INVESTIGATOR'S BROCHURE

OBELDESIVIR (GS-5245) CORONAVIRUS DISEASE

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~~28 July 2022~~

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SYNOPSIS OF CHANGES TO THE INVESTIGATOR'S BROCHURE

GS-5245Obeldesivir for Coronavirus Disease Edition 34: 28 July 202206 June 2023

Previous Edition Editions	Date
3	28 July 2022
2	28 April 2022
1	26 October 2021

The investigator's brochure (IB) for obeldesivir (ODV; GS-5245) was updated to reflect new information from the ongoing development program of Gilead Sciences (Gilead) as summarized below. The reference safety information (RSI) for GS-5245ODV is provided in Appendix 8.19.1.

Section 21: Executive Summary was added.

Section 3: Physical, Chemical, and Pharmaceutical Properties and Formulation

-Section 2.23.2 (Formulation) was updated with the new name for an excipient in the GS-5245 and placebo formulations.

Section 3: Nonclinical Studies
-Section 3.3.5 (Reproductive and Developmental Toxicity) and Section 3.3.8 (Toxicology Conclusions) were updated to include preliminary results from embryo-fetal development studies in rabbits (Study TX-611-2005 and Study TX-611-2009) and rats (Study TX-611-2009)to include information for the 175 mg ODV formulation.

Section 4: Clinical Studies was updated to state there is an on-going Phase 1 clinical study in healthy volunteers.

Section 6: Summary of Data and Guidance for the Investigator
-Section 6.1.1 (Nonclinical FindingsStudies) was updated to include preliminarykey results from embryo fetal developmentnonclinical studies in rats (Study TX-611-2005 and Study TX-611-2009) and rabbits (Study TX-611-2008).as follows:

- Study PC-611-2026: antiviral activity of ODV against Omicron subvariants B.1.1.529/BA.1, BA.2, BA.2.12.1, BA.4, BA.5, BA.2.75, BA.4.6, BF.5, XBB, and BQ.1.1 in transformed human lung epithelial small cell carcinoma cells overexpressing human angiotensin-converting enzyme 2 (A549-hACE2)-TMPRSS2 cells

- Study PC-611-2029: the in vitro drug combination antiviral activity of GS-441524 or ODV in combination with molnupiravir, nirmatrelvir, or nirmatrelvir and ritonavir against SARS-CoV-2

- Study of the in vivo therapeutic efficacy of ODV against SARS-CoV-2 Omicron variant BA.1. in K18-hACE2 mice {Martinez 2023}

- Study of the in vivo therapeutic efficacy of ODV in combination with nirmatrelvir against SARS-CoV-2 in Balb/c mice {Martinez 2023}
- Study PC-554-2001: the in vivo therapeutic efficacy of ODV against SARS-CoV-2 in African green monkeys
- Study TX-611-2015: a 4-week oral gavage toxicity and toxicokinetic study with ODV in Wistar Han rats with a 4-week recovery period
- Study TX-611-2016: a 4-week oral gavage toxicity and toxicokinetic study with ODV in beagle dogs with a 4-week recovery period
- Study TX-611-2011: oral (gavage) study of the effects of ODV on fertility and early embryonic development to implantation in Wistar Han rats
- Study TX-611-2012: oral (gavage) study of the effects of ODV on prenatal and postnatal development, including maternal function in Wistar Han rats (preliminary data)
- Study TX-611-2026: phototoxicity study using mouse fibroblasts
- Study TX-611-2006: repeat-dose toxicity study to compare the toxicity of different manufactured lots of ODV administered once daily by oral (gavage) to Wistar Hans rats

Section 6.1.25 (Clinical FindingsStudies) was updated to state there is an on-goinginclude key results from clinical studies as follows:

- Study GS-US-611-6248: results are summarized from this Phase 1 study evaluating the safety, tolerability, and pharmacokinetics (PK) of ODV, and the effect of food and formulation on ODV PK in healthy participants.
- Study GS-US-611-6408: results are summarized from this Phase 1 clinical study evaluating the PK, metabolism, and excretion of ODV in healthy volunteersparticipants.

Section 6.2.5 (Adverse Drug Reactions) was updated to include laboratory changes (transiently decreased creatinine clearance that was reversible).Section 6.2.9 (Pregnancy, Contraception and Lactation) was updated based on the findings from the nonclinical embryo-fetal development studies.

Section 8.2: Tablular Summary of Nonclinical Safety Studies
Appendix Table 4: Summaries of Reproductive Toxicity Studies was added

Appendix 9.2 (Tabular Summary of Nonclinical Studies) was updated to include the nonclinical studies added to Section 4.

Appendix 9.3 (Tabular Summary of Clinical Studies) was updated to include completed clinical studies as of 12 May 2023.

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GLOSSARY OF ABBREVIATIONS AND DEFINITIONDEFINITIONS OF TERMS

5-FU	5-fluorouracil
	A549-hACE2 transformed human lung epithelial small cell carcinoma cells overexpressing human angiotensin-converting enzyme 2
ADR	adverse drug reaction
AE	adverse event
AGM	African green monkey
ALP	alkaline phosphatase
aPTT	activated partial thromboplastin time
ATP	adenosine triphosphate
AUC	area under the concentration versus time curve
AUC_{inf}^{inf}	area under the concentration versus time curve extrapolated to infinite time, calculated as $AUC_{last}^{AUC_{last}} + (C_{last}^{C_{last}}/\lambda_z^{\lambda_z})$
AUC_{last}^{last}	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"
BALF	bronchoalveolar lavage fluid
BCRP	breast cancer resistance protein
~~BID~~	~~twice daily~~
~~BLQ~~	~~below limit of quantitation~~
~~BMS~~	~~Bristol-Myers Squibb~~
CC_{50}	half-maximal cytotoxic concentration
CL_{cr}	creatinine clearance
CL_r	renal clearance
C_{max}	maximum observed concentration of drug
CNS	central nervous system
CNT	concentrative nucleoside transporter
CoV	coronavirus
COVID-19	coronavirus disease 2019
CYP	cytochrome P450 enzyme
DDI	drug-drug interaction
DMSO	dimethylsulfoxide
DNA	deoxyribonucleic acid
dpi	days postinfection
EC_{50}	half-maximal effective concentration
ECG	electrocardiogram
E_H	hepatic extraction
~~EIDD-2801~~	~~molnupiravir~~
ENT	equilibrative nucleoside transporter
~~F~~	~~bioavailability~~
~~FC~~	~~functional class~~
FDA	Food and Drug Administration
GD	gestation day
GI	gastrointestinal
Gilead	Gilead Sciences/Gilead Sciences, Inc.
GLP	Good Laboratory Practice
~~hACE2~~	~~human angiotensin-converting enzyme 2~~
HAE	human airway epithelial
~~HED~~	~~human equivalent dose~~
hERG	human ether-a-go-go-related gene
hpi	hours postinfection
IC_{50}	half-maximal inhibitory concentration
ICH	~~ICH~~ International Council for Harmonisation (of Technical Requirements for Pharmaceuticals for Human Use)
IUPAC	International Union of Pure and Applied Chemistry
IV	intravenous
~~k_a~~	~~first order absorption rate constant~~LD lactation day

~~K_m~~	~~concentration at half-maximum nonlinear elimination~~
MATE	multidrug and toxin extrusion
MERS	Middle East respiratory syndrome
MN	micronuclei
~~mRNA~~	~~messenger RNA~~
MRP	multidrug resistance-associated protein
mRNA	messenger RNA
mtDNA	mitochondrial DNA
mtRNA	mitochondrial RNA
NHBE	normal human bronchial epithelial
NMP	nucleoside monophosphate
NOAEL	no observed adverse effect level
NOEL	no observed effect level
NTCP	sodium-taurocholate cotransporting polypeptide
OAT	organic anion transporter
OATP	organic anion transporting polypeptide
ODV	obeldesivir (GS-5245)
OECD	Organization for Economic Co-Operation and Development
PBMC	peripheral blood mononuclear cell
PCE	polychromatic erythrocyte
P-gp	P-glycoprotein
PHH	primary human hepatocyte
PK	pharmacokinetic(s)
PND	postnatal day
PR (interval)	electrocardiographic interval occurring between the onset of the P wave and the QRS complex representing time for atrial and ventricular depolarization, respectively
PTM	placebo-to-match
QRS	electrocardiographic deflection between the beginning of the Q wave and termination of the S wave, representing time for ventricular depolarization
QT (interval)	electrocardiographic interval between the beginning of the Q wave and termination of the T wave, representing the time for both ventricular depolarization and repolarization to occur
~~electrocardiographic interval between the beginning of the Q wave and termination of~~ QT	electrocardiographic deflection between the beginning of the Q wave and termination of the S wave, representing time for ventricular depolarization
RBC	red blood cell
RdRp	RNA-dependent RNA polymerase
RDV	remdesivir (Veklury®)
RNA	ribonucleic acid

RSV	respiratory syncytial virus
SARS	severe acute respiratory syndrome
SARS-CoV-2	severe acute respiratory syndrome coronavirus 2
SARS-CoV-2 (MA10)	mouse-adapted severe acute respiratory syndrome coronavirus 2
SD	standard deviation
SI	selectivity index
$t_{1/2}$	terminal elimination half-life
$TCID_{50}$	50% tissue culture infectious dose
~~TE~~	~~total erythrocyte~~
TK	toxicokinetic(s)
T_{max}	time (observed time point) of C_{max}
UGT	uridine diphosphate glucuronosyltransferase
US	United States
V_d	volume of distribution
V_{max}	maximum rate of nonlinear elimination
V_{ss}	volume of distribution at steady state
WBC	white blood cell

1. EXECUTIVE SUMMARY

Obeldesivir (ODV; GS-5245) is being developed for the treatment of coronavirus disease.

Obeldesivir is a mono-5'-isobutyryl ester prodrug of GS-441524. Following oral administration, ODV is extensively hydrolyzed presystemically to the parent nucleoside GS-441524, which can then enter cells where it is subsequently anabolized to the same active triphosphate metabolite (GS-443902) as remdesivir (RDV; Veklury®). Obeldesivir has been developed with the intent to deliver consistent and high systemic exposures of GS-441524 following oral administration. At targeted therapeutic doses of ODV, GS-441524 exposures are anticipated to be approximately 14-fold higher as compared to exposures with RDV.

Table 1 presents a comparison of steady-state pharmacokinetics (PK) of metabolites (GS-443902 and GS-441524) following administration of the Phase 3 dosing regimens for oral ODV (350 mg twice daily) and intravenous (IV) RDV (200/100 mg once daily).

Table 1. Comparison of Steady-State PK of Metabolites Following Administration of Oral ODV and IV RDV

Metabolite PK Parameter[a]	ODV[b] Phase 3 Regimen 350 mg BID (N = 6)	RDV[c] Approved Regimen 200/100 mg QD (N = 26)
GS-443902 in PBMCs		
AUC_{0-24}, h•µM[d]	591 (49.1)	240 (25.4)
C_{tau}, µM	32.2 (33.4)	10.2 (49.5)

GS-441524 in plasma

AUC_{0-24}, h•ng/mL	30,100 (19.3)	2230 (18.4)
C_{max}, ng/mL	3240 (18.2)	145 (19.3)

%CV = percentage coefficient of variation; BID = twice daily; IV = intravenous; ODV = obeldesivir (GS-5245); PBMC = peripheral blood mononuclear cell; PK = pharmacokinetic(s); QD = once daily; RDV = remdesivir a Data presented as mean (%CV).

b Reference: GS-US-611-6248 multiple-dose PK parameters following 500 mg BID oral administration for 5 days of ODV to healthy participants (Cohort 5) were scaled to 350 mg BID, as supported by dose proportionality in this range.

c Reference: Humeniuk R, et al. Clinical Pharmacokinet 2021;60:569-583; GS-US-399-5505, multiple-dose PK parameters following IV administration of RDV 100 mg for 5-10 days to healthy participants.

d AUC_{0-24} calculated as 2 × AUC_{tau} for ODV.

2. ~~1.~~INTRODUCTION

2.1. ~~1.1.~~Background Information

~~Coronaviruses (CoVs) are positive-sense, single-stranded enveloped RNA viruses, many of which are commonly found in humans and cause mild symptoms. Over the past 2 decades, emerging pathogenic CoVs that can cause life-threatening disease in humans and animals have been identified, namely severe acute respiratory syndrome (SARS)-CoV {de Wit 2016, Ksiazek 2003}, Middle East respiratory syndrome (MERS)-CoV {Assiri 2013, Choi 2016}, and SARS-CoV-2 {Zhu 2020}.~~

~~In December 2019, a series of pneumonia cases of unknown cause emerged in Wuhan, Hubei province, China {Huang 2020}. Sequencing analyses from respiratory tract samples of patients identified a novel CoV, which was named SARS-CoV-2. Cases of COVID-19, the disease caused by SARS-CoV-2, rapidly increased throughout the world {Zhou 2020}, and on 11 March 2020, the World Health Organization declared COVID-19 a pandemic {World Health Organization (WHO) 2020}. The clinical spectrum of COVID-19 ranges from mild viral illness without evidence of pneumonia to moderate or severe pneumonia to critical illness with acute respiratory distress syndrome and/or multiple organ dysfunction {COVID-19 Treatment Guidelines Panel 2021, Kwok 2021}, and while most patients infected with SARS-CoV-2 experience a mild clinical course, disease progression requiring hospitalization is possible.~~

~~The SARS-CoV-2 pandemic continues, causing high numbers of deaths and taxing healthcare systems worldwide. New variants have evolved that challenge our ability to end the pandemic. Despite the availability of vaccines that reduce the risk of hospitalization or death from COVID-19, hospitalizations and deaths remain high, and as of 22 July 2022, a total of more than 565,207,160 confirmed cases and 6,373,739 associated deaths were reported worldwide {World Health Organization (WHO) }. The development of therapies that can effectively treat COVID-19 is urgently needed.~~

~~1.2. Rationale for Development of GS-5245~~

~~Remdesivir (RDV; GS-5734; Veklury®) is a monophosphoramidate prodrug administered by intravenous (IV) infusion once daily over 3 to 10 days that is approved for the treatment of patients who are hospitalized with COVID-19 or who are not hospitalized and have mild to moderate COVID-19 and are at high risk of progression to severe COVID-19 {VEKLURY 2021, VEKLURY 2022}. The pharmacologically active metabolite of RDV within lung cells is the triphosphate (GS-~~

443902), which is generated by the enzymatic breakdown of the prodrug in lung cells to the monophosphate metabolite and subsequent phosphorylation by cellular kinases to the active triphosphate. The triphosphate metabolite of RDV is recognized and incorporated in SARS-CoV-2 viral RNA by viral RNA-dependent RNA polymerase (RdRp) as an alternate substrate, thereby selectively inhibiting the synthesis of viral RNA {Gordon 2020c, Tchesnokov 2020}.

The SARS-CoV-2 RdRp nucleotide binding site is highly conserved across known SARS-CoV-2 variants. Sequencing data from more than 90,000 globally circulating SARS-CoV-2 clinical isolates showed low diversity and high genetic stability of the RNA replication complex, with no substitutions near the established polymerase active site or sites critical for the RDV mechanism of inhibition {Martin 2021}. In vitro resistance selection experiments with CoVs have demonstrated a high barrier to development of resistance to RDV, with high passage numbers required to develop nucleotide substitutions and minimal-fold decreases in activity associated with these substitutions (Studies PC-540-2028 and PC-540-2029; {Agostini 2018, Szemiel 2021}). Recent in vitro studies confirmed potent antiviral activity of the RDV triphosphate metabolite against SARS-CoV-2 variants of interest, and it is anticipated that future CoV strains are likely to remain susceptible to RDV, as it has potent activity in vitro and in vivo toward both SARS-CoV and MERS-CoV, whose polymerase active sites are 100% conserved (Studies PC-540-2021, PC-540-2024, and PC-540-2026; {de Wit 2020, Sheahan 2017}).Remdesivir is approved for the treatment of COVID-19 in hospitalized and nonhospitalized patients in the United States (US), the European Union, Japan, and other countries for pediatric and adult patients {Veklury. Gilead Sciences Inc. 2023, VEKLURY® Gilead Sciences 2023} The broader utility of RDV for the treatment of early infection with SARS-CoV-2 and other potential emerging CoV strains is limited due to the IV route of administration; therefore,. Currently available oral therapies have limitations in pill burden and drug-drug interactions (DDIs). Clinically relevant DDIs need to be managed by dose reduction, use of alternative agents, increased monitoring for adverse events (AEs) and/or drug levels, temporary discontinuation or avoidance of coadministration of certain concomitant medications. This complicates the use of these treatments in a broader population of COVID-19 patients.
Therefore, the availability of more convenientoral treatment options using other routes of administration is desirable. In nonclinical studies, oral RDV was found to have very low bioavailability (< 1% in cynomolgus monkey) and exposure of the parent nucleoside (GS-441524) was also very low, indicating extensive first-pass hepatic extraction (E_H) of the prodrug {Mackman 2021}. An alternative approach to generating the same triphosphate metabolite in the lung is through systemic exposure of GS-441524. However, GS-441524 is metabolized to the active triphosphate less efficiently than RDV, and direct oral delivery of GS-441524 at high systemic exposures is hampered by low and variable oral bioavailability across nonclinical species.with fewer DDIs and a reduced pill burden is crucial to increase patient access to early antiviral therapy, which may confer benefits such as decreased hospitalization and death, a reduction in the duration of infectiousness, a shorter duration of illness, and/or a more rapid return to normal activities.

GS-5245

2.2. Rationale for Development of ODV

Obeldesivir is a mono-5'-isobutyryl ester prodrug of GS-441524. Following oral administration, GS-5245ODV is extensively hydrolyzed presystemically to the parent nucleoside GS-441524, which can then enter cells where it is subsequently anabolized to the same active triphosphate

metabolite (GS-443902) as RDV. ~~GS-5245 is being~~Obeldesivir has been developed with the intent to deliver consistent and high systemic exposures ~~to~~of GS-441524 following oral administration ~~and therefore~~.

Availability of a highly effective oral treatment with a high barrier to resistance, similar to that of RDV, with minimal DDIs and a reduced pill burden (ie, the dose of ODV selected for Phase 3 is one 350-mg tablet twice daily) has the potential to address a critical public health need in the ongoing COVID-19 pandemic.

Obeldesivir represents a promising oral option for the treatment of COVID-19 that is anticipated to fulfill an unmet medical need.

3. ~~2.~~PHYSICAL, CHEMICAL, AND PHARMACEUTICAL PROPERTIES AND FORMULATION

3.1. ~~2.1.~~Chemistry

The official designations and chemical and physical characteristics of ~~GS-5245~~ODV are summarized below:

Generic Name:	~~Not available~~Obeldesivir
Gilead Product No.:	GS-5245
Physical Appearance:	White- to off-white-colored solid
Solubility:	For aqueous media at room temperature, ~~GS-5245~~ODV is soluble at pH 2 (≥ 40 mg/mL) and very slightly soluble at pH 7 (1 mg/mL).
International Union of Pure and Applied Chemistry (IUPAC) Name:	[(2*R*,3*S*,4*R*,5*R*)-5-(4-aminopyrrolo[2,1-*f*][1,2,4]triazin-7-yl)-5-cyano-3,4-dihydroxytetrahydrofuran-2-yl]methyl 2-methylpropanoate
Molecular Formula:	$C_{16}H_{19}N_5O_5$
Molecular Weight:	361.4

3.2. ~~2.2.~~Formulation

~~GS-5245~~Obeldesivir tablets are available in strengths of 100 mg, 175 mg, 350 mg, and 500 mg. The 100- mg tablets are round, plain faced, film-coated purplish-pink. The 175 mg tablets are capsule shaped, debossed either with "175" on one side and "GSI" on the other side, or debossed with a bisecting score line on one side and "GSI" on the other side, and film-coated white. The 350- mg tablets are oval shaped, debossed with "GSI" on one side and "5245" on the other side, and film-coated light yellow. The 500- mg tablets are capsule shaped, plain faced, film-coated purplish-pink

or the 500- mg tablets are capsule shaped, debossed with "GSI" on one side and "5245" on the other side, and film-coated light yellow. In addition to the active ingredient, GS-5245ODV purplish-pink tablets also contain the following inactive ingredients: microcrystalline cellulose, crospovidone, magnesium stearate, polyvinyl alcohol, titanium dioxide, polyethylene glycol, talc, iron oxide red, and ferrosoferric oxide. In addition to the active ingredient, GS-5245ODV white tablets contain the following inactive ingredients: microcrystalline cellulose, crospovidone, magnesium stearate, macrogol polyvinyl alcohol graft copolymer, talc, titanium dioxide, glyceryl mono and dicaprylocaprate (glyceryl monocaprylocaprate type I, mono/diglycerides of fatty acids), and polyvinyl alcohol. In addition to the active ingredient, ODV light-yellow tablets contain the following inactive ingredients: microcrystalline cellulose, crospovidone, magnesium stearate, macrogol polyvinyl alcohol graft copolymer, talc, titanium dioxide, glyceryl mono and dicaprylocaprate (glyceryl monocaprylocaprate type I, mono/diglycerides of fatty acids), polyvinyl alcohol, and yellow iron oxide.

Placebo-to-match (PTM) GS-5245ODV purplish-pink tablets are identical in size, shape, color, and appearance to the corresponding active-strength GS-5245ODV purplish-pink tablets. The PTM GS-5245ODV purplish-pink tablets contain lactose monohydrate, microcrystalline cellulose, croscarmellose sodium, magnesium stearate, polyvinyl alcohol, titanium dioxide, polyethylene glycol, talc, iron oxide red, and ferrosoferric oxide. Placebo-to-match GS-5245ODV light-yellow tablets are identical in size, shape, color, and appearance to the corresponding active-strength GS-5245ODV light-yellow tablets. The PTM GS-5245ODV light-yellow tablets contain commonly used excipients, including lactose monohydrate, microcrystalline cellulose, croscarmellose sodium, magnesium stearate, macrogol polyvinyl alcohol graft copolymer, talc, titanium dioxide, glyceryl mono and dicaprylocaprate (glyceryl monocaprylocaprate type I, mono/diglycerides of fatty acids), polyvinyl alcohol, and yellow iron oxide.

3.3. 2.3. Packaging, Storage, and Handling

GS-5245Obeldesivir purplish-pink tablets, 100 mg and 500 mg, and corresponding PTM tablets are packaged in white, high-density polyethylene bottles. Each bottle contains 5 tablets and polyester packing material. Each bottle is enclosed with a white, continuous- thread, child-resistant polypropylene screw cap with an induction-sealed and aluminum-faced liner.

GS-5245Obeldesivir white tablets, 175 mg, with both debossing options are packaged in white, high-density polyethylene bottles. Each bottle contains 10 tablets and polyester packing material. Each bottle is enclosed with a white, continuous thread, child-resistant polypropylene screw cap with an induction-sealed and aluminum-faced liner.

Obeldesivir light-yellow tablets, 350 mg and 500 mg, and corresponding PTM tablets are packaged in white, high-density polyethylene bottles. Each bottle contains 10 tablets and polyester packing material. Each bottle is enclosed with a white, continuous thread, child-resistant polypropylene screw cap with an induction-sealed and aluminum-faced liner.

GS-5245Obeldesivir and PTM tablets should be stored below 30 °C (86 °F). Storage conditions are specified on the label.

Measures that minimize drug contact with the body should always be considered during handling, preparation, and disposal procedures. Any unused study druginvestigational product should be disposed of in accordance with local requirements.

3.4. 2.4.Stability

Gilead's policy is to monitor the stability of each formulation of study drug investigational product until the shelf life or a reevaluation period is established for the drug product. The stability studies cover the labeled storage conditions and, as appropriate, accelerated conditions. The stability is tested every 3 months for the first year, every 6 months for the second year, and annually thereafter for the minimum of the clinical study duration. Studies under accelerated conditions serve to identify any stability trend ahead of its occurrence under the labeled storage conditions.

Through the combined approach of accelerated condition studies and long-term stability monitoring with frequent testing, the purported identity, strength, and quality will be assured for all drug product lots used in clinical studies.

4. 3.NONCLINICAL STUDIES

The nonclinical pharmacology, PK, and toxicology of ODV for COVID-19 are described below. Where applicable, safety pharmacology and toxicology studies have been conducted in accordance with Good Laboratory Practice (GLP) requirements. Study; study details are provided in Appendix 8.29.2.

4.1. 3.1.Nonclinical Pharmacology

4.1.1. 3.1.1.Primary Pharmacodynamics

4.1.1.1. 3.1.1.1.In Vitro Studies

4.1.1.1.1. 3.1.1.1.1.Antiviral Activity Against SARS-CoV-2

The in vivo antiviral efficacy following GS-5245ODV administration is expected to be driven by circulating systemic levels of GS-441524. Therefore, assessment of GS-441524 in vitro is the appropriate measure of antiviral potency that would translate into in vivo efficacy. The in vitro antiviral profile of GS-5245ODV is also presented to provide a complete dataset of the drug administered, in case the intact GS-5245ODV prodrug is detected systemically in humans. The in vitro potencies of GS-5245ODV and GS-441524 were assessed against a recombinant SARS-CoV-2 virus (WA1 strain) expressing the nanoluciferase reporter gene (SARS-CoV-2-NLucNluc) in relevant lung airway cell types, using RDV as a positive control inhibitor of these viruses (Study PC-611-2009). In all antiviral assays, RDV demonstrated antiviral activities similar to previously reported values {Mackman 2021, Xie 2020}.

GS-5245Obeldesivir and GS-441524 demonstrated potent antiviral activities against SARS-CoV-2 in the A549-transformed human lung epithelial small cell carcinoma cells overexpressing human angiotensin-converting enzyme 2 (A549-hACE2) transformed lung alveolar epithelial cell line, with half-maximal effective concentration (EC_{50}) values of 1.27 and 2.10 µM after 48 hours of treatment (Table 12), respectively. The cytotoxicity values (half-maximal cytotoxic concentration [CC_{50}]) of GS-5245ODV and GS-441524 in uninfected cultures of A549-hACE2 cells after 48 hours of treatment were > 50 µM, yielding a selectivity index (ratio of CC_{50} to IC_{50}) (SI) of > 39 for GS-5245ODV and > 23.8 for GS-441524. GS-

~~5245~~
Obeldesivir was comparatively more potent against SARS-CoV-2 in primary cultures of normal human bronchial epithelial (NHBE) cells, with an EC_{50} of 0.339 µM after a 24-hour treatment. The EC_{50} of GS-441524 against SARS-CoV-2 in NHBE cells was 2.45 µM, which is similar to that observed in A549-hACE2 cells.

Table ~~1~~2. Potencies of ~~GS-5245~~ODV and GS-441524 Against SARS-CoV-2 in Human Cells

Cells	~~GS-5245[a]~~ ODV[a]		GS-441524[a]		Remdesivir[a]	
	EC_{50} (µM)	CC_{50} (µM)	EC_{50} (µM)	CC_{50} (µM)	EC_{50} (µM)	CC_{50} (µM)
A549-hACE2	1.27 ± 0.20	> 50	2.10 ± 0.63	> 50	0.056 ± 0.021	19.5
NHBE	0.339 ± 0.037	NA[b]	2.45 ± 0.06	NA[b]	0.0371 ± 0.004	NA[b]

A549-hACE2 = transformed human lung epithelial small cell carcinoma cells overexpressing human angiotensin-converting enzyme 2; CC_{50} = half-maximal cytotoxic concentration; EC_{50} = half-maximal effective concentration; NA = not applicable; NHBE = normal human bronchial epithelia; ODV = obeldesivir (GS-5245); SARS-CoV-2 = severe acute respiratory syndrome coronavirus 2

a The data represent the mean ± standard deviation of at least 2 independent experiments performed in duplicate.

b Under conditions matching antiviral assay.

4.1.1.1.2. Antiviral Activity Against SARS-CoV-2 Variants

New variants of SARS-CoV-2 have emerged since the first ancestral Wuhan and WA1 strains, some with an increased transmission rate compared to the original strain. Variants of concern, variants of interest, and variants being monitored, classified by the WHO and Centers of Disease Control, have emerged as circulating strains over time. The in vitro antiviral activity of ODV against Omicron B.1.1.529/BA.1, BA.2, BA.2.12.1, BA.4, BA.5, BA.2.75, BA.4.6, BF.5, XBB, and BQ.1.1 subvariants was assessed and compared to its activity against the SARS-CoV-2 reference strain, WA1, using a nucleoprotein ELISA performed in A549-hACE2–TMPRSS2 cells. In this assay, ODV inhibition was assessed by N expression, which served as a marker of SARS-CoV-2 replication. The mean ODV EC_{50} was 2.19 µM against the reference WA1 strain and ranged from 0.40 to 3.13 µM against the Omicron subvariants tested. These Omicron subvariants exhibited EC_{50} fold-changes of 0.26 to 1.33 compared to reference strain WA1 (Table 3), confirming that these subvariants are as susceptible to ODV as the ancestral WA1 isolate of SARS-CoV-2. These results support that ODV maintains potent antiviral activity against Omicron subvariants (Study PC-611-2026, {Mackman 2023}).

Table 3. ODV EC_{50} Against SARS-CoV-2 Omicron B.1.1.529/BA.1, BA.2, BA.2.12.1, BA.4, BA.5, BA.2.75, BA.4.6, BF.5, XBB, and BQ.1.1 Subvariants Based on Anti-Nucleoprotein ELISA Results

Variant	Lineage	ODV EC_{50} (µM)													Mean ± Standard Deviation[a]	EC_{50} Fold-Change From WA1[b]
		Replicate														
		1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th	11th	12th	13th		
WA1	A	2.28	2.18	1.45	2.11	1.74	1.72	1.43	1.87	2.07	4.17	3.75	1.94	1.74	2.19 ± 0.83	1.0
Omicron	BA.1	0.84	0.66	0.44	—	—	—	—	—	—	—	—	—	—	0.65 ± 0.20	0.32
Omicron	BA.2	—	—	—	—	0.64	—	—	—	—	—	—	—	—	0.71 ± 0.10	0.37
Omicron	BA.2.12.1	—	—	—	—	—	0.35	0.54	—	—	—	—	—	—	0.44 ± 0.13	0.29
Omicron	BA.4	—	—	—	—	—	0.42	0.38	—	—	—	—	—	—	0.40 ± 0.02	0.26
Omicron	BA.5	—	—	—	—	—	1.40	1.21	—	—	—	—	—	—	1.30 ± 0.13	0.83
Omicron	BA.2.75	—	—	—	—	—	—	—	0.59	0.79	—	—	—	—	0.69 ± 0.14	0.35
Omicron	BA.4.6	—	—	—	—	—	—	—	—	—	2.25	2.65	—	—	2.45 ± 0.28	0.62
Omicron	BF.5	—	—	—	—	—	—	—	—	—	2.88	3.39	—	—	3.13 ± 0.36	0.80
Omicron	XBB	—	—	—	—	—	—	—	—	—	—	—	2.43	1.63	2.03 ± 0.56	1.10
Omicron	BQ.1.1	—	—	—	—	—	—	—	—	—	—	—	2.51	2.38	2.44 ± 0.09	1.33

EC_{50} = half-maximal effective concentration; ELISA = enzyme-linked immunosorbent assay; ODV = obeldesivir (GS-5245); SARS-CoV-2 = severe acute respiratory syndrome coronavirus 2

a Values are the mean ± standard deviation of the results of independent experiments (number of replicate experiments shown).
b A fold-change was calculated for each experiment and a mean fold-change was calculated with these values.

4.1.1.1.3. Combination Antiviral Activity Against SARS-CoV-2

The in vitro SARS-CoV-2 antiviral activity of GS-441524 or ODV in combination with either molnupiravir or nirmatrelvir were assessed in A549-hACE2 cells (Study PC-611-2029). Bliss independence consensus scores were used to evaluate the drug combinations by interpreting any score between −10 and 10 as additive, greater than 10 are synergistic, and below −10 are antagonistic. The Bliss consensus score summary for 2-drug combinations is reported in Table 4. For 2-drug combinations of GS-441524 and nirmatrelvir, we observed an additive effect with an average Bliss score of 0.96 ± 0.67 for 3 replicates. For ODV and nirmatrelvir, we observed additive effects with and average Bliss score of 2.77 ± 0.07 across replicates. Additive effects are observed for the combinations of GS-441524 with molnupiravir and ODV with molnupiravir, demonstrated by average Bliss scores of −0.22 ± 0.20 and −1.36 ± 1.44, respectively. Therefore, GS-441524 and ODV each exhibited additive in vitro potencies when combined with either nirmatrelvir or molnupiravir.

Table 4. Two-Drug Combination Bliss Independence Consensus Scores for the A549-hACE2 SARS-CoV-2 Antiviral Analyses

2-Compound Combinations	GS-441524+ Nirmatrelvir	ODV+ Nirmatrelvir	GS-441524+ Molnupiravir	ODV+ Molnupiravir
Replicate 1	1.622	2.782	−0.414	−1.328
Replicate 2	0.287	2.703	−0.013	0.059
Replicate 3	0.977	2.836	−0.228	−2.814
Average of replicates	0.96	2.77	−0.22	−1.36
Standard deviation of replicates	0.67	0.07	0.20	1.44

A549-hACE2 = transformed human lung epithelial small cell carcinoma cells overexpressing human angiotensin-converting enzyme 2; ODV = obeldesivir (GS-5245); SARS-CoV-2 = severe acute respiratory syndrome coronavirus 2

To further assess potential DDIs with Paxlovid on in vitro SARS-CoV-2 antiviral activity, 3-drug combinations of either GS-441524 or ODV and nirmatrelvir and ritonavir were also assessed in A549-hACE2 cells (Study PC-611-2029). For the 3-drug combinations of GS-441524, nirmatrelvir, and ritonavir, additive effects are observed with an average Bliss score of 2.80 ± 3.95, as shown in Table 5. Alongside the triple drug combination, 2-drug combinations of nirmatrelvir with ritonavir, GS-441524 with ritonavir, and GS-441524 with nirmatrelvir demonstrate additive effects with average Bliss scores of 0.63 ± 5.65, −2.47 ± 3.00, and 1.67 ± 0.53, respectively as shown in Table 5. Likewise, 3-drug combinations of ODV, nirmatrelvir, and ritonavir, demonstrate additive effects with an observed average Bliss score of 5.33 ± 4.76 across replicates (Table 6). Alongside this 3-drug combination, 2-drug combinations of nirmatrelvir with ritonavir, ODV with ritonavir, and ODV with nirmatrelvir, demonstrate additive effects with observed average Bliss scores of 6.31 ± 5.38, 0.75 ± 6.96, and 2.01 ± 0.79, respectively, as shown in Table 6.

Table 5. Bliss Independence Consensus Scores for the A549-hACE-2 SARS-CoV-2 Fluc Antiviral Analyses of 3-Drug Combination with GS-441524 and Each Internal 2-Drug Combination

3 Compound Combinations	GS-441524+ Nirmatrelvir+ Ritonavir	Nirmatrelvir+ Ritonavir	GS-441524+ Ritonavir	GS-441524+ Nirmatrelvir
Replicate 1	4.122	1.994	−1.189	2.135
Replicate 2	5.923	5.479	−0.322	1.77
Replicate 3	−1.637	−5.578	−5.898	1.098
Average of replicates	2.80	0.63	−2.47	1.67
Standard deviation of replicates	3.95	5.65	3.00	0.53

A549-hACE2 = transformed human lung epithelial small cell carcinoma cells overexpressing human angiotensin-converting enzyme 2; SARS-CoV-2 = severe acute respiratory syndrome coronavirus 2

Table 6. Bliss Independence Consensus Scores for the A549-hACE2 SARS-CoV-2 Fluc Antiviral Analyses of 3-Drug Combination with ODV and Each Internal 2-Drug Combination

3 Compound Combinations	ODV+ Nirmatrelvir+ Ritonavir	Nirmatrelvir+ Ritonavir	ODV+ Ritonavir	ODV+ Nirmatrelvir
Replicate 1	1.714	2.309	−2.675	1.244
Replicate 2	3.546	4.207	−3.827	2.813
Replicate 3	10.726	12.426	8.766	1.958
Average of replicates	5.33	6.31	0.75	2.01
Standard deviation of replicates	4.76	5.38	6.96	0.79

A549-hACE2 = transformed human lung epithelial small cell carcinoma cells overexpressing human angiotensin-converting enzyme 2; ODV = obeldesivir (GS-5245); SARS-CoV-2 = severe acute respiratory syndrome coronavirus 2

The drug combinations of GS-441524 or ODV with either molnupiravir, nirmatrelvir, and/or ritonavir all demonstrate additive effects against SARS-CoV-2 in A549-hACE2 cells. No antagonistic in vitro DDIs were observed between GS-441524 or ODV and molnupiravir, nirmatrelvir alone, or nirmatrelvir and ritonavir.

4.1.1.1.4. ~~3.1.1.1.2.~~ Mechanism of Action

~~GS-5245~~Obeldesivir is extensively metabolized presystemically to the parent nucleoside GS-441524 which distributes into cells where it is further metabolized to form the pharmacologically active nucleoside triphosphate metabolite, GS-443902. Metabolism of GS-441524 to GS-443902 has been demonstrated in multiple cell types, including NHBE cells, human airway epithelial (HAE),
EpiAlveolar, and the human alveolar basal epithelial adenocarcinoma cell line A549 (Study AD-611-2030).

Biochemical studies demonstrate that the nucleoside triphosphate GS-443902 acts as an analog of adenosine triphosphate (ATP) and competes with the natural ATP substrate, selectively inhibiting CoV viral RNA-dependent RNA polymerases (RdRps) by 2 mechanisms of action. One mechanism of inhibition is the utilization of the nucleoside triphosphate GS-443902 as a

substrate by the viral RdRp during nascent RNA strand synthesis, which results in delayed (i+3) RNA chain termination during replication of the viral RNA {Gordon 2020a}. Delayed chain termination has been shown to be a mechanism of action of GS-443902 inhibition of the SARS-CoV-2 {Gordon 2020b}, SARS-CoV, Middle East respiratory syndrome (MERS)-CoV {Gordon 2020a}, Ebola virus {Tchesnokov 2019}, respiratory syncytial virus (RSV) {Warren 2016}, and Nipah virus {Jordan 2018} polymerases.

Delayed chain termination was the first mechanism of action identified for GS-443902, but there is a second inhibitory mechanism. Further biochemical analysis demonstrated that, at physiological levels of natural nucleoside triphosphates, nascent strand elongation could continue beyond the i+3 position and complete nascent strand synthesis. A second mechanism of inhibition of SARS-CoV-2 replication by GS-443902 was later shown to be template-dependent inhibition due to the presence of the incorporated nonnatural nucleotide as a monophosphate (nucleoside monophosphate [NMP]) in the daughter RNA strand. When the RdRp encounters an incorporated nonnatural NMP while reading the daughter strand template, it is unable to pair a native uridine triphosphate with the incorporated nonnatural NMP due to a steric clash between the SARS-CoV-2 RdRp Ala-558 and the 1'-CN group of the modified NMP, halting nascent strand synthesis {Tchesnokov 2020.}. This template-dependent mechanism of inhibition means that the incorporated nonnatural NMP would not be a substrate for the viral exoribonuclease excision as this enzyme can only cleave mismatched incorporated nucleotides in the nascent strand. The low recognition capacity of CoV proofreading exoribonucleases combined with their interaction at highly conserved active sites favors a high barrier to resistance for ~~GS-5245~~ODV and similar analogs.

The ratio of Michaelis-Menten steady-state kinetic parameters for single nucleotide incorporation (V_{max}/K_m) of a natural nucleotide substrate over a nucleotide analog defines its selectivity. Based on the steady-state kinetic parameters for single nucleotide incorporations in comparison with ATP, the coronaviruses SARS-CoV-2, SARS-CoV, and MERS-CoV were shown to utilize GS-443902 more efficiently than ATP. GS-443902 selectivity values were 0.26, 0.32, and 0.35, respectively, compared with ATP {Gordon 2020a}. GS-443902 inhibited MERS-CoV RNA polymerase with a half-maximal inhibitory concentration (IC_{50}) value of 0.032 µM {Gordon 2020a}.

4.1.1.1.5. ~~3.1.1.1.3.~~Viral Resistance

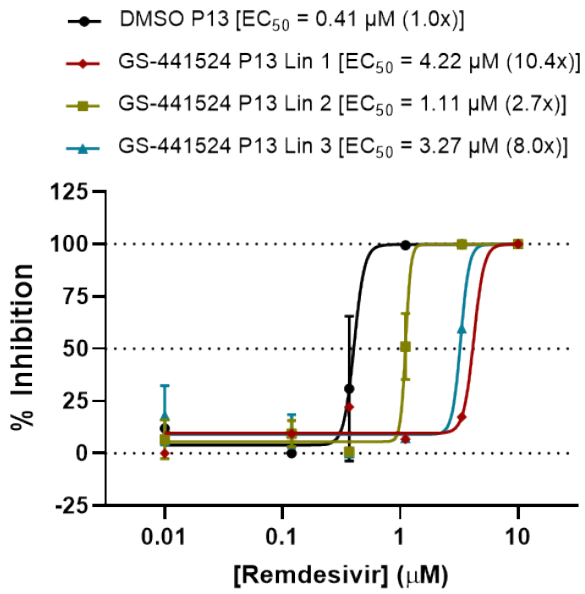
As ~~GS-5245~~ODV is an orally bioavailable 5′ monoester of the parent nucleoside GS-441524 and yields the same active triphosphate species as RDV, characterization of the resistance profiles of GS-441524 and RDV is critical for understanding the potential for development of drug resistance to ~~GS-5245~~ODV.

4.1.1.1.5.1. ~~3.1.1.1.3.1.~~SARS-CoV-2 In Vitro Resistance Selection Experiments With GS-441524 or Remdesivir

Three independent lineages of SARS-CoV-2 WA-1 were serially passaged in the presence of increasing concentrations of GS-441524 in Vero E6 cells (Study PC-540-2028). After 13 passages, lineage 2 was able to maintain viral replication in the presence of 3 µM GS-441524 while lineages 1 and 3 were able to replicate at 9 µM GS-441524. The RDV EC_{50} values of the GS-441524-selected virus populations were 4.22, 1.11, and 3.27 µM in Vero E6 cells for lineages 1, 2, and 3, respectively (Figure 1, Table ~~2~~7). Compared with control

virus passaged in the absence of any inhibitor (EC_{50} = 0.41 µM), this resulted in fold changes of 10.4, 2.7, and 8.0 for lineages 1, 2, and 3, respectively (Figure 1, Table ~~2~~7). GS-441524 was not evaluated against pooled virus samples of each lineage.

Figure 1. Antiviral Activity of Remdesivir Against SARS-CoV-2 Lineages Selected in the Presence of GS-441524



DMSO = dimethylsulfoxide; EC_{50} = half-maximal effective concentration; Lin = lineage; P13 = passage 13; SARS-CoV-2 = severe acute respiratory syndrome coronavirus 2

Table ~~2~~7. Remdesivir EC_{50} Values and Nsp12 Mutations Present at > 50% of Virus Population in SARS-CoV-2 Selected in the Presence of GS-441524

Virus Lineage	Remdesivir EC_{50} (µM)	EC_{50} Fold -Change From DMSO Control	Nsp12 Substitutions Detected at Passage 13
Wild-type control	0.41	1.0	—
1	4.22	10.4	V166A, N198S, S759A[a], C799F
2	1.11	2.7	V166A, N198S, C799R
3	3.27	8.0	V792I, S759A[a]

DMSO = dimethylsulfoxide; EC_{50} = half-maximal effective concentration; SARS-CoV-2 = severe acute respiratory syndrome coronavirus 2

a S759A was preexisting at < 1% in the WA1 virus stock prior to serial passaging.

Sequencing of the GS-441524-selected virus population from each lineage identified several nonsynonymous substitutions in the nsp12 polymerase present at > 50% of the virus population that were not detected in the dimethylsulfoxide (DMSO)-passaged viruses (Table ~~2~~7). After 13 passages in the presence of GS-441524, amino acid changes in nsp12 were identified as V166A, N198S, S759A, V792I, C799F, and C799R. These mutations were engineered into a SARS-CoV-2 recombinant virus using reverse genetics and evaluated for susceptibility to GS-441524 and RDV in A549-hACE2 cells (Studies PC-540-2029, PC-540-2028) (Table ~~3~~8).

Phenotypic testing of recombinant SARS-CoV-2 viruses containing the individual amino acid substitutions resulted in GS-441524 EC_{50} values between 1.79 and 5.94 µM (1.84- to 6.04-fold change relative to the WA1 reference) and RDV EC_{50} values between 0.095 to 0.199 µM (1.67- to 3.49-fold change relative to the WA1 reference), indicating low-level reduced susceptibility to GS-441524 or RDV.

Table 38. Phenotypic Profiling of SARS-CoV-2-NLucNluc Recombinant Viruses Expressing Nsp12 Mutations Selected by GS-441524

	GS-441524		Remdesivir	
Nsp12 Substitution	**Average EC_{50} ± Standard Deviation (µM)**	**EC_{50} Fold Change From Reference**	**Average EC_{50} ± Standard Deviation (µM)**	**EC_{50} Fold Change From Reference**
WA1 reference	0.975 ± 0.070	1.00	0.057 ± 0.011	1.00
V166A	2.44 ± 0.079	2.51	0.168 ± 0.042	2.95
N198S	1.79 ± 0.05	1.84	0.095 ± 0.026	1.67
S759A	3.23 ± 0.39	3.34	0.160 ± 0.024	2.81
V792I	4.22 ± 1.00	4.30	0.171 ± 0.070	3.00
C799F	5.94 ± 1.88	6.04	0.199 ± 0.058	3.49
C799R	2.75 ± 0.85	2.86	0.141 ± 0.021	2.47

EC_{50} = half-maximal effective concentration; SARS-CoV-2 = severe acute respiratory syndrome coronavirus 2; SARS-CoV-2-NLucNluc = recombinant SARS-CoV-2 virus expressing the nanoluciferase reporter gene

The SARS-CoV-2 nsp12 F480L and V557L substitutions corresponding to the residues identified during mouse hepatitis virus (a CoV family virus) resistance selection experiments conducted previously {Agostini 2018} were also individually engineered into SARS-CoV-2 using the recombinant virus system and the mutant viruses were evaluated for susceptibility to GS-441524 and RDV (Studies PC-611-2010, PC-540-2028) (Table 49). Phenotypic evaluation in SARS-CoV-2 resulted in GS-441524 EC_{50} values of 2.78 µM for F480L and 2.50 µM for V557L, with fold changes of 2.85 and 2.54, respectively, compared with the reference. The RDV EC_{50} values for the F480L and V557L viruses were 0.118 and 0.112 µM, respectively, or 2.07- and 1.96-fold change from the WA1 reference strain EC_{50}. Minimal changes in susceptibility to GS-441524 and RDV were observed in the F480L and V557L nsp12 substitutions.

Table 49. Phenotypic Profiling of SARS-CoV-2-NLucNluc Recombinant Viruses Expressing Orthogonal Nsp12 Substitutions Identified From Mouse Hepatitis Virus Resistance Selection

	GS-441524		Remdesivir	
Nsp12 Substitution	**Average EC_{50} ± Standard Deviation (µM)**	**EC_{50} Fold Change From Reference**	**Average EC_{50} ± Standard Deviation (µM)**	**EC_{50} Fold Change From Reference**
WA1 reference	0.975 ± 0.070	1.00	0.057 ± 0.011	1.00
F480L	2.78 ± 0.12	2.85	0.118 ± 0.026	2.07
V557L	2.50 ± 0.85	2.54	0.112 ± 0.027	1.96

EC_{50} = half-maximal effective concentration; SARS-CoV-2 = severe acute respiratory syndrome coronavirus 2;

SARS-CoV-2-~~NLuc~~Nluc = recombinant SARS-CoV-2 virus expressing the nanoluciferase reporter gene

Furthermore, in vitro resistance selection experiments were conducted by serially passaging SARS-CoV-2 strain 21697 containing the P323L nsp12 substitution in the presence of RDV in Vero E6 cells. After 9, 11, 17, and 23 passages in the presence of RDV, the single amino acid substitution, V166L in nsp12, was identified (Study PC-540-2029). Phenotypic testing of recombinant SARS-CoV-2 viruses containing P323L alone or P323L+V166L in combination resulted in GS-441524 EC_{50} values of 1.49 and 1.59 µM, or 1.52- and 1.64-fold change from the WA1 reference strain, respectively, indicating minimal loss of susceptibility to GS-441524 (Study PC-611-2010) (Table ~~5~~10). The RDV EC_{50} values for the P323L single- and V166L+P323L double-mutant viruses were 0.074 and 0.088 µM, respectively, or 1.30- and 1.54-fold change from the WA1 reference strain EC_{50} (Table ~~5~~10).

Table ~~5~~10. GS-441524 and Remdesivir EC_{50} Values Against SARS-CoV-2 WA1 Reference and Mutant Viruses

	GS-441524		Remdesivir	
Nsp12 Substitution	**Average EC_{50} ± Standard Deviation (µM)**	**EC_{50} Fold - Change From Reference**	**Average EC_{50} ± Standard Deviation (µM)**	**EC_{50} Fold - Change From Reference**
WA1 reference	0.975 ± 0.070	1.00	0.057 ± 0.011	1.00
P323L	1.49 ± 0.34	1.52	0.074 ± 0.016	1.30
V166L+P323L	1.59 ± 0.04	1.64	0.088 ± 0.001	1.54

EC_{50} = half-maximal effective concentration; SARS-CoV-2 = severe acute respiratory syndrome coronavirus 2

The relatively high number of passages at which substitutions were identified either by selection with GS-441524 or RDV and minimal loss in GS-441524 susceptibility associated with these substitutions suggests a high barrier to resistance to ~~GS-5245~~ODV during treatment of SARS-CoV-2 infection.

4.1.1.2. ~~3.1.1.2.~~In Vivo Studies

4.1.1.2.1. ~~3.1.1.2.1.~~In Vivo Efficacy of ~~GS-5245~~ODV Against Mouse-Adapted SARS-CoV-2 in Balb/c Mice

Efficacy of ~~GS-5245~~ODV was assessed against a mouse-adapted strain of SARS-CoV-2 in Balb/c mice (Study PC-611-2003). All animal husbandry services, efficacy studies, and analyses were performed at the University of North Carolina (Chapel Hill, NC) in the laboratories of Ralph Baric, PhD. Female mice (7-8 weeks old; 10 animals/group) were inoculated intranasally with a mouse-adapted SARS-CoV-2 (MA10). As shown in Table ~~6~~11, mice were dosed twice daily for 4 days by oral dosing of 3 to 30 mg/kg ~~GS-5245~~ODV or 100 mg/kg ~~EIDD-2801 (~~molnupiravir~~)~~ beginning at 12 hours postinfection (hpi). A separate group of mice was treated with 30 mg/kg ~~GS-5245~~ ODV twice daily initiated at 24 hpi. Weight loss was evaluated daily on all animals while daily pulmonary function was determined by plethysmography on 4 consistently selected mice per group. At 4 days after infection, animals were euthanized and lung tissues were evaluated for lung congestion (hemorrhage) score and infectious viral titers of SARS-CoV-2 in the lung homogenate. Lung samples were evaluated for SARS-CoV-2 infectious viral titers by plaque assay and histopathology.

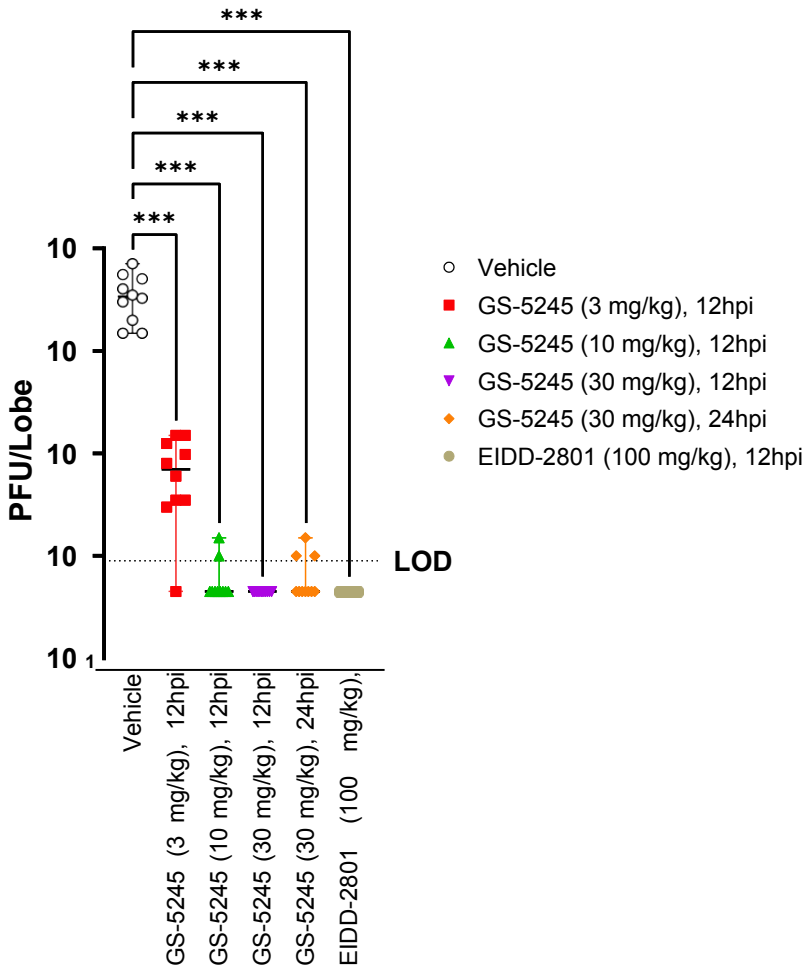
Table ~~6~~11. Study Design to Evaluate Efficacy of ~~GS-5245~~ODV Against SARS-CoV-2 (MA10) in Balb/c Mice

Group	Treatment	Dose (mg/kg), BID	Treatment Initiation
1	Vehicle	-	+ 12 hpi
2	~~GS-5245~~ODV	3	+ 12 hpi
3	~~GS-5245~~ODV	10	+ 12 hpi
4	~~GS-5245~~ODV	30	+ 12 hpi
5	~~GS-5245~~ODV	30	+ 24 hpi
6	EIDD-2801	100	+ 12 hpi

BID = twice daily; EIDD-2801 = molnupiravir; hpi = hours postinfection; ODV = obeldesivir (GS-5245);
SARS-CoV-2 =(MA10) = mouse-adapted severe acute respiratory syndrome coronavirus 2

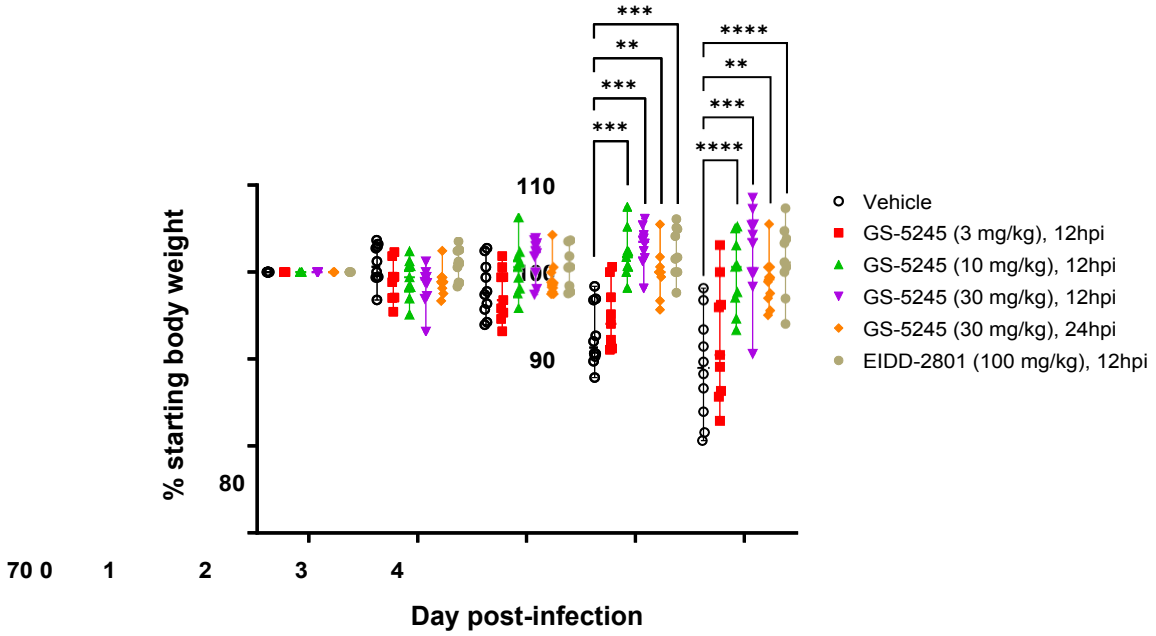
The reduction in infectious virus in lung homogenates was dependent on ~~GS-5245~~ODV dose, with maximal virus titer reductions in mice treated twice daily with 30 mg/kg ~~GS-5245~~ODV with treatment initiated at 12 hpi (Figure 2). Mean body weights of mice infected with SARS-CoV-2 were maintained in the 10 and 30 mg/kg twice-daily ~~GS-5245~~ODV dose groups when treatment was initiated at 12 hpi as well as when 30 mg/kg ODV was administered starting at +24 hpi (Figure 3). The effect of SARS-CoV-2 on pulmonary function was significantly reduced when mice were treated with 30 mg/kg ~~GS-5245~~ODV at 12 hpi (Figure 4). Congestion scores (of lung hemorrhage histopathology; also referred to as lung discoloration score) were significantly lower in all treatment groups, with less significant effects in mice treated with ~~GS-5245~~ODV at 3 mg/kg twice daily at 12 hpi or 30 mg/kg with dosing starting at 24 hpi (Figure 5). Overall, maximal efficacy of
~~GS-5245~~ ODV by physiological metrics was observed in mice dosed with 10 or 30 mg/kg twice daily.
Compared with mice dosed with vehicle, delaying the 30 mg/kg ~~GS-5245~~ODV treatment initiation to 24 hpi significantly reduced weight loss, congestion scores, and lung virus titers; however, lung function was not significantly different than vehicle-treated mice. Seven of 10 mice treated with 30 mg/kg ~~GS-5245~~ODV at 24 hpi had undetectable levels of SARS-CoV-2, while virus was not detected in all 10 mice treated with 30 mg/kg ~~GS-5245~~ODV at 12 hpi. Overall, ~~GS-5245~~ODV exhibited potent in vivo efficacy in a pathogenic mouse model of SARS-CoV-2 infection at doses ≥ 10 mg/kg.

Figure 2. SARS-CoV-2 Infectious Titers in Lungs of Mice



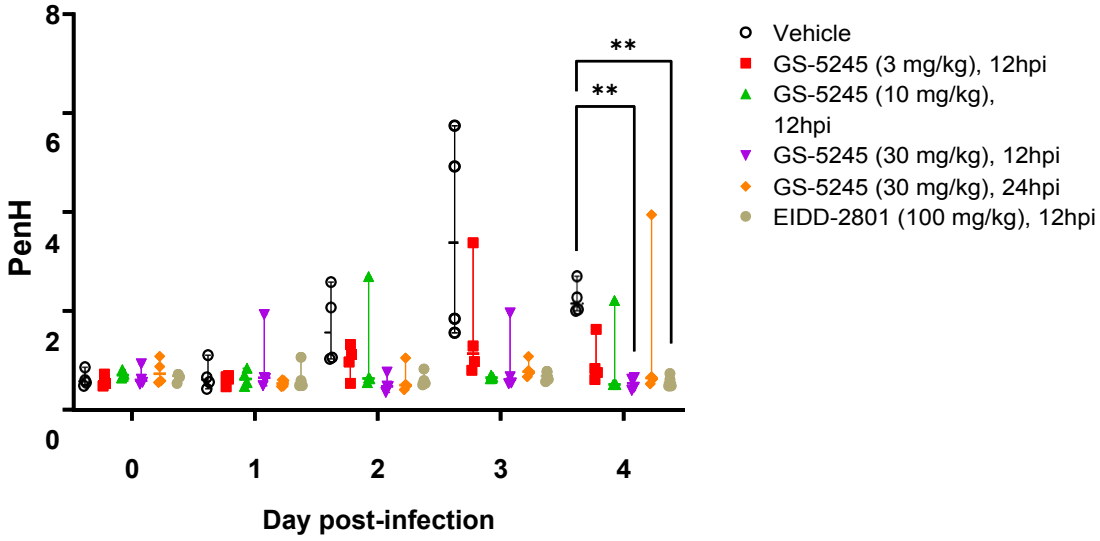
ANOVA = analysis of variance; BLQ = below the limit of quantitation; GS-5245 = obeldesivir (ODV); EIDD-2801 = molnupiravir; hpi = hours postinfection; LOD = limit of detection; PFU = plaque- forming unit; SARS-CoV-2 = severe acute respiratory syndrome coronavirus 2 *** $P = 0.0007$.
BLQ values were assigned as one-half the LOD (90 PFU/lobe).
Statistics by Sidak's multiple comparisons test following 2-way ANOVA.

Figure 3. Weight Loss in Mice Infected With SARS-CoV-2



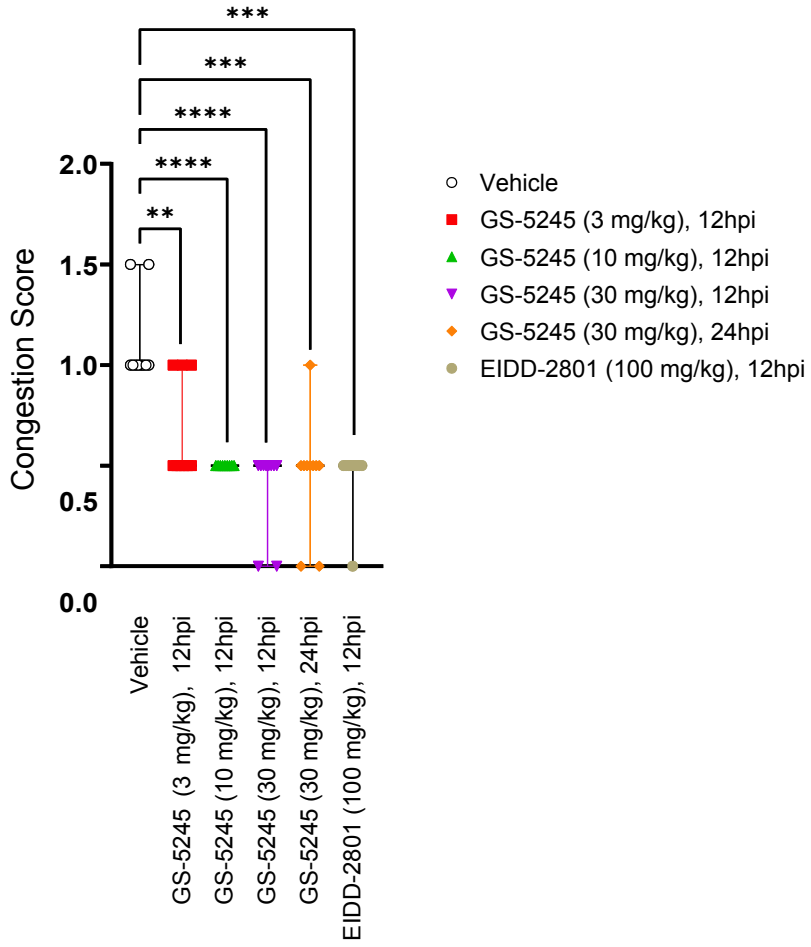
EIDD-2801 = molnupiravir; GS-5245 = obeldesivir (ODV); hpi = hours postinfection; SARS-CoV-2 = severe acute respiratory syndrome coronavirus 2 ** $P = \leq 0.01$.
*** $P = \leq 0.001$.
**** $P = \leq 0.0001$.
Statistics by Dunnett's multiple comparisons test.

Figure 4. Lung Function (Airway Resistance) in Mice Infected With SARS-CoV-2



EIDD-2801 = molnupiravir; GS-5245 = ODV; hpi = hours postinfection; PenH = pulmonary function; SARS-CoV-2 = severe acute respiratory syndrome coronavirus 2 ** $P = < 0.01$.
Statistics by Dunnett's multiple comparisons test.

Figure 5. Congestion Scores in Mice Infected With SARS-CoV-2

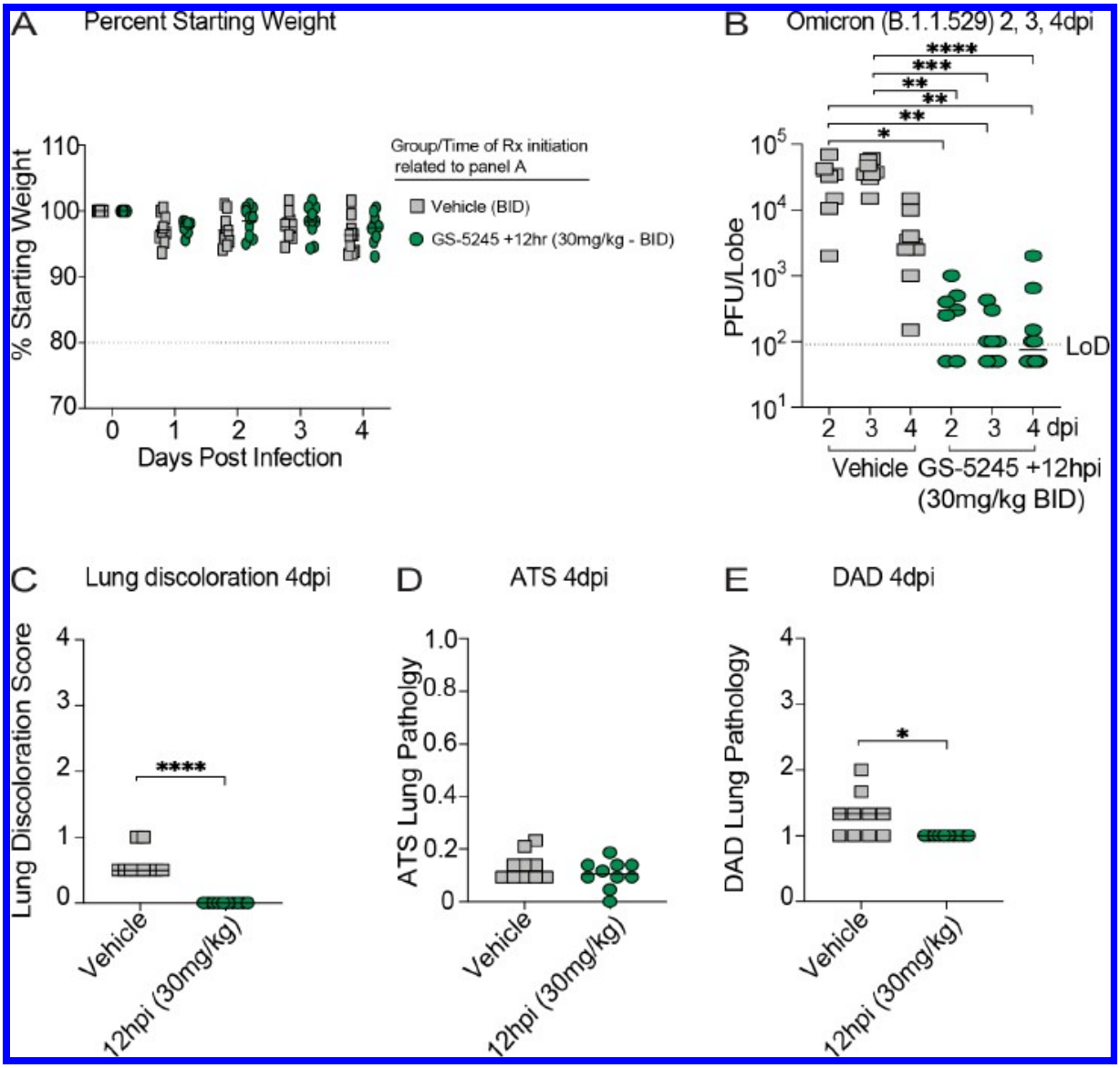


ANOVA = analysis of variance; EIDD-2801 = molnupiravir; GS-5245 = obeldesivir (ODV); hpi = hours postinfection; SARS- CoV-2 = severe acute respiratory syndrome coronavirus 2 ** P =<≤ 0.01.
*** P =<≤ 0.001.
**** P =<≤ 0.0001.
Statistics by Sidak's multiple comparisons test following 2-way ANOVA.

4.1.1.2.2. ~~3.1.1.2.2.In Vivo Efficacy of GS-5245~~In Vivo Therapeutic Efficacy of ODV Against SARS-CoV-2 Omicron BA.1. in K18-hACE2 Mice

The therapeutic efficacy of ODV was determined in K18-hACE2 transgenic mice infected with the highly-transmissible SARS-CoV-2 Omicron variant B.1.1.529/BA.1 ({Martinez 2023}, submitted). As previous studies did not find that BA.1 caused severe disease in K18-hACE2 mice including weight loss or lung pathology {Halfmann 2022}, we performed a therapeutic efficacy study where the main readout was BA.1 replication in the lung. Consistent with prior observations {Martinez 2023}, weight loss was not observed through Day 4 postinfection nor was severe lung pathology in vehicle-treated mice (Figure 6A and Figure 6B). Mice treated with ODV at 30 mg/kg 12 hpi had significantly lower lung infectious viral titers relative to vehicle-treated groups at 2-, 3-, and 4-days postinfection (dpi) (Figure 6B), and protection from macroscopic and microscopic lung pathology compared to vehicle (Figure 6C, Figure 6D, and Figure 6E). Therefore, ODV demonstrates a high degree of protection against lung viral replication in vivo against the highly transmissible BA.1 variant.

Figure 6. Therapeutic Efficacy of ODV Against SARS-CoV-2 BA.1 (Omicron B.1.1.529) in K18-hACE2 Mice



ANOVA = analysis of variance; ATS = American Thoracic Society scoring system; BID = twice daily; DAD = Diffuse Alveolar Damage scoring system; dpi = days postinfection; hpi = hours postinfection; LoD = limit of detection; ODV = obeldesivir (GS-5245); PFU = plaque forming units; Rx = drug; SARS-CoV-2 = severe acute respiratory syndrome coronavirus 2

BA.1 is a subset of the Omicron B.1.1.529 lineage.
* $P < 0.05$.
** $P < 0.01$.
*** $P < 0.001$.
**** $P < 0.0001$.
Statistics by 2-way ANOVA after Dunnett's multiple comparisons test (A); Kluskal-Wallis test after Dunnett's multiple comparisons test (B); Mann-Whitney test (C, D, and E).

4.1.1.2.3. Combination Therapy of Nirmatrelvir against SARS-CoV-2 in Balb/c Mice

The effect of combining ODV with the SARS-CoV-2 main protease inhibitor nirmatrelvir (PF-07321332; the active antiviral of Paxlovid®), on SARS-CoV-2 infectious levels was determined in Balb/c mice {Martinez 2023}. In preliminary studies, doses of ODV > 4 mg/kg or nirmatrelvir > 40 mg/kg reduced virus levels below the limit of detection when administered twice daily at 12 hpi, obfuscating the ability to assess the effect of combinations of these compounds at these doses on viral load reductions. Thus, as shown in Table 12, to assess the

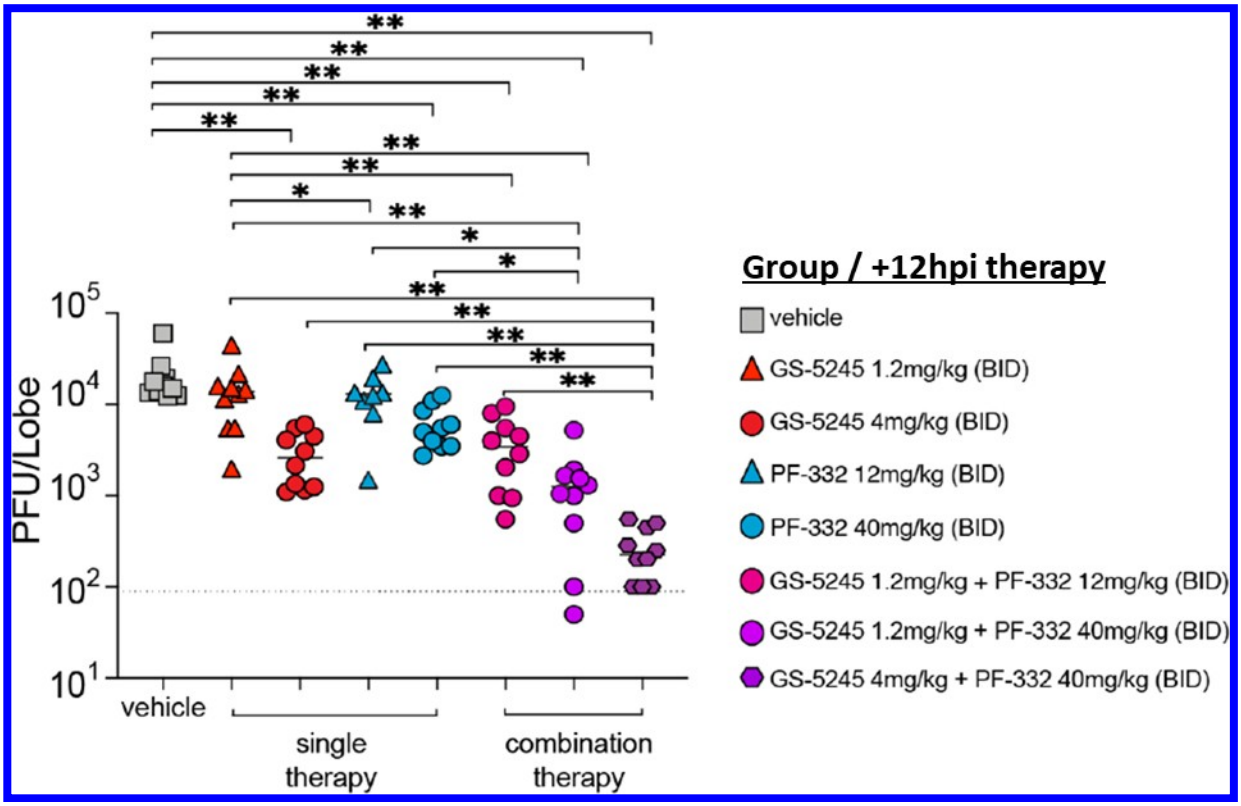
impact on combination efficacy of ODV and nirmatrelvir, a therapeutic efficacy study was conducted in mice infected with SARS-CoV-2 (MA10) and treated with suboptimal doses of single agents administered twice daily (ODV at 1.2 or 4 mg/kg, nirmatrelvir at 12 or 40 mg/kg) or combinations of the 2 administered twice daily: 1.2 mg/kg ODV + 12 mg/kg nirmatrelvir (low dose combo), 1.2 mg/kg ODV + 40 mg/kg nirmatrelvir (medium-dose combo), or 4 mg/kg ODV + 40 mg/kg nirmatrelvir (high-dose combo). Compared with vehicle-treated animals, SARS-CoV-2 infectious lung titers at 4 dpi were not reduced following therapy with suboptimal doses of 1.2 mg/kg of ODV or nirmatrelvir at 12 mg/kg (Figure 7). In contrast, intervention of either ODV at 4 mg/kg or nirmatrelvir at 40 mg/kg alone significantly reduced infectious viral titers in the lung. Combination of ODV at 1.2 mg/kg and nirmatrelvir at 40 mg/kg resulted in significantly more profound levels of lung viral replication compared to vehicle and single agent groups. Similarly, by increasing the concentration of each component of combination therapy (4 mg/kg ODV and 40 mg/kg nirmatrelvir), we observed a marked reduction in infectious viral titers in the lung that was significantly lower than either single agent (Figure 7). Altogether, these data suggest that combination therapy of ODV and nirmatrelvir is highly effective at suppressing lung viral replication in mice even when combined at suboptimal doses.

Table 12. Study Design to Evaluate Efficacy of ODV in Combination With Nirmatrelvir Against SARS-CoV-2 (MA10) in Balb/c Mice

Group	Treatment	Dose (mg/kg), BID	Treatment Initiation
1	Vehicle	—	+ 12 hpi
2	ODV	1.2	+ 12 hpi
3	ODV	4	+ 12 hpi
4	PF-07321332	12	+ 12 hpi
5	PF-07321332	40	+ 12 hpi
6	Low dose combo of ODV / PF-07321332	1.2 / 12	+ 12 hpi
7	Medium dose combo of ODV / PF-07321332	1.2 / 40	+ 12 hpi
8	High dose combo of ODV / PF-07321332	4 / 40	+12 hpi

BID = twice daily; hpi = hours postinfection; ODV = obeldesivir (GS-5245); SARS-CoV-2 (MA10) = mouse-adapted severe acute respiratory syndrome coronavirus 2; PF-07321332 = nirmatrelvir

Figure 7. Effect of ODV and Nirmatrelvir Combination Therapy on SARS-CoV-2 (MA10) Infectious Viral Titers in Balb/c Mice



BID = twice daily; GS-5245 = obeldesivir (ODV); PF-07321332 = nirmatrelvir; PFU = plaque forming units; hpi = hours postinfection; SARS-CoV-2 (MA10) = mouse-adapted severe acute respiratory syndrome coronavirus 2 * $P < 0.05$.
** $P < 0.01$.
Dotted line = limit of detection.
Statistics by Kruskal-Wallis test was used to compare the group samples, and Dwass-Steel-Critchlow-Fligner procedure was used for pairwise comparisons.

4.1.1.2.4. In Vivo Therapeutic Efficacy of ODV Against SARS-CoV-2 in Ferrets

The therapeutic efficacy of ~~GS-5245~~ODV was determined in ferrets infected with SARS-CoV-2 (Study PC-611-2001). All animal husbandry services, efficacy studies, and analyses were performed at the Georgia State University (Atlanta, GA) in the laboratories of Richard Plemper, PhD. As displayed in Table ~~7~~13, female ferrets (4 animals per dose group; 6-10 months old) were infected intranasally with 10^5 plaque~~-~~ forming units of SARS-CoV-2 and treated twice daily with either vehicle, 5 mg/kg molnupiravir, or ~~GS-5245~~ODV at 5, 10, or 20 mg/kg; or once daily with ~~GS-5245~~ODV at 20 or 40 mg/kg. Treatment was initiated 12 hpi and ferrets were euthanized on Day 4.
SARS-CoV-2 infection of ferrets did not cause physiological effects on weight or lung function, and viral infection was limited to the nasopharynx with no consistent detection of virus in the lungs. SARS-CoV-2 infectious viral titers were measured from daily nasal lavage fluid samples, as well as terminal nasal turbinates and lung tissue.

Table ~~7~~13. Study Design to Evaluate ~~GS-5245~~ODV Efficacy Against SARS-CoV-2 in Ferrets

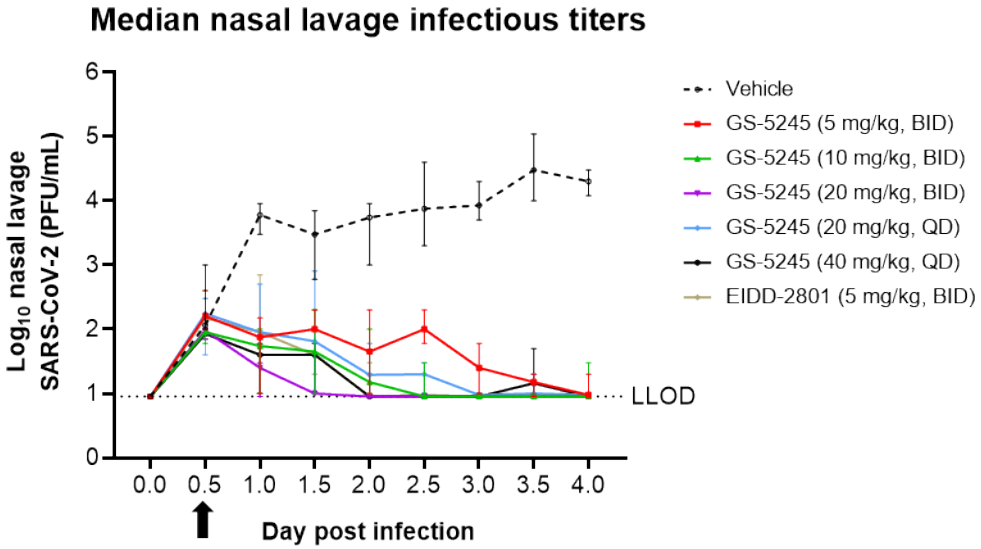
Group	Treatment	Dose (mg/kg)	Frequency of dosing
1	Vehicle	NA	BID

2	GS-5245 ODV	5	BID
3	GS-5245 ODV	10	BID
4	GS-5245 ODV	20	BID
5	GS-5245 ODV	20	QD
6	GS-5245 ODV	40	QD
7	EIDD-2801	5	BID

BID = twice daily; EIDD-2801 = molnupiravir; NA = not applicable; ODV = obeldesivir (GS-5245); QD = once daily; SARS-CoV-2 = severe acute respiratory syndrome coronavirus 2

Twelve hours after treatment initiation (ie, 24 hpi), median infectious viral titers of SARS-CoV-2 in ferret nasal lavages were lower in all treatment groups compared with the vehicle group (Figure 68). Dose-dependent reductions in nasal lavage infectious viral titers (Figure 68) and viral RNA copies (Figure 79) were observed in ferrets treated with oral GS-5245ODV twice daily, with undetectable viral titers at Day 1.5 onward in the 20 mg/kg GS-5245ODV twice-daily group. Overall, the reductions in nasal lavage virus RNA and infectious viral titers of the twice-daily dose groups were similar to their daily equivalent once-daily dose groups (Table 814). However, reductions in SARS-CoV-2 RNA and infectious viral loadstiter in terminal nasal turbinates at the end of the study by both viral load metrics were greater in the once-daily dose groups than their total daily twice-daily equivalent dose groups (Table 814). GS-5245Obeldesivir treatment groups at 20 mg/kg twice daily and 40 mg/kg once daily had numerically superior reductions in virus RNA and infectious viral titers in most analyses as compared with ferrets treated twice daily with 5 mg/kg molnupiravir (Figure 68, Figure 79; Table 814).

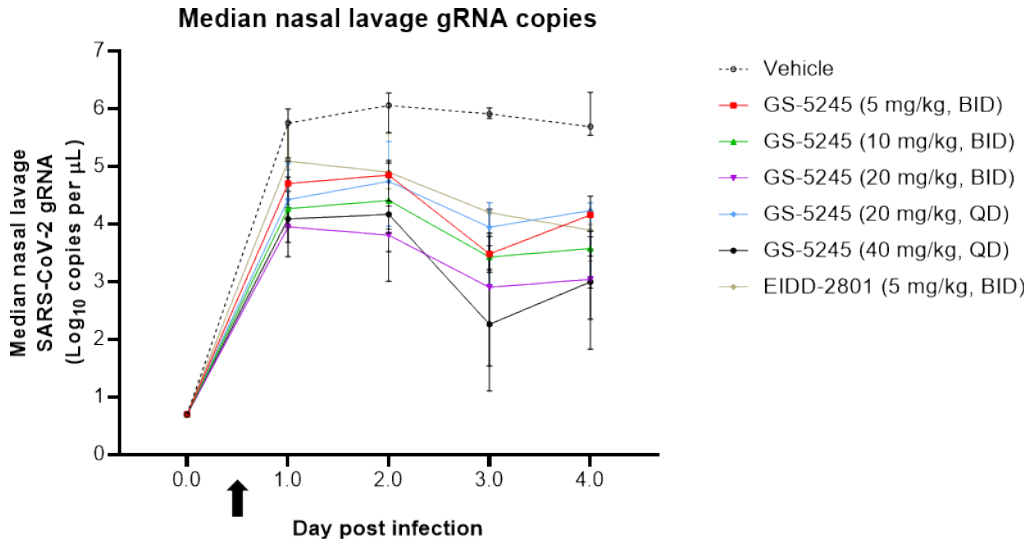
Figure 68. SARS-CoV-2 Infectious Viral Titers in Ferret Nasal Lavage Fluid



BID = twice daily; EIDD-2801 = molnupiravir; GS-5245 = obeldesivir (ODV); LLOD = lower limit of detection; PFU = plaque- forming unit; QD = once daily; SARS-CoV-2 = severe acute respiratory syndrome coronavirus 2 Arrow indicates time of first treatment regardless of BID or QD dose schedule.
Data shown are the $\log_{10}$ median and range of infectious virus (PFU/mL) in nasal lavages at each time point.

LLOD = 9 PFU/mL.

Figure 79. SARS-CoV-2 Genomic RNA in Ferret Nasal Lavage Fluid



BID = twice daily; EIDD-2801 = molnupiravir; gRNA = genomic RNAribonucleic acid; GS-5245 = obeldesivir (ODV); QD = once daily; SARS-CoV-2 = severe acute respiratory syndrome coronavirus 2 Arrow indicates time of first treatment regardless of BID or QD dose schedule.
Data shown are the $\log_{10}$ median and range of SARS-CoV-2 gRNA copies/µL in nasal lavages at each time point.

Table 814. Reductions in SARS-CoV-2 Infectious Viral Titers and Viral RNA Levels in Ferret Nasal Lavages Compared to Vehicle-Dosed Animals

Sample	Analysis	Change From Vehicle ($\log_{10}$)					
		GS-5245 5ODV 5 mg/kg BID	GS-5245 10 ODV 10 mg/kg BID	GS-5245 20ODV 20 mg/kg BID	GS-5245 20 ODV 20 mg/kg QD	GS-5245 40 ODV 40 mg/kg QD	EIDD-2801 5 mg/kg BID
Nasal lavage	$AUC_{0\text{-}96h}$ PFU/mL	−2.21	−2.50	−3.00	−2.21	−2.80	−2.34
	$AUC_{0\text{-}96h}$ RNA copies/µL	−1.33	−1.51	−2.22	−1.26	−1.91	−0.87
Nasal turbinate	Terminal PFU/mL[a]	−1.68 (P = 0.0004)	−2.20 (P < 0.0001)	−3.78 (P < 0.0001)	−3.42 (P < 0.0001)	−3.79 (P < 0.0001)	−3.79 (P < 0.0001)
	Terminal RNA copies/µL[b]	−2.14 (P = 0.0002)	−1.79 (P = 0.0024)	−3.04 (P < 0.0001)	−2.37 (P < 0.0001)	−4.01 (P < 0.0001)	−2.68 (P < 0.0001)

ANOVA = analysis of variance; BID = twice daily; EIDD-2801 = molnupiravir; ODV = obeldesivir (GS-5245); PFU = plaque- forming unit; QD = once daily; RNA = ribonucleic acid; SARS-CoV-2 = severe acute respiratory syndrome coronavirus 2 a
a Statistics by Tukey's multiple comparisons test after ordinary 1-way ANOVA.
b Statistics by Dunnett's multiple comparisons test after ordinary 1-way ANOVA.

4.1.1.2.5. 3.1.1.2.3. Therapeutic Efficacy of GS-441524 and a Related Analog of GS-5245ODV Against SARS-CoV-2 in African Green Monkeys

While GS-5245 has not been evaluated for in vivo efficacy in a nonhuman primate model, GS-441524 and GS-621763ODV were studied in SARS-CoV-2-infected African green monkeys (AGMs) at Lovelace Biomedical ({Mackman 2023}, submitted; Study PC-554-2001; PC-554-2003). GS-621763 is the tri-isobutyryl ester prodrug analog of GS-441524 and was also designed to be presystemically cleaved following oral delivery to generate systemic GS-441524). GS-441524 is the major circulating metabolite that results from oral dosing of both GS-5245 and GS-621763, and from IV dosing of GS-441524. Efficacy data from the AGM SARS-CoV-2 model with oral GS-621763 and IV GS-441524 serves as a surrogate for GS-5245ODV.

Animals were challenged by combined intranasal and intratracheal SARS-CoV-2 inoculation. Treatment began approximately 8 hpi and continued once daily for an additional 5 days. Viral replication was measured from serially collected nasal and throat swabs, and from bronchoalveolar lavage fluid (BALF). At 6 days postinfection (dpi), animals were euthanized for evaluation of gross and microscopic pathology and viral load in upper and lower respiratory tract tissues. Clinical pathology was also evaluated at baseline and at 3 and 6 dpi.

4.1.1.2.5.1. 3.1.1.2.3.1. Efficacy of GS-441524 by Intravenous Infusion Against SARS-CoV-2 Infection in African Green Monkeys

Efficacy of IV GS-441524 and RDV was assessed in AGMs by monitoring viral load and tissue pathology following SARS-CoV-2 infection (Study PC-554-2001). As displayed in Table 915, IV administration of GS-441524 or RDV was evaluated in parallel with vehicle control (n = 6 animals per treatment group).

Treatment of animals with GS-441524 (20 or 7.5 mg/kg) or RDV (10/5 mg/kg) was well tolerated by all animals. Pulmonary inflammation was mild overall and similar among all treatment groups. There were no treatment-related effects on lung weight ratios collected at necropsy.

Table 915. Study Design to Evaluate Intravenous GS-441524 and Remdesivir Efficacy Against SARS-CoV-2 in African Green Monkeys

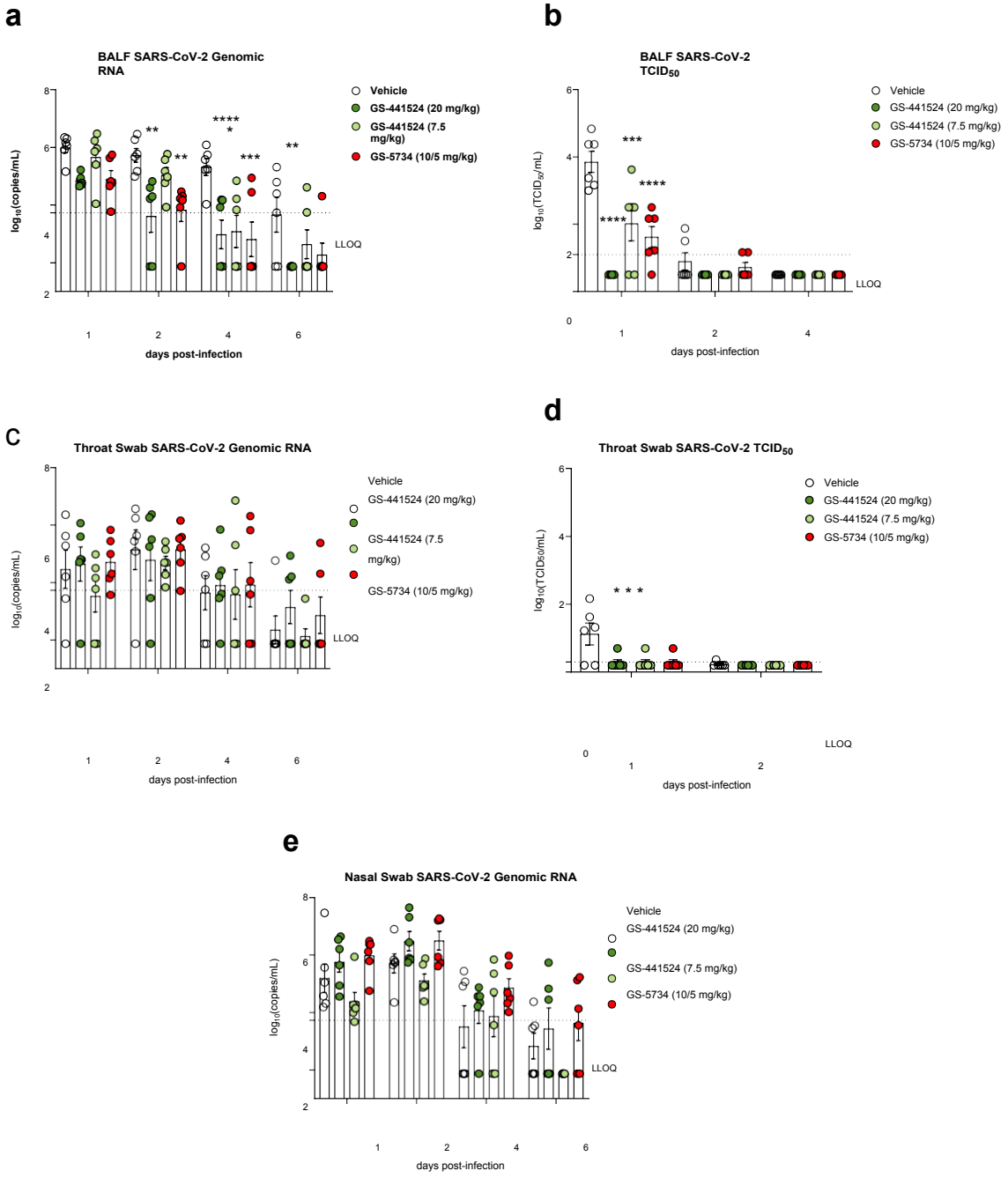
Group	Treatment	Dose[a] (mg/kg)
1	Vehicle	NA
2	GS-441524	20 mg/kg
3	GS-441524	7.5 mg/kg
4	RDV	10 mg/kg on Day 0 followed by 5 mg/kg daily thereafter

IV = intravenous; NA = not applicable; RDV = remdesivir; SARS-CoV-2 = severe acute respiratory syndrome coronavirus 2
a Administered as 30-minute IV infusion once daily.

The 20 mg/kg GS-441524 treatment resulted in a significant reduction in SARS-CoV-2 RNA in BALF at 2, 4, and 6 dpi, and a significant reduction in infectious SARS-CoV-2 titers in BALF at 1 dpi (Figure 810a and b). The magnitude of this effect in BALF was similar to or greater than that of RDV (GS-5734) treatment. Infectious SARS-CoV-2 titers in BALF were detectable in all vehicle-treated animals at 1 dpi, with titers ranging from 10^3 to 10^5 50% tissue culture infectious dose ($TCID_{50}$)/mL (Figure 810b). In contrast, infectious SARS-CoV-2 titers were undetectable

in all animals treated with 20 mg/kg GS-441524 at 1 dpi and were significantly reduced in animals treated with 7.5 mg/kg GS-441524 or RDV (Figure 810b), as compared with vehicle. At 2 dpi, detection of infectious virus in BALF samples was infrequent (Figure 810b) despite the presence of viral RNA (Figure 810a), and all animals, regardless of treatment group, demonstrated no infectious SARS-CoV-2 in BALF by 4 dpi (Figure 810b). In the throat, while no significant differences in SARS-CoV-2 genomic RNA were detected in the treatment groups (Figure 810c), both 20 and 7.5 mg/kg doses of GS-441524 resulted in reductions in infectious SARS-CoV-2 titers that were similar to RDV treatment (Figure 810d). Infectious viral titers in throat swabs were detectable in 4 of 6 animals in the vehicle treatment group at 1 dpi (Figure 810d). In contrast, animals in each of the 20 mg/kg GS-441524, 7.5 mg/kg GS-441524, and RDV treatment groups had only 1 animal with detectable SARS-CoV-2 by $TCID_{50}$ assay, which was significantly reduced in comparison with vehicle-treated controls (Figure 810d). By 2 dpi, all but 1 vehicle-treated animal were negative for infectious SARS-CoV-2 (Figure 810d). In nasal swabs, no treatment-associated effects on SARS-CoV-2 RNA viral load were observed (Figure 810e).

Figure ~~8~~10. Effect of IV GS-441524 and Remdesivir ~~(GS-5734)~~ on SARS-CoV-2 RNA and Infectious Viral Titers in Bronchoalveolar Lavage Fluid, Throat Swabs, and Nasal Swabs



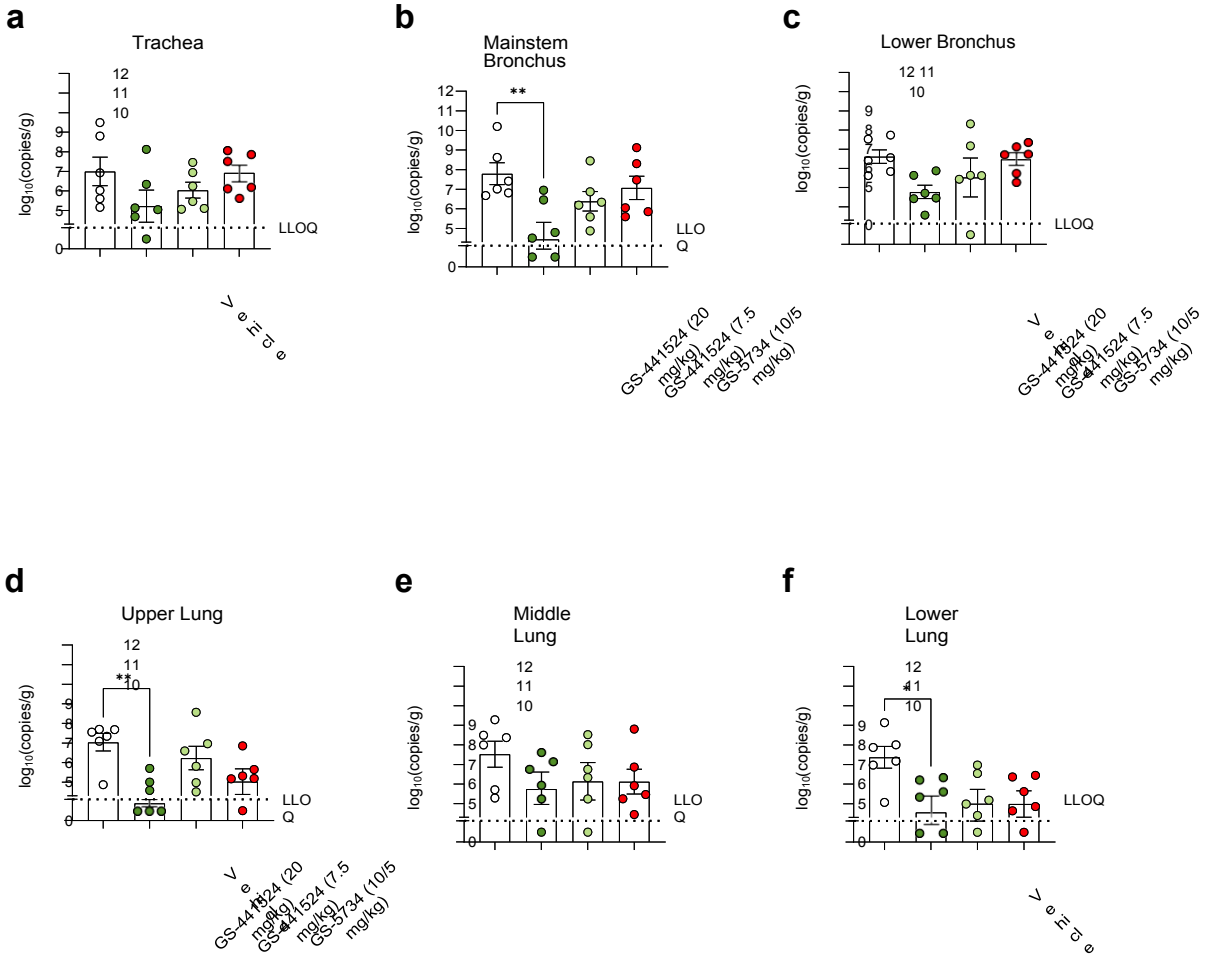
ANOVA = analysis of variance; BALF = bronchoalveolar lavage fluid; dpi = days postinfection; IV = intravenous; LLOQ = lower limit of quantitation; PCR = polymerase chain reaction; RDV = remdesivir ~~(GS-5734)~~; RNA = ribonucleic acid; SARS-CoV-2 = severe acute respiratory syndrome coronavirus 2; SEM = standard error of the mean; $TCID_{50}$ = 50% tissue culture infectious dose

African green monkeys were inoculated with SARS-CoV-2 and treated with GS-441524, RDV ~~(GS-5734)~~, or vehicle by IV infusion. BALF (a, b), throat swabs (c, d), and nasal swabs (e) were collected at 1, 2, 4, and 6 dpi. SARS-CoV-2 genome copies were quantified by quantitative PCR on Days 1, 2, 4, and 6 (a, c, e). Infectious SARS-CoV-2 titers were quantified by median $TCID_{50}$ assay (b, d). Throat swabs were not analyzed for infectious viral titers beyond Day 2 since all animals in all groups were

LLOQ by 2 dpi. Samples that were below the LLOQ for the $TCID_{50}$ assay were assigned a value of half the LLOQ. Individual animal data with group means (± SEM) are presented for all groups. Data were analyzed using repeated measures 2-way ANOVA with Bonferroni post hoc correction compared with vehicle, and a corrected $P < 0.05$ was considered statistically significant. Asterisks indicate $P < 0.05$ (*), $P < 0.01$ (**), $P < 0.001$ (***), and $P < 0.0001$ (****).

In respiratory tissues collected at 6 dpi, GS-441524 at 20 mg/kg reduced both genomic (Figure ~~9~~11) and subgenomic (Figure ~~10~~12) SARS-CoV-2 RNA in mainstem bronchus, ~~lower bronchus,~~ upper lung tissue, and lower lung tissue, and reduced infectious SARS-CoV-2 titers in mainstem bronchus, lower bronchus, and lower lung (Figure ~~11~~13). GS-441524 at 7.5 mg/kg also reduced subgenomic SARS-CoV-2 RNA copies in mainstem bronchus and lower lung~~,~~ and reduced infectious SARS-CoV-2 in lower lung (Figure ~~10~~12). Overall, the effect of the 20 mg/kg GS-441524 dose on reducing SARS-CoV-2 RNA and infectious virus in the respiratory tract was greater than that of RDV.

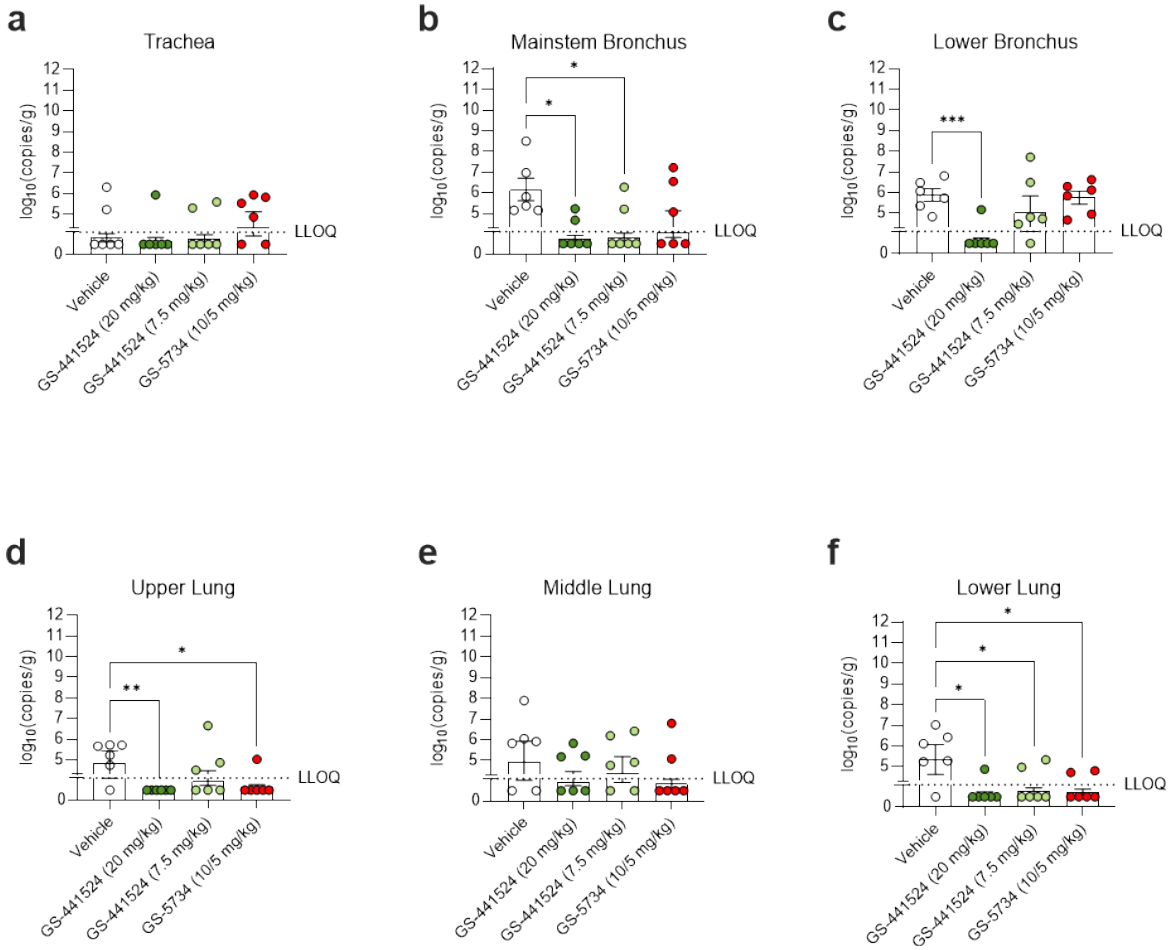
Figure ~~9~~11. Effect of Intravenous GS-441524 and Remdesivir ~~(GS-5734)~~ on SARS-CoV-2 Genomic RNA in Respiratory Tract Tissues



ANOVA = analysis of variance; dpi = days postinfection; IV = intravenous; LLOQ = lower limit of quantitation; PCR = polymerase chain reaction; RDV = remdesivir ~~(GS-5734)~~; RNA = ribonucleic acid; SARS-CoV-2 = severe acute respiratory syndrome coronavirus 2; SEM = standard error of the mean African green monkeys were inoculated with SARS-CoV-2 and treated with GS-441524, RDV ~~(GS-5734)~~, or vehicle by IV infusion. At 6 dpi, animals were euthanized, and respiratory tissues were collected for quantification of SARS-CoV-2 genomic RNA copies by quantitative PCR. Individual animal data with group means (± SEM) are presented for trachea (a) mainstem bronchus (b), lower bronchus (c), and for upper (d), middle (e), and lower (f) lung. Samples that were below the LLOQ for the assay were assigned a value of half the LLOQ. Data were analyzed using a 1-way ANOVA with Bonferroni's post hoc correction compared with vehicle, and a corrected $P < 0.05$ was considered statistically

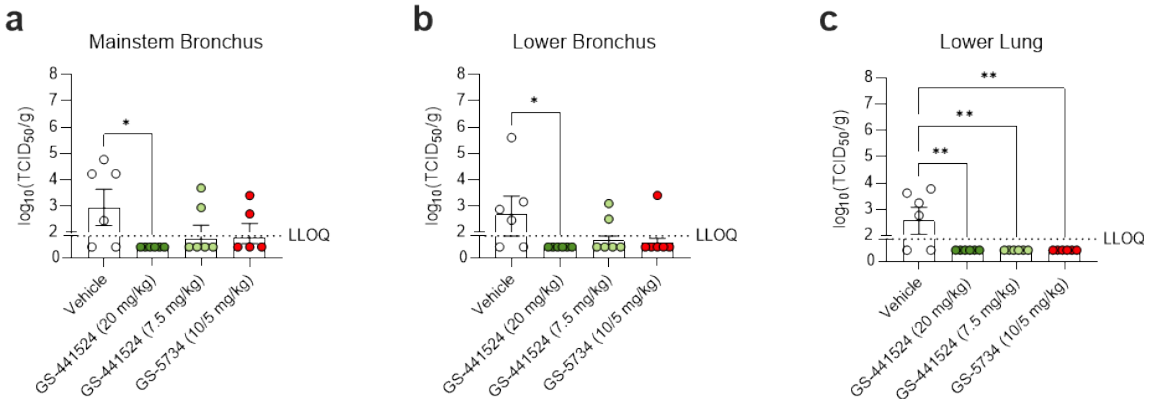
significant. Asterisks indicate $P < 0.05$ (*) and $P < 0.01$ (**).

Figure ~~10~~12. Effect of Intravenous GS-441524 and Remdesivir ~~(GS-5734)~~ on SARS-CoV-2 Subgenomic RNA in Respiratory Tract Tissues



ANOVA = analysis of variance; dpi = days postinfection; IV = intravenous; LLOQ = lower limit of quantitation; PCR = polymerase chain reaction; RDV = remdesivir ~~(GS-5734)~~; RNA = ribonucleic acid; SARS-CoV-2 = severe acute respiratory syndrome coronavirus 2; SEM = standard error of the mean African green monkeys were inoculated with SARS-CoV-2 and treated with GS-441524, RDV ~~(GS-5734)~~, or vehicle by IV infusion. At 6 dpi, animals were euthanized, and respiratory tissues were collected for quantification of SARS-CoV-2 subgenomic RNA copies by quantitative PCR. Individual animal data with group means (± SEM) are presented for trachea (a), mainstem bronchus (b), lower bronchus (c), and for upper (d), middle (e), and lower (f) lung. Samples that were below the LLOQ for the assay were assigned a value of half the LLOQ. Data were analyzed using a 1-way ANOVA with Bonferroni's post hoc correction compared with vehicle, and a corrected $P < 0.05$ was considered statistically significant. Asterisks indicate $P < 0.05$ (*), $P < 0.01$ (**), and $P < 0.001$ (***).

Figure ~~11~~13. Effect of GS-441524 and Remdesivir ~~(GS-5734)~~ on Infectious SARS-CoV-2 Titers in Respiratory Tissues



ANOVA = analysis of variance; dpi = days postinfection; IV = intravenous; LLOQ = lower limit of quantitation; RDV = remdesivir ~~(GS-5734)~~; SARS-CoV-2 = severe acute respiratory syndrome coronavirus 2; SEM = standard error of the mean; $TCID_{50}$ = 50% tissue culture infectious dose African green monkeys were inoculated with SARS-CoV-2 and treated with GS-441524, RDV ~~(GS-5734)~~, or vehicle by IV infusion, n = 6 animals per treatment. At 6 dpi, animals were euthanized, and respiratory tissues were collected for quantification of infectious SARS-CoV-2 titers by median $TCID_{50}$ assay. Individual animal data with group means (± SEM) are presented for mainstem bronchus (a), lower bronchus (b), and lower lung (c). Samples that were below the LLOQ for the assay were assigned a value of half the LLOQ. Data were analyzed using a 1-way ANOVA with Bonferroni's post hoc correction compared with vehicle, and a corrected $P < 0.05$ was considered statistically significant. Asterisks indicate $P < 0.05$ (*) and $P < 0.01$ (**).

The results of this study indicate that the antiviral effects of IV administered 20 mg/kg GS-441524 are comparable to or greater than those of IV treatment with 10/5 mg/kg RDV and resulted in a significant decrease in SARS-CoV-2 viral load in both upper and lower respiratory tract. The 7.5 mg/kg GS-441524 treatment also demonstrated efficacy by some viral endpoints in a more limited subset of samples.

4.1.1.2.6. ~~3.1.1.2.3.2.~~Therapeutic Efficacy of ~~GS-621763 by Oral Administration~~ODV Against SARS-CoV-2 ~~Infection~~ in African Green Monkeys

The efficacy of ~~orally administered GS-621763 was assessed in AGMs by viral load and tissue pathology following SARS-CoV-2 infection (PC-554-2003). As displayed in Table 10, oral administration of GS-621763 (120 mg/kg or 60 mg/kg) was evaluated in parallel with vehicle control (n = 6 animals per treatment group)~~ODV against SARS-CoV-2 was evaluated in African green monkeys at Lovelace Biomedical (Albuquerque, NM). As shown in the study design in Table 14, oral treatment with ODV was initiated at 8 hpi with 60 or 120 mg/kg doses and then continued daily for 5 days. The doses were selected to provide a daily systemic exposure of GS-441524 that bracketed the exposure from IV administered GS-441524 at 20 mg/kg, which demonstrated strong efficacy relative to RDV in the earlier study (Section 4.1.1.2.5.1). The viral loads (genomic RNA and infectious virus titer) were evaluated on BALF, nasal, and throat swab samples, collected on 1, 2, 4, and 6 dpi (Figure 14). At the end of the study, terminal respiratory tissues were harvested for evaluation of tissue viral RNA loads {Mackman 2023}.

Table ~~10~~16. Study Design to Evaluate Oral ODV Efficacy ~~of Orally Administered GS-621763~~ Against SARS-CoV-2 in African Green Monkeys

Group	Treatment	Dose (mg/kg)[a]
1	Vehicle	NA

2	ODV	60
23	GS-621763 ODV	120 mg/kg
3	GS-621763	60 mg/kg

NA = not applicable; ODV = obeldesivir (GS-5245); SARS-CoV-2 = severe acute respiratory syndrome coronavirus 2 a Administered once daily by oral gavage.

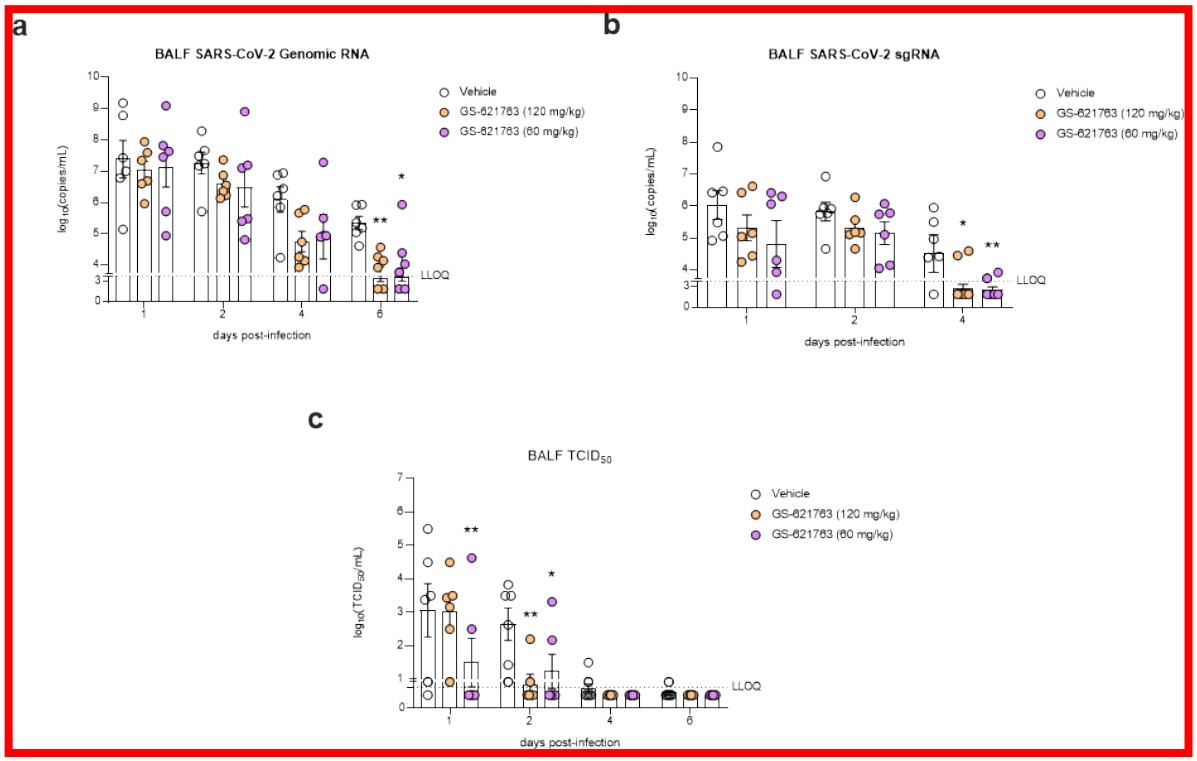
Both doses of GS-621763 (120 and 60 mg/kg) treatments resulted in significant reductions in infectious SARS-CoV-2 titers in BALF as early as 1 to 2 dpi (Figure 12c) and significant reductions in subgenomic SARS-CoV-2 RNA in BALF at 4 dpi (Figure 12b). Corresponding reductions in genomic SARS-CoV-2 RNA in BALF were observed at 6 dpi (Figure 12a) (PC-554-2003). In nasal swabs on 2 and 4 dpi, there were trends toward reduced genomic SARS-CoV-2 RNA, in both 60 and 120 mg/kg GS-621763 treatment groups as compared with vehicle, suggesting some attenuation of viral load in the upper respiratory tract. In respiratory tissues collected on 6 dpi, genomic SARS-CoV-2 RNA was highly variable. Respiratory tissues collected from animals treated with 120 mg/kg GS-621763 demonstrated non-statistically significant trends toward reduced genomic viral RNA relative to vehicle. Subgenomic SARS-CoV-2 RNA was below the limit of detection in the majority of animals, and thus, treatment-associated effects could not be evaluated.

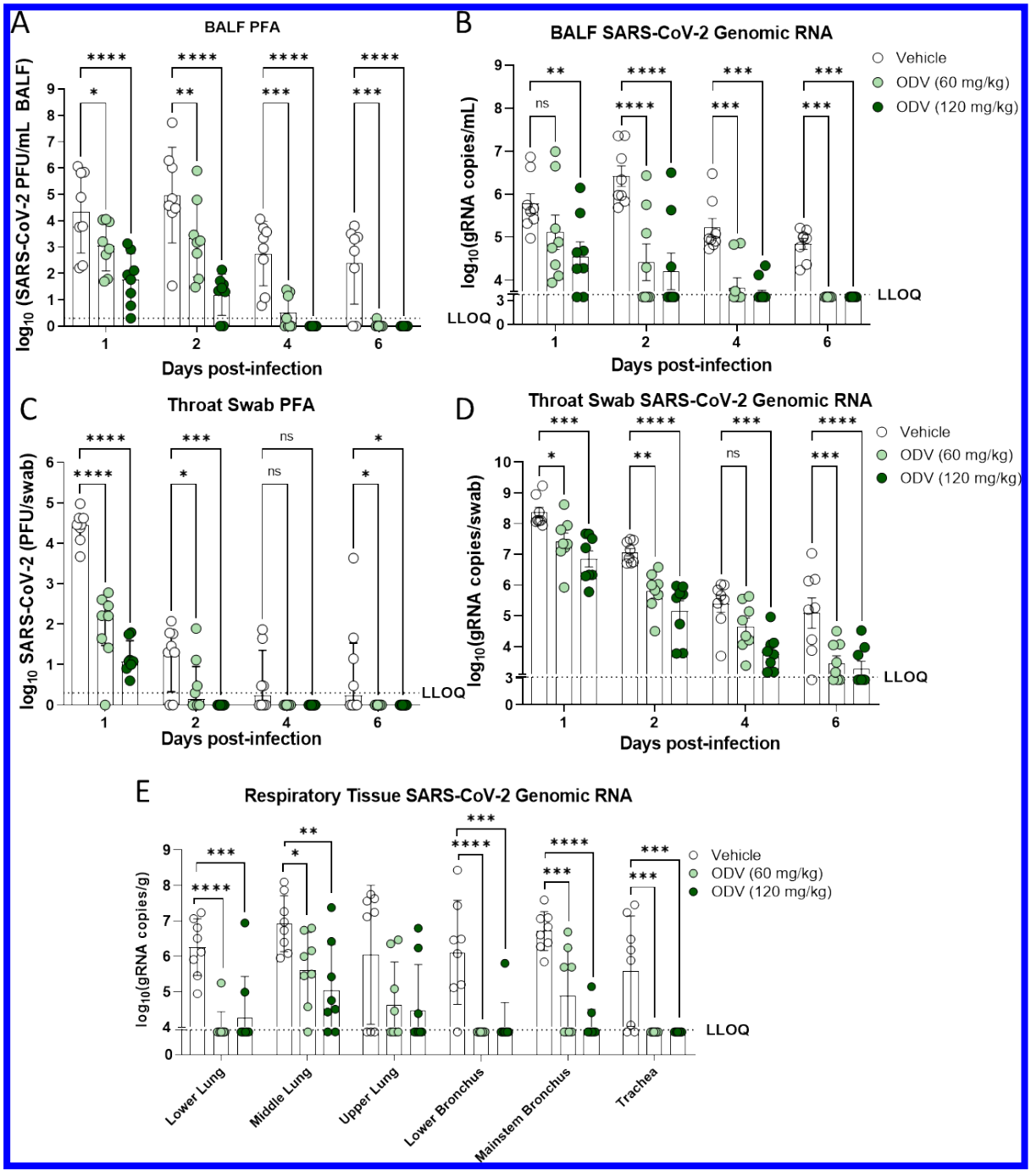
Figure 12. Effect of Orally Administered GS-621763 on SARS-CoV-2 Infectious Titers, Genomic RNA, and Subgenomic RNA in Bronchoalveolar Lavage Fluid Treatment of infected AGMs with either 60 or 120 mg/kg ODV resulted in statistically significant decreases in infectious viral loads in the BALF throughout infection (Figure 14A). Treatment with ODV resulted in only 1 animal (in the 60 mg/kg group) with infectious SARS-CoV-2 in the BALF at or above the limit of quantification at Day 6 postinfection, meanwhile 6 of the 8 vehicle animals had quantifiable infectious viral loads at this time point (Figure 14A). The extent of infectious viral load reductions was dose-dependent, with larger reductions of infectious viral load observed for the group receiving ODV at 120 mg/kg over the sampling period. In line with reductions in infectious viral loads in the BALF, genomic RNA loads in BALF of ODV treated animals were also found to be significantly reduced at nearly all study time points assessed, with the one exception being the 60 mg/kg ODV treatment group at 1 dpi (Figure 14B). The results from RNA and infectious viral loads in the BALF highlights the significant effect of treatment with ODV in the lower airway.

In the upper airway, infectious viral titers and RNA were quantified from throat (Figure 14C-D) and nasal swabs (data not shown). In throat swabs, significant reductions in SARS-CoV-2 infectious (Figure 14C) and RNA viral loads (Figure 14D) were observed from the throat swabs at 1, 2, and 6 dpi from both dose groups. Animals in the 120 mg/kg group showed additional reductions in viral RNA at 4 dpi. Notably, in both throat and nasal swabs, no detectable infectious virus was observed for any animal in either group receiving ODV after 4 dpi. Vehicle control animals at 1 dpi had a higher viral RNA load than that observed in BALF, but only half of the vehicle control animals had detectable levels of infectious virus. This difference highlights that viral RNA levels do not always correlate with infectious virus, and that viral RNA levels in the nasal cavity may, in part, be due to non-infectious viral RNA released from dead or dying cells. At the end of the study (Day 6), terminal respiratory tissues were also assessed for SARS-CoV-2 RNA loads; both doses resulted in significant reductions of viral RNA levels in 5 out of the 6 respiratory tissues evaluated (Figure 14E). Collectively, these data demonstrate that oral ODV at 60 and 120 mg/kg are both highly efficacious at reducing infectious virus and viral RNA loads throughout the upper and lower respiratory tract of AGMs and similarly efficacious to

RDV (Section 4.1.1.2.5.1).

Figure 14. Antiviral Effect of Oral ODV on Respiratory Tract Lavage, Swab, and Tissue Samples in the African Green Monkey SARS-CoV-2 Infection Model





~~ANOVA = analysis of variance; BALF = bronchoalveolar lavage fluid; dpi = days postinfection~~BALF = bronchoalveolar lavage fluid; LLOQ = lower limit of ~~quantitation~~quantification; ~~PCR~~ODV = ~~polymerase chain reaction~~obeldesivir (GS-5245); PFA = plaque forming assay; PFU = plaque forming unit; RNA = ribonucleic acid; SARS-CoV-2 = severe acute respiratory syndrome coronavirus 2~~;RNA = ribonucleic acid; SEM = standard error of the mean; sgRNA = subgenomic RNA; $TCID_{50}$ = 50% tissue culture infectious dose African green monkeys were inoculated with SARS-CoV-2 and treated once daily with GS-621763 or vehicle by oral gavage. BALF was collected at 1, 2, 4, and 6 dpi. SARS-CoV-2 genomic RNA (a) and sgRNA (b) copies were quantified by quantitative PCR. Infectious SARS-CoV-2 titers were quantified by $TCID_{50}$ assay (c). Infectious titer samples that were below the LLOQ for the assay were assigned a value of half the LLOQ. Data were analyzed using repeated measures 2-way ANOVA with Bonferroni post hoc correction compared with vehicle, and a corrected $P < 0.05$ was considered statistically significant. Asterisks indicate $P < 0.05$ (*) and $P < 0.01$ (**).~~

The results of this study show that daily oral treatment with either 60 or 120 mg/kg GS-621763 beginning approximately 8 hours following SARS-CoV-2 challenge had significant antiviral effects in the lower airways, which was evident as early as 1 to 2 days after infection in the AGM model. The antiviral effects in the upper airways could not be assessed with certainty in this study, but trends in SARS-CoV-2 RNA levels in nasal swabs across the study suggest an effect of GS-621763 in the upper respiratory tract that may reach statistical significance with a larger sample size.

(A) Infectious virus in BALF; (B) Genomic RNA in BALF; (C) Infectious viral titer in throat swabs; (D) Genomic RNA in throat swabs; (E) Genomic RNA in respiratory tissue samples harvested at Day 6.
*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; ****$P < 0.0001$.

4.1.2. 3.1.2. Secondary Pharmacodynamics

4.1.2.1. 3.1.2.1. Antiviral Activity Against Other RNA and DNA Viruses

The GS-441524, the parent nucleoside of both GS-5245ODV and RDV share the same, demonstrates a broad-spectrum antiviral profilesprofile against numerous virus families, including CoVs {Agostini 2018, Lo 2017, Warren 2016}.

In a 72-hour antiviral assay in NHBE cells, GS-5245 inhibited RSV with an EC_{50} value of 0.410 µM (Table 11; PC-611-2009). The cytotoxicity potential of GS-5245 was also assessed in NHBE cells after a 72-hour treatment; the CC_{50} value was 30.3 µM (SI = 74.0). Additionally, GS-5245 was evaluated for inhibition of RSV in HEp-2 cervical epithelial cells after 72 hours of treatment (EC_{50} = 0.391 µM) (Table 11).

Table 11. Activity of GS-5245, GS-441524, and Remdesivir Against Respiratory Syncytial Virus

Cell culture (assay)	GS-5245[a]		GS-441524[a]		Remdesivir[a]	
	EC_{50} (µM)	CC_{50} (µM)	EC_{50} (µM)	CC_{50} (µM)	EC_{50} (µM)	CC_{50} (µM)
NHBE	0.410 ± 0.155	30.3 ± 0.2	2.68 ± 1.68	> 50	0.0323 ± 0.0290	> 12.8
HEp-2	0.391 ± 0.122	ND	0.947 ± 0.495	> 50	0.0210 ± 0.0073	5.63 ± 1.13

CC_{50} = half-maximal cytotoxic concentration; EC_{50} = half-maximal effective concentration; HEp-2 = human cervical carcinoma derived; ND = not done; NHBE = normal human bronchial epithelial; SD = standard deviation
a The data represent the mean of at least 2 independent experiments ± SD.

4.1.2.2. 3.1.2.2. In Vitro Cytotoxicity

4.1.2.2.1. 3.1.2.2.1. Cytotoxicity in Human Cell Lines

The cytotoxicity profiles of GS-5245ODV and the nucleoside analog GS-441524 were determined in several immortalized human cell lines, including hepatoma, hepatoblastoma, prostate, lymphoblastoid, and lung fibroblast transformed cell lines (Table 1217; Study PC-611-2009). The CC_{50} values for GS-5245ODV ranged from 19.2 to > 44 µM in the tested cell lines. GS-441524 showed no cytotoxicity up to the highest concentrations evaluated in the tested cell lines, with the exception of the MT-4 T-cell line, a model with the shortest doubling time among all cell types tested. Control compound puromycin showed cytotoxic effects consistent with historical data, with CC_{50} values of 0.12 to 0.73 µM.

Table ~~12~~17. In Vitro Cytotoxicity of ~~GS-5245~~ODV in Human Cell Lines

	CC_{50} ± SD (µM)[a]				
	Huh7	HepG2 (Galactose)	PC-3 (Galactose)	MT-4	MRC5
Compound	Hepatoma Cells	Hepatoma Cells	Prostate Cancer Cells	Lymphoblast T Cells	Lung Fibroblast Cells
~~GS-5245~~ ODV	> 44	19.2 ± 9.6	> 41	> 44	> 44
GS-441524	> 44	> 100	> 100	69.3 ± 25.7	> 44
Puromycin	0.42	0.73 ± 0.01	0.52 ± 0.11	0.12 ± 0.03	0.41

CC_{50} = half-maximal cytotoxic concentration; ODV = obeldesivir (GS-5245); SD = standard deviation
a All CC_{50} values represent the mean ± SD of at least 2 independent experiments, unless otherwise noted.

4.1.2.2.2. ~~3.1.2.2.2.~~Cytotoxicity in Primary Human Cells

The cytotoxicity of ~~GS-5245~~ODV and ~~the nucleoside analog~~ GS-441524 was tested against primary human NHBE cultures, hepatocytes, and quiescent peripheral blood mononuclear cells (PBMCs) (Study PC-611-2009). The CC_{50} values for ~~GS-5245~~ODV ranged from ~~to~~ 18.6 µM in unstimulated PBMCs to 30.3 µM in NHBE cultures. GS-441524 showed no cytotoxicity up to the highest concentration tested in all primary cells (Table ~~13~~18).

Table ~~13~~18. In Vitro Cytotoxicity of ~~GS-5245~~ODV in Primary Human Cells

	CC_{50} ± SD (µM)[a]				
Compound	NHBE Cultures	Pneumocytes	Primary Hepatocytes	PBMCs (Donor 1)	PBMCs (Donor 2)
~~GS-5245~~ ODV	30.3 ± 0.2	ND	ND	18.6 ± 2.5	25.9 ± 3.8
GS-441524	> 50	> 50	ND	> 44	> 44
Puromycin	ND	ND	1.11 ± 0.39	0.66	0.58

CC_{50} = half-maximal cytotoxic concentration; ND = not done; NHBE = normal human bronchial epithelial; ODV = obeldesivir (GS-5245); PBMC = peripheral blood mononuclear ~~cells~~cell; SD = standard deviation
a All CC_{50} values represent the mean ± SD of at least 2 independent experiments.

4.1.2.2.3. ~~3.1.2.2.3.~~In Vitro Effect on Human Hematopoietic Progenitor Cells

GS-441524~~, RDV,~~ and control compound 5-fluorouracil ~~(5-FU)~~ were tested in vitro for their effects on the proliferation of erythroid, myeloid, and megakaryoid progenitors from 3 independent donors (Study PC-399-2018). The study was performed at Stemcell Technologies (Vancouver, BC, Canada). Following an 11- to 14-day incubation, GS-441524 exhibited CC_{50} values ranging from 5.9 to 22.7 µM across the 3 progenitor cell types from the 3 tested donors (Table ~~14~~19). ~~Remdesivir exhibited slightly stronger antiproliferative effects with CC_{50} values ranging from 2.3 to 10.5 µM, and the control 5-FU was the most potent inhibitor of progenitor cell proliferation among the 3 compounds tested.~~ No major differences in the sensitivity to GS-441524 were observed among the different progenitor cell types and across the 3 tested donors.

Table ~~14~~19. In Vitro Effect of GS-441524 ~~and Remdesivir~~ on Human

Hematopoietic Progenitor Cells

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

CC_{50} = half-maximal cytotoxic concentration
a The CC_{50} values represent a mean and a range from testing of human progenitor cells isolated from 3 independent donors.

4.1.2.2.4. ~~3.1.2.2.4.~~Effect on Renal Organic Anion Transporters

The active transport of GS-441524 ~~and RDV~~ was studied in cells overexpressing human or rat renal organic anion transporter (OAT)1 or OAT3 to determine their potential for OAT-mediated intracellular uptake and cytotoxicity (Studies AD-540-2025, PC-399-2020).

The expression of neither rat nor human OATs significantly changed the cytotoxicity or intracellular triphosphate accumulation upon incubation with GS-441524 ~~or RDV~~ compared with
mock-transfected control cells. These data indicate that GS-441524 ~~and RDV are~~is not an effective ~~substrates~~substrate of rat OAT1 or OAT3 and ~~do~~does not exhibit rat OAT3-dependent cytotoxicity.

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

4.1.2.3. ~~3.1.2.3.~~In Vitro Mitochondrial Toxicity

4.1.2.3.1. ~~3.1.2.3.1.~~Cytotoxicity Under Aerobic Metabolic Conditions

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

Using intracellular ATP quantification, the effects of glucose and galactose as a source of energy on the cytotoxicity of GS-441524 ~~and RDV were~~was assessed in the HepG2 hepatoma cell line that has previously been identified as a suitable model for testing compounds under aerobic conditions (Study PC-399-2013) {Marroquin 2007}. In addition, the same conditions were tested in the PC-3 prostate-derived cell line as a model of quickly proliferating cells. GS-441524 did not show any cytotoxicity in either PC-3 or HepG2 cells at the highest concentrations tested (100 µM), irrespective of the metabolic conditions (Table ~~15~~20).

Puromycin, used as general cytotoxic control, exhibited similar cytotoxicity in both cell types in the presence of glucose or galactose, matching historical data from these assays.

Table 1520. In Vitro Cytotoxicity of GS-441524 and Remdesivir Under Anaerobic and Aerobic Metabolic Conditions

Compound	CC_{50} ± SD (µM)[a]			
	HepG2 Cells		PC-3 Cells	
	Anaerobic Conditions (Glucose)	Aerobic Conditions (Galactose)	Anaerobic Conditions (Glucose)	Aerobic Conditions (Galactose)
GS-441524	> 100	> 100	> 100	> 100
Remdesivir	3.7 ± 0.2	11.1 ± 1.2	8.9 ± 1.6	1.4 ± 0.1
Puromycin	0.73 ± 0.01	0.96 ± 0.13	0.52 ± 0.11	0.48 ± 0.01

CC_{50} = half-maximal cytotoxic concentration; HepG2 = hepatoma; PC-3 = prostate cancer; SD = standard deviation
a All CC_{50} values represent the mean ± SD of at least 2 independent experiments.

4.1.2.3.2. 3.1.2.3.2. Effect on Mitochondrial Proteosynthesis

The effect of GS-441524 and RDV on mitochondrial protein synthesis was assessed following a 5-day incubation with the human cell line PC-3 (Study PC-399-2016). This particular cell model was chosen because of a high rate of cell division and protein synthesis. The selective effect of tested compounds on mitochondrial protein synthesis was determined by parallel quantification of the level of cytochrome oxidase subunit 1 (encoded by mitochondrial DNA [mtDNA]) and succinate dehydrogenase A (encoded by nuclear DNA). GS-441524 showed no effect on proteosynthesis up to the highest concentration tested (100 µM) (Table 1621). Chloramphenicol was used as a positive control and its specific effect on mitochondrial proteosynthesis was consistent with historical data.

Table 1621. In Vitro Effect of GS-441524 and Remdesivir on Mitochondrial Proteosynthesis

Compounds	Mitochondrial and Cellular Protein Synthesis 5-Day CC_{50} ± SD (µM)[a]		
	COX-1	SDH-A	ATP
GS-441524	> 100	> 100	> 100
Remdesivir	8.9 ± 1.1	8.6 ± 1.3	11.3 ± 3.3
Chloramphenicol (positive control)	2.6 ± 0.6	> 25	14.1 ± 3.6

ATP = adenosine triphosphate; CC_{50} = half-maximal cytotoxic concentration; COX-1 = cytochrome oxidase subunit 1; SD = standard deviation; SDH-A = succinate dehydrogenase A
a CC_{50} values were reported as average of 2 or more independent experiments ± SD.

4.1.2.3.3. 3.1.2.3.3. Effect on Mitochondrial Respiration

GS-441524 and RDV werewas further evaluated for theiritsits effects on mitochondrial respiration in multiple human cell types including PC-3 (prostate cancer) cells, HepG2 cells, and primary renal proximal tubule epithelial cells by measuring the rate of oxygen consumption using a Seahorse Extracellular Flux Analyzer (Study PC-399-2016). GS-441524 showed no effect on mitochondrial protein synthesis or respiration at the highest concentration tested (100 µM) (Table 1722).

Table ~~17~~22. Effect of GS-441524 ~~and Remdesivir~~ on Mitochondrial Respiration

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

ATP = adenosine triphosphate; CC_{50} = half-maximal cytotoxic concentration; DNA = deoxyribonucleic acid; HepG2 = hepatoma; PC-3 = prostate cancer; RPTEC = renal proximal tubule epithelial cell; SD = standard deviation
a CC_{50} values represent mean ± SD from at least 2 independent experiments following a 3-day incubation with the tested compounds.

To assess the risk of mitochondrial toxicity in liver, GS-441524 ~~and RDV were~~was studied for ~~their~~its effect on mitochondrial spare respiration in primary human hepatocytes (PHHs) after a 4-hour or 3-day incubation (Study PC-399-2028). GS-441524 did not exhibit any effects on mitochondrial spare respiration, cellular ATP level, or total DNA level at the highest concentration tested (100 µM) after either a 4-hour or a 3-day treatment. GS-441524 did not exhibit any degree of toxicity in PHH at concentrations substantially exceeding anticipated systemic levels in humans.

4.1.2.3.4. ~~3.1.2.3.4.~~Effect on Mitochondrial DNA

The in vitro potential of GS-441524 to affect mtDNA was assessed by quantitative real-time polymerase chain reaction analysis following continual treatment of HepG2 cells (Study PC-399-2015). HepG2 cells were treated with 1.0, 10.0, or 100 µM GS-441524 for 10 days with a fresh compound addition every 3 days. No significant decrease in mtDNA was observed at either 1 or 10 µM GS-441524, and only a slight reduction of approximately 17% in mtDNA content was detected at 100 µM GS-441524 (Table ~~18~~23). In parallel, the treatment of HepG2 cells with 0.2, 2.0, and 20 µM dideoxycytidine, resulted in a 43.0%, 74.9%, and 93.1% reduction in mtDNA levels, respectively, compared with the DMSO control.

Table ~~18~~23. Effect of GS-441524 on the Levels of Mitochondrial DNA in HepG2 Cells

Compound	Compound Concentration (µM)	Relative Amount of mtDNA (% Control)[a]	*P* ~~value~~Value Compared With DMSO ~~(vs Control)~~[b]Control[b]
DMSO (control)	—	100.0 ± 8.8	—
GS-441524	1.0	96.1 ± 31.5	0.70
	10.0	90.3 ± 21.9	0.22

	100	83.3 ± 11.7	0.003
ddC	0.2	57.0 ± 10.4	< 0.001
	2.0	25.1 ± 7.8	< 0.001
	20.0	6.9 ± 2.9	< 0.001

ddC = dideoxycytidine; DMSO = dimethylsulfoxide; DNA = deoxyribonucleic acid; mtDNA = mitochondrial DNA; SD = standard deviation
a The data represent the mean ± SD of 3 independent experiments performed in triplicate.
b Paired, 2-tailed student *t* test.

4.1.2.4. 3.1.2.4. Interaction With Host RNA and DNA Polymerases

GS-443902, the active triphosphate metabolite of GS-5245,ODV and GS-441524, and RDV, is a potent inhibitor of SARS-CoV-2, SARS-CoV, and MERS-CoV RdRps {Gordon 2020a}. GS-443902 has been tested in multiple in vitro biochemical assays to assess its interaction with important host DNA and RNA polymerases (Study PC-399-2017). The enzymatic activities of human DNA polymerases α and β, as well as that of RNA polymerase II, were unaffected by GS-443902 up to 200 µM, the highest concentration tested (Table 1924).

Table 1924. Inhibition of Host DNA and RNA Polymerases by the Active Triphosphate Metabolite GS-443902

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

dGTP = deoxyguanosine triphosphate; DNA = deoxyribonucleic acid; dTTP = deoxythymidine triphosphate; GTP = guanosine triphosphate; IC_{50} = half-maximal inhibitory concentration; mtRNA = mitochondrial RNA; Pol = polymerase; RNA = ribonucleic acid; SD = standard deviation
a IC_{50} values represent mean ± SD from at least 2 independent experiments.

In addition to direct inhibition of DNA and RNA polymerases, GS-443902 was tested for its incorporation into nucleic acids by host mtDNA and mitochondrial RNA (mtRNA) polymerases using a single nucleotide incorporation assay (Table 2025) (Study PC-399-2017). GS-443902 was not incorporated into DNA by mtDNA polymerase γ and was a poor substrate for mtRNA polymerase, with a rate of incorporation equal to 5.8% relative to ATP (Table 2025). This result contrasts with the significantly higher incorporation rates observed with the triphosphates of BMS-986094 and balapiravir (R1626-TP), 2 anti-hepatitis C virus nucleosides associated with clinical toxicity. Together, these data further support the low potential of GS-5245ODV to be associated with selective mitochondrial toxicity.

Table 2025. Relative Rate of Incorporation of the Active Triphosphate Metabolite GS-443902 by Human Mitochondrial DNA and RNA Polymerases

	Rate of Incorporation (% of Natural dNTP or NTP)[a]	
Nucleoside Triphosphate	**DNA Polymerase γ**	**mtRNA Polymerase**
GS-443902	0%	5.8% ± 1.4%

Decitabine-TP	79% ± 8%	ND
BMS-986094-TP	ND	92% ± 33%
Balapiravir~~/RG~~ (R1626-TP)	ND	112% ± 10%

DNA = deoxyribonucleic acid; dNTP = deoxyribonucleoside triphosphate; mtRNA = mitochondrial RNA; ND = not determined; NTP = nucleoside triphosphate; RNA = ribonucleic acid; SD = standard deviation; TP = triphosphate

a The rate of single nucleotide incorporation was measured in the presence of 500 µM nucleotide analog and expressed as percentage of the natural NTP incorporation at the same concentration. Data are presented as mean ± SD from at least 2 independent experiments.

4.1.2.5. ~~3.1.2.5.~~Molecular Target Screen of the Nucleoside Analog GS-441524

The potential for off-target pharmacological activity of ~~GS-5245 (referred to as GS-1167944 in the report)~~ODV and the parent nucleoside GS-441524 were evaluated against a panel of up to 87 targets consisting of receptors, ion channels, and transporters (Studies PC-611-2002, PC-399-2001). There were no responses (> 50% inhibition of ligand binding) considered related to either test article at a concentration of 10 µM.

4.1.3. ~~3.1.3.~~Safety Pharmacology

The nonclinical safety profile of ~~GS-5245~~ODV was characterized in studies evaluating its potential pharmacologic effects on specific organ systems. Study designs and evaluated parameters were consistent with accepted principles and practices as outlined in International ~~Conference~~Council for Harmonisation (ICH) and ~~United States (~~US~~)~~ Food and Drug Administration (FDA) guidelines. All pivotal studies were conducted in accordance with US FDA GLP regulations.

The rat and dog were selected for in vivo investigations based on the formation of the same metabolites as expected in humans. As no sex-specific ~~pharmacokinetic (~~PK~~)~~ differences have been observed in rat and dog studies (Section ~~3.3.2~~4.3.2), the use of only males or females for the cardiovascular system, respiratory system, and central nervous system (CNS) safety pharmacology assessment was acceptable. Consistent with its intended route of administration in humans, studies were conducted by the oral gavage route of administration. Formulations of ~~GS-5245~~ODV were prepared in 0.5% methylcellulose in deionized water for all in vivo studies.

4.1.3.1. ~~3.1.3.1.~~Cardiovascular System

4.1.3.1.1. ~~3.1.3.1.1.~~In Vitro

4.1.3.1.1.1. ~~3.1.3.1.1.1.~~Effect of ~~GS-5245~~ODV on Cloned hERG Potassium Channels Expressed in Human Embryonic Kidney Cells

The in vitro effect of ~~GS-5245~~ODV on the human ether-a-go-go-related gene (hERG) channel current (a surrogate for IKr, the rapidly activating delayed rectifier cardiac potassium current) was assessed at near-physiological temperature (Study PC-611-2005). ~~GS-5245~~Obeldesivir inhibited hERG current by 3.3% at 30.48 µM and 8.0% at 304.9 µM versus −0.9% in vehicle control. The IC_{50} or concentration that resulted in 20% inhibition ~~(IC_{20})~~ could not be calculated but ~~were~~was estimated to be greater than 304.9 µM.

4.1.3.1.1.2. ~~3.1.3.1.1.2.~~Effect of GS-441524 on Cloned hERG Potassium Channels Expressed in Chinese Hamster Ovary ~~(CHO)~~-hERG DUO Cells

In a non-GLP study, the hERG IC_{50} value was > 30 µM for GS-441524 (Study PC-399-2025).

4.1.3.1.2. ~~3.1.3.1.2.~~In Vivo

4.1.3.1.2.1. ~~3.1.3.1.2.1.~~Effect of ~~GS-5245~~ODV on Telemetry-Instrumented Conscious Beagle Dogs

The objective of this study was to determine the potential acute effects of orally administered ~~GS-5245~~ODV on heart rate, arterial blood pressure (systolic, diastolic, mean arterial, and pulse pressure), body temperature, and electrocardiograms (ECGs) in conscious radiotelemetry-instrumented male beagle dogs (Study PC-611-2004). Blood samples collected 4 and 24 hours postdosing following each dosing day confirmed exposure of ~~GS-5245~~ODV and GS-441524.

Four male beagle dogs were administered a single dose of vehicle or ~~GS-5245~~ODV in a Latin square crossover design at dose levels of 0, 30, 100, or 300 mg/kg with a washout period of 4 days between doses. The following parameters and endpoints were evaluated: clinical observations, heart rate, arterial blood pressure (systolic, diastolic, and mean), pulse pressure, body temperature, ECG waveforms (from which ECG intervals PR, QRS, QT, and heart rate-corrected QT were derived and ECG waveform morphologies).

There were no ~~GS-5245~~ODV-related effects on mortality, clinical observations, body weight, or food consumption. Statistically significant higher heart rate (up to 18.0 beats per minute) was noted at 300 mg/kg; this change was considered to be ~~GS-5245~~ODV-related due to magnitude of change and duration of effect. Lower systolic, diastolic, and arterial blood pressures at 300 mg/kg were considered ~~GS-5245~~ODV-related based on the magnitude and duration of effect, and correlation (compensatory in nature) to the observed changes in heart rate. Statistically significant higher body temperature at 300 mg/kg during the dark photoperiod (9 through 18 hours postdosing) was considered to be ~~GS-5245~~ODV-related due to duration of effect and correlation to higher heart rate. There were no ~~GS-5245~~ODV-related qualitative changes in ECG waveforms. Changes in PR and QT intervals were considered secondary effects to the observed changes in heart rate.

Based on the results of this study, a single oral administration of ~~GS-5245~~ODV to male beagle dogs at dose levels of 30, 100, and 300 mg/kg resulted in increased heart rate and body temperature, and compensatory decreases in systolic, diastolic, mean arterial, and pulse pressure at 300 mg/kg.
Therefore, the no observed effect level (NOEL) was considered to be 100 mg/kg. Exposures to ~~GS-5245~~ODV and GS-441524 at the NOEL were approximately 1280 and 36,700 ng/mL, respectively, based on Day 1 C_{max} values in male dogs administered 100 mg/kg in the 2-week repeat-dose toxicity study (Section ~~3.3.2.2.1~~4.3.2.2.1, Study TX-611-2002).

4.1.3.2. ~~3.1.3.2.~~Respiratory System

4.1.3.2.1. ~~3.1.3.2.1.~~Effect of ~~GS-5245~~ODV on the Respiratory System of Male Wistar Han Rats Using Head-Out Plethysmography

Ten male Wistar Han rats were administered a single dose of vehicle or ~~GS-5245~~ODV at 0, 200, or 500 mg/kg via oral gavage (Study TX-611-2001). Respiratory data (respiratory rate, tidal volume, and minute volume) were collected for at least 1 hour prior to dosing

(baseline) and continuously for at least the first 5 hours postdose and at 22 hours (± 1 hour) postdose for at least 1.5 hours. Although no changes were identified for either respiratory rate or tidal volume, a statistically significant higher minute volume was noted at 200 and 500 mg/kg. The increases in minute volume from 0 to 2 hours postdose were considered to be GS-5245ODV-related based on magnitude and duration of change. There was no clear dose-dependent response between the 200 and 500 mg/kg dose groups. GS-441524 exposures in male rats administered 200 mg/kg were approximately 37,900 ng/mL, based on the Day 1 C_{max} values.

4.1.3.3. 3.1.3.3. Central Nervous System

4.1.3.3.1. 3.1.3.3.1. Effect of GS-5245ODV on the Central Nervous System of Female

Wistar Han Rats Ten female Wistar Han rats were administered a single dose of vehicle or

GS-5245ODV at 0, 200, or
500 mg/kg via oral gavage (Study TX-611-2001). Neurobehavioral assessments were conducted during the pretreatment period (Day −6) and on Day 1 at 1 to 2, 6, and 22 hours (± 1 hour) postdose. The neurobehavioral evaluations consisted of assessments for activity, excitability, autonomic function, neuromuscular function, sensorimotor responses, and physiological function. There were no effects on autonomic, neuromuscular, sensorimotor, or behavioral domains, and the NOEL for neurological effects was 500 mg/kg. GS-441524 exposure at the NOEL was approximately 67,900 ng/mL, based on the Day 1 C_{max} in female rats administered 500 mg/kg.

4.1.4. 3.1.4. Pharmacodynamic Drug Interactions

No studies of pharmacodynamic drug interactions have been conducted to date.

4.1.5. 3.1.5. Nonclinical Pharmacology Conclusions

GS-5245Obeldesivir is a mono-5'-isobutyryl ester prodrug that is extensively metabolized presystemically to the parent nucleoside, GS-441524, an unnatural adenine *C*-nucleoside analog. GS-441524 in the bloodstream can distribute into tissues including the lung. In cells, including cell types relevant for CoV replication, GS-441524 undergoes conversion to the pharmacologically active triphosphate GS-443902, the same active triphosphate generated by RDV (Section 3.2.3.2.24.2.3.2.2). The mechanisms of inhibition result from the incorporation of GS-443902 into nascent RNA chains by the viral RdRp, which causes template-dependent inhibition of daughter strand synthesis and delayed RNA chain termination during viral replication of nascent strands. The SARS-CoV-2, SARS-CoV, and MERS-CoV RdRps were shown to incorporate GS-443902 more efficiently than ATP, with selectivity values of 0.26, 0.32, and 0.35, respectively, compared with ATP. In contrast, GS-443902 does not inhibit host RNA and DNA polymerases, including mitochondrial polymerases, at concentrations as high as 200 µM.

GS-5245Obeldesivir and GS-441524 are potent inhibitors of SARS-CoV-2 in the A549-hACE2 transformed lung alveolar epithelial line and primary cultures of human bronchial epithelial cells. In uninfected A549-hACE2 cells, the CC_{50} values of GS-5245ODV and GS-441524 after 48 hours of treatment were > 50 µM, resulting in SI values of > 39 for GS-5245ODV and > 23.8 for GS-441524. The low cytotoxicity profile of GS-5245ODV in a panel of diverse cell types was

similar to ~~its effects in~~ lung-derived cell cultures, with CC_{50} values ranging from 18.6 to > 44 µM. Cytotoxicity of GS-441524 in rapidly dividing MT-4 lymphoblast T cells was observed with a CC_{50} value of 69.3 µM but was not detected in other primary cultures at the highest concentrations tested, which ranged from 44 to 100 µM. The parent nucleoside analog GS-441524 did not show in vitro mitochondrial toxicity in multiple relevant cell types and diverse functional assays.

Obeldesivir retain potent antiviral activity against SARS-CoV-2 Omicron subvariants (B.1.1.529/BA.1, BA.2, BA.2.12.1, BA.4, BA.5, BA.2.75, BA.4.6, BF.5, XBB, and BQ.1.1), with EC_{50} fold-changes of 0.26 to 1.33 compared to reference strain WA1.

In studies with SARS-CoV-2, a selection of in vitro resistance by treatment with either GS-441524 or RDV occurred at relatively high numbers of passages. The resulting substitutions in nsp12 RdRp conferred up to a 10.4-fold reduction in susceptibility to GS-441524 and RDV. Together, these results suggest a high barrier to the emergence of resistance to ~~GS-5245~~ODV and the systemic metabolite GS-441524.

In rodent efficacy models, ~~GS-5245~~ODV effectively delivered GS-441524 into the plasma leading to reduced infectious viral titers of an early strain of SARS-CoV-2 in mouse lung homogenates in a dose-dependent manner, with maximal virus titer reductions in 8 of 10 mice treated twice daily with 10 mg/kg ~~GS-5245~~ODV and in all mice administered 30 mg/kg ~~GS-5245~~ODV twice daily starting at 12 hpi. Mean body weights, pulmonary function, and healthy lung physiology of mice infected with SARS-CoV-2 were maintained in the 10 and 30 mg/kg twice-daily ~~GS-5245~~ODV dose groups when treatment was initiated at 12 hpi. ~~GS-5245~~ODV demonstrated effective antiviral responses at doses ranging from 10 to 20 mg/kg twice daily and 20 to 40 mg/kg once daily in the SARS-CoV-2 ferret model. Overall, based on the assessments of viral titer in both the nasal lavage and nasal turbinates, the 20 mg/kg once-daily dosing regimen was most similar in efficacy to molnupiravir dosed at 5 mg/kg twice daily. The highest efficacy was observed with ~~GS-5245~~ODV at 20 mg/kg twice daily or 40 mg/kg once daily, both of which were numerically superior to molnupiravir.

In the AGM model of SARS-CoV-2, once-daily IV administration of 20 mg/kg parent nucleoside GS-441524 beginning at 8 hpi demonstrated significant reductions of BALF genomic RNA and infectious virus that was comparable to RDV IV following the standard 10/5 mg/kg infusion. Based on genomic RNA analysis in terminal lung tissue, GS-441524 showed significant effects in multiple ~~parts~~regions of the lower respiratory tract that were superior to RDV IV. ~~Once-daily oral administration of~~Oral ODV at 60 and 120 ~~or 60~~ mg/kg ~~GS-621763, a tool compound that is also an ester prodrug of GS-441524, reduced~~are both highly efficacious at reducing SARS-CoV-2 infectious ~~titers~~virus and viral RNA ~~in BALF, demonstrating efficacy of an orally dosed surrogate ester prodrug~~loads throughout the upper and lower respiratory tracts of AGMs and similarly efficacious to RDV.

In the cardiovascular safety pharmacology study in beagle dogs, oral dosing of ~~GS-5245~~ODV increased heart rate and body temperature, with compensatory decreases in systolic, diastolic, mean arterial, and pulse pressure at 300 mg/kg; there were no effects at 100 mg/kg. GS-441524 C_{max} exposures in dogs at 100 mg/kg were approximately ~~8~~11-fold higher than anticipated clinical exposures at the projected efficacious dose (~~Section 3.2.6~~Table 1). In vitro, the hERG IC_{50} values were > 304 µM for ~~GS-5245~~ODV and > 30 µM for GS-441524. In rats, there were no effects on respiratory rate or tidal volume, although overall respiration (ie, minute volume) was increased at 200 and 500 mg/kg. Exposures at 200 mg/kg in male rats

were approximately 89-fold higher than the anticipated clinical exposures at the projected efficacious dose (Section 3.2.6). GS-5245. Obeldesivir had no effects on the CNS of rats at 500 mg/kg. Taken together, the risk for cardiovascular, respiratory, or CNS effects in the clinic is considered low and readily monitorable.

In conclusion, GS-5245ODV is a novel small-molecule inhibitor of SARS-CoV-2 replication with potent antiviral activity in vitro and robust efficacy in several animal models. The overall nonclinical pharmacology profile of GS-5245ODV supports its further development as a novel oral agent for the treatment of COVID-19.

4.2. 3.2. Pharmacokinetics (Absorption, Distribution, Metabolism, and Excretion)

4.2.1. 3.2.1. Absorption

4.2.1.1. 3.2.1.1. In Vitro Absorption Studies

4.2.1.1.1. 3.2.1.1.1. Permeability

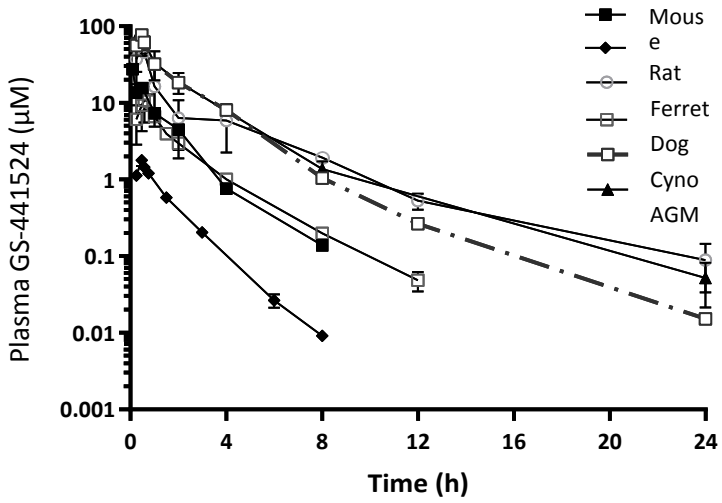
The apparent permeability of GS-441524 and GS-5245ODV was assessed in monolayers of Caco-2 cells (AD-611-2001). GS-441524 (10 µM) exhibited low forward permeability (P_{app} 0.12×10^{-6} cm/sec) and a high efflux ratio of 13. Results with GS-5245ODV also showed low forward permeability (P_{app} 0.12×10^{-6} cm/sec) in Caco-2 cells; however, after accounting for the conversion of parent drug to GS-441524, the permeability was high at 2.4×10^{-6} cm/sec with a lower efflux ratio of 2.9.

4.2.1.2. 3.2.1.2. Single-Dose In Vivo Studies

4.2.1.2.1. 3.2.1.2.1. Plasma Pharmacokinetics Following Intravenous Administration of GS-441524

GS-441524 plasma PK was determined following an IV slow bolus (mouse only) or a 30-minute infusion in Sprague-Dawley rat, ferret, beagle dog, cynomolgus monkey, and AGM (Studies AD-611-2002; AD-611-2003; AD-611-2004; AD-611-2005; AD-611-2006; AD-611-2007). The representative plasma concentration-time profiles are shown in Figure 1315 with accompanying PK parameters summarized in Table 2126. GS-441524 exhibited low-to-moderate CL, a moderate V_d, and a short estimated $t_{1/2}$ in plasma across species.

Figure ~~13~~15. Plasma Concentration Versus Time Profiles Across Species Following Intravenous Administration of GS-441524



AGM = African green monkey; cyno = cynomolgus monkey

Table ~~21~~26. Plasma Pharmacokinetic Parameters Across Species Following Intravenous Administration of GS-441524

	GS-441524 Plasma PK Parameters, Mean ± SD (N = 3)					
Species	**Mouse**	**Rat**	**Ferret**	**Dog**	**Cyno**	**AGM**
Dose (mg/kg)~~c~~	5[a]	1[a]	20[a]	1[b]	20[a]	20[a]
$t_{1/2}$ (h)	0.95 ± 0.13	1.03 ± 0.02	3.40 ± 0.63	1.82 ± 0.11	2.68 ± 0.07	2.63 ± 0.19
CL (L/h/kg)	0.67 ± 0.20	1.36 ± 0.02	0.86 ± 0.16	0.18 ± 0.01	0.56 ± 0.05	0.49 ± 0.12
V_{ss} (L/kg)	0.78 ± 0.22	1.66 ± 0.06	2.48 ± 0.65	0.33 ± 0.06	1.03 ± 0.09	0.89 ± 0.26
C_{max} (µM)	27.4 ± 13.8	1.78 ± 0.28	54.2 ± 10.0	13.5 ± 2.9	79.2 ± 6.7	87.3 ± 37.4
AUC_{inf} (µM•h)	28.2 ± 10.8	2.52 ± 0.04	81.5 ± 14.8	18.9 ± 1.4	123 ± 11	147 ± 36

AGM = African green monkey; cyno = cynomolgus monkey; EtOH = ethanol; HCl = hydrochloric acid; PEG = polyethylene glycol; PG = propylene glycol; PK = pharmacokinetic(s); SD = standard deviation

a Formulated in 5% EtOH, 45% PEG 300, 30% PG, and 20% water, pH ~2.

b Formulated in 5% EtOH, 30% PG, 45% PEG 400, and 20% water + 1 equivalent of HCl.

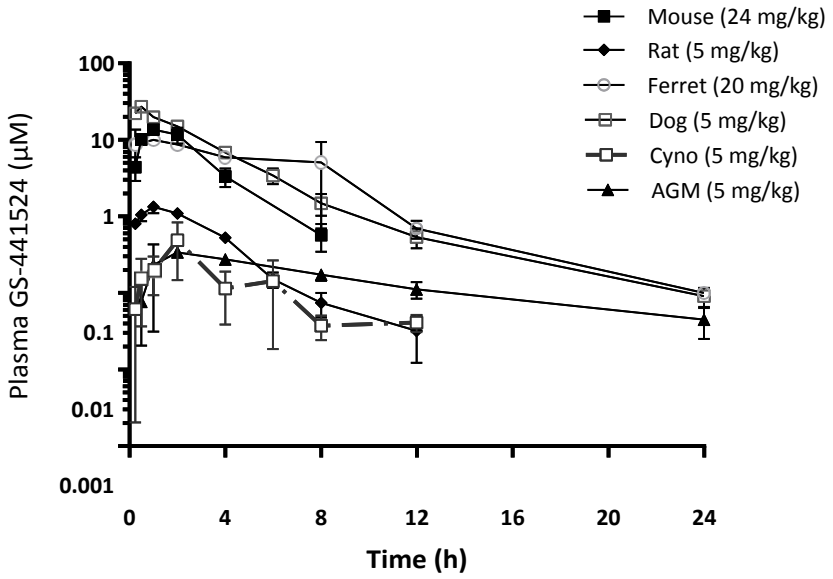
c Intravenous dose administered as either slow bolus injection in mouse or as a 30-minute infusion in all other species.

4.2.1.2.2. ~~3.2.1.2.2.~~Plasma Pharmacokinetics Following Oral Administration of GS-441524

GS-441524 oral PK was assessed in Balb/c mouse, Sprague-Dawley rat, ferret, beagle dog, cynomolgus monkey, and AGM (Studies AD-611-2002; AD-611-2003; AD-611-2004; AD-611-2005; AD-611-2006; AD-611-2007). The plasma PK parameters of GS-441524 for these studies are summarized in Table ~~22~~27 and representative concentration-time profiles are shown in Figure ~~14~~16. GS-441524 was rapidly absorbed and cleared from the systemic circulation with similar $t_{1/2}$ (1.4 to ~~4.2~~6.7 hours) as those observed following IV administration (0.7 to
3.4 hours). However, oral bioavailability across species was highly variable ranging from low in

monkeys (mean F < 10%) to moderate in rodents and high in ferret and dog (mean F = 87% to 89%).

Figure ~~14~~16. Plasma Concentration-Time Profiles of GS-441524 Across Species Following Oral Administration of GS-441524



AGM = African green monkey; cyno = cynomolgus monkey

Table ~~22~~27. Plasma Pharmacokinetic Parameters Across Species Following a Single Oral Administration of GS-441524

	GS-441524 Plasma PK Parameters, Mean ± SD (N = 3)					
Species	**Mouse**	**Rat**	**Ferret[a]**	**Dog**	**Cyno**	**AGM**
Dose (mg/kg)	24	5	20	5	5	5
$t_{1/2}$ (h)	1.39 ± 0.16	1.87 ± 0.27	3.35	4.18 ± 0.64	3.31 ± 0.65	6.65 ± 1.82
T_{max} (h)	0.88 ± 0.25	1 ± 0	0.63	0.50 ± 0	1.50 ± 0.87	1.67 ± 0.58
C_{max} (µM)	13.7 ± 1.5	1.34 ± 0.23	11.7	27.3 ± 3.5	0.54 ± 0.28	0.36 ± 0.05
AUC_{inf} (µM•h)	45.1 ± 5.4	4.98 ± 0.43	70.9	83.9 ± 14.3	1.86 ± 0.74	3.69 ± 0.96
F (%)	33.3 ± 4.0	39.6 ± 3.4	87.0	89 ± 15	5.4 ± 2.5	10.0 ± 2.1

AGM = African green monkey; cyno = cynomolgus monkey; PK = pharmacokinetic(s); SD = standard deviation
a N = 2 in ferret.

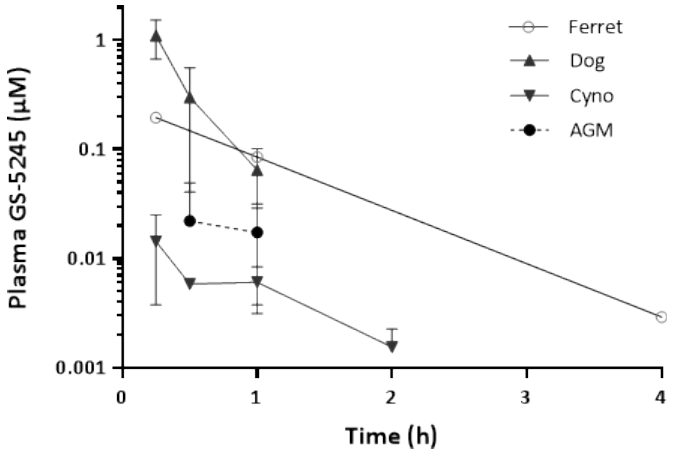
4.2.1.2.3. ~~3.2.1.2.3.~~Plasma Pharmacokinetics Following Oral Administration of ~~GS-5245~~ODV Across Species

The PK following oral administration of ~~GS-5245~~ODV as a solution was assessed in Balb/c mouse, Sprague-Dawley rat, ferret, beagle dog, cynomolgus monkey, and AGM (Studies AD-611-2008; AD-611-2009; AD-611-2010; AD-611-2011; AD-611-2012; AD-611-2013). Representative ~~GS-5245~~ODV and GS-441524 plasma concentration-time profiles are shown in Figure ~~15~~17 and Figure ~~16~~18, respectively, and plasma PK parameters are summarized in Table ~~23~~28. Intact ~~GS-5245~~ODV was below the limit of quantification in rodent species and was

transiently observed at very low levels in nonrodent species. Relative to oral administration of GS-441524, plasma exposures to
GS-441524 following GS-5245ODV administration were more consistent and higher within studies and

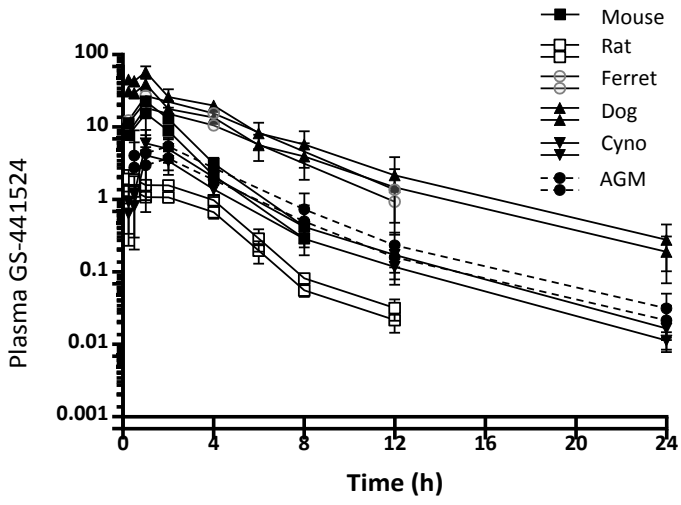
resulted in bioavailability improvements across species that showed low-to-moderate bioavailability with GS-441524. In dog and ferret, the high GS-441524 bioavailability was maintained with oral GS-5245ODV administration. Taken together, the plasma data following orally administered GS-5245ODV indicate extensive presystemic conversion to parent nucleoside and maintained or improved bioavailability of GS-441524 across species.

Figure 1517. Plasma Concentration-Time Profiles of GS-5245ODV Across Species Following Oral Administration of GS-5245[a]ODV[a]



AGM = African green monkey; cyno = cynomolgus monkeya ; GS-5245 = obeldesivir (ODV) a ODV plasma concentrations were below the limit of quantitation in mouse and rat.

Figure 1618. Plasma Concentration-Time Profiles of GS-441524 Across Species Following Oral Administration of GS-5245ODV



AGM = African green monkey; cyno = cynomolgus monkey; ODV = obeldesivir (GS-5245)

Table 2328. Plasma Pharmacokinetic Parameters of GS-5245ODV and GS-441524 Across Species Following a Single Oral Solution Administration of GS-5245ODV

Species		Mouse	Rat	Ferret	Dog	Cyno	AGM
Dose	(mg/kg)	30	6	30	14.4	11.7	14.4
	(mg-eq 524GS-441524/kg)	24	4.8	24	11.6	9.3	11.6
GS-5245ODV Plasma PK Parameters, Mean ± SD (N = 3)[a]							
$T_{1/2}$ (h)		NC	NC	0.62	0.20 ± 0.07	0.68 ± 0.09	0.52 ± 0.21
T_{max} (h)		NC	NC	0.25	0.25 ± 0	0.25 ± 0	0.67 ± 0.29
C_{max} (µM)		BLQ	BLQ	0.20	1.10 ± 0.43	0.1 ± 0.01	0.03 ± 0.02
AUC_{last} (µM•h)		BLQ	BLQ	0.26	0.40 ± 0.04	0.0 ± 0.01	0.1 ± 0.02
GS-441524 Plasma PK Parameters, Mean ± SD (N = 3)[a]							
$T_{1/2}$ (h)		1.12 ± 0.18	2.02 ± 0.33	2.50	3.62 ± 0.23	2.62 ± 0.55	3.19 ± 0.10
T_{max} (h)		1 ± 0	0.42 ± 0.14	1	1 ± 0	1.67 ± 0.58	1.50 ± 0.87
C_{max} (µM)		22.7 ± 4.9	2.10 ± 0.49	27.0	57.8 ± 11.3	7.57 ± 6.07	6.81 ± 3.08
AUC_{inf} (µM•h)		55.2 ± 2.4	7.67 ± 0.57	147 [b]	204 ± 45	21.8 ± 10.9	26.7 ± 12.2
F (%)		40.5 ± 1.8	63.4 ± 4.7	157	92.9 ± 20.4	37.6 ± 18.7	31.3 ± 14.3

AGM = African green monkey; BLQ = below the limit of quantitation; cyno = cynomolgus monkey; eq = equivalent; NC = not calculated; ODV = obeldesivir (GS-5245); PK = pharmacokinetic(s); SD = standard deviation

a Composite PK sampling for ferret; serial PK sampling for all other nonclinical species.

b $AUC_{0\text{-}12h}$ for ferret.

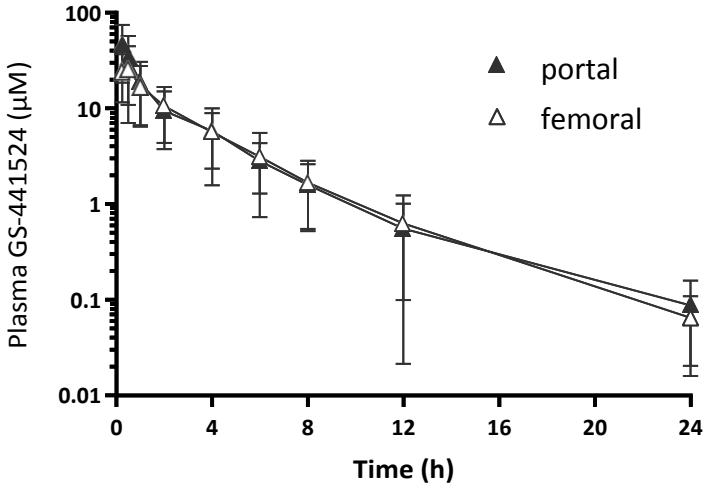
4.2.1.2.4. 3.2.1.2.4. Plasma Pharmacokinetics Following Oral Administration of GS-5245ODV in Portal Vein-Cannulated Beagle Dog and Cynomolgus Monkey

The PK following oral administration of GS-5245ODV was also assessed in portal vein-cannulated beagle dog and cynomolgus monkey (Studies AD-611-2016; AD-611-2017). Intact GS-5245ODV was transiently observed in the portal vein at low levels (AUC_{last}: 1.99 µM•h in dog, 0.153 µM•h in monkey) and at lower and similarly transient exposures in systemic circulation (AUC_{last}: 0.281 µM•h in dog, 0.005 µM•h in monkey). These exposures were consistent with the in vitro intestinal S9 instability of GS-5245ODV (Section 3.2.3.1.24.2.3.1.2). The plasma PK parameters of the major systemic metabolite, GS-441524, are summarized in Table 2429. Representative GS-441524 concentration-time profiles are shown for dog in Figure

1719 and for cynomolgus monkey in Figure 1820. The E_H (hepatic extraction) of GS-5245ODV was high at 84% and 96% in dog and monkey,

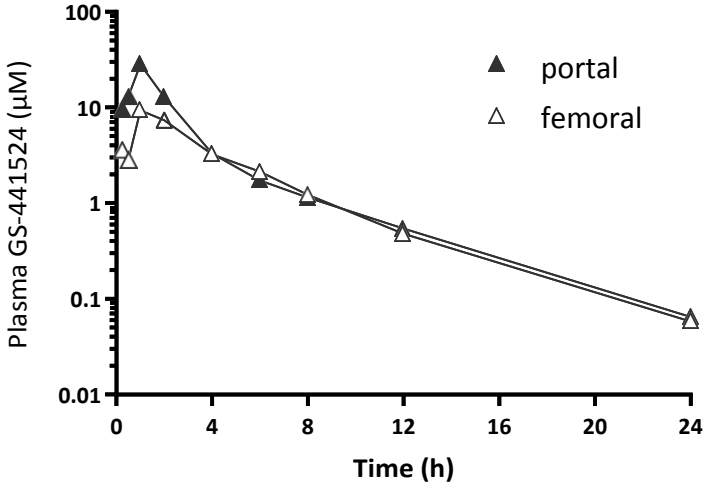
respectively; these values were consistent with in vitro predicted E_H (Section 3.2.3.1.24.2.3.1.2). The E_H for GS-441524 following oral administration of GS-5245ODV was variable in dog (8%) and monkey (41%).

Figure 1719. Plasma Concentration-Time Profiles of GS-441524 in Portal Vein-Cannulated Beagle Dog Following Oral Administration of GS-5245ODV



ODV = obeldesivir (GS-5245)

Figure 1820. Plasma Concentration-Time Profiles of GS-441524 in a Portal Vein-Cannulated Cynomolgus Monkey Following Oral Administration of GS-5245ODV



ODV = obeldesivir (GS-5245)

Table 2429. Plasma Pharmacokinetic Parameters in Portal Vein-Cannulated

Beagle Dog and Cynomolgus Monkey Following Oral Administration of ~~GS-5245~~ODV

Dose (mg/kg)	**14.4**		**14.4**	
Species	**Dog (N = 3; Mean ± SD)**		**Cynomolgus Monkey (N = 1)**	
Sampling route	**Portal**	**Systemic**	**Portal**	**Systemic**
~~GS-5245~~ODV Plasma PK Parameters				
C_{max} (µM)	5.05 ± 4.52	0.677 ± 0.598	0.193	0.013
AUC_{last} (µM•h)	1.99 ± 1.66	0.281 ± 0.230	0.153	0.005
E_H (%)	84		96	
GS-441524 Plasma PK Parameters				
C_{max} (µM)	46.8 ± 28.0	28.9 ± 15.2	28.3	9.38
AUC_{last} (µM•h)	79.7 ± 48.5	72.6 ± 42.4	65.8	38.8
E_H (%)	8.4		41	

E_H = hepatic extraction; ODV = obeldesivir (GS-5245); PK = pharmacokinetic(s); SD = standard deviation

~~3.2.1.2.5.Pharmacokinetics in In Vivo Efficacy Study Models~~

~~The efficacious dose levels following oral administration of GS-5245 in mouse and ferret, as well as for the tool prodrug, GS-621763 (also a prodrug of GS-441524; triester analog of GS-5245), and IV administered GS-441524 in AGM were determined in 3 SARS-CoV-2 challenge models (PC-611-2001; PC-611-2003; PC-554-2001; PC-554-2003). The GS-441524 plasma exposures (Table 25) at the efficacious dose levels were either determined or extrapolated from single-dose PK studies in uninfected animals.~~

~~Table 25. Measured or Projected GS-441524 Plasma Exposures in Mouse, Ferret, and African Green Monkey at Efficacious Doses~~

~~PK Parameter~~	~~Mouse~~	~~Ferret~~	~~African Green Monkey~~	
	~~GS-5245 PO 10 mg/kg, BID~~	~~GS-5245 PO 20 mg/kg, QD~~	~~Tool Prodrug[a] PO 120 mg/kg, QD~~	~~GS-441524 IV 20 mg/kg, QD~~
~~C_{max} (µM)~~	~~8~~	~~18~~	~~24~~	~~87~~
~~AUC_{0-24h} (µM•h)~~	~~36~~	~~98~~	~~125~~	~~147~~

~~BID = twice daily; IV = intravenous; PK = pharmacokinetic(s); PO = orally; QD = once daily~~

~~[a]Tool prodrug is an ester prodrug of GS-441524 that is different to GS-5245 but essentially performed the same in delivering GS-441524 into systemic circulation.~~

4.2.1.2.5. ~~3.2.1.2.6.~~Dose Escalation Pharmacokinetics

A summary of the oral formulation studies performed with ~~GS-5245~~ODV to support the GLP 14-day toxicology studies in rat and dog (Studies AD-611-2014; AD-611-2015), with accompanying GS-441524 plasma PK parameters is provided in Table ~~26~~30. When formulated in 0.5% methylcellulose and administered as a single dose to rats, dose-

proportional increases in GS-441524 plasma exposure (AUC_{0-24h}) were observed up to 1000 mg/kg. In the same formulation administered at the same 1000 mg/kg total dose to rats, exposures were 50% greater when administered twice as 500 mg/kg dosed approximately 12 hours apart, compared with a single administration. As shown in Table ~~23~~28, GS-441524 plasma exposures were significantly higher in dog compared with cynomolgus monkey following ~~GS-5245~~ODV oral administration, supporting toxicological evaluation in dogs. GS-441524 plasma exposures in dogs were highest when ~~GS-5245~~ODV was formulated in 0.5% methylcellulose and increased dose proportionally up to 300 mg/kg but showed evidence of saturation at the next higher dose (1000 mg/kg).

Table ~~26~~30. Plasma GS-441524 Pharmacokinetic Parameters in Rats and Dogs Following Oral Administration of ~~GS-5245~~ODV Using Different Formulations

Formulation	Dose (mg/kg)	C_{max} (µM)	AUC_{0-24h} (µM•h)
Rat			
0.5% Methylcellulose suspension (pH ~6-7)	300	164 ± 22[a]	542 ± 236[a]
	1000	548 ± 103	1760 ± 160
	500 × 2, BID[b]	372 ± 16	2610 ± 320
12% Captisol solution, pH 2	1000	315 ± 24	1520 ± 350
	500 × 2, BID[b]	187 ± 47	955 ± 154
Dog			
0.5% Methylcellulose suspension (pH ~6-7)	100	249 ± 98	1050 ± 240
	300	405 ± 123	2670 ± 960
	1000	252 ± 74	2070 ± 420
12% Captisol solution, pH 2	100	226 ± 49	893 ± 121
	300	326 ± 25	2010 ± 60
2.5% Cremophor RH40, 10% PEG 300, 87.5% water solution (pH 2-3)	100	124 ± 34	631 ± 96

BID = twice daily; ODV = obeldesivir (GS-5245); PEG = polyethylene glycol; SD = standard deviation
a Mean ± SD from 2 independent studies.
b The 2 BID doses were administered 12 hours apart.

4.2.1.3. ~~3.2.1.3.~~Repeat-Dose In Vivo Studies

Detailed results for all aspects of the GLP 14-day and 28-day repeat-dose toxicity studies in Wistar Han rat and beagle dog are presented in Section ~~3.3.2~~4.3.2 so they can be interpreted in the context of each study.

4.2.2. ~~3.2.2.~~Distribution

4.2.2.1. ~~3.2.2.1.~~In Vitro Plasma Protein Binding and Blood Distribution

The extent of ~~GS-5245~~ODV and GS-441524 binding to plasma proteins was determined in

nonclinical species and human (Studies AD-611-2020; AD-611-2028). GS-5245Obeldesivir showed high plasma protein-free fraction across all species tested (Table 2731); the free fraction in human was 64%. GS-441524 was virtually completely free in all species; the free fraction in human was 98%.

Table 2731. Protein Binding for GS-5245ODV and GS-441524 in Plasma From Different Species (Mean, n = 3)

Species	Free Fraction (%)	
	GS-5245 ODV	GS-441524
Balb/c mouse	67	103
Rat	56[a]	90[b]
Beagle dog	72	108
Cynomolgus monkey	76	99
Rhesus monkey	ND	85
Human	64	98

ND = not determined; ODV = obeldesivir (GS-5245)
a Sprague-Dawley. b —
b Wistar Han.

The distribution of GS-441524 between the cellular and soluble fractions of blood from rhesus monkey and human was assessed (AD-540-2007). GS-441524 (0.5 µM) showed slight association with the cellular fraction with a mean blood/plasma ratio of 1.36 and 1.19 in monkey and human, respectively.

4.2.3. 3.2.3.Metabolism

4.2.3.1. 3.2.3.1.In Vitro Metabolism

4.2.3.1.1. 3.2.3.1.1.Plasma Stability

The plasma stability of GS-5245ODV in plasma in nonclinical species and human, and of GS-441524 from Sprague-Dawley rat, beagle dog, cynomolgus monkey, and human was determined (Studies AD-611-2025; AD-611-2029). GS-5245ODV was unstable in mouse and rat plasma ($t_{1/2} \leq 3$ minutes), consistent with the presence of high esterase activity in plasma in many rodent species. GS-5245Obeldesivir was more stable in nonrodent species with $t_{1/2}$ ranging from 9 minutes in cynomolgus monkey to 417 minutes in dog. GS-441524 was stable with $t_{1/2}$ greater than 636 minutes in all species tested.

4.2.3.1.2. 3.2.3.1.2.Metabolic Stability

The metabolic stability of GS-5245ODV and GS-441524 was assessed in intestinal and hepatic S9 from nonclinical species and human (Study AD-611-2024). Across species, GS-5245ODV was moderately stable in intestinal extract ($t_{1/2}$ = 13.3-203 minutes) but was unstable in hepatic extract ($t_{1/2}$ < 4.3 minutes), with time-concentration profiles showing concomitant formation of GS-441524 with loss of GS-5245ODV in all species. GS-441524 was stable in both intestinal ($t_{1/2}$ > 789 minutes) and hepatic ($t_{1/2}$ > 592 minutes) extract. In

humans, upon oral administration of GS-5245ODV, it is anticipated that low levels of intact absorbed GS-5245ODV will also be cleaved efficiently to GS-441524 by hepatic enzymes.

4.2.3.2. 3.2.3.2.Enzymatic Metabolism

4.2.3.2.1. 3.2.3.2.1.Turnover of GS-441524 by Cytochrome P450

To assess the potential of GS-441524 metabolism to be affected by coadministration with cytochrome P450 enzyme (CYP) inducers, incubation with individual recombinant human CYP enzymes was performed and its rates of metabolism were quantified (Study AD-540-2018). The turnover rates of GS-441524 in all tested metabolizing enzymes were below the limits of quantification, as summarized in Table 2832.

Table 2832. Reaction Phenotyping of GS-441524 for Turnover by Cytochrome P450 Enzymes

	Metabolism Rate, min^{-1}								
CYP enzyme	1A1	1A2	2B6	2C8	2C9	2C19	2D6	3A4	3A5
GS-441524	< 0.09	< 0.02	< 0.02	< 0.05	< 0.09	< 0.02	< 0.05	< 0.09	< 0.09
Control substrate	18.1	3.7	0.41	1.7	23.9	7.5	14.7	19.1	13.1

CYP = cytochrome P450 enzyme Control substrates for individual enzymes are CYP1A1 (granisetron), CYP1A2 (tacrine), CYP2B6 (efavirenz), CYP2C8 (paclitaxel), CYP2C9 (diclofenac), CYP2C19 (omeprazole), CYP2D6 (dextromethorphan), and CYP3A4/5 (simvastatin).

4.2.3.2.2. 3.2.3.2.2.Intracellular

Metabolism 3.2.3.2.2.14.2.3.2.2.1. In

Vitro Metabolism

The intracellular loading and subsequent anabolism to phosphorylated metabolites was assessed in NHBE, HAE, lung adenocarcinoma (A549), and EpiAlveolar cells in vitro following continuous incubation of GS-441524 at 1 µM (AD-611-2030). In NHBE at 24 hours, GS-443902 (the pharmacologically active triphosphate metabolite) was formed. Levels of intracellular triphosphate were also observed in other respiratory cells including HAE, A549, and EpiAlveolar cells (Table 2933). In summary, GS-441524, the anticipated major circulating metabolite following oral administration of GS-5245ODV in human, would be able to load respiratory cells with GS-443902.

Table 2933. Average GS-443902 Concentrations Following Incubation With GS-441524 in Various Respiratory Cell Cultures

Cell Culture	Average GS-443902 Concentration (pmol/million cells)[a]
NHBE	0.91 ± 0.31
HAE	2.03 ± 0.51
A549	2.89 ± 0.83
EpiAlveolar	0.66 ± 0.05

HAE = human airway epithelial; NHBE = normal human bronchial epithelial

a Average GS-443902 concentration over 24 hours for NHBE, over 48 hours for all others.

4.2.3.2.2.2. 3.2.3.2.2.2.In Vivo Metabolism

4.2.3.2.2.2.1. 3.2.3.2.2.2.1.Lung GS-443902 Levels in Ferret and African Green Monkey Following Intravenous Administration of GS-441524

Formation of the GS-443902 in respiratory target tissue (lung) and PBMC was also confirmed in ferret and AGM following IV infusion administration of GS-441524 at 20 mg/kg (Study AD-611-2004; AD-611-2007). GS-441524 and its phosphorylated metabolites were observed in the lung.

4.2.4. 3.2.4.Excretion

4.2.4.1. 3.2.4.1.Studies in Bile Duct-Cannulated Animals

The excretion of GS-441524 following an IV 30-minute infusion was determined in beagle dog (Study AD-611-2018). The dose excreted by matrix (represented as a percentage of dose; 0 to 72 hours postdose) are summarized in Table 3034. Intact GS-441524 was predominantly eliminated by renal excretion in dog, accounting for 90% of the dose.

Table 3034. Dose Excreted by Matrix Following Intravenous Administration of GS-441524 to Bile Duct-Cannulated Beagle Dogs

	% Dose Excreted as Intact GS-441524
Dose (mg/kg)	5
Bile	1.6 ± 1.3
Urine	90.1 ± 9.8
Feces	0.36 ± 0.42

4.2.5. 3.2.5.Pharmacokinetic Drug Interactions

The systemic exposure of GS-5245ODV following oral administration of GS-5245ODV is anticipated to be low and transient. Therefore, the drug-drug interaction (DDI) potential of GS-5245ODV with intestinal transporters was assessed. The DDI potential for the parent nucleoside GS-441524 was comprehensively assessed in vitro for all relevant intestinal, hepatic, and renal transporters. The anticipated clinical C_{max} for GS-441524 is 16.8 µM (Section 3.2.6Table 1), with no impact expected by protein binding.

4.2.5.1. 3.2.5.1.Enzyme Inhibition

4.2.5.1.1. 3.2.5.1.1.Cytochrome P450 Inhibition

To test the potential to cause DDIs when GS-5245ODV is coadministered with CYP substrates, inhibition studies were conducted in vitro with GS-5245ODV and GS-441524 (AD-540-2013; AD-540-2014; AD-611-2027). GS-5245Obeldesivir was tested for reversible inhibition of CYP3A (midazolam as substrate) and showed undetectable inhibition at the highest tested concentration (25 µM). The parent nucleoside, GS-441524 exhibited weak (IC_{50} > 25 µM; Table

3135) or undetectable reversible inhibition. The greatest effect observed for GS-441524 was with CYP3A testosterone

6β-hydroxylase activity (30.2% inhibition at 25 µM). The potential for GS-441524 to be a mechanism-based inhibitor of the CYP enzymes (CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, and CYP3A) was also assessed and did not show time-dependent inhibition of the human CYPs. Overall, GS-5245ODV and GS-441524 are unlikely to cause CYP-mediated DDI at their anticipated clinical exposures.

Table 3135. Inhibition of Cytochrome P450 by GS-441524

Enzyme	Substrate	Calculated IC_{50} (µM)		Inhibition at 25 µM (%)
		Control[a]	GS-441524	GS-441524
CYP1A2	Phenacetin	0.12	> 25	15.5%
CYP2B6	Buproprion	0.45	> 25	0%
CYP2C8	Paclitaxel	0.99	> 25	1.4%
CYP2C9	Tolbutamide	0.54	> 25	0%
CYP2C19	S-Mephenytoin	12.7	> 25	0%
CYP2D6	Dextromethorphan	0.07	> 25	0%
CYP3A	Midazolam	0.04	> 25	7.5%
	Testosterone	0.07	> 25	30.2%

CYP = cytochrome P450 enzyme; IC_{50} = half-maximal inhibitory concentration; NADPH = nicotinamide adenine dinucleotide phosphate, reduced

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).

Test compounds were incubated with human liver microsomes and NADPH in the presence of probe substrates.

4.2.5.1.2. 3.2.5.1.2. Interactions With UGT Enzymes

The potential for GS-441524 to inhibit glucuronidation reactions catalyzed by 6 human uridine diphosphate glucuronosyltransferase (UGT) enzymes was assessed (AD-540-2015). Weak inhibition of only UGT1A9 was observed with GS-441524 (IC_{50} 85.5 µM). Phenotyping (AD-540-2019) also revealed no detectable formation of a direct glucuronide metabolite of GS-441524 by 7 recombinant human UGT enzymes. GS-441524 is unlikely to cause UGT-mediated DDI at its anticipated clinical exposure.

4.2.5.2. 3.2.5.2. Assessment of Induction Liability

The potential for GS-441524 to induce CYP enzymes (CYP1A2, CYP2B6, and CYP3A4) was assessed in human hepatocytes. Following incubation with GS-441524, both messenger RNA (mRNA) levels and CYP enzyme activities were quantified (AD-399-2027). GS-441524 showed no induction of either the mRNA or activity of all 3 CYP isoforms. Drug-drug interaction assessment using even the most sensitive donor in this assay predicts no liability for GS-441524.

4.2.5.3. 3.2.5.3. Interactions With Drug Transporters

4.2.5.3.1. 3.2.5.3.1. Interactions With Intestinal and Hepatic Transporters

GS-5245Obeldesivir was tested for interaction with intestinal transporters and the parent nucleoside GS-441524 was tested for interaction with intestinal and hepatic transporters (Studies AD-399-2035; AD-540-2009; AD-540-2026; AD-611-2026); the results are summarized in Table 3236. GS-5245Obeldesivir showed minimal to no inhibition of efflux transporters P-glycoprotein
(P-gp) (no inhibition at 833 µM) and breast cancer resistance protein (BCRP) (34% at 889 µM). GS-441524 also showed weak to no inhibition of efflux transporters P-gp (50% at 178 µM) and BCRP (no inhibition at 200 µM). GS-441524 (1 µM) was not a substrate of organic anion transporting polypeptide (OATP)1B1 or OATP1B3. GS-441524 was a weak inhibitor at the highest tested concentration for OATP1B1 (IC_{50} of 241 µM) or OATP1B3 (35% at 241 µM). GS-441524
(1 µM) was not a substrate of organic anion transporting polypeptide (OATP)1B1 or OATP1B3. Inhibitors or inducers of P-gp and BCRP are unlikely to elicit a clinical DDI because GS-5245ODV is rapidly metabolized resulting in low and transient exposure. Overall, GS-5245ODV and GS-441524 have low victim and perpetrator DDI potential mediated by these efflux or hepatic uptake transporters.

Table 3236. Interaction of GS-5245ODV and GS-441524 With Hepatic and Intestinal Transporters

	IC_{50} (µM) (% Inhibition at the Highest Tested Concentration)	
	GS-5245[a]ODV[a]	GS-441524[b]
OATP1B1	NA	241 µM
OATP1B3	NA	> 241 (35% at 241 µM)
P-gp	> 833 (no inhibition at 833 µM)	178 µM
BCRP	> 889 (34% at 889 µM)	> 200 (no inhibition at 200 µM)

BCRP = breast cancer resistance protein; IC_{50} = half-maximal inhibitory concentration; NA = not applicable; OATP = organic anion transporting polypeptide; ODV = obeldesivir (GS-5245); P-gp = P-glycoprotein
a IC_{50} values determined using the model substrates digoxin (P-gp) and prazosin (BCRP).
b IC_{50} values determined using the model substrates pravastatin (OATP1B1/1B3), digoxin (P-gp), and estrone 3-sulfate (BCRP).

4.2.5.3.2. 3.2.5.3.2. Interactions With Renal Transporters

Studies were also conducted to assess whether GS-441524 is a substrate for (AD-540-2025) or inhibitor of (Study AD-540-2011) human renal transporters OAT1, OAT3, OCT2, multidrug and toxin extrusion (MATE)1, and MATE2-K in cell-based assays. GS-441524 was not a substrate of OAT1, OAT3, OCT2, MATE1, and MATE2-K transporters. GS-441524 also showed no inhibition of MATE1, MATE2-K, or OCT2 at the highest tested concentration; minimal inhibition was observed (21%-27% at the highest tested concentration) for OAT1, OAT3, and OCT1. GS-441524 was also assessed for interaction with sodium-taurocholate cotransporter protein (NTCP), bile salt export pump, multidrug resistance-associated protein (MRP)2, MRP3, and MRP4 efflux transporters (Studies AD-399-2029; AD-399-2035) and was found to only minimally inhibit NTCP (24% at 100 µM, the highest tested concentration). Drug-drug interactions mediated by these transporters appear to be unlikely.

Table 3337. Interaction of GS-441524 With Renal Transporters

	IC_{50} (µM) (% Inhibition at the Highest Tested Concentration)
OAT1	> 136 (21%)
OAT3	> 186 (27%)
OCT1	> 136 (26%)
OCT2	> 186
MATE1	> 162
MATE2-K	> 162
NTCP	> 100 (24%)
BSEP	> 100
MRP2	> 100
MRP3	> 100
MRP4	> 100

BSEP = bile salt export pump; DHEAS = dehydroepiandrosterone sulfate; $E_2$17βG = estradiol-17b-glucuronide; IC_{50} = half-maximal inhibitory concentration; MATE = multidrug and toxin extrusion; MRP = multidrug resistance-associated protein; MTX = methotrexate; NTCP = sodium-taurocholate cotransporting polypeptide; OAT = organic anion transporter; OCT = organic cation transporter IC_{50} values determined using the model substrates taurocholate (BSEP, NTCP), $E_2$17βG (MRP2/3), DHEAS (MRP4), metformin (MATE1/2-K, OCT1/2), tenofovir (OAT1), and MTX (OAT3).

4.2.5.3.3. ~~3.2.5.3.3.~~Interactions With Nucleoside Transporters

GS-441524 was tested as a substrate for human uptake nucleoside transporters (AD-611-2032). GS-441524 (3 µM) showed uptake in specific equilibrative nucleoside transporter (ENT1 and ENT2)- and concentrative nucleoside transporter (CNT3)-transfected cells. GS-441524 was a substrate for ENT1, ENT2, and CNT3, but not a substrate of CNT1, CNT2, or ENT4 (less than 2-fold difference in uptake compared with control).

~~3.2.6.Predicted Pharmacokinetics in Human~~

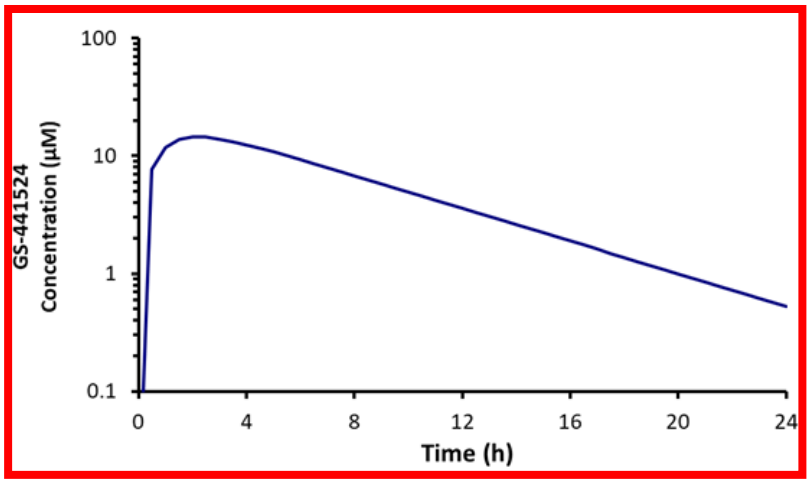
~~The efficacy of GS-441524 delivered directly by IV administration or orally via GS-5245 and the related ester prodrug GS-621763 in mouse, ferret, and AGM models are described in Section 3.1.1.2. The plasma exposure levels of GS-441524 were determined in noninfected animals in the same species as these 3 nonclinical models. The efficacious GS-441524 exposures in the mouse, ferret, and AGM models of SARS-CoV-2 infection are summarized in Table 25.~~
~~An efficacious plasma AUC exposure of 125 µM•h, which is sufficient for efficacy in all 3 models, was set as the target efficacious plasma AUC exposure for human.~~

~~The PK parameters used to predict the human GS-441524 PK following GS-5245 administration is shown in Table 34. The human CL was predicted by body surface area-based allometric scaling; scaled values from the 2 monkey species or an average of scaled values from rat, dog, and monkey (Table 21) yielded similar CL values (approximately 0.16 L/h/kg). The V_{ss}—the average of the 2 monkey species or the average of rat, dog, and monkey—yielded the same V_{ss} values (~1 L/kg). The average GS-441524 oral bioavailability following a GS-5245 solution formulation administration in rat, dog, and monkey was 63%. The k_a was rapid across all species and a value of 1 $hour^{-1}$ was assumed. Using these PK parameters, the GS-5245 orally delivered dose is predicted to achieve the target plasma exposure (AUC) of 125 µM•h and a C_{max} of 16.8 µM. The predicted human PK profile is shown in Figure 19.~~

Table 34. Predicted GS-441524 Human Pharmacokinetics Following Oral Administration of GS-5245

Predicted Parameter	GS-441524 Parameters
GS-5245 oral dose (mg)	710
Oral bioavailability (%)	63
CL (L/h/kg)	0.16
V_{ss} (L/kg)	1
k_a (h^{-1})	1
C_{max}	16.8 µM (4890 ng/mL)
$AUC_{0\text{-}24h}$	125 µM•h (36,400 ng•h/mL)

Figure 19. Predicted Human Plasma Profile Following a Single Oral Administration of GS-5245 at 710 mg



4.2.6. 3.2.7. Pharmacokinetic Conclusions

GS-5245Obeldesivir is a mono-5'-isobutyryl ester prodrug that is extensively hydrolyzed both presystemically and systemically to GS-441524. GS-5245Obeldesivir is being developed with the intent to deliver consistent systemic exposures of GS-441524 following oral administration that are consistent with SARS-CoV-2 efficacy in nonclinical models.

The plasma PK profiles following oral administration of GS-5245ODV across species consistently show very low systemic levels of intact GS-5245ODV, ranging from undetectable in rodents to transiently low micromolar levels in dog, that are orders of magnitude lower than GS-441524, confirming extensive metabolism during absorption and first-pass metabolism. Compared with GS-441524, GS-5245ODV has improved intestinal absorption and delivers a higher amount of GS-441524 with less variability into systemic circulation. Plasma exposure of GS-441524 after oral administration was either maintained or improved by the ester prodrug across all species tested.

GS-5245Obeldesivir is metabolically unstable, while in comparison, GS-441524 is stable across species including human in all biological matrices tested. In vitro, GS-441524 demonstrated ability to penetrate cells and generate GS-443902 in a variety of lung cell types, while in vivo, GS-443902 levels were measured in gross lung tissue of ferrets and AGM following IV administration of GS-441524.

Following IV administration of GS-441524 to dogs, nearly 100% of the administered dose was eliminated by renal excretion as intact GS-441524. Both GS-5245ODV and GS-441524 have low potential for DDIs with either the human CYP enzymes or transporters.

4.3. 3.3.Toxicology

To support initial clinical trials, the nonclinical safety profile of GS-5245ODV has been characterized in 14-day repeat-dose toxicity studies in rats and dogs, and in in vitro and in vivo genotoxicity studies, in developmental and reproductive toxicity studies, in a photosafety study, and in an impurity qualification study.
Safety pharmacology studies in rats and dogs are described in Section 3.1.3. In addition, preliminary findings from embryo-fetal development studies in pregnant rats and rabbits are described4.1.3. Consistent with its intended route of administration in humans, repeat-dose toxicity and reproductive toxicity studies were conducted by the oral (gavage) route of administration. The vehicle used in all in vivo studies was 0.5% methylcellulose in deionized water. Study designs and parameters evaluated were consistent with accepted principles and practices as outlined in ICH, Organization for Economic Co-Operation and Development (OECD), and national regulations (US FDA and European Community Directives). All pivotal studies were conducted in accordance with US FDA or OECD GLP regulations. As expected, the plasma PK profiles following oral administration of GS-5245ODV across species consistently show very low systemic levels of intact GS-5245ODV, ranging from undetectable to nanomolar levels in rodents and rabbits, to transiently low micromolar levels in dogs that are orders of magnitude lower than GS-441524. Dose-response relationships and margins of exposure are therefore based on the toxicokinetics (TK) of the parent nucleoside, GS-441524.

4.3.1. 3.3.1.Single-Dose Toxicity Studies

Single-dose toxicity studies with GS-5245ODV have not been conducted.

4.3.2. 3.3.2.Repeat-Dose Toxicity Studies

4.3.2.1. 3.3.2.1.Rat

4.3.2.1.1. 3.3.2.1.1.A 14-Day Oral Gavage Toxicity and Toxicokinetic Study With GS-5245ODV in Wistar Han Rats With a Bone Marrow Micronucleus Assay

The objective of this study was to evaluate the potential toxicity and determine the TK of GS-5245ODV and GS-441524 (parent nucleoside) after administration of GS-5245ODV once or twice daily by oral gavage to Wistar Han rats for 14 days (Study TX-611-2001). In addition, bone marrow micronucleus, respiratory, and CNS evaluations were conducted.

Four groups of Crl:WI(Han) Wistar Han rats (10 animals/sex/group in the toxicity phase; 3 to 12 animals/sex/group in the TK phase) were administered vehicle or GS-5245ODV at 200, 500, or 1000 mg/kg/day for up to 14 days. Vehicle and 1000 mg/kg/day groups were dosed

twice daily approximately 12 hours apart. Due to GS-5245ODV-related toxicity and unscheduled deaths, surviving females at 1000 mg/kg/day were euthanized on Day 8. In addition, 6 animals/sex were administered 60 mg/kg cyclophosphamide once via oral gavage on Day 14 as a positive control for micronucleus assessment.

GS-5245Obeldesivir administration resulted in mortality of 4 toxicity phase females at 1000 mg/kg/day (Day 4 to Day 7), 1 toxicity phase female at 500 mg/kg/day (Day 15), and euthanasia of all surviving toxicity phase females administered 1000 mg/kg/day by Day 8 following signs of overt toxicity. In 4 of the toxicity phase females at ≥ 500 mg/kg/day that were found dead, the cause of mortality was attributed to GS-5245ODV-related renal findings of crystal formation in the renal papilla associated with tubular degeneration and regeneration and/or mixed cell inflammation in renal pelvis and urinary bladder, and ureter crystals and dilatation. In addition, 1 toxicity phase female in the early termination group (1000 mg/kg/day, Day 8) had similar kidney findings. These renal microscopic findings generally correlated with macroscopic findings of renal enlargement and discoloration. The cause of death for 1 toxicity phase female at 1000 mg/kg/day was considered accidental due to gavage error. Three TK phase females at 500 mg/kg/day and 5 TK phase females at 1000 mg/kg/day were found dead or euthanized early; all these deaths were considered related to administration of GS-5245ODV with the exception of 1 animal at 500 mg/kg/day and 1 animal at 1000 mg/kg/day that had esophageal perforations (gavage error).

All other animals survived to the scheduled necropsy. There were no GS-5245ODV-related ophthalmic or neurobehavioral effects, or effects on urinalysis. A slight increase in overall ventilation (minute volume) was noted from 0 to 2 hours postdose on Day 1 at 200 and 500 mg/kg/day. GS-5245Obeldesivir was negative for clastogenic activity and/or disruption of the mitotic apparatus in the micronucleus assay.

GS-5245Obeldesivir-related clinical observations noted in the females at 500 mg/kg/day included abnormal breathing sounds, erected fur, red fur staining on forepaws and lower jaw, thin body, hunched posture, and cold to touch. Clinical observations noted in the males and females at 1000 mg/kg/day included labored breathing (females only), abnormal breathing sounds, erected fur, red fur staining on forepaws and lower jaw (males only), thin body, cold to touch, slight salivation (males only), and wet fur (mouth and muzzle; males only).

GS-5245Obeldesivir-related decreases in body weights and/or body weight gains were noted in the males and females at 500 and 1000 mg/kg/day. These differences correlated with lower food consumption and were considered adverse at 1000 mg/kg/day.

In the 1000 mg/kg/day females terminated early at Day 8, clinical pathology changes consisted of mildly increased white blood cell (WBC) count due to mildly increased neutrophil, lymphocyte, and monocyte counts; minimally prolonged activated partial thromboplastin time (aPTT); minimally increased alkaline phosphatase (ALP); and minimally decreased glucose and cholesterol concentrations. The changes in leukocytes were consistent with inflammation with the prolonged aPTT also attributed to a secondary effect of inflammation. The decreased glucose and cholesterol were most likely associated with decreased food consumption.

In animals that survived to the terminal euthanasia, several changes in clinical pathology parameters were noted. GS-5245Obeldesivir-related minimally increased WBC count due to minimally increased neutrophil and monocyte counts were observed in males at ≥ 200 mg/kg/day with increased monocytes also noted in the 500 mg/kg/day group females; these

changes were consistent with inflammation. In males at 1000 mg/kg/day, there was minimally increased red blood cell (RBC) count, hemoglobin, and hematocrit likely due to hemoconcentration, and moderately decreased reticulocyte count. One male and 2 females at 500 mg/kg/day were also noted with ~~GS-5245~~ODV-related minimally to mildly increased RBC count, hemoglobin, and hematocrit, and moderately to markedly decreased reticulocyte counts. The decrease in reticulocytes correlated with the microscopic finding of decreased hematopoietic cells in the sternal bone marrow and was attributed to a stress response and decreased food consumption. ~~GS-5245~~ Obeldesivir-related clinical chemistry group effects at Day 15 were limited to mildly decreased triglycerides concentration in males at ≥ 200 mg/kg/day.

One female at 500 mg/kg/day and 1 female at 1000 mg/kg/day had changes in clinical chemistry values consistent with acute kidney injury (moderately to markedly increased urea nitrogen, creatinine, phosphorus, and with increased potassium also noted in the 500 mg/kg/day female); these changes correlated with the microscopic findings in the kidney of tubular degeneration, inflammation, and/or renal papillary crystals. These changes were considered ~~GS-5245~~ODV related and adverse.

There were no ~~GS-5245~~ODV-related effects on coagulation in animals that survived to the terminal euthanasia and no ~~GS-5245~~ODV-related changes in urinalysis parameters were observed at any dose level or time point.

At the terminal euthanasia (Day 15), ~~GS-5245~~ ODV-related primary findings were observed in kidneys of a single female at 500 mg/kg/day. Renal changes consisted of crystal formation in the renal papilla and cortex associated with tubular degeneration and regeneration, fibrosis and inflammation in renal pelvis, and ureter dilation. Degeneration and regeneration in the kidneys correlated with macroscopic findings of renal enlargement and discoloration, and clinical chemistry values consistent with acute kidney injury.

~~GS-5245~~The chemical composition of the renal deposits in ODV-treated rats (500 and 1000 mg/kg/day) were identified as parent nucleoside, GS-441524. Concentrations of GS-441524 were above the upper limit of quantitation but extrapolated to be > 1 mg/g tissue. Obeldesivir-related secondary (stress-related) findings in males (1000 mg/kg/day) or females (≥ 500 mg/kg/day) were noted in the thymus (lower mean weights, decreased lymphoid cellularity), adrenal gland (higher mean weights, hypertrophy), spleen (lower mean weights, decreased cellularity of white pulp), mesenteric lymph node (decreased lymphoid cellularity), sternal bone marrow (decreased hematopoietic cells), prostate/seminal vesicle (lower mean weights, seminal vesicle secretory depletion), cecum and/or rectum (crypt degeneration and/or regeneration), and/or ovary (increased atretic follicles). Microscopic findings in females at ≥ 500 mg/kg/day and in males at 1000 mg/kg/day were considered adverse. There were no ~~GS-5245~~ODV-related changes at 200 mg/kg/day (both sexes) or 500 mg/kg/day (males only).

Exposure to ~~GS-5245~~ODV as assessed by $AUC_{0\text{-}24h}$ was generally low and highly variable (Table ~~35~~38), limiting interpretation of ~~GS-5245~~ODV TK parameters. Overall, sex-based differences were generally less than 2-fold in ~~GS-5245~~ODV C_{max} and $AUC_{0\text{-}24h}$ values with no discernible trend. The $AUC_{0\text{-}24h}$ metabolite-to-parent ratios indicated that ~~GS-5245~~ODV was extensively converted to GS-441524 following oral gavage administration of ~~GS-5245~~ODV. Exposure to GS-441524 increased with the increase in ~~GS-5245~~ODV dose level from 200 to 1000 mg/kg/day for males and females on Day 1 (Table ~~36~~39). Exposure also increased from

200 to 1000 mg/kg/day on Day 14 in males and from 200 to 500 mg/kg/day in females on Day 14, and to 1000 mg/kg/day on Day 8. The increases in C_{max} and AUC_{0-24h} were approximately dose proportional for males and females. Sex-based differences were less than 2-fold in GS-441524 C_{max} and AUC_{0-24h} values. There was evidence of possible accumulation of GS-441524 at ~~GS-5245~~ODV doses of ≥ 500 mg/kg/day.

Table ~~35~~38. Mean Toxicokinetic Parameters for ~~GS-5245~~ODV in Rats Administered ~~GS-5245~~ODV for 14 Days

Dose (mg/kg/~~dose~~day)	Sex	AUC_{0-24h} (ng•h/mL)		C_{max} (ng/mL)	
		Day 1	Day 14	Day 1	Day 14
200	Male	12.4	57.5	14.7	16.7
	Female	7.49	39.8	6.83	9.74
	Combined sex	10.5	63.9	9.44	11.3
500	Male	21.4	57.8	8.23	11.4
	Female	41.8	50.7	11.1	10.8
	Combined sex	23.5	55.1	7.50	11.2
1000	Male	32.8	561	9.12	75.7
	Female	70.8	124[a]	8.38	34.7[a]
	Combined sex	59.7	—	7.11	—

ODV = obeldesivir (GS-5245)

a Samples were collected from 1000 mg/kg/day females on Day 8 following the second dose. AUC_{0-24h} was calculated from these values ($AUC_{12-24h} \times 2$).

Table ~~36~~39. Mean Toxicokinetic Parameters for GS-441524 in Rats Administered ~~GS-5245~~ODV for 14 Days

Dose (mg/kg/~~dose~~day)	Sex	AUC_{0-24h} (ng•h/mL)		C_{max} (ng/mL)	
		Day 1	Day 14	Day 1	Day 14
200	Male	84,000	77,900	37,900	28,000
	Female	88,200	129,000	40,800	32,400
	Combined sex	86,300	104,000	39,400	30,200
500	Male	157,000	203,000	51,200	41,400
	Female	207,000	301,000	67,900	64,100
	Combined sex	182,000	243,000	59,500	48,800
1000	Male	293,000	539,000	63,600	75,100
	Female	319,000	628,000[a]	56,100	68,700[a]
	Combined sex	306,000	—	56,400	—

ODV = obeldesivir (GS-5245)

a Samples were collected from 1000 mg/kg/day females on Day 8 following the second dose. AUC_{0-24h} was calculated from these values ($AUC_{12-24h} \times 2$).

In conclusion, administration of ~~GS-5245~~ODV by once- or twice-daily oral gavage to

Crl:WI(Han) Wistar Han rats at dose levels of 200, 500, and 1000 mg/kg/day for 14 consecutive days resulted in lethality at 500 and 1000 mg/kg/day in females. GS-5245Obeldesivir-related lower body weights noted in males at 1000 mg/kg/day were considered adverse. In addition, GS-5245ODV-related adverse findings were observed in kidneys of a single 500 mg/kg/day female which included crystal formation in the renal papilla and cortex with tubular degeneration and regeneration, fibrosis, and inflammation associated with crystals (identified as GS-441524) and ureter dilatation.
Degeneration and regeneration in the kidney correlated with adverse macroscopic findings of renal enlargement and discoloration.
GS-5245 Obeldesivir-related secondary (stress-related) findings in males (1000 mg/kg/day) or females (≥ 500 mg/kg/day) were noted in the thymus, adrenal gland, spleen, mesenteric lymph node, sternal bone marrow, prostate/seminal vesicle, cecum and/or rectum, and ovary. Based on these results, the no observed adverse effect level (NOAEL) was considered to be 500 mg/kg/day in males and 200 mg/kg/day in females. These doses corresponded to mean GS-441524 $AUC_{0\text{-}24h}$ and C_{max} values of 203,000 ng•h/mL and 41,400 ng/mL, respectively, in males at 500 mg/kg/day, and $AUC_{0\text{-}24h}$ and C_{max} values of 129,000 ng•h/mL and 32,400 ng/mL, respectively, in females at 200 mg/kg/day on Day 14.

4.3.2.1.2. A 4-Week Oral Gavage Toxicity and Toxicokinetic Study With ODV in Wistar Han Rats With a 4-Week Recovery Period

The objectives of this study were to determine the potential toxicity and determine the TK of ODV and GS-441524 when ODV was administered once daily by oral gavage to Wistar Han rats for 28 days and to evaluate the potential reversibility of any findings following a 28-day recovery period (Study TX-611-2015). Four groups of rats (10 animals/sex/group in the toxicity phase; 3 to 6 animals/sex/group in the TK phase) were administered ODV at 0, 50 (females, only), 100, 200 or 500 (males, only) mg/kg/day for up to 4 weeks. Five additional animals/sex in vehicle and high dose male and female dose groups were retained for an additional 4 weeks to assess recovery from any observed effects.

All animals survived to the scheduled euthanasia. There were no ODV-related clinical observations or effects on body weight, body weight gains, food consumption, coagulation, or urinalysis. There were no ODV-related ophthalmic, macroscopic, or microscopic findings.

ODV-related effects on hematology parameters at the terminal necropsy (Day 29) included increased WBC and monocytes in males at 500 mg/kg/day and females at 200 mg/kg/day, and increased neutrophils in males at 500 mg/kg/day. At the recovery necropsy (Day 57), the increase in neutrophils was partially reversed in males at 500 mg/kg/day, and WBC and monocyte changes were completely reversed.

ODV-related effects on clinical chemistry parameters included decreased glucose in males at ≥ 200 mg/kg/day and females at 200 mg/kg/day on Day 21, and in males at 500 mg/kg/day and females at 200 mg/kg/day on Day 29. On Day 57, all clinical chemistry changes were completely reversed.

ODV-related effects on organ weights on Day 29 included higher adrenal gland weights in males at 500 mg/kg/day. These changes lacked any correlative macroscopic or microscopic findings and were not observed at the end of the recovery period.

Exposure to ODV was generally low and highly variable. C_{max} and AUC_{0-24h} metabolite to parent ratios indicated that ODV was rapidly and extensively converted to GS-441524. Exposure to GS-441524 increased with the increase in ODV dose level from 100 to 500 mg/kg/day for males and from 50 to 200 mg/kg/day for females on Days 1 and 28 (Table 40). The increase in C_{max} and AUC_{0-24h} values were approximately dose-proportional from 100 to 500 mg/kg/day for males and from 50 to 200 mg/kg/day for females. Sex-based differences were less than 2-fold in GS-441524 C_{max} and AUC_{0-24h} values at 100 and 200 mg/kg/day. No notable accumulation of GS-441524 was observed after multiple doses of ODV.

Table 40. Mean Toxicokinetic Parameters for GS-441524 in Rats Administered ODV for 4 Weeks

	AUC_{0-24h} (ng•h/mL)		C_{max} (ng/mL)	
Dose (mg/kg/day)	**Day 1**	**Day 28**	**Day 1**	**Day 28**
Males				
100	45,600	50,800	14,300	19,800
200	97,700	125,00	28,400	31,500
500	183,000	242,000	40,800	46,600
Females				
50	21,900	29,900	7900	16,700
100	40,700	56,100	14,900	23,200
200	75,800	128,000	29,500	43,100

ODV = obeldesivir (GS-5245)

In conclusion, administration of ODV by once daily oral gavage to rats at dose levels of 100, 200, and 500 mg/kg/day to males and 50, 100, and 200 mg/kg/day to females for 28 days was well tolerated at all doses with no mortality or adverse findings. Based on these results, the NOAELs were 500 mg/kg/day for males and 200 mg/kg/day for females. These doses corresponded to GS-441524 mean AUC_{0-24h} values of 242,000 and 128,000 ng•h/mL and mean C_{max} values of 46,600 and 43,100 ng/mL for males and females, respectively, on Day 28.

4.3.2.2. ~~3.3.2.2.~~Dog

4.3.2.2.1. ~~3.3.2.2.1.~~A 14-Day Oral Gavage Toxicity and Toxicokinetic Study ~~With GS-5245~~with ODV in Beagle Dogs

The objective of this study was to determine the potential toxicity and determine the TK of ~~GS-5245~~ODV and GS-441524 ~~(parent nucleoside)~~ after administration of ~~GS-5245~~ODV by oral gavage to beagle dogs once daily for 14 days (TX-611-2002). Beagle dogs (4 animals/sex/group) were administered vehicle or ~~GS-5245~~ODV at 30, 100, or 300 mg/kg/day via oral gavage once daily for up to 14 days. Due to clinical condition, several 300 mg/kg/day animals were given dose holidays and all surviving 300 mg/kg/day animals were euthanized early on Day 7/6 (males/females) due to evidence of overt toxicity.

One female administered 300 mg/kg/day was euthanized for humane reasons on Day 4. On Day 3, clinical signs included liquid feces, decreased activity, hunched posture, intermittent partial eye closure, signs of dehydration (mild delay in skin turgor and tacky mucous

membranes), and moderate reddened sclera in both eyes. During examination by the veterinary staff on Day 4, observations included mild delay in skin turgor, tachycardia at 160 beats per minute, increased respiratory rate and effort, moderately reddened sclera in both eyes, and standing only with assistance. Blood for hematology and clinical chemistry analysis was collected prior to euthanasia and compared with baseline values; changes in hematology values included increased neutrophil and decreased lymphocyte counts, minimally increased RBC count, hemoglobin and hematocrit with increased reticulocyte count, and mildly decreased platelet count. Coagulation changes were characterized by mildly prolonged prothrombin time and aPTT. Clinical chemistry changes consisted of increases in ALP activity, total and direct bilirubin, urea nitrogen, creatinine, total protein, albumin, globulins, calcium, phosphorus, and potassium, and decreases in glucose, triglycerides, sodium, and chloride. The majority of the clinical pathology changes in this female were consistent with acute kidney injury, hemoconcentration/dehydration, inflammation and/or stress, and electrolyte loss, and correlated with the microscopic findings in the kidney (crystal formation in the renal papilla and tubular necrosis) and gastrointestinal (GI) tract (neutrophilic inflammation), and the clinical observations of delayed skin turgor, tachycardia, and tacky mucous membranes.

All animals administered vehicle or 30 or 100 mg/kg/day GS-5245ODV survived to the scheduled necropsy. There were no GS-5245ODV-related effects on ECG, urinalysis, pupillary light response, or intraocular pressure, and there were no GS-5245ODV-related ophthalmic findings in these animals.

GS-5245Obeldesivir-related clinical observations of liquid feces (moderate); food partly digested (slight to severe); slight-to-moderate dry, foamy, or liquid material in the cage (yellow, brown, or white); and/or decreased activity were noted at 300 mg/kg/day. Additionally, two 300 mg/kg/day group males were noted with corneal cloudiness on Day 5 during veterinary examinations.

GS-5245Obeldesivir-related effects on overall body weight, body weight gains, and food consumption were noted at 100 and 300 mg/kg/day. GS-5245Obeldesivir-related lower body weight was noted in the 300 mg/kg/day males and females on Day 5 (−12.83% and −16.55% compared with vehicle, respectively). GS-5245Obeldesivir-related lower body weight was noted in the 100 mg/kg/day males and females on Day 14 (−8.75% and −13.75% compared with vehicle, respectively) with body weight gains at 100 mg/kg/day typically lower throughout the study. The effect on overall body weight and body weight gains correlated to overall lower food consumption in these groups.
Food consumption was decreased throughout the study in the 100 mg/kg/day group.

GS-5245Obeldesivir-related changes in clinical pathology values were observed at ≥ 100 mg/kg/day. In 300 mg/kg/day animals euthanized on Days 6/7, hematology effects included mildly decreased lymphocyte count in females; mildly increased RBC count, hemoglobin, and hematocrit in males and females; and mildly to moderately decreased platelet count in males and females. Minimally prolonged aPTT was noted in both sexes. Minimally increased ALP activity and decreased cholesterol and triglycerides were observed in females with mildly decreased chloride concentration evident in both sexes. The increases in erythrocyte parameters were consistent with hemoconcentration and correlated with clinical observations of dehydration. Two 300 mg/kg/day females had GS-5245ODV-related clinical pathology changes consistent with acute kidney injury (moderately to markedly increased urea nitrogen, creatinine, and phosphorus, and minimally to mildly increased neutrophil and monocyte counts [1 female

only]), which correlated with the kidney microscopic findings of crystal formation in the renal papilla, tubular necrosis, tubular dilatation, degeneration/regeneration of the transitional epithelium of the renal pelvis, and in the female with increased neutrophil and monocyte counts, neutrophilic inflammation of the renal pelvis and stomach.

At 100 mg/kg/day, GS-5245ODV-related hematology effects were limited to moderately decreased platelet count with mildly increased mean platelet volume, and mildly increased neutrophil and/or monocyte counts consistent with inflammation in 1 male and 1 female that correlated with the microscopic findings of pancreatic necrosis and neutrophilic infiltration in the liver.
Minimally prolonged aPTT was noted in both sexes. GS-5245Obeldesivir-related effects on clinical chemistry parameters at 100 mg/kg/day included decreased triglycerides in both sexes, decreased total protein and albumin in females, decreased globulins in males, and decreased calcium in females (attributed to the decreased albumin). The decrease in albumin may have reflected a negative acute phase response and/or loss.

There were no GS-5245ODV-related effects on clinical pathology parameters at 30

mg/kg/day. Exposure to GS-5245ODV and GS-441524 increased with the increase in GS-

5245ODV dose level from 30 to
300 mg/kg/day on Day 1 and from 30 to 100 mg/kg/day on Day 14 (Table 3741 and Table 3842). The increases in mean C_{max} and $AUC_{0\text{-}24h}$ were approximately dose -proportional between the 30 and 300 mg/kg/day dose levels on Day 1 and between the 30 and 100 mg/kg/day dose levels on

Day 14. Sex-based differences were less than 2-fold in GS-5245ODV and GS-441524 mean C_{max} and $AUC_{0\text{-}24h}$ values. No accumulation of GS-5245ODV or GS-441524 was observed after multiple doses of GS-5245ODV at 30 and 100 mg/kg/day. The mean $AUC_{0\text{-}24h}$ metabolite-to-parent ratios indicated that GS-5245ODV was extensively converted to GS-441524. Toxicokinetic samples taken at least 24 hours after the last dose administered to 300 mg/kg/day animals (prior to euthanasia) showed notably higher (approximately 14- and 18-fold higher) GS-441524 levels in 2 female dogs compared with expected values; these 2 females had evidence of GS-5245ODV-related kidney damage based on clinical and anatomic pathology changes.

Table 3741. Mean Toxicokinetic Parameters for GS-5245ODV in Beagle Dogs Administered GS-5245ODV for 2 Weeks

Dose (mg/kg/doseday)	Sex	$AUC_{0\text{-}24h}$ (ng•h/mL)		C_{max} (ng/mL)	
		Day 1	Day 14	Day 1	Day 14
30	Male	341	379	358	541
	Female	369	315	599	565
	Combined sex	355	347	478	553
100	Male	1090	1220	1280	1700
	Female	938	1330	1140	1670
	Combined sex	1010	1270	1210	1690

300	Male	4700	NA	2460	NA
	Female	5420	NA	2710	NA
	Combined sex	5060	NA	2590	NA

NA = not applicable; ODV = obeldesivir (GS-5245)

Table ~~38~~42. Mean Toxicokinetic Parameters for GS-441524 in Beagle Dogs Administered ~~GS-5245~~ODV for 2 Weeks

Dose (mg/kg/~~dose~~day)	Sex	$AUC_{0\text{-}24h}$ (ng•h/mL)		C_{max} (ng/mL)	
		Day 1	Day 14	Day 1	Day 14
30	Male	54,900	61,900	11,300	15,800
	Female	69,100	63,700	13,800	15,400
	Combined sex	62,000	62,800	12,500	15,600
100	Male	202,000	251,000	36,700	46,300
	Female	205,000	290,000	39,100	61,900
	Combined sex	203,000	271,000	37,900	54,100
300	Male	711,000	NA	104,000	NA
	Female	839,000	NA	115,000	NA
	Combined sex	775,000	NA	109,000	NA

NA = not applicable; ODV = obeldesivir (GS-5245)

Morbidity at 300 mg/kg/day was attributed to kidney (female only), pancreatic (male and female), and/or GI changes (female only). Changes in the kidneys in females consisted of crystal formation in the renal papilla associated with tubular necrosis and secondary changes in the renal cortex (tubular dilatation) and pelvis (neutrophilic inflammation and degeneration/regeneration of transitional epithelium). Changes in the pancreas consisted of minimal-to-marked acinar (exocrine) degeneration/necrosis. The acinar changes in the pancreas were generally associated with low number of inflammatory cells suggesting degeneration/necrosis resulting from increased autophagocytic activity and not acute inflammation. Gastrointestinal changes in females consisted of ulceration (esophagus and stomach), epithelial degeneration (esophagus, stomach, and duodenum), and/or intestinal neutrophilic inflammation. Other primary ~~GS-5245~~ODV-related findings were observed in the eyes (anterior uvea inflammation, corneal edema, and endothelial degeneration) and liver (vascular/perivascular neutrophilic inflammation) without any associated liver enzyme changes.

At the terminal euthanasia (Day 15), primary ~~GS-5245~~ODV-related changes were present in the pancreas and liver, and secondary (stress-related) findings were present in the thymus. ~~GS-5245~~ Obeldesivir-related changes in the pancreas consisted of minimal-to-moderate acinar degeneration/necrosis in 100 mg/kg/day males and females. Acinar degeneration/necrosis was considered adverse at 100 mg/kg/day given a severity grade of moderate in 1 female. ~~GS-5245~~ Obeldesivir-related changes in the liver consisted of minimal perivascular neutrophilic infiltrate in 1 male and 1 female at 100 mg/kg/day; there were no associated liver enzyme changes. ~~GS-5245~~ Obeldesivir-related lower mean thymic weights were observed in 100 mg/kg/day males and females and correlated microscopically with decreased lymphoid cellularity in all animals and grossly with a small thymus in 1 female. Thymic changes were considered secondary to stress.

In conclusion, administration of GS-5245ODV by once-daily oral gavage to beagle dogs at dose levels of 30, 100, and 300 mg/kg/day for up to 14 days was not tolerated at 300 mg/kg/day. At 100 mg/kg/day, GS-5245ODV-related microscopic changes were present in the pancreas and liver, with secondary (stress-related) findings present in the thymus. Pancreatic findings were considered adverse in the 100 mg/kg/day group while the liver findings were considered nonadverse. Based on these results, the NOAEL was considered to be 30 mg/kg/day in male and females. This dose corresponded to mean (sex-combined) GS-5245ODV $AUC_{0\text{-}24h}$ and C_{max} values of 347 ng•h/mL and 553 ng/mL, respectively, and GS-441524 $AUC_{0\text{-}24h}$ and C_{max} values of 62,800 ng•h/mL and 15,600 ng/mL, respectively, on Day 14.

4.3.2.2.2. A 4-Week Oral Gavage Toxicity and Toxicokinetic Study With ODV in Beagle Dogs With a 4-Week Recovery Period

The objectives of this study were to determine the potential toxicity and determine the TK of ODV and GS-441524 when administered once daily by oral gavage to beagle dogs for at least 28 days and to evaluate the potential reversibility of any findings after a 28-day recovery period (Study TX-611-2016). Three groups of dogs (3 main study animals/sex/group and 2 recovery study animals/sex in the vehicle control and high-dose groups) were administered ODV via oral gavage at 0, 25, and 50 mg/kg/day once daily for 28 consecutive days. Two animals/sex in the vehicle control and high dose groups were retained for an additional 4 weeks to assess recovery from any observed effects.

All animals survived to the scheduled necropsies. There were no ODV-related clinical observations or effects on electrocardiography, hematology, coagulation, clinical chemistry, urinalysis, or organ weights. There were no ODV-related ophthalmic, macroscopic, or microscopic findings.

ODV-related lower body weights were noted in the 50 mg/kg/day group females generally throughout the dosing period, which correlated with lower food consumption. During the recovery period, higher body weights gains were noted, indicating recovery, which correlated with higher food consumption during the recovery period.

Exposure to ODV and GS-441524 increased with the increase in ODV dose level from 25 to 50 mg/kg/day (Table 43). The increases in C_{max} and $AUC_{0\text{-}24h}$ were approximately dose-proportional from 25 to 50 mg/kg/day for both analytes. Sex-based differences were less than 2-fold in ODV and GS-441524 mean C_{max} and $AUC_{0\text{-}24h}$ values. No accumulation of ODV and GS-441524 was observed after multiple doses of ODV in dogs. The mean $AUC_{0\text{-}24h}$ metabolite to parent ratios indicates that ODV was extensively converted to GS-441524 in dogs following oral gavage administration of ODV.

Table 43. Mean Toxicokinetic Parameters for GS-441524 in Beagle Dogs Administered ODV for 4 Weeks

Dose (mg/kg/day)	Sex	$AUC_{0\text{-}24h}$ (ng•h/mL)		C_{max} (ng/mL)	
		Day 1	Day 28	Day 1	Day 14
25	Male	41,100	45,600	10,300	12,500
	Female	41,100	45,600	12,300	15,200

	Combined sex	48,000	48,700	11,300	13,800
50	Male	94,500	88,500	21,000	17,800
	Female	118,000	99,400	24,600	21,700
	Combined sex	106,000	94,000	22,800	19,700

ODV = obeldesivir (GS-5245)

In conclusion, administration of ODV by once daily oral gavage to beagle dogs at dose levels of 25 and 50 mg/kg/day for 28 days was well tolerated at all doses with no effects on survival or adverse findings. Based on these results, the NOAEL was considered to be 50 mg/kg/day. This dose corresponded mean (sex combined) ODV $AUC_{0\text{-}24h}$ and C_{max} values of 524 ng•h/mL and 578 ng/mL, respectively, and GS-441524 $AUC_{0\text{-}24h}$ and C_{max} values of 94,000 ng•h/mL and 19,700 ng/mL, respectively, on Day 28.

4.3.3. ~~3.3.3.~~Genotoxicity

4.3.3.1. ~~3.3.3.1.~~In Vitro

4.3.3.1.1. ~~3.3.3.1.1.~~Bacterial Reverse Mutation Assay

~~GS-5245~~Obeldesivir was evaluated for mutagenic activity in the in vitro bacterial reverse mutation assay using the plate incorporation method (Study TX-611-2003). Four tester strains of *Salmonella typhimurium* (TA98, TA100, TA1535, and TA1537) and 1 *Escherichia coli* strain (WP2 *uvrA*) were used for mutagenicity testing. ~~GS-5245~~Obeldesivir was prepared as a stock formulation in DMSO at a concentration of 50 mg/mL for each assay. Mutagenicity testing was performed in triplicate at each concentration with and without a phenobarbital/5,6-benzoflavone-induced rat liver S9 metabolic activation system.

In the mutagenicity assays with ~~GS-5245~~ODV, criteria for a negative response were met for all tester strains with and without metabolic activation. The data from the vehicle and positive controls demonstrated the validity and sensitivity of this test system for detecting chemical mutagens with and without metabolic activation. These data support the conclusion that ~~GS-5245~~ODV is negative for mutagenic activity in the *S typhimurium* strains TA98, TA100, TA1535, and TA1537 and in the *E coli* strain WP2 *uvrA*, with and without metabolic activation.

4.3.3.1.2. ~~3.3.3.1.2.~~In Vitro Mammalian Chromosomal Aberration Assay in Human Peripheral Blood Lymphocytes

~~GS-5245~~Obeldesivir was evaluated for the potential to induce chromosome aberrations in human peripheral blood lymphocytes during short (3-hour) and long (22-hour) incubations with or without an exogenous metabolic activation system (Study TX-611-2004). ~~GS-5245~~Obeldesivir was prepared as a stock formulation in DMSO at a target concentration of 36.2 mg/mL for the range- finding and aberration assays. Human peripheral blood lymphocyte cultures were treated with ~~GS-5245~~ODV, positive control, or vehicle control in the presence and absence of a phenobarbital/ 5,6-benzoflavone-induced rat liver S9 microsomal fraction.

In the 3-hour treatment assay without metabolic activation, chromosomal aberrations were analyzed from the cultures treated with 37.5, 100, and 135 µg/mL. In the 22-hour treatment assay without metabolic activation, chromosomal aberrations were analyzed from the cultures treated

with 4.17, 12.5, and 30.0 µg/mL. In the 3-hour treatment assay with metabolic activation, chromosomal aberrations were analyzed from the cultures treated with 75, 220, and 362 µg/mL. Structural chromosome aberrations were scored for each concentration from a total of 300 metaphase cells or ≥ 50 aberrant cells. Numerical aberrations were evaluated in 400 metaphase cells per concentration.

No statistically significant differences in the percentage of cells with structural chromosome aberrations or the percentage of cells with greater than 1 aberration were noted under any assay condition. GS-5245ODV was considered negative for inducing structural or numerical (polyploidy or endoreduplication) aberrations in human peripheral blood lymphocytes with or without metabolic activation.

4.3.3.2. 3.3.3.2. In Vivo

4.3.3.2.1. 3.3.3.2.1. A 14-Day Oral Gavage Toxicity and Toxicokinetic Study in Wistar Han Rats Withwith a Bone Marrow Micronucleus Assay

The objective of the micronucleus portion of thisthe 14-day study was to evaluate GS-5245ODV for in vivo clastogenic activity and/or disruption of the mitotic apparatus by counting micronuclei (MN) in polychromatic erythrocytes (PCEs) in bone marrow following oral gavage to male and female rats (TX-611-2001). Four groups of Wistar Han rats were administered vehicle or GS-5245ODV at 200, 500, or 1000 mg/kg/day for up to 14 days. Due to GS-5245ODV-related toxicity and unscheduled deaths, surviving females at 1000 mg/kg/day were euthanized on Day 8. The positive control, cyclophosphamide, was administered orally via gavage as a single dose on Day 14. Bone marrow was analyzed from 5 animals/sex/group.

GS-5245Obeldesivir did not produce statistically significant or dose-dependent higher percentages of micronucleated PCEs (%MN PCEs) in male or female rats at any dose level as compared to the vehicle controls. A statistically significant analysis of variance response in bone marrow cytotoxicity (lower polychromatic to total erythrocytes [PCE:TE] ratio) was noted in male rats. The follow-up Dunnett's test produced a statistically significant decrease in PCE:TE ratio in 1000 mg/kg/day males ($P \leq 0.01$) when compared to the vehicle control. In addition, both male and female rats displayed a trend in higher bone marrow cytotoxicity. Group mean values for
%MN PCEs and PCE:TE ratios for the vehicle and positive controls demonstrated the acceptability of the assay.

GS-5245Obeldesivir was concluded to be negative for clastogenic activity and/or disruption of the mitotic apparatus in the rodent micronucleus assay when administered orally at daily doses up to 1000 mg/kg/day. At 1000 mg/kg/day, $AUC_{0\text{-}24h}$ exposures were 628,000 ng•h/mL in females on Day 8 and 539,000 ng•h/mL in males on Day 14.

4.3.4. 3.3.4. Carcinogenicity

No carcinogenicity studies have been conducted.

4.3.5. 3.3.5. Reproductive and Developmental Toxicity

~~Embryo-fetal development studies with GS-5245 have been performed in rats (Studies TX-611-~~

2005 and TX-611-2009) and rabbits (Study TX-611-2008). Final results for Study TX-911-2005 and preliminary results for Studies TX-611-2009 and TX-611-2008 are described below.

3.3.5.1.Study TX-611-2005: Enhanced Dose Range Finding and Toxicokinetic

Embryo-FetalOral (Gavage) Study of the Effects of ODV on Fertility and Early Embryonic Development Study of GS-5245 by Oral Gavage Administrationto Implantation in Wistar Han Rats

The objectives of this study were to determine the potential adverse effects/disturbances in the reproductive process resulting from oral administration of ODV to male and female Wistar Han rats from pre-mating to conception and from conception to implantation (Study TX-611-2011). This included identification of deficits in estrous cycling, tubal transport, implantation, development of the preimplantation stages of the embryo in the female, and functional reproductive effects (alterations in libido and epididymal sperm maturation) in the male. In addition, the TK of ODV and GS-441524 were determined from a satellite group of non-mated animals.

Four groups of male rats (25 main study and 6 TK phase/group) were administered ODV by oral gavage once daily at doses of 0, 125, 250, or 500 mg/kg/day. Males in the main study were dosed for 14 days prior to mating and continuing through Study Day 50 or 51 (1 day prior to euthanasia on Study Day 51 or 52). Three groups of female rats (25 main and 6 TK/group) were administered ODV at doses of 0, 125 or 250 mg/kg/day. An additional 25 females (main study) were not dosed but used for breeding purposes only for the high dose 500 mg/kg/day males. Females in the main study were dosed for 14 days prior to mating and continuing through gestation day (GD) 7. Toxicokinetic phase animals were dosed for 14 days.

The following parameters and endpoints were evaluated in this study: mortality, clinical signs, body weights, body weight gains, food consumption, estrous cycles, reproductive performance, toxicokinetic parameters, intrauterine survival (postimplantation loss and number of live embryos), macroscopic findings, sperm parameters, and organ weights.

There were no ODV-related effects on male survival, clinical and macroscopic observations, body weights, body weight gains, food consumption, and organ weights at ≤ 250 mg/kg/day. Male reproductive performance (mating, fertility, and pregnancy indices and pre-coital intervals) and spermatogenic parameters were unaffected by ODV administration at ≤ 500 mg/kg/day. There were no ODV-related effects on female survival or clinical observations at 125 mg/kg/day or on pre-mating and gestation body weights, body weight gains, food consumption, estrous cycle length, reproductive performance, macroscopic findings, or organ weights at ≤ 250 mg/kg/day. Intrauterine survival was also not affected by ODV administration at any dose level. All parameters in the untreated females (mated with 500 mg/kg/day males) were comparable to the vehicle control group.

Obeldesivir-related mortality and/or moribundity was noted at 500 mg/kg/day in males and 250 mg/kg/day in females. Four males in the 500 mg/kg/day group were found dead and/or euthanized in extremis during Study Day 31 through Study Day 42 and 3 females in the 250 mg/kg/day group were euthanized in extremis on Study Day 16 or 23 and GD 7. These animals had body weight loss, low food consumption and adverse clinical observations. At necropsy, enlarged, pale kidneys were noted in 1 male and pale kidneys with rough surface were

noted in 2 females. Early euthanasia of one female at 250 mg/kg/day and one TK female at 125 mg/kg/day were unrelated to ODV administration. All other animals survived to the scheduled necropsy.

Obeldesivir-related clinical observations consisted of abnormal breathing sounds, erect fur, hunched posture, thin body condition, and/or decreased activity in the 250 mg/kg/day group females and the 500 mg/kg/day group males beginning on Study Day 13. In addition, single occurrences of salivation were noted in 5 males in the 500 mg/kg/day group at the detailed clinical or postdosing observations during Study Day 44 through Study Day 51 and were possibly ODV-related.

Obeldesivir-related adverse mean body weight losses and lower mean body weight gains and food consumption were noted in the 500 mg/kg/day group males following the mating period when compared to the vehicle control group. As a result, mean absolute body weight in this group was 23.8% lower than the vehicle control group on Study Day 51.

At the scheduled necropsy, macroscopic findings attributed to ODV administration consisted of enlarged, pale kidneys and small prostate gland, seminal vesicles, and coagulating glands in the 500 mg/kg/day group males. The kidney findings were considered adverse because they were noted in animals that died or were euthanized early. Lower mean absolute epididymis, cauda epididymis, pituitary gland, prostate gland, and testes weights in the 500 mg/kg/day group males were considered secondary to the lower mean final body weights and not considered adverse as there were no effects on male reproductive performance or sperm parameters noted at 500 mg/kg/day.

Exposure to OBV was generally low and highly variable. The increase in C_{max} and $AUC_{0\text{-}24h}$ values were generally dose proportional in combined sexes from 125 to 250 mg/kg/day and in males from 250 to 500 mg/kg/day. There was no notable sex-based difference with GS-441524 C_{max} and $AUC_{0\text{-}24h}$ values generally less than 2-fold; however, C_{max} and $AUC_{0\text{-}24h}$ values were higher in females at 250 mg/kg/day. No accumulation of GS-441524 was observed after multiple doses of OBV. The C_{max} and $AUC_{0\text{-}24h}$ metabolite to parent ratios indicate that OBV was extensively converted to GS-441524.

Based on the lack of effects on reproductive performance and spermatogenic parameters, a dose level of 500 mg/kg/day (the highest dose level tested in males) was considered to be the NOAEL for male reproductive toxicity of ODV when administered orally by gavage to rats. Based on the lack of effects on female reproductive performance, estrous cyclicity, and intrauterine survival, the NOAEL for female reproductive toxicity and embryonic toxicity was considered to be 250 mg/kg/day (the highest dose level tested in females). GS-441524 exposures at the reproductive NOAELs were 172,000 and 203,000 h•ng/mL in males and females, respectively.

Based on mortality, clinical observations, body weight losses, and lower body weight gains and food consumption at 500 mg/kg/day in males, a dose level of 250 mg/kg/day was considered to be the NOAEL for male systemic toxicity. Based on mortality and clinical observations, body weight losses, and lower body weight gains and food consumption in moribund animals at 250 mg/kg/day in females, a dose level of 125 mg/kg/day was considered to be the NOAEL for female systemic toxicity.

4.3.5.1. Enhanced Dose Range-Finding and Toxicokinetic Embryo-Fetal Development

Study of Obeldesivir by Oral Gavage Administration in Wistar Han Rats

The objective of this GLP range-finding study was to provide a preliminary evaluation of the effects of GS-5245ODV on pregnant Wistar Han rats and development of the embryo consequent to exposure of the female from implantation to closure of the hard palate. GS-5245 (Study TX-611-2005). Obeldesivir was administered to 4 groups of pregnant rats (8 animals/group) at doses of 0, 125, 250 and 500 mg/kg/day once daily via oral gavage during organogenesis (gestation days [GDs] 6 through 17).

Systemic exposure to GS-5245ODV was generally low and variable. Exposure to GS-441524 increased with the increase in GS-5245ODV dose level from 125 to 500 mg/kg/day. The high C_{max} and AUC_{0-24h} metabolite to parent ratios indicate that GS-5245ODV was extensively converted to GS-441524 in pregnant rats.

Animals administered 500 mg/kg/day exhibited severe clinical signs, including decreased activity, hunched posture, thin appearance, low food consumption, and body weight loss. Macroscopic observations of enlarged and discolored kidneys and enlarged and discolored adrenal glands were noted at necropsy in multiple animals. Due to excessive maternal toxicity prompting early termination of animals at 500 mg/kg/day, fetal parameters were not evaluated for this group.

There were no remarkable clinical signs or effects on body weight, food consumption, or macroscopic observations at 125 or 250 mg/kg/day. No GS-5245 ODV-related effects were observed for embryo-fetal viability parameters, litter and fetal weights, or fetal pathology at 125 or 250 mg/kg/day. The NOAEL of GS-5245ODV on maternal toxicity and embryo-fetal development was 250 mg/kg/day (GD 17 GS-441524 C_{max} of 43,700 ng/mL and AUC_{0-24h} of 183,000 h•ng/mL).

4.3.5.2. 3.3.5.2.Study TX-611-2009: An Embryo-Fetal Development Study of GS-5245ODV by Oral Gavage in Wistar Han Rats (Preliminary Results)

The objectives of this GLP study were to determine the potential of GS-5245ODV to induce developmental toxicity after maternal exposure during the critical period of organogenesis (GDs 6 through 17), to characterize maternal toxicity at the exposure levels tested, and to determine a NOAEL for maternal toxicity and developmental toxicity following oral administration to Wistar Han rats. GS-5245 (Study TX-611-2009). Obeldesivir was administered to
3 groups of pregnant rats (25 animals/group) at doses of 0 (0.5% methylcellulose in deionized water), 125 and 250 mg/kg/day once daily via oral gavage. Assessment of toxicity was based on mortality, clinical observations, body weight, food consumption, necropsy findings, laparohysterectomy data, and fetal external, visceral, and skeletal evaluations. Preliminary findings from this study are summarized below.

All animals survived to scheduled necropsy on GD 21. There were no GS-5245ODV-related effects on body weight and food consumption. Mean numbers of corpora lutea and implantation sites, the percentage of pre-implantation losses and mean numbers of viable fetuses were generally comparable across all groups. Mean fetal weights were similar across all groups.

There were no GS-5245 ODV-related effects on clinical observations, maternal body

weights, food consumption, gravid uterine weight, gross necropsy, intrauterine growth and survival, or fetal morphology (external, visceral, or skeletal).

In summary, GS-5245ODV dose levels of 125 and 250 mg/kg/day were well tolerated in maternal animals, and there were no remarkable effects on fetal morphology. Based on TK analysis from athe enhanced dose range-finding study in pregnant rats (Section 3.3.5.14.3.5.2), mean GS-441524 exposures at 250 mg/kg/day on GD 17 were C_{max} of 43,700 ng/mL and $AUC_{0\text{-}24h}$ of 183,000 h•ng/mL.

4.3.5.3. 3.3.5.3.Study TX-611-2008: Oral Gavage Embryo-Fetal Development and Toxicokinetics Study With GS-5245ODV in Rabbits (Preliminary Results)

The purpose of this GLP study was to evaluate the effects of GS-5245ODV on pregnant New Zealand White rabbits and the development of the embryo consequent to exposure of the female from implantation to closure of the hard palate (GDs 7 through 19; Study TX-611-2008). GS-5245

Obeldesivir was administered to 4 groups of pregnant rabbits (20 animals/group) at doses of 0, 125, 250, and 500 mg/kg/day once daily via oral gavage. Satellite groups (4 animals/group) were sampled for TK evaluation of GS-5245ODV and metabolite, GS-441524. Assessment of toxicity was based on mortality, clinical observations, body weight, food consumption, necropsy findings, laparohysterectomy data, and fetal external, visceral, and skeletal evaluations. Preliminary findings from this study are summarized below.

Systemic exposure to GS-5245ODV was generally low and highly variable. Exposure to the metabolite GS-441524 increased with the increase in GS-5245ODV dose level from 125 to 500 mg/kg/day.
Metabolite-to-parent AUC ratios indicated that GS-5245ODV was extensively converted to GS-441524 in pregnant rabbits.

GS-5245Obeldesivir–related moribund condition and early termination occurred for 1 animal administered 250 mg/kg/day and 5 main study and 2 TK animals administered 500 mg/kg/day. In addition, abortion was noted in 2 main study and 2 TK animals administered 500 mg/kg/day. Due to excessive maternal toxicity observed at 500 mg/kg/day, the group was terminated early between GDs 17 and 22, and fetal parameters were not evaluated for this group.

An adverse reduction in food consumption was noted for animals administered 250 mg/kg/day, which correlated with reduced body weight gain relative to controls during the dosing period. Reduced maternal body weight gain at 250 mg/kg/day correlated with a reduction in fetal and uterine weight. No notable effect on maternal parameters was observed in animals administered 125 mg/kg/day.

Increased rates of adverse fetal effects, including postimplantation loss and fetal visceral malformations related to the development of the heart, blood vessels, and liver, were noted in animals administered 250 mg/kg/day. Visceral malformations noted at 250 mg/kg/day included retroesophageal subclavian artery, ventricular septum defect, large or small heart ventricle, dilated or malpositioned aorta, retroesophageal aortic arch, and abnormal lobulation of the liver. No GS-5245 ODV-related skeletal malformations were noted. Visceral and skeletal variations included increased rates of supernumerary liver lobe and absent intermediary lung lobe at 250 mg/kg/day, a non–dose-responsive increase in the rate of adrenal cysts at 125 and 250 mg/kg/day, and increased rates of supernumerary sternebra sites, supernumerary lumbar

vertebra, and isolated ossification sites on the nasal/premaxilla bone at 250 mg/kg/day.

In summary, administration of 500 mg/kg/day was not tolerated in pregnant rabbits. At the 250 mg/kg/day dose, maternal toxicity was evident, along with an increase in postimplantation loss and fetal visceral malformations primarily related to the development of the heart, blood vessels, and liver. Based on these findings, the NOAEL of ~~GS-5245~~ODV on maternal toxicity and embryo-fetal development was 125 mg/kg/day. This NOAEL corresponds to GS-441524 GD 19 C_{max} of 24,100 ng/mL and AUC_{0-24h} of 64,100 h•ng/mL.

4.3.5.4. Oral (Gavage) Study of the Effects of ODV on Prenatal and Postnatal Development, Including Maternal Function in Wistar Han Rats (preliminary data)

The objectives of this study were to determine the potential adverse effects of maternal ODV exposure when given by oral administration from implantation to weaning on pregnancy, parturition, and lactation of the maternal (F_0) rats and on the growth, viability, and development

of the F_1 neonates (Study TX-611-2012). Reproductive performance of the F_1 generation was also assessed. The TK of ODV and GS-441524 was assessed in maternal animals (F_0) and F_1 neonates.

Three groups (25 or 26 main study and 3 or 6 TK phase females/group) of Wistar Han rats were administered ODV by oral gavage once daily at doses of 0, 100, or 200 mg/kg/day. Females in the main study were dosed from GD 6 through lactation day (LD) 20. One female weanling/sex/litter was randomly selected to form the F_1 generation (behavioral/reproductive phase; 25 animals/sex/group, when possible) and were not directly administered ODV. At approximately 85 days of age, the F_1 generation was assigned to a 15-day cohabitation period. All F_1 females were allowed to deliver and rear their pups until postnatal day (PND) 4. Toxicokinetic phase females were dosed from GD 6 through LD 10. Animals that failed to deliver were dosed until euthanasia on postmating Day 25.

There were no ODV-related effects on survival of the F_0 females at any dose level. All F_0 females survived to the scheduled necropsies, and no ODV-related clinical findings were observed in the F_0 generation. No ODV-related effects on mean body weights, body weight gains, food consumption, gestation length, the process of parturition, and macroscopic findings were noted.

No ODV-related effects on mean numbers of F_1 pups born, live litter size, percent of males per litter, and postnatal survival and growth were noted. No observations in F_1 males or females could be attributed to maternal ODV administration during clinical observations or at scheduled necropsy for nonselected pups on PND 21.

Mean days of attainment, and mean body weight on day of attainment were similar across all groups for pinnal detachment, eye opening, pupillary reflex, balanopreputial separation, and vaginal patency for the F_1 generation. Auditory startle response, motor activity, and learning and memory assessments in F_1 animals were also unaffected by maternal ODV administration.

A single F_1 male in the 200 mg/kg group was found dead on PND 66; no remarkable observations prior to death or internal findings at necropsy were noted. In the absence of any other effects during the F_1 generation that could be attributed to maternal ODV administration, this death was not considered ODV-related.

Mean F_1 body weights, body weight gains, reproductive performance, pre-coital interval, gestation length, the process of parturition, and macroscopic findings were unaffected by F_0 maternal ODV administration.

No ODV-related effects on mean numbers of F_2 pups born, live litter size, percent of males per litter, and postnatal survival and growth were noted. No observations in F_2 males or females could be attributed to F_0 maternal ODV administration during clinical observations.

Exposure to ODV was generally low and highly variable in maternal F_0 rats and F_1 pups. For maternal F_0 rats and F_1 pups, exposure to GS-441524 increased with the increase in ODV dose level from 100 to 200 mg/kg/day. The increase in C_{max} and AUC_{0-24h} values were approximately dose proportional from 100 to 200 mg/kg/day for maternal F_0 rats on GD 6 and LD 10 and for

F_1 pups on PND 10. No accumulation of GS-441524 was observed after multiple doses of ODV in maternal F_0 rats. The AUC_{0-24h} metabolite to parent ratios ranged from 5030 to 18,800 for maternal F_0 rats and was 125 for the 200 mg/kg/day group F_1 pups. For combined males and females F_1 pups, the AUC_{0-24h} F_0 to F_1 ratios were 31.6 and 23.2 at 100 and 200 mg/kg/day, respectively, indicating that F_1 pups were exposed to ODV and GS-441524 in utero and/or during lactation, but at notably lower levels relative to maternal exposure.

In conclusion, no ODV-related systemic effects were noted at any dose level in the F_0 or F_1 generation. Therefore, a dose level of 200 mg/kg/day, the highest level tested, was considered to be the NOEL for F_0 maternal systemic, F_1 developmental/neonatal, F_1 parental systemic, F_1 reproductive, and F_2 developmental/neonatal toxicity of ODV when administered orally to Crl:WI(Han) rats. At the NOEL, F_0 maternal GS-441524 AUC_{0-24h} and C_{max} values were 100,000 h•ng/mL and 37,300 ng/mL, respectively, on LD 10.

4.3.6. ~~3.3.6.~~Local Tolerance

~~Dedicated local tolerance studies with GS-5245 have not been performed. GS-5245~~ODV is intended for oral administration and evaluation of local tolerance for this route was conducted during the repeat-dose studies (Section ~~3.3.2~~4.3.2).

4.3.6.1. Phototoxicity

Results of a 3T3 neutral red uptake test with GS-441524 using mouse fibroblasts showed that GS-441524 was not cytotoxic and did not display a IC_{50} with or without ultraviolet radiation exposure, up to the highest soluble concentration tested (100 µg/mL) (Study TX-611-2026). Administration of ODV is therefore not considered to exhibit a photosafety risk.

4.3.7. ~~3.3.7.~~Other Toxicity Studies

~~No other toxicity studies with GS-5245 have been conducted.~~

4.3.7.1. Impurity Qualification

A repeat dose toxicity study was conducted to compare the toxicity of different manufactured lots of ODV (a purified lot and a lot containing high levels of process-related impurities) when administered once daily by oral gavage to rats for 14 days (Study TX-611-2006). Wistar Han rats (5/sex/group for toxicology, 3-6/sex for TK) were administered vehicle, ODV at 200 mg/kg/day, or ODV-A (impurities-containing lot) at 100 or 200 mg/kg/day.

All animals survived to the scheduled euthanasia. There were no ODV- or ODV-A-related

clinical observations, effects on body weight or food consumption, or ophthalmic findings. There were no ODV-related effects on hematology, coagulation, clinical chemistry, urinalysis, or organ weights, or macroscopic findings. ODV-A-related adverse effects were limited to 2 female animals administered 200 mg/kg/day and were indicative of decreased renal function and associated microscopic kidney changes resulting from renal papillary crystals with secondary inflammatory changes. These changes were similar to findings previously observed in the 14-day repeat dose rat toxicity study (Section 4.3.2.1.1). The NOAEL was considered to be 200 mg/kg/day for ODV in males and females and 200 mg/kg/day for ODV-A in males and 100 mg/kg/day in females. These doses corresponded to mean GS-441524 AUC_{0-24h} values of 87,500 and 169,000 h•ng/mL for males and females in the 200 mg/kg/day ODV group, respectively, and mean AUC_{0-24h} values of 90,000 (in males at 200 mg/kg/day) and 58,300 h•ng/mL (in females 100 mg/kg/day) in the ODV-A groups on Day 14.

4.3.8. ~~3.3.8.~~ Toxicology Conclusions

~~GS-5245~~Obeldesivir is a mono-5'-isobutyryl ester prodrug of an adenosine analog GS-441524 that is being developed for oral administration as a potential treatment for COVID-19. The nonclinical toxicology profile of ~~GS-5245~~ODV has been characterized through the conduct of repeat-dose studies in rats and dogs of up to ~~14~~28 days in duration, and studies to evaluate ~~the genotoxic potential of the compound~~genotoxicity, developmental and reproductive toxicity, and phototoxicity. Obeldesivir is not considered a phototoxic risk.

Early mortality/morbidity was noted in both 14-day rat and dog repeat-dose studies, with females in both species being more sensitive. In female rats and dogs, early mortality was attributed to ~~GS-5245~~ODV-related acute renal failure due to ~~crystal formation~~formations of GS-441524-containing crystals in the renal papilla associated with tubular degeneration and necrosis. In dogs, mortality/morbidity was also attributed to GI (female only) and/or pancreatic (male and female) changes. Additional target organs, observed in dogs only, included the eyes and liver. In the 28-day studies, there were no unexpected toxicities or evidence of cumulative toxicity; GS-441524 exposure margins at the NOAELs were 4.3- to 8-fold higher in female and male rats, respectively, and 3.1-fold higher in dogs compared to projected exposures at the 350 mg twice daily human dose.

In embryo-fetal development studies, administration of ~~GS-5245~~ODV to pregnant rabbits was associated with an increase in postimplantation loss and fetal visceral malformations primarily related to the development of the heart, blood vessels, and liver. ~~Preliminary findings indicate~~There were no remarkable ~~GS-5245~~ ODV-related effects on embryo-fetal development in pregnant rats, no adverse effects on fertility and early embryonic development, and no effects on prenatal and postnatal development. At the NOAELs for embryo-fetal development, AUC exposures in rats and rabbits were approximately ~~5~~6- and ~~1.8~~2.1-fold higher, respectively, than predicted steady-state exposure in humans at the projected clinically efficacious 350 mg twice daily dose of ~~GS-5245 (Section 3.2.6)~~ODV (Table 1). In the fertility study, exposures at the NOAELs for reproductive effects were approximately 6- to 7-fold higher than projected at the 350 mg twice daily dose. In the prenatal and postnatal development study, exposures at the NOEL were approximately 3-fold higher than projected at the 350 mg twice daily dose.

4.3.8.1. ~~3.3.8.1.~~ Target Organ Toxicity

Kidney changes consistent with acute renal failure in both rats and dogs were secondary to crystal formation, mostly observed in the renal papilla, and were considered the cause of early

mortality/morbidity in both species. These changes were only observed in female animals; a mechanism for this apparent sex difference in sensitivity is unknown. Crystals and associated pathological changes were noted in female rats at ≥ 500 mg/kg/day, and in 2 female dogs at 300 mg/kg/day. In the absence of crystals, changes in the kidney were generally unremarkable. Clinical pathology changes supported the microscopic changes, with increases in urea nitrogen, creatinine and/or phosphorus noted. Although definitive evidence is lacking, it is believed thatThe chemical composition of the crystalsrenal deposits were likely drug-related material based on their birefringent appearance. Following an IV 30-minute infusion of GS-441524 in dogsidentified as the parent nucleoside, intact GS-441524 waswhich is predominantly eliminated by renal excretion, accounting for 90% of the dose in dogs (Section 3.2.4.14.2.4.1). A similar excretion profile is anticipated in rats. Crystal formation can occur in all mammalian species, and the toxicological effects related to these crystals is a well-defined threshold effect related to the solubility of the agent in urine {Cohen 2018}. In rats and dogs, renal crystals occurred at C_{max} levels approximately 20- and 34-fold higher, and AUC exposures approximately 810- and 2326-fold higher, respectively, than predicted steady-state exposure in humans at the projected clinically efficacious dose of GS-5245 (Section 3.2.6ODV (Table 1).

GS-5245Obeldesivir-related GI changes were observed in 2 female dogs at 300 mg/kg/day; both females also had crystals in the renal papilla and associated renal findings. These GI changes consisted of ulceration (esophagus and stomach), epithelial degeneration (esophagus, stomach, and duodenum), and/or intestinal neutrophilic inflammation at exposures approximately 2326-fold higher than predicted steady-state exposure in humans at the projected 710-350 mg clinically

efficaciousstwice daily dose of GS-5245. In rats, secondary findings related to GS-5245ODV-related debilitation were noted in the small and large intestine with minimal-to-marked crypt degeneration observed in the jejunum, ileum, cecum, colon, and/or rectum of 2 females at 1000 mg/kg/day. There were no notable GI changes in rats at ≤ 500 mg/kg/day and dogs at ≤ 100 mg/kg/day.

Changes in the pancreas consisted of minimal-to-marked acinar (exocrine) degeneration/necrosis in dogs only. Acinar changes in pancreas were generally associated with a low number of inflammatory cells suggesting degeneration/necrosis resulted from increased autophagocytic activity and not acute inflammation. In animals euthanized early by Day 7 at the 300 mg/kg/day dose, pancreatic findings were observed in all males and in 1 female. Pancreatic findings were generally considered the cause of moribundity in these animals. At the 100 mg/kg/day dose, pancreatic changes were similar to those observed by Day 7 at 300 mg/kg/day, but were generally of lesser severity and were considered adverse in only 1 female given a moderate severity grade. The pancreatic finding occurred at exposures at least 79-fold higher than predictedprojected steady-state AUC exposure in humans at the projected efficacious350 mg twice daily dose.

Ocular changes were only observed in dogs at the 300 mg/kg/day dose that was not tolerated. Changes were noted as anterior uvea inflammation (minimal severity), corneal edema (minimal-to-mild severity), and endothelial degeneration (minimal-to-mild severity) at exposures at least 21-fold higher than predicted steady-state exposure in humans at the projected clinically efficacious dose. In dogs at ≤ 100 mg/kg/day, there were no microscopic changes and no abnormal ophthalmic findings, and animals had normal pupillary light reflexes and intraocular pressure readings. No ocular changes were noted in rats.

Hepatic changes were observed at the 300-mg/kg/day dose in dogs, and in 1 male and 1 female dog at 100 mg/kg/day. Vascular/perivascular neutrophilic inflammation was noted in high dose animals. Hepatic findings at 100 mg/kg/day on Day 15 differed from findings in the 300 mg/kg/day animals in that Day 15 findings lacked secondary changes indicative of inflammation and were more consistent with a diagnosis of neutrophilic infiltrate at the high dose; these changes were considered not adverse given their minimal-to-mild severity grade and the absence of elevated liver enzymes that would suggest a functional disruption.

4.3.8.2. 3.3.8.2. Genetic Toxicology

GS-5245Obeldesivir was nonmutagenic in the bacterial reverse mutation assay, was negative in the chromosome aberrations assay with human lymphocytes, was negative in the rat micronucleus assay, and is not considered genotoxic.

4.3.8.3. 3.3.8.3. Comparative Exposures and Safety Margins

The NOAELs in the repeat dose toxicology studies and estimated margins of exposure for GS-441524 based on the proposed starting GS-5245 dose and projected therapeuticexposures at the 350 mg twice daily human dose are presented in Table 39. The margin based on converting the NOAEL (100 mg/kg) from the 14-day dog study to a human equivalent dose (HED) (1000 mg) is 10-fold higher than the proposed first-in-human dose of 100 mg. Given the adequate safety margins based on HED in the 2-week repeat-dose toxicity studies (19- to 48-fold in rats, 10-fold in dogs), the nonclinical toxicology studies are considered adequate for supporting the proposed first-in-human study. In addition, GS-441524 exposures (AUC) at the NOAELs in the 14-day repeat-dose rat and dog studies are expected to be at least 25- and 12-fold higher, respectively, than exposures at the 100-mg dose, and at least 1.7-fold higher than the predicted steady-state exposure in humans at the projected clinically efficacious GS-5245 dose (Section 3.2.6)44.

Table 3944. Estimated Exposure Margins for GS-441524 Based on GS-5245 Human Equivalent Dose Relative to GS-5245 First-in-Human Dose and Projected GS-441524 Exposure at the First-in-Human 100 mg GS-5245 Dose and Anticipated Therapeutic Human GS-5245350 mg Twice Daily ODV Dose

Species	Duration	Route	GS-5245 ODV NOAEL (mg/kg/day)	GS-5245 HED[a] (mg/day)	NOAEL GS-441524 AUC_{0-24h} (h•ng•h/mL)	Margins Exposure Margin[a]		
						HED[b]	AUC[c]	AUC[d]
Rat	1428 days	Oral	500 (M)	4838 (M)	203,000 (M)242,000 (M)	48	40	5.6 8.0
			200 (F)	1935 (F)	129,000 (F) 128,000 (F)	19	25	3.5

								4.3
Dog	1428 days	Oral	3050	1000	62,800[e] 94,000[b]	10	12	1.7 3.1

BID = twice daily; F = female; HED = human equivalent dose; M = male; NOAEL = no observed adverse effect level

aGS-5245 HED was calculated by dividing the NOAEL by 6.2 for rats and 1.8 for dogs and then multiplying by average body weight of 60 kg.

bBased on a proposed first-in-human starting dose of 100 mg GS-5245.c; ODV = obeldesivir (GS-5245) a Margins of exposure were calculated using an estimateda projected steady state GS-441524 exposure (AUC_{0-24h}) in humans of 5126 ng•h/mL at 100 mg GS-5245, assuming dose linearly below 710 mg (Section 3.2.7).

dMargins of exposure were calculated using an estimated steady-state GS-441524 exposure (AUC_{0-24h}) in humans of 36,400 ng•h/mL at 710 mg GS-5245 (Section 3.2.7).e
30,100 h•ng/mL at 350 mg BID ODV (Table 1).

b Day 1428; males and females, combined.

5. 4.CLINICAL STUDIES

There is no previous human experience with GS-5245. As of 22 July 2022, there is 1 on-going Phase 1 (first-in-human) clinical study (Study GS-US-611-6248).As of 12 May 2023, 2 Phase 1 clinical studies have been completed in which 64 healthy participants have been dosed with ODV.

5.1. Phase 1 Safety and Pharmacokinetics of ODV in Healthy Adult Participants

5.1.1. Clinical Study GS-US-611-6248 (Single and Multiple Ascending Dose, Food and Formulation Effect Study)

5.1.1.1. Study Design

Study GS-US-611-6248 was a Phase 1 study evaluating the safety, tolerability, PK, and the effect of food and formulation on PK in healthy participants following administration of ODV.

Single and Multiple Ascending Doses (Cohorts 1 to 7): a randomized, blinded, placebo-controlled, single- and multiple-dose study with staggered dose escalation and adaptive ODV dose selection to evaluate the safety, tolerability, and plasma PK of escalating single and multiple doses of ODV in healthy normal participants.

Cohort 7 was planned but not initiated as the doses evaluated in Cohorts 1 to 6 allowed for characterization of the PK of ODV across a sufficiently wide dose range.

Food and Formulation Effect (Cohorts 8 and 9): an open-label, single-dose cohort for evaluation of the food effect on ODV Phase 3 formulation.

5.1.1.2. Participants Disposition and Demographics

A total of 70 participants were randomized or enrolled into the study across 8 cohorts, and all participants completed the study. Of the 70 participants, 58 participants received ODV tablets, and 12 participants received placebo tablets.

A similar proportion of male and female participants (52.9% and 47.1%, respectively) were

randomized/enrolled in this study. The mean (SD) age was 32 (7.5) years (range: 20-45). The majority of participants were Black or White (44.3% and 41.4%, respectively), and not Hispanic or Latino (88.6%). The median (Q1, Q3) body mass index was 25.9 (23.4, 27.8) kg/m^2.

5.1.1.3. Pharmacokinetic Results

5.1.1.3.1. Plasma

Following single dose administration of ODV at doses of 100, 300, 900, or 1600 mg under fasted conditions, dose-proportional increases in the GS-441524 plasma PK parameters (AUC_{last}, AUC_{inf}, and C_{max}) were observed across the dose range of 100 to 900 mg, and less than dose-proportional increase between the 900 mg and 1600 mg doses. The median T_{max} of GS-441524 in plasma was 0.75 hours postdose, and the median $t_{1/2}$ ranged from 6 to 7 hours across the 100 to 900 mg single doses (Table 45).

Table 45. GS-US-611-6248 (SAD Cohorts): Plasma Pharmacokinetic Parameters of GS-441524 Following Single Dose Administration of ODV (GS-441524 PK Analysis Set)

PK Parameter[a] Mean (%CV)	SAD			
	100 mg ODV (Cohort 1) (N = 6)	300 mg ODV (Cohort 2) (N = 6)	900 mg ODV (Cohort 3) (N = 6)	1600 mg ODV (Cohort 4) (N = 6)
C_{max} (ng/mL)	570.2 (30.5)	1833.3 (32.6)	5940.0 (44.6)	7170.0 (26.7)
T_{max} (h)	0.75 (0.50, 0.75)	0.78 (0.50, 1.50)	0.75 (0.75, 1.50)	1.50 (0.75, 1.53)
C_{last} (ng/mL)	25.3 (43.7)	71.5 (125.3)	17.9 (29.6)	12.6 (42.8)
T_{last} (h)	24.0 (24.00, 24.00)	24.0 (24.00, 48.08)	48.1 (48.00, 72.00)	84.0 (72.00, 96.00)
AUC_{last} (h•ng/mL)	3479.3 (35.0)	9856.8 (26.2)	34,408.9 (35.0)	48,036.8 (22.5)
AUC_{inf} (h•ng/mL)	3698.7 (34.7)	10,405.2 (21.4)	34,595.4 (34.7)	48,299.0 (22.4)
AUC_{exp} (%)	6.0 (42.3)	6.0 (121.5)	0.6 (46.3)	0.6 (41.1)
$t_{1/2}$ (h)	5.85 (5.57, 6.26)	6.10 (4.86, 6.93)	7.36 (6.89, 7.81)	15.69 (14.05, 17.56)

%CV = percentage coefficient of variation; CSR = clinical study report; ODV = obeldesivir (GS-5245); PK = pharmacokinetic(s); Q1 = first quartile; Q3 = third quartile; SAD = single ascending dose a Data presented as mean (%CV), except for T_{max}, T_{last}, and $t_{1/2}$ as median (Q1, Q3).
Source: Study GS-US-611-6248 CSR, Table 15.10.1.2.2

Following ODV (500 mg) administration twice daily for 5 days under fasted conditions, accumulation of GS-441524 in plasma (approximately 35% based on AUC values) was observed and reached steady state at 5 days (Table 46). Minimal (approximately 12% based on AUC values) accumulation of GS-441524 was observed after once-daily dosing.

Table 46. GS-US-611-6248 (MAD Cohorts): Plasma Pharmacokinetic Parameters of GS-441524 Following Multiple Dose Administration of ODV (GS-441524 PK Analysis Set)

	MAD

PK Parameter[a] Mean (%CV)	500 mg BID ODV (Cohort 5) (N = 6)		900 mg QD ODV (Cohort 6) (N = 6)	
	Day 1	Day 5	Day 1	Day 5
C_{max} (ng/mL)	3815.0 (32.7)	4623.3 (18.2)	6230.0 (14.5)	5183.3 (19.6)
T_{max} (h)	0.75 (0.75, 1.50)	0.75 (0.50, 1.50)	0.75 (0.75, 1.50)	1.50 (1.50, 3.00)
C_{last} (ng/mL)	443.3 (22.5)	532.8 (17.0)	171.0 (31.2)	10.2 (14.2)
T_{last} (h)	11.92 (11.92, 11.92)	11.92 (11.92, 11.92)	23.92 (23.92, 23.92)	96.0 (72.00, 96.00)
$AUC_{0\text{-}12h/0\text{-}24h/tau}$ (h•ng/mL)[b]	15,902.3 (18.2)	21,481.5 (19.3)	31,968.8 (11.6)	35,700.6 (11.6)
$C_{12h/24h/tau}$ (ng/mL)[c]	443.3 (22.5)	532.8 (17.0)	171.0 (31.2)	158.0 (29.5)
$t_{1/2}$ (h)	—	—	5.09 (4.73, 5.15)	17.18 (12.77, 18.78)

%CV = percentage coefficient of variation; BID = twice daily; CSR = clinical study report; MAD = multiple ascending dose; ODV = obeldesivir (GS-5245); PK = pharmacokinetic(s); Q1 = first quartile; Q3 = third quartile; QD = once daily a Data presented as mean (%CV), except for T_{max}, T_{last}, and $t_{1/2}$ as median (Q1, Q3).
b $AUC_{0\text{-}12h}$ and $AUC_{0\text{-}24h}$ are presented on Day 1 for BID and QD, respectively. AUC_{tau} is presented on Day 5 for both. c C_{12h} and C_{24h} are presented on Day 1 for BID and QD, respectively. C_{tau} is presented on Day 5 for both.
Source: Study GS-US-611-6248 CSR, Table 15.10.1.2.2

5.1.1.3.2. Urine

The GS-441524 metabolite renal excretion rate (CL_r) was consistent across all single doses studied (100 mg to 1600 mg) and ranged from 144 to 189 mL/min. Approximately 41% to 50% of the ODV dose was recovered in urine as GS-441524 sampled between 96 to 120 hours postdose (within the linear dose range of 100 to 900 mg ODV).

5.1.1.3.3. Peripheral Blood Mononuclear Cells

The activation of GS-441524 to the pharmacologically active intracellular triphosphate metabolite (GS-443902) was assessed in PBMCs. The median T_{max} ranged from 12 to 24 hours postdose and the median $t_{1/2}$ was approximately 21 to 70 hours across all single doses studied.

5.1.1.3.4. Food and Formulation Effect

In the food and formulation effect part of the study, administration of ODV with a high-fat meal increased the plasma GS-441524 T_{max} from 0.75 to 3.0 hours but had no effect on its overall plasma exposure (C_{max} and AUC) (Table 47). The Phase 1 and Phase 3 formulations demonstrated similar PK at the 500 mg twice daily dose and 500 mg single dose (Table 47 vs Day 1 in Table 46).

Table 47. GS-US-611-6248 (Food Effect Cohorts): Plasma Pharmacokinetic Parameters of GS-441524 Following Single Dose Administration of ODV (GS-441524 PK Analysis Set)

	Food Effect (500 mg ODV Phase 3 Formulation)

PK Parameter[a] Mean (%CV)	Fasted (Cohort 8) (N = 11)	Fed (Cohort 9) (N = 11)	%GLSM Ratio (90%CI)
C_{max} (ng/mL)	3596.4 (33.3)	3426.4 (35.3)	94.06 (73.22, 120.83)
T_{max} (h)	0.75 (0.75, 1.53)	3.0(3.00, 4.05)	—
C_{last} (ng/mL)	11.9 (27.6)	15.6 (28.2)	—
T_{last} (h)	48.0 (48.00, 48.08)	48.0 (36.27, 48.00)	—
AUC_{last} (h•ng/mL)	20,733.9 (18.0)	22,969.7 (13.5)	111.83 (98.25, 127.28)
AUC_{inf} (h•ng/mL)	20,857.7 (17.9)	23,104.4 (13.4)	111.81 (98.28, 127.20)
AUC_{exp} (%)	0.6 (46.7)	0.6 (45.1)	—
$t_{1/2}$ (h)	6.30 (5.45, 7.09)	6.23 (4.69, 6.49)	—

%CV = percentage coefficient of variation; ANOVA = analysis of variance; CSR = clinical study report; GLSM = geometric least-squares mean; ODV = obeldesivir (GS-5245); PK = pharmacokinetics; Q1 = first quartile; Q3 = third quartile
a Data presented as mean (%CV), except for T_{max}, T_{last}, and $t_{1/2}$ as median (Q1, Q3).
Mixed model covariance parameter estimates: rMSE = square root of mean squared error.
ANOVA model includes fasting status as a fixed effect.
Source: Study GS-US-611-6248 CSR, Table 15.10.1.2.2, Table 15.10.1.3.7

5.1.1.4. Safety Results

Administration of ODV or placebo was safe and well tolerated.

Adverse events were reported for 14 of 70 (20%) participants. Most AEs were Grade 1. There was 1 Grade 2 AE (vertigo not attributed to study drug), and no Grade 3 or higher AEs.

The only AEs reported in more than 1 participant were headache (4 participants, 3 who received ODV, 1 who received placebo), rash (2 participants who both received ODV), and contact dermatitis (2 participants, 1 each who received ODV or placebo). Three of the 4 Grade 1 headaches were the only AEs considered to be related to study drug; observed in 1 of 6 (16.7%) participants in Cohort 5 (500 mg twice daily for 5 days), 1 of 4 (25%) participants in the pooled placebo group for the multiple ascending doses, and in 1 of 11 (9.1%) participants in Cohort 8 (500 mg Phase 3 formulation, single dose in the fasted state).

There were no SAEs, AEs leading to premature discontinuation of study drug, pregnancies, or deaths.

The majority of laboratory abnormalities were Grade 1 or 2 in severity. The most frequently reported graded laboratory abnormality was Grade 2 decreased creatinine clearance (there is no Grade 1 for this abnormality in the Division of AIDS [DAIDS] Scale Version 2.1), which was reported for 21 of 70 participants (30.0%) overall, 3 of 12 (25.0%) in the participants who received placebo, and 18 of 58 (31.0%) in the participants who received ODV; 1 of 6 participants (16.7%) in Cohort 2 (300 mg single dose in the fasted state), 2 of 6 participants (33.3%) in Cohort 3 (900 mg single dose in the fasted state), 6 of 6 participants (100.0%) in Cohort 4 (1600 mg single dose in the fasted state), 2 of 6 participants (33.3%) in Cohort 6 (900 mg once daily for 5 days in the fasted state), 2 of 11 participants (18.2%) in Cohort 8 (500 mg Phase 3 formulation,

single dose in the fasted state), 5 of 11 participants (45.5%) in Cohort 9 (500 mg Phase 3 formulation, single dose in the fed state). Creatinine clearance decreases were generally transient and returned to baseline levels. There was only 1 Grade 3 or higher laboratory abnormality: 1 participant in Cohort 2 (300 mg single dose) who experienced a lipase elevation at Day 3 which returned to normal levels at Day 5. No Grade 4 laboratory abnormalities were reported in any cohort.

There were no clinically relevant changes in vital signs, ECGs, or ophthalmologic examinations reported during the study.

5.1.1.5. Conclusions

The conclusions from Study GS-US-611-6248 are as follows:

- Obeldesivir administered as the Phase 1 or Phase 3 formulation was safe and well tolerated with comparable PK.
- Administration of ODV resulted in quantifiable and robust plasma exposures to GS-441524 metabolite, with low or no detectable prodrug itself.
- GS-441524 plasma PK was linear and dose-proportional with similar terminal phases across 100 mg to 900 mg doses.
- Efficient intracellular activation of GS-441524 to the active triphosphate metabolite GS-443902 was observed.
- Administration of ODV with a high-fat meal increased the plasma GS-441524 T_{max} from 0.75 to 3.0 hours compared to fasting but had no effect on GS-441524 plasma exposure (C_{max} and AUC).

5.1.2. Clinical Study GS-US-611-6408 (Human Absorption, Distribution, Metabolism, and Excretion Study)

5.1.2.1. Study Design

Study GS-US-611-6408 was a single-center, open-label, mass-balance Phase 1 study of ODV administered as a single oral dose of mixture of unlabeled and radiolabeled [^{14}C]-ODV in healthy participants. The purpose of this study was to evaluate the PK, metabolism, and excretion of

ODV following a single oral dose of 500 mg ODV containing a mixture of unlabeled and radiolabeled [^{14}C]-ODV in healthy male participants. The study was conducted at a single center in the US.

5.1.2.2. Participant Disposition and Demographics

A total of 6 participants were enrolled in the study. All 6 participants were administered study drug and completed the study. All 6 participants were assigned male at birth; 3 Black, 2 White, and 1 Asian. The median age was 34 years (range: 28 to 46 years).

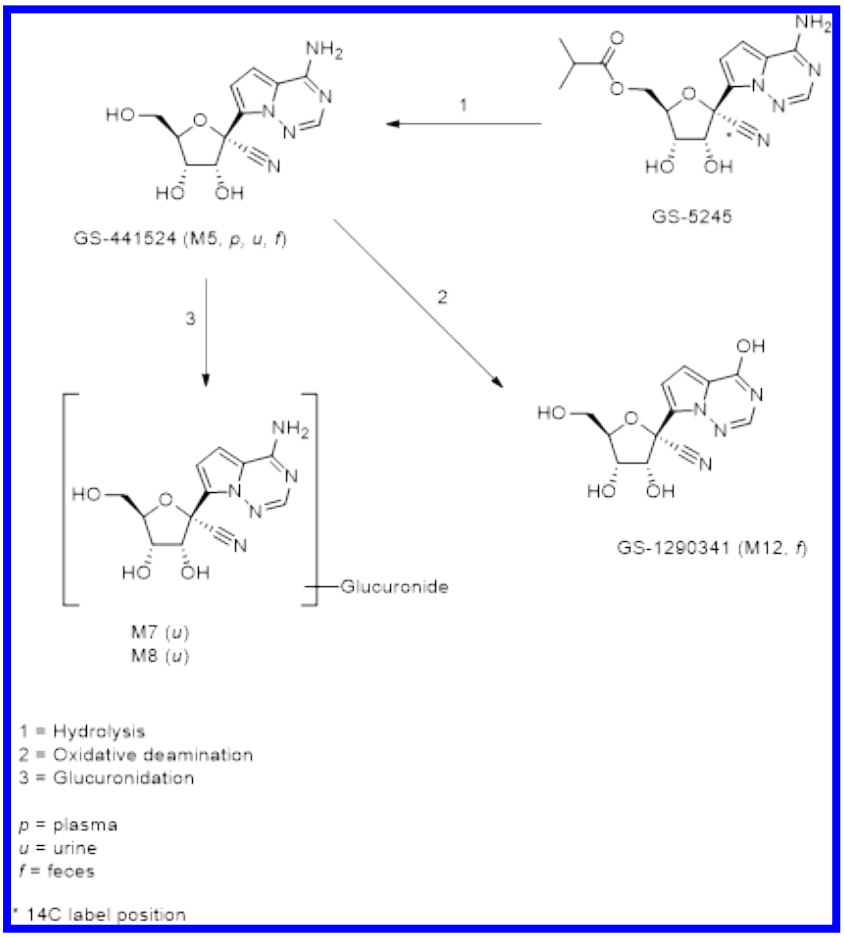
5.1.2.3. Pharmacokinetic Results

The mass-balance study demonstrated that the total cumulative combined mean (%CV) recovery of [^{14}C]-radioactivity in urine and feces was 90.7% (3.1%), with most of the radioactive dose recovered from urine (58.5% [6.1%]) and with 32.2% (17.4%) recovered from feces.

The predominant species detected in urine were [^{14}C]-M5 (GS-441524, hydrolysis of ODV) (> 95%), followed by [^{14}C]-M7 (GS-441524–glucuronide-1) and [^{14}C]-M8 (GS-441524–glucuronide-2), each accounting for < 1% of total radioactive dose. In feces, the abundance of [^{14}C]-M5 (GS-441524) and [^{14}C]-M12 (GS-1290341, oxidative-deamination of M5) metabolites varied across individual participants, each accounting for means of 11.6 and 16.6% of the administered radioactive dose with %CV of 10.2% and 6.4%, respectively.

The proposed human biotransformation pathways of ODV are shown in Figure 21.

Figure 21. GS-US-611-6408: Proposed Biotransformation Pathways for Metabolism of ODV in Humans



ODV = obeldesivir (GS-5245)

The totality of the data on ODV plasma metabolite profiling indicated that systemic exposure was almost exclusively attributable to GS-441524, the major metabolite of ODV, at all times postdose.

The mean whole blood-to-plasma concentration ratio of [^{14}C]-radioactivity through 24 hours

ranged from 1.2 to 1.4, indicating slight association of total radioactivity with blood cells.

The plasma and urine PK of GS-441524 was consistent with previous studies in healthy participants.

The human biotransformation pathways of ODV were consistent with the known nonclinical profile of ODV.

5.1.2.4. Safety Results

There were no clinically relevant safety findings in this study. No AE was considered related to study drug by the investigator. No deaths or SAEs occurred during this study, and no participant discontinued the study due to an AE.

Five of 6 participants (83.3%) experienced at least 1 laboratory abnormality and 1 participant had a Grade 3 laboratory abnormality. There were no clinically relevant changes from baseline in median values for any hematology or chemistry parameter assessed in this study. One participant had a Grade 3 laboratory abnormality of decreased creatinine clearance (Grade 0 at screening) considered unrelated to ODV by the investigator and the abnormality improved by the next day.

No clinically relevant changes in vital signs or clinically significant ECG results were observed during this study.

5.1.2.5. Conclusions

The conclusions from Study GS-US-611-6408 are as follows:

- Following oral administration of ODV, the predominant species circulating in plasma was GS-441524 and the primary route of elimination was renal excretion.
- Following administration of [^{14}C]-ODV, mean total recovery of the radioactive dose was 90.7%, consisting of approximately 58.5% and 32.2% recovered in urine and feces, respectively.
- A single oral dose of 500 mg ODV, containing a mixture of unlabeled and radiolabeled [^{14}C]-ODV, was generally safe and well tolerated in healthy male participants with no clinically relevant safety findings.

6. ~~5.~~MARKETING EXPERIENCE

No application for marketing approval or registration has been made for ~~GS-5245~~ODV.
There is no previous marketing experience with ~~GS-5245~~ODV.

7. ~~6.~~SUMMARY OF DATA AND GUIDANCE FOR THE INVESTIGATOR

7.1. ~~6.1.~~Summary of Data

7.1.1. 6.1.1.Nonclinical Findings

7.1.1.1. 6.1.1.1.Nonclinical Pharmacology of GS-5245ODV

GS-5245Obeldesivir and GS-441524 are potent against SARS-CoV-2 in the A549-hACE2 transformed lung alveolar epithelial line, with EC_{50} values of 1.27 and 2.10 µM after 48 hours of treatment, respectively. In uninfected A549-hACE2 cells, the CC_{50} values of GS-5245ODV and GS-441524 after 48 hours of treatment were each > 50 µM, resulting in SIs of > 39 and > 23.8, respectively. In a 24-hour antiviral assay in primary cultures of NHBE cells, GS-5245ODV inhibited SARS-CoV-2 with an EC_{50} value of 0.339 µM, while GS-441524 inhibited SARS-CoV-2 in these cultures with an EC_{50} value of 2.45 µM.
GS-5245 and Combinations of GS-441524 inhibited RSV in NHBE cultures with EC_{50} values of 0.410 and 2.68 µM after 72 hours of treatment, respectively. The CC_{50} of GS-5245or ODV with either molnupiravir, nirmatrelvir, and/or ritonavir all demonstrate additive effects against SARS-CoV-2 in NHBEA549-hACE2 cells after a 72-hour treatment was 30.3 µM (SI = 74.0), and the CC_{50} of GS-441524 in these cultures was > 50 µM (SI > 18.6). No antagonistic in vitro DDIs were observed between ODV or GS-441524 and molnupiravir, nirmatrelvir alone, or nirmatrelvir and ritonavir.

The cytotoxicity profile of GS-5245ODV in a panel of diverse human cell types was similar to its effects in lung-derived cell cultures, with CC_{50} values ranging from 18.6 to > 44 µM. Cytotoxicity of GS-441524 in rapidly dividing MT-4 lymphoblast T cells was observed with a CC_{50} value of 69.3 µM but was not detected in other primary cultures at the highest concentrations tested, which ranged from 44 to 100 µM.

Biochemical studies demonstrate that the nucleoside triphosphate GS-443902 acts as an analog of ATP and competes with the natural ATP substrate, selectively inhibiting coronavirus viral RdRps by 2 mechanisms of action. One mechanism of inhibition is the utilization of the modified nucleoside triphosphate GS-443902 as a substrate by the viral RdRp during nascent RNA strand synthesis, which results in delayed (i+3) RNA chain termination during replication of the viral RNA. Under physiological conditions of natural nucleoside triphosphates, the SARS-CoV-2 RdRp uses GS-443902 as a substrate and incorporates the nonnatural NMP but does not terminate strand synthesis, which results in a daughter strand containing the nonnatural NMP. When the RdRp encounters an incorporated nonnatural NMP while reading the daughter strand template, it is unable to pair a native uridine triphosphate with the incorporated nonnatural NMP due to a steric clash between the SARS-CoV-2 RdRp Ala-558 and the 1'-CN group of the modified NMP, halting nascent strand synthesis.

Obeldesivir retains potent antiviral activity against SARS-CoV-2 Omicron subvariants (B.1.1.529/BA.1, BA.2, BA.2.12.1, BA.4, BA.5, BA.2.75, BA.4.6, BF.5, XBB, and BQ.1.1), with EC_{50} fold-changes of 0.26 to 1.33 compared to reference strain WA1.

In vitro SARS-CoV-2 resistance selection in the presence of either GS-441524 or RDV resulted in substitutions in nsp12 (RdRp) after multiple passages of the virus. The selected variants had up to a 10.4-fold loss in susceptibility to GS-441524 and RDV. Together, these results suggest a

high barrier to resistance for the GS-441524 prodrug GS-5245ODV during treatment of SARS-CoV-2 infection.

In a mouse model of SARS-CoV-2 infection, GS-5245, ODV reduced infectious virus titers of

an early strain of SARS-CoV-2 in mouse lung homogenates in a dose-dependent manner, with maximal virus titer reductions in all mice treated with 30 mg/kg GS-5245ODV at 12 hpi. Mean body weights, pulmonary function, and healthy lung physiology of mice infected with SARS-CoV-2 were maintained in the 10 and 30 mg/kg twice-daily GS-5245ODV dose groups when treatment was initiated at 12 hpi.
GS-5245 In a separate study, the combination of ODV and the SARS-CoV-2 main protease inhibitor PF-07321332 (nirmatrelvir; Paxlovid active component) reduced viral lung titers of SARS-CoV-2 in mice significantly more than each drug alone. Furthermore, ODV reduced replication of the highly transmissible SARS-CoV-2 BA.1 variant in mouse lungs.

Obeldesivir demonstrated effective antiviral responses at doses 10 to 20 mg/kg twice daily and 20 to 40 mg/kg once daily in the ferret model. Overall, based on the assessments of viral titer in both the nasal lavage and nasal turbinates, the 20 mg/kg once-daily dosing regimen was most similar in efficacy to molnupiravir dosed at 5 mg/kg twice daily. Optimal efficacy was observed with GS-5245ODV dosed either at 20 mg/kg twice daily or 40 mg/kg once daily, both of which were superior to molnupiravir.

In the AGM model of SARS-CoV-2, once daily treatment with either 60 or 120 mg/kg ODV beginning at 8 hpi for 6 doses resulted in statistically significant decreases in infectious viral loads in the BALF throughout infection. Viral load reductions were dose-dependent, with larger reductions of infectious viral load the group receiving 120 mg/kg ODV. Similarly, genomic RNA loads in BALF of ODV-treated animals were also found to be significantly reduced at nearly all study time points assessed. Significant reductions in SARS-CoV-2 infectious and RNA loads were observed from the throat swabs at 1, 2, and 6 dpi from both dose groups, with additional reductions in viral RNA at 4 dpi in animals dosed with 120 mg/kg ODV. In both throat and nasal swabs, no detectable infectious virus was observed for any animal in either group receiving ODV after 4 dpi. Both 60 and 120 mg/kg ODV doses significantly reduced viral RNA levels in 5 out of 6 respiratory tissues evaluated. These data demonstrate that oral ODV at 60 and 120 mg/kg was highly efficacious at reducing infectious virus and viral RNA loads throughout the upper and lower respiratory tract of AGMs.

A SARS-CoV-2 efficacy study in AGMs demonstrated that once-daily IV delivery of 20 mg/kg parent nucleoside GS-441524 beginning at 8 hpi for 6 doses demonstrated significant reductions of BALF genomic RNA and infectious virus that was comparable to RDV IV. In terminal lung tissue genomic RNA analysis, GS-441524 showed significant effects in the lower and upper lung, and mainstem and lower bronchus samples that were superior to RDV IV. Daily oral treatment with GS-621763, a tri-isobutyryl ester tool prodrug of GS-441524, at either 60 mg/kg or 120 mg/kg, significantly reduced SARS-CoV-2 levels in the lower airways, as early as 1 to 2 dpi.

MolecularOff target screening studies with GS-5245ODV and GS-441524 showed no responses (> 50% inhibition of ligand binding) at 10 µM.

7.1.1.2. 6.1.1.2. Nonclinical Pharmacokinetics of GS-5245ODV

GS-5245Obeldesivir is extensively hydrolyzed both presystemically and systemically to GS-441524. GS-5245Obeldesivir is being developed with the intent to deliver consistent systemic exposures of GS-441524 following oral administration that are consistent with SARS-CoV-2 efficacy in nonclinical models.

The plasma PK profiles following oral administration of GS-5245ODV across species consistently show very low systemic levels of intact GS-5245ODV, ranging from undetectable in rodents to transiently low micromolar levels in dog, that are orders of magnitude lower than GS-441524, confirming extensive metabolism during absorption and first-pass metabolism. Compared with GS-441524, GS-5245ODV has improved intestinal absorption and delivers a higher amount of GS-441524 with less variability into systemic circulation. Plasma exposure of GS-441524 after oral administration was either maintained or improved by the ester prodrug across all species tested.

GS-5245Obeldesivir is metabolically unstable, while in comparison, GS-441524 is stable across species including human, in all biological matrices tested. In vitro, GS-441524 demonstrated ability to penetrate cells and generate GS-443902 in a variety of lung cell types, while in vivo, GS-443902 levels were measured in gross lung tissue of ferrets and AGM following IV administration of GS-441524.

Following IV administration of GS-441524 to dogs, nearly 100% of the administered dose was eliminated by renal excretion as intact GS-441524. Both GS-5245ODV and GS-441524 have low potential for DDIs with either the human CYP enzymes or transporters.

7.1.1.3. 6.1.1.3. Nonclinical Safety of GS-5245ODV

Safety pharmacology studies were conducted to examine the potential effects of GS-5245ODV on the cardiovascular system, respiratory system, and CNS after oral administration. In rats, there were no effects on respiratory rate or tidal volume, although overall respiration (ie, minute volume) was increased at 200 and 500 mg/kg. GS-5245ODV had no effects on the CNS of rats at 500 mg/kg. In dogs, oral dosing of GS-5245ODV increased heart rate and body temperature, with compensatory decreases in blood and pulse pressures at 300 mg/kg; there were no effects at 100 mg/kg. Taken together, the risk for cardiovascular, respiratory, or CNS effects in the clinic is considered low.

Early mortality/morbidity was noted in both rat and dog 14-day repeat-dose studies, with females in both species more sensitive than males. In female rats and dogs, early mortality was attributed to GS-5245ODV-related acute renal failure due to crystal formationformations of GS-441524–containing crystals in the renal papilla and associated tubular degeneration and necrosis. In dogs, mortality/morbidity was also attributed to GI (female only) and/or pancreatic (male and female) changes. The target organs identified were the kidney in both rats and dogs, and the pancreas, GI system, eyes, and liver in dogs. Changes in the pancreas in dogs consisted of acinar (exocrine) degeneration/necrosis. Gastrointestinal changes consisted of ulceration (esophagus and stomach), epithelial degeneration (esophagus, stomach, and duodenum), and/or intestinal neutrophilic inflammation. Changes in dogs were also observed in the eyes (anterior uvea inflammation, corneal edema, and endothelial degeneration) and liver (vascular/perivascular neutrophilic inflammation and/or infiltration). GS-5245 study participants will be monitored for these events as specified in the protocol for each clinical studyIn the 28-day studies, there were no unexpected toxicities or evidence of cumulative toxicity; GS-441524 exposure margins at the NOAELs in the 28-day

studies were 4.3- to 8-fold higher in female and male rats, respectively, and 3.1-fold higher in dogs compared to projected exposures at the 350 mg twice daily human dose.

In embryo-fetal development studies, the effects of GS-5245ODV have been assessed in rats and rabbits that received oral administration of GS-5245ODV during organogenesis. Administration of GS-5245ODV to pregnant rabbits was associated with an increase in postimplantation loss and visceral malformations primarily related to the development of the heart, blood vessels, and liver. Preliminary findings indicated no remarkable GS-5245There were no ODV-related effects on embryo-fetal development in pregnant rats, no adverse effects on fertility and early embryonic development, and no effects on prenatal and postnatal development in rats. At the NOAELs for embryo-fetal development, AUC exposures in rats and rabbits were approximately 56- and 1.82.1-fold higher, respectively, than predicted steady-state exposure in humans at the projected clinically efficacious 350 mg twice daily dose of GS-5245. GS-5245ODV. In the fertility study, exposures at the NOAELs for reproductive effects were approximately 6- to 7-fold higher than projected at the 350 mg twice daily dose. In the prenatal and postnatal development study, exposures at the NOEL were approximately 3-fold higher than projected at the 350 mg twice daily dose

Obeldesivir was nonmutagenic in the bacterial reverse mutation assay, negative in the chromosome aberrations assay with human lymphocytes, negative in the rat micronucleus assay, and is not considered genotoxic. Obeldesivir is not considered a phototoxic risk.

7.1.2. 6.1.2.Clinical Findings

As of 22 July 2022, there is 1 on-going Phase 1 (first-in-human) clinical study (Study GS-US-611-6248).12 May 2023, 2 Phase 1 clinical studies have been completed in which 64 healthy participants have been dosed with ODV.

Final results from Studies GS-US-611-6248 and GS-US-611-6408 indicate ODV is generally safe and well tolerated at a single dose of 100 to 1600 mg (Studies GS-US-611-6248 and GS-US-611-6408) and multiple doses of 500 mg twice daily or 900 mg once daily for 5 days (Study GS-US-611-6248).

In Study GS-US-611-6248, the most frequently reported graded laboratory abnormality was Grade 2 decreased creatinine clearance (there is no Grade 1 for this abnormality in the DAIDS Scale V2.1), which was reported for 21 of 70 participants (30.0%) overall, 3 of 12 (25.0%) in the participants who received placebo, and 18 of 58 (31.0%) in the participants who received ODV; 1 of 6 participants (16.7%) in Cohort 2 (300 mg single dose in the fasted state), 2 of 6 participants (33.3%) in Cohort 3 (900 mg single dose in the fasted state), 6 of 6 participants (100.0%) in Cohort 4 (1600 mg single dose in the fasted state), 2 of 6 participants (33.3%) in Cohort 6 (900 mg once daily for 5 days in the fasted state), 2 of 11 participants (18.2%) in Cohort 8 (500 mg Phase 3 formulation, single dose in the fasted state), 5 of 11 participants (45.5%) in Cohort 9 (500 mg Phase 3 formulation, single dose in the fed state). Creatinine clearance decreases were generally transient and returned to baseline levels. There was only 1 Grade 3 or higher laboratory abnormality: 1 participant in Cohort 2 (300 mg single dose) who experienced a lipase elevation at Day 3 which returned to normal levels at Day 5. No Grade 4 laboratory abnormalities were reported in any cohort.

One participant in Study GS-US-611-6408 had a Grade 3 laboratory abnormality of decreased creatinine clearance (Grade 0 at screening) considered unrelated to ODV by the investigator and the abnormality improved by the next day.

Overall, no other clinically relevant consistent patterns of laboratory abnormalities or changes from baseline in laboratory parameters were noted during the studies. No patterns of clinically relevant changes in vital signs or shifts in 12-lead ECGs were observed during the studies.

Following single-dose administration of ODV at doses of 100, 300, 900, or 1600 mg under fasted conditions (Study GS-US-611-6248), dose-proportional increases in the GS-441524 plasma PK parameters (AUC_{last}, AUC_{inf}, and C_{max}) were observed across the dose range of 100 to 900 mg, and less than dose-proportional increase between the 900 mg and 1600 mg doses. The median T_{max} of GS-441524 in plasma was 0.75 hours postdose, and the median $t_{1/2}$ ranged from 6 to 7 hours across the 100 to 900 mg single doses.

Following ODV (500 mg) administration twice daily for 5 days under fasted conditions, accumulation of GS-441524 in plasma (approximately 35% based on AUC values) was observed and reached steady state at 5 days. Minimal (approximately 12% based on AUC values) accumulation of GS-441524 was observed after once-daily dosing.

Administration of ODV with a high-fat meal increased the plasma GS-441524 T_{max} from 0.75 to 3.0 hours but had no effect on its overall plasma exposure (C_{max} and AUC). The Phase 1 and Phase 3 formulations demonstrated similar PK.

The mass-balance study (Study GS-US-611-6408) demonstrated that the total cumulative combined mean (%CV) recovery of [^{14}C]-radioactivity in urine and feces was 90.7% (3.1%), with most of the radioactive dose recovered from urine (58.5% [6.1%]) and with 32.2% (17.4%) recovered from feces.

The totality of the data on ODV plasma metabolite profiling indicated that systemic exposure was almost exclusively attributable to GS-441524, the major metabolite of ODV, at all times postdose.

The plasma and urine PK of GS-441524 was consistent with previous studies in healthy participants.

7.2. ~~6.2.~~Guidance for Investigators

7.2.1. ~~6.2.1.~~Indication(s)

~~GS-5245~~Obeldesivir is being developed for the treatment of ~~CoV diseases~~coronavirus disease.

7.2.2. ~~6.2.2.~~Dosage and Administration

~~GS-5245~~Obeldesivir is available as 100~~-~~ mg, 175 mg, 350~~-~~ mg, and 500~~-~~ mg strength tablets based on ~~GS-5245~~ODV content. ~~GS-5245~~Obeldesivir tablets are to be administered orally.

7.2.3. ~~6.2.3.~~Contraindications

~~GS-5245~~Obeldesivir is contraindicated in participants with previously demonstrated hypersensitivity to any of the components of the ~~products~~product and in participants who develop resistance to any of the components.

7.2.4. ~~6.2.4.~~Warnings and Precautions

Currently there are no warnings or precautions for the use of GS-5245ODV. No previous investigational experience elucidating the clinical safety profile of GS-5245ODV is available at this time. Therefore, GS-5245ODV should only be administered according to clinical study protocol-specified monitoring.

7.2.5. 6.2.5. Adverse Drug Reactions

This section refers to events considered to be adverse drug reactions (ADRs) to GS-5245ODV at the current stage of development. For the purposes of this investigator's brochureIB, ADRs are those deemed by the sponsor to be causally related to the drug. Of ADRs provided in this section, those that are serious adverse reactions and considered expected for the purpose of global regulatory reporting for GS-5245ODV are provided in the reference safety informationRSI in Appendix 8.19.1 of this investigator's brochureIB.

Laboratory Changes:

In a blinded study in healthy volunteers (Study GS-US-611-6248), preliminary, in healthy volunteers, data showed transiently decreased creatinine clearance (Grade 2) reported across cohorts, most prominently (6 of 8 participants) at the supratherapeutic dose of 1600 mg, which was reversible.

7.2.6. 6.2.6. Other Important Safety Information

7.2.6.1. Important Identified and Potential Risks

No important identified risks have been identified.

Important potential risks for GS-5245ODV that are under monitoring by Gilead are as follows:

- Renal dysfunction
- Pancreatitis
- Gastrointestinal ulceration
- Uveitis
- Embryo-fetal toxicity

7.2.7. 6.2.7. Drug Interactions and Other Forms of Interactions

NoA clinical DDI studies havestudy has been conducted with GS-5245ODV but no final report is available at this time.

7.2.8. 6.2.8. Overdosage

There is no known antidote for GS-5245ODV. In the case of overdose, the study participants should receive standard treatment for overdose and supportive therapy based on the participant's signs and symptoms.

7.2.9. 6.2.9.Pregnancy, Contraception, and Lactation

7.2.9.1. 6.2.9.1.Pregnancy and Contraception

GS-5245Obeldesivir has not been studied in pregnant women. In animal reproduction studies, GS-5245ODV has been shown to have adverse effects on embryo-fetal development, including teratogenic effects, (Section 4.3.5) when administered at exposures similar to what wasis being studied in the development program (Section 3.3.5). Therefore, in GS-5245ODV clinical studies, women of childbearing potential should be advised of the risk of fetal harm if GS-5245ODV is taken during pregnancy. If enrolling into studies with GS-5245ODV, women of childbearing potential must have a negative pregnancy test result, and should use highly effective contraception from enrollment until 2 weeks after stopping treatment with GS-5245. Pregnancy tests during treatment with GS-5245 are recommended as clinically indicatedand pregnancy testing are required in accordance with protocol guidance. Women of childbearing potential should be advised to contact their physician immediately if they become pregnant or suspect they may be pregnant. Other pregnancy and contraception details are specified in the protocol.

The risk of fetal harm from exposure in female sexual partners of men taking GS-5245ODV is unknown.

7.2.9.2. 6.2.9.2.Lactation

It is not known whether GS-5245ODV is secreted in human milk. Therefore, breastfeeding (nursing) women should be excluded from GS-5245ODV clinical studies.

7.2.10. 6.2.10.Effect on Ability to Drive and Use Machines

No studies on the effects of GS-5245ODV on the ability to drive and use machines have been performed.

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9. 8.APPENDICES

9.1. 8.1.Reference Safety Information for Assessment of Expectedness of Serious Adverse Reactions

The RSI includes a list of expected serious adverse reactions (SARs) to GS-5245 used by the sponsor to assess expectedness for global regulatory reporting of suspected unexpected serious adverse reactions (SUSARs). Fatal and life-threatening adverse reactions are always regarded as unexpected. The RSI does not present a comprehensive overview of the safety profile of GS-5245; a summary of the safety profile of GS-5245 is presented in Section ~~6~~7 Summary of Data and Guidance for the Investigator of the IB.

No SARs are considered expected by the sponsor for the purpose of expedited reporting of SUSARs and for identification of SUSARs in the Cumulative Summary Tabulation of Serious Adverse Reactions in the Development Safety Update Report (DSUR) for GS-5245.

9.2. 8.2.

Gilead Study No. (CRO Study No.)	Title/Description	Species/Strain	Duration of Dosing and Route	Dose (mg/kg)	Major Findings
TX-611-2001 (Charles River 00604533)	Effect of GS-5245 on central nervous system of rats	Rat, Crl:WI(Han) 10 females/group	Single dose, oral gavage	0[a], 200, 500	None NOEL = 500 mg/kg
TX-611-2001 (Charles River 00604533)	Effect of GS-5245 on respiratory system of rats	Rat, Crl:WI(Han) 10 males/group	Single dose, oral gavage	0[a], 200, 500	Slight increase in overall ventilation as measured by minute volume from 0 to 2 hours postdose at ≥ 200 mg/kg NOEL not determined
PC-611-2004 (Charles River 00604535)	Effect of GS-5245 on cardiovascular system of dogs	Dog, beagle 4 males/group	Single dose (Latin Square Design), oral gavage	0[a], 30, 100, 300	Increased heart rate (≤ 18.0 beats per minute) and body temperature (≤ 0.59 °C), and compensatory decreases in systolic (≤ 10.9 mm Hg), diastolic (≤ 5.5 mm Hg), mean arterial pressure (≤ 7 mm Hg), and pulse pressure (≤ 5.6 mm Hg) at 300 mg/kg NOEL = 100 mg/kg
PC-611-2005 (Charles River 210517.HJT)	Effect of GS-5245 on hERG channel currents	HEK293 cells expressing hERG potassium channels	In vitro	GS-5245: 0, 30, and 300 µM	IC_{50} > 300 µM
PC-399-2025 (non-GLP)	Effect of GS-441524 on the hERG Channel	CHO-hERG DUO cells (CHO cells stably expressing the hERG channel)	In vitro	GS-441524: 0 and 30 µM	IC_{50} > 30 µM

9.3. Tabular Summary of Nonclinical Safety Studies

Appendix Table 1. Summaries of GLP Safety Pharmacology Studies

Gilead Study No. (CRO Study No.)	Title/Description	Species/Strain	Duration of Dosing and Route	Dose (mg/kg)	Major Findings

TX-611-2001 (Charles River 00604533)	Effect of ODV on central nervous system of rats	Rat, Crl:WI(Han) 10 females/group	Single dose, oral gavage	0[a], 200, 500	None NOEL = 500 mg/kg
TX-611-2001 (Charles River 00604533)	Effect of ODV on respiratory system of rats	Rat, Crl:WI(Han) 10 males/group	Single dose, oral gavage	0[a], 200, 500	Slight increase in overall ventilation as measured by minute volume from 0 to 2 hours postdose at ≥ 200 mg/kg NOEL not determined
PC-611-2004 (Charles River 00604535)	Effect of ODV on cardiovascular system of dogs	Dog, beagle 4 males/group	Single dose (Latin Square Design), oral gavage	0[a], 30, 100, 300	Increased heart rate (≤ 18.0 beats per minute) and body temperature (≤ 0.59 °C), and compensatory decreases in systolic (≤ 10.9 mm Hg), diastolic (≤ 5.5 mm Hg), mean arterial pressure (≤ 7 mm Hg), and pulse pressure (≤ 5.6 mm Hg) at 300 mg/kg NOEL = 100 mg/kg
PC-611-2005 (Charles River 210517.HJT)	Effect of ODV on hERG channel currents	HEK293 cells expressing hERG potassium channels	In vitro	ODV: 0, 30, and 300 µM	IC_{50} > 300 µM
PC-399-2025 (non-GLP)	Effect of GS-441524 on the hERG Channel	CHO-hERG DUO cells (CHO cells stably expressing the hERG channel)	In vitro	GS-441524: 0 and 30 µM	IC_{50} > 30 µM

Crl:WI(Han) = Wistar Han; CHO = Chinese hamster ovary; CRO = contract research organization; GLP = Good Laboratory Practice; HEK293 = human embryonic kidney 293 cell line; hERG = human ether-a-go-go-related gene; IC_{50} = half-maximal inhibitory concentration; NOEL = no observed effect level; ODV = obeldesivir (GS-5245)
a Vehicle: 0.5% Methocel A4M Premium in deionized water.

Appendix Table 2. Summaries of Repeat-Dose Toxicity Studies

Species/Strain	Method of Administration	Duration of Dosing	Dose (mg/kg/day)	Sex, No. per Group	NOAEL (mg/kg/day)	GLP[a]	Notable Findings	Gilead Study No. (CRO Name and Study No.)

Rat/ Crl:WI(Han)	Oral gavage	14 days	0, 200, 500, 1000[b]	10 male/ 10 female	200 in females 500 in males	Yes	200 mg/kg: minimal changes in hematology consistent with inflammation; mildly ↓TRIG 500 mg/kg: Asas above, plus mortality in 1 F on Day 15 attributed to acute kidney injury (marked changes in clinical chemistry with crystal formation in renal papilla associated with tubular degeneration/ inflammation); Clinical observations (abnormal breathing sounds, erected/stained fur, thin body, hunched posture, cold to touch) ↓BW/FC; Changes in hematology likely due to hemoconcentration correlating to ↓cells in bone marrow attributed to stress response and ↓FC; One F noted with adverse renal changes (crystal in papilla/cortex, tubular degeneration/ regeneration, fibrosis and inflammation in renal pelvis and ureter dilation); secondary, stress-related findings in F only (↓ cellularity in immune tissues, ↓ weights/secretory depletion in prostate/seminal vesicle, crypt degeneration/regeneration in cecum/rectum, and ↑ atretic follicles in ovary) 1000 mg/kg: Asas above, plus mortality (3 F, Days 4 to 7) 7) attributed to acute kidney injury, remaining F euthanized on Day 8; ↓BW/FC; Minimally prolonged aPTT attributed to secondary effect of inflammation, minimal changes in CHOL and GLU associated with ↓FC; Single female with renal changes (acute kidney injury due to crystals in papilla/cortex; as above); secondary, stress-related findings in	TX-611-2001 (Charles River 604532)

							M and F (as above) NOAEL = 200 mg/kg/day in females (GS-441524 AUC_{0-24h} 129,000 ng•h/mL; C_{max} 32,400 ng/mL) and 500 mg/kg/day in males (GS-441524 AUC_{0-24h} 203,000 ng•h/mL; C_{max} 41,400 ng/mL)	

Rat/Crl:WI(Han)	Oral gavage	4 weeks plus 4 weeks recovery	0, 100, 200, 500 (males); 50, 100 and 200 (females)	10 male/ 10 female (main study) and 5/vehicle and high-dose groups (recovery groups)	200 in females 500 in males	Yes	100 mg/kg: no notable findings > 200 mg/kg (males/females): ↓glucose (reversed) 500 mg/kg (males); ↑neutrophils (partially reversed); ↑adrenal weight (reversed) 500 mg/kg (males)/200 mg/kg (females): ↑WBC, monocytes (reversed) NOAEL(males) = 500 mg/kg/day (GS-441524 AUC_{0-24h} 242,000 ng•h/mL; C_{max} 46,600 ng/mL) NOAEL(females) = 200 mg/kg/day (GS-441524 AUC_{0-24h} 128,000 ng•h/mL; C_{max} 43,100 ng/mL)	TX-611-2015 (Charles River 604687)

Dog/beagle	Oral gavage	14 days	0, 30, 100, 300	4 male/ 4 female	30	Yes	30 mg/kg: Nono notable findings 100 mg/kg: ↓BW/FC; Mild to moderate changes in hematology consistent with inflammation; ↓platelets/↑platelet volume; minimally prolonged aPTT; minimal ↓ in TRIG, total protein/albumin and/or globulins; Microscopic changes in pancreas (minimal-to-moderate acinar cell degeneration/necrosis) and liver (minimal perivascular neutrophilic infiltrate); secondary (stress-related) changes in thymus (↓ weights/lymphoid cellularity). 300 mg/kg: Overtovert toxicity, all M/F animals euthanized early by Day 7; morbidity associated with kidney (F), pancreatic (M+F) and/or GI (F) changes; liquid feces, evidence of vomitus, decreased activity, corneal cloudiness ↓BW/↓FC; Mild to moderate changes in hematology (hemoconcentration, correlated with signs of dehydration); mild prolonged aPTT; minimal changes ALP, CHOL, TRIG (F only) and mild change in chloride; Two F with acute kidney injury: up to marked changes in clinical pathology, microscopic findings of crystal formation in renal papilla and secondary changes in renal cortex and pelvis; minimal-to-marked acinar (exocrine) degeneration/necrosis in pancreas; ulceration, epithelial degeneration and/or intestinal neutrophilic inflammation in GI tract; edema, inflammation, and endothelial degeneration in eyes; and neutrophilic inflammation in liver. NOAEL = 30 mg/kg/day (GS-	TX-611-2002 (Charles River 604533)

							441524 AUC_{0-24h} 62,800 ng•h/mL; C_{max} 15,600 ng/mL; sex combined)	

Dog/beagle	Oral gavage	4 weeks plus 4 weeks recovery	0, 25, 50	3/sex and 2/sex/vehicle and high dose groups (recovery groups)	50	Yes	50 mg/kg (females): ↓BW/↓FC (recovered) NOAEL = 50 mg/kg/day (GS-441524 AUC_{0-24h} 94,000 ng•h/mL; C_{max} 19,700 ng/mL; sex combined)	TX-611-2016 (Charles River 604688)

ALP = alkaline phosphatase; aPTT = activated partial thromboplastin time; ~~AUC_{0-24h} = partial area under the concentration versus time curve from 0 to 24 hours~~; BID = twice daily; BW = body weight; CHOL = total cholesterol; ~~C_{max} = maximum observed concentration of drug~~; Crl:WI(Han) = Wistar Han; CRO = contract research organization; F = female; FC = functional class; GI = gastrointestinal; GLP = Good Laboratory Practice; GLU = glucose; M = male; NOAEL = no observed adverse effect level; TRIG = triglyceride
a "Yes" indicates study included a GLP compliance statement.
b Vehicle control and high -dose level (500 mg/kg/dose; total of 1000 mg/kg/day) were administered BID.

Appendix Table 3. Summaries of Genotoxicity Studies

Species/Strain	Method of Administration	Duration of Dosing	Dose	GLP[a]	Notable Findings	Gilead Study No. (CRO Name and Study No.)
Bacterial mutation/ *Salmonella typhimurium, Escherichia coli*	In vitro	2 days	100 to 5000 µg/plate with and without S9[b]	Yes	There was no evidence of mutagenic activity.	TX-611-2003 (Charles River 604548)
Chromosome aberration/Primary human lymphocytes	In vitro	3 to 22 hours	4.17 to 362 µg/mL with and without S9[b]	Yes	Negative for inducing structural aberrations	TX-611-2004 (Charles River 604549)
In vivo micronucleus/ Rat/Crl:WI(Han)[c]	Oral gavage	14 days	0, 200, 500, 1000 mg/kg/day	Yes	Negative for genotoxic effects	TX-611-2002 (Charles River 604533)

Crl:WI(Han) = Wistar Han; CRO = contract research organization; GLP = Good Laboratory Practice
a "Yes" indicates study included a GLP compliance statement.
b Metabolic activation: ~~Phenobarbital~~phenobarbital/5,6-benzoflavone-induced rat liver S9 fraction.
c In vivo micronucleus study was incorporated into the 14-day repeat-dose toxicity study.

Appendix Table 4. Summaries of Reproductive Toxicity Studies

Species/Strain	Method of Administration	Duration of Dosing	Dose (mg/kg/day)	Sex, No. per Group	NOAEL (mg/kg/day)	GLP[a]	Notable Findings	Gilead Study No. (CRO Name and Study No.)
Rat/Crl:WI(Han)	Oral gavage	Males: 14 days prior to mating, throughout mating, and continuing until the day prior to euthanasia on Days 51/52 Females: 14 days prior to mating through GD 7	0, 125, 250, 500 (males) 0, 125, 250 (females)	25/sex plus 6/sex/TK	Reproductive toxicity: 500 (males)/250 (females) Systemic toxicity: 250 (males)/125 (females)	Yes	Males: no effects on reproductive performance and spermatogenic parameters Females: no effects on reproductive performance, estrous cyclicity and intrauterine survival 500 (males/250 females): mortality/moribundity; enlarged pale kidneys 500 (males): ↓BW/↓FC	TX-611-2011 (Charles River 604636)
Rat/Crl:WI(Han)	Oral gavage	GDs 6- 17	0, 125, 250, 500	8 females plus 3-9/TK	250 (maternal and fetal)	Yes	≤ 250 mg/kg/day: ~~No~~no remarkable maternal changes or effects on embryo-fetal development 500 mg/kg/day: ~~Animals~~ animals euthanized early due to excessive toxicity (severe clinical signs, body weight loss); fetal parameters not evaluated	TX-611-2005 (LabCorp 8485750)

Rat/Crl:WI(Han)	Oral gavage	GDs 6- 17	0, 125, 250	25 females	250 (maternal and fetal)	Yes	≤ 250 mg/kg/day: ~~N~~no remarkable maternal changes or effects on embryo-fetal development NOAEL (embryo-fetal development) = 250 mg/kg/day (GS-441524 $AUC_{0\text{-}24h}$ 183,000 ng•h/mL; C_{max} 43,700 ng/mL)	TX-611-2009 (Charles River 604635)

Rabbit/New Zealand White (NZW)	Oral gavage	GDs 7- 19	0, 125, 250, 500	20 females	125 (maternal and fetal)	Yes	125 mg/kg/day: Nono remarkable maternal changes or effects on embryo-fetal development 250 mg/kg/day: maternal toxicity (1 animal terminated early due to moribund condition; ↓food consumption and body weight gain); ↓fetal and uterine weights at necropsy; ↑post-implantation loss/early resorptions; ↑fetal visceral malformations related to the development of the heart, blood vessels, and liver; ↑visceral variations (supernumerary liver lobe and missing intermediate lung lobe); ↑skeletal variations (isolated ossification sites on the nasal/premaxilla bone; supernumerary sternebra sites and supernumerary lumbar vertebra) 500 mg/kg/day: Animals animals euthanized early due to moribund condition (severe clinical signs, minimal food consumption, body weight loss, and abortion); fetal parameters not evaluated NOAEL (embryo-fetal development) = 250125 mg/kg/day (GS-441524	TX-611-2008 (LabCorp 8485751)

							AUC_{0-24h} 64,100 ng•h/mL; C_{max} 24,100 ng/mL)	

Rat/Crl:WI(Han)	Oral gavage	GD 6-LD 20	0, 100, 200	25-26 females (main); 3-6/TK	200	Yes	No effects NOEL (F0 maternal systemic, F1 developmental/neonatal, F1 parental systemic, F1 reproductive, and F2 developmental/neonatal toxicity) = 200 mg/kg/day (GS-441524 AUC_{0-24h} 100,000 ng•h/mL; C_{max} 37,300 ng/mL)	TX-611-2012 (Charles River 604651)

BW = body weight; FC = functional class; Crl:WI(Han) = Wistar Han; CRO = contract research organization; GD = gestation day; GLP = Good Laboratory Practice; NOAELLD = lactation day; NOAEL = no observed adverse effect level; NOEL = no observed effect level; TK = toxicokinetics a "Yes" indicates study included a GLP compliance statement.

Appendix Table 5. Summaries of Local Tolerance and Impurity Qualification Studies

Species/Strain	Method of Administration	Duration of Dosing	Dose (mg/kg/day)	Sex, No. per Group	NOAEL (mg/kg/day)	GLP[a]	Notable Findings	Gilead Study No. (CRO Name and Study No.)
Fibroblasts/ Mouse/Balb/c 3T3 cells	In vitro	60 min	1.77-100 µg/mL	NA	IC50 > 100 µg/mL	Yes	GS-441524 did not show cytotoxicity or phototoxicity (did not achieve an IC50 with or without UVR exposure). GS-441524 did not demonstrate phototoxic potential.	TX-611-2026 (Charles River 20427519)

Rat/Crl:WI(Han)	Oral gavage	14 days	0, 200 (ODV) 0, 100, 200 (ODV+impurities)	5 (main); 3-6 (TK)	200 (ODV) 100 (ODV+impurities)	Yes	Adverse effects were limited to 2 females administered 200 mg/kg/day ODV+impurities indicative of decreased renal function and associated microscopic kidney changes resulting from renal papillary crystals with secondary inflammatory changes	TX-611-2006 (Charles River 604621)

Crl:WI(Han) = Wistar Han; CRO = contract research organization; GLP = Good Laboratory Practice; IC_{50} = half-maximal inhibitory concentration; NOAEL = no observed adverse effect level; ODV = obeldesivir (GS-5245); TK = toxicokinetics; UVR = ultraviolet radiation a "Yes" indicates study included a GLP compliance statement..

9.4. Tabular Summary of Completed Clinical Studies

Appendix Table 6. Gilead-Sponsored Completed Clinical Studies of ODV

Study Number	Country	Study Title
GS-US-611-6248	United States	A Phase 1 Study in Healthy Volunteers to Evaluate the Safety, Tolerability, and Pharmacokinetics of GS-5245[a]
GS-US-611-6408	United States	A Phase 1 Study to Evaluate the Pharmacokinetics, Metabolism, and Excretion of GS-5245[a] in Healthy Participants

a GS-5245 is now known as obeldesivir (ODV).