLQ	BLQ	145	189	398	450	1660	1950
GS-704277	BLQ	BLQ	58.8	66.1	172	214	770	807
Week 4								
GS-5734	BLQ	BLQ	101	103	265	265	1260	1400
GS-441524	BLQ	BLQ	167	213	404	482	1890	2240
GS-704277	BLQ	BLQ	65.7	68.6	160	209	794	903
Toxicokinetics: C_{max} (ng/mL)								
Day 1								
GS-5734	BLQ	BLQ	86.3	72.8	223	235	1030	1020
GS-441524	BLQ	BLQ	24.4	30.5	63.0	68.3	268	316
GS-704277	BLQ	BLQ	65.9	76.2	206	237	857	780
Week 4								
GS-5734	BLQ	BLQ	98.9	101	240	245	1120	1200
GS-441524	BLQ	BLQ	26.7	31.1	60.5	73.3	274	301
GS-704277	BLQ	BLQ	62.0	66.4	166	170	668	692

Test Article: Remdesivir

Daily Dose (mg/kg/day)	**0 (Control)**		**1 (Low)**		**3 (Mid)**		**10 (High)**	
Sex: Number of Animals	**M: 4**	**F: 4**	**M: 4**	**F: 4**	**M: 4**	**F: 4**	**M: 4**	**F: 4**
Noteworthy Findings								
Died or sacrificed moribund	0	0	0	0	0	0	0	0
Body Weight (%)	No test article-related findings							
Food Consumption (%)	No test article-related findings							
Clinical Observations	No test article-related findings							
Ophthalmoscopy	No test article-related findings							
Hematology and Coagulation	No test article-related findings							
Electrocardiogram	No test article-related findings							
Clinical Chemistry								
Cholesterol concentration								
Day 4	-	143	-	132	-	142	-	97*
Day 29	-	150	-	147	-	150	-	109*
Urinalysis	No test article-related findings							
Organ Weights	No test article-related findings							
Urine Chemistry	No test article-related findings							
Urine Biomarkers								
beta2microglobulin:creatinine								
Day 29	-	0	-	0	-	0	-	0.019
Gross Pathology	No test article-related findings							
Histopathology								
Number examined	4	4	4	4	4	4	4	4
Kidney								
Vacuolation, transitional cell								
Minimal	4	3	4	4	3	4	4	4
Slight	0	0	0	0	1	0	0	0
Vacuolation, tubule cell								
Minimal	4	3	3	3	4	3	4	4
Slight	0	0	1	1	0	1	0	0
Urinary Bladder								

Test Article: Remdesivir

Daily Dose (mg/kg/day)	0 (Control)		1 (Low)		3 (Mid)		10 (High)	
Sex: Number of Animals	**M: 4**	**F: 4**	**M: 4**	**F: 4**	**M: 4**	**F: 4**	**M: 4**	**F: 4**
Vacuolation, transitional cell								
Minimal	0	1	0	0	0	1	3	0
Slight	4	3	4	4	4	3	1	4
Ureter								
Vacuolation, transitional cell								
Minimal	0	2	1	2	2	3	3	2
Slight	4	2	2	2	2	1	1	2
Urethra								
Vacuolation, transitional cell								
Minimal	3	3	4	1	3	3	3	1
Slight	1	1	0	2	1	1	0	2

NA = Not Applicable; BLQ = below the lower limit of quantitation (< 2.00 ng/mL for GS-5734 and GS-441524 and <5.00 ng/mL for GS-704277 ng/mL); AUC = area under the concentration-time curve; C_{max} = maximum observed concentration

− Comparable to control animals or no test article-related findings; * = $p \leq 0.05$

8. GENOTOXICITY: IN VITRO

8.1. Ames

Test Article: Remdesivir

Report Title: Bacterial Reverse Mutation Assay Plate Incorporation with Remdesivir		**Gilead Study No.:** TX-399-2005 [redacted]
Test for Induction of: Reverse mutation in bacterial cells	**No. of Independent Assays:** 1	**Location in CTD:** 4.2.3.3.1
Strains: *Salmonella typhimurium* and *Escherichia coli*	**No. of Replicate Cultures:** 2	**GLP Compliance:** Yes
Metabolizing System: Aroclor™-induced rat liver S9	**No. of Cells Analyzed/Cultured:** ~10^9	**Lot Number:** [redacted]
Vehicle: Dimethylsulfoxide (DMSO)	**Vehicle for Positive Controls:** Deionized (DI) Water (sodium azide only); DMSO (all other positive controls)	
Treatment: Plate incorporation for 52 ± 4 hours	**Date of Treatment:** [redacted]20[redacted]	
Cytotoxic Effects: None	**Genotoxic Effects:** None	

Test Article: Remdesivir

		Revertant Colony Counts (Mean ± SD)				
		Strain				
Treatment	**Dose (µg/plate)**	**TA98**	**TA100**	**TA1535**	**TA1537**	**WP2*uvr*A**
Without Metabolic Activation						
DMSO		32 ± 10 N	93 ± 8 N	13 ± 4 N	12 ± 3 N	19 ± 1 M N
Remdesivir	5.00	35 ± 6 N	97 ± 6 N	13 ± 1 N	10 ± 6 N	22 ± 2 M N
	16.0	33 ± 10 N	102 ± 15 N	10 ± 7 N	13 ± 5 N	18 ± 2 M N
	50.0	35 ± 4 N	94 ± 11 N	12 ± 2 N	12 ± 3 N	19 ± 4 N
	160	27 ± 7 N	85 ± 6 N	12 ± 2 N	13 ± 3 N	18 ± 5 M N
	500	28 ± 9 N	81 ± 0 N	12 ± 3 N	14 ± 9 N	18 ± 2 M N
	1600	29 ± 2 N	85 ± 13 N	16 ± 3 N	9 ± 7 N	16 ± 1 M N
	5000	24 ± 6 M P N	77 ± 19 P N	12 ± 5 P N	8 ± 2 P N	19 ± 3 M P N
2-nitrofluorene	1.0	165 ± 29 N	—	—	—	—
sodium azide	2.0	—	1055 ± 16 N	747 ± 24 N	—	—
ICR-191	2.0	—	—	—	476 ± 36 N	—
4-nitroquinoline-N-oxide	1.0	—	—	—	—	184 ± 55 N

Test Article: Remdesivir

Treatment	Dose (µg/plate)	Revertant Colony Counts (Mean ± SD)				
		Strain				
		TA98	TA100	TA1535	TA1537	WP2*uvr*A
With Metabolic Activation						
DMSO		37 ± 9 N	105 ± 12 M N	11 ± 5 N	12 ± 3 N	25 ± 7 M N
Remdesivir	5.00	34 ± 6 N	113 ± 8 N	16 ± 2 N	11 ± 3 M N	23 ± 1 N
	16.0	40 ± 7 N	109 ± 2 N	10 ± 3 N	15 ± 2 M N	20 ± 2 N
	50.0	37 ± 1 N	106 ± 9 N	11 ± 4 N	8 ± 1 N	22 ± 5 N
	160	40 ± 11 N	102 ± 12 N	12 ± 3 N	13 ± 3 N	21 ± 2 M N
	500	31 ± 5 N	89 ± 7 N	12 ± 4 N	12 ± 2 N	22 ± 8 M N
	1600	46 ± 4 N	93 ± 9 N	14 ± 3 N	10 ± 5 N	20 ± 3 N
	5000	36 ± 1 P N	93 ± 10 P N	14 ± 5 M P N	11 ± 6 M P N	18 ± 10 M P N
benzo[a]pyrene	2.5	370 ± 31 N	—	—	—	—
2-aminoanthracene	2.5	—	1285 ± 176 N	156 ± 13 N	136 ± 29 N	—
2-aminoanthracene	25.0	—	—	—	—	371 ± 54 N

GLP = Good Laboratory Practice; M = Plate counted manually; N = Normal background bacterial lawn; P = Precipitation of test article observed; SD = Standard Deviation

8.2. Chromosome Aberrations

Test Article: Remdesivir

Report Title: Chromosomal Aberrations in Cultured Human Peripheral Blood Lymphocytes with Remdesivir		**Gilead Study No.:** TX-399-2006 [redacted]
Test for Induction of: Chromosome aberrations	**No. of Independent Assays:** 2	**Location in CTD:** 4.2.3.3.1
Strains: Primary human lymphocytes	**No. of Replicate Cultures:** 2	**GLP Compliance:** Yes
Metabolizing System: Aroclor™-induced rat liver S9	**No. of Cells Analyzed/Culture:** 100 (or ≥ 25 if > 25% cells with aberrations)	**Lot Number:** [redacted]
Vehicle for Test Article: Dimethylsulfoxide (DMSO)	**Vehicle for Positive Controls:** Deionized (DI) Water	
Treatment: Continuous treatment 24 hour without S9; pulse treatment 3 hour and recovery time 21 hour with and without S9		**Date of Treatment:** [redacted] 20[redacted]
Cytotoxic Effects: Yes		
Genotoxic Effects: Negative at 3- and 24-hour treatment without S9, equivocal at 3-hour treatment with S9		

Test Article: Remdesivir

	Initial Assay						
Metabolic Activation	**Test Article**	**Concentration (µg/mL)**	**Cytotoxicity (% of Control)[a]**	**% Aberrant Cells (Mean -g)**	**% Aberrant Cells (Mean +g)**	**% Polyploid Cells**	**% Endoreduplicate Cells**
3-hour without Activation	Vehicle	10.0 µL/mL	100	0.5	2.0	0	0
	Remdesivir	117	86	0.5	2.0	0	0
		146	73	1.0	1.5	0	0
		171	42	0.0	1.5	0	0
	Mitomycin C	1.00	32	55*	57	0	0
24-hour without Activation	Vehicle	10.0 µL/mL	100	0.0	0.5	0.0	0.0
	Remdesivir	41.2	79	1.0	2.5	0.5	0.0
		58.8	62	0.5	3.0	0.0	0.0
		84.0	41	0.5	2.0	0.0	0.0
	Mitomycin C	0.300	55	37.0*	47.0	0.0	0.0
3-hour with Activation	Vehicle	10.0 µL/mL	100	0.0	0.5	0.0	0.0
	Remdesivir	120	72	0.0	0.0	0.5	0.0
		172	66	0.0	0.0	0.5	0.0
		245	52	4.0*	5.5	0.5	0.0
	Cyclophosphamide	15.0	38	55.0*	56.0	0.0	0.0

-g = % of cells with chromosome aberrations; +g = % of cells with chromosome aberrations + % of cells with gaps
* Significantly greater in -g than the vehicle control; $p \leq 0.01$
Based on mitotic index

9. GENOTOXICITY: IN VIVO

9.1. In vivo Micronucleus Assay

Test Article: Remdesivir

Report Title: 2-Week Intravenous (Slow Bolus) Toxicity and Toxicokinetic Study with GS-5734 in Wistar-Han Rats with a 4 Week Recovery Phase and Bone Marrow Micronucleus Assay		**Gilead Study No.:** TX-399-2003 [redacted]
Test for Induction of: GS-5734 (previously identified as GS-643134)	**Treatment Schedule:** Once daily, for at least 2 weeks	**Location in CTD:** 4.2.3.3.2
Species/Strain: Crl:WI(Han) Rats	**Sampling Time:** 24 hours after last dose	**GLP Compliance:** Yes
Age: 6-8 weeks	**Method of Administration:** Intravenous (slow bolus)	**Date of Treatment:** [redacted] 20[redacted]
No. of Cells Evaluated / Animal: 2000	**Vehicle:** 12% (w/v) sulfobutylether-b-cyclodextrin in Sterile water for Injection, USP, pH 3.5 ± 0.1	
Special Features: None	**Positive Control Vehicle:** RO Water	
Toxic/Cytotoxic Effects: None	**Genotoxic Effects:** None	

Daily Dose (mg/kg/day)	**Saline Control**		**Vehicle Control**		**5**		**20**		**50**	
Gender: Number of Animals (TK)	**M: 3**	**F: 3**	**M: 9**	**F: 9**	**M: 9**	**F: 9**	**M: 9**	**F: 9**	**M: 9**	**F: 9**
GS-5734 Toxicokinetics: AUC_{0-24} (µg•hr/mL)										
Day 1	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
Day 15	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	385	BLQ
GS-5734 Toxicokinetics: C_{max} (µg/mL)										
Day 1	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	79.5	157
Day 15	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	8.46	520	233
GS-441524 Toxicokinetics: AUC_{0-24} (µg•hr/mL)										
Day 1	BLQ	BLQ	BLQ	BLQ	1190	942	4560	3350	12900	14300
Day 15	BLQ	BLQ	BLQ	BLQ	1880	1420	10000	12700	21700	19000
GS-441524 Toxicokinetics: C_{max} (µg/mL)										
Day 1	BLQ	BLQ	BLQ	BLQ	315	294	1240	879	2750	2170
Day 15	BLQ	BLQ	BLQ	BLQ	371	324	1760	1690	3560	2900

Test Article: Remdesivir

Test Article	Dose (mg/kg/day)	No. of Animals/Sex	Sampling Time (Hours)	Mean Ratio PCE: NCE (± SD) Males	Mean Ratio PCE: NCE (± SD) Females	Mean % MN PCEs (± SD) Males	Mean % MN PCEs (± SD) Females
Saline Control	0	5	24	0.96 ± 0.04	0.97 ± 0.02	0.10 ± 0.04	0.07 ± 0.07
Vehicle Control	0	5	24	0.93 ± 0.09	0.97 ± 0.03	0.05 ± 0.04	0.06 ± 0.04
Remdesivir	5	5	24	1.53 ± 1.36	1.00 ± 0.24	0.11 ± 0.12	0.08 ± 0.07
	20	5	24	0.96 ± 0.05	0.90 ± 0.08	0.08 ± 0.09	0.07 ± 0.06
	50	5	24	0.93 ± 0.05	0.96 ± 0.02	0.06 ± 0.02	0.05 ± 0.04
Cyclophosphamide	30	5	24	0.75 ± 0.10**	0.85 ± 0.06**	2.08 ± 0.67**	1.68 ± 0.65**

AUC_{0-24} = Area under concentration-time curve from time 0 to 24 hours postdose; BLQ = Below the limit of quantitation for rat (< 2.00 ng/mL for GS-5734 and GS-441524); C_{max} = Maximum (peak) observed drug concentration; Sterile Saline = 0.9% Sodium chloride for Injection, USP (sterile saline); M = Male; F = Female
Vehicle = 12% (w/v) sulfobutylether-β-cyclodextrin in Sterile water for Injection, USP, pH 3.5 ± 0.1; PCE = Polychromatic erythrocyte; MN PCE = Micronucleated PCE; NCE = Normochromatic erythrocyte
** Significantly less than the corresponding vehicle control, $p \leq 0.01$

10. CARCINOGENICITY

Not applicable.

11. REPRODUCTIVE AND DEVELOPMENTAL TOXICITY (NONPIVOTAL STUDIES)

Not applicable.

12. REPRODUCTIVE AND DEVELOPMENTAL TOXICITY: FERTILITY AND EARLY EMBRYONIC DEVELOPMENT TO IMPLANTATION

Test Article: Remdesivir

Report Title: An Intravenous (Slow Push) Study of Fertility and Early Embryonic Development to Implantation of GS-5734 in Sprague Dawley Rats		**Gilead Study No.** TX-399-2012 [redacted]
Species / Strain: Rat/Crl:CD(SD) Initial Age: Males: 8 weeks; Females: 9 weeks	**Duration of Dosing:** Males: 28 days prior to mating, throughout mating, and continuing until the day prior to euthanasia Females: 14 days prior to mating through day 7 of gestation	**Location in CTD:** 4.2.3.5.1 **GLP Compliance:** yes
Date of First Dose: [redacted]20[redacted]	**Day of Mating:** SD 27	**Lot Number:** [redacted]
No-Observed-Adverse-Effect Level **F_0 Males - Systemic:** 3 mg/kg/day **F_0 Males - Reproductive:** 10 mg/kg/day **F_0 Females:** 3 mg/kg/day **F_1 Litters:** 3 mg/kg/day	**Day of C-Section:** GD 15 **Method of Administration:** Intravenous (slow push) **Vehicle:** 12% (w/v) sulfobutylether-β-cyclodextrin in sterile water for injection, pH 3.5 ± 0.1 **Special Features:** NA	**Design Similar to ICH 4.1.1?** yes

Daily Dose (mg/kg/day)		**0 (Control)**	**1**	**3**	**10**
Males	**Number of Animals**	**25**	**25**	**25**	**25**
	No. Died or Sacrificed Moribund	0	0	0	0
	Clinical Observations				
	Decreased Defecation	-	-	-	Increased incidence
	Body Weight, SD 63 (g, %)[a]	502	504 (0.4)	477 (-5.0)*	392 (-21.9)**
	Body Weight Gain (g)				
	SD 0-27 (Pre-Mating Period)	131	133	117	59**
	SD 0-63 (Entire Treatment Period)	220	223	196*	111**
	Food Consumption (g/animal/day)				
	SD 24-27	23	22	20**	16**
	SD 59-63	25	24	23	19**

Test Article: Remdesivir

Daily Dose (mg/kg/day)		**0 (Control)**	**1**	**3**	**10**
Males	**Number of Animals**	**25**	**25**	**25**	**25**
	Necropsy Observations	No test article related findings			
	Organ Weights (Absolute Weights, g) [a]				
	Left Cauda Epididymis	0.3124	0.3159 (1.1)	0.3029 (-3.0)	0.2821 (-9.7)**
	Right Cauda Epididymis	0.3182	0.3209 (0.8)	0.3062 (-3.8)	0.2935 (-7.8)*
	Left Epididymis	0.67	0.69 (3.0)	0.66 (-1.5)	0.62 (-7.5)**
	Right Epididymis	0.70	0.71 (1.4)	0.67 (-4.3)	0.65 (-7.1)**
	Pituitary	0.0152	0.0149 (-2.0)	0.0150 (-1.3)	0.0129 (-15.1)**
	Prostate	1.16	1.20 (3.4)	1.06 (-8.6)	0.84 (-27.6)**
	Seminal Vesicle/Coagulating Gland/Accessory Fluid	2.22	2.17 (-2.3)	2.00 (-9.9)*	1.70 (-23.4)**
	No. of Males that Mated	25	23	25	25
	No. of Fertile Males	25	22	24	25
	Spermatogenic Evaluations	No test article related findings			
Females	**Number of Animals**	**25**	**25**	**25**	**25**
	No. Died or Sacrificed Moribund	0	0	0	0
	Clinical Observations	No test article related findings			
	Pre-mating Body Weight, SD 27, g (%)[a]	240	246 (2.5)	244 (1.7)	227 (-5.4)**
	Pre-mating Body Weight Gain, SD 14-27 (g)	17	22*	21	7**
	Gestation Body Weight, GD 7, g (%)[a]	274	277 (1.1)	277 (1.1)	248 (-9.5)**
	Gestation Body Weight Gain, GD 0-7 (g)	29	30	26	15**
	Gestation Body Weight, GD 15, g (%)[a]	320	324 (1.3)	330 (3.1)	306 (-4.4)*
	Gestation Body Weight Gain, GD 7-15 (g)	45	46	52**	58**
Females	Pre-mating Food Consumption, SD 24-27 (g/animal/day)	14	15	14	11**
	Gestation Food Consumption, GD 0-7 (g/animal/day)	19	20	19	15**
	Gestation Food Consumption, GD 7-15 (g/animal/day)	22	22	22	21
	Necropsy Observations	No test article related findings			
	Organ Weights (Absolute Weights, g (%) [a]				

Test Article: Remdesivir

Daily Dose (mg/kg/day)		0 (Control)	1	3	10
	Ovaries	0.1319	0.1342 (1.7)	0.1295 (-1.8)	0.1101 (-16.5)**
	Uterus/Cervix/Oviducts	19.30	18.70 (-3.1)	19.81 (2.6)	16.72 (-13.4)**
	Mean Length of Estrous Cycle (days)	4.3	4.2	4.5	4.1
	Mean No. Days Prior to Mating	2.3	3.0	2.7	2.2
	No. of Females Sperm-Positive	25	25	25	25
	No. of Pregnant Females	25	24	24	25
	Mean No. of Corpora Lutea	16.4	16.2	15.7	13.2**
	Mean No. of Implantation Sites	15.0	14.3	14.5	12.1**
	Mean No. of Viable Embryos	14.3	13.5	14.0	11.6**
	Mean Litter Proportion of Pre-implantation Loss (% per Litter)	7.3	10.7	7.3	7.5
	Mean Litter Proportion of Postimplantation Loss (% per Litter)	4.7	5.5	3.2	5.1
	Mean Litter Proportion of Viable Embryos (% per Litter)	95.3	94.5	96.8	94.9

M = Male; F = Female; SD = Study day; GD = Gestation day; - = No noteworthy findings.
* = $p<0.05$, ** = $p<0.01$
[a] = For all groups, group means are shown. For treated groups, percent differences or fold change from controls are also shown in brackets. Statistical significance is based on actual data not on the percent.

13. REPRODUCTIVE AND DEVELOPMENTAL TOXICITY: EFFECTS ON EMBRYO-FETAL DEVELOPMENT

13.1. Rat

Test Article: Remdesivir

Report Title: An Intravenous (Slow Push) Study of the Effects of GS-5734 on Embryo/Fetal Development with a Toxicokinetic Phase in Sprague Dawley Rats					**Gilead Study No.** TX-399-2013 [redacted]
Species / Strain: Rat/Crl:CD(SD) **Initial Age:** 13 weeks **Date of First Dose:** [redacted]20[redacted]	**Duration of Dosing:** GD 6-17 **Day of Mating:** GD 0 **Day of C-Section:** GD 21				**Location in CTD:** 4.2.3.5.2 **GLP Compliance:** Yes **Lot Number:** [redacted]
No-Observed-Adverse-Effect Level **F_0 Females:** 20 mg/kg/day **F_1 Litters:** 20 mg/kg/day	**Method of Administration:** Intravenous (slow push)				**Design Similar to ICH 4.1.3?** Yes
	Vehicle: 12% (w/v) sulfobutylether-β-cyclodextrin in sterile water for injection, pH 3.5 ± 0.1				
	Special Features: Toxicokinetics				
Daily Dose (mg/kg/day)	**0 (Control)**	**2.5**	**5**	**10**	**20**
Dams: Number of Animals (TK)	**3**	**9**	**9**	**9**	**9**
GS-5734 Toxicokinetics:					
C_{max} (ng/mL) – GD 6	BLQ	BLQ	BLQ	BLQ	BLQ
C_{max} (ng/mL) – GD 17	BLQ	BLQ	BLQ	BLQ	BLQ
AUC_{0-24} (ng•h/mL) – GD 6	BLQ	BLQ	BLQ	BLQ	BLQ
AUC_{0-24} (ng•h/mL) – GD 17	BLQ	BLQ	BLQ	BLQ	BLQ
GS-441524 Toxicokinetics:					

Test Article: Remdesivir

Daily Dose (mg/kg/day)	**0 (Control)**	**2.5**	**5**	**10**	**20**
Dams: Number of Animals (TK)	**3**	**9**	**9**	**9**	**9**
C_{max} (ng/mL) – GD 6	NA	126	249	462	799
C_{max} (ng/mL) – GD 17	NA	137	272	676	1580
AUC_{0-24} (ng•h/mL) – GD 6	NA	453	961	1800	3510
AUC_{0-24} (ng•h/mL) – GD 17	NA	642	1210	3680	8740
GS-704277 Toxicokinetics:					
C_{max} (ng/mL) – GD 6	NA	415	882	1910	3810
C_{max} (ng/mL) – GD 17	NA	458	1020	2850	8620
AUC_{0-24} (ng•h/mL) – GD 6	NA	158	328	812	1420
AUC_{0-24} (ng•h/mL) – GD 17	NA	333	391	1180	3420
Dams: Number of Animals (Main)	**25**	**25**	**25**	**25**	**25**
No. Pregnant	25	24	25	25	23
No. Died or Sacrificed Moribund	0	0	0	0	0
No. Aborted or with Total Resorption of Litter	0	0	0	0	0
Clinical Observations	No test article related findings				
Body Weight, GD 18 (g, %)[a]	364	1.6	0.8	-3.3	-3.8
Body Weight Change (g)					
GD 6-9	8	12	8	-4**	-13**
GD 9-12	10	14	13	13	14
GD 12-18	55	57	59	55	55
GD 6-18	74	82	80	64	57**
GD 18-21	54	50	58	65**	62*
Net Body Weight (g)	304.5	312.4	309.5	302.3	303.3
Net Body Weight Change (g)	48.3	58.0*	54.1	46.7	46.3
Gravid Uterine Weight (g)	113.2	108.4	115.2	115.1	109.3
Food Consumption (g/animal/day)					
GD 6-9	20	21	19	15**	12**
GD 9-12	20	20	20	17**	14**

Test Article: Remdesivir

Daily Dose (mg/kg/day)		**0 (Control)**	**2.5**	**5**	**10**	**20**
	GD 12-18	23	24	24	22	19**
	GD 6-18	21	22	22	19**	16**
	GD 18-21	25	25	25	26	26
	Necropsy Observations	No test article related findings				
	Mean No. Corpora Lutea	16.9	15.8	16.9	16.9	16.7
	Mean No. Implantations	15.7	15.0	15.9	16.2	15.4
	Mean % Preimplantation Loss	6.3	6.3	5.7	4.2	6.9
Litters:	**Litters Evaluated**	**25**	**24**	**25**	**25**	**23**
	Live Fetuses	377	337	382	385	334
Litters:	**Litters Evaluated**	**25**	**24**	**25**	**25**	**23**
	Dead Fetuses	0	0	0	0	0
	Mean No. Total Resorptions	0.6	0.9	0.6	0.8	0.9
	Mean % Postimplantation Loss	3.9	6.0	3.6	4.8	5.9
	Mean Fetal Body Weight (g, sexes combined)[a]	5.7	1.8	-1.8	-1.8	0.0
	Fetal Sex Ratios (% Male)	55.3	44.7+	50.0	53.4	51.7
Litters:	Fetal Anomalies	No test article related findings				

$AUC_{0\text{-}24}$= Area under the concentration-time curve from hour 0 to hour 24, estimated by the linear trapezoidal rule; BLQ = below the lower limit of quantitation (< 2.00 ng/mL for GS-5734 and GS-441524 and < 5.00 ng/mL for GS-704277); C_{max}= Maximum observed concentration; GLP = Good Laboratory Practice; GD = gestation day; TK = toxicokinetic; NA = not applicable; * = $p \leq 0.05$ (Dunnett's test), ** = $p \leq 0.01$ (Dunnett's test); + = $p \leq 0.05$ (Dunn's test)

a For controls, group means are shown. For treated groups, percent differences or fold change from controls are shown. Statistical significance is based on actual data not on the percent.

13.2. Rabbit

Test Article: Remdesivir

Report Title: An Intravenous (Slow Push) Study of the Effects of GS-5734 on Embryo/Fetal Development with a Toxicokinetic Phase in Rabbits		**Gilead Sciences Study No.** TX-399-2018 [redacted]
Species / Strain: Rabbit / New Zealand White	**Duration of Dosing:** GD 7-20	**Location in CTD:** 4.2.3.5.2
Initial Age: 6-7 months	**Day of Mating:** GD 0	**GLP Compliance:** Yes
Date of First Dose: [redacted]20[redacted]	**Day of C-Section:** GD 29	**Lot Number:** [redacted]
Special Features: Toxicokinetics	**Method of Administration:** Intravenous (slow push)	**Design Similar to ICH 4.1.3?** Yes
No-Observed-Adverse-Effect Level **F_0 Females:** 10 mg/kg/day **F_1 Litters:** 20 mg/kg/day	**Vehicle:** 12% (w/v) sulfobutylether-β-cyclodextrin in sterile water for injection, pH 3.5 ± 0.1	

Daily Dose (mg/kg/day)	**0 (Control)**	**2.5**	**5**	**10**	**20**
Dams/Does: Number of Animals (TK)	**4**	**4**	**4**	**4**	**4**
GS-5734 Toxicokinetics:					
C_{max} (ng/mL) – GD 7	BLQ	18.1	81.6	226	604
C_{max} (ng/mL) – GD 20	BLQ	204	558	1600	2950
AUC_{0-24} (ng•hr/mL) – GD 7	BLQ	28.2	72.7	214	586
AUC_{0-24} (ng•hr/mL) – GD 20	BLQ	196	643	2110	2830
GS-441524 Toxicokinetics:					
C_{max} (ng/mL) – GD 7	BLQ	150	308	526	1330
C_{max} (ng/mL) – GD 20	BLQ	119	247	380	1680

Test Article: Remdesivir

Daily Dose (mg/kg/day)	**0 (Control)**	**2.5**	**5**	**10**	**20**
Dams/Does: Number of Animals (TK)	**4**	**4**	**4**	**4**	**4**
AUC_{0-24} (ng•hr/mL) – GD 7	BLQ	462	1030	1710	4960
AUC_{0-24} (ng•hr/mL) – GD 20	BLQ	429	1090	1790	8930
GS-704277 Toxicokinetics:					
C_{max} (ng/mL) – GD 7	BLQ	1560	3060	5270	14800
C_{max} (ng/mL) – GD 20	BLQ	1140	2050	3630	13200
AUC_{0-24} (ng•hr/mL) – GD 7	BLQ	946	1870	3180	9860
AUC_{0-24} (ng•hr/mL) – GD 20	BLQ	657	1280	2860	11700
Dams/Does: Number of Animals (Main)	**22**	**22**	**22**	**22**	**22**
No. Pregnant	22	20	22	22	22
No. Died or Sacrificed Moribund	1	0	0	0	1
No. Aborted or with Total Resorption of Litter	0	0	0	0	1
Clinical Observations[a]					
Decreased Defecation	3/1	2/1	5/2	2/1	48/11
Body Weight (g, %)[b]					
GD 7	3394	3356 (-1.1)	3359 (-1.0)	3382 (-0.4)	3360 (-1.0)
GD 14	3533	3496 (-1.0)	3481 (-1.5)	3442 (-2.6)	3241 (-8.3)**
GD 21	3689	3604 (-2.3)	3649 (-1.1)	3589 (-2.7)	3490 (-5.4)

Test Article: Remdesivir

Daily Dose (mg/kg/day)		**0 (Control)**	**2.5**	**5**	**10**	**20**
Dams/Does: Number of Animals (Main)		**22**	**20**	**22**	**22**	**22**
Body Weight Change (g)						
GD 7-10		65	43	46	-56**	-90**
GD 10-13		40	56	42	64	-51**
GD 13-21		185	149	202	199	258*
GD 7-21		297	247	290	207	128**
GD 21-29		97	119	60	142	206**
Gravid Uterine Weight (g)		489.6	508.0	485.4	519.8	462.7
Food Consumption (g/animal/day)						
GD 7-10		176	159	164	111**	93**
GD 10-13		164	150	141	95**	43**
GD 13-21		162	147	159	146	135
GD 7-21		166	150	156	128**	107**
GD 21-29		121	110	109	119	154**
Necropsy Observations						
Litters:	**Litters Evaluated**	**21**	**20**	**22**	**22**	**21**
Mean No. Corpora Lutea		9.6	10.0	9.8	10.3	9.7
Mean No. Implantations		8.6	9.6	8.9	9.5	8.8
Live Fetuses (no. fetuses/litter)		8.6	9.1	8.6	9.2	8.2
Dead Fetuses (no. fetuses/litter)		0.0	0.0	0.0	0.0	0.0
Mean % Viable Fetuses		99.4	94.5	98.0	96.5	93.1
Mean No. Total Resorptions		0.0	0.5	0.2	0.4	0.6
Mean % Preimplantation Loss		11.0	4.0	9.0	7.1	8.2

Test Article: Remdesivir

Daily Dose (mg/kg/day)	0 (Control)	2.5	5	10	20
Mean % Postimplantation Loss	0.6	5.5	2.0	3.5	6.9
Mean Fetal Body Weight (g)[b]					
Male	42.8	42.2 (-1.4)	41.3 (-3.5)	40.5 (-5.4)	41.5 (-3.0)
Female	41.2	40.2 (-2.4)	40.3 (-2.2)	40.6 (-1.5)	39.8 (-3.4)
Combined	41.9	41.0 (-2.1)	40.9 (-2.4)	40.5 (-3.3)	40.7 (-2.9)
Fetal Sex Ratios (% Male)	53.4	48.4	50.0	50.6	48.2
Fetal Anomalies	No test article related findings				

GD = gestation day; * = $p \leq 0.05$ using Dunnett's test; ** = $p \leq 0.01$ using Dunnett's; g = grams; Remdesivir = GS-5734

a Presented as total occurrence/number of animals

b For controls, group means are shown. For treated groups, means are presented with percent differences or fold change from controls shown in parentheses. Statistical significance is based on actual data not on the percent.

14. REPRODUCTIVE AND DEVELOPMENTAL TOXICITY: EFFECTS ON PRE- AND POSTNATAL DEVELOPMENT, INCLUDING MATERNAL FUNCTION

Test Article: Remdesivir

Report Title: An Intravenous (Slow Push) Study of the Effects of GS-5734 on Pre- and Postnatal Development, Including Maternal Function in Sprague Dawley Rats		**Gilead Study No.** TX-399-2014 [redacted]
Species / Strain: Rat/Crl:CD(SD)	**Duration of Dosing:** GD6-LD20 **Day of Mating:** GD0	**Location in CTD:** 4.2.3.5.3
Initial Age: 14 weeks		**GLP Compliance:** Yes
Date of First Dose: [redacted]20[redacted]	**Method of Administration:** Intravenou injection	**Lot Number:** [redacted]
No-Observed-Adverse-Effect Level **F_0 systemic:** 10 mg/kg/day **F_1 developmental/neonatal:** 10 mg/kg/day **F_1 parental systemic:** 10 mg/kg/day **F_1 reproductive:** 10 mg/kg/day **F_2 neonatal/early postnatal:** 10 mg/kg/day	**Vehicle:** 12% [w/v] sulfobutylether-β-cyclodextrin in sterile water for injection, pH 3.5 ± 0.1	**Design Similar to ICH 4.1.2?** Yes
	Litters Culled / Not Culled: Culled	
	Special Features: Toxicokinetics	

Daily Dose (mg/kg/day)	**0 (Control)**	**1**	**3**	**10**
F_0 Females:	**4**	**10**	**10**	**10**
GS-5734 Toxicokinetics:				
GD6	BLQ	BLQ	BLQ	BLQ
C_{max} (ng/mL)	BLQ	BLQ	BLQ	BLQ
AUC_{0-24} (ng•h/mL)	BLQ	BLQ	BLQ	BLQ
LD10				
C_{max} (ng/mL)	BLQ	BLQ	BLQ	BLQ
AUC_{0-24} (ng•h/mL)	BLQ	BLQ	BLQ	BLQ

Test Article: Remdesivir

Daily Dose (mg/kg/day)	0 (Control)	1	3	10
GS-441524 Toxicokinetics:				
GD6				
C_{max} (ng/mL)	BLQ	49.5	142	477
AUC_{0-24} (ng•h/mL)	BLQ	137	415	1610
LD10				
C_{max} (ng/mL)	BLQ	60.3	149	572
AUC_{0-24} (ng•h/mL)	BLQ	349	538	2310
PND 10				
C_{max} (ng/mL)	BLQ	BLQ	3.50[a]	4.51[b]
AUC_{0-24} (ng•h/mL)	BLQ	BLQ	NR	NR
GS-704277 Toxicokinetics:				
GD6				
C_{max} (ng/mL)	BLQ	190	576	2320
AUC_{0-24} (ng•h/mL)	BLQ	71.2	213	775
LD10				
C_{max} (ng/mL)	BLQ	196	570	2680
AUC_{0-24} (ng•h/mL)	BLQ	70.7	343	1190
Dams: Number of Animals (Main)	**25**	**25**	**25**	**25**
No. Pregnant	25	25	24	24
Dams: Number of Animals (Main)	**25**	**25**	**24**	**24**
No. Died or Sacrificed Moribund	0	0	0	0
No. Aborted or with Total Resorption of Litter	0	0	0	0
Clinical Observations	No test article related findings			
Necropsy Observations	No test article related findings			
Gestation Body Weight - GD 20 (%)[c]	443	441 (-0.5)	441 (-0.5)	415(-6.3**)
Lactation Body Weight - LD 21 (%)[c]	373	366 (-1.9)	373 (0.0)	367 (-1.6)
Gestation Food Consumption - GD 6-20 (g/animal/day)	26	26	26	23**

Test Article: Remdesivir

Daily Dose (mg/kg/day)		**0 (Control)**	**1**	**3**	**10**
Lactation Food Consumption - LD 1-21 (g/animal/day)		63	62	61	62
Mean Duration of Gestation (days)		22.1	22.0	22.0	22.1
Abnormal Parturition		No test article related findings			
F_1 Litters:	**No. Litters Evaluated**	**25**	**25**	**24**	**24**
(preweaning)	Mean No. of Implantations	16.1	15.6	16.3	16.1
	Mean No. Pups/Litter	15.1	14.7	15.5	15.1
	Mean No. Liveborn Pups/Litter	14.8	14.4	15.0	14.7
	Mean No Stillborn Pups/Litter	0.3	0.3	0.5	0.4
	Postnatal Survival to Day 4 (% per litter)	96.5	96.5	94.4	95.1
	Postnatal Survival to Weaning (% per litter)	100.0	100.0	100.0	100.0
	No. of Total Litter Losses	0	0	0	0
	Change in Pup Body Weights (g)[c]	No test article related findings			
	Pup Sex Ratios (% males per litter)	47.3	54.1	56.4	51.4
	Pup Clinical Signs	No test article related findings			
	Pup Necropsy Observations	No test article related findings			
F_1 Males	**No. Evaluated Postweaning Per Litter**	**50**	**50**	**47**	**48**
(postweaning)	No. Died or Sacrificed Moribund	1	1	0	0
	Clinical Observations	No test article related findings			
	Body Weight Change - PND 21-126 (g)	578	601	583	577
	Necropsy Observations	No test article related findings			
	Mean Age of Preputial Separation (days)	42.8	42.6	42.6	42.5
	Sensory Function	No test article related findings			
	Motor Activity	No test article related findings			
	Learning and Memory				
	Mean No. Days Prior to Mating	2.9	3.2	2.5	2.9
	Mating Index (%)	96.0	100.0	100.0	91.3
	Fertility Index (%)	88.0	100.0	100.0	82.6

Test Article: Remdesivir

Daily Dose (mg/kg/day)		**0 (Control)**	**1**	**3**	**10**
	Copulation Index (%)	91.7	100.0	100.0	90.5
	No. of Males that Mated	24	24	24	21
	No. of Fertile Males	22	24	24	19
F_1 Females	**No. Evaluated Postweaning**	**50**	**50**	**47**	**48**
(postweaning)	No. Died or Sacrificed Moribund	0	1	0	1
	Clinical Observations	No test article related findings			
	Premating Body Weight Change (g)	237	235	232	238
	Gestating Body Weight Change (g)	162	160	162	164
	Necropsy Observations				
	Mean Age of Vaginal Patency (days)	32.3	32.6	33.4**	32.5
	Sensory Function	No test article related findings			
	Motor Activity	No test article related findings			
	Learning and Memory	No test article related findings			
	Mean No. Days Prior to Mating	2.9	3.2	2.5	2.9
	No. of Females Sperm Positive	24	24	24	21
	No. of Pregnant Females	22	24	24	19
	Mating Index (%)	96.0	100.0	100.0	91.3
	Fertility Index (%)	88.0	100.0	100.0	82.6
	Conception Index (%)	91.7	100.0	100.0	90.5
	Mean No. Corpora Lutea	18.7	17.8	17.7	18.7
	Mean No. Implantations	16.3	16.1	16.0	16.6
F_2 Litters	No. of Litters Evaluated	**22**	**24**	**24**	**19**
	Mean No. of Pups Born/Litter	15.4	15.0	15.2	15.9
	Mean No. Liveborn Pups/Litter	15.3	14.7	14.8	15.9
	Postnatal Survival to Day 4 (% per Litter)	97.8	96.1	95.2	96.7
	Pup Body Weight Change - PND 1-4 (g)				
	Males	3.2	3.2	3.3	3.4

Test Article: Remdesivir

Daily Dose (mg/kg/day)		0 (Control)	1	3	10
	Females	3.2	3.1	3.1	3.3
	Pup Sex Ratio (% Males per Litter)	48.6	53.4	46.6	51.7
	Pup Clinical Observations	No test article related findings			
	Pup Necropsy Observations	No test article related findings			

AUC_{0-24}= Area under the concentration-time curve from hour 0 to hour 24, estimated by the linear trapezoidal rule; BLQ = below the lower limit of quantitation (< 2.00 ng/mL for GS-5734 and GS-441524 and < 5.00 ng/mL for GS-704277); C_{max}= Maximum observed concentration; GLP = Good Laboratory Practice; TK = toxicokinetic; - No noteworthy findings; GD = gestation day; LD = lactation day; NA = not applicable; NR = not reported; Remdesivir = GS-5734

** = $p \leq 0.01$ (Dunnett's test)

a Females only

b Males and females combined

c For controls, group means are shown. For treated groups, means are presented with percent differences or fold change from controls shown in parentheses. Statistical significance is based on actual data not on the percent.

15. LOCAL TOLERANCE

Test Article: Remdesivir

Type of Study	Species / Strain	Method of Administration	Duration of Dosing	Dose	Gender and No. per Group	Noteworthy Findings	Gilead Study No. (CRO Name and Study No.)
Ocular irritation assay	Bovine cornea	In vitro	Opacity assessment: 4 hours Permeability assessment: 90 minutes	750 µL 20% (w/v) Remdesivir in 0.9% saline	3 Corneas	Nonirritant	TX-399-2025 [redacted]
Skin irritation assay	EpiDerm™ human skin	In vitro	15 minutes	10 mg Remdesivir	3 tissues per test article	Nonirritant	TX-399-2023 [redacted]

16. OTHER TOXICITY STUDIES

Test Article: Test Article: Remdesivir

Type of Study	Species/Strain	Method of Administration (Vehicle)	Duration of Dosing	Dose (mg/kg)	Gender and No. per Group	Noteworthy Findings	Gilead Study No. (CRO Name and Study No.)
Hemolytic Potential and Plasma Compatibility Study of Remdesivir in Human, Cynomolgus Monkey, and Rat Blood and Plasma	Human, Cynomolgus monkey (*Macaca fascicularis*), Wistar- Han Rat (Crl:WI[Han])	In Vitro (12% (w/v) sulfobutylether-β-cyclodextrin (in Sterile water for Injection, USP, pH 3.5+ 0.1)	41 minutes	0, 1, 3, 10 mg/mL	In Vitro	No noteworthy findings. GS-5734 formulations were compatible with monkey, rat and human whole blood and plasma.	TX-399-2008 [redacted]
Subcutaneous Tolerability Study	Mouse/Ces1c$^{-/-}$	Subcutaneous Injection (12% (w/v) sulfobutylether-β-cyclodextrin (in Sterile water for Injection)	1-week	10, 50	Main: 5/sex/group; TK: 9/sex/group	Decreases in body weight and food consumption occurred in animals administered 50 mg/kg.	TX-399-2019 [redacted]
Vehicle Investigative Toxicity and Toxicokinetic Study	Rat/Crl:WI(Han)	Intravenous (slow bolus) injection (Vehicle 1 (12% [w/v] sulfobutylether-β-cyclodextrin in Sterile water for Injection, USP, pH 3.0 to 3.5) and (Vehicle 2 (90% normal saline/5% ethanol/5% polysorbate 80 [v/v/v] and hydrochloric acid, pH 3.0)	1-week	0, 0, 20, 50	Main: 5 males/group; TK: 9 males /group; Vehicle 1 and 2: 3 male/group	Mortality (Vehicle 2 at 50 mg/kg/day): One toxicity male (Animal No. B32559) was sacrificed in moribund condition on Day 7 due to severe tail lesions resulting from test article administration. One toxicokinetic animal (Animal No. B32565) was found dead on Day 7. Adverse decreases in body weight gain and food consumption and clinical pathology and microscopic findings indicative of kidney injury and/or dysfunction in males administered ≥ 20 mg/kg/day in either vehicle. In general, the severity of renal changes and	TX-399-2009 [redacted]

Test Article: Test Article: Remdesivir

Type of Study	Species/Strain	Method of Administration (Vehicle)	Duration of Dosing	Dose (mg/kg)	Gender and No. per Group	Noteworthy Findings	Gilead Study No. (CRO Name and Study No.)
						injection site reactions were greater in animals administered GS-5734 in vehicle 2 versus vehicle 1 NOAEL: Not Identified	
Tolerability Study	Rabbit/Hra:(NZW) SPF	Intravenous (slow bolus) injection (12% (w/v) sulfobutylether-β-cyclodextrin (in Sterile water for Injection, USP, pH 3.5+ 0.1)	1-week	0, 5, 15, 50	3 females/group	One female in the 50 mg/kg/day group was found dead on study day 6. Clinical observations noted for females in the 50 mg/kg/day group included impaired equilibrium for female found dead, ataxia, partial closure of the eyes, impaired use of the hindlimbs, decreased or shallow respiration rate, constricted pupils, clear or white discharge of the eyes, and/or exophthalmos. Severe body weight losses with corresponding minimal food consumption were noted for the 50 mg/kg/day group.	TX-399-2010 [redacted]
Intramuscular Injection Toxicity and Toxicokinetic Study	Monkey / Cynomolgus	Intramuscular Injection (5% ethanol, 95% propylene glycol [v/v])	1-week	GS-466547[d] 0, 2.5, 7.5, 15	3 males/group	≥ 2.5 mg/kg/day: local injection site inflammation correlating with ↑ fibrinogen and platelet counts, ↓ serum albumin and A:G, macroscopic injection site firmness correlating with myofiber necrosis and neutrophilic or mixed-cell inflammation ≥ 7.5 mg/kg/day (NOAEL): ↑ neutrophil and monocyte counts compatible with inflammation, ↑ kidney weights	TX-399-2001 [redacted]

Test Article: Test Article: Remdesivir

Type of Study	Species/Strain	Method of Administration (Vehicle)	Duration of Dosing	Dose (mg/kg)	Gender and No. per Group	Noteworthy Findings	Gilead Study No. (CRO Name and Study No.)
						15 mg/kg/day: ↓ food consumption, ↑ serum creatinine, ↓ urine concentration, 1 or 2+ urine protein compatible with renal dysfunction, ↓ reticulocyte counts, ↓ serum triglyceride concentrations, macroscopically pale renal cortex correlating with proximal tubular epithelial cell degeneration/necrosis, ↓ thymus weights with one animal with correlating ↓ thymic lymphocytes considered secondary stress response NOAEL (systemic) = 7.5 mg/kg/day (Day 7 AUC_{0-24}: GS-466547 4350 ng•h/mL; GS-441524 3370 ng•h/mL; GS-704277 2980 ng•h/mL) NOAEL for local tolerance not identified	
Impurity qualification study	Monkey/Cynomolgus	Intravenous (slow bolus) Injection (12% (w/v) sulfobutylether-β-cyclodextrin (in Sterile water for Injection, USP, pH 3.5+ 0.1)	2 weeks	0 (saline), 0 (vehicle), 5[b], 5[c], 10[c], 10[b], 10[a]	3 males /group	No noteworthy findings. Day 14: GS-5734-I 10 mg/kg/day NOAEL $AUC_{(0-24)}$ 1880 ng·hr/mL Day 14: GS-5734-D 10 mg/kg/day NOAEL $AUC_{(0-24)}$ 1130 ng·hr/mL	TX-399-2015 [redacted]
Reverse mutation assay	*Salmonella typhimurium* and *Escherichia coli*	In Vitro	52 ± 4 hours	[redacted][e] 5.0 to 5000 µg/plate	In Vitro	Non-mutagenic	TX-399-2020 [redacted]

Test Article: Test Article: Remdesivir

Type of Study	Species/Strain	Method of Administration (Vehicle)	Duration of Dosing	Dose (mg/kg)	Gender and No. per Group	Noteworthy Findings	Gilead Study No. (CRO Name and Study No.)
Reverse mutation assay	*Salmonella typhimurium* and *Escherichia coli*	In Vitro	72 hours	GS-441524[f] 0.075 to 250 µg/well	In Vitro	Non-mutagenic	TX-195-2006 [redacted]
Hepatotoxic profile in 3D Liver Tissues (GS-5734 and Metabolites GS-704277, and GS-441524)	Human Liver Tissue	In Vitro (0.1% DMSO)	2 weeks	GS-5734: 7.5-0.3µM GS-704277: 3-0.3µM GS-441524: 3-0.3µM	5 tissues/treatment	No conclusive evidence of toxicity in the exVive3D human liver tissues	TX-399-2022 [redacted]
Hepatotoxic profile (GS-5734 and Metabolites GS-704277, and GS-441524)	Rat, Monkey, and Human Hepatocytes	In Vitro (0.1% DMSO)	2 weeks	GS-5734: 30-0.12µM GS-704277: 100-0.4µM GS-441524: 300-1.2µM	In Vitro	GS-5734 IC_{50} values based on the decrease in albumin secretion were < 0.12, 0.96, and 2.1 µM in human, rat, and monkey hepatocytes. The 14-day IC50 values of GS-5734 in human hepatocytes measured by albumin production, culture integrity, and ATP levels were < 0.12, < 0.12, and 0.68 µM, respectively. GS-5734 was substantially more toxic in the in-vitro assay than its metabolites GS-704277 and GS-441524 across the 3 tested species, and particularly in human hepatocytes	PC-399-2027 [redacted]

DMSO = Dimethylsulfoxide; NOAEL = no observed adverse effect level; Remdesivir = GS-5734. AUC_{0-24}= Area under the concentration-time curve from hour 0 to hour 24; TK = Toxicokinetics; ↑ = increased from vehicle control; ↓ = decreased from vehicle control

a Remdesivir
b Remdesivir-I (with impurities)
c Remdesivir-D (with degradants and impurities)
d GS-466547 (diastereomeric prodrug mixture containing Remdesivir)
e [redacted] is an impurity of Remdesivir
f GS-441524 is a metabolite of Remdesivir