SECTION 2.4—NONCLINICAL OVERVIEW

LENACAPAVIR

Gilead Sciences

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CONFIDENTIAL AND PROPRIETARY INFORMATION

TABLE OF CONTENTS

SECTION 2.4—NONCLINICAL OVERVIEW 1
TABLE OF CONTENTS 2
LIST OF IN-TEXT TABLES 3
LIST OF IN-TEXT FIGURES 3
GLOSSARY OF ABBREVIATIONS AND DEFINITION OF TERMS 4
NOTE TO THE REVIEWER 7
1. OVERVIEW OF THE NONCLINICAL TESTING STRATEGY 8
2. PHARMACOLOGY 12
2.1. Primary Pharmacodynamics 12
2.2. Secondary Pharmacodynamics 13
2.3. Safety Pharmacology 13
2.4. Pharmacodynamic Drug Interactions 14
2.5. Summary of Pharmacology 14
3. PHARMACOKINETICS 15
3.1. Analytical Methods 15
3.2. Absorption and Pharmacokinetics 15
3.2.1. Absorption 15
3.2.2. Single Dose Pharmacokinetics 15
3.3. Repeat Dose Pharmacokinetics 18
3.4. Distribution 18
3.5. Metabolism 19
3.6. Excretion 20
3.7. Distribution in Pregnant or Nursing Animals 21
3.8. Pharmacokinetic Drug Interactions 21
3.8.1. CYP Reaction Phenotyping 21
3.8.2. UGT Phenotyping 21
3.8.3. Cytochrome P450 and UGT1A1 Inhibition 22
3.8.4. Assessment of Induction Liability 22
3.8.5. Interactions with Transporters 23
3.9. Summary of Pharmacokinetics 23
4. TOXICOLOGY 25
4.1. Brief Summary 25
4.2. Single Dose Toxicity 29
4.3. Repeat Dose Toxicity 29
4.3.1. Liver 29
4.4. Genotoxicity 31
4.5. Carcinogenicity 31
4.6. Reproductive Toxicity 32
4.7. Local Tolerance 33
4.8. Other Toxicity Studies 33
4.8.1. Antigenicity 33
4.8.2. Immunotoxicity 33
4.8.3. Dependence 34
4.8.4. Studies on Metabolites 34
4.8.5. Impurities/Degradation Products 34
4.8.6. Other Toxicity Studies 35

4.8.7. 7-Day Once Daily Oral (Gavage) Tolerability and Toxicokinetic Study of LEN in Female Nonpregnant New Zealand White Rabbits 36
4.8.8. Assessment of Subcutaneous Depot Following a Single Subcutaneous Infusion of LEN in the Wistar Han Rat 37
4.9. Summary of Nonclinical Safety 38
4.9.1. Relationship of Findings to Exposure 38
4.9.2. Target Organ Effects 42
5. INTEGRATED OVERVIEW AND CONCLUSIONS 45
5.1. Integrated Overview 45
5.2. Justification for Text in Labeling 47
5.3. Overall Conclusion 47
6. REFERENCES 49

LIST OF IN-TEXT TABLES

Table 1. Overview of Lenacapavir Toxicology Program 25
Table 2. Genotoxicity Studies Conducted with Lenacapavir 31
Table 3. Concentrations of Lenacapavir Associated with Key Responses[a] 38
Table 4. Systemic Exposure of Dogs and Humans to LEN compared to BSEP IC_{50} 44

LIST OF IN-TEXT FIGURES

Figure 1. Proposed Biotransformation of Lenacapavir in Rat, Dog and Human 20

GLOSSARY OF ABBREVIATIONS AND DEFINITION OF TERMS

AhR	aryl hydrocarbon receptor
API	active pharmaceutical ingredient
AUC	area under the plasma concentration versus time curve
AUC_{τ}/AUC_{tau}	area under the concentration versus time curve over the dosing interval
BCRP	breast cancer resistance protein
BCS	Biopharmaceutics Classification System
BDC	bile duct cannulated
BSEP	bile salt export pump
CA	capsid
Caco-2	human colorectal carcinoma cells
CAR	constitutive androstane receptor
C_{ave}	average concentration
CC_{50}	cytotoxic concentration giving 50% killing of the cells
C_{max}	the maximum observed concentration of drug in serum, plasma
$C_{max\ Day1\text{-}Week\ 26SC}$	C_{max} after 1500 mg oral dosing in first 2 weeks and 927 mg SC administration
C_{min}	clinical plasma trough concentration
CNS	central nervous system
COBI	cobicistat
CPSF6	cleavage and polyadenylation specificity factor subunit 6
CYP	cytochrome P450
DDI	drug-drug interaction
DMSO	dimethyl sulfoxide
DRV	darunavir
DTG	dolutegravir
EC_{50}	effective concentration giving 50% inhibition of virus replication
ECG	electrocardiogram
EFV	efavirenz
F%	bioavailability
FDA	Food and Drug Administration
FOB	functional observational battery
FOC	fold over control
GD	gestation day
GLP	Good Laboratory Practice
h	hours
HIV-1	human immunodeficiency virus type 1
HPBL	human blood peripheral lymphocytes
ICH	International Conference on Harmonization
INSTI	integrase strand transfer inhibitor
ISR	injection site reaction

IV	intravenous
kg	kilogram
K_I	inhibition constant
K_{inact}	rate of enzyme inactivation
OECD	Organization for Economic Co-Operation and Development
LC-MS/MS	liquid chromatography-tandem mass spectrometry
LD	lactation day
LE rat	Long Evans rat
LEN	lenacapavir
MATE	multidrug and toxin extrusion protein
MI	maturation inhibitor
MDCK	Madin-Darby canine kidney
MDCKII	Madin-Darby canine kidney strain II
MDZ	midazolam
MDR	multidrug resistant
MRI	magnetic resonance imaging
µg	microgram
mg	milligram
mL	milliliter
mM	millimolar
MN	micronucleus
min	minutes
M, mol	molar
N	Normal
NADPH	nicotinamide adenine dinucleotide phosphate, reduced
NaOH	sodium hydroxide
NNRTI	non-nucleotide reverse transcriptase inhibitor
NOEL	no-observed-effect-level
NOAEL	no-observed-adverse-effect level
NRTI	nucleoside reverse transcriptase inhibitor
ng	nanogram
NUP153	nucleoporin 153
NUP358	nucleoporin 358
PI	protease inhibitor
PBMC	peripheral blood mononuclear cell
PBS	phosphate buffered saline
PEG	polyethylene glycol
PCE	polychromatic erythrocyte
PK	pharmacokinetic
PG	propylene glycol
P-gp	P-glycoprotein

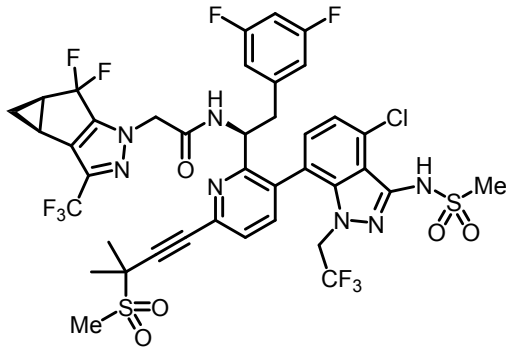
PND	postnatal day
PO	oral
PR	interval between the peak of the P wave and the peak of the R wave on ECG
PXR	pregnane X receptor
S9	post-mitochondrial supernatant fraction from homogenized liver
SC	subcutaneous
SWI	sterile water for injection
$t_{1/2}$	estimate of the terminal elimination half-life of the drug in plasma/serum, calculated by dividing the natural log of 2 by the terminal elimination rate constant (λ_z)
TAF	tenofovir alafenamide
T_{max}	the time of maximum observed concentration
UDPGA	uridine diphosphate glucuronic acid
UGT	uridine diphosphate glucuronosyltransferase
UGT1A1	uridine diphosphate glucuronosyltransferase 1A1
US	United States
USP	United States Pharmacopeia
$AUC_{Day1\text{-}Week\ 26SC}$	AUC after 1500 mg oral dosing in first 2 weeks and 927 mg SC administration
UVR	ultraviolet radiation
WH rat	Wistar Han rat
WT	wild-type
v/v	volume to volume
w/w	weight for weight

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

NOTE TO THE REVIEWER

The structure of lenacapavir (LEN, GS-6207) is shown below. The following conversions are provided to aid the reviewer.

LEN (GS-6207): 1 nM = 0.968 ng/mL; 1 ng/mL = 1.03 nM

Name	Structure
LEN	

1. OVERVIEW OF THE NONCLINICAL TESTING STRATEGY

This overview provides all relevant nonclinical information for the assessment of lenacapavir (LEN: GS-6207 or GS-6707-02 [sodium salt]). Nonclinical pharmacology, pharmacokinetic, and toxicology data for LEN are provided in m2.6 and nonclinical study reports are provided in m4 and m5. The proposed indication for LEN is for the treatment of HIV-1 infection. A critical assessment of the completeness and relevance of the nonclinical testing program and the key findings are included.

Lenacapavir is a first-in-class and selective inhibitor of human immunodeficiency virus type 1 (HIV-1) capsid function. Lenacapavir inhibits HIV-1 at multiple points in the viral lifecycle, including interfering with capsid-mediated nuclear uptake of preintegration complexes and impairing virion production and proper capsid core formation. Virus produced in the presence of LEN display aberrantly shaped capsids. These malformed virus particles can still infect a new target cell but cannot replicate as they are unable to support reverse transcription without a properly formed capsid core. Lenacapavir displays potent antiviral activity, a novel resistance profile, low human clearance, and low aqueous solubility. These combined characteristics allow LEN to be well suited for a long-acting parenteral formulation. Lenacapavir is active against all major types of HIV-1, including those that have developed resistance to existing major classes of antiretrovirals (e.g. nucleoside reverse transcriptase inhibitors (NRTI), nonnucleoside reverse transcriptase inhibitors (NNRTI), protease inhibitors (PI), and integrase strand-transfer inhibitors (INSTI)). Two dosage forms of LEN are currently in development, a solution formulation for subcutaneous (SC) injection once every 6 months and a film-coated tablet formulation for oral administration. The oral dosage form is used as loading doses to rapidly achieve the target plasma PK concentration.

Nonclinical studies required to support the proposed chronic use of LEN in people with HIV-1 infection have been completed. These included a comprehensive set of primary and secondary pharmacodynamics studies; core safety pharmacology studies; complete pharmacokinetic (PK) evaluation including absorption, distribution, metabolism, excretion, and drug interaction potential; single dose intravenous toxicity studies in rats and dogs; repeat-dose subcutaneous toxicity studies of up to 26 weeks duration in rats, and 39-weeks in dogs; in vitro and in vivo genotoxicity studies; assessment of fertility, early embryonic development, and pre- and postnatal development toxicity in rats; and 1-month oral toxicity studies in rats and dogs. Additional toxicology studies conducted include assessments of local tolerance, antigenicity potential and genotoxicity and toxicity studies for qualification of impurities. The 6-month transgenic mouse (TgrasH2) and the 2-year rat carcinogenicity studies are in progress. The final reports will be submitted when available; approximately the end of 20[redacted] for the mouse study and early 20[redacted] for the rat study. The rat and dog were the appropriate species for the repeat-dose toxicology studies because high systemic exposures were achieved and the disposition of LEN in these species was similar to human.

The nonclinical pharmacology program demonstrated that LEN is a potent inhibitor of HIV-1 replication, is fully active against a broad spectrum of HIV-1 mutants and clinical isolates resistant to currently approved antiretrovirals, has similar antiviral activity across clinical isolates from all major HIV-1 subtypes and has synergistic in vitro antiviral activity when combined with other antiretrovirals. These data demonstrate the potent, selective and multi-stage inhibition of HIV-1 capsid function by LEN, a mechanism that is orthogonal and complementary to existing anti-HIV therapies. In safety pharmacology evaluations, LEN had no clinically relevant effect on the central nervous, cardiovascular, or respiratory systems.

Lenacapavir is a Biopharmaceutics Classification System (BCS) Class IV molecule with low solubility in biorelevant fluids and low permeability. Lenacapavir bioavailability (F%) following SC administration to rat, dog and human was high. In nonclinical testing, sustained LEN release was demonstrated following SC administration. The terminal half-life ($t_{1/2}$) following SC administration was substantially longer than the $t_{1/2}$ following IV administration indicating "flip-flop" PK (i.e. the rate of absorption is significantly slower than its rate of elimination from the body). Neither a prominent initial burst release nor an unintended rapid drug release was observed in nonclinical PK profiles. Lenacapavir bioavailability following oral administration in nonclinical species was low, consistent with low F% in humans, likely due to low permeability and efflux by P-gp.

Lenacapavir exhibits very high in vitro metabolic stability across species with a predicted human hepatic extraction < 1%. This high stability was reflected in vivo, with low clearance (CL) values in nonclinical species and humans. Lenacapavir displayed a high volume of distribution; thus, resulting in a long PK $t_{1/2}$ across all nonclinical species. Lenacapavir also exhibits a low plasma free fraction across all species. Whole-body autoradiography studies following intravenous (IV) administration of [^{14}C]LEN to rats revealed exclusion of radioactivity from the brain (consistent with LEN being a P-gp substrate) and no preferential association with melanin-containing tissues. Lenacapavir was detected in rat plasma of nursing pups.

Intact LEN exists as a mixture of atropisomers, henceforth referred to as LEN.1 and LEN.2. In vitro, the ratios of LEN.1 to LEN.2 were stable over time and were not influenced by plasma binding proteins or enzymes.

In vivo, the major route of elimination of LEN in rats and dogs was excretion in bile and intestinal secretion as intact LEN. Intestinal secretion appeared to be mediated by P-gp as it was inhibited by coadministration of elacridar (a P-gp inhibitor). Metabolism played a lesser role in both nonclinical species. The major route of elimination of LEN in humans was consistent with excretion as intact LEN (to bile and secretion to intestine, likely mediated by P-gp) and to a lesser extent via metabolism (oxidation by CYP3A and/or glucuronidation by UGT1A1). The LEN metabolites that were identified were formed via oxidation or direct conjugation. Intact LEN was the only major circulating plasma component in rat, dog and human. Lenacapavir derived products with low abundance (each < 10%) were observed in human plasma and excreta. All human metabolites were detected in nonclinical species.

Assessment of LEN as a victim of drug-drug interactions (DDIs) showed inhibitors or inducers of CYP3A and UGT1A1 may affect the PK of LEN. In a clinical DDI study, CYP3A/P-gp inhibitor, cobicistat, increased exposure of orally administered LEN, but the CYP3A selective inhibitor, voriconazole, had less effect. The potent CYP3A and P-gp inducer, rifampicin, greatly reduced exposure to oral LEN.

Assessment of LEN as a perpetrator of drug interactions indicates low potential for reversible inhibition of CYP enzymes or UGT1A1 at the clinical systemic concentrations observed following oral or SC administration. Inhibition of intestinal efflux transporters (P-gp and BCRP) following oral dosing of LEN cannot be ruled out from in vitro data as the maximum concentration that could be tested in vitro was 1 μM wherein no inhibition of either transporter was observed. In a clinical drug-drug interaction (DDI) study, there was no clinically meaningful effect of orally administered LEN on tenofovir alafenamide (a P-gp substrate) or rosuvastatin (a BCRP substrate). While LEN was a potent inhibitor of hepatic uptake transporters OATP1B1 and OATP1B3 in vitro, there was no effect of orally administered LEN on the PK of pitavastatin in humans. From potencies determined in vitro, LEN exhibits no potential for inhibition of renal transporters (organic anion transporter 1 (OAT1), OAT3, organic cation transporter 1 (OCT2), multidrug and toxin extrusion protein 2K (MATE2K) and weak inhibition of MATE1. No clinically relevant changes in glomerular filtration rate or changes in serum creatinine were observed in clinical studies.

With respect to induction, there was no detectable activation of the human xenobiotic receptors, aryl hydrocarbon receptor (AhR) and constitutive androstane receptor (CAR), and weak activation of pregnane X receptor (PXR) by LEN in cell systems. Lenacapavir was also tested to its toxicity limit as an inducer in human hepatocytes (n = 3 donors). There was no change in mRNA expression for CYP1A2 or CYP2B6 (sensitive targets for AhR and CAR activation, respectively). Increased expression of CYP3A4 mRNA (a sensitive target for PXR) was observed in one of the three donors. Slight changes in mRNA expression for secondary induction markers (CYP2C9 and UGT1A1; maximum ≤ 2.5-fold higher than the vehicle control) were seen in one donor only. There were no changes in P-gp mRNA expression in any of the donors.

Drug-related adverse effects in dogs related to dysfunction of the hepatobiliary system were observed and was likely the result of LEN inhibition of the dog bile salt export pump (BSEP) transport protein. Lenacapavir inhibition of the human BSEP is 10-fold less potent compared to the dog BSEP and the clinical plasma concentrations are substantially less than the dog plasma concentration. This results in a human exposure margin of 8-fold at the no-adverse-effect level (NOAEL) and at least 51-fold at the effect dose.

The chronic granulomatous inflammation reaction at the injection site in rats, dogs and rabbits is a foreign body response to the LEN SC depot and is an expected host response. Granulomatous inflammation has been observed after SC and intramuscular implantation of bioactive material, including those for HIV treatment {Anderson 1997, Shah 2017, Van't Klooster 2010}.

The definitive safety pharmacology, toxicology, and toxicokinetic studies with LEN were conducted in accordance with guidelines issued by the International Conference on Harmonization (ICH) and with Good Laboratory Practice (GLP) or other applicable regulations promulgated by international Health Authorities. Pilot, exploratory, and mechanistic studies were either conducted in full compliance with GLP regulations or were conducted using appropriate protocols and documentation to ensure data integrity. Exposure margins comparing nonclinical toxicology species to the population PK data were calculated using total drug concentration.

The nonclinical data discussed within this document support the favorable benefit/risk profile for the proposed use of LEN for the treatment of HIV-1 infection. All information from nonclinical studies that is relevant to the prescriber and patient has been included in the proposed prescribing information.

2. PHARMACOLOGY

2.1. Primary Pharmacodynamics

In acutely infected cells, LEN binding to capsid results in potent suppression of both early- and late-stage processes essential for HIV-1 replication, with EC_{50} values of 0.023 nM and 0.439 nM, respectively (PC-200-2021). This dual mechanism of action is unique among all clinically used antiretroviral classes. Lenacapavir interferes with late-stage virus production and capsid core formation events. Lenacapavir also interferes with an early-stage process occurring after reverse transcription but before the integration of viral DNA (PC-200-2024). Lenacapavir reduces the accumulation of integration products and abortive two-long-terminal-repeat (2-LTR)-containing circles, both of which form exclusively in the nucleus {Bukrinsky 1993}. Lenacapavir interacts with capsid at a binding site shared by multiple host proteins implicated in HIV-1 nuclear entry, including cleavage and polyadenylation specificity factor 6 (CPSF6), nucleoporin 153 (NUP153) and nucleoporin 358 (NUP358) {Bhattacharya 2014, Matreyek 2013, Price 2012, Price 2014} (PC-200-2022). Thus, LEN binding is expected to prevent these host cell factors from binding to HIV-1 capsid (CA) protein and thereby interfere with capsid-mediated nuclear import of viral pre-integration complexes, an essential step in the formation of an integrated provirus.

Lenacapavir demonstrated potent antiretroviral activity in the MT-4 T-lymphoblastoid cell line, in primary human $CD4^+$ T-lymphocytes, and in monocyte-derived macrophages, with EC_{50} and selectivity (CC_{50}/EC_{50}) values ranging from 0.030 to 0.190 nM and 140,000 to >1,670,000, respectively (PC-200-2018, PC-200-2019). The cytotoxicity of LEN in unstimulated and stimulated human PBMCs from three independent donors was low, with respective CC_{50} values of >44 µM and >50 µM, indicating that the compound has a selectivity for HIV-1 of at least >888,000 in human PBMCs (PC-200-2019). Lenacapavir also showed potent antiviral activity against two HIV-2 isolates but was 15- to 25-fold less active relative to HIV-1 (PC-200-2020). Similar to other antiretroviral drugs, the in vitro antiviral activity of LEN is affected by the level of infection. However, even at the highest multiplicity-of-infection tested, the EC_{50} value of LEN was 5- to >100-fold lower than any of the inhibitors tested from other antiretroviral classes (PC-200-2031).

Lenacapavir displayed similar antiviral activity across clinical isolates from all major HIV-1 subtypes with a mean EC_{50} value of 0.05 nM (ranging from 0.02 to 0.16 nM) in PBMCs, and a mean EC_{50} value of 0.24 nM (ranging from 0.15 to 0.36) in the HEK293T cell line (human cell line, derived from the HEK 293 cell line, that expresses a mutant version of the SV40 large T antigen). Consistent with these findings, the capsid protein sequence is highly conserved across 8 major HIV-1 subtypes, including the majority of residues within the LEN binding site. Most capsid polymorphisms near the LEN binding site displayed WT susceptibility to LEN (PC-200-2032, PC-200-2038).

Lenacapavir maintains full antiviral activity against a broad spectrum of HIV-1 mutants and HIV-1 clinical isolates resistant to currently approved antiretrovirals from the nucleotide and NRTI, NNRTI, INSTI and PI classes (PC-200-2027, PC-200-2044).

Lenacapavir also maintains full antiviral activity against patient-derived HIV-1 isolates containing naturally-occurring Gag polymorphisms, in contrast to compounds from the maturation inhibitor (MI) class which displayed strongly reduced susceptibility in the presence of most naturally-occurring Gag polymorphisms. This confirms that LEN and MIs exert their antiviral activity through different mechanisms (PC-200-2036). The antiviral activity of LEN is not affected by the presence of Gag cleavage site mutations associated with PI treatment or MI resistance, further underlining the lack of cross-resistance between existing drug classes and LEN (PC-200-2037).

Lenacapavir selects for single and double capsid amino acid substitutions, including L56I, M66I, Q67H, K70N, N74D/S and T107N (PC-200-2025). HIV-1 variants encoding these individual mutations confer resistance to LEN (4- to >3,226-fold) but remain fully sensitive to drugs from other classes (NNRTI: efavirenz [EFV], NRTI: tenofovir alafenamide [TAF], PI: darunavir [DRV], INSTI: dolutegravir [DTG]) (PC-200-2026, PC-200-2045, PC-200-2046). The majority of these capsid HIV-1 mutants, including those conferring moderate to high-level LEN resistance, display substantially reduced replication capacity relative to the wild type (WT) virus in $CD4^+$ T-lymphocytes and macrophages (PC-200-2026). Capsid population-level and deep sequence analyses suggest that all known LEN resistance associated mutations are present in <1% of the patient population (PC-200-2032).

2.2. Secondary Pharmacodynamics

Lenacapavir showed low cytotoxicity in primary human hepatocytes (CC_{50} > 50 µM) and in several non-target human cell lines of different tissue origin (CC_{50} values > 44 µM), including two cell lines that were adapted for growth in galactose-containing medium to increase their sensitivity to mitochondrial toxicity (PC-200-2028).

Lenacapavir showed low potential for off-target effects when tested against a panel of 87 molecular targets at a concentration of 10 µM, at least 5000-fold above the estimated free drug concentration (1.98 ng/mL) at C_{max} at the anticipated clinically efficacious dose (PC-200-2012, CTRA-2021-1054 LEN PopPK).

2.3. Safety Pharmacology

Lenacapavir DMSO/buffer formulations ≥ 0.1 µM were suspensions and not solutions and thus a GLP hERG assay was not conducted with LEN (solutions are required for this assay). No LEN-related changes in central nervous system (CNS) endpoints were observed up to 100 mg/kg in rats (TX-200-2005). In dogs, no electrocardiogram (ECG) or blood pressure changes were attributed to LEN after a single SC dose of 100 mg/kg or after repeated SC doses of 30 mg/kg (TX-200-2006). The free C_{max} at the no-observed-(adverse)-effect levels (NO(A)ELs) for CNS (2.7 ng/mL) and cardiovascular (39 ng/mL) endpoints were 1.4 and 20-fold higher than exposures at the $C_{max\ Day1\text{-}Week26}$ (1.98 ng/mL, the free C_{max} after 1500 mg oral dosing in first 2 weeks and 927 mg SC administration).

In a single SC dose safety pharmacology study in rats, LEN was associated with a minor non-statistically significant increase in tidal volume and a decrease in respiration rate at 100 mg/kg (TX-200-2014). No LEN-related changes in tidal volume or respiration rate were noted in animals administered ≤ 30 mg/kg up to 72 hours postdose, and there were no LEN-related changes in minute volume in animals administered ≤ 100 mg/kg.

2.4. Pharmacodynamic Drug Interactions

Lenacapavir demonstrated synergistic in vitro antiviral activity when combined with antiretrovirals from other classes, including TAF, EFV, DTG and DRV.

The in vitro anti-HIV-1 activity of LEN was examined in combination with clinically approved agents from each of the four major classes of antiretrovirals (NRTIs, NNRTIs, INSTIs, and PIs) (PC-200-2030). Pair-wise combination of LEN with an NRTI (TAF) showed moderate synergistic anti-HIV-1 activity when tested in vitro using MT-2 cells infected with HIV-1 (IIIB strain). Lenacapavir exhibited highly synergistic activity when combined with an NNRTI (EFV), an INSTI (DTG), or an HIV-PI (DRV). Importantly, no antagonistic antiviral interaction was found between LEN and these four clinically relevant classes of antiretrovirals. These results support the clinical investigation of LEN in combination with other antiretroviral agents.

2.5. Summary of Pharmacology

The nonclinical pharmacology program demonstrated that LEN is a potent inhibitor of HIV-1 replication, is fully active against a broad spectrum of HIV-1 mutants and clinical isolates resistant to currently approved antiretrovirals, has similar antiviral activity across clinical isolates from all major HIV-1 subtypes and has synergistic in vitro antiviral activity when combined with other antiretrovirals. These data demonstrate the potent, selective and multi-stage inhibition of HIV-1 capsid function by LEN, a mechanism that is orthogonal and complementary to existing anti-HIV therapies.

Overall, the favorable pharmacodynamic and pharmacological assessment of LEN supports the effective and safe use of LEN for the treatment of HIV-1 infection.

3. PHARMACOKINETICS

The absorption, distribution, metabolism, and excretion of LEN were evaluated in vitro and in a variety of animal models in vivo. In addition, the DDI profile was evaluated.

3.1. Analytical Methods

The plasma concentrations of LEN were quantified in GLP repeat-dose toxicology studies in rat and dog using fully validated high-performance liquid chromatography-tandem mass spectrometry (LC-MS/MS) methods. The plasma LEN concentrations in nonclinical PK studies in rat, dog and monkey were quantified by LC-MS/MS. These methods did not strictly conform to Good Laboratory Practice (GLP) guidelines but were evaluated for selectivity, sensitivity, and linearity, as well as intra-assay accuracy and precision. All bioanalytical methods were conducted using appropriate protocols and documentation to assure data integrity.

3.2. Absorption and Pharmacokinetics

3.2.1. Absorption

The bidirectional permeability of LEN (1.0 μM) was studied across Caco-2 monolayers (AD-200-2009). Lenacapavir showed low forward (absorptive) permeability of 0.3 x 10^{-6} cm/sec and high reverse permeability of 2.5 x 10^{-6} cm/sec through Caco-2 monolayers with an efflux ratio of 7.8.

3.2.2. Single Dose Pharmacokinetics

3.2.2.1. Intravenous Administration

Lenacapavir PK following a single IV infusion administration was determined in male rat, dog, and monkey (AD-200-2010, AD-200-2011, AD-200-2012, AD-200-2013). The plasma CL of LEN was low across all tested species (1% to 12% of hepatic blood flow). The V_{ss} in all species was larger than that of total body water. The elimination half-life ($t_{1/2}$) ranged from 14.6 to 38.1 hours across species.

3.2.2.2. Subcutaneous Administration

Lenacapavir PK following a single SC administration was evaluated in rat and dog. Multiple factors affecting LEN absorption from the SC injection site (release) were evaluated, including LEN salt form, formulation state (i.e., suspension or solution), formulation vehicle, dose, dose concentration and dose volume. The plasma exposure to LEN was characterized using sensitive LC-MS/MS assays until concentrations were below the lower limit of quantification of 1 nM (0.968 ng/mL). Overall, PK following SC administration showed a sustained drug release at all administered doses in both rat and dog. Neither a prominent initial burst release nor unintended rapid drug release was observed. The mean $t_{1/2}$ following SC administration was substantially longer than the mean $t_{1/2}$ following IV administration indicating a flip-flop SC PK. Lenacapavir bioavailability (F%) following SC administration was high.

3.2.2.2.1. Subcutaneous - Rat

Lenacapavir free acid, formulated as an aqueous suspension with 2% poloxamer 188 in normal saline, was administered subcutaneously to male Wistar Han (WH) rats (AD-200-2014). Rats were administered either a single injection at three dose levels (10 to100 mg/kg) or two equal-volume injections at 100 mg/kg for a total of 200 mg/kg. Lenacapavir PK following SC administration showed a sustained drug release at all administered doses. Neither a prominent initial burst release nor unintended rapid drug release was observed. F% was at least 55% in all dose groups. The mean $t_{1/2}$ following SC administration was substantially longer than the mean $t_{1/2}$ following IV administration indicating a flip-flop SC PK. Lenacapavir plasma C_{max} values were similar between 10 and 30 mg/kg but increased dose proportionally from 30 to 100 mg/kg. Plasma C_{max} nearly doubled following 2 doses of 100 mg/kg compared to one dose of 100 mg/kg. Total LEN plasma exposure (AUC_{inf}) increased with dose in the range of 10 to 100 mg/kg but the increase was less than dose proportional. Lenacapavir AUC_{inf} observed following 2 doses of 100 mg/kg each (for a total of 200 mg/kg) was greater than the exposure achieved with one 100 mg/kg dose, however the increase was less than dose proportional.

Lenacapavir free acid or LEN sodium salt, formulated as a solution in 77:10:13 w/w/w polyethylene glycol (PEG) 200:ethanol:water (solution formulation A) at up to 3 dose concentrations, was administered to male WH rats via a single SC injection (AD-200-2038). Following a single SC administration of LEN free acid solution formulations at up to 3 dose levels, LEN PK showed a sustained slow drug release at all administered doses. Neither a prominent initial burst release nor unintended rapid drug release was observed. F% was at least 77% in all dose groups. Lenacapavir exposure (C_{max} and AUC_{inf}) increased with dose from 10 to 50 mg/kg (at 200 mg/mL) but the increase was less than dose proportional. At the 50 mg/kg dose, mean C_{max} was lower at 400 mg/mL dose concentration but a higher mean systemic concentration was observed on Day 154 as compared to the 200 mg/mL solution. This indicated a more sustained LEN release at a higher dose concentration. Following a single SC administration of LEN sodium salt solution formulations, LEN AUC_{inf} increase was approximately dose proportional and the increase in C_{max} was less than dose proportional as the dose was increased from 50 to 100 mg/kg. Administering a constant 10 mg/kg dose while increasing the dose concentration from 100 to 200 mg/mL resulted in a similar mean C_{max} and a similar mean systemic concentration on Day 56; these results demonstrate that dose concentration had minimal impact on the LEN PK. The impact of LEN salt form (also in a solution formulation) was assessed at 50 mg/kg (200 mg/mL). Administration of LEN free acid resulted in a lower mean C_{max} but a comparable AUC_{inf} relative to administration of LEN sodium salt.

3.2.2.2.2. Subcutaneous - Dog

Lenacapavir free acid, formulated as an aqueous suspension with 2% poloxamer 188 in normal saline, was administered via SC injections to male beagle dogs (AD-200-2015). The SC dose was administered as a single injection at 4 dose levels (3 to 100 mg/kg) or as two equal-volume 3 mg/kg injections for a total of 6 mg/kg. Following a single SC administration, LEN PK showed a sustained slow drug release. Neither a prominent initial burst release nor unintended rapid drug release was observed. F% was at least 56% in all dose groups. The mean $t_{1/2}$ following SC administration was substantially longer than the mean $t_{1/2}$ following IV administration, indicating flip-flop SC PK. Following a single SC injection of the 100 mg/mL formulation, the average LEN AUC_{inf} increased with dose in the range of 3 to 100 mg/kg. The increase in average AUC_{inf} was more than dose proportional from 3 to 6 mg/kg but less than dose proportional from 6 to 100 mg/kg. The increase in average C_{max} was more than dose proportional from 3 to 30 mg/kg and less than dose proportional from 30 to 100 mg/kg. The average LEN AUC_{inf} following a single SC injection at 6 mg/kg was higher, but more variable compared to that from two equal-volume injections of 3 mg/kg. The average C_{max} was similar and the percent of total exposure by Day 28 was higher in the two equal-volume injection group. Following a single SC injection at 6 mg/kg and at two different concentrations (100 mg/mL, 0.06 mL/kg vs. 200 mg/mL, 0.03 mL/kg), the average LEN plasma exposure (AUC_{inf} and C_{max}) values were higher and the average T_{max} achieved earlier in animals dosed at lower concentration (i.e., 100 mg/mL) than at higher concentration (i.e., 200 mg/mL).

Lenacapavir free acid or LEN sodium salt was formulated as a solution in 77:10:13 w/w/w PEG200:ethanol:water (solution formulation A) for each form at up to 3 dose concentrations. The formulated solution was administered to male beagle dogs at up 3 dose levels via a single SC injection (AD-200-2039). Lenacapvir PK showed a sustained slow drug release at all administered doses. F% was at least 45% in all dose groups. No unintended rapid drug release was observed in all groups. Lenacpavir exposure increased as the dose increased from 6 to 12 mg/kg (at 200 mg/mL); the C_{max} increase was approximately dose proportional and the AUC_{inf} increase was less than dose proportional. The effect of LEN free acid at the three dose concentrations (200, 300 and 400 mg/mL) was assessed at the 12 mg/kg dose − higher LEN dose concentration resulted in a similar mean C_{max} and a similar mean systemic concentration on Day 112; these results demonstrate that dose concentration had minimal impact on the LEN PK. Following a single administration of LEN sodium salt at the three dose concentrations (100, 200 and 300 mg/mL) was assessed at 6 mg/kg − the 300 mg/mL concentration resulted in the lowest mean C_{max}. The impact of LEN salt form (free acid vs sodium salt) was compared at 6 mg/kg (200 mg/mL) − administration of LEN free acid resulted in lower mean C_{max} but a comparable AUC_{inf} relative to administration of LEN sodium salt.

Lenacapavir free acid was also formulated as a solution in 65:25.2:9.8 w/w/w PEG300:LEN:water (solution formulation B) at a single 300 mg/mL concentration (AD-200-2051). The formulated solution was administered to male beagle dogs via a single SC injection at 12 mg/kg. Lenacapavir PK showed a sustained slow release and no unintended rapid drug release was observed. PK profiles of solution formulations A and B were similar, suggesting presence or absence of ethanol in formulations did not significantly alter the LEN release profile.

3.2.2.3. Oral Administration

Lenacapavir bioavailability (F%) following oral administration in nonclinical species was investigated.

Following oral administration of LEN in a solution formulation, the PK was determined in rat, dog and monkey (AD-200-2010, AD-200-2011, AD-200-2012, AD-200-2013). Lenacapavir was slowly absorbed with average T_{max} range from 8.7 to 18.7 h. F% ranged from 2.2% to 22%.

To understand the potential CYP mediated metabolism contribution to fraction of the administered oral dose escaping first-pass gut metabolism (F_g) and hepatic metabolism (F_h), the PK of LEN in solution following oral administration was evaluated in the portal vein cannulated dogs (AD-200-2016) and monkeys (AD-200-2017) without and with pretreatment of 1-aminobenzotriazole (ABT), a pan CYP inhibitor. ABT plasma concentrations were quantified by a LC-MS/MS method and were consistent with historic values. Pretreatment with ABT did not alter LEN oral exposure in dogs and monkeys. This suggested negligible gut metabolism and a minor role for intestinal CYPs in LEN metabolism. Data collectively showed that the observed F% was consistent with limited absorption.

The PK of LEN was also determined in rat and dog following single increasing oral doses to inform dose and formulation selection for repeat dose toxicology studies (AD-200-2018, AD-200-2019, AD-200-2036 and AD-200-2042). In rat, the AUC_{inf} of LEN increased with doses from 30 to 100 mg/kg, but the increase was less than dose proportional (AD-200-2018).

A single dose of LEN, formulated in solution with three different vehicles (formulation A, B and C), was administered orally at 30 and/or 100 mg/kg to male beagle dogs (AD-200-2019). The C_{max} and AUC_{inf} increased with increasing doses from 30 to 100 mg/kg for both formulations B and C, but the increases were less than dose proportional. Formulation B (10% dimethyl sulfoxide, 10% Vitamin E TPGS, 80% PG) achieved higher exposure at 100 mg/kg and was chosen for repeat dose toxicology studies.

3.3. Repeat Dose Pharmacokinetics

Repeat-dose pharmacokinetics following SC or oral administration was assessed in support of toxicology studies in rat and dog; the toxicokinetic data are presented in the Toxicology Tabulated Summary (m2.6.7). The toxicokinetic data after repeat-dose in nonclinical species did not show any gender differences. Accumulation of LEN was observed after multiple monthly subcutaneous doses in dogs, and after daily oral doses in rats and dogs.

3.4. Distribution

The binding of LEN (2 µM) to plasma proteins was high, with less than 1.5% free in all tested species (AD-200-2020, AD-200-2044). The highest free fraction of 1.46% was observed in human plasma. Lenacapavir (2 µM) binding to human hepatic microsomes was high with mean fraction bound of 89.2% (AD-200-2032). Lenacapavir (0.5 µM) blood to plasma ratio was similar across species with mean values ranging from 0.59 to 0.67. The human blood-plasma ratio was 0.64, showing minimal binding to blood cells (AD-200-2021).

The tissue distribution of LEN following a single IV bolus administration of [^{14}C]LEN by quantitative whole-body autoradiography (AD-200-2006). [^{14}C]LEN-derived radioactivity was widely distributed to most tissues by the first collection time point (0.5 hours postdose) in both Wistar Han (non-pigmented) and Long Evans(pigmented) male rats and the distribution of radioactivity was similar in both species. Most of the tissues reached C_{max} by 0.5 hours. Liver achieved the highest concentration of radioactivity. Low levels of radioactivity were detected in brain and testes suggesting the distribution of [^{14}C]LEN-derived radioactivity was restricted by the blood-to-brain and blood-to-testes barriers. Radioactivity was cleared from all tissues in both strains by 1344 hours (56 days) postdose. No significant binding to melanin-containing tissues was observed.

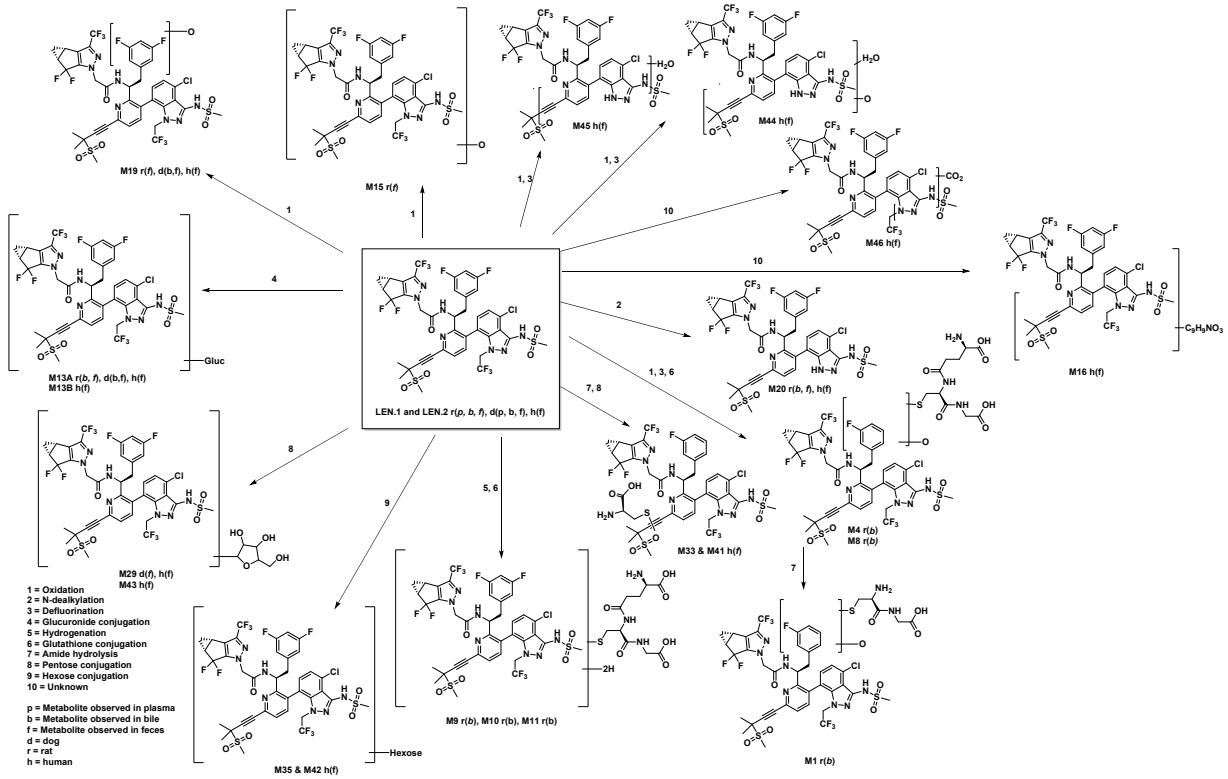
3.5. Metabolism

The relative abundance of LEN atropisomers, LEN.1 and LEN.2, was assessed in vitro in phosphate buffered saline (PBS) at pH 7.4, and plasma from rat, dog and human (AD-200-2047). The ratios of abundances were similar and remained unchanged during the study duration in PBS and across all examined plasma. This indicates that the LEN atropisomer pattern was stable over time and is not influenced by binding proteins or enzymes in plasma. This was consistent with the lack of changes in the atropisomer ratios in rat and dog plasma after administration of [^{14}C]LEN (AD-200-2002 and AD-200-2004).

[^{3}H]LEN (0.25 µM) showed high metabolic stability across all species in both hepatic microsomes (with appropriate co-factors), hepatocytes, and Hµrel® hepatocyte co-cultures (AD-200-2022, AD-200-2008 and AD-200-2037). The turnover of LEN was extremely low with unchanged LEN observed as the predominant component across all in vitro systems and species. No turnover of [^{14}C]LEN was observed in human microsomes; LEN was predicted to have a low hepatic metabolic clearance in human. In hepatic microsomes from rat, dog and monkey, [^{14}C]LEN was metabolized to a total of 4 metabolites formed via oxidation and subsequent glutathione conjugation. In the Hµrel® system, [^{14}C]LEN was metabolized via phase 2 conjugation with glutathione, pentose, hexose, and glucuronic acid; no oxidative metabolites were detected. No unique human metabolite was observed.

[^{14}C]LEN metabolism was determined following a single IV administration to rat, dog, human and an oral administration to dog (Figure 1). A comprehensive listing of the identified metabolites in plasma and excreta are provided in m2.6.5 in bile duct intact and bile duct cannulated (BDC) rats (AD-200-2002), bile duct intact and BDC dogs (AD-200-2004), and healthy human subjects (GS-US-200-4329). Urine was not profiled because of low radioactivity (< 1 % of dose) across all species. Lenacapavir was the predominant component in plasma of animal species and human. A few minor (each < 10%) late eluting peaks were identified in human AUC plasma profiles; there were also observed in dog plasma and in in vitro (rat, dog , and human) profiles – these late eluting minor peaks were likely formed due to photolysis and/or radiolysis of LEN.

Figure 1. Proposed Biotransformation of Lenacapavir in Rat, Dog and Human



3.6. Excretion

Both intestinal excretion and biliary elimination are major disposition pathways in rat and dog while renal excretion (< 1.0% of dose) is a minor disposition pathway for [^{14}C]LEN. A comprehensive listing of the extent, routes, and rates of excretion are provided in m2.6.5 including intact and BDC WH rats (AD-200-2006); intact and BDC dogs (AD-200-2007). The average cumulative recovery of administered ^{14}C dose was > 87% in rat and dog. The excretion routes in bile duct intact animals were consistent across species, with a majority of the excreted [^{14}C]LEN dose in feces (> 86% of dose) and with minor amounts in urine (< 1.0% of dose). In BDC rat and dog, the administered ^{14}C dose was excreted in bile (rat, 41.7%; dog, 32.4%) and feces (rat, 35.2%; dog, 63.0%). In combination with metabolite profiling, excretion of unchanged LEN in feces from BDC rat and dog following IV administration confirmed significant intestinal secretion of parent LEN.

Excretion of [^{14}C]LEN-derived radioactivity was also determined following a single IV administration to healthy human subjects with data reported in m2.7.2 (GS-US-200-4329).

A mechanistic study was designed to understand the excretion of intact LEN in feces that was observed in the rat [^{14}C]LEN ADME study (AD-200-2002). Lenacapavir was administered by IV infusion to bile duct intact WH rats or BDC WH rats without or with pretreatment of elacridar, a P-gp and BCRP inhibitor (AD-200-2045 and AD-200-2046). The combined data indicated that a P-gp and BCRP inhibitor decreased LEN intestinal excretion by 2- to 3-fold while having no effect on the negligible excretion in bile or urine. Systemic exposure (AUC) was increased; the overall impact on LEN CL was less than 2-fold. In vitro, LEN was a substrate for human P-gp, but not BCRP. Collectively, these data strongly suggest that intestinal secretion of LEN by P-gp was the primary mechanism for fecal excretion and a significant overall clearance mechanism of unchanged drug in rats.

3.7. Distribution in Pregnant or Nursing Animals

The plasma exposure of LEN in nursing pups was determined in a prenatal and postnatal development (PND) study in rats (TX-200-2049). A solution formulation of LEN was administered SC once to mated female SD rats at 30 and 300 mg/kg on gestation day 6. Lenacapavir was detected in the plasma of neonates on lactation day 10. Lenacapavir exposure increased with the increase in dose level from 30 to 300 mg/kg in both dams and pups. The increases in C_{max} and $AUC_{0\text{-}192h}$ were less than dose proportional from 30 to 300 mg/kg in dams. Sex-based differences were less than 2-fold. The exposure profile was similar between pregnant and nonpregnant rats. Lenacapavir present in systemic circulation of maternal rat was distributed to nursing pups via either milk or via placental transfer from maternal systemic circulation.

3.8. Pharmacokinetic Drug Interactions

3.8.1. CYP Reaction Phenotyping

There was no detectable metabolism of [^{3}H]LEN by recombinant expressed CYPs 1A2, 2B6, 2C8, 2C9, 2C19, 2D6 or 3A4 (AD-200-2030). Very slow metabolism of LEN by CYP3A5 was observed with the average rate of formation of LEN metabolite at 0.09 pmol/h/pmole enzyme which was close to the detection limit of 0.03 pmol/h/pmol enzyme. In addition, there was no clear difference in oxidative metabolites observed in hepatic microsomal fraction from a CYP3A5 *3/*3 donor compared to a CYP3A5 *1/*1 donor (AD-200-2054). These in vitro findings were consistent with the results from a clinical DDI study in healthy subjects while co-administration of oral LEN with voriconazole, a strong CYP3A inhibitor, resulted in an approximately 1.3-fold increase in LEN AUC_{inf} and no change in C_{max}. These data indicate that LEN is a substrate of CYP3A. Also, in a clinical DDI study, LEN AUC_{inf} and C_{max} were 6.7-fold and 4-fold lower, respectively, upon co-administration with rifampin, a prototypical strong inducer of multiple enzymes and transporters (GS-US-200-4333).

3.8.2. UGT Phenotyping

Low rates of metabolism of [^{3}H]LEN (2 μM) by UGT1A1 were observed with the average rate of formation of LEN metabolite was 7.2 pmol/h/mg microsomal protein (AD-200-2033). There was no detectable metabolism of [^{3}H]LEN by UGT enzymes 1A3, 1A4, 1A6, 1A7, 1A8, 1A9, 1A10, 2B4, 2B7, 2B15 or 2B17. Inhibitors or inducers of UGT1A1 and pharmacogenetic variability may potentially affect the PK of LEN. Clinical DDI studies of LEN in combination with a strong CYP3A/P-gp/UGT1A1 inhibitor atazanavir/cobicistat (COBI) confirmed that LEN is a UGT1A1 substrate (GS-US-200-4333).

3.8.3. Cytochrome P450 and UGT1A1 Inhibition

Lenacapavir (≤ 25 µM) had little or no inhibitory effect on the reversible activities of human hepatic microsomal CYPs 1A2, 2B6, 2C8, 2C9, 2C19, or 2D6 (IC_{50} > 25 µM; AD-200-2028, AD-200-2031). Clinically relevant interactions with substrates of these enzymes are unlikely. Lenacapavir showed moderate substrate-dependent, competitive inhibition of CYP3A with midazolam hydroxylase and an IC_{50} of 5.4 µM. Further testing based on IC_{50} shift analysis revealed that LEN is a mechanism-based inhibitor of this enzyme with an IC_{50} shift value of 5.2-fold (AD-200-2050). Characterization of CYP3A inactivation kinetics revealed the rate of enzyme inactivation (k_{inact}) of 0.021 min^{-1} and the inhibition constant (K_I) of 1.14 µM. The k_{inact}/K_I ratio of 0.0175 $min^{-1}\mu M^{-1}$ indicates that LEN is a relatively weak mechanism-based inhibitor. In a clinical study, AUC and C_{max} of midazolam (MDZ), a probe substrate for CYP3A, were approximately 2- to 4- fold higher, and 1-OH-MDZ AUC and C_{max} were correspondingly lower following co-administration with LEN, suggesting LEN was a moderate inhibitor of CYP3A (GS-US-200-4333).

Lenacapavir inhibited recombinant human UGT1A1 with an IC_{50} of 3.2 µM (AD-200-2031). The human plasma concentration of LEN is low (0.112 µM); SC or oral (PO) administration of LEN is unlikely to cause marked drug interactions through inhibition of this enzyme based on the regulatory guideline assessment (AD-200-2055).

3.8.4. Assessment of Induction Liability

Lenacapavir (0 - 50 µM) did not activate human xenobiotic receptors AhR or CAR (< 2-fold activation) at the highest concentration tested (AD-200-2029). Lenacapavir was a weak activator of human PXR reaching 32% of the positive control (rifampicin) at concentrations up to 50 µM and no activation was observed at concentrations ≤ 0.5 µM.

Lenacapavir (0.01– 10 µM; 7 concentrations) was evaluated in human hepatocytes (3 donors) for its potential to induce CYP1A2, CYP2B6, CYP3A4, CYP2C9, P-gp, and UGT1A1 (AD-200-2049). Changes in morphology, in parallel with increases in lactate dehydrogenase leakage, and decreases in ATP content suggested that LEN was cytopathic at 3 µM and cytotoxic at 10 µM. P-gp and CYP1A2 mRNA and activity were unchanged suggesting there was no potential for induction of CYP1A2 and AhR-regulated gene products at LEN concentrations up to 10 µM. There were no changes in CYP2B6 mRNA that were considered positive (increase >2-fold over dimethyl sulfoxide (DMSO) vehicle control (fold over control, FOC) and >20% of the positive control). For CYP2B6 activity, two donors achieved FOC values >2-fold (FOC ≤ 2.29) only at 0.3 µM LEN and both exceeded 20% of the respective positive control. However, there was no clear concentration-response relationship as FOC values at 1 µM LEN (a non-toxic concentration) were all <2-fold. The results indicate that if LEN is an inducer of CYP2B6 and CAR-regulated gene products, the maximum effect would be very weak. CYP3A4 mRNA was unchanged in two of the three donors; the mRNA FOC increased > 2-fold in the third donor and reached a maximum of 23% of the positive control at 3 µM LEN (a cytopathic dose). Thus, while this effect was only seen in one donor it suggests that induction of CYP3A4 and PXR-regulated gene products by LEN cannot be ruled out completely. CYP3A activity was reduced in all three donors in a concentration-dependent manner, likely reflecting an

inhibitory effect of LEN on CYP3A enzymes. In a clinical study, LEN was identified as a moderate inhibitor of CYP3A (GS-US-200-4333). Secondary markers for induction, CYP2C9 and UGT1A1 mRNA, increased in only one of the three donors, reaching > 2-fold at 3 µM – maximum FOC 2.38-fold and 2.48-fold, respectively; the relevance of this isolated change is unknown. The potential for LEN to cause clinically relevant drug interactions through induction is low, given the maximal LEN (C_{max}) concentration observed following single SC or oral administration was approximately 140 nM (137 ng/mL; CTRA-2021-1054).

3.8.5. Interactions with Transporters

Lenacapavir (0.1 µM) was a substrate for human P-gp, but not BCRP (AD-200-2005 and AD-200-2025). These results are consistent with efflux transport observed in Caco-2 cells. Lenacpavir was not a substrate of uptake transporters OATP1B1 or OATP1B3. Co-administration of oral LEN with strong dual CYP3A/P-gp inhibitors, COBI and darunavir/COBI, resulted in an approximately 2-fold increase in LEN AUC_{inf} and C_{max}. Collectively, these data, along with data from co-administration of oral LEN with voriconazole, indicate that LEN is also a substrate of P-gp (GS-US-200-4333).

The potential for LEN to inhibit human drug transporters was determined in cell lines transfected with individual transporters (AD-200-2026 and AD-200-2035). Lenacapavir (1 µM) did not inhibit P-gp or BCRP-mediated transport. Lenacapavir (up to 10 µM) did not inhibit OAT1-, OAT3-, OCT1-, OCT2-, and MATE2-K-mediated transport. Lenacapavir showed concentration-dependent inhibition of MATE1 and BSEP mediated transport with IC_{50} values of 2.39 and 1.21 µM, respectively. These data suggest a low potential for LEN to cause clinically relevant DDI mediated by MATE1 and BSEP inhibition. No clinically relevant changes in glomerular filtration rate or changes in serum creatinine were observed in clinical studies (GS-US-200-4625, GS-US-200-4334). Lenacapavir showed dose dependent inhibition of OATP1B1 and OATP1B3 mediated uptake with IC_{50} values of 0.021 and 0.049 µM, respectively. However, in clinical DDI studies, AUC and C_{max} of pitavastatin, an OATP substrate, were not affected following administration with LEN, suggesting LEN does not inhibit OATP transporters at clinically relevant concentrations (GS-US-200-4333).

Lenacapavir showed a concentration dependent inhibition of dog Bsep with an IC_{50} value of 0.12 µM (AD-200-2053). These data suggest that LEN can inhibit dog Bsep at the concentrations observed in safety evaluation studies (TX-200-2017 and TX-200-2047).

3.9. Summary of Pharmacokinetics

Comprehensive nonclinical studies were carried out on LEN to assess its absorption, distribution, metabolism, excretion and drug interaction potential.

Low solubility, low clearance and high potency make LEN well suited as a long acting SC injectable which was demonstrated in extensive studies in rat and dog. All factors that could influence the SC PK of LEN were assessed and supported the drug substance and formulations evaluated in safety studies and in the clinic. The oral absorption of LEN is low; however, the absorption is sufficient to support oral dosing and as a lead-in prior to initiating the SC route.

Lenacapavir atropisomers are stable in vitro and in vivo. Lenacapavir is highly metabolically stable which contributes to the very low clearance and minimal excretion as metabolites. Lenacapavir is the main component in plasma of animal species and human.

Lenacapavir is mainly cleared by biliary excretion and intestinal secretion by P-gp and to a lesser extent by metabolism, providing multiple pathways for elimination. This was supported by the modest interaction with CYP3A and P-gp inhibition in the clinic.

Lenacapavir has a favorable DDI profile as a perpetrator. CYP3A inhibition was noted in vitro and a moderate inhibition was observed in a clinical DDI study. Regulatory assessment of other drug metabolizing enzymes and transporters show no further clinical assessment is required. The most potent inhibition of transporters in vitro by LEN was with OATPs; however, no in vivo DDI was noted in a clinical DDI study.

4. TOXICOLOGY

4.1. Brief Summary

A comprehensive toxicology program has been conducted with LEN. Studies included safety pharmacology studies; single dose intravenous toxicity studies in rats and dogs; repeat-dose subcutaneous toxicity studies of up to 26 weeks duration in rats, and 39-weeks in dogs; in vitro and in vivo genotoxicity studies; assessment of fertility, early embryonic development, and pre- and postnatal development toxicity in rats; and 1-month oral toxicity studies in rats and dogs. Additional toxicology studies conducted include assessments of local tolerance, antigenicity potential and genotoxicity and toxicity studies for qualification of impurities. The completed safety pharmacology studies are described in detail in m2.6.2. The cardiovascular evaluation was included as an endpoint in the dog repeat dose toxicity study (TX-200-2006); LEN did not alter electrocardiogram (ECG) or blood pressure parameters after a single SC dose of 100 mg/kg or after repeated SC doses of 30 mg/kg.

The completed toxicology studies are described in detail in m2.6.6 and are listed in Table 1 below. For exposure margin calculations, AUC plasma values in the toxicology studies were adjusted to match the duration of the measured clinical exposure and divided by the AUC plasma values from the population PK analysis presented in m2.7.2 (CTRA-2021-1054 LEN PopPK). This data includes the C_{max} and AUC after 1500 mg oral dosing in first 2 weeks and 927 mg SC administration ($C_{max\ Day1\text{-}week\ 26}$ =136 ng/mL; free Cmax 1.98 ng/mL; $AUC_{Day1\text{-}week26}$ = 277,903 ng•h/mL)

Table 1. Overview of Lenacapavir Toxicology Program

Study	Study Number	Concentration or Dose	Tabulated Summary
Single-Dose Toxicity			
Intravenous infusion toxicity and toxicokinetic study in rats	TX-200-2029	0, 3, 10, 30 mg/kg	2.6.7.5
Intravenous infusion toxicity and toxicokinetic study in dogs	TX-200-2030	0, 3, 10, 30 mg/kg	2.6.7.5
Repeat-Dose Toxicity			
Non-Pivotal Studies			
7-Day subcutaneous and intramuscular dose range finding toxicity study in rats	TX-200-2002	Subcutaneous: 0, 5, 30, 100 mg/kg Intramuscular: 0, 5 mg/kg	2.6.7.6
7-Day subcutaneous injection dose range finding toxicity study in dog	TX-200-2004	0, 3, 10, 30 mg/kg	2.6.7.6
Pivotal Studies			
4-Dose (twice monthly) subcutaneous injection repeat dose toxicity in rats with a 6-week observation period	TX-200-2005	0, 10, 30, 100 mg/kg	2.6.7.7

Study	Study Number	Concentration or Dose	Tabulated Summary
A single- and two-dose subcutaneous injection toxicity and toxicokinetic study in rats with 3 formulations with a 13-week observation period after each dose	TX-200-2014	0, 100 mg/kg	2.6.7.7
A 4-week oral gavage toxicity and toxicokinetic study in rats	TX-200-2011	0, 3, 10, 30 mg/kg	2.6.7.7
4-Dose (twice monthly) repeat dose toxicity study in dogs with a 6-week observation period	TX-200-2006	0, 10, 30, 100 mg/kg	2.6.7.7
A 9-month (once monthly) subcutaneous injection toxicity and toxicokinetic study in dogs	TX-200-2017	0, 20, 20, 40 mg/kg	2.6.7.7
A 9-month (once monthly) subcutaneous injection toxicity and toxicokinetic study in dogs	TX-200-2047	0, 130, 411 mg/kg	2.6.7.7
A 4-week oral gavage toxicity and toxicokinetic study in dogs	TX-200-2012	0, 1, 5, 30 mg/kg	2.6.7.7
Genotoxicity			
In Vitro Studies			
Bacterial reverse mutation assay	TX-200-2007	With and without activation: 5-5000 µg/plate	2.6.7.8
Chromosome aberration assay in human peripheral blood lymphocytes	TX-200-2008	3h without activation: 84 – 172 µg/mL 24h without activation: 41.2 – 172 µg/mL 3h with activation: 120-245 µg/mL	2.6.7.8
In vivo Studies			
In vivo micronucleus assay	TX-200-2005	0, 10, 30, 100 mg/kg	2.6.7.9
Developmental and Reproductive Toxicity			
Non-Pivotal Studies			
An oral (gavage) dose range finding study of the effects on embryo/fetal development in rats	TX-200-2031	0, 3, 30, 300 mg/kg	2.6.7.11
A 30-minute intravenous infusion dose range finding study of the effects on embryo/fetal development in rabbits	TX-200-2032	0, 5, 10, 20 mg/kg	2.6.7.11
Pivotal Studies			
A subcutaneous injection study of the effects on fertility and early embryonic development to implantation in rats	TX-200-2043	0, 20, 100 mg/kg	2.6.7.12

Study	Study Number	Concentration or Dose	Tabulated Summary
An oral (gavage) study of the effects on embryo/fetal development in rats	TX-200-2036	0, 3, 10, 30 mg/kg	2.6.7.13
A 30-minute intravenous infusion study of the effects on embryo/fetal development in rabbits	TX-200-2037	0, 5, 10, 20 mg/kg	2.6.7.13
Subcutaneous prenatal and postnatal development, including a postnatal behavioral/functional and reproduction evaluation	TX-200-2049	0, 30, 300 mg/kg	2.6.7.14
Local Tolerance			
Non-Pivotal Studies			
A single dose study by subcutaneous injection in rabbits	TX-200-2009	0, 12.5, 20, 25, 50, 100, 150 mg	N/A
A subcutaneous or intramuscular injection local tolerance study in rabbits	TX-200-2015	0, 200, 300, 400 mg	N/A
Pivotal Studies			
Neutral red uptake phototoxicity assay in BALB/c 3T3 mouse fibroblasts	TX-200-2013	1.78-100 µg/mL	2.6.7.16
Bovine corneal opacity and permeability assay	TX-200-2051	750 mg	2.6.7.16
An assessment of in vitro skin corrosion using EpiDerm™	TX-200-2052	25 mg	2.6.7.16
In vitro hemocompatibility with rat, dog and human plasma	TX-200-2034	0, 0.2, 2, 3 mg/mL (Vehicle 1, rat, dog human) 0, 30 mg/mL (Vehicle 2, dog, human)	2.6.7.16
1- Month subcutaneous irritation study	TX-200-2010	0, 50, 100 mg via 100 mg/mL	2.6.7.16
1- Month subcutaneous irritation study	TX-200-2033	0, 150, 300 mg via 300 mg/mL	2.6.7.16
1- Month subcutaneous irritation study	TX-200-2048	0 or 300 mg via 500 mg/mL	2.6.7.16
3- Month subcutaneous irritation study	TX-200-2038	0 or 300 mg via 300 mg/mL	2.6.7.16
9- Month subcutaneous irritation study	TX-200-2019	0, 100, 200, 400 mg/kg via 200 mg/mL or 400 mg/mL	2.6.7.16
Other Toxicity Studies			
In Chemico skin sensitization (Direct peptide reactivity assay)	TX-200-2071	100 mM	2.6.7.17
In vitro skin sensitization (ARE-Nrf2 luciferase test method)	TX-200-2072	0.98 – 2000 µM	2.6.7.17

Study	Study Number	Concentration or Dose	Tabulated Summary
In vitro sensitization (human cell line activation test	TX-200-2073	17.5-62.8 µg/mL	2.6.7.17
Local lymph node assay in the mouse	TX-200-2053	10, 25, 50% v/v	2.6.7.17
Impurity bacterial reverse mutation assay with GS-833244	TX-200-2025	5-5000 µg/plate with and without activation	2.6.7.17
Impurity bacterial reverse mutation assay with LENA-2078 (GS-832078)	TX-200-2041	25-5000 µg/plate with and without activation	2.6.7.17
Impurity bacterial reverse mutation assay with LENA-0738 (GS-830738)	TX-200-2023	5-5000 µg/plate with and without activation	2.6.7.17
Bacterial reverse mutation assay with GS-831396 (LENA-1396)	TX-200-2024	5-5000 µg/plate with and without activation	2.6.7.17
Impurity chromosome aberration assay in human peripheral blood lymphocytes with GS-833244	TX-200-2075	4 h without S9: 10, 25, 70 µg/mL 20 h without S9: 5, 15, 25 µg/mL 4 h with S9: 5, 25, 50 µg/mL	2.6.7.17
Impurity chromosome aberration assay in human peripheral blood lymphocytes with GS-941756	TX-200-2076	4 hours with and without activation or 20 hours without activation: 21.9, 43.9, 87.8 µg/mL	2.6.7.17
Single dose subcutaneous injection impurity qualification study in rats with a 4-week observation period	TX-200-2042	0, 100 mg/kg	2.6.7.17
Single dose subcutaneous injection impurity qualification study in rats with a 3-month observation period	TX-200-2050	0, 100 mg/kg	2.6.7.17
Transgenic mouse dose range finding toxicity study	TX-200-2045	0, 30, 99, 300 mg/kg	2.6.7.17
Single dose oral gavage pharmacokinetic study in rats	TX-200-2026	0, 3, 30 mg/kg	2.6.7.17
A 7-day once daily oral gavage tolerability and toxicokinetic study in female nonpregnant rabbits	TX-200-2035	0, 100, 300, 1000 mg/kg	2.6.7.17
Assessment of a subcutaneous depot following a single subcutaneous infusion in rats	TX-200-2027	0, 6, 30, 240 mL/h	2.6.7.17

4.2. Single Dose Toxicity

Single dose IV toxicity studies in rat (TX-200-2029) and dog (TX-200-2030) with LEN have been conducted; no mortality occurred at up to 30 mg/kg, the highest dose tested. The intravenous NOAEL in rats is 30 mg/kg; the IV NOAEL in dogs is 3 mg/kg.

Male and female WH rats were administered a single dose of vehicle control article or 3, 10, or 30 mg/kg LEN by 30-minute IV infusion via tail vein and observed over 2 weeks. No LEN-related adverse effects occurred.

Male and female dogs were administered a single dose of vehicle control article or 3, 10, or 30 mg/kg LEN via 30-minute IV infusion, and animals were observed over 2 weeks. In animals administered ≥10 mg/kg, LEN-related adverse liver changes were observed on Day 2 in clinical pathology parameters (mildly to markedly increased alanine and aspartate aminotransferase, gamma glutamyl transferase, alkaline phosphatase activity, and total bilirubin) with correlative microscopic findings (hepatocyte degeneration and necrosis, and mixed cell infiltrate; hemorrhage around central veins and in connective tissue between the liver and gallbladder). At the 15-day terminal sacrifice, clinical pathology changes had partially or fully reversed and there were no LEN-related microscopic findings. Therefore, the NOAEL is 3 mg/kg. Based on the time course, the liver changes appear to be C_{max} related. The C_{max} reached after IV dosing of 10 and 30 mg/kg was 15.4 and 38.9 µg/mL, respectively. Based on the $C_{max\ Day1\text{-}Week\ 26SC}$ (CTRA-2021-1054 LEN PopPK) of 0.136 µg/mL, the C_{max} achieved where liver effects were observed (10 mg/kg) are ≥ 113-fold higher than the clinical C_{max}. The C_{max} at the 3 mg/kg NOAEL (5.1 µg/mL) is 38-fold higher than the clinical C_{max}.

4.3. Repeat Dose Toxicity

Repeat-dose subcutaneous toxicity studies were conducted of up to 26 weeks duration in rats (TX-200-2002, TX-200-2005, TX-200-2014 and 39-weeks in dogs (TX-200-2004, TX-200-2006, TX-200-2017, TX-200-2047) and 28-day oral toxicity studies in rats (TX-200-2011) and dogs (TX-200-2012). Expected local granulomatous inflammation was observed at the subcutaneous injection sites due to the depot formation. No systemic toxicity was observed in rats after subcutaneous or oral administration of LEN or in dogs after oral administration.

4.3.1. Liver

Two repeat toxicity studies in dogs have been conducted via monthly SC injection of LEN for up to 9 months (10 doses). Clinical, clinical pathology and anatomic pathology data from the second study indicate hepatobiliary changes indicative of cholestasis.

LEN was well tolerated in male and female beagle dogs when administered as 10 monthly subcutaneous injections of 20 mg/kg/dose with or without NaOH or 40 mg/kg/dose without NaOH in the vehicle (TX-200-2017). No LEN-related systemic toxicity was observed; LEN-related subcutaneous injection site reactions were expected and included microscopic observations of necrosis, granulomatous inflammation, mixed cell inflammation, and/or macrophage infiltrate. Adverse subcutaneous injection site necrosis was noted in animals

administered 20 mg/kg/dose with NaOH or 40 mg/kg/dose without NaOH. Overall, an increased interval since dosing was generally associated with a successive decrease in the overall incidence/severity of microscopic findings. Lenacapavir was present in plasma at all time points analyzed, indicating exposure for the 9-month study duration. On Day 225, the sex-combined plasma C_{max} and $AUC_{0\text{-}672h}$ values were 494 ng/mL and 267,000 ng·h/mL, respectively, in animals administered 200 mg/mL LEN without NaOH; 555 ng/mL and 277,000 ng·h/mL, respectively, in animals administered 200 mg/mL LEN with NaOH; and 658 ng/mL and 352,000 ng·h/mL, respectively, in animals administered 400 mg/mL LEN without NaOH. These LEN exposures are up to 8-fold higher than clinical exposure based on the $AUC_{Day1\text{-}Week\ 26SC}$ (CTRA-2021-1054 LEN PopPK) of 277903 ng•h/mL.

In the second study (TX-200-2047), three groups of male and female Beagle dogs (4 or 6 animals/sex/group) were administered GS-6207 at dosages of 0 (vehicle control article: 68:32 w/w/w PEG300:sterile water for injection [SWI]), 130, or 411 mg/kg/dose at a dose volume of 1.33, 0.42 or 1.33 mL/kg, respectively. Animals were administered up to 10 doses via subcutaneous injection in the dorsal region as a single dose on Days 1, 29/32, 57, 85, 113, 141, 169, 197, 225, and 253. On Day 29, only controls and one 130 mg/kg/dose male were dosed due to transient discomfort postdose; all remaining animals in the 130 and 411 mg/kg/dose groups were dosed on Day 32 when dosing under anesthesia was initiated.

Monthly subcutaneous administration of 411 mg/kg/dose LEN to dogs was not tolerated and resulted in adverse clinical observations that necessitated cessation of dosing after 2 or 3 doses. All vehicle and 130 mg/kg/dose animals survived to their scheduled interim or terminal sacrifice. The clinical observations that preceded the unscheduled sacrifice of 1 male and 2 females administered 411 mg/kg/dose included yellow color of oral mucosa or conjunctiva, thin or hunched appearance, and/or 19 to 28% drop in body weight from peak and were attributed to hepatobiliary degeneration. Lenacapavir-related clinical pathology changes in animals administered 411 mg/kg/dose were consistent with cholestasis and hepatobiliary injury. These clinical pathology changes correlated with clinical observations (icteric sclera, oral mucosa, and inner pinna) and microscopic findings in the liver of vacuolar hepatocyte and bile duct epithelial degeneration, bile pigment, and/or oval cell/bile ductule hyperplasia. Lenacapavir-related microscopic findings in animals administered 411 mg/kg/dose were noted in the liver, gall bladder, kidney, and stomach; these microscopic findings were partially (liver and gall bladder) or fully reversible (kidney and stomach). Compared to the 411 mg/kg/dose group, monthly administration of 130 mg/kg/dose GS-6207 for 9 months (10 doses) resulted in less severe changes in clinical pathology test results, and fewer and less severe liver and gall bladder microscopic findings, but included periductular fibrosis in one dog, considered adverse. Subcutaneous injection sites had nonadverse minimal to marked granulomatous and/or mixed cell inflammation, adipose tissue necrosis, fibrosis, and/or pseudocyst. A NOAEL was not established for LEN in this study. The dose level of 130 mg/kg/dose corresponded to Study Day 225 mean C_{max} and $AUC_{0\text{-}336}$ values of 5480 ng/mL and 1540000 ng•h/mL, respectively and Day 253 C_{max} value of 4860 ng/mL, for combined sexes. These LEN exposures are ≥ 51fold higher than clinical exposure based on the $AUC_{Day1\text{-}Week\ 26SC}$ (CTRA-2021-1054 LEN PopPK) of 277903 ng•h/mL.

4.4. Genotoxicity

Lenacapavir was not genotoxic in the bacterial reverse mutation test (TX-200-2007), the in vitro chromosomal aberrations assay using cultured human peripheral blood lymphocytes (TX-200-2008) and the in vivo micronucleus assay in rats conducted as part of the repeat dose toxicity study (TX-200-2005).

Table 2. Genotoxicity Studies Conducted with Lenacapavir

Test	Lot Number	Test System	Metabolic activation[a]	Dose/Concentration	Results
In vitro Microbial reverse mutation assay	14044-28-002	S. typhimurium and E. coli	Without and With	5-5000 µg/plate	(–)
In vitro chromosomal aberrations	14044-28-002	Human lymphocytes (in vitro) 3-hour 24-hour 3 hour	 Without Without With	 84-172 µg/mL 41.2 - 172 µg/mL 120-245 µg/mL	 (–) (–) (–)
Rat micronucleus	14044-66-003	Rat bone marrow cells (in vivo)[b]	N/A	10, 30, 100 mg/kg	(–)

N/A – Not applicable.
a Aroclor-induced rat liver S-9 fraction from male SD rats.
b The mean C_{max} and $AUC_{(0\text{-}336h)}$ values (males + females) for LEN after the 4th SC injection of 100 mg/kg/dose was 2080 ng/mL and 583000 ng·h/mL, respectively.

4.5. Carcinogenicity

The 6-month transgenic mouse (TgrasH2) and the 2-year rat carcinogenicity studies with LEN via subcutaneous injection are in progress. Doses were agreed upon with the US FDA. The final reports will be submitted when available; approximately the end of 20[redacted] for the mouse study and early 20[redacted] for the rat study. The nonclinical data support the overall positive benefit-risk of LEN for the proposed population in the absence of carcinogenicity reports at the time of the initial application. See Section 5.1 for additional information.

The 6-month transgenic mouse carcinogenicity study is being conducted to evaluate the carcinogenic potential and toxicokinetics of LEN when administered once every 13 weeks by a subcutaneous injection to 001178-T (hemizygous) RasH2 mice for at least 26 weeks (TX-200-2068). Four groups of 25 male and 25 female 001178-T (hemizygous) RasH2 mice are administered LEN solution via subcutaneous injection every 13 weeks at dose levels of 0 (vehicle control: 64:30:6 w/w/w PEG 300:SWI:poloxamer 188), 30, 100, 300 mg/kg and observed for an additional 13 weeks. An additional group of 001178-T (hemizygous) RasH2 mice (10 animals/sex) were administered one intraperitoneal dose of N-methyl-N-nitrosourea on Day 1 and served as a positive control to ensure animals supplied appropriately expressed oncogenes and responded to carcinogenic insult. Assessment of carcinogenic potential is based on anatomic pathology. Mortality, clinical observations, body weights, and food consumption are also assessed. Blood samples will be collected from toxicokinetic animals for toxicokinetic evaluations of LEN.

The 104-week rat carcinogenicity study is being conducted to evaluate the carcinogenic potential of LEN administered once every 13 weeks (via 1 or 2 injections) to Wistar Han rats for up to 104 weeks (TX-200-2046). Four groups of 55 male and 55 female Crl:WI(Han) rats are administered LEN solution via subcutaneous injection every 13 weeks at dose levels of 0 (vehicle control: 68:32 w/w PEG 300:SWI), 102, 309, or 927 mg/kg. Assessment of carcinogenic potential is based upon anatomic pathology. In addition, mortality, clinical observations, body weight, and food consumption are evaluated. Toxicokinetic assessment is conducted for LEN.

4.6. Reproductive Toxicity

The rat fertility (TX-200-2043) and peri/postnatal (TX-200-2049) studies administered LEN via single SC injection because it is the primary clinical route and adequate exposures were reached for the duration of critical development phases. The rat embryofetal development studies (TX-200-2031, TX-200-2036) administered LEN daily via the oral route because C_{max} is reached faster after oral administration than SC, ensuring exposure during organogenesis. (T_{max} is ~2-4 weeks after a single SC dose). The rabbit embryofetal development studies (TX-200-2032, TX-200-2037) administered LEN daily via IV infusion because adequate exposure to LEN was not reached with oral administration (TX-200-2035).

In the subcutaneous rat fertility study, no adverse effects occurred in male or female fertility study at 100 mg/kg, the highest dose administered. No LEN-related effects on mean body weights, body weight gains, and food consumption were noted, and no effects on male and female reproductive performance (mating, fertility, and pregnancy), male spermatogenesis (sperm numbers, sperm production rate, and motility), and male or female organ weights were noted. No LEN-related macroscopic findings were noted. Intrauterine survival of the embryos was unaffected by LEN administration at all dose.

Oral administration of LEN to pregnant rats during gestation day (GD) 6 through 17 at dose levels of 3, 10, and 30 mg/kg/day resulted in no LEN-related effects on maternal animals or embryo/fetal development. Intravenous administration of LEN to pregnant rabbits during GD 7 through 19 at dose levels of 5, 10 and 20 mg/kg/day also resulted in no LEN- related effects on maternal animals or embryo/fetal development.

In the subcutaneous rat SC peri- and postnatal toxicity study, pregnant rats were administered a single dose of LEN on GD6 of 30 or 300 mg/kg. Parameters evaluated included pregnancy, parturition, and lactation of the maternal (F_0) animals and on the growth, viability, and development of the F_1 neonates. Reproductive performance of the F_1 generation was also assessed. No LEN-related systemic effects were noted at any dose level tested (30, 300 mg/kg) in the F_0 or F_1 generation.

4.7. Local Tolerance

Lenacapavir was negative in the *in vitro* mouse fibroblast assay for phototoxicity potential (TX-200-2013). The results of the bovine corneal opacity assay indicate that LEN does not require classification for eye irritation (TX-200-2051). Lenacapavir was noncorrosive in the *in vitro* corrosion assay using EpiDerm™ (TX-200-2052). A vehicle was identified as compatible for single dose intravenous administration in a hemocompatibility assay using rat, dog and human blood samples (TX-200-2034).

Several rabbit subcutaneous irritation studies were conducted to evaluate injection site reactions to potential clinical formulations. Injection sites were evaluated for 1 month (TX-200-2010, TX-200-2033, TX-200-2048), 3 months (TX-200-2038) or 9 months (TX-200-2019). Varying degrees of erythema and edema were observed. The macroscopic observation of thickening and the microscopic observations of mixed cell or granulomatous inflammation, necrosis, and mononuclear cell infiltrates at the subcutaneous injection site of animals administered LEN in local tolerance studies were expected reactions of subcutaneous depots. Similar macroscopic and microscopic observations occurred at the injection sites in repeat dose subcutaneous toxicity studies.

4.8. Other Toxicity Studies

4.8.1. Antigenicity

Three in vitro assays were conducted as part of an integrated approach to testing and assessment to support the discrimination between skin sensitizers and non-sensitizers for the purpose of hazard classification and labelling. An In Chemico skin sensitization study (Direct Peptide Reactivity Assay) was conducted to quantify the reactivity of LEN towards model synthetic peptides containing either lysine or cysteine (TX-200-2071). Lenacapavir was positive with low reactivity in the Direct Peptide Reactivity Assay. The potential of LEN to induce genes that are regulated by the antioxidant response element (ARE) (TX-200-2072) was evaluated. Lenacapavir was negative in the ARE-Nrf2 Luciferase assay. The potential of LEN to activate monocytes and dendritic cells in the human monocytic leukemia cell line THP-1 was evaluated by quantifying changes in the expression of cell surface markers (CD86 and CD54) (TX-200-2073). Lenacapavir was positive in the human Cell Line Activation Test.

The in vivo assay for assessment of sensitization potential was the mouse lymph node assay (individual method, TX-200-2053). Lenacapavir was prepared for topical administration at 10, 25 and 50% v/v in dimethylforamamide. Lenacapavir was not a sensitizer in the local lymph node assay.

4.8.2. Immunotoxicity

Data from repeat-dose toxicity studies with LEN (hematology, lymphoid organ weights, microscopy of lymphoid tissues, bone marrow cellularity) did not suggest immunotoxic potential. No further studies were deemed necessary with LEN.

4.8.3. Dependence

No specific studies on dependency of LEN were conducted. There was no evidence of development of dependence in nonclinical studies with LEN. Tissue distribution studies using radiolabeled LEN in both rat and dog (m2.6.4, Section 4.3) indicated that very low concentrations of radioactivity at C_{max} were observed in the central nervous system. Consequently, dependency studies with LEN are not necessary.

4.8.4. Studies on Metabolites

No specific toxicology studies on LEN metabolites were conducted. In vivo, LEN is primarily cleared as an unchanged parent drug (m2.6.4, Section 5.2). There is no major human metabolite.

4.8.5. Impurities/Degradation Products

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

Two repeat-dose toxicity studies were conducted in rats to determine if there were unexpected adverse effects from LEN-related process impurities (TX-200-2042, TX-200-2050). Male and female rats were administered a single SC dose of 100 mg/kg LEN solutions using a pure (at least 98.7%) LEN batch or batches with added impurities and observed for 4 weeks (TX-200-2042) or 13 weeks (TX-200-2050). The vehicle used in both toxicology studies was 67.24% [w/w] PEG-300/31.40% [w/w] SWI/1.36 % [w/w] poloxamer 188. Lenacapavir plasma exposure was confirmed for the duration of observation. No adverse LEN-related effects were observed and there were no differences in findings in animals treated with lots containing LEN-related impurities to those observed in previous studies, or to a comparator lot. The NOAEL for both studies was 100 mg/kg.

Four GLP reverse mutation assays were conducted with impurities in S. typhimurium strains in the absence and in the presence of a rat liver metabolic activation system (S9). GS-833244 (TX-200-2025) and LENA-2078 (GS-832078) (TX-200-2041) were negative for the ability to induce reverse mutations at selected loci of several strains of Salmonella typhimurium and at the tryptophan locus of Escherichia coli strain WP2 uvrA in the presence and absence of S9.

LENA-0738 (GS-830738) induced mutation in three histidine-requiring strains TA98, TA100 and TA1537, and one tryptophan-requiring strain WP2 *uvrA* of *Escherichia coli* in the presence of S-9 when tested under the conditions of this study (TX-200-2023).

LENA-1396 (GS-831396) induced mutation in histidine-requiring strain *Salmonella typhimurium* TA100 when tested in the presence of a rat liver metabolic activation system (S-9) under the conditions of this study (TX-200-2024).

Two GLP studies were conducted to evaluate the potential of two LEN impurities and/or its metabolites to induce structural chromosomal aberrations in human peripheral blood lymphocytes in the presence and absence of an exogenous metabolic activation system (S-9). GS-833244 did not induce chromosomal aberrations in the absence and/or presence of S-9 when tested under the conditions of this study (TX-200-2075). GS-941756 did not induce chromosomal aberrations in the absence and/or presence of S-9 when tested under the conditions of this study (TX-200-2076).

Based on their impurity profiles, the multiple batches of LEN tested in the GLP toxicology program, in composite, are considered to be representative of the Good Manufacturing Process material and support the specified limits of impurities for commercial production. The nongenotoxic compound specifications are discussed in m3.2.S.4.5, Justification of Specification, and the specified limits of degradation products proposed, m3.2.P.5.6, Justification of Specifications). The genotoxic compound controls are discussed in m3.2.S.2.6 of the filing Section 1.3.3.5. Both GS-830738 (i.e., LENA-0738) and GS-831396 (i.e., LENA-1396) are controlled below the threshold of toxicological concern (TTC) in LEN sodium as discussed in Section 1.3.3.5.6.2 and 1.3.3.5.7.1, respectively.

4.8.6. Other Toxicity Studies

4.8.6.1. Transgenic Mouse Dose Range Finding Toxicity Study

The purpose of this study was to evaluate the toxicity and determine the toxicokinetics of LEN when administered once via SC injection to rasH2 transgenic mice with a 13-week observation period (TX-200-2045).

Four groups of 10 male and 10 female RasH2 (001178-T [hemizygous], CByB6F1-Tg[HRAS]2Jic) mice were administered LEN sodium solution once via SC injection at dose levels of 0 (vehicle control article: 63.7% w/w PEG 300, 29.7% w/w SWI, 6.6% w/w poloxamer 188), 30, 99, or 300 mg/kg at a dose volume of 1000 µL/kg (vehicle and 300 mg/kg group), 100 µL/kg (30 mg/kg group), or 330 µL/kg (99 mg/kg group). A subgroup of animals (3 vehicle control animals/sex, 27 animals/sex in LEN-treated groups) was included for toxicokinetic evaluation.

Assessment of toxicity was based on mortality, clinical observations, body weights, body weight changes, quantitative food consumption, ophthalmic observations, and clinical and anatomic pathology. Blood samples were collected for toxicokinetic evaluation.

The toxicokinetic data indicate that the mice were exposed to LEN for the 13-week observation period. Exposure to LEN increased with the increase in dose level from 30 to 300 mg/kg. The increases in C_{max} and $AUC_{0\text{-}336h}$ were less than dose proportional. Sex-based differences were less than 2-fold.

Lenacapavir-related clinical observations included palpable masses and abnormal skin color at the dose site for animals administered 300 mg/kg, which correlated with microscopic observations of minimal to marked granulomatous inflammation at the SC injection site. Minimal to moderate granulomatous inflammation was also observed at the SC injection site in

animals administered 30 or 99 mg/kg. Lenacapavir-related increases in ALT activities were noted in one female each administered 30, 99, or 300 mg/kg. Lenacapavir-related slight increases in mean liver weight values were noted in both sexes administered 300 mg/kg. Increased liver weights in males administered 300 mg/kg correlated with the microscopic finding of slight centrilobular hepatocyte vacuolation. Thus, the LEN systemic NOAEL in rasH2 transgenic mice is 300 mg/kg. The local tolerance/toxicity NOAEL is 300 mg/kg for males and 99 mg/kg for females

4.8.6.2. Single Dose Oral Gavage Pharmacokinetic Study of LEN in Wistar Han Rats

The purpose of this study was to investigate the pharmacokinetics of LEN solution in Capmul® when administered as a single dose via oral gavage to male and female Wistar Han rats. Three groups (n = 6 animals/sex/group) of Wistar Han (Crl:WI[Han]) rats were administered a single oral dose of LEN solution in 100% Capmul MCM at dose levels of 3 or 30 mg/kg via 2 mL/kg, or 30 mg/kg via 0.6 mL/kg, respectively (TX-200-2026).

After oral gavage administration, LEN was slowly absorbed with an average T_{max} values ranging from 8.00 to 24.0 hours. Lenacapavir concentrations were measurable through 120 hours in all groups. The mean concentration-time profiles for males, females, and combined sexes showed that exposure to LEN increased with the increase in dose levels from 3 to 30 mg/kg. Exposure was generally similar in animals administered 30 mg/kg at a dose volume of either 2 or 0.6 mL/kg. The increases in LEN C_{max}, $AUC_{0\text{-}24h}$, and $AUC_{0\text{-}120h}$ values were less than dose proportional between 3 and 30 mg/kg at a dose volume of 2 mL/kg. Sex differences were less than 2-fold.

4.8.7. 7-Day Once Daily Oral (Gavage) Tolerability and Toxicokinetic Study of LEN in Female Nonpregnant New Zealand White Rabbits

The objective of this nonGLP study was to determine the tolerability of LEN in nonpregnant female New Zealand White rabbits (TX-200-2035). A LEN solution was administered at doses of 0 (100% Capmul MCM), 100, 300, and 1000 mg/kg/day once daily via oral gavage to nonpregnant female New Zealand White rabbits (3/group) for 7 days at dose volumes of 1.3, 0.2, 0.6, and 2 mL/kg, respectively. Assessment of toxicity was based on mortality, clinical signs, body weights, body weight gains, food consumption, toxicokinetic parameters, and gross necropsy findings.

All plasma concentration values of LEN in the vehicle control group were below the lower limit of quantification. After the first dose, individual $AUC_{0\text{–}24h}$ values in animals administered 100 and 300 mg/kg increased with the increase in dose level from 100 to 300 mg/kg/day, and C_{max} increased approximately dose-proportionally. A single dose of 1000 mg/kg/day did not result in an increase in exposure compared to 300 mg/kg.

No LEN-related adverse effects occurred upon daily dosing of 100, 300, and 1000 mg/kg/day for 7 days. Based on these results, 1000 mg/kg/day was considered the NOAEL, corresponding to Study Day 6 C_{max} and $AUC_{0\text{-}96h}$ values of 103 ng/mL and 5270 ng·h/mL, respectively.

4.8.8. Assessment of Subcutaneous Depot Following a Single Subcutaneous Infusion of LEN in the Wistar Han Rat

The objective of this non-GLP study was to determine the physical characteristics of a SC depot when delivered to rats over various infusion durations (TX-200-2027).

Male Wistar-Han rats were assigned to 6, 30 or 240-mL/hour infusion rate groups (7 groups total, 3 rats/group). Rats were administered a single dose of either vehicle 1 (77:10:13 w/w/w PEG300:ethanol:SWI), Vehicle 2 (85:15 w/w PEG300:SWI) or LEN in Vehicle 1 or 2 at a dose concentration of 300 mg/mL and a fixed volume of 0.25 mL.

Post dose evaluations consisted of manual depot measurements and magnetic resonance imaging (MRI) evaluation through 7 days post dose. Manual depot measurements were recorded twice on Day 1 as soon as possible after dosing and after MRI and at 24 hours and 7 days post dose. MRI evaluations were conducted on anesthetized rats immediately post dose, approximately 24 hours and 7 days post dose. In the MRI evaluation animals were imaged in the ventral position using a small animal 7T MRI system equipped with a 210/105 mm Magnex gradient coils and a 63 mm volume coil. Injection sites were collected following the last MRI evaluation to quantify the mineralization site by micro-computerized tomography (micro-CT).

All animals survived until study termination. In general, similar depot volumes were observed immediately post dose in animals administered either Vehicle 1 or 2 or LEN groups as assessed by manual depot size measurements and MRI. Manual depot size measurements were slightly higher immediately post MRI timepoint than measurements taken immediately post dose.

As assessed quantitatively by MRI, both control and all treated groups had similar depot volume on Day 1 as soon as possible after dosing. Following Day 1, decreases in depot volume were noted in all groups. A higher rate of decrease in depot volume was noted at 0 mg/mL, leading to lower depot volume compared to treated groups at 24h and 7 days post dose. Magnetic resonance images suggested that this process was concomitant with an organization of the depot, as characterized by decreased intensity of the signal over time, and the development of an inflammatory process, as characterized by the presence of hyperintensity zones. These observations were confirmed by the objective assessment of the depot as the mean intensity of the signal of the depot decreased over time, but the heterogeneity of the depot, as assessed by the coefficient of variation of the signal intensity into the depot, remained relatively stable over time.

Micro-computed tomography was not conducted as the depots were not clearly detected in the spare animals used for method development.

In conclusion, both reference items and all LEN dosed groups presented similar depot volume immediately following dosing. Twenty-four hours and 7-days post dose decreases in depot volume were noted in all groups. Reference Item groups presented a higher rate of decrease in depot volume leading to lower depot volumes in Reference Item groups compared to LEN-dosed groups at 24 hours and 7 days post dose. At 24 hours and 7 days post dose reference item groups presented similar depot volume and no meaningful differences were noted between LEN-dosed groups. All groups presented similar heterogeneity of the depot over the time course of the study and there was less leaking in groups receiving PEG300:ethanol:SWI formulation. Based on results obtained, the infusion rate and needle gauge didn't seem to impact depot size or heterogeneity.

4.9. Summary of Nonclinical Safety

4.9.1. Relationship of Findings to Exposure

The plasma concentrations of LEN associated with key responses and the associated exposure margins are presented in Table 3, below. Margins of exposure presented are based $C_{max\ Day1\text{-}week26}$ (136 ng/mL) and $AUC_{Day1\text{-}week26}$ (277903 ng•h/mL) after 1500 mg oral dosing in first 2 weeks and 927 mg SC administration (CTRA-2021-1054 LEN PopPK).

Table 3. Concentrations of Lenacapavir Associated with Key Responses[a]

Key Response(s)	Dose (mg/kg)	AUC[b] (ng•h/mL)	C_{max}[b] (ng/mL)	Exposure Margin[c]
Safety Pharmacology Studies				
Respiratory evaluation using head-out plethysmography via SC injection in male rats (8/dose)				
No noteworthy findings	10	ND	ND	N/A[d]
No noteworthy findings	30 (NOEL)	ND	ND	N/A[d]
Minor increase in covariate adjusted mean tidal volume and a minor decrease in covariate-adjusted mean respiration rate (not statistically significant)	100 (NOAEL)	ND	ND	N/A[d]
Behavioral/CNS evaluation as part of 6-Week (4 dose) SC toxicity study in rats with a 6-week observation period - 20/sex/dose				
		$AUC_{0\text{-}336h}$	Free C_{max}	Free C_{max} Margin[c]
No noteworthy CNS findings	10	120,000	0.545	0.27
No noteworthy CNS findings	30	279,000	1.23	0.62
No noteworthy CNS findings	100 (NOEL)	583,000	2.7	1.4
ECG evaluation as part of 6-Week (4 dose) SC toxicity study in dogs (6/sex/dose)				
		$AUC_{0\text{-}336h}$		
No noteworthy ECG findings	10	118,000	5.30	2.7
No noteworthy ECG findings	30	436,000	18.5	9.3
No noteworthy ECG findings (single dose)	100 (NOEL)	1,030,000	39.0	20
Single-Dose Toxicity Studies				
Rat intravenous infusion (30 min) (15/sex/dose)		$AUC_{0\text{-}168.5h}$		
No LEN-related findings	3	32,500	1280	3[e]
No LEN-related findings	10	99,300	4630	9[e]
↑liver weight	30 (NOAEL)	224,000	12700	21[e]

Key Response(s)	Dose (mg/kg)	AUC[b] (ng•h/mL)	C_{max}[b] (ng/mL)	Exposure Margin[c]
Dog intravenous infusion (30 min) (5/sex/dose) –				
↓Body weight, hemorrhage present around central veins and in the connective tissue between the liver and gallbladder	3 (NOAEL)	35,200	5140	3[e]
↓Body weight, changes in coagulation and liver parameters in clinical pathology associated with hepatocyte degeneration, individual hepatocyte necrosis, mixed cell infiltration 2 days post dosing; hemorrhage present around central veins and in the connective tissue between the liver and gallbladder. Clinical pathology changes less severe 14 days post dose. No histopathology 14 days postdose.	10	194,000	15400	18[e]
↓Body weight, changes in coagulation and liver parameters in clinical pathology associated with hepatocyte degeneration, individual hepatocyte necrosis, mixed cell infiltration 2 days post dosing; hemorrhage present around central veins and in the connective tissue between the liver and gallbladder Clinical pathology changes less severe 14 days post dose. No histopathology 14 days postdose.	30	611,000	38900	57[e]
Repeat-Dose Toxicity Studies				
6-Week (4 dose) SC toxicity study in rats with a 6-week observation period (20/sex/dose)				
		$AUC_{0\text{-}336h}$		
No noteworthy findings	10	120,000	419	6[f]
No noteworthy findings	30	279,000	945	13[f]
No noteworthy findings	100 (NOAEL)	583,000	2080	27[f]
26-week (2 dose) SC toxicity study in rats with a 13-week interim necropsy (10/sex/dose)				
		$AUC_{0\text{-}672h}$		
No noteworthy findings – 200 mg/mL, 0 mol-eq NaOH	100 (NOAEL)	307,000	742	7[g]
No noteworthy findings – 200 mg/mL, 1 mol-eq NaOH	100 (NOAEL)	284,000	780	6[g]
No noteworthy findings – 400 mg/mL, 0 mol-eq NaOH	100 (NOAEL)	211,000	451	5[g]
4-Week oral toxicity study in rats (10/sex/dose)				
		$AUC_{0\text{-}24h}$		
No noteworthy findings	3	15,300	712	9[h]
No noteworthy findings	10	27,800	1480	17[h]
↑ CYP3A activity in females	30 (NOAEL)	34,500	1700	21[h]

Key Response(s)	Dose (mg/kg)	AUC[b] (ng•h/mL)	C_{max}[b] (ng/mL)	Exposure Margin[c]
6-Week (4 dose) SC toxicity study in dogs (6/sex/dose)				
		$AUC_{0\text{-}336h}$		
Adverse behavioral reactions to injection; anesthesia used for last 2 doses; adverse local injection site reactions	10	118,000	641	6[f]
Adverse behavioral reactions to injection; anesthesia used for last 2 doses; adverse local injection site reactions	30	436,000	2230	20[f]
Adverse behavioral reactions to injection; group discontinued after 1 dose	100	1,030,000	4700	48[f]
9-Month (10 Doses) SC toxicity study in dogs (9/sex/group)				
		$AUC_{0\text{-}672h}$		
No noteworthy findings – 200 mg/mL, 0 mol-eq NaOH	20	267,000	494	6[g]
No noteworthy findings – 200 mg/mL, 1 mol-eq NaOH	20	277,000	555	6[g]
No noteworthy findings – 400 mg/mL, 0 mol-eq NaOH	40 (NOAEL)	352,000	658	8[g]
9-Month (10 Doses) SC toxicity study in dogs (9/sex/group)				
		$AUC_{0\text{-}672h}$		
Changes in the liver (oval cell/bile ductule hyperplasia, periductular fibrosis attributed to the longer dosing duration, and/or increased pigmented macrophages), gall bladder (epithelial hyperplasia, increased lumen secretion, and/or lumen pigment),	130	3,200,000	5,480	69[g]
Dosing discontinued after 2 or 3 doses. Vacuolar degeneration of hepatocytes and/or bile duct epithelium, bile pigment, mixed cell infiltrate, and/or oval cell/bile ductule hyperplasia), gall bladder (epithelial hyperplasia, mucosal edema, increased lumen secretion, and/or lumen pigment), kidney (tubule degeneration and/or dilatation, and/or pigment), and stomach (mucosa degeneration/atrophy).	411	2,380,000	4,920	51[g]
4-Week oral toxicity study in dogs (4/sex/group)				
		$AUC_{0\text{-}24h}$		
No noteworthy findings	1	3440	204	2[h]
No noteworthy findings	5	19,300	1,230	12[h]
No noteworthy findings	30 (NOAEL)	75,900	4,390	46[h]

Key Response(s)	Dose (mg/kg)	AUC[b] (ng•h/mL)	C_{max}[b] (ng/mL)	Exposure Margin[c]
Developmental and Reproductive Toxicity Studies				
Subcutaneous male and female fertility and early embryonic development study in Rats (25/sex/dose)				
		$AUC_{0\text{-}672h}$ M/F	C_{max} M/F	
No noteworthy findings	20	103,000/91,100	188/153	0.4/0.3
No noteworthy findings	100 (NOAEL)	270,000/192,000	577/597	1/0.7
Oral embryo-fetal development study in rats (25 females/dose)				
		$AUC_{0\text{-}24h}$		
No noteworthy findings	3	9,200	435	6[h]
No noteworthy findings	10	17,800	1050	11[h]
No noteworthy findings	30 (NOAEL)	22,000	1210	13[h]
Intravenous embryo-fetal development study in rabbits (22 females/dose)				
		$AUC_{0\text{-}24.5}$		
No noteworthy findings	5	26,600	7,120	16[h]
No noteworthy findings	10	89,500	22,300	54[h]
No noteworthy findings	20 (NOAEL)	178,000	45,700	108[h]
Subcutaneous peri- and postnatal Development Study in rats (24 females/dose)				
		$AUC_{0\text{-}192h}$		
No noteworthy findings	30	10,900	88	1[i]
No noteworthy findings	300 (NOAEL for F_0, F_1 and F_2)	54,800	412	4[i]

NaOH = sodium hydroxide
N/A = Not applicable, TK not done.
ND = Not determined
NOEL = No Observed Effect Level.
NOAEL = No Observed Adverse Effect Level.

a Noteworthy responses do not include nonadverse injection site reactions in toxicity studies; expected granulomatous inflammation occurred at subcutaneous depot injection sites
b In repeat-dose studies, AUC and Cmax values indicate mean serum concentrations. Reported values were obtained near termination, or as specified.
c Exposure calculation methods: margins based on total AUC except safety pharmacology which is based on free Cmax. Margins based on the $C_{max\ Day1\text{-}week\ 26}$ (136 ng/mL; free Cmax 1.98 ng/mL) and $AUC_{Day1\text{-}week\ 26}$ (277903 ng•h/mL) after 1500 mg oral dosing in first 2 weeks and 927 mg SC administration; CTRA-2021-1054 LEN PopPK)
d Although TK not collected on the respiratory safety pharmacology study, the doses are the same as for the CNS evaluation and therefore, the margins can be used for most.
e Calculated using $AUC_{0\text{-}168.5h}$ x 26 weeks (to adjust 1 week to 26 weeks) / 277903 ng•h/mL
f Calculated using $AUC_{0\text{-}336h}$ x 13 weeks (to adjust 2 weeks to 26 weeks) / 277903 ng•h/mL
g Calculated using $AUC_{0\text{-}672h}$ x 6 (to adjust 1 month to 6 months)/ 277903 ng•h/mL
h Calculated using $AUC_{0\text{-}24h}$ x 28 x 6 (to adjust 24 hours to 6 months)/ 277903 ng•h/mL
i Calculated using $AUC_{0\text{-}192h}$ x 21 (to modify 8 days to 168d/6 months)/ 277903 ng•h/mL

4.9.2. Target Organ Effects

A comprehensive toxicology program has been conducted with LEN including single dose intravenous toxicity studies in rats and dogs; repeat-dose subcutaneous toxicity studies of up to 26-weeks duration in rats, and 39-weeks in dogs; in vitro and in vivo genotoxicity studies; assessment of fertility, early embryonic development, and pre- and postnatal development toxicity in rats; and 1-month oral toxicity studies in rats and dogs. Additional toxicology studies conducted include assessments of local tolerance, antigenicity potential and genotoxicity and toxicity studies for qualification of impurities.

Two dosage forms of LEN are currently in development, a solution formulation for SC injection once every 6 months and a film-coated tablet formulation for oral administration. The oral dosage form is used as loading doses to rapidly achieve the target plasma PK concentration. The toxicology studies generally used the SC route of administration. To achieve multiples over a clinically relevant plasma concentration of LEN, the rats were dosed SC every 3 months; the dogs were dosed SC monthly. Rats and dogs in repeat toxicity studies via the oral route were dosed daily. Large exposure margins were achieved in the SC and oral studies (Section 4.9). These studies, therefore, have adequately assessed the potential systemic toxicity and local oral tolerance of LEN and support the clinical regimen for the treatment of HIV-1 infection.

Several potential SC clinical formulations were utilized in the LEN toxicology studies; the clinical formulation used in the Phase 2/3 study in heavily treatment experienced subjects (GS-US-200-4625) was administered in the second 9-month dog study (TX-200-2047), the rat peri- and postnatal reproductive toxicity study (TX-200-2049), a local tolerance study (TX-200-2033) and is being administered in the ongoing rat carcinogenicity study (TX-200-2046).

LEN was well tolerated in male and female Wistar Han rats when administered via subcutaneous injection once or twice at a dose level of 100 mg/kg/dose with or without 1 mol-eq NaOH in the formulation followed by 13-week observation period after each injection (up to 26 weeks). No adverse effects occurred after daily oral administration of LEN to rats or dogs at up to 30 mg/kg/day. Therefore, no target organ toxicity has been observed in rats after 6 months of exposure, via subcutaneous administration or after 28 days of daily oral administration that achieved exposure levels up to 7-fold and 21-fold higher, respectively, than clinical exposure. No target organ toxicity has been observed in dogs after 28 days of daily oral administration that achieved exposure levels up to 46-fold higher than clinical $AUC_{Day1\text{-}Week\ 26SC}$ (CTRA-2021-1054 LEN PopPK). Lenacapavir is not genotoxic. Nonclinical reproductive toxicity studies with LEN have demonstrated no adverse effect on fertility, embryo-fetal development or growth, viability, and development of F_1 neonates. In the rat SC peri- and postnatal toxicity study, low levels of LEN were measured in the neonates on postnatal day 10. The chronic granulomatous inflammation reaction at the injection site in rats, dogs and rabbits is a foreign body response to the LEN SC depot and is an expected host response.

4.9.2.1. Effects on the Liver

Reversible changes in liver function were observed in dogs after very large single IV doses and repeat SC doses and consistent with the inhibition of the BSEP transporter.

In dogs after a single IV dose of 10 or 30 mg/kg, LEN-related serum chemistry changes were observed 24 hours after dosing and included mildly to markedly increased serum alanine and aspartate aminotransferase, gamma glutamyl transferase, alkaline phosphatase activity, and total bilirubin with correlative microscopic findings of hepatocyte degeneration and hepatocyte necrosis (TX-200-2030). At the 14-day terminal sacrifice, clinical pathology changes had partially or fully reversed and there were no LEN-related microscopic findings. The NOAEL was determined to be 3 mg/kg. Based on the time course, the liver changes appear to be C_{max} related. The C_{max} reached after IV dosing of 10 and 30 mg/kg was 15.4 and 38.9 µg/mL, respectively. Based on the $C_{max\ Day1\text{-}Week\ 26SC}$ (CTRA-2021-1054 LEN PopPK) of 0.136 µg/mL, the C_{max} achieved where liver effects were observed (≥ 10 mg/kg) are ≥ 113-fold higher than the clinical C_{max}. The C_{max} at the 3 mg/kg NOAEL (5.1 µg/mL) is 38-fold higher than the clinical C_{max}.

Two repeat toxicity studies in dogs have been conducted via monthly SC injection of LEN for up to 9 months (10 doses). No systemic toxicity was observed in the first study (TX-200-2017) that achieved LEN exposures of up to 8-fold higher based on the $AUC_{Day1\text{-}Week\ 26SC}$ (CTRA-2021-1054 LEN PopPK) of 278 µg•h/mL. The second dog study (TX-200-2047) achieved mean LEN exposures ≥ 51fold higher than $AUC_{Day1\text{-}Week\ 26SC}$. Clinical observations, clinical pathology and anatomic pathology data from the second study indicate hepatobiliary changes indicative of cholestasis.

The transient acute toxicity observed in the IV dog study and the presence of icteric clinical signs, increased liver bile pigment, increased serum bile acids and increased serum conjugated bilirubin in the chronic dog study are consistent with disruption in secretion of bilirubin into bile. Inhibition of the BSEP transporter induces hepatotoxicity by causing intracellular concentrations of bile acids to rise above a toxic threshold. Lenacapavir is a much more potent inhibitor of dog BSEP (dBsep) transporter (IC_{50} = 0.124 µg/mL) than the human BSEP transporter (IC_{50} =1.15 µg/mL) (AD-200-2053). Table 4 summarizes the dog and human exposure data. The mean plasma LEN C_{max} achieved at 10 mg/kg in the single dose IV dog study of 15.4 µg/mL is 124-fold and 13-fold higher than the dBsep and hBSEP IC_{50}s, respectively.

The average dog plasma LEN concentration (C_{ave}) after 9 months of 130 mg/kg/dose or 3 months of 411 mg/kg is at least 39-fold higher than the dBsep transporter IC_{50}.

The average clinical plasma LEN concentration is 27-fold lower than the hBSEP transporter IC_{50}. Hepatic metabolism is not a major elimination pathway for LEN. No adjustment of LEN is recommended in patients with mild to moderate hepatic impairment (GS-US-200-4331).

Table 4. Systemic Exposure of Dogs and Humans to LEN compared to BSEP IC_{50}

Study	BSEP IC_{50} (µg/mL)	Dosing Regimen	Dose (mg/kg/dose)	Mean C_{max} (µg/mL)	C_{ave} (µg/mL)
IV dog study	Dog IC_{50} = 0.124	Single dose	10 (Lowest effect dose)	15.4	1.15[a]
Chronic dog SC toxicity studies		Monthly for 9 months	40 (NOAEL)	0.658	0.52[b]
		Monthly for 9 months	130	5.48	4.8[c]
		Monthly for 3 months	411	9.23	6.7[d]
GS-US-200-4538	Human IC_{50} = 1.15	Single dose for 9 months	927 mg	0.071	0.042

C_{ave} = average plasma concentration
a Calculated by dividing mean (males/females combined) $AUC_{0\text{-}168.5h}$ by 168.5 (TX-200-2030)
b Calculated by dividing mean (males/females combined) $AUC_{0\text{-}672h}$ on day 225 by 672 (TX-200-2017)
c Calculated by dividing mean (males/females combined) $AUC_{0\text{-}672h}$ on day 225 by 672 (TX-200-2047)
d Calculated by dividing mean (males/females combined) $AUC_{0\text{-}336h}$ on day 57 by 336 (TX-200-2047)

5. INTEGRATED OVERVIEW AND CONCLUSIONS

5.1. Integrated Overview

The pharmacologic basis to recommend LEN for the treatment of HIV-1 infection is scientifically sound based on the nonclinical in vitro and in vivo efficacy data. Lenacapavir has potent antiviral activity against all tested subtypes of HIV-1 in vitro and no overlapping resistance with other approved agents. The pharmacokinetic and toxicologic profiles of LEN are well characterized in multiple animal species. Adverse pharmacokinetic interactions that would negatively affect safety or pharmacological efficacy are not anticipated. This was confirmed in a clinical drug interaction study with LEN. Cumulative data from nonclinical and clinical studies demonstrated acceptable tolerability and safety profiles to support use of LEN in patients.

Reversible changes in liver function were observed in dogs after very large IV single doses and SC repeat doses and consistent with the inhibition of the dog BSEP transporter. Lenacapavir is a much more potent inhibitor of dog BSEP transporter than the human BSEP transporter and large exposure margins support the safety of LEN.

The chronic granulomatous inflammation reaction at the injection site in rats, dogs and rabbits is a foreign body response to the LEN SC depot and is an expected host response. Granulomatous inflammation has been observed after SC and intramuscular implantation of bioactive material, including those for HIV treatment {Anderson 1997, Shah 2017, Van't Klooster 2010}.

The reports for the carcinogenicity studies are expected at the end of 20[redacted] for the mouse study and early 20[redacted] for the rat study. The proposal that carcinogenicity studies be submitted post approval is supported by 1) the unmet medical need that LEN provides, 2) the significant limitations of alternative therapies and 3) the weight of evidence that indicates that LEN is highly likely not to be tumorigenic in rats or humans.

1) Unmet medical need: Gilead has assessed the clinical efficacy and safety of LEN in people infected with multidrug resistant HIV for whom it is otherwise not possible to construct a suppressive antiviral regimen (Study GS-US-200-4625), a population of high unmet medical need with limited treatment options. The results demonstrate that LEN is an effective treatment option for this population providing overwhelming positive benefit/risk.

2) The significant limitations of alternate therapies: For patients previously treated with NRTIs, NNRTIs, PIs, and INSTIs who subsequently develop viral resistance to at least 3 classes, there are few options with which to create an active regimen. Many agents that can be used in patients with multidrug resistant (MDR) HIV have limitations, which include safety concerns and suboptimal efficacy, as well as modes of administration which contribute to limited adherence by patients. Some examples are described below:

- Enfuvirtide: SC injection twice daily with 98% of patients experiencing injection site reactions {Hoffman La Roch Inc. 2018}
- Ibalizumab: IV infusion every 2 weeks, risk of immunogenicity {TaiMed Biologics USA Corp. 2018}

- Maraviroc: oral twice daily, boxed warnings for hepatotoxicity sometimes associated with systemic allergic reactions, significant drug interactions, need for testing CCR5 tropism {Pfizer Manufacturing Deutschland GmbH 2015}

- Etravirine: oral twice daily, severe potentially life-threatening and fatal skin reactions not labeled for use in patients who have experienced virologic failure taking an NNRTI containing regimen {Janssen Pharmaceuticals Inc. 2018}

As such, there is a great unmet medical need for new safe and effective treatment options for heavily TE patients with MDR HIV.

3) The weight of evidence that indicates that the LEN is highly likely not to be tumorigenic in rats or humans: This includes the LEN pharmacologic, pharmacokinetic and toxicologic data:

- The LEN site of action is at the conserved interface between capsid protein monomers, where it interferes with capsid-protein-mediated interactions between proteins that are essential for multiple phases of the viral replication cycle as they are unable to support reverse transcription without a properly formed capsid core. Lenacapavir exhibited minimal cytotoxicity (CC_{50} > 50 μM) in human cell lines and primary cells.

- Lenacapavir has low potential for off-target effects when tested against a panel of 87 molecular targets at a concentration of 10 μM, at least 5,000-fold above the estimated free drug concentration at the clinical maximum plasma concentration (C_{max}).

- Lenacapavir is metabolically stable. Lenacapavir has a favorable DDI profile as a perpetrator. Inhibition of CYP3A was noted in vitro and moderate inhibition noted in the clinic. Further assessment of other drug metabolizing enzymes and transporters is not required.

- Oral (1-month) and subcutaneous (6/9 month) repeat dose toxicity studies were conducted in rats and dogs. No histopathologic risk factors for neoplasia were identified.

- Lenacapavir is not genotoxic in the bacterial reverse mutation test, the in vitro chromosomal aberrations assay using cultured human peripheral blood lymphocytes, and the in vivo micronucleus assay in rats conducted as part of a repeat dose toxicity study.

- No evidence of hormonal perturbation was observed in the repeat dose or reproductive toxicity studies. No weight or histopathological changes occurred in male or female reproductive organs. No functional changes occurred in the fertility or peri/postnatal studies.

- No clinical or anatomic pathology indicative of immunosuppression was observed in the repeat dose studies.

In conclusion, the current clinical safety and efficacy data demonstrate the unmet medical need filled by LEN, the significant limitations of alternative therapies and the pharmacology, pharmacokinetic and toxicologic dataset for LEN does not suggest carcinogenic potential arising from on-target or off-target factors.

5.2. Justification for Text in Labeling

The proposed Prescribing Information for LEN include all relevant nonclinical safety findings. Based on findings in the nonclinical studies the key safety points for consideration include potential DDI with potent inhibitors for P-gp, use by women of childbearing potential and during pregnancy, and use during lactation. The following should be considered:

- Lenacapavir is cleared by intestinal secretion by P-gp and metabolism, providing multiple pathways for elimination. This was supported by the modest interaction with CYP3A and P-gp inhibition in the clinic. Lenacapavir has a favorable DDI profile as a perpetrator. Inhibition of CYP3A was noted in vitro and moderate inhibition noted in the clinic. Further assessment of other drug metabolizing enzymes and transporters is not required.

- Animal data indicate that LEN does not cause reproductive or fetal toxicity.

- LEN was detected in the plasma of nursing rat pups. It is not known if LEN is secreted in human milk.

- Data indicate that LEN is nongenotoxic.

- Long-term carcinogenicity studies of LEN are in progress.

5.3. Overall Conclusion

The data from nonclinical LEN studies are adequate to support the efficacy and safety of the LEN subcutaneous and oral products. Lenacapavir is a potent inhibitor of HIV-1 replication, is fully active against a broad spectrum of HIV-1 mutants and clinical isolates resistant to currently approved antiretrovirals, has similar antiviral activity across clinical isolates from all major HIV-1 subtypes and has synergistic in vitro antiviral activity when combined with other antiretrovirals. These data demonstrate the potent, selective and multi-stage inhibition of HIV-1 capsid function by LEN, a mechanism that is orthogonal and complementary to existing anti-HIV therapies.

Comprehensive nonclinical studies were carried out with LEN to assess its absorption, distribution, metabolism, excretion and drug interaction potential. Low solubility, low clearance and high potency make LEN well suited as a long acting SC injectable which was demonstrated in extensive studies in rat and dog. Lenacapavir is highly metabolically stable which contributes to the very low clearance and minimal excretion as metabolites. Lenacapavir is the main component in plasma of animal species and human which is consistent with the high metabolic stability. Lenacapavir is cleared by intestinal secretion by P-gp and metabolism. This was supported by the modest interaction with CYP3A and P-gp inhibition in the clinic. Lenacapavir has a favorable DDI profile as a perpetrator. Inhibition of CYP3A was noted in vitro and moderate inhibition was observed in the clinic. The most potent inhibition of transporters in vitro by LEN was with OATPs, but no in vivo DDI was noted in the clinic. Further assessment of other drug metabolizing enzymes and transporters is not required.

A comprehensive toxicology program has been conducted with LEN. Lenacapavir was well tolerated in rats and dogs exposed to LEN after subcutaneous injections or oral gavage; cholestatic changes were observed in dogs only at high exposures that inhibited the dog BSEP transporter. Lenacapavir was not genotoxic and did not show any adverse effects in reproductive and developmental toxicity studies. Identified impurities and degradants have been assessed in LEN standard toxicology and qualification studies.

6. REFERENCES

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Justification for Absent Nonclinical Data in Module 4

Section	Comment
4.2.3.4 Carcinogenicity (including supportive toxicokinetics evaluations) 4.2.3.4.1 Long-term studies (in order by species; including range finding studies that cannot appropriately be included under repeat-dose toxicity or pharmacokinetics) 4.2.3.4.2 Short- or medium-term studies (including range-finding studies that cannot appropriately be included under repeat dose toxicity or pharmacokinetics) 4.2.3.4.3 Other studies	The 6-month transgenic mouse (TgrasH2) and the 2-year rat carcinogenicity studies with lenacapavir (LEN) via subcutaneous injection are in progress. Doses were agreed upon with the US FDA. The final reports will be submitted when available; approximately the end of 20[redacted] for the mouse study and early 20[redacted] for the rat study. The nonclinical data support the overall positive benefit-risk of LEN for the proposed population in the absence of carcinogenicity reports at the time of the initial application. See m2.4 Section 5.1 for additional information. Provision of these reports when available, or post approval has been discussed with the EMA and Rapporteurs during the [redacted] meeting, [redacted] 20[redacted]. The Agency agreed to assess this request during review of the LEN MAA.
Reproductive and Developmental Toxicity 4.2.3.5.4 Studies in which the offspring (juvenile animals) are dosed and/or further evaluated.	As agreed with PDCO, based on the available data, no paediatric specific concerns have been identified that would require the conduct of a juvenile animal study to support the clinical development of LEN in children from 2 years of age, see m1.10.