CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

214787Orig1s000

NON-CLINICAL REVIEW(S)

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

PHARMACOLOGY/TOXICOLOGY NDA REVIEW AND EVALUATION

Application number:	214787
Applicant's letter date:	04/08/2020
CDER stamp date:	08/07/2020
Product:	Veklury™ (Remdesivir [RDV]/ GS-5734)
Indication:	COVID19
Applicant:	Gilead
Review Division:	DAV
Reviewer:	John Dubinion, Ph.D.
Supervisor/Team Leader:	Hanan Ghantous, Ph.D., DABT
Division Director:	Debra B. Birnkrant, M.D.
Project Manager:	Christine Kim, Pharm.D.

Disclaimer

Except as specifically identified, all data and information discussed below and necessary for approval of NDA #214787 are owned by Gilead Sciences, Inc. or are data for which Gilead has obtained a written right of reference.

Any information or data necessary for approval of NDA #214787 that Gilead does not own or have a written right to reference constitutes one of the following: (1) published literature, or (2) a prior FDA finding of safety or effectiveness for a listed drug, as reflected in the drug's approved labeling. Any data or information described or referenced below from reviews or publicly available summaries of a previously approved application is for descriptive purposes only and is not relied upon for approval of NDA #214787.

TABLE OF CONTENTS

Table of Tables

Table of Figures

Executive Summary

Introduction

Veklury™ is intended (b) (4)

Veklury™ comes in two dosage forms: a lyophilized powder at 100 mg for reconstitution in 19 mL saline and a concentrate at 5 mg/mL. The vehicle used in the formulation of Veklury™ contains sulfobutyl-β-cyclodextrin (SBECD). SBECD is used in several approved parenteral formulations, including both IV and intramuscular (IM) products. SBECD is cited in the US FDA list of Inactive Pharmaceutic Ingredients and is generally considered safe.

Remdesivir (RDV, GS-5374, Veklury™) is a SARS-CoV-2 ribonucleic acid nucleotide analog polymerase inhibitor. RDV is a prodrug that distributes into cells where it is metabolized to form the pharmacologically active nucleoside triphosphate metabolite GS-443902. The predominant circulating metabolite of RDV that was used for measuring exposure was GS-441524.

The nonclinical safety profile of RDV has been evaluated in: safety pharmacology studies in rats and Cynomolgus macaques; repeat-dose toxicology studies in rats and Cynomolgus macaques for up to a month; two follow-up 1-week toxicology studies in Cynomolgus and Rhesus macaques; 2-week repeat-dose toxicology studies to qualify impurities; fertility and pre- and post-natal developmental studies in rats; embryo-fetal developmental studies in rats and rabbits; and genetic toxicology studies (Ames, *in vitro* chromosomal aberration and *in vivo* rat micronucleus assays). In addition, numerous *in vitro* and *in vivo* nonclinical pharmacokinetic studies evaluating the absorption, distribution, metabolism and excretion of RDV have been conducted in mice, rats, rabbits, and monkeys.

Brief Discussion of Nonclinical Findings

The kidney was the target organ of toxicity identified in repeat-dose toxicology studies in rats and monkeys. In 2-week studies, RDV of doses up to 50 and 10 mg/kg/day, respectively in rats and monkeys, resulted in tubule cell vacuolation. However, only rats had adverse kidney effects with basophilic staining accompanied with body weight loss and alterations in clinical chemistry and urinalysis. In 4-week studies, RDV up to 10 mg/kg also resulted in tubule cell vacuolation in both species; vacuolation was more widespread (liver, lymph node, adrenal, spleen) with longer dosing and attributed to the vehicle, SBECD.

While SBECD is generally recognized as safe and can be found in an approved product up to (b) (4) g, it has also been characterized to cause acute kidney injury consistent with the finding of tubule cell vacuolation in the repeat-dose studies. The European Medicines Agency (EMA) has published a review summarizing the safety of cyclodextrins. SBECD has been found to cause vacuolation of the kidney tubular cells without loss/change of kidney function. The effect appeared to be dose- and duration- dependent; however, all effects were reversable. In the absence of detrimental changes to kidney function, this effect is predicted to be a physiological response representing a sequestration process and appears to be non-adverse.

In repeat-dose studies, rat kidneys transitioned from non-adverse vehicle-mediated vacuolation to adverse vehicle-mediated vacuolation with accompanied effects indicative of kidney functional changes. This transition demonstrated the additive effect of RDV to exacerbate the vehicle mediated vacuolation resulting in kidney dysfunction. As the observed kidney injury (vacuolation) in monkeys at 10 mg/kg was not accompanied with body weight loss and signs of kidney dysfunction, the sponsor performed an additional 7-day study in Rhesus macaques at higher doses to achieve adverse RDV toxicity. In this study, monkeys that were administered ≥ 10 mg/kg had kidney injury accompanied with signs of kidney dysfunction. The sensitivity of rats to kidney injury preclude using this model to elucidate the dose/exposure where administration of RDV transitions from non-adverse renal injury, likely due to SBECD, to adverse kidney dysfunction. In monkeys, this effect appears to go from minimal to moderate from 5 to 10 mg/kg and this dose range appears to be the point in monkeys where kidney effects become adverse due to RDV. Exposure at 10 mg/kg in monkeys is 2.7 times the exposure in humans in the Phase 1 clinical trial.

Tissue distribution studies following IV administration showed RDV-derived material present in many tissues, including lungs. RDV rapidly declined in plasma and was accompanied by a sequential appearance of the metabolites, GS-704277 and GS-441524. RDV also showed broad distribution and efficient activation of RDV to active nucleoside analog, GS-443902, in respiratory tissues of monkeys. Similarly, high intracellular levels of GS-441524 and its phosphorylated metabolites were also observed in surrogate cells (peripheral blood mononuclear cells [PBMC]) from studies in monkeys. A half-life of 22 and > 24 hours was observed for GS-443902 in lung and PBMC, respectively, supporting once-daily administration.

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

Remdesivir was not genotoxic in in vivo and in vitro assays. In the reproductive and development toxicity studies, there was a decrease in corpora lutea, a consequent decrease in implantation sites and viable embryos, and lower ovary and uterus/cervix/oviduct weights in the rats at 10 mg/kg; these changes were observed at a systemically toxic dose. There were no remarkable findings in male rats in the fertility study, no adverse findings in embryo-fetal studies in rats and rabbits, and no adverse changes in the pre- and postnatal study in rats.

Table 1: Exposure Margin for Pivotal Toxicity Studies

Species	Study	NOAEL (mg/kg)	AUC (ng*h/mL)	Exposure Margin AUC*
Rat	2-week	5[+]	1420	**<1 (0.64)**
Rat	4-week	3	748	**<1 (0.34)**
Monkey	2-week	10	2390	**1.1**
Monkey	4-week	10	2070	**<1 (0.92)**
Monkey[¤]	7-day	10[¤]	6050	**2.7**
Rat	Fertility (Female)	3	748^	**<1 (0.34)**
	Fertility (Male)	10	4210	**2**
Rat	Embryofetal	20	8740	**3.9**
Rabbit	Embryofetal (Maternal)	10	1790	**<1 (0.80)**
	Embryofetal (Fetus)	20	8930	**4**
Rat	PPND	10	2310	**1.0**

*200 mg Day 1 followed by 100 mg Day 2-9 in HV patients, AUC 2229 ng*h/mL

[+] NOAEL could not be identified in males in this study. Threshold is for females only.

[¤] This study was done in Rhesus not Cynomolgus.; The threshold is a LOAEL for RDV exacerbated kidney injury.

^ No toxicokinetics were performed in this study; AUC is from 4-week study.

Recommendations

Approvability

Yes. The sponsor provided sufficient nonclinical safety information on remdesivir in support of approval for marketing in the U.S. The identified safety concern appears to be both monitorable and reversable. Furthermore, the indication is life threatening and the benefit of Veklury™ outweighs the potential risk identified in the nonclinical program.

Labeling

The sponsor's draft product label adequately described risks identified nonclinical program.

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

(b) (4)

8 USE IN SPECIFIC POPULATIONS

8.1. Pregnancy

Risk Summary

(b) (4)

In nonclinical reproductive toxicity studies, remdesivir demonstrated no adverse effect on embryofetal development when administered to pregnant animals at systemic exposures (AUC) of the predominant circulating metabolite of remdesivir (GS-441524) that were 4 times (rats and rabbits) the exposure in humans at the recommended human dose (RHD) (see Data).

The estimated background risk of major birth defects and miscarriage for the indicated population is unknown. All pregnancies have a background risk of birth defect, loss, or other adverse outcomes. In the U.S. general population, the estimated background risk of major birth defects and miscarriage in clinically recognized pregnancies is 2 to 4% and 15 to 20%, respectively.

Data

Animal Data

Remdesivir was administered via intravenous injection to pregnant rats and rabbits (up to 20 mg/kg/day) on Gestation Days 6 through 17, and 7 through 20, respectively, and also to rats from Gestation Day 6 to Lactation/Post-partum Day 20. No adverse effects on embryo-fetal (rats and rabbits) or pre/postnatal (rats) development were observed in rats and rabbits at nontoxic doses in pregnant animals. During organogenesis, exposures to the predominant circulating metabolite (GS-441524) were 4 (rats and rabbits) times higher than the exposure in humans at the RHD. In a pre/postnatal development study, exposures to the predominant circulating metabolite of remdesivir (GS-441524) were similar to the human exposures at the RHD.

8.2. Lactation

Risk Summary

There is no available data on the presence of remdesivir in human milk, the effects on the breastfed infant, or the effects on milk production. In animal studies, remdesivir and metabolites have been detected in the nursing pups of mothers given remdesivir, likely due to the presence of remdesivir in milk (see Data). The developmental and health benefits of breastfeeding should be considered along with the mother's clinical need for VEKLURY and any potential adverse effects on the breastfed child from VEKLURY or from the underlying maternal condition.

Data

Animal Data

Remdesivir and its metabolites were detected in the plasma of nursing rat pups, likely due to the presence of remdesivir and/or its metabolites in milk, following daily intravenous administration of remdesivir to pregnant rats from Gestation Day 6 to Lactation Day 20. Exposures in nursing pups were approximately 1% that of maternal exposure on Lactation Day 10.

13 NONCLINICAL TOXICOLOGY

13.1. Carcinogenesis, Mutagenesis, Impairment of Fertility

Carcinogenesis and Mutagenesis

Given the short-term administration of VEKLURY for the treatment of COVID-19, long-term animal studies to evaluate the carcinogenic potential of remdesivir were not conducted.

Remdesivir was not genotoxic in a battery of assays, including bacterial mutagenicity, chromosome aberration using human peripheral blood lymphocytes, and in vivo rat micronucleus assays.

Impairment of Fertility

Nonclinical toxicity studies in rats demonstrated no adverse effect on male fertility at exposures of the predominant circulating metabolite (GS-441524) approximately 2 times the exposure in humans at the RHD.

Reproductive toxicity, including decreases in corpora lutea, numbers of implantation sites, and viable embryos, was seen when remdesivir was administered by daily intravenous administration at a systemically toxic dose (10 mg/kg) in female rats 14 days prior to mating and during conception; exposures of the predominant circulating metabolite (GS-441524) were 1.3 times the exposure in humans at the RHD.

13.2. Animal Toxicology and/or Pharmacology

Intravenous administration (slow bolus) of remdesivir to male rhesus monkeys at dosage levels of 5, 10, and 20 mg/kg/day for 7 days resulted, at all dose levels, in increased mean urea nitrogen and increased mean creatinine, renal tubular atrophy, and basophilia and casts.

Intravenous administration (slow bolus) of remdesivir to rats at dosage levels of ≥3 mg/kg/day for up to 4 weeks resulted in findings indicative of kidney injury and/or dysfunction.

Kidney-related effects in rats and monkeys were observed at exposures of the predominant circulating metabolite (GS-441524) that are lower than the exposure in humans at the RHD.

Summary Review of Studies Submitted Under IND

Remdesivir (GS-5734; RDV) initiated under IND 125566 as a treatment for Ebola Virus Disease. In addition to EVD, in vitro studies showed activity of RDV against a broad number of viruses, including coronaviruses. As a coronavirus (SARS-CoV-2) was identified as the cause of the COVID-19 pandemic, RDV transitioned to also be an investigative agent for COVID-19. All nonclinical safety studies conducted in support of intravenous RDV were submitted to the NDA for COVID-19 and are reviewed in the following sections. All tables in this review are the Applicant's unless otherwise stated.

Pharmacology (Primary and Secondary)

Remdesivir (GS-5734; RDV) is a nucleotide prodrug that efficiently distributes into cells and is intracellularly metabolized into an analog of adenosine triphosphate, GS-443902, that inhibits viral RNA polymerases. RDV has potent activity against coronaviruses (SARS-CoV-2, SARS-

CoV, MERS-CoV), filoviruses (EBOV and MARV) and paramyxoviruses (RSV, NiV, and Hendra virus). See the virology review for additional details on all cell-based efficacy studies.

In vivo efficacy of RDV was assessed via mouse models of SARS-CoV and MERS-CoV infection and non-human primate models of SARS-CoV-2 and MERS-CoV infection. None of these studies were performed in a well characterized model of COVID-19. RDV exhibited potential activity against all three viruses with milder disease symptoms and reduced viral RNA levels in treated animals.

Table 2: Summary of In Vivo Studies Evaluating the Efficacy of Remdesivir

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

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

Summary of Exploratory *in vivo* Animal Model of SARS-CoV-2 Infection

In the most clinically relevant animal model of SARS-CoV-2 infection, RDV was evaluated in a single exploratory study using rhesus macaques. Vehicle or RDV (10 mg/kg first dose, followed

by 5 mg/kg thereafter) was administered once daily using IV bolus injection beginning 12 hours after inoculation (2.6 x 10^6 tissue culture infectious dose 50 (TCID50) of SARS-CoV-2 via the intranasal, ocular, oral, and intratracheal routes) through Day 6 post-inoculation. Treatment was associated with reduced clinical signs, gross lung lesions, and viral loads. Unfortunately, the sponsor failed to adequately establish the natural progression of the disease in this animal model. Therefore, results from this study are generally not sufficient to draw any definitive conclusions concerning RDV's potential to treat COVID-19 in humans.

Secondary Pharmacology / Off Target Liability

The off-target liability of RDV and its metabolites was evaluated in a series of in vitro studies. No clinically meaningful findings were noted. The following is a list of RDV and its diastereomers and metabolites referenced in the summary of findings from the Secondary Pharmacology Studies. Of note, the key metabolites for the evaluation of the safety of RDV in animals were the parent drug, GS-5734, the active metabolite, GS-443902, and the metabolite by-product used to determine exposure in plasma, GS-441524.

Table 3: Remdesivir and its Diastereomers and Metabolites

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

Table 4. Secondary Pharmacology Studies

Study Title (Study No.)	Findings
Off-Target Receptors, Ion channels, and Transports (Study PC-399-2002, PC-399-2001)	87 targets consisting of receptors, ion channels, and transporters were assessed with diastereomeric mixture GS-466547 and the nucleoside analog GS-441524 at a concentration of 10 µM. No inhibition of any ligand binding at a concentration of 10 µM.
Cytotoxicity Assessment (Study PC-399-2013)	HEp-2, HepG2, PC-3, MT-4, and primary hepatocyte cell lines with or without stimulation were assessed with GS-5734 and GS-441524 up to 100 µM. The CC_{50} values of GS-5734 ranged from 1.7 to >20 µM, and GS-441524 showed no cytotoxicity up to 100 µM (except within MT-4 T cell line where the CC_{50} value was 69 µM). GS-5734 inhibits SARS-CoV-2 with an EC_{50} value of 0.0099 µM and systemic exposure at RDV is much lower than the effects observed.

Study Title (Study No.)	Findings
Human Bone Marrow Assessment (Study PC-399-2018)	GS-5734 and GS-441524 related effects on human bone marrow derived erythroid and myeloid progenitor proliferation and megakaryoid progenitor proliferation were assessed up to 30 µM. GS-5734 exhibited CC_{50} values ranging from 2.3 to 10.5 µM and GS-441524 exhibited CC_{50} values of 5.9 to 22.7 µM Systemic concentration of GS-441524 at 10 mg in Cynomolgus Monkeys remains at or below 1 µM.
Mitochondrial Toxicity (Study PC-399-2015; PC-399-2016)	GS-5734 effects on mitochondrial DNA was evaluated in HepG2 cells at concentrations up to 10 µM. Mitochondrial protein synthesis and mitochondrial respiration was also evaluated in human PC-3 and PC-3 and HepG2 cells, respectively, up to 100 µM. GS-5734 had no effect on mtDNA and a mild effect on mitochondrial respiration (CC_{50} values ranging from 2.5 to 24 µM).
Interaction with Host RNA/DNA Polymerases (Study PC-399-2017)	Evaluation of the potential of GS-443902, the active triphosphate metabolite, to interact with human DNA polymerases α, β, and γ, as well as RNA polymerase II and mitochondrial RNA polymerase was performed in biochemical in vitro assays up to 200 µM. A nucleotide incorporation assay also determined GS-443902 incorporated in DNA or RNA. GS-443902 did not inhibit any DNA or RNA polymerase. It was a poor substrate for human mitochondria RNA polymerases with incorporation rates below 6%.

HEp-2= Human epithelial Type 2; HepG2= Human liver cancer; PC-3= Human prostate cancer; MT-4= Human T-Cell leukemia; CC_{50}= 50% cytotoxic concentration; EC_{50} = effective concentration [inhibiting 50% activity]

Safety Pharmacology

Table 5. Safety Pharmacology Studies

Study Title (Study No.)	Findings
hERG Assay (Study PC-399-2006, PC-399-2025)	hERG-transfected HEK-293 cells were treated with up to 30 µM GS-5734. Potassium current was inhibited 50.4% at the highest concentration, and an IC_{20} and IC_{50} was identified as 7.5 and 28.9 µM. GS-441524 and GS-704277, were also assessed in CHO-hERG DUO cells stably expressing hERG up to 30 µM. GS-441524 and GS-704277 inhibited I_{hERG} peak tail current by 21 and 2.5%, respectively, at 30µM. The IC_{50} of GS-441524 and GS-704277 were estimated to be greater than 30 µM. No significant effect on hERG channel.
Cardiovascular Assessment in Monkeys (Study PC-399-2005)	Heart rate, blood pressure, electrocardiography parameters, and body temperature were evaluated via telemetry in cynomolgus monkeys (4 males/group) up to 19 hours after single intravenous injection of GS-5734 (0, 1, 3 & 10 mg/kg). No significant test-article effect was noted. NOAEL =10 mg/kg.

Study Title (Study No.)	Findings
Respiratory Assessment in Rats (Study PC-399-2004)	Respiratory parameters (respiratory rate, tidal volume, minute volume, and total pulmonary resistance) and body temperature were evaluated in Crl:WI(Han) rats (8 males/group) up to 24 hours after single intravenous injection of GS-5734 (0, 5, 20 & 50 mg/kg). A tendency of increased respiration rate was evident in animals administered ≥20 mg/kg starting at 0.75 hours post dose and reached statistical significance at 3 hours post dose. NOAEL =5 mg/kg.
Central Nervous System Assessment in Rats (Study PC-399-2003)	A battery of behavioral tests and observations (via a modified Irwin Test) were evaluated in Crl:WI(Han) rats (8 males/group) up to 24 hours after a single intravenous injection of GS-5734 (0, 5, 20 & 50 mg/kg). No significant test-article effects were noted. NOEL= 50 mg/kg

AUC = area under the curve; IC_{50} = concentration inhibiting 50% activity; NOEL = no observed effect level; NOAEL= no observed adverse effect level

Pharmacokinetics

Pharmacokinetics of RDV (GS-5734) and its metabolites [see Table 2: Remdesivir and its Diastereomers and Metabolites] were characterized using high performance liquid chromatography coupled to tandem mass spectrometry (LC/MS/MS) in:

- pharmacokinetic (PK) studies following single administration in rats and monkeys (African green, marmoset, cynomolgus and rhesus monkeys),
- non-GLP toxicokinetic (TK) single/repeat dose studies in cynomolgus/rhesus monkey, rat, rabbit and transgenic (Ces1c-/-) mouse,
- GLP repeat dose toxicity studies in rat, and cynomolgus monkeys, and
- non-GLP repeat dose toxicity studies in rabbits and rhesus monkeys.

The clearance of GS-5734 exceeded liver blood flow in all species. Disappearance was followed by transient exposure to the intermediate metabolite GS-704277 and more persistent exposure to the nucleoside metabolite GS-441524. The pharmacologically active nucleoside triphosphate metabolite, GS-443902, is found within cells and tissues after remdesivir is converted by hydrolase and phosphoramidase cleavage to the nucleoside analog monophosphate. The intracellular levels of GS-443902 were, therefore, also assessed in

- PBMC and lung tissue from PK/TK studies,
- in combination with GS-441524 and its phosphorylated metabolites in PBMC, macrophages, monocytes, HeLa, and HMVEC cells, and
- in select respiratory and non-respiratory tissues in marmoset and African green monkeys.

The methods used to measure intracellular concentrations did not strictly conform to regulatory guidelines but were evaluated robustly.

Absorption:

Single-dose absorption studies were conducted in rats and monkeys using intravenous RDV (in 12% sulfobutyl-β-cyclodextrin (SBECD)). Pharmacokinetic parameters, from 5 studies (AD-399-2001, AD-399-2002, AD-399-2003, AD-399-2022, AD-399-2033), following administration to three animals per study are presented in the following tables.

Table 6: Pharmacokinetic Parameters for Remdesivir in Male Wistar Han Rat, Cynomolgus Monkey, and Rhesus Monkey After IV RDV

Parameter (units)	Rat	Monkey (Cynomolgus)	Monkey (Rhesus)	Monkey (Rhesus)
Dose (mg/kg)	50	10	3	10
Formulation	12% (w/v) SBECD in water (w/v; pH = 4)	12% (w/v) SBECD and 98% water (pH = 4)	12% (w/v) SBECD in water, pH 3.5-4.0	12% (w/v) SBECD in water, pH 3.5-4.0
C_{max} (µM)	17.5	16.0	2.39	5.07
$t_{1/2}$ (h)	0.05	0.29	0.35	0.39
AUC_{0-24} (µM•h)	6.20	4.76	0.80	2.09
CL (L/h/kg)	13.5	3.50	6.37	7.96

Source: AD-399-2001; AD-399-2002; AD-399-2003; AD-399-2022

Table 7: Pharmacokinetic Parameters for GS-704277 in Male Wistar Han Rat, Cynomolgus Monkey, and Rhesus Monkey After IV RDV

Parameter (units)	Rat	Monkey (Cynomolgus)	Monkey (Rhesus)	Monkey (Rhesus)
Dose (mg/kg)	50	10	3	10
Formulation	12% (w/v) SBECD in water (w/v; pH = 4)	12% (w/v) SBECD and 98% water (pH = 4)	12% (w/v) SBECD in water, pH 3.5-4.0	12% (w/v) SBECD in water, pH 3.5-4.0
T_{max} (h)	0.48	0.08	0.33	0.19
C_{max} (µM)	40.6	3.43	0.71	1.80
$t_{1/2}$ (h)	0.25	0.84	3.59	0.99
AUC_{0-24} (µM•h)	25.1	2.73	0.84	2.38

Source: AD-399-2001; AD-399-2002; AD-399-2003; AD-399-2022

Table 8: Pharmacokinetic Parameters for GS-441524 in Male Wistar Han Rat, Cynomolgus Monkey, and Rhesus Monkey After IV RDV

Parameter (units)	Rat	Monkey (Cynomolgus)	Monkey (Rhesus)	Monkey (Rhesus)
Dose (mg/kg)	50	10	3	10
Formulation	12% (w/v) SBECD in water (w/v; pH = 4)	12% (w/v) SBECD and 98% water (pH = 4)	12% (w/v) SBECD in water, pH 3.5-4.0	12% (w/v) SBECD in water, pH 3.5-4.0
T_{max} (h)	1.25	1.33	0.50	1.33
C_{max} (µM)	7.82	1.15	0.33	1.14
$t_{1/2}$ (h)	6.21	7.16	8.71	8.55
AUC_{0-24} (µM•h)	63.7	8.23	2.11	8.73

Source: AD-399-2001; AD-399-2002; AD-399-2003; AD-399-2022

Table 9: Pharmacokinetic Parameters of GS-5734 and its Metabolites Following a 10 mg/kg IV Injection in Cynomolgus Monkeys

	PK Parameter		
Metabolite	GS-5734	GS-704277	GS-441524
C_{max} (µM)	7.29 ± 0.78	0.80 ± 0.11	0.68 ± 0.35
T_{max} (h)	0.083 ± 0	0.39 ± 0.53	2.08 ± 1.88
AUC_{0-24} (µM•h)	3.15 ± 0.99	1.36 ± 0.53	7.02 ± 3.55
$T_{1/2}$ (h)	0.57 ± 0.08	1.30 ± 0.10	6.42 ± 0.58
Cl (L/h/kg)	5.64 ± 1.87	NA[a]	NA
V_{ss} (L/kg)	2.21 ± 0.50	NA	NA

Source: AD-399-2033

GS-441524 and its phosphorylated metabolites were also measured in PBMC in the rhesus single dose study (AD-399-2022). Levels of the pharmacologically active metabolite, GS-443902 in PBMC achieved a C_{max} of 33.3 µM at 2 h and had an apparent intracellular terminal elimination $t_{1/2}$ of approximately 14 h.

Table 10: Concentration of the GS-441524 and its Phosphorylated Metabolites in PBMC Following 10 mg/kg IV RDV

Time (h)	PBMC Concentration (µM)			
	GS-441524	GS-719700	GS-719699	GS-443902
2	6.19 ± 4.03	0.11 ± 0.04	10.3 ± 4.3	33.3 ± 9.7
4	3.26 ± 1.47	0.07 ± 0.02	8.73 ± 2.65	32.8 ± 6.2
8	1.63 ± 0.48	0.05 ± 0.03	8.07 ± 2.93	26.0 ± 8.0
24	0.93 ± 0.17	BLQ	4.23 ± 2.44	11.4 ± 4.5

a Data represent the mean ± SD of 3 animals.
BLQ = below lower limit of quantitation; IV = intravenous; M = male; NA = not applicable; PBMC = peripheral blood mononuclear cells

Both plasma and PBMC exposures to all metabolites showed roughly dose-proportional increases between 3 and 10 mg/kg following IV bolus administration. Intramuscular injection

was also assessed in rhesus monkeys (AD-399-2016); however, IM administration was not pursued and further studies to identify a dosing rational for this route were not performed.

Distribution:

The in vitro plasma protein binding of GS-5734, GS-704277 and GS-441524 was determined in plasma from rat, monkey, and human (AD-399-2013). GS-5734 had moderate protein binding in all species with a free fraction ranging from 8.0-14.2% (free fraction in human was 12.1%). GS-704277 and GS-441524 exhibited very low protein binding in plasma from all species.

The distribution of GS-5734 and GS-441524 between the cellular and soluble fractions of blood from monkey and human (AD-540-2007) found GS-5734 was excluded from the blood cellular fraction in both species, with mean whole blood/plasma concentration ratios of 0.71 and 0.76 for monkey and human, respectively. GS-441524 showed some association with the cellular fraction with respective mean blood/plasma ratios of 1.36 and 1.19 for monkey and human.

In monkey (marmoset, rhesus, and African green) studies, tissue distribution of GS-443902, the pharmacologically active nucleoside triphosphate, was measured in PBMC and lung. As described in absorption studies, GS-5734 was rapidly eliminated followed by the sequential appearance of GS-704277 and GS-441524 as well as efficient formation of GS-443902 within cells. In marmosets (AD-399-2023), GS-443902 persists with an approximate half-life of > 24 h and 22 h in PBMC and lung, respectively, following a single dose of GS-5374. In rhesus (AD-399-2030), GS-443902 appeared to accumulate in PBMC after 7 days of once daily dosing. C_{max} AUC_{0-24} increase by 10% and 38%, respectively. In African green (AD-540-2003), GS-443902 persisted in respiratory tissues 24 h postdose although GS-441524 was no longer measurable.

Table 11: Tissue Concentrations of GS-441524 and its Phosphorylated Metabolites in Respiratory System of African Green Monkey Following 20 mg/kg IV RDV

Tissue	GS-441524	GS-441524-MP	GS-441524-DP	GS-443902	Total Nucleotide
Lower Lung Lobe	BLQ	BLQ	0.27 ± 0.04	1.03 ± 0.19	1.30 ± 0.23
Upper Trachea	BLQ	BLQ	0.27 ± 0.08	0.54 ± 0.15	0.81 ± 0.14
Lower Trachea	BLQ	BLQ	0.26 ± 0.08	0.53 ± 0.36	0.78 ± 0.44
Mainstem Bronchi	BLQ	BLQ	0.40 ± 0.03	0.81 ± 0.47	1.21 ± 0.50
Lower Bronchi	BLQ	BLQ	0.45 ± 0.06	1.12 ± 0.12	1.57 ± 0.16

Whole body autoradiography was performed in pigmented and nonpigmented male rats (AD-399-2017). Distribution was similar in both species where all tissues examined had exposure of ^{14}GS-5734. The tissues examined included the following.

Adrenal gland(s)	Large intestine (including cecum)	Small intestine (including duodenum, jejunum, and ileum)
Bile (from gall bladder)	Liver	Small intestine contents and wash
Bone (both femurs)	Lungs	Spinal cord
Bone marrow (from both femurs)	Lymph node(s), axillary	Spleen
Brain	Lymph node(s), iliac	Stomach
Cerebrospinal fluid (collected prior to exsanguination)	Lymph node(s), inguinal	Stomach contents and wash
Epididymis	Lymph node(s), mesenteric	Testis(es)
Eyes (both)	Muscle (biceps femoris)	Thymus
Gall bladder	Pancreas	Thyroid (including parathyroid)
Heart	Pituitary gland	Urinary bladder
Kidney(s)	Prostate gland	
Large intestine and cecum contents and wash	Salivary glands (mandibular)	
	Seminal vesicle(s)	
	Skin (dorsal, shaved)	

Most tissues reached C_{max} by the first collection time point (0.167 h postdose). The majority of the tissues eliminated ^{14}GS-5734 by 96 hours postdose. No melanin binding was observed. ^{14}GS-5734 was cleared from the majority of tissues by 96 hours postdose; however, at 168 hours postdose, ^{14}GS-5734 was still quantifiable in kidney, kidney cortex, kidney medulla, liver, and skin.

The distribution of remdesivir was also determined following a single IV administration of 10 mg/kg ^{14}C-GS-5734 to male cynomolgus monkeys (AD-399-2019). ^{14}C-GS-5734 was widely distributed throughout all tissues by 4 hours. Total exposure declined over 168, but 8.26% of the administered dose was retained in the tissues, mostly in liver and muscle.

Metabolism:

The stability of GS-5734 in plasma from Sprague-Dawley rat, beagle dog, cynomolgus monkey, rhesus monkey, and human was determined (AD-399-2012). GS-5734 was unstable in rat plasma ($t_{1/2} \leq 0.9$ min). GS-5734 was substantially more stable in non-rodent species with $t_{1/2}$ ranging from 68.5 min in human to 630 min in dog.

The metabolic stability of GS-5734 was assessed in intestinal and hepatic subcellular fractions from Sprague-Dawley rat, beagle dog, cynomolgus monkey, rhesus monkey, and human (AD-399-2014). GS-5734 was moderately stable in intestinal extract ($t_{1/2}$ = 40.3 - 114.1 min) from human, dog, and rat, but was unstable in hepatic extract ($t_{1/2}$ < 3.9 min) in all species. Of the species tested, GS-5734 was relatively stable in human intestinal S9 ($t_{1/2}$ = 114.1 min).

The metabolism of ^{14}C-GS-5734 was assessed *in vitro* (mouse, rat, monkey, and human cryopreserved hepatocytes) at 1 and 10 µM 14C-GS-5734 (AD-399-2024). ^{14}C-GS-5734 was metabolized by mouse, rat, monkey, and human hepatocytes, primarily via hydrolysis. The rate

of biotransformation in mouse and rat was relatively faster compared to monkey and human. The remaining unchanged ^{14}C-GS-5734 accounted for <10% across all the species at 120 minutes. In all four species, most of the ^{14}C-GS-5734 was associated with three major metabolites; GS-704277 (M5), GS-441524 (M15), and GS-441524-phosphate (M4) generated via hydrolysis. GS-704277 (M5) was the predominant component. Other minor components tentatively identified were dihydroxy-GS-5734 (M30) and hydroxy-GS-5734 (M32). GS-704277 (M5), GS-441524 (M15), and GS-441524-phosphate (M4) were found in all species tested. M30 (dihydroxy-GS-5734) and M32 (hydroxy-GS-5734) were found in monkeys and humans. A summary of metabolites identified, and the proposed biotransformation pathways are presented in the sponsor's following table and figure.

Table 12: Metabolites Identified in Mouse, Rat, Monkey, and Human Hepatocytes Following Dosing up to 10 µM GS-5734

Metabolite Designation	Retention Time (Minutes)[a]	Proposed Identification	Species			
			Mouse	Rat	Monkey	Human
M4	8.83-9.00	GS-441524-phosphate	x	x	x	x
GS-704277 (M5)	12.5-12.67	GS-704277	x	x	x	x
GS-441524 (M15)	28.83	GS-441524	x	x	x	x
M30	51.67-51.83	Dihydroxy-GS-5374			x	x
M32	54.33	Hydroxy-GS-5374			x	x
GS-5734	58.00	GS-5734	x	x	x	x

a Retention time ranges from analysis of all matrices.

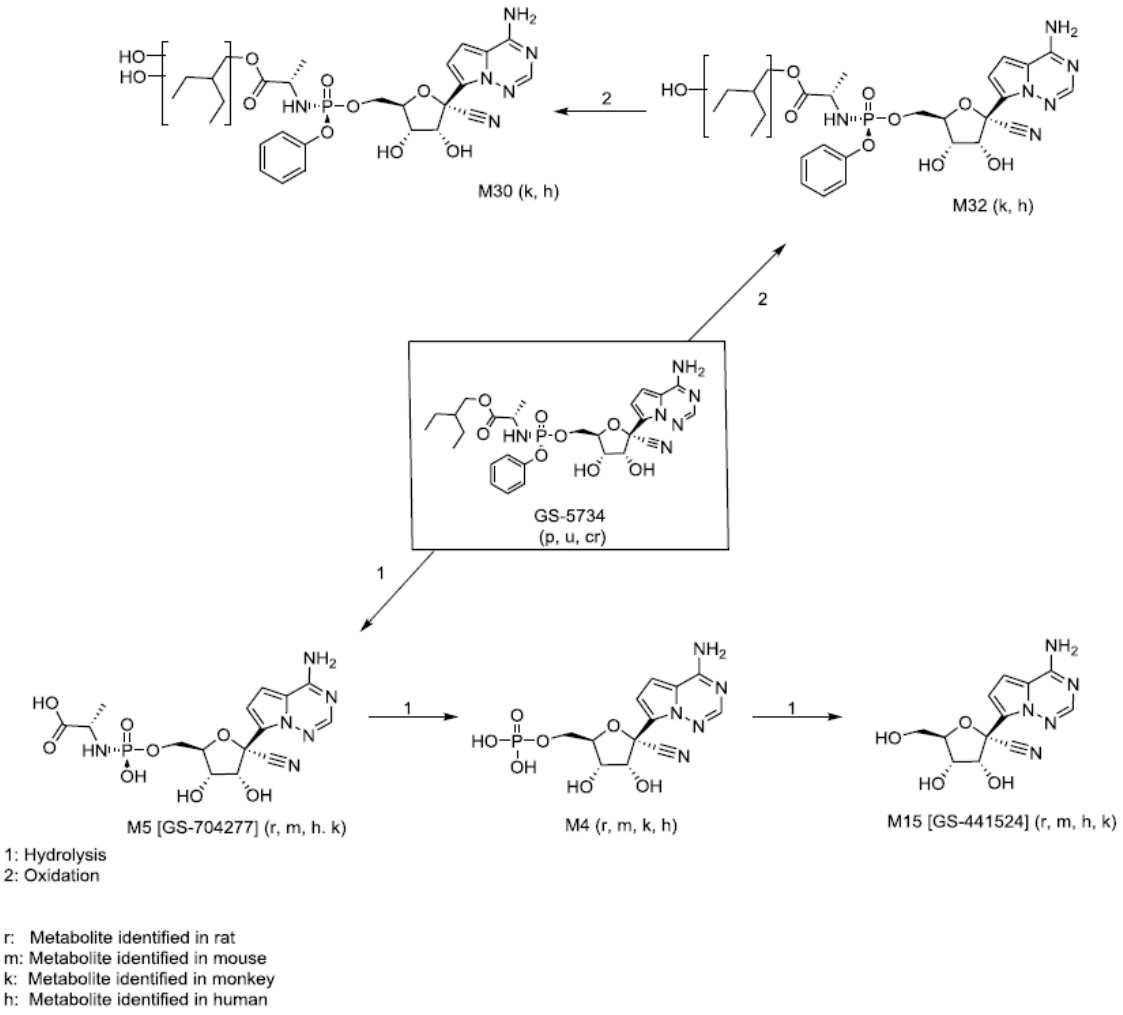

Figure 1: Proposed Biotransformation Pathways in Hepatocytes of Mouse, Rat, Monkey, and Human

Plasma, urine, feces, and cage rinse samples were profiled for parent and metabolites of ^{14}C-GS-5734 from monkeys after administration of a single intravenous dose of 10 mg/kg (AD-399-2020). Metabolites were identified by comparison with reference standards and by liquid chromatography-mass spectrometry. The profile of an AUC-pooled plasma sample showed GS-441524 was the only circulating component in plasma through 96 hours (AUC_{0-96}). ^{14}C-GS-5734 underwent rapid biotransformation with renal elimination being the major route of excretion (~31%). Less than 1% of the dose was recovered as unchanged GS-5734 in urine in the first 24 hrs. GS-441524 and GS-441524-glucuronide (M18) were the major components in urine, accounting for 15.6 and 8.43% of the dose, respectively. A large amount of radioactivity was eliminated in feces (25%). No unchanged GS-5734 was recovered in feces. Desamino-hydroxy-GS-441524 (M14) was the major component in feces, accounting for 19.9%. 15% of the administered dose was recovered in cage rinse; GS-441524 and GS-441524-glucuronide (M18), accounted for 6.17 and 3.18%, respectively. A summary of metabolites identified in plasma,

urine, feces, and cage rinse, and the proposed biotransformation pathways in monkeys are presented in the sponsor's following table and figure.

Table 13: Metabolites Identified in Plasma, Urine, Feces, and Cage Rinse from Monkeys Dosed 10 mg/kg GS-5734

Metabolite Designation	Retention Time (Minutes)	Proposed Identification	Matrix			
			Plasma	Urine	Feces	Cage Rinse
GS-704277 (M5)	11.67-12.00[a]	GS-704277	X	X		X
M18	12.83-13.00[a]	GS-441524-glucuronide	X	X		X
M14	25.50-25.67	Desamino-hydroxy-GS-441524		X	X	X
GS-441524 (M15)	29.00-29.17	GS-441524	X	X	X	X
GS-5734	58.33	GS-5734	X	X		X

Notes: Retention time ranges are from profiling analyses of all matrices.
Metabolite is found in matrix designated with "X".
a Due to peak splitting, plasma retention times not included in the overall retention time for this metabolite.

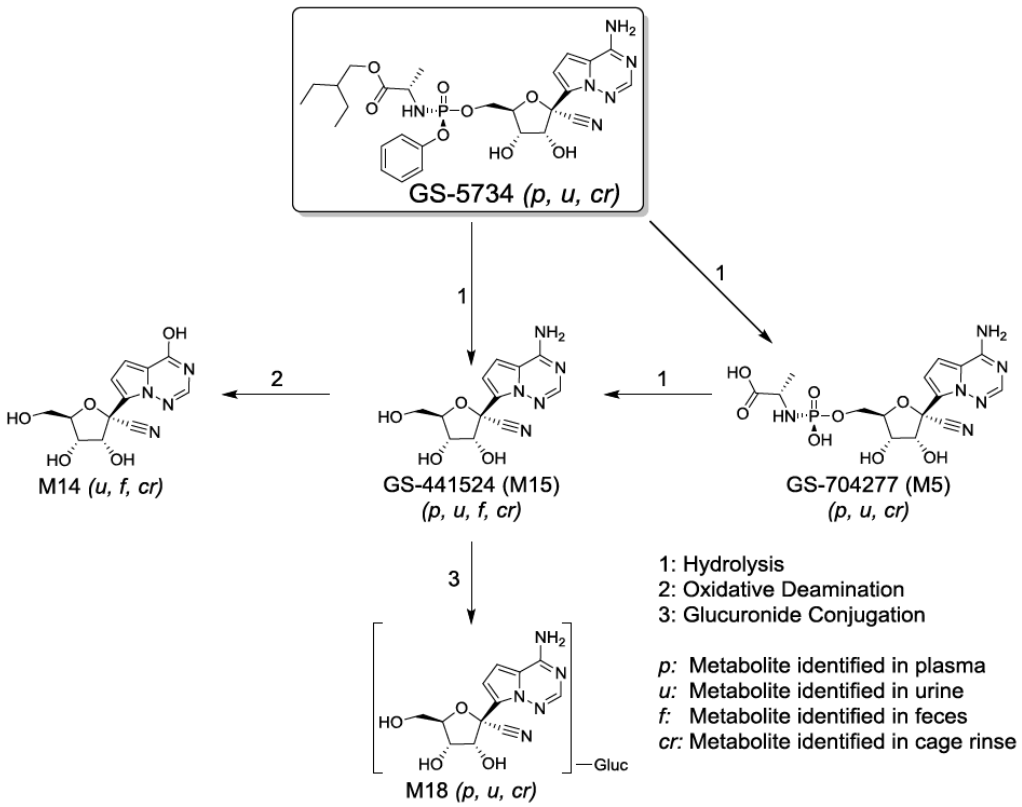

Figure 2: Proposed Biotransformation Pathways in Monkeys

Plasma, urine, feces, and cage rinse samples were profiled for parent and metabolites of ^{14}C-GS-5734 from female rabbits after a single intravenous dose of 10 mg/kg (AD-399-2026). ^{14}C-GS-5734 underwent rapid biotransformation in rabbits after IV dosing. The profile of an AUC-pooled plasma sample showed unchanged GS-5734 contributed <1% through 96 hours. GS-441524 (M15) was the major circulating metabolite (45%) and Desamino-hydroxy-GS-441524 (M14) contributed approximately 25% of the exposure through 96 hours. Other components identified in plasma, oxy-GS-441524 (M24) and GS-441524-sulfate (M25), contributed approximately 8 and 11% of the total exposure, respectively. Renal elimination was the major route of excretion, with 64% of the dose recovered in urine through 72 hours postdose. Hepato-biliary excretion was a less significant route of elimination (10%), and no unchanged parent was detected in feces. ^{14}C-GS-5734 was rapidly metabolized in rabbits after intravenous administration via hydrolysis, oxidation, deamination, and sulfation. Hydrolysis was the major pathway. Four metabolites were identified, in addition to previously identified metabolites GS-441524 and GS-704277.

Table 14: Metabolites Identified in Plasma, Urine, Feces, and Cage Rinse from Female Rabbits Dosed 10 mg/kg GS-5734

Metabolite Designation	Retention Time (Minutes)	Proposed Identification	Matrix			
			Plasma	Urine	Feces	Cage Rinse
M4	9.00	GS-441524-phosphate	X			
GS-704277 (M5)	11.67-12.50	GS-704277	X	X		X
M24	15.83-16.33	Oxy-GS-441524	X			
M25	16.00-16.83	GS-441524-sulfate	X	X	X	X
M14	25.67-26.17	Desamino-hydroxy-GS-441524	X	X	X	X
GS-441524 (M15)	29.00-29.33	GS-441524	X	X	X	X
GS-5734	58.33-58.50	GS-5734	X	X		

Notes: Retention time ranges are from profiling analyses of all matrices.
Metabolite is found in matrix designated with "X".

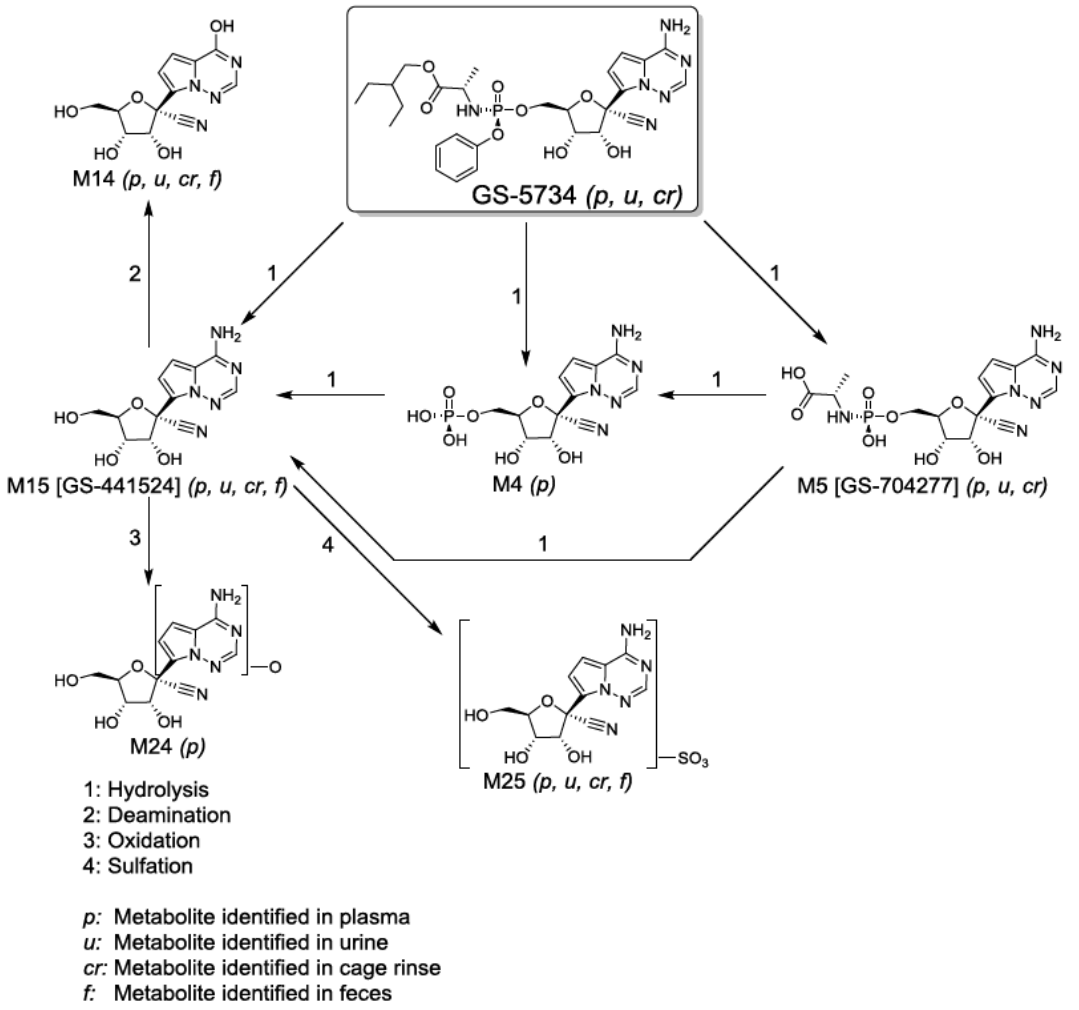

Figure 3: Proposed Biotransformation Pathways in Rabbits

In order to assess any differences in metabolism between monkey and human, the metabolism of GS-5734 was compared in human and rhesus PBMC and monocytes (AD-399-2015). Intracellular triphosphate, GS-443902, concentrations were higher in human PBMC (3.64x) and monocytes (4.24x).

The kinetics of GS-5734 loading in human macrophages (differentiated from monocytes *in vitro*) and subsequent metabolism were studied following a 2hr pulse incubation with 1 μM GS-466547, the mixture containing GS-5734 (AD-399-2004). The pulse incubation was designed to mimic the transient exposure to prodrug following IV administration. The active analog, GS-443902, was efficiently formed in human macrophage cells (accounting for 70% of intracellular metabolites) and persisted with a $t_{1/2}$ of 11 hr.

The proposed intracellular metabolic pathway is presented in **Figure 4**. GS-5734 is activated to the active nucleoside analog triphosphate, GS-443902, by a sequential metabolic activation

pathway: (i) hydrolase activity removes the ester releasing 2-ethyl-butanol; (ii) a spontaneous chemical step releases phenol and forms GS-704277; (iii) phosphoramidase activity cleaves the phosphoramidate bond, liberating the nucleoside analog monophosphate and alanine; and (iv) nucleotide kinases catalyze the conversion to the active triphosphate, GS-443902. Dephosphorylation of the nucleoside analog monophosphate results in the formation of the nucleoside analog, GS-441524 (PC-399-2007 and PC-399-2008, studies reviewed by Virology).

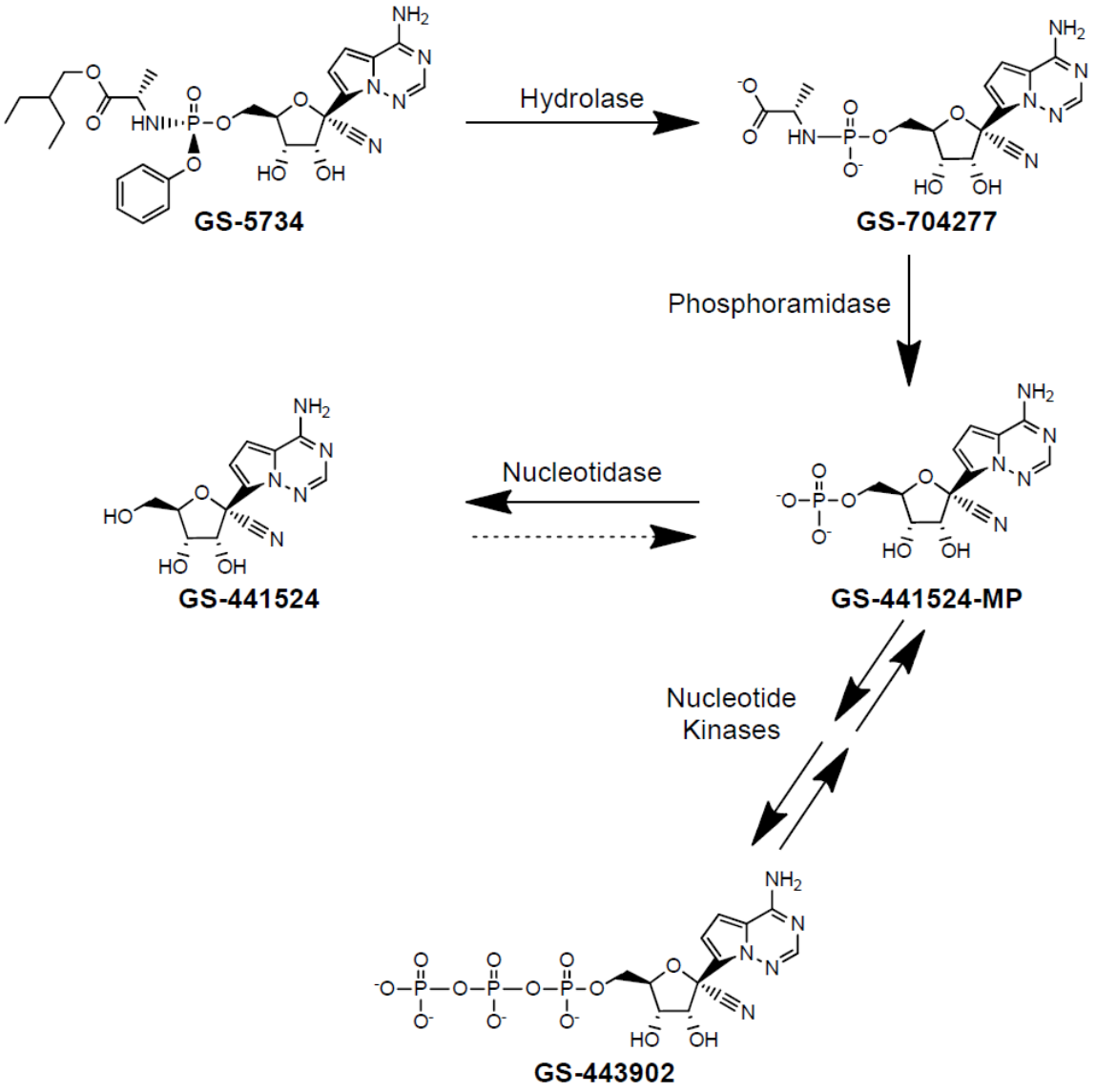

Figure 4: Proposed Biotransformation Pathways in Rabbits

Excretion:

The routes and extent of excretion were determined after 10 mg/kg IV ^{14}CGS-5734 to rats (AD-399-2017). 63.0% and 27.8% of the administered dose were excreted in urine and feces,

respectively, by 168 hours postdose. After IV dosing in bile duct cannulated rats, 63.4%, 22.7%, and 3.26% of the administered dose were excreted in urine, bile, and feces, respectively, by 168 hours postdose, indicating renal and biliary excretion as the major routs of elimination. Overall, recovery was 95.1 and 95.3%, respectively.

The routes and extent of excretion were determined after 10 mg/kg IV ^{14}CGS-5734 to rabbits (AD-399-2025). 67.0 and 11.9% of the administered radioactivity were excreted in urine and feces, respectively, by 168 hours postdose. Overall mean recovery of radioactivity after IV dosing to rabbits was 91.7%.

The routes and extent of excretion were determined after 10 mg/kg IV ^{14}CGS-5734 to monkeys (AD-399-2019). By 168 hours postdose, 33.6% and 25.6% of the administered dose were recovered in urine and feces, respectively, indicating that renal and biliary excretion were the major routes of elimination. Overall mean recovery in monkeys was 78.8% (16.9% was found in cage rinse).

Toxicology

Overview of Safety Margins Based on Exposure

Table 15: Safety Margin/Toxicokinetic Data

Study Title (Study No.)	Major Findings
General Toxicology Studies	
2-Week Intravenous Toxicity Study in Rats (Study #TX-399-2003) Sample collection times: 0.25, 0.5, 1, 3, 12, and 24 (Days 1 & 15) NOAEL =Not identified in males; 5 mg/kg/day (AUC_{0-24} = 1420 ng*h/mL at Day 15, females) **<u>Exposure multiple = 0.64</u>** Based on mean steady-state exposures in healthy human volunteers (**Study GS-US-399-5505**) receiving 200 mg Day 1 and 100 mg Days 2-9 (AUC_{tau} =2229 ng*h/mL)	GS-5734

Interval	Dose Group	Dose Level (mg/kg/day)	Sex	C_{max} (ng/mL)	T_{max} (h)	AUC_{0-t} (ng·h/mL)	AUC_{0-24} (ng·h/mL)	C_{last} (ng/mL)	T_{last} (h)
Day 1	3	5	M	315	0.500	1190	1190	2.71	24.0
			F	294	0.250	942	942	2.68	24.0
			MF	295	0.500	1080	1080	2.70	24.0
	4	20	M	1240	0.500	4560	4560	15.1	24.0
			F	879	0.250	3350	3350	14.9	24.0
			MF	1040	0.500	3950	3950	15.0	24.0
	5	50	M	2750	0.500	12900	12900	103	24.0
			F	2170	1.00	14300	14300	133	24.0
			MF	2360	0.500	13500	13500	118	24.0
Day 15	3	5	M	371	0.500	1880	1880	8.29	24.0
			F	324	0.500	1420	1420	4.53	24.0
			MF	347	0.500	1650	1650	6.41	24.0
	4	20	M	1760	0.500	10000	10000	51.7	24.0
			F	1690	3.00	12700	12700	54.4	24.0
			MF	1520	0.500	11400	11400	53.1	24.0
	5	50	M	3560	0.500	21700	21700	131	24.0
			F	2900	0.500	19000	19000	72.6	24.0
			MF	3230	0.500	20300	20300	102	24.0

Note: MF data are based on the analysis of the combined concentration data for both males and females.

Study Title (Study No.)

Major Findings

4-Week Intravenous Toxicity Study in Rats (Study #TX-399-2016)

Sample collection times: 0.25, 0.5, 1, 3, 12, and 24 (Days 1 & 28)

NOAEL =3 mg/kg/day (AUC_{0-24} = 748 ng*h/mL at Day 28, gender-averaged)

Exposure multiple = 0.34

Based on mean steady-state exposures in healthy human volunteers (**Study GS-US-399-5505**) receiving 200 mg Day 1 and 100 mg Days 2-9 (AUC_{tau} =2229 ng*h/mL)

Interval	Dose Group	GS-5734 Dose Level (mg/kg/day)	Sex	C_{max} (ng/mL)	T_{max} (h)	AUC_{0-t} (ng·h/mL)	AUC_{0-24} (ng·h/mL)	C_{last} (ng/mL)	T_{last} (h)
Day 1	2	1	M	44.1	0.250	85.8	151	14.5	3.00
			F	36.0	0.500	72.0	121	10.9	3.00
			MF	40.0	0.500	78.9	136	12.7	3.00
	3	3	M	124	0.250	444	473	4.72	12.0
			F	115	0.500	390	419	4.84	12.0
			MF	119	0.250	417	446	4.78	12.0
	4	10	M	448	0.250	1880	1880	5.96	24.0
			F	397	0.250	1700	1700	4.79	24.0
			MF	423	0.250	1790	1790	5.38	24.0
Day 28	2	1	M	50.1	0.500	188	209	3.46	12.0
			F	38.8	0.500	74.8	140	14.4	3.00
			MF	44.5	0.500	170	190	3.21	12.0
	3	3	M	208	1.00	1000	1000	3.84	24.0
			F	127	0.500	493	493	2.17	24.0
			MF	156	0.500	748	748	3.17	24.0
	4	10	M	754	1.00	4210	4210	14.5	24.0
			F	521	0.500	2940	2940	7.63	24.0
			MF	615	1.00	3570	3570	11.1	24.0

Note: MF data are based on the analysis of the combined concentration data for both males and females.

7-Day Intravenous Toxicity Study in Rhesus Monkeys (Study #TX-399-2021)

Sample collection times: 0.25, 0.5, 1, 3, 6, 12, and 24 (Days 0 & 6)

NOAEL =Not identified

LOAEL for RDV exacerbated kidney effects: 10 mg/kg (AUC_{0-24} = 6050 ng*h/mL at Day 6)

Exposure multiple = 2.7

Based on mean steady-state exposures in healthy human volunteers (**Study GS-US-399-5505**) receiving 200 mg Day 1 and 100 mg Days 2-9 (AUC_{tau} =2229 ng*h/mL)

GS-5734 (mg/kg/day)	Cmax (ng/mL)		AUC0-24 (ng·h/mL)	
	Day 0	Day 6	Day 0	Day 6
GS-5734				
5	389	537	361	653
10	755	1380	815	2120
20	2210	3960	2700	5600
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

Study Title (Study No.)	Major Findings

2-Week Intravenous Toxicity Study in Monkeys (Study #TX-399-2004)

Sample collection times: 0.25, 0.5, 1, 3, 12, and 24 (Days 1 & 15)

NOAEL =10 mg/kg/day (AUC_{0-24} = 2390 ng*h/mL at Day 15, gender-averaged)

Exposure multiple = 1.1

Based on mean steady-state exposures in healthy human volunteers (**Study GS-US-399-5505**) receiving 200 mg Day 1 and 100 mg Days 2-9 (AUC_{tau} =2229 ng*h/mL)

Dose Group	GS-5734 Dose Level (mg/kg/day)	Sex		C_{max} (ng/mL)	T_{max} (h)	AUC_{0-t} (ng·h/mL)	AUC_{0-24} (ng·h/mL)	C_{last} (ng/mL)	T_{last} (h)	M:P
3	1	M	Mean	26.5	0.625	192	192	4.50	24.0	2.41
			SD	5.32	0.250	16.5	16.5	0.550	0	0.739
			N	4	4	4	4	4	4	4
		F	Mean	27.5	0.750	214	214	4.26	24.0	2.17
			SD	2.14	0.289	23.8	23.8	0.533	0	0.971
			N	4	4	4	4	4	4	4
		MF	Mean	27.0	0.688	203	203	4.38	24.0	2.29
			SD	3.80	0.259	22.3	22.3	0.517	0	0.809
			N	8	8	8	8	8	8	8
4	3	M	Mean	100	0.750	804	804	15.4	24.0	2.48
			SD	4.63	0.289	88.1	88.1	2.66	0	1.05
			N	4	4	4	4	4	4	4
		F	Mean	81.6	0.563	577	577	11.4	24.0	1.70
			SD	17.6	0.315	127	127	4.27	0	0.0761
			N	4	4	4	4	4	4	4
		MF	Mean	90.8	0.656	690	690	13.4	24.0	2.09
			SD	15.5	0.297	158	158	3.92	0	0.806
			N	8	8	8	8	8	8	8
5	10	M	Mean	363	0.667	2450	2450	40.3	24.0	1.43
			SD	68.9	0.258	679	679	8.70	0	0.329
			N	6	6	6	6	6	6	6
		F	Mean	335	0.750	2330	2330	33.5	24.0	1.34
			SD	57.9	0.274	555	555	9.83	0	0.302
			N	6	6	6	6	6	6	6
		MF	Mean	349	0.708	2390	2390	36.9	24.0	1.38
			SD	62.4	0.257	594	594	9.55	0	0.304
			N	12	12	12	12	12	12	12

4-Week Intravenous Toxicity Study in Monkeys (Study #TX-399-2017)

Sample collection times: 0.25, 0.5, 1, 3, 12, and 24 (Days 1 & 28)

NOAEL =10 mg/kg/day (AUC_{0-24} = 2070 ng*h/mL at Day 28, gender-averaged)

Exposure multiple = 0.92

Based on mean steady-state exposures in healthy human volunteers (**Study GS-US-399-5505**) receiving 200 mg Day 1 and 100 mg Days 2-9 (AUC_{tau} =2229 ng*h/mL)

Interval	Dose Group	GS-5734 Dose Level (mg/kg/day)	Sex	C_{max} (ng/mL)	AUC_{0-t} (ng·h/mL)	AUC_{0-24} (ng·h/mL)	M:P C_{max}	M:P AUC_{0-24}
Day 1	2	1	M	24.4	135	145	0.297	1.89
			F	30.5	175	189	0.427	2.92
			MF	27.4	155	167	0.362	2.41
	3	3	M	63.0	398	398	0.292	1.97
			F	68.3	450	450	0.297	2.27
			MF	65.6	424	424	0.295	2.12
	4	10	M	268	1660	1660	0.260	1.62
			F	316	1950	1950	0.343	2.16
			MF	292	1810	1810	0.301	1.89
Day 28	2	1	M	26.7	163	167	0.297	1.74
			F	31.1	213	213	0.306	2.10
			MF	28.9	188	190	0.302	1.92
	3	3	M	60.5	404	404	0.261	1.57
			F	73.3	482	482	0.316	1.89
			MF	66.9	443	443	0.289	1.73
	4	10	M	274	1890	1890	0.255	1.56
			F	301	2240	2240	0.251	1.58
			MF	288	2070	2070	0.253	1.57

7-Day Intramuscular Toxicity Study in Monkeys (Study #TX-399-2001)

Sample collection times: 0.25, 1, 3, 12, and 24 (Days 1 & 7)

NOAEL = 7.5 mg/kg/day (AUC_{0-24} = 3370 ng*h/mL at Day 7, male)

Exposure multiple = 1.5

Based on mean steady-state exposures in healthy human volunteers (**Study GS-US-399-5505**) receiving 200 mg Day 1 and 100 mg Days 2-9 (AUC_{tau} =2229 ng*h/mL)

Dose Level (mg/kg/day)	Day	C_{max} (ng/mL) Male	AUC_{0-24} (ng·h/mL) Male
2.5	1	49.5 ± 29.1	437 ± 143
	7	58.7 ± 18.3	771 ± 162
7.5	1	83.0 ± 32.3	1350 ± 330
	7	239 ± 105	3370 ± 1180
15	1	105 ± 26	1930 ± 370
	7	923 ± 257	18,200 ± 4000

Study Title (Study No.)	Major Findings
Reproductive and Developmental Toxicity Studies	
Embryofetal Development Study in Rats (Study #TX-399-2013) Sample collection times: 0.25, 0.5, 1, 3, 12, and 24 (GD 6 & 17) NOAEL =20 mg/kg/day (AUC_{0-24} = 8740 ng*h/mL at GD 17) **Exposure multiple = 3.9** Based on mean steady-state exposures in healthy human volunteers (**Study GS-US-399-5505**) receiving 200 mg Day 1 and 100 mg Days 2-9 (AUC_{tau} =2229 ng*h/mL)	

Interval	Dose Group	GS-5734 Dose Level (mg/kg/day)	C_{max} (ng/mL)	T_{max} (h)	AUC_{0-t} (ng·h/mL)	AUC_{0-24} (ng·h/mL)	C_{last} (ng/mL)	T_{last} (h)
GD 6	2	2.5	126	0.250	432	453	3.55	12.0
	3	5	249	0.250	961	961	2.70	24.0
	4	10	462	0.500	1800	1800	4.15	24.0
	5	20	799	0.250	3510	3510	13.6	24.0
GD 17	2	2.5	137	0.500	642	642	3.36	24.0
	3	5	272	0.250	1210	1210	4.73	24.0
	4	10	676	0.500	3680	3680	29.9	24.0
	5	20	1580	1.00	8740	8740	41.3	24.0

Embryofetal Development Study in Rabbits (Study #TX-399-2018) Sample collection times: 0.25, 0.5, 1, 3, 12, and 24 (GD 7 & 20) NOAEL = 10 mg/kg/day (AUC_{0-24} = 1790 ng*h/mL at GD 20) **Exposure multiple = 0.80** Based on mean steady-state exposures in healthy human volunteers (**Study GS-US-399-5505**) receiving 200 mg Day 1 and 100 mg Days 2-9 (AUC_{tau} =2229 ng*h/mL)	

Dose Group	GS-5734 Dose Level (mg/kg/day)	C_{max} (ng/mL)	T_{max} (h)	AUC_{0-t} (ng·h/mL)	AUC_{0-24} (ng·h/mL)	C_{last} (ng/mL)	T_{last} (h)	M:P C_{max}	M:P AUC_{0-24}
GD 7									
2	2.5	150	0.438	462	462	3.30	24.0	8.76	26.4
3	5	308	0.500	1030	1030	6.23	24.0	4.36	16.4
4	10	526	0.438	1710	1710	11.2	24.0	2.97	10.7
5	20	1330	0.500	4960	4960	32.2	24.0	2.25	8.70
GD 20									
2	2.5	119	0.500	429	429	4.91	24.0	0.814	2.86
3	5	247	0.438	1090	1090	11.4	24.0	0.441	1.73
4	10	380	0.750	1790	1790	16.9	24.0	0.323	1.34
5	20	1680	0.875	8930	8930	53.1	24.0	0.583	3.29

Pre- and Postnatal Development Study in Rats (Study #TX-399-2014)

Sample collection times: 0.25, 0.5, 1, 3, 12, and 24 (GD 6 & LD 10)

NOAEL =10 mg/kg/day (AUC_{0-24} = 2310 ng*h/mL at Day 28, gender-averaged)

Exposure multiple = 1.0

Maternal/Pup C_{max} Ratio = 114

Based on mean steady-state exposures in healthy human volunteers (**Study GS-US-399-5505**) receiving 200 mg Day 1 and 100 mg Days 2-9 (AUC_{tau} =2229 ng*h/mL)

Interval	Dose Group	GS-5734 Dose Level (mg/kg/day)	C_{max} (ng/mL)	T_{max} (h)	AUC_{0-t} (ng·h/mL)	AUC_{0-24} (ng·h/mL)	C_{last} (ng/mL)	T_{last} (h)
GD 6	2	1	49.5	0.250	117	137	6.47	6.00
	3	3	142	0.250	390	415	4.21	12.0
	4	10	477	0.250	1610	1610	6.34	24.0
LD 10	2	1	60.3	0.250	335	349	2.26	12.0
	3	3	149	0.500	499	538	6.52	12.0
	4	10	572	0.500	2310	2310	9.60	24.0

F_1 generation Exposure

Dose Group	GS-5734 Dose Level (mg/kg/day)	Sex	C_{max} (ng/mL)	T_{max} (h)	AUC_{0-t} (ng·h/mL)	AUC_{0-24} (ng·h/mL)	C_{last} (ng/mL)	T_{last} (h)	Mat:Pup C_{max}
3	3	F	3.50	3.00	NR	NR	NR	NR	42.6
4	10	M	3.99	3.00	NR	NR	3.92	6.00	143
		F	5.03	3.00	NR	NR	3.74	6.00	114
		MF	4.51	3.00	NR	NR	3.83	6.00	127

NR Not reported
Note: MF data are based on the analysis of the combined concentration data for both males and females.

General Toxicology

2-Week Intravenous Toxicity Study in Rats (Study #TX-399-2003):

Key Study Findings

- NOAEL = Unidentified in male/ 5 mg/kg/day in females
- AUC_{tau} =1420 ng.h/mL; C_{max} =324 ng/mL
- GS-5734 resulted in adverse body weight gain and decreased food consumption.
- Clinical pathology and microscopic findings were indicative of kidney injury and /or dysfunction in males at ≥ 5mg/kg/day and in females at ≥ 20 mg/kg/day.

Conducting laboratory: (b) (4)

GLP compliance: Yes

Table 16: 2-Week Rat Intravenous Toxicity Study Design

Methods	Details
Dose and frequency of dosing:	0 (saline), 0 (vehicle), 5, 20, 50 mg/kg/day
Route of administration:	Intravenous; Slow bolus with 0.3 mL flush
Formulation/vehicle:	12% (w/v) sulfobutylether-b-cyclodextrin ((b) (4) SBE-b-CD]) in Sterile water for Injection, USP (SWFI), pH (b) (4)
Species/strain:	Wistar Han Rats
Number/sex/group:	10 5 for 4 week Recovery (Vehicle and High Dose)
Age:	6/7 weeks
Satellite groups/unique design:	TK; n=9 Bone Marrow Micronucleus Assay
Deviations affecting interpretation:	None

Table 17: 2-Week Rat Intravenous Toxicity Study Findings

Parameters	Major findings
Mortality	All toxicity animals survived to their scheduled sacrifice. Among toxicokinetic animals, one female administered 5 mg/kg/day died and one female administered 20 mg/kg/day was sacrificed in moribund condition. These deaths were attributed to the blood collection procedure and were considered accidental.
Clinical signs	Examined at least once daily. GS-5734-related clinical observations were limited to red discoloration of tail skin in males ≥20 mg/kg/day and females ≥5 mg/kg/day. These observations were not present in recovery groups and considered nonadverse.

Parameters	Major findings
Body weights	Measured predose and Days 1, 4, 7, 11, and 15. Dose-related decreases in mean body weight were observed in all groups between days 1 and 4. Between days 4 and 15, all dose groups gained body weight. Over the entire dosing period adverse and statistically significant decreases in mean body weight gain versus vehicle controls were noted in males at ≥5 mg/kg/day (approximately 18 to 66%) and in females at ≥20 mg/kg/day (approximately 71%). This effect completely reversed following 2 weeks of recovery.
Food consumption	Recorded on days 1 to 4, Days 4 to 7, Days 7 to 11, and Days 11 to 15. Animals administered ≥20 mg/kg/day had decreased food consumption that reversed during the recovery phase.
Ophthalmoscopy	Evaluated pretreatment and during week 2 of dosing. No drug-related findings.
Hematology	Blood samples were collected on day of scheduled sacrifice. GS-5734-related effects included • lower absolute reticulocyte count for males at all dose levels (-20 to -34%) and females administered ≥20 mg/kg/day (-16 to -40%), • lower red blood cell count for females (-6%), hemoglobin concentration for males and females (-3 and -7%, respectively), hematocrit for males and females (-4 and -6%, respectively; not reversible during recovery), and mean corpuscular volume for males (-3%) at 50 mg/kg/day, and • mildly shorter activated partial thromboplastin time for males administered 50 mg/kg/day (-19%) and females administered ≥20 mg/kg/day (-20 to -25%). All effects were considered secondary to SBECD-related kidney effects but exhibited reversibility.

Parameters	Major findings
Clinical chemistry	Blood samples were collected on day of scheduled sacrifice. The most relevant clinical chemistry effects were those indicating GS-5734/SBECD-related effects on kidney function.

	Males					Females				
	Saline Vehicle					Saline Vehicle				
GS-5734 dose (mg/kg/day)	0	0	5	20	50	0	0	5	20	50
Urea nitrogen concentration (mg/dL)	11	11	14*	13*	12	17	14	15	16	15
% difference from control	0	—	+27	+18	+9	+21	—	+7	+14	+7
Creatinine concentration (mg/dL)	0.5	0.5	0.6*	0.7*	0.7*	0.6	0.6	0.6	0.8*	0.7*
% difference from control	0	—	+20	+40	+40	0	—	0	+33	+17
Inorganic phosphorus concentration (mg/dL)	9.5	9.1	9.2	9.3	8.6	8.3	7.6†	7.7	8.2*	8.6*
% difference from control	+4	—	+1	+2	-5	+9	—	+1	+8	+13
Chloride concentration (mmol/L)	101	101	100	101	103*	102	102	100	103	105*
% difference from control	0	—	-1	0	+2	0	—	-2	+1	+3

+/- = 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.

These additional effects were likely secondary to alter kidney function

- higher total protein concentration (+5 to +7%) and higher albumin concentration (+7 to +14%) for males at all dose levels,
- lower globulin concentration for animals administered 50 mg/kg/day (-17%),
- higher albumin:globulin ratio for males administered ≥20 mg/kg/day (+21 to +33%) and females administered 50 mg/kg/day (+23%),
- lower cholesterol concentration for animals administered 50 mg/kg/day (-25 to -30%),
- lower triglyceride concentration for males administered ≥20 mg/kg/day (-36 to -41%), and
- higher alkaline phosphatase activity for animals administered 50 mg/kg/day (+27 to +35%).

The only GS-5734-related clinical chemistry effect at 50 mg/kg/day that did not exhibit reversibility was minimally lower cholesterol concentration (-31%) for females.

Urinalysis

Urine samples were collected on Days 4 and 7 and day of sacrifice. GS-5734-related effects on these parameters indicated kidney injury and/or dysfunction. Transient effects included

- higher urine volume on Day 4 of the dosing phase for males administered 50 mg/kg/day (+92%) and females administered ≥20 mg/kg/day (+63 to +93%),
- lower urine pH on Day 4 of the dosing phase for males administered 50 mg/kg/day (-13%), and
- increased incidence of white blood cells and granular casts in urine sediment on Days 4 and/or 7 of the dosing phase for animals administered ≥20 mg/kg/day.

Effects on Days 4, 7, and 16 of the dosing phase included

- increased incidence of positive urine protein for males at all dose levels and females administered 50 mg/kg/day,
- increased incidence of positive urine blood for animals administered 50 mg/kg/day, and
- increased incidence of positive urine glucose for males administered 50 mg/kg/day.

Increased incidence of positive urine ketones on Days 4, 7, and 16 of the dosing phase for males administered ≥20 mg/kg/day was likely secondary to reduced food consumption and utilization of fat for energy. The only GS-5734-related urinalysis effect at 50 mg/kg/day that did not exhibit reversibility was increased incidence of positive urine protein (4 out of 5 at 50 mg/kg/day) for males.

Urine chemistry findings are presented below, and the effects that did not exhibit reversibility was minimally higher urine chloride excretion (+42%) for females and higher (+188%) urine total protein:urine creatinine ratio for males administered 50 mg/kg/day.

	Males					Females				
	Saline	Vehicle				Saline	Vehicle			
GS-5734 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
Sodium excretion (mmol)	0.66	0.56	0.64	0.93*	0.81*	0.39	0.39	0.42	0.82*	0.63*
% difference from control	+18	—	+14	+66	+45	0	—	+8	+110	+62
Potassium excretion (mmol)	0.76	0.66	0.61	0.87*	1.07*	0.50	0.43	0.46	0.64*	0.78*
% difference from control	+15	—	-8	+32	+62	+16	—	+7	+49	+81
Chloride excretion (mmol)	0.52	0.38†	0.36	0.38	0.44	0.29	0.23	0.25	0.38*	0.33*
% difference from control	+37	—	-5	0	+16	+26	—	+9	+65	+43
Day 7										
Total protein:creatinine ratio	0.47	0.59	1.39*	2.39*	3.17*	0.36	0.39	0.42	0.56*	2.04*
% difference from control	-20	—	+136	+305	+437	-8	—	+8	+44	+423
NAG:creatinine ratio	0.5	0.4†	0.5*	1.0*	1.5*	0.4	0.4	0.4	0.6*	1.0*
% difference from control	+25	—	+25	+150	+275	0	—	0	+50	+150
Sodium excretion (mmol)	0.58	0.51	0.50	0.44	0.73*	0.31	0.36	0.39	0.34	0.36
% difference from control	+14	—	-2	-14	+43	-14	—	+8	-6	0
Potassium excretion (mmol)	0.80	0.64†	0.59	0.61	0.75	0.42	0.45	0.46	0.46	0.46
% difference from control	+25	—	-8	-5	+17	-7	—	+2	+2	+2
Chloride excretion (mmol)	0.44	0.31†	0.28	0.27	0.43*	0.26	0.23	0.23	0.16	0.19
% difference from control	+42	—	-10	-13	+39	+13	—	0	-30	-17
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
Sodium excretion (mmol)	0.62	0.55	0.61	0.70	1.12*	0.41	0.42	0.38	0.50	0.54*
% difference from control	+13	—	+11	+27	+104	-2	—	-10	+19	+29
Potassium excretion (mmol)	0.89	0.69	0.78	0.70	0.82	0.55	0.49	0.45	0.54	0.53
% difference from control	+29	—	+13	+1	+19	+12	—	-8	+10	+8
Chloride excretion (mmol)	0.46	0.38	0.33	0.43	0.59*	0.33	0.27	0.23	0.28	0.41*
% difference from control	+21	—	-13	+13	+55	+22	—	-15	+4	+52

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

	Males					Females				
	Saline	Vehicle				Saline	Vehicle			
GS-5734 dose (mg/kg/day)	0	0	5	20	50	0	0	5	20	50
Recovery Day 29										
Total protein:creatinine ratio	—	0.67	—	—	1.93	—	0.27	—	—	0.32
% difference from control	—	—	—	—	+188	—	—	—	—	+19
NAG:creatinine ratio	—	0.3	—	—	0.3	—	0.3	—	—	0.3
% difference from control	—	—	—	—	0	—	—	—	—	0
Sodium excretion (mmol)	—	0.67	—	—	0.62	—	0.53	—	—	0.59
% difference from control	—	—	—	—	-7	—	—	—	—	+11
Potassium excretion (mmol)	—	0.79	—	—	0.68	—	0.51	—	—	0.70
% difference from control	—	—	—	—	-14	—	—	—	—	+37
Chloride excretion (mmol)	—	0.47	—	—	0.57	—	0.45	—	—	0.64
% difference from control	—	—	—	—	+21	—	—	—	—	+42

+/- = 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.

GS-5734 administration had reversible effects on all urine biomarkers, as well.

	Males					Females				
GS-5734 dose	Saline	Vehicle				Saline	Vehicle			
(mg/kg/day)	0	0	5	20	50	0	0	5	20	50
Day 4										
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	0.96x	—	2.2x	44.9x	302.5x	0.9x	—	1.7x	8.4x	134.0x
Beta-2-microglobulin: 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	0.66x	—	2.7x	16.2x	32.7x	1.2x	—	1.4x	14.4x	135.4x
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	1.1x	—	2.5x	20.3x	62.1x	1.2x	—	1.7x	19.8x	75.0x
Day 7										
Cystatin C:creatinine ratio	1.9	2.1	7.6*	41.7*	114.0*	0.9	0.9	1.6*	6.2*	47.6*
multiple of control mean	0.90x	—	3.6x	19.9x	54.3x	1.0x	—	1.8x	6.9x	52.9x
Beta-2-microglobulin: creatinine ratio	43.53	50.23	307.12*	932.48*	1128.36*	13.14	11.73	18.35*	131.63*	449.37*
multiple of control mean	0.87x	—	6.1x	18.6x	22.5x	1.1x	—	1.6x	11.2x	38.3x
KIM-1:creatinine ratio	0.010	0.008	0.017*	0.639*	0.758*	0.009	0.009	0.010	0.172*	0.753*
multiple of control mean	1.3x	—	2.1x	79.9x	94.8x	1.0x	—	1.1x	19.1x	83.7x
Day 16										
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	1.1x	—	13.3x	43.9x	164.7x	1.1x	—	3.3x	15.6x	40.1x
Beta-2-microglobulin: 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	0.86x	—	9.2x	15.6x	17.0x	1.1x	—	2.1x	30.7x	41.7x
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	1.4x	—	1.9x	9.0x	19.7x	1.1x	—	1.1x	4.3x	10.8x

— = 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 biomarker:urine creatinine.

Parameters	Major findings

	Males					Females				
GS-5734 dose	Saline	Vehicle				Saline	Vehicle			
(mg/kg/day)	0	0	5	20	50	0	0	5	20	50
Recovery Day 29										
Cystatin C: creatinine ratio	—	1.2	—	—	2.2	—	0.9	—	—	0.6
multiple of control mean	—	—	—	—	1.8x	—	—	—	—	0.67x
Beta-2-microglobulin: creatinine ratio	—	32.51	—	—	64.61	—	7.94	—	—	7.00
multiple of control mean	—	—	—	—	2.0x	—	—	—	—	0.88x
KIM-1:creatinine ratio	—	0.005	—	—	0.005	—	0.005	—	—	0.006
multiple of control mean	—	—	—	—	1.0x	—	—	—	—	1.2x

— = Not applicable.
Note: Results presented are group mean values. Ratios are expressed as urine biomarker:urine creatinine.

Gross pathology: Evaluated at necropsy (Day 16). No drug-related findings.

Organ weights: Evaluated at necropsy (Day 16). There was a GS-5734-related increase in absolute and relative kidney weights at 50 mg/kg/day. Females kidney weights remained 31% increased at recovery.

Histopathology
Adequate battery: Yes
Peer review: Yes

Evaluated at necropsy. GS-5734-related microscopic findings were limited to the kidney and included basophilic tubules and tubule cell vacuolation. Vehicle control SBECD-related microscopic findings in the kidney included tubule cell and focal or multifocal tubule cell hypertrophy.

Tissue/finding Sex	Males					Females				
	Saline	Vehicle				Saline	Vehicle			
GS-5734 Dose (mg/kg/day)	0	0	5	20	50	0	0	5	20	50
No. Examined	10	10	10	10	10	10	10	10	10	10
Kidney										
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

4-Week Intravenous Toxicity Study in Rats (Study #TX-399-2016):

Key Study Findings

- NOAEL =**3** mg/kg/day
- AUC_{tau} =747 ng.h/mL; C_{max} =168 ng/mL
- GS-5734 at 10 mg/kg/day resulted in decreased body weight gain and food consumption.
- 2 females had unscheduled deaths Day 14 (found dead) and Day 19 (moribund).
    - Clinical observation prior to death included pale body and/or ears and eyes, labored respiration, piloerection, ataxia, tremors or convulsions.
    - Microscopic findings were noted in kidney; Cause of death unclear.
- SBECD (vehicle) related microscopic findings were noted in the urinary tract, spleen, lymph nodes, adrenal cortex, liver, and stifle joint of animals.

Conducting laboratory: (b) (4)

GLP compliance: Yes

Table 18: 4-Week Rat Intravenous Toxicity Study Design

Methods	Details
Dose and frequency of dosing:	0, 1, 3, 10 mg/kg/day
Route of administration:	Intravenous; Slow bolus with 0.4 mL flush
Formulation/vehicle:	12% (w/v) sulfobutylether-b-cyclodextrin ((b) (4) SBE-b-CD]) in Sterile water for Injection, USP (SWFI), pH (b) (4)
Species/strain:	Wistar Han Rats
Number/sex/group:	10
Age:	6/7 weeks
Satellite groups/unique design:	TK; n=6
Deviations affecting interpretation:	None

Table 19: 4-Week Rat Intravenous Toxicity Study Findings

Parameters	Major findings
Mortality	2 females administered 10 mg/kg/day; 1 found dead on D14, and 1 euthanized moribund on Day 19. No cause of death could be identified, but animals were noted with pale body and eyes, labored respiration, piloerection, ataxia, and convulsions. No macroscopic findings were noted and SBECD related kidney injury was present. Finding is unlikely due to GS-5734-related toxicity.
Clinical signs	Examined at least once daily. GS-5734-related clinical observations were limited to the unscheduled death animals.
Body weights	Measured predose and Days 1, 4, 7, 11, and 15. Dose-related decreases in mean body weight and body weight gain were observed in the 10 mg/kg/day animals. Body weights were decreased on Day 29 (-12 to -9%). Body weight change was also decreased from Days 1 through 29 (-34%). These effects were considered adverse due to their magnitude.
Food consumption	Recorded on days 1 to 4, Days 4 to 7, Days 7 to 11, and Days 11 to 15. Animals administered 10 mg/kg/day had decreased food consumption.
Ophthalmoscopy	Evaluated pretreatment and during week 2 of dosing. No drug-related findings.
Hematology	Blood samples were collected on day of scheduled sacrifice. GS-5734-related hematology effects were limited to lower hemoglobin concentration (-4%), hematocrit (-3%), and corpuscular volume (-2%) and lower absolute reticulocyte count (-30%) for males administered 10 mg/kg/day. This effect was considered secondary to the SBECD-related kidney effects.

Parameters	Major findings
Clinical chemistry	Blood samples were collected on day of scheduled sacrifice. There was a GS-5734-related increase in creatinine concentration (+17%) on Day 30 for animals administered 10 mg/kg/day. This finding, along with lower globulin concentration (-11 to -16%), higher albumin:globulin ratio (+14 to +20%), and higher alkaline phosphatase activity (+27% in males), appear to be secondary to SBECD kidney injury and correlated with urinalysis, urine chemistry, and urine biomarker effects.
Urinalysis	Urine samples were collected on Days 4 and day of sacrifice. Males (10 mg/kg/day) observed an increase in protein and glucose in the urine and increased urine sodium and chloride excretion. Urine chemistry findings included

	Males				Females			
	Vehicle				Vehicle			
GS-5734 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.

Urine Biomarker findings included

	Males				Females			
GS-5734 dose	Vehicle				Vehicle			
(mg/kg/day)	0	1	3	10	0	1	3	10
Day 4								
Cystatin C:creatinine ratio	2.1	2.2	3.8*	8.2*	1.0	1.0	1.2	1.7*
multiple of control mean	-	1.0x	1.8x	3.9x	-	1.0x	1.2x	1.7x
Beta-2- microglobulin: creatinine ratio	30.01	20.84	56.20	168.22*	NC	NC	6.99	8.81
multiple of control mean	-	0.69x	1.9x	5.6x	-	NC	NC	NC
KIM-1:creatinine ratio	0.007	0.008	0.011*	0.087*	0.009	0.011	0.009	0.036*
multiple of control mean	-	1.1x	1.6x	12.4x	-	1.2x	1.0x	4.0x
Day 30								
Cystatin C:creatinine ratio	1.5	2.5	12.5*	42.9*	0.9	1.1	1.2	6.0*
multiple of control mean	-	1.7x	8.3x	28.6x	-	1.2x	1.3x	6.7x
Beta-2-microglobulin: creatinine ratio	25.51	47.32	299.35*	575.93*	6.48	14.75	6.49	130.19*
multiple of control mean	-	1.9x	11.7x	22.6x	-	2.3x	1.0x	20.1x
KIM-1:creatinine ratio	0.003	0.003	0.005*	0.012*	0.004	0.005	0.005	0.007*
multiple of control mean	-	1.0x	1.7x	4.0x	-	1.3x	1.3x	1.8x

- = Not applicable. NC = Not calculable.
* = Statistically significant at $p \leq 0.05$ versus control group.
Note: Results presented are group mean values. Ratios are expressed as urine biomarker:urine creatinine.

These effects indicated kidney injury and/or dysfunction.

Gross pathology	Evaluated at necropsy (Day 30). No drug-related findings.

Parameters	Major findings
Organ weights	Evaluated at necropsy (Day 30). There was a GS-5734-related increase in absolute and relative kidney weights >3 mg/kg/day in males.

Sex	Males				Females			
GS-5734 (mg/kg/day)	0	1	3	10	0	1	3	10
No. examined	10	10	10	10	10	10	10	8
Body weight mean (g)	261	255	265	227*	181	175	177	168
(% difference)	—	-2	+2	-13	—	-3	-2	-7
Brain weight mean (g)	1.9939	2.0130	2.0005	1.9958	1.9016	1.8993	1.8892	2.0263
(% difference)	—	+1	0	0	—	0	-1	+7
Kidney								
Absolute mean (g)	1.8040	1.8529	2.0088*	1.8860	1.4464	1.4116	1.3598	1.2498
(% difference)	—	+3	+11	+5	—	-2	-6	-14
Relative to body weight (%)	0.6934	0.7268	0.7603*	0.8335*	0.7990	0.8060	0.7681	0.7379
(% difference)	—	+5	+10	+20	—	+1	-4	-8
Relative to brain weight (%)	90.6404	91.8626	100.4357*	94.6153	76.1143	74.2282	71.9715	65.2225
(% difference)	—	+1	+11	+4	—	-2	-5	-14

+/ - = Increase/Decrease; — = Not applicable.
* = Based on statistical analysis of group means, values are significantly different from control at $P \leq 0.05$. Refer to data tables for actual significance levels and tests used.
(% difference) = percent difference between group means relative to vehicle-treated control group.

Histopathology
Adequate battery: Yes
Peer review: Yes

Evaluated at necropsy. GS-5734-related microscopic findings were limited to the kidney and included basophilic tubules and karyomegaly.

Tissue/finding Sex	Males				Females			
GS-5734 Dose (mg/kg/day)	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

Vehicle control SBECD-related microscopic findings in the kidney included tubule cell and transitional cell vacuolation and focal or multifocal tubule cell hypertrophy.

Tissue/finding Sex	Males				Females			
GS-5734 Dose (mg/kg/day)	0	1	3	10	0	1	3	10
Kidney								
No. examined:	10	10	10	10	10	10	10	8
Vacuolation, tubule cell								
Total number affected	10	10	10	10	10	10	10	8
Minimal	2	3	9	6	2	3	1	8
Slight	7	7	1	4	8	7	9	0
Moderate	1	0	0	0	0	0	0	0
Vacuolation, transitional cell								
Total number affected	10	10	10	10	8	10	9	6
Minimal	9	10	10	10	7	9	9	5
Slight	1	0	0	0	1	1	0	1
Hypertrophy, tubule cell								
Total number affected	5	3	2	1	4	3	2	3
Minimal	5	2	2	1	4	3	2	3
Slight	0	1	0	0	0	0	0	0
Urinary Bladder								
No. examined:	10	0	0	10	10	0	0	8
Vacuolation, transitional cell								
Total number affected	10	NE	NE	10	10	NE	NE	7
Minimal	5	NE	NE	4	6	NE	NE	3
Slight	5	NE	NE	6	4	NE	NE	4
Ureter								
No. examined:	10	0	0	10	10	0	0	8
Vacuolation, transitional cell								
Total number affected	10	NE	NE	10	9	NE	NE	6
Minimal	5	NE	NE	6	7	NE	NE	5
Slight	5	NE	NE	4	2	NE	NE	1
Urethra								
No. examined:	10	0	0	8	10	0	0	8
Vacuolation, transitional cell								
Total number affected	1	NE	NE	1	1	NE	NE	2
Minimal	1	NE	NE	1	1	NE	NE	2

Abbreviation: NE = Not examined.

Parameters	Major findings

Tissue/finding Sex	Males				Females			
GS-5734 Dose (mg/kg/day)	0	1	3	10	0	1	3	10
Spleen								
No. examined:	10	0	0	10	10	0	0	8
Infiltrate, macrophages, vacuolated								
Total number affected	10	NE	NE	10	8	NE	NE	8
Minimal	4	NE	NE	5	3	NE	NE	2
Slight	6	NE	NE	5	5	NE	NE	6
Lymph Node, Inguinal								
No. examined:	10	0	0	10	9	0	0	8
Infiltrate, macrophages, vacuolated								
Total number affected	10	NE	NE	10	9	NE	NE	8
Minimal	5	NE	NE	5	6	NE	NE	6
Slight	5	NE	NE	5	2	NE	NE	1
Moderate	0	NE	NE	0	1	NE	NE	1
Lymph Node, Mandibular								
No. examined:	10	0	0	10	10	0	0	8
Infiltrate, macrophages, vacuolated								
Total number affected	10	NE	NE	10	10	NE	NE	7
Minimal	7	NE	NE	6	9	NE	NE	6
Slight	3	NE	NE	4	1	NE	NE	1
Lymph Node, Mesenteric								
No. examined:	10	0	0	10	10	0	0	8
Infiltrate, macrophages, vacuolated								
Total number affected	10	NE	NE	10	6	NE	NE	5
Minimal	8	NE	NE	7	6	NE	NE	5
Slight	2	NE	NE	3	0	NE	NE	0
Lymph Node, Popliteal								
No. examined:	10	0	0	9	10	0	0	8
Infiltrate, macrophages, vacuolated								
Total number affected	10	NE	NE	9	10	NE	NE	8
Minimal	1	NE	NE	0	2	NE	NE	2
Slight	7	NE	NE	8	7	NE	NE	5
Moderate	2	NE	NE	1	1	NE	NE	1
Joint, Stifle								
No. examined:	10	0	0	10	10	0	0	8
Vacuolation, epithelial cell								
Total number affected	10	NE	NE	10	7	NE	NE	6
Minimal	6	NE	NE	6	7	NE	NE	6
Slight	4	NE	NE	4	0	NE	NE	0

Abbreviation: NE = Not examined.

Tissue/finding Sex	Males				Females			
GS-5734 Dose (mg/kg/day)	0	1	3	10	0	1	3	10
Adrenal, cortex								
No. examined:	10	0	0	10	10	0	0	8
Vacuolation, increased, zona fasciculata								
Total number affected	0	NE	NE	0	3	NE	NE	3
Minimal	0	NE	NE	0	3	NE	NE	3
Liver								
No. examined:	10	10	10	10	10	10	10	8
Vacuolation, Kupffer cell								
Total number affected	4	3	3	4	1	2	1	1
Minimal	4	3	3	4	1	2	1	1

Abbreviation: NE = Not examined.

In general, it appears that SBECD, the vehicle, is driving the renal injury. As this model over predicts renal injury, it is unclear how this finding translates to clinical use. RDV is expected to cause acute renal injury ≥ 3 mg/kg in rats which is equivalent to a human dose of 0.48 mg/kg.

7-Day Intravenous Toxicity Study in Rhesus Monkeys (Study #TX-399-2021):

Key Study Findings

- NOAEL = Not identified
- A single death at 20 mg/kg/day Day 6 due to kidney findings.
- GS-5734 administration resulted in adverse kidney injury with effects on body weight, hematology, coagulation, serum chemistry, and urinalysis.
- GS-5734-related microscopic findings of tubular atrophy and basophilic tubules with casts were observed in all dose groups.

Conducting laboratory: (b) (4)

GLP compliance: No

Table 20: 7-Day Monkey Intravenous Toxicity Study Design

Methods	Details
Dose and frequency of dosing:	0, 5, 10, 20 mg/kg/day
Route of administration:	Intravenous
Formulation/vehicle:	12% (w/v) sulfobutylether-b-cyclodextrin ((b) (4) SBE-b-CD]) in Sterile water for Injection, USP (SWFI), pH (b) (4)
Species/strain:	Rhesus Monkey
Number/sex/group:	3 Males only
Age:	3/4 years
Satellite groups/unique design:	3 Recovery animals in Vehicle and High Dose
Deviations affecting interpretation:	None

Table 21: 7-Day Monkey Intravenous Toxicity Study Findings

Parameters	Major findings
Mortality	A male in the 20 mg/kg/day was euthanized early due to moribundity attributed to test article-related kidney findings (tubular atrophy with slight tubular basophilia and casts in the kidney). Clinical chemistry, histopathology, and hematological changes were suggestive of altered kidney function. All other animals survived to their scheduled sacrifice.
Clinical signs	Examined at least once daily. No test article-related clinical observations were attributed to GS-5734.
Body weights	Recorded predose and Days 0, 3, 5, 6, 9, 12, and 16. No significant GS-5734-related effects were observed. Body weight losses and lower gains were observed Days 3 to 5 at ≥ 10 mg/kg.
Hematology	Blood samples were collected predose, on days 2, 4, and on day of scheduled sacrifice (Days 6 and 16). GS-5734 administration was associated with increased neutrophil and monocyte counts and decreased lymphocyte counts at 20 mg/kg/day, starting D4; partial recovery was observed. This effect was considered nonadverse due to recovery and additional signs of being stress induced (histopathology findings in lymph nodes, spleen, and thymus). Increases in PT and APTT and decreases in fibrinogen started as early as Day 2 in animals treated with 20 mg/kg/day. 10 mg/kg/day imitated similar findings starting day 4. No correlative parameters were affected. This effect was nonadverse as the magnitude of change was not considered meaningful.

Clinical chemistry

Blood samples were collected predose, on days 4, 7, and 13, and on day of scheduled sacrifice. GS-5734-related changes in serum chemistry consisted of increased mean urea nitrogen, creatinine, and chloride, and decreased mean albumin with consequently decreased mean total protein, decreased mean cholesterol, and phosphorus. These effects appear to be indicative of a GS-5734-related effect on kidney function. Effects were more pronounced in the 20 mg/kg/day group showing a clear dose-response relationship.

Sex	Males			
GS-5734 Dose (mg/kg/day)	0	5	10	20
No. examined	6	3	3	5
Creatinine (mg/dL)				
Day -18	0.92	1.09	0.95	0.98
(% difference [a])	-	+18.5	+3.3	+6.5
Day -11	0.85	1.05*	0.93	0.95
(% difference [a])	-	+23.5	+9.4	+11.8
Day 2	0.75	1.01*	0.94	1.11*
(% difference [a])	-	+34.7	+25.3	+48.0
Day 4	0.73	1.03	1.30	2.11*
(% difference [a])	-	+41.1	+78.1	+189.0
Day 6	0.73	1.18	1.84	3.89*
(% difference [a])	-	+61.6	+152.1	+432.9
Day 9	0.67	NA	NA	4.34
(% difference [a])	-	NA	NA	+547.8
Day 16	0.67	NA	NA	1.13
(% difference [a])	-	NA	NA	+68.7
Albumin (mg/dL)				
Day -18	5.0	5.3	5.0	5.0
(% difference [a])	-	+6.0	0.0	0.0
Day -11	4.8	4.9	4.9	4.9
(% difference [a])	-	+2.1	+2.1	+2.1
Day 2	4.6	4.4	4.7	4.8
(% difference [a])	-	-4.3	+2.2	+4.3
Day 4	4.7	4.4	4.7	4.6
(% difference [a])	-	-6.4	0.0	-2.1
Day 6	4.7	4.6	4.6	4.1*
(% difference [a])	-	-2.1	-2.1	-12.8
Day 9	4.7	NA	NA	4.0
(% difference [a])	-	NA	NA	-14.9
Day 16	4.7	NA	NA	4.2
(% difference [a])	-	NA	NA	-10.6

[a] (% difference) = percent difference between group means relative to vehicle-treated control group.
* Based on statistical analysis of group means, values are significantly different from vehicle control. Refer to data tables for actual significance levels and tests used.

Sex	Males			
GS-5734 Dose (mg/kg/day)	0	5	10	20
No. examined	6	3	3	5
Urea nitrogen (mg/dL)				
Day -18	17.7	18.6	18.9	19.1
(% difference [a])	-	+5.1	+6.8	+7.9
Day -11	18.3	19.5	19.9	21.5
(% difference [a])	-	+6.6	+8.7	+17.5
Day 2	17.0	21.4	16.8	21.5
(% difference [a])	-	+25.9	-1.2	+26.5
Day 4	16.3	20.4	18.7	29.3*
(% difference [a])	-	+25.2	+14.7	+79.8
Day 6	17.3	27.3	22.4	55.0*
(% difference [a])	-	+57.8	+29.5	+217.9
Day 9	16.9	NA	NA	63.0
(% difference [a])	-	NA	NA	+272.8
Day 16	17.3	NA	NA	30.7
(% difference [a])	-	NA	NA	+77.5

[a] (% difference) = percent difference between group means relative to vehicle-treated control group.
* Based on statistical analysis of group means, values are significantly different from vehicle control. Refer to data tables for actual significance levels and tests used.

Parameters	Major findings

Sex	Males			
GS-5734 Dose (mg/kg/day)	0	5	10	20
No. examined	6	3	3	5
Total Protein (g/dL)				
Day -18	8.3	9.1	8.5	8.2
(% difference [a])	-	+9.6	+2.4	-1.2
Day -11	8.0	8.3	8.1	7.9
(% difference [a])	-	+3.8	+1.3	-1.3
Day 2	7.6	7.6	7.7	7.6
(% difference [a])	-	0.0	+1.3	0.0
Day 4	7.7	7.5	7.8	7.6
(% difference [a])	-	-2.6	+1.3	-1.3
Day 6	7.8	8.0	7.8	6.9*
(% difference [a])	-	+2.6	0.0	-11.5
Day 9	7.8	NA	NA	7.0
(% difference [a])	-	NA	NA	-10.3
Day 16	7.9	NA	NA	7.6
(% difference [a])	-	NA	NA	-3.8
Cholesterol (mg/dL)				
Day -18	175	169	164	164
(% difference [a])	-	-3.4	-6.3	-6.3
Day -11	158	141	146	146
(% difference [a])	-	-10.8	-7.6	-7.6
Day 2	144	95	110	93
(% difference [a])	-	-34.0	-23.6	-35.4
Day 4	158	94*	106*	83*
(% difference [a])	-	-40.5	-32.9	-47.5
Day 6	151	97*	99*	70*
(% difference [a])	-	-35.8	-34.4	-53.6
Day 9	174	NA	NA	76
(% difference [a])	-	NA	NA	-56.3
Day 16	188	NA	NA	149
(% difference [a])	-	NA	NA	-20.7

[a] (% difference) = percent difference between group means relative to vehicle-treated control group.
* Based on statistical analysis of group means, values are significantly different from vehicle control. Refer to data tables for actual significance levels and tests used.

Sex	Males			
GS-5734 Dose (mg/kg/day)	0	5	10	20
No. examined	6	3	3	5
Chloride (mEq/L)				
Day -18	112	109	113	110
(% difference [a])	-	-2.7	+0.9	-1.8
Day -11	110	107	111	108
(% difference [a])	-	-2.7	+0.9	-1.8
Day 2	110	108	110	109
(% difference [a])	-	-1.8	0.0	-0.9
Day 4	108	107	112*	115*
(% difference [a])	-	-0.9	+3.7	+6.5
Day 6	109	108	114*	116*
(% difference [a])	-	-0.9	+4.6	+6.4
Day 9	111	NA	NA	113
(% difference [a])	-	NA	NA	+1.8
Day 16	109	NA	NA	107
(% difference [a])	-	NA	NA	-1.8
Phosphorous (mg/dL)				
Day -18	5.8	7.8	5.7	5.8
(% difference [a])	-	+34.5	-1.7	0.0
Day -11	5.6	6.8	6.1	5.9
(% difference [a])	-	+21.4	+8.9	+5.4
Day 2	6.0	6.2	5.8	6.4
(% difference [a])	-	+3.3	-3.3	+6.7
Day 4	5.0	5.2	5.1	4.1
(% difference [a])	-	+4.0	+2.0	-18.0
Day 6	5.8	6.2	4.7	3.7*
(% difference [a])	-	+6.9	-19.0	-36.2
Day 9	6.3	NA	NA	4.2
(% difference [a])	-	NA	NA	-33.3
Day 16	6.2	NA	NA	5.3
(% difference [a])	-	NA	NA	-14.5

[a] (% difference) = percent difference between group means relative to vehicle-treated control group.
* Based on statistical analysis of group means, values are significantly different from vehicle control. Refer to data tables for actual significance levels and tests used.

Parameters	Major findings
Urinalysis	Urine samples were collected on days 2, 6, and 16. GS-5734-related changes in urinalysis consisted of increased urine volume, decreased pH, lack of increased urine specific gravity and positive protein reaction. These effects appear to be associated with GS-5734-related effect on kidney function. Effects were more pronounced in the 20 mg/kg/day group suggesting dose-response relationship.

Sex	Males			
GS-5734 Dose (mg/kg/day)	0	5	10	20
No. examined	6	3	3	5
Specific Gravity				
Day -18	1.009	1.013	1.006	1.010
Day 2	1.015	1.016	1.008	1.013
Day 6	1.016	1.017	1.008	1.014
Day 16	1.010	NA	NA	1.006
pH				
Day -18	7.7	8.2	7.3	7.3
Day 2	8.0	7.3	7.7	7.7
Day 6	8.4	8.3	7.7	6.1*
Day 16	7.7	NA	NA	7.8
Total Volume (mL)				
Day -18	395.0	290.0	450.0	380.0
Day 2	224.8	114.3	396.7	251.0
Day 6	218.3	180.0	453.3	310.0
Day 16	303.3	NA	NA	435.0

* Based on statistical analysis of group means, values are significantly different from vehicle control. Refer to data tables for actual significance levels and tests used.

Gross pathology	Evaluated at necropsy (Day 16). No drug-related findings.
Organ weights	GS-5734-related increases in kidney and spleen weights were noted at the terminal sacrifice. Only the 20 mg/kg/day groups had microscopic correlates.

Histopathology
Adequate battery: Yes
Peer review: Yes

Evaluated at necropsy. GS-5734/SBECD-related microscopic findings were in the kidney, liver, lymph node, spleen, and thymus. Kidney and thymus effects were still present at recovery sacrifice.

Main animal findings on Day 6.

Tissue/finding	Sex	Males			
	GS-5734 Dose (mg/kg/day)	0	5	10	20
Kidney	No. examined:	3	3	3	4
Atrophy, Tubule					
	Total number affected	0	3	2	4
	Minimal	0	3	1	0
	Mild	0	0	1	0
	Moderate	0	0	0	1
	Marked	0	0	0	3
Basophilic Tubule					
	Total number affected	0	3	2	4
	Minimal	0	3	0	0
	Mild	0	0	0	1
	Moderate	0	0	2	1
	Marked	0	0	0	2
Basophilic Tubule with Karyomegaly					
	Total number affected	0	0	1	0
	Moderate	0	0	1	0
Casts, Amphophilic					
	Total number affected	0	2	2	4
	Minimal	0	2	2	3
	Mild	0	0	0	1
Casts, Cellular					
	Total number affected	0	0	1	4
	Minimal	0	0	1	2
	Mild	0	0	0	2
Casts, Granular					
	Total number affected	0	0	3	4
	Minimal	0	0	3	0
	Mild	0	0	0	2
	Moderate	0	0	0	2
Casts, Hyaline					
	Total number affected	0	0	2	4
	Minimal	0	0	2	2
	Moderate	0	0	0	2
Liver	No. examined:	3	3	3	4
Vacuolation, Ito Cell					
	Total number affected	3	3	3	4
	Minimal	3	3	3	4
Lymph Node, Inguinal	No. examined:	3	3	3	4
Decreased Cellularity, Lymphoid Follicle					
	Total number affected	0	0	0	4
	Mild	0	0	0	2
	Moderate	0	0	0	2
Lymph Node, Mandibular	No. examined:	3	3	3	4
Decreased Cellularity, Lymphoid Follicle					
	Total number affected	0	0	0	2
	Mild	0	0	0	1
	Moderate	0	0	0	1

Parameters	Major findings

Tissue/finding	Sex	Males			
	GS-5734 Dose (mg/kg/day)	0	5	10	20
Lymph Node, Mesenteric	No. examined:	3	3	3	4
Decreased Cellularity, Lymphoid Follicle					
	Total number affected	0	0	0	3
	Mild	0	0	0	2
	Moderate	0	0	0	1
Lymph Node, Popliteal	No. examined:	3	3	3	4
Decreased Cellularity, Lymphoid Follicle					
	Total number affected	0	0	0	3
	Mild	0	0	0	1
	Moderate	0	0	0	1
	Marked	0	0	0	1
Spleen	No. examined:	3	3	3	4
Decreased Cellularity, Lymphoid Follicle					
	Total number affected	0	0	0	4
	Mild	0	0	0	1
	Moderate	0	0	0	3
Thymus	No. examined:	3	3	3	4
Decreased Lymphocytes					
	Total number affected	2	2	3	4
	Minimal	1	0	2	0
	Mild	1	1	1	1
	Moderate	0	1	0	1
	Marked	0	0	0	1
	Severe	0	0	0	1

Recovery animal findings on Day 16.

Tissue/finding	Sex	Males			
	GS-5734 Dose (mg/kg/day)	0	5	10	20
Kidney	No. examined:	3	NA	NA	2
Atrophy, Tubule					
	Total number affected	0	NA	NA	1
	Mild	0	NA	NA	1
Basophilic Tubule					
	Total number affected	0	NA	NA	1
	Moderate	0	NA	NA	1
Casts, Cellular					
	Total number affected	0	NA	NA	1
	Minimal	0	NA	NA	1
Casts, Granular					
	Total number affected	0	NA	NA	1
	Mild	0	NA	NA	1
Fibrosis, Interstitial					
	Total number affected	0	NA	NA	1
	Moderate	0	NA	NA	1
Thymus	No. examined:	3	NA	NA	2
Decreased Lymphocytes, Cortex					
	Total number affected	0	NA	NA	2
	Moderate	0	NA	NA	1
	Marked	0	NA	NA	1

NA = Not applicable

2-Week Intravenous Toxicity Study in Monkeys (Study #TX-399-2004):

Key Study Findings

- NOAEL = **10** mg/kg/day
- AUC_{tau} = 2390 ng.h/mL; C_{max} =349 ng/mL
- GS-5734 administration resulted in kidney injury (SBECD related).

Conducting laboratory: [redacted] (b) (4)

GLP compliance: Yes

Table 22: 2-Week Monkey Intravenous Toxicity Study Design

Methods	Details
Dose and frequency of dosing:	0 (saline), 0 (vehicle), 1, 3, 10 mg/kg/day

Methods	Details
Route of administration:	Intravenous
Formulation/vehicle:	12% (w/v) sulfobutylether-b-cyclodextrin ((b) (4) SBE-b-CD]) in Sterile water for Injection, USP (SWFI), pH (b) (4)
Species/strain:	Cynomolgus Monkey
Number/sex/group:	4
Age:	2/4 years
Satellite groups/unique design:	2 Recovery (4 Weeks) animals in Vehicle and High Dose
Deviations affecting interpretation:	None

Table 23: 2-Week Monkey Intravenous Toxicity Study Findings

Parameters	Major findings
Mortality	All animals survived to their scheduled sacrifice.
Clinical signs	Examined at least once daily. No test article-related clinical observations were attributed to GS-5734. One female administered GS-5734 at 10 mg/kg/day was noted with mucoid red feces on day 15 of dosing, but no possible cause for the condition was identified.
Body weights	Recorded predose and Days 1, 4, 7, 11, and 15. No GS-5734-related effects were observed.
Food consumption	Measured predose, Day 1, and then weekly. No test article-related effects were observed on body weights.
Ophthalmoscopy	Evaluated pretreatment and during week 2 of dosing. No drug-related findings.
ECG	Evaluated pretreatment and during week 2 of dosing. No drug-related findings.
Hematology	Blood samples were collected predose, on days 7 and 13, and on day of scheduled sacrifice. GS-5734 administration had no effect on hematology or coagulation test results.
Clinical chemistry	Blood samples were collected predose, on days 4, 7, and 13, and on day of scheduled sacrifice. There was a GS-5734-related decrease in cholesterol concentration on days 4, 7 and 13 in animals administered 10 mg/kg/day (-18 to -26%). Effect did not appear toxicologically meaningful.
Urinalysis	Urine samples were collected on days 4, 7, and 13. GS-5734 administration had no effect on urinalysis, urine chemistry or urine biomarkers.
Gross pathology	Evaluated at necropsy (Day 16). No drug-related findings.
Organ weights	No GS-5734-related organ weight differences were noted at the terminal sacrifice.

Parameters	Major findings
Histopathology Adequate battery: Yes Peer review: Yes	Evaluated at necropsy. GS-5734/SBECD-related microscopic findings were limited to the kidney and included tubule cell vacuolation. Effect was still present at recovery sacrifice.

Tissue/finding Sex	Males					Females				
	Saline	Vehicle				Saline	Vehicle			
GS-5734 Dose (mg/kg/day)	0	0	1	3	10	0	0	1	3	10
No. Examined	4	4	4	4	4	4	4	4	4	4
Kidney Vacuolation, tubule cell										
Total number affected	0	1	1	2	1	0	1	1	0	1
Minimal	0	1	1	2	1	0	1	1	0	1

Tissue/finding Sex	Males		Females	
	Vehicle		Vehicle	
GS-5734 Dose (mg/kg/day)	0	10	0	10
No. Examined	2	2	2	2
Kidney Vacuolation, tubule cell				
Total number affected	1	2	1	1
Minimal	1	2	1	1

4-Week Intravenous Toxicity Study in Monkeys (Study #TX-399-2017):

Key Study Findings

- NOAEL =**10** mg/kg/day
- AUC_{tau} =2070 ng.h/mL; C_{max} =288 ng/mL
- GS-5734 resulted in no adverse findings in monkeys, but clinical pathology and microscopic findings were indicative of kidney injury (SBECD related).

Conducting laboratory: (b) (4)

GLP compliance: Yes

Table 24: 4-Week Monkey Intravenous Toxicity Study Design

Methods	Details
Dose and frequency of dosing:	0, 1, 3, 10 mg/kg/day
Route of administration:	Intravenous; Slow bolus with 0.3 mL flush
Formulation/vehicle:	12% (w/v) sulfobutylether-b-cyclodextrin ((b) (4) SBE-b-CD]) in Sterile water for Injection, USP (SWFI), pH (b) (4)
Species/strain:	Cynomolgus Monkeys
Number/sex/group:	4
Age:	2/3 years
Satellite groups/unique design:	None
Deviations affecting interpretation:	None

Table 25: 4-Week Monkey Intravenous Toxicity Study Findings

Parameters	Major findings
Mortality	All animals survived to their scheduled sacrifice.

Parameters	Major findings
Clinical signs	Examined at least once daily. No test article-related clinical observations were noted.
Body weights	Measured predose, Day 1, and then weekly. No test article-related effects were observed on body weights.
Food consumption	Recorded daily. No test article-related alterations were apparent.
Ophthalmoscopy	Evaluated pretreatment and during week 4 of dosing. No drug-related findings.
ECG	Recorded during the predose phase and week 4 of dosing. No qualitative ECG abnormalities with GS-5734 administration.
Hematology	Blood samples were collected predose, on day 4, and on day of scheduled sacrifice. GS-5734 administration had no effect on hematology or coagulation test results.
Clinical chemistry	Blood samples were collected predose, on day 4, and on day of scheduled sacrifice. There was a GS-5734-related decrease in cholesterol concentration on days 4, and 29 in females administered 10 mg/kg/day (-31 to -22%). Effect did not appear toxicologically meaningful.
Urinalysis	Urine samples were collected on day 4 and day of sacrifice. GS-5734 administration had no effect on urinalysis, urine chemistry or urine biomarkers.
Gross pathology	Evaluated at necropsy (Day 29). No drug-related findings.
Organ weights	No GS-5734-related organ weight differences were noted at the terminal sacrifice.

Histopathology
Adequate battery: Yes
Peer review: Yes

No GS-5734-related microscopic findings were noted in animals at the terminal sacrifice. A vehicle (SBECD)-related microscopic finding of vacuolation was observed in organs of the urinary tract, lymph nodes, and liver of animals in all groups, including controls. The findings and 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.

Tissue/finding Sex	Males				Females			
GS-5734 Dose (mg/kg/day)	0	1	3	10	0	1	3	10
Kidney								
No. examined:	4	4	4	4	4	4	4	4
Vacuolation, transitional cell								
Total number affected	4	4	4	4	3	4	4	4
Minimal	4	4	3	4	3	4	4	4
Slight	0	0	1	0	0	0	0	0
Vacuolation, tubule cell								
Total number affected	4	4	4	4	3	4	4	4
Minimal	4	3	4	4	3	3	3	4
Slight	0	1	0	0	0	1	1	0
Urinary Bladder								
No. examined:	4	4	4	4	4	4	4	4
Vacuolation, transitional cell								
Total number affected	4	4	4	4	4	4	4	4
Minimal	0	0	0	3	1	0	1	0
Slight	4	4	4	1	3	4	3	4
Ureter								
No. examined:	4	4	4	4	4	4	4	4
Vacuolation, transitional cell								
Total number affected	4	3	4	4	4	4	4	4
Minimal	0	1	2	3	2	2	3	2
Slight	4	2	2	1	2	2	1	2
Urethra								
No. examined:	4	4	4	4	4	4	4	4
Vacuolation, transitional cell								
Total number affected	4	4	4	3	4	3	4	3
Minimal	3	4	3	3	3	1	3	1
Slight	1	0	1	0	1	2	1	2

Parameters	Major findings

Tissue/finding Sex	Males				Females			
GS-5734 Dose (mg/kg/day)	0	1	3	10	0	1	3	10
Lymph Node, Inguinal								
No. examined:	4	4	4	4	4	4	4	4
Infiltrate, macrophages, vacuolated								
Total number affected	4	4	4	4	4	4	4	4
Minimal	0	0	1	3	2	1	2	1
Slight	4	4	3	1	2	3	2	3
Lymph Node, Mandibular								
No. examined:	4	4	4	4	4	4	4	4
Infiltrate, macrophages, vacuolated								
Total number affected	3	4	4	4	4	4	4	3
Minimal	3	3	1	3	3	3	3	2
Slight	0	1	3	1	1	1	1	1
Lymph Node, Mesenteric								
No. examined:	4	4	4	4	4	4	4	4
Infiltrate, macrophages, vacuolated								
Total number affected	4	4	4	4	4	4	3	4
Minimal	0	1	1	2	3	1	0	2
Slight	4	3	3	2	1	3	3	2
Lymph Node, Popliteal								
No. examined:	2	4	4	4	4	4	4	4
Infiltrate, macrophages, vacuolated								
Total number affected	2	4	4	3	4	4	2	4
Minimal	2	3	3	1	4	3	1	3
Slight	0	1	1	2	0	1	1	1
Liver								
No. examined:	4	4	4	4	4	4	4	4
Vacuolation, Kupffer cell								
Total number affected	4	3	4	3	3	2	4	2
Minimal	4	3	4	3	3	2	4	2

7-Day Intramuscular Toxicity Study in Monkeys (Study #TX-399-2001):

Key Study Findings

- Systemic NOAEL = 7.5 mg/kg/day
- AUC_{tau} = 3370 ng.h/mL; C_{max} =239 ng/mL
- GS-5734 at 15 mg/kg/day resulted in significant renal injury with increase serum creatinine, proteinuria, increased kidney weight, and proximal tubular epithelial necrosis.
- Local injection site reactions were noted at all doses.

Conducting laboratory: (b) (4)

GLP compliance: No

Table 26: 7-Day Monkey Intramuscular Toxicity Study Design

Methods	Details
Dose and frequency of dosing:	0, 2.5, 7.5, 15 mg/kg/day
Route of administration:	Intramuscular
Formulation/vehicle:	5% ethanol, 95% Propylene Glycol (v/v)
Species/strain:	Cynomolgus Monkey
Number/sex/group:	3 Males
Age:	3/4 years
Satellite groups/unique design:	None
Deviations affecting interpretation:	None

Table 27: 7-Day Monkey Intramuscular Toxicity Study Findings

Parameters	Major findings
Mortality	All animals survived to their scheduled sacrifice.
Clinical signs	Unremarkable, except local injection site reactions.
Body weights	A nonadverse decrease in body weight (up to 7% at 15 mg/kg/day).
Food consumption	Reduced food consumption of over 25% from days 4 to 7.
Hematology	Unremarkable, except injection site induced inflammation (increased neutrophil and monocyte counts at 7.5 mg/kg/day).
Clinical chemistry	Unremarkable, except for an increase serum creatinine (15 mg/kg/day). Injection site induced inflammation was noted (decreased albumin and albumin:globulin ratio at all doses).
Urinalysis	Findings at 15 mg/kg/day were indicative of renal injury and included increased urine protein and low urine-specific gravity.
Gross pathology	Animals administered 15 mg/kg/day had adverse kidney changes visible as pale kidney cortices.
Organ weights	Increased kidney weight and decrease thymus weight were noted.
Histopathology Adequate battery: Yes Peer review: Yes	

Sex	Males			
GS-466547 Dose (mg/kg/day)	0	2.5	7.5	15
No. Examined	3	3	3	3
Tissue/finding				
Kidney				
Degeneration/necrosis, proximal tubule				
Slight	0	0	0	1
Moderate	0	0	0	2
Injection site, right				
Inflammation, neutrophilic				
Moderate	0	1	3	2
Necrosis, myofiber				
Slight	1	0	1	2
Moderate	0	3	1	1
Inflammation, mixed cell				
Slight	0	1	0	1
Moderate	0	1	0	0
Injection site, left				
Inflammation, neutrophilic				
Moderate	0	1	3	3
Necrosis, myofiber				
Minimal	1	0	0	1
Slight	0	0	1	2
Moderate	0	1	2	0
Inflammation, mixed cell				
Minimal	0	2	0	0
Slight	1	0	0	0

Genetic Toxicology

Table 28: Genetic Toxicology Studies

Study Title (Study No.)	Key Study Findings
In Vitro Bacterial Reverse Mutation Assay (Ames Test) **(Study #TX-399-2005)** GLP compliance: Yes Study is valid: Yes	No toxicity was observed at any tested dose level (5.00, 16.0, 50.0, 160, 500, 1600, and 5000 µg/plate) in any strain (TA98, TA100, TA1535, TA1537, WP2*uvr*A) activated or not. There were no increases in mean number of revertant colonies. All control values were within acceptable ranges. GS-5734 was **negative** in this AMES assay.

Study Title (Study No.)	Key Study Findings
In Vitro Bacterial Reverse Mutation Assay (Ames Test) for Metabolite **(Study #TX-195-2006)** GLP compliance: No Study is valid: Yes	No toxicity was observed at any tested dose level (up to 250 µg/well of GS-441524) in any strain (Salmonella, TA98, TA100, TA1535, TA97a, WP2*uvr*A) activated or not. There were no increases in mean number of revertant colonies. All control values were within acceptable ranges. Metabolite GS-441524 was **negative** in this AMES assay.
In Vitro Chromosomal Aberrations Assay **(Study #TX-399-2006)** GLP compliance: Yes Study is valid: Yes	Cultured human peripheral blood lymphocytes were examined with GS-5734 at 38.0, 60.0, 117, 146, 162, 171, 180, 189, 199, 210, 221, 232, 244, 271, and 300 µg/mL. There were no increases in the number of cells with chromosomal aberrations, polyploidy, or endoreduplication observed in the cultures analyzed under 24-hour treatment without metabolic activation. With 3 hours of metabolic activation, a slight increase in cells with aberrations was observed at 245 µg/mL. However, this increase was seen at a high level of cytotoxicity that was just under the acceptable limit (48%). The biological relevance was questionable as there were no observed increases at lower concentrations, and thus, was considered **equivocal**.
In Vivo Clastogenicity Assay in Rats **(Study #TX-399-2003)** GLP compliance: Yes Study is valid: Yes	GS-5734 did not induce increases in micronucleated PCEs in rats at any dose level examined (5, 20, and 50 mg/kg). GS-5734 did not show signs of bone marrow cytotoxicity as evident by the lack of statistically significant or biologically relevant decreases in the PCE:NCE ratios. Therefore, GS-5734 is **negative** for clastogenicity.

Carcinogenicity

Carcinogenicity studies are not necessary for the proposed indication with a dosing duration of less than 3 months.

Reproductive / Developmental Toxicology

Fertility and Early Embryonic Development Study in Rats (Study #TX-399-2012):

Key Study Findings

- NOAEL=3 mg/kg/dose (TK not evaluated).
- Adverse effects of lower mean body weights, body weight gains, and food consumption were observed at 10 mg/kg/day.
- Females at 10 mg/kg/day also had lower number of corpora lutea, and consequently lower numbers of implantation sites and viable embryos; lower ovary and uterus/cervix/oviduct weights were also noted.

Conducting laboratory: (b) (4)

GLP compliance: Yes

Table 29: Rat Fertility Study Design

Methods	Details
Dose and frequency of dosing:	0, 1, 3, 10 mg/kg/day
Route of administration:	Intravenous
Formulation/vehicle:	12% (w/v) sulfobutylether-β-cyclodextrin in sterile water for injection, pH (b) (4)
Species/strain:	Sprague-Dawley rats
Number/sex/group:	25
Satellite groups:	None
Study design:	• Males were dosed for 28 days before cohabitation and until necropsy. • Females were dosed for 14 days before cohabitation and through GD7. • Female laparohysterectomy on GD15.
Deviations affecting interpretation:	None

Table 30: Rat Fertility Study Findings

Parameters	Major findings
Mortality	All animals survived to their scheduled necropsy.
Clinical signs	Assessed at least once daily. A slight increase in the incidence of decreased defecation was noted in the 10 mg/kg/day group males. Other findings that were considered incidental included hair loss on various body surfaces, red material around the eyes and/or nose, or swollen scabbed or blackened tails. All were considered nonadverse.
Body weights	Measured once daily in males and GD 0, 3, 7, 10, 13, and 15 in females. Body weight gains were adversely lower in the 10 mg/kg/day (Males by 22% and females by up to 10%). Males in 3 mg/kg/day group had a 11% lower body weight gain. In pregnant females, lower maternal body weight gains were noted in the 10 mg/kg/day during the gestation treatment period; the differences were significant ($p<0.01$) during GD 0-3 and when the entire gestation treatment period (GD 0-7) was evaluated. The lower mean body weight gains in these females were considered adverse. As a result of the decrements in mean body weight gains during the pre-mating and gestation treatment periods, mean body weights were significantly ($p<0.05$ or $p<0.01$) lower throughout gestation, although amelioration of the effects occurred during the post-treatment period; mean body weights were 9.5% lower at the end of the treatment period (GD 7) and 4.4% lower on GD 15. No effects were noted at 1 and 3 mg/kg in females and at 1 mg/kg in males.
Food consumption	Measured once weekly in males and on GD 0, 3, 7, 10, 13, and 15 in females. Food consumption in the 10 mg/kg/day group was lower and considered adverse. In pregnant females, maternal food consumption in the 10 mg/kg/day group was lower throughout the gestation treatment period (days 0-7) and continued into the first 3 days of the post-treatment period (GD 7-10). The differences were significant ($p<0.01$). Mean food consumption recovered during post-treatment period. No effects were observed in the 1 and 3 mg/kg females and 1 mg/kg males.

Parameters	Major findings
Fertility Parameters	
Reproductive Indices:	The animals were cohabited on a 1:1 basis and each mating pair was examined daily. GS-5734 did not affect female reproductive performance. Male reproductive performance was not affected by GS-5734 administration; mating, fertility, and copulation indices were similar in all groups.
Estrous Cycle and Cesarean section	No GS-5734-related effects on estrous cycle were observed. A lower number of corpora lutea was noted in the 10 mg/kg/ group (13.2 per dam vs. 16.4 per dam), resulting in lower numbers of implantation sites and viable embryos (12.1 and 11.6 per dam, respectively, compared to control at 15.0 and 14.3 per dam, respectively). These findings were attributed to the test article and considered adverse. The mean litter proportions of pre-implantation loss, post-implantation loss, and viable embryos in the 10 mg/kg/day group were unchanged.
Organ weights	In 10 mg/kg males, all organ weights were lower with the exception of testis, pituitary, prostate, and seminal vesicles. This trend correlated with the loss in body weight. In 10 mg/kg females, ovary and uterus/cervix/oviduct weights were lower (~16%). .
Sperm evaluation	No GS-5734-related effects on sperm motility or sperm concentration were noted.
Necropsy findings	No test article-related effects were noted on examination.

Embryofetal Development Study in Rats (Study #TX-399-2013):

Key Study Findings

- NOAEL for embryofetal and maternal toxicity = 20 mg/kg/day.
- AUC_{tau} = 8740 ng.h/mL; C_{max} =1580 ng/mL
- No adverse, drug-related effects were noted.

Conducting laboratory: (b) (4)

GLP compliance: Yes

Table 31: Rat Embryofetal Developmental Study Design

Methods	Details
Dose and frequency of dosing:	0, 2.5, 5, 10, 20 mg/kg/day
Route of administration:	Intravenous
Formulation/vehicle:	12% (w/v) sulfobutylether-β-cyclodextrin in sterile water for injection, pH (b) (4)
Species/strain:	Sprague-Dawley rats
Number/sex/group:	25
Satellite groups:	Toxicokinetics; n=9
Study design:	Animals dosed GD 6-17
Deviations affecting interpretation:	None

Table 32: Rat Embryofetal Developmental Study Findings

Parameters	Major findings
Mortality	No unscheduled deaths.
Clinical signs	Examined at least once daily. No drug-related findings.
Body weights	Measured once daily. Body weight losses were observed in the 10 and 20 mg/kg/day groups during GD 6-9. As a result, body weight gains at 10 and 20 mg/kg/day were lower the entire treatment period; the difference in the 20 mg/kg/day group (decreased 23% versus vehicle control) was significant ($p<0.01$). In addition, body weights in the 10 and 20 mg/kg/day groups were lower by 5.6% and 6.3%, respectively. Given that body weights were comparable at the end of the treatment period (GD 17) and at the time of scheduled laparohysterectomy (GD 21), the effects on body weight were not considered adverse
Food consumption	Measured once daily. Maternal food consumption in the 10 and 20 mg/kg/day groups was lower during GD 6-12 (10 mg/kg/day) or throughout the treatment period (20 mg/kg/day). Because the resulting effects on body weight were of small magnitude, these test article-related effects on food consumption were not considered adverse.
Cesarean Data	All animals produced viable litters with no drug-related findings.
Necropsy findings	No drug-related findings.
Cesarean sections	No drug-related findings.
Fetal examinations	No drug-related fetal variations or malformations. Incidental findings were noted where affected fetuses had multiple malformations, or anomalies within the ranges of historical controls.

Embryofetal Development Study in Rabbits (Study #TX-399-2018):

Key Study Findings

- NOAEL for maternal toxicity: 10 mg/kg/day
- NOAEL for embryofetal effects: 30 mg/kg/day.
- AUC_{tau} = 1790 ng.h/mL; C_{max} =380 ng/mL
- Adverse body weight loss and reduced food consumption in dams at 20 mg/kg/day.

Conducting laboratory and location: (b) (4)

GLP compliance: Yes

Table 33: Rabbit Embryofetal Developmental Study Design

Methods	Details
Dose and frequency of dosing:	0, 2.5, 5, 10, 20 mg/kg/day
Route of administration:	Intravenous
Formulation/vehicle:	12% (w/v) sulfobutylether-β-cyclodextrin in sterile water for injection, pH (b) (4)
Species/strain:	New Zealand White Rabbits
Number/sex/group:	22
Satellite groups:	Toxicokinetics; n=4
Study design:	Animals dosed GD 7-20
Deviations affecting interpretation:	None

Table 34: Rabbit Embryofetal Developmental Study Findings

Parameters	Major findings
Mortality	A female in the 20 mg/kg/day group and a female in the vehicle control group were euthanized *in extremis* on GD 16 and 17, respectively, following severe body weight losses (16.0% and 13.2%, respectively, of GD 7 body weight) during the treatment period and periods of minimal food consumption (≤ 13 g feed/day during GD 10-16 and ≤ 3 g feed/day during GD 13-17, respectively). Clinical findings of decreased defecation were noted for these females for 2-4 days prior to euthanasia. Because the moribundity was noted in both the high dose and control group, the moribundity was not considered test article-related. All other females survived to the scheduled necropsy on GD 29.
Clinical signs	Examined at least once daily. Test article-related clinical findings of decreased defecation were noted for females in the 20 mg/kg/day group at the daily examinations during GD 11-18. No other test article-related clinical findings were noted.
Body weights	Measured once daily. Body weight losses were noted in the 20 mg/kg/day group during GD 7-13. These results were considered test article-related and resulted in lower (up to 8.3%) body weights during GD 11-16. When the entire treatment period (GD 7-21) was evaluated, mean body weight gain in this group was lower and considered adverse.
Food consumption	Measured once daily. Maternal food consumption in the 10 and 20 mg/kg/day groups was lower during GD 7-10 (both dose groups) and 10 -13 (20 mg/kg/day only). When the entire treatment period (GD 7-21) was evaluated, mean food consumption in these groups were lower. Because the resulting effects on body weight were only adverse at 20 mg/kg/day, these test article-related effects on food consumption were only considered adverse in the high dose group.
Cesarean Data	Most animals produced viable litters with no drug-related findings. 2 dams in the 2.5 mg/kg group were nongravid.
Necropsy findings	No drug-related findings.
F_1 Offspring Data	
Terminal Observations	The numbers of fetuses (litters) available for morphological evaluation were 180(21), 181(20), 190(22), 202(22), and 172(20), respectively. Malformations were observed in 11(5), 6(6), 6(5), 3(3), and 10(7) fetuses (litters) in these same respective dosage groups and were considered spontaneous in origin.

Parameters	Major findings
Incidental Findings	A number of findings were not attributed to test article administration as they were not noted in a dose-related manner, were noted infrequently, were similar to vehicle control group, or the anomaly were within the ranges of historical control data. These findings included: 20 mg/kg/day group • Omphalocele was noted for a single fetus • Lobular agenesis of the lungs 4 fetuses (controls had n=8) • Absent right kidney/ureter in a single fetus 10 mg/kg/day group • a fetus had a malpositioned right subclavian artery (arose from the pulmonary trunk [no brachiocephalic trunk]) • a fetus had persistent truncus arteriosus (pulmonary arteries arose from ascending aorta; right pulmonary artery coursed retroesophageal; interventricular septal defect [a 1 mm opening in anterior portion of the septum]) 5 mg/kg/day group • a fetus had a malpositioned right kidney (right kidney located more posterior than normal) • a fetus had an absent left or right thyroid gland 2.5 mg/kg/day group • a fetus had an absent left or right thyroid gland • a fetus had an interventricular septal defect (1 mm opening in the anterior portion of the septum), coarctation of the aortic arch, and dilated right atria ventricular ostium
Fetal Necropsy	No drug-related fetal variations or malformations.

Intravenous Pre- and Postnatal Developmental Study in Rats (Study #TX-399-2014):

Key Study Findings

- NOAEL for F_0 and F_1 =10 mg/kg/dose
- No adverse effects were noted at any dosage level to dams or their offspring.

Conducting laboratory: (b) (4)

GLP compliance: Yes

Table 35: Rat Intravenous PPND Study Design

Methods	Details
Dose and frequency of dosing:	0, 1, 3, 10 mg/kg/day
Route of administration:	Intravenous
Formulation/vehicle:	12% (w/v) sulfobutylether-β-cyclodextrin in sterile water for injection, pH (b) (4)
Species/strain:	Sprague-Dawley rats
Number/sex/group:	25 females/group
Satellite groups:	None
Study design:	• Pregnant F_0 females were treated daily from GD 6 to LD 20 then euthanized on PND21.

Methods	Details
	• F_1 and F_2 animals were untreated. F_1 animals were split into 2 groups. F_1 pups not selected for breeding phase were sacrificed after attainment of developmental landmarks (PND21). • Laparohysterectomies were performed for F_1 animals on LD 4, post-mating day 25, or post -cohabitation day 25. • F_2 animals were examined externally and euthanized on PND4.
Deviations affecting interpretation:	None

Table 36: Rat Intravenous PPND Study Findings (F_0 generation)

Parameters	Major findings
Mortality	All F_0 maternal animals survived to scheduled necropsy.
Clinical signs	Examined at least once daily. No drug-related findings.
Body weights	Measured once daily. No adverse findings were noted. Body weight loss was noted in the 10 mg/kg/day group (GD 6-9); when the entire gestation treatment period (GD 6-20) was evaluated, body weight gain was lower. As a result, body weights in this group were lower (up to 6.3%).
Food consumption	Measured once daily. Food consumption was also lower during treatment in the 10 mg/kg/day, but was considered nonadverse.
Pregnancy status	No test article-related effects were noted.
Necropsy findings	No test article-related internal findings were observed, and at the LD 21 necropsy, no test article-related effects were observed on the number of former implantation sites and the number of unaccounted-for sites.

Table 37: Rat Intravenous PPND Study Findings (F_1 generation)

Parameters	Major Findings
Mortality	No drug-related findings on number of F_1 pups born, live litter size, percentage of males per litter at birth, and postnatal survival of F_1.
Clinical signs	Examined at least once daily. No test article-related findings.
Body weights	No drug-related findings.
Food consumption	No drug-related findings.
Early Necropsy of F_1	• Found Dead: The numbers of F1 pups (litters) found dead from PND 0 through the selection of the F1 generation were 9(7), 12(9), 15(5), and 12(8) in the vehicle control, 1, 3, and 10 mg/kg/day groups, respectively. No internal macroscopic findings that could be attributed to maternal administration to the test article were noted. • Nonselected animals: No internal findings that could be attributed to maternal administration were noted at the necropsy of F1 pups euthanized on PND 21.
Sexual maturation	Parameters included balanopreputial separation in males and vaginal patency in females. No drug-related findings.
Behavior and activity	Acoustic startle habituation was evaluated on PND 20 and 60. Motor activity was evaluated on PND 21 and 61. Learning and retention of a spatial navigation task were evaluated with a Biel water maze on PND 22 and 62. No drug-related findings.
Fertility parameters	No drug-related findings.
Pregnancy parameters	No drug-related findings.
Necropsy observations	No drug-related findings.

Table 38: Rat Intravenous PPND Study Findings (F_2 generation)

Parameters	Major Findings
Mortality	No drug-related findings on number of F_2 pups born, live litter size, percentage of males per litter at birth, and postnatal survival of F_1.
General observations	No drug-related findings.
Body weights	Measured on PND 1 and 4. No drug-related findings.
Necropsy	The numbers of F_2 pups (litters) found dead were 3(2), 9(7), 12(8), and 2(2) in the vehicle control, 1, 3, and 10 mg/kg/day groups, respectively. There were no internal findings that could be attributed to F_0 maternal administration of the test article.

Other Toxicology/Specialized Studies

Assessment of Dermal Irritation (TX#-399-2023):

The irritation potential of GS-5734 was evaluated using the EpiSkin *in vitro* irritation test. 10 mg ± 2 mg of GS-5734 to the exposed surface for 15 min, at an application rate of 26.3 mg/cm^2, resulted in an EpiSkin viability of 107.23% ± 4.97% of the negative control value, and therefore a United Nations Globally Harmonized System of Classification and Labelling of Chemicals classification of "No Category". Thus, GS-5734 is not a dermal irritant.

Assessment of Ocular Irritation (TX#-399-2025):

The ocular irritation potential was evaluated Bovine Corneal Opacity and Permeability (BCOP) in vitro assay. GS-5734 (20%, w/v in saline, ca 750 µL) applied to corneas, resulted in an IVIS score of 0.96 which is an UN GHS classification of "No Category." Thus GS-5732 is not an ocular irritant.

Follow-up Study for Clinically Identified Liver Toxicity: Cross-species hepatotoxicity profile (PC#-399-2027):

To evaluate the *in vitro* cross-species hepatotoxic profile of GS-5734 and its systemic metabolites GS-704277, and GS-441524, human, monkey, and rat primary hepatocyte co-cultures ((b) (4) Report GIL-160418) were incubated with GS-5734 (up to 30 µM), GS-704277 (up to 100 µM), and GS-441524 (up to 300 µM). After 14 days of continuous exposure in culture, the IC_{50} based on the decrease in albumin secretion were < 0.12, 0.96, and 2.1 µM in human, rat, and monkey hepatocytes, respectively. The 14-day IC_{50} 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. This may indicate that GS-5734 is likely the drug species associated with the low-grade changes in liver enzymes observed in humans treated with multiple doses of GS-5734.

Follow-up Study for Clinically Identified Liver Toxicity: exVive3D™ Liver Assay (TX#-399-2022):

To evaluate the hepatoxicity of GS-5734 and its metabolites, three-dimensional (3D) bioprinted liver tissues where treated with GS-5734, GS-441524, and GS-704277 at up to 7.5, 3, and 3 µM, respectively. Treatment with GS-5734 and GS-441524 resulted in damage to hepatocytes evidenced by the increased ALT observed and decreased albumin in tissues treated with GS-5734. Histologically there were subtle morphological changes observed, mostly with regard to the non-parenchymal cell regions of the tissues. These data indicate that treatment resulted in some effects different than vehicle treatment; However, this assay does not provide any conclusive evidence of clinically relevant toxicity and does not support drawing conclusions regarding mechanism of the identified clinical liver toxicity.

Excipients/Impurities/Degradants

No novel excipients are used in the manufacture of RDV and it contains no excipients of human or animal origin. RDV drug product is prepared as a concentrate formulation (5 mg/mL) and a reconstituted formulation (100 mg). The quantitative composition of RDV formulations are described here.

Table 39: Quantitative Composition of Concentrated RDV Injection, 5 mg/mL

Components	% w/v	% w/w[a]	Quantity per 20 mL solution[b] (mg)	Quality Standard	Function
Remdesivir[c]	(b) (4)		100	In-House	Active ingredient
Betadex Sulfobutyl Ether Sodium			(b) (4)	USP	(b) (4)
Hydrochloric Acid[d]				NF/Ph. Eur.	pH adjustment
Sodium Hydroxide[d]				NF/Ph. Eur.	pH adjustment
Water for Injection[e]				USP/Ph. Eur.	(b) (4)
Total	100.0	100.0	20	—	—
(b) (4)					

Table 40: Quantitative Composition of Reconstituted RDV for Injection, 100 mg

Components	Quantity per Vial (g)	% w/w	Quality Standard	Function
Remdesivir[b]	(b) (4)	(b) (4)	In-House	Active ingredient
Betadex Sulfobutyl Ether Sodium			USP	(b) (4)
Hydrochloric Acid[c]			NF/Ph. Eur.	pH adjustment
Sodium Hydroxide[c]			NF/Ph. Eur.	pH adjustment
Water for Injection[d]			USP/Ph. Eur.	(b) (4)
Total		100.0	—	—

(b) (4)

Formulation Excipient - Sulfobutylether-β-cyclodextrin sodium (SBECD)

The vehicle, SBECD, used in RDV is an excipient with known effects on the kidney (vacuolation and hypertropy). The toxicity of SBECD has been well characterized in toxicology studies and peer-reviewed publications. SBECD-related microscopic findings in the kidney described in all toxicity studies were not considered adverse as they were reversible and had no major signs of induced kidney dysfunction. These effects have been previously described in toxicity studies for SBECD and for other SBECD- containing products. There was no notable exacerbation of the SBECD-related effects when administered with RDV.

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 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.

To support use of the product in those < 2 years of age during clinical trials and the emergency use authorization, Gilead provided toxicity data for SBECD administered to juvenile animals in DMF # (b) (4). IV doses of up to 4500 mg/kg/day for 2 weeks were well tolerated in juvenile rats. SBECD produced known renal effects; tubular vacuolation was similar to that produced in adult rats. There was no evidence that the juvenile kidney was more sensitive than adult kidney, and effects were fully reversible.

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.

Impurities

The qualification of specified and unspecified impurities within the RDV drug substances, and degradants in the RDV drug products are described below. Overall, the proposed specifications, or lack of specifications, are considered acceptable from a pharmacology/toxicology perspective. This conclusion is based on the general toxicology studies, data from literature, and/or Ames test.

In Vitro Bacterial Reverse Mutation Assay for unspecified impurity (b) (4) (starting material)

An Ames assay was conducted in four *S. typhimurium* strains (TA98, TA100, TA1535 and TA1537) and E. coli WP2*uvr*A with and without S9 for (b) (4) (a starting material in the manufacturing of RDV). The assay met acceptance criteria and (b) (4) was negative for mutagenicity.

2-Week Intravenous Impurity Toxicity Study in Monkeys (Study #TX-399-2015):

Key Study Findings

- 3 lots of GS-5734: GS-5734, (b) (4) (impurities), (b) (4) (degradants)
- NOAEL =**10** mg/kg/day
- AUC_{tau} =1400, 1880, and 1130 ng.h/mL; C_{max} =1130, 1660, and 1110 ng/mL, respectively
- No adverse findings were noted.

Table 41: Content of Drug Lots Included in Impurity Toxicity Study

	GS-5734	(b) (4)	(b) (4)
Lot #	**6660-077-05**	**10148-40-07**	**TX5734-15-01**
GS-5734 Content	(b) (4) %	(b) (4) %	(b) (4) %
Impurity Content	(b) (4) %	(b) (4) %	(b) (4) %

Listed Impurities >(b) (4)%	(b) (4)

Conducting laboratory: (b) (4)

GLP compliance: Yes

Table 42: 2-Week Monkey Intravenous Impurity Toxicity Study Design

Methods	Details
Lot 1 (comparator):	GS-5734, lot #6660-077-05; (b) (4)% Purity
Lot 2 (impurities):	(b) (4), lot #10148-40-07; (b) (4)% Purity
Lot 3 (degradants):	(b) (4), lot #TX5734-15-01; (b) (4)% Purity
Dose and frequency of dosing:	0, 5, 10 mg/kg/day
Route of administration:	Intravenous; Slow bolus
Formulation/vehicle:	12% (w/v) sulfobutylether-b-cyclodextrin ((b) (4) SBE-b-CD]) in Sterile water for Injection, USP (SWFI), pH (b) (4)
Species/strain:	Cynomolgus Monkeys
Number/sex/group:	3 males
Age:	2/3 years
Satellite groups/unique design:	None
Deviations affecting interpretation:	None

Table 43: 4-Week Monkey Intravenous Toxicity Study Findings

Parameters	Major findings
Mortality	All animals survived to their scheduled sacrifice.
Clinical signs	Examined at least once daily. No test article-related clinical observations were noted.
Body weights	Measured predose, and on Days 1, 4, 7, 11, and 14. No test article-related effects were observed on body weights.
Food consumption	Recorded daily. No test article-related alterations were apparent.
Ophthalmoscopy	Evaluated pretreatment on Day 13 of dosing. No drug-related findings.
ECG	Recorded during the predose phase and on Day 11. No qualitative ECG abnormalities.

Parameters	Major findings
Hematology	Blood samples were collected predose and on day of scheduled sacrifice. No effect on hematology or coagulation test results.
Clinical chemistry	Blood samples were collected predose and on day of scheduled sacrifice. There was a GS-5734-related decrease in cholesterol concentration across all doses and formuations (-13 to -32%). Effect did not appear toxicologically meaningful.
Urinalysis	Urine samples were collected on day of sacrifice. No effect on urinalysis test results.
Gross pathology	Evaluated at necropsy (Day 15). No drug-related findings.
Organ weights	No organ weight differences were noted at the terminal sacrifice.
Histopathology Adequate battery: Yes Peer review: Yes	No microscopic findings were noted. Injection site findings (in all groups) consisted of fibrosis, hemorrhage, edema, neutrophil infiltrates, and mixed cell infiltrates.
Toxicokinetic	

		Day 1				Day 14			
Dose Level (mg/kg/day)		C_{max} (ng/mL)	AUC_{0-24} (ng·hr/mL)	M:P C_{max}	M:P AUC_{0-24}	C_{max} (ng/mL)	AUC_{0-24} (ng·hr/mL)	M:P C_{max}	M:P AUC_{0-24}
10	Mean	282	1860	0.358	2.2	300	2260	0.294	1.87
GS-5734	SD	58.2	383	0.102	0.564	70.2	593	0.0916	0.729
5	Mean	170	1090	0.307	1.59	152	969	0.256	1.35
GS-5734-I	SD	2.89	94.1	0.0752	0.473	17.2	106	0.051	0.298
10	Mean	338	2270	0.284	1.49	281	1880	0.169	0.995
GS-5734-I	SD	41	263	0.0357	0.154	35.4	356	0.00892	0.111
5	Mean	263	1360	0.699	3.44	290	1610	0.613	3.08
GS-5734-D	SD	58.7	447	0.0969	0.8	58.3	588	0.143	0.395
10	Mean	548	2330	0.696	3.03	552	2780	0.544	2.62
GS-5734-D	SD	18	220	0.241	1.08	39	441	0.181	0.721

Specified Organic Impurities:

Eight specified impurities have been identified in the RDV drug. The qualified levels are calculated from the NOAEL determined in toxicological studies, TX-399-2004 and TX-399-2015, or set below the maximum allowable concentration. The acceptance limits for each specified impurity are justified and pose no safety concerns as all acceptance limits are below the toxicologically qualified level. Summary information is provided in the following tables. All proposed specifications for the specified impurities are therefore considered acceptable.

Table 44: Specified Organic Impurities in the RDV Drug Substance

Impurity	Maximum Observed in Toxicological Studies (%)	NOAEL[a] (mg/kg/day)	Qualified Level (%)	Batch Number	Toxicological Study Number	Acceptance Limit (%)
[redacted]	[redacted]	[redacted]	[redacted] (b) (4)	6225-123-31	TX-399-2004	NMT [redacted] (b) (4)
				TX5734-15-01[c]	TX-399-2015	NMT
				TX5734-15-01[c]	TX-399-2015	NMT
				10148-40-07	TX-399-2015	NMT

(b) (4)	10148-40-07	TX-399-2015	NMT	(b) (4)
	10148-40-07	TX-399-2015	NMT	
	10148-40-07	TX-399-2015	NMT	

a Study conducted in cynomolgus monkeys.

b (b) (4)

c Remdesivir injection, 5 mg/mL (b) (4)

Table 45: Maximum Allowable Content for Specified Impurity (b) (4)

PDE (mg/day)[a]	Maximum Allowable Content (%) at 200 mg/day Dose of Remdesivir	Acceptance Limit (%)
(b) (4)		

Residual Solvents:

The acceptance criteria for the residual solvents take into consideration the ICH Q3C(R6) PDE at a maximum daily dose of 200 mg of remdesivir. The acceptance limits provide a safety margin of at least (b) (4) when compared to ICH Q3C, Option 2 using the solvent PDE and 200 mg remdesivir. The acceptance limit of NMT (b) (4) ppm for (b) (4) was established in accordance with the recommendation in ICH Q3C. Although (b) (4) is not used in the manufacturing process for remdesivir, it may be present as an impurity in some solvents that are used in the process. Summary information is provided in the following table.

Table 46: Permitted Daily Exposure and Acceptance Limits of Residual Solvents in RDV

Solvent	ICH Classification	ICH PDE (mg/day)	Maximum Allowable Content (%) at 200 mg/day Dose of Remdesivir	Acceptance Limit (%)
(b) (4)				

Elemental Impurities:

There are no specified elemental impurities in the RDV drug substance. The manufacturing process for remdesivir does not utilize metal catalysts or reagents, therefore an intentional test for elemental impurities is not necessary.

Unspecified Impurities:

The acceptance limit for any unspecified impurity is NMT (b) (4)% each. This acceptance limit was justified using a literature based approach, a less-than-lifetime qualification threshold (such as described in ICH M7), and the short-term duration of RDV. The proposed specification is considered acceptable.

During the review of 6-month stability studies and mass balance data, Gilead identified (b) (4) for unspecified impurities. Based on Study #TX-399-2015, is qualified to %. The calculated levels (b) (4) are below the toxicologically qualified level and pose no safety risk to patients.

Drug Product Specifications:

The specified degradation products. (b) (4) are formed (b) (4) (b) (4) The acceptance limits (NMT (b) (4)% for both) were consistent with the acceptance criteria for these organic impurities also found in the drug subtance (Specified Organic Impurities in the RDV Drug Substance). The acceptance limit for any unspecified impurity in drug product is NMT (b) (4)%. This acceptance limit was justified using a literature based approach, a less-than-lifetime qualification threshold (such as described in ICH M7), and the short-term duration of RDV. The proposed specification is considered acceptable. Summary information is provided in the following table.

Table 47: Impurity Specifications in the RDV Drug Product

Impurities/Degradants	Qualified Levels	Proposed Specifications	
		Release	Shelf Life
Remdesivir, injection			
(b) (4)			

a Study #TX-399-2015 (TX5734-15-01) where RDV drug product (b) (4)

b (b) (4)

See the product quality review for additional details on the drug product excipients and impurities.

Extractables/Leachables

RDV 100mg

Concentrate Container Closure System

Identified and theoretical extractable compounds were reported for the drug product container closure system and manufacturing equipment train for RDV 100 mg that is to be reconstituted (see container system components below).

Table 48: RDV Concentrate 100 mg Container Closure System

Component	Description	Specification	Supplier
Vial	(b) (4)		
Elastomeric Closure			
Seal			

Target leachable compounds were selected for the leachable study based on compounds with low/concerning Permitted Daily Exposure (PDE) values and estimated high solubility in drug product (**Table 48**).

Table 49: Leachable Targets for RDV Concentrate 100 mg Drug Product Container Closure System

Leachable Target	CAS#	Potential Source	PDE[a] (µg/day)
(b) (4)			

In addition to these target compounds, four leachables were detected above the analytical evaluation threshold (AET: (b) (4) µg/mL) in the remdesivir drug product samples in leachable studies 20-VR- 1067, 20-VR-1068, 20-VR-1069, and 20-VR-1079. The AET was appropriate and based on a qualification threshold of (b) (4) µg/day, a drug concentration of 5 mg/mL and a maximum dose of 200 mg, which is the loading dose (200 mg x 1 mL/5 mg = 40 mL; (b) (4) µg/day / 40 mL = (b) (4) µg/mL). Leachable study **20-VR-1067** was performed on the remdesivir injection, 5 mg/mL, solution formulation manufactured at Gilead Sciences, La Verne, and packaged in a 20 mL (b) (4) glass vial (b) (4) with the (b) (4) stopper. Leachable study **20-VR-1068** was performed on the remdesivir for injection, 100 mg, lyophilized

formulation manufactured (b) (4) and packaged in a 30 mL (b) (4) (b) (4) glass vial (b) (4) with the (b) (4) stopper. Leachable study **20-VR-1069** was performed on remdesivir for injection, 100 mg, lyophilized formulation manufactured (b) (4) and packaged in a 30 mL (b) (4) glass vial (b) (4) with the (b) (4) stopper. Leachable study **20-VR-1079** was performed on remdesivir for injection, 100 mg, lyophilized formulation manufactured (b) (4) and packaged in the (b) (4) 30 mL (b) (4) glass vial with the (b) (4) stopper. The list of the compounds above the AET are below.

Table 50: Leachables Identified in Extractable Studies Above the AET ((b) (4) µg/mL)

Identified Leachate	CAS#	Study	PDE[a] (µg/day)
(b) (4)			

The toxicological assessments were conducted in a manner consistent with (ICH) M7(R1) (Assessment and Control of DNA Reactive (Mutagenic) Impurities in Pharmaceuticals to Limit Potential Carcinogenic Risk), ICH Q3C(R6) (Guideline for Residual Solvents), ICH Q3D(R1) (Guideline for Elemental Impurities) and guidelines set by the Product Quality Research Institute Extractables and Leachables Working. The calculated PDEs appear to be appropriate based on the reference data provided. Assumptions for calculating the safety concern threshold and AET were also accurate. The maximum allowed concentration of all compounds at time zero was

below the calculated PDE for each compound (provided in the tables above) and no toxicity concerns were identified. Stability testing at longer storage times are pending.

RDV 5mg/kg

Infusion Components Compatibility Testing

To evaluate the potential for extractable substances, compatibility testing was performed on representative infusion components at conditions intended to simulate drug product infusion. Study components included IV infusion bags and infusion set tubing manufactured from polyvinyl chloride (PVC) or manufactured from non-PVC plastic from three manufacturers. The IV bags and tubing were extracted with (b) (4) the sample extracts were analyzed by GC/MS, LC/UV/MS, and ICP-OES and a toxicological assessment of the results was performed.

The maximum daily volume of administration was used to calculate estimated daily exposures for the detected extractable substances (b) (4). A permissible daily exposure (PDE) assessment was performed to determine the margin of safety for identified substances. Methodology used to derive the PDEs was based on ICH Q3C (R7) guidelines. The margin of safety was at least (b) (4) times greater than the estimated PDE for all other extractable substances detected. The potential exposure to extractables from the IV bags and infusion tubing that may come into contact with the remdesivir drug product is not of toxicological concern.

Container Closure Compatibility Testing

To evaluate the potential for extractable substances, compatibility testing was performed on stoppers used for initial clinical supplies. The stopper was manufactured from (b) (4) rubber, (b) (4) Two extractions were performed on the stoppers and one extraction was performed on the glass vials. For the first extraction, five stoppers (b) (4) were exposed (b) (4). The sample extract was analyzed by GC/MS and LC/UV/MS. For the second extraction, the stoppers and vials were exposed (b) (4) the sample solution was analyzed by ICP-OES for elemental impurities.

PDE assessment was performed to determine the margin of safety for the identified extractable in the stopper. The extractable (b) (4) was observed with an estimated maximum daily exposure of (b) (4) mg, which is (b) (4) times below the compound PDE (b) (4) mg/day. Therefore,

exposure to extractables via the representative stopper is not of toxicological concern. No extractables were detected in the glass vials.

Referenced NDAs, BLAs, DMFs

Not applicable.

Individual Reviews of Studies Submitted to the NDA

Not applicable.

This is a representation of an electronic record that was signed electronically. Following this are manifestations of any and all electronic signatures for this electronic record.

/s/

JOHN H DUBINION
09/17/2020 02:27:29 PM

HANAN N GHANTOUS
09/17/2020 02:55:59 PM
I concur with Dr. Dubinion on the decision to approve remdesivir for marketing in the U.S.