SECTION 2.6.4—PHARMACOKINETICS WRITTEN SUMMARY

LENACAPAVIR

Gilead Sciences

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CONFIDENTIAL AND PROPRIETARY INFORMATION

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LIST OF ABBREVIATIONS

AAG	alpha1-acid glycoprotein
ABT	aminobenzotriazole
AhR	aryl hydrocarbon receptor
AUC	area under the plasma concentration versus time curve
$AUC_{0\text{-}72h}$	area under the time-concentration curve from time zero to 72 h (Day 3)
$AUC_{0\text{-}336h}$	area under the time-concentration curve from time zero to 336 h (Day 14)
$AUC_{0\text{-}672h}$	area under the time-concentration curve from time zero to 672 h (Day 28)
AUC_{last}	area under the time-concentration curve from time zero to last measured time-point
AUC_{inf}	area under the concentration versus time curve extrapolated to infinite time, calculated as $AUC_{last} + (C_{last}/\lambda_z)$
BPR	blood to plasma ratio
BCRP	breast cancer resistance protein
BDC	bile duct cannulated
BLQ	below the limit of quantitation
BSEP	bile salt export pump
Caco-2	human colorectal carcinoma cells
CAR	constitutive androstane receptor
CCM	cell culture medium
CHO	Chinese hamster ovary
CL	systemic clearance
C_{max}	maximum observed concentration of drug in plasma
CsA	cyclosporine (cyclosporin A)
CYP	cytochrome P450 enzyme
DDI	drug-drug interaction
DMSO	dimethyl sulfoxide
GLP	Good Laboratory Practice
HRMS	high resolution mass spectrometry
HSA	human serum albumin
IC_{50}	half-maximal inhibitory concentration
IV	Intravenous
K_I	the inhibition constant
k_{inact}	the rate of enzyme inactivation
LC	liquid chromatography
LC-MS/MS	liquid chromatography-tandem mass spectrometry
LEN	Lenacapavir
MATE	multidrug and toxin extrusion protein
MDCK	Madin-Darby canine kidney
MDCKII	Madin-Darby canine kidney strain II
MRT	mean residence time

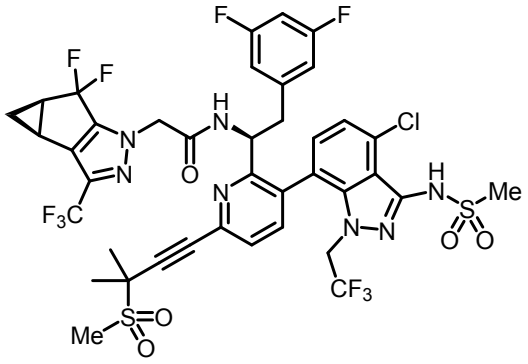
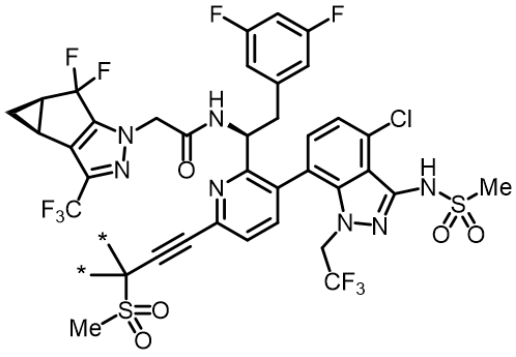
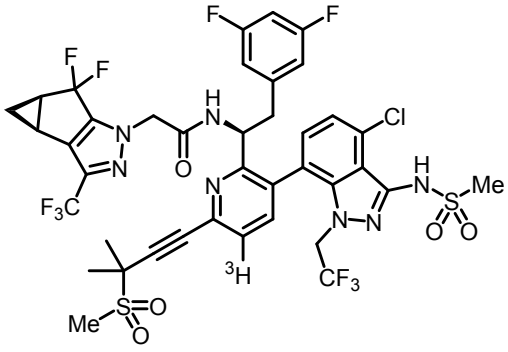
min	minute(s)
NA	not applicable
NADPH	nicotinamide adenine dinucleotide phosphate, reduced
ND	not detectable
OAT	organic anion transporter
OATP	organic anion transporting polypeptide
OCT	organic cation transporter
P_{app}	apparent permeability coefficient
PBS	phosphate buffered saline
PEG	polyethylene glycol
P-gp	P-glycoprotein
PK	pharmacokinetic(s)
PXR	pregnane X receptor
SC	subcutaneous
SD	standard deviation
$t_{1/2}$	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)
T_{max}	time (observed time point) of C_{max}
UDPGA	uridine diphosphate glucuronic acid
UGT	uridine diphosphate glucuronosyltransferase
UGT1A1	uridine diphosphate glucuronosyltransferase 1A1
V_{ss}	volume of distribution at steady state
WH rat	Wistar Han rat

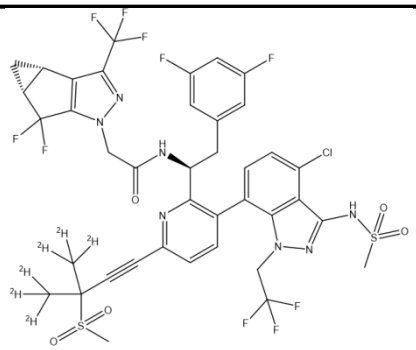
NOTE TO REVIEWER

The structure of Lenacapavir (LEN, GS-6207) and related compounds are illustrated in Table 1. The following conversions are provided to aid the reviewer.

LEN: 1 nM = 0.968 ng/mL; 1 ng/mL = 1.03 nM

Table 1. Names and Structures of LEN and Related Compounds

Name	Alternative Names	Identity	Structure
LEN (LEN.1 and LEN.2)	GS-6207	Parent Compound (LEN.1 and LEN.2 are atropisomers, ie, interconvertible isomers due to restricted rotation about a single bond in the molecule)	
[^{14}C]LEN	[^{14}C]GS-6207	Radiolabeled parent	 (* = ^{14}C)
[^{3}H]LEN	[^{3}H]GS-6207	Radiolabeled parent	

Name	Alternative Names	Identity	Structure
$[D_6]$LEN	$[D_6]$GS-6207	Stable deuterium labeled parent	 (^{2}H = Deuterium (D))

1. BRIEF SUMMARY

Lenacapavir (LEN, GS-6207), is a novel, first-in-class, selective inhibitor of HIV-1 capsid function, characterized by potent antiviral activity, novel resistance profile, low human clearance (CL) and low aqueous solubility. These properties allow LEN to be well suited for an extended-release parenteral formulation that can be administered once every six months.

The absorption, distribution, metabolism, excretion and drug interaction characteristics of LEN were assessed through in vitro and in vivo studies. Good Laboratory Practice (GLP)-compliant safety studies were supported by fully validated bioanalytical methods utilizing [D_6]LEN as the mass spectrometric internal standard.

The intended route of administration for LEN is subcutaneous (SC) with oral pharmacokinetic (PK) loading. LEN is a Biopharmaceutics Classification System Class IV molecule with low solubility in biorelevant fluids and exhibits low permeability across monolayers of human colorectal carcinoma cells (Caco-2) with measurable efflux. Further studies in Madin-Darby canine kidney strain II (MDCKII) cells demonstrated that LEN is a substrate for efflux by P-glycoprotein (P-gp) but not breast cancer resistance protein (BCRP). Consistent with this, following oral administration of LEN as a low dose in solution, bioavailability (F%) in nonclinical species was low, consistent with low F% in humans. LEN oral exposure was not altered with aminobenzotriazole (ABT, a pan-CYP inhibitor) pretreatment in dog and monkey, indicating negligible intestinal cytochrome P450 enzymes (CYPs) in these species contributed to LEN metabolism. Data collectively showed that the observed oral F% was consistent with limited absorption.

Lenacapavir F% was high following SC administration to rat, dog and human. During nonclinical testing of various LEN salt forms, formulations, doses, dose volumes and dose concentrations, potential was demonstrated for sustained release following SC administration, with no evidence for an undesired initial burst.

Lenacapavir exhibits a low free fraction in plasma from all species, with albumin being the preferred binding partner in human plasma (lower binding to α_1-acid glycoprotein (AAG)). Relative binding in human plasma and cell culture medium (CCM) was used to adjust the in vitro EC_{95} for efficacy to allow calculation of the PK target. Lenacapavir is modestly excluded from the cellular fraction of blood with whole blood to plasma concentration ratios of ≤ 0.67. The high steady-state volume of distribution (V_{ss}) of LEN in nonclinical species and humans likely reflects the compound's lipophilic nature. 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. There was strong preferential distribution of [^{14}C]LEN-derived radioactivity to the liver, but in vitro studies showed that LEN was not a substrate for human organic anion transporting polypeptide 1B1 (OATP1B1) and OATP1B3 hepatic uptake transporters, and there was no evidence for human hepatic uptake of LEN in vitro. All radioactivity was cleared from rat tissues by the conclusion of the study. Lenacapavir was detected in rat plasma of nursing pups after administration to the mother post-fertilization.

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

In vitro, LEN exhibits very high metabolic stability in multiple systems with predicted human hepatic extraction < 1%. This high stability was reflected in vivo, with low CL values in nonclinical species and humans. Slow metabolism of [^{3}H]LEN by recombinant CYP3A5 and uridine diphosphate glucuronosyltransferase 1A1 (UGT1A1) was detected. However, there was no clear difference in oxidative metabolism by hepatic microsomal fraction from a CYP3A5 *3/*3 donor compared to a CYP3A5 *1/*1 donor. [^{14}C]LEN in Hμrel® hepatic co-cultures was metabolized via glutathione conjugation, pentose conjugation, hexose conjugation, and glucuronidation. All human metabolites were detected in nonclinical species.

In vivo, the major route of elimination of LEN in rats and dogs was excretion in bile and direct intestinal secretion as parent. Intestinal secretion appeared to be mediated by P-gp as it was inhibited by elacridar. Metabolism played a lesser role in both species. 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. Low abundance of metabolites was observed in human excreta.

With respect to drug interactions, elimination of LEN is likely due to a combination of metabolism (oxidation by CYP3A and/or glucuronidation by UGT1A1) and parent drug excretion (to bile and secretion to intestine, likely mediated by P-gp). Inhibitors or inducers of these proteins may affect the PK of LEN. In support of this, the CYP3A/P-gp inhibitor cobicistat increased exposure of orally administered LEN, but the CYP3A selective inhibitor voriconazole (VORI) had less effect in the clinical drug-drug interaction (DDI) study (m2.7.6, GS-US-200-4333). The potent CYP3A and P-gp inducer rifampicin greatly reduced exposure to oral LEN in the clinical DDI study (m2.7.6, GS-US-200-4333).

Assessment of LEN as a perpetrator of drug interactions indicates low potential for reversible inhibition of CYP enzymes or UGT1A1 at the low systemic concentrations observed following oral or SC administration. Briefly, inhibition of intestinal CYP enzymes is possible following oral administration of LEN. Lenacapavir is a weak mechanism-based inhibitor of CYP3A enzymes so interactions with substrates of these enzymes would be predicted. This was confirmed clinically where a moderate interaction with the sensitive CYP3A substrate, midazolam, was observed following oral administration of LEN. 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 (no inhibition of either transporter was observed). In a clinical DDI study, there was no clinically meaningful effect of orally administered LEN on either 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. 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 creatinine clearance 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.

In conclusion, the observed clinical behavior of LEN is reflected in the properties determined in vitro and in nonclinical species in vivo. The properties include F% which is high following SC dosing but low following oral administration, likely due to low permeability and efflux by P-gp. The compound has very high metabolic stability and a high volume of distribution resulting in a long PK half-life ($t_{1/2}$). Elimination is likely due to a combination of metabolism and excretion in bile and intestinal secretion. This renders it sensitive to drug interaction with potent inducers but comparatively resistant to the effects of potent CYP3A inhibitors. Parent compound was the only drug-related component in plasma and low abundance of metabolites were observed in human excreta. Lenacapavir exhibits low potential to cause drug interactions with little effect on most CYP enzymes. It is a weak mechanism-based inhibitor of CYP3A, exhibiting only a modest effect on the sensitive CYP3A substrate, midazolam. No inhibition of transporters would be expected at systemic concentrations and there appears to be little effect on P-gp or OATP activity following oral administration in clinical DDI studies.

2. METHODS OF ANALYSIS

The in vivo PK, distribution, metabolism and excretion of LEN were assessed in rat, dog, and monkey. The in-vitro absorption, distribution, metabolism, excretion, and potential for CYP or transporter mediated DDI were studied in standard model systems.

2.1. Bioanalytical Methods Supporting In Vivo Studies

2.1.1. Pharmacokinetic Studies

The plasma LEN concentrations in nonclinical PK studies in rat, dog and monkey were quantified by high-performance liquid chromatography-tandem mass spectrometry (LC-MS/MS) (m2.6.5, Section 3, AD-200-2009, AD-200-2010, AD-200-2011, AD-200-2012, AD-200-2013, AD-200-2014, AD-200-2015, AD-200-2016, AD-200-2017, AD-200-2018, AD-200-2019, AD-200-2036, AD-200-2038, AD-200-2039, AD-200-2042, AD-200-2051, m2.6.5, Section 7, AD-200-2045, AD-200-2046). These methods did not strictly conform to GLP guidelines but were evaluated for selectivity, sensitivity, and linearity, as well as intra-assay accuracy and precision. The PK parameters were determined by non-compartmental analysis.

2.1.2. Toxicokinetic Studies

The plasma concentrations of LEN were quantified in GLP repeat-dose toxicology studies in rat (m2.6.6, Section 3, TX-200-2005, TX-200-2011 and TX-200-2014) and dog (m2.6.6, Section 3, TX-200-2006, TX-200-2012, TX-200-2017 and TX-200-2047) using fully validated LC-MS/MS methods with Deuterium (^{2}H) labeled LEN (m2.6.5, Section 2, BA-200-2002 and BA-200-2003). Validated parameters included selectivity, sensitivity, linearity, recovery, carryover, intra- and inter-assay precision and accuracy, sample collection stability, stock solution stability, injection medium integrity, short-term matrix stability, freeze-thaw matrix stability, long-term matrix stability, dilution integrity, and re-injection reproducibility. The lower limit of quantitation for LEN was 2 ng/mL (2.06 nM) and the upper limit of quantitation for LEN was 2,000 ng/mL (2066 nM) in rat and dog plasma. The results of incurred sample reproducibility analyses in toxicology studies confirmed the repeatability of the methods. The toxicokinetic parameters were determined by non-compartmental analysis.

2.1.3. Other In Vivo Studies

The distribution, excretion and mass balance of LEN were assessed in intact and bile-duct cannulated (BDC) male Wistar Han (WH) rats following a single IV administration of [^{14}C]LEN (Table 1; m2.6.5, Section 7.4, AD-200-2006). The tissue distribution of [^{14}C]LEN-derived material was assessed in male non-pigmented WH and pigmented Long Evans rats using quantitative whole-body autoradiography. The excretion and mass balance of LEN were assessed in intact and BDC male dogs following a single IV and oral administration of [^{14}C]LEN (m2.6.5, Section 7, AD-200-2007). [^{14}C]LEN derived radioactivity was profiled in pooled rat and dog plasma, bile and feces (m2.6.5, Section 5, AD-200-2002 and AD-200-2004). Radiocounting in the study matrices were determined by liquid scintillation counting. Radiochromatograms of plasma, urine, bile, and feces were generated by liquid chromatography

(LC) with fraction collection followed by offline radio-detection and the identity of the radiopeaks were determined by simultaneous inline high-resolution mass spectrometry (HRMS).

The excretion of LEN was assessed in bile duct intact and BDC male WH rats following a single IV administration of LEN without and with elacridar (a P-gp and BCRP inhibitor) treatment (m2.6.5, Section 7, AD-200-2045 and AD-200-2046). Excretion of intact LEN in bile, feces and urine were quantified by a LC-MS/MS method.

2.2. In Vitro Methods

2.2.1. Permeability

The bi-directional permeability of LEN (1 µM) was assessed in human Caco-2 cell monolayers (m2.6.5, Section 3, AD-200-2009).

2.2.2. Protein Binding

The extent of LEN (2 µM) binding in plasma was assessed by equilibrium dialysis for 3 hours at 37°C (m2.6.5, Section 4, AD-200-2020 and AD-200-2044). The relative protein binding of LEN (2 µM) between human plasma and CCM containing 10% fetal bovine serum was assessed in a competitive equilibrium dialysis assay at 37°C (m2.6.5, Section 4, AD-200-2020). The extent of LEN (2 µM) binding to human hepatic microsomal fraction (0.5 mg protein/mL) was assessed by equilibrium dialysis at 37°C (m2.6.5, Section 4, AD-200-2032). The free fractions of LEN (2 µM) in purified human serum albumin (HSA, 40 mg/mL) and AAG, 0.8 mg/mL) were determined by equilibrium dialysis at 37°C (m2.6.5, Section 4, AD-200-2048).

2.2.3. Blood to Plasma Ratio

The whole blood to plasma ratios (BPR) were determined following LEN (0.5 µM) incubation in whole blood from nonclinical species and human at 37°C for 1 hour (m2.6.5, Section 4, AD-200-2021).

2.2.4. Metabolism

The potential for changes in the relative proportions of the two atropisomers (LEN.1 and LEN.2) was assessed following incubation of LEN (0.1 or 1.0 µM) for 24 hr in phosphate buffered saline (PBS) or in plasma from rat, dog or human (m2.6.5, Section 5, AD-200-2047). Peak area ratios of LEN.1 to LEN.2 were quantified by a LC-MS/MS method.

Metabolic stability was determined by measuring the rates of appearance of radiolabeled metabolites from the [^{3}H]LEN (0.25 µM) substrate following incubation (2, 12, 25, 45 and 65 minutes) in the pooled hepatic microsomal fractions in the presence of a cofactor mixture that consisted of nicotinamide adenine dinucleotide phosphate (NADPH), uridine 5'-diphospho-glucuronic acid (UDPGA) and incubation (0, 1, 3 and 6 hours) in cryopreserved hepatocytes from Sprague-Dawley rat, beagle dog, cynomolgus and rhesus monkey, and human (m2.6.5, Section 5, AD-200-2022). CYP reaction phenotyping was performed by incubating [^{3}H]LEN (2 µM) with individual human recombinant CYPs (1A2, 2B6,

2C8, 2C9, 2C19, 2D6, and 3A4) co-expressed with NADPH CYP reductase (m2.6.5, Section 6, AD-200-2030 and AD-200-2054). The rates of metabolite formation were determined following LC separation followed by online radioflow detection. [^{3}H]LEN was also incubated with hepatic microsomal fractions from single donors characterized as homozygous CYP3A5 wild type (*1/*1) or homozygous deficient (*3/*3).

UGT phenotyping was performed by incubating [^{3}H]LEN (2 µM) with individual human recombinant UGTs (1A1, 1A3, 1A4, 1A6, 1A7, 1A8, 1A9, 1A10, 2B4, 2B7, 2B15 and 2B17) (m2.6.5, Section 6, AD-200-2033). The rates of metabolite formation were determined following LC separation followed by inline radioflow detection.

Metabolism of [^{14}C]LEN (10 µM) was determined following incubation (2 hours) in pooled hepatic microsomes in the presence of NADPH, UDPGA and glutathione from WH rat, beagle dog, cynomolgus and rhesus monkey, and human (m2.6.5, Section 5, AD-200-2008). The rates of metabolism and the metabolite profiles of LEN were also assessed using Hµrel® rat, dog, monkey and human hepatic co-cultures (m2.6.5, Section 5, AD-200-2037). Hµrel® hepatic co-cultures were incubated with [^{14}C]LEN (10 µM) for 7 days with sample collection at 0, 72 and 168 hours. Metabolite formation and identity was determined following LC separation by offline radio-detection and simultaneous inline HRMS.

2.2.5. Inhibition of Cytochrome P450 Enzymes and UGT1A1

The potential for LEN (0 – 25 µM) to reversibly inhibit the major human drug metabolizing CYP enzymes (1A2, 2B6, 2C8, 2C9, 2C19, 2D6, and 3A4) was assessed in human hepatic microsomal fractions (m2.6.5, Section 6, AD-200-2028). Isoform specific probe substrates were incubated individually with pooled human liver microsomes in the presence and absence of LEN. The production of the enzyme-specific metabolites was measured and, where possible, the IC_{50} values were determined. The potential for LEN (0 - 50 µM) to inhibit UGT1A1 was determined in recombinant human UGT1A1 (Supersomes™) fortified with alamethicin and UDP-glucuronic acid, with ethyl estradiol as the substrate (m2.6.5, Section 6, AD-200-2031). Appropriate positive control inhibitors were included in each experiment.

The potential for LEN to act as a mechanism-based inhibitor of human CYP3A was assessed using a method based on the reduction in the IC_{50} for its activity following preincubation of LEN with hepatic microsomal fraction and NADPH cofactor for 30 min (m2.6.5, Section 6, AD-200-2028). The inactivation kinetics of human hepatic microsomal CYP3A (midazolam 1'-hydroxylase activity by LEN were further characterized using Kitz-Wilson analysis (m2.6.5, Section 6, AD-200-2050).

2.2.6. Induction Potential

The potential for LEN to induce metabolizing enzymes through activation of xenobiotic receptors, AhR, CAR and PXR, was assessed in reporter cell lines (m2.6.5, Section 6, AD-200-2029). DPX2 cells, stably transformed with an expression vector for human PXR and a reporter gene vector containing the enhancer regions of CYP3A4 linked to luciferase, were used for assessment of PXR activation. Dioxin response element (DRE) 12.6 cells, transformed with an expression vector for human AhR and the DRE of the human CYP1A2 gene linked to a luciferase reporter, were used to determine AhR activation. HepG2 cells, transfected with an expression vector for human CAR1, plus retinoid X receptor α and the human CYP2B6 enhancers and promoters linked to the luciferase reporter were used to assess CAR activation. Appropriate control compounds were also analyzed to confirm the responsiveness of the cells.

The induction potential of LEN was also assessed in cryopreserved hepatocytes (m2.6.5, Section 6, AD-200-2049). In a pilot study (with hepatocytes from one donor), cytotoxicity (by morphology and ATP content) was noted following treatment with 5 or 10 µM LEN for 48 or 72 h. Thus, the LEN upper concentration limit was set to 10 µM for the main study. Hepatocytes from 3 individual donors were incubated with vehicle control, appropriate positive controls, or LEN (0.01 – 10 µM; 7 concentrations) for a total of 3 days. Induction of CYPs (1A2, 2B6 and 3A) was measured by in situ catalytic activity assays selective for each CYP enzyme. Induction of mRNA expression was determined for CYPs (1A2, 2B6, 3A4 and 2C9), UGT1A1 and P-gp by quantitative reverse transcription-polymerase chain reaction analysis.

2.2.7. Interaction with Transporters

The bi-directional permeability of LEN (1 µM) was assessed in MDCKII cell monolayers of wild type and in MDCKII cells overexpressing either P-gp or BCRP (m2.6.5, Section 8, AD-200-2005).

Lenacapavir (0.1 µM) was assessed as a substrate for efflux transporters in transwell assays using P-gp- and BCRP-transfected MDCKII cell monolayers (m2.6.5, Section 8, AD-200-2005). Transporter expression-dependent changes in the bidirectional permeability assay were confirmed using control inhibitors. LEN (0.025 µM) was also evaluated as a potential substrate for uptake transporters OATP1B1 and OATP1B3 using Chinese hamster ovary (CHO) cells transfected with the individual transporters (m2.6.5, Section 8, AD-200-2025). The uptake rate of LEN in OATP1B1- and OATP1B3-overexpressing cells was determined in the absence or presence of a control inhibitor. Hepatic uptake of LEN was also assayed in cryopreserved human hepatocytes (m2.6.5, Section 8, AD-200-2027). Rosuvastatin was used as the positive control in this study and rifamycin was used as the inhibitor of hepatic uptake by OATP transporters.

Lenacapavir (0 – 2.8 µM) was assessed as an inhibitor of influx and efflux using cell lines transfected with the individual transporters or membrane vesicle preparations (m2.6.5, Section 8, AD-200-2026). Inhibition of OATP1B1 and OATP1B3 was studied in transfected CHO cells. Inhibition of P-gp and BCRP was studied in transfected MDCKII cells. Inhibition of the transporter specific probe substrates was assessed in the presence of increasing concentrations of

LEN. The inhibition of the transporter specific substrates was measured and, where possible, IC_{50} values were determined. Appropriate positive control compounds were included in each assessment.

The inhibition of the human uptake transporters OAT1, OAT3, OCT1, OCT2, MATE1, MATE2K, and BSEP by LEN (0 - 10 µM) was studied *in vitro* in transfected cell lines or membrane vesicles (m2.6.5, Section 8, AD-200-2035). The assays used model substrates and known positive control inhibitors of the respective transporters.

The inhibition of the human BSEP and dog Bsep by LEN (0.014 - 10 µM) was studied *in vitro* in membrane vesicles (m2.6.5, Section 8, AD-200-2053). The assays used model substrate.

3. ABSORPTION

3.1. In Vitro Studies

The bidirectional permeability of LEN (1.0 µM) was studied across Caco-2 monolayers (m2.6.5, Section 3, AD-200-2009). LEN 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. Single Dose in Vivo Studies

3.2.1. Intravenous Administration

Lenacapavir PK following a single IV infusion administration was determined in male rat, dog, and monkey (m2.6.5, Section 3, AD-200-2010, AD-200-2011, AD-200-2012 and AD-200-2013). The plasma concentration-time profiles of LEN are shown in Figure 1. The plasma PK parameters are summarized in Table 2.

The average plasma CL of LEN was low across all tested species (1% to 12% of hepatic blood flow). The average V_{ss} in all species was larger than that of total body water. The elimination $t_{1/2}$ ranged from 14.6 to 38.1 hours across species.

Table 2. Plasma PK Parameters for LEN Following a Single Intravenous Infusion Administration at 1 mg/kg to Rat, Dog and Monkey

Species[a]	n	$AUC_{0\text{-}72h}$ (nM•h)	AUC_{inf} (nM•h)	CL (L/h/kg)	V_{ss} (L/kg)	$t_{1/2}$ (h)	MRT (h)
Sprague-Dawley Rat	3	17533 ± 3182	22867 ± 2011	0.045 ± 0.004	2.22 ± 0.56	38.1 ± 7.9	48.8 ± 9.6
Beagle Dog	3	13467 ± 2281	16667 ± 6296	0.070 ± 0.025	1.96 ± 0.91	30.2 ± 24.4	34.7 ± 27.8
Cynomolgus Monkey	3	4573 ± 1174	4673 ± 1258	0.21 ± 0.06	2.86 ± 0.54	14.6 ± 5.5	14.0 ± 2.7
Rhesus Monkey	6	4502 ± 1579	5198 ± 2204	0.25 ± 0.08	5.75 ± 2.24	37.7 ± 11.4	25.9 ± 11.3

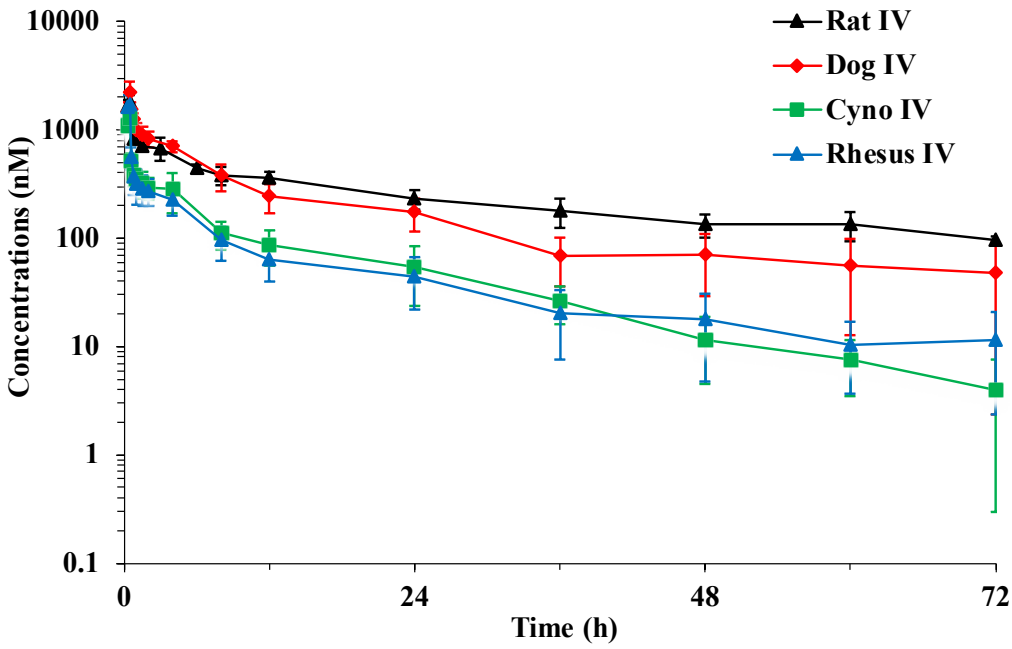
$AUC_{0\text{-}72h}$ = area under the plasma concentration-time curve from 0 to 72 h; AUC_{inf} = area under the plasma concentration-time curve extrapolated to time infinity; CL = systemic clearance; MRT = mean residence time; $t_{1/2}$ = estimated plasma elimination half-life; SD = standard deviation; V_{ss} = volume of distribution at steady state

a Formulated as a solution in 5% ethanol, 20% propylene glycol, 45% polyethylene glycol 300 and 30% 10 mM phosphate buffer; Doses were administered as a 30-minute IV infusion.

Values are the mean ± SD

Source: m2.6.5, Section 3, AD-200-2010, AD-200-2011, AD-200-2012 and AD-200-2013

Figure 1. Plasma Concentration-Time Profiles of LEN Following Intravenous Administration of LEN to Rat, Dog and Monkey



3.2.2. 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 (ie, 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). A comprehensive PK summary is provided in M2.6.5 (Section 3). A summary of rat and dog PK from key formulations are provided in this section (Table 3). Solution formulation B is currently being evaluated in clinical studies and seeking approval for heavily treatment experienced people with HIV.

Table 3. Formulations Evaluated Following Single Subcutaneous Administration in Rat and Dog

Formulation Type	Formulations Details from Single Dose PK Studies	Relevant Toxicology Studies	Relevant Clinical Studies
Aqueous suspension	2% poloxamer 188 in normal saline AD-200-2014 and AD-200-2015 (m2.6.5, Section 3)	TX-200-2005 and TX-200-2006 (m2.6.7, Section 7)	GS-US-200-4070
Solution Formulation A	77:10:13 w/w/w polyethylene glycol 200:ethanol:water AD-200-2038 and AD-200-2039 (m2.6.5, Section 3)	TX-200-2014 and TX-200-2017 (m2.6.7, Section 7)	Not dosed
Solution Formulation B	65:25.2:9.8 w/w/w PEG300:LEN:water AD-200-2051 (m2.6.5, Section 3)	TX-200-2047 (m2.6.7, Section 7)	GS-US-200-4538

3.2.2.1. Rat

3.2.2.1.1. Aqueous Suspension Formulation

Lenacapavir free acid, formulated as an aqueous suspension with 2% poloxamer 188 in normal saline, was administered subcutaneously to male WH rats (m2.6.5, Section 3, AD-200-2014). Rats were administered either a single injection at 10, 30 and 100 mg/kg or two equal-volume injections at 100 mg/kg for a total of 200 mg/kg. Plasma PK samples were collected up to 154 days postdose. The plasma concentration-time profiles of LEN in rats following SC administration of LEN in aqueous suspension are shown in Figure 2. The plasma PK parameters are summarized in Table 4.

Lenacapavir PK following SC administration showed a sustained drug release at all administered doses. F% was at least 55% in all dose groups. The mean $t_{1/2}$ following SC administration in rat ranged from 219 to 403 hours, which are substantially longer than the mean $t_{1/2}$ following IV administration (Table 2), indicating flip-flop PK following SC administration. LEN concentrations remained detectable for at least 70 days after dose administration.

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. LEN 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.

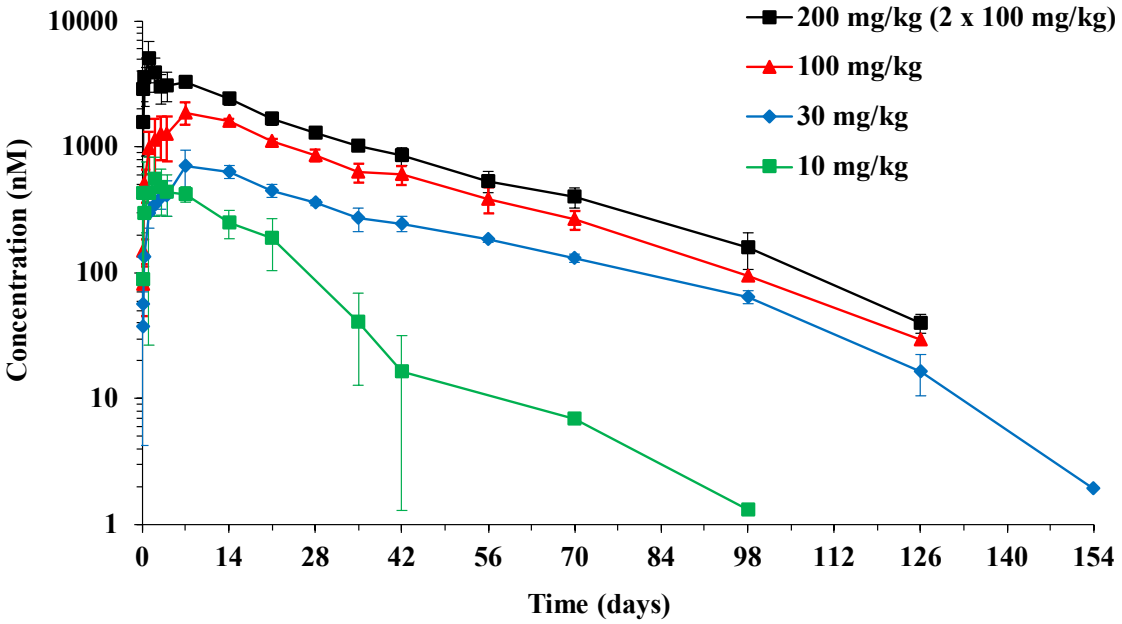
Neither a prominent initial burst release nor unintended rapid drug release was observed. By Day 3 (72 h postdose), approximately 14% of the bioavailable dose was released at the 10 mg/kg dose and a lower percent (3 to 7%) of the bioavailable dose was released at the higher dose levels. By Day 28 (672 h postdose), approximately 82% of bioavailable dose was released at the 10 mg/kg dose and a lower percent of AUC_{inf} at Day 28 (52% to 60%) was released at the higher dose levels.

Table 4. PK Parameters of LEN Following Subcutaneous Administration of LEN in Aqueous Suspension to Male WH Rats

Group ID	Dose (mg/kg)[a]	Dose Volume (mL/kg)	$AUC_{0\text{-}72h}$ (µM•h)	$AUC_{0\text{-}672h}$ (µM•h)	AUC_{last} (µM•h)	AUC_{inf} (µM•h)	C_{max} (µM)	T_{max} (h)	$t_{1/2}$ (h)	% of AUC_{inf} on Day 3	% of AUC_{inf} on Day 28	Mean % F[d]
1	10	0.10	32.5 ± 20.8	194 ± 14	236 ± 36	236 ± 36	0.671 ± 0.198	25 ± 23	219 ± 81	14%	82%	103
2	30	0.30	20.6 ± 3.9	343 ± 63	663 ± 83	664 ± 83	0.724 ± 0.222	224 ± 97	263 ± 31	3%	52%	97
3	100	1.0	68.0 ± 27.2	900 ± 89	1,545[b]	1,554[b]	1.91 ± 0.33	224 ± 97	397[b]	4%	58%	68
4	200	1.0 x 2[c]	180 ± 93	1498 ± 223	2499 ± 188	2517 ± 194	3.81 ± 1.08	120 ± 83	403 ± 50	7%	60%	55

a Formulated as aqueous suspension with 2% poloxamer 188 in normal saline
b Only mean value was reported because one animal died at 2112 h postdose. This animal had appeared normal at the daily regular check time of 2088 h.
c Two injections
d Calculated with mean IV AUC_{inf} = 22867 nM•h at 1 mg/kg from Table 2.
$AUC_{0\text{-}72h}$ is 3-day AUC; $AUC_{0\text{-}672h}$ is 28-day AUC
Values are the mean ± SD from 3 animals.
Source: m2.6.5, Section 3, AD-200-2014

Figure 2. Plasma Concentration-Time Profiles of LEN Following Subcutaneous Administration of LEN in Aqueous Suspension to Male WH Rats



3.2.2.1.2. Solution Formulation A

Lenacapavir free acid or LEN sodium salt formed in situ with 1 mole equivalent of sodium hydroxide were formulated as a solution in 77:10:13 w/w/w PEG200:ethanol:water (solution formulation A). The formulated solution was administered to male WH rats via a single SC injection (m2.6.5, Section 3, AD-200-2038).

Multiple factors affecting LEN release following SC administration of a solution formulation were evaluated, including LEN salt form, dose, dose concentration and dose volume. The plasma concentration-time profiles of LEN in rats are shown in Figure 3 and Figure 4. The plasma PK parameters are summarized in Table 5.

Following a single SC administration in solution, LEN PK showed a sustained slow drug release at all administered doses. F% was at least 77% in all dose groups. By Day 3 (72 h postdose), less than 10% of the bioavailable dose was released, indicating no unintended rapid drug release in all groups.

Following a single SC administration of LEN free acid in solution formulation A at 200 and 400 mg/mL dose concentrations, LEN concentrations remained detectable for at least 126 days after dose administration. LEN 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 formed in situ with 1 mole equivalent of sodium hydroxide, formulated at 100 and 200 mg/mL dose concentrations, LEN remained detectable for at least 56 days after dose administration. 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 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.

Table 5. Pharmacokinetic Parameters of LEN Following a Single Subcutaneous Administration of LEN in Solution Formulation A to Male Wistar Han Rats

LEN Ionization State	Group ID	Dose (mg/kg)	Dose Concentration (mg/mL)	Dose Volume (mL/kg)	$AUC_{0\text{-}72h}$ (µM•h)	AUC_{last} (µM•h)	AUC_{inf} (µM•h)	C_{max} (µM)	T_{max} (h)	% of AUC_{inf} on Day 3	Mean % F[c]
Free Acid[a]	1	10	200	0.05	2.80 ± 0.41	220 ± 22	222 ± 23	0.193 ± 0.049	504 ± 0	1.3	97
	2	50	200	0.25	4.81 ± 0.48	858 ± 56	886 ± 59	0.537 ± 0.033	672 ± 291	0.5	77
	3	50	400	0.125	5.53 ± 4.23	632 ± 155	NC	0.283 ± 0.119	840 ± 291	0.9[d]	NC
Sodium Salt[b]	4	10	100	0.10	18.3 ± 7.2	189 ± 34	190 ± 35	0.392 ± 0.057	232 ± 180	9.6	83
	5	10	200	0.05	5.87 ± 0.89	237 ± 31	NC	0.286 ± 0.086	560 ± 257	2.5[d]	NC
	6	50	200	0.25	19.5 ± 6.7	874 ± 68	876 ± 66	0.823 ± 0.158	504 ± 291	2.2	77
	7	100	200	0.50	41.3 ± 12.0	1798 ± 149	1825 ± 177	1.188 ± 0.183	616 ± 194	2.3	80

a. Formulated as solution in 77:10:13 w/w/w PEG200: ethanol: water .
b. Formulated as solution in 77:10:13 w/w/w PEG200: ethanol: water with 1 mole equivalent NaOH.
c. Calculated with mean IV AUC_{inf} = 22867 nM•h at 1 mg/kg from Table 2.
d. % of AUC_{last} on Day 3.

NC: not calculated due to insufficient data.
$AUC_{0\text{-}72h}$ is 3-day AUC
Values are the mean ± standard deviation from 3 animals.
Source: m2.6.5, Section 3, AD-200-2038

Figure 3. **Plasma Concentration-Time Profiles of LEN Following a Single Subcutaneous Administration of LEN Free Acid in Solution Formulation A to Male Wistar Han Rats (Mean ± SD, n = 3)[a]**

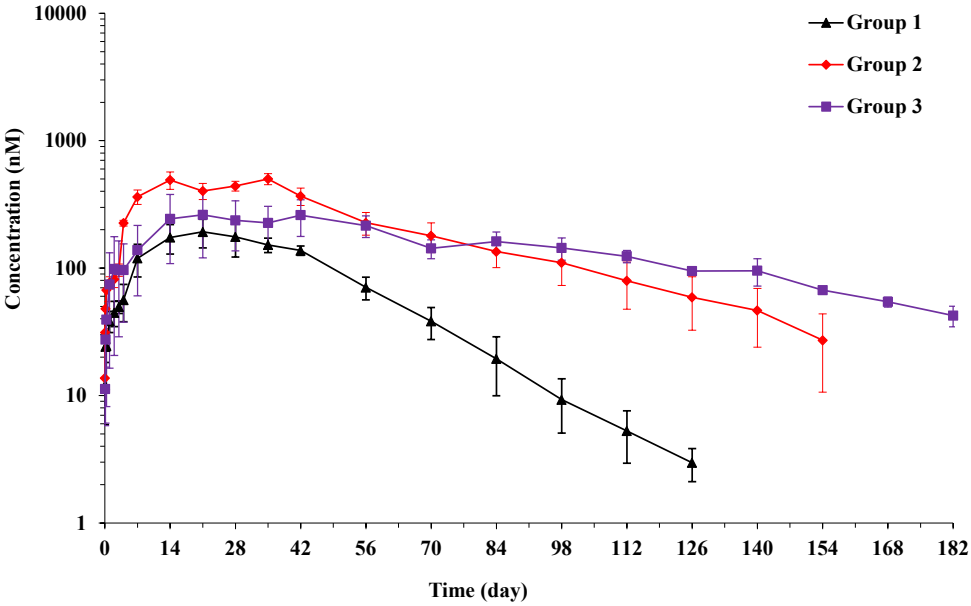
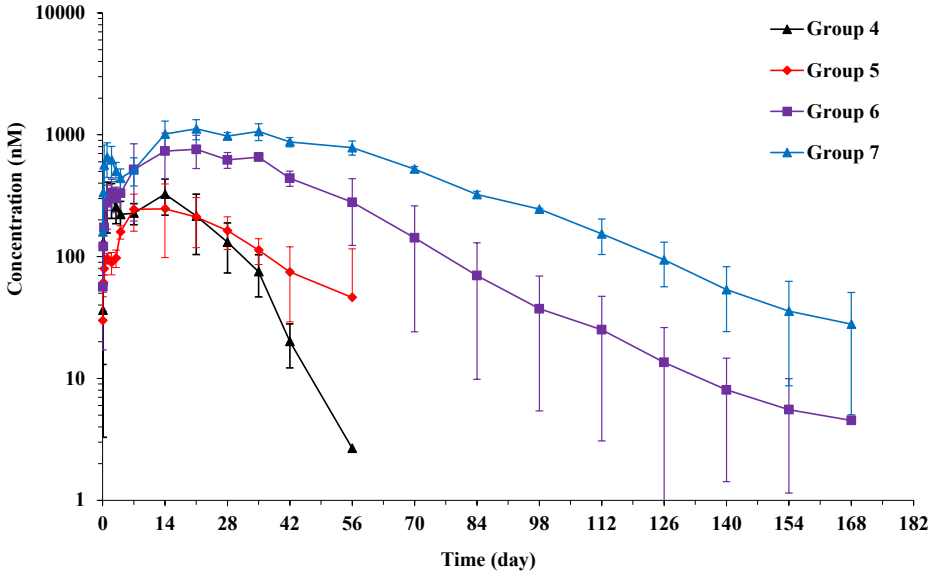


Figure 4. **Plasma Concentration-Time Profiles of LEN Following a Single Subcutaneous Administration of LEN Sodium Salt in Solution Formulation A to Male Wistar Han Rats (Mean ± SD, n = 3)[a]**



a Group assignment in Table 5

3.2.2.2. Dog

3.2.2.2.1. Aqueous Suspension Formulation

Lenacapavir free acid, formulated as an aqueous suspension with 2% poloxamer 188 in normal saline, was administered via SC injections to male beagle dogs (m2.6.5, Section 3, AD-200-2015). The SC dose was administered as a single injection at 3, 6, 30, 100 mg/kg or as two equal-volume 3 mg/kg injections for a total of 6 mg/kg. Plasma PK samples were collected up to 70 days postdose. The plasma concentration-time profiles of LEN in dogs following SC administration of LEN in aqueous suspension are shown in Figure 5, Figure 6, and Figure 7. The plasma PK parameters are summarized in Table 6.

Following a single SC administration, LEN PK showed a sustained slow drug release at all administered doses with measurable LEN concentrations for at least 672 h (28 days). F% was at least 56% in all dose groups. The mean $t_{1/2}$ following SC administration in dog ranged from 66 to 525 hours, which are substantially longer than the mean $t_{1/2}$ following IV administration (Table 2), indicating flip-flop PK following SC administration. No unintended rapid drug release was observed, ie, less than 30% of the bioavailable dose ($AUC_{0\text{-}72h}$ compared to AUC_{inf}) was released by Day 3 (72 h postdose). The average T_{max} ranged from 32 to 96 h.

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 (Figure 5). 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. By Day 28, > 90% of the total drug exposure (AUC_{inf}) was achieved in all three groups except the 3 mg/kg SC dose group. 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 (Figure 7). The average C_{max} was similar and the percent of total exposure by Day 28 was higher in the two equal-volume injection group. LEN systemic concentrations were detectable for at least 672 h (28 days).

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 (ie, 100 mg/mL) than at higher concentration (ie, 200 mg/mL). The percent of AUC_{inf} by Day 28 was similar across both groups. LEN systemic concentrations were detectable for at least 1344 h (56 days) in both groups (Figure 6).

Table 6. PK Parameters of LEN Following Subcutaneous Administration of LEN in Aqueous Suspension to Male Beagle Dogs

Group ID	Dose (mg/kg)[a]	Dose volume (mL/kg)	$AUC_{0\text{-}72h}$ (µM•h)	$AUC_{0\text{-}672h}$ (µM•h)	AUC_{last} (µM•h)	AUC_{inf} (µM•h)	C_{max} (µM)	T_{max} (h)	$t_{1/2}$ (h)	% of AUC_{inf} on Day 3	% of AUC_{inf} on Day 28	Mean % F[b]
1	3	0.03	3.0 ± 2.1	18.6 ± 9.0	25.4 ± 7.5	28.0 ± 6.4	0.056 ± 0.037	72 ± 83	525 ± 147	11%	66%	56
2	6	0.06	20.8 ± 7.4	117 ± 37	128 ± 41	129 ± 42	0.410 ± 0.141	40 ± 28	322 ± 51	16%	91%	129
3	6	0.03 x 2	18.8 ± 2.4	85.0 ± 7.0	88.4 ± 6.2	88.9 ± 6.2	0.417 ± 0.014	64 ± 28	174 ± 38	21%	96%	89
4	6	0.03	6.6 ± 3.4	63.9 ± 27.3	75.9 ± 34.6	79.1 ± 32.2	0.175 ± 0.068	96 ± 63	354 ± 329	8%	96%	79
5	30	0.30	145 ± 9	496 ± 49	497 ± 51	497 ± 51	2.960 ± 0.295	40 ± 14	66 ± 5	29%	100%	99
6	100	1.0	340 ± 76	1336 ± 504	1356 ± 500	1357 ± 500	7.243 ± 1.279	32 ± 14	94 ± 59	25%	100%	81

a Formulated as a suspension in 2% poloxamer 188 in normal saline
b Calculated with mean IV AUC_{inf} = 16667 nM•h at 1 mg/kg from Table 2.
$AUC_{0\text{-}72h}$ is 3-day AUC; $AUC_{0\text{-}672h}$ is 28-day AUC
Values are the mean ± standard deviation from 3 animals.
Source: m2.6.5, Section 3, AD-200-2015

Figure 5. **Plasma Concentration-Time Profiles of LEN Following Subcutaneous Administration of a 100 mg/mL Formulation of LEN in Aqueous Suspension at 3 mg/kg, 6 mg/kg, 30 mg/kg and 100 mg/kg to Male Beagle Dogs**

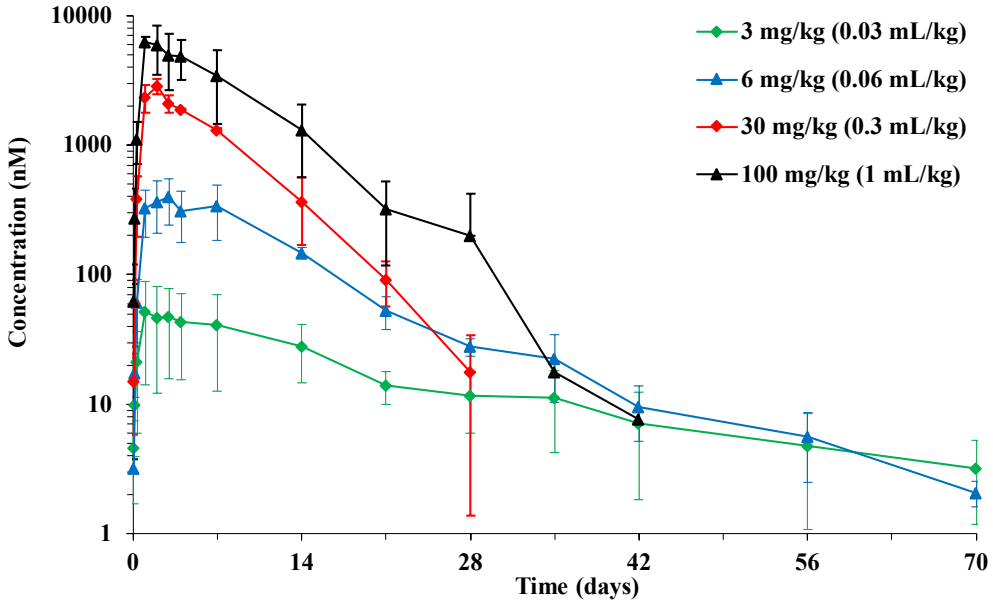


Figure 6. **Plasma Concentration-Time Profiles of LEN Following Subcutaneous Administration of LEN in Aqueous Suspension at 6 mg/kg to Male Beagle Dogs with Formulation Concentration at 100 mg/mL vs. 200 mg/mL**

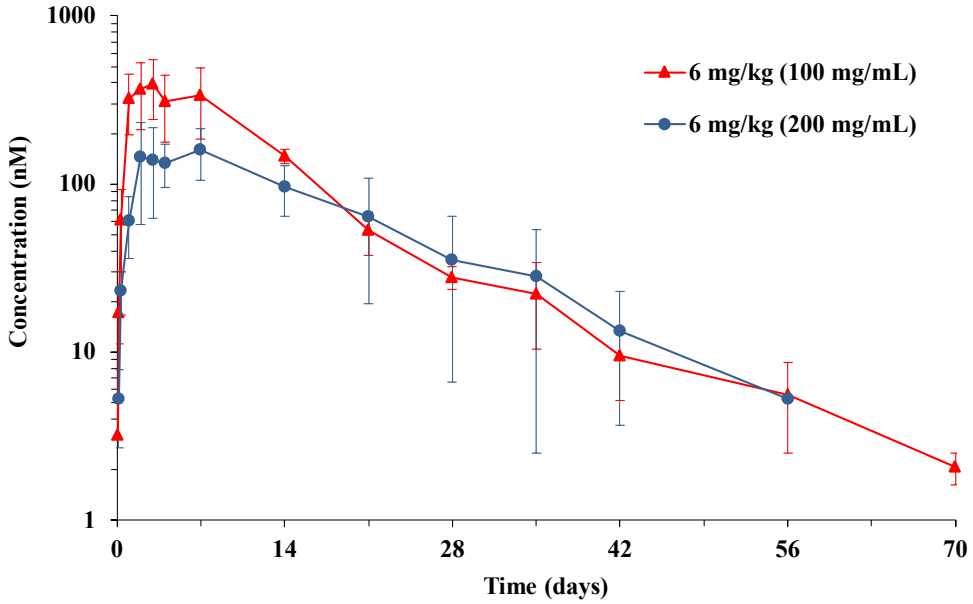
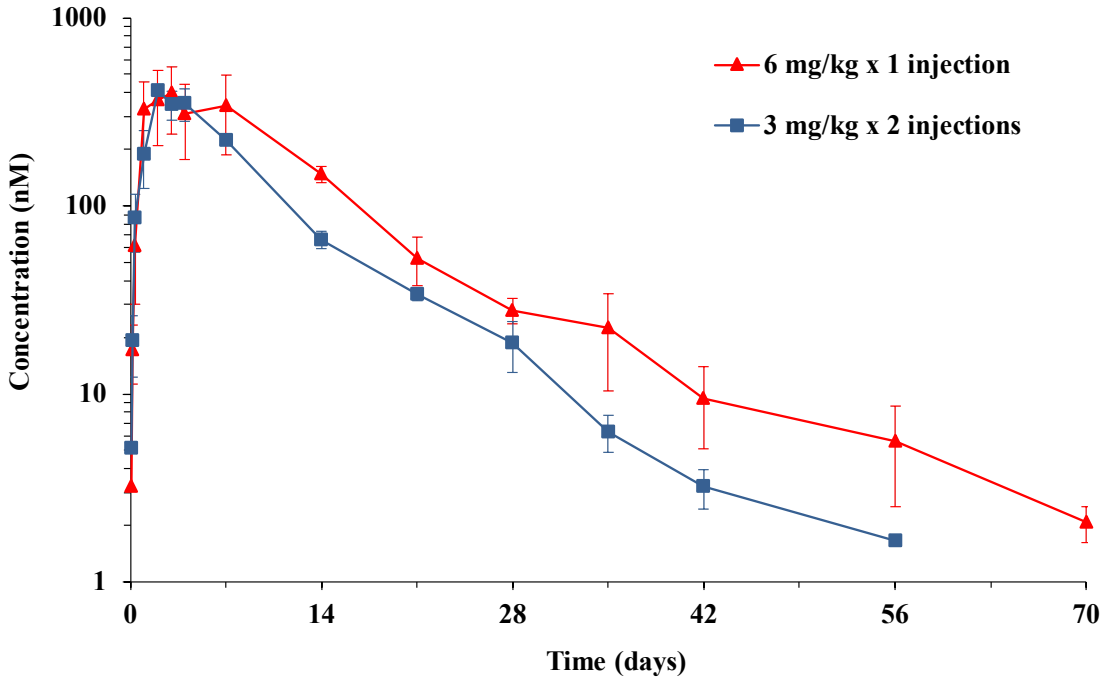


Figure 7. Plasma Concentration-Time Profiles of 100 mg/mL LEN Following Subcutaneous Administration of LEN in Aqueous Suspension at 6 mg/kg as One or Two Equal Volume Injections to Male Beagle Dogs



3.2.2.2.2. Solution Formulation A

Lenacapavir free acid or LEN sodium salt formed in situ with 1 mole equivalent of sodium hydroxide were formulated as a solution in 77:10:13 w/w/w PEG200:ethanol:water (solution formulation A). The formulated solution was administered to male beagle dogs via a single SC injection (m2.6.5, Section 3, AD-200-2039).

Multiple factors affecting LEN release following SC administration were evaluated, including LEN salt form, dose, dose concentration and dose volume. The plasma concentration-time profiles of LEN in dogs following SC administration of LEN solution are shown in Figure 8 and Figure 9. The plasma PK parameters are summarized in Table 7.

Following a single SC administration LEN in solution, LEN PK showed a sustained slow drug release at all administered doses. F% was at least 45% in all dose groups. By Day 3 (72 h postdose), less than 11% of the bioavailable dose was released, indicating no unintended rapid drug release in all groups. LEN concentrations remained detectable for at least 56 days after dose administration.

Following a single SC administration of LEN free acid, formulated at 200, 300 and 400 mg/mL dose concentrations, LEN concentrations remained detectable for at least 56 days after dose administration. LEN 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 dose concentration (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 6 mg/kg, the effect of LEN formulated at dose concentrations of 100, 200 and 300 mg/mL was assessed. LEN concentrations remained detectable for at least 56 days after dose administration. Administration at the 300 mg/mL concentration resulted in the lowest mean C_{max}.

The impact of LEN salt form 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.

Table 7. Pharmacokinetic Parameters of LEN Following a Single Subcutaneous Administration of LEN in Solution Formulation A to Male Beagle Dogs

LEN Ionization State	Group ID	Dose (mg/kg)	Dose Concentration (mg/mL)	Dose Volume (mL/kg)	$AUC_{0\text{-}72h}$ (µM•h)	AUC_{last} (µM•h)	AUC_{inf} (µM•h)	C_{max} (µM)	T_{max} (h)	% of AUC_{inf} on Day 3	Mean % F[d]
Free Acid	1	6[a]	200	0.03	0.98 ± 0.16	72.7 ± 20.8	88.9 ± 28.0	0.063 ± 0.019	392 ± 97	1.1	89
	2	12[b]	200	0.06	2.24 ± 0.36	124 ± 24	138 ± 19	0.144 ± 0.034	448 ± 97	1.6	69
	3	12[b]	300	0.04	1.09 ± 0.51	126 ± 72	NC	0.143 ± 0.082	392 ± 97	0.9[e]	NC
	4	12[b]	400	0.03	0.83 ± 0.36	111 ± 14	NC	0.107 ± 0.013	336 ± 0	0.7[e]	NC
Sodium Salt	5	6[c]	100	0.06	9.90 ± 6.06	91.7 ± 4.1	91.9 ± 4.1	0.259 ± 0.069	240 ± 166	10.8	92
	6	6[c]	200	0.03	4.92 ± 0.58	83.6 ± 18.0	84.4 ± 18.0	0.392 ± 0.097	392 ± 97	5.8	84
	7	6[c]	300	0.02	2.98 ± 1.91	43.2 ± 50.8	44.6 ± 50.2	0.122 ± 0.154	144 ± 166	6.7	45

a. Formulated as solution in 78:10:12 w/w/w PEG200: ethanol: water.
b. Formulated as solution in 77:10:13 w/w/w PEG200: ethanol: water .
c. Formulated as solution in 77:10:13 w/w/w PEG200: ethanol: water with 1 mole equivalent NaOH.
d Calculated with mean IV AUC_{inf} = 16667 µM•h at 1 mg/kg from Table 2.
e % of AUC_{last} on Day 3.
$AUC_{0\text{-}72h}$ is 3-day AUC.
NC: not calculated due to insufficient data.
Values are the mean ± standard deviation from 3 animals.
Source: m2.6.5, Section 3, AD-200-2039

Figure 8. **Plasma Concentration-Time Profiles of LEN Following a Single Subcutaneous Administration of LEN Free Acid in Solution Formulation A to Male Beagle Dogs (Mean ± SD, n = 3)[a]**

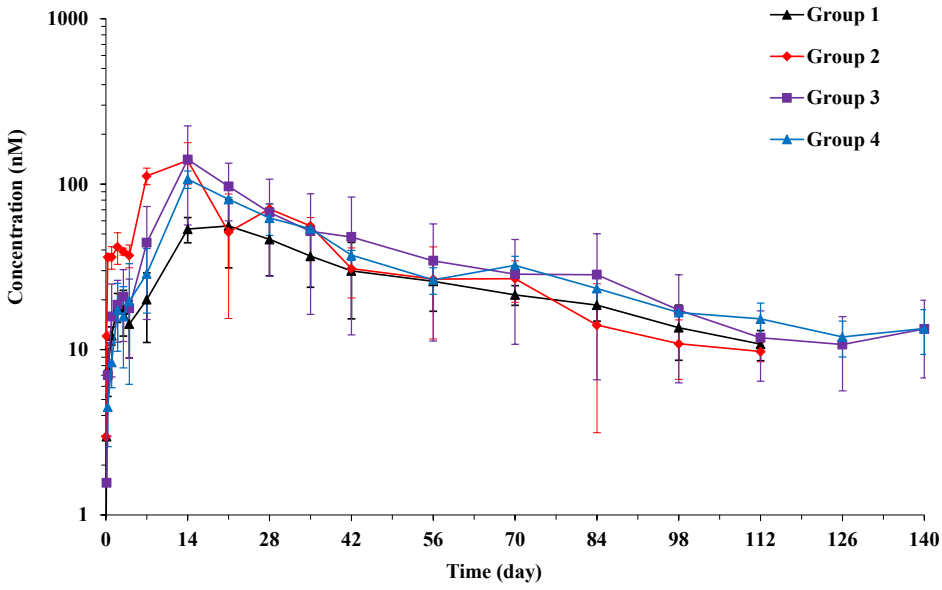
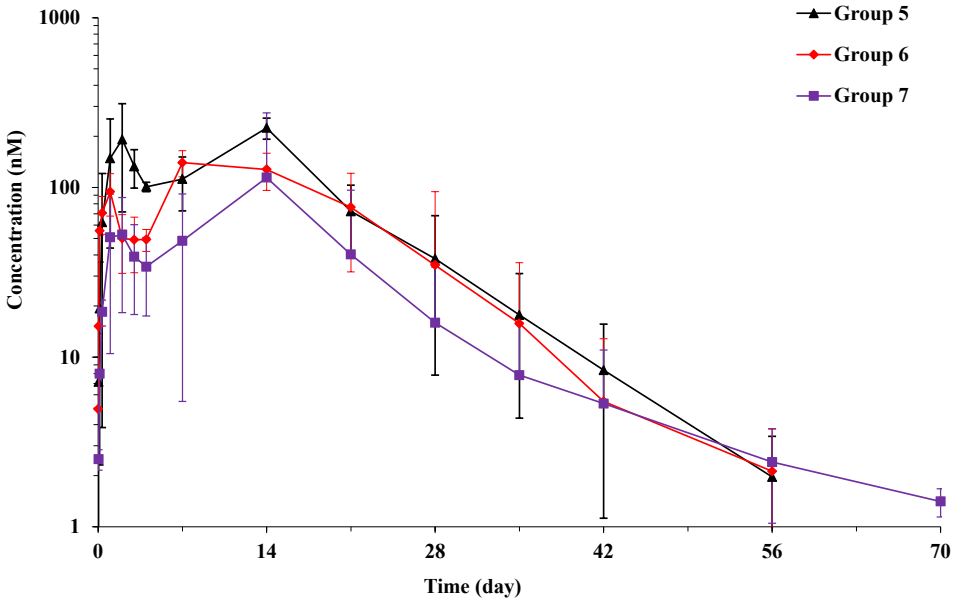


Figure 9. **Plasma Concentration-Time Profiles of LEN Following a Single Subcutaneous Administration of LEN Sodium Salt in Solution Formulation A to Male Beagle Dogs (Mean ± SD, n = 3)**

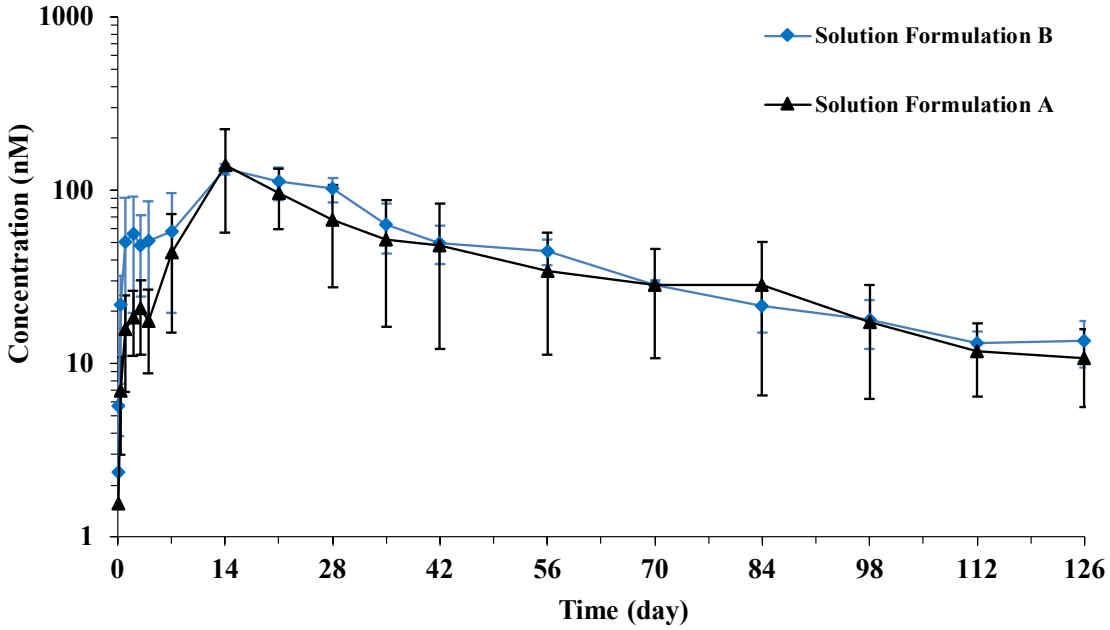


a. Group assignment in Table 7

3.2.2.2.3. Solution Formulation B

Lenacapavir free acid was formulated as a solution in 65:25.2:9.8 w/w/w PEG300:LEN:water (solution formulation B, m2.6.5, Section 3, AD-200-2051). The formulated solution was administered to male beagle dogs via a single SC injection at 12 mg/kg (300 mg/mL). LEN PK were monitored for at least 126 days. An overlay of LEN PK following administration of LEN in solution formulations A and B is shown in Figure 10. LEN PK showed a sustained slow release and no unintended rapid drug release was observed (Figure 10). PK profiles up to 126 days were similar, suggesting presence or absence of ethanol in formulations did not significantly alter the LEN release profile following SC administration.

Figure 10. Plasma Concentration-Time Profiles of LEN Following a Single Subcutaneous Administration of LEN in Solution Formulation A and Solution Formulation B (12 mg/kg; 300 mg/mL) to Male Beagle Dogs (Mean ± SD, n = 3)



3.2.3. Oral Administration

3.2.3.1. Solution PK

Following oral administration of LEN in a solution formulation, the PK was determined in rat, dog and monkey (m2.6.5, Section 3, AD-200-2010, AD-200-2011, AD-200-2012, and AD-200-2013). The plasma concentration-time profiles of LEN following PO administration are shown in Figure 11. The plasma PK parameters are summarized in Table 8. LEN was slowly absorbed with average T_{max} range from 8.7 to 18.7 h. F% ranged from 2.2% to 22%.

Table 8. Plasma PK Parameters of LEN Following a Single Oral Administration in Solution to Rat, Dog, Cynomolgus Monkey and Rhesus Monkey (Mean ± SD, n = 3)

Species	Dose[a] (mg/kg)	$AUC_{0\text{-}72h}$ (nM•h)	AUC_{inf} (nM•h)	C_{max} (nM)	T_{max} (h)	F (%)
Sprague-Dawley Rat	5.0	13467 ± 3927	25200 ± 10221	336 ± 116	10.0 ± 3.5	21.7 ± 8.9
Beagle Dog	4.0	11410 ± 4324	16533 ± 10366	471 ± 203	10.7 ± 11.5	22.1 ± 8.4
Cynomolgus Monkey	4.0	298 ± 85[b]	ND	17.7 ± 5.6	8.7 ± 3.1	2.2 ± 0.6[b]
Rhesus Monkey	4.0	972 ± 367	1133 ± 446	29.4 ± 7.5	18.7 ± 9.2	4.4 ± 1.7

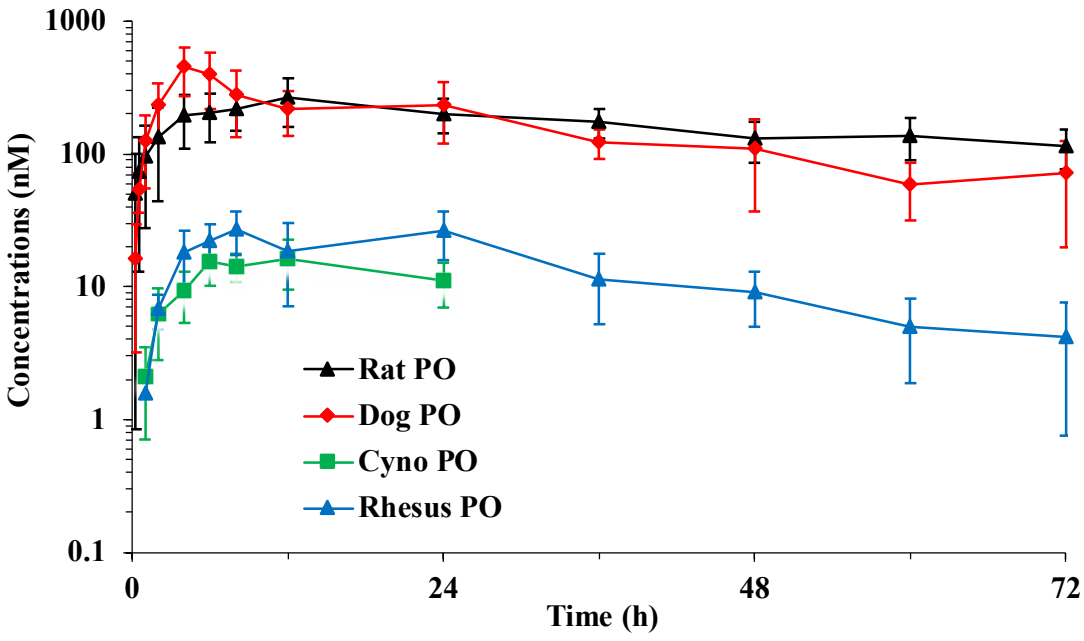
$AUC_{0\text{-}24h}$ = area under the plasma concentration-time curve from 0 to 24 h; $AUC_{0\text{-}72h}$ = area under the plasma concentration-time curve from 0 to 72 h; AUC_{inf} = area under the plasma concentration-time curve extrapolated to time infinity; C_{max} = maximum observed plasma concentration; F = oral bioavailability; ND = not determined; T_{max} = time of the observed maximum plasma concentration.

a Formulated as a solution in 5% ethanol, 20% propylene glycol, 45% polyethylene glycol 300 and 30% 0.01 N hydrochloric acid

b $AUC_{0\text{-}24h}$; F% Calculated based on $AUC_{0\text{-}24h}$.

Source: m2.6.5, Section 3, AD-200-2010, AD-200-2011, AD-200-2012 and AD-200-2013

Figure 11. Plasma Concentration-Time Profiles of LEN Following Single Oral Administration of LEN to Rat, Dog and Monkey



3.2.3.2. Solution PK in Portal Vein Cannulated Dogs and Monkeys

To understand the potential CYP mediated metabolism contribution to fraction of the administered PO 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 (m2.6.5, Section 3, AD-200-2016) and monkeys (m2.6.5, Section 3, AD-200-2017) without and with pretreatment of ABT, a pan CYP inhibitor. ABT plasma concentrations were quantified by a LC-MS/MS method and were consistent with historic values. These studies demonstrated that pretreatment with ABT did not alter LEN oral exposure in dogs (Table 9) and monkeys (Table 10). 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.

Table 9. Plasma Pharmacokinetic Parameters of LEN Following an Oral Dose at 2 mg/kg to Portal Vein Cannulated Dogs without or with ABT Pretreatment

PK Parameters	Without ABT Pretreatment[a]		With ABT Pretreatment[a]	
	Jugular Vein	Portal Vein	Jugular Vein	Portal Vein
$AUC_{0\text{-}120h}$ (nM•h)	9041 ± 2175	8053 ± 1780	8746 ± 7337	7291 ± 5509
AUC_{inf} (nM•h)	9297 ± 2428	8264 ± 1962	9042 ± 7712	7583 ± 5871
C_{max} (nM)	273 ± 23	256 ± 28.2	282 ± 143	260 ± 109
T_{max} (h)	10.7 ± 11.6	4.0 ± 2.0	4.0 ± 2.0	3.3 ± 1.2
F	23.7% ± 6.2%[b]	ND	23.0% ± 19.6%[b]	ND
F_h	1.0 ± 0.0		1.0 ± 0.0	
E_h	0.0 ± 0.0		0.0 ± 0.0	
F_{abs}	23.7% ± 6.2%		23.0% ± 19.6%	

E_h = hepatic extraction ratio; calculated as 1 – F_h following oral dose; calculated as CL/Q_h following IV administration; F = oral bioavailability calculated as the ratio of $(Dose_{(IV)} \times AUC_{inf, PO})/(Dose_{(PO)} \times AUC_{inf, IV})$, $F = F_g \times F_{abs} \times F_h$; F_{abs} = fraction (%) absorbed in the gut calculated as $F_{abs} = F/(F_h * F_g)$; F_g = fraction of the dose escaping the first-pass gut metabolism; F_h = fraction of the dose escaping the liver metabolism; calculated as $F_h = AUC_{inf}$ from jugular vein over AUC_{inf} from portal vein; Q_h = the hepatic blood flow; ND = not determined.

a LEN was formulated as a solution in 5% ethanol, 20% propylene glycol, 40% polyethylene glycol 300 and 35% pH 7 phosphate buffer; ABT was formulated in phosphate buffer saline (pH 7.4) and administered orally at 20 mg/kg 2 hours before LEN dose.

b F% Calculated based on AUC_{inf} = 19631 ± 7871 nM•h at 1 mg/kg IV infusion from the same 3 animals.

Values are the mean ± standard deviation from 3 animals.

Source: m2.6.5, Section 3, AD-200-2016

Table 10. Plasma Pharmacokinetic Parameters of LEN Following an Oral Dose at 2 mg/kg to Portal Vein Cannulated Monkeys without or with ABT Pretreatment

PK Parameters	Without ABT Pretreatment[a]		With ABT Pretreatment[a,b]	
	Jugular Vein	Portal Vein	Jugular Vein	Portal Vein
$AUC_{0\text{-}120h}$ (nM•h)	882 ± 453	950 ± 77	704 ± 331	1021 ± 262
AUC_{inf} (nM•h)	909 ± 458	981 ± 85	730 ± 333	1046 ± 260
C_{max} (nM)	38.7 ± 16.0	133 ± 85	38.4 ± 17	90.7 ± 7.28
T_{max} (h)	10.7 ± 2.3	4.3 ± 2.9	10.7 ± 2.3	5.3 ± 2.3
F	11.4% ± 5.7%[c]	ND	9.1% ± 4.2%[c]	ND
F_h	0.80 ± 0.18		0.68 ± 0.16	
E_h	0.20 ± 0.18		0.32 ± 0.16	
F_{abs}	13.7% ± 3.8%		13.1% ± 3.1%	

a LEN was formulated as a solution in 5% ethanol, 20% propylene glycol, 45% polyethylene glycol 300 and 30% pH 2 water
b ABT was formulated in phosphate buffer saline (pH 7.4) and administered orally at 20 mg/kg 2 hours before LEN dose.
c F% Calculated based on AUC_{inf} = 4001 ± 1057 nM•h at 1 mg/kg IV infusion from the same 3 animals.
Values are the mean ± standard deviation from 3 animals.
Source: m2.6.5, Section 3, AD-200-2017

3.2.3.3. PK at Multiple Dose Levels

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 (m2.6.5, Section 3, AD-200-2018 and AD-200-2019).

3.2.3.3.1. Rat

Following oral administration of LEN to male WH rats at 30 and 100 mg/kg in a solution formulation, LEN was slowly absorbed with a mean T_{max} of 9.0 and 20.0 h at 30 and 100 mg/kg, respectively (m2.6.5, Section 3, AD-200-2018). The AUC_{inf} of LEN increased with doses from 30 to 100 mg/kg, but the increase was less than dose proportional (Table 11).

Table 11. Plasma PK Parameters of LEN Following a Single Oral Dose in Solution to Male Wistar Han Rats at Multiple Dose Levels

Dosage (mg/kg)[a]	AUC_{last} (nM•h)	AUC_{inf} (nM•h)	C_{max} (nM)	T_{max} (h)
30	73843 ± 14148	83410 ± 17982	1292 ± 377	9.0 ± 2.6
100	90083 ± 34147	120428 ± 62292	1133 ± 406	20.0 ± 6.9

a Formulated in a solution in 10% dimethyl sulfoxide, 10% Vitamin E d-α-tocopheryl polyethylene glycol 1000 succinate and 80% propylene glycol.
Values are the mean ± standard deviation from 3 animals.
Source: AD-200-2018

3.2.3.3.2. Dog

A single dose of LEN, formulated in solution with three different vehicles, was administered orally at 30 and/or 100 mg/kg to male beagle dogs (m2.6.5, Section 3, 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 (Table 12). Formulation B achieved higher exposure at 100 mg/kg and was chosen for repeat dose toxicology studies.

Table 12. Plasma PK Parameters of LEN Following a Single Oral Administration in Various Solution Formulations to Male Beagle Dogs

Formulation[a]	Dose (mg/kg)	Number of Animals	AUC_{last} (μM•h)	AUC_{inf} (μM•h)	C_{max} (μM)	T_{max} (h)
A	30	3	59.7 ± 82.4	60.9 ± 84.3	2.97 ± 4.19	3.3 ± 1.2
B	30	3	69.6 ± 58.6	70.6 ± 58.5	2.47 ± 1.96	16.7 ± 12.7
C	30	6	92.9 ± 33.6	93.1 ± 33.5	3.23 ± 1.61	10.0 ± 8.0
B	100	6	148 ± 37	148 ± 37	4.25 ± 0.73	12.0 ± 0.0
C	100	6	120 ± 52	121 ± 53	2.89 ± 0.86	15.7 ± 9.7

a Formulation A: solution formulation in 5% ethanol, 10% Vitamin E d-α-tocopheryl polyethylene glycol 1000 succinate (TPGS), 85% propylene glycol (PG).
Formulation B: solution formulation in 10% dimethyl sulfoxide, 10% Vitamin E TPGS, 80% PG.
Formulation C: solution formulation in 10% ethanol, 10% Vitamin E TPGS, 25% PEG 200, 55% PG.
Values are the mean ± standard deviation.
Source: m2.6.5, Section 3, AD-200-2019

3.3. Repeat Dose In Vivo Studies

3.3.1. Intravenous Administration

The TK profiles of LEN following repeat IV administration of LEN in solution were examined in rabbit (m2.6.7, Section 1, TX-200-2032) as part of GLP toxicology studies (m2.6.6 Section 3).

Lenacapavir was formulated in a solution of 6.12% w/w PEG 300, 93.88% w/w sterile water and dosed once daily via IV infusion over 30 minutes at 5, 10 and 20 mg/kg/day to pregnant female rabbits during Gestation Day 7 to 19 (m2.6.6, Section 2, TX-200-2032). LEN exposure increased with the increase in dose level from 5 to 20 mg/kg/day and the increases in C_{max} and $AUC_{0\text{-}24.5h}$ were generally greater than dose proportional. No accumulation of LEN occurred. LEN was measurable 96 hours after the last dose.

3.3.2. Subcutaneous Administration

The TK profiles of LEN following repeat SC administration of LEN in solution were examined in rat (TX-200-2014) and dog (TX-200-2017 and TX-200-2047) as part of GLP toxicology studies (m2.6.7, Section 2).

3.3.2.1. Rat

Lenacapavir was administered to rats once on Day 1 only or twice on both Day 1 and Day 92 at 100 mg/kg/dose via SC injection in a solution formulation of 77:10:13 w/w/w PEG200:ethanol:water (m2.6.7, Section 2, TX-200-2014). LEN plasma concentrations were quantifiable at the last time point (2184 hours) in all non-vehicle groups. Following a single SC dose on Day 1, the mean C_{max} and AUC_{last} values were higher in groups dosed at 200 mg/mL with 1 molar-equivalent (mol-eq) NaOH than those dosed at 200 mg/mL with 0 mol-eq NaOH. Following a repeat SC dose on Day 92, the mean C_{max} and AUC_{last} values were similar in all groups administered 200 mg/mL. Following a single SC dose on Day 1 and Day 92, the mean C_{max} and AUC_{last} values were lower in groups dosed at 400 mg/mL than those dosed at 200 mg/mL with 0 mol-eq NaOH. No accumulation was observed after a repeat SC dose on Day 92. No sex differences were observed.

3.3.2.2. Dog

Lenacapavir was dosed to dogs in 77:10:13 w/w/w PEG200:ethanol:water at 20 mg/kg/dose (200 mg/mL with or without 1-mol-eq NaOH) or 40 mg/kg/dose (400 mg/mL without NaOH). Animals were dosed via SC injection in the dorsal region as a single dose on Days 1, 29, 58, 85, 113, 141, 169, 197, 225, and 253 at a volume of 0.1 mL/kg (m2.6.7, Section 2, TX-200-2017). LEN was quantifiable in plasma at all time points, indicating sustained exposure for the entire 9-month study duration. Sex-based differences were less than 2-fold. LEN steady-state exposures (C_{max} and $AUC_{0\text{-}672h}$) were reached after four monthly SC doses on Day 85. Mean C_{max} and $AUC_{0\text{-}672h}$ values were higher in animals administered 20 mg/kg/dose (200 mg/mL, 1 mol-eq NaOH) and 40 mg/kg/dose (400 mg/mL, 0 mol-eq NaOH) than 20 mg/kg/dose (200 mg/mL, 0 mol-eq NaOH) after multiple doses.

Lenacapavir was formulated in a solution of 68.20% (w/w) PEG 300, 31.80% (w/w) sterile water and administered once every 4 weeks via SC injection to dogs at 130 to 411 mg/kg/dose for up to 37 weeks (m2.6.7, Section 2, TX-200-2047). LEN was quantifiable at all time points, indicating sustained exposure through the 13-week observation period (Day 143) after the last dose for the 411 mg/kg/dose group on Day 57 and through the 13-week observation period (Day 253) after the last dose for the 130 mg/kg/dose group on Day 225. LEN exposure increased with the increase in dose level from 130 to 411 mg/kg/dose. The increases in $C_{max,}$ $AUC_{0\text{-}336h}$ and $AUC_{0\text{-}672h}$ were less than dose proportional between 130 and 411 mg/kg/dose on Days 1, 32, and 57. Sex differences were less than 2-fold. Accumulation of LEN was observed after multiple doses in dogs. The accumulation ratios were 3.2 and 2.3 for the 130 mg/kg/dose group on Day 225 and the 411 mg/kg/dose group on Day 57, respectively.

3.3.3. Oral Administration

The TK profiles of LEN following repeat oral administration of LEN in solution were examined in rat (TX-200-2011 and TX-200-2036) and dog (TX-200-2012) as part of GLP toxicology studies (m2.6.7, Section 2).

3.3.3.1. Rat

Lenacapavir was formulated in a solution of 10% volume-to-volume (v/v) dimethyl sulfoxide (DMSO), 10% v/v Vitamin E d-α-tocopheryl polyethylene glycol 1000 succinate (TPGS), 80% v/v propylene glycol (PG) and dosed to WH rats via oral gavage once daily for 4 weeks at 3, 10 and 30 mg/kg/day (m2.6.7, Section 2, TX-200-2011). LEN exposure increased with increase in dose levels from 3 to 30 mg/kg/day. The increases in C_{max} and $AUC_{0\text{-}24h}$ on Day 1 were approximately dose proportional between the 3 and 10 mg/kg/day dose levels and less than dose proportional between the 10 and 30 mg/kg/day dose levels. The increases in C_{max} and $AUC_{0\text{-}24h}$ on Day 28 were less than dose proportional between the 3 to 30 mg/kg/day dose levels. No sex-based differences were observed. Accumulation ratios for LEN were 5.47, 2.71, and 2.24-fold at the 3, 10, and 30 mg/kg/day dose levels, respectively.

Lenacapavir was formulated in a solution of 10% DMSO, 10% vitamin E TPGS, 80% PG and administered at doses of 0, 3, 10, and 30 mg/kg/day once daily by oral gavage to time-mated SD rats during organogenesis (Gestation Day 6 to 17) (m2.6.7, Section 2, TX-200-2036). LEN exposures (C_{max} and $AUC_{0\text{-}24h}$) increased with the increase in dose levels from 3 to 30 mg/kg/day, but the increases were less than dose proportional. Approximately 2-fold accumulation was observed after multiple doses of 3 mg/kg/day in pregnant rats.

3.3.3.2. Dog

Lenacapavir was formulated in a solution of 10% v/v DMSO, 10% v/v Vitamin E TPGS, 80% v/v PG and dosed to beagle dogs via oral gavage once daily for 4 weeks at 1, 5 and 30 mg/kg/day (m2.6.7, Section 2, TX-200-2012). LEN exposure increased with the increase in dose levels from 1 to 30 mg/kg/day. The increases in C_{max} and $AUC_{0\text{-}24h}$ were approximately dose proportional between the 1 to 30 mg/kg/day dose levels on Day 1, with the exception of C_{max} between the 5 and 30 mg/kg/day dose level where the increases were less than dose proportional. On Day 28, the increases in C_{max} and $AUC_{0\text{-}24h}$ were greater than dose proportional between the 1 to 5 mg/kg/day and less than dose proportional between the 5 to 30 mg/kg/day dose levels. Sex-based differences were less than 2-fold. Mean accumulation ratio values for LEN were 2.27, 3.43, and 1.59 at the 1, 5, and 30 mg/kg/day dose levels, respectively.

3.4. Summary of Absorption

Extensive studies were conducted to characterize the absorption of LEN.

In Caco-2 assay, LEN showed low forward permeability with evidence for efflux transport.

Lenacapavir systemic plasma CL was low and V_{ss} was high in all nonclinical species.

Lenacapavir demonstrated high F% following SC administration. Factors affecting LEN release were extensively assessed. LEN PK in rat and dog showed a sustained slow drug release no matter which parameters were varied.

Lenacapavir demonstrated low F% following oral administration. The observed oral F% was consistent with limited absorption.

4. DISTRIBUTION

4.1. In Vitro Protein Binding

4.1.1. Plasma Protein Binding

The binding of LEN (2 µM) to plasma proteins was high, with less than 1.5% free in all tested species (m2.6.5, Section 4, AD-200-2020 and AD-200-2044). The highest free fraction of 1.46% was observed in human plasma (Table 13).

Lenacapavir (2 µM) demonstrated very high binding to HSA with 0.01% free in 40 mg/mL of HSA (m2.6.5, Section 4, AD-200-2048). LEN (2 µM) bound moderately to AAG with mean of 7.0% free at a typical level of 0.8 mg/mL AAG and 1.78% free at a more pathological level (5 fold) of 4.0 mg/mL AAG. LEN is expected to be bound primarily to albumin in human plasma.

Table 13. Binding of LEN in Plasma (Mean ± SD, n = 3)

Species	Free Fraction (%)[a]
CD-1 Mouse	0.18 ± 0.02
Sprague-Dawley Rat	0.13 ± 0.02
Beagle Dog	0.83 ± 0.19
Cynomolgus Monkey	1.11 ± 0.22
Rhesus Monkey	1.45 ± 0.18
Human	1.46 ± 0.23

Source: AD-200-2020 and AD-200-2044

4.1.2. Relative Protein Binding in Human Plasma and Cell Culture Medium

The relative protein binding of LEN (2 µM) between 10% (v/v) human plasma and CCM was determined in vitro (m2.6.5, Section 4, AD-200-2020). Binding to CCM was expected because it contained 10% v/v fetal bovine serum. At equilibrium, the concentration of LEN in human plasma, corrected for dilution, was 17.4-fold higher than in CCM. This relative protein binding value was used to obtain the plasma protein binding-adjusted effective concentration to achieve 95% inhibition ($paEC_{95}$ = 17.4 × in vitro EC_{95}; Section 2.1.3 in m2.6.2).

4.1.3. Protein Binding to Human Microsomal Fraction

Lenacapavir (2 µM) binding to human hepatic microsomes was high with mean fraction bound of 89.2% (m2.6.5, Section 4, AD-200-2032).

4.2. Blood to Plasma Ratio

Lenacapavir (0.5 µM) BPR was similar across species with mean values ranging from 0.59 to 0.67. The human BPR was 0.64, showing minimal binding to blood cells (m2.6.5, Section 4, AD-200-2021).

4.3. Tissue Distribution Studies

The tissue distribution of LEN following a single IV bolus administration of [^{14}C]LEN at 3 mg/kg to male WH (non-pigmented) and Long Evans (pigmented) rats was determined by quantitative whole-body autoradiography (m2.6.5, Section 4, AD-200-2006). A total of 52 tissues was examined and results from representative tissues at select time-points are shown in Table 14 and Table 15. A complete listing is provided in Sections 4.5 and 4.6 in m2.6.5.

[^{14}C]LEN-derived radioactivity was widely distributed to most tissues by the first collection time point (0.5 hours postdose) in both WH and LE male rats and the distribution of radioactivity was similar in both species. Representative autoradiographs from WH and LE rats obtained 0.5 hour postdose are shown in Figure 12 and Figure 13, respectively. Most of the tissues reached C_{max} by 0.5 hours. Liver achieved the highest concentration of radioactivity of the tissues sampled. 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.

Table 14. Concentrations of Radioactivity in Blood and Select Tissues After a Single Intravenous Administration of [^{14}C]LEN to Male WH Rats at 3 mg/kg (172 µCi/kg)

	[^{14}C]LEN ng-Equivalents/g Tissue									
Tissue/Matrix	**0.5 h**	**2 h**	**24 h**	**72 h**	**168 h**	**336 h**	**672 h**	**840 h**	**1008 h**	**1344 h**
Adrenal gland	1120[a]	553[a]	292[a]	132[a]	81.4	BLQ[a]	BLQ	ND	ND	ND
Blood	719	534	224	169	75.9	ND	ND	ND	ND	ND
Bone	43.1	27.9	BLQ	ND	ND	ND	ND	ND	ND	ND
Bone marrow	885	423	247	95.8	44.6	ND	ND	ND	ND	ND
Brain cerebrum/medulla	BLQ	BLQ	ND	ND	ND	ND	ND	ND	ND	ND
Eye	68.0	44.7	33.4	BLQ	BLQ	ND	ND	ND	ND	ND
Fat (brown)	1870	945	454	169	87.5	ND	ND	ND	ND	ND
Kidney	2490	1650	1070	391	185	31.0	ND	ND	ND	ND
Large intestine	458	381	191	71.7	BLQ	BLQ	ND	ND	ND	ND
Liver	53400	54700	34000	15000	5610	1010	52.9	BLQ	BLQ	ND
Lung(s)	809	539	333	172	69.9	ND	ND	ND	ND	ND
Pancreas	2210	1060	593	209	95.3	BLQ	ND	ND	ND	ND
Skin (nonpigmented)	316	310	177	52.6	BLQ	ND	ND	ND	ND	ND
Small intestine	1150	626	471	427	72.8	29.7	ND	ND	ND	ND
Spinal cord	BLQ	BLQ	ND	ND	ND	ND	ND	ND	ND	ND
Spleen	724	491	252	88.6	59.7	BLQ	BLQ	ND	ND	ND
Stomach	977	475	260	112	41.8	BLQ	ND	ND	ND	ND
Testis	73.3	99.6	196	101	37.6	ND	ND	ND	ND	ND
Urine	36.9	NR	38.3	ND	ND	ND	ND	ND	ND	ND

BLQ = below the limit of quantitation (< 28.0 ng equivalents [^{14}C]LEN/g); h = hours; ND = not detected.
a Tissue appeared to be fat soaked.
Source: m2.6.5, Section 4, AD-200-2006

Figure 12. Annotated Whole-Body Autoradiograph at 0.5 Hour After a Single Intravenous Administration of [^{14}C]LEN to a Male WH Rat (3 mg/kg, 172 µCi/kg, Animal R0201)

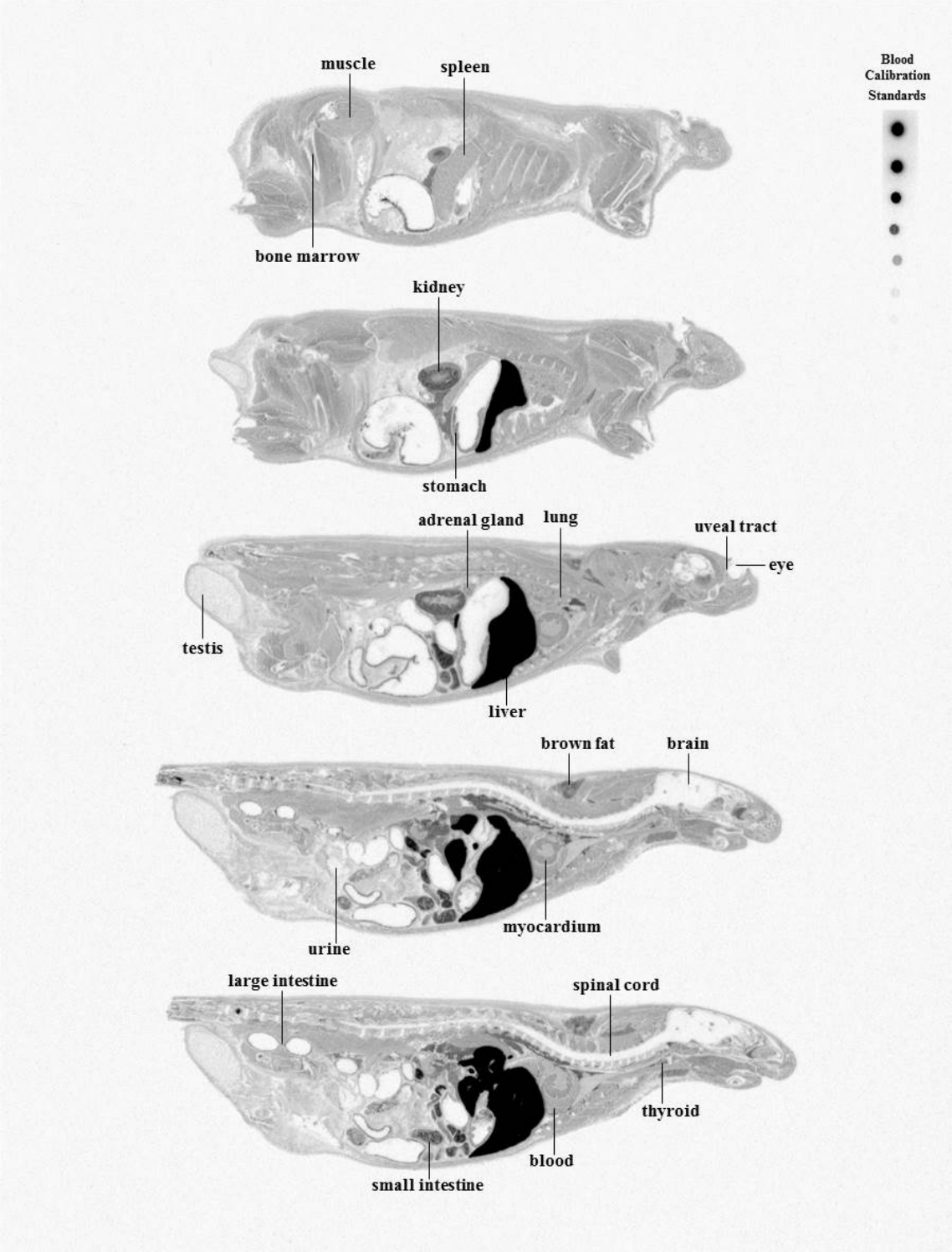
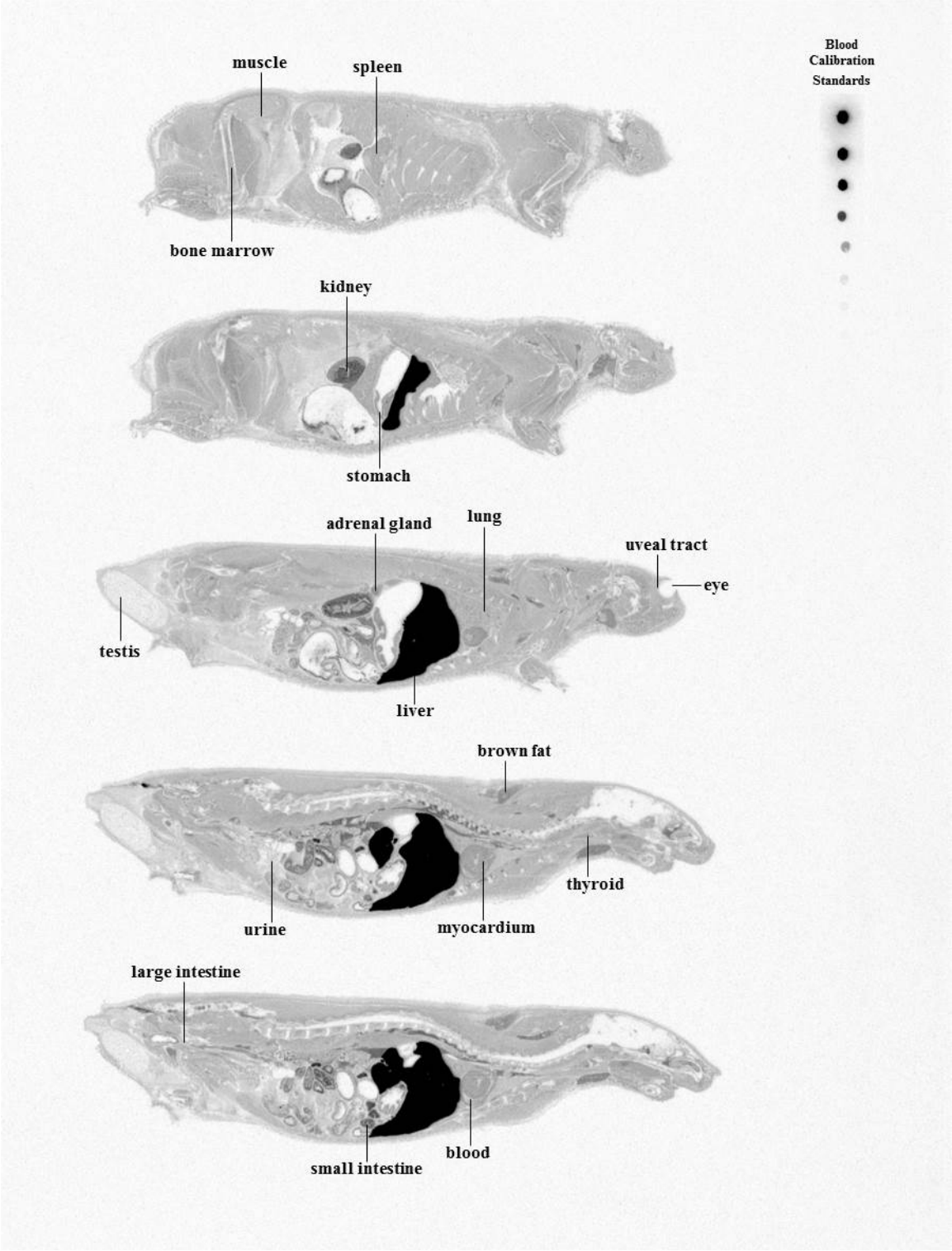


Table 15. Concentrations of Radioactivity in Blood and Selected Tissues After a Single Intravenous Administration of [^{14}C]LEN to Male LE Rats at 3 mg/kg (172 µCi/kg)

	[^{14}C]LEN ng-Equivalents/g Tissue									
Tissue/Matrix	**0.5 h**	**2 h**	**24 h**	**72 h**	**168 h**	**336 h**	**672 h**	**840 h**	**1008 h**	**1344 h**
Adrenal gland	1060[a]	1280[a]	308[a]	162	171[a]	35.9[a]	BLQ	ND	ND	ND
Blood	467	988	315	135	75.9	ND	ND	ND	ND	ND
Bone	58.2	54.6	39.7	ND	ND	ND	ND	ND	ND	ND
Bone marrow	573	461	211	98.4	63.0	ND	ND	ND	ND	ND
Brain cerebrum/medulla	BLQ	BLQ	BLQ	ND	ND	ND	ND	ND	ND	ND
Eye	36.2	51.9	30.2	BLQ	BLQ	ND	ND	ND	ND	ND
Fat (brown)	130	506	618	228	159	BLQ	ND	ND	ND	ND
Kidney	2100	1870	1080	510	306	62.6	ND	ND	ND	ND
Large intestine	774	690	374	422	156	46.8	ND	ND	ND	ND
Liver	40300	52800	34900	17800	9670	1960	36.9	41.8	36.3	ND
Lung(s)	562	686	306	128	86.1	ND	ND	ND	ND	ND
Pancreas	1500	1130	581	231	169	34.9	ND	ND	ND	ND
Skin (pigmented)	263	371	214	75.2	49.1	ND	ND	ND	ND	ND
Small intestine	931	818	477	242	154	32.3	ND	ND	ND	ND
Spinal cord	BLQ	BLQ	BLQ	ND	ND	ND	ND	ND	ND	ND
Spleen	625	483	228	103	63.2	BLQ	BLQ	ND	ND	ND
Stomach	691	607	280	125	81.6	BLQ	ND	ND	ND	ND
Testis	48.7	162	184	103	72.7	28.6	ND	ND	ND	ND
Urine	62.3	44.5	31.0	BLQ	BLQ	ND	ND	ND	ND	ND

a Tissue appeared to be fat soaked.
Source: m2.6.5, Section 4, AD-200-2006

Figure 13. Annotated Whole-Body Autoradiograph at 0.5 Hour After a Single Intravenous Administration of [^{14}C]LEN to a Male LE Rat (3 mg/kg, 172 µCi/kg, Animal R0301)



4.4. Studies in Pregnant or Nursing Animals

4.4.1. Placental and Nursing Animal Distribution

The plasma exposure of LEN in nursing pups was determined in a prenatal and postnatal development (PND) study in rats (m2.6.7, Section 12, TX-200-2043). LEN was formulated in a solution of 68.2% PEG 300, 31.8% sterile water and dosed to mated female SD rats via a single SC injection at 30 and 300 mg/kg on Gestation Day 6. LEN was detected in the plasma of neonates on Lactation Day 10. LEN 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. The mean maternal concentration on Lactation Day 10 after a dose of 30 or 300 mg/kg was 36.7 and 196 ng/mL, respectively, with the respective mean pup concentration of 7.12 and 32.8 ng/mL on PND Day 10. The mean maternal to mean pup concentration ratios were 5.15 and 5.96 at 30 mg/kg and 300 mg/kg, respectively. Sex-based differences were less than 2-fold. These data suggested that LEN present in systemic circulation of maternal rat was distributed to nursing pups via either milk or via placental transfer from maternal systemic circulation.

5. METABOLISM

Metabolism of LEN was studied in vitro and in vivo (also see Section 8). Intact LEN exists as a mixture of atropisomers. Consequently, two distinct peaks were observed for intact LEN in metabolite profiles which are identified as LEN.1 and LEN.2, respectively.

5.1. In Vitro Metabolism

5.1.1. In Vitro Stability of LEN Atropisomers

The relative abundance of LEN atropisomers, LEN.1 and LEN.2, was assessed in vitro in PBS (pH 7.4), and plasma from rat, dog and human (m2.6.5, Section 5, AD-200-2047). The ratios of abundances were similar and remained unchanged during the study duration in PBS and across all examined plasma (Table 16). This indicates that the LEN atropisomer pattern is stable over time and is not influenced by binding proteins or enzymes in plasma. This is consistent with the lack of atropisomer ratio changes in vivo in rat and dog plasma with [^{14}C]LEN (m2.6.5, Section 5, AD-200-2002 and AD-200-2004).

Table 16. Atropisomer Distribution of LEN in Plasma and Buffer

	Atropisomer LEN.1 as a Proportion of Total LEN (Mean ± SD, n = 3)			
	LEN (0.1 µM)		LEN (1.0 µM)	
Matrix	0 hr	24 hr	0 hr	24 hr
Rat plasma	20% ± 2%	18% ± 2%	16% ± 5%	19% ± 1%
Dog plasma	21% ± 1%	18% ± 2%	20% ± 5%	20% ± 2%
Human plasma	20% ± 1%	21% ± 4%	22% ± 5%	23% ± 2%
Phosphate buffered saline	18% ± 6%	15% ± 5%	18% ± 5%	23% ± 3%

Source: AD-200-2047

5.1.2. Rates of Hepatic Metabolism

The rate of metabolism of [^{3}H]LEN (0.25 µM), assessed by appearance of metabolites, was determined in pooled hepatic microsomal fractions in presence of cofactors and hepatocyte suspensions from human and nonclinical species (m2.6.5, Sections 5, AD-200-2022). [^{3}H]LEN showed high metabolic stability across all species in both microsomes and hepatocytes (Table 17). LEN was predicted to have a low hepatic metabolic clearance in human.

Table 17. In Vitro Rates of Metabolism of [^{3}H]LEN in Hepatic Microsomes in the Presence of NADPH and UDPGA and Hepatocytes

	Predicted Hepatic Clearance (L/h/kg)		Predicted Hepatic Extraction (% of liver blood flow)
Species	Microsomes	Hepatocyte	
Sprague-Dawley Rat	0.01	0.02	< 1
Beagle Dog	0.02	0.06	≤ 3
Cynomolgus Monkey	0.04	0.05 ± 0.01	≤ 3
Rhesus Monkey	0.13	0.15 ± 0.03	≤ 6
Human	0.01	0.01 ± 0.00	< 1

Values represent mean value of n = 2 or mean ± standard deviation of n = 3 measurements.
Source: AD-200-2022

5.1.3. Metabolic Pathways

Metabolism of [^{14}C]LEN was assessed in hepatic microsomes in presence of NADPH and UDPGA and Hμrel® hepatocyte co-cultures (m2.6.5, Section 5, 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 (Table 18 and Table 19).

No turnover of [^{14}C]LEN was observed in human microsomes. 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.

Table 18. Metabolism of [^{14}C] LEN in Hepatic Microsomes

Analyte[a]	MH^+ (m/z)	Identity	Percent of Radiochromatogram					
			Wister Han Rat	Beagle Dog	Cynomolgus Monkey	Rhesus Monkey	Human	Control[b]
LEN.1	968.1485	LEN	14.2	14.2	14.4	13.4	14.0	14.4
LEN.2	968.1488	LEN	79.7	79.6	77.2	77.1	80.5	80.3
M4	1271.2198	Hydroxy-desfluoro-LEN-glutathione conjugate-1	ND	ND	1.1	1.8	ND	ND
M8	1271.2202	Hydroxy-desfluoro-LEN-glutathione conjugate-2	ND	ND	0.2	0.3	ND	ND
M9	1275.2321	Dihydro-LEN-glutathione conjugate-1	0.3	ND	ND	ND	ND	ND
M19	984.1431	Oxy-LEN-2	ND	0.3	ND	0.2	ND	ND
M31[c]	906.1672	Impurity	2.2	2.5	2.4	2.4	2.4	2.4
M32[c]	920.1472	Impurity	1.5	1.5	2.0	2.4	1.5	1.2
Total	NA	NA	97.9	98.1	97.2	97.5	98.3	98.3

MH^+ = protonated molecular ion
a LEN.1 and LEN.2 refer to the first-eluting (minor) and second-eluting (major) atropisomers, respectively.
b Control incubations included incubation of [^{14}C]LEN and hepatic microsomes in the absence of cofactors.
c M31 (*不純物9) and M32 (M31 + 14 Da): impurities present in radiolabel solution; likely degradant products formed via photolysis and/or radiolysis of LEN (AD-200-2056)
Source: AD-200-2008, AD-200-2056

Due to the high in vitro metabolic stability of LEN in human systems, the turnover of [^{14}C]LEN was further investigated in Hμrel® hepatic co-cultures from rat, dog, monkey and human with a 7 day incubation period. 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.

Table 19. Metabolism in Hμrel® Hepatic Co-Cultures

Metabolite Designation	MH^+ Mass (m/z)	Proposed Identification	% of Radioactivity at 168 Hour				
			Stromal	Rat	Dog	Monkey	Human
M9	1275.2330	Dihydro-LEN-glutathione conjugate-1	ND	0.64	ND	ND	ND
M10	1275.2358	Dihydro-LEN-glutathione conjugate-2	ND	0.48	ND	ND	ND
M11	1275.2325	Dihydro-LEN-glutathione conjugate-3	ND	1.37	ND	ND	0.29
M13	1144.1831	LEN-glucuronide	ND	0.73	ND	1.46	ND
M16	1147.2084	Unknown	ND	ND	ND	0.85[a]	ND
M29	1100.1923	LEN-pentose conjugate	ND	ND	ND	0.85[a]	ND
M33	1089.1681	Dihydro-LEN-cysteine conjugate	ND	1.69	ND	0.54	0.41
M35	1130.2051	LEN-hexose-conjugate	ND	ND	ND	D	0.41
LEN.1	968.1495	LEN.1	15.7	14.5	16.0	14.5	14.6
LEN.2	968.1491	LEN.2	76.3	72.2	77.5	75.7	77.1

D = detected by mass spectrometry; MH^+ = protonated molecular ion; ND = not detected.
a Metabolites co-eluted.
Source: AD-200-2037

5.2. In Vivo Metabolism

[^{14}C]LEN metabolism was determined following a single IV administration to rat, dog, human and an oral administration to dog. Pooled plasma, bile, and fecal samples obtained following in vivo oral administration of [^{14}C]LEN were profiled and a comprehensive listing of the identified metabolites are provided in m2.6.5 in bile duct intact and BDC rats (m2.6.5, Section 5, AD-200-2002), intact and BDC dogs (m2.6.5, Sections 5, AD-200-2004), and healthy human subjects (m2.7.2, Section 3, GS-US-200-4329). Urine was not profiled because of low radioactivity (< 1 % of dose) across all species.

5.2.1. [^{14}C]LEN Derived Components in Plasma

The components observed in ^{14}C AUC-pooled plasma are summarized in Table 20. The AUC-pooled plasma profile showed that predominant component of the total radioactivity was associated with unchanged LEN (combined LEN.1 and LEN.2 atropisomers). No LEN-derived metabolite was identified in plasma in rat and dog. The LEN.1 to LEN.2 ratios in plasma were similar in rat, dog and remained unchanged in both species. There is no major circulating

metabolite in human. Minor peaks (P9 and P11; each < 10% of ^{14}C AUC) observed in human plasma were also present in dog plasma and in vitro; they are likely degradation products formed via photolysis and/or radiolysis of LEN (m2.6.5, Sections 5, AD-200-2056).

Table 20. Plasma Profiles Following Intravenous Administration of [^{14}C]LEN

Metabolite Designation	% of radioactivity in AUC Pooled Plasma[a]		
	Rat (AUC_{0-7d})[b]	Dog (AUC_{0-3d})[b]	Human (AUC_{0-49d})[c]
LEN.1	11.9	14.0	14.2
LEN.2	87.5	85.3	54.6
Other	none	< 1 (P9), < 1 (P11)[e]	8.7 (P9[e]); 9.3 (P11[e]); < 2 (other[d])
Total	99.4	99.3	100

d = day
a AUC pooled plasma = area under the plasma ^{14}C concentration-time curve
b Radioprofile generated with Topcount ^{14}C detection
c Radioprofile generated with accelerator mass spectrometry ^{14}C detection
d each component < 1% in rat, < 1% in dog, < 2% in human
e P9 = M32; P11 = two coeluting peaks (*不純物8 and *不純物32)
Source: AD-200-2002, AD-200-2004, AD-200-2056 and GS-US-200-4329

5.2.2. [^{14}C]LEN Derived Components in Excreta

The combined results demonstrate that unchanged LEN was the main component in excreta and was cleared as unchanged parent drug in all species. Excretion of appreciable amounts (30.5% to 59.6% of ^{14}C dose) as intact LEN in feces from BDC rat and BDC dog following IV administration suggested significant intestinal secretion of unchanged LEN (Table 21). Metabolism played a lesser role in both species; metabolites included those formed via oxidation, direct glutathione conjugation, oxidation followed by glutathione conjugation products and direct conjugation to pentose, hexose and glucuronide moieties. Low abundance of metabolites was observed in human excreta (m2.7.2, Section 3, GS-US-200-4329).

Following a single IV administration [^{14}C]LEN in rat (3 mg/kg, 172 µCi/kg), unchanged LEN was the major component recovered in feces from both intact and BDC rats accounting for 64.2% and 30.5% of the dose, respectively. Biliary excretion represented approximately 42% of the administered dose recovered in bile. Less than 0.1% of dose represented unchanged LEN in bile. Eighteen metabolites were identified in the bile radioprofile.

Following a single IV administration [^{14}C]LEN in dog (1 mg/kg, 15 µCi/kg), unchanged LEN was the major component in radioactivity recovered in feces from both intact and BDC dogs and accounted for 65.1% and 59.6% of the dose, respectively. Biliary excretion represented approximately 31% of the administered dose in bile through 96 hours postdose. Most of the radioactivity recovered in bile was unchanged LEN and accounted for approximately 21.4% of the dose. LEN-glucuronide (M13) and Oxy-LEN (M19) accounted for 2.4% and 2.6% of the dose in bile, respectively.

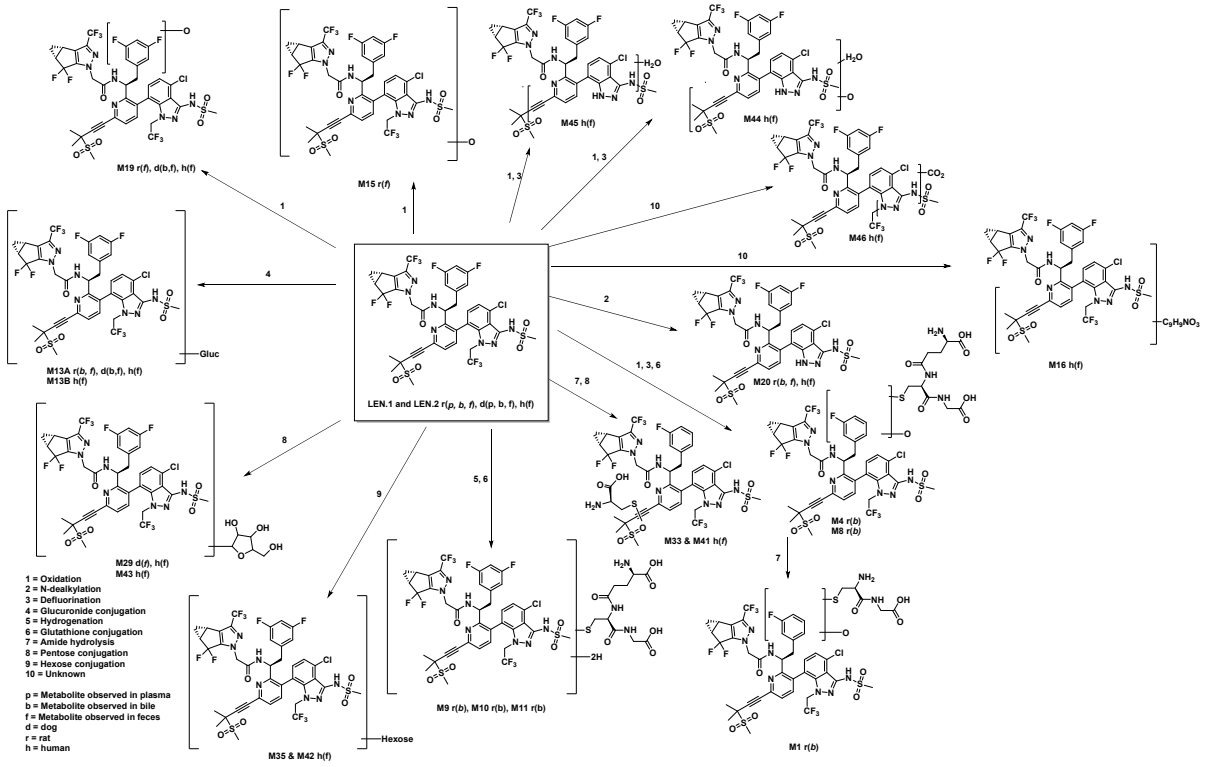
Table 21. Percentage of Radioactive Dose in Pooled Excreta after a Single Intravenous Administration of [^{14}C]LEN

Analyte	MH^+ Mass (m/z)	Identification[b]	% Abundance[a]						
			Intact Rats	BDC Rats		Intact Dogs	BDC Dogs		Human
			Feces	Feces	Bile	Feces	Feces	Bile	Feces[d,e]
LEN	968.1490	LEN.1 and LEN.2	64.2	30.5	0.08	65.1	59.6	21.4	43.4
M1	1142.1797	LEN-cysteine-glycine conjugate	ND	ND	1.7	ND	ND	ND	ND
M4, M8	1271.2218	Hydroxy-desfluoro-LEN-glutathione conjugate.1 and Hydroxy-desfluoro-LEN-glutathione conjugate.2	ND	ND	16.8	ND	ND	ND	ND
M9	1275.2329	Dihydro-LEN-glutathione conjugate-1	ND	ND	3.9	ND	ND	ND	ND
M10	1275.2329	Dihydro-LEN-glutathione-conjugate-2	ND	ND	1.8	ND	ND	ND	ND
M11	1275.2333	Dihydro-LEN-glutathione-conjugate-3	ND	ND	2.0	ND	ND	ND	ND
M13A, M13B	1144.1821	LEN-glucuronide.1 and LEN-glucuronide.2	2.2[c]	ND	5.7	4.2	ND	2.4[c]	4.0[c]
M15	984.1444	Oxy-LEN-1	NC[c]	ND	ND	ND	ND	ND	ND
M19	984.1440	Oxy-LEN-2	1.2	ND	ND	4.8	ND	2.6	ND
M20	886.1456	*N*-destrifluoroethyl-LEN	1.5	ND	0.3	ND	ND	ND	ND
M29, M43	1100.1910	LEN-pentose conjugate.1 and LEN-pentose conjugate.2	ND	ND	ND	1.4	ND	ND	7.9[c]
M26, M27	1130.2051	LEN-hexose-conjugate.1 and LEN-hexose-conjugate.2	ND	ND	ND	D	ND	2.4[c]	8.7

BDC = bile-duct cannulated; D = detected by mass spectrometry; MH^+ = protonated molecular ion; ND = not detected.
a abundance = % administered [^{14}C]LEN dose in bile and feces; % [^{14}C]-radiochromatogram in AUC-pooled plasma. Only metabolites identified with assigned structures and with > 1% of the dose in either feces or bile are listed.
b atropisomer pairs designated as .1 and .2.
c co-eluted with other metabolite(s).
d % excreted dose calculated from total ^{14}C AUC in the collection intervals (spanning 0 to 56 days) that were profiled.
e additional metabolites, each < 1.2%, were observed in human feces.
Source: m2.6.5, Section 5, AD-200-2002 and AD-200-2004; m2.7.2, Section 3, GS-US-200-4329

Following a single oral administration of [^{14}C]LEN (2 mg/kg; 15 µCi/kg) to male beagle dogs, the majority of the radioactivity recovered in feces was associated with unchanged LEN, accounting for 80.8% of the radioactivity. Minimal [^{14}C]LEN metabolism via oxidation (< 1% of dose) was observed.

Figure 14. Proposed Biotransformation Pathways of LEN Following Single Intravenous Administration of [^{14}C]LEN in Rat, Dog and Human



6. EXCRETION

6.1. Excretion of [^{14}C]LEN in Bile Duct Intact and Bile Duct Cannulated Animals

Excretion of [^{14}C]LEN-derived radioactivity was determined following a single IV administration to rat, dog and oral administration to dog. A comprehensive listing of the extent, routes, and rates of excretion are provided in m2.6.5 including intact and BDC WH rats (m2.6.5, Section 7, AD-200-2006); intact and BDC dogs (m2.6.5, Sections 7, AD-200-2007). The cumulative excretion over the duration of the study is summarized in Table 22. 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 (Section 5.2.2; Table 21). These results demonstrate that both intestinal excretion and biliary elimination are major disposition pathways in rat and dog while renal excretion was minor disposition pathway for [^{14}C]LEN (< 1.0% of dose).

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 (Section 3, GS-US-200-4329).

Table 22. Cumulative Dose Recovery Following a Single Dose of [^{14}C]LEN to Rat and Dog

Group	Route of Administration	Collection Time (h)	Cumulative Recovery of Radioactivity (% Dose)			
			Urine	Feces	Bile	Total[a]
Intact Rats	IV	0–24	0.05 ± 0.02	12.6 ± 2.6	NA	NA
		0–168	0.13 ± 0.04	79.6 ± 5.3	NA	NA
		0–816	0.16 ± 0.04	94.4 ± 0.5	NA	94.8 ± 0.4
BDC Rats	IV	0–24	0.05 ± 0.03	7.5 ± 4.4	13.5 ± 4.0	NA
		0–72	0.10 ± 0.03	23.0 ± 5.7	28.4 ± 4.4	NA
		0–168	0.15 ± 0.03	35.2 ± 2.9	41.7 ± 2.8	91.7 ± 1.7
Intact Dogs	IV	0–24	0.09 ± 0.02	0.06 ± 0.11	NA	NA
		0–168	0.33 ± 0.08	85.7 ± 5.0	NA	NA
		0–792	0.42 ± 0.15	86.3 ± 4.6	NA	87.3 ± 4.1
BDC Dogs	IV	0–24	0.25 ± 0.11	14.9 ± 12.8	21.4 ± 9.1	NA
		0–168	0.53 ± 0.27	62.8 ± 7.1	32.2 ± 10.0	NA
		0–336	0.54 ± 0.29	63.0 ± 7.1	32.4 ± 10.0	96.0 ± 2.6
Intact Dogs	Oral	0–24	0.05 ± 0.02	25.1 ± 43.5	NA	NA
		0–72	0.13 ± 0.02	82.7 ± 9.4	NA	NA
		0–624	0.22 ± 0.08	87.7 ± 6.4	NA	88.7 ± 5.8

NA = not applicable.
a Total recovery includes radioactivity in excreta carcass, cage rinses, cage wash, cage wipe, cage debris, bile cannula rinse, and jacket rinse.
Values represent the mean ± SD obtained from 3 animals.
Source: m2.6.5, Section 7, AD-200-2006 and AD-200-2007

6.2. Excretion and PK of LEN Following IV Infusion in Bile Duct Intact and Bile Duct Cannulated Rats without or with Elacridar Pretreatment

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 (m2.6.5, Section 5, AD-200-2002). LEN in solution was administered by a 30-min IV infusion to male bile-duct intact WH rats at 1.0 mg/kg or BDC WH rats at 10.0 mg/kg without or with pretreatment of elacridar, a P-gp and BCRP inhibitor (m2.6.5, Section 7, AD-200-2045 and AD-200-2046). The combined data (Table 23) 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 is a substrate for human P-gp, but not BCRP (Table 25). Collectively, these data strongly suggest that intestinal secretion of LEN by P-gp is the primary mechanism for fecal excretion and a significant overall clearance mechanism of unchanged drug in rats.

Table 23. Excretion and Pharmacokinetic Parameters of LEN Following Intravenous Administration of LEN without or with Elacridar Pretreatment in Bile Duct Intact or Bile Duct Cannulated Rats (mean ± SD, n = 3, 4 or 6)

	Intact Rats at 1.0 mg/kg (n = 3 or 4)				BDC Rats at 10.0 mg/kg (n = 4 or 6)			
	Without Elacridar Pretreatment[a]		With Elacridar Pretreatment[a,b]		Without Elacridar Pretreatment[a]		With Elacridar Pretreatment[a,b]	
PK Parameter	Mean	SD	Mean	SD	Mean	SD	Mean	SD
AUC_{inf} (μM•h)	11.30	1.37	20.20	2.92	227	34	381	73
C_{max} (μM)	0.804	0.114	0.644	0.139	8.93	1.71	13.4	15.0
MRT (h)	71.4	21.6	90.5	14.2	40.1	5.5	52.7	3.4
$t_{1/2}$ (h)	51.2	21.2	58.1	10.1	61.2	5.7	47.0	8.9
V_{ss} (L/kg)	6.45	1.50	4.67	0.72	1.83	0.40	1.44	0.24
CL (L/h/kg)	0.09	0.01	0.05	0.01	0.046	0.000	0.028	0.005
% excretion as LEN in bile	NA	NA	NA	NA	0.2	0.1	0.2	0.1
% excretion as LEN in urine	NA	NA	NA	NA	BLQ	NC	BLQ	NC
% excretion as LEN in feces	21.9	1.1	7.4	1.2	42.0	5.3	19.6	2.5

BLQ: below the limit of quantitation (< 0.1% of dose); NA = not applicable; NC: not calculated
a Formulation contained w/w 68.1% PEG 300, 0.3% 1N NaOH and 31.6% water (pH 8)
b Animals received oral doses of elacridar twice daily (approximately 12 hours apart) for 4 days (total of 7 doses). The first elacridar dose was administered approximately 15 minutes prior to the LEN infusion dose on Day 1. Elacridar was formulated as a solution in 10% N-methyl-2-pyrrolidone, 20% PEG 300 and 70% water.
Source: m2.6.5, Section 7, AD-200-2045 and AD-200-2046

7. PK DRUG INTERACTIONS

Discussions of DDI liability are made by reference to current industry, United States, European and Japan regulatory guidelines{Bjornsson 2003, European Medicines Agency 2012, Giacomini 2010, Pharmaceuticals and Medical Devices Agency (PMDA) 2019, U. S. Department of Health & Human Services (DHHS) 2020}. A comprehensive summary of guidance calculated results are provided (m2.6.5, Section 8, AD-200-2055). DDI assessments are placed in context with the observed LEN clinical concentrations. In clinical studies, LEN $t_{1/2}$ ranges from 10 to 13 days following single dose oral administration (m2.7.2, Section 3, GS-US-200-4071). The LEN clinical regimen for use in people with HIV with multidrug resistance entailed a 2 week oral lead-in regimen of 600 mg on Days 1 and 2, 300 mg on Day 8, followed by a SC maintenance dose of 927 mg at 309 mg/mL, to be administered once every 6 months starting from Day 15. The clinical LEN C_{max} value was ~ 136 ng/mL (140 nM) following a single SC administration at 927 mg with oral lead-in at 600 mg on Days 1 and 2 (m2.7.2, Section 3, GS-US-200-4538 and GS-US-200-4071). A C_{max} value at 140 nM, a fraction of dose absorbed (F_a) at 6.24%, an absorption rate constant (k_a) at 0.0287 h^{-1}, the maximum equilibrium solubility in simulated intestinal fluid under fasted state at 3.9 µg/mL and under fed state at 4.1 µg/mL (m3, Section 3.2.S.1.3) were used to assess the intestinal and hepatic DDI risk.

7.1. Enzymology of Metabolism

7.1.1. CYP Reaction Phenotyping

Due to the extremely high metabolic stability of LEN in in vitro systems, standard approaches to study reaction phenotyping are very challenging. In order to evaluate the human CYP enzymes responsible for CYP-mediated metabolism of LEN, high specific activity [^{3}H]LEN was utilized. Metabolites were separated with gradient reverse phase chromatography. Appearance of metabolite formation was measured with a radioflow detector. The human CYP isoforms responsible for CYP-mediated metabolism of [^{3}H]LEN (2 µM) were assessed using 8 individual recombinant expressed CYP450 enzyme preparations co-expressed with human NADPH CYP reductase (m2.6.5, Section 6, AD-200-2030). There was no detectable metabolism of [^{3}H]LEN by CYPs 1A2, 2B6, 2C8, 2C9, 2C19, 2D6 or 3A4. 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 metabolism by hepatic microsomal fraction from a CYP3A5 *3/*3 donor compared to a CYP3A5 *1/*1 donor (m2.6.5, Section 6, AD-200-2054). These in vitro findings were consistent with the results from a clinical DDI study in healthy subjects where co-administration of oral LEN with VORI, a strong CYP3A inhibitor, resulted in an approximately 1.4-fold increase in LEN AUC_{inf} and no change in C_{max}. These data indicate that LEN is a substrate of CYP3A. Clinical data indicate LEN AUC_{inf} and C_{max} were 84% and 55% lower, respectively, upon co-administration with rifampin, a prototypical strong inducer of multiple enzymes and transporters (m2.7.2, Section 3, GS-US-200-4333).

7.1.2. UGT Phenotyping

A panel of human UGT enzymes was tested for their potential to metabolize LEN (m2.6.5, Section 6, AD-200-2033). 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. 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 (m2.7.2, Section 2.5, GS-US-200-4333).

7.2. Cytochrome P450 and UGT1A1 Inhibition

The potential for LEN to reversibly inhibit major human drug metabolizing CYP enzymes and UGT1A1 was determined in human hepatic microsomal fractions with known substrates of individual enzymes and results are summarized in Table 24 (m2.6.5, Section 6, AD-200-2028 and AD-200-2031). The potential for LEN to be a time-dependent inhibitor of CYP3A was characterized (m2.6.5, Section 6, AD-200-2050)

At concentrations up to 25 µM, LEN 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). Clinically relevant interactions with substrates of these enzymes are unlikely. LEN 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. 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 $min^{-1}µM^{-1}$ indicates that LEN is a relatively weak mechanism-based inhibitor. The guidance calculated minimum AUC ratios (AUCR) were 5.0 for LEN following oral administration and 2.75 for LEN following SC administration (m2.6.5, Section 8, AD-200-2055). In a clinical study, AUC and C_{max} of MDZ, a probe substrate for CYP3A, were approximately 3.6- and 1.9- fold higher, respectively and 1-OH-MDZ AUC and C_{max} were correspondingly lower following co-administration with LEN, suggesting LEN was a moderate inhibitor of CYP3A (m2.7.2, Section 3, GS-US-200-4333).

Table 24. CYP Inhibition Potential of LEN

Enzyme	Activity	% inhibition at 25 µM LEN	LEN IC_{50} (µM)
CYP1A2	Phenacetin O-deethylase	0%	> 25
CYP2B6	Bupropion 4-hydroxylase	0%	> 25
CYP2C8	Paclitaxel 6α-hydroxylase	24%	> 25
CYP2C9	Tolbutamide 4-hydroxylase	0%	> 25
CYP2C19	S-Mephenytoin 4'-hydroxylase	9%	> 25
CYP2D6	Dextromethorphan O-demethylase	0%	> 25
CYP3A	Midazolam 1'-hydroxylase	66%	5.4
CYP3A	Testosterone 6β-hydroxylase	0%	> 25

CYP = cytochrome P450; IC_{50} = concentration resulting in 50% inhibition
Values are the mean from 7 independent measurements
Source: AD-200-2028

The potential for LEN (0 – 50 µM) to inhibit UGT1A1 was determined in recombinant expressed human UGT1A1 (Supersomes™) with ethyl estradiol as the probe substrate (m2.6.5, section 6, AD-200-2031). LEN inhibited human UGT1A1 with an IC_{50} of 3.2 µM. The human plasma concentration of LEN is low (0.112 µM); SC or PO administration of LEN is unlikely to cause marked drug interactions through inhibition of this enzyme based on the regulatory guideline assessment (m2.6.5. Section 8, AD-200-2055).

7.3. Assessment of Induction Liability

The potential for LEN (0 - 50 µM) to induce human drug metabolizing enzymes through the transactivation of xenobiotic receptors AhR, CAR and PXR was assessed in reporter cell lines (m2.6.5, Section 6, AD-200-2029). There was no activation of AhR or CAR (< 2-fold activation) at the highest concentration tested (50 µM). LEN 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 (m2.6.5, Section 6, 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 DMSO vehicle 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 (m2.7.2, Section 3, 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 with maximum FOC 2.38-fold and 2.48-fold, respectively; the relevance of this isolated change is unknown. The guidance calculated LEN AUCR values indicated LEN would not be classified as an inducer following either SC or oral administration (m2.6.5. Section 8, AD-200-2055). The potential for LEN to cause clinically relevant drug interactions through induction is low.

7.4. Interactions with Transporters

The potential for LEN (0.1 µM) to be a substrate of human efflux and uptake transporters was determined in transfected cellular systems (m2.6.5, Section 8, AD-200-2005 and AD-200-2025) and the results are summarized in Table 25 and Table 26. LEN is a substrate for human P-gp, but not BCRP. These results are consistent with efflux transport observed in Caco-2 cells (Section 3.1). LEN is 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 VORI, indicate that LEN is also a substrate of P-gp (m2.7.2, Section 3, GS-US-200-4333).

Lenacapavir (0.025 µM) uptake into cryopreserved human hepatocytes at 37º C was not inhibited by rifamycin (100 µM), further confirming that LEN was not a substrate of hepatic uptake transporters (m2.6.5, Section 8, AD-200-2027).

Table 25. Permeability of LEN in P-gp- or BCRP-Overexpressing MDCKII Cells

P_{app} ($\times 10^{-6}$ cm/sec) of LEN	P-gp-overexpressing MDCKII		BCRP-overexpressing MDCKII	
	- inhibitor	+ inhibitor[a]	- inhibitor	+ inhibitor[a]
Forward (A to B)	0.25	4.69	0.73	0.80
Reverse (B to A)	8.85	5.18	9.06	10.2
Efflux Ratio	35	1.1	12	13

BCRP = breast cancer resistance protein; Efflux Ratio = P_{app} (Reverse)/P_{app} (Forward); MDCKII = Madin-Darby canine kidney cell line; P_{app} = apparent permeability.
a Control inhibitor: P-gp, valspodar (1 µM); BCRP, Ko134 (0.5 µM).
Source: AD-200-2005

Table 26. Uptake Rates for LEN in Non-transfected Wild Type CHO Cells and CHO Cells Transfected with OATP1B1 and OATP1B3

	Uptake Rate (pmole/minute/1.0x10^6 cells)		
	CHO	CHO-OATP1B1	CHO-OATP1B3
- Inhibitor	8.4	9.8	11
+ Inhibitor[a]	9.7	10.0	14

a Rifampicin (40 µM) was used as an OATP inhibitor. Atorvastatin (0.1 µM), a known OATP substrate, was used a positive control and antipyrine (10 µM) was used as the lack of uptake of the passive permeability control.
CHO = Chinese hamster ovary; OATP = organic anion-transporting polypeptide
Source: AD-200-2025

The potential for LEN to inhibit human drug transporters was determined in cell lines transfected with individual transporters (m2.6.5, Section 8, AD-200-2026 and AD-200-2035) and the results are summarized in Table 27. LEN did not inhibit P-gp or BCRP-mediated transport at the highest concentration tested (1 µM). LEN did not inhibit OAT1-, OAT3-, OCT1-, OCT2-, and MATE2-K-mediated transport when tested up to 10 µM. LEN 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. The guidance calculated results indicated no interaction with MATE1 (m2.6.5. Section 8, AD-200-2055). No clinically relevant changes in glomerular filtration rate or creatinine clearance were observed in clinical studies (m2.7.2, Section 3, GS-US-200-4625). LEN showed dose dependent inhibition of OATP1B1 and OATP1B3 mediated uptake with IC_{50} values of 0.021 and 0.049 µM, respectively. The guidance calculated results indicated little or no interaction with either OATP1B1 or OATP1B3 (AD-200-2055). 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 (m2.7.2, Section 3, GS-US-200-4333).

To support toxicology studies of LEN in dogs, the effect of LEN (0.014 - 10 µM) on canine Bsep was also determined *in vitro* in membrane vesicles (m2.6.5, Section 8, AD-200-2053). LEN showed a concentration dependent inhibition of dog Bsep with an IC_{50} value of 0.12 µM. These data suggest that LEN can inhibit dog Bsep at the concentrations observed in safety evaluation studies (m2.6.7, Sections 2, TX-200-2017 and TX-200-2047).

Table 27. Inhibition Potential of Human Transporters by LEN

Transporter	Maximum Inhibition (Highest LEN Concentration Tested)	IC_{50} (µM)
P-gp	NIO (1.0 µM)	>1.0
BCRP	NIO (1.0 µM)	>1.0
OATP1B1	94% (2.8 µM)	0.021
OATP1B3	91% (2.8 µM)	0.049
OAT1	NIO (10 µM)	>10
OAT3	NIO (10 µM)	>10
OCT1	NIO (10 µM)	>10
OCT2	NIO (10 µM)	>10
MATE1	56% (10 µM)	2.39
MATE2K	NIO (10 µM)	>10
BSEP	75% (10 µM)	1.21

NIO = no inhibition observed.
Source: m2.6.5, Section 8, AD-200-2026 and AD-200-2035

8. OTHER STUDIES

None.

9. DISCUSSION AND CONCLUSIONS

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.

Although the oral absorption of LEN is low, the absorption is sufficient to support oral dosing.

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 which is consistent with the high metabolic stability.

Lenacapavir is cleared by metabolism, excretion into bile and intestinal secretion by P-gp, providing multiple pathways for elimination. This was supported by the modest interaction with CYP3A and P-gp inhibitors in the clinical DDI study.

Lenacapavir has a favorable DDI profile as a perpetrator. CYP3A inhibition was noted in vitro and moderate inhibition noted in the clinical DDI study. Regulatory assessment of other drug metabolizing enzymes and transporters do not require further assessment.

The most potent inhibition of transporters in vitro by LEN was with OATPs but no in vivo DDI was noted in the clinic.

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SECTION 2.6.5—PHARMACOKINETICS TABULATED SUMMARY

LENACAPAVIR

Gilead Sciences Inc.,

[redacted] **20**[redacted]

CONFIDENTIAL AND PROPRIETARY INFORMATION

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LIST OF ABBREVIATIONS

BCRP	breast cancer resistance protein
BSEP	bile salt export pump
CYP	cytochrome P450 enzyme
Gilead	Gilead Sciences/Gilead Sciences, Inc.
IM	intramuscular
IV	intravenous
LEN	Lenacapavir
m	male
PBS	Phosphate buffered saline
PEG	polyethylene glycol
PG	propylene glycol
P-gp	P-glycoprotein
PXR	pregnane X receptor
SC	subcutaneous
TPGS	Tocopheryl polyethylene glycol 1000 succinate
UDP	uridine diphosphate
UGT1A1	uridine diphosphate glucuronosyltransferase 1A1

1. PHARMACOKINETICS: OVERVIEW

Test Article: LEN

Type of Study/Description	GLP[a]	Test System	Method of Administration	Testing Facility	Gilead Study No. (CRO Study No.)
Analytical Methods and Validation					
Validation of a method for the determination of LEN in mouse plasma by HPLC with MS/MS Detection	Yes	NA	In Vitro	[redacted]	BA-200-2012 (8410258)
Validation of a method for the determination of LEN in rat plasma by HPLC with MS/MS Detection	Yes	NA	In Vitro	[redacted]	BA-200-2002 (8353633)
Validation of a method for the determination of LEN in rabbit plasma by HPLC with MS/MS Detection	Yes	NA	In Vitro	[redacted]	BA-200-2008 (8388918)
Validation of a method for the determination of LEN in dog plasma by HPLC with MS/MS Detection	Yes	NA	In Vitro	[redacted]	BA-200-2003 (8353634)
Absorption After a Single Dose					
Permeability across Caco-2 cell monolayers	No	Caco-2 cells	In Vitro	Gilead Sciences, Inc. Foster City, CA	AD-200-2009
Pharmacokinetics of LEN in male Sprague-Dawley rats	No	Rat	IV; PO	[redacted]	AD-200-2010 (8327395 & 8327396)
Pharmacokinetics of LEN in male beagle dogs	No	Dog	IV; PO	[redacted]	AD-200-2011 (15GILE6R1S10 & 8327428)
Pharmacokinetics of LEN in male cynomolgus monkeys	No	Cynomolgus monkey	IV; PO	[redacted]	AD-200-2012 (8330175 & B880-15-Leg H)
Pharmacokinetics of LEN in male rhesus monkeys	No	Rhesus monkey	IV; PO	[redacted]	AD-200-2013 (8334447, 8334428 & 8339402)

Test Article: LEN

Type of Study/Description	GLP[a]	Test System	Method of Administration	Testing Facility	Gilead Study No. (CRO Study No.)
Pharmacokinetics of LEN following subcutaneous administration of LEN suspension formulated with 2% Poloxamer 188 in normal saline in male Wistar Han rats	No	Rat	SC	[redacted]	AD-200-2014 (BG-1371-DA-RI & BG-1347-DA-RI)
Pharmacokinetics of LEN following a single subcutaneous administration of LEN solution formulated with 77:10:13 w/w/w PEG200:ethanol:water in male Wistar Han rats	No	Rat	SC	[redacted]	AD-200-2038 (BG-1600-DA-RI, BG-1615-DA-RI, BG-1678-DA-RI, BG-1696-DA-RI and BG-1753-DA-RI)
Pharmacokinetics of LEN following subcutaneous and intramuscular administration of LEN suspension formulated with 2% Poloxamer 188 in normal saline in male beagle dogs	No	Dog	SC; IM	[redacted]	AD-200-2015 (15GILE6R1S34, 15GILE6R1S35, 8358844, BG-1362-DA-DI, BG1348-DA-DI and BG-1276-DA-DI)
Pharmacokinetics of LEN following a single subcutaneous administration of LEN solution formulated with 77:10:13 w/w/w PEG200: ethanol: water in male beagle dogs	No	Dog	SC	[redacted]	AD-200-2039 (15GILEP6R1S37, 8368197, BG-1529-DA-DI and BG-1601-DA-DI)
Pharmacokinetics of LEN following subcutaneous administration of LEN in solution in male beagle dogs	No	Dog	SC	[redacted]	AD-200-2051 (BG-2167-DA-DI)
Pharmacokinetics of LEN following single oral doses in male Wistar Han rats	No	Rat	PO	[redacted]	AD-200-2018 (8368167)
Pharmacokinetics of LEN following single oral administration in various formulations in male beagle dogs	No	Dog	PO	[redacted]	AD-200-2019 (BG-1458-DA-DI and BG-1570-DA-DI)

Test Article: LEN

Type of Study/Description	GLP[a]	Test System	Method of Administration	Testing Facility	Gilead Study No. (CRO Study No.)
Pharmacokinetics of LEN following single oral administration in liquid filled capsules to male beagle dogs	No	Dog	PO	[redacted]	AD-200-2036 (BG-1642-DA-DI, 15GILEP6R1S40, 15GILEP6R1S42 and 8376024abb)
Pharmacokinetics of LEN following single oral administration as tablets to male beagle dogs	No	Dog	PO	[redacted]	AD-200-2042 (BG-2472-DA-DI and BG-2607-DA-DI)
Pharmacokinetics of LEN following intravenous or oral administration to portal vein cannulated beagle dogs	No	PVC dog	PO	[redacted]	AD-200-2016 (8356608)
Pharmacokinetics of LEN following intravenous or oral administration to portal vein cannulated cynomolgus monkeys	No	PVC cynomolgus monkey	PO	[redacted]	AD-200-2017 (8356610)
Distribution					
Plasma protein binding	No	Plasma (Rat, Dog, Monkey and Human); Cell culture medium	In Vitro	[redacted]; Gilead Sciences, Inc. Foster City, CA	AD-200-2020 (60D-1595; 60D-15120 & EXP-15-AB6489)
Plasma protein binding	No	Plasma (Mouse)	In Vitro	[redacted]; Gilead Sciences, Inc. Foster City, CA	AD-200-2044 (60D-2009)
Microsomal binding	No	Human liver microsomes	In Vitro	Gilead Sciences, Inc. Foster City, CA	AD-200-2032
Binding to human serum albumin and α_1-acid glycoprotein	No	Human serum albumin and α_1-acid glycoprotein	In Vitro	Gilead Sciences, Inc. Foster City, CA	AD-200-2048
Blood to plasma ratio	No	Whole Blood (Rat, Dog, Monkey and Human)	In Vitro	[redacted] UK	AD-200-2021 (174-R384)

Test Article: LEN

Type of Study/Description	GLP[a]	Test System	Method of Administration	Testing Facility	Gilead Study No. (CRO Study No.)
Tissue distribution and excretion of [^{14}C] radioactivity	No	Intact and BDC Wistar Han rats; Intact Long Evans rats	IV	[redacted]	AD-200-2006 (8356497)
Metabolism					
In vitro stability of LEN atropisomers in PBS and in plasma from human and nonclinical species	No	Plasma (Rat, Dog and Human); PBS	In Vitro	Gilead Sciences, Inc. Foster City, CA	AD-200-2047 (EXP-20-BG4392)
[^{3}H] Stability in hepatic microsomal fractions and hepatocytes	No	Pooled hepatic microsomes (Rat, Dog, Monkey and Human); Cryopreserved hepatocytes (Rat, Dog, Monkey and Human)	In Vitro	Gilead Sciences, Inc. Foster City, CA	AD-200-2022
In vitro metabolism of [^{14}C]LEN in rat, dog, monkey, and human hepatic microsomes	No	Pooled hepatic microsomes (Rat, Dog, Monkey and Human)	In Vitro	[redacted]	AD-200-2008 (8367522)
In vitro metabolic stability of [^{14}C]LEN in rat, dog, monkey and human using Hμrel system	No	Hμrel® hepatic co-cultures (Rat, Dog, Monkey and Human)	In Vitro	[redacted]	AD-200-2037 (8389160)
[^{14}C] Metabolite profiling and identification in rat plasma, urine, bile and feces	No	Rat	IV	[redacted]	AD-200-2002 (8346944)
[^{14}C] Metabolite profiling and identification in dog plasma, urine, bile and feces	No	Dog	IV; PO	[redacted]	AD-200-2004 (8346949)
Identification of lenacapavir late-eluting peaks in human plasma and nonclinical samples	No	Rat, dog and human	IV	[redacted]	AD-200-2056 (8465299)

Test Article: LEN

Type of Study/Description	GLP[a]	Test System	Method of Administration	Testing Facility	Gilead Study No. (CRO Study No.)
Excretion					
Pharmacokinetics of LEN following intravenous infusion to male bile duct cannulated rats	No	BDC rat	IV	[redacted]	AD-200-2024 (8332771)
Excretion and Pharmacokinetics of LEN following IV infusion in rats pretreated without and with elacridar pretreatment	No	Rat	IV	[redacted]	AD-200-2045 (8390236 & 8403597)
Excretion and Pharmacokinetics of LEN following IV infusion in BDC rats pretreated without and with elacridar	No	BDC Rat	IV	[redacted]	AD-200-2046 (8407052)
Pharmacokinetics, distribution, and excretion of [^{14}C]LEN following intravenous administration to Dogs	No	Bile Duct Intact and BDC dog	IV; PO	[redacted]	AD-200-2007 (8359730)
Pharmacokinetic Drug Interactions					
In vitro assessment of human CYP inhibition potential of LEN	No	Human liver microsomes	In Vitro	[redacted] UK; Gilead Sciences, Inc., Foster City, CA	AD-200-2028 (174-R361, 174-R384 & 174-R389)
Human CYP3A mechanism-based inhibition kinetics of LEN	No	Human liver microsomes	In Vitro	Gilead Sciences, Inc. Foster City, CA	AD-200-2050 (EXP-21-BA7659 & EXP-21-BA7660)
UGT1A1 inhibition potential of LEN	No	Human liver microsomes	In Vitro	[redacted] UK	AD-200-2031 (174-R361)
CYP phenotyping of LEN	No	Recombinant human CYP enzymes	In Vitro	Gilead Sciences, Inc. Foster City, CA	AD-200-2030
Further analysis of CYP metabolic reaction phenotyping of LEN	No	Recombinant human CYP enzymes	In Vitro	Gilead Sciences, Inc. Foster City, CA	AD-200-2054
UGT phenotyping of LEN	No	Recombinant human UGT enzymes	In Vitro	Gilead Sciences, Inc. Foster City, CA	AD-200-2033

Test Article: LEN

Type of Study/Description	GLP[a]	Test System	Method of Administration	Testing Facility	Gilead Study No. (CRO Study No.)
In vitro assessment of the induction potential of LEN	No	Reporter cell-lines	In Vitro	[redacted]	AD-200-2029 (GIL-2015-125 and GIL-2016-109)
In vitro characterization of LEN for CYP induction potential using sandwich-cultured transporter-certified™ hepatocytes	No	Human hepatocytes	In Vitro	[redacted]	AD-200-2049 (2004IND-GILD-005)
Bi-directional permeability of LEN through monolayers of P-gp and BCRP over-expressing cells	No	Transfected MDCKII cells	In Vitro	[redacted]	AD-200-2005
In vitro assessment of LEN as substrate for human OATP1B1 and OATP1B3	No	Transfected CHO cells	In Vitro	Gilead Sciences, Inc. Foster City, CA	AD-200-2025
LEN uptake in human hepatocytes	No	Cryopreserved human hepatocytes	In Vitro	Gilead Sciences, Inc. Foster City, CA	AD-200-2027
Inhibition of human P-gp, BCRP, OATP1B1 and OATP1B3 by LEN	No	Transfected MDCKII and CHO cells	In Vitro	Gilead Sciences, Inc. Foster City, CA	AD-200-2026
Inhibition of human OAT1, OAT3, OCT1, OCT2, MATE1, MATE2K, and BSEP Transporters by LEN	No	Transfected cell lines	In Vitro	[redacted] Hungary	AD-200-2035
In vitro interaction of LEN with human and dog BSEP transporter	No	Transfected cell lines	In Vitro	[redacted] Hungary	AD-200-2053
Pharmacokinetic drug-drug interaction liability assessment for LEN	No	NA	In Vitro	Gilead Sciences, Inc. Foster City, CA	AD-200-2055
Other Pharmacokinetic Studies: None					

BCRP = breast cancer resistance protein; BDC = bile duct-cannulated; Caco-2 = human colon carcinoma cell line; CHO = Chinese hamster ovary; CRO = contract research organization; CsA = cyclosporine A; CYP = cytochrome P 450 enzyme; Gilead = Gilead Sciences; GLP = Good Laboratory Practice; IV = intravenous; IM = intramuscular; MDCKII = Madin-Darby canine kidney cell line; MRP = multidrug resistance-associated protein; NA = not applicable; OATP = organic anion-transporting polypeptide; P-gp = P-glycoprotein; PO = Oral gavage; PBS = phosphate buffered saline; PVC = portal vein cannulated; SC = subcutaneous; UDP = uridine diphosphate; UGT = UDP glucuronosyl transferase

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

2. PHARMACOKINETICS: ANALYTICAL METHODS AND VALIDATION REPORTS

Test Article: LEN

Type of Study:	**Analytical Method Validation in Rat and Dog Plasma**		**Location in CTD: 4.2.2.1**
Study No.	**Matrix**	**Analyte**	**Analytical Method**
BA-200-2012	Mouse plasma	LEN	LC-MS/MS
BA-200-2002, Final Report and Addendum	Rat Plasma	LEN	LC-MS/MS
BA-200-2008, Final Report and Addendum	Rabbit plasma	LEN	LC-MS/MS
BA-200-2003, Final Report and Addendum	Dog Plasma	LEN	LC-MS/MS

LC-MS/MS = high performance liquid chromatography coupled to tandem mass spectrometry

3. PHARMACOKINETICS: ABSORPTION AFTER A SINGLE DOSE

3.1. AD-200-2009: In Vitro Membrane Permeability of LEN

Test Article: LEN

Report Title	Study Type	Test Article	Report Number
Bi-directional Permeability of LEN through Caco-2 Cell Monolayers	**Absorption Study (in vitro)**	**LEN**	**AD-200-2009**
Study System	Caco-2 cell monolayers		
Method	The bi-directional permeability of LEN through monolayers of Caco-2 cells in 24-well transwell plates was determined at 1 µM of LEN.		

Incubation Concentration (µM)	P_{app} (× 10^{-6} cm/sec)		Efflux Ratio
	Forward (A→B) Direction	Reverse (B→A) Direction	
1.0	0.3	2.5	7.8

A = apical; B = basolateral; Caco-2 = human colon carcinoma cell line; P_{app} = apparent permeability coefficient
Experiment done with duplicate wells and values reported were the mean of two wells.
Assay control compounds: atenolol ($P_{app} < 1.0 \times 10^{-6}$ cm/s), propranolol ($P_{app} \geq 6 \times 10^{-6}$ cm/s), and vinblastine (efflux ratio ≥ 10)

3.2. AD-200-2010: Pharmacokinetics of LEN in Solution Following Intravenous and Oral Administration in Rats

Test Article: LEN

Report Title	Study Type	Test Article	Report Number
Pharmacokinetics of LEN in Male Sprague-Dawley Rats	**Single-Dose Pharmacokinetics**	**LEN**	**AD-200-2010**
Species/Strain	Rat/Sprague-Dawley		Rat/Sprague-Dawley
Sex/ No. of Animals per group	Male/3		Male/3
Feeding Condition	Fasted		Fasted
Sample	Plasma		Plasma
Analyte	LEN		LEN
Assay	LC-MS/MS		LC-MS/MS
Method of Administration	30-minute intravenous infusion		Oral gavage
Dose (mg/kg)	1.0		5.0
Vehicle/Formulation	5% ethanol, 20% PG, 45% PEG 300 and 30% 10 mM phosphate buffer		5% ethanol, 20% PG, 45% PEG 300 and 30% 0.01 N hydrochloric acid
PK Parameters			
T_{max} (h)	0.48 ± 0.00		10.0 ± 3.5
C_{max} (nM)	1783 ± 238		336 ± 116
MRT (h)	48.8 ± 9.6		NA
$t_{1/2}$ (h)	38.1 ± 7.9		ND
$AUC_{0\text{-}72h}$ (nM•h)	17533 ± 3182		13467 ± 3927
AUC_{inf} (nM•h)	22867 ± 2011		25200 ± 10221
CL (L/h/kg)	0.045 ± 0.004		NA
V_{ss} (L/kg)	2.22 ± 0.56		NA
F (%)[a]	NA		21.7 ± 8.9

$AUC_{0\text{-}72h}$ = area under the plasma concentration-time curve from zero to 72 h; AUC_{inf} = area under the plasma concentration-time curve extrapolated to time infinity; CL = plasma clearance; C_{max} = maximum plasma concentration; Fasted = animals were fasted overnight prior to dose administration and up to 4 hours after dosing; LC-MS/MS = high performance liquid chromatography coupled to tandem mass spectrometry; MRT = mean residence time; NA = not applicable; ND = not determined; PEG = polyethylene glycol; PG = propylene glycol; PK = pharmacokinetic; $t_{1/2}$ = estimated plasma elimination half-life; T_{max} = time to reach the maximum plasma concentration; V_{ss} = volume of distribution at steady state

a Calculated based on $AUC_{0\text{-}72h}$.

3.3. AD-200-2011: Pharmacokinetics of LEN in Solution Following Intravenous and Oral Administration in Dogs

Test Article: LEN

Report Title	Study Type	Test Article	Report Number
Pharmacokinetics of LEN in Male Beagle Dogs	**Single-Dose Pharmacokinetics**	**LEN**	**AD-200-2011**
Species/Strain	Dog/Beagle		Dog/Beagle
Sex/ No. of Animals per group	Male/3		Male/3
Feeding Condition	Fasted		Fasted
Sample	Plasma		Plasma
Analyte	LEN		LEN
Assay	LC-MS/MS		LC-MS/MS
Method of Administration	30-minute intravenous infusion		Oral gavage
Vehicle/Formulation	5% ethanol, 20% PG, 45% PEG 300 and 30% 10 mM phosphate buffer		5% ethanol, 20% PG, 45% PEG 300 and 30% 0.01 N hydrochloric acid
Dose (mg/kg)	1.0		4.0
PK Parameters			
T_{max} (h)	0.48 ± 0.00		10.7 ± 11.5
C_{max} (nM)	2053 ± 641		471 ± 203
MRT (h)	34.7 ± 27.8		NA
$t_{1/2}$ (h)	30.2 ± 24.4		ND
$AUC_{0\text{-}72h}$ (nM•h)	13467 ± 2281		11410 ± 4324
AUC_{inf} (nM•h)	16667 ± 6296		16533 ± 10366
CL (L/h/kg)	0.070 ± 0.025		NA
V_{ss} (L/kg)	1.96 ± 0.91		NA
F (%)[a]	NA		22.1 ± 8.4

$AUC_{0\text{-}72h}$ = area under the plasma concentration-time curve from zero to 72 h; AUC_{inf} = area under the plasma concentration-time curve extrapolated to time infinity; CL = plasma clearance; C_{max} = maximum plasma concentration; Fasted = animals were fasted overnight prior to dose administration and up to 4 hours after dosing; LC-MS/MS = high performance liquid chromatography coupled to tandem mass spectrometry; MRT = mean residence time; NA = not applicable; ND = not determined; PEG = polyethylene glycol; PG = propylene glycol; PK = pharmacokinetic; $t_{1/2}$ = estimated plasma elimination half-life; T_{max} = time to reach the maximum plasma concentration; V_{ss} = volume of distribution at steady state

a Calculated based on $AUC_{0\text{-}72h}$.

3.4. AD-200-2012: Pharmacokinetics of LEN in Solution Following Intravenous and Oral Administration in Cynomolgus Monkeys

Test Article: LEN

Report Title	Study Type	Test Article	Report Number
Pharmacokinetics of LEN in Male Cynomolgus Monkeys	**Single-Dose Pharmacokinetics**	**LEN**	**AD-200-2012**
Species/Strain	Monkey/Cynomolgus		Monkey/Cynomolgus
Sex/ No. of Animals	Male/3		Male/3
Feeding Condition	Fasted		Fasted
Method of Administration	30-minute intravenous infusion		Oral gavage
Sample	Plasma		Plasma
Analyte	LEN		LEN
Assay	LC-MS/MS		LC-MS/MS
Vehicle/Formulation	5% ethanol, 20% propylene glycol, 45% polyethylene glycol 300 and 30% 10 mM phosphate buffer		5% ethanol, 20% propylene glycol, 45% polyethylene glycol 300 and 30% 0.01 N hydrochloric acid
Dose (mg/kg)	1.0		4.0
PK Parameters			
T_{max} (h)	ND		8.7 ± 3.1
C_{max} (nM)	1236 ± 354		17.7 ± 5.6
MRT (h)	14.0 ± 2.7		NA
$t_{1/2}$ (h)	14.6 ± 5.5		NC
AUC_{0-24h} (nM•h)	3670 ± 793		298 ± 85
AUC_{0-72h} (nM•h)	4573 ± 1174		NA
AUC_{inf} (nM•h)	4673 ± 1258		ND
CL (L/h/kg)	0.21± 0.06		NA
V_{ss} (L/kg)	2.86 ± 0.54		NA
F (%)[a]	NA		2.2 ± 0.6

AUC_{0-24h} = area under the plasma concentration-time curve from zero to 24 h; AUC_{0-72h} = area under the plasma concentration-time curve from zero to 72 h; AUC_{inf} = area under the plasma concentration-time curve extrapolated to time infinity; CL = plasma clearance; C_{max} = maximum plasma concentration; Fasted = animals were fasted overnight prior to dose administration and up to 4 hours after dosing; LC-MS/MS = high performance liquid chromatography coupled to tandem mass spectrometry; MRT = mean residence time; NA = not applicable; ND = not determined; PK = pharmacokinetic; $t_{1/2}$ = estimated elimination half-life; T_{max} = time to reach the maximum plasma concentration; V_{ss} = volume of distribution at steady state

a Calculated based on AUC_{0-24h}.

3.5. AD-200-2013: Pharmacokinetics of LEN in Solution Following Intravenous and Oral Administration in Rhesus Monkeys

Test Article: LEN

Report Title	Study Type	Test Article	Report Number
Pharmacokinetics of LEN in Male Rhesus Monkeys	**Single-Dose Pharmacokinetics**	**LEN**	**AD-200-2013**
Species/Strain	Monkey/Rhesus		Monkey/Rhesus
Feeding Condition	Fasted		Fasted
Method of Administration	30-minute intravenous infusion		Oral gavage
Sample	Plasma		Plasma
Analyte	LEN		LEN
Assay	LC-MS/MS		LC-MS/MS
Sex/ No. of Animals	Male/6		Male/3
Vehicle/Formulation	5% ethanol, 20% PG, 45% PEG 300 and 30% 10 mM phosphate buffer		5% ethanol, 20% PG, 45% PEG 300 and 30% 0.01 N hydrochloric acid
Dose (mg/kg)	1.0		4.0
PK Parameters			
T_{max} (h)	ND		18.7 ± 9.2
C_{max} (nM)	1992 ± 424		29.4 ± 7.5
MRT (h)	25.9 ± 11.3		NA
$t_{1/2}$ (h)	37.7 ± 11.4		ND
$AUC_{0\text{-}72h}$ (nM•h)	4502 ± 1579		972 ± 367
AUC_{inf} (nM•h)	5198 ± 2204		1133 ± 446
CL (L/h/kg)	0.25 ± 0.08		NA
V_{ss} (L/kg)	5.75 ± 2.24		NA
F (%)[a]	NA		4.4 ± 1.7

$AUC_{0\text{-}72h}$ = area under the plasma concentration-time curve from zero to 72 h; AUC_{inf} = area under the plasma concentration-time curve extrapolated to time infinity; CL = plasma clearance; C_{max} = maximum plasma concentration; Fasted = animals were fasted overnight prior to dose administration and up to 4 hours after dosing; LC-MS/MS = high performance liquid chromatography coupled to tandem mass spectrometry; MRT = mean residence time; NA = not applicable; ND = not determined; PEG = polyethylene glycol; PG = propylene glycol; PK = pharmacokinetic; $t_{1/2}$ = estimated elimination half-life; T_{max} = time to reach the maximum plasma concentration; V_{ss} = volume of distribution at steady state

a Calculated based on $AUC_{0\text{-}72h}$.

3.6. AD-200-2014: Pharmacokinetics of LEN in Suspension Following Subcutaneous Administration in Rats

Test Article: LEN

Report Title	**Study Type**	**Test Article**	**Report Number**
Pharmacokinetics of LEN in Male Wistar Han Rats	**Single-Dose Pharmacokinetics**	**LEN**	**AD-200-2014**

Species/Strain	Rat/Wistar Han			
Sex/ No. of Animals per group	Male/3			
Feeding Condition	Non-fasted			
Sample	Plasma			
Analyte	LEN			
Assay	LC-MS/MS			
Method of Administration	Subcutaneous injection			
Vehicle/Formulation	Suspension in 2% Poloxamer 188 in normal saline			
Dose (mg/kg)	10	30	100	200
Dose Concentration (mg/mL)	100	100	100	100
Number of Injection	1	1	1	2
Dose Volume (mL/kg)	0.10	0.30	1.0	1.0 x 2
PK Parameters				
$AUC_{0\text{-}72h}$ (µM•h)	32.5 ± 20.8	20.6 ± 3.9	68.0 ± 27.2	180 ± 93
$AUC_{0\text{-}672h}$ (µM•h)	194 ± 14	343 ± 63	900 ± 89	1498 ± 223
AUC_{last} (µM•h)	236 ± 36	663 ± 83	1545	2499 ± 188
AUC_{inf} (µM•h)	236 ± 36	664 ± 83	1554	2517 ± 194
C_{max} (µM)	0.671 ± 0.198	0.724 ± 0.222	1.91 ± 0.33	3.81 ± 1.08
T_{max} (h)	25 ± 23	224 ± 97	224 ± 97	120 ± 83
$t_{1/2}$ (h)	219 ± 81	263 ± 31	397	403 ± 50
% of total exposure by Day 3	14%	3%	4%	7%
% of total exposure by Day 28	82%	52%	58%	60%

$AUC_{0\text{-}72h}$ = area under the plasma concentration-time curve from zero to 72 h; $AUC_{0\text{-}672h}$ = area under the plasma concentration-time curve from zero to 672 h; AUC_{inf} = area under the plasma concentration-time curve extrapolated to time infinity; AUC_{last} = area under the plasma concentration vs. time curve from 0 h to the last quantifiable value; C_{max} = maximum plasma concentration; LC-MS/MS = high performance liquid chromatography coupled to tandem mass spectrometry; PK = pharmacokinetic; $t_{1/2}$ = estimated plasma elimination half-life; T_{max} = time to reach the maximum plasma concentration; % of total exposure by Day 3 was calculated as 100% x average $AUC_{0\text{-}72h}$ / average AUC_{inf}; % of total exposure by Day 28 was calculated as 100% x average $AUC_{0\text{-}672h}$ / average AUC_{inf}.

3.7. AD-200-2038: Pharmacokinetics of LEN in Solution Following Subcutaneous Administration in Rats

Test Article: LEN

Report Title			**Study Type**		**Test Article**		**Report Number**
Pharmacokinetics of LEN in Male Wistar Han Rats			**Single-Dose Pharmacokinetics**		**LEN**		**AD-200-2038**
Species/Strain	Rat/Wistar Han						
Sex/ No. of Animals per group	Male/3						
Feeding Condition	Non-fasted						
Sample	Plasma						
Analyte	LEN						
Assay	LC-MS/MS						
Method of Administration	Subcutaneous injection						
Vehicle/Formulation	Solution in 77:10:13 w/w/w PEG200:ethanol:water			Sodium salt formed in situ in solution of 77:10:13 w/w/w PEG200: ethanol: water with 1 molar equivalent sodium hydroxide			
LEN Ionization State	Free Acid			Sodium Salt			
Dose (mg/kg)	10	50	50	10	10	50	100
Dose Concentration (mg/mL)	200	200	400	100	200	200	200
Dose Volume (mL/kg)	0.05	0.25	0.125	0.10	0.05	0.25	0.50
PK Parameters							
AUC_{last} (µM•h)	220 ± 22	858 ± 56	632 ± 155	189 ± 34	237 ± 31	874 ± 68	1798 ± 149
AUC_{inf} (µM•h)	222 ± 23	886 ± 59	NC	190 ± 35	NC	876 ± 66	1825 ± 177
C_{max} (µM)	0.193 ± 0.049	0.537 ± 0.033	0.283 ± 0.119	0.392 ± 0.057	0.286 ± 0.086	0.823 ± 0.158	1.188 ± 0.183
T_{max} (h)	504 ± 0	672 ± 291	840 ± 291	232 ± 180	560 ± 257	504 ± 291	616 ± 194

AUC_{inf} = area under the plasma concentration-time curve extrapolated to time infinity; AUC_{last} = area under the plasma concentration vs. time curve from 0 h to the last quantifiable value; C_{max} = maximum plasma concentration; LC-MS/MS = high performance liquid chromatography coupled to tandem mass spectrometry; NC = not calculated due to insufficient data; PEG = polyethylene glycol; PK = pharmacokinetic; T_{max} = time to reach the maximum plasma concentration.

3.8. AD-200-2015: Pharmacokinetics of LEN in Suspension Following Subcutaneous and Intramuscular Administration in Dogs

Test Article: LEN

Report Title			**Study Type**		**Test Article**		**Report Number**
Pharmacokinetics of LEN in Male Beagle Dogs			**Single-Dose Pharmacokinetics**		**LEN**		**AD-200-2015**
Species/Strain	Dog/Beagle						
Sex/ No. of Animals per group	Male/3						
Feeding Condition	Non-fasted						
Sample	Plasma						
Analyte	LEN						
Assay	LC-MS/MS						
Vehicle/Formulation	Suspension in 2% Poloxamer 188 in normal saline						
Dose (mg/kg)	3	6	6	6	30	100	6
Dose Concentration (mg/mL)	100	100	100	200	100	100	100
Number of Injection	1	1	2	1	1	1	1
Dose Volume (mL/kg)	0.03	0.06	0.03 x 2	0.03	0.30	1.0	0.06
Method of Administration	SC injection	SC injection	SC injection	SC injection	SC injection	SC injection	IM injection
PK Parameters							
$AUC_{0\text{-}72h}$ (µM•h)	3.0 ± 2.1	20.8 ± 7.4	18.8 ± 2.4	6.6 ± 3.4	145 ± 9	340 ± 76	7.9 ± 1.8
$AUC_{0\text{-}672h}$ (µM•h)	18.6 ± 9.0	117 ± 37	85.0 ± 7.0	63.9 ± 27.3	496 ± 49	1336 ± 504	43.8 ± 10.6
AUC_{last} (µM•h)	25.4 ± 7.5	128 ± 41	88.4 ± 6.2	75.9 ± 34.6	497 ± 51	1356 ± 500	53.7 ± 11.5
AUC_{inf} (µM•h)	28.0 ± 6.4	129 ± 42	88.9 ± 6.2	79.1 ± 32.2	497 ± 51	1357 ± 500	55.2 ± 11.4
C_{max} (µM)	0.056 ± 0.037	0.410 ± 0.141	0.417 ± 0.014	0.175 ± 0.068	2.960 ± 0.295	7.243 ± 1.279	0.136 ± 0.040
T_{max} (h)	72 ± 83	40 ± 28	64 ± 28	96 ± 63	40 ± 14	32 ± 14	40 ± 28
$t_{1/2}$ (h)	525 ± 147	322 ± 51	174 ± 38	354 ± 329	66 ± 5	94 ± 59	335 ± 46
% of total exposure by Day 3	11%	16%	21%	8%	29%	25%	14%
% of total exposure by Day 28	66%	91%	96%	96%	100%	100%	97%

$AUC_{0\text{-}72h}$ = area under the plasma concentration-time curve from zero to 72 h; $AUC_{0\text{-}672h}$ = area under the plasma concentration-time curve from zero to 672 h; AUC_{inf} = area under the plasma concentration-time curve extrapolated to time infinity; AUC_{last} = area under the plasma concentration vs. time curve from 0 h to the last quantifiable value; C_{max} = maximum plasma concentration; IM = intramuscular; LC-MS/MS = high performance liquid chromatography coupled to tandem mass spectrometry; PK = pharmacokinetic; SC = subcutaneous; $t_{1/2}$ = estimated plasma elimination half-life; T_{max} = time to reach the maximum plasma concentration; % of total exposure by Day 3 was calculated as 100% x average $AUC_{0\text{-}72h}$ / average AUC_{inf}; % of total exposure by Day 28 was calculated as 100% x average $AUC_{0\text{-}672h}$ / average AUC_{inf}.

3.9. AD-200-2039: Pharmacokinetics of LEN in Solution Following Subcutaneous Administration in Dogs

Test Article: LEN

Report Title	**Study Type**	**Test Article**	**Report Number**
Pharmacokinetics of LEN in Male Beagle Dogs	**Single-Dose Pharmacokinetics**	**LEN**	**AD-200-2039**

Species/Strain	Dog/Beagle						
Sex/ No. of Animals per group	Male/3						
Feeding Condition	Non-fasted						
Sample	Plasma						
Analyte	LEN						
Assay	LC-MS/MS						
Method of Administration	Subcutaneous injection						
Vehicle/Formulation	Solution in 78:10:12 w/w/w PEG200:ethanol:water	Solution in 77:10:13 w/w/w PEG200: ethanol: water			Sodium salt formed in situ in solution of 77:10:13 w/w/w PEG200: ethanol: water with 1 mole equivalent sodium hydroxide		
LEN Ionization State	Free Acid	Free Acid			Sodium Salt		
Dose (mg/kg)	6	12	12	12	6	6	6
Dose Concentration (mg/mL)	200	200	300	400	100	200	300
Dose Volume (mL/kg)	0.03	0.06	0.04	0.03	0.06	0.03	0.02
PK Parameters							
AUC_{last} (µM•h)	72.7 ± 20.8	124 ± 24	126 ± 72	111 ± 14	91.7 ± 4.1	83.6 ± 18.0	43.2 ± 50.8
AUC_{inf} (µM•h)	88.9 ± 28.0	138 ± 19	NC	NC	91.9 ± 4.1	84.4 ± 18.0	44.6 ± 50.2
C_{max} (µM)	0.063 ± 0.019	0.144 ± 0.034	0.143 ± 0.082	0.107 ± 0.013	0.259 ± 0.069	0.392 ± 0.097	0.122 ± 0.154
T_{max} (h)	392 ± 97	448 ± 97	392 ± 97	336 ± 0	240 ± 166	392 ± 97	144 ± 166

AUC_{inf} = area under the plasma concentration-time curve extrapolated to time infinity; AUC_{last} = area under the plasma concentration vs. time curve from 0 h to the last quantifiable value; C_{max} = maximum plasma concentration; LC-MS/MS = high performance liquid chromatography coupled to tandem mass spectrometry; NC = not calculated due to insufficient data; PEG = polyethylene glycol; PK = pharmacokinetic; T_{max} = time to reach the maximum plasma concentration.

3.10. AD-200-2051: Pharmacokinetics of LEN Following Subcutaneous Administration of LEN in Solution in Male Beagle Dogs

Test Article: LEN

Report Title		**Study Type**	**Test Article**	**Report Number**
Pharmacokinetics of LEN Following Subcutaneous Administration of LEN in Solution in Male Beagle Dogs		**Single-Dose Pharmacokinetics**	**LEN**	**AD-200-2051**
Species/Strain	Dog/Beagle			
Sex/ No. of Animals per group	Male/3			
Feeding Condition	Non-fasted			
Sample	Plasma			
Analyte	LEN			
Assay	LC-MS/MS			
Method of Administration	Subcutaneous injection			
Vehicle/Formulation	65% w/w polyethylene glycol 300, 25.2% w/w LEN, and 9.8% w/w water			
Dose (mg/kg)	12			
Dose Concentration (mg/mL)	300			
Dose Volume (mL/kg)	0.04			
PK Parameters				
AUC_{last} (µM•h)	139 ± 8			
C_{max} (µM)	0.133 ± 0.010			
T_{max} (h)	336 ± 0			

AUC_{last} = area under the plasma concentration vs. time curve from 0 h to the last quantifiable value; C_{max} = maximum plasma concentration; LC-MS/MS = high performance liquid chromatography coupled to tandem mass spectrometry; T_{max} = time to reach the maximum plasma concentration.

3.11. AD-200-2018: Pharmacokinetics of LEN in Rats Following Oral Administration in Solution at Multiple Dose Levels

Test Article: LEN

Report Title	Study Type	Test Article	Report Number
Pharmacokinetics of LEN in Male Wistar Han Rats Following Oral Administration in Solution at Multiple Dose Levels	**Single-Dose Pharmacokinetics**	**LEN**	**AD-200-2018**
Species/Strain	Rat/Wistar Han		
Sex/ No. of Animals per group	Male/3		
Feeding Condition	Fasted		
Sample	Plasma		
Analyte	LEN		
Assay	LC-MS/MS		
Method of Administration	Oral gavage		
Vehicle/Formulation	10% dimethyl sulfoxide, 10% Vitamin E TPGS and 80% PG		
Dose (mg/kg)	30	100	
PK Parameters			
T_{max} (h)	9.0 ± 2.6	20.0 ± 6.9	
C_{max} (nM)	1292 ± 377	1133 ± 406	
$AUC_{0\text{-}24h}$ (nM•h)	24667 ± 7003	19551 ± 4821	
AUC_{last} (nM•h)	73843 ± 14148	90083 ± 34147	
AUC_{inf} (nM•h)	83410 ± 17982	120428 ± 62292	

$AUC_{0\text{-}24h}$ = area under the plasma concentration-time curve from zero to 24 h; AUC_{last} = area under the plasma concentration-time curve from zero to the last quantifiable concentration; PG = propylene glycol; TPGS = d-α-Tocopheryl polyethylene glycol 1000 succinate.

3.12. AD-200-2019: Pharmacokinetics of LEN in Dogs Following Oral Administration in Solution at Multiple Dose Levels

Test Article: LEN

Report Title			**Study Type**	**Test Article**	**Report Number**
Pharmacokinetics of LEN in Male Beagle Dogs Following Oral Administration in Solution at Multiple Dose Levels			**Single-Dose Pharmacokinetics**	**LEN**	**AD-200-2019**
Species/Strain	Dog/Beagle				
Feeding Condition	Fasted				
Sample	Plasma				
Analyte	LEN				
Assay	LC-MS/MS				
Method of Administration	Oral gavage				
Sex/ No. of Animals per group	Male/3	Male/3	Male/6	Male/6	Male/6
Vehicle/Formulation	A	B	C	B	C
Dose (mg/kg)	30	30	30	100	100
PK Parameters					
T_{max} (h)	3.3 ± 1.2	16.7 ± 12.7	10.0 ± 8.0	12.0 ± 0.0	15.7 ± 9.7
C_{max} (µM)	2.967 ± 4.189	2.472 ± 1.957	3.225 ± 1.614	4.253 ± 0.732	2.890 ± 0.860
AUC_{last} (µM•h)	59.7 ± 82.4	69.6 ± 58.6	92.9 ± 33.6	148 ± 37	120 ± 52
AUC_{inf} (µM•h)	60.9 ± 84.3	70.6 ± 58.5	93.1 ± 33.5	148 ± 37	121 ± 53

AUC_{last} = area under the plasma concentration-time curve from 0 to last; AUC_{inf} = area under the plasma concentration-time curve extrapolated to time infinity; C_{max} = maximum plasma concentration; T_{max} =time to reach the maximum plasma concentration
Formulation A: solution in 5% ethanol, 10% Vitamin E d-α-Tocopheryl polyethylene glycol 1000 succinate (TPGS), 85% propylene glycol; Formulation B: solution in 10% dimethyl sulfoxide, 10% Vitamin E TPGS, 80% propylene glycol; Formulation C: solution in 10% ethanol, 10% Vitamin E TPGS, 25% polyethylene glycol 200, 55% propylene glycol

3.13. AD-200-2036: Pharmacokinetics of LEN in Dogs Following Oral Administration in Liquid-Filled Capsules

Test Article: LEN

Report Title				**Study Type**		**Test Article**		**Report Number**	
Pharmacokinetics of LEN in Male Beagle Dogs Following Oral Administration in Liquid-Filled Capsules				**Single-Dose Pharmacokinetics**		**LEN**		**AD-200-2036**	
Species/Strain	Dog/Beagle								
Sample	Plasma								
Analyte	LEN								
Assay	LC-MS/MS								
Method of Administration	Oral gavage								
Sex/ No. of Animals per group	Male/6								
Dose	7.5 mg fixed								
Vehicle/Formulation[a]	A	B	B	B	B	B	A	C	D	D
Feeding Condition[b]	Fasted	Fasted	Fasted	Fasted	Fed	Fasted	Fasted	Fasted	Fasted	Fasted
Pretreatment[c]	None	None	Yes	Yes	None	Yes	Yes	Yes	Yes	Yes
Dose (mg/kg)	10	10	10	50	50	100	100	100	100	50
PK Parameters										
T_{max} (h)	7.3 ± 8.2	11.0 ± 10.1	4.7 ± 1.6	4.8 ± 1.8	4.7 ± 1.6	5.3 ± 2.1	5.3 ± 2.1	7.7 ± 8.0	5.0 ± 1.1	4.0 ± 0.0
C_{max} (nM)	147 ± 29	118 ± 43	114 ± 45	64.8 ± 12.5	70.3 ± 19.6	51.4 ± 16.6	59.7 ± 20.2	53.2 ± 16.7	71.2 ± 25.8	82.7 ± 32.1
$AUC_{0\text{-}72h}$ (nM•h)	2447 ± 1124	2539 ± 1128	2792 ± 703	1993 ± 767	1778 ± 856	1197 ± 465	1346 ± 390	1181 ± 575	1667 ± 1045	2178 ± 1243
AUC_{inf} (nM•h)	2591 ± 1196	2700 ± 1222	3339 ± 864	2256 ± 987	2155 ± 1126	1791 ± 1334	1953 ± 823	1303 ± 634	1867 ± 1188	2329 ± 1348
F%[d]	25 ± 12	26 ± 13	35 ± 8	26 ± 11	21 ± 9	15 ± 7	17 ± 5	11 ± 4	16 ± 9	22 ± 10

$AUC_{0\text{-}72h}$ = area under the plasma concentration-time curve from zero to 72 h; AUC_{inf} = area under the plasma concentration-time curve extrapolated to time infinity; C_{max} = maximum plasma concentration; LFC = liquid filled capsule; T_{max} = time to reach maximum plasma concentration

a Formulation A: LFC in 100% caprylocaproyl polyoxyl-8 glycerides; Formulation B: LFC in 20% caprylocaproyl polyoxyl-8 glycerides and 80% PEG 400; Formulation C: LFC in 20% caprylocaproyl polyoxyl-8 glycerides and 80% glycerol monocaprylocaprate type I; Formulation D: LFC in 100% glycerol monocaprylocaprate type I.

b Under fasted condition, animals were fasted at least 12 hours prior to dosing and fed 4 hours post dose; Under fed condition, animals were fed 2 hours prior to dosing.

c An entry of "Yes" = pentagastrin pretreatment. In the pentagastrin-pretreated dog model, pentagastrin (6 µg/kg) was administered intramuscularly ~ 30 minutes prior to dosing of LEN to ensure an acidic pH in the gastrointestinal tract.

d %F calculated based on $AUC_{0\text{-}72h}$ with average IV $AUC_{0\text{-}72h}$ = 13467 nM•h at 1 mg/kg from report AD-200-2011.

3.14. AD-200-2042: Pharmacokinetics of LEN in Dogs Following Oral Administration in Tablets

Test Article: LEN

Report Title	**Study Type**	**Test Article**	**Report Number**
Pharmacokinetics of LEN in Male Beagle Dogs Following Oral Administration in Tablets	**Single-Dose Pharmacokinetics**	**LEN**	**AD-200-2042**

Species/Strain	Dog/Beagle				
Sample	Plasma				
Analyte	LEN				
Assay	LC-MS/MS				
Method of Administration	Oral gavage				
Sex/ No. of Animals per group	Male/6				
Vehicle/Formulation[a]	A	B	C	C	C
Feeding Condition[b]	Fasted	Fasted	Fed	Fasted	Fasted
Pretreatment[c]	Pentagastrin	Pentagastrin	None	Pentagastrin	Pentagastrin
Dose (mg fixed)	40	40	40	80	300
PK Parameters					
T_{max} (h)	8.7 ± 8.2	14.3 ± 10.6	11.7 ± 9.6	4.0 ± 0.0	5.3 ± 3.3
C_{max} (nM)	166 ± 64	151 ± 79	162 ± 93	437 ± 108	718 ± 409
$AUC_{0\text{-}72h}$ (nM•h)	4495 ± 3408	3776 ± 2945	4573 ± 3348	6945 ± 1652	15841 ± 1000
AUC_{inf} (nM•h)	5030 ± 3783	4440 ± 3527	5548 ± 4149	7704 ± 1791	18554 ± 11070

$AUC_{0\text{-}72h}$ = area under the plasma concentration-time curve from zero to 72 h; AUC_{inf} = area under the plasma concentration-time curve extrapolated to time infinity; C_{max} = maximum plasma concentration; T_{max} = time to reach maximum plasma concentration

a Formulation A: liquid filled capsule in 100% Capmul MCM, pH 7; Formulation B: tablet: 8% Croscarmellose sodium, 1.5% Magnesium stearate; 31% mannitol; 15.5 % Microcrystalline cellulose; 17.6% Povidone; Formulation C: tablet: 5.34% Copovidone; 8% Croscarmellose sodium; 1.5% Magnesium stearate; 42.55% Mannitol; 21.28% Microcrystalline cellulose; 1.33% Poloxamer 407

b Under fasted condition, animals were fasted at least 12 hours prior to dosing and fed 4 hours post dose. Under fed condition, animals were fed 30 minutes prior to dosing with slurry of canned food (~100g).

c In the pentagastrin-pretreated dog model, pentagastrin (6 µg/kg) was administered intramuscularly ~ 30 minutes prior to dosing of LEN to ensure an acidic pH in the gastrointestinal tract.

3.15. AD-200-2016: Pharmacokinetics of LEN Following Oral Administration in Portal Vein Cannulated Dogs

Test Article: LEN

Report Title	**Study Type**	**Test Article**	**Report Number**
Pharmacokinetics of LEN Following Oral Administration in Portal Vein Cannulated Male Beagle Dogs	**Single-Dose Pharmacokinetics**	**LEN**	**AD-200-2016**

Species/Strain	Dog/Beagle			
Sex/ No. of Animals per group	Male/3			
Feeding Condition	Fasted			
Sample	Plasma			
Analyte	LEN			
Assay	LC-MS/MS			
Method of Administration	Oral gavage			
Vehicle/Formulation	5% ethanol, 20% PG, 45% PEG 300 and 30% 0.01 N hydrochloric acid			
LEN Dose (mg/kg)	2.0			
1-Aminobenzotriazole Dose (mg/kg)[a]	0		20	
PK Parameters				
	Jugular Vein	**Portal Vein**	**Jugular Vein**	**Portal Vein**
$AUC_{0\text{-}120h}$ (nM•h)	9041 ± 2175	8053 ± 1780	8746 ± 7337	7291 ± 5509
AUC_{inf} (nM•h)	9297 ± 2428	8264 ± 1962	9042 ± 7712	7583 ± 5871
C_{max} (nM)	273 ± 23	256 ± 28.2	282 ± 143	260 ± 109
T_{max} (h)	10.7 ± 11.6	4.0 ± 2.0	4.0 ± 2.0	3.3 ± 1.2
F[b]	23.7% ± 6.2%	NA	23.0% ± 19.6%	NA
F_h	1.0 ± 0.0		1.0 ± 0.0	
E_h	0.0 ± 0.0		0.0 ± 0.0	
F_{abs}	23.7% ± 6.2%		23.0% ± 19.6%	

$AUC_{0\text{-}120h}$ = area under the plasma concentration-time curve from zero to 120 h; CL = plasma clearance; E_h = hepatic extraction ratio; calculated as 1 – F_h following oral dose; calculated as CL/Q_h following IV administration; $F = F_g \times F_{abs} \times F_h$; F_{abs} = fraction (%) absorbed in the gut, calculated as $F_{abs} = F/(F_h * F_g)$; F_g = fraction of the dose escaping the first-pass gut metabolism; F_h = fraction of the dose escaping the liver metabolism, calculated as $F_h = AUC_{inf}$ from jugular vein over AUC_{inf} from portal vein. Values greater than 1 will be treated as 1; NA = not applicable; PEG = polyethylene glycol; PG = propylene glycol; Q_h = hepatic blood flow.

a The 1-aminobenzotriazole dose was administered by oral gavage at approximately 2 hours prior to the LEN administration.

b Calculated based on AUC_{inf}.

3.16. AD-200-2017: Pharmacokinetics of LEN Following Oral Administration in Portal Vein Cannulated Cynomolgus Monkeys

Test Article: LEN

Report Title	**Study Type**		**Test Article**	**Report Number**
Pharmacokinetics of LEN Following Oral Administration in Portal Vein Cannulated Male Cynomolgus Monkeys	**Single-Dose Pharmacokinetics**		**LEN**	**AD-200-2017**
Species/Strain	Monkey/Cynomolgus			
Sex/ No. of Animals per group	Male/3			
Feeding Condition	Fasted			
Sample	Plasma			
Analyte	LEN			
Assay	LC-MS/MS			
Method of Administration	Oral gavage			
Vehicle/Formulation	5% ethanol, 20% PG, 45% PEG 300 and 30% 0.01 N hydrochloric acid			
LEN Dose (mg/kg)	2.0			
1-Aminobenzotriazole Dose (mg/kg)[a]	0		20	
PK Parameters				
	Jugular Vein	**Portal Vein**	**Jugular Vein**	**Portal Vein**
$AUC_{0\text{-}120h}$ (nM•h)	882 ± 453	950 ± 77	704 ± 331	1021 ± 262
AUC_{inf} (nM•h)	909 ± 458	981 ± 85	730 ± 333	1046 ± 260
C_{max} (nM)	38.7 ± 16.0	133 ± 85	38.4 ± 17	90.7 ± 7.28
T_{max} (h)	10.7 ± 2.3	4.3 ± 2.9	10.7 ± 2.3	5.3 ± 2.3
F[b]	11.4% ± 5.7%	NA	9.1% ± 4.2%	NA
F_h	0.80 ± 0.18		0.68 ± 0.16	
E_h	0.20 ± 0.18		0.32 ± 0.16	
F_{abs}	13.7% ± 3.8%		13.1% ± 3.1%	

NA = not applicable; PEG = polyethylene glycol; PG = propylene glycol.
a The 1-aminobenzotriazole dose was administered by oral gavage at approximately 2 hours prior to the LEN administration.
b Calculated based on AUC_{inf}.

4. PHARMACOKINETICS: DISTRIBUTION

4.1. AD-200-2020: Plasma Protein Binding of LEN in Mouse, Rat, Dog, Monkey and Human

Test Article: LEN

Report Title	Study Type	Test Article	Report Number
Plasma Protein Binding of LEN	**Distribution**	**LEN**	**AD-200-2020 & AD-200-2044**
Study System	Plasma from CD-1 mouse, Sprague-Dawley rat, beagle dog, cynomolgus monkey, rhesus monkey and human		
Method	Plasma (1 mL) containing LEN (2 µM) and compound-free phosphate buffer (1 mL) were placed into opposite sides of the assembled dialysis cells separated by a semipermeable membrane. The dialysis was carried out at 37°C for 24 hours. Lenacapavir concentrations in each cell was determined by LC-MS/MS.		
Species	**LEN Free Fraction (%) (Mean ± SD, n=3)**		
CD-1 mouse	0.18 ± 0.02		
Sprague-Dawley Rat	0.13 ± 0.02		
Beagle Dog	0.83 ± 0.19		
Cynomolgus Monkey	1.11 ± 0.22		
Rhesus Monkey	1.45 ± 0.18		
Human	1.46 ± 0.23		
Study System	Human plasma and CCM		
Method	CCM and 10% human plasma (1 mL) containing LEN (2 µM) were placed in opposite sides of the assembled dialysis cells separated by a semipermeable membrane. The dialysis was carried out at 37°C for 24 hours. Lenacapavir concentration in each cell was determined by LC-MS/MS.		
Human Plasma to CCM Ratio[a]	17.4 ± 0.9		

CCM = cell culture medium containing 10% v/v fetal bovine serum; LC-MS/MS = high performance liquid chromatography coupled to tandem mass spectrometry
a Values are the Mean ± standard deviation with n = 5 determinations.

4.2. AD-200-2021: In Vitro Assessment of Blood Distribution of LEN

Test Article: LEN

Report Title	Study Type	Test Article	Report Number
In Vitro Assessment of Blood Distribution of LEN	**Distribution**	**LEN**	**AD-200-2021**
Study System	Heparinized blood samples from Sprague-Dawley rat, beagle dog, cynomolgus monkey, rhesus monkey, and human		
Method	Lenacapavir and control compounds were incubated in triplicate at an initial concentration of 0.5 µM in blood, plasma or cell fraction for 60 minutes at 37°C and then chilled on ice. Blood samples were then centrifuged at 4°C to separate the cellular and plasma fractions. Test article concentration in each fraction was determined by LC-MS/MS.		

	LEN[a]		**Control[a]**	
Species	**CPR**	**B/P ratio**	**Compound**	**B/P ratio**
Sprague-Dawley Rat	0.08 ± 0.00	0.59 ± 0.00	Chlorthalidone	28.95 ± 5.42
Beagle Dog	0.17 ± 0.07	0.67 ± 0.03	Chloroquine	3.2 ± 0.50
Cynomolgus Monkey	0.16 ± 0.02	0.60 ± 0.01	Methazolamide	87.2 ± 11.56
Rhesus Monkey	0.26 ± 0.06	0.64 ± 0.03	Methazolamide	50.6 ± 22.85
Human	0.31 ± 0.11	0.64 ± 0.05	Methazolamide	34.3 ± 3.12

B/P ratio = Whole Blood to Plasma Concentration Ratios; CPR = Cell to Plasma Concentration Ratios; LC-MS/MS = high performance liquid chromatography coupled to tandem mass spectrometry

a Values are the Mean ± standard deviation (n = 3 determinations using pooled blood samples from each species)

4.3. AD-200-2032: Human Hepatic Microsomal Binding of LEN

Test Article: LEN

Report Title	Study Type	Test Article	Report Number
Human Hepatic Microsomal Binding of LEN	**Distribution**	**LEN**	**AD-200-2032**

Method	Pooled human hepatic microsomal fraction (0.5 mg protein/mL) containing LEN (2 μM) or positive control (amitriptyline, 2 μM) was dialyzed in triplicate against buffer at 37°C for 3 hours. Test compound concentrations in the buffer and microsomal fraction were determined by LC-MS/MS.	
Species	**Fraction Unbound (%)[a]**	**Mean Recovery (%)[a]**
Human	10.8 ± 1.1	72

LC-MS/MS = high performance liquid chromatography coupled to tandem mass spectrometry
a Values are the Mean ± standard deviation (n = 3)

4.4. AD-200-2048: Binding of LEN to Human Serum Albumin and α_1-Acid Glycoprotein

Test Article: LEN

Report Title	Study Type	Test Article	Report Number
Binding of LEN to Human Serum Albumin and α_1-Acid Glycoprotein	**Distribution**	**LEN**	**AD-200-2048**

Method	The free fraction of LEN (2 μM) was determined, by equilibrium dialysis, using physiological concentrations of purified human serum albumin (HSA, 40 mg/mL) and purified α_1-acid glycoprotein (AAG, 0.8 mg/mL). Warfarin (2 μM) and disopyramide (2 μM) were used as the positive control ligands for HSA and AAG, respectively. The dialysis cells were incubated in a 37°C water bath for 24 hours. Test compound concentrations were determined by LC-MS/MS.

Compound	Identity	Free Fraction (%) (Mean ± SD, n = 3, Unless Otherwise Noted)			
		AAG[a]	5×AAG[b]	HSA[c]	Plasma[d]
LEN	Test compound	7.0[e]	1.8 ± 0.1	0.01 ± 0.0	0.70 ± 0.1
Disopyramide	AAG ligand	37.1 ± 5.0	3.7 ± 0.4	79.2 ± 5.9	22.1 ± 1.1
Warfarin	HSA ligand	3.8 ± 0.7	0.45 ± 0.0	0.57 ± 0.0	0.58 ± 0.0

LC-MS/MS = high performance liquid chromatography coupled to tandem mass spectrometry
a α_1-Acid glycoprotein, 0.8 mg/mL
b α_1-Acid glycoprotein, 4.0 mg/m
c Human serum albumin 40 mg/mL
d Pooled human plasma with α_1-acid glycoprotein concentration of 0.86 mg/mL
e Mean (n = 2), Study EXP-20-BB0738

4.5. AD-200-2006: Distribution in Wistar Han Rats Following a Single Intravenous Dose of [^{14}C]LEN

Test Article: LEN

Report Title	**Study Type**	**Test Article**	**Report Number**
Pharmacokinetics, Absorption, Distribution, and Excretion of [^{14}C]LEN Following Intravenous Administration to Rats	**Distribution**	**[^{14}C]LEN**	**AD-200-2006**

Species	Wistar Han (non-pigmented) Rat												
Gender /No. of Animals	Male/13 (1 per time point)												
Feeding Condition	Certified rodent diet was provided												
Vehicle/Formulation	5% ethanol, 20% propylene glycol, 45% polyethylene glycol 300 and 30% sterile water for injection, pH 9												
Method of Administration	Intravenous bolus injection												
Dose	3 mg/kg (172 µCi/kg)												
Radionuclide	Carbon-14												
Specific Activity	113 mCi/mmol												
Specific Activity of Formulation	57.3 µCi/mg												
Sampling Time	0.5, 2, 4, 24, 48, 72, 168, 336, 672, 840, 1008, 1344, and 1680 hours postdose												
Analyte/Assay	Carbon-14/Quantitative Whole Body Autoradiography												
Tissues/Organs ↓	**Tissue Concentration of Radioactivity (ng Equivalents [^{14}C]LEN/g tissue)**												
Time-point	**0.5 h**	**2 h**	**4 h**	**1 d**	**2 d**	**3 d**	**7 d**	**14 d**	**28 d**	**35 d**	**42 d**	**56 d**	**70 d**
Adrenal gland(s)	1120[a]	553[a]	612[a]	292[a]	171[a]	132[a]	81.4	BLQ	BLQ	ND	ND	ND	ND
Bile	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Blood	719	534	552	224	245	169	75.9	ND	ND	ND	ND	ND	ND
Bone	43.1	27.9	30.7	24.3	20.1	ND	ND	ND	ND	ND	ND	ND	ND
Bone marrow	885	423	401	247	147	95.8	44.6	ND	ND	ND	ND	ND	ND
Brain cerebellum	23.2	BLQ	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Brain cerebrum	BLQ	BLQ	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

Test Article: LEN

Tissues/Organs ↓	Tissue Concentration of Radioactivity (ng Equivalents [^{14}C]LEN/g tissue)												
Time-point	**0.5 h**	**2 h**	**4 h**	**1 d**	**2 d**	**3 d**	**7 d**	**14 d**	**28 d**	**35 d**	**42 d**	**56 d**	**70 d**
Brain choroid plexus	847	346	284	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Brain medulla	BLQ	BLQ	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Brain olfactory lobe	BLQ	BLQ	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Bulbo-urethral gland	1530	1150	993	604	294	216	97.3	ND	ND	ND	ND	ND	ND
Cecum	1120	946	636	388	201	162	57.4	43.7	ND	ND	ND	ND	ND
Diaphragm	1180	575	412	269	139	139	51.1	ND	ND	ND	ND	ND	ND
Epididymis	202	216	213	203	157[a]	94.0	42.1	BLQ	ND	ND	ND	ND	ND
Esophagus	506	251	250	231	109	36.7	30.3	ND	ND	ND	ND	ND	ND
Exorbital lacrimal gland	756	743	633	332	170	83.1	37.6	ND	ND	ND	ND	ND	ND
Eye lens	BLQ	BLQ	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Eye uveal tract	333	145	178	121	61.7	53.1	BLQ	ND	ND	ND	ND	ND	ND
Eye(s)	68.0	44.7	34.1	33.4	22.5	BLQ	BLQ	ND	ND	ND	ND	ND	ND
Fat (abdominal)	183	337	635	579	433	208	53.6	ND	ND	ND	ND	ND	ND
Fat (brown)	1870	945	941	454	309	169	87.5	ND	ND	ND	ND	ND	ND
Fat (reproductive)	219	401	606	605	442	187	66.4	ND	ND	ND	ND	ND	ND
Fat (subcutaneous)	182	465	624	568	360	162	46.1	ND	ND	ND	ND	ND	ND
Harderian gland	577	941	1220	672	328	197	61.8	ND	ND	ND	ND	ND	ND
Intra-orbital lacrimal gland	597	664	543	256	170	115	ND	ND	ND	ND	ND	ND	ND
Kidney cortex	2900	1960	1880	1100	649	478	208	36.7	ND	ND	ND	ND	ND
Kidney medulla	2010	1310	1420	673	425	314	145	BLQ	ND	ND	ND	ND	ND
Kidney(s)	2490	1650	1810	1070	606	391	185	31.0	ND	ND	ND	ND	ND
Large intestine	458	381	265	191	163	71.7	BLQ	BLQ	ND	ND	ND	ND	ND
Liver	53400	54700	57800	34000	29000	15000	5610	1010	52.9	BLQ	BLQ	ND	ND
Lung(s)	809	539	496	333	193	172	69.9	ND	ND	ND	ND	ND	ND

Test Article: LEN

Tissues/Organs ↓	Tissue Concentration of Radioactivity (ng Equivalents [^{14}C]LEN/g tissue)												
Time-point	**0.5 h**	**2 h**	**4 h**	**1 d**	**2 d**	**3 d**	**7 d**	**14 d**	**28 d**	**35 d**	**42 d**	**56 d**	**70 d**
Lymph node(s)	449	517	315	167	154	56.8[a]	37.5	BLQ	ND	ND	ND	ND	ND
Muscle	771	265	212	120	79.9	34.5	BLQ	ND	ND	ND	ND	ND	ND
Myocardium	1190	625	548	322	203	141	45.1	ND	ND	ND	ND	ND	ND
Nasal turbinates	253	123	180	227	265	244	32.4	BLQ	ND	ND	ND	ND	ND
Pancreas	2210	1060	920	593	429	209	95.3	BLQ	ND	ND	ND	ND	ND
Pituitary gland	998	581	524	280	214	110	52.5	ND	ND	ND	ND	ND	ND
Preputial gland	419[a]	607[a]	431[a]	529[a]	174[a]	121[a]	49.9[a]	BLQ	ND	ND	ND	ND	ND
Prostate gland	346	433	291	190	118	70.4	33.5	ND	ND	ND	ND	ND	ND
Salivary gland(s)	1610	993	895	540	328	234	105	BLQ	ND	ND	ND	ND	ND
Seminal vesicle(s)	87.6[a]	75.3[a]	81.4[a]	45.1[a]	57.0[a]	BLQ	BLQ	ND	ND	ND	ND	ND	ND
Skin (nonpigmented)	316	310	349	177	104	52.6	BLQ	ND	ND	ND	ND	ND	ND
Small intestine	1150	626	762	471	233	427	72.8	29.7	ND	ND	ND	ND	ND
Spinal cord	BLQ	BLQ	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Spleen	724	491	413	252	163	88.6	59.7	BLQ	BLQ	ND	ND	ND	ND
Stomach	977	475	500	260	135	112	41.8	BLQ	ND	ND	ND	ND	ND
Testis(es)	73.3	99.6	160	196	141	101	37.6	ND	ND	ND	ND	ND	ND
Thymus	390	452	292	162	92.9	68.3	34.4	ND	ND	ND	ND	ND	ND
Thyroid	1780	1030	802	444	315	234	62.9	ND	ND	ND	ND	ND	ND
Urinary bladder	598[a]	491	224	258	110	92.5	47.9	ND	ND	ND	ND	ND	ND
Urine	36.9	NR	92.8	38.3	BLQ	ND	ND	ND	ND	ND	ND	ND	ND

BLQ = below the limit of quantitation (< 17.8 ng equivalents [^{14}C]LEN/g); d = day; h = hours; ND = not detectable (sample shape not discernible from background or surrounding tissue); NR = not represented (tissue not present in section).
a Tissue appeared to be fat soaked.

4.6. AD-200-2006: Distribution in Long Evans Rats Following a Single Intravenous Dose of [^{14}C]LEN

Test Article: LEN

Report Title	**Study Type**	**Test Article**	**Report Number**
Pharmacokinetics, Absorption, Distribution, and Excretion of [^{14}C]LEN Following Intravenous Administration to Rats	**Distribution**	**[^{14}C]LEN**	**AD-200-2006**

Species	Long Evans (pigmented) Rat												
Gender /No. of Animals	Male/13 (1 per time point)												
Feeding Condition	Certified rodent diet was provided												
Vehicle/Formulation	5% ethanol, 20% propylene glycol, 45% polyethylene glycol 300, and 30% sterile water for injection, pH 9												
Method of Administration	Intravenous bolus injection												
Dose	3 mg/kg (172 µCi/kg)												
Radionuclide	Carbon-14												
Specific Activity	113 mCi/mmol												
Specific Activity of Formulation	57.3 µCi/mg												
Sampling Time	0.5, 2, 4, 24, 48, 72, 168, 336, 672, 840, 1008, 1344, and 1680 hours postdose												
Analyte/Assay	Carbon-14/Quantitative Whole Body Autoradiography												
Tissues/Organs ↓	**Tissue Concentration of Radioactivity (ng Equivalents [^{14}C]LEN/g tissue)**												
Time-point	**0.5 h**	**2 h**	**4 h**	**1 d**	**2 d**	**3 d**	**7 d**	**14 d**	**28 d**	**35 d**	**42 d**	**56 d**	**70 d**
Adrenal gland(s)	1060[a]	1280[a]	768	308[a]	226	162	171[a]	35.9[a]	BLQ	ND	ND	ND	ND
Bile	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Blood	467	988	490	315	278	135	75.9	ND	ND	ND	ND	ND	ND
Bone	58.2	54.6	54.8	39.7	ND	ND	ND	ND	ND	ND	ND	ND	ND
Bone marrow	573	461	377	211	159	98.4	63.0	ND	ND	ND	ND	ND	ND
Brain cerebellum	BLQ	BLQ	BLQ	BLQ	ND	ND	ND	ND	ND	ND	ND	ND	ND

Test Article: LEN

Tissues/Organs ↓	Tissue Concentration of Radioactivity (ng Equivalents [^{14}C]LEN/g tissue)												
Time-point	**0.5 h**	**2 h**	**4 h**	**1 d**	**2 d**	**3 d**	**7 d**	**14 d**	**28 d**	**35 d**	**42 d**	**56 d**	**70 d**
Brain cerebrum	BLQ	BLQ	BLQ	BLQ	ND	ND	ND	ND	ND	ND	ND	ND	ND
Brain choroid plexus	524	655	223	232	162	112	91.2	ND	ND	ND	ND	ND	ND
Brain medulla	BLQ	BLQ	BLQ	BLQ	ND	ND	ND	ND	ND	ND	ND	ND	ND
Brain olfactory lobe	BLQ	BLQ	BLQ	BLQ	ND	ND	ND	ND	ND	ND	ND	ND	ND
Bulbo-urethral gland	1100	1080	1040	612	420	318	177	ND	ND	ND	ND	ND	ND
Cecum	914	444	567	414	303[a]	245	152	28.8	ND	ND	ND	ND	ND
Diaphragm	645	539	425	257	150	98.6	81.0	BLQ	ND	ND	ND	ND	ND
Epididymis	124	162	223[a]	233[a]	196	97.3[a]	58.2[a]	BLQ	ND	ND	ND	ND	ND
Esophagus	324	265	220	156	115	58.0	84.2	31.5	ND	ND	ND	ND	ND
Exorbital lacrimal gland	942	853	640	298	219	131	72.2	BLQ	ND	ND	ND	ND	ND
Eye lens	BLQ	BLQ	BLQ	BLQ	ND	ND	ND	ND	ND	ND	ND	ND	ND
Eye uveal tract	125	217	122	103	88.1	43.9	29.7	ND	ND	ND	ND	ND	ND
Eye(s)	36.2	51.9	37.2	30.2	37.3	BLQ	BLQ	ND	ND	ND	ND	ND	ND
Fat (abdominal)	130	506	577	618	410	228	159	BLQ	ND	ND	ND	ND	ND
Fat (brown)	1230	940	963	465	340	198	110	BLQ	ND	ND	ND	ND	ND
Fat (reproductive)	158	397	572	643	386	271	132	36.1	ND	ND	ND	ND	ND
Fat (subcutaneous)	229	555	680	602	336	200	135	BLQ	ND	ND	ND	ND	ND
Harderian gland	605	848	1120	283	289	196	115	ND	ND	ND	ND	ND	ND
Intra-orbital lacrimal gland	826	874	634	279	240	125	91.6	ND	ND	ND	ND	ND	ND
Kidney cortex	2100	1990	1640	1230	845	584	340	76.5	ND	ND	ND	ND	ND
Kidney medulla	1770	1460	1510	842	505	441	194	46.8	ND	ND	ND	ND	ND
Kidney(s)	2100	1870	1680	1080	809	510	306	62.6	ND	ND	ND	ND	ND

Test Article: LEN

Tissues/Organs ↓	**Tissue Concentration of Radioactivity (ng Equivalents [^{14}C]LEN/g tissue)**												
Time-point	**0.5 h**	**2 h**	**4 h**	**1 d**	**2 d**	**3 d**	**7 d**	**14 d**	**28 d**	**35 d**	**42 d**	**56 d**	**70 d**
Large intestine	774	690	492	374	328	422	156	46.8	ND	ND	ND	ND	ND
Liver	40300	52800	47700	34900	26100	17800	9670	1960	36.9	41.8	36.3	ND	ND
Lung(s)	562	686	432	306	251	128	86.1	ND	ND	ND	ND	ND	ND
Lymph node(s)	456	485	361	230	146	94.4	69.9	32.9	ND	ND	ND	ND	ND
Muscle	497	289	250	129	92.3	47.8	28.5	BLQ	ND	ND	ND	ND	ND
Myocardium	845	797	611	275	213	116	73.2	ND	ND	ND	ND	ND	ND
Nasal turbinates	97.9	119	199	361	219	212	173	BLQ	ND	ND	ND	ND	ND
Pancreas	1500	1130	972	581	416	231	169	34.9	ND	ND	ND	ND	ND
Pituitary gland	716	687	436	265	194	126	88.1	ND	ND	ND	ND	ND	ND
Preputial gland	539[a]	622[a]	485[a]	277	169[a]	127[a]	67.1[a]	36.1[a]	ND	ND	ND	ND	ND
Prostate gland	277	427	324	173	98.2	77.1	63.3	ND	ND	ND	ND	ND	ND
Salivary gland(s)	1340	1140	847	610	429	312	176	47.0	ND	ND	ND	ND	ND
Seminal vesicle(s)	44.7[a]	128[a]	59.6	63.1[a]	61.5[a]	30.5	25.1	ND	ND	ND	ND	ND	ND
Skin (nonpigmented)	232	294	285	140	130	74.4	44.5	ND	ND	ND	ND	ND	ND
Skin (pigmented)	263	371	321	214	133	75.2	49.1	ND	ND	ND	ND	ND	ND
Small intestine	931	818	749	477	352	242	154	32.3	ND	ND	ND	ND	ND
Spinal cord	BLQ	BLQ	BLQ	BLQ	ND	ND	ND	ND	ND	ND	ND	ND	ND
Spleen	625	483	379	228	159	103	63.2	BLQ	BLQ	ND	ND	ND	ND
Stomach	691	607	506	280	152	125	81.6	BLQ	ND	ND	ND	ND	ND
Testis(es)	48.7	162	228	184	161	103	72.7	28.6	ND	ND	ND	ND	ND
Thymus	350	387	330	158	119	75.6	56.6	BLQ	ND	ND	ND	ND	ND

Tissues/Organs ↓	Tissue Concentration of Radioactivity (ng Equivalents [^{14}C]LEN/g tissue)												
Time-point	**0.5 h**	**2 h**	**4 h**	**1 d**	**2 d**	**3 d**	**7 d**	**14 d**	**28 d**	**35 d**	**42 d**	**56 d**	**70 d**
Thyroid	822	927	778	432	301	215	119	29.1	ND	ND	ND	ND	ND
Urinary bladder	470	514	401	277	143	125	96.6	ND	ND	ND	ND	ND	ND
Urine	62.3	44.5	64.6	31.0	27.1	18.0	18.0	ND	ND	ND	ND	ND	ND

BLQ = below the limit of quantitation (< 17.8 ng equivalents [^{14}C]LEN/g); d = day; h = hours; ND = not detectable (sample shape not discernible from background or surrounding tissue); NR = not represented (tissue not present in section).
a Tissue appeared to be fat soaked.

5. PHARMACOKINETICS: METABOLISM IN VITRO AND IN VIVO

5.1. AD-200-2047: In Vitro Stability of LEN Atropisomers in PBS and in Plasma from Human and Nonclinical Species

Test Article: LEN

Report Title	Study Type	Test Article	Report Number	Location in CTD
In Vitro Stability of LEN Atropisomers in PBS and in Plasma from Human and Nonclinical Species	**Other**	**LEN**	**AD-200-2047**	**4.2.2.4**
Study System	PBS and plasma from Sprague-Dawley rat, beagle dog and human			
Method	Triplicate aliquots of plasma and PBS were warmed to 37°C and the incubations were initiated by the addition of LEN to obtain final substrate concentrations of 0.1 and 1 µM and the incubations continued at 37°C for 24 hr. Aliquots (25 µL) of the reaction mixtures were transferred into 96-well plates. Lenacapavir concentrations were determined by LC-MS/MS.			

	Atropisomer LEN.1 as Proportion of Total LEN (Mean ± SD, n = 3)			
	LEN @ 0.1 µM		**Len @ 1.0 µM**	
Matrix	**0 hr**	**24 hr**	**0 hr**	**24 hr**
Rat plasma	20% ± 2%	18% ± 2%	16% ± 5%	19% ± 1%
Dog plasma	21% ± 1%	18% ± 2%	20% ± 5%	20% ± 2%
Human plasma	20% ± 1%	21% ± 4%	22% ± 5%	23% ± 2%
Phosphate buffer	18% ± 6%	15% ± 5%	18% ± 5%	23% ± 3%
Water/acetonitrile[a]	18% ± 3%	16% ± 0%	Not determined	

CCM = cell culture medium containing 10% v/v fetal bovine serum; LC-MS/MS = high performance liquid chromatography coupled to tandem mass spectrometry; PBS = phosphate buffered saline

a Unincubated sample (analyzed immediately or stored in autosampler at 4°C for 24 hr)

5.2. AD-200-2022: In Vitro Metabolic Stability of [^{3}H]LEN in Hepatic Microsomes with Mixed Co-factors

Test Article: LEN

Report Title	Study Type	Test Article	Report Number	Location in CTD
In Vitro Metabolism of [^{3}H]LEN in Hepatic Microsomes and Hepatocytes	**Metabolism**	**[^{3}H]LEN**	**AD-200-2022**	**4.2.2.4**

Study System	Pooled hepatic microsomes from rat, dog, cynomolgus and rhesus monkeys, and human
Method	[^{3}H]LEN (0.25 µM) was incubated for up to 65 minutes at 37°C in hepatic microsomes in the presence of NADPH and UDPGA co-factors. Quantification of [^{3}H]LEN was performed by radio flow chromatography.

Species	Mean Predicted Hepatic Clearance (L/h/kg)[a]	Mean Predicted Hepatic Extraction (%)[a]
Sprague-Dawley Rat	0.01	< 1
Beagle Dog	0.02	1
Cynomolgus Monkey	0.04	2
Rhesus Monkey	0.13	5
Human	0.01	1

NADPH = nicotinamide adenine dinucleotide phosphate, reduced; UDPGA = uridine diphosphate glucuronic acid.
a Values are expressed as mean with n = 2.

5.3. AD-200-2022: In Vitro Metabolic Stability of [^{3}H]LEN in Cryopreserved Hepatocytes

Test Article: LEN

Report Title	**Study Type**	**Test Article**	**Report Number**	**Location in CTD**
In Vitro Metabolism of [^{3}H]LEN in Hepatic Microsomes and Hepatocytes	**Metabolism**	**[^{3}H]LEN**	**AD-200-2022**	**4.2.2.4**

Study System	Cryopreserved hepatocytes from mouse, rat, dog, cynomolgus and rhesus monkeys, and human
Method	[^{3}H]LEN (0.25 µM) was incubated for up to 6 hours at 37°C in hepatocytes. Quantification of [^{3}H]LEN was performed by radio flow chromatography.

Species	**n**	**Predicted Hepatic Clearance (L/h/kg)**	**Mean Predicted Hepatic Extraction (%)**
C57BL/6 Mouse	2	0.03	< 1
Sprague-Dawley Rat	2	0.02	< 1
Beagle Dog	2	0.06	3
Cynomolgus Monkey	3	0.05 ± 0.01	3
Rhesus Monkey	3	0.15 ± 0.03	6
Human	3	0.01 ± 0.00	1

5.4. AD-200-2008: Metabolites of [^{14}C]LEN Detected in Hepatic Microsomes

Test Article: LEN

Report Title	Study Type	Test Article	Report Number	Location in CTD
In Vitro Metabolism of [^{14}C]LEN in Rat, Dog, Monkey and Human Hepatic Microsomes	**Metabolism**	**[^{14}C]LEN**	**AD-200-2008**	**4.2.2.4**

Study System	Pooled hepatic microsomes from rats, dogs, cynomolgus and rhesus monkeys, and humans
Method	[^{14}C]LEN (10 µM) was incubated in rat, dog, rhesus monkey, cynomolgus monkey, and human microsomes at 37ºC for 0 and 120 minutes in the presence of NADPH, UDPGA, and GSH co-factors.

Analyte	Measured MH^+ Accurate Mass (m/z)	Identity	Percent of Injected Radioactivity					
			Wister Han Rat	Beagle Dog	Cynomolgus Monkey	Rhesus Monkey	Human	Control[a]
LEN.1	968.1485	Parent	14.2	14.2	14.4	13.4	14.0	14.4
LEN.2	968.1488	Parent	79.7	79.6	77.2	77.1	80.5	80.3
M4	1271.2198	Hydroxy-desfluoro-LEN-glutathione conjugate-1	ND	ND	1.1	1.8	ND	ND
M8	1271.2202	Hydroxy-desfluoro-LEN-glutathione conjugate-2	ND	ND	0.2	0.3	ND	ND
M9	1275.2321	Dihydro-LEN-glutathione conjugate-1	0.3	ND	ND	ND	ND	ND
M19	984.1431	Oxy-LEN-2	ND	0.3	ND	0.2	ND	ND
M31	NA	Impurity	2.2	2.5	2.4	2.4	2.4	2.4
M32	NA	Impurity	1.5	1.5	2.0	2.4	1.5	1.2
Total	NA	NA	97.9	98.1	97.2	97.5	98.3	98.3

GSH = glutathione; MH^+ = protonated molecular ion; ND = peak not detected or below the established limit of quantitation (1% of run and 10 cpm peak height)
a Control incubations included incubation of [^{14}C]LEN and hepatic microsomes in the absence of cofactors.
LEN.1 and LEN.2 refer to the first-eluting (minor) and second-eluting (major) atropisomers, respectively.

5.5. AD-200-2037: Metabolites of [^{14}C]LEN Detected in Hμrel® Hepatic Co-cultures

Test Article: LEN

Report Title	Study Type	Test Article	Report Number	Location in CTD
In Vitro Metabolism of [14C]LEN in Rat, Dog, Monkey and Human Using Hμrel® Hepatic Co-cultures Systems	**Metabolism**	**[^{14}C]LEN**	**AD-200-2037**	**4.2.2.4**

Study System	HμREL®'s cell-based hepatic models are comprised of primary cryopreserved hepatocytes cultured together with cells of a non-parenchymal, stromal type which have demonstrated cellular competence to persist for weeks. HμREL hepatocyte co-culture plates including pooled cryopreserved hepatocytes from a female pool of Sprague Dawley rats, a male pool of Beagle dogs, male pool of cynomolgus monkeys and a mixed-sex human pool were obtained from Hμrel® Corporation (North Brunswick, New Jersey). HμREL plates were stored according to the supplier's information.
Method	Metabolic activity of Hμrel® hepatic co-cultures was assessed by quantitating warfarin (1 μM) metabolism and by characterizing warfarin metabolites. The metabolism of LEN was determined using Hμrel® hepatic co-cultures at a concentration of 10 μM (0.222 μCi/mL) at 37°C in an atmosphere of 5% CO_2 for 0, 72, and 168 hours in duplicate. Incubations were initiated by the addition of test article containing fortification solution. Incubations were terminated by the addition of acetonitrile.

Analyte	MH^+ Mass (m/z)	Retention Time (minutes)	Proposed Identification	Percent of Radioactivity at 168 Hour				
				Stromal	Rat	Dog	Monkey	Human
M9	1275.2330	38.17	Dihydro-LEN-GSH conjugate-1	ND	0.64	ND	ND	ND
M33	1089.1681	38.50-38.83	Dihydro-LEN-cysteine conjugate	ND	1.69	ND	0.54	0.41
M10	1275.2358	39.17	Dihydro-LEN-GSH conjugate-2	ND	0.48	ND	ND	ND
M11	1275.2325	39.50-39.83	Dihydro-LEN-GSH conjugate-3	ND	1.37	ND	ND	0.29
M13	1144.1831	41.67-41.83	LEN-glucuronide	ND	0.73	ND	1.46	ND
M35	1130.2051	42.17	LEN-hexose-conjugate	ND	ND	ND	ND	0.41
M16	1147.2084	43.50-43.67	Unknown	ND	ND	ND	0.85[a]	ND
M29	1100.1923	43.50-43.67	LEN-pentose conjugate	ND	ND	ND	0.85[a]	ND
LEN.1	968.1495	49.50-49.83	LEN.1	15.66	14.53	16.04	14.51	14.58
LEN.2	968.1491	51.00-51.33	LEN.2	76.30	72.19	77.49	75.71	77.09

GSH = glutathione; MH^+ = protonated molecular ion; ND = peak not detected or below the established limit of quantitation (1% of run and 10 cpm peak height)
a Metabolites co-eluted.
LEN.1 and LEN.2 refer to the first-eluting (minor) and second-eluting (major) atropisomers, respectively.

5.6. AD-200-2002: In Vivo Metabolite Profiling of [^{14}C]LEN in Rats Following Intravenous Administration

Test Article: LEN

Report Title	Study Type	Test Article	Report Number	Location in CTD
Metabolite Profiling and Identification of Metabolites in Rat Plasma, Urine, Bile, and Feces Following a Single Intravenous Administration of [^{14}C]LEN	**Metabolism**	**[^{14}C]LEN**	**AD-200-2002**	**4.2.2.4**

Study System	Plasma, urine, bile, and feces from intact and bile-duct cannulated Sprague-Dawley rats
Method	Each sample was pooled by collection time point/interval. Metabolite profiling of [^{14}C]LEN derived radioactivity with high resolution mass spectrometry with radio detection.

Analyte	Measured Accurate MH^+ Mass (m/z)	Modifications	% Abundance[a]			
			Intact Rats		BDC Rats	
			Plasma	Feces	Feces	Bile
LEN.1	968.1490	Parent	11.9	6.5	2.8	0.03
LEN.2	968.1496	Parent	87.5	57.7	27.7	0.05
M1	1142.1797	LEN-cysteine-glycine conjugate	ND	ND	ND	1.7
M4	1271.2218	Hydroxy-desfluoro-LEN-glutathione conjugate-1	ND	ND	ND	13.9
M8	1271.2218	Hydroxy-desfluoro-LEN-glutathione conjugate-2	ND	ND	ND	2.9
M9	1275.2329	Dihydro-LEN-glutathione conjugate-1	ND	ND	ND	3.9
M10	1275.2329	Dihydro-LEN-glutathione-conjugate-2	ND	ND	ND	1.8
M11	1275.2333	Dihydro-LEN-glutathione-conjugate-3	ND	ND	ND	2.0
M13	1144.1821	LEN-glucuronide	ND	2.2[b]	ND	5.7
M15	984.1444	Oxy-LEN-1	ND	ND	ND	ND
M19	984.1440	Oxy-LEN-2	ND	1.2	ND	ND
M20	886.1456	*N*-destrifluoroethyl-LEN	ND	1.5	ND	0.3

a Abundance = % radioactive dose in urine and feces and % [^{14}C]-peak area in AUC-pooled plasma. Only metabolites identified with assigned structures and with > 1% of the dose in either feces or bile are listed.
b Co-eluted with M15.
MH^+, protonated molecular ion; ND: not detected.

5.7. AD-200-2004: In Vivo Metabolite Profiling of [^{14}C]LEN in Dogs Following Intravenous Administration

Test Article: LEN

Report Title	Study Type	Test Article	Report Number	Location in CTD
Metabolite Profiling and Identification of Metabolites in Dog Plasma, Urine, Bile, and Feces Following a Single Intravenous Administration of [^{14}C]LEN	**Metabolism**	**[^{14}C]LEN**	**AD-200-2004**	**4.2.2.4**

Study System	Plasma, urine, bile, and feces from intact and bile-duct cannulated dogs
Method	Each sample was pooled by collection time point/interval. Metabolite profiling of [^{14}C]LEN derived radioactivity with high resolution mass spectrometry with radio detection.

Analyte	Measured MH^+ Accurate Mass (m/z)	Modifications	% Abundance[a]			
			Intact Dogs		BDC Dogs	
			Plasma	Feces	Feces	Bile
LEN.1	968.1490	LEN	14.0	9.2	7.6	4.4
LEN.2	968.1496	LEN	85.3	55.9	52.0	17.0
M13	1144.1821	LEN-glucuronide	ND	4.2	ND	2.4
M19	984.1440	Oxy-LEN-2	ND	4.8	ND	2.6
M29	1100.1910	LEN-pentose conjugate	ND	1.4	ND	ND

a Abundance = % radioactive dose in urine and feces and % [^{14}C]-peak area in AUC-pooled plasma. Only metabolites identified with assigned structures and with > 1% of the dose in either feces or bile are listed.

MH^+, protonated molecular ion; ND: not detected.

5.8. AD-200-2004: In Vivo Metabolic Pathways of [^{14}C]LEN in Dogs Following Oral Administration

Test Article: LEN

Report Title	**Study Type**	**Test Article**	**Report Number**	**Location in CTD**
Metabolite Profiling and Identification of Metabolites of [^{14}C]LEN in Selected Plasma, Urine, and Feces Samples Following a Single Oral Dose of [^{14}C]LEN in Dogs	**Metabolism**	**[^{14}C]LEN**	**AD-200-2004**	**4.2.2.4**

Study System	Plasma, urine and feces from dogs
Method	Each sample was pooled by collection time point/interval. Metabolite profiling of [^{14}C]LEN derived radioactivity with high resolution mass spectrometry with radio detection.

Analyte	**Measured MH$^+$ Accurate Mass (m/z)**	**Modifications**	**% Abundance[a]**	
			Plasma	**Feces**
LEN.1	968.1490	LEN	12.7	11.0
LEN.2	968.1496	LEN	85.8	69.8
M19	984.1440	Oxy-LEN	ND	0.53
M31	NA	Impurity	ND	0.66
M32	NA	Impurity	ND	0.15

ND = peak not detected.
a Abundance = % administered [^{14}C]LEN dose in feces; % [^{14}C]-radiochromatogram in AUC-pooled plasma. Only metabolites identified with assigned structures and with > 0.1% of the dose in feces are listed.

5.9. AD-200-2056: Identification of Lenacapavir Late-eluting Peaks in Human Plasma and Nonclinical Samples

Test Article: LEN

Report Title	Study Type	Test Article	Report Number	Location in CTD
Identification of lenacapavir late-eluting peaks in human plasma and nonclinical samples	**Metabolism**	**[^{14}C]LEN**	**AD-200-2056**	**4.2.2.4**

Study System	Plasma from human ^{14}C ADME study (GS-US-200-4329); nonclinical samples from previous studies (see notes below); control samples
Method	Human plasma samples were pooled to form a ^{14}C $AUC_{0\text{-}168h}$ representative sample. Metabolite profiling of [^{14}C]LEN derived radioactivity with high resolution mass spectrometry with radio detection.

Summary of Late Eluting Components in Human Plasma and in Nonclinical Samples

Component Designation	Retention Time (Minutes)	m/z	Human		Dog (AD-200-2004)				Rat (AD-200-2002)		
			% ^{14}C $AUC_{0\text{-}168h}$[b]	Plasma[b]	Plasma	Feces	Bile	Dose Solution	Plasma	Feces	Bile
*不純物9 (M31)[a]	52.60-54.50	910	1.04	X	X[c,d]	X[c,d]	X[c,d]	X[c,d]	-	-	-
M32 (dioxy-dihydro-*不純物32)[a]	59.40-59.50	888	3.35	X	X[c,d]	-	-	X[c,d]	-	-	-
*不純物8 [a]	59.40-60.20	890		X	X[c]	-	-	X[c]	-	-	-
*不純物32 [a]	59.10-59.20	924		X	X[c]	-	-	X[c]	-	X[c]	X[c]
Result	The identified components each eluted after LEN (retention time 49.28 – 50.69 min) and are referred to as late-eluting peaks. These late-eluting peaks were also observed in LEN photolyzed (under ultraviolet light) solution and in the radiolysed [^{14}C]LEN stock solution. The combined data suggest that the late-eluting components in human plasma and nonclinical samples were likely due to the impurities generated by radiolysis and/or the exposure of these samples to light and not due to LEN metabolism.										

NA = not applicable. An entry of "X" indicates component detected.
a All four components were also detected in Hurel hepatic co-culture systems (AD-200-2037) and in liver microsomes in vitro.
b Plasma $AUC_{0\text{-}168h}$ pooled sample from study GS-US-200-4329.
c Components detected in select plasma and excreta. Identity was based on full-scan high-resolution mass spectrometry data.
d Radiolabeled analyte is the dominant component.

6. PHARMACOKINETICS: INDUCTION/INHIBITION OF DRUG METABOLIZING ENZYMES

6.1. AD-200-2028: CYP Inhibition Potential of LEN

Test Article: LEN

Report Title	Study Type	Test Article	Report Number	Location in CTD
In Vitro Assessment of Human Cytochrome P450 Inhibition Potential of LEN	**Metabolism**	**LEN**	**AD-200-2028**	**4.2.2.6**

Study System	Human liver microsomes
Method	Lenacapavir (0 – 25 µM) or control inhibitors were incubated with human liver microsomes and NADPH in the presence of probe substrates for individual enzymes. All assays were conducted under conditions that were linear with respect to time and protein concentration. The enzyme-specific probe substrate metabolites were quantified by LC-MS/MS.

		Control Inhibitor[a]	LEN	
CYP Enzyme	**Activity**	**IC_{50} (µM)[b]**	**% inhibition at 25 µM**	**IC_{50} (µM)[b]**
CYP1A2	Phenacetin *O*-deethylase	0.06	0%	> 25
CYP2B6	Bupropion hydroxylase	1.3	0%	> 25
CYP2C8	Paclitaxel 6α-hydroxylase	0.7	23.8%	> 25
CYP2C9	Tolbutamide 4-hydroxylase	0.5	0%	> 25
CYP2C19	*S*-Mephenytoin 4'-hydroxylase	6.9	8.8%	> 25
CYP2D6	Dextromethorphan *O*-demethylase	0.05	0%	> 25
CYP3A	Midazolam 1'-hydroxylase	0.07	66%	5.4
	Testosterone 6β-hydroxylase	0.3	0%	> 25

CYP = cytochrome P450 enzyme; LC-MS/MS = high performance liquid chromatography coupled to tandem mass spectrometry; NADPH = nicotinamide adenine dinucleotide phosphate, reduced

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

b Values are the mean of 7 determinations.

6.2. AD-200-2028: CYP3A Mechanism-Based Inhibition Potential of LEN

Test Article: LEN

Report Title	Study Type	Test Article	Report Number	Location in CTD
In Vitro Assessment of Human CYP3A Mechanism Based Inhibition Potential of LEN	**Metabolism**	**LEN**	**AD-200-2028**	**4.2.2.6**

Study System	Human liver microsomes
Method	Two-stage incubation was used to test the potential for LEN (25 µM) to act as a mechanism-based inhibitor of human CYP3A. The first stage contained pooled human hepatic microsomal fractions diluted in 50 mM potassium phosphate buffer pH 7.4 to a final concentration of 1 mg/mL. Lenacapavir was added to a final concentration of 25 µM. The incubation was carried out at 37°C for 30 minutes in the absence or presence of 1 mM NADPH as the CYP cofactor. After this time, the incubation mixture was diluted 10-fold with 50 mM potassium phosphate buffer pH 7.4, plus midazolam substrate (final concentration 12.5 µM which is ~5× the apparent K_M) and fresh NADPH (1 mM final). The second-stage incubation was carried out at 37°C and then terminated by the addition of five volumes of methanol. The enzyme-specific metabolite, 1'-hydroxy-midazolam was quantified by LC-MS/MS. The inhibitory potencies (IC_{50} values) were measured following pre-incubation of LEN with hepatic microsomal fraction in the absence or presence of NADPH. The IC_{50} shift is the ratio of the +NADPH IC_{50} to the -NADPH IC_{50}. A value greater than 2 indicates potential for mechanism-based inhibition.

	IC_{50} (nM)		
Compound	**-NADPH**	**+NADPH**	**IC_{50} Shift (Fold)**
LEN	1560	298	5.2
Triacetyloleandomycin	5640	231	24.4

NADPH = nicotinamide adenine dinucleotide phosphate, reduced
Values are the mean of duplicate measurement.

6.3. AD-200-2050: Human CYP3A Mechanism-Based Inhibition Kinetics of LEN

Test Article: LEN

Report Title	Study Type	Test Article	Report Number	Location in CTD
In Vitro Assessment of Human CYP3A Mechanism Based Inhibition Kinetics of LEN	**Metabolism**	**LEN**	**AD-200-2050**	**4.2.2.6**

Study System	Human liver microsomes
Method	Assays were performed in triplicate using a two-step incubation protocol. The first-step incubations were performed in a final volume of 750 µL containing 2 mg protein/mL of human hepatic microsomal fraction, LEN and 1 mM NADPH in 0.1 M potassium phosphate buffer, pH 7.4. After incubation with mixing at 37°C for 0, 5, 10, 15, 20, 25, and 30 min, 25 µL aliquots were transferred to the second-step incubation (total volume: 250 µL) consisting of pre-warmed (37°C) phosphate buffer, 1 mM NADPH and midazolam substrate (2,5 µM). Following incubation at 37°C for 6 min, the second-step incubation was terminated by the addition of 150 µL of an ice-cold acetonitrile and methanol mixture (9:1, v/v) containing 200 nM labetalol as the mass spectrometric internal standard. Following centrifugation, aliquots of the supernatants were analyzed by LC-MS/MS. The parameters, k_{inact} and K_I, characterizing the inactivation kinetics were determined by Kitz-Wilson analysis.

Parameter	Best Fit Value	Standard Error	95% Confidence Interval
k_{inact} (min^{-1})	0.021	0.001	0.019 – 0.024
K_I (µM)	1.14	0.32	0.60 – 2.10

LC-MS/MS = high performance liquid chromatography coupled to tandem mass spectrometry; NADPH = nicotinamide adenine dinucleotide phosphate, reduced
Values are the mean of duplicate measurement.

6.4. AD-200-2031: UGT1A1 Inhibition Potential of LEN

Test Article: LEN

Report Title	Study Type	Test Article	Report Number	Location in CTD
In Vitro Assessment of Human UGT1A1 Inhibition Potential of LEN	**Metabolism**	**LEN**	**AD-200-2031**	**4.2.2.6**

Study System	Insect cell microsomal fraction containing baculovirus-expressed human UGT1A1 (Supersomes™)
Method	Lenacapavir (0.2 – 50 µM) or the control inhibitor (atazanavir) was incubated with insect cell microsomal fraction containing baculovirus-expressed human UGT1A1 (Supersomes™) (final protein concentration 0.25 mg/mL), alamethicin (25 µg/mL) and UDP-glucuronic acid (5 mM), and the substrate, estradiol (10 µM)). The UGT1A1-selective metabolite, estradiol 3-glucuronide, was monitored by LC-MS/MS and a decrease in the formation of the metabolite compared to the vehicle control was used to calculate an IC_{50} value.

		Calculated IC_{50} (µM)	
Enzyme	**Activity**	**Atazanavir**	**LEN**
UGT1A1	Estradiol-3-glucuronidation	0.3	3.2

LC-MS/MS = high performance liquid chromatography coupled to tandem mass spectrometry; UDP = uridine diphosphate; UGT = uridine diphosphate glucuronosyltransferase

6.5. AD-200-2030 and AD-200-2054: Cytochrome P450 Reaction Phenotyping of [^{3}H]LEN

Test Article: LEN

Report Title	Study Type	Test Article	Report Number	Location in CTD
Cytochrome P450 Metabolic Reaction Phenotyping of LEN	**Metabolism**	**[^{3}H]LEN**	**AD-200-2030 AD-200-2054**	**4.2.2.6**

Study System	Individual cDNA expressed human CYP enzyme preparations (Supersomes™)
Method	[^{3}H]LEN (2 µM) was incubated with individual cDNA expressed human CYP enzyme preparations co-expressed with human NADPH CYP reductase (Supersomes™) for 60 min. Compounds known to be metabolized by each CYP enzyme were used as positive controls.

	Positive Control Metabolism			LEN Metabolism	
		Rate of metabolite formation (PAR/min)		Rate of formation of CYP-M1 (pmol/h/pmol)	
Enzyme	**Substrate**	**Rep 1**	**Rep 2**	**Rep 1**	**Rep 2**
CYP1A2	Phenacetin	0.19	0.19	ND	ND
CYP2B6	Bupropion	0.69	0.71	ND	ND
CYP2C8	Paclitaxel	0.03	0.03	ND	ND
CYP2C9	Diclofenac	0.05	0.06	ND	ND
CYP2C19	S Mephenytoin	0.01	0.01	ND	ND
CYP2D6	Dextromethorphan	0.38	0.42	ND	ND
CYP3A4	Terfenadine	0.14	0.14	ND	ND
CYP3A5	Terfenadine	0.06	0.06	0.07	0.11
None	-	-	-	ND	ND

CYP3A5	Further analysis of the chromatograms obtained following incubation of [^{3}H]LEN with hepatic microsomal fractions from donors genotyped as homozygous CYP3A5 wild type (*1/*1) or homozygous deficient (*3/*3) was conducted. While rates of metabolism were below the limit of quantification, examination of the radiochromatograms revealed that the formation of CYP-M1 was very similar for the CYP3A5 *1/*1 and CYP3A5 *3/*3 individuals. This indicates that the metabolism of LEN is unlikely to display CYP3A5 pharmacogenetic variability.

NADPH = nicotinamide adenine dinucleotide phosphate, reduced; ND = not detectable if peak area ≤ 1 mV × min (rate < 0.03 pmol/hr/pmol); PAR = peak area ratio.
Positive Control Metabolites: CYP1A2 Acetaminophen, CYP2B6 Hydroxybupropion, CYP2D6 Dextrorphan, CYP2C8 6α-Hydroxypaclitaxel, CYP2C19 Hydroxymephenytoin, CYP2C9 4'-Hydroxydiclofenac, CYP3A4 fexofenadine, CYP3A5 fexofenadine

6.6. AD-200-2033: UDP Glucuronosyl Transferase Metabolic Reaction Phenotyping of [^{3}H]LEN

Test Article: LEN

Report Title	Study Type	Test Article	Report Number	Location in CTD
UDP Glucuronosyl Transferase Metabolic Reaction Phenotyping of LEN	**Metabolism**	**[^{3}H]LEN**	**AD-200-2033**	**4.2.2.6**

Study System	Individual cDNA expressed human UGT enzyme preparations (Supersomes^{TM})
Method	[^{3}H]LEN (2 µM) was incubated with individual cDNA expressed human UGT enzyme preparations (Supersomes^{TM}) in the presence of UDPGA for 60 min. Compounds known to be metabolized by each UGT enzyme were used as positive controls.

	Positive Control					UGT-M1 formation from [^{3}H]LEN (pmol/hr/mg)	
		% remaining at 60 min		Metabolite formation (PAR/hr)			
Enzyme	**Substrate**	**#1**	**#2**	**#1**	**#2**	**#1**	**#2**
UGT1A1	Raloxifene	24	22	-	-	7.5	6.8
UGT1A3	Raloxifene	68	61	-	-	ND	ND
UGT1A4	Trifluoperazine	-	-	0.01	0.01	ND	ND
UGT1A6	7-Hydroxycoumarin	<10	< 10	-	-	ND	ND
UGT1A7	7-Hydroxycoumarin	48	63	-	-	ND	ND
UGT1A8	7-Hydroxycoumarin	56	62	-	-	ND	ND
UGT1A9	7-Hydroxycoumarin	<10	< 10	-	-	ND	ND
UGT1A10	Raloxifene	14	19	-	-	ND	ND
UGT2B4	4-Hydroxyestradiol	50	57	-	-	ND	ND
UGT2B7	4-Hydroxyestradiol	<10	< 10	-	-	ND	ND
UGT2B15	Scopoletin	<10	< 10	-	-	ND	ND
UGT2B17	4-Hydroxyestradiol	47	51	-	-	ND	ND

ND = not detectable if peak area ≤ 1 mV × min (rate < 0.5 pmol/hr/mg); PAR = peak area ratio; UDPGA = uridine diphosphate glucuronic acid; UGT = uridine diphosphate glucuronosyltransferase
Positive control substrates: raloxifene (3 µM) for UGT1A1, UGT1A3 and UGT1A10; trifluoperazine (3 µM) for UGT1A4; 7-hydroxy-coumarin (10 µM) for UGT1A6, UGT1A7, UGT1A8 and UGT1A9; 4-hydroxyestradiol (10 µM) for UGT2B4, UGT2B7 and UGT2B17; scopoletin (10 µM) for UGT2B15.

6.7. AD-200-2029: In Vitro Assessment of the Effect of LEN on AhR, CAR and PXR

Test Article: LEN

Report Title	Study Type	Test Article	Report Number	Location in CTD
In Vitro Assessment of the Induction Potential of LEN	**Metabolism**	**LEN**	**AD-200-2029**	**4.2.2.6**

Study System	For AhR activation assay, the Dioxin Response Element 12.6 cells were transformed with an expression vector for human AhR and the DRE of the human CYP1A2 gene linked to a luciferase reporter. For PXR activation assay, DPX2 cells were stably transformed with an expression vector for human PXR and a reporter gene vector containing the enhancer regions of CYP3A4 linked to luciferase. For CAR activation assay, HepG2 cells were transfected with an expression vector for human CAR1.
Method	Lenacapavir (0.15 – 50 µM) or positive controls were incubated with the cells for 24 h, after which the luciferase substrate 5'-fluoroluciferin was added and the luminescence was read in a luminometer. The average luminescent units for three replicates were divided by the average for the DMSO solvent control to determine the fold-activation.

Concentration (µM)	**Fold Activation Over 0.1-0.5% DMSO Control (Mean, n=2)**					
	AhR		**CAR**		**PXR**	
	LEN	**3-Methylcholanthrene[a]**	**LEN**	**CITCO[a]**	**LEN**	**Rifampicin[a]**
0.005	-	-	-	1.6	-	-
0.025	-	-	-	-	-	1.1
0.05	-	2.2	-	-	-	-
0.1	-	-	-	2.7	-	1.5
0.15	1.0	-	1.1	-	1.3	-
0.5	1.0	4.2	1.4	5.5	1.5	3.3
1.0	-	7.2	-	10.0	-	5.2
1.5	1.2	-	1.4	-	3.3	-
2.5	-	-	-	-	-	9.1
5.0	1.2	32.1	1.1	13.4	5.5	-
10	-	41.5	-	19.4	-	13.4
15	1.3	-	0.9	-	4.6	-

Test Article: LEN

Concentration (µM)	Fold Activation Over 0.1% DMSO Control (Mean, n=2)					
	AhR		CAR		PXR	
	LEN	3-Methylcholanthrene[a]	LEN	CITCO[a]	LEN	Rifampicin[a]
20	-	46.4	-	18.0	-	-
25	-	-	-	-	-	15.0
50	1.5	-	0.7	-	4.9[b]	-
EC_{50} (µM)	NA	2.7	NA	1.3	9.9	2.0

AhR = aryl hydrocarbon receptor; CAR = constitutive androstane receptor; CITCO = 6-(4-chlorophenyl)imidazo[2,1-b]thiazole-5-carbaldehyde O-(3,4-dichlorobenzyl)oxime; CYP = cytochrome P450 enzyme; DMSO = dimethyl sulfoxide; NA = not applicable (activation < 2-fold at all concentrations); PXR = pregnane X receptor.
a 3-Methylcholanthrene is a positive control for AhR activation; CITCO is a positive control for CAR activation; rifampicin is a positive control for PXR activation.
b Precipitation noted

6.8. AD-200-2049: In Vitro Characterization of LEN for CYP Induction Potential Using Sandwich-Cultured Transporter-Certified™ Hepatocytes

Test Article: LEN

Report Title	Study Type	Test Article	Report Number	Location in CTD
In Vitro Characterization of LEN for CYP Induction Potential Using Sandwich-Cultured Transporter-Certified™ Hepatocytes	**Metabolism**	**LEN**	**AD-200-2049**	**4.2.2.6**

Study System	Lenacapavir was evaluated for its potential to induce CYP1A2, CYP2B6, CYP3A4, CYP2C9, P-gp and UGT1A1. Transporter Certified™, cryopreserved human hepatocytes from three donors were cultured in a sandwich configuration to maximize the function of drug transporters and CYP enzymes.
Method	The cytotoxicity potential of LEN in sandwich-cultured human hepatocytes was evaluated following 48 and 72 hours of exposure to LEN. Fresh dosing solutions containing LEN were applied every 24 hours at seven concentrations (0.01, 0.03, 0.1, 0.3, 1, 3, and 10µM). Morphology was evaluated daily for each donor, exposure time (24, 48, and 72 hours), and dose concentration. The potential to reduce hepatocyte cell viability was assessed using leakage of lactate dehydrogenase from the hepatocytes and quantitation of intracellular adenosine triphosphate content. The experiments were performed in 24-well format using three lots of Transporter-Certified™ cryopreserved human hepatocytes. A cytotoxicity assessment of a positive control toxicant (tamoxifen) was performed in parallel using the same donors and study design. Each test condition was performed in three wells to provide triplicate data, expressed as mean ± standard deviation.

Enzyme	Concentration (μM)	Mean mRNA Changes in Hepatocyte Donors BXW, EGO and WID Treated for 48 Hours					
		Donor BXW		Donor EGO		Donor WID	
		Fold Over Control (FOC)	% Positive Control[a]	FOC	% Positive Control[a]	LEN	% Positive Control[a]
CYP1A2	0.1	0.819	-1.13	1.27	0.896	1.53	2.95
	1.0	0.702	-1.85	0.888	-0.371	1.03	0.166
	10	0.456	-3.38	0.930	-0.232	0.431	-3.20
CYP2B6	0.1	0.825	-1.94	1.41	3.11	1.53	4.48
	1.0	0.737	-2.91	2.33	10.0	1.19	1.59
	10	0.357	-7.12	1.60	4.53	0.203	-6.92
CYP3A4	0.1	1.62	4.53	3.16	5.63	2.05	10.0
	1.0	1.33	2.37	5.62	12.1	1.56	5.25
	10	1.93	6.74	9.51	22.2	0.954	-0.521
CYP2C9	0.1	0.845	-10.4	1.51	22.5	1.55	25.0
	1.0	0.684	-21.0	1.75	33.2	1.23	10.0
	10	0.782	-14.5	2.29	57.0	0.622	-17.8
P-gp	0.1	0.660	-26.7	0.893	-10.2	1.18	12.8
	1.0	1.23	18.3	1.18	17.0	1.48	34.8
	10	1.04	2.71	1.74	69.8	0.911	-6.92
UGT1A1	0.1	0.828	-5.58	1.09	6.51	1.31	22.1
	1.0	1.47	15.2	1.36	26.7	1.27	19.4
	10	2.48	47.8	1.59	43.6	1.16	11.6

CYP = cytochrome P450 enzyme; P-gp = P-glycoprotein; UGT1A1 = uridine diphosphate glucuronosyltransferase 1A1
a Omeprazole (50 μM) is a positive control for CYP1A2 induction; Phenobarbital (1000 μM) is a positive control for CYP2B6/CYP3A4/P-gp/UGT1A1 induction; Rifampicin (10 μM) is a positive control for CYP3A4/CYP2C9 induction.

7. PHARMACOKINETICS: EXCRETION

7.1. AD-200-2024: Excretion of LEN in Bile Duct Cannulated Rats Following Intravenous Administration

Test Article: LEN

Report Title	Study Type	Test Article	Report Number	Location in CTD
Pharmacokinetics of LEN Following Intravenous Infusion to Male Bile Duct Cannulated Rats	**Excretion**	**LEN**	**AD-200-2024**	**4.2.2.5**

Species	Sprague-Dawley rat (bile duct cannulated)	
Sex / No. of Animals	Male/3	
Feeding Condition	Fasted	
Vehicle / Formulation	5% ethanol, 20% propylene glycol, 45% polyethylene glycol 300 and 30% 0.01 N hydrochloric acid	
Method of Administration	30-minute intravenous infusion	
Dose (mg/kg)	1.0	
Analyte	LEN	
Assay	LC-MS/MS	
	Excretion Route	
Collection Period (h)	**Bile**	**Urine**
0–4	BLQ	BLQ
0–8	BLQ	BLQ
0–12	BLQ	BLQ
0–24	BLQ	BLQ

BLQ = below the lower limit of quantitation (< 0.1% of dose); h = hours; LC-MS/MS = high performance liquid chromatography coupled to tandem mass spectrometry

7.2. AD-200-2045: Excretion and Pharmacokinetics of LEN in Solution Following Intravenous Administration in Rats

Test Article: LEN

Report Title	Study Type	Test Article	Report Number	Location in CTD
Excretion and Pharmacokinetics of LEN Following IV Infusion in Male Wistar Han Rats Pretreated Without and With Elacridar	**Other**	**LEN**	**AD-200-2045**	**4.2.2.5**
Species/Strain	Rat/Wistar Han		Rat/Wistar Han	
Sex/ No. of Animals per group	Male/3		Male/4	
Feeding Condition	Fasted		Fasted	
Sample	Plasma, feces		Plasma, feces	
Analyte	LEN		LEN	
Assay	LC-MS/MS		LC-MS/MS	
Method of Administration	30-minute intravenous infusion		30-minute intravenous infusion	
LEN Dose (mg/kg)	1.0		1.0	
Elacridar Dose (mg/kg)	0		30 (PO BID, total 7 doses)	
Vehicle/Formulation	67.8% PEG 300, 31.6% water, 0.3% LEN and 0.3% 1N NaOH		68.1% PEG 300, 31.6% water and 0.3% 1N NaOH	
PK Parameters				
AUC_{inf} (nM•h)	11300 ± 1370		20200 ± 2920	
C_{max} (nM)	804 ± 114		644 ± 139	
MRT (h)	71.4 ± 21.6		90.5 ± 14.2	
$t_{1/2}$ (h)	51.2 ± 21.2		58.1 ± 10.1	
V_{ss} (L/kg)	6.45 ± 1.50		4.67 ± 0.72	
CL (L/h/kg)	0.09 ± 0.01		0.05 ± 0.01	
% excretion as LEN in feces	21.9 ± 1.1		7.4 ± 1.2	

AUC_{inf} = area under the plasma concentration-time curve extrapolated to time infinity; CL = plasma clearance; C_{max} = maximum plasma concentration; Fasted = animals were fasted overnight prior to dose administration and up to 4 hours after dosing; LC-MS/MS = high performance liquid chromatography coupled to tandem mass spectrometry; MRT = mean residence time; PEG = polyethylene glycol; PK = pharmacokinetic; $t_{1/2}$ = estimated plasma elimination half-life; T_{max} = time to reach the maximum plasma concentration; V_{ss} = volume of distribution at steady state.

7.3. AD-200-2046: Excretion and Pharmacokinetics of LEN in Solution Following Intravenous Administration in Bile Duct Cannulated Rats

Test Article: LEN

Report Title	Study Type	Test Article	Report Number	Location in CTD
Excretion and Pharmacokinetics of LEN Following IV Infusion in Male BDC Wistar Han Rats Pretreated Without and With Elacridar	**Other**	**LEN**	**AD-200-2046**	**4.2.2.5**
Species/Strain	Bile-duct Cannulated Rat/Wistar Han		Bile-duct Cannulated Rat/Wistar Han	
Sex/ No. of Animals per group	Male/6		Male/4	
Feeding Condition	Fasted		Fasted	
Sample	Plasma, bile, urine, feces		Plasma, bile, urine, feces	
Analyte	LEN		LEN	
Assay	LC-MS/MS		LC-MS/MS	
Method of Administration	30-minute intravenous infusion		30-minute intravenous infusion	
LEN Dose (mg/kg)	10		10	
Elacridar Dose (mg/kg)	0		30 (PO twice daily, total 7 doses)	
Vehicle/Formulation	67.8% PEG 300, 31.6% water, 0.3% LEN and 0.3% 1N NaOH		68.1% PEG 300, 31.6% water and 0.3% 1N NaOH	
PK Parameters				
AUC_{inf} (µM•h)	227 ± 34		381 ± 73	
C_{max} (µM)	8.93 ± 1.71		13.4 ± 15.0	
V_{ss} (L/kg)	1.83 ± 0.40		1.44 ± 0.24	
CL (L/h/kg)	0.046 ± 0.000		0.028 ± 0.005	
% excretion as LEN in bile	0.2 ± 0.1		0.2 ± 0.1	
% excretion as LEN in urine	BLQ		BLQ	
% excretion as LEN in feces	42.0 ± 5.3		19.6 ± 2.5	

AUC_{inf} = area under the plasma concentration-time curve extrapolated to time infinity; BLQ: below the limit of quantitation (< 0.1% of dose); CL = plasma clearance; C_{max} = maximum plasma concentration; Fasted = animals were fasted overnight prior to dose administration and up to 4 hours after dosing; LC-MS/MS = high performance liquid chromatography coupled to tandem mass spectrometry; MRT = mean residence time; PEG = polyethylene glycol; PK = pharmacokinetic; $t_{1/2}$ = estimated plasma elimination half-life; T_{max} = time to reach the maximum plasma concentration; V_{ss} = volume of distribution at steady state.

7.4. AD-200-2006: Excretion of [^{14}C]LEN in Urine, Bile, and Feces Following Intravenous Administration to Rats

Test Article: LEN

Report Title	**Study Type**	**Test Article**	**Report Number**	**Location in CTD**
Pharmacokinetics, Distribution, and Excretion of [^{14}C]LEN Following Intravenous Administration to Rats	**Excretion**	**[^{14}C]LEN**	**AD-200-2006**	**4.2.2.5**
Species	Wistar Han Rat (bile duct-intact)			
Gender /No. of Animals	Male/3			
Feeding Condition	Nonfasted			
Vehicle/Formulation	5% ethanol, 20% propylene glycol, 45% polyethylene glycol 300, and 30% sterile water for injection, pH 9			
Method of Administration	Intravenous bolus injection			
Dose	3.0 mg/kg (172 µCi/kg)			
Analyte	Carbon-14			
Specific Activity	113 mCi/mmol			
Specific Activity of Formulation	57.3 µCi/mg			
Assay	Liquid scintillation counting			
	Cumulative Recovery of % Administered [^{14}C] Dose (Mean ± SD)			
Collection Period (h)	**Urine**	**Feces**	**Cage (residual)**	**Total[a]**
0 - 24	0.05 ± 0.02	12.6 ± 2.6	-	-
0 - 72	0.09 ± 0.03	52.1 ± 8.7	-	-
0 - 168	0.13 ± 0.04	79.6 ± 5.3	-	-
0 - 336	0.16 ± 0.04	91.1 ± 2.0	-	-
0 - 504	0.16 ± 0.04	93.6 ± 0.7	-	-
0 - 672	0.16 ± 0.04	94.2 ± 0.5	-	-
0 - 816	0.16 ± 0.04	94.4 ± 0.5	0.2 ± 0.0	94.8 ± 0.4

Test Article: LEN

Report Title	Study Type	Test Article	Report Number	Location in CTD
Pharmacokinetics, Distribution, and Excretion of [^{14}C]LEN Following Intravenous Administration to Rats	**Excretion**	**[^{14}C]LEN**	**AD-200-2006**	**4.2.2.5**

Species	Wistar Han rat (bile duct-cannulated)
Gender /No. of Animals	Male/3
Feeding Condition	Nonfasted
Vehicle/Formulation	5% ethanol, 20% propylene glycol, 45% polyethylene glycol 300, and 30% sterile water for injection, pH 9
Method of Administration	Intravenous bolus injection
Dose	3.0 mg/kg (172 µCi/kg)
Analyte	Carbon-14
Specific Activity	113 mCi/mmol
Specific Activity of Formulation	57.3 µCi/mg
Assay	Liquid scintillation counting

	Cumulative Recovery of % Administered [^{14}C] Dose (Mean ± SD)				
Collection Period (h)	**Urine**	**Bile**	**Feces**	**Cage (residual)**	**Total[a]**
0 - 24	0.05 ± 0.03	13.5 ± 4.0	7.5 ± 4.4	-	-
0 - 48	0.08 ± 0.03	22.3 ± 4.7	16.9 ± 5.9	-	-
0 - 72	0.10 ± 0.03	28.4 ± 4.4	23.0 ± 5.7	-	-
0 - 96	0.12 ± 0.03	32.6 ± 3.5	26.8 ± 5.7	-	-
0 - 120	0.13 ± 0.03	36.2 ± 3.0	30.6 ± 4.5	-	-
0 - 144	0.14 ± 0.03	39.3 ± 2.8	33.2 ± 3.6	-	-
0 - 168	0.15 ± 0.03	41.7 ± 2.8	35.2 ± 2.9	14.6 ± 5.2	91.8 ± 1.7

Nonfasted = Certified Rodent Diet #2016C and 2016CM (Envigo RMS, Inc.) were provided ad libitum; SD = standard deviation
a Total recovery of radioactivity includes cage rinses, cage wash, cage wipe, bile cannula rinse, jacket rinse and carcass

7.5. AD-200-2007: Excretion of [^{14}C]LEN in Urine, Bile, and Feces Following Intravenous Administration to Dogs

Test Article: LEN

Report Title	Study Type	Test Article	Report Number	Location in CTD
Pharmacokinetics, Absorption, Distribution, and Excretion of [^{14}C]LEN Following Intravenous Administration to Dogs	**Excretion**	**[^{14}C]LEN**	**AD-200-2007**	**4.2.2.5**

Species	Beagle Dogs (bile duct-intact)
Gender /No. of Animals	Male/3
Feeding Condition	Fed
Vehicle/Formulation	5% ethanol, 20% propylene glycol, 45% polyethylene glycol 300, and 30% pH 2 water
Method of Administration	Intravenous bolus injection
Dose	1.0 mg/kg (15 µCi/kg)
Analyte	Carbon-14
Specific Activity	113 mCi/mmol
Specific Activity of Formulation	15 µCi/mg
Assay	Liquid scintillation counting

Test Article: LEN

Collection Period (h)	Cumulative Recovery of % Administered [^{14}C] Dose (Mean ± SD)			
	Urine	**Feces**	**Cage (residual)**	**Total[a]**
0 - 24	0.09 ± 0.02	0.06 ± 0.11	-	-
0 - 72	0.21 ± 0.04	72.3 ± 11.8	-	-
0 - 168	0.33 ± 0.08	85.7 ± 5.0	-	-
0 - 336	0.41 ± 0.14	86.3 ± 4.6	-	-
0 - 504	0.42 ± 0.15	86.3 ± 4.6	-	-
0 - 672	0.42 ± 0.15	86.3 ± 4.6	-	-
0 - 792	0.42 ± 0.15	86.3 ± 4.6	0.7 ± 0.5	87.5 ± 4.1
Species	Beagle dogs (bile duct-cannulated)			
Gender /No. of Animals	Male/3			
Feeding Condition	Fed			
Vehicle/Formulation	5% ethanol, 20% propylene glycol, 45% polyethylene glycol 300, and 30% pH 2 water			
Method of Administration	Intravenous bolus injection			
Dose	1.0 mg/kg (15 µCi/kg)			
Analyte	Carbon-14			
Specific Activity	113 mCi/mmol			
Specific Activity of Formulation	15 µCi/mg			
Assay	Liquid scintillation counting			

Test Article: LEN

Collection Period (h)	Cumulative Recovery of % Administered [^{14}C] Dose (Mean ± SD)				
	Urine	Bile	Feces	Cage (residual)	Total[a]
0 - 24	0.25 ± 0.11	21.4 ± 9.1	14.9 ± 12.8	-	-
0 - 48	0.38 ± 0.18	27.6 ± 10.1	47.8 ± 1.6	-	-
0 - 72	0.45 ± 0.24	30.2 ± 10.2	56.0 ± 4.9	-	-
0 - 96	0.48 ± 0.26	31.3 ± 10.1	60.9 ± 6.5	-	-
0 - 120	0.51 ± 0.27	31.8 ± 10.0	62.0 ± 6.7	-	-
0 - 144	0.52 ± 0.27	32.0 ± 10.0	62.6 ± 7.0	-	-
0 - 168	0.53 ± 0.27	32.2 ± 10.0	62.8 ± 7.1	-	-
0 - 336	0.54 ± 0.29	32.4 ± 10.0	63.0 ± 7.1	0.1 ± 0.1	96.0 ± 2.6

Fed = High Protein Diet 5L66 (PMI, Inc.) was provided to BDC animals and Certified Canine Diet #5007 (PMI, Inc.) was provided to bile duct-intact animals; SD = standard deviation
a Total recovery of radioactivity includes cage rinses, cage wash, cage wipe, bile cannula rinse, jacket rinse and carcass

7.6. AD-200-2007: Excretion of [^{14}C]LEN Following Oral Administration to Dogs

Test Article: LEN

Report Title	Study Type	Test Article	Report Number	Location in CTD
Pharmacokinetics, Absorption, Distribution and Excretion of [^{14}C]LEN Following Oral Administration to Dogs	**Excretion**	**[^{14}C]LEN**	**AD-200-2007**	**4.2.2.5**

Species	Beagle dogs
Gender /No. of Animals	Male/3
Feeding Condition	Fasted
Vehicle/Formulation	5% ethanol, 20% propylene glycol, 45% polyethylene glycol 300, and 30% pH 2 water
Method of Administration	Oral
Dose	2.0 mg/kg (15 µCi/kg)
Analyte	Carbon-14

Test Article: LEN

Report Title	**Study Type**	**Test Article**	**Report Number**	**Location in CTD**
Pharmacokinetics, Absorption, Distribution and Excretion of [^{14}C]LEN Following Oral Administration to Dogs	**Excretion**	**[^{14}C]LEN**	**AD-200-2007**	**4.2.2.5**

Specific Activity	113 mCi/mmol			
Specific Activity of Formulation	7.5 µCi/mg			
Assay	Liquid scintillation counting			
	Cumulative Recovery of % Administered [^{14}C] Dose (Mean ± SD)			
Collection Period (h)	**Urine**	**Feces**	**Cage (residual)**	**Total Recovery[a]**
0 - 24	0.05 ± 0.02	25.1 ± 43.5	NA	NA
0 - 72	0.13 ± 0.02	82.7 ± 9.4	NA	NA
0 - 624	0.22 ± 0.08	87.7 ± 6.4	0.7 ± 0.6	88.7 ± 5.8

SD = standard deviation.
a Total recovery of radioactivity includes cage rinses, cage wash, cage wipe, bile cannula rinse, jacket rinse and carcass.

8. PHARMACOKINETICS: OTHER DRUG-DRUG INTERACTIONS

8.1. AD-200-2005: In Vitro Assessment of LEN as a Human P-gp or BCRP Substrate

Test Article: LEN

Report Title	Study Type	Test Article	Report Number	Location in CTD
Bi-Directional Permeability of LEN Through Monolayers of P-glycoprotein and BCRP Over-expressing MDCK Cells	**Drug Transport**	**LEN**	**AD-200-2005**	**4.2.2.6**

Study System	Monolayers of P-gp- or BCRP-overexpressing MDCKII Cells in 24-well transwell plates			
Method	Bi-directional permeability of LEN (0.1 µM) across monolayers of wild type MDCKII cells and P-gp- or BCRP-overexpressing MDCKII cells was determined in the presence and absence of known P-gp or BCRP inhibitors. Lenacapavir concentrations in the transwell were quantified by LC-MS/MS.			
	P_{app} (x 10^{-6} cm/sec) of LEN			
	P-gp-overexpressing MDCKII		**BCRP-overexpressing MDCKII**	
Results	**- inhibitor**	**+ inhibitor[a]**	**- inhibitor**	**+ inhibitor[a]**
Forward (A to B)	0.25	4.69	0.73	0.80
Reverse (B to A)	8.85	5.18	9.06	10.2
Efflux Ratio	35	1.1	12	13

BCRP = breast cancer resistance protein; LC-MS/MS = high performance liquid chromatography coupled to tandem mass spectrometry; MDCKII = Madin-Darby canine kidney cell line; P-gp = P-glycoprotein; P_{app} = apparent permeability
a Control inhibitor: P-gp, valspodar (1 µM); BCRP, Ko134 (0.5 µM)

8.2. AD-200-2025: In Vitro Assessment of LEN as a Substrate for Human OATP1B1 and OATP1B3

Test Article: LEN

Report Title	Study Type	Test Article	Report Number	Location in CTD
In Vitro Assessment of LEN as Substrate for Human OATP1B1 and OATP1B3	**Drug Transport**	**LEN**	**AD-200-2025**	**4.2.2.6**

Study System	CHO cells and CHO cells transfected with mammalian expression plasmids encoding human OATP1B1 and OATP1B3		
Method	The uptake rate of LEN (0.025 µM) in CHO cells versus CHO cells overexpressing human OATP1B1 or OATP1B3 transporters was determined in the presence and absence of a known OATP inhibitor. Uptake rates were also measured with positive (atorvastatin) and negative (antipyrine) control compounds. Lenacapavir and control compound concentrations were quantified by LC-MS/MS.		
	Uptake Rate (pmole/minute/1.0 x 10^6 cells)		
Results	**LEN 0.025 µM**	**Atorvastatin 0.1 µM**	**Antipyrine 10 µM**
CHO	8.4	0.17	7.0
CHO-OATP1B1	9.8	1.25	7.1
CHO-OATP1B3	11	1.30	8.3
CHO-OATP1B1 / CHO Ratio	1.2	7.4	1.0
CHO-OATP1B3 / CHO Ratio	1.3	7.6	1.2

CHO = Chinese Hamster Ovary; LC-MS/MS = high performance liquid chromatography coupled to tandem mass spectrometry; OATP = organic anion-transporting polypeptide

8.3. AD-200-2027: In Vitro Assessment of LEN as a Substrate for Human Hepatic Uptake Transporters

Test Article: LEN

Report Title	Study Type	Test Article	Report Number	Location in CTD
In Vitro Assessment of LEN as Substrate for Human Hepatic Uptake Transporters	**Drug Transport**	**LEN**	**AD-200-2027**	**4.2.2.6**

Study System	Cryopreserved plateable human hepatocytes (single male donor, lot YTW). Cells thawed from cryopreserved vial are seeded onto a collagen I coated 96-well plate. The plates are incubated at 37º C, 90% humidity and 5% CO2 for 2 hours to allow the hepatocytes to attach.
Method	After the hepatocytes have attached, the plating media is removed, cells are rinsed once with DPBS containing calcium and magnesium. LEN (0.1 µM) are prepared in Krebs-Henseleit buffer with 10% human serum with or without rifamycin (100 µM). After the 10 minute pre-treatment of the cells, the dosing solution is added and uptake is performed in triplicates at various time points (0.25, 0.5, 1, 3, 5, 30 minutes for rosuvastatin and 1, 3, 5, 10, 15, 30 minutes for LEN). The uptake reaction is terminated by aspirating the dosing solutions and then adding ice cold DPBS containing calcium and magnesium to the wells. Cells are washed three times with ice cold DPBS containing calcium and magnesium. Cells are lysed with 70% methanol/30% acetonitrile/30 nM labetalol (internal standard). The intracellular concentration of LEN and control compound is quantified by LC-MS/MS.
Results	Hepatic OATP transporters are not involved in LEN transport.

DPBS = Dulbecco's phosphate-buffered saline; LC-MS/MS = high performance liquid chromatography coupled to tandem mass spectrometry; OATP = organic anion-transporting polypeptide

8.4. AD-200-2026 and AD-200-2035: In Vitro Inhibition of Human Transporters by LEN

Test Article: LEN

Report Title	Study Type	Test Article	Report Number	Location in CTD
In Vitro Inhibition of Human P-gp, BCRP, OATP1B1, OATP1B3, OAT1, OAT3, OCT1, OCT2, MATE1, MATE2K and BSEP by LEN	**Drug Transport**	**LEN**	**AD-200-2026 AD-200-2035**	**4.2.2.6**

Study System	Monolayers of P-gp- or BCRP-overexpressing MDCKII cells in 96-well black cell culture plates with clear bottoms, and CHO cells transfected with the genes encoding human OATP1B1 and OATP1B3 were seeded in poly D lysine coated 96-well plates. OAT1-, OAT3-, OCT1 over-expressing HEK293FT cells, OCT1-over-expressing CHO cells, and MATE1-/MATE2k-over-expressing MDCKII cells seeded in 96-well plates. BSEP assays were conducted in HEK293FT vesicle system.
Method	Lenacapavir (0.004 – 1.0 µM) was incubated for 1 hour with P-gp-overexpressing MDCKII cells in the presence of probe substrate (10 µM calcein AM). Lenacapavir (0.004 – 1.0 µM) was incubated for 18 hours with BCRP-overexpressing MDCKII cells in the presence of probe substrate (1 µM pheophorbide A). Each well was analyzed for fluorescence after washing and lysing the cells. Positive control experiments were also performed in parallel with known inhibitors (Verapamil & Kol143). Lenacapavir (0.0038 – 2.8 µM) was incubated with OATP1B1 and OATP1B3 overexpressing cells for 1 h in the presence of probe substrate (2 µM Fluo-3). Each well was analyzed for Fluo-3 fluorescence after washing and lysing the cells. Positive control experiments were also performed in parallel with a known inhibitor (Rifampicin). OAT1, OAT3, OCT1, OCT2, MATE1 and MATE2K inhibition experiments were carried out at 37°C without pre-incubation in Krebs-Henseleit buffer pH 7.3 (or pH 8.0 for MATE1 and MATE2K) containing the radiolabeled probe substrate and LEN (0.01 – 10 µM) or the solvent. Organic solvent concentration was equal in each well and did not exceed v/v 1%. After the experiment, the cells were rinsed with Krebs-Henseleit buffer and lysed with 0.1 M NaOH. The amount of substrate inside the cells was determined by liquid scintillation counting. Lenacapavir was incubated with membrane vesicle preparations (total protein: 50 µg/well) and themodel substrate (^{3}H-taurocholate) in the absence or presence of ATP. Vesicles were washed and substrate accumulated inside the vesicles were quantified by liquid scintialltion counting.

Transporter	Maximum Inhibition (Highest LEN Concentration Tested)	IC_{50} (µM)
P-gp	NIO (1.0 µM)	>1.0
BCRP	NIO (1.0 µM)	>1.0
OATP1B1	94% (2.8 µM)	0.021
OATP1B3	91% (2.8 µM)	0.049
OAT1	NIO (10 µM)	>10
OAT3	NIO (10 µM)	>10
OCT1	NIO (10 µM)	>10
OCT2	NIO (10 µM)	>10
MATE1	56% (10 µM)	2.39
MATE2K	NIO (10 µM)	>10
BSEP	75% (10 µM)	1.21

BCRP = breast cancer resistance protein; BSEP = bile salt export pump; CHO = Chinese Hamster Ovary; MATE = multidrug and toxin extrusion protein; MDCKII = Madin-Darby canine kidney cell line; NA = not applicable; NIO = no inhibition observed; OAT = organic anion transporter; OATP = organic anion-transporting polypeptide; OCT = organic cation transporter; P-gp = P-glycoprotein.

8.5. AD-200-2053: In Vitro Inhibition of Human and Dog BSEP by LEN

Test Article: LEN

Report Title	Study Type	Test Article	Report Number	Location in CTD
In vitro Interaction of LEN with the Human and Dog BSEP Transporter	**Drug Transport**	**LEN**	**AD-200-2053**	**4.2.2.6**

Study System	For inhibition assessment, vesicles were incubated in the respective assay buffer containing the probe substrate in the presence of LEN (0.014-10 µM). The reaction was initiated with the addition of ATP or AMP to the solution and following the incubation time the reaction was stopped by the addition of 200 µL of ice cold washing buffer and immediate filtration via glass fiber filters mounted to a 96-well plate (filter plate). The filters were washed, dried and the amount of substrate inside the filtered vesicles was determined by liquid scintillation counting.
Method	Lenacapavir was incubated with membrane vesicle preparations containing human BSEP or dog Bsep (total protein: 50 µg/well) and the model substrate (3H-taurocholate) in the absence or presence of ATP. Vesicles were washed and substrate accumulated inside the vesicles were quantified by liquid scintialltion counter.

Transporter	Maximum Inhibition (Highest LEN Concentration Tested)	IC_{50} (µM)
Human BSEP	75% (10.8 µM)	1.15
Dog Bsep	92% (10.7 µM)	0.12

BSEP or Bsep = bile salt export pump

8.6. AD-200-2055: Inhibition Liability Assessment for LEN

Test Article: LEN

Report Title	Study Type	Test Article	Report Number	Location in CTD
Pharmacokinetic Drug-Drug Interaction Liability Assessment for LEN	**Drug-Drug Interaction**	**LEN**	**AD-200-2055**	**4.2.2.6**

Report Number	Enzyme	Isoform	IC_{50} (µM)	CYP UGT R Value[a]				Transporters R Value[a]					
				Hepatic		Gut		Gut	Uptake		Renal		
				FDA and PMDA	EMA	FDA and PMDA	EMA	FDA, PMDA (EMA)	FDA and PMDA	EMA	FDA	EMA 50x $C_{max,u}$ (µM)	PMDA
AD-200-2028	CYP	CYP1A2	> 25	< 1.001	< 0.001	NA	NA	NA	NA	NA	NA	NA	NA
		CYP2B6	> 25	1.000	0.000	NA	NA	NA	NA	NA	NA	NA	NA
		CYP2C8	> 25	< 1.001	< 0.001	NA	NA	NA	NA	NA	NA	NA	NA
		CYP2C9	> 25	< 1.003	< 0.003	NA	NA	NA	NA	NA	NA	NA	NA
		CYP2C19	> 25	< 1.002	< 0.002	NA	NA	NA	NA	NA	NA	NA	NA
		CYP2D6	> 25	< 1.002	< 0.002	NA	NA	NA	NA	NA	NA	NA	NA
		CYP3A M[b]	5.4	1.004	0.004	9.0	8.0	NA	NA	NA	NA	NA	NA
		CYP3A T[c]	> 25	< 1.002	< 0.002	< 4.1	< 3.1	NA	NA	NA	NA	NA	NA
	CYP3A time dependent inhibition			**2.75**		**≥ 5.0**		NA	NA	NA	NA	NA	NA
AD-200-2031	UGT	1A1	3.2	1.007	0.007	NA	NA	NA	NA	NA	NA	NA	NA
AD-200-2026 & AD-200-2035	Transporter	P-gp	> 1.0	NA	NA	NA	NA	< 4.2 (> 0.24)	NA	NA	NA	NA	NA
		BCRP	> 1.0	NA	NA	NA	NA	< 4.2 (> 0.24)	NA	NA	NA	NA	NA
		OATP1B1	> 0.021	NA	NA	NA	NA	NA	**1.11**	**9.1**	NA	NA	NA
		OATP1B3	> 0.049	NA	NA	NA	NA	NA	1.05	**21.2**	NA	NA	NA
		OCT1	> 10	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
		OCT2	> 10	NA	NA	NA	NA	NA	NA	NA	0.0002	0.103	1.0002
		OAT1	> 10	NA	NA	NA	NA	NA	NA	NA	0.0002	0.103	1.0002
		OAT3	> 10	NA	NA	NA	NA	NA	NA	NA	0.0002	0.103	1.0002
		MATE1	2.39	NA	NA	NA	NA	NA	NA	NA	0.0010	0.103	1.0010
		MATE2K	> 10	NA	NA	NA	NA	NA	NA	NA	0.0002	0.103	1.0002

$C_{max,u}$ = unbound C_{max}; NA = not applicable.
a The clinical LEN C_{max} value was ~ 136 ng/mL (140 nM) following a single SC administration at 927 mg with oral lead-in at 600 mg on Days 1 and 2 (CTRA-2021-1054). A C_{max} value at 140 nM, a fraction of dose absorbed (F_a) at 6.24%, an absorption rate constant (k_a) at 0.0287 h^{-1}, the maximum equilibrium solubility in simulated intestinal fluid under fasted state at 3.9 µg/mL and under fed state at 4.1 µg/mL (m3, Section 3.2.S.1.3) were used to assess the intestinal and hepatic DDI risk. R values in bold indicate potential risk.
b IC_{50} determined with midazolam 1'-hydroxylase.
c IC_{50} determined with testosterone 6β-hydroxylase.
Human plasma protein binding: 1.46% free (Source AD-200-2020)
Human hepatic microsomal binding: 10.7% free (Source AD-200-2032)

8.7. AD-200-2055: Induction Liability Assessment for LEN

Test Article: LEN

Report Title	Study Type	Test Article	Report Number	Location in CTD
Pharmacokinetic Drug-Drug Interaction Liability Assessment for LEN	**Drug-Drug Interaction**	**LEN**	**AD-200-2055**	**4.2.2.6**

	Summary of Induction Liability Calculations for LEN Using mRNA Results from the Most Sensitive Hepatocytes (Donor 1)[a]		
Report Number	**Intestinal[b]**	**Hepatic[b]**	**Oral AUCR[b]**
AD-200-2049	1.1 / 1.2	1.0 / 1.0	0.98 / 0.90

AUCR = AUC ratio.
a A fraction of dose absorbed (F_a) at 6.24% and an absorption rate constant (k_a) at 0.0287 h^{-1} were used to assess the DDI risk.
b Value calculated using full agonist model / value calculated using partial agonist model

9. PHARMACOKINETICS: OTHER STUDIES

None.