# SECTION 2.6.4—PHARMACOKINETICS WRITTEN SUMMARY

REMDESIVIR (GS-5734™)

**Gilead Sciences**

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**CONFIDENTIAL AND PROPRIETARY INFORMATION**

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# LIST OF ABBREVIATIONS

| | |
|---|---|
| AGM | African Green Monkey |
| BCRP | breast cancer resistance protein |
| CHO | Chinese hamster ovary (cells) |
| CYP | cytochrome P450 |
| EBOV | Ebola virus |
| g | gram |
| h | hours |
| HMVEC | human microvascular endothelial cells |
| IM | intramuscular |
| IV | intravenous |
| kg | kilogram |
| m | meter |
| µg | microgram |
| µl | microliter |
| µm | micrometer |
| µM | micromolar |
| LC/MS/MS | high performance liquid chromatography coupled to tandem mass spectrometry |
| MDCKII | Madin-Darby canine kidney cells |
| mg | milligram |
| ml | milliliter |
| mm | millimeter |
| mM | millimolar |
| min | minutes |
| M | Molar |
| mol | mole |
| N | Normal |
| NADPH | β-nicotinamide adenine dinucleotide phosphate (reduced form) |
| ng | nanogram |
| NHP | non-human primate |
| nm | nanometer |
| OATP | organic anion transporting polypeptide |
| PBMC | peripheral blood mononuclear cells |
| P-gp | P-glycoprotein |
| s | seconds |
| SBECD | sulfobutylether-β-cyclodextrin |
| SD | standard deviation |
| UDPGA | uridine 5'-diphosphoglucorinic acid |

# PHARMACOKINETIC ABBREVIATIONS

| | |
|---|---|
| AUC | the area under the concentration versus time curve |
| $AUC_{last}$ | the area under the concentration versus time curve from time zero to the last quantifiable concentration |
| $AUC_{x-xx}$ | partial area under the concentration versus time curve from time "x" to time "xx" |
| CL | clearance |
| $C_{max}$ | the maximum observed concentration of drug |
| MRT | mean residence time |
| $t_{1/2}$ | estimate of the terminal elimination half-life of the drug in serum/plasma/PBMC, calculated by dividing the natural log of 2 by the terminal elimination rate constant ($\lambda_z$) |
| $T_{max}$ | time of observed maximal concentration |
| $V_{ss}$ | volume of distribution at distribution steady state |

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

# 1. NOTE TO REVIEWER

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

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

| Gilead No. | Description | Conversion Factors |
|---|---|---|
| Remdesivir (GS-5734, 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 |

# 2. BRIEF SUMMARY

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

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

Remdesivir was selected for its ability to distribute into cells, including those within tissues, where it is metabolized to form the pharmacologically active nucleoside triphosphate metabolite, GS-443902. In cells and tissues, remdesivir is converted by hydrolase and phosphoramidase cleavage to the nucleoside analog monophosphate. Further phosphorylation by nucleotide kinases results in formation of GS-443902. Dephosphorylation of nucleotide metabolites results in conversion to GS-441524 that itself is not well rephosphorylated. Efficient formation of the pharmacologically active triphosphate, GS-443902, has also been observed in a number of human lung cell types *in vitro* including normal human bronchial epithelial (NHBE) and Calu-3 as well as in PBMC, macrophages, monocytes, and human microvascular endothelial cells (HMVEC). Once formed, GS-443902 has been observed to have a half-life in excess of 15 h in the various cell types mentioned, following incubation with remdesivir *in vitro*.

Consistent with the high esterase activity in the plasma of rodent species, remdesivir was unstable in rat plasma but significantly more stable in human plasma and other nonrodent species. *In vitro* hepatic stability of remdesivir in rat, dog, monkey and human showed that across species, remdesivir was largely unstable and metabolized primarily via hydrolysis. The rate of biotransformation in rodents was relatively fast compared to non-rodents. In all species tested, remdesivir hydrolysis was associated predominantly with the formation of GS-704277 and to a lesser degree, formation of GS-441524.

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

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

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

Remdesivir has a low potential for drug-drug interactions. While a substrate for cytochrome P450 (CYP) 2C8, 2D6, and 3A4 *in vitro*, coadministration with inhibitors of these CYP isoforms is unlikely to markedly increase remdesivir levels as its metabolism is predominantly mediated by hydrolase activity. Remdesivir is a substrate for the organic anion transporter 1B1 (OATP1B1) and P-glycoprotein (P-gp); however, the impact of these transporters on remdesivir disposition is likely minimized by the parenteral route of administration. Remdesivir is an inhibitor of CYP3A, and OATP1B1- and OATP1B3-mediated transport *in vitro.* but its potential to be the perpetrator of clinically significant drug-drug interactions is limited by its transient exposure at clinically relevant concentrations and its rapid

clearance. In human microsomes, remdesivir was a weak inhibitor of CYP1A2, 2B6, 2C8, 2C9, 2C19, and 2D6. The most potent effects of remdesivir were upon CYP3A activities, with an $IC_{50}$ of 11.0 μM being determined with testosterone 6β-hydroxylase activity, and an $IC_{50}$ of 1.6 μM with midazolam 1'-hydroxylase. Further analysis showed that there was no evidence for remdesivir to be a mechanism-based inhibitor of CYP3A. Assessment of remdesivir, GS-704277 and GS-441524 in human hepatocytes indicated they were unlikely to pose any induction risk.

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

# 3. METHODS OF ANALYSIS

## 3.1. Bioanalytical Methods

### 3.1.1. Bioanalytical Methods Supporting *In Vitro* Cellular Metabolism and Pharmacokinetic Studies

Analyses of remdesivir and its metabolites in plasma and PBMC were performed during pharmacokinetic (PK) studies following single administration in Wistar Han rat, African green, marmoset, cynomolgus and rhesus monkeys (m2.6.5, Section 3; AD-399-2001; AD-399-2002; AD-399-2003; AD-399-2016; AD-399-2022; AD-399-2033; and m2.6.5, Section 5; AD-399-2023; AD-399-2028; AD-399-2030; AD-540-2003), single or repeat dose non-Good Laboratory Practice (GLP) toxicokinetic (TK) studies in cynomolgus monkey, rat, rabbit and Ces1c$^{-/-}$ mouse (m2.6.7, Section 16; TX-399-2001; TX-399-2009; TX-399-2010; TX-399-2019), in rat and cynomolgus monkeys (m2.6.7, Section 7; TX-399-2003; TX-399-2016; TX-399-2004; TX-399-2017), in rat and rabbit (m2.6.7, Section 13; TX-399-2013; TX-399-2018), in rat (m2.6.7, Section 14; TX-399-2014) and rhesus monkey (m2.6.7, Section 6; TX-399-2021) used high performance liquid chromatography coupled to tandem mass spectrometry (LC/MS/MS). Methods used to analyze intracellular levels of the pharmacologically active GS-443902 in PBMC and lung tissue (m2.6.5, Section 3; AD-399-2001 and m2.6.7, Section 16; TX-399-2019) in PK/TK studies were also applied to analysis of GS-441524 and its phosphorylated metabolites in PBMC, macrophages, monocytes, HeLa, and HMVEC cells incubated *in vitro* (m2.6.5, Section 7; AD-399-2004; AD-399-2006; AD-399-2015) and select respiratory and non-respiratory tissues in marmoset and African green monkeys (m2.6.5, Section 5; AD-399-2023; AD-540-2003). These methods did not strictly conform to regulatory guidelines but were evaluated for appropriate selectivity, sensitivity, linearity, as well as intra-assay accuracy and precision.

### 3.1.2. Bioanalytical Methods Supporting GLP Toxicokinetic Studies

Plasma concentrations of remdesivir and its metabolite GS-441524 were quantified in repeat dose toxicology studies in Wistar Han rat (m2.6.7, Section 7; TX-399-2003) and cynomolgus monkey (m2.6.7, Section 7; TX-399-2004) using fully validated LC/MS/MS methods (Table 1, m2.6.5, Section 2; BA-399-2002 and BA-399-2003). The anticoagulant was $K_2$EDTA and the sample volume was 50 µL. The extraction was via protein precipitation and the detection was by LC-MS/MS. The above-mentioned methods used structurally related analogs as the internal standards: GS-465124 and GS-441285 (for remdesivir and GS-441524, respectively). Subsequent methods (Table 1, m2.6.5, Section 2; BA-399-2004, BA-399-2006 and BA-399-2007) were upgraded to also include the metabolite GS-704277. The internal standards used were the following stable isotope labeled compounds: GS-829143, GS-828840, and GS-829466 ($^{13}C_3$- remdesivir, $^{13}C_3$-GS-441524 and $^{13}C_3$-GS-704277, respectively). The $K_2$EDTA plasma matrix was also treated with dichlorvos 80 mM aqueous solution (100:2.5). Validation parameters for all methods in Table 2 conformed to the applicable regulatory guidance and documents on bioanalytical method validation {European Medicines Agency (EMA) 2011, Shah 2000, U. S. Department of Health & Human Services (DHHS) 2018,

Viswanathan 2007} and included selectivity, sensitivity, linearity, carryover, intra- and inter-assay precision and accuracy, stock solution stability, injection medium integrity, short-term matrix stability, freeze-thaw matrix stability, long-term matrix stability, dilution integrity and extraction recovery. Results of Incurred Sample Reanalysis (ISR) conducted during the toxicology studies confirmed the repeatability of the methods.

**Table 2. Calibration Range and Lower Limit of Quantification for the Validated Bioanalytical Methods used to Support the GLP Toxicokinetic Studies**

| Validated Method Reference Number | Species | Calibration Range (ng/mL) | LLOQ (ng/mL) | Toxicology Studies using the Validated Method |
|---|---|---|---|---|
| BA-399-2002 | Rat | Remdesivir: 2.00 to 1000<br>GS-441524: 2.00 to 500 | 2.00<br>2.00 | TX-399-2003 |
| BA-399-2003 | Monkey | Remdesivir: 4.00 to 4000<br>GS-441524: 2.00 to 500 | 4.00<br>2.00 | TX-399-2004 |
| BA-399-2004 | Rabbit | Remdesivir: 2.00 to 500<br>GS-441524: 2.00 to 1000<br>GS-704277: 5.00 to 5000 | 2.00<br>2.00<br>5.00 | TX-399-2018 |
| BA-399-2006 | Rat | Remdesivir: 2.00 to 500<br>GS-441524: 2.00 to 1000<br>GS-704277: 5.00 to 5000 | 2.00<br>2.00<br>5.00 | TX-399-2013<br>TX-399-2014<br>TX-399-2016 |
| BA-399-2007 | Monkey | Remdesivir: 2.00 to 500<br>GS-441524: 2.00 to 1000<br>GS-704277: 5.00 to 5000 | 2.00<br>2.00<br>5.00 | TX-399-2015<br>TX-399-2017<br>TX-399-2021 |

## 3.2. *In Vitro* Methods

### 3.2.1. Plasma Binding

The extents of protein binding of remdesivir, GS-704277 and GS-441524 in plasma from Wistar Han rat, cynomolgus monkey, rhesus monkey and human were assessed by equilibrium dialysis by incubating 2 µM compound for 3 h at 37 °C in a Dianorm® Equilibrium Dialyser device with 1 mL Teflon cells (Harvard Apparatus, Holliston MA; m.2.6.5, Section 5.1; AD-399-2013; AD-399-2031).

### 3.2.2. Distribution within Blood

The distribution of remdesivir and GS-441524 between the soluble and cellular fractions of blood from rhesus monkeys and humans was determined at a compound concentration of 0.5 µM. After incubation at 37°C for 1 h the concentrations in the plasma and cellular fractions were determined by LC-MS. Methazolamide, a compound exhibiting preferential distribution to blood cells, was tested in parallel as an assay control (m2.6.5, Section 5.2; AD-540-2007),

### 3.2.3. Stability

The stability of remdesivir has been assessed in plasma, extracts, and enzyme preparations. In these studies the disappearance or metabolism of remdesivir was assessed by LC/MS/MS. The stability of 2 µM remdesivir was assessed over a 4 h incubation in plasma from Sprague-Dawley rat, beagle dog, cynomolgus monkey, rhesus monkey, and human (m.2.6.5, Section 7.1; AD-399-2012). The stability of 2 µM remdesivir was assessed in pooled post-mitochondrial supernatant (S9) isolated from the intestine of Sprague-Dawley rat, beagle dog, and human, and liver of Sprague-Dawley rat, beagle dog, cynomolgus monkey, rhesus monkey, and human (m2.6.5, Section 7.2; AD-399-2014). Stability studies in hepatic S9 were initiated by addition of cofactors (NADPH and UDPGA) and monitored over 90 min. Stability studies in intestinal S9 were initiated by addition of remdesivir and monitored over 120 min. The metabolism of [$^{14}$C]remdesivir was assessed in vitro using mouse, rat, monkey, and human cryopreserved hepatocytes (m.2.6.5, Section 7.6; AD-399-2024). Approximately 750,000 hepatocytes/mL were incubated with 1 and 10 µM [$^{14}$C]remdesivir for 0, 30, 60, and 120 minutes.

### 3.2.4. Intracellular Metabolism

The intracellular metabolism of the diastereomeric mixture, GS-466547, containing remdesivir was studied following a pulse incubation in primary human macrophages *in vitro* (m2.6.5, Section 7.4; AD-399-2004). Macrophages were differentiated from monocytes isolated from the blood of three healthy human donors. Following a 2 h incubation of GS-466547 at 1 µM with human macrophages, the compound-containing media was changed with fresh drug-free media and intracellular metabolites were measured by LC/MS/MS over 24 h.

Intracellular levels of the pharmacologically active triphosphate, GS-443902, were studied during a 72 h continuous incubation with 1 µM GS-466547, the diastereomeric mixture containing remdesivir, with cells permissive to EBOV infection, HMVEC, HeLa cells, or primary human macrophages *in vitro* (m2.6.5, Section 7.5; AD-399-2006). Concentrations of GS-443902 were determined at 2, 24, 48 and 72 h by LC/MS/MS and the intracellular concentrations were estimated based on cell number.

Species differences between rhesus monkeys and humans in the intracellular metabolism of 1 µM remdesivir were assessed following a 2 h incubation in PBMC and monocytes (m2.6.5, Section 7.3; AD-399-2015). After a 2 h continuous incubation of 1 µM remdesivir with PBMC and monocytes from human and rhesus monkey, the intracellular concentrations of GS-441524 (nucleoside analog) and its mono-, di-, and tri-phosphate metabolites were determined by LC/MS/MS analysis.

### 3.2.5. Interaction with Enzymes

Cytochrome P450 (CYP) reaction phenotyping was determined by incubating 5 µM remdesivir with Bactosomes™, cDNA expressed human CYP enzyme preparation co-expressed with human NADPH CYP reductase and monitoring compound disappearance (m2.6.5, Section 11.1; AD-399-2011). The enzymes studied were CYP1A2, 2B6, 2C8, 2C9, 2C19, 2D6, and 3A4.

The potential for CYP inhibition was assessed in pooled liver microsomal fraction at remdesivir concentrations up to 100 µM (m2.6.5, Section 9; AD-399-2010, AD-540-2004). Specific CYP-catalyzed transformations of probe substrates were monitored over 5 to 60 min depending on the CYP isoform. The CYP1A2-mediated formation of acetaminophen from phenacetin (30 µM) as well as the CYP2B6-, 2C8-, 2C9-, 2C19-, 2D6-mediated conversion of buproprion (110 µM) to hydroxybuproprion, paclitaxel (7.5 µM) to 6α-hydroxypaclitaxel, tolbutamide (120 µM) to 4-hydroxytolbutamide, S-mephenytoin (25 µM) to 4'-hydroxymephenytoin, dextromethorphan (5 µM) to dextrorphan, respectively, as well as the CYP3A-mediated conversion of midazolam (2.5 µM) to 1'-hydroxymidazolam and testosterone (50 µM) to 6β-hydroxytestosterone were monitored by LC/MS/MS. To test the potential for remdesivir to act as a mechanism-based inhibitor of human CYP3A a two-stage incubation protocol was used, with the first stage allowing inactivation of the enzyme in the absence of substrate, and the second stage being used to assay remaining enzyme activity with conversion of midazolam (12.5 µM) to 1'-hydroxymidazolam monitored by LC/MS/MS. A 10-fold dilution was performed between the two stages to reduce any direct inhibitory effects of the test compounds. Effects of remdesivir upon human UGT1A1 were tested using insect cell microsomal fraction containing baculovirus-expressed enzyme. Enzyme activity was determined by estradiol 3-glucuronidation by LC/MS/MS and atazanavir was run in parallel as a positive control (m2.6.5, Section 9.3; AD-540-2005).

### 3.2.6. Induction Potential

The extent of induction of specific human cytochrome P450 (CYP) enzymes (CYP1A2, CYP2B6, and CYP3A4) was measured following exposure of human hepatocytes (n = 3 donors) to remdesivir, GS-441524, and GS-704277, and to compare the effects of the test articles with those of prototypical inducers. Induction of CYP enzymes were assessed by quantitating mRNA levels and CYP enzyme activities (m2.6.5, Section 9.4; AD-399-2027).

### 3.2.7. Interaction with Transporters

The interaction of remdesivir with membrane transporters either as an inhibitor or substrate was assessed in appropriate model systems. Remdesivir was tested for its interaction as a substrate and inhibitor with OATP1B1 (SLC21A6), OATP1B3 (SLC21A8), BCRP (ABCG2), and P-gp (MDR1, ABCB1). Additional testing of remdesivir, GS-704277 or GS-441524 as inhibitors of human BSEP, MRP2, MRP4, and NTCP transporters was evaluated using model substrates and transfected cell lines or membrane vesicles (m2.6.5, Section 11; AD-399-2005; AD-399-2007; AD-399-2008; AD-399-2029). Assay systems, probe substrates and model inhibitors are summarized in Table 3. Also investigated was the potential interaction of GS-441524, GS-719700, GS-719699, GS-443902 and GS-704277 with the human BCRP, BSEP, MRP2 and MRP3 efflux transporters in a vesicular transporter inhibition assay (m2.6.5, Section 11.5; AD-399-2035).

**Table 3. Cells and Experimental Conditions for Transporter Assays**

| Transporter (System) | Negative Control | Probe Substrate | Reference Inhibitor |
|---|---|---|---|
| BCRP (MDCKII) | parental MDCKII | pheophorbide A | fumitremorgin C |
| P-gp (MDCKII) | parental MDCKII | calcein AM | verapamil |
| OATP1B1 (CHO) | parental CHO cells | Fluo 3 | rifampicin |
| OATP1B3 (CHO) | parental CHO cells | Fluo 3 | rifampicin |
| human BSEP | betagal-Sf9 | taurocholate | cyclosporin A |
| human MRP2 | defMRP-Sf9 | $E_2$17βG | benzbromarone |
| human MRP4 | HEK293-CTRL | DHEAS | MK571 |
| Human NTCP (CHO) | Na+ free buffer | taurocholate | TCDC |

Source: AD-399-2005; AD-399-2007; AD-399-2008; AD-399-2029

### 3.3. Other in vivo methods

Absorption, distribution, metabolism, and excretion studies were performed in rat, rabbit and monkey following a single intravenous dose of [$^{14}$C]remdesivir. Tissue distribution was determined following a single 10 mg/kg dose of [$^{14}$C]remdesivir administration in non-pigmented and pigmented rats by quantitative whole body autoradiography (QWBA) and in rats and monkeys by scintillation counting (m2.6.5, Sections 5 and 10; AD-399-2017; AD-399-2019). Samples for absorption and excretion were determined in rabbits by scintillation counting (m2.6.5, Section 10; AD-399-2025) Plasma, urine, bile and feces samples were analyzed by LC-MS with eluent fractions collected at 10-second intervals into 96-well plates containing solid scintillant. Radioactivity in each well was determined using TopCount analysis, and radiochemical profiles were generated based on radioactivity counts. (m2.6.5, Section 6; AD-399-2018; AD-399-2020; AD-399-2026).

# 4. ABSORPTION

Plasma pharmacokinetics following a single dose of remdesivir have been assessed after intravenous (IV) administration to Wistar Han rat, cynomolgus monkey, and rhesus monkey or intramuscular (IM) injection to rhesus monkey. Intracellular pharmacokinetics in peripheral blood mononuclear cells (PBMC) were also assessed following a single dose in rhesus monkeys. The multiple dose toxicokinetics of remdesivir were assessed in the 2-week and 4-week IV administration studies in Wistar Han rat and cynomolgus monkey. The multiple dose toxicokinetics of GS-466547, the diastereomeric mixture containing remdesivir, were also assessed in plasma and PBMC during 7-day IM administration to cynomolgus monkey. Additional plasma, PBMC and select tissue pharmacokinetics were also conducted following single-dose IV remdesivir in marmoset, rhesus and African green monkey and are discussed in Section 5.2 (Tissue Distribution Studies), below.

## 4.1. *In Vitro* Absorption Studies

None. Remdesivir is being developed for IV administration as it has insufficient hepatic stability for oral delivery (Section 6.1.2).

## 4.2. Single Dose *In Vivo* Studies

### 4.2.1. Intravenous Administration

The plasma pharmacokinetics of remdesivir and its metabolites following a single IV administration of remdesivir in 12% sulfobutyl-β-cyclodextrin (SBECD) have been assessed in Wistar Han rat, cynomolgus monkey, and rhesus monkey (m2.6.5, Section 3; AD-399-2001; AD-399-2002; AD-399-2003; AD-399-2022; AD-399-2033). The plasma pharmacokinetic parameters for remdesivir, GS-704277, and GS-441524 are summarized in Table 4, Table 5, and Table 6, respectively. The clearance of remdesivir exceeded liver blood flow in all species. Disappearance of remdesivir was followed by transient exposure to the intermediate metabolite GS-704277 and more persistent exposure to the nucleoside metabolite GS-441524. Dose normalized exposure to remdesivir and its metabolites were similar in cynomolgus and rhesus monkeys. In rats, remdesivir had the shortest half-life and relatively high systemic levels of the metabolites GS-704277 and GS-441524. The different pharmacokinetic profile in rat likely reflects high levels of plasma esterase activity in some rodent species and extracellular prodrug clearance by hydrolysis.

**Table 4. Pharmacokinetic Parameters for Remdesivir in Male Wistar Han Rat, Cynomolgus Monkey, and Rhesus Monkey after Single Dose Intravenous Bolus Administration of Remdesivir (Mean, n = 3)**

| 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 5. Pharmacokinetic Parameters for Metabolite GS-704277 in Male Wistar Han Rat, Cynomolgus Monkey, and Rhesus Monkey after Single Dose Intravenous Bolus Administration of Remdesivir (Mean, n = 3)**

| 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

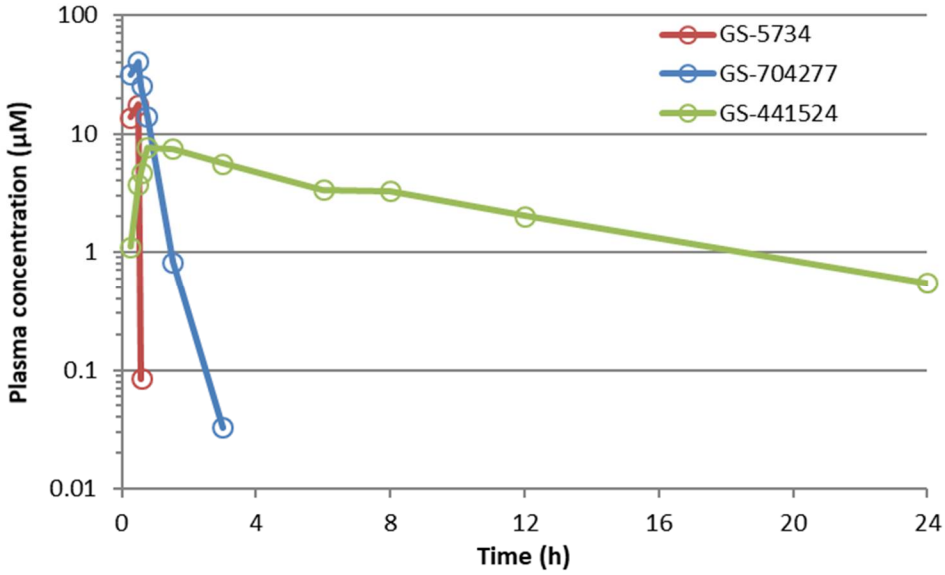
**Table 6. Pharmacokinetic Parameters for Metabolite GS-441524 in Male Wistar Han Rat, Cynomolgus Monkey, and Rhesus Monkey after Single Dose Intravenous Bolus Administration of Remdesivir (Mean, n = 3)**

| 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

The plasma pharmacokinetics of remdesivir and its metabolites following a single, 30-minute IV infusion of 50 mg/kg remdesivir were assessed in male Wistar Han rats (m2.6.5, Section 3.2; AD-399-2003). The plasma profile of remdesivir, GS-704277, and GS-441524, are summarized in Figure 1. Remdesivir and GS-704277 achieved maximal concentrations in plasma during the infusion and exhibited short terminal elimination half-lives of 0.05 and 0.25 h, respectively. The nucleoside analog, GS-441524, achieved maximal concentration in plasma of 7.82 µM and persisted with a terminal half-life of 6.2 h.

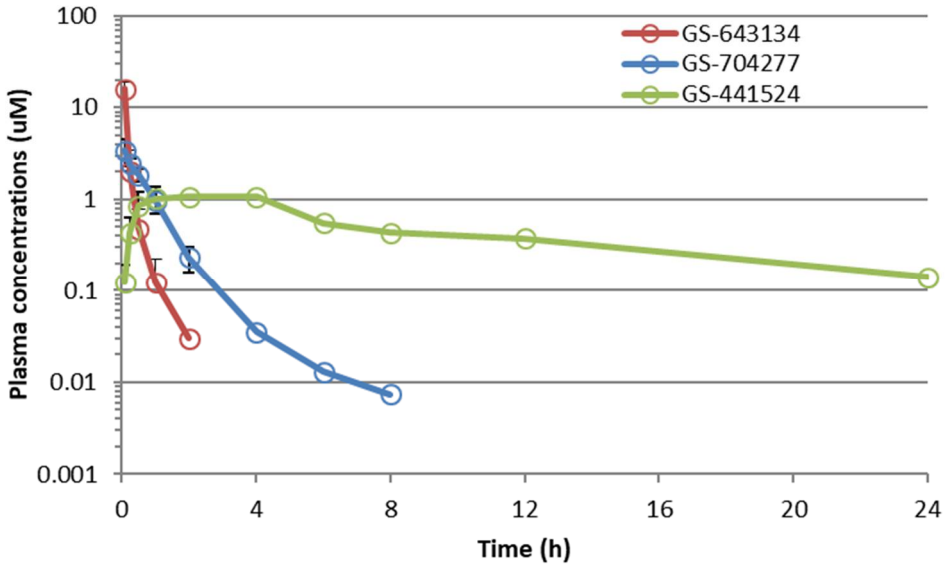
**Figure 1. Concentration-Time Profiles of Remdesivir and its Metabolites GS-704277 and GS-441524 in Plasma Following Intravenous Administration of Remdesivir at 50 mg/kg as a 30-minute Infusion in Rats (Mean, n = 3)**




Source: AD-399-2003

The plasma pharmacokinetics of remdesivir and its metabolites following a single IV administration of 10 mg/kg remdesivir was assessed in male cynomolgus monkeys (m2.6.5, Section 3.3; AD-399-2001). The plasma profile of remdesivir, GS-704277 and GS-441524, are summarized in Figure 2. GS-5734 and GS-704277 achieved maximal concentrations in plasma during the infusion and exhibited short terminal elimination half-lives of 0.29 and 0.84 h, respectively. The nucleoside analog, GS-441524, achieved maximal concentration in plasma of 1.15 µM and persisted with a terminal half-life of around 7.2 h. Similar results were obtained in a later plasma pharmacokinetic study following IV remdesivir at the same dose (m2.6.5, Section 3.6; AD-399-2033).

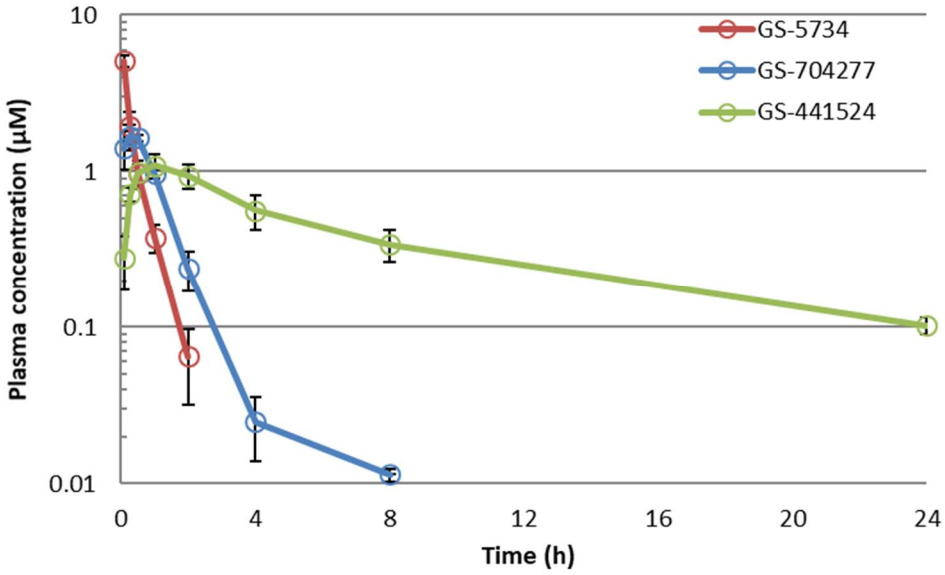
**Figure 2.** **Concentration-Time Profiles of Remdesivir and its Metabolites GS-704277 and GS-441524 in Plasma Following Intravenous Administration of Remdesivir at 10 mg/kg in Cynomolgus Monkeys (Mean, n = 3)**




Source: AD-399-2001

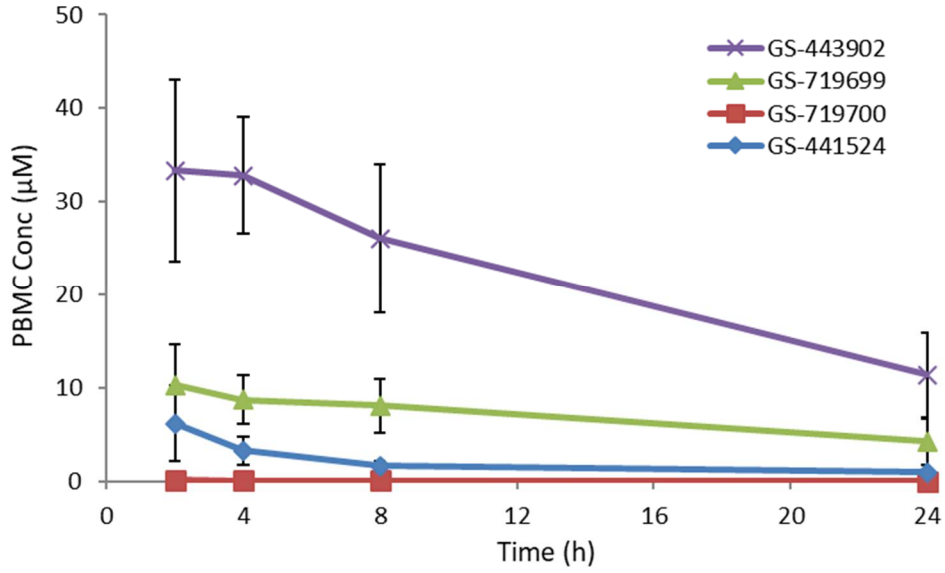
The plasma and PBMC pharmacokinetics of remdesivir and its metabolites following a single IV administration of 3 or 10 mg/kg remdesivir were assessed in male rhesus monkeys (m2.6.5, Section 3.4 and 3.5; AD-399-2002; AD-399-2022). Remdesivir was rapidly eliminated followed by the sequential appearance of GS-704277 and GS-441524. The plasma profiles of remdesivir, GS-704277, and GS-441524, following 10 mg/kg remdesivir are summarized in Figure 3, consistent with those seen in cynomolgus monkeys above. GS-441524 and its phosphorylated metabolites in PBMC are summarized in Figure 4. 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. Both plasma and PBMC exposures to all metabolites showed roughly dose-proportional increases between 3 and 10 mg/kg following IV bolus administration.

**Figure 3.** **Concentration-Time Profiles of Remdesivir and its Metabolites GS-704277 and GS-441524 in Plasma Following Intravenous Injection of Remdesivir at 10 mg/kg in Rhesus Monkeys (Mean, n = 3)**




Source: AD-399-2022

**Figure 4.** **Concentration-Time Profile of GS-441524 and its Phosphorylated Metabolites in Peripheral Blood Mononuclear Cells Following Intravenous Injection of Remdesivir at 10 mg/kg in Rhesus Monkeys (Mean, n = 3)**

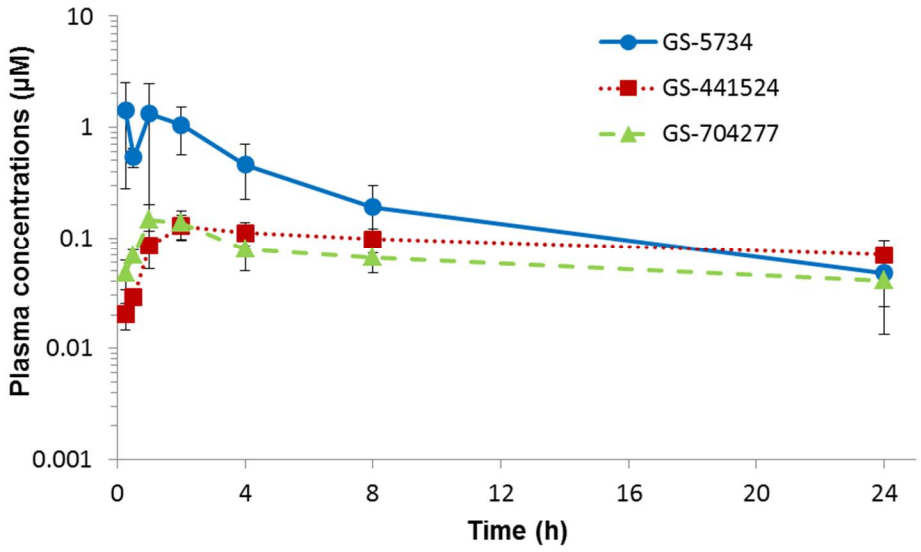



Source: AD-399-2022

### 4.2.2. Intramuscular Injection

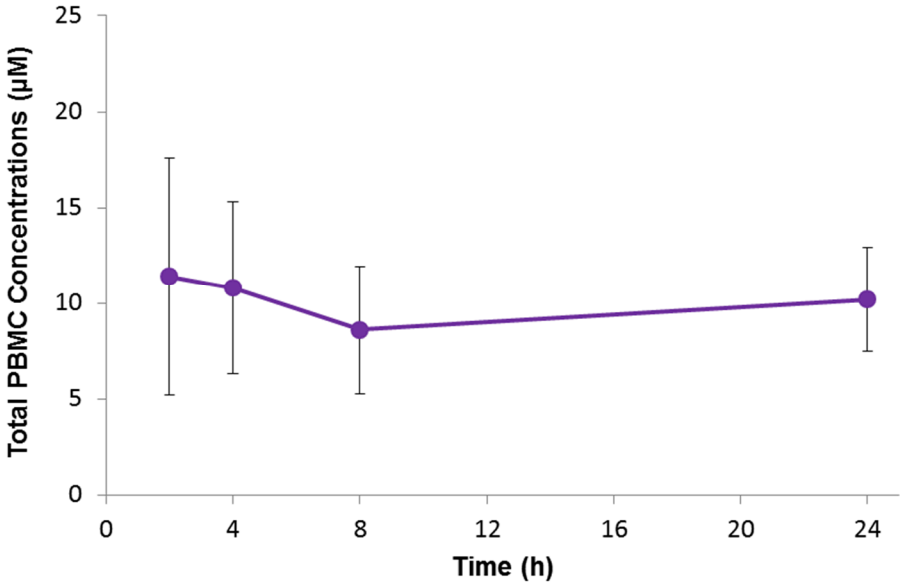
The plasma and PBMC pharmacokinetics of remdesivir and its metabolites following a single IM injection of 3 mg/kg remdesivir were assessed in male rhesus monkeys (m2.6.5, Section 3.1; AD-399-2016). The plasma profile of remdesivir, its intermediate metabolite GS-704277, and its nucleoside metabolite GS-441524 are summarized in Figure 5. Slow and variable release of remdesivir was observed from muscle. Once released, remdesivir was rapidly metabolized to GS-704277 and GS-441524 which persisted over 24 h. High ($C_{max}$ > 10 µM) and persistent ($t_{1/2}$ > 24 h) levels of GS-441524 and its phosphorylated metabolites were also observed in PBMC (Figure 6). Further studies to optimize IM administration were not pursued.

**Figure 5. Concentration-Time Profiles of Remdesivir and its Metabolites GS-704277 and GS-441524 in Plasma Following Intramuscular Injection of Remdesivir at 3 mg/kg in Rhesus Monkeys (Mean, n = 3)**




Source: AD-399-2016

**Figure 6. Concentration-Time Profile of Total GS-441524 and its Phosphorylated Metabolites in Peripheral Blood Mononuclear Cells Following Intramuscular Injection of Remdesivir at 3 mg/kg in Rhesus Monkeys (Mean, n = 3)**




Source: AD-399-2016

### 4.3. Multiple Dose *In Vivo* Studies

Summaries of toxicokinetic findings from the GLP 4-week repeat dose toxicity studies in Wistar Han rat and cynomolgus monkey, and from the GLP reproduction and developmental toxicity studies in rats and rabbits are presented. Additional summaries from non-GLP studies in mice and rhesus monkeys are also included. Detailed results for all aspects of these toxicokinetic studies as well as those with shorter duration assessments are presented in m2.6.6 so they can be interpreted in the context of each study.

#### 4.3.1. Mouse Toxicokinetics

The toxicokinetics (TK) of remdesivir and its metabolites was assessed in carboxylesterase 1c-deficient (Ces1c$^{-/-}$) mice following daily subcutaneous injection of GS-5734 for 7 days at 10 mg/kg and 50 mg/kg (m2.6.7, Section 16; TX-399-2019). In this study, the nucleoside metabolite, GS-441524, was the major compound observed in plasma with lower and more transient exposure to remdesivir and the intermediate metabolite GS-704277. The pharmacologically active triphosphate (GS-443902) was efficiently formed in mouse lung tissue and increased proportionally with dose. Lung maximal concentrations were measured at 4 h post-dose of 0.477 and 2.53 nmol/gram tissue following administration at 10 and 50 mg/kg, respectively. Lung levels of GS-443902 were substantially lower at 24 hours suggesting a short half-life (≤ 10 h). No significant gender difference in lung GS-443902 levels was observed.

### 4.3.2. Rat Toxicokinetics

The toxicokinetics of remdesivir and the nucleoside metabolite GS-441524 were assessed on Day 1 and during week 4 in male and female Wistar Han rat administered remdesivir via IV injection (slow bolus) once daily for 28 days at doses of 0 (vehicle), 1, 3, 10 mg/kg/day (m2.6.7, Section 7.1; TX-399-2016). Remdesivir was rapidly cleared and extensively metabolized to GS-441524 and GS-704277 in rats following intravenous administration. Exposure to GS-441524 increased with the increase in remdesivir dose level from 1 to 10 mg/kg/day. The increases in GS-441524 $C_{max}$ and $AUC_{0-24}$ values were generally greater than dose proportional on Days 1 and 28, with differences generally within 2-fold. Sex-based differences were generally less than 2-fold in GS-441524 $C_{max}$ and $AUC_{0-24}$ values. No accumulation of GS-441524 was observed after multiple doses of remdesivir in rats. Exposure to GS-704277 increased with the increase in remdesivir dose level from 1 to 10 mg/kg/day. The increases in GS-704277 $C_{max}$ and $AUC_{0-24}$ values were, in general, approximately dose proportional between the 3 and 10 mg/kg/day remdesivir dose levels on Day 1, and slightly greater than dose proportional between 1 and 10 mg/kg/day on Day 28. Males had higher GS-704277 $C_{max}$ and $AUC_{0-24}$ values than females, with sex-based differences generally greater than 2-fold for $AUC_{0-24}$ values. No accumulation of GS-704277 was observed after multiple doses of remdesivir in rats.

The toxicokinetics of remdesivir, GS-704277 and GS-441524 were assessed on GD 6 and GD 17 in female pregnant rats administered remdesivir at doses of 0 (vehicle), 2.5, 5, 10, and 20 mg/kg/day via once daily IV injection (slow-push over 1-2 minutes to pregnant Crl:CD(SD) rats (25/group) during organogenesis (GD 6 to 17) (m2.6.7, Section 13.1, TX-399-2013). All concentration values of remdesivir were below the lower limit of quantitation on GD 6 and 17 therefore no toxicokinetic parameters were calculated. These results indicated that remdesivir was rapidly cleared and extensively metabolized in pregnant rats following IV administration of remdesivir. Exposure to GS-441524 and GS-704277 increased with the increase in remdesivir dose level from 2.5 to 20 mg/kg/day. For GS-441524, the increase in $C_{max}$ and $AUC_{0-24}$ values were approximately dose proportional between the 2.5 to 20 mg/kg/day remdesivir dose levels. For GS-704277, the increase in $C_{max}$ and $AUC_{0-24}$ values were, in general, approximately dose proportional between the 2.5 to 20 mg/kg/day remdesivir dose levels, with the exception of GD 17 where the increases were greater than dose proportional from 5 to 20 mg/kg/day. $AUC_{0-24}$ values were slightly higher on GD 17 than on GD 6 for GS-441524 (1.3- to 2.5-fold) and GS-704277 (1.2- to 2.4-fold) after multiple doses of remdesivir in pregnant rats.

The toxicokinetics of remdesivir, GS-704277 and GS-441524 were assessed on GD 6 and lactation day (LD) 20 in four groups of mated female Crl:CD(SD) rats following administration of remdesivir at 0 (vehicle), 1, 3, and 10 mg/kg/day, once daily via IV injection (slow bolus) from GD 6 through LD 20, and on PND 10 from 1 pup/sex/litter from litters whose dams were used for the same collection time points on LD 10 (m2.6.7, Section 14, TX-399-2014). All concentration values of remdesivir, GS-441524, and GS-704277 for maternal and pup rats in the vehicle control group were below the lower limit of quantitation. All concentration values of remdesivir on GD 6 and LD 10 for maternal rats and the majority of concentration values of remdesivir on PND 10 for pups were below the lower limit of quantitation; therefore, no toxicokinetic parameters were calculated. Exposure to GS-441524 and GS-704277 increased with the increase in maternal remdesivir dose level from 1 to 10 mg/kg/day for maternal rats and

exposure to GS-441524 slightly increased with the increase in maternal remdesivir dose level from 3 to 10 mg/kg/day for pups. The increase in $C_{max}$ and $AUC_{0-24}$ values were approximately dose proportional between the 1 and 10 mg/kg/day remdesivir dose levels for maternal rats on GD 6 and LD 10, and were slightly greater than dose proportional for GS-704277 on LD 10. No accumulation of GS-441524 and GS-704277 was observed after multiple doses of remdesivir in maternal rats. Due to limited data in pups for GS-441524, assessment of dose proportionality and gender were not possible. Exposure to GS-441524, in regards to $C_{max}$, was higher in maternal rats than in pups, with maternal:pup $C_{max}$ ratios of 42.6 for females at the 3 mg/kg/day remdesivir dose level and 143 and 114 for males and females, respectively, at the 10 mg/kg/day remdesivir dose level. Concentrations of GS-704277 were not measurable in pups.

### 4.3.3. Rabbit Toxicokinetics

The toxicokinetics of remdesivir, GS-704277 and GS-441524 were assessed in plasma on GD7 and GD 20 in pregnant, time-mated New Zealand white rabbits when administered remdesivir at doses of 0 (vehicle), 2.5, 5, 10, and 20 mg/kg/day via once daily IV injection (slow bolus) during organogenesis (m2.6.7, Section 13.2, TX-399-2018). Exposure to remdesivir increased with dose level from 2.5 to 20 mg/kg/day. The increases in $C_{max}$ and $AUC_{0-24}$ were generally greater than dose proportional between 2.5 and 20 mg/kg/day. Values for mean $C_{max}$ and $AUC_{0-24}$ were approximately 5- to 13-fold higher on GD 20 than on GD 7; however, concentration values were generally below the lower limit of quantitation by 12 hours postdose, indicating no accumulation of remdesivir after multiple doses. Exposure to metabolites GS-441524 and GS-704277 increased with the increase in remdesivir dose level from 2.5 to 20 mg/kg/day. The increases in $C_{max}$ and $AUC_{0-24}$ were approximately dose proportional between the 2.5 and 10 mg/kg/day dose levels and greater than dose proportional between the 10 and 20 mg/kg/day dose levels. No accumulation of GS-441524 and GS-704277 was observed after multiple doses of remdesivir. Mean $T_{max}$ values and $C_{max}$ and $AUC_{0-24}$ metabolite to parent ratios indicate that remdesivir was rapidly and extensively metabolized to GS-441524 and GS-704277 in rabbits.

### 4.3.4. Monkey Toxicokinetics

The toxicokinetics of GS-466547, the diastereomeric mixture containing remdesivir, GS-704277 and GS-441524 were assessed in plasma on Day 1 and 7, together with GS-441524 and its phosphorylated metabolites in PBMC collected 24 h post-dose on Day 7 in male cynomolgus monkey administered GS-466547 via IM injection once daily for 7 days at doses of 0 (vehicle), 2.5, 7.5, and 15 mg/kg/day (m2.6.7, Section 16; TX-399-2001). On Day 1 the rate of GS-466547 absorption from muscle was dose-dependent with $T_{max}$ values of 1.17, 2.42, and 5.08 h at 2.5, 7.5, and 15 mg/kg/day, respectively. The increases in exposures of GS-466547 and its metabolites, GS-704277 and GS-441524, were approximately dose proportional between 2.5 and 7.5 mg/kg/day but less than dose proportional between 7.5 and 15 mg/kg/day on Day 1. No evidence for accumulation of GS-466547 was observed between Day 1 and Day 7. In the 2.5 and 7.5 mg/kg/day dose groups, the metabolites accumulated approximately 2-fold between Day 1 and Day 7. Marked accumulation of metabolites was observed in the 15 mg/kg/day dose group between Day 1 and Day 7 with increases in GS-704277 and GS-441524 $AUC_{0-24}$ of 5.7- and 9.4-fold, respectively. Total levels of GS-441524 and its phosphorylated metabolites in PBMCs increased with dose achieving 122 µM 24 h post Day 7 dosing in the 15 mg/kg/day group.

The toxicokinetics of remdesivir, GS-704277 and GS-441524 were assessed in plasma on Day 1 and Day 28 in male and female cynomolgus monkeys administered remdesivir via IV injection (slow bolus) once daily for 15 days at doses of 0 (vehicle), 1, 3, or 10 mg/kg/day (m2.6.7, Section 7.4; TX-399-2017). Exposures to remdesivir, GS-441524, and GS-704277 increased with the increase in remdesivir dose level from 1 to 10 mg/kg/day. The increases in remdesivir, GS-441524, and GS-704277 $C_{max}$ and $AUC_{0-24}$ were approximately dose proportional between the 1 and 10 mg/kg/day remdesivir dose levels. Sex-based differences were less than 2-fold in remdesivir, GS-441524, and GS-704277 $C_{max}$ and $AUC_{0-24}$ values. No accumulation of remdesivir, GS-441524, and GS-704277 was observed after multiple doses of remdesivir in monkeys. The mean $AUC_{0-24}$ metabolite to parent ratios indicate that remdesivir was extensively metabolized to GS-441524 and GS-704277 in monkeys.

The toxicokinetics of remdesivir, GS-704277 and GS-441524 were assessed in plasma on Day 0 and Day 6 in male Indian-origin rhesus monkeys administered remdesivir via IV injection (slow bolus) once daily for 7 days at doses of 0 (vehicle), 5, 10, and 20 mg/kg/day (m2.6.7, Section 6; TX-399-2021). Exposure to remdesivir increased with the increase in dosage level from 5 to 20 mg/kg/day. The increases in $C_{max}$ and $AUC_{0-24}$ were generally greater than dose proportional between the 5 and 20 mg/kg/day. No accumulation of remdesivir was observed after multiple doses. Exposure to GS-441524 and GS-704277 increased with the increase in remdesivir dose from 5 to 20 mg/kg/day. The increases in $C_{max}$ and $AUC_{0-24}$ were approximately dose proportional between the 5 and 20 mg/kg/day on Day 0 for GS-441524 and GS-704277 and between the 5 and 10 mg/kg/day on Day 6 for GS-704277. The increases in $C_{max}$ and $AUC_{0-24}$ were greater than dose proportional between the 5 and 20 mg/kg/day on Day 6 for GS-441524 and between the 10 and 20 mg/kg/day on Day 6 for GS-704277. No accumulation of GS-441524 was observed after multiple doses of remdesivir in monkeys at 5 mg/kg/day; however, accumulation was observed at the 10 and 20 mg/kg/day. No accumulation of GS-704277 was observed after multiple doses of remdesivir in monkeys. The mean $C_{max}$ and $AUC_{0-24}$ metabolite to parent ratios indicate that remdesivir was rapidly and extensively metabolized to GS-441524 and GS-704277 in monkeys following intravenous (slow bolus) injection administration of remdesivir.

# 5. DISTRIBUTION

## 5.1. *In Vitro* Plasma Protein Binding and Blood Distribution

The extent of remdesivir, GS-704277 and GS-441524 binding to plasma proteins was determined in plasma from Wistar Han rat, cynomolgus monkey, rhesus monkey and human (m2.6.5, Section 5; AD-399-2013; AD-399-2031). Remdesivir had moderate protein binding in all species with a free fraction ranging from 8.0% in rat to 14.2% in cynomolgus monkey (Table 7). The free fraction in human was 12.1%. GS-704277 and GS-441524 exhibited very low protein binding in plasma from Wistar Han rat, cynomolgus monkey, rhesus monkey and human.

**Table 7. Protein Binding for Remdesivir, GS-704277 and GS-441524 at 2 µM in Plasma from Different Species (Mean, n = 2)**

| | Free Fraction (%) | | |
|---|---|---|---|
| **Species** | **Remdesivir** | **GS-704277** | **GS-441524** |
| Wistar Han Rat | 8.0 | 95 | 90 |
| Cynomolgus monkey | 14.2 | 127 | 99 |
| Rhesus Monkey | 13.5 | 106 | 85 |
| Human | 12.1 | 99 | 98 |

Source: AD-399-2013; AD-399-2031

The distribution of remdesivir and GS-441524 between the cellular and soluble fractions of blood from rhesus monkey and human was assessed (m2.6.5, Section 5.2; AD-540-2007). After incubation of blood with the compounds (initial concentration 0.5 µM) remdesivir was found to be somewhat excluded from the blood cellular fraction in both species, with mean whole blood/plasma concentration ratios of 0.71 and 0.76 for monkey and human, respectively, while GS-441524 showed some association with the cellular fraction with respective mean blood/plasma ratios of 1.36 and 1.19 for monkey and human.

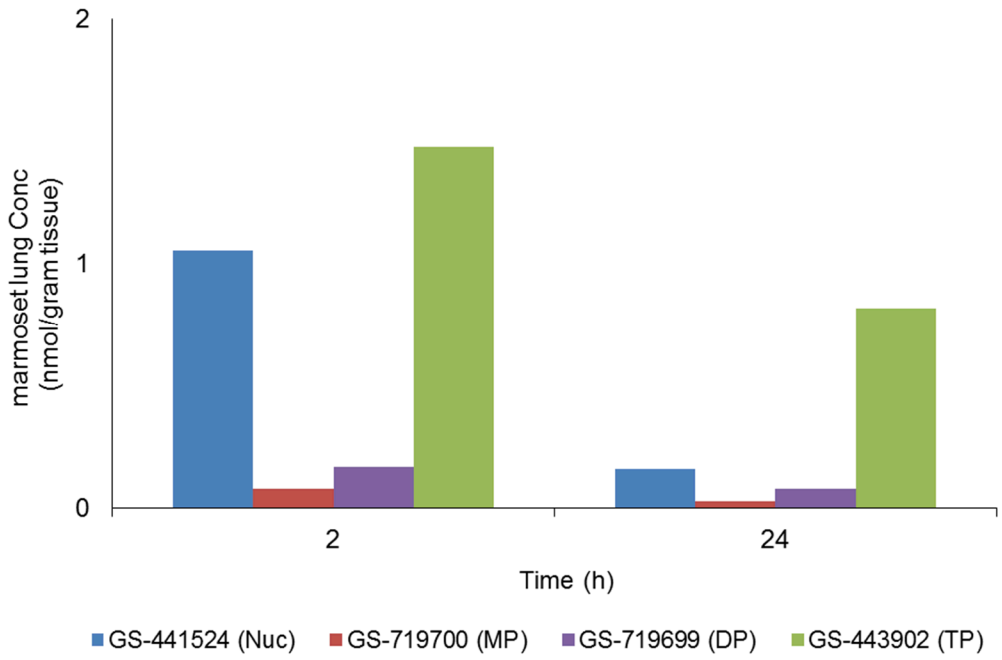
## 5.2. Tissue Distribution Studies

### 5.2.1. Studies Involving GS-443902 measurements

#### 5.2.1.1. Marmoset

A single dose pharmacokinetic study with remdesivir was conducted in male marmosets (m2.6.5, Section 5.6; AD-399-2023). Remdesivir was administered by intravenous (IV) injection (slow bolus) at 10 mg/kg and formulated in 12% SBECD in water, pH 3.5-4.0. As seen in previous monkey studies, remdesivir was rapidly eliminated followed by the sequential appearance of GS-704277 and GS-441524 as well as efficient formation of the pharmacologically active triphosphate, GS-443902, observed in PBMCs. Additionally, profiles of GS-441524 and its phosphorylated species in marmoset lung are shown in Figure 7. Once formed, GS-443902 appeared to persist with an approximate half-life of > 24 h and 22 h in PBMC and lung, respectively.

**Figure 7. Concentration-time Profile of GS-441524 and Its Phosphorylated Metabolites in Lung Following Intravenous Injection of Remdesivir at 10 mg/kg in Marmosets (mean, n=2 per time point)**

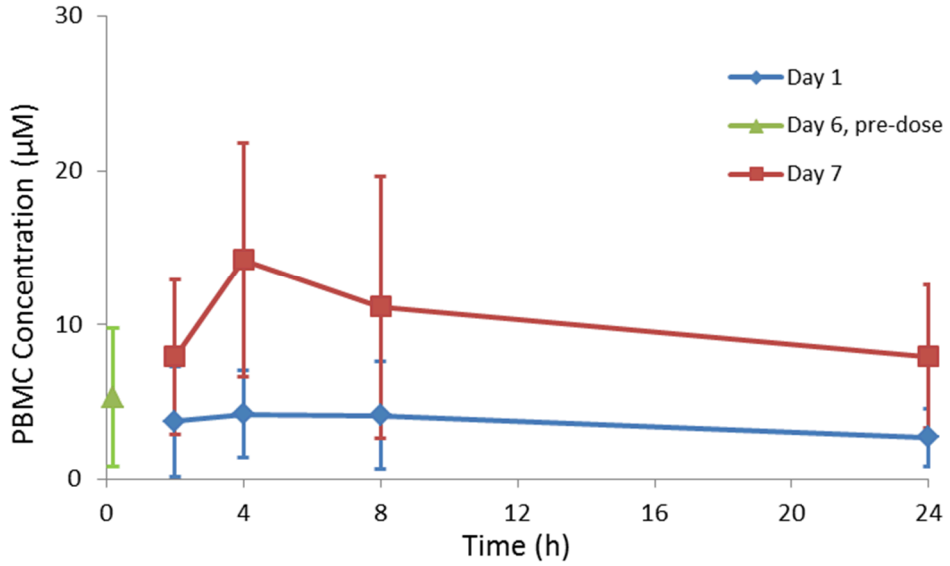



Source: AD-399-2023

5.2.1.2. Rhesus

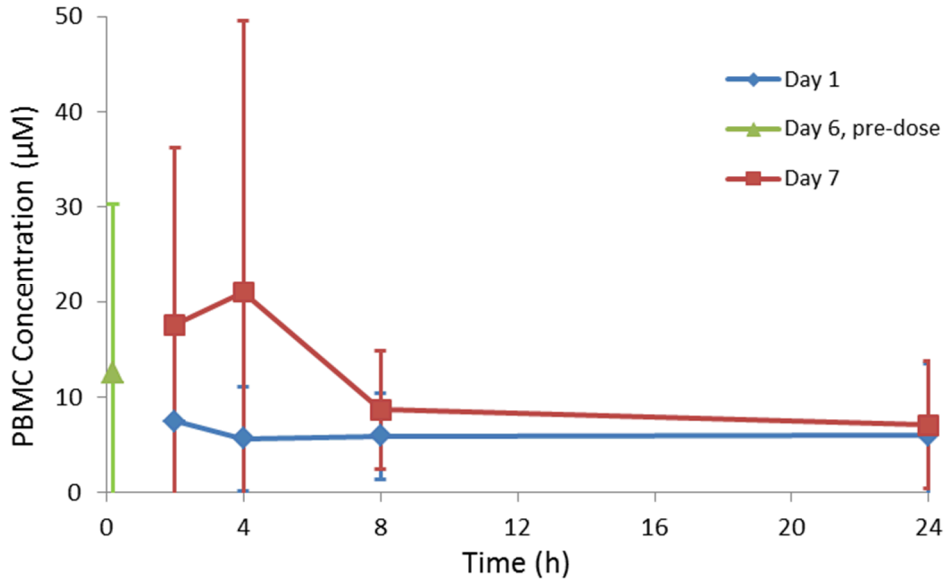
A multiple dose pharmacokinetic study with remdesivir was conducted in rhesus monkeys (m2.6.5, Section 5.8; AD-399-2030). Remdesivir was administered either by intravenous (IV) injection (slow bolus) or as a 30-minute IV infusion at 5 mg/kg, daily for 7 days (qd x7). The bolus vehicle was 12% SBECD in water and sterile saline reconstitution of lyophilized powder for infusion administration. Following IV administration as either bolus or infusion at 5 mg/kg, plasma pharmacokinetic profiles were similar to previously reported studies in rhesus (described previously in Section 4.2). Efficient formation of the pharmacologically active triphosphate, GS-443902, was observed in PBMCs, with accumulation to steady-state concentrations achieved by day 7. No marked difference in exposure of GS-443902 in PBMCs was observed between bolus or infusion administration following daily dosing for 7 days (Figure 8 and Figure 9, respectively).

**Figure 8.** **Concentration-time Profiles of GS-443902 in PBMC Following Daily Intravenous Administration of Remdesivir at 5 mg/kg for 7 days as Slow-Push Bolus in Rhesus Monkeys (mean ± SD, n=8)**




Source: AD-399-2030

**Figure 9.** **Concentration-time Profiles of GS-443902 in PBMC Following Daily Intravenous Administration of Remdesivir at 5 mg/kg for 7 days as a 30-minute Infusion in Rhesus Monkeys (mean ± SD, n=8)**




Source: AD-399-2030

5.2.1.3. African Green Monkey

A single dose pharmacokinetic study with remdesivir was conducted in African green monkeys (m2.6.5, Section 5.9; AD-540-2003). Remdesivir was administered by constant rate intravenous infusion over 30 minutes at 10 mg/kg formulated in 12% SBECD in water (pH 3.5). The plasma profiles of remdesivir, GS-704277, and GS-441524, are summarized in Figure 10. Profiles for GS-441524 and its phosphorylated metabolites in PBMC and respiratory tissues in are provided in Figure 11 and Figure 12, respectively, and for total GS-441524 phosphorylated metabolites in liver, gastrointestinal (GI) tract, kidney, testis and eye in Figure 13. At 24 h postdose, levels of the pharmacologically active metabolite, GS-443902 persisted in respiratory tissues which is suggestive of broad and efficient loading within these tissues following IV administration. Consistent with previous tissue distribution results in cynomolgus monkey, but reflective of specific intracellular activation, high levels of GS-441524 phosphorylated metabolites were observed in kidney and liver, moderate levels in GI tract with more limited distribution observed in testis and eye.

**Figure 10. Concentration-Time Profiles of Remdesivir and its Metabolites in Plasma Following a 30-minute Intravenous Infusion of Remdesivir at 10 mg/kg in AGM (Mean ± SD, n=3 per time point [remdesivir 15 min time point, n=1])**

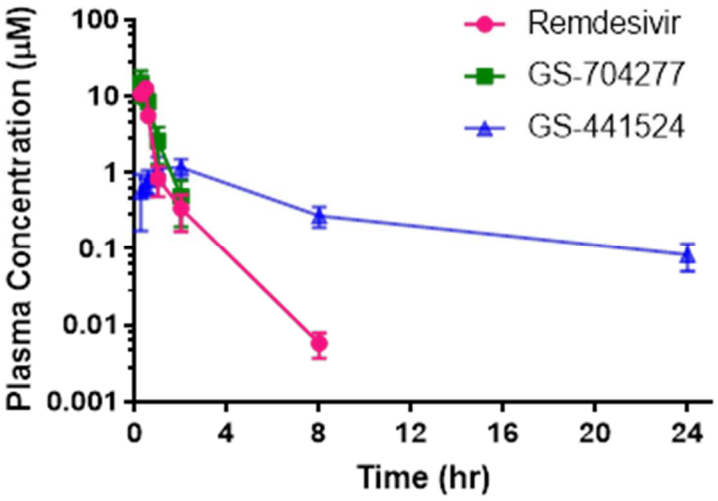


**Figure 11.** **Profiles of GS-441524 and its Phosphorylated Metabolites in PBMC Following Intravenous Administration of Remdesivir at 10 mg/kg as a 30-minute Infusion in African Green Monkeys (Mean, n = 3)**

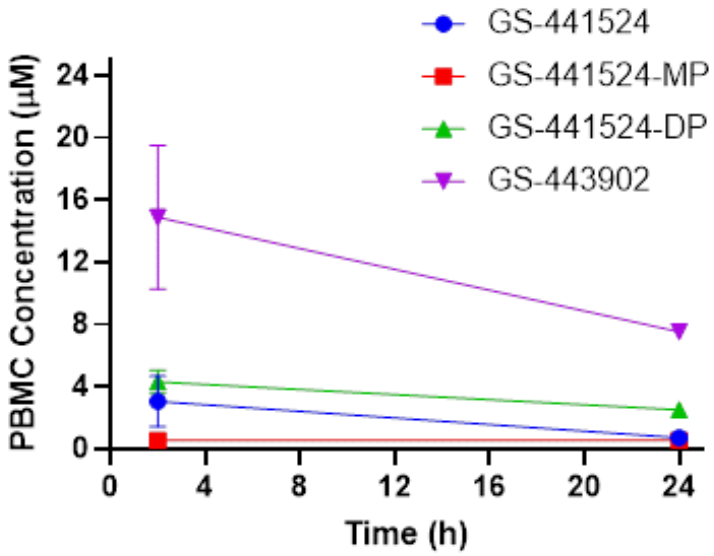



**Figure 12.** **Profiles of GS-441524 and its Phosphorylated Metabolites in Respiratory Tissues Following Intravenous Administration of Remdesivir at 10 mg/kg as a 30-minute Infusion in African Green Monkeys (Mean, n = 3)**

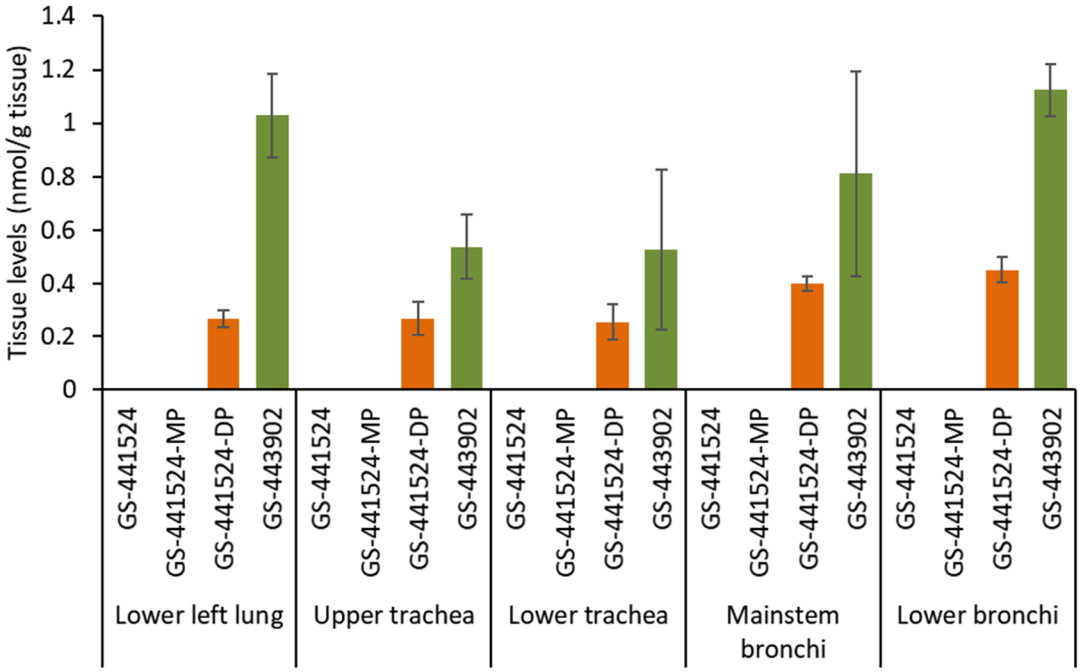
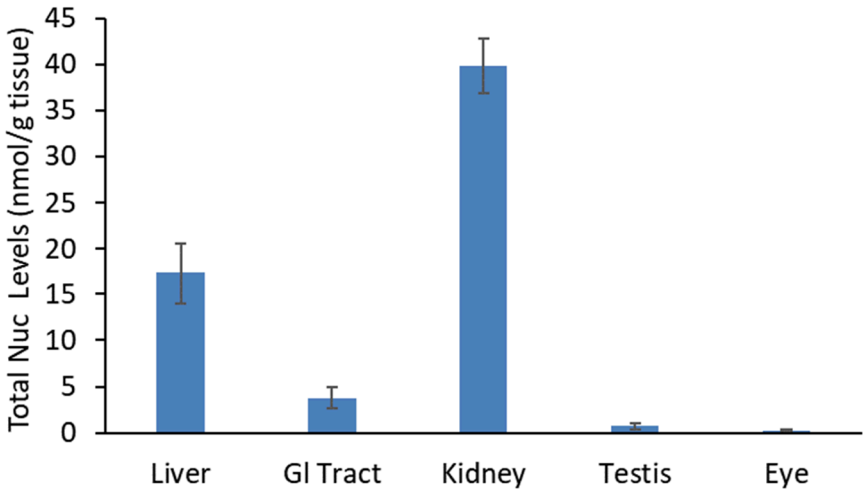


**Figure 13. Profiles of GS-441524 and its Phosphorylated Metabolites in Select Tissues Following Intravenous Administration of Remdesivir at 10 mg/kg as a 30-minute Infusion in African Green Monkeys (Mean, n = 3)**




## 5.2.2. Radiolabeled Tissue Distribution

5.2.2.1. Rat

The tissue distribution following a single intravenous dose of [$^{14}$C]remdesivir at 10 mg/kg to male Sprague Dawley (SD; non-pigmented) and Long Evans (LE; pigmented) rats was determined by quantitative whole body autoradiography (QWBA; AD-399-2017). A total of 52 tissues were examined and results from select tissues are shown in Table 8 and Table 9; a complete listing is provided in m2.6.5, Section 5.3 and 5.4. [$^{14}$C]remdesivir-derived radioactivity was widely distributed to most tissues by the first collection time point (0.167 hours postdose) in both SD and LE male rats. Distribution of radioactivity was similar in both SD and LE rats. Most of the tissues reached maximum concentration ($C_{max}$) by the first collection time point for both SD and LE rats. Low levels of radioactivity were detected in testes, suggesting [$^{14}$C]remdesivir-derived radioactivity crossed the blood to testes barrier. Radioactivity was eliminated from majority of the tissues by 96 hours postdose in both SD and LE rats. No melanin binding was observed.

In SD rats (Figure 14), the tissues showing the highest maximum concentrations of radioactivity included kidney cortex, kidney, kidney medulla, liver, arterial wall, nonpigmented skin, cecum, urinary bladder, and esophagus (Table 8). The tissues with the lowest $C_{max}$ values were brain medulla, spinal cord, brain cerebellum, brain cerebrum, and bone. Radioactivity was cleared from the majority of tissues by 96 hours postdose, with the exception of cecum, kidney, kidney cortex, kidney medulla, liver, nonpigmented skin, stomach, and urinary bladder. At 168 hours postdose, radioactivity was still quantifiable in kidney, kidney cortex, kidney medulla, liver, and nonpigmented skin.

In LE rats (Figure 15), the distribution of radioactivity was similar to SD rats, and the tissues showing the highest maximum concentrations of radioactivity included kidney cortex, kidney, kidney medulla, liver, cecum, urinary bladder, arterial wall, and pigmented skin (Table 9). The tissues with the lowest $C_{max}$ values were brain cerebellum, brain olfactory lobe, eye lens, abdominal fat, and bone. Radioactivity was cleared from the majority of tissues by 96 hours postdose, with the exception of kidney, kidney cortex, kidney medulla, liver, and pigmented skin. At 168 hours postdose, radioactivity was still quantifiable in kidney, kidney cortex, and pigmented skin. No melanin binding was observed.

**Table 8. Individual Concentrations of Radioactivity in Blood and Select Tissues Determined After a Single Intravenous Administration of [$^{14}$C]Remdesivir to Male Sprague Dawley Rats (10 mg/kg)**

| Tissue | Concentration (ng Equivalents $^{14}$C-GS-5734/g) | | | | | |
|---|---|---|---|---|---|---|
| | 0.167 h | 1 h | 4 h | 12 h | 48 h | 168 h |
| Adrenal gland(s) | 3310[a] | 1140 | 364[a] | 159[a] | ND | ND |
| Bile | 189000 | ND | ND | ND | ND | ND |
| Blood | 6690 | 1130 | 384 | 187 | ND | ND |
| Bone | 367 | BLQ | BLQ | ND | ND | ND |
| Bone marrow | 2010 | 892 | 395 | 121 | ND | ND |
| Brain choroid plexus | 1220 | 241 | ND | ND | ND | ND |
| Brain medulla | 158 | BLQ | ND | ND | ND | ND |
| Cecum | 3300 | 1140 | 6380 | 4710 | 223 | ND |
| Diaphragm | 1960 | 1080 | 605 | 398 | 147 | BLQ |
| Epididymis | 3850[a] | 819 | 326 | 138[a] | BLQ | ND |
| Esophagus | 4680 | 960 | 506 | 407 | 257 | BLQ |
| Eye(s) | 906 | 212 | 146 | BLQ | ND | ND |
| Fat (brown) | 2130 | 751 | 399 | 244 | BLQ | ND |
| Kidney(s) | 115000 | 158000 | 120000 | 78500 | 10700 | 745 |
| Liver | 51000 | 29400 | 22200 | 5620 | 501 | 123 |
| Lung(s) | 3800 | 1280 | 605 | 268 | ND | ND |
| Lymph node(s) | 3540 | 1330 | 491 | 275 | ND | ND |
| Muscle | 959 | 927 | 376 | 226 | 122 | ND |
| Myocardium | 3210 | 1310 | 604 | 346 | ND | ND |
| Nasal turbinates | 2010 | 354 | 227 | 121 | ND | ND |
| Pancreas | 2810[a] | 1750[a] | 1120 | 772 | BLQ | ND |
| Preputial gland | 2210[a] | 593[a] | 287[a] | 185[a] | 234[a] | ND |
| Prostate gland | 3030[a] | 570 | 394 | 190 | BLQ | ND |

| Tissue | Concentration (ng Equivalents $^{14}$C-GS-5734/g) | | | | | |
|---|---|---|---|---|---|---|
| | **0.167 h** | **1 h** | **4 h** | **12 h** | **48 h** | **168 h** |
| Seminal vesicle(s) | 846[a] | 188[a] | BLQ[a] | ND | ND | ND |
| Skin (nonpigmented) | 7150 | 1130 | 675 | 936 | 840 | 509 |
| Spleen | 1610 | 1160 | 439 | 202 | BLQ | ND |
| Stomach | 3820 | 1190 | 629 | 410 | 201 | ND |
| Testis(es) | 1250 | 451 | 298 | 146 | ND | ND |
| Thyroid | 3290 | 1150 | 549 | ND | ND | ND |
| Urinary bladder | 5470 | 1360 | 5580 | 5820 | 761 | ND |
| Urine | 171000 | 117000 | 89700 | 16900 | 1730 | ND |

BLQ = Below the limit of quantitation (<112 ng equivalents $^{14}$C-GS-5734/g); h = hours; ND = not detectable (sample shape not discernible from background or surrounding tissue)
a Tissue appeared to be fat-soaked.
Source: AD-399-2017

**Table 9. Individual Concentrations of Radioactivity in Blood and Select Tissues After a Single Intravenous Administration of [$^{14}$C]Remdesivir to Male Long Evans Rats (10 mg/kg)**

| | Concentration (ng Equivalents 14C-GS-5734/g) | | | | | |
|---|---|---|---|---|---|---|
| Tissue | 0.167 h | 1 h | 4 h | 12 h | 48 h | 168 h |
| Adrenal gland(s) | 1720 | 941 | 518[a] | ND | ND | ND |
| Bile | 255000 | ND | ND | ND | ND | ND |
| Blood | 5360 | 1060 | 583 | 131 | ND | ND |
| Bone | 162 | BLQ | 144 | BLQ | ND | ND |
| Bone marrow | 1180 | 1020 | 508 | 114 | ND | ND |
| Brain choroid plexus | 718 | 132 | ND | ND | ND | ND |
| Cecum | 2070 | 985 | 12000 | ND[b] | ND | ND |
| Diaphragm | 1800 | 1240 | 622 | 230 | BLQ | ND |
| Epididymis | 2700[a] | 626[a] | 451[a] | 119a | ND | ND |
| Esophagus | 3330 | 1260 | 921 | 255 | ND | ND |
| Eye uveal tract | 2780 | 1330 | 639 | 578 | 144 | ND |
| Eye(s) | 694 | 277 | 219 | 119 | BLQ | ND |
| Fat (brown) | 1020 | 583 | 327 | 119 | ND | ND |
| Kidney(s) | 62400 | 134000 | 119000 | 55900 | 7810 | 407 |
| Liver | 47500 | 34200 | 32200 | 6830 | 392 | BLQ |
| Lung(s) | 3670 | 991 | 552 | 167 | ND | ND |
| Lymph node(s) | 2190 | 1370 | 585 | 141 | ND | ND |
| Muscle | 719 | 979 | 454 | 144 | BLQ | ND |
| Myocardium | 2030 | 1270 | 798 | 256 | ND | ND |
| Nasal turbinates | 653 | 410 | 202 | ND | ND | ND |
| Pancreas | 2560 | 2030 | 1240 | 419 | BLQ | ND |
| Preputial gland | 1610[a] | 399[a] | 243[a] | ND[a] | ND | ND |
| Prostate gland | 1670 | 860 | 581 | 124 | ND | ND |
| Seminal vesicle(s) | 731 | 147[a] | 134 | BLQ[a] | ND | ND |
| Skin (pigmented) | 4560 | 1000 | 730 | 626 | 664 | 316 |
| Spleen | 1120 | 1180 | 560 | 165 | ND | ND |
| Stomach | 3170 | 1220 | 817 | 254 | BLQ | ND |
| Testis(es) | 1030 | 386 | 395 | BLQ | ND | ND |
| Thyroid | 1930 | 1170 | 671 | ND | ND | ND |
| Urinary bladder | 7890 | 6240 | 11100 | ND[b] | ND | ND |
| Urine | 288000 | 95000 | 45400 | 31500 | 1060 | ND |

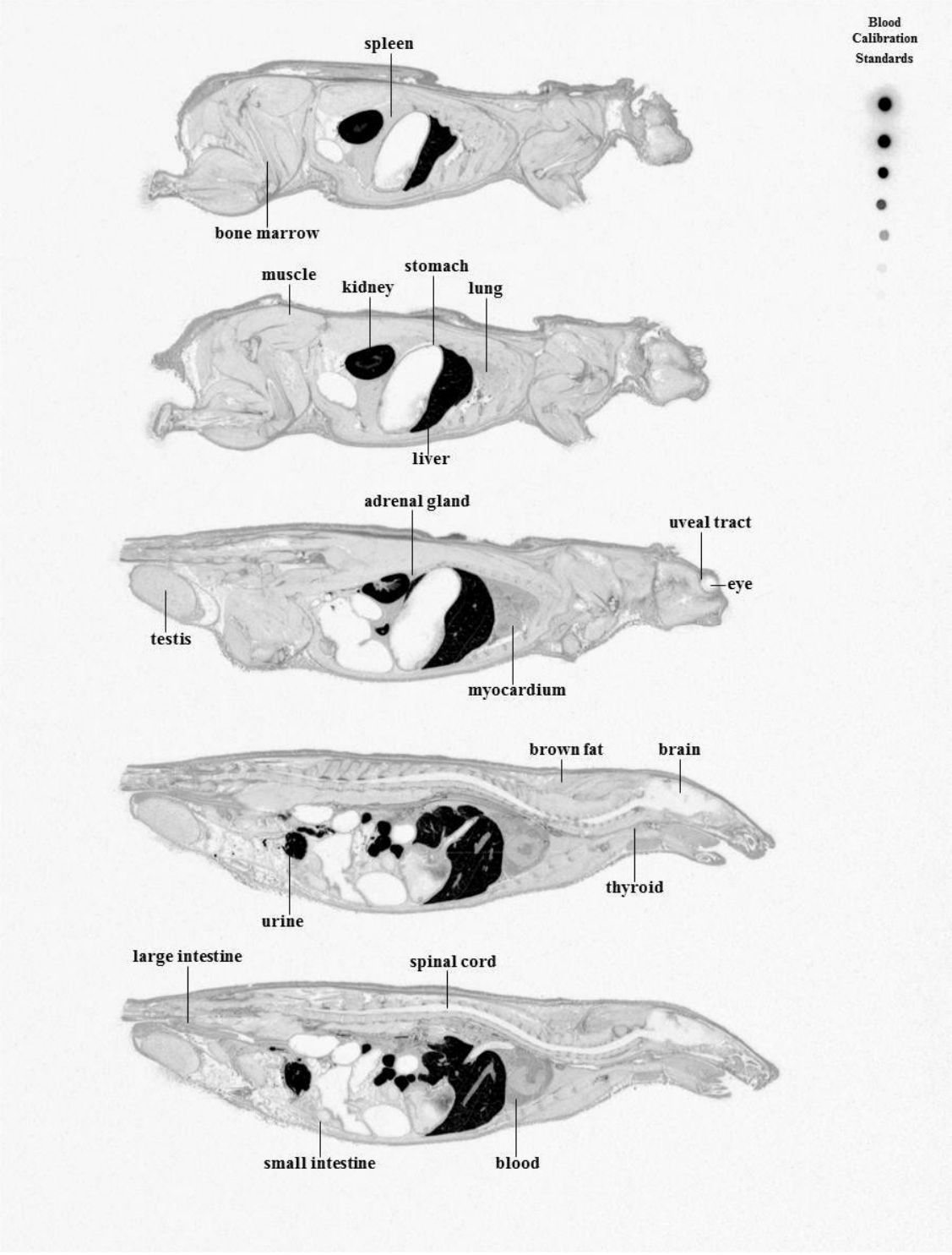
BLQ = Below the limit of quantitation (<112 ng equivalents $^{14}$C-GS-5734/g); h = hours; ND = not detectable (sample shape not discernible from background or surrounding tissue)

a Tissue appeared to be fat-soaked;

b Tissue was not detectable due to flare from gastrointestinal contents/urine

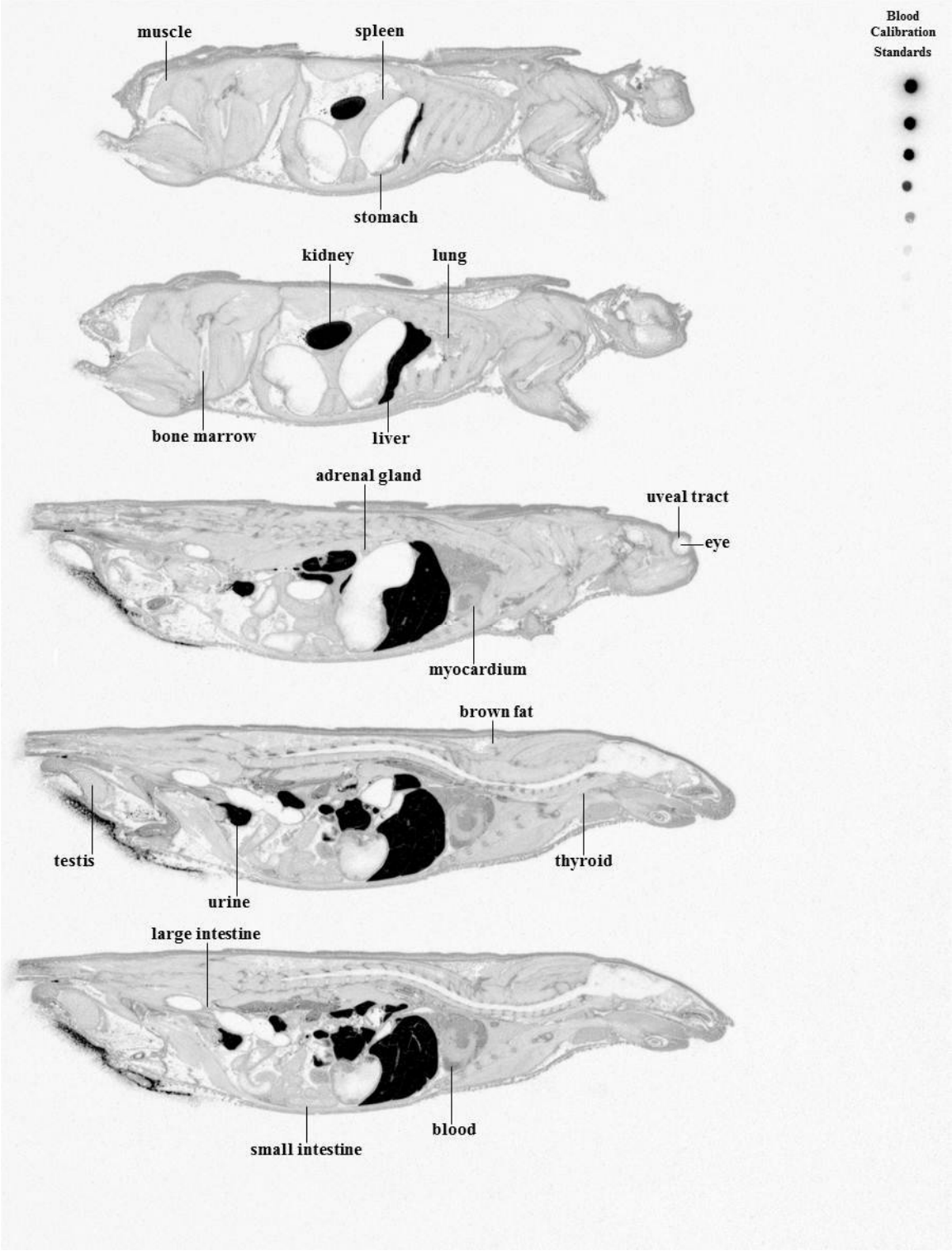
**Source:** AD-399-2017

**Figure 14. Representative Quantitative Whole-body Autoradiograph for Male Sprague Dawley Animal No. B50194 0.167 Hours After a Single Intravenous Administration of [$^{14}$C]Remdesivir (10 mg/kg)**




Source: AD-399-2017

**Figure 15. Representative Quantitative Whole-body Autoradiograph for Male Long Evans Animal No. B50205 0.167 Hours After a Single Intravenous Administration of [$^{14}$C]Remdesivir (10 mg/kg)**




Source: AD-399-2017

### 5.2.2.2. Monkey

The distribution of remdesivir was also determined by liquid scintillation counting following a single IV administration of 10 mg/kg [$^{14}$C]remdesivir to male cynomolgus monkeys (m2.6.5, Section 5.5; AD-399-2019). A total of 38 tissues were examined and results from select tissues are shown in Table 10; a complete listing is provided in m2.6.5, Section 5.5. Tissues showing the highest mean concentrations of radioactivity at 4 hours postdose, excluding GI tract, were gall bladder, kidneys, liver, prostate gland, salivary gland (mandibular), pancreas, and seminal vesicle(s). Notably, appreciable levels of radioactivity were also found in lung tissue, while some of the lowest levels were seen in bone, brain, eye and testis(es). Total radioactivity declined over 168 h; elimination of radioactivity was not complete and radioactivity was still quantifiable in most tissues. At 168 hours, a mean of 8.26% of the administered dose was retained in the tissues, mostly in liver and muscle.

**Table 10. Mean Concentrations of Radioactivity in Blood and Select Tissues Determined After a Single Intravenous Administration of [$^{14}$C]Remdesivir to Male Cynomolgus Monkey at 10 mg/kg (n = 3)**

| Tissues/Organs ↓ | Mean Tissue Concentration of Radioactivity (ng Equivalents [$^{14}$C]GS-5734/g tissue) | |
|---|---|---|
| | 4 h | 168 h |
| Adrenal gland(s) | 2470 | 81.9 |
| Bile (from gall bladder) | 359000 | 513 |
| Blood (sac) | 750 | 87.4 |
| Bone (femur) | 291 | NA |
| Bone marrow (femur) | 1610 | NA |
| Brain | 99.3 | 114 |
| Cerebrospinal fluid | NA | NA |
| Epididymis | 1430 | NA |
| Eye(s) | 378 | NA |
| Gall bladder | 123000 | 393 |
| Heart | 4920 | 400 |
| Kidney (s) | 72900 | 2400 |
| Large intestine | 15400 | 157 |
| Liver | 67900 | 2880 |
| Lungs | 3540 | 293 |
| Lymph node(s), axillary | 5730 | NA |
| Muscle (biceps femoris) | 2460 | 1460 |
| Pancreas | 12800 | 222 |
| Pituitary gland | 7290 | 243 |

| Tissues/Organs ↓ | Mean Tissue Concentration of Radioactivity (ng Equivalents [$^{14}$C]GS-5734/g tissue) | |
|---|---|---|
| | **4 h** | **168 h** |
| Plasma (sac) | 822 | 76.1 |
| Prostate gland | 14300 | 338 |
| Salivary gland | 13800 | 424 |
| Seminal vesicle(s) | 12800 | 160 |
| Skin (dorsal, shaved) | 1570 | NA |
| Small intestine | 5670 | 174 |
| Spinal cord | NA | NA |
| Spleen | 7510 | 523 |
| Stomach | 4740 | 253 |
| Testis(es) | 1060 | NA |
| Thymus | 2960 | 83.6 |
| Thyroid (including parathyroid) | 3100 | 399 |
| Urinary bladder | 6110 | 225 |

BLQ = below the limit of quantitation (<112 ng equivalents $^{14}$C-GS-5734/g); h = hours; NA = not applicable
Source: AD-399-2019

# 6. METABOLISM (INTERSPECIES COMPARISON)

## 6.1. *In Vitro* Metabolism

The metabolism of remdesivir has been characterized through *in vitro* studies. The interaction of remdesivir with pathways commonly associated with the metabolism of xenobiotics has been characterized.

### 6.1.1. Plasma Stability

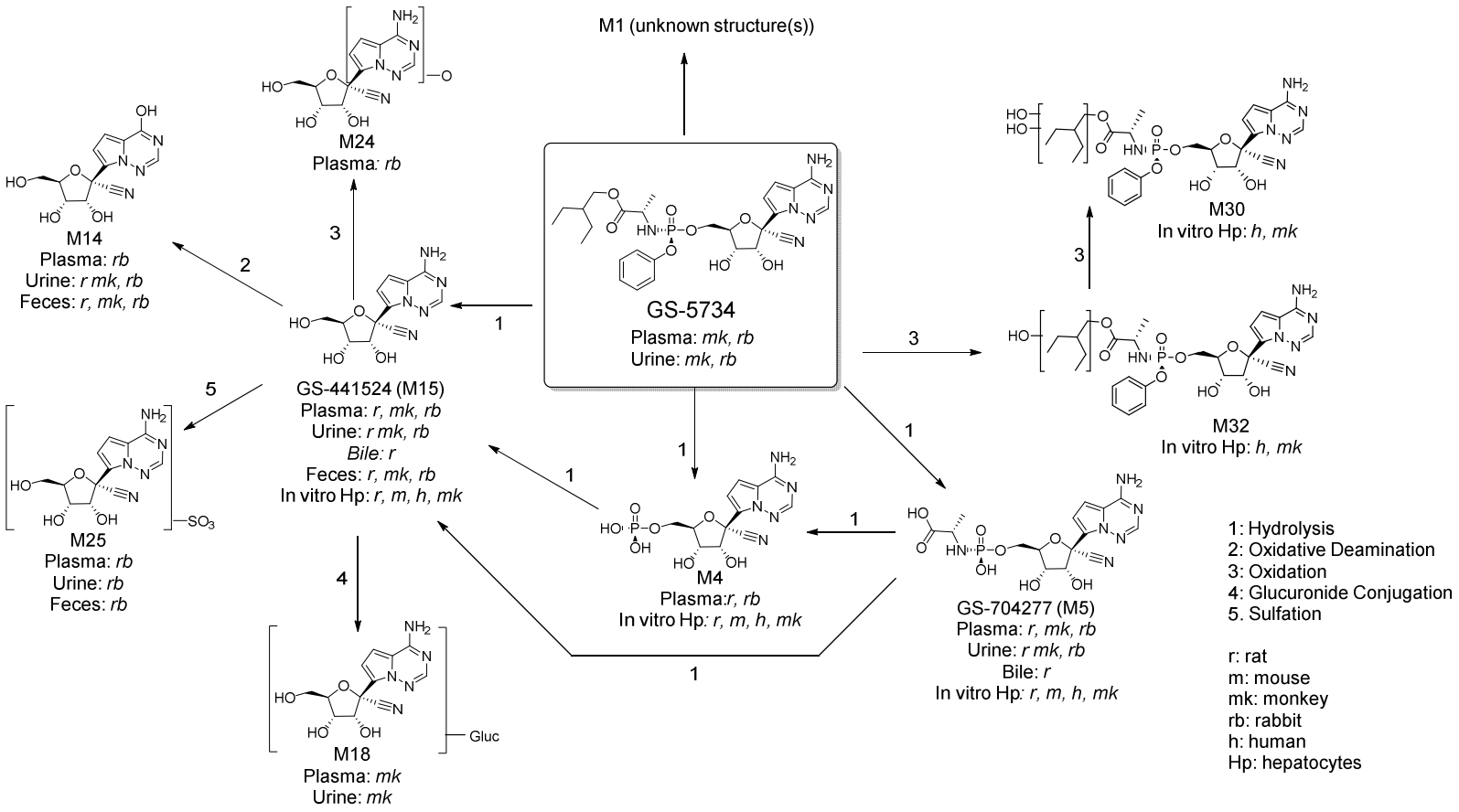
The stability of remdesivir in plasma from Sprague-Dawley rat, beagle dog, cynomolgus monkey, rhesus monkey, and human was determined (m2.6.5, Section 7.1; AD-399-2012). Consistent with the presence of high esterase activity in plasma in many rodent species, remdesivir was unstable in rat plasma ($t_{1/2}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

### 6.1.2. Metabolic Stability

The metabolic stability of remdesivir was assessed in intestinal S9 from Sprague-Dawley rat, beagle dog, and human and hepatic S9 from Sprague-Dawley rat, beagle dog, cynomolgus monkey, rhesus monkey, and human (m2.6.5, Section 7.2; AD-399-2014). Across species, remdesivir was moderately stable in intestinal extract ($t_{1/2}$ = 40.3 - 114.1 min) but was unstable in hepatic extract ($t_{1/2} < 3.9$ min). Of the species tested, remdesivir was relatively stable in human intestinal S9 ($t_{1/2}$ = 114.1 min).

The metabolism of [$^{14}$C]remdesivir was assessed *in vitro* using mouse, rat, monkey, and human cryopreserved hepatocytes (m2.6.5, Section 7.6; AD-399-2024); identified metabolites are shown in Figure 16. [$^{14}$C]remdesivir was metabolized by mouse, rat, monkey, and human hepatocytes, primarily via hydrolysis. The rate of biotransformation in mouse and rat was faster relative to monkey and human at both 1 and 10 µM concentrations. The remaining unchanged [$^{14}$C]remdesivir accounted for <10% of the total radioactivity in samples across all the species at 120 minutes. In all four species, most of the [$^{14}$C]remdesivir-derived radioactivity was associated with three major metabolites; GS-704277, GS-441524, and GS-441524-phosphate generated via hydrolysis. GS-704277 was the predominant component.

**Figure 16. Proposed Biotransformation Pathways of Remdesivir [a]**



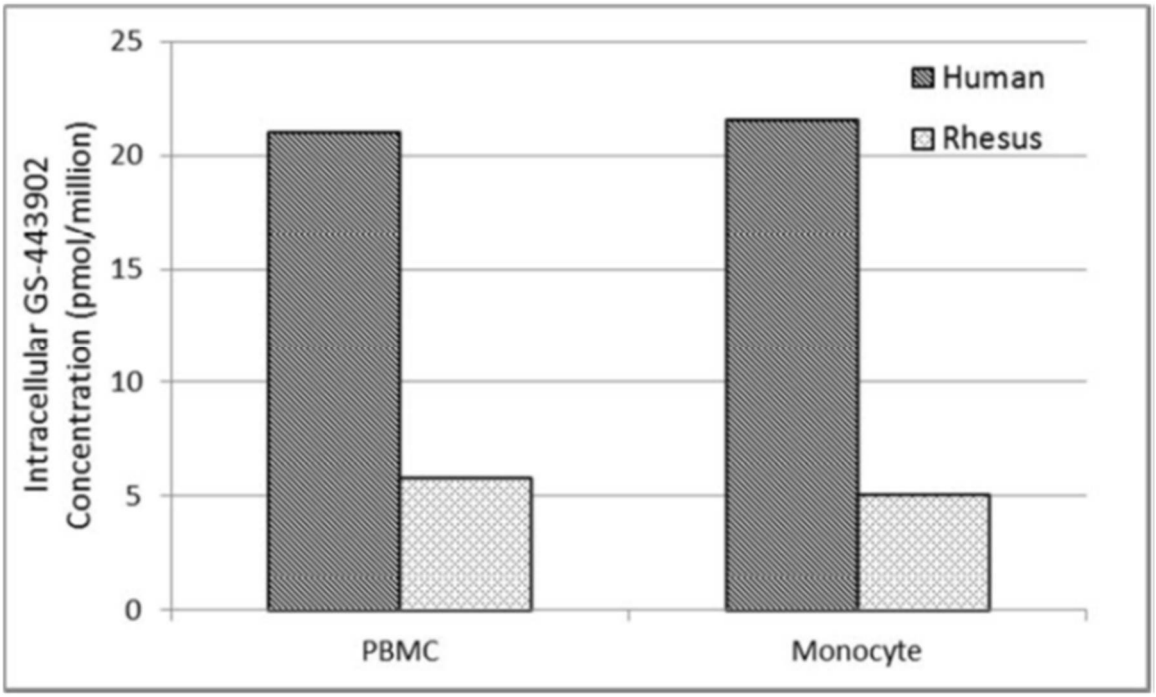

a The proposed pathways represent biotransformation products formed through one or more steps and are based on general knowledge of xenobiotic metabolism. The pathways were not established experimentally. Human - in vivo metabolites not listed; metabolite identification from the radiolabeled ADME study (GS-US-399-4231) in progress.

Source: M2.6.5, Section 8.1

### 6.1.3. Intracellular Metabolism

One of the main target tissues for SARS-CoV-2 is the lung, which is largely inaccessible for routine sampling. Therefore, a sampling surrogate could potentially be used to assess target distribution and metabolism. Circulating PBMCs may be appropriate as they are currently collected to assess active metabolite exposure and antiviral potency of nucleos(t)-ide analogs, or respective prodrugs, in human patients. To support nonclinical evaluations, and to assess potential differences in metabolism between monkey and human, the metabolism of remdesivir was compared in human and rhesus PBMC and monocytes (m2.6.5, Section 7.3; AD-399-2015). Intracellular triphosphate concentrations were higher in human PBMC (3.64-fold) and monocytes (4.24-fold) compared to respective monkey cell types (Figure 17) in vitro.

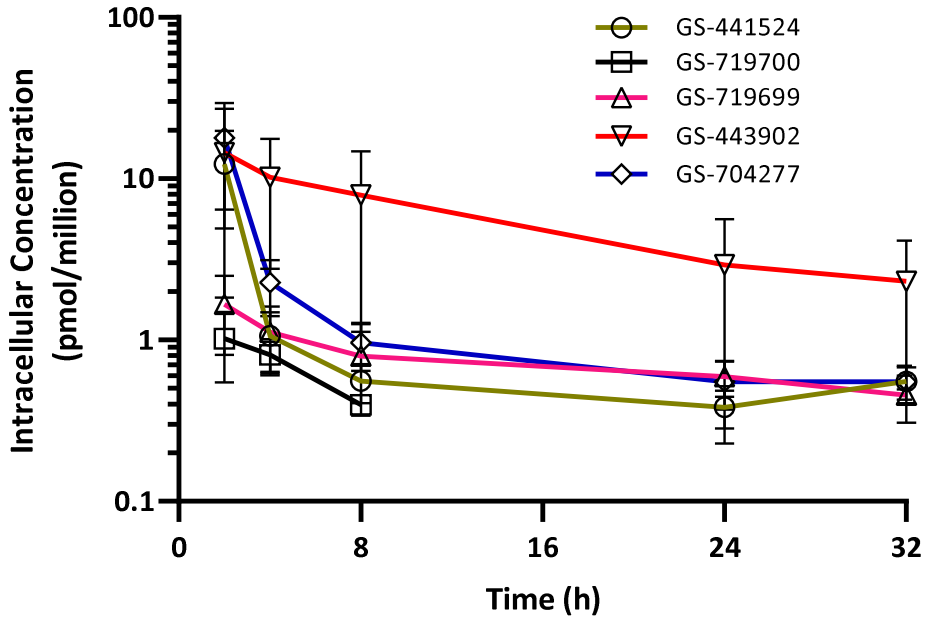
**Figure 17. Comparison of Intracellular GS-443902 Concentrations Formed in Peripheral Blood Mononuclear Cells and Monocytes from Human and Rhesus Monkey During Incubation with Remdesivir**




Concentrations measured following a 2 h incubation with 1 µM remdesivir. Values are the average of n = 2 donors with duplicate measurements in the cells from each donor.
Source: AD-399-2015

NHBE are representative target cells for SARS-CoV-2 infection. Therefore, the kinetics of loading of NHBE by remdesivir and subsequent metabolism to GS-441524 and its phosphorylated metabolites, followed by their elimination, were studied following a 2 h pulse incubation with 1 µM remdesivir, *in vitro* (m2.6.5, Section 7.8; AD-540-2001). The pulse incubation is designed to mimic the transient exposure to prodrug following IV administration. The pharmacologically active triphosphate analog, GS-443902, was efficiently formed in NHBE cells from three different donors (mean GS-443902 $C_{max}$ of 14.5 ± 12.7 pmol/million cells and persisted with a $t_{1/2}$ of approximately 15 h (Figure 18).

**Figure 18. Mean Intracellular Concentrations of GS-441524 and Metabolites in NHBE cells Following a 2-hour Continuous Incubation with Remdesivir (1 µM) and for up to 30 hours Following Removal from Extracellular Media (Mean, n=3 experiments)**




Calu-3 is a lung cell line which may be used to assess antiviral activity against SARS-CoV-2. An experiment was carried out to characterize GS-443902 concentrations following continuous incubation for 48 h with either 1 µM remdesivir or its nucleoside metabolite, GS-441524 at 10 µM in Calu-3 cells (m2.6.5, Section 7.9; AD-540-2002). GS-443902 was again efficiently formed intracellularly, with almost 3-fold higher levels following incubation with remdesivir compared to GS-441524, on a dose-normalized basis.

## 6.2. In Vivo Metabolism

*In vivo* biotransformation pathways described for the select species assessed below are also represented as described above in Figure 16.

Following IV administration of 10 mg/kg [$^{14}$C]remdesivir to intact and bile duct-cannulated (BDC) Sprague Dawley rats, most of the radioactivity in plasma, bile, and urine was associated with the nucleoside analog GS 441524 (m2.6.5, Section 6.1; AD-399-2018). No unchanged remdesivir was detected in any of these matrices. The other major systemic metabolite, GS-704277, was also an abundant component in plasma (Table 11) and bile, contributing approximately 19% and 20.3% of the total radioactivity exposure through 36 and 24 hours, respectively. A minor unidentified component, M1, contributed approximately 6% of the total radioactivity exposure through 36 hours. Desamino-hydroxy-GS-441524 was a major component in feces from intact rats, accounting for 22.6% of the dose through 48 hours.

Following IV administration of 10 mg/kg [$^{14}$C]remdesivir to monkeys, the nucleoside analog GS-441524 was the only circulating component in the AUC-pooled plasma sample and accounted for 100% of the total radioactivity exposure through 96 hours (Table 11; m2.6.5, Section 6.3; AD-399-2020). However, profiles of individual plasma samples showed radioactive peaks associated with remdesivir, GS-704277, GS-441524 and GS-441524-glucuronide. Less than 1% of the dose was recovered as unchanged remdesivir in urine in the first collection interval, 0 to 24 hours. GS-441524 and GS-441524-glucuronide were the major components in urine, accounting for 15.6% and 8.43% of the dose, respectively, through 72 hours postdose. No unchanged remdesivir was recovered in feces. Desamino-hydroxy-GS-441524 was the major component in feces, accounting for 19.9% of the dose through 72 hours.

Following IV administration of 10 mg/kg [$^{14}$C]remdesivir to rabbits, unchanged remdesivir contributed <1% of the total radioactivity through 96 hours in plasma (m2.6.5, Section 6.5; AD-399-2026). GS-441524 was the major circulating metabolite in plasma and accounted for approximately 45% and desamino-hydroxy-GS-441524 (M15) contributed approximately 25% of the total radioactivity exposure through 96 hours (Table 11), while profiles of individual plasma samples also showed peaks associated with GS-704277. Renal elimination was the major route of excretion, with approximately 64% of the dose recovered in urine through 72 hours postdose and less than 1% of the dose recovered as unchanged remdesivir. Hepatobiliary excretion was a minor route of elimination of radioactivity, with approximately 10% of radioactivity eliminated in feces after IV dosing through 72 hours postdose, with no unchanged parent detected.

**Table 11. Plasma Profile Following IV Administration of [$^{14}$C]Remdesivir**

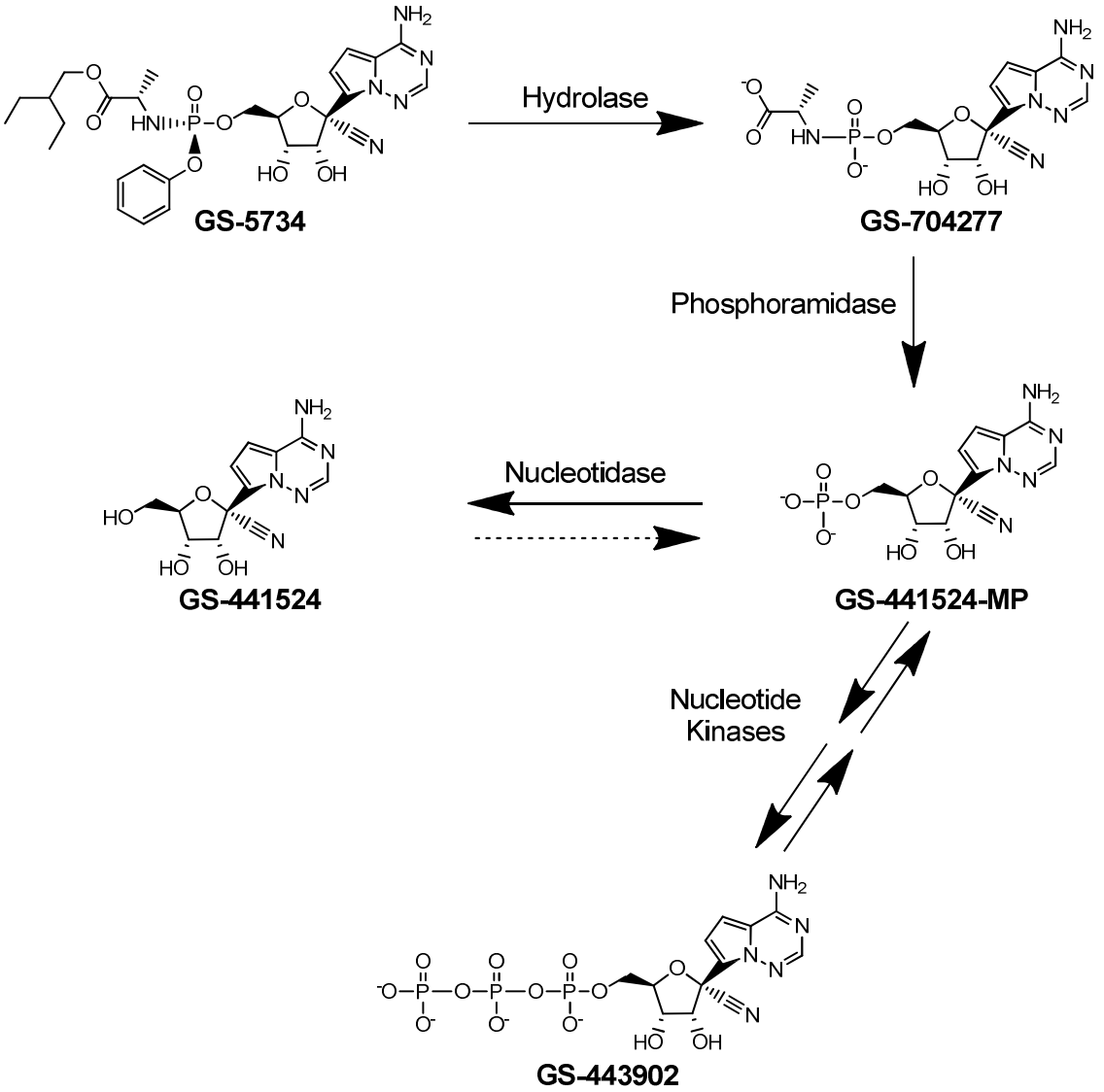
| Component | % of Total Radioactivity in AUC Pooled Plasma [a] | | | |
|---|---|---|---|---|
| | Rat | Rabbit | Monkey | Human |
| M1 | 6.03 | ND | ND | Ongoing Analysis |
| GS-704277 (M5) | 19.0 | ND [b] | ND [b] | |
| M14 | ND | 24.8 | ND | |
| GS-441524 (M15) | 70.3 | 44.5 | 100 | |
| M24 | ND | 8.03 | ND | |
| M25 | ND | 11.0 | ND | |
| Remdesivir | ND | 0.73 | ND [b] | |

ND = not detected or below the limit of quantitation
a AUC pool plasma = area under the plasma $^{14}$C concentration-time curve from time zero to 36 hours post dose in Sprague Dawley rats, from time zero to 96 hours post dose in rats, from time zero to 96 hours post dose in cynomolgus monkeys
b Profiles of individual plasma samples showed radioactive peaks associated with these metabolites
c Metabolite identification from the human ADME study (GS-US-399-4231) is on-going
Source: AD-399-2018, AD-399-2020, AD-399-2026

## 6.3. Intracellular Metabolic Pathways

Combined results from pharmacology and pharmacokinetic studies have led to the proposed intracellular metabolic pathway presented in Figure 19. Remdesivir is activated to the pharmacologically active nucleoside analog triphosphate, GS-443902, by a sequential metabolic activation pathway: (i) hydrolase activity removes the ester, resulting in the release of 2-ethyl-butanol; (ii) a spontaneous chemical step that releases phenol and forms the intermediate metabolite, GS-704277; (iii) phosphoramidase activity cleaves the phosphoramidate bond, liberating the nucleoside analog monophosphate and alanine; and (iv) nucleotide kinases likely catalyze the conversion to the active triphosphate, GS-443902. Dephosphorylation of the nucleoside analog monophosphate results in the formation of the nucleoside analog, GS-441524, that is not efficiently rephosphorylated.

**Figure 19. Intracellular Metabolic Pathway of Remdesivir**



# 7. EXCRETION

## 7.1. Rat

The routes and extent of remdesivir excretion were determined after IV administration of 10 mg/kg [$^{14}$C]remdesivir to Sprague-Dawley rats (m2.6.5, Section 10.1; AD-399-2017). Most of the radioactivity was excreted within 48 hours after IV administration to intact rats. Means of 63.0% and 27.8% of the administered radioactivity were excreted in urine and feces, respectively, by 168 hours postdose. After IV dosing in BDC rats, means of 63.4%, 22.7%, and 3.26% of the administered radioactivity were excreted in urine, bile, and feces, respectively, by 168 hours postdose, indicating renal and biliary excretion were the major routes of elimination of [$^{14}$C]-remdesivir-derived radioactivity. Mean overall recoveries of radioactivity after intravenous dosing to intact and bile duct-cannulated rats were 95.1 and 95.3%, respectively.

## 7.2. Rabbit

The routes and extent of remdesivir excretion were also determined after IV administration of 10 mg/kg [$^{14}$C]remdesivir to rabbits (m2.6.5, Section 10.4; AD-399-2025). Most of the [$^{14}$C]remdesivir-derived radioactivity was excreted after IV administration by 48 hours postdose, primarily in urine. Means of 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%.

## 7.3. Monkey

The routes and extent of remdesivir excretion were also determined after IV administration of 10 mg/kg [$^{14}$C]remdesivir to monkeys (m2.6.5, Section 10.3; AD-399-2019). By 168 hours postdose, means of 33.6% and 25.6% of the administered radioactivity were recovered in urine and feces, respectively, indicating that renal and biliary excretion were the major routes of elimination. Significant radioactivity was recovered in cage rinses, accounting for a mean of 16.9% of the dose. Overall mean recovery in monkeys was 78.8%.

# 8. PHARMACOKINETIC DRUG INTERACTIONS

## 8.1. Clinical Pharmacokinetic Parameters

Human plasma $C_{max}$ values for remdesivir, GS-704277 and GS-441524 are presented in Table 12 as reference values for comparison to relative enzyme or transporter interactions.

**Table 12. Plasma $C_{max}$ values for Remdesivir, GS-704277 and GS-441524 Following Daily Intravenous Administrations of Remdesivir in Healthy Humans (Mean (%CV))**

| | cohort 1 and 2 Day 1 (n=28) (200 mg, 30-minutes IV) | cohort 1 and 2 Day 5 and 10 (n=26) (100 mg QD, 30-minutes IV) |
|---|---|---|
| **Species** | **$C_{max}$ (ng/mL)** | |
| Remdesivir | 4380 (23.5) | 2230 (19.2) |
| GS-704277 | 370 (29.3) | 246 (33.9) |
| GS-441524 | 143 (21.5) | 69.2 (18.2) |

Source: (Study GS-US-399-5505)

## 8.2. Interactions with Enzymes

### 8.2.1. Enzymology of Metabolism

In order to determine if remdesivir is a substrate for CYP enzymes, remdesivir (5 µM) was incubated with seven individual cDNA expressed human CYP enzyme preparations co-expressed with human NADPH CYP reductase (m2.6.5, Section 11.1; AD-399-2011). Rates of CYP-mediated metabolism of remdesivir and control substrates are summarized in Table 13. There was no detectable metabolism of remdesivir by recombinant CYP1A2, 2B6, 2C9, or 2C19. Remdesivir was metabolized by CYP2C8, 2D6, and 3A4. The rate of CYP3A4 metabolism was considerably greater than CYP2C8 and 2D6 and was 42.1% of that of the positive control simvastatin.

**Table 13. Rates of Metabolism of Remdesivir by Cytochrome P450 Isoforms**

| Compound | Metabolism Rate ($min^{-1}$) | | | | | | |
|---|---|---|---|---|---|---|---|
| | CYP1A2 | CYP2B6 | CYP2C8 | CYP2C9 | CYP2C19 | CYP2D6 | CYP3A4 |
| Remdesivir (% Positive Control Rate) | < 0.12 (2.2%) | < 0.12 (< 12%) | 3.9 (16.1%) | < 0.47 (< 1.9%) | < 0.12 (< 0.6%) | 1.9 (16.4%) | 18.3 (42.1%) |
| Tacrine | 4.6 | NA | NA | NA | NA | NA | NA |
| Efavirenz | NA | 1.77[a] | NA | NA | NA | NA | NA |
| Amodiaquine | NA | NA | 24.5 | NA | NA | NA | NA |
| Diclofenac | NA | NA | NA | 17.2 | NA | NA | NA |
| Omeprazole | NA | NA | NA | NA | 21.8 | NA | NA |
| Dextromethorphan | NA | NA | NA | NA | NA | 11.6 | NA |
| Simvastatin | NA | NA | NA | NA | NA | NA | 43.5 |

NA = not applicable
Efavirenz is a selective substrate for CYP2B6 but is metabolized relatively slowly.
Source: AD-399-2011

### 8.2.2. Cytochrome P450 Inhibition

The potential for remdesivir to inhibit CYP enzymes was evaluated using human hepatic microsomes and monitoring specific CYP-mediated transformations of probe substrates (m2.6.5, Section 9.1 and 9.2; AD-399-2010; AD-540-2004). The results of the CYP inhibition study are provided in Table 14. Remdesivir was a weak inhibitor of CYP1A2, 2B6, 2C8, 2C9, 2C19, and 2D6. The most potent effects of remdesivir were upon CYP3A activities, with an $IC_{50}$ of 11.0 µM being determined with testosterone 6β-hydroxylase activity, and an $IC_{50}$ of 1.6 µM with midazolam 1'-hydroxylase. Further analysis showed that there was no evidence for remdesivir to be a mechanism-based inhibitor of CYP3A using the most sensitive activity.

Due to its weak potency, remdesivir is unlikely to cause clinical drug interactions through inhibition of human CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19 or CYP2D6. Effects upon CYP3A activity would be dependent upon plasma exposure and the plasma free fraction with clinical data summarized above suggesting limited interaction based on the transient exposure to low concentration of intact, unbound remdesivir following a 30-minute IV infusion.

**Table 14. $IC_{50}$ Values for Major Human CYP Enzymes for Remdesivir and Positive Control Inhibitors (Mean, n = 7 data points)**

| Enzyme | Activity | Calculated $IC_{50}$ (μM) | | % Inhibition by Remdesivir at 100 μM |
|---|---|---|---|---|
| | | Control[a] | Remdesivir | |
| CYP1A2 | Phenacetin O-deethylase | 0.15 | > 100 | 15.2% |
| CYP2B6 | Bupropion hydroxylase | 1.3 | 77.8 | 54.6% |
| CYP2C8 | Paclitaxel 6α-hydroxylase | 0.98 | 54.9 | 68.8% |
| CYP2C9 | Tolbutamide 4-hydroxylase | 0.66 | 63.3 | 60.3% |
| CYP2C19 | S Mephenytoin 4'-hydroxylase | 7.7 | 68.3 | 64.3% |
| CYP2D6 | Dextromethorphan O-demethylase | 0.05 | 73.0 | 61.0% |
| CYP3A | Midazolam 1'-hydroxylase | 0.04 | 1.6 | 87.7% |
| | Testosterone 6β-hydroxylase | 0.21 | 11.0 | 88.8% |

a Control Inhibitors: CYP1A2 α-Naphthoflavone (0–3 μM); CYP2B6 Ticlopidine (0-25 μM); CYP2C8 Montelukast (0–10 μM); CYP2C9, Sulfaphenazole (0–10 μM); CYP2C19, Tranylcypromine (0–50 μM); CYP2D6, Quinidine (0–3 μM); CYP3A, Ketoconazole (0–3 μM).
Source: AD-399-2010; AD-540-2004

### 8.2.3. UGT Inhibition

The potential for remdesivir to inhibit the catalytic activity of human UGT1A1 was also assessed (m2.6.5, Section 9.3; AD-540-2005). The rate of enzyme-specific metabolite formation from estradiol, the probe substrate, was quantified in the presence and absence of test compound and the $IC_{50}$ value was determined. Remdesivir inhibited UGT1A1 activity with an $IC_{50}$ of 9.78 μM.

## 8.3. Assessment of Induction Liability

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

## 8.4. Interactions with Transporters

### 8.4.1. Transporter Substrate

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

non-transfected and OATP1B3-transfected cells and not significantly changed in the presence of rifampicin.

Remdesivir was assessed as a substrate for the efflux transporters P-gp and BCRP (m2.6.5, Section 11.2; AD-399-2007). Remdesivir was found to be a substrate for P-gp but not BCRP based on the changes in its permeability observed in P-gp but not BCRP over-expressing MDCKII cells. Consistent with P-gp dependent transport, the efflux ratio of remdesivir decreased in the presence of the P-gp inhibitor cyclosporin A in P-gp over-expressing cells. In contrast the efflux ratio observed for remdesivir remained similar in the presence and absence of BCRP inhibitor Ko143 in BCRP over-expressing cells.

In cells overexpressing human organic anion transporters (OAT) 1 and 3, no evidence for transport of remdesivir and its major systemic metabolites, GS-704277 and the nucleoside analog GS-441524, was observed (m2.6.3, Section 3; PC-399-2020). Similarly, rat OAT1 did not transport remdesivir or its metabolites, and rat OAT3 did not transport remdesivir or GS-441524. In contrast to human OAT3, cells overexpressing rat OAT3 were observed to transport GS-704277 and showed increased cytotoxicity compared to mock-transfected control cells. Concomitant with the increase in GS-704277 transport, significant increases in GS-443902 accumulation were also observed in these cells, compared to those overexpressing human OAT3.

### 8.4.2. Transporter Inhibition

The potential for remdesivir to inhibit drug transporters has been assessed *in vitro* in transfected cell lines expressing OATP1B1, OATP1B3, Pgp, and BCRP (m2.6.5, Section 11.4; AD-399-2005). Inhibition constants for tested transporters are presented in Table 15. Remdesivir did not inhibit P-gp transport of calcein AM or BCRP transport of pheophorbide A at the highest concentration tested (40 µM). Remdesivir inhibited OATP1B1- and OATP1B3-dependent transport of Fluo-3, with $IC_{50}$ values of 2.8 and 2.1 µM, respectively. The potential for clinically significant drug-drug interactions caused by inhibition of OATP1B1 and OATP1B3 is limited by the transient exposure to low concentration of intact, unbound remdesivir following IV infusion.

**Table 15. Inhibition of Transporters by Remdesivir**

| Transporter | Maximum Inhibition (Concentration) | $IC_{50}$ (µM) |
|---|---|---|
| P-gp | 0 (40 µM) | > 40 |
| BCRP | 0 (40 µM) | > 40 |
| OATP1B1 | 88.5% (40 µM) | 2.8 |
| OATP1B3 | 95.2% (40 µM) | 2.1 |

AD-399-2005

Studies were also performed to investigate whether remdesivir or its major systemic metabolites, GS-704277 and the nucleoside analog GS-441524, are inhibitors of human bile salt export pump (BSEP), multidrug resistance-associated protein 2 (MRP2), MRP4, and sodium-taurocholate co-transporting polypeptide (NTCP) transporters using model substrates and transfected cell lines or membrane vesicles (m2.6.5, Section 11.6; AD-399-2029). Remdesivir inhibited BSEP-,

MRP4- and NTCP-mediated probe substrate transport, with calculated $IC_{50}$ values of 22, 5.1, and 72 µM, respectively. No interaction of remdesivir with MRP2 was observed at up to 100 µM. GS-704277 showed 25% and 44% inhibition of MRP2- and NTCP-mediated transport, respectively, at the 100 µM test concentration. No interaction of GS-704277 with BSEP or MRP4 was observed at up to 100 µM. GS-441524 showed 24% inhibition of NTCP-mediated transport at the 100 µM test concentration. No interaction of GS-441524 with BSEP, MRP2, or MRP4 was observed at up to 100 µM.

Remdesivir metabolites GS-441524, GS-719700 (GS-441524-MP), GS-719699 (GS-441524-DP), GS-443902 and GS-704277 were investigated for interaction with the human BCRP, BSEP, MRP2 and MRP3 efflux transporters in the vesicular transporter inhibition assay (m2.6.5, Section 11.5; AD-399-2035). Transporter inhibition was only observed in the case of GS-719699 and GS-443902. GS-719699 inhibited BCRP (26%), while GS-443902 had an inhibitory effect on MRP2 (24%), both at 200 µM, thus no IC50 values were calculated. The remaining transporters were not affected by these test articles. GS-719700, GS-704277 and GS-441524 did not have an effect on the investigated transporters.

## 8.5. Drug-Drug Interaction Liability Assessment

The liability for remdesivir to cause pharmacokinetic drug interactions was assessed using current FDA Guidelines ({U. S. Department of Health & Human Services (DHHS) 2020}; and m2.6.5, Section 11.7; AD-540-2006) and representative clinical pharmacokinetic data (Table 12). Due to instability of remdesivir in microsomal fractions during dialysis the microsomal binding was estimated using an in silico model ($f_{u,m}$ 45.3% at a microsomal concentration of 0.5 mg/mL). Inhibitory effects upon enzyme activities were determined using the Basic model and the Mechanistic Static model (Table 16). For the mechanistic model remdesivir is predicted to not be a clinically relevant inhibitor of CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6 or UGT1A1. Remdesivir is a weak inhibitor of CYP3A enzymes. The pharmacokinetic half-life of remdesivir is < 1 h, so concentrations would lie in the inhibitory range for < 2.5 h post-infusion on dosing Day 1 and < 1.5 h on subsequent days.

**Table 16. Regulatory Calculations for Enzyme Inhibition by Remdesivir**

| Enzyme | $K_{i,u}$ (µM) | Basic Model | Mechanistic Static Model | |
|---|---|---|---|---|
| | | $R_1$ | Hepatic Component | AUCR |
| CYP1A2 | > 32.3 | **< 1.027** | > 0.97 | < 1.02 |
| CYP2B6 | 31.8 | **1.028** | 0.97 | 1.02 |
| CYP2C8 | 15.0 | **1.059** | 0.94 | 1.05 |
| CYP2C9 | 12.6 | **1.070** | 0.93 | 1.06 |
| CYP2C19 | 21.3 | **1.041** | 0.96 | 1.04 |
| CYP2D6 | 17.8 | **1.049** | 0.95 | 1.05 |
| CYP3A M | 0.6 | **2.445** | 0.41 | **2.11** / **1.61**[a] |
| CYP3A T | 2.4 | **1.367** | 0.73 | **1.31** / 1.16[a] |
| UGT1A1 | 3.1 | **1.285** | 0.78 | 1.22 |

Values potentially exceeding the threshold are in **bold**
a Value calculated using Day 1 $C_{max}$ / Value calculated using Day 5 $C_{max}$

Remdesivir exhibited little effect as an inducer in the hepatocyte assay, with no induction effect upon CYP1A2, CYP2B6 or CYP3A activities at concentrations up to 25 µM (the cytotoxicity limit). Remdesivir also had no effect upon CYP3A4 mRNA. Remdesivir was negative for effects upon CYP1A2 and CYP2B6 in two of the three donors and weakly positive in the third (Donor 1). $EC_{50}$ values for remdesivir for CYP1A2 and CYP2B6 mRNA were estimated using data solely from Donor 1 (the most sensitive) and the Basic model and Mechanistic Static model calculations performed (Table 17). Using the mechanistic model remdesivir is predicted to have no liability as an inducer.

**Table 17. Summary of Induction Liability Calculations for Remdesivir Using mRNA Results from the Most Sensitive Hepatocytes (Donor 1)**

| | CYP1A2 | CYP2B6 |
|---|---|---|
| $EC_{50,u}$ (µM) | 83.9 | 80.6 |
| $E_{max}$ (fold over control) | 22.5 | 20.1 |
| $R_3$ | **0.32** | **0.34** |
| Hepatic component | 1.23 | 1.22 |
| AUCR | 0.84 | 0.88 |

Values potentially exceeding the associated threshold are in **bold**

The inhibitory effects of remdesivir upon transporters was assessed and the data are summarized in Table 18. At systemic plasma concentrations achieved following intravenous infusion dosing, remdesivir has no liability as an inhibitor of P-gp and BCRP but is predicted to be a weak inhibitor of OATP1B1 and OATP1B3. As noted above, the short pharmacokinetic half-life of remdesivir means that inhibitory concentrations would only last < 2.5 h post-infusion on dosing Day 1 and < 1.5 h on subsequent days.

**Table 18. Guidance Calculation Results for Transporter Interactions for Remdesivir**

| Transporter | $IC_{50,u}$ (µM) | $K_{i,u}$ (µM) | Guidance Metric |
|---|---|---|---|
| P-gp | > 40 | > 40 | < 0.022 |
| BCRP | > 40 | > 40 | < 0.022 |
| OATP1B1 | 2.8 | 2.8 | **0.31** |
| OATP1B3 | 2.1 | 2.1 | **0.42** |

Values potentially exceeding the respective threshold are in **bold**

In conclusion, the only drug interaction liabilities identified for remdesivir are inhibition of CYP3A, OATP1B1 and OATP1B3. The inhibitory effects are weak and, due to the short half-life of remdesivir, the effects would only be manifest briefly. Further evaluation of GS-704277 and GS-441524 for possible interactions with drug metabolizing enzymes and transporters is ongoing.

# 9. OTHER PHARMACOKINETIC STUDIES

There are no other pharmacokinetic studies to include.

# 10. DISCUSSION AND CONCLUSIONS

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

Intracellular metabolism studies conducted *in vitro* illustrated effective activation in NHBE and Calu-3, relevant cell types for SARS-CoV-2 infection. Additionally, high plasma stability in non-rodent species demonstrated sufficient systemic exposure to intact prodrug to load target tissues and cells supporting SARS-CoV-2 replication. *In vivo* studies performed in cynomolgus monkey also showed distribution of [$^{14}$C]remdesivir-equivalents to the lungs, which are a target tissue. Additional distribution studies confirmed rapid delivery and efficient formation of the active triphosphate metabolite in marmoset and African green monkey lungs following intravenous administration of remdesivir. Finally, extensive studies in nonhuman primates demonstrated the utility of monitoring the active triphosphate in PBMC as an accessible surrogate for exposure in target tissues. The half-life of the pharmacologically active triphosphate GS-443902 observed *in vitro* in NHBE (15 h) and confirmed *in vivo* in marmoset lung and PBMC following IV administration (at least 22 h) supports once daily administration of remdesivir. Collectively, these results corroborate utility of remdesivir as an optimal prodrug for parenteral delivery, and in the context of a rapidly progressing viral infection, IV administration may be preferred.

While remdesivir is a substrate for cytochrome P450 (CYP) 2C8, 2D6, 3A4 *in vitro*, coadministration with inhibitors of these CYP isoforms is unlikely to markedly increase remdesivir levels as its metabolism is predominantly mediated by hydrolase activity. Remdesivir is a substrate for the organic anion transporter 1B1 (OATP1B1) and P-glycoprotein (P-gp). However, the impact of inhibition of these transporters on remdesivir disposition is likely minimized by the parenteral route of administration. Remdesivir is an inhibitor of CYP3A, OATP1B1 and OATP1B3 *in vitro* but its potential to be the perpetrator of clinically significant drug-drug interactions is limited by its rapid clearance. No potential of a clinical induction risk for remdesivir, GS-704277 or GS-441524 was observed in human hepatocyte assays. The once daily administration and short duration of remdesivir treatment may allow for temporary dose modification of other drugs as needed.

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

# 11. TABLES AND FIGURES

Tables and figures have been integrated into the summary above.

# 12. REFERENCES

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# SECTION 2.6.5—PHARMACOKINETICS TABULATED SUMMARY

REMDESIVIR (GS-5734™)

**Gilead Sciences**

[redacted] **2020**

**CONFIDENTIAL AND PROPRIETARY INFORMATION**

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## NOTE TO REVIEWER

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

**Table 1. Description of GS-5734 and its Diastereomers and Metabolites Referenced in the Text**

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

# 1. PHARMACOKINETICS: OVERVIEW

**Test Article: GS-5734**

| Type of Study / Description | GLP[a] | Test System | Method of Administration | Testing Facility | Gilead Study No. (CRO Study No.) |
|---|---|---|---|---|---|
| Analytical Methods and Validation | | | | | |
| Method Validation | Yes | Rat | *In Vitro* | [redacted], USA | BA-399-2002 |
| Method Validation | Yes | Monkey | *In Vitro* | [redacted], USA | BA-399-2003 |
| Method Validation | Yes | Rabbit | *In Vitro* | [redacted], USA | BA-399-2004 |
| Method Validation | Yes | Rat | *In Vitro* | [redacted], USA | BA-399-2006 |
| Method Validation | Yes | Monkey | *In Vitro* | [redacted], USA | BA-399-2007 |
| Absorption | | | | | |
| Single Dose Pharmacokinetics | No | Rhesus Monkey | IM | [redacted], USA | AD-399-2016 |
| Single Dose Pharmacokinetics | No | Wistar Han Rats | IV | [redacted], USA | AD-399-2003 |
| Single Dose Pharmacokinetics | No | Cynomolgus Monkey | IV | [redacted], USA | AD-399-2001 |
| Single Dose Pharmacokinetics | No | Rhesus Monkey | IV | [redacted], USA | AD-399-2002 |
| Single Dose Pharmacokinetics | No | Rhesus Monkey | IV | [redacted], USA | AD-399-2022 |
| Single Dose Pharmacokinetics | No | Cynomolgus Monkey | IV | [redacted], USA | AD-399-2033 |

**Test Article: GS-5734**

| Type of Study / Description | GLP[a] | Test System | Method of Administration | Testing Facility | Gilead Study No. (CRO Study No.) |
|---|---|---|---|---|---|
| Distribution | | | | | |
| Plasma Protein Binding | No | Wistar Han rat, cynomolgus monkey, rhesus monkey, and human | *In Vitro* | Gilead Sciences, Inc., Foster City, CA, USA | AD-399-2013 and AD-399-2031 |
| Tissue distribution | No | Sprague Dawley Rat | IV | [redacted], USA | AD-399-2017 |
| Tissue distribution | No | Cynomolgus Monkey | IV | [redacted], USA | AD-399-2019 |
| Single Dose Pharmacokinetics | No | Marmosets | IV | [redacted], USA | AD-399-2023 |
| Single Dose Pharmacokinetics | No | Rhesus Monkey | IV | [redacted], USA | AD-399-2028 |
| Repeat Dose Pharmacokinetics | No | Rhesus Monkey | IV | [redacted], USA | AD-399-2030 |
| Single Dose Pharmacokinetics | No | African Green Monkey | IV | [redacted], USA | AD-540-2003 |
| Blood/plasma ratio | No | Blood from rhesus monkeys and human | *In Vitro* | [redacted], UK | AD-540-2007 (CYP0174-R468) |
| Metabolism | | | | | |
| Metabolite identification and profiling of radioactivity | No | Sprague-Dawley rat | IV | [redacted], USA | AD-399-2018 |
| Metabolite identification and profiling of radioactivity | No | Cynomolgus Monkey | IV | [redacted], USA | AD-399-2020 |
| Metabolite identification and profiling of radioactivity | No | New Zealand White Rabbit | IV | [redacted], USA | AD-399-2026 |
| Plasma Stability | No | Sprague-Dawley rat, beagle dog, cynomolgus monkey, rhesus monkey, and human | *In Vitro* | Gilead Sciences, Inc., Foster City, CA, USA | AD-399-2012 |

**Test Article: GS-5734**

| Type of Study / Description | GLP[a] | Test System | Method of Administration | Testing Facility | Gilead Study No. (CRO Study No.) |
|---|---|---|---|---|---|
| Hepatic and Intestinal S9 Stability | No | Sprague-Dawley rat, beagle dog, cynomolgus monkey, rhesus monkey, and human | *In Vitro* | Gilead Sciences, Inc., Foster City, CA, USA | AD-399-2014 |
| Metabolism in PBMCs and Monocytes | No | Human | *In Vitro* | Gilead Sciences, Inc., Foster City, CA, USA | AD-399-2015 |
| Metabolism in Macrophages | No | Human | *In Vitro* | Gilead Sciences, Inc., Foster City, CA, USA | AD-399-2004 |
| Metabolism in Macrophages, HMVECs, and HeLa cells | No | Human | *In Vitro* | Gilead Sciences, Inc., Foster City, CA, USA | AD-399-2006 |
| Metabolism in Hepatocytes | No | C57BL6 Mouse, Wistar Han Rat, Cynomolgus Monkey, and Human Primary Hepatocytes | *In Vitro* | [redacted], USA | AD-399-2024 |
| Metabolism in NHBE | No | Human | *In Vitro* | Gilead Sciences, Inc., Foster City, CA, USA | AD-540-2001 |
| Metabolism in Calu-3 cells | No | Human | *In Vitro* | Gilead Sciences, Inc., Foster City, CA, USA | AD-540-2002 |
| Excretion | | | | | |
| Excretion of radioactivity | No | Sprague Dawley Rat | IV | [redacted], USA | AD-399-2017 |
| Excretion of radioactivity | No | Cynomolgus Monkey | IV | [redacted], USA | AD-399-2019 |
| Excretion of radioactivity | No | New Zealand White Rabbit | IV | [redacted], USA | AD-399-2025 |
| Pharmacokinetic Drug Interactions | | | | | |
| CYP Phenotyping | No | Recombinant human CYP enzymes | *In Vitro* | [redacted], UK | AD-399-2011 |
| Cytochrome P450 Inhibition | No | Human hepatic microsomal fraction | *In Vitro* | [redacted], UK | AD-399-2010 |

**Test Article: GS-5734**

| Type of Study / Description | GLP[a] | Test System | Method of Administration | Testing Facility | Gilead Study No. (CRO Study No.) |
|---|---|---|---|---|---|
| Hepatocyte Induction | No | Human hepatocytes | *In Vitro* | [redacted] | AD-399-2027 (8344953) |
| Effect on P-gp and BCRP | No | Human | *In Vitro* | Gilead Sciences, Inc., Foster City, CA, USA | AD-399-2007 |
| Substrate Potential on OATP1B1 and OATP1B3 | No | Human | *In Vitro* | Gilead Sciences, Inc., Foster City, CA, USA | AD-399-2008 |
| Inhibition Potential on OATP1B1 and OATP1B3 | No | Human | *In Vitro* | Gilead Sciences, Inc., Foster City, CA, USA | AD-399-2005 |
| Inhibition Potential on BCRP, BSEP, MRP2 and MRP3 | No | Human | *In Vitro* | [redacted], Hungary | AD-399-2035 |
| Inhibition Potential on BSEP, MRP2, MRP4, and NTCP | No | Human | *In Vitro* | [redacted], Hungary | AD-399-2029 |
| Cytochrome P450 Inhibition | No | Human hepatic microsomal fraction | *In Vitro* | [redacted], UK | AD-540-2004 (CYP0174-R333) |
| UGT1A1 Inhibition | No | Recombinant human UGT1A1 | *In Vitro* | [redacted], UK | AD-540-2005 |
| DDI report | No | In Vitro | *In Vitro* | Gilead Sciences, Inc., Foster City, CA, USA | AD-540-2006 |
| Other Pharmacokinetic Studies | | | | | |
| None | — | — | — | — | — |

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

# 2. PHARMACOKINETICS: ANALYTICAL METHODS AND VALIDATION REPORTS

**Location in CTD: 4.2.2.1**

| Type of Study | Test Articles | GLP[a] | Test System | Analytical Method | Testing Facility | Study Number |
|---|---|---|---|---|---|---|
| Method Validation | GS-5734<br>GS-441524 | Yes | Rat Plasma | LC/MS/MS | [redacted], USA | BA-399-2002 |
| Method Validation | GS-5734<br>GS-441524 | Yes | Monkey Plasma | LC/MS/MS | [redacted], USA | BA-399-2003 |
| Method Validation | GS-5734<br>GS-441524<br>GS-704277 | Yes | Rabbit Plasma | LC/MS/MS | [redacted], USA | BA-399-2004 |
| Method Validation | GS-5734<br>GS-441524<br>GS-704277 | Yes | Rat Plasma | LC/MS/MS | [redacted], USA | BA-399-2006 |
| Method Validation | GS-5734<br>GS-441524<br>GS-704277 | Yes | Monkey Plasma | LC/MS/MS | [redacted], USA | BA-399-2007 |

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

# 3. PHARMACOKINETICS: ABSORPTION AFTER A SINGLE DOSE

## 3.1. AD-399-2016: Pharmacokinetics of GS-5734 and Metabolites Following Intramuscular Administration to Male Rhesus Monkeys

| Report Title | Study Type | Test Article | Report Number |
|---|---|---|---|
| Pharmacokinetics of GS-5734 and Metabolites Following Intramuscular Administration to Male Rhesus Monkeys | Absorption | GS-5734 | AD-399-2016 |

| **Species** | **Rhesus Macaque Monkey** | | |
|---|---|---|---|
| Gender (M/F) / N of Animals | M / 6 | | |
| Feeding Condition | Non-fasted | | |
| Vehicle / Formulation | 5% ethanol / 95% propylene glycol (v/v) | | |
| Method of Administration | IM | | |
| Dose (mg/kg) | 3 | | |
| Sample | Plasma and PBMCs | | |
| Analyte | GS-5734, GS-704277, and GS-441524 for plasma and GS-441524, -MP, -DP and GS-443902 for PBMCs | | |
| Assay | LC/MS/MS | | |
| **Plasma PK Parameters [a]** | **GS-5734** | **GS-704277** | **GS-441524** |
| $T_{max}$ (h) | 0.79 ± 0.70 | 1.50 ± 0.55 | 2.33 ± 0.82 |
| $C_{max}$ (µM) | 2.08 ± 1.04 | 0.16 ± 0.05 | 0.13 ± 0.03 |
| $AUC_{0-t}$ (µM•h) | 6.80 ± 2.68 | 1.58 ± 0.43 | 2.13 ± 0.52 |
| $t_{1/2}$ (h) | 5.40 ± 1.86 | 16.4 ± 8.9 | > 24 |
| **Time (h)** | **Total PBMC Concentrations (µM)[b]** | | |
| 2 | 11.4 ± 6.2 | | |
| 4 | 10.8 ± 4.5 | | |
| 8 | 8.62 ± 3.33 | | |
| 24 | 10.2 ± 2.7 | | |

a Data represent the mean ± SD of 6 animals.
b Sum of GS-441524, -MP, -DP and GS-443902 from PBMC samples, n = 6 per time point for 2, 4 and 8 h and n = 3 for 24 h
IM = intramuscular; M = male; PBMC = peripheral blood mononuclear cells

## 3.2. AD-399-2003: Pharmacokinetics of GS-5734 and its Metabolites Following Intravenous Administration to Male Wistar Han Rats

| Report Title | Study Type | Test Article | Report Number |
|---|---|---|---|
| Pharmacokinetics of GS-5734 and Its Metabolites Following Intravenous Administration to Male Wistar Han Rats | Absorption | GS-5734 | AD-399-2003 |

| **Species** | **Wistar Han Rat** | | |
|---|---|---|---|
| Gender (M/F) / N of Animals | M / 3 | | |
| Feeding Condition | Non-fasted | | |
| Vehicle / Formulation | 12% Sulfobutylether-β-cyclodextrin in water (w/v; pH = 4) | | |
| Method of Administration | IV | | |
| Dose (mg/kg) | 50 | | |
| Sample | Plasma | | |
| Analyte | GS-5734, GS-704277, and GS-441524 | | |
| Assay | LC/MS/MS | | |
| **PK Parameters [a]** | **GS-5734** | **GS-704277** | **GS-441524** |
| $T_{max}$ (h) | 0.48 ± 0.00 | 0.48 ± 0.00 | 1.25 ± 0.43 |
| $C_{max}$ (µM) | 17.5 ± 0.65 | 40.6 ± 2.66 | 7.82 ± 1.31 |
| $AUC_{0-t}$ (µM•h) | 6.20 ± 0.68 | 25.1 ± 2.52 | 63.7 ± 7.42 |
| $t_{1/2}$ (h) | 0.05 ± 0.01 | 0.25 ± 0.02 | 6.21 ± 0.56 |
| CL (L/h/kg) | 13.5 ± 1.41 | NA | NA |

a Data represent the mean ± SD of 3 animals.
IV = intravenous; M = male; NA = not applicable

## 3.3. AD-399-2001: Pharmacokinetics of GS-5734 in Male Cynomolgus Monkeys

| Report Title | Study Type | Test Article | Report Number |
|---|---|---|---|
| Pharmacokinetics of GS-5734 in Male Cynomolgus Monkeys | Absorption | GS-5734 | AD-399-2001 |

| | | | |
|---|---|---|---|
| **Species** | **Cynomolgus Monkey** | | |
| Gender (M/F) / N of Animals | M / 3 | | |
| Feeding Condition | Non-fasted | | |
| Vehicle / Formulation | 12% (w/v) Sulfobutylether-β-cyclodextrin and 98% water (pH = 4) | | |
| Method of Administration | IV Bolus | | |
| Dose (mg/kg) | 10 | | |
| Sample | Plasma | | |
| Analyte | GS-5734, GS-704277, and GS-441524 | | |
| Assay | LC/MS/MS | | |
| **PK Parameters [a]** | **GS-5734** | **GS-704277** | **GS-441524** |
| $T_{max}$ (h) | 0.08 ± 0.00 | 0.08 ± 0.00 | 1.33 ± 0.58 |
| $C_{max}$ (µM) | 16.0 ± 2.84 | 3.43 ± 1.14 | 1.15 ± 0.49 |
| $AUC_{0-t}$ (µM•h) | 4.76 ± 0.50 | 2.73 ± 0.79 | 8.23 ± 6.80 |
| $t_{1/2}$ (h) | 0.29 ± 0.10 | 0.84 ± 0.36 | 7.16 ± 3.19 |
| CL (L/h/kg) | 3.50 ± 0.37 | NA | NA |

a Data represent the mean ± SD of 3 animals.
IV = intravenous; M = male; NA = not applicable

## 3.4. AD-399-2002: Single Dose Pharmacokinetic Study with GS-5734 in Male Rhesus Monkeys

| **Report Title** | **Study Type** | **Test Article** | **Report Number** |
|---|---|---|---|
| Single Dose Pharmacokinetic Study with GS-5734 in Male Rhesus Monkeys | Absorption | GS-5734 | AD-399-2002 |

| **Species** | **Rhesus Macaque Monkey** |
|---|---|
| Gender (M/F) / N of Animals | M / 3 |
| Feeding Condition | Non-fasted |
| Vehicle / Formulation | 12% Sulfobutylether-β-cyclodextrin in water, pH 3.5-4.0 |
| Method of Administration | IV |
| Dose (mg/kg) | 3 |
| Sample | Plasma and PBMCs |
| Analyte | GS-5734, GS-704277, and GS-441524 for plasma and GS-441524, -MP, -DP and GS-443902 for PBMCs |
| Assay | LC/MS/MS |

| **Plasma PK Parameters [a]** | **GS-5734** | **GS-704277** | **GS-441524** |
|---|---|---|---|
| $T_{max}$ (h) | 0.083 ± 0.000 | 0.33 ± 0.14 | 0.50 ± 0.43 |
| $C_{max}$ (µM) | 2.39 ± 0.60 | 0.71 ± 0.24 | 0.33 ± 0.11 |
| $AUC_{0-t}$ (µM•h) | 0.80 ± 0.18 | 0.84 ± 0.25 | 2.11 ± 0.25 |
| $t_{1/2}$ (h) | 0.35 ± 0.07 | 3.59 ± 2.11 | 8.71 ± 0.45 |
| CL (L/h/kg) | 6.37 ± 1.59 | NA | NA |

| **Time (h)** | **Total PBMC Concentrations (µM)[b]** |
|---|---|
| 2 | 26.3 ± 5.0 |
| 4 | 22.9 ± 0.7 |
| 8 | 19.9 ± 3.0 |
| 24 | 12.8 ± 4.8 |

IV = intravenous; M = male; NA = not applicable; PBMC = peripheral blood mononuclear cells; MP = monophosphate; DP = diphosphate
a Data represent the mean ± SD of 3 animals.
b Sum of GS-441524, -MP, -DP and GS-443902 from PBMC samples; -MP and -DP concentrations were estimated based on calibration curve for GS-443902.

## 3.5. AD-399-2022: Single Dose Pharmacokinetic Study with GS-5734 in Male Rhesus Monkeys

| Report Title | Study Type | Test Article | Report Number |
|---|---|---|---|
| Single Dose Pharmacokinetic Study with GS-5734 in Male Rhesus Monkeys | Absorption | GS-5734 | AD-399-2022 |

| | |
|---|---|
| Species | Rhesus Macaque Monkey |
| Gender (M/F) / N of Animals | M / 3 |
| Feeding Condition | Non-fasted |
| Formulation | 12% Sulfobutylether-β-cyclodextrin in water, pH 3.5-4.0 |
| Method of Administration | IV (slow bolus) |
| Dose (mg/kg/day) | 10 |
| Sample | Plasma and PBMCs |
| Analyte | GS-5734, GS-704277, and GS-441524 for plasma<br>GS-441524 (Nuc), GS-719700 (MP), GS-719699 (DP), and GS-443902 (TP) for PBMCs |
| Assay | LC/MS/MS |

| | Plasma Pharmacokinetics | | |
|---|---|---|---|
| **Parameters** [a] | **GS-5734** | **GS-704277** | **GS-441524** |
| $T_{max}$ (h) | 0.08 ± 0.000 | 0.19 ± 0.10 | 1.33 ± 0.58 |
| $C_{max}$ (µM) | 5.07 ± 0.42 | 1.80 ± 0.13 | 1.14 ± 0.18 |
| $AUC_{0-24}$ (µM•h) | 2.09 ± 0.31 | 2.38 ± 0.07 | 8.73 ± 1.10 |
| $t_{1/2}$ (h) | 0.39 ± 0.08 | 0.99 ± 0.01 | 8.55 ± 1.05 |
| CL (L/h/kg) | 7.96 ± 1.11 | NA | NA |
| $V_{ss}$ (L/kg) | 2.97 ± 0.30 | NA | NA |

| **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

## 3.6. AD-399-2033: Single Dose Pharmacokinetic Study with GS-5734 in Male Cynomolgus Monkeys

| Report Title | Study Type | Test Article | Report Number |
|---|---|---|---|
| Single Dose Pharmacokinetic Study with GS-5734 in Male Cynomolgus Monkeys | Absorption | GS-5734 | AD-399-2033 |

| Species | Cynomolgus Monkey | | |
|---|---|---|---|
| Gender (M/F) / N of Animals | M / 3 | | |
| Feeding Condition | Non-fasted | | |
| Vehicle / Formulation | 12% Sulfobutylether-β-cyclodextrin in water, pH 3.5 | | |
| Method of Administration | IV (slow bolus) | | |
| Dose (mg/kg) | 10 | | |
| Sample | Plasma | | |
| Analyte | GS-5734, GS-704277, and GS-441524 | | |
| Assay | LC/MS/MS | | |
| **Plasma PK Parameters [a]** | **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 | 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 | NA |
| $V_{ss}$ (L/kg) | 2.21 ± 0.50 | NA | NA |

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

# 4. PHARMACOKINETICS: ABSORPTION AFTER REPEATED DOSES

Repeated dose pharmacokinetic studies of GS-5734 are described in m2.6.7 as a part of toxicology studies.

# 5. PHARMACOKINETICS: IN VITRO AND IN VIVO DISTRIBUTION

## 5.1. AD-399-2013 and AD-399-2031: Plasma Binding of GS-5734

| Report Title | Study Type | Test Article | Report Number |
|---|---|---|---|
| Plasma Protein Binding of GS-5734 | Distribution | GS-5734 | AD-399-2013<br>AD-399-2031 |

**Method**

The equilibrium dialysis assay was conducted for 3 hr at 37 °C in duplicate using plasma from Wistar Han rat, cynomolgus monkey, rhesus monkey, or human spiked with GS-5734 to a final concentration of 2 µM (initial concentration in protein-containing dialysis cell). Analysis of remdesivir, GS-704277 and GS-441524 was done by LC/MS/MS.

| | % Free Fraction [a] (Recovery) | | |
|---|---|---|---|
| **Matrix** | **Remdesivir** | **GS-704277** | **GS-441524** |
| Human Plasma | 12.1 ± 0.7 (132) | 99 ± 10 (72) | 98 ± 6 (74) |
| Wistar Han Rat Plasma | 8.0 ± 0.1 (87) | 95 ± 13 (72) | 90 ± 0 (70) |
| Cynomolgus Monkey Plasma | 14.2 ± 0.6 (112) | 127 ± 20 (67) | 99 ± 8 (71) |
| Rhesus Monkey Plasma | 13.5 ± 0.6 (108) | 106 ± 18 (69) | 85 ± 10 (75) |

a Mean ± Standard Deviation (n =3)

## 5.2. AD-540-2007: Distribution within Blood from Rhesus Monkeys and Human

| Report Title | | Study Type | Report Number |
|---|---|---|---|
| In Vitro Assessment of Human Blood Distribution of Remdesivir and GS-441524 | | Distribution | AD-540-2007 |

**Method**

Remdesivir, GS-441524 or positive control (methazolamide) spiked into pooled blood from rhesus monkey or human to a final concentration of 0.5 µM. Assay n = 6 for methaazolamide in rhesus monkey and n = 3 otherwise. Incubated at 37°C for 1 h and then concentrations of the compounds determined in the soluble (plasma) and cellular fractions by LC-MS. Cell to plasma concentration ratios (CPR) calculated and then whole blood to plasma ratios (λ) calculated using CPR and hematocrit.

| | Remdesivir [a] | | GS-441524 [a] | | Methazolamide |
|---|---|---|---|---|---|
| Species | CPR | λ | CPR | λ | λ |
| Rhesus Monkey | 0.33 ± 0.08 | 0.71 ± 0.03 | 1.79 ± 0.30 | 1.36 ± 0.14 | 68.67 ± 42.49 [b] |
| Human | 0.42 ± 0.17 | 0.76 ± 0.07 | 1.46 ± 0.29 | 1.19 ± 0.12 | 14.95 ± 4.00 [a] |

a N = 3
b N = 6

## 5.3. AD-399-2017: Distribution in Sprague Dawley Rats Following a Single Intravenous Dose of [$^{14}$C]GS-5734

| Report Title | Study Type | Test Article | Report Number |
|---|---|---|---|
| Pharmacokinetics, Distribution, and Excretion of $^{14}$C-GS-5734 Following a Single Intravenous Bolus Administration to Rats | Distribution | [$^{14}$C]GS-5734 | AD-399-2017 |

| | | | |
|---|---|---|---|
| Species | Sprague Dawley (non-pigmented) Rat | | |
| Gender /No. of Animals | Male / 11 (1 per time point) | | |
| Feeding Condition | Non-fasted | | |
| Vehicle/Formulation | 12% Sulfobutylether-β-cyclodextrin in water, pH 3.0 | | |
| Method of Administration | Intravenous slow-bolus | | |
| Dose | 10 mg/kg (100 µCi/kg) | | |
| Radionuclide | Carbon-14 | | |
| Specific Activity | 58.0 mCi/mmol | Specific Activity of Formulation | 8.45 µCi/mg |
| Sampling Time | 0.167, 0.5, 1, 2, 4, 8, 12, 24, 48, 96, and 168 h post-dose | | |
| Analyte/Assay | Carbon-14/Quantitative Whole Body Autoradiography | | |

| **Tissues/Organs** | **Tissue Concentration of Radioactivity (ng Equivalents [$^{14}$C]GS-5734/g tissue)** | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Time-point (h) →** | **0.167** | **0.50** | **1** | **2** | **4** | **8** | **12** | **24** | **48** | **96** | **168** |
| Adrenal gland(s) | 3310[a] | 1560[a] | 1140 | 882 | 364[a] | 398[a] | 159[a] | ND | ND | ND | ND |
| Arterial wall | 9360 | 2630 | 1590 | 1250 | 618 | 811 | 404 | ND | ND | ND | ND |
| Bile | 189000 | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| Blood | 6690 | 1740 | 1130 | 811 | 384 | 478 | 187 | BLQ | ND | ND | ND |
| Bone | 367 | 245 | BLQ | 136 | BLQ | ND | ND | ND | ND | ND | ND |
| Bone marrow | 2010 | 1360 | 892 | 797 | 395 | 224 | 121 | BLQ | ND | ND | ND |
| Brain cerebellum | 197 | BLQ | BLQ | BLQ | BLQ | ND | ND | ND | ND | ND | ND |
| Brain cerebrum | 287 | 178 | BLQ | BLQ | ND | ND | ND | ND | ND | ND | ND |
| Brain choroid plexus | 1220 | 723 | 241 | ND | ND | ND | ND | ND | ND | ND | ND |
| Brain medulla | 158 | BLQ | BLQ | BLQ | ND | ND | ND | ND | ND | ND | ND |

| Report Title | | | | | | | Study Type | | Test Article | Report Number | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Pharmacokinetics, Distribution, and Excretion of $^{14}$C-GS-5734 Following a Single Intravenous Bolus Administration to Rats | | | | | | | Distribution | | [$^{14}$C]GS-5734 | AD-399-2017 | |
| Brain olfactory lobe | 437 | BLQ | BLQ | 123 | BLQ | BLQ | ND | ND | ND | ND | ND |
| Bulbo-urethral gland | 3670[a] | 2070 | 1110 | 893 | 453 | 404 | 269 | ND | ND | ND | ND |
| Cecum | 3300 | 1450 | 1140 | 5830 | 6380 | 3950 | 4710 | 2820 | 223 | 344 | ND |
| Diaphragm | 1960 | 1450 | 1080 | 986 | 605 | 499 | 398 | 206 | 147 | BLQ | BLQ |
| Epididymis | 3850[a] | 1120[a] | 819 | 540 | 326 | 240 | 138[a] | BLQ | BLQ | ND | ND |
| Esophagus | 4680 | 1440 | 960 | 1130 | 506 | 450 | 407 | 184 | 257 | ND | BLQ |
| Exorbital lacrimal gland | 2860 | 1440 | 1190 | 951 | 568 | 393 | 284 | BLQ | ND | ND | ND |
| Eye lens | 270 | 427 | 137 | ND | BLQ | BLQ | BLQ | ND | ND | ND | ND |
| Eye uveal tract | 2670 | 961 | 571 | 467 | 291 | 276 | 192 | ND | ND | ND | ND |
| Eye(s) | 906 | 535 | 212 | 189 | 146 | BLQ | BLQ | ND | ND | ND | ND |
| Fat (abdominal) | 381 | BLQ | BLQ | BLQ | ND | ND | ND | ND | ND | ND | ND |
| Fat (brown) | 2130 | 1480 | 751 | 669 | 399 | 294 | 244 | BLQ | BLQ | ND | ND |
| Harderian gland | 1240 | 882 | 849 | 608 | ND | ND | ND | ND | ND | ND | ND |
| Intra-orbital lacrimal gland | 2140 | 1430 | 1000 | 870 | ND | ND | ND | ND | ND | ND | ND |
| Kidney cortex | 133000 | 153000 | 163000 | 159000 | 113000 | 124000 | 83700 | 40900 | 11900 | 1930 | 757 |
| Kidney medulla | 49800 | 69000 | 20300 | 26800 | 40600 | 50300 | 18000 | 3640 | 3250 | 619 | 114 |
| Kidney(s) | 115000 | 141000 | 158000 | 154000 | 120000 | 118000 | 78500 | 39500 | 10700 | 1890 | 745 |
| Large intestine | 4230 | 1730 | 955 | 927 | 467 | 467 | 433 | 225 | ND | ND | ND |
| Liver | 51000 | 34000 | 29400 | 28100 | 22200 | 13700 | 5620 | 2500 | 501 | 187 | 123 |
| Lung(s) | 3800 | 2650 | 1280 | 758 | 605 | 628 | 268 | BLQ | ND | ND | ND |
| Lymph node(s) | 3540 | 1670 | 1330 | 881 | 491 | 318 | 275 | BLQ | ND | ND | ND |
| Muscle | 959 | 775 | 927 | 875 | 376 | 291 | 226 | 117 | 122 | ND | ND |

| Report Title | | | | | | | Study Type | | Test Article | Report Number | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Pharmacokinetics, Distribution, and Excretion of $^{14}$C-GS-5734 Following a Single Intravenous Bolus Administration to Rats | | | | | | | Distribution | | [$^{14}$C]GS-5734 | AD-399-2017 | |
| Myocardium | 3210 | 1760 | 1310 | 1020 | 604 | 760 | 346 | 158 | ND | ND | ND |
| Nasal turbinates | 2010 | 703 | 354 | 369 | 227 | 192 | 121 | ND | ND | ND | ND |
| Pancreas | 2810[a] | 2230 | 1750[a] | 1810 | 1120 | 892 | 772 | 274 | BLQ | ND | ND |
| Pituitary gland | 2360 | 1400 | 1290 | 927 | 444 | 441 | 115 | ND | ND | ND | ND |
| Preputial gland | 2210[a] | 1250[a] | 593[a] | 713[a] | 287[a] | 225[a] | 185[a] | BLQ[a] | 234[a] | ND | ND |
| Prostate gland | 3030[a] | 930 | 570 | 533 | 394 | 225 | 190 | BLQ | BLQ | ND | ND |
| Salivary gland(s) | 3480 | 1710 | 1310 | 999 | 516 | 514 | 307 | 139 | BLQ | ND | ND |
| Seminal vesicle(s) | 846[a] | 602[a] | 188[a] | 324[a] | BLQ[a] | ND | ND | ND | ND | ND | ND |
| Skin (nonpigmented) | 7150 | 1930 | 1130 | 949 | 675 | 1010 | 936 | 511 | 840 | 828 | 509 |
| Small intestine | 2770 | 2000 | 1090 | 1120 | 970 | 549 | 532 | 507 | ND | ND | ND |
| Spinal cord | 172 | BLQ | BLQ | ND | ND | ND | ND | ND | ND | ND | ND |
| Spleen | 1610 | 1490 | 1160 | 988 | 439 | 320 | 202 | BLQ | BLQ | ND | ND |
| Stomach | 3820 | 1610 | 1190 | 1080 | 629 | 821 | 410 | 215 | 201 | 159 | ND |
| Testis(es) | 1250 | 562 | 451 | 459 | 298 | 215 | 146 | BLQ | ND | ND | ND |
| Thymus | 1680 | 1510 | 1290 | 1050 | 553 | 447 | 226 | BLQ | ND | ND | ND |
| Thyroid | 3290 | 1650 | 1150 | 663 | 549 | ND | ND | ND | ND | ND | ND |
| Urinary bladder | 5470 | 4210 | 1360 | 977 | 5580 | 1390 | 5820 | 4100 | 761 | 113 | ND |
| Urine | 171000 | 129000 | 117000 | 111000 | 89700 | 19500 | 16900 | 5640 | 1730 | 169 | ND |

BLQ = below the limit of quantitation (<112 ng equivalents $^{14}$C-GS-5734/g); h = hours; ND = not detectable (sample shape not discernible from background or surrounding tissue)
a Tissue appeared to be fat soaked

## 5.4. AD-399-2017: Distribution in Long Evans Rats Following a Single Intravenous Dose of [$^{14}$C]GS-5734

| Report Title | Study Type | Test Article | Report Number |
|---|---|---|---|
| Pharmacokinetics, Distribution, and Excretion of $^{14}$C-GS-5734 Following a Single Intravenous Bolus Administration to Rats | Distribution | [$^{14}$C]GS-5734 | AD-399-2017 |

| | | | |
|---|---|---|---|
| Species | Long Evans (pigmented) Rat | | |
| Gender /No. of Animals | Male / 11 (1 per time point) | | |
| Feeding Condition | Non-fasted | | |
| Vehicle/Formulation | 12% Sulfobutylether-β-cyclodextrin in water, pH 3.0 | | |
| Method of Administration | Intravenous slow-bolus | | |
| Dose | 10 mg/kg (100 µCi/kg) | | |
| Radionuclide | Carbon-14 | | |
| Specific Activity | 58.0 mCi/mmol | Specific Activity of Formulation | 8.45 µCi/mg |
| Sampling Time | 0.167, 0.5, 1, 2, 4, 8, 12, 24, 48, 96, and 168 h post-dose | | |
| Analyte/Assay | Carbon-14/Quantitative Whole Body Autoradiography | | |

| **Tissues/Organs ↓** | **Tissue Concentration of Radioactivity (ng Equivalents [$^{14}$C]GS-5734/g tissue)** | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Time-point (h) →** | **0.3** | **0.50** | **1** | **2** | **4** | **8** | **12** | **24** | **48** | **96** | **168** |
| Adrenal gland(s) | 1720 | 1470[a] | 941 | 789[a] | 518[a] | 193[a] | ND | ND | ND | ND | ND |
| Arterial wall | 5920 | 3420 | 1610 | 1060 | 796 | 448 | 209 | ND | ND | ND | ND |
| Bile | 255000 | 56500 | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| Blood | 5360 | 2450 | 1060 | 709 | 583 | 206 | 131 | BLQ | ND | ND | ND |
| Bone | 162 | 293 | BLQ | BLQ | 144 | BLQ | BLQ | ND | ND | ND | ND |
| Bone marrow | 1180 | 1900 | 1020 | 668 | 508 | 164 | 114 | BLQ | ND | ND | ND |
| Brain cerebellum | BLQ | 122 | BLQ | BLQ | 115 | BLQ | ND | ND | ND | ND | ND |
| Brain cerebrum | BLQ | BLQ | BLQ | BLQ | BLQ | ND | BLQ | ND | ND | ND | ND |
| Brain choroid plexus | 718 | 1190 | 132 | 218 | ND | ND | ND | ND | ND | ND | ND |

| Tissues/Organs ↓ | Tissue Concentration of Radioactivity (ng Equivalents [$^{14}$C]GS-5734/g tissue) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Time-point (h) →** | **0.3** | **0.50** | **1** | **2** | **4** | **8** | **12** | **24** | **48** | **96** | **168** |
| Brain medulla | BLQ | BLQ | BLQ | BLQ | BLQ | ND | ND | ND | ND | ND | ND |
| Brain olfactory lobe | BLQ | 188 | BLQ | BLQ | BLQ | ND | ND | ND | ND | ND | ND |
| Bulbo-urethral gland | 2950 | 2540 | 1070 | 749 | ND | 274 | ND | ND | ND | ND | ND |
| Cecum | 2070 | 2650 | 985 | 857 | 12000 | 16900 | ND[b] | 1320 | ND | ND | ND |
| Diaphragm | 1800 | 1700 | 1240 | 795 | 622 | 284 | 230 | 192 | BLQ | ND | ND |
| Epididymis | 2700[a] | 1430[a] | 626[a] | 543[a] | 451[a] | 186 | 119[a] | BLQ[a] | ND | ND | ND |
| Esophagus | 3330 | 2730 | 1260 | 836 | 921 | 310 | 255 | 234 | ND | ND | ND |
| Exorbital lacrimal gland | 1590 | 2060 | 1180 | 824 | 634 | 341 | 230 | 171 | ND | ND | ND |
| Eye lens | BLQ | 197 | BLQ | 114 | ND | BLQ | ND | BLQ | ND | ND | ND |
| Eye uveal tract | 2780 | 2110 | 1330 | 1360 | 639 | 503 | 578 | 439 | 144 | ND | ND |
| Eye(s) | 694 | 590 | 277 | 329 | 219 | 145 | 119 | 120 | BLQ | ND | ND |
| Fat (abdominal) | 244 | 225 | BLQ | BLQ | BLQ | ND | ND | ND | ND | ND | ND |
| Fat (brown) | 1020 | 1140 | 583 | 421 | 327 | 141 | 119 | ND | ND | ND | ND |
| Harderian gland | 1030 | 1360 | 933 | 838 | 310 | ND | ND | ND | ND | ND | ND |
| Intra-orbital lacrimal gland | 2140 | 2060 | 1192 | 824 | 490 | ND | ND | ND | ND | ND | ND |
| Kidney cortex | 58900 | 153000 | 146000 | 127000 | 142000 | 80600 | 74600 | 50300 | 10600 | 1730 | 488 |
| Kidney medulla | 57800 | 59000 | 40800 | 44700 | 50000 | 21300 | 26300 | 21900 | 3070 | 670 | BLQ |
| Kidney(s) | 62400 | 121000 | 134000 | 107000 | 119000 | 70900 | 55900 | 37900 | 7810 | 1900 | 407 |
| Large intestine | 3270 | 2270 | 1360 | 674 | 796 | 625 | 193 | 319 | ND | ND | ND |
| Liver | 47500 | 42800 | 34200 | 23600 | 32200 | 15800 | 6830 | 1970 | 392 | 180 | BLQ |
| Lung(s) | 3670 | 2100 | 991 | 733 | 552 | 179 | 167 | BLQ | ND | ND | ND |
| Lymph node(s) | 2190 | 1990 | 1370 | 849 | 585 | 274 | 141 | ND | ND | ND | ND |
| Muscle | 719 | 1350 | 979 | 779 | 454 | 212 | 144 | 119 | BLQ | BLQ | ND |
| Myocardium | 2030 | 2240 | 1270 | 951 | 798 | 347 | 256 | BLQ | ND | ND | ND |

| Tissues/Organs ↓ | Tissue Concentration of Radioactivity (ng Equivalents [$^{14}$C]GS-5734/g tissue) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Time-point (h) →** | **0.3** | **0.50** | **1** | **2** | **4** | **8** | **12** | **24** | **48** | **96** | **168** |
| Nasal turbinates | 653 | 856 | 410 | 289 | 202 | 202 | ND | ND | ND | ND | ND |
| Pancreas | 2560 | 2850 | 2030 | 1770 | 1240 | 550 | 419 | ND[b] | BLQ | ND | ND |
| Pituitary gland | 1520 | 1650 | 1050 | 676 | 505 | 275 | ND | ND | ND | ND | ND |
| Preputial gland | 1610[a] | 1050[a] | 399[a] | 474[a] | 243[a] | 120[a] | ND[a] | ND | ND | ND | ND |
| Prostate gland | 1670 | 1260 | 860 | 616 | 581 | 205 | 124 | ND | ND | ND | ND |
| Salivary gland(s) | 2180 | 2130 | 1180 | 826 | 757 | 297 | 222 | BLQ | ND | ND | ND |
| Seminal vesicle(s) | 731 | 321[a] | 147[a] | 147 | 134 | BLQ[a] | BLQ[a] | ND | ND | ND | ND |
| Skin (nonpigmented) | 2520 | 2120 | 962 | 789 | 591 | 487 | ND | ND | ND | ND | ND |
| Skin (pigmented) | 4560 | 2240 | 1000 | 602 | 730 | 657 | 626 | 929 | 664 | 367 | 316 |
| Small intestine | 1740 | 2160 | 1570 | 986 | 2790 | 318 | 328 | ND | ND | ND | ND |
| Spinal cord | BLQ | BLQ | BLQ | BLQ | BLQ | BLQ | ND | ND | ND | ND | ND |
| Spleen | 1120 | 1970 | 1180 | 845 | 560 | 231 | 165 | BLQ | ND | ND | ND |
| Stomach | 3170 | 1850 | 1220 | 876 | 817 | 510 | 254 | 200 | BLQ | BLQ | ND |
| Testis(es) | 1030 | 748 | 386 | 418 | 395 | 150 | BLQ | BLQ | ND | ND | ND |
| Thymus | 1290 | 1800 | 1350 | 881 | 693 | 277 | 179 | ND | ND | ND | ND |
| Thyroid | 1930 | 1450 | 1170 | 656 | 671 | ND | ND | ND | ND | ND | ND |
| Urinary bladder | 7890 | 6600 | 6240 | 809 | 11100 | ND[c] | ND[c] | 1350 | ND | ND | ND |
| Urine | 288000 | 347000 | 95000 | 127000 | 45400 | 51800 | 31500 | 7900 | 1060 | 158 | ND |

BLQ = below the limit of quantitation (<112 ng equivalents $^{14}$C-GS-5734/g); h = hours; ND = not detectable (sample shape not discernible from background or surrounding tissue)

a Tissue appeared to be fat soaked

b Tissue was not detectable due to flare from gastrointestinal contents.

c Tissue was not detectable due to flare from urine

## 5.5. AD-399-2019: Distribution in Cynomolgus Monkeys Following a Single Intravenous Dose of [$^{14}$C]GS-5734

| Report Title | Study Type | Test Article | Report Number |
|---|---|---|---|
| Pharmacokinetics, Distribution, and Excretion of $^{14}$C-GS-5734 Following an Intravenous Administration to Monkeys | Distribution | [$^{14}$C]GS-5734 | AD-399-2019 |

| | | | |
|---|---|---|---|
| Species | cynomolgus monkey | | |
| Gender /No. of Animals | Male / 6 (3 per time point) | | |
| Feeding Condition | Non-fasted | | |
| Vehicle/Formulation | 12% Sulfobutylether-β-cyclodextrin in water, pH 3.0 | | |
| Method of Administration | Intravenous slow-bolus | | |
| Dose | 10 mg/kg (25 µCi/kg) | | |
| Radionuclide | Carbon-14 | | |
| Specific Activity | 58.0 mCi/mmol | Specific Activity of Formulation | 2.38 µCi/mg |
| Sampling Time | 4 and 168 h post-dose | | |
| Analyte/Assay | Carbon-14/Liquid Scintillation Counting | | |

| **Tissues/Organs ↓** | **Tissue Concentration of Radioactivity (ng Equivalents [$^{14}$C]GS-5734/g tissue)** | | | |
|---|---|---|---|---|
| | **4 h** | | **168 h** | |
| | **Mean** | **SD** | **Mean** | **SD** |
| Adrenal gland(s) | 2470 | 914 | 81.9 | 13.3 |
| Bile (from gall bladder) | 359000 | 301000 | 513 | 128 |
| Blood (sac) | 750 | 192 | 87.4 | 23.0 |
| Bone (femur) | 291 | 45.0 | NA | NA |
| Bone marrow (femur) | 1610 | 390 | NA | NA |
| Brain | 99.3 | NA | 114 | 82.6 |
| Cerebrospinal fluid | NA | NA | NA | NA |
| Epididymis | 1430 | 222 | NA | NA |
| Eye(s) | 378 | 55.5 | NA | NA |
| Gall bladder | 123000 | 86100 | 393 | 43.9 |
| Heart | 4920 | 1160 | 400 | 79.8 |
| Kidney (s) | 72900 | 18800 | 2400 | 1020 |
| Large intestine | 15400 | 2700 | 157 | 37.8 |

| Report Title | | | Study Type | Test Article | Report Number |
|---|---|---|---|---|---|
| Pharmacokinetics, Distribution, and Excretion of $^{14}$C-GS-5734 Following an Intravenous Administration to Monkeys | | | Distribution | [$^{14}$C]GS-5734 | AD-399-2019 |
| Large intestine contents and wash | 16900 | 5800 | 49.4 | | 8.23 |
| Liver | 67900 | 14600 | 2880 | | 321 |
| Lungs | 3540 | 318 | 293 | | 18.7 |
| Lymph node(s), axillary | 5730 | 1310 | NA | | NA |
| Lymph node(s), iliac | 4450 | 712 | NA | | NA |
| Lymph node(s), inguinal | 2970 | 840 | 194 | | NA |
| Lymph node(s), mesenteric | 2910 | 271 | 120 | | NA |
| Muscle (biceps femoris) | 2460 | 425 | 1460 | | 469 |
| Pancreas | 12800 | 3870 | 222 | | 55.9 |
| Pituitary gland | 7290 | 3160 | 243 | | 16.2 |
| Plasma (sac) | 822 | 238 | 76.1 | | 30.6 |
| Prostate gland | 14300 | 11800 | 338 | | 62.3 |
| Salivary glands (mandibular) | 13800 | 2720 | 424 | | 118 |
| Seminal vesicle(s) | 12800 | 14100 | 160 | | 6.87 |
| Skin (dorsal, shaved) | 1570 | 203 | NA | | NA |
| Small intestine | 5670 | 2200 | 174 | | 25.7 |
| Small intestine contents and wash | 2440 | 677 | 21.6 | | NA |
| Spinal cord | NA | NA | NA | | NA |
| Spleen | 7510 | 1120 | 523 | | 206 |
| Stomach | 4740 | 290 | 253 | | 19.2 |
| Stomach contents and wash | 436 | 189 | 60.9 | | 20.0 |
| Testis(es) | 1060 | 7.94 | NA | | NA |
| Thymus | 2960 | 332 | 83.6 | | NA |
| Thyroid (including parathyroid) | 3100 | 156 | 399 | | 250 |
| Urinary bladder | 6110 | 6900 | 225 | | 38.8 |

BLQ = below the limit of quantitation (<112 ng equivalents $^{14}$C-GS-5734/g); h = hours; NA = not applicable

## 5.6. AD-399-2023: Single Dose Plasma, PBMC, and Lung PK

| Report Title | Study Type | Test Article | Report Number |
|---|---|---|---|
| Single Dose Pharmacokinetic Study with Intravenous GS-5734 in Male Marmosets | Distribution | GS-5734 | AD-399-2023 |

| | |
|---|---|
| Species | Marmosets |
| Gender (M/F) / N of Animals | M / 7 (Group1: N=2, Group2: N=2, Group3: N=3) |
| Feeding Condition | Non-fasted |
| Formulation | 12% Sulfobutylether-β-cyclodextrin in water, pH 3.0 |
| Method of Administration | IV slow bolus |
| Dose (mg/kg/day) | 10 |
| Sample | Plasma, PBMC, and Lung |
| Analyte | GS-5734, GS-704277, and GS-441524 for plasma (Group 3)<br>GS-441524, -MP, -DP and GS-443902 for PBMC and lung (Group 1 and 2) |
| Assay | LC/MS/MS |

| | Plasma Pharmacokinetics | | |
|---|---|---|---|
| **Parameters** | **GS-5734** | **GS-704277** | **GS-441524** |
| $C_{max}$ (µM) | 5.8 | 2.33 | 1.74 |
| $T_{max}$ (h) | 0.25 | 0.25 | 1.33 |
| $AUC_{0-24}$ (µM•h) | 7.72 | 2.96 | 11.2 |
| $T_{1/2}$ (h) | 1.02 | 0.64 | 5.87 |
| Cl (L/h/kg) | 7.74 | NA | NA |

| **Time (h)** | PBMC Concentration (µM) | | | |
|---|---|---|---|---|
| | **GS-441524** | **GS-719700** | **GS-719699** | **GS-443902** |
| 2 | 2.42 | BLQ | 1.63 | 5.92 |
| 24 | 0.53 | BLQ | 1.45 | 5.99 |
| **Time (h)** | **Lung Concentration (µM)** | | | |
| | **GS-441524** | **GS-719700** | **GS-719699** | **GS-443902** |
| 2 | 1.05 | 0.08 | 0.17 | 1.48 |
| 24 | 0.16 | 0.03 | 0.08 | 0.81 |

BLQ = below the limit of quantitation; NA = not applicable

## 5.7. AD-399-2028: Single Dose Plasma and PBMC PK

| Report Title | Study Type | Test Article | Report Number |
|---|---|---|---|
| GS-5734 Plasma and Lymphocyte Pharmacokinetics Following Two Hour Infusion in Male Rhesus Monkeys | Distribution | GS-5734 | AD-399-2028 |

| | |
|---|---|
| **Species** | Rhesus Monkey |
| Gender (M/F) / N of Animals | M / 4 |
| Feeding Condition | Non-fasted |
| Vehicle / Formulation | 88:12 water:sulfobutylether-ß-cyclodextrin pH 3.0 |
| Method of Administration | IV 2 h infusion |
| Dose (mg/kg) | 10 |
| Sample | Plasma and PBMC |
| Analyte | GS-5734, GS-704277, and GS-441524 for plasma and GS-441524, -MP, -DP and GS-443902 for PBMC |
| Assay | LC/MS/MS |

| **Plasma PK Parameters[a]** | **GS-5734** | **GS-704277** | **GS-441524** |
|---|---|---|---|
| $C_{max}$ (µM) | 4.20 ± 2.25 | 1.85 ± 0.37 | 0.95 ± 0.21 |
| $T_{max}$ (h) | 0.86 ± 0.71 | 2.00 ± 0.09 | 2.17 ± 0.10 |
| $AUC_{0-24}$ (µM•h) | 6.82 ± 3.01 | 3.91 ± 0.66 | 6.25 ± 1.61 |
| $t_{1/2}$ (h) | 0.36 ± 0.05 | 0.84 ± 0.32 | 15.2 ± 1.6 |
| CL (L/h/kg) | 2.73 ± 0.93 | 4.32 ± 0.74 | 1.99 ± 0.41 |
| $V_{ss}$ (L/kg) | 0.48 ± 0.25 | 3.48 ± 0.78 | NA |

| | **PBMC Concentration (µM)** | | | | | |
|---|---|---|---|---|---|---|
| **Time (h)** | **GS-441524** | **GS-719700** | **GS-719699** | **GS-443902** | **Summed Total** | **GS-441524 after dephosphorylation[b]** |
| 1.92 | 6.22 | 0.212 | 12.5 | 19.9 | 38.9 | 33.0 |
| 4 | 1.44 | 0.164 | 16.6 | 29.5 | 47.8 | 27.5 |
| 8 | 0.750 | 0.092 | 6.88 | 11.4 | 19.1 | 19.1 |
| 24 | 0.595 | 0.054 | 7.71 | 11.8 | 20.2 | 18.6 |

a Nucleotide metabolites were converted to GS-441524 (Nuc) by treatment with calf intestinal alkaline phosphatase; NA = not applicable

## 5.8. AD-399-2030: Multiple Dose Plasma and PBMC PK

| Report Title | Study Type | Test Article | Report Number |
|---|---|---|---|
| Pharmacokinetics of GS-5734 in Healthy Rhesus Monkeys Following Administration as Either IV Bolus or 30-minute IV Infusion | Distribution | GS-5734 | AD-399-2030 |

| | |
|---|---|
| Species | Marmosets |
| Gender (M/F) / N of Animals | 4 Males, 4 Females / 16 (8 per group) |
| Feeding Condition | Non-fasted |
| Formulation | IV slow bolus: 88:12- water:sulfobutyl ether beta-cyclodextrin with hydrogen chloride/sodium hydroxide, pH- 3.5 (Group 1)<br>IV infusion: lyophilized powder dissolved in saline (Group 2) |
| Method of Administration | IV slow bolus (Group 1) or IV 30-min infusion (Group 2) |
| Dose (mg/kg/day) | 5 |
| Duration (days) | 7, QD |
| Sample | Plasma and PBMC |
| Analyte | GS-5734, GS-704277, and GS-441524 for plasma and GS-443902 (TP) for PBMC |
| Assay | LC/MS/MS |

| | **Plasma Pharmacokinetics - Group 1: Slow Bolus** | | | | | |
|---|---|---|---|---|---|---|
| | **Day 1** | | | **Day 7** | | |
| **Parameters** | **GS-5734** | **GS-704277** | **GS-441524** | **GS-5734** | **GS-704277** | **GS-441524** |
| $C_{max}$ (ng/mL) | 2,620 ± 320 | 290 ± 76 | 175 ± 40 | 3,080 ± 870 | 294 ± 43 | 193 ± 47 |
| $T_{max}$ (h) | 0.083 ± 0 | 0.083 ± 0 | 0.38 ± 0.13 | 0.083 ± 0 | 0.10 ± 0.06 | 0.44 ± 0.12 |
| $AUC_{0-24}$ (ng•h/mL) | 559 ± 89 | 184 ± 48 | 899 ± 255 | 690 ± 177 | 205 ± 45 | 1,240 ± 360 |
| $T_{1/2}$ (h) | 0.33 ± 0.03 | 0.40 ± 0.10 | 6.23 ± 0.60 | 0.38 ± 0.02 | 0.44 ± 0.08 | 7.41 ± 0.90 |
| Cl (L/h/kg) | 9.05 ± 1.37 | NA | NA | 7.58 ± 2.04 | NA | NA |

NA = not applicable

| | Plasma Pharmacokinetics - Group 2: 30-min Infusion | | | | | |
|---|---|---|---|---|---|---|
| | Day 1 | | | Day 7 | | |
| **Parameters** | **GS-5734** | **GS-704277** | **GS-441524** | **GS-5734** | **GS-704277** | **GS-441524** |
| $C_{max}$ (ng/mL) | 3,660 ± 400 | 165 ± 25 | 127 ± 28 | 3,350 ± 390 | 155 ± 18 | 140 ± 36 |
| $T_{max}$ (h) | 0.28 ± 0.08 | 0.48 ± 0 | 0.91 ± 0.13 | 0.25 ± 0 | 0.48 ± 0 | 0.83 ± 0.19 |
| $AUC_{0-24}$ (ng•h/mL) | 1,480 ± 240 | 137 ± 34 | 837 ± 195 | 1,430 ± 230 | 143 ± 27 | 1,060 ± 240 |
| $T_{1/2}$ (h) | 0.31 ± 0.05 | 0.38 ± 0.07 | 7.34 ± 1.90 | 0.50 ± 0.10 | 0.51 ± 0.09 | 7.58 ± 0.88 |
| Cl (L/h/kg) | 3.43 ± 5.65 | NA | NA | 3.57 ± 0.62 | NA | NA |

| | Group 1: PBMC GS-443902 (TP) Concentrations (µM) | | |
|---|---|---|---|
| **Time (h)** | **Day 1** | **Day 6** | **Day 7** |
| 0 (pre-dose) | NC [a] | 5.28 ± 4.47 | NC |
| 2 | 3.7 ± 3.5 | NC | 7.9 ± 5.0 |
| 4 | 4.18 ± 2.83 | NC | 14.2 ± 7.6 |
| 8 | 4.1 ± 3.5 | NC | 11.1 ± 8.5 |
| 24 | 2.68 ± 1.87 | NC | 7.9 ± 4.6 |

| | Group 2: PBMC GS-443902 (TP) Concentrations (µM) | | |
|---|---|---|---|
| **Time (h)** | **Day 1** | **Day 6** | **Day 7** |
| 0 (pre-dose) | NC | 12.6 ± 17.7 | NC |
| 2 | 7.5 ± 10.0 | NC | 17.6 ± 18.6 |
| 4 | 5.6 ± 5.4 | NC | 21.0 ± 28.5 |
| 8 | 5.9 ± 4.5 | NC | 8.7 ± 6.2 |
| 24 | 6.0 ± 7.4 | NC | 7.1 ± 6.7 [a] |

Data represent the mean ± SD of 8 animals
NA: Not applicable; NC: Not collected
a 24h PBMC data from animal RA1972 excluded as outlier
Unit conversions - GS-5734: 1 µM = 0.603 µg/mL; GS-704277: 1 µM = 0.442 µg/mL; GS-441524: 1 µM = 0.291 µg/mL

## 5.9. AD-540-2003: Single Dose Pharmacokinetic Study with Remdesivir in Male African Green Monkeys

| Report Title | Study Type | Test Article | Report Number |
|---|---|---|---|
| Single Dose Pharmacokinetic Study with Remdesivir in Male African Green Monkeys | Distribution | Remdesivir | AD-540-2003 |

| | |
|---|---|
| Species | African Green Monkey |
| Gender (M/F) / N of Animals | M / 3 |
| Feeding Condition | Non-fasted |
| Vehicle / Formulation | 12% Sulfobutylether-β-cyclodextrin in water, pH 3.5 |
| Method of Administration | IV (30-minute infusion) |
| Dose (mg/kg) | 10 |
| Sample | Plasma, peripheral blood mononuclear cells (PBMCs), Respiratory Tissues (lung, trachea, bronchi), Non-respiratory tissues (liver gastrointestinal tract, kidney, testis, and eye) |
| Analyte | Remdesivir, GS-704277, and GS-441524 for plasma<br>GS-441525, GS-441524-MP, GS-441524-DP, GS-443902 for PBMC and Tissues |
| Assay | LC/MS/MS |

| | Plasma Pharmacokinetics | | |
|---|---|---|---|
| **Parameters** | **Remdesivir** | **GS-704277** | **GS-441524** |
| $C_{max}$ (µM) | 12.6 ± 1.0 | 15.9 ± 4.9 | 1.23 ± 0.36 |
| $T_{max}$ (h) | 0.48 ± 0.00 | 0.33 ± 0.13 | 1.67 ± 0.58 |
| $AUC_{0-24}$ (µM•h) | 7.26 ± 1.29 | 9.47 ± 1.07 | 9.06 ± 2.60 |
| $T_{1/2}$ (h) | 1.00 ± 0.07 | 0.35 ± 0.05 | 6.24 ± 0.70 |

| | PBMC Concentrations (µM) | | | |
|---|---|---|---|---|
| **Time (h)** | **GS-441524** | **GS-441524-MP** | **GS-441524-DP** | **GS-443902** |
| 2 | 3.05 ± 1.63 | 0.56 ± 0.05 | 4.30 ± 0.76 | 14.9 ± 4.6 |
| 24 [a] | 0.71 | 0.55 | 2.51 | 7.54 |

| Tissue | Respiratory Tissue Concentrations (nmol/g 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 |
| **Tissue** | **Non-respiratory Tissue Concentrations (nmol/g tissue)** | | | | |
| | **Total Metabolite Concentrations[b]** | | | | |
| Liver | 17.3 ± 2.7 | | | | |
| Gastrointestinal Tract | 3.78 ± 0.93 | | | | |
| Kidney | 39.8 ± 2.5 | | | | |
| Testis | 0.71 ± 0.31 | | | | |
| Eye | 0.26 ± 0.09 | | | | |

Data represent the mean ± SD of 3 animals
a Mean calculated based on n=2
b Summed total of GS-441524 and its phosphorylated metabolites
IV = intravenous; M = male; PBMC = peripheral blood mononuclear cells
BLQ = below lower limit of quantitation in respiratory tissue: For GS-441524: 0.154 nmol/g tissue; for GS-719700: 0.463 nmol/g tissue
GS-441524-MP (GS-719700) and GS-441524-DP (GS-719699) were quantified using authentic standard

# 6. PHARMACOKINETICS: METABOLISM IN VIVO

## 6.1. AD-399-2018: Metabolite Profiling of Samples from Rat After Administration of [$^{14}$C]GS-5734

| Report Title | Study Type | Test Article | Report Number |
|---|---|---|---|
| Metabolite Profiling and Identification of Metabolites of $^{14}$C-GS-5734 in Selected Rat Plasma, Urine, Bile, and Feces Samples after a Single Intravenous Bolus Administration of $^{14}$C-GS-5734 from [redacted] Study No. 8328793 | Metabolism | [$^{14}$C]GS-5734 | AD-399-2018 |

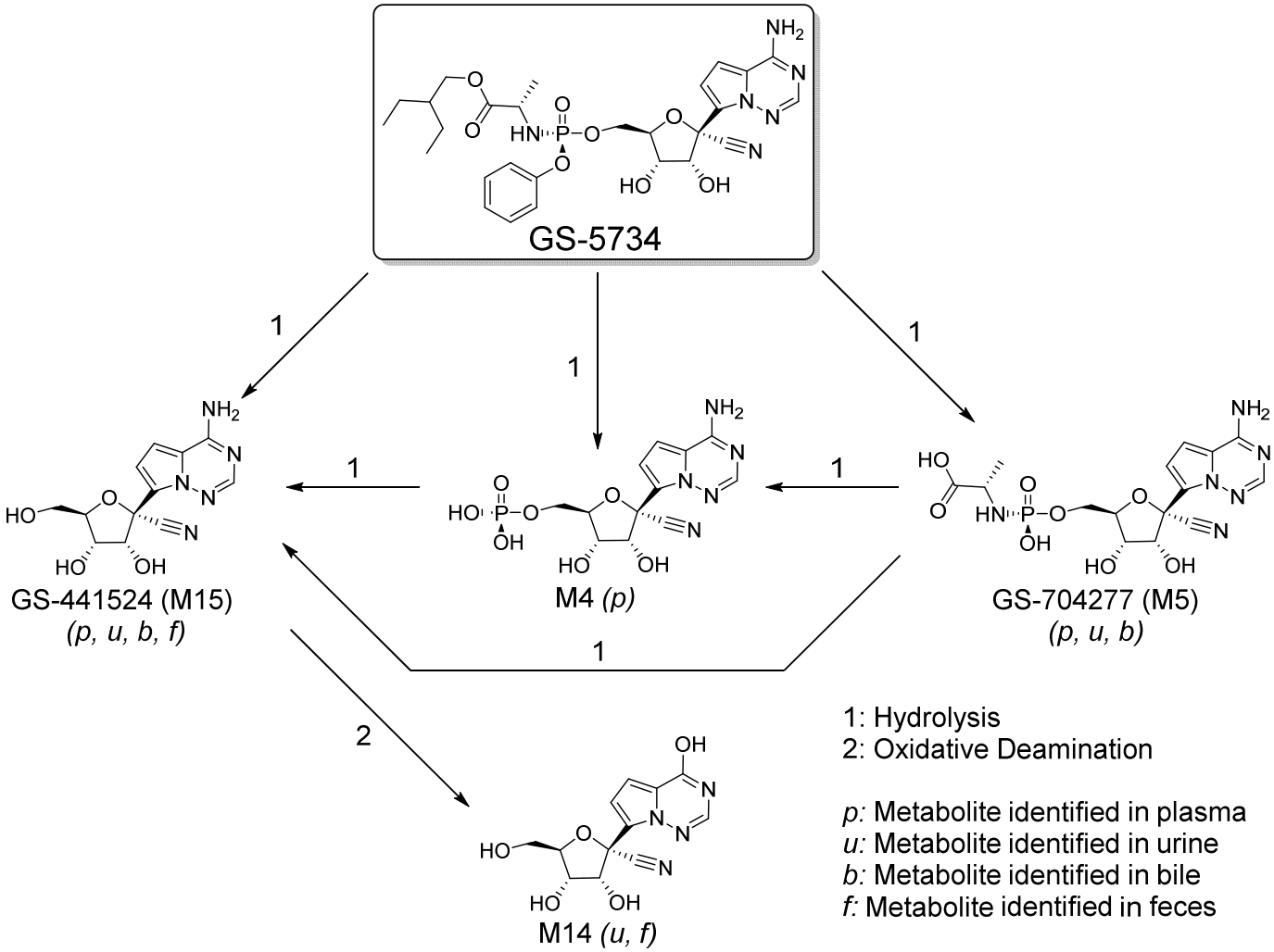
| **Study System** | Metabolite profiling of [$^{14}$C]GS-5734 in plasma, urine, bile, and feces from bile duct-intact and bile duct-cannulated male Sprague Dawley rats following a 10 mg/kg intravenous dose | |
|---|---|---|
| | **Plasma Profile (0-36 h pool)** | |
| **Component[a]** | **$AUC_{0\text{-}36h}$ (ng [$^{14}$C]GS-5734 eq·h/g)** | **% $^{14}$C in Plasma AUC pool** |
| M1 | 688 | 6.03 |
| GS-704277 (M5) | 2163 | 19.0 |
| GS-441524 (M15) | 8011 | 70.3 |
| Total in Plasma | 11402 | 100 |
| | **Urine Profile from Bile Duct-Intact Rats** (0-48 h pool) | **Urine Profile from Bile Duct-Cannulated Rats** (0-48 h pool) |
| **Component[a]** | **% Administered $^{14}$C Dose** | |
| GS-704277 (M5) | 4.20 | 10.6 |
| M7 | 0.0919 | 0.854 |
| M14 | 9.31 | 0.123 |
| GS-441524 (M15) | 43.8 | 45.6 |
| Other | 2 components detected, each < 0.5 % | 4 components detected, each < 0.5 % |
| Total $^{14}$C Dose | 61.2 | 62.2 |

| Report Title | Study Type | Test Article | Report Number |
|---|---|---|---|
| Metabolite Profiling and Identification of Metabolites of $^{14}$C-GS-5734 in Selected Rat Plasma, Urine, Bile, and Feces Samples after a Single Intravenous Bolus Administration of $^{14}$C-GS-5734 from [redacted] Study No. 8328793 | Metabolism | [$^{14}$C]GS-5734 | AD-399-2018 |

| Component[a] | Feces Profile from Bile Duct-Intact Rats (0-48 h pool) | Feces Profile from Bile Duct-Cannulated Rats (0-48 h pool) | Bile Profile (0-24h pool) |
|---|---|---|---|
| | % Administered $^{14}$C Dose | | |
| GS-704277 (M5) | ND | ND | 20.3 |
| M11 | 0.0596 | ND | 0.546 |
| M14 | 22.6 | 2.29 | ND |
| GS-441524 (M15) | 0.348 | 0.129 | 0.58 |
| Other | 3 components detected, each < 0.2% | 1 component detected, each < 0.1% | 2 components detected, each < 0.5 % |
| Total $^{14}$C Dose | 26.7 | 2.78 | 22.6 |

ND = not detected or below the limit of quantitation
a Proposed structures are shown in Section 6.2 and summarized across species in Section 8.1

## 6.2. AD-399-2018: Proposed Biotransformation Pathways of [$^{14}$C]GS-5734 in Rat



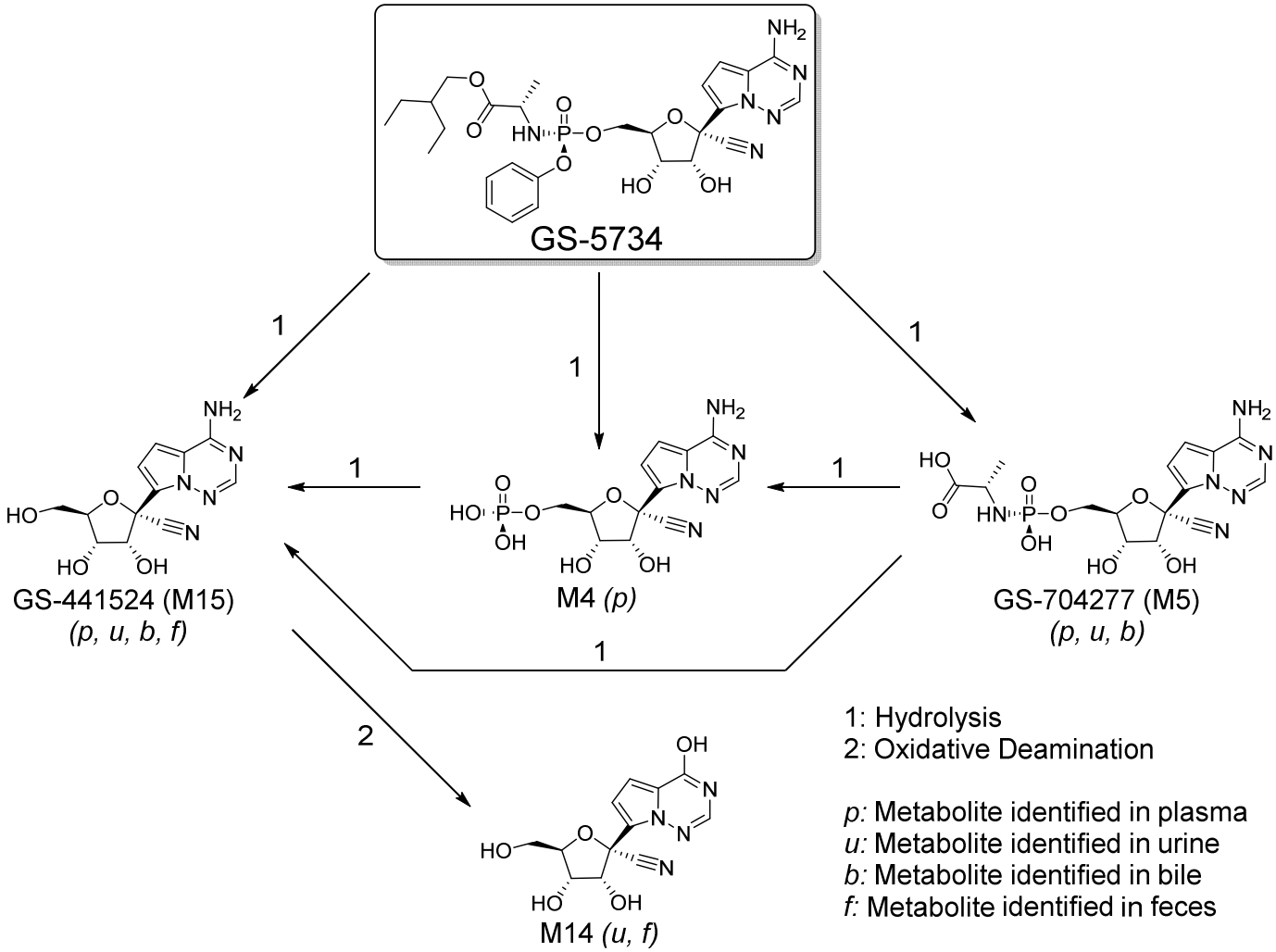
## 6.3. AD-399-2020: Metabolite Profiling of Samples from Monkey After Administration of [$^{14}$C]GS-5734

| Report Title | Study Type | Test Article | Report Number |
|---|---|---|---|
| Metabolite Profiling and Identification of Metabolites of $^{14}$C-GS-5734 in Selected Monkey Plasma, Urine, and Feces Samples after a Single Intravenous Administration of $^{14}$C-GS-5734 from [redacted] Study No. 8328795 | Metabolism | [$^{14}$C]GS-5734 | AD-399-2020 |

| **Study System** | Metabolite profiling of [$^{14}$C]GS-5734 in plasma, urine, and feces from cynomolgus monkeys following a 10 mg/kg intravenous dose | | |
|---|---|---|---|
| | **Plasma Profile (0-96 h pool)[a]** | | |
| **Component[b]** | **$AUC_{0\text{-}96h}$ (ng [$^{14}$C]GS-5734 eq·h/g)** | **% $^{14}$C in Plasma AUC pool [a]** | |
| GS-441524 (M15) | 31273 | 100 | |
| Total $^{14}$C in Plasma | 31273 | 100 | |
| | **Urine** (0-72 h pool) | **Feces** (0-72 h pool) | **Cage Rinse** (0-48 h pool) |
| **Component[b]** | **% Administered $^{14}$C Dose** | | |
| GS-704277 (M5) | 0.884 | ND | 1.55 |
| M14 | 2.65 | 19.9 | 0.509 |
| GS-441524 (M15) | 15.6 | 0.221 | 6.17 |
| M18 | 8.43 | ND | 3.18 |
| M19 | 0.276 | ND | ND |
| M20 | 0.333 | ND | ND |
| GS-5734 | 0.950 | ND | 1.19 |
| Other | 1 component detected < 0.2% | ND | 2 components detected, each < 0.1 % |
| Total $^{14}$C Dose | 30.9 | 24.5 | 14.6 |

ND = not detected or below the limit of quantitation
a Individual time point radiochromatograms showed up to 4 peaks. Composition at 0.5 h: GS-704277 (M5) = 29.4%; M18 = 35.9%; GS-441524 (M15) = 36.3%; GS-5734 = 15.2%
b Proposed structures are shown in Section 6.4 and summarized across species in Section 8.1

## 6.4. AD-399-2020: Proposed Biotransformation Pathways of [$^{14}$C]GS-5734 in Monkey




M19 – structure unknown

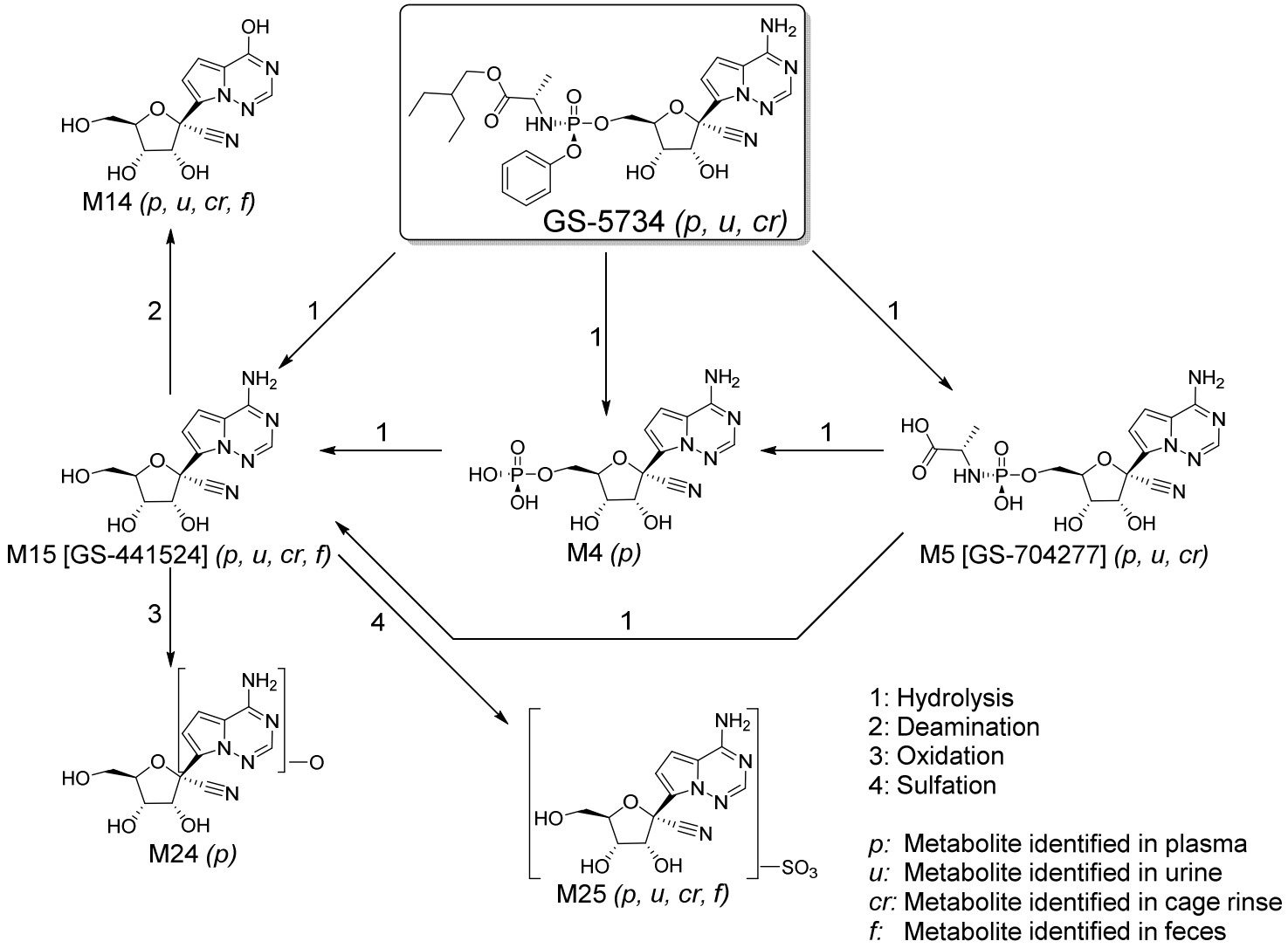
## 6.5. AD-399-2026: Metabolite Profiling of Samples from Rabbit After Administration of [$^{14}$C]GS-5734

| Report Title | Study Type | Test Article | Report Number |
|---|---|---|---|
| Metabolite Profiling and Identification of Metabolites of $^{14}$C-GS-5734 in Selected Rabbit Plasma, Urine, and Feces Samples after a Single Intravenous Dose of $^{14}$C-GS-5734 from [redacted] Study No. 8341988 | Metabolism | [$^{14}$C]GS-5734 | AD-399-2026 |

| **Study System** | Metabolite profiling of [$^{14}$C]GS-5734 in plasma, urine, and feces from NZW rabbit following a 10 mg/kg intravenous dose | | |
|---|---|---|---|
| | **Plasma Profile (0-96 h pool)** | | |
| **Component[a]** | **$AUC_{0-96h}$ (ng [$^{14}$C]GS-5734 eq·h/g)** | **% $^{14}$C in Plasma AUC pool** | |
| M14 | 7049 | 24.8 | |
| GS-441524 (M15) | 12647 | 44.5 | |
| M24 | 2281 | 8.03 | |
| M25 | 3110 | 11.0 | |
| GS-5734 | 207 | 0.73 | |
| Total $^{14}$C in Plasma | 28400 | 100 | |
| | **Urine** (0-72 h pool) | **Feces** (0-72 h pool) | **Cage Rinse** (0-48 h pool) |
| **Component[a]** | **% Administered $^{14}$C Dose** | | |
| M1 | 0.199 | 1.39 | ND |
| M2 | 0.324 | 0.196 | ND |
| GS-704277 (M5) | 14.8 | ND | 2.40 |
| M14 | 11.9 | 3.96 | 3.02 |
| GS-441524 (M15) | 16.2 | 0.117 | 2.68 |
| M22 | 0.258 | ND | ND |
| M25 | 15.0 | 1.05 | 2.20 |
| GS-5734 | 0.669 | ND | ND |
| Other | ND | ND | ND |
| Total $^{14}$C Dose | 64.4 | 9.50 | 10.9 |

ND = not detected or below the limit of quantitation; NZW = New Zealand white
a Proposed structures are shown in Section 6.6 and summarized across species in Section 8.1

## 6.6. AD-399-2026: Proposed Biotransformation Pathways of [$^{14}$C]GS-5734 in Rabbit




M1, M2 – structures unknown

# 7. PHARMACOKINETICS: METABOLISM IN VITRO

## 7.1. AD-399-2012: *In Vitro* Plasma Stability of GS-5734

| Report Title | Study Type | Test Article | Report Number |
|---|---|---|---|
| *In Vitro* Plasma Stability of GS-5734 | Metabolism, *In Vitro* | GS-5734 | AD-399-2012 |

**Method**

Duplicate aliquots of 2 µM GS-5734 were incubated with pooled plasma from Sprague-Dawley rat, beagle dog, cynomolgus monkey, rhesus monkey, and human at 37 °C up to 4 hours. Rates of metabolism (loss of GS-5734 expressed as *in vitro* half-life values) were determined. Analysis of GS-5734 and GS-7340 was done by LC/MS/MS.

| | Plasma Stability, $T_{1/2}$ (min)[a] | | | | |
|---|---|---|---|---|---|
| Compound | Human | Dog | Rat | Cynomolgus Monkey | Rhesus Monkey |
| GS-5734 | 68.5 ± 3.1 | 630 ± 96 | ≤ 0.9 | 385 ± 14 | 467 ± 12 |
| GS-7340 (Control)[b] | 90.0 ± 7.5 | 105 ± 6 | ≤ 0.9 | 151 ± 3 | 154 ± 3 |

a Mean ± Standard Deviation (n =2)
b GS-7340: Tenofovir Alafenamide

## 7.2. AD-399-2014: *In Vitro* Stability of GS-5734 in Hepatic and Intestinal Subcellular Fractions

| Report Title | Study Type | Test Article | Report Number |
|---|---|---|---|
| *In Vitro* Stability of GS-5734 in Hepatic and Intestinal Subcellular Fractions | Metabolism, *In Vitro* | GS-5734 | AD-399-2014 |

**Method**

Duplicate aliquots of 2 µM GS-5734 were incubated with hepatic S9 fraction from Sprague-Dawley rat, beagle dog, cynomolgus monkey, rhesus monkey, or human at a final protein concentration of 2.4 mg/ml, 1.25 mM NADP, 3.3 mM glucose-6-phosphate, 0.4 U/mL glucose-6-phosphate dehydrogenase, 1.9 mM UDPGA and 3.3 mM $MgCl_2$ in 100 mM potassium phosphate buffer, pH 7.4. Duplicate aliquots of GS-5734 were incubated with intestinal S9 fraction from Sprague-Dawley rat, beagle dog, or human at a final protein concentration of 1.0 mg/ml in potassium phosphate buffer, pH 7.4. Rates of metabolism (loss of GS-5734 expressed as *in vitro* half-life values) were determined. Analysis of GS-5734 and controls was done by LC/MS/MS.

| | Hepatic S9 Stability, $T_{1/2}$ (min)[a] | | | | |
|---|---|---|---|---|---|
| **Compound** | **Human** | **Rhesus Monkey** | **Cynomolgus Monkey** | **Beagle Dog** | **Sprague-Dawley Rat** |
| GS-5734 | < 3.9 | < 3.9 | < 3.9 | < 3.9 | < 3.9 |
| Propranolol (Control) | 81.5 ± 3.2 | 30.2 ± 1.4 | 39.9 ± 2.6 | 60.0 ± 5.1 | < 3.9 |

| | Intestinal S9 Stability, $T_{1/2}$ (min)[a] | | |
|---|---|---|---|
| **Compound** | **Human** | **Beagle Dog** | **Sprague-Dawley Rat** |
| GS-5734 | 114.1 ± 9.1 | 88.2 ± 10.0 | 40.3 ± 1.9 |
| GS-7340 (Control) | 35.5 ± 2.0 | 25.6 ± 2.5 | 14.0 ± 0.7 |

a Mean ± SE, N = 12 datapoints from duplicate determinations

## 7.3. AD-399-2015: *In Vitro* Activation of GS-5734 in Peripheral Blood Mononuclear Cell (PBMC) and Monocyte from Human and Monkey

| Report Title | Study Type | Test Article | Report Number |
|---|---|---|---|
| *In Vitro* Activation of GS-5734 in Peripheral Blood Mononuclear Cell (PBMC) and Monocyte from Human and Monkey | Metabolism | GS-5734 | AD-399-2015 |

**Method**

PBMCs and monocytes from rhesus monkey and human were incubated with GS-5734 at 1 μM for 2 hours. Following oil spin extraction, intracellular concentrations of GS-441524 and its phosphorylated metabolites were determined. Analysis was done by LC/MS/MS.

| | | Intracellular Concentrations (pmol/million)[a] | | | | |
|---|---|---|---|---|---|---|
| Cells | Species | GS-441524 | GS-441524-MP[b] | GS-441524-DP[b] | GS-443902 (GS-441524-TP) | Total[c] |
| PBMCs | Human | BLQ[c] | 2.30 | 47.4 | 21.0 | 70.7 |
| | Rhesus Monkey | 4.92 | BLQ | 0.70 | 5.77 | 11.4 |
| Monocytes | Human | 3.22 | 0.83 | 6.88 | 21.5 | 32.4 |
| | Rhesus Monkey | 7.70 | BLQ[d] | 0.33 | 5.07 | 13.1 |

MP = nucleoside analog monophosphate; DP = nucleoside analog diphosphate
a Mean from 2 donors determined in duplicate experiments
b GS-441524-MP and -DP concentrations were estimated based on calibration curve for GS-443902.
c Sum of GS-441524, GS-441524-MP, GS-441524-DP, and GS-443902
d BLQ = Below the lower limit of quantitation (LOQ for GS-441524 and GS-443902: 0.4 μM)

## 7.4. AD-399-2004: *In Vitro* Activation of GS-466547 in Human Macrophage Cells

| Report Title | Study Type | Test Article | Report Number |
|---|---|---|---|
| *In Vitro* Activation of GS-466547 in Human Macrophage Cells | Metabolism | GS-466547 | AD-399-2004 |

**Method**

Human macrophage cells were incubated with GS-466547 (isomeric mixture containing remdesivir) at 1 µM for 2 hours. After 2 h the compound containing media was removed and cells were washed two times in 37 °C media. Compound-free media was then added and cells were incubated for an additional 22 h. At select time points (2, 4, 8, 24), cells were harvested and intracellular concentrations of GS-443902 were determined. Analysis was done by LC/MS/MS.

| | Intracellular GS-443902 Concentration (pmol/million) | | | |
|---|---|---|---|---|
| **Time** | **Donor 1** | **Donor 2** | **Donor 3** | **Mean ± SD** |
| 2 | 53.5 | 71.6 | 147 | 90.8 ± 49.7 |
| 4 | 55.8 | 97.4 | 241 | 131 ± 97 |
| 8 | 50.1 | 76.2 | 191 | 106 ± 75 |
| 24 | 20.8 | 26.9 | 50.7 | 32.8 ± 15.8 |

| PK Parameters | Donor 1 | Donor 2 | Donor 3 | Mean ± SD |
|---|---|---|---|---|
| $T_{max}$ (h) | 4.0 | 4.0 | 4.0 | 4.0 ± 0.0 |
| $C_{max}$ (pmol/million) | 55.8 | 97.4 | 241 | 131 ± 97 |
| $C_{24}$ (pmol/million) | 20.8 | 26.9 | 50.7 | 32.8 ± 15.8 |
| $AUC_{0-24}$ (pmol*h/million) | 942 | 1413 | 3328 | 1890 ± 1260 |
| $t_{1/2}$ (h) | 13.6 | 10.8 | 8.70 | 11.0 ± 2.5 |

## 7.5. AD-399-2006: *In Vitro* Activation and Intrinsic Potency of GS-466547 in Human Macrophage Cells, Human Microvascular Endothelial Cells (HMVEC) and HeLa Cells

| Report Title | Study Type | Test Article | Report Number |
|---|---|---|---|
| *In Vitro* Activation and Intrinsic Potency of GS-466547 in Human Macrophage Cells, Human Microvascular Endothelial Cells (HMVEC) and HeLa Cells | Metabolism | GS-466547 | AD-399-2006 |

**Method**

Human macrophage cells were incubated with GS-466547 (isomeric mixture containing remdesivir) at 1 µM for 72 hours. At select time points (2 or 4, 24, 48, 72 h), cells were harvested and intracellular concentrations of GS-443902 were determined. Analysis was done by LC/MS/MS. Based on the observed mean trihosphate levels and $EC_{50}$ values, the triphosphate levels required fifty percent inhibition ($IIC_{50}$) were calculated.

| | Intracellular GS-443902 Concentration (pmol/million)[a] | | |
|---|---|---|---|
| Time | Macrophage | HMVEC (n = 2) | HeLa |
| 2 | 93.5 | 36.1 | 7.30 |
| 24 | 299 | 103 | 90.8 |
| 48 | 140 | 109 | 62.8 |
| 72 | 93.2 | 64.9 | 43.0 |

| | Macrophage | HMVEC (n = 2) | HeLa |
|---|---|---|---|
| Mean Triphosphate (pmol/million)[b] | 156 | 76.0 | 51.0 |
| $EC_{50}$ (µM) | 0.100[d] | 0.12[c] | 0.18[d] |
| $IIC_{50}$ (pmol/million) | 15.6 | 9.20 | 9.38 |

a LOQ: 0.549 pmol/million (Macrophages), 1.37 pmol/million (HMVEC), and 0.229 pmol/millions (HeLa). Human macrophage results are from cells from a single donor done in duplicate. HMVEC results from 2 different donors done in duplicate. HeLa cells are from a single experiment done in duplicate.
b Values are average of intracellular concentrations of GS-443902 at 2, 24, 48, and 72 h.
c Source: PC-399-2007
d Source: PC-399-2008

## 7.6. AD-399-2024: Metabolites of GS-5734 in Cryopreserved Hepatocytes

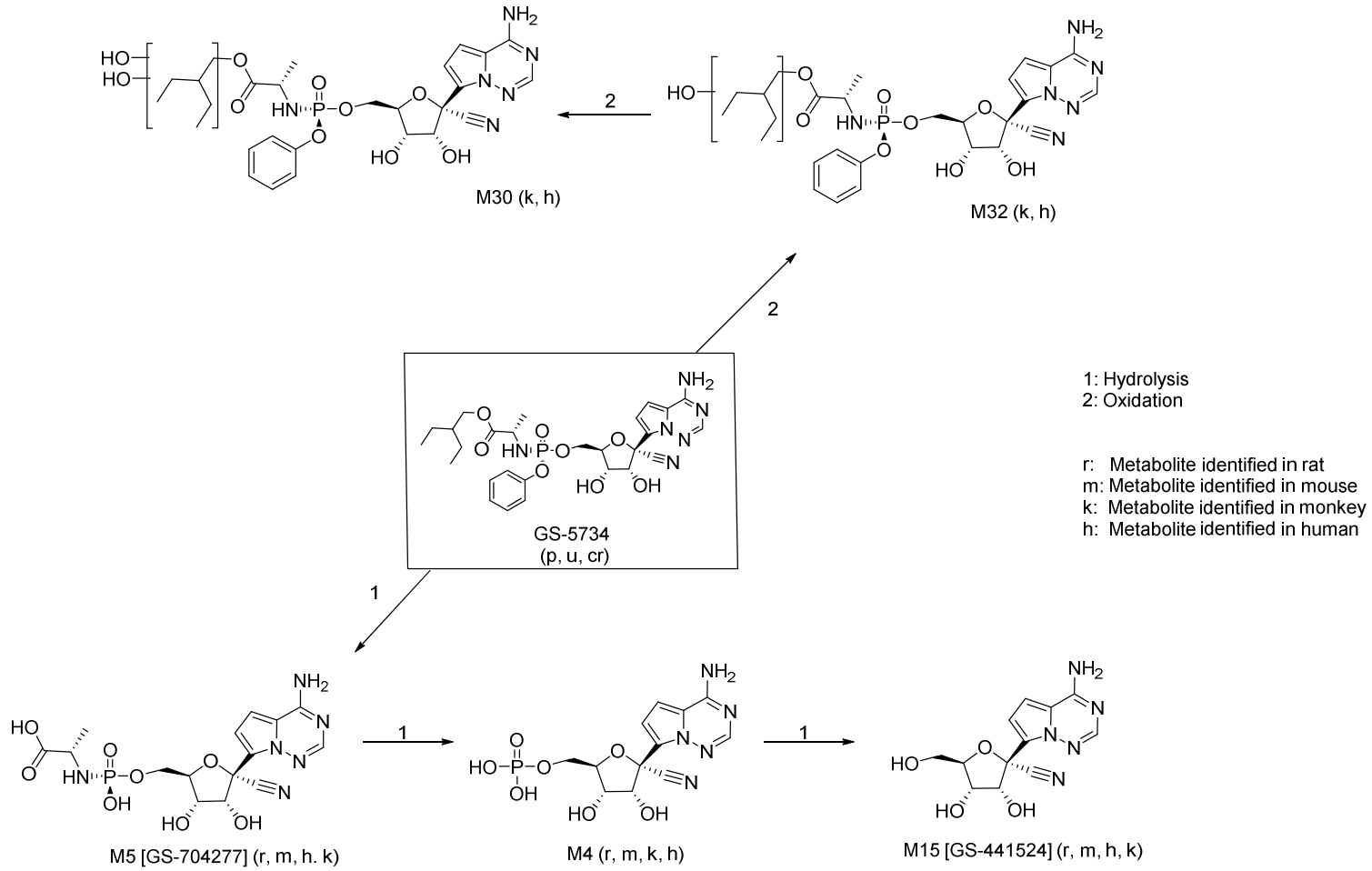
| Report Title | Study Type | Test Article | Report Number |
|---|---|---|---|
| Metabolism of 14C-GS-5734 in Mouse, Rat, Monkey, and Human Primary Hepatocytes | Metabolism | [$^{14}$C]GS-5734 | AD-399-2024 |

| | |
|---|---|
| Study System | Cryopreserved hepatocytes mixed-sex pool from C57BL6 mouse, Wistar Han rat, cynomolgus monkey, and human |
| Method | [$^{14}$C]GS-5734 (1 & 10 µM) was incubated (n =3) in hepatocyte suspensions (750,000 cells/mL) at 37ºC for 0, 0.5, 1, and 2 h. |

| | **Percent Relative Abundance at 2 Hours Following [$^{14}$C]GS-5734 (10 µM) Incubation [a]** | | | |
|---|---|---|---|---|
| **Component [c]** | **Mouse** | Rat | **Monkey** | **Human** |
| M1 | 1.22 | 4.86 | 2.97 | ND |
| M2 | ND | 2.70 | ND | ND |
| M4 | 14.9 | 7.92 | 1.43 | 3.49 |
| GS-704277 (M5) | 64.2 | 58.8 | 31.1 | 54.3 |
| GS-441524 (M15) | 6.05 | 10.7 | 12.2 | 18.2 |
| M16 | ND | 1.57 | ND | ND |
| M17 | ND | 1.70 | ND | ND |
| M19 | ND | 1.20 | 1.09 | ND |
| M27 | ND | 1.06 | ND | ND |
| M28 | ND | ND | 1.50 | 1.11 |
| M30 | ND | ND | 3.67 | 1.22 |
| M31b | ND | 1.01 | 8.25 | 5.66 |
| M32 | ND | ND | 3.31 | 1.54 |
| M35 | ND | ND | 4.74 | 1.35 |
| M38 | ND | ND | ND | ND |
| GS-5734 | 5.51 | 1.54 | 5.65 | 5.13 |
| Total | 91.9 | 93.0 | 63.7 | 92.0 |

ND = Peak not detected or below the limit of quantitation
a Determined by comparison of radiochromatographic peak area.
b M31 shown to be an artifact of ethanol
c Proposed structures are shown in Section 7.7

## 7.7. AD-399-2024: Proposed Biotransformation Pathways of [$^{14}$C]GS-5734 in Cryopreserved Hepatocytes from Mouse, Rat, Monkey and Human[a]




a The structures of following minor metabolites are unknown - M1, M2, M16, M17, M19, M27, M28, M32, M38

## 7.8. AD-540-2001: *In Vitro* Activation of GS-5734 in Normal Human Bronchial Epithelial (NHBE) cells

| Report Title | Study Type | Test Article | Report Number |
|---|---|---|---|
| *In Vitro* Activation of GS-5734 in Normal Human Bronchial Epithelial (NHBE) cells | Metabolism | GS-5734 | AD-540-2001 |
| **Method** | | | |

NHBE cells were incubated with GS-5734 at 1 µM for 2 hours. After 2 hours, the compound containing media was removed and cells were washed two time with 37□ media. Compound-free media was added and cells were incubated for an additional 30 hours. At selected time point (2,4,8,24 and 32), cells were collected and intracellular concentrations of GS-441524 and its phosphorylated metabolites were measured. Analysis was done by LC/MS/MS.

| | Mean Intracellular Concentration (pmol/million) | | | | |
|---|---|---|---|---|---|
| **Time** | **GS-441524** | **GS-719700** | **GS-719699** | **GS-443902** | **GS-704277** |
| 2 | 12.4 ± 7.43 | 1.02 ± 0.470 | 1.66 ± 0.849 | 14.5 ± 12.7 | 17.9 ±11.5 |
| 4 | 1.06 ± 0.420 | 0.811 ± 0.205 | 1.12 ± 0.498 | 10.2 ± 7.40 | 2.27 ± 0.860 |
| 8 | 0.558 ± 0.152 | 0.397 ± 0.020 | 0.796 ± 0.457 | 7.94 ± 6.80 | 0.962 ±0.317 |
| 24 | 0.384 ± 0.101 | BLQ | 0.595 ± 0.150 | 2.92 ± 2.69 | 0.552 ±0.182 |
| 32 | 0.554 ± 0.125 | BLQ | 0.455 ± 0.146 | 2.31 ± 1.82 | 0.551 ± 0.144 |

Data represent the mean ± SD of 3 independent experiments
BLQ = Below lower limit of quantitation (All analytes: 0.274 pmol/million)

## 7.9. AD-540-2002: *In Vitro* Activation of GS-5734 and GS-441524 in Calu-3, human lung adenocarcinoma cell line

| Report Title | Study Type | Test Article | Report Number |
|---|---|---|---|
| *In Vitro* Activation of GS-5734 and GS-441524 in Calu-3, human lung adenocarcinoma cell line | Metabolism | GS-5734, GS-441524 | AD-540-2002 |

**Method**

Calu-3 cells were incubated with remdesivir at 1 µM or GS-441524 at 10 µM for 48 hours. At select time points (2, 8, 24, 36 and 48 h), cells were harvested and intracellular concentrations of GS-704277 (For remdesivir incubation only), GS-441524 and its phosphorylated metabolites were measured. Analysis was done by LC/MS/MS

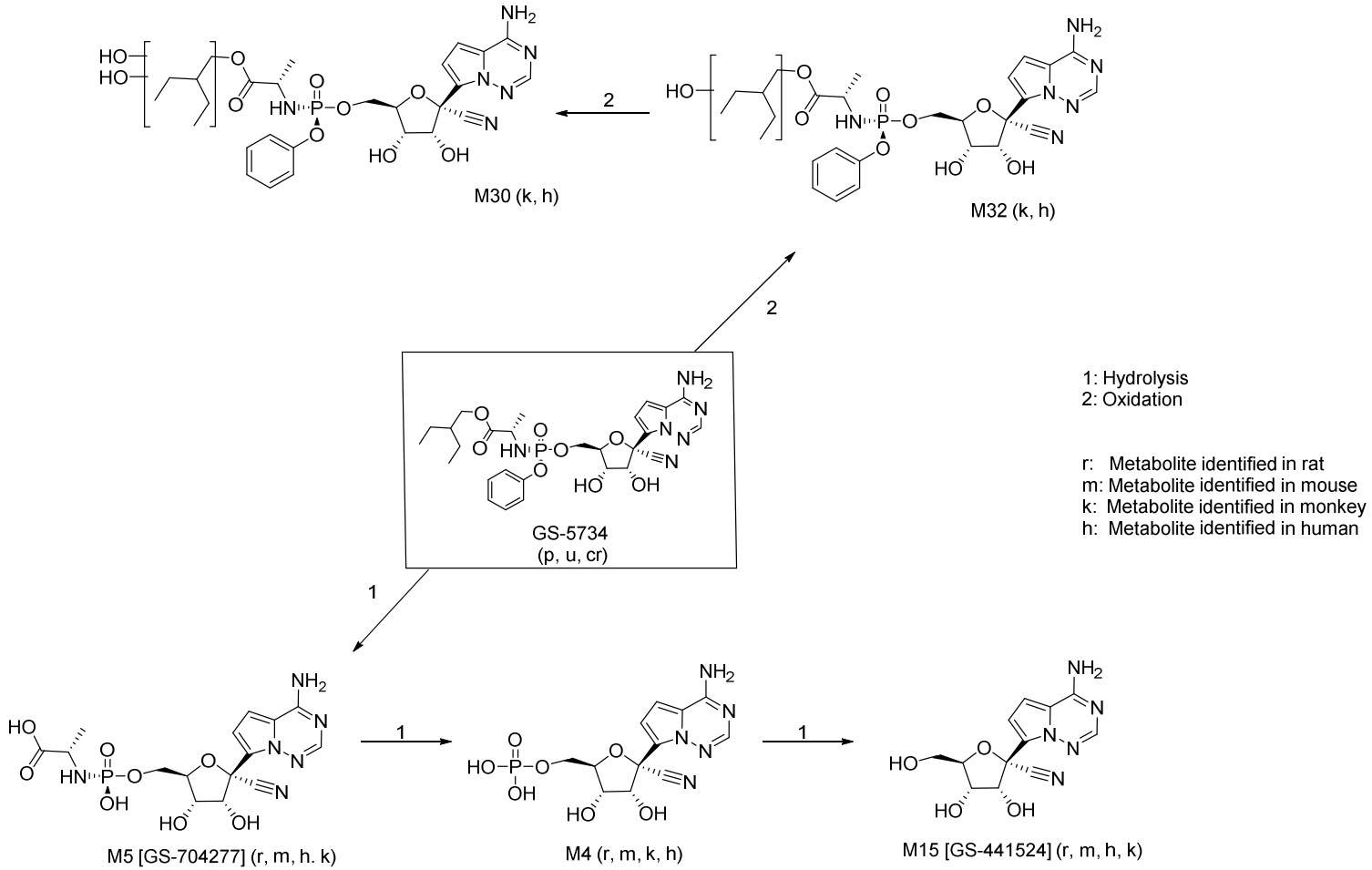
| | Mean Intracellular Concentrations in Calu-3 cells Following a 48-hour Continuous Incubation with 1 µM Remdesivir (Mean ± SD) [b,c] | | | | |
|---|---|---|---|---|---|
| **Time** | **GS-441524** | **GS-719700** | **GS-719699** | **GS-443902** | **GS-704277** |
| 2 | 2.86 ± 0.85 | 0.169 ± 0.026 | 0.517 ± 0.442 | 1.37 ± 0.37 | 1.44 ± 0.34 |
| 8 | 4.01 ± 0.42 | 0.363 ± 0.030 | 0.932 ± 0.517 | 3.88 ± 0.47 | 1.50 ± 0.29 |
| 24 | 6.52 ±3.47 | 0.678 ± 0.104 | 1.69 ± 0.90 | 8.33 ± 1.72 | 1.93 ± 0.26 |
| 36 [a] | 10.0 ± 0.4 | 0.727 ± 0.100 | 1.81 ± 0.68 | 8.36 ± 1.31 | 1.80 ± 0.10 |
| 48 | 4.31 ± 1.62 | 0.325 ± 0.009 | 1.14 ± 0.50 | 5.72 ± 1.10 | 0.959 ± 0.183 |

| | Mean Intracellular Concentration of GS-441524 and its Phosphorylated Metabolites in Calu-3 cells Following a 48- hour Continuous Incubation with 10 µM GS-441524 (Mean ± SD) [b,c] | | | |
|---|---|---|---|---|
| **Time** | **GS-441524** | **GS-719700** | **GS-719699** | **GS-443902** |
| 2 | 71.4 ±20.2 | 0.550 ± 0.108 | 0.715 ±0.146 | 3.69 ± 1.28 |
| 8 | 201 ± 98 | 3.83 ± 0.20 | 3.76 ± 0.64 | 14.6 ± 2.4 |
| 24 | 238 ± 150 | 4.05 ± 0.01 | 6.95 ± 1.43 | 26.8 ± 4.6 |
| 36 [a] | 316 ± 43 | 4.08 ± 0.02 | 7.82 ± 0.99 | 30.3 ± 8.1 |
| 48 | 157 ± 63 | 1.99 ± 0.01 | 4.01 ± 1.22 | 17.2 ± 4.5 |

a 36-hour time point samples were not collected during experiment 1.
b Time points from each experiment were collected in duplicate.
c Mean of 3 independent experiments performed in duplicate, total n=6 replicates except for 36 h with n=4 replicates

# 8. PHARMACOKINETICS: POSSIBLE METABOLIC PATHWAYS

## 8.1. Proposed Biotransformation Pathways of GS-5734 in Mouse, Rat, Monkey, and Human




The proposed pathways represent biotransformation products formed through one or more steps and are based on general knowledge of xenobiotic metabolism. The pathways were not established experimentally. Human (in vivo) metabolites not listed; metabolite identification from the radiolabeled ADME study (study GS-US-399-4231) in progress.

# 9. PHARMACOKINETICS: INDUCTION/INHIBITION OF DRUG METABOLIZING ENZYMES

## 9.1. AD-399-2010 and AD-540-2004: *In Vitro* Assessment of Human Cytochrome P450 Inhibition Potential of GS-5734

| Report Title | Study Type | Test Article | Report Number |
|---|---|---|---|
| *In Vitro* Assessment of Human Cytochrome P450 Inhibition Potential of GS-5734 | Drug-drug interaction | GS-5734 | AD-399-2010<br>AD-540-2004 |

**Method**

The inhibitory effect of GS-5734 on human P450 enzymes was investigated using human liver microsomes in the presence of NADPH at concentrations of GS-5734 up to 100 µM.

| Enzyme | Report | Activity | Calculated $IC_{50}$ (µM) | | Maximum Inhibition by GS-5734 (%) |
|---|---|---|---|---|---|
| | | | Control Inhibitor[a] | GS-5734 | |
| CYP1A2 | AD-540-2004 | Phenacetin O-deethylase | 0.15 | > 100 | 15.2% |
| CYP2B6 | AD-540-2004 | Bupropion hydroxylase | 1.3 | 77.8 | 54.6% |
| CYP2C8 | AD-540-2004 | Paclitaxel 6α-hydroxylase | 0.98 | 54.9 | 68.8% |
| CYP2C9 | AD-399-2010 | Tolbutamide 4-hydroxylase | 0.66 | 63.3 | 60.3% |
| CYP2C19 | AD-399-2010 | (S) Mephenytoin 4'-hydroxylase | 7.7 | 68.3 | 64.3% |
| CYP2D6 | AD-399-2010 | Dextromethorphan O-demethylase | 0.05 | 73.0 | 61.0% |
| CYP3A | AD-399-2010 | Midazolam 1'-hydroxylase | 0.04 | 1.6 | 87.7% |
| | AD-540-2004 | Testosterone 6β-hydroxylase | 0.21 | 11.0 | 88.8% |

a Control Inhibitors: CYP1A2 α Naphthoflavone (0-3 µM); CYP2B6 Ticlopidine (0-25 µM); CYP2C8 Montelukast (0-10 µM); CYP2C9, Sulfaphenazole (0-10 µM); CYP2C19, Tranylcypromine (0-50 µM); CYP2D6, Quinidine (0-3 µM); CYP3A, Ketoconazole (0-3 µM).

## 9.2. AD-540-2004: CYP3A Mechanism-Based Inhibition Potential of Remdesivir

| Report Title | Study Type | Report Number |
|---|---|---|
| Further Assessment of Human Cytochrome P450 Inhibition Potential of Remdesivir | Drug-drug interactions | AD-540-2004 |

**Method**

Human hepatic microsomal fraction spiked with remdesivir (final concentration 100 µ), positive control (mifepristone, 25 µM) or DMSO vehicle. Aliquots incubated ± NADPH cofactor at 37°C for 30 min. Incubations then diluted 10-fold and remaining CYP3A activity determined using midazolam 1'-hydroxylase with LC-MS detection. The %Change value was calculated from the incubation with compound + NADPH in comparison to that without NADPH and corrected for changes in incubations with DMSO vehicle. A %Change value of > 30% is considered positive.

| | | %Change (Mean ± SD) | |
|---|---|---|---|
| Enzyme | Positive control | Positive Control | Remdesivir |
| CYP3A (midazolam) | Mifepristone | 79.6 ± 1.0 | 26.1 ± 6.3 |

## 9.3. AD-540-2005: Human UGT1A1 Inhibition Potential of Remdesivir

| Report Title | | Study Type | Report Number |
|---|---|---|---|
| In Vitro Assessment of Human UGT1A1 Inhibition Potential of Remdesivir | | Drug-drug interactions | AD-540-2005 |
| **Method** | | | |
| Remdesivir or positive control inhibitor (atazanavir) incubated with insect cell microsomal fractions containing baculovirus-expressed human UGT1A1 and UDP-glucuronic acid cofactor. Activity determined by estradiol 3-glucuronidation. Activity remaining compared to incubations with DMSO vehicle. $IC_{50}$ values calculated by nonlinear regression (best-fit value ± standard error, n = 7 data points). | | | |
| **Compound** | **Role** | **$IC_{50}$ (µM)** | |
| Remdesivir | Test compound | 9.78 ± 2.11 | |
| Atazanavir | Clinically relevant positive control | 0.37 ± 0.02 | |

## 9.4. AD-399-2027: *In Vitro* Assessment of Induction Potential of GS-5734 and Metabolites in Humans

| Report Title | Study Type | Test Articles | Report Number |
|---|---|---|---|
| Evaluation of Cytochrome P450 Induction Following Exposure of Primary Cultures of Human Hepatocytes to GS-5734, GS-441524 and GS-704277 | Drug-drug interactions | GS-5734<br>GS-704277<br>GS-441524 | AD-399-2027 |

| Method |
|---|
| Human hepatocytes (n = 3 donors) incubated with test compounds, DMSO vehicle, positive controls and negative control (flumazenil) for three days (media changed every 24 h). Activity for CYP1A2 (phenacetin O-deethylase), CYP2B6 (bupropion hydroxylase) and CYP3A (testosterone 6β-hydoxylase) then determined by LC-MS and mRNA for CYP1A2, CYP2B6 and CYP3A4 determined by qRT-PCR. Concentrations of test compounds used were shown to be non-toxic using the MTS assay using a single hepatocyte donor in a prestudy. |

| | | Results for GS-5734 (1, 10 and 25 µM) | | | | | |
|---|---|---|---|---|---|---|---|
| | | mRNA | | | Enzyme Activity | | |
| Endpoint | Donor | Fold Change | %Emax | Induction | Fold Change | %Emax | Induction |
| CYP1A2 | 1 | 7.19 | 4.19 | Yes | 0.552 | 0 | No |
| | 2 | 1.40 | 1.05 | No | 1.13 | 1.12 | No |
| | 3 | 0.750 | 3.73 | No | 0.784 | 2.77 | No |
| CYP2B6 | 1 | 7.19 | 4.19 | Yes | 0.552 | 0 | No |
| | 2 | 1.40 | 1.05 | No | 1.13 | 1.12 | No |
| | 3 | 0.750 | 3.73 | No | 0.784 | 2.77 | No |
| CYP3A4 | 1 | 7.19 | 4.19 | No | 0.552 | 0 | No |
| | 2 | 1.40 | 1.05 | No | 1.13 | 1.12 | No |
| | 3 | 0.750 | 3.73 | No | 0.784 | 2.77 | No |

| Endpoint | Donor | Results for GS-441524 (5, 20 and 50 µM) | | | | | |
|---|---|---|---|---|---|---|---|
| | | mRNA | | | Enzyme Activity | | |
| | | Fold Change | %Emax | Induction | Fold Change | %Emax | Induction |
| CYP1A2 | 1 | 1.76 | 6.11 | No | 2.11 | 2.46 | No |
| | 2 | 1.28 | 1.61 | No | 2.17 | 0.964 | No |
| | 3 | 1.15 | 1.84 | No | 1.21 | 0.714 | No |
| CYP2B6 | 1 | 1.16 | 4.11 | No | 1.66 | 5.39 | No |
| | 2 | 0.911 | 3.18 | No | 0.929 | 0 | No |
| | 3 | 1.40 | 6.80 | No | 0.833 | 0 | No |
| CYP3A4 | 1 | 0.939 | 0.670 | No | 0.918 | 0 | No |
| | 2 | 0.750 | 0.441 | No | 1.10 | 1.17 | No |
| | 3 | 1.01 | 5.50 | No | 1.03 | 0.449 | No |

| Endpoint | Donor | Results for GS-704277 (1, 10 and 25 µM) | | | | | |
|---|---|---|---|---|---|---|---|
| | | mRNA | | | Enzyme Activity | | |
| | | Fold Change | %Emax | Induction | Fold Change | %Emax | Induction |
| CYP1A2 | 1 | 7.19 | 4.19 | Yes | 0.552 | 0 | No |
| | 2 | 1.40 | 1.05 | No | 1.13 | 1.12 | No |
| | 3 | 0.750 | 3.73 | No | 0.784 | 2.77 | No |
| CYP2B6 | 1 | 7.19 | 4.19 | Yes | 0.552 | 0 | No |
| | 2 | 1.40 | 1.05 | No | 1.13 | 1.12 | No |
| | 3 | 0.750 | 3.73 | No | 0.784 | 2.77 | No |
| CYP3A4 | 1 | 7.19 | 4.19 | No | 0.552 | 0 | No |
| | 2 | 1.40 | 1.05 | No | 1.13 | 1.12 | No |
| | 3 | 0.750 | 3.73 | No | 0.784 | 2.77 | No |

# 10. PHARMACOKINETICS: EXCRETION

## 10.1. AD-399-2017: Excretion in Bile Duct-Intact Rats Following Intravenous Administration of [$^{14}$C]GS-5734

| Report Title | Study Type | Test Article | Report Number |
|---|---|---|---|
| Pharmacokinetics, Distribution, and Excretion of $^{14}$C-GS-5734 Following a Single Intravenous Bolus Administration to Rats | Excretion | [$^{14}$C]GS-5734 | AD-399-2017 |

| | |
|---|---|
| Species | Sprague Dawley Rat (bile duct-intact) |
| Gender /No. of Animals | Male/3 |
| Feeding Condition | Non-fasted |
| Vehicle/Formulation | 12% Sulfobutylether-β-cyclodextrin in water, pH 3.0 |
| Method of Administration | Intravenous slow-bolus |
| Dose | 10 mg/kg (100 µCi/kg) |
| Analyte | Carbon-14 |
| Specific Activity | 58.0 mCi/mmol |
| Specific Activity of Formulation | 8.45 µCi/mg |
| Assay | Liquid scintillation counting |

| | Cumulative Recovery of % Administered $^{14}$C Dose | | | | % Administered $^{14}$C Dose | |
|---|---|---|---|---|---|---|
| | Urine | | Feces | | Cage Rinse | |
| Collection Period (h) | Mean | SD | Mean | SD | Mean | SD |
| 0–8 | 37.0 | 3.93 | NA | NA | NA | NA |
| 0–24 | 54.5 | 1.94 | 20.0 | 1.82 | 1.44 | 0.793 |
| 0–48 | 61.2 | 0.882 | 26.7 | 0.896 | 1.92 | 0.709 |
| 0–72 | 62.2 | 0.672 | 27.3 | 1.11 | 2.08 | 0.756 |
| 0–96 | 62.6 | 0.614 | 27.6 | 1.15 | 2.16 | 0.779 |
| 0–120 | 62.8 | 0.677 | 27.7 | 1.14 | 2.24 | 0.770 |
| 0–144 | 62.9 | 0.660 | 27.7 | 1.14 | 2.28 | 0.783 |
| 0–168 | 63.0 | 0.666 | 27.8 | 1.13 | NA | NA |
| Total Recovery (%)[a] | 95.1 | | | | | |

a Mean recovery of radioactivity from excreta and cage rinse.
NA = not applicable; SD = standard deviation

## 10.2. AD-399-2017: Excretion in Bile Duct Cannulated Rats Following Intravenous Administration of [$^{14}$C]GS-5734

| Report Title | Study Type | Test Article | Report Number |
|---|---|---|---|
| Pharmacokinetics, Distribution, and Excretion of $^{14}$C-GS-5734 Following a Single Intravenous Bolus Administration to Rats | Excretion | [$^{14}$C]GS-5734 | AD-399-2017 |

| | |
|---|---|
| Species | Sprague Dawley Rat (bile duct-cannulated) |
| Gender /No. of Animals | Male/3 |
| Feeding Condition | Non-fasted |
| Vehicle/Formulation | 12% Sulfobutylether-β-cyclodextrin in water, pH 3.0 |
| Method of Administration | Intravenous slow-bolus |
| Dose | 10 mg/kg (100 µCi/kg) |
| Analyte | Carbon-14 |
| Specific Activity | 58.0 mCi/mmol |
| Specific Activity of Formulation | 8.45 µCi/mg |
| Assay | Liquid scintillation counting |

| Collection Period (h) | Cumulative Recovery of % Administered $^{14}$C Dose | | | | | | % Administered $^{14}$C Dose | |
|---|---|---|---|---|---|---|---|---|
| | Urine | | Bile | | Feces | | Cage Rinse | |
| | Mean | SD | Mean | SD | Mean | SD | Mean | SD |
| 0-4 | 32.5 | 13.3 | 22.0 | 19.1 | NA | NA | NA | NA |
| 0–8 | 42.3 | 15.5 | 22.3 | 19.2 | NA | NA | NA | NA |
| 0–24 | 58.0 | 16.1 | 22.6 | 19.5 | 1.58 | 1.25 | 1.13 | 0.393 |
| 0–48 | 62.2 | 18.0 | 22.7 | 19.6 | 2.78 | 1.75 | 1.40 | 0.330 |
| 0–72 | 62.9 | 17.5 | 22.7 | 19.6 | 3.05 | 1.98 | 1.57 | 0.393 |
| 0–96 | 63.1 | 17.2 | 22.7 | 19.6 | 3.15 | 2.07 | 1.62 | 0.389 |
| 0–120 | 63.2 | 17.2 | 22.7 | 19.6 | 3.20 | 2.11 | 1.64 | 0.403 |
| 0–144 | 63.3 | 17.1 | 22.7 | 19.6 | 3.23 | 2.14 | 1.67 | 0.425 |
| 0–168 | 63.4 | 17.1 | 22.7 | 19.6 | 3.26 | 2.16 | NA | NA |
| Total Recovery (%)[a] | 95.3 | | | | | | | |

a Mean recovery of radioactivity from excreta, bile cannula & jacket, and cage rinse.
NA = not applicable; SD = standard deviation

## 10.3. AD-399-2019: Excretion in Cynomolgus Monkey Following Intravenous Administration of [$^{14}$C]GS-5734

| Report Title | Study Type | Test Article | Report Number |
|---|---|---|---|
| Pharmacokinetics, Distribution, and Excretion of $^{14}$C-GS-5734 Following a Single Intravenous Bolus Administration to Monkeys | Excretion | [$^{14}$C]GS-5734 | AD-399-2019 |

| | |
|---|---|
| Species | Cynomolgus monkey (bile duct-intact) |
| Gender /No. of Animals | Male/3 |
| Feeding Condition | Non-fasted |
| Vehicle/Formulation | 12% Sulfobutylether-β-cyclodextrin in water, pH 3.0 |
| Method of Administration | Intravenous slow-bolus |
| Dose | 10 mg/kg (25 µCi/kg) |
| Analyte | Carbon-14 |
| Specific Activity | 58.0 mCi/mmol |
| Specific Activity of Formulation | 2.37 µCi/mg |
| Assay | Liquid scintillation counting |

| Collection Period (h) | Cumulative Recovery of % Administered $^{14}$C Dose | | | | % Administered $^{14}$C Dose | |
|---|---|---|---|---|---|---|
| | Urine | | Feces | | Cage Rinse | |
| | Mean | SD | Mean | SD | Mean | SD |
| 0-4 | 4.44 | 3.26 | NA | NA | NA | NA |
| 0–8 | 7.34 | 7.17 | NA | NA | NA | NA |
| 0–24 | 22.1 | 4.30 | 10.0 | 1.26 | 12.1 | 4.97 |
| 0–48 | 29.0 | 3.64 | 22.1 | 1.81 | 14.6 | 5.92 |
| 0–72 | 30.9 | 3.81 | 24.6 | 2.16 | 15.5 | 5.82 |
| 0–96 | 31.9 | 4.10 | 25.1 | 2.06 | 15.9 | 5.76 |
| 0–120 | 32.5 | 4.25 | 25.3 | 1.99 | 16.4 | 5.64 |
| 0–144 | 33.2 | 4.19 | 25.5 | 2.00 | 16.9 | 5.64 |
| 0–168 | 33.6 | 4.28 | 25.6 | 2.00 | NA | NA |
| Total Recovery (%)[a] | 78.8 | | | | | |

a Mean recovery of radioactivity from excreta, carcass (8.26%), and cage rinse.
NA = not applicable; SD = standard deviation

## 10.4. AD-399-2025: Excretion in New Zealand White Rabbit Following Intravenous Administration of [$^{14}$C]GS-5734

| Report Title | Study Type | Test Article | Report Number |
|---|---|---|---|
| Pharmacokinetics, Distribution, and Excretion of $^{14}$C-GS-5734 Following a Single Intravenous Bolus Administration to New Zealand White Rabbits | Excretion | [$^{14}$C]GS-5734 | AD-399-2025 |

| | |
|---|---|
| Species | Cynomolgus monkey (bile duct-intact) |
| Gender /No. of Animals | Male/3 |
| Feeding Condition | Non-fasted |
| Vehicle/Formulation | 12% Sulfobutylether-β-cyclodextrin in water, pH 3.0 |
| Method of Administration | Intravenous |
| Dose | 10 mg/kg (30 µCi/kg) |
| Analyte | Carbon-14 |
| Specific Activity | 57.9 mCi/mmol |
| Specific Activity of Formulation | 2.45 µCi/mg |
| Assay | Liquid scintillation counting |

| Collection Period (h) | Cumulative Recovery of % Administered $^{14}$C Dose | | | | % Administered $^{14}$C Dose | |
|---|---|---|---|---|---|---|
| | Urine | | Feces | | Cage Rinse | |
| | Mean | SD | Mean | SD | Mean | SD |
| 0-4 | 0.00 | 0.00 | NA | NA | NA | NA |
| 0–8 | 0.00 | 0.00 | NA | NA | NA | NA |
| 0–24 | 48.5 | 3.12 | 3.30 | 0.505 | 9.11 | 4.66 |
| 0–48 | 60.8 | 5.68 | 7.38 | 1.01 | 10.9 | 5.36 |
| 0–72 | 64.4 | 6.43 | 9.50 | 1.45 | 11.8 | 5.39 |
| 0–96 | 65.6 | 6.57 | 10.5 | 1.49 | 12.1 | 5.37 |
| 0–120 | 66.4 | 6.82 | 11.1 | 1.50 | 12.4 | 5.42 |
| 0–144 | 66.7 | 6.84 | 11.6 | 1.54 | 12.4 | 5.46 |
| 0–168 | 67.0 | 6.90 | 11.9 | 1.57 | NA | NA |
| Total Recovery (%)[a] | 91.7 | | | | | |

a Mean recovery of radioactivity from excreta and cage rinse.
NA = not applicable; SD = standard deviation

# 11. PHARMACOKINETICS: OTHER DRUG-DRUG INTERACTIONS

## 11.1. AD-399-2011: Cytochrome P450 Metabolic Reaction Phenotyping of GS-5734

| Report Title | Study Type | Test Article | Report Number |
|---|---|---|---|
| Cytochrome P450 Metabolic Reaction Phenotyping of GS-5734 | Metabolism, *In Vitro* | GS-5734 | AD-399-2011 |

**Method**

Rates of metabolism, as assessed by the loss of GS-5734 (5 µM) catalyzed by cDNA expressed major human cytochrome P450 enzyme preparations co-expressed with NADPH CYP450 reductase. GS-5734 was assayed by LC/MS/MS.

| | Metabolism Rate ($min^{-1}$) | | | | | | |
|---|---|---|---|---|---|---|---|
| **Test Compound** | **CYP1A2** | **CYP2B6** | **CYP2C8** | **CYP2C9** | **CYP2C19** | **CYP2D6** | **CYP3A4** |
| GS-5734<br>(% Positive Control Rate) | < 0.12<br>(< 2.2%) | < 0.12<br>(< 2.2%) | 3.9<br>(16.1%) | < 0.47<br>(< 1.9%) | < 0.12<br>(< 0.6%) | 1.9<br>(16.4%) | 18.3<br>(42.1%) |
| Tacrine | 4.6 | — | — | — | — | — | — |
| Efavirenz | — | 1.77[a] | — | — | — | — | — |
| Amodiaquine | — | — | 24.5 | — | — | — | — |
| Diclofenac | — | — | — | 17.2 | — | — | — |
| Omeprazole | — | — | — | — | 21.8 | — | — |
| Dextromethorphan | — | — | — | — | — | 11.6 | — |
| Simvastatin | — | — | — | — | — | — | 43.5 |

a Efavirenz is a selective substrate for CYP2B6 but is metabolized relatively slowly.

## 11.2. AD-399-2007: Bidirectional Permeability of GS-5734 through Monolayers of P-gp and BCRP Over-Expressing Cells

| Report Title | Study Type | Test Article | Report Number |
|---|---|---|---|
| Bidirectional Permeability of GS-5734 through Monolayers of P-glycoprotein and Breast Cancer Resistance Protein Over-expressing Cells | Drug-drug interaction | GS-5734 | AD-399-2007 |

**Method**

The potential for GS-5734 to act as a substrate for P-gp (MDR1) and BCRP was tested in monolayers of either wild type, MDR1-transfected or BCRP-transfected Madin-Darby canine kidney (MDCK II) cells (MDCK II-WT, MDCK II-MDR1 and MDCK II-BCRP, respectively). The effects of transporter-selective inhibitors were also assessed. GS-5734 was assayed by LC/MS/MS.

| Cell Type | Target Conc. (µM) | Direction | Initial Conc. (µM) | Recovery (%) | $P_{app}$ ($10^{-6}$ cm/s) | | | Efflux Ratio |
|---|---|---|---|---|---|---|---|---|
| | | | | | Replicate 1 | Replicate 2 | Average | |
| MDCKII-WT | 1 | Cell-Free | 0.7 | 94 | 39 | — | 39 | — |
| | | Forward | 0.7 | 110 | 0.4 | 0.2 | 0.3 | 20 |
| | | Reverse | 0.7 | 110 | 5.7 | 6.5 | 6.1 | |
| MDCKII-MDR1 | | Forward | 0.5 | 170 | 0.2 | 0.1 | 0.1 | 68 |
| | | Reverse | 0.5 | 160 | 9.6 | 10 | 9.9 | |
| MDCKII-MDR1 (10 µM CsA) | | Forward | 0.3 | 170 | 0.5 | 0.3 | 0.4 | 6.6 |
| | | Reverse | 0.4 | 170 | 3.0 | 2.3 | 2.6 | |
| MDCKII-BCRP | | Forward | 0.5 | 170 | 0.9 | 0.4 | 0.7 | 16 |
| | | Reverse | 0.5 | 150 | 12 | 9.4 | 11 | |
| MDCKII-BCRP (10 µM Ko143) | | Forward | 0.3 | 160 | 0.6 | 1.3 | 1.0 | 12 |
| | | Reverse | 0.4 | 130 | 14 | 9.3 | 12 | |

BCRP = breast cancer resistance protein; MDR1 = P-glycoprotein (P-gp, ABCB1)

## 11.3. AD-399-2008: *In Vitro* Assessment of GS-5734 as Substrate for Human OATP1B1 and OATP1B3

| Report Title | Study Type | Test Article | Report Number |
|---|---|---|---|
| *In Vitro* Assessment of GS-5734 as Substrate for Human OATP1B1 and OATP1B3 | Drug-drug interaction (*in vitro*) | GS-5734 | AD-399-2008 |

**Method**

The potential of GS-5734 as a substrate in human OATP1B1 and OATP1B3 was assessed in Chinese Hamster Ovary (CHO) cells, either wild type or transfected with the genes encoding human OATP1B1 or OATP1B3 in the presence and absence of 40 µM rifampicin (OATP inhibitor). Atorvastatin and antipyrine were used as positive and passive permeability controls, respectively. Following removal of media, the cell extracts were analyzed by LC/MS/MS.

| | Uptake Rate (pmole/min/million cells) | | | | | |
|---|---|---|---|---|---|---|
| **Test Compound** | **0.5 µM GS-5734** | **0.5 µM GS-5734 + Rifampicin** | **0.1 µM Atorvastatin** | **0.1 µM Atorvastatin + Rifampicin** | **10 µM Antipyrine** | **10 µM Antipyrine + Rifampicin** |
| CHO-WT | 0.080 | 0.067 | 0.093 | 0.094 | 9.0 | 9.4 |
| CHO-OATP1B1 | 0.14 | 0.070 | 3.3 | 0.29 | 8.7 | 8.9 |
| CHO-OATP1B3 | 0.072 | 0.061 | 4.6 | 0.36 | 8.8 | 9.0 |
| OATP1B1/WT Ratio | 1.8 | NA | 35 | NA | 1.0 | NA |
| OATP1B3/WT Ratio | 0.90 | NA | 50 | NA | 1.0 | NA |

NA = not applicable; OATP = organic anion transporting polypeptide (SLCO or SLC22A gene products)

## 11.4. AD-399-2005: *In Vitro* Inhibition Assessment of GS-5734 with Human P-gp, BCRP, OATP1B1 and OATP1B3

| Report Title | Study Type | Test Article | Report Number |
|---|---|---|---|
| *In Vitro* Inhibition Assessment of GS-5734 with Human P-gp, BCRP, OATP1B1 and OATP1B3 | Drug-drug interaction (*in vitro*) | GS-5734 | AD-399-2005 |

**Method**

The inhibition potential of GS-5734 of human P-gp and BCRP was assessed in Madin Darby Canine Kidney (MDCKII) cells, either wild type or transfected with the genes encoding human P-gp or BCRP. The incubation was carried out in cell culture medium (without FBS) containing 10 µM Calcein AM (P-gp) or 1 µM pheophorbide a (PhA) (BCRP). Following removal of media containing calcein AM or PhA, the cells were analyzed immediately for calcein fluorescence at an excitation of 494 nm and an emission of 517 nm or PhA fluorescence at an excitation of 415 nm and an emission of 675 nm.

The inhibition potential of GS-5734 of human OATP1B1 and OATP1B3 was assessed in Chinese Hamster Ovary (CHO) cells, either wild type or transfected with the genes encoding human OATP1B1 or OATP1B3. GS-5734 and positive control compound were diluted in assay buffer containing 2 µM Fluo 3. Following removal of media containing Fluo 3, the cells were immediately analyzed for Fluo 3 fluorescence at an excitation of 485 nm and emission of 530 nm.

| | Efflux Transporters $IC_{50}$ (µM) | | Uptake Transporters $IC_{50}$ (µM) | |
|---|---|---|---|---|
| **Test Compound** | **P-gp** | **BCRP** | **OATP1B1** | **OATP1B3** |
| GS-5734 | > 40 | > 40 | 2.8 ± 0.6 | 2.1 ± 0.6 |
| Verapamil | 4.9 ± 2.0 | NA | NA | NA |
| Fumitremorgin C (FTC) | NA | 1.01 | NA | NA |
| Rifampicin | NA | NA | 4.1 ± 0.5 | 3.1 ± 0.6 |

BCRP = breast cancer resistance protein; NA = not applicable; OATP = organic anion transporting polypeptide (SLCO or SLC22A gene products); P-gp = P-glycoprotein

## 11.5. AD-399-2035: *In Vitro* inhibition assessment of GS-441524, GS-719700, GS-719699, GS-443902 and GS-704277 with the BCRP, BSEP, MRP2 and MRP3 Efflux Transporters

| Report Title: | Study Type | Test Article | Report Number |
|---|---|---|---|
| *In Vitro* inhibition assessment of GS-441524, GS-719700, GS-719699, GS-443902 and GS-704277 with the BCRP, BSEP, MRP2 and MRP3 Efflux Transporters | Drug-drug interaction (in vitro) | GS-441524, GS-719700, GS-719699, GS-443902 and GS-704277 | AD-399-2035 |

**Method** The potential for test compounds to inhibit the human breast cancer resistance protein (BCRP), bile salt export pump (BSEP), and multidrug resistance associated proteins (MRP2 and MRP3) was assessed in vitro in membrane vesicular transport assays. In BCRP vesicular transport assay, test compounds were incubated with membrane vesicle preparations (total protein: 25 µg/well) and probe substrate estrone-3-sulfate (1 µM) in the absence or presence of ATP. In BSEP vesicular transport assay, test compounds were incubated with membrane vesicle preparations (total protein: 50 µg/well) and probe substrate taurocholate (2 µM) in the absence or presence of ATP. In MRP2 vesicular transport assay, test compounds were incubated with membrane vesicle preparations (total protein: 50 µg/well) and probe substrate E217βG (50 µM) in the absence or presence of ATP. In MRP3 vesicular transport assay, test compounds were incubated with membrane vesicle preparations (total protein: 50 µg/well) and probe substrate E217βG (10 µM) in the absence or presence of ATP. The amount of substrate inside the vesicles was determined by liquid scintillation counting for all vesicular transport assays.

| | GS-441524 | | GS-719700 | | GS-719699 | | GS-443902 | | GS-704277 | |
|---|---|---|---|---|---|---|---|---|---|---|
| Transporter | $IC_{50}$ (µM) | Maximum inhibition (%) | $IC_{50}$ (µM) | Maximum inhibition (%) | $IC_{50}$ (µM) | Maximum inhibition (%) | $IC_{50}$ (µM) | Maximum inhibition (%) | $IC_{50}$ (µM) | Maximum inhibition (%) |
| BCRP | NA | NIO up to 200µM | NA | NIO up to 200µM | NA | 26% at 200µM | NA | NIO up to 200µM | NA | NIO up to 200µM |
| BSEP | NA | NIO up to 200µM | NA | NIO up to 200µM | NA | NIO up to 200µM | NA | NIO up to 200µM | NA | NIO up to 200µM |
| MRP2 | NA | NIO up to 200µM | NA | NIO up to 200µM | NA | NIO up to 200µM | NA | 24% at 200µM | NA | NIO up to 200µM |
| MRP3 | NA | NIO up to 200µM | NA | NIO up to 200µM | NA | NIO up to 200µM | NA | NIO up to 200µM | NA | NIO up to 200µM |

BCRP = breast cancer resistance protein; BSEP = bile salt export pump; MRP2 = multidrug resistance associated protein 2; MRP2 = multidrug resistance associated protein 3; E217βG = estradiol 17β-D-glucuronide; ND = Not Applicable; NIO = No Inhibition Observed

## 11.6. AD-399-2029: *In Vitro* Inhibition Assessment of GS-5734 and its Metabolites with human BSEP, MRP2, MRP4, and NTCP Transporters

| Report Title: | Study Type | Test Article | Report Number |
|---|---|---|---|
| *In Vitro* Inhibition Assessment of GS-5734 and its Metabolites with human BSEP, MRP2, MRP4, and NTCP Transporters | Drug-drug interaction (in vitro) | GS-5734, GS-704277, GS-441524 | AD-399-2029 |

**Method** The potential for test compounds to inhibit the human bile salt export pump (BSEP), multidrug resistance associated proteins (MRP2 and MRP4), and Na/Taurocholate co-transporting polypeptide (NTCP) transporter was assessed in vitro in membrane vesicular transport or cell-based transporter assays. In BSEP vesicular transport assay, test compounds were incubated with membrane vesicle preparations (total protein: 50 µg/well) and probe substrate taurocholate (2 µM) in the absence or presence of ATP. In MRP2 vesicular transport assay, test compounds were incubated with membrane vesicle preparations (total protein: 50 µg/well) and probe substrate E217βG (50 µM) in the absence or presence of ATP. In MRP4 vesicular transport assay, test compounds were incubated with membrane vesicle preparations (total protein: 50 µg/well) and probe substrate DHEAS (0.5 µM) in the absence or presence of ATP. In NTCP transport assay, test compounds were incubated with CHO cells transfected with NTCP transporters and probe substrate taurocholate (2 µM) in the presence and absence of sodium. The amount of substrate inside the vesicles or cells was determined by liquid scintillation counting for all vesicular transport assays.

| | GS-5734 | | GS-704277 | | GS-441524 | |
|---|---|---|---|---|---|---|
| Transporter | $IC_{50}$ (µM) | Maximum inhibition (%) | $IC_{50}$ (µM) | Maximum inhibition (%) | $IC_{50}$ (µM) | Maximum inhibition (%) |
| BSEP | 22 | 86% at 100µM | >100 | 25% at 100µM | >100 | NIO up to 100µM |
| MRP2 | >100 | NIO up to 100µM | >100 | NIO up to 100µM | >100 | NIO up to 100µM |
| MRP4 | 5.1 | 91% at 100µM | >100 | NIO up to 100µM | >100 | NIO up to 100µM |
| NTCP | 72 | 58% at 100µM | >100 | 44% at 100µM | >100 | 24% at 100µM |

BSEP = bile salt export pump; MRP2 = multidrug resistance associated protein 2; MRP4 = multidrug resistance associated protein 4; NTCP = Na/Taurocholate co-transporting polypeptide; E217βG = estradiol 17β-D-glucuronide; DHEAS = dehydroepiandrosterone sulfate; CHO = Chinese Hamster Oocyte; NIO = No Inhibition Observed

## 11.7. AD-540-2006: Regulatory DDI Calculations

| Report Title | | Study Type | Report Number |
|---|---|---|---|
| Drug-Drug Interaction Liability Assessment for Remdesivir | | Drug-drug interactions | AD-540-2006 |

**Method**

Using current FDA Guidelines, representative plasma Cmax values for remdesivir were compared with its potency to act as an inducer and its potency to be an enzyme inhibitor.

**Inhibitory Effects of Remdesivir upon Enzyme Activities: Basic Model**

| Enzyme | $K_{i,u}$ (µM) | $R_1$ |
|---|---|---|
| CYP1A2 | > 32.3 | **< 1.027** |
| CYP2B6 | 31.8 | **1.028** |
| CYP2C8 | 15.0 | **1.059** |
| CYP2C9 | 12.6 | **1.070** |
| CYP2C19 | 21.3 | **1.041** |
| CYP2D6 | 17.8 | **1.049** |
| CYP3A M | 0.6 | **2.445** |
| CYP3A T | 2.4 | **1.367** |
| UGT1A1 | 3.1 | **1.285** |

Values in **bold** potentially exceed the threshold for liability

**Inhibitory Effects of Remdesivir upon Enzyme Activities: Mechanistic Static Model**

| Enzyme | Hepatic Component | AUCR |
|---|---|---|
| CYP1A2 | > 0.97 | < 1.02 |
| CYP2B6 | 0.97 | 1.02 |
| CYP2C8 | 0.94 | 1.05 |
| CYP2C9 | 0.93 | 1.06 |
| CYP2C19 | 0.96 | 1.04 |
| CYP2D6 | 0.95 | 1.05 |
| CYP3A M | 0.41 | **2.11** / **1.61**[a] |
| CYP3A T | 0.73 | **1.31** / 1.16[a] |
| UGT1A1 | 0.78 | 1.22 |

Values in **bold** potentially exceed the threshold for liability
a Value calcuated using Day 1 $C_{max}$ (200 mg dose) / Value calculated using Day 5 $C_{max}$ (100 mg dose)

**Basic and Mechanistic Static Model Calculations for Induction Liability for Remdesivir for the Hepatocytes (Donor 1) and Endpoints (CYP1A2 and CYP2B6 mRNA) Exhibiting a Positive Signal**

| Parameter | CYP1A2 | CYP2B6 |
|---|---|---|
| $EC_{50,u}$ (μM) | 83.9 | 80.6 |
| $E_{max}$ (fold over control) | 22.5 | 20.1 |
| $R_3$ | **0.32** | **0.34** |
| Hepatic component | 1.23 | 1.22 |
| AUCR | 0.84 | 0.88 |

Values in **bold** potentially exceed the threshold for liability

**Inhibitory Effects of Remdesivir upon Transporters**

| Transporter | $IC_{50,u}$ (µM) | $K_{i,u}$ (µM) | Guidance Metric |
|---|---|---|---|
| P-gp | > 40 | > 40 | < 0.022 |
| BCRP | > 40 | > 40 | < 0.022 |
| OATP1B1 | 2.8 | 2.8 | **0.31** |
| OATP1B3 | 2.1 | 2.1 | **0.42** |

Values in **bold** potentially exceed the threshold for liability

# 12. PHARMACOKINETICS: OTHER

No other pharmacokinetic studies have been conducted.