SECTION 2.6.6—TOXICOLOGY WRITTEN SUMMARY

LENACAPAVIR

Gilead Sciences

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CONFIDENTIAL AND PROPRIETARY INFORMATION

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LIST OF ABBREVIATIONS

AUC	area under the plasma concentration versus time curve
AUC_t/AUC_{tau}	area under the concentration versus time curve over the dosing interval
BSEP or Bsep	bile salt export pump
C_{max}	the maximum observed concentration of drug in serum, plasma
CYP	cytochrome P450
DMSO	dimethyl sulfoxide
ECG	electrocardiogram
EOW	every-other-week
F	female
FDA	Food and Drug Administration
FOB	functional observational battery
GD	gestation day
GLP	Good Laboratory Practice
h	hours
HIV-1	human immunodeficiency virus type 1
HPBL	human blood peripheral lymphocytes
HTE	heavily treatment experienced
ICH	International Conference on Harmonization
IC_{50}	concentration of drug required for 50% inhibition
IM	intramuscular
ISR	injection site reaction
IV	intravenous
kg	kilogram
OECD	Organization for Economic Co-Operation and Development
LD	lactation day
µg	microgram
M	male
mg	milligram
mL	milliliter
mM	millimolar
MN	micronucleus
min	minutes
M, mol	molar
N	Normal
NaOH	sodium hydroxide
NOEL	no-observed-effect-level
NOAEL	no-observed-adverse-effect level
ng	nanogram
PEG	polyethylene glycol

PCE	polychromatic erythrocyte
PG	propylene glycol
PR	interval between the peak of the P wave and the peak of the R wave on ECG
QRS	interval between the start of Q wave to the end of the S wave on ECG
QT	interval between the start of the Q wave and the end of the T wave on ECG
cQT	corrected QT interval
S9	post-mitochondrial supernatant fraction from homogenized liver
SC	subcutaneous
SWI	sterile water for injection
TK	Toxicokinetic(s)
T_{max}	the time of maximum observed concentration
US	United States
USP	United States Pharmacopeia
UVR	ultraviolet radiation
W	week
v/v	volume to volume
w/w	weight for weight

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

1. BRIEF SUMMARY

Lenacapavir (LEN, GS-6207 or GS-6707-02 [sodium salt]) is a novel, first-in-class, selective inhibitor of human immunodeficiency virus type 1 (HIV-1) capsid function. Lenacapavir inhibits HIV-1 at multiple points in the viral lifecycle, including interfering with capsid-mediated nuclear uptake of preintegration complexes and impairing virion production and proper capsid core formation. Virus produced in the presence of LEN display aberrantly shaped capsids. These malformed virus particles can still infect a new target cell but cannot replicate as they are unable to support reverse transcription without a properly formed capsid core. Lenacapavir is characterized by potent antiviral activity, a novel resistance profile, low human clearance, and low aqueous solubility. These combined characteristics allow LEN to be well suited for a long-acting parenteral formulation. Lenacapavir is anticipated to be active against all major types of HIV-1, including those that have developed resistance to existing major classes of antiretrovirals (e.g. nucleoside reverse transcriptase inhibitors (NRTI), nonnucleoside reverse transcriptase inhibitors (NNRTI), protease inhibitors (PI), and integrase strand-transfer inhibitors (INSTI)). Two dosage forms of LEN are currently in development, a solution for subcutaneous (SC) injection, for administration once every 6 months and a film-coated tablet formulation for oral administration used for initial loading.

A comprehensive program of nonclinical subcutaneous, oral and intravenous (IV) toxicology studies with LEN has been conducted (see Table 1, below). This program includes GLP single dose IV toxicity studies in rat and dog, repeat-dose subcutaneous toxicity studies of up to 26 weeks duration in rats and 39-weeks in dogs, 28-day oral toxicity studies in rats and dogs, in vitro and in vivo genotoxicity studies; assessment of fertility, early embryonic development, and a pre- and postnatal development toxicity in rats. Toxicokinetics of plasma LEN was included in the toxicity studies. Additional toxicology studies conducted include assessments of local tolerance, antigenicity potential, and genotoxicity and toxicity studies for qualification of impurities. The 6-month transgenic mouse (TgrasH2) and the 2-year rat carcinogenicity studies are in progress; final reports will be submitted when completed. The rat and dog were the appropriate species for the repeat-dose toxicology studies because of the similar disposition of LEN in these species compared to humans and the ability to achieve high systemic exposure.

Clinical and potential clinical formulations were utilized in toxicology studies; the clinical formulation used in the Phase 2/3 study (309 mg/ml LEN sodium in polyethylene glycol [PEG]/water) in heavily treatment experienced (HTE) people with HIV (GS-US-200-4625) was administered in the second 9-month dog study, the rat peri- and postnatal reproductive toxicity study, a rabbit local tolerance study and is being administered in the ongoing rat carcinogenicity study. During development, the density of LEN was modified slightly which changed the calculated concentration of LEN (i.e. 300 mg/mL to 309 mg/mL). This amended value had no impact on the studies and the reports were not amended. LEN free acid, LEN sodium or LEN sodium formed in situ by adding 1 mole equivalent of sodium hydroxide were administered.

Study designs and parameters evaluated were consistent with accepted principles and practices as outlined in International Conference on Harmonization (ICH), Organization for Economic Co-Operation and Development (OECD), and national regulations (United States Food and Drug Administration [US FDA], and European Community Directives). All pivotal studies were conducted in accordance with US FDA Good Laboratory Practice (GLP) regulations.

Table 1. Lenacapavir Toxicology Program

Study Type and Duration	Route of Administration (Formulation)	Species
Single-Dose Toxicity		
Intravenous infusion with a 2-week observation period	Intravenous infusion (30 min)	Rat, Dog
Repeat-Dose Toxicity		
4-Dose (EOW) repeat dose toxicity with a 6-week observation period	Subcutaneous	Rat, Dog
A single- and two-dose subcutaneous injection toxicity and toxicokinetic study with 3 formulations of len with a 13-week observation period after each dose	Subcutaneous	Rat
A 9-month (once monthly) subcutaneous injection toxicity and toxicokinetic study (2 studies)	Subcutaneous	Dog
A 4-week oral gavage toxicity and toxicokinetic study	Oral	Rat, Dog
Genotoxicity		
In vitro reverse mutation assay	In vitro	Bacteria (Salmonella typhimurium, Escherichia coli)
In vitro chromosome aberration assay	In vitro	Human Lymphocytes
In vivo micronucleus assay	Subcutaneous (Suspension)	Rat
Carcinogenicity		
26 weeks (study in progress; report will be submitted when available)	Subcutaneous	C6BF-1 TgrasH2 Mouse
104 weeks (study in progress; report will be submitted when available)	Subcutaneous	Rat
Reproductive and Developmental Toxicity		
Fertility and early embryonic development	Subcutaneous	Rat
Embryo-fetal development	Oral, Intravenous infusion (30 minutes)	Rat, Rabbit
Prenatal and postnatal development, including a postnatal behavioral/functional and reproduction evaluation	Subcutaneous	Rat

Study Type and Duration	Route of Administration (Formulation)	Species
Local Tolerance		
Phototoxicity	In vitro	BALB/c 3T3 mouse fibroblasts
Ocular irritation	Ex vivo	Bovine corneas
Skin irritation	In vitro	Human keratinocytes
Hemolytic Potential and Plasma Compatibility	Ex vivo	Rat, Dog, Human/Blood and Plasma
Injection Site Reaction	Subcutaneous	Rabbit
Other Studies		
Antigenicity	In silico, In vitro, Topical	Human monocytes (THP-1 cell line), Mouse
Impurities	In vitro, Subcutaneous	Bacteria (Salmonella typhimurium, Escherichia coli), Human Lymphocytes, Rabbit
Other	Subcutaneous, oral	Mouse, Rat, Rabbit

EOW = every other week

The nonclinical safety program for LEN identified expected injection site reactions. Dose related, reversible hepatobiliary changes in dogs were consistent with inhibition of the Bile Salt Export Pump (BSEP). After IV administration of 10 and 30 mg/kg, LEN-related serum chemistry changes were observed 24 hours after dosing and included mildly to markedly increased serum alanine and aspartate aminotransferase, gamma glutamyl transferase, alkaline phosphatase activity, and total bilirubin with correlative microscopic findings of minimal to moderate hepatocyte degeneration and minimal to slight hepatocyte necrosis. At the 14-day terminal sacrifice, clinical pathology changes had partially or fully reversed and there were no LEN-related microscopic findings. The no-adverse-effect-level (NOAEL) was determined to be 3 mg/kg.

The 9-month dog SC toxicity data indicate no systemic toxicity at 8-fold the expected human plasma exposure. The presence of icteric clinical signs, bile pigment, increased bile acids and increased conjugated bilirubin observations at plasma exposures at least 51-fold higher than human exposure indicate hepatobiliary changes indicative of cholestasis.

The transient acute toxicity and the clinical, clinical pathology and histopathology observations in the chronic dog study are consistent with disruption in secretion of bilirubin into bile. Inhibition of the BSEP transporter induces hepatotoxicity by causing intracellular concentrations of bile acids to rise above a toxic threshold. Lenacapavir is a much more potent inhibitor of dog BSEP (dBsep) transporter (IC_{50} = 0.124 µg/mL) than the human BSEP transporter (IC_{50} =1.15 µg/mL).

The mean LEN plasma Cmax achieved at 10 mg/kg in the single dose IV dog study of 15.4 µg/mL is 124-fold and 13-fold higher than the dBsep and hBSEP IC_{50}s, respectively.

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

The average clinical plasma LEN concentration is 27-fold lower than the hBSEP transporter IC_{50}. Hepatic metabolism is not a major elimination pathway for LEN.

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

The nonclinical toxicology studies provide an adequate basis to evaluate potential toxicity of LEN. All information from the nonclinical toxicology studies that is of relevance to the prescriber and patient has been included in the proposed prescribing information. Overall, the nonclinical toxicological assessment of LEN supports its safe and effective use in the treatment of HIV-1 infection.

2. SINGLE DOSE TOXICITY

Single dose IV toxicity studies with LEN have been conducted. A single subcutaneous dose of 100 mg/kg was administered as part of the repeat dose dog toxicity study with LEN suspension formulation (Section 3.2.1). The 26-week rat study (2 doses, Section 3.1.2) and the rat fertility study includes data after a single subcutaneous dose of LEN (Section 6.1.1).

2.1. Rat – Intravenous Injection

The purpose of this study was to evaluate the toxicity and determine the toxicokinetics (TK) of LEN when administered over 30 minutes via IV infusion to rats and to assess any effects after a 2-week observation period (m2.6.7, Section 5.1, TX-200-2029).

Male and female Crl:WI(Han) rats (15 toxicity animals/sex/group) were assigned to four groups. Animals were administered vehicle control article (67.0 % w/w PEG 300 and 33.0% w/w SWI, USP) or 3, 10, or 30 mg/kg LEN in vehicle. Doses were administered by IV infusion via tail vein over approximately 30 minutes using 0.20, 0.67 or 2 mg/mL LEN at a dose volume of 15 mL/kg. A subgroup of animals (3 animals/sex in the vehicle group and 9 animals/group/sex in LEN-treated groups) were included for toxicokinetic evaluation on Day 1.

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

No LEN-related mortality occurred. Vehicle related mortality and transient clinical findings of hemolysis were observed. No LEN-related changes in mean body weights, body weight gains, food consumption, or ophthalmic observations were noted. No LEN-related changes in hematology, coagulation, clinical chemistry, or urinalysis occurred.

Plasma exposure to LEN increased with the increase in dose level from 3 to 30 mg/kg. The increases in C_{max} and AUC_{inf} were approximately dose proportional between the 3 and 30 mg/kg dose levels. After completion of the 30-minute IV infusion administration, LEN concentrations declined, generally in a bi-exponential manner. No sex-based differences were observed.

At the interim sacrifice on Day 2, liver weight parameters (mean absolute weight and mean relative to body weight) were increased in animals administered 30 mg/kg/day and were considered LEN related. No macroscopic or microscopic observations or clinical pathology correlates were present in animals at the interim sacrifice.

At the terminal sacrifice, liver weight parameters in animals administered 30 mg/kg were comparable with control values. No LEN-related macroscopic or microscopic findings were present in animals at the terminal sacrifice.

In conclusion, no LEN-related adverse effects occurred in male and female rats administered a single dose of vehicle control article or 3, 10, or 30 mg/kg LEN via 30-minute IV infusion. The no observed adverse effect level (NOAEL) is 30 mg/kg. This dose level corresponded to gender-combined mean maximum observed concentration (C_{max}) and area under the concentration-time curve ($AUC_{0\text{-}168.5h}$) values of 12,700 ng/mL and 224,000 ng·h/mL, respectively.

Table 2. Mean Toxicokinetic Parameters for LEN Solution in Rats After IV Infusion

Dose (mg/kg)	Sex	$AUC_{0\text{-}168.5h}$ (ng•h/mL)	C_{max} (ng/mL)
3	Male	33,600	1340
	Female	31,400	1240
	MF Combined	32,500	1280
10	Male	99,000	4830
	Female	98,700	4590
	MF Combined	99,300	4630
30	Male	235,000	14,100
	Female	210,000	12,300
	MF Combined	224,000	12,700

2.2. Dog – Intravenous Injection

The purpose of this study was to evaluate the toxicity and determine the TK of LEN when administered as a single IV infusion to dogs and to assess any effects after a 2-week observation period (m2.6.7, Section 5.2, TX-200-2030).

Male and female purebred Beagle dogs (5 animals/sex/group) were assigned to 4 groups. Animals were administered vehicle control article (6.12% w/w PEG 300 and 93.88% w/w SWI, USP) or 3, 10, or 30 mg/kg LEN in vehicle with 1 mole equivalent of sodium hydroxide as a single dose on Day 1. Doses were administered via IV infusion over 30 minutes using 30 mg/mL LEN.

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

Plasma exposure to LEN generally increased with the increase in dose level from 3 to 30 mg/kg. The increases in mean maximum observed concentration (C_{max}) values were generally less than dose proportional between the 3 to 30 mg/kg dose levels for both males and females. The increases in mean area under the concentration-time curve to infinity (AUC_{inf}) values for males were greater than dose proportional between the 3 and10 mg/kg dose levels and less than dose proportional between the 10 and 30 mg/kg dose levels.

All animals survived to their scheduled sacrifice. No adverse LEN-related clinical observations were observed.

Lenacapavir-related decreases in mean body weights (up to -13.6% in males and -29% for females) were observed in animals administered ≥3 mg/kg compared with the control group after the 2-week observation period. Mean body weight changes for males and females generally decreased between dosing Days 1 through 8 compared to controls and recovered for males from Days 8 through 14 and decreased for females between Days 8 through 14 without a correlation to dose. A corresponding decrease in mean food consumption was mostly noted for females administered ≥3 mg/kg.

No LEN-related clinical pathology changes occurred in animals administered 3 mg/kg. Adverse LEN-related changes in coagulation and liver parameters on Day 2 (approximately 24 hours postdose) in animals administered ≥10 mg/kg occurred. These changes were generally of a higher magnitude in animals administered 30 mg/kg, and correlated with liver microscopic findings (hepatocyte degeneration, individual hepatocyte necrosis, and mixed cell infiltrate).

Lenacapavir dose related coagulation changes on Day 2 were consistent with reduced production of coagulation factors and fibrinogen associated with the liver effect in animals administered ≥10 mg/kg. Effects included minimally prolonged prothrombin time and/or activated partial thromboplastin time and/or minimally decreased fibrinogen concentration in animals administered 10 (1 female only) or 30 mg/kg. Other LEN-related changes noted in males administered 30 mg/kg included minimally increased red cell mass consistent with hemoconcentration (increased red blood cell count, hemoglobin concentration, and hematocrit) and minimally increased cholesterol concentration. The coagulation and hematology changes were reversible.

Lenacapavir dose related liver parameter increases on Day 2 included mildly to markedly increased alanine and aspartate aminotransferase and gamma glutamyl transferase activities in animals administered ≥10 mg/kg, and mildly to moderately increased alkaline phosphatase activity and total bilirubin (direct and/or indirect) concentration in animals administered 30 mg/kg. For animals undergoing a two-week observational period (n=2/sex/dose), liver enzyme increases of lower magnitude compared to Day 2 were still noted on Day 15 but were not always dose related. Changes included mildly to moderately increased alanine aminotransferase activity in all males administered ≥10 mg/kg, all females administered 10 mg/kg, and 1 female administered 30 mg/kg and gamma glutamyl transferase activity in all males and 1 female administered 30 mg/kg. The aspartate aminotransferase and/or alkaline phosphatase activities and/or total bilirubin (direct and/or indirect) concentration on Day 15 had returned to predose values in animals administered ≥10 mg/kg.

Adverse LEN-related microscopic findings were noted at the interim sacrifice in the liver of animals administered ≥10 mg/kg and included slight to moderate hepatocyte degeneration. All males administered 30 mg/kg and 1 female administered 10 mg/kg also had individual hepatocyte necrosis. Mixed cell infiltrates were present in portal areas and sinusoids of animals administered ≥10 mg/kg, and hemorrhage was present around central veins and in the connective tissue between the liver and gallbladder of animals administered ≥3 mg/kg.

No LEN-related microscopic findings were observed at the terminal sacrifice. No LEN-related mortality, organ weight changes or macroscopic observations were noted at the interim or terminal sacrifice.

In conclusion, male and female dogs were administered a single dose of vehicle control article or 3, 10, or 30 mg/kg LEN via 30-minute IV infusion, and animals were observed over 2 weeks. LEN-related adverse liver changes were observed on Day 2 in clinical pathology parameters (mildly to markedly increased alanine and aspartate aminotransferase, gamma glutamyl transferase, alkaline phosphatase activity, and total bilirubin) with correlative microscopic findings (hepatocyte degeneration and necrosis, and mixed cell infiltrate) in animals administered ≥10 mg/kg. At the 15-day terminal sacrifice, clinical pathology changes had partially or fully reversed and there were no LEN-related microscopic findings. Therefore, the NOAEL is 3 mg/kg, and this dose level corresponded to sex-combined mean C_{max} and $AUC_{0\text{-}168.5h}$ values of 5140 ng/mL and 35200 ng·h/mL, respectively.

Table 3. Mean Toxicokinetic Parameters for LEN Solution in Dogs After IV Infusion

Dose (mg/kg)	Sex	AUC $_{(0\text{-}168.5h)}$ (ng•h/mL)	C_{max} (ng/mL)
3	Male	39,000	5140
	Female	31,500	5140
	MF Combined	35,200	5140
10	Male	265,000	15,200
	Female	124,000	15,600
	MF Combined	194,000	15,400
30	Male	460,000	32,700
	Female	762,000	45,200
	MF Combined	611,000	38,900

3. REPEAT DOSE TOXICITY

GLP repeat-dose toxicity studies with LEN utilized SC administration, the proposed clinical route of administration. As presented in the original IND 136260, LEN was administered to Wistar Han rats and beagle dogs by SC injection in an aqueous suspension of LEN in the clinical vehicle containing 2% w/v poloxamer 188, 0.9% w/v sodium chloride in SWI, and 400 mM phosphate buffer. LEN chronic toxicity studies in Wistar Han rats and beagle dogs by SC injection using PEG 200:Ethanol:Water solution test articles have been completed – administering the LEN free acid or LEN sodium formed in situ with 1 mole equivalent of sodium hydroxide.

3.1. Rat – Subcutaneous Injection

3.1.1. 7-Day Subcutaneous and Intramuscular Dose Range Finding Toxicity Study

The purpose of this nonGLP study was to evaluate the toxicity and TK of LEN in male Wistar Han rats given once weekly doses (2 doses) via subcutaneous (SC) or intramuscular (IM) injections.

Male Wistar Han rats were assigned to ten groups and were administered LEN suspension at a dose level of 0 (vehicle, 2% w/v poloxamer 338 and 98% w/v SWI), or 5, 30, or 100 mg/kg for the subcutaneous injections at a dose level of 0 (vehicle) or 5 mg/kg for the IM injections at a dose volume of 1 mL/kg (m2.6.7, Section 6.1, TX-200-2002).

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

The increase in LEN total exposure was dose-proportional following an increase in the SC dose from 5 to 100 mg/kg. After a twice weekly SC dose of 5, 30, or 100 mg/kg LEN, the area under the concentration-time curve from hour 0 to 672 hours postdose ($AUC_{0\text{-}672h}$; Day 28) was 164 ± 35, 525 ± 136, and 932 ± 138 h•µg/mL, respectively. After a twice weekly IM dose of 5 mg/kg LEN, the $AUC_{0\text{-}672h}$ was 109 ± 20.7 h•µg/mL.

Test article-related clinical findings included injection site reactions on Day 1 in animals administered 5 mg/kg via IM injection. Coagulation and serum chemistry findings consisted of changes consistent with inflammation in animals administered ≥30 mg/kg via SC or 5 mg/kg via IM injection. LEN-related anatomic pathology changes were limited to local adverse injection site reactions in animals administered the test article by SC injection. Due to these injection site reactions the NOAEL for LEN when administered via SC injection was not achieved. The NOAEL for LEN when administered via IM injection is 5 mg/kg (corresponding to an $AUC_{0\text{-}24h}$ value in males of 11.9 ± 1.1 h•µg/mL after the last dose).

3.1.2. 6-Week Subcutaneous Toxicity with a 6-Week Observation Period (Suspension)

The purpose of this study was to evaluate the toxicity and determine the TK of LEN when administered once every other week via subcutaneous injection to rats for a total of four doses and to monitor animals for at least 6 weeks after the last dose. Four groups of 20 male and 20 female Wistar Han rats were administered a 100 mg/mL LEN suspension via SC injection at dose levels of 0 (vehicle control article: 2% w/v poloxamer 188 prepared in 0.9% w/v sodium chloride in SWI), 10, 30, or 100 mg/kg/dose in a volume of 1, 0.1, 0.3, or 1 mL/kg, respectively (m2.6.7, Section 7.1, TX-200-2005). Rats were administered LEN once every other week (EOW) for 4 doses (on Study Days 1, 15, 29, and 43 in 4 separate dorsal dose sites). On each day of dosing, the pH of the LEN dose formulations was adjusted with 400 mM phosphate buffer. A separate group of animals (5 animals/sex) was included as a positive control group for the micronucleus (MN) assay evaluation and were administered a single oral gavage dose of 60 mg/kg cyclophosphamide (positive control article) on Study Day 56 (24 hours [± 1.5 hours] prior to bone marrow collection on Day 57) at a volume of 10 mL/kg.

Animals administered LEN and designated for Terminal Sacrifice 1 (up to 10 animals/sex/group dependent on survival) were necropsied on Study Day 57. Animals administered LEN designated for Terminal Sacrifice 2 (remaining animals/group) were monitored for 6 weeks after the last dose and necropsied on Study Day 85. Additionally, subgroups of animals (3 animals/sex/group in vehicle control and 12 animals/sex/group in LEN-treated groups) were included for TK evaluation and were administered the vehicle control or LEN in the same manner as the toxicity animals. Toxicokinetic animals were sacrificed on Study Day 113 following the final blood collection.

Assessment of toxicity was based on mortality, clinical observations, body weights, food consumption, ophthalmic observations, functional observational battery (FOB) tests, locomotor activity assessment, bone marrow MN formation, and clinical and anatomic pathology. Liver samples were taken at the first necropsy for hepatic microsomal enzyme analysis.

No LEN-related mortality occurred. There were 2 unscheduled deaths on study: one toxicity male administered the vehicle control and one TK female administered 10 mg/kg/dose. The death of 1 toxicity male in the vehicle group on Study Day 21 was considered related to the procedure of shaving, and the death of 1 TK female on Study Day 15 was considered unrelated to LEN since no other deaths occurred at this or higher dose levels.

No LEN-related adverse changes in clinical observations, body weights, food consumption, ophthalmic observations, FOB tests, or locomotor activity assessments occurred. LEN had no adverse effect on hematology, coagulation, clinical chemistry or urinalysis test results. No LEN-related organ weight differences occurred.

Dose-related macroscopic and microscopic findings occurred at the SC injection sites and consisted of macroscopic thickening, which correlated with microscopic granulomatous inflammation. The incidence and severity of granulomatous inflammation varied between injection sites, but the overall incidence, severity, and morphology were not notably different at Terminal Sacrifice 2 (Day 85), compared with Terminal Sacrifice 1 (Study Day 57). Granulomatous inflammation was considered adverse when the severity was moderate or marked; adverse inflammation occurred rarely in animals administered 10 mg/kg/dose and more often in animals administered doses of ≥ 30 mg/kg/dose. No systemic organ toxicity occurred at any dose.

No notable changes in microsomal protein yield, total cytochrome P450 (CYP) content, and CYP1A, CYP2B, CYP3A, CYP2E, CYP4A, and uridine 5'-diphospho-glucuronosyltransferase (UDPGT) activities were observed for any LEN-treated group when compared with the vehicle control group, with the exception of a notable increase (2.3x) in CYP3A activity observed in females administered 100 mg/kg/dose.

LEN did not induce micronuclei in the polychromatic erythrocytes of the bone marrow of both sexes when administered up to 100 mg/kg/dose every other week for 4 doses and under the experimental conditions employed.

All concentration values of LEN in the vehicle control group were below the lower limit of quantitation. Exposure to LEN increased with the increase in dose levels from 10 to 100 mg/kg/dose. The increases in C_{max} and $AUC_{0\text{-}336h}$ were less-than-dose proportional between10 and 100 mg/kg. The time of maximum observed concentration (T_{max}) ranged from 48.0 to 168 hours. Values for C_{max} and $AUC_{0\text{-}336h}$ were 1.65 to 2.15-fold higher on Day 43 than on Day 1, indicating minimal accumulation after multiple doses. No sex differences were observed.

In conclusion, LEN was well tolerated clinically in male and female rats when administered subcutaneously EOW for 4 doses at doses up to 100 mg/kg/dose. LEN-related adverse macroscopic and microscopic findings occurred at the SC injection sites on Study Days 57 and 85 and consisted of macroscopic thickening, which correlated with microscopic granulomatous inflammation at all dose levels. No systemic organ toxicity occurred at any dose level. Therefore, a NOAEL for LEN for local injection site effects was not established. The NOAEL for systemic effects is 100 mg/kg/dose.

Table 4. Mean Toxicokinetic Parameters for LEN in Rats After Subcutaneous Injection

Dose (mg/kg/dose)	Sex	$AUC_{0\text{-}336h}$ (ng•h/mL)		C_{max} (ng/mL)	
		Day 1	Day 43	Day 1	Day 43
10	Male	64,100	134,000	241	410
	Female	55,700	106,000	215	451
	MF Combined	59,900	120,000	203	419
30	Male	146,000	314,000	586	1060
	Female	124,000	244,000	445	843
	MF Combined	135,000	279,000	516	945
100	Male	338,000	608,000	1230	2500
	Female	338,000	559,000	1290	2130
	MF Combined	338000	583,000	1260	2080

3.1.3. 26-Week Subcutaneous Toxicity Study (Solution)

The purpose of this study was to evaluate the toxicity and determine the TK of 3 formulations of LEN solution when administered at the same dose either once or twice via subcutaneous injection to Wistar Han rats (m2.6.7, Section 7.2, TX-200-2014). Animals were observed for 13 weeks following each dose (up to 26 weeks, 183 days).

Eight groups of 10 male and 10 female Crl:WI(Han) rats for toxicity evaluation were administered LEN at dose levels of 0 or 100 mg/kg/dose via subcutaneous injection using dose concentrations of 0 mg/mL (vehicle control, 77:10:13 w/w/w PEG 200:ethanol:water), 200 mg/mL with or without 1 mol-eq sodium hydroxide (NaOH), or 400 mg/mL without NaOH. Four groups were necropsied 13 weeks after a single dose. Replicate groups were necropsied 13 weeks after a second dose on Day 92 (13 weeks between doses). The dose volume administered to the vehicle control and the 200 mg/mL formulation groups was 0.5 mL/kg; the dose volume administered to the 400 mg/mL formulation group was 0.25 mL/kg. Toxicokinetic animals were euthanized on Days 92 or 183 following the final blood collection.

Assessment of toxicity was based on mortality, clinical observations, body weights, body weight change, food consumption, ophthalmic observations, dermal observations, and clinical and anatomic pathology.

All plasma concentration values of LEN in the vehicle control groups were below the lower limit of quantitation. LEN plasma concentrations were quantifiable at the last time point (2184 hours, 91 days postdose) 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 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 (< 2-fold) was observed after a repeat SC dose on Day 92. The mean T_{max} values for pooled combined sexes were 1040, 1060, and 742 hours in 200 mg/mL with 0 mol-eq NaOH, 200 mg/mL with 1 mol-eq NaOH, and 400 mg/mL with 0 mol-eq NaOH, respectively, on Day 1. The mean T_{max} values for combined sexes were 728, 1230, and 1060 hours in 200 mg/mL with 0 mol-eq NaOH, 200 mg/mL with 1 mol-eq NaOH, and 400 mg/mL with 0 mol-eq NaOH, respectively on Day 92. No sex-based differences were observed.

All animals survived to their scheduled sacrifice. No LEN-related clinical or ophthalmic observations or alterations in body weight, body weight gain, food consumption, clinical pathology, or organ weights were noted.

Lenacapavir-related nonadverse dermal observations of very slight to moderate edema were noted throughout the observation periods for animals administered 200 (with or without NaOH) or 400 (without NaOH) mg/mL LEN. Edema correlated with the macroscopic observation of subcutaneous injection site thickening and the microscopic observations of granulomatous inflammation at the injection site.

Lenacapavir-related nonadverse granulomatous inflammation (of minimal to moderate severity) and correlative macroscopic thickening and/or discoloration were present at the subcutaneous injection site(s) of animals administered LEN. The microscopic findings in the first of two injection sites per animal indicated LEN-related granulomatous inflammation and/or macrophage infiltrate was present 26 weeks after dosing, but reversibility was suggested by decreased overall severity (relative to findings) compared to injection sites evaluated 13 weeks after dosing. At the 26-week necropsy, 2 animals also had minimal adipose tissue necrosis within the focus of granulomatous inflammation; additionally, a few animals administered 200 mg/mL (with or without NaOH) LEN had an observation of macrophage infiltrate, considered an intermediate finding between minimal granulomatous inflammation and complete recovery. Subcutaneous injection site granulomatous inflammation, with or without necrosis, and macrophage infiltrate were considered not adverse based on the localized nature and absence of severe granulomatous inflammation and of clinical observations considered indicative of discomfort. The incidence and overall severity of these findings were highest for animals administered 400 mg/mL LEN, and the lowest incidence and severity occurred in animals administered 200 mg/mL LEN without NaOH. Evidence of LEN-related granulomatous inflammation reversibility included decreased severity relative to subcutaneous injection site(s) used more recently, and/or resolution to a minimal macrophage infiltrate.

In conclusion, LEN was well tolerated in male and female Wistar Han rats when administered via subcutaneous injection once or twice at a dose level of 100 mg/kg/dose with or without 1 mol-eq NaOH in the formulation followed by 13-week observation period after each injection (up to 26 weeks, 183 days). LEN-related nonadverse edema, macroscopic thickening and/or tan discoloration, correlative granulomatous inflammation (with or without necrosis), and macrophage infiltrate were present at the subcutaneous injection sites of animals administered LEN. The incidence and overall severity of these findings were highest for animals administered 400 mg/mL LEN without NaOH, and the lowest incidence and severity occurred in animals

administered 200 mg/mL LEN without NaOH. Evidence of LEN-related granulomatous inflammation reversibility was present. After subcutaneous injection, LEN was slowly absorbed; LEN plasma concentrations were quantifiable at the last time point (2184 hours; 91 days postdose) in all LEN groups. The mean concentration-time profiles for males, females, and combined sexes show that exposure to LEN were generally similar at 100 mg/kg/dose among the three different formulations. The Day 92 sex-combined C_{max} and $AUC_{0\text{-}672h}$ values were 742 ng/mL and 307,000 ng h/mL, respectively, in animals administered 200 mg/mL LEN without NaOH; 780 ng/mL and 284,000 ng·h/mL, respectively, in animals administered 200 mg/mL LEN with NaOH; and 451 ng/mL and 211,000 ng·h/mL, respectively, in animals administered 400 mg/mL LEN without NaOH.

Table 5. Mean Toxicokinetic Parameters for LEN in Rats after 2 Subcutaneous Injections 13 Weeks Apart

Dose (mg/kg/dose) (formulation)	Sex	$AUC_{0\text{-}672h}$ (ng•h/mL)		C_{max} (ng/mL)	
		After 1 injection	After 2 injections	After 1 injection	After 2 injections
100 (200 mg/mL, 0 mol-eq NaOH)	Male	155,000	338,000	427	846
	Female	98,500	276,000	331	637
	MF Combined	127,000	307,000	379	742
100 (200 mg/mL, 1 mol-eq NaOH)	Male	184,000	276,000	544	856
	Female	166,000	293,000	539	703
	MF Combined	175,000	284,000	542	780
100 (400 mg/mL, 0 mol-eq NaOH)	Male	133,000	265,000	318	570
	Female	102,000	156,000	254	332
	MF Combined	117,000	211,000	286	451

3.2. Rat - Oral

3.2.1. 4-Week Oral Gavage Toxicity and Toxicokinetic Study of LEN

The purpose of this study was to evaluate the toxicity and determine the TK of LEN when administered daily via oral gavage to rats for at least 4 weeks (m2.6.7, Section 7.3, TX-200-2011).

Four groups of 10 male and 10 female Crl:WI(Han) rats were administered LEN solution via oral gavage once daily for 4 weeks at dose levels of 0 (vehicle control article: 10% v/v dimethyl sulfoxide [DMSO], 10% v/v vitamin E TPGS, and 80% v/v propylene glycol PG), 3, 10, or 30 mg/kg/day at a volume of 2 mL/kg. A subgroup of animals (3 vehicle control animals/sex, 9 animals/sex in LEN-treated groups) was included for toxicokinetic evaluation on Days 1 and 28.

Assessment of toxicity was based on mortality, clinical observations, body weights, body weight change, food consumption, ophthalmic observations, and clinical and anatomic pathology. Blood samples were collected for toxicokinetic evaluations. Liver samples were collected at the terminal sacrifice on Day 29 for hepatic microsomal fraction analysis.

Exposure to LEN increased with the increase in dose levels from 3 to 30 mg/kg/day. The increases in C_{max} and AUC_{0-24h} 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_{0-24h} on Day 28 were less than dose proportional between the 3 to 30 mg/kg/day dose levels. No sex-based differences were observed. Mean accumulation ratio values for LEN were 5.47, 2.71, and 2.24-fold at the 3, 10, and 30 mg/kg/day dose levels, respectively.

No LEN-related deaths occurred. No adverse clinical or ophthalmic observations, body weight or food consumption alterations, clinical pathology, organ weight changes, macroscopic observations, or microscopic observations were noted.

Minor, nonadverse LEN-related clinical pathology changes included a minimally prolonged (5%) activated partial prothrombin time on Day 29 in males administered ≥ 10 mg/kg/day and minimally increased cholesterol concentration on Days 7 and 29 in females administered ≥ 3 mg/kg/day (up to 36%). The changes were of unclear mechanism and were considered not adverse based on their small magnitude and absence of correlating clinical or microscopic findings.

A notable increase in CYP3A activity (2.7-fold) was observed in females administered 30 mg/kg/day; no decrease in LEN systemic exposure occurred.

In conclusion, no adverse LEN-related effects were noted in male and female rats administered 3, 10, or 30 mg/kg/day LEN via oral gavage once daily for 4 weeks. Thus, the NOAEL is 30 mg/kg/day. This dose level corresponded to sex-combined mean maximum observed concentration (C_{max}) and area under the curve (AUC_{0-24h}) values of 1700 ng/mL and 34500 ng·h/mL, respectively, on Day 28.

Table 6. Mean Toxicokinetic Parameters for LEN in Rats After Daily Oral Administration

Dose (mg/kg/dose)	Sex	AUC_{0-24h} (ng•h/mL)		C_{max} (ng/mL)	
		Day 1	Day 28	Day 1	Day 28
3	Male	2890	16,400	178	829
	Female	2700	14,200	149	658
	MF Combined	2790	15,300	144	712
10	Male	12,000	27,800	727	1500
	Female	8560	27,800	439	1460
	MF Combined	10,300	27,800	583	1480
30	Male	16,200	33,700	792	1650
	Female	14,700	35,400	889	2110
	MF Combined	15,400	34,500	790	1700

3.3. Dog – Subcutaneous Injection

3.3.1. 7-Day Subcutaneous Injection Dose Range Finding Toxicity Study of LEN

The purpose of this study was to determine the potential toxicity of LEN, when given weekly (2 doses) via subcutaneous injection to male beagle dogs (m2.6.7, Section 6.2 TX-200-2004). In addition, TK of LEN was determined.

Male beagle dogs were assigned to four groups and were administered LEN suspension at dose levels of 0 (vehicle, 2% w/v poloxamer 338 and 98% w/v SWI), or 3, 10, or 30 mg/kg via subcutaneous injection at a dose volume of 0.75 mL/kg.

The following parameters and end points were evaluated in this study: clinical signs, body weights, food consumption, electrocardiology, clinical pathology parameters (hematology, coagulation, clinical chemistry, and urinalysis), toxicokinetic parameters, gross necropsy findings, organ weights, and histopathologic examinations.

All dogs survived to scheduled necropsy, 3 days after the last injection. Following each dose administration, LEN-related clinical signs were observed at the injection site of animals given 30 mg/kg/dose and consisted of swelling (soft to firm).

Blood samples for clinical pathology were collected approximately 72 hours after the last dose. Hematology changes consisted minimal increases in neutrophil and monocyte counts, reflected in total white blood cell counts, in males at 30 mg/kg. Coagulation changes consisted of minimal increases in fibrinogen concentration in males dosed at ≥ 10 mg/kg.

Clinical chemistry changes consisted of minimal increases alkaline phosphatase activity in males at 30 mg/kg and minimal increases in cholesterol concentrations in males dosed at ≥ 10 mg/kg. In animals at ≥ 10 mg/kg, minimal increases in globulin concentrations, reflected in mildly decreased A/G ratio, were observed at ≥ 10 mg/kg. These findings are consistent with histopathological changes indicative of inflammation.

All animals dosed with LEN were exposed to LEN and no test item was detected in analyzed samples taken from vehicle control animals. The T_{max} for the first dose was between 24 and 40 hours postdosing. Accumulation of LEN occurred after the second dose.

Administration of LEN to male beagle dogs resulted in macroscopic and microscopic findings at the injection site and in the thymus. At the injection site, a dose-dependent increased severity of mixed cell infiltration (correlating with swelling, thickening and mass) and hemorrhage as well as presence of fibrin exudates and vascular inflammation was observed at all dose levels. In the 10 mg/kg and 30 mg/kg dose groups, in animals with moderate to marked mixed cell infiltration there was evidence of abscess formation characterized by a center of necrotic debris and fibrin that was surrounded by granulation tissue at the site of the first injection. Decreased cellularity in the thymus that correlated with small size (at ≥ 10 mg/kg) and decreased thymus weights were observed at all dose levels without evidence of a dose relationship.

In conclusion, administration of LEN to male beagle dogs by two subcutaneous injections over 7 days was well tolerated in dog at levels of 3, 10 and 30 mg/kg/dose. Clinical signs (soft to firm swelling) were observed at the injection sites of animals given 30 mg/kg/dose. Clinical pathology changes noted in the 10 and 30 mg/kg/day groups were related to inflammation and correlated with macroscopic and microscopic changes observed at the injection sites. In addition, LEN-related thymus changes were observed at all dose levels tested during this study. Based on the abscess formation observed in histopathology in animals given ≥ 10 mg/kg/dose, the NOAEL was considered to be the lowest dose level of 3 mg/kg/dose, which corresponds to a C_{max} of 0.63 µg/mL and $AUC_{0\text{-}72h}$ of 32.2 h•µg/mL following dosing on Day 7.

3.3.2. 6-Week Subcutaneous Toxicity Study with a 6-Week Observation Period (Suspension)

The purpose of this study was to evaluate the toxicity and determine the TK of LEN when administered once every 2 weeks via subcutaneous injection to dogs for a total of 4 doses and to monitor animals for 6 weeks after the last dose. Effects on the cardiovascular system using safety pharmacology endpoints were also evaluated.

Four groups of Beagle dogs (6 animals/sex/group) were administered a 100 mg/mL LEN suspension via SC injection at dose levels of 0 (vehicle control: 2% w/v Poloxamer 188 prepared in 0.9% w/v sodium chloride, in SWI), 10, 30 or 100 mg/kg/dose (m2.6.7, Section 7.4, TX-200-2006). Except for the high dose, dogs were administered vehicle or LEN at unique dorsal dose sites once EOW for 4 doses. The high dose males were administered 2 SC doses and two high dose females were administered one SC injection; 4 high dose females were not dosed. Dose volumes for the vehicle, 10, 30 and 100 mg/kg doses were 1, 0.1, 0.3 or 1 mL/kg respectively. Vehicle control volumes were decreased to 0.3 mL/kg on Study Days 29 and 43 (males) or Study Days 15, 29, and 43 (females). Animals administered LEN and designated for Terminal Sacrifice 1 (3 animals/sex/group dependent on survival) were necropsied on Study Day 57. Animals administered LEN designated for Terminal Sacrifice 2 (remaining animals/group) were monitored for 6 weeks after the last dose and necropsied on Study Day 85. Assessment of toxicity was based on mortality, clinical observations, body weights, body weight change, food consumption (dry and canned), ophthalmic observations, jacketed external telemetry electrocardiography (ECG) measurements, blood pressure (BP) determination, and clinical and anatomic pathology. Blood samples were collected for LEN TK evaluation. Liver samples were collected for microsomal enzyme analysis.

All animals administered vehicle control article or 10 or 30 mg/kg/dose survived to their scheduled sacrifice on Study Day 57 or 85. No LEN-related body weight, body weight gain, ophthalmic observations or alterations to food consumption, blood pressure, clinical pathology or organ weight changes occurred in dogs administered the vehicle, or 10 and 30 mg/kg/dose.

No LEN-related changes in PR interval, QRS duration, QT interval, corrected QT (QTc,) interval (using Fridericia QT/[RR]1/3), or heart rate were observed on Study Days 1, 15, 29, 43, or 82 in animals administered 10 or 30 mg/kg/dose or on Study Day 1 in animals administered 100 mg/kg/dose (6 males, 2 females). No abnormal ECG waveforms or arrhythmias attributed to LEN were observed during qualitative assessment of the ECGs.

All animals administered 100 mg/kg/dose (6 of 6 males and 2 of 6 females) were sacrificed and necropsied early (on Study Day 22 [males] or 15 [females]) due to adverse reactions to the SC injection. The remaining 4 (undosed) females in this group were returned to stock on Study Day 20. Adverse LEN-related behavioral reactions to the 1.0 mL/kg SC injections included vocalization and struggling during dosing, and barrel rolling (observed up to approximately 6 minutes postdose). Upon observing the signs of pain and distress, the LEN aqueous suspension was adjusted to a more neutral pH (from 2.98 to ~5) and the dogs were administered prophylactic analgesics for doses administered on Study Day 15. Excessive struggling and vocalization were noted again in males at 100 mg/kg/dose resulting in the discontinuation of dosing of this dose level on Study Day 15 for males and a decision to terminate dosing such that females were not dosed at 100 mg/kg/day beginning on Study Day 15. Other clinical observations of raised areas, redness, and scabs were noted at the dose sites at 100 mg/kg/dose. No LEN-related behavioral clinical observations occurred between doses or after the last dose at 100 mg/kg/dose. Macroscopic observations of thickening or scab were noted at SC injection sites. No microscopic examination of tissues was performed at 100 mg/kg/dose.

Despite pH adjustment of the 100 mg/mL aqueous suspension, and palliative analgesics, dose administration to females on Study Day 15 resulted in struggling, vocalization during dosing, or barrel rolling at 10 or 30 mg/kg/dose. As a result, all animals of all remaining groups were anesthetized in order to administer doses on Study Days 29 and 43. Behavioral reactions (vocalization and struggling during dosing and barrel rolling) prior to anesthesia use, were considered adverse since anesthesia was required to complete dosing. Dosing under anesthesia resolved all behavioral observations previously associated with dose administration.

Lenacapavir-related clinical observations for anesthetized animals administered 10 or 30 mg/kg/dose were limited to raised or red area on the dose site of some animals administered 30 mg/kg/dose and scab on the dose site of 2 females administered 10 mg/kg/dose.

Lenacapavir-related microscopic finding of minimal to marked granulomatous inflammation at the SC injection sites occurred at both terminal sacrifices in animals administered ≥ 10 mg/kg/dose and frequently correlated with the macroscopic finding of raised area. This was considered adverse when marked in severity. A small overall decreased incidence at Terminal Sacrifice 2, as compared with Terminal Sacrifice 1, suggested partial reversibility.

No systemic organ toxicity occurred at doses of 10 and 30 mg/kg/dose (100 mg/kg/dose was not assessed histologically).

Exposure to LEN increased with the increase in dose level from 10 to 100 mg/kg/dose on Study Day 1 and from 10 to 30 mg/kg/dose on Study Day 43. The increases in C_{max} were approximately dose proportional between the 10 to 30 mg/kg dose levels and less than dose proportional between the 30 to 100 mg/kg (Study Day 1 only) dose levels. The increase in $AUC_{0\text{-}336h}$ was approximately dose proportional between the 10 to 100 mg/kg (Study Day 1 only) dose levels. After SC injection, LEN was slowly absorbed, with mean T_{max} ranging from 39.0 to 64.0 hours on Study Day 1 and from 50.0 to 54.0 hours on Study Day 43. No accumulation of LEN was observed after multiple doses in dogs. No sex-based differences were observed.

In conclusion, administration of LEN once EOW via SC injection to male and female beagle dogs for a total of 4 doses caused adverse behavioral reactions (vocalization and struggling) at the time of injection at all dose levels ≥ 10 mg/kg/dose. Administration of 100 mg/kg/dose was terminated early (after 1 or 2 doses), while the 3rd and 4th doses of 10 and 30 mg/kg/dose were completed under anesthesia. LEN-related microscopic findings of granulomatous inflammation at the SC injection sites were considered adverse at marked severity; this finding occurred at both terminal sacrifices in animals administered ≥10 mg/kg/dose, with a small overall decreased incidence at Terminal Sacrifice 2, as compared with Terminal Sacrifice 1, which suggested partial reversibility. No ECG changes occurred after multiple doses of 10 and 30 mg/kg and a single dose of 100 mg/kg. No systemic organ toxicity occurred at doses of 10 and 30 mg/kg/dose. Based on adverse behavioral observations at the time of injection, and adverse injection site findings for animals administered ≥10 mg/kg/dose, a NOAEL was not established for LEN in this study.

Table 7. Mean Toxicokinetic Parameters for LEN in Dogs After Subcutaneous Injection

Dose (mg/kg/dose)	Sex	AUC_{0-336h} (ng•h/mL)		C_{max} (ng/mL)	
		Study Day 1	Study Day 43	Study Day 1	Study Day 43
10	Male	112,000	127,000	632	634
	Female	79,900	110,000	507	648
	MF Combined	96,100	118,000	570	641
30	Male	311,000	391,000	1730	2210
	Female	339,000	481,000	2040	2240
	MF Combined	325,000	436,000	1890	2230
100	Male	925,000	NA[b]	4260	NA[b]
	Female	1,350,000[a]	NA[b]	6020	NA[b]
	MF Combined	1,030,000[a]	-	4700	-

NA = Not applicable

a Females were euthanized on Study Day 15; therefore, the 336-hour samples are from the unscheduled collection (approximately 333 hours) .

b All males and 2/6 females in Group 4 (the only Group 4 females that were dosed) underwent an early sacrifice on Study Day 22.

3.3.3. 39-Week Subcutaneous Toxicity Study (Solution)

The purpose of this study was to evaluate the toxicity and determine the TK of 3 formulations of LEN when administered once monthly via subcutaneous injection to Beagle dogs for 9 months (10 doses) (m2.6.7, Section 7.5, TX-200-2017).

Male and female purebred Beagles (9 animals/sex/group) were administered vehicle control article (77:10:13 w/w/w PEG 200:ethanol:SWI or 20 mg/kg/dose LEN solution (200 mg/mL with or without 1-molar equivalent NaOH) or 40 mg/kg/dose LEN (400 mg/mL without NaOH). Animals were dosed via subcutaneous 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.

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

All animals survived to their scheduled sacrifice. No LEN-related alterations in ophthalmic examinations, body weight, body weight gain, food consumption, clinical pathology test results, organ weights, or microscopic findings suggestive of systemic toxicity were noted. Nonadverse clinical observations (related to discomfort upon administration of the vehicle control article and LEN formulations) and dermal observations (edema and erythema) were noted.

At each sacrifice, LEN-related necrosis and granulomatous inflammation (minimal to marked severity), mixed cell inflammation (minimal to moderate severity), and/or macrophage infiltrate (minimal or slight severity) were observed in multiple subcutaneous injection sites and often correlated with macroscopic thickening and/or discoloration. At Interim Sacrifice 1, epidermal ulcer (slight or moderate) or erosion (minimal) rarely occurred in the overlaying skin and were considered secondary findings, most likely induced by scratching or biting at the subcutaneous injection site. Marked subcutaneous injection site necrosis was considered adverse based on severity and was observed at a low incidence in animals administered 20 mg/kg/dose with 1 molar equivalent NaOH or 40 mg/kg/dose without NaOH. Considering all sacrifice intervals, the overall incidence and severity of microscopic findings were highest for animals administered 40 mg/kg/dose without NaOH, and the lowest incidence and severity was observed in animals administered 20 mg/kg/dose with 1 molar equivalent NaOH. At each sacrifice interval, granulomatous inflammation, with variable central necrosis, was generally observed in all but the last site dosed and was considered indicative of the maturation/recovery process. In the oldest injection sites at Interim Sacrifice 2 and the Terminal Sacrifice, inflammation had recovered to a macrophage infiltrate in some animals. Overall, an increased interval since dosing was generally associated with a successive decrease in the overall incidence/severity of microscopic findings.

All concentration plasma values of LEN in the vehicle control group were below the lower limit of quantitation. 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 in LEN C_{max} and $AUC_{0\text{-}672h}$ values. Values for mean C_{max} and $AUC_{0\text{-}672h}$ were generally greater than 2-fold higher on Days 29, 58, 85, 113, 141, 169, 197, and 225 than on Day 1. LEN steady-state exposures (C_{max} and $AUC_{0\text{-}672h}$) were reached after four monthly SC doses on Day 85. Values for mean C_{max} and $AUC_{0\text{-}672h}$ were generally similar between Days 85, 113, 141, 169, 197, and 225. 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.

In conclusion, no systemic toxicity was noted, and LEN was well tolerated in male and female beagle dogs when administered as 10 monthly subcutaneous injections of 20 mg/kg/dose with or without NaOH or 40 mg/kg/dose without NaOH in the vehicle. LEN-related microscopic observations of necrosis, granulomatous inflammation, mixed cell inflammation, and/or macrophage infiltrate were observed at the subcutaneous injection sites of animals administered LEN. Adverse subcutaneous injection site necrosis was noted in animals administered 20 mg/kg/dose with NaOH or 40 mg/kg/dose without NaOH. Overall, an increased interval since

dosing was generally associated with a successive decrease in the overall incidence/severity of microscopic findings. LEN was present in plasma at all time points analyzed, indicating exposure for the entire 9-month study duration. On Day 225, the sex-combined plasma C_{max} and $AUC_{0\text{-}672h}$ values were 494 ng/mL and 267,000 ng·h/mL, respectively, in animals administered 200 mg/mL LEN without NaOH; 555 ng/mL and 277,000 ng·h/mL, respectively, in animals administered 200 mg/mL LEN with NaOH; and 658 ng/mL and 352,000 ng·h/mL, respectively, in animals administered 400 mg/mL LEN without NaOH.

Table 8. Mean Toxicokinetic Parameters up to Study Day 85 for LEN in Dogs After Monthly Subcutaneous Injections

Dose (mg/kg/dose) (formulation)	Sex	$AUC_{0\text{-}672h}$ (ng•h/mL)				C_{max} (ng/mL)			
		Study Day 1	Study Day 29	Study Day 58[a]	Study Day 85	Study Day 1	Study Day 29	Study Day 58	Study Day 85
20 (200 mg/mL, 0 mol-eq NaOH)	Male	50,300	122,000	150,000	215,000	110	218	295	383
	Female	64,100	129,000	148,000	175,000	149	257	282	311
	MF Combined	57,200	126,000	149,000	195,000	129	238	288	347
20 (200 mg/mL, 1 mol-eq NaOH)	Male	108,000	262,000	217,000	266,000	294	494	408	479
	Female	143,000	232,000	226,000	292,000	357	445	464	558
	MF Combined	125,000	247,000	222,000	279,000	325	470	436	518
40 (400 mg/mL, 0 mol-eq NaOH)	Male	67,800	176,000	185,000	246,000	180	322	319	434
	Female	65,100	187,000	224,000	263,000	171	362	452	458
	MF Combined	66,500	182,000	205,000	255,000	176	342	386	446

a Data is $AUC_{0\text{-}648h}$

Table 9. Mean Toxicokinetic Parameters for Study Days 113 to 169 for LEN in Dogs After Monthly Subcutaneous Injections

Dose (mg/kg/dose) (formulation)	Sex	AUC_{0-672h} (ng•h/mL)			C_{max} (ng/mL)		
		Study Day 113	Study Day 141	Study Day 169	Study Day 113	Study Day 141	Study Day 169
20 (200 mg/mL, 0 mol-eq NaOH)	Male	219,000	246,000	265000	404	413	416
	Female	185,000	191,000	183,000	332	327	316
	MF Combined	202,000	218,000	224,000	368	370	366
20 (200 mg/mL, 1 mol-eq NaOH)	Male	223,000	280,000	274,000	456	467	492
	Female	185,000	314,000	300,000	390	577	581
	MF Combined	204,000	297,000	287,000	423	522	537
40 (400 mg/mL, 0 mol-eq NaOH)	Male	269,000	330,000	367,000	487	624	607
	Female	332,000	367,000	417,000	662	634	719
	MF Combined	301,000	349,000	392,000	574	629	663

Table 10. Mean Toxicokinetic Parameters for Study Days 197 and 225 for LEN in Dogs After Monthly Subcutaneous Injections

Dose (mg/kg/dose) (formulation)	Sex	AUC_{0-672h} (ng•h/mL)		C_{max} (ng/mL)	
		Study Day 197	Study Day 225	Study Day 197	Study Day 225
20 (200 mg/mL, 0 mol-eq NaOH)	Male	311,000	312,000	511	571
	Female	167,000	221,000	294	416
	MF Combined	239,000	267,000	402	494
20 (200 mg/mL, 1 mol-eq NaOH)	Male	276,000	301,000	472	580
	Female	309,000	254,000	625	530
	MF Combined	292,000	277,000	549	555
40 (400 mg/mL, 0 mol-eq NaOH)	Male	355,000	363,000	612	723
	Female	335,000	340,000	589	592
	MF Combined	345,000	352,000	601	658

3.3.4. 39-Week Subcutaneous Toxicity Study (Solution)

The purpose of this study was to evaluate the toxicity and determine the toxicokinetics of LEN) when administered once every 4 weeks via subcutaneous injection to dogs for up to 37 weeks followed by a 2 or 13-week observation period (m2.6.7, Section 7.6, TX-200-2047).

Three groups of male and female Beagle dogs (4 or 6 animals/sex/group) were administered LEN at free form equivalent (FFE) dosages of 0 (vehicle control article: 68.17% [w/w] polyethylene glycol 300 [PEG-300]/31.83% [w/w] sterile water for injection), 130, or 411 mg/kg/dose at a dose volume of 1.33, 0.42 or 1.33 mL/kg, respectively. Assessment of toxicity was based on mortality, clinical observations, ophthalmic observations, body weights, food consumption, dermal observations, and clinical and anatomic pathology. Blood samples were collected for toxicokinetic evaluation. Animals were administered up to 10 doses via subcutaneous injection in the dorsal region as a single dose on Days 1, 29/32, 57, 85, 113, 141, 169, 197, 225, and 253. Each dose was administered at a unique dose site identified as Dose Site A through J. On Day 29, only controls and one 130 mg/kg/dose male were dosed due to transient discomfort postdose; all remaining animals in the 130 and 411 mg/kg/dose groups were dosed on Day 32 when dosing under anesthesia was initiated.

Dosing was discontinued for animals administered 411 mg/kg/dose after Day 57 due to adverse clinical observations. One male and two females administered 411 mg/kg/dose were euthanized on Days 51, 71 and 82, respectively. An interim necropsy of a subset of 411 mg/kg/dose animals (3 males, 2 females) occurred 2 weeks after the dose, Day 73. The terminal necropsy for remaining animals administered 411 mg/kg/dose occurred 12 weeks after the last dose, on Day 143 (2 animals/sex/group). Two vehicle controls/sex last dosed on Day 141 were necropsied on Day 143. All remaining animals from the vehicle and 130 mg/kg/dose groups last dosed on Day 253 were necropsied following a 2-week observation period, on Day 268.

All vehicle and 130 mg/kg/dose animals survived to their scheduled interim or terminal sacrifice. The clinical observations that preceded the unscheduled sacrifice of 1 male and 2 females administered 411 mg/kg/dose included yellow color of oral mucosa or conjunctiva, thin or hunched appearance, and/or 19 to 28% drop in body weight from peak and were attributed to hepatobiliary degeneration.

Transient observations, associated with discomfort of dosing, were noted in some animals immediately postdose (generally within 5 to 30 minutes) following administration of the vehicle control article or LEN on Days 1, 29, and 32. These observations were generally not noted in animals dosed after anesthetization, beginning on Day 32. No LEN-related alterations in ophthalmic examinations were noted.

LEN-related decreased body weight (up to 19 % from Day 1 body weight) and decreased food consumption was noted for animals administered 411 mg/kg/dose and sacrificed at an unscheduled interval. This effect on body weight and food consumption was secondary to LEN-related moribund condition. No other LEN-related alterations in body weight, body weight gain or food consumption were noted.

LEN-related dermal observations of edema and erythema were noted at the injection site for animals administered 130 or 411 mg/kg/dose LEN. Dermal observations of very slight to severe erythema were noted at an incidence that was less than that noted for edema, and the erythema observations tended to resolve sooner than edema. Edema and erythema were considered not adverse because they did not impact the health and wellbeing of animals.

LEN-related effects on clinical pathology test results in animals administered 411 mg/kg/dose were consistent with cholestasis and hepatobiliary injury in animals necropsied at an unscheduled interval or at Interim Necropsy 1 (Day 73). These changes consisted of moderately to markedly increased total bilirubin concentration, predominantly composed of direct (conjugated) bilirubin concentration; total bile acid concentration, alanine aminotransferase (ALT), alkaline phosphatase (ALP), and gamma glutamyltransferase (GGT) activities; total bile acid concentration; and mildly increased aspartate aminotransferase (AST) activity. These clinical pathology changes correlated with clinical observations (icteric sclera, oral mucosa, and inner pinna) and microscopic findings in the liver of vacuolar hepatocyte and bile duct epithelial degeneration, bile pigment, and/or oval cell/bile ductule hyperplasia. Increases in these serum chemistry indicators of cholestasis and hepatobiliary injury, as well as increased cholesterol concentrations (males only), were noted on Days 60 and 73 and in individual animals on Days 87 and 115 that survived until Day 143 (Terminal Necropsy 1). These findings were not present on Day 143, and therefore, these effects were reversible (with the exception of increased cholesterol concentration in one male) (Terminal Necropsy 1) and consistent with the reversibility of the vacuolar bile duct epithelium degeneration and bile pigment in the liver, noted in animals necropsied at an unscheduled interval or at Interim Necropsy 1.

Other LEN-related effects on clinical pathology test results in animals administered 411 mg/kg/dose, which were necropsied at an unscheduled interval or at Interim Necropsy 1 (Day 73) were considered likely secondary to adipose tissue necrosis and granulomatous inflammation associated with the injection site of animals. These effects in clinical pathology test results were reversible in animals that survived until Day 143.

LEN-related effects on clinical pathology test results in animals administered 130 mg/kg/dose were similar to, but of a lesser magnitude than, those in animals administered 411 mg/kg/dose and consisted of minimal to mild increases in 1 or more of the following: increased bile acid concentrations, and increased ALT, GGT, and ALP activities and bile acid concentrations. These effects were noted in individual animals administered 130 mg/kg/dose, starting on Day 87, and at 1 or more subsequent time points up to Day 268 (Terminal Necropsy 2), and were associated with microscopic observations of oval cell/bile ductule hyperplasia, increased pigmented macrophages, and, in 1 male, periductular fibrosis at Terminal Necropsy 2. Increased total bilirubin concentrations were not noted in animals administered this dose. Other LEN-related effects in individual animals administered 130 mg/kg/dose consisted of minimally and often transiently increased fibrinogen concentrations, which potentially correlated with cell infiltrate associated with the injection site(s) noted at Terminal Necropsy 2.

LEN-related macroscopic findings included thickened and/or discolored subcutaneous injection sites for animals administered ≥130 mg/kg/dose (correlated with microscopic findings). LEN-related macroscopic findings in animals administered 411 mg/kg/dose were limited to 2 animals necropsied in a moribund condition on Day 51 or 71; these included bilateral yellow eye sclera discoloration for 1 animal and the other had yellow discoloration of the oral mucosa, liver, kidney, and aorta; and thickened gall bladder (associated with microscopic findings in the liver and/or gall bladder). No LEN-related alterations in organ weights were noted.

LEN-related microscopic findings were noted in the liver, gall bladder, and/or subcutaneous injection sites of animals administered ≥130 mg/kg/dose and in the kidney and/or stomach of animals administered 411 mg/kg/dose.

LEN-related adverse findings in animals administered 411 mg/kg/dose were noted in the liver (vacuolar degeneration of hepatocytes and/or bile duct epithelium, bile pigment, mixed cell infiltrate, and/or oval cell/bile ductule hyperplasia), gall bladder (epithelial hyperplasia, mucosal edema, increased lumen secretion, and/or lumen pigment), kidney (tubule degeneration and/or dilatation, and/or pigment), and stomach (mucosa degeneration/atrophy). The overall incidence and severity (minimal to marked) of LEN-related microscopic findings were similar for animals necropsied at an unscheduled interval (Days 51 to 82) or at Interim Necropsy 1 (Day 73), and the findings were generally partially (liver, gall bladder, and Subcutaneous Injection Sites A through C) or fully (kidney and stomach) reversed by Terminal Necropsy 1 (Day 143).

LEN-related findings in animals administered 130 mg/kg/dose were noted in the liver (oval cell/bile ductule hyperplasia, periductular fibrosis attributed to the longer dosing duration, and increased pigmented macrophages), gall bladder (epithelial hyperplasia, increased lumen secretion, and/or lumen pigment), and Subcutaneous Injection Sites A through J (minimal to marked granulomatous and/or mixed cell inflammation, adipose tissue necrosis, fibrosis, and/or pseudocyst). The overall incidence of LEN-related liver and gall bladder microscopic findings were generally less severe at Terminal Necropsy 2 (minimal or slight) than those noted for animals administered 411 mg/kg/dose and necropsied at earlier intervals. The periductular fibrosis in liver of one animal was considered adverse. All other liver, gall bladder and skin injection site findings were considered nonadverse.

All concentration values of LEN in the vehicle control group were below the lower limit of quantitation. 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. Exposure to LEN 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-based differences were generally less than 2-fold in LEN $C_{max,}$ $AUC_{0\text{-}336h}$ and $AUC_{0\text{-}672h}$ values. Accumulation of LEN was observed after multiple doses in dogs. The accumulation ratios (AR) 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.

In conclusion, monthly subcutaneous administration of 411 mg/kg/dose LEN to dogs was not tolerated and resulted in adverse clinical observations that necessitated cessation of dosing after 2 or 3 doses. LEN-related clinical pathology changes in animals administered 411 mg/kg/dose were consistent with cholestasis and hepatobiliary injury. LEN-related microscopic findings in animals administered 411 mg/kg/dose were noted in the liver, gall bladder, kidney, and stomach; these microscopic findings were partially (liver and gall bladder) or fully reversible (kidney and stomach). Compared to the 411 mg/kg/dose group, monthly administration of 130 mg/kg/dose LEN for 9 months (10 doses) resulted in less severe changes in clinical pathology test results, and fewer and less severe liver and gall bladder microscopic findings, but included periductular fibrosis in one dog, considered adverse. A no-observable-adverse-effect level (NOAEL) was not established for LEN in this study. The dose level of 130 mg/kg/dose corresponded to Study Day 225 mean C_{max} and AUC_{0-336} values of 5480 ng/mL and 1540000 ng•h/mL, respectively and Day 253 C_{max} value of 4860 ng/mL, for combined sexes.

Table 11. Mean Toxicokinetic Parameters up to Study Day 57 for LEN in Dogs After Monthly Subcutaneous Injections

Dose (mg/kg/dose)	Sex	AUC_{0-672h} (ng•h/mL)				C_{max} (ng/mL)			
		Day 1	Day 29[a]	Day 32[b]	Day 57	Day 1	Day 29	Day 32	Day 57
130	Male	814,000	1,970,000	NA	1,630,000	1910	3720	2120	2670
	Female	1,210,000	NA	NA	2,440,000	3240	NA	3510	4120
	MF Combined	1,010,000	NA	NA	2,030,000	2580	NA	2910	3400
411	Male	2,480,000	NA	NA	NA[c]	5090	NA	9530	9950
	Female	2,290,000	NA	NA	NA[c]	4760	NA	7550	8620
	MF Combined	2,380,000	NA	NA	NA[c]	4920	NA	8540	9230

a One male animal had samples collected for TK on day 29
b A sample was not collected 672 h postdose on day 32. Predose on day 57 = 600 h postdose
c Animals not administered 4th dose, therefore no data available for day 57

Table 12. Mean Toxicokinetic Parameters for Study Days 85 to 141 for LEN in Dogs After Monthly Subcutaneous Injections

Dose (mg/kg/dose)	Sex	AUC_{0-672h} (ng•h/mL)			C_{max} (ng/mL)		
		Day 85	Day 113	Day 141	Day 85	Day 113	Day 141
130	Male	1,940,000	2,300,000	2,370,000	3460	4040	4180
	Female	2,910,000	2,650,000	3,010,000	4900	4610	5340
	MF Combined	2,430,000	2,470,000	2,690,000	4180	4320	4760

Table 13. Mean Toxicokinetic Parameters for Study Days 169 to 225 for LEN in Dogs After Monthly Subcutaneous Injections

Dose (mg/kg/dose)	Sex	$AUC_{0\text{-}672h}$ (ng•h/mL)			C_{max} (ng/mL)		
		Day 169	Day 197	Day 225	Day 169	Day 197	Day 225
130	Male	2,480,000	2,640,000	2,850,000	4540	4300	4640
	Female	2,990,000	3,650,000	3,540,000	5430	6220	6320
	MF Combined	2,730,000	3,150,000	3,200,000	4980	5260	5480

3.4. Dog - Oral

3.4.1. 4-Week Oral Gavage Toxicity and Toxicokinetic Study of LEN

The purpose of this study was to evaluate the toxicity and determine the TK of LEN when administered daily via oral gavage to Beagle dogs for at least 4 weeks (m2.6.7, Section 7.7, TX-200-2012).

Four groups of purebred Beagle dogs (4/sex/group) were administered LEN solution at dose levels of 0 (vehicle control article: 10% v/v DMSO, 10% v/v vitamin E TPGS, and 80% v/v PG), 1, 5, or 30 mg/kg/day at a volume of 2 mL/kg.

Assessment of toxicity was based on mortality, clinical observations, body weights, body weight change, food consumption, ophthalmic observations, and clinical and anatomic pathology. Blood samples were collected for toxicokinetic evaluations. Liver samples were collected at the terminal necropsy on Day 29 for hepatic microsomal fraction analysis.

Exposure to LEN increased with the increase in dose level 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, except for 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 generally less than 2-fold in LEN C_{max} and $AUC_{0\text{-}24h}$ values. 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.

No LEN-related mortality occurred. One male administered 30 mg/kg/day was sacrificed early due to gavage error. No adverse LEN-related clinical observations were noted. The nonadverse clinical observation of struggling during dosing was noted for all groups administered LEN and was of uncertain relationship to the test article due to the sporadic incidence.

No LEN-related changes in body weight, body weight change, food consumption, hematology, coagulation, or urinalysis test results or ophthalmic, macroscopic, or microscopic observations were noted.

Potential, nonadverse LEN-related clinical pathology changes included liver enzyme increases (minimally to mildly increased aspartate and alanine aminotransferase, alkaline phosphatase, and/or gamma glutamyl transferase activities) on Day 29 in 1 male and 1 female administered 30 mg/kg/day. These findings were suggestive of a minor hepatobiliary alteration and correlated with slight biliary hyperplasia in the female.

A notable increase in CYP1A and CYP2B activities (2.6 and 2-fold, respectively) was observed in females administered 1 and 30 mg/kg/day, respectively.

In conclusion, no adverse LEN-related effects were noted in male and female Beagle dogs administered 1, 5 or 30 mg/kg/day via once daily oral gavage for 4 weeks; therefore, the NOAEL is 30 mg/kg/day. This dose level corresponds to sex-combined C_{max} and $AUC_{0\text{-}24h}$ values of 4390 ng/mL and 75900 ng·h/mL, respectively, on Day 28.

Table 14. Mean Toxicokinetic Parameters for LEN in Dogs After Daily Oral Administration

Dose (mg/kg/dose)	Sex	$AUC_{0\text{-}24h}$ (ng•h/mL)		C_{max} (ng/mL)	
		Study Day 1	Study Day 28	Study Day 1	Study Day 28
1	Male	1920	4610	129	263
	Female	1400	2270	92.7	146
	MF Combined	1660	3440	111	204
5	Male	9510	20,400	721	1310
	Female	6500	18,200	467	1160
	MF Combined	8010	19,300	594	1230
30	Male	54,700	82,900	3110	4520
	Female	45,700	70,600	2470	4290
	MF Combined	50,200	75,900	2790	4390

4. GENOTOXICITY

4.1. In Vitro

4.1.1. *Salmonella-E. Coli*/Mammalian Microsome Reverse Mutation Assay with LEN

The purpose of this study was to evaluate the ability of LEN to induce reverse mutations in histidine requiring strains of *Salmonella typhimurium* and tryptophan requiring strain of *Escherichia coli* in the presence and absence of a rat liver metabolizing system (S9) (m2.6.7, Section 8.1, TX-200-2007).

The test article, LEN, was evaluated in the bacterial reverse mutation assay in all five tester strains at concentrations of 5.00, 16.0, 50.0, 160, 500, 1600, and 5000 µg/plate in the presence and absence of S9. Precipitate was observed at ≥500 µg/plate in all tester strains in the presence and absence of S9 except WP2*uvr*A, where precipitate was observed at ≥1600 µg/plate in the absence of S9. No toxicity was observed at any tested concentration in any tester strain in the presence or absence of S9.

No increase in the mean number of revertant colonies occurred in the presence or absence of S9. All vehicle and positive controls were within the acceptable range. All acceptance criteria were met, and the study was accepted as valid.

The results indicate LEN is negative in the bacterial reverse mutation assay when tested in the presence and absence of S9.

4.1.2. In Vitro Chromosome Aberration Test in Cultured Human Peripheral Blood Lymphocytes with LEN

The purpose of this study was to evaluate the ability of LEN to induce chromosomal aberrations in cultured human peripheral blood lymphocytes (HPBL) with and without an exogenous metabolic activation system (Aroclor 1254 induced rat liver S9) (m2.6.7, Section 8.2, TX-200-2008). DMSO was used as vehicle in this study. Different concentrations of LEN were used with a dosing volume of 1% (v/v). Vehicle and positive controls were used concurrently. Based on the current ICH S2(R1) guideline, the highest concentration of LEN used in the initial assay was the limit dose of 500 µg/mL.

In the initial chromosomal aberration assay, LEN was evaluated in HPBL at concentrations ranging from 3.39 to 500 µg/mL for 3 and approximately 24 hours without metabolic activation and for 3 hours with metabolic activation. The vehicle control cultures were treated with an equal volume (100 µL) of vehicle (DMSO). All cultures were harvested approximately 24 hours after the initiation of treatment. In the 3-hour treatment without S9 assay, 172 µg/mL was the lowest LEN concentration where precipitate was observed; 172, 120 and 84.0 µg/mL produced 3%, 5% and 1% mitotic inhibition, respectively, and were analyzed for chromosomal aberrations. In the 3-hour treatment with S9 assay, 245 µg/mL was the lowest concentration where precipitate was observed; 245, 172 and 120 µg/mL produced 31%, 19% and 25% mitotic inhibition, respectively, and were analyzed for chromosomal aberrations.

In the approximate 24-hour treatment without S9 assay, the mitotic index for the vehicle control cultures did not meet the assay criteria and the assay was repeated. In the repeat assay, 172 µg/mL was the lowest LEN concentration where precipitate was observed; 172, 120 and 41.2 µg/mL produced 49%, 35% and 21% mitotic inhibition, respectively, and were analyzed for chromosomal aberrations.

No statistically significant increase in the aberrant cells excluding gaps was observed with or without S9. All vehicle and positive controls were within the acceptable range. All acceptance criteria were met, and the study was accepted as valid.

Lenacapavir was considered negative for inducing chromosomal aberrations in HPBL when tested up to the limit of cytotoxicity in the approximate 24-hour treatment without S9 and up to the limit of solubility in the 3-hour treatments with and without S9.

4.2. In Vivo

Micronuclei in bone marrow were taken from the study "Four-Dose (Twice Monthly) Subcutaneous Injection Toxicity and Toxicokinetic Study of LEN in Wistar Han Rats" (m2.6.7, Section 7.1, TX-200-2005). Micronuclei were assessed following SC injection to male and female rats at 0 (vehicle control), 10, 30 and 100 mg/kg/dose EOW for 4 injections. Positive control animals were administered 60 mg/kg cyclophosphamide once by oral gavage approximately 24 hours prior to harvest time-point. Femoral bone marrow smears were prepared from the first 5 animals/sex/group on Day 57) and from all animals administered cyclophosphamide. Cytotoxicity was assessed by scoring the number of polychromatic erythrocytes (PCEs) and normochromatic erythrocytes in at least 2,000 total erythrocytes for each animal.

All vehicle control data (% PCE and MN PCE) were comparable with the laboratory's historical vehicle control data. The positive control treatments for both sexes resulted in a statistically significant increases in MN PCE over the concurrent vehicle controls. The assay was therefore accepted as valid. For both sexes, all animals administered LEN exhibited group mean % PCE that were similar to the vehicle control group. All values fell within the historical vehicle control observed range. As such, there was no evidence of any test article-induced toxicity to the bone marrow. All male and female animals administered LEN exhibited MN PCE frequencies that were similar to the vehicle control group. Group mean and individual MN PCE values fell within the historical vehicle control range.

In conclusion, LEN was negative in the male and female rat bone marrow MN assay when administered subcutaneously at doses of up to 100 mg/kg/dose EOW for a total of 4 injections. This dose corresponded to Study Day 43 combined sexes mean C_{max} and $AUC_{0\text{-}336h}$ values of 2080 ng/mL and 583000 ng.h/mL, respectively.

5. CARCINOGENICITY

The 6-month transgenic mouse (TgrasH2) and the 2-year rat carcinogenicity studies with LEN via subcutaneous injection are in progress. Doses were agreed upon with US FDA. The final reports will be submitted when available; approximately the end of 20[redacted] for the mouse study and early 20[redacted] for the rat study.

5.1. A 26-Week Subcutaneous Carcinogenicity Study in 001178-T (Hemizygous) RasH2 Mice

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

5.2. 104 Week Subcutaneous Injection Carcinogenicity and Toxicokinetic Study of LEN Administered Every 13 Weeks in Wistar-Han Rats

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

6. REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

6.1. Fertility and Early Embryonic Development

6.1.1. Subcutaneous Injection Study of the Effects of LEN on Fertility and Early Embryonic Development to Implantation in Sprague Dawley Rats

The purpose of this study was to determine the potential adverse effects in the reproductive process resulting from a single subcutaneous administration of a long acting LEN formulation in male and female Sprague Dawley rats (m2.6.7, Section 12, TX-200-2043). Exposure from the single dose included the premating period through conception and implantation. This included identification of potential deficits in estrous cycling, tubal transport, implantation, development of the pre-implantation stages of the embryo in the female, and functional reproductive effects (alterations in libido and epididymal sperm maturation) in the male. Male rats were dosed 6 weeks prior to mating. Female rats were dosed 4 weeks prior to mating. The duration of the exposure prior to mating was selected to ensure delivery of sufficient drug from the subcutaneous drug depot to achieve relevant LEN plasma levels. A TK assessment of LEN was performed to confirm exposure for the duration of the in-life phase.

Lenacapavir solution was administered at doses of 0 (77% w/w PEG 200, 10% w/w ethanol, and 13% w/w SWI), 20, or 100 mg/kg once via subcutaneous injection to male and female Crl:CD(SD) rats (N = 25/sex/group) via 200 mg/mL LEN (0.5, 0.1, and 0.5 mL/kg, respectively). Assessment of toxicity was based on mortality, clinical signs, body weights, body weight gains, food consumption, estrous cycles, reproductive performance, intrauterine survival, gross necropsy findings, sperm parameters, and organ weights. A satellite group of animals (3/sex control, 6/sex/group in LEN groups) was included for determination of toxicokinetic parameters.

No LEN-related effects on survival or clinical observations were noted for males and females at any dose. No LEN-related effects on mean body weights, body weight gains, and food consumption were noted, and no effects on male and female reproductive performance (mating, fertility, and pregnancy), male spermatogenesis (sperm numbers, sperm production rate, and motility), and male or female organ weights were noted. No LEN-related macroscopic findings were noted. Intrauterine survival of the embryos was unaffected by LEN administration at all dose.

Exposure to LEN was confirmed for the duration of the in-life phase and increased with the increase in dose from 20 to 100 mg/kg. The increases in C_{max} and $AUC_{0\text{-}672h}$ were less than dose proportional. Sex-based differences were less than 2-fold in C_{max} and $AUC_{0\text{-}672h}$ values.

Based on the lack of effects, a dose of 100 mg/kg, the highest dose evaluated, was considered to be the no-observed-effect level (NOEL) for male and female systemic toxicity, male and female reproductive toxicity, and embryonic toxicity of LEN when administered as a single subcutaneous dose to Crl:CD(SD) rats. At the NOEL (100 mg/kg), the C_{max} and $AUC_{0\text{-}1008h}$ values were 577 ng/mL and 441,000 h•ng/mL, respectively, for males and the C_{max} and $AUC_{0\text{-}672h}$ values were 597 ng/mL and 192000 h•ng/mL, respectively, for females.

Table 15. Mean Toxicokinetic Parameters for LEN in Mated Rats after Subcutaneous Injection

Dose (mg/kg)	Sex	C_{max} (ng/mL)	T_{max} (h)	AUC_{0-t} (ng•h/mL)	AUC_{0-672h} (ng•h/mL)	$AUC_{0-1008h}$ (ng•h/mL)	C_{last} (ng/mL)	T_{last} (h)
20	M	188	672	161,000	103,000	161,000	157	1008
	F	153	336	91,100	91,100	NC	123	672
	MF	168	336	150,000	97,200	N/A	157	1008
100	M	577	672	441,000	270,000	441,000	439	1008
	F	597	24.0	192,000	192,000	NC	290	672
	MF	493	24.0	378,000	231,000	N/A	439	1008

N/A Not applicable,
NC Not calculated as samples were only collected to 672 hours.
Note: MF data are based on the analysis of the combined concentration data for both males and females, where applicable. Additionally, only male samples were collected out to 1008 hours because this includes the 6 weeks prior to mating; female sample collections ended at 672 hours because this includes the 4 weeks prior to mating.

6.2. Embryofetal Development

6.2.1. Oral (Gavage) Dose Range Finding Study of the Effects of LEN on Embryo/Fetal Development in Sprague Dawley Rats

The purpose of this study was to determine dose levels of LEN to be evaluated in a definitive developmental toxicity study in rats. LEN solution was administered at oral doses of 0 (10% v/v DMSO, 10% v/v vitamin E TPGS, and 80% v/v propylene glycol), 3, 30, and 300 mg/kg/day via once daily oral gavage to time-mated Crl:CD(SD) rats (8/group) during organogenesis (Gestation Day [GD] 6 to 17) at a dose volume of 2 mL/kg. Assessment of toxicity was based on mortality, clinical observations, body weights, body weight gains, gravid uterine weights, food consumption, gross necropsy findings, intrauterine growth and survival, and fetal external and visceral morphology. Additional time-mated rats (3 rats in the vehicle control group and 6 rats/LEN-treated group) were used for toxicokinetic analysis on GD 6 and 17 (m2.6.7, Section 11, TX-200-2031).

All females in the vehicle control, 3, 30, and 300 mg/kg/day groups survived to the scheduled necropsy on GD 21.

There were no LEN-related effects on clinical or post-dose observations, mean body weights, body weight gains, net body weights, net body weight gains, gravid uterine weights, or food consumption at any dose.

There were no LEN-related macroscopic findings at the scheduled GD 21 necropsy, and no effects on intrauterine growth and survival or fetal external and visceral morphology at any dose level.

All LEN concentrations at the last time point in the LEN dosing groups were quantifiable (T_{last} = 24.0 h). Exposure (AUC_{0-24h} and C_{max}) to LEN increased with the increase in dose from 3 to 30 mg/kg/day but did not increase with the further dose increase from 30 to 300 mg/kg/day. The increases in C_{max} and AUC_{0-24h} were less than dose proportional between the 3 and 30 mg/kg/day dose levels. Accumulation ranging from 1.8 to 2.8-fold was observed at all dose levels after multiple doses of LEN in pregnant rats.

In conclusion, administration of LEN to pregnant rats during GD 6 through 17 at dose levels of 3, 30, and 300 mg/kg/day resulted in no LEN-related effects on maternal animals or embryo/fetal development. Therefore, the NOEL for maternal and embryo/fetal developmental toxicity was determined to be 300 mg/kg/day, which corresponded to GD 17 LEN C_{max} and AUC_{0-24h} values of 1240 ng/mL and 26300 ng•h/mL, respectively.

Table 16. Mean Toxicokinetic Parameters for LEN in Pregnant Rats After Daily Oral Administration: GD 6 and 17

Interval (GD)	Dose (mg/kg/day)	C_{max} (ng/mL)	$AUC_{(0-24h)}$ (ng•h/mL)
6	3	211	4090
	30	861	16,500
	300	677	13,800
17	3	562	11,600
	30	1570	29,300
	300	1240	26,300

6.2.2. Oral (Gavage) Study of the Effects of LEN on Embryo/Fetal Development in Sprague Dawley Rats

The objectives of this study were to determine the potential of LEN to induce developmental toxicity after maternal exposure during the critical period of organogenesis, to characterize maternal toxicity at the exposure levels tested, and to determine a NOAEL for maternal toxicity and developmental toxicity (m2.6.7, Section 13.1,TX-200-2036). In addition, a toxicokinetic assessment of plasma levels of LEN was performed. LEN was administered at doses of 0 (10% DMSO, 10% vitamin E TPGS, and 80% propylene glycol) 3, 10, and 30 mg/kg/day once daily by oral gavage to time-mated Crl:CD(SD) rats (25/group) during organogenesis (Gestation Day [GD] 6 to 17) at a dose volume of 2 mL/kg. Assessment of toxicity was based on mortality, clinical signs, body weights, body weight gains, gravid uterine weight, food consumption, gross necropsy findings, intrauterine growth and survival, and external, visceral, and skeletal morphology. Additional time-mated rats (3 rats in the vehicle control group and 6 rats in each LEN-treated group) were used for toxicokinetic analysis on GD 6 and 17.

In the 3 mg/kg/day group, 1 female was found dead on GD 18. This death was not considered LEN-related based on the lack of clinical findings or effects on body weight for this female and because there was no LEN-related effect on survival at 10 and 30 mg/kg/day. All other females in the vehicle control, 3, 10, and 30 mg/kg/day groups survived to the scheduled necropsy on GD 21. A single female in the 10 mg/kg/day group delivered on the day of scheduled euthanasia, which was not considered LEN-related because GD 21 is the anticipated day of delivery.

There were no LEN-related effects on post-dose clinical observations, mean maternal body weights, body weight gains, gravid uterine weights, corrected body weights, corrected body weight gains, or food consumption at any dose level.

There were no LEN-related macroscopic findings noted at the scheduled necropsy on GD 21 and no effects on intrauterine growth and survival or fetal morphology at any dose level.

Exposure (C_{max} and AUC_{0-24h}) to LEN increased with the increase in dose level from 3 to 30 mg/kg/day; however, the increases were less than dose proportional. Slight accumulation (approximately 2-fold) of LEN was observed after multiple doses of 3 mg/kg/day in pregnant rats. The daily oral doses maintained systemic exposure of LEN for at least 24 hours/day (no values BLQ) and were still relatively high at the end of dosing. The T_{max} ranged from 3.00 to 8.00 hours.

In conclusion, administration of LEN to pregnant rats during GD 6 through 17 at dose levels of 3, 10, and 30 mg/kg/day resulted in no LEN-related effects on maternal animals or embryo/fetal development. Therefore, NOEL for maternal toxicity and embryo/fetal developmental toxicity was determined to be 30 mg/kg/day, which corresponded to LEN GD 17 mean C_{max} and AUC_{0-24h} values of 1210 ng/mL and 22,000 ng•h/mL, respectively.

Table 17. Mean Toxicokinetic Parameters for LEN in Pregnant Rats After Daily Oral Administration: GD 6 and 17

GD	Dose (mg/kg/day)	C_{max} (ng/mL)	AUC_{0-24h} (ng•h/mL)
6	3	209	4410
	10	529	10,700
	30	692	13,300
17	3	435	9200
	10	1050	17,800
	30	1210	22,000

6.2.3. A 30-Minute IV Infusion Dose Range Finding Study of the Effects of LEN on Embryo/Fetal Development in New Zealand White Rabbits

The purpose of this study was to determine IV dose levels of LEN to be evaluated in a definitive developmental toxicity study in New Zealand white rabbits (6 presumed pregnant females/group). LEN solution was administered at doses of 0 (6.12% w/w PEG 300 and 93.88% w/w SWI, 5, 10, or 20 mg/kg/day via IV infusion over approximately 30 minutes via the marginal ear vein once daily during Gestation Days (GD) 7 to 19 at dose volumes of 0.667, 0.167, 0.333, and 0.667 mL/kg, respectively. All surviving animals were necropsied on GD 29. Assessment of toxicity was based on clinical signs, body weights, body weight gains, gravid uterine weights, food consumption, toxicokinetic parameters, gross necropsy findings, intrauterine growth and survival, and fetal morphology (m2.6.7, Section 11, TX-200-2032).

There were no LEN-related adverse effects on clinical observations, mean corrected body weights, corrected body weight gains, and gravid uterine weights at any dose level. Except for 1 female in the vehicle control group, all females were determined to be gravid. There were no LEN-related adverse effects on macroscopic findings at the scheduled necropsy, and there were no effects on intrauterine growth and survival, or external and visceral fetal morphology noted at any dose level.

One female in the 10 mg/kg/day group was euthanized in extremis on GD 13 following body weight loss and reduced food consumption from GD 8 through 13. Clinical observations noted for this female prior to euthanasia included decreased fecal output, soft or mucoid feces, and brown staining on the tail and hind limbs. All other females survived to the scheduled euthanasia on GD 29 with no LEN-related findings, or deaths when dosed up to 20 mg/kg/day.

All plasma concentration values of LEN in the vehicle control group were below the lower limit of quantitation. Exposure to LEN increased with the increase in LEN dose level from 5 to 20 mg/kg/day. The increases in C_{max} and $AUC_{0\text{-}24.5h}$ were greater than dose proportional between the 5 to 20 mg/kg/day dose levels, with the exception of the increases between 10 and 20 mg/kg/day on GD 19 where the increases were approximately dose proportional. No accumulation of LEN occurred. LEN was measurable 96 hours after the last dose.

In conclusion, IV administration of LEN to time-mated New Zealand White rabbits during GD 7 to 19 resulted in no LEN-related adverse maternal or embryo/fetal effects up to 20 mg/kg/day, the highest dose evaluated. Therefore, a dose level of 20 mg/kg/day was NOAEL for maternal toxicity and the NOEL for embryo/fetal development. This dose level corresponded to C_{max} and $AUC_{0\text{-}24.5h}$ values of 45,700 ng/mL and 178,000 ng•hr/mL, respectively, on Gestation Day 19.

Table 18. Mean Toxicokinetic Parameters for LEN in Pregnant Rabbits After Intravenous Infusion: GD 7 and 19

Dose	$AUC_{(0\text{-}24.5h)}$ (ng•hr/mL)		$AUC_{(0\text{-}96.5h)}$ (ng•hr/mL)		C_{max} (ng/mL)	
	GD 7	GD 19	GD 7	GD 19	GD 7	GD 19
5 mg/kg/day	13,700	26,600	NA	31,200	4330	7120
10 mg/kg/day	44,200	89,500	NA	101,000	15,100	22,300
20 mg/kg/day	134,000	178,000	NA	204,000	46,700	45,700

NA = Not applicable.

6.2.4. A 30-Minute IV Infusion Study of the Effects of LEN on Embryo/Fetal Development in New Zealand White Rabbits

The objectives of this study were to determine the potential of LEN to induce developmental toxicity after maternal exposure during the critical period of organogenesis, to characterize maternal toxicity at the exposure levels tested, and to determine a NOAEL for maternal toxicity and developmental toxicity in New Zealand white rabbits (m2.6.7, Section 13.2,TX-200-2037).

Lenacapavir solution (30 mg/mL) was administered to mated rabbits (22/group) at doses of 0 (6.12% w/w PEG 300 and 93.88% w/w SWI), 5, 10, and 20 mg/kg/day by IV infusion over approximately 30 minutes via the marginal ear vein once daily during Gestation Days (GD) 7 to 19 at dose volumes of 0.667, 0.167, 0.333, and 0.667 mL/kg, respectively. All surviving animals were necropsied on GD 29. Assessment of toxicity was based on clinical signs, body weights, body weight gains, gravid uterine weights, food consumption, gross necropsy findings, intrauterine growth and survival, and fetal morphology.

There were no adverse LEN-related effects on mean body weights, corrected body weights, corrected body weight gains, food intake and gravid uterine weights at any dose level. During the treatment period (GD 7-20), statistically significantly lower mean body weight gains occurred in a dose related manner compared to the vehicle control group, but mean body weights were not significantly different. There were no LEN-related effects on macroscopic findings at the scheduled necropsy, and there were no effects on intrauterine growth and survival or external, visceral, or skeletal fetal morphology at any dose level.

One female in the 20 mg/kg/day group was found dead on GD 19; this mortality was attributed to mechanical injury possibly from the restraint or dosing procedures, and therefore was not considered LEN-related. All other females survived to the scheduled euthanasia on GD 29. Local skin observations at the daily examinations and/or approximately 4 hours post-dose for females in all LEN-administered groups included skin discoloration, bruising, and scabbing at the injection sites. No other LEN-related clinical observations were noted at the daily examinations or approximately 4 hours following dose administration at any dose level.

Based on the lack of adverse maternal effects and lack of embryo/fetal effects following administration of LEN via IV injection to pregnant rabbits during GD 7 through 19 at dose levels of 5, 10, and 20 mg/kg/day, a dose level of 20 mg/kg/day was considered to be the NOAEL for maternal toxicity and NOEL for embryo/fetal development.

6.3. Prenatal and Postnatal Development

6.3.1. Subcutaneous Developmental and Perinatal/Postnatal Reproduction Toxicity Study of LEN in Sprague Dawley Rats, Including a Postnatal Behavioral/Functional and Reproduction Evaluation

The purpose of this study was to determine the potential adverse effects of maternal LEN exposure from implantation to weaning on pregnancy, parturition, and lactation of the maternal (F_0) animals and on the growth, viability, and development of the F_1 neonates (m2.6.7, Section 14, TX-200-2049). Reproductive performance of the F_1 generation was also assessed. A toxicokinetic (TK) assessment of LEN was performed to confirm exposure of the maternal animals over the duration of the in-life phase (up to Postmating Day 25 or Lactation Day (LD) 21).

Four groups of mated female Sprague Dawley rats (24/group) were administered LEN solution at doses of 0 (68.2% PEG 300 and 31.8% SWI), 30, or 300 mg/kg via a single subcutaneous injection at dose volumes of 1.0, 0.1, and 1.0 mL/kg, respectively, on Gestation Day (GD) 6. Assessment of F_0 toxicity was based on mortality, clinical signs, body weights, body weight gains, food consumption, parturition, gestation length, and gross necropsy findings; assessment of F_1 toxicity was based on litter viability and survival, mortality, clinical signs, body weights, body weight gains, pre- and postweaning developmental landmarks, neurobehavior, estrous cycles, reproductive performance, intrauterine survival, and gross necropsy findings. Satellite groups of maternal animals (3/sex in the vehicle control group, 6/sex/group in LEN groups) were included for determination of LEN toxicokinetic parameters. Blood samples were also collected for LEN analyses from F_1 pups (2 pups/sex/litter) on PND 10.

There were no LEN-related effects on survival of the F_0 females at any dosage level. One female in the 300 mg/kg group did not deliver and was euthanized on postmating day 25 and determined to be non-gravid based on macroscopic examination. No LEN-related clinical findings were observed in the F_0 generation except for at the injection site. LEN-related swollen dorsal trunk and scabbing on the dorsal trunk associated with the injection site were noted at 300 mg/kg at the daily examinations throughout the study. In the absence of any other signs of systemic toxicity, these observations were considered nonadverse. No LEN-related effects on mean body weights, body weight gains, food consumption, gestation length, and the process of parturition were noted. At the scheduled necropsy, LEN-related macroscopic findings of thickened injection sites in the dorsal scapular area were noted in the 30 and 300 mg/kg groups in a dose-responsive manner; these findings corresponded to the aforementioned clinical observations noted in the 300 mg/kg group and were attributed to LEN administration but were considered nonadverse in the absence of any signs of systemic toxicity.

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

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

A single F_1 male in the 300 mg/kg group was euthanized in extremis on PND 56 following a body weight loss and observations of labored respiration and salivation; no internal findings were noted at necropsy. In the absence of any other effects during the F_1 generation that could be attributed to maternal LEN administration, this death was not considered LEN-related.

Mean F_1 body weights, body weight gains, estrous cycle length, reproductive performance, pre-coital index, gross pathology, mean numbers of corpora lutea and implantation sites, and intrauterine survival of the F_2 embryos were unaffected by F_0 maternal LEN administration.

All concentration values of plasma LEN in the vehicle control group were below the lower limit of quantitation. Dams were exposed to LEN for the duration of gestation and to LD 10. Exposure to LEN 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 LD 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 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 on PND 10 plasma values for pups.

In conclusion, no LEN-related systemic effects were noted at any dose level in the F_0 or F_1 generation; nonadverse local injection site reactions were observed in the F_0 LEN-dosed dams. Therefore, a dose level of 300 mg/kg, the highest level tested, was considered to be the NOAEL for F_0 maternal systemic toxicity, and the NOEL for F_1 developmental/neonatal, F_1 parental systemic, F_1 reproductive toxicity, and F_2 embryo intrauterine survival of LEN when administered subcutaneously as a single dose to Sprague-Dawley rats on GD 6. This dose level corresponds to F_0 maternal $AUC_{0–192h}$ and C_{max} values of 54,800 ng·h/mL and 412 ng/mL, respectively.

Table 19. Mean Toxicokinetic Parameters for LEN in Pregnant Rats After Subcutaneous Injection: GD 6

Dose (mg/kg)	C_{max} (ng/mL)	T_{max} (h)	$AUC_{0\text{-}t}$ (ng•h/mL)	$AUC_{0\text{-}192h}$ (ng•h/mL)	C_{last} (ng/mL)	T_{last} (h)
30	87.6	96.0	10,900	10,900	78.5	192
300	412	72.0	54,800	54,800	395	192

Table 20. Mean Maternal to Pup Mean Concentration Ratios: LD 10 and PND 10

Dose (mg/kg)	Mean Maternal Concentration (ng/mL)	Mean Pup Concentration (ng/mL)	Maternal Pup Ratio
30	36.7	7.12	5.15
300	196	32.8	5.96

7. LOCAL TOLERANCE

7.1. Phototoxicity

7.1.1. Neutral Red Uptake Phototoxicity Assay of LEN in BALB/c 3T3 Mouse Fibroblasts

The purpose of this study was to evaluate the phototoxic potential of LEN as measured by the relative reduction in viability of BALB/c 3T3 mouse fibroblasts exposed to LEN and ultraviolet radiation (+UVR), as compared with the viability of fibroblasts exposed to LEN in the absence of ultraviolet radiation (-UVR) (m2.6.7, Section 16, TX-200-2013). Promethazine was used as the positive control. All OECD 432-recommended cell survival and OD_{540} criteria and promethazine cytotoxicity and phototoxicity criteria were met, indicating that the assay was valid. LEN did not demonstrate phototoxic potential in this assay.

7.2. Ocular Irritation

7.2.1. Assessment of Ocular Irritation using the Bovine Corneal Opacity and Permeability Assay

The purpose of this study was to determine whether the test article LEN sodium causes serious eye damage or does not require classifying for eye irritation, using the bovine corneal opacity and permeability (BCOP) assay, in accordance with OECD Guidelines for Testing of Chemicals Method 437 (m2.6.7, Section 16, TX-200-2051).

A weight of 750 mg of the undiluted test article was applied to each of three corneas followed by a four-hour incubation (incubator set to 32°C). After this incubation, each cornea was washed with media containing phenol red followed by media without phenol red and then the chamber was re-filled, before the corneas were assessed for opacity.

For the permeability endpoint, sodium fluorescein solution was added into the anterior chamber and the corneas were incubated in the horizontal position for 1 hour and 30 minutes (incubator set to 32°C). Following this period, the media in the posterior chamber was removed and three 350 µL aliquots of this media (per cornea) were analysed for optical density at 490 nanometers (OD_{490}).

A volume of 750 µL of the negative or positive control was similarly applied to further groups of three corneas. These corneas were subject to the procedures detailed above.

The mean corrected opacity reading for the test article was -1.7, for the negative control was 0.0 and for the positive control was 73.7.

The corneas treated with the positive control were noted to be cloudy following treatment.

The mean group corrected optical density for the test article was 0.017, for the negative control was 0.000 and for the positive control was 1.387.

The test article, LEN sodium, produced an IVIS score of -1.41 and does not require classification for eye irritation.

7.3. Skin Irritation

7.3.1. An assessment of *in vitro* skin corrosion using EpiDerm™

The purpose of this study was to determine whether LEN sodium causes corrosion in the *in vitro* skin model EpiDerm™, in accordance with OECD Guidelines for Testing of Chemicals Method 431 (m2.6.7, Section 16, TX-200-2052). Human keratinocytes are used to construct the epithelium.

Duplicate EpiDerm™ inserts were treated with 25 mg LEN, purified water (negative control) and 8N potassium hydroxide (positive control) for 3 minutes and 1 hour. At the end of the treatment period, the tissues were washed with phosphate buffered saline (PBS) and cell viability was assessed using the MTT assay. The skin corrosivity potential was classified according to the remaining cell viability obtained after test material treatment with either of the two treatment times.

Skin viability after a three minute or one-hour exposure to the test article was 96.1% and 97.5%, respectively.

The OD values for the negative controls met the acceptance criteria.

Skin viability after a three minute or one-hour exposure to the positive control article was 1.4% and -3.4%, respectively, demonstrating appropriate performance of the assay.

LEN sodium was non-corrosive according to the UN GHS classification system.

7.4. In vitro Hemocompatibility

The purpose of this study was to assess LEN solutions prepared in 2 different vehicles for hemolytic potential and plasma compatibility. Lenacapavir solution formulations were prepared in 2 different vehicles. Lenacapavir solution formulations in Vehicle 1 (67.0% w/w PEG 300 and 33.0% w/w SWI, with 1 mol equivalent of NaOH), prepared at concentrations of 0.2 and 2 mg/mL, were evaluated for hemolytic potential and plasma compatibility in rat and dog whole blood/plasma (m2.6.7, Section 16, TX-200-2034). Lenacapavir formulation, in Vehicle 1 prepared at concentration of 3 mg/mL, was evaluated for hemolytic potential and plasma compatibility in human whole blood/plasma. LEN formulation in Vehicle 2 (5.74% PEG 300 and 88.14% SWFI with 3.17% 1N NaOH), prepared at a concentration of 30 mg/mL, was evaluated for hemolytic potential and plasma compatibility in dog and human whole blood/plasma. Additionally, Vehicle 1 and Vehicle 2 controls (without NaOH) were also evaluated for hemolytic potential and plasma compatibility.

In conclusion, Vehicle 1 control and 0.2, 2, and 3 mg/mL LEN in Vehicle 1 (67.0% PEG300 and 33.0% SWFI, with 1 mol equivalent of NaOH) resulted in minimal to moderate hemolysis of rat, dog and human whole blood; Vehicle 2 control and 30 mg/mL LEN in Vehicle 2 (5.74% PEG 300 and 88.14% SWFI with 3.17% 1N NaOH), resulted in minimal to moderate hemolysis of dog blood but no hemolysis in human blood. In plasma compatibility testing, LEN in Vehicle 1 and Vehicle 1 control alone resulted in a cloudy appearance and microscopic flocculation in rat, dog, and human plasma. Lenacapavir in Vehicle 2 resulted in a cloudy appearance and microscopic flocculation in dog and human plasma, but Vehicle 2 control alone did not.

7.5. Injection Site Reactions

7.5.1. Irritation screening studies

Single dose nonGLP injection site reaction studies in New Zealand white rabbits were conducted early in the development of LEN. The goal of these studies was to evaluate SC and/or IM LEN formulations at different concentrations and using different vehicle. These included TX-200-2009, and TX-200-2015 and are not included in this submission but may be requested.

7.5.2. 1-Month Subcutaneous Irritation Study – Suspension Formulation

The purpose of this study was to evaluate the local tolerance of two pre-formulated 100-mg/mL LEN suspension formulations when administered via SC injection to male New Zealand white rabbits on a single dosing day; animals were observed postdose for approximately 72 hours (Study Day 4, Terminal Sacrifice 1) or 28 days (Terminal Sacrifice 2, Study Day 29) (m2.6.7, Section 16, TX-200-2010).

Lenacapavir was well tolerated clinically in male rabbits when administered as a SC injection at 50 or 100 mg in Vehicle 1 (2% w/v poloxamer 188, 0.76% w/v sodium chloride in 20 mM phosphate buffer) or Vehicle 2 (2% w/v poloxamer 188, 4.74% mannitol w/v in 20 mM Phosphate buffer). LEN-related microscopic findings on Day 4 (Terminal Sacrifice 1) included slight to marked granulomatous inflammation in animals administered 50 or 100 mg TA 1 or 2. This finding was considered adverse when noted as moderate or marked, although showed evidence of reversibility.

7.5.3. 1-Month Subcutaneous Irritation Study – Solution Formulation

The purpose of this study was to evaluate the local tolerance of two LEN solution formulations when administered once via SC injection to male New Zealand white rabbits; after dosing, animals were observed postdose for approximately 72 hours (Day 4 Terminal Sacrifice 1) or 28 days (Day 29 Terminal Sacrifice 2) (m2.6.7, Section 16, TX-200-2033).

Five groups of 6 male Hra:(NZW)SPF rabbits were administered doses of 0, 150, or 300 mg LEN solution via SC injection using dose concentrations of 0 or 300 mg/mL with or without NaOH (*in situ* LEN sodium) and varying volumes of 0.5 or 1.0 mL. The vehicle control article was 67:33 w/w PEG 300:SWI). Animals were dosed via SC injection in the left dorsal region as a single dose on Day 1. After dosing, three males/group were necropsied on Day 4 (Terminal Sacrifice 1), and three males/group were necropsied on Day 29 (Terminal Sacrifice 2).

Assessment of local tolerance was based on mortality, clinical observations, body weights, qualitative food consumption, dermal observations, and anatomic pathology.

All animals survived to their scheduled terminal sacrifice on Day 4 or Day 29, and no clinical observations or effects on qualitative food consumption, body weight, or body weight change occurred.

Very slight to severe edema was noted from 72 hours postdose through Day 29 in animals administered 300 mg LEN free base (no NaOH) and 150 or 300 mg LEN with NaOH. Edema correlated with the macroscopic observation of thickening and the microscopic finding of inflammation noted at the SC injection site. The observations did not impact the health of the animals and therefore observations were not considered adverse.

At Terminal Sacrifice 1, LEN-related microscopic findings at the injection site consisted of slight to marked necrosis, minimal to moderate mixed cell inflammation, and minimal to slight mononuclear cell infiltrates, that correlated with the macroscopic observation of thickening in all animals administered LEN. At Terminal Sacrifice 2, LEN-related microscopic findings of marked granulomatous inflammation and slight to marked necrosis correlated with the macroscopic observation of thickening in all animals administered LEN. Overall, the incidence and severity of mixed cell and/or granulomatous inflammation and necrosis were similar between dose levels and formulations.

In conclusion, no adverse findings occurred in male rabbits administered a single SC injection of 0, 150, or 300 mg LEN or LEN sodium (in situ) in PEG 300/SRI. LEN-related nonadverse edema correlated with the macroscopic observation of thickening and the microscopic observations of mixed cell or granulomatous inflammation, necrosis, and mononuclear cell infiltrates at the subcutaneous injection site of animals administered LEN.

7.5.4. 1-Month Subcutaneous Irritation Study – Solution Formulation

The purpose of this study was to evaluate the local tolerance of a LEN solution when administered once via SC injection to male New Zealand white rabbits; after dosing, animals were observed for approximately 72 hours (Terminal Euthanasia 1) or 28 days (Terminal Euthanasia 2, m2.6.7, Section 16, TX-200-2048).

Six male rabbits were assigned to one group, and doses were administered via SC (slow bolus) injection over 1 to 5 minutes at a volume of 0.6 mL/animal. Animals were administered vehicle control article (52.81% w/w PEG 300, 24.66% w/w SWI, 8.78% w/w ethanol, and 13.75% w/w poloxamer 188, hereafter referred to as vehicle) to Dose Site A (Dorsal region, lower left) followed by test article (LEN) to Dose Site B (Dorsal region, upper right).

Assessment of toxicity was based on mortality, clinical observations, body weights, qualitative food consumption, dermal observations, and anatomic pathology.

All animals survived to their scheduled necropsy. No LEN-related effects on body weight or qualitative food consumption were noted.

No vehicle-related clinical or dermal observations were noted at Dose Site A.

Lenacapavir-related non adverse dermal observations of very slight edema and very slight erythema were noted at Dose Site B as early as 72 hours postdose and resolved by Day 15. Corresponding LEN-related non adverse clinical observations included scab and abnormal color (red or purple) at Dose Site B. No clinical observations were noted after 72 hours postdose.

At Terminal Necropsy 1 (72 hours postdose), LEN-related microscopic findings in Dose Site B consisted of mixed cell inflammation in the subcutis, with associated findings of ulcer/necrosis and acanthosis in the epidermis, mixed cell infiltrate in the dermis, inflammation in the panniculus muscle, foreign material in the subcutis and dermis. These findings correlated with macroscopic findings of discoloration, raised area, and/or scab.

At Terminal Necropsy 2 (Day 29), LEN-related microscopic findings in Dose Site B consisted of granulomatous inflammation in the subcutis and dermis and associated findings acanthosis of the epidermis, and foreign material and mixed cell infiltrates in the dermis. These findings correlated with the macroscopic finding thickening.

In conclusion, male rabbits were administered a single, SC (slow bolus) injection of vehicle to Dose Site A and 0.6 mL of 500 mg/mL LEN to Dose Site B. Dermal findings included very slight edema and very slight erythema which resolved by Day 15. Based on the resolution of dermal observations by Day 15, transient clinical observations, and the minimal severity of microscopic findings from Day 29, the dose level of 500 mg/mL (0.6 mL) LEN was considered tolerated.

7.5.5. 3-Month Subcutaneous Irritation Study – Solution Formulation

The purpose of this study was to evaluate the local tolerance of a LEN solution formulation when administered once via SC injection to female New Zealand white rabbits; after dosing, animals were observed postdose for approximately 72 hours (Day 4 Terminal Sacrifice 1), 28 days (Day 29 Terminal Sacrifice 2), or 91 days (Day 92 Terminal Sacrifice 3, m2.6.7, Section 16, TX-200-2038).

Two groups of female Hra:(NZW)SPF rabbits (n=9/group) were administered LEN at doses of 0 or 300 mg/mL via a single 1 mL SC injection in the dorsal region. The vehicle control article was 63.0% w/w PEG 300, 30.8% w/w SWI, and 6.2% w/w poloxamer 188. After dosing, 3 females/group were observed for approximately 72 hours and necropsied on Day 4 (Terminal Sacrifice 1); 3 females/group were observed for 28 days and necropsied on Day 29 (Terminal Sacrifice 2), and 3 females/group were observed for 91 days and necropsied on Day 92 (Terminal Sacrifice 3).

Assessment of local tolerance was based on mortality, clinical observations, body weights, qualitative food consumption, dermal observations, and anatomic pathology.

All animals survived to their scheduled terminal sacrifice. No adverse effects on clinical observations, body weights or food consumption occurred. Intermittent non adverse (very slight to severe) edema correlated with the macroscopic observation of thickening at the injection site of most animals administered 300 mg/mL LEN, and this correlated with microscopic observations of granulomatous inflammation.

At the Terminal Sacrifice 1, LEN-related microscopic observations of marked necrosis and minimal mixed cell inflammation were observed within the subcutaneous injection sites that were associated with the macroscopic observation of tan discoloration. At Terminal Sacrifice 2 (Day 29), LEN-related microscopic observation of marked or severe granulomatous inflammation was observed in the SC injection site. At Terminal Sacrifice 3 (Day 92), granulomatous inflammation of the SC injection site was observed in animals administered 300 mg/mL LEN, but at slight to moderate severity, which indicated partial reversal of the inflammation.

In conclusion, no adverse local tolerance findings were observed in female rabbits administered a single 1 mL SC injection of 300 mg/mL LEN solution in PEG 300, poloxamer 188, sodium hydroxide and SWI. Test article-related edema correlated with the macroscopic observation of thickening at the injection site, which correlated with microscopic observations of granulomatous inflammation. Partial reversal of granulomatous inflammation was observed at 92 days post dose.

7.5.6. 9-Month Subcutaneous Irritation Study – Solution Formulation

The purpose of this study was to evaluate the local tolerance of three solution formulations of LEN when administered via a single SC injection to male New Zealand white rabbits; after a single dose, animals were observed for approximately 72-hours (Day 4 sacrifice) or 13, 26, or 39 weeks (m2.6.7, Section 16, TX-200-2019).

Seven groups of 12 male Hra:(NZW)SPF rabbits were administered SC injections using dose concentrations of 0 (vehicle), 200 (with or without 1 molar equivalent NaOH), or 400 mg/mL (without NaOH). Each of the three formulations was administered a total volume of 0.5 or 1 mL at a dose of 100, 200, or 400 mg/kg. The vehicle control article consisted of 77:10:13 w/w/w PEG 200:Ethanol:SWI and was administered as a 1 mL total volume SC injection. three rabbits/groups were sacrificed at each of the scheduled sacrifices (Day 4 or 13, 26, or 39 weeks postdose).

Assessment of local tolerance was based on dermal observations and anatomic pathology. Mortality, clinical observations, body weights, body weight change, and food consumption were also evaluated.

All animals survived to their scheduled terminal sacrifice, and no effects on body weight or body weight change were noted.

The dermal observation of very slight to moderate edema and the correlating clinical observation of raised area within the dose site were noted for animals administered LEN from Days 4 through 141. The highest incidence of observations was noted for animals administered 200 mg (of the 200 mg/mL formulation without NaOH) or 400 mg LEN without NaOH and persisted the longest in animals administered 400 mg LEN without NaOH. Edema was considered not adverse as it did not require veterinary intervention or impact the overall health of animals. Lenacapavir-related microscopic findings at Terminal Sacrifice 1 (Day 4) consisted predominantly of necrosis surrounded by an acute inflammatory reaction (mixed cell inflammation). The necrosis had resolved by Terminal Sacrifice 2 (Day 92) and was replaced by

granulomatous inflammation, which decreased in incidence and/or severity at Terminal Sacrifice 3 (Day 183) and Terminal Sacrifice 4 (Day 274) indicative of the maturation/recovery process. At Terminal Sacrifices 1, 2, 3, and 4, a LEN-related macroscopic finding of thickened skin was noted at the SC injection site of animals administered LEN with or without NaOH, which generally correlated with microscopic findings. Overall, the microscopic findings were not dose-dependent and did not differ between different LEN formulations at Terminal Sacrifice 1, 2, 3, or 4.

In conclusion, LEN was well tolerated in male rabbits when administered as a single SC injection of 0 (vehicle control article), 100, 200, or 400 mg LEN with or without 1 molar equivalent NaOH. Lenacapavir-related nonadverse edema and microscopic observations of necrosis and mixed cell inflammation (at 72 hours postdose) and granulomatous inflammation (on Days 92, 183, and 274) were present at the SC injection site of animals administered LEN.

8. OTHER TOXICITY STUDIES

8.1. Antigenicity

8.1.1. In Chemico Skin Sensitisation (Direct Peptide Reactivity Assay)

The study was conducted to quantify the reactivity of LEN sodium towards model synthetic peptides containing either lysine or cysteine (m2.6.7, Section 17, TX-200-2071). The data may be used as part of an integrated approach to testing and assessment (IATA) to support the discrimination between skin sensitisers and non-sensitisers for the purpose of hazard classification and labelling.

The test article was dissolved in 1:1 mixture water:acetonitrile at a concentration of 100 mM.

The test solutions were incubated at 1:10 and 1:50 ratios with the cysteine and lysine peptides, respectively, for 24±2 hours in glass autosampler vials, protected from light, in an incubator set to 25°C.

The remaining concentration of cysteine- or lysine-containing peptide following the 24-hour incubation period was measured by high performance liquid chromatography (HPLC) with gradient elution and UV detection at 220 nm.

The cysteine depletion value was 0%, the lysine depletion value was 33.31% and the mean of the cysteine and lysine depletion values was 16.65%.

The LEN sodium was considered to be positive with low reactivity in the Direct Peptide Reactivity Assay.

8.1.2. In Vitro Skin Sensitisation (ARE-Nrf2 Luciferase Test Method)

The study was conducted to investigate the potential of LEN sodium to induce genes that are regulated by the antioxidant response element (ARE) (m2.6.7, Section 17, TX-200-2072). The data may be used as part of an integrated approach to testing and assessment (IATA) to support the discrimination between skin sensitisers and non-sensitisers for the purpose of hazard classification and labelling.

The test article was dissolved in DMSO to the final stock concentration (200 mM). Serial dilutions were then made using DMSO to obtain 12 master concentrations of the test article in the range 0.098 to 200 mM.

The master concentrations were then further diluted 25-fold into culture medium containing serum and finally used for treatment with a further 4-fold dilution factor so that the final concentrations ranged from 0.98 to 2000 µM.

Aliquots of 50 µL of each of the final concentrations were transferred to give three luciferase replicates on a white-walled plate and a single viability replicate on a clear-walled plate. Each plate was sealed using a plate sealer and then incubated for 48±1 hours in a humidified incubator set to 37ºC, 5% CO2.

After the 48-hour exposure period, the medium in the viability plate was replaced with fresh medium containing 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT). The plate was sealed and incubated for 4 hours in an incubator set to 37°C, 5% CO2. The MTT medium was then removed and SDS (at 10% w/v) added per well. The plate was sealed and placed into an incubator set to 37°C, 5% CO2, and left overnight. After the overnight incubation, the plate was shaken to ensure homogeneity of the solution in the wells and then absorption read at 600 nm.

After the 48-hour exposure period, the cells in the luciferase plate were washed with phosphate buffered saline and lysis buffer for luminescence readings was added to each well. The plate was then incubated for 20 minutes in an incubator set to 25°C, loaded into the luminescence plate reader and read.

The I_{max} value in both Experiments was less than 1.5.

All acceptance criteria were met, with the exception that in Experiment 1 the EC1.5 for the positive control was below two standard deviations of the historical mean and the average induction for the positive control at 64 µM was above the range. As the EC1.5 was within the range seen at this laboratory and a clear positive dose response was observed for luciferase induction, these data were considered to be acceptable.

The I_{max} values were less than 1.5 in both experiments; therefore, LEN sodium was negative in the ARE-Nrf2 Luciferase Test.

8.1.3. In Vitro Skin Sensitisation (human Cell Line Activation Test)

The study was conducted to investigate the potential of LEN sodium to activate monocytes and dendritic cells in the human monocytic leukemia cell line THP-1, by quantifying changes in the expression of cell surface markers (CD86 and CD54) (m2.6.7, Section 17, TX-200-2073). The data may be used as part of an integrated approach to testing and assessment (IATA) to support the discrimination between skin sensitisers and non-sensitisers for the purpose of hazard classification and labelling.

The human Cell Line Activation Test (h-CLAT) was performed according to OECD TG 442E.

For the dose finding assay, the test article was dissolved in DMSO at a concentration of 31.4 mg/mL giving a maximum test concentration of 62.80 µg/mL. No reduction in viability was observed.

For the expression measurements, test concentrations in a range from 17.53 to 62.80 µg/mL (after dilution in medium) were used.

Aliquots of 500 µL of each of the working solutions were mixed 1:1 with cell suspensions at 1 x 10^6 cells per well.

After blocking, the cells were stained with FITC-labelled anti-CD86, anti-CD54 or mouse IgG1 antibodies on ice for 30 minutes.

The stained cells were washed, re-suspended in FACS buffer and propidium iodide solution was added. The expression levels of CD86 and CD54 and cell viability were analysed using flow cytometry.

In Experiment 1, all RFI Values for CD86 and CD54 were <150% and <200%, respectively. In Experiment 2 all RFI values for CD54 were also <200%, however, for CD86 the RFI values were greater than 150% at 7 out of 8 concentrations. This therefore required a third experiment to be conducted. In Experiment 3, all RFI values for CD54 were <200%, but there were several concentrations where the RFI value for CD86 was >150%.

The EC150 value for CD86 was calculated to be 24.85 µg/mL.

All acceptance criteria of the h-CLAT assay parameters were met in each experiment.

Lenacapavir sodium was considered to be positive in the human Cell Line Activation Test, at the maximum attainable concentration, as the RFI of CD86 was ≥150% in 2 of 3 independent runs.

8.1.4. Local Lymph Node Assay in the Mouse (Individual Method)

This study was conducted to assess the potential of LEN sodium to cause skin sensitisation in the mouse (m2.6.7, Section 17, TX-200-2053). Following a preliminary screening test using a 50% w/v formulation, LEN sodium was prepared for administration at 10, 25 and 50% w/v in dimethylformamide.

Groups of five female CBA / CaCrl mice were subjected to topical applications of positive control, vehicle control or of one of the test formulations to the outer aspect of the auditory pinnae once daily on Days 1, 2 and 3. On Day 6 a 20 µCi dose of tritiated ^{3}H-methyl thymidine was injected intravenously into each mouse. Approximately five hours later the auricular lymph nodes were recovered from each animal. The nodes from each animal were placed into individual petri dishes and suspensions of the cellular components of the lymph nodes were prepared in 5% w/v trichloroacetic acid and processed through a scintillation counter.

Test results are expressed in terms of Stimulation Indices, the ratios of the mean scintillation counts obtained from the test groups relative to the corresponding mean scintillation count obtained from controls. The threshold level for the Stimulation Index to be considered a positive indicator of the potential to cause skin sensitisation is 3.0. The positive control article produced a Stimulation Index of 12.90, demonstrating adequate performance of the assay. The Stimulation Index for 10%, 25% and 50% LEN sodium was 1.22, 1.75 and 1.77, respectively.

The Local Lymph Node Assay demonstrated that LEN sodium does not have the potential to cause skin sensitisation.

8.2. Dependence

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

8.3. Studies on Metabolites

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

8.4. Studies on Impurities

8.4.1. Stereoisomeric Impurities

Lenacapavir sodium has three stereocenters and is produced as a single stereoisomer by process design. Only one stereochemical impurity is possible from *不純物33 because diastereomers possessing a *trans*-orientation within a cyclopropyl ring are not energetically favorable. Therefore, potential stereochemical impurities of LEN include the enantiomer and two diastereomeric impurities. The potential stereochemical impurities are managed through controls for the starting materials, and a process that has been designed to assure stereochemical purity and purge of undesired stereoisomeric impurities.

Two distinct peaks were observed for intact LEN in metabolite profiles. The stability of LEN atropisomers, LEN.1 and LEN.2, was assessed in vitro in phosphate buffered saline buffer (pH 7.4) and in plasma (rat, dog, human) – the ratio of LEN.1 to LEN.2 was stable over time and was not influenced by binding proteins or enzymes in plasma (m2.6.5 Section 8.1). For additional details on LEN atropisomer chemistry refer to 3.2.S.3.1, Section 1.1.7.

8.4.2. In vitro Genotoxicity Studies with Impurities

Four GLP reverse mutation assays were conducted with impurities in S. typhimurium strains in the absence and in the presence of a rat liver metabolic activation system (S9). *不純物1 (m2.6.7, Section 17, TX-200-2025) and *不純物2 (m2.6.7, Section 17, TX-200-2041) were negative for the ability to induce reverse mutations at selected loci of several strains of Salmonella typhimurium and at the tryptophan locus of Escherichia coli strain WP2 uvrA in the presence and absence of S9.

*不純物3 induced mutation in three histidine-requiring strains TA98, TA100 and TA1537, and one tryptophan-requiring strain WP2 *uvrA* of *Escherichia coli* in the presence of S-9 when tested under the conditions of this study (m2.6.7, Section 17, TX-200-2023).

*不純物4 induced mutation in histidine-requiring strain *Salmonella typhimurium* TA100 when tested in the presence of a rat liver metabolic activation system (S-9) under the conditions of this study (m2.6.7, Section 17, TX-200-2024).

Two GLP studies were conducted to evaluate the potential of two LEN impurities and/or its metabolites to induce structural chromosomal aberrations in human peripheral blood lymphocytes in the presence and absence of an exogenous metabolic activation system (S-9).

*不純物1 did not induce chromosomal aberrations in the absence and/or presence of S-9 when tested under the conditions of this study (m2.6.7, Section 17, TX-200-2075).

*不純物5 did not induce chromosomal aberrations in the absence and/or presence of S-9 when tested under the conditions of this study (m2.6.7, Section 17, TX-200-2076).

8.4.3. In vivo Qualification Studies with Impurities

8.4.3.1. A Single-Dose Subcutaneous Injection Impurity Qualification Study with LEN in Wistar Han Rats With a 4-Week Observation Period

The purpose of this study was to qualify new impurities in LEN when administered as a single dose via SC injection to rats; after dosing, animals were observed for 4 weeks (Day 29 terminal euthanasia) to assess the reversibility, persistence, or delayed occurrence of effects (m2.6.7, Section 17, TX-200-2042).

Male and female Crl:WI(Han) rats (10 toxicity animals/sex/group) were administered solution formulations of LEN (pure lot), LEN-A (with impurities), or LEN-B (with impurities) in vehicle control article (67.24% w/w PEG 300/31.40% w/w SWI, 1.36% w/w poloxamer 188) at a dose level of 0 (vehicle), or 100 mg/kg/dose (LEN-treated groups). A subgroup of animals (3 animals/sex in the control group and 6 animals/sex in the LEN-treated groups) were included for toxicokinetic evaluation. Animals were given a single SC injection at a dose volume of 1.34 mL/kg on Day 1.

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

No LEN-related mortality occurred. An accidental death occurred to one female administered 100 mg/kg/day LEN (pure lot) on Day 14.

All remaining animals survived to their scheduled euthanasia. No adverse clinical observations were observed.

No LEN-related ophthalmic observations or changes in mean body weight or food consumption were noted.

Relative to controls, animals administered LEN (pure lot), LEN-A (with impurities), or LEN-B (with impurities) had no effect on hematology, coagulation, or urinalysis results on Days 5 and 29. Minor, nonadverse clinical chemistry differences on Day 5 suggested a minor inflammatory response and included minimally increased globulin concentration and mildly decreased albumin:globulin ratio in animals administered LEN (pure lot), LEN-A (with impurities), or LEN-B (with impurities). Males administered LEN-A (with impurities) or LEN-B (with impurities) also had minimally decreased albumin concentration. These nonadverse clinical chemistry differences were reversed on Day 29 and had a potential microscopic correlate (i.e., granulomatous inflammation at the SC injection site). Relative to animals administered LEN (pure lot), animals administered LEN-A (with impurities) or LEN-B (with impurities) had no clear differences in clinical pathology results.

No LEN-related or impurity-related organ weight changes, or macroscopic findings were noted. No impurity-related microscopic findings were noted. A LEN-related microscopic finding of granulomatous inflammation was observed at the SC injection site of a low number of animals administered LEN (pure lot) or LEN-A (with impurities).

Exposure to LEN was similar between the three lots administered at 100 mg/kg/dose. Sex-based differences were less than 2-fold in LEN mean C_{max} and $AUC_{0\text{-}672h}$ values.

In conclusion, single dose of SC administered 100 mg/kg LEN (pure lot), LEN-A, or LEN-B to male and female Crl:WI(Han) rats was well tolerated. There were no significant differences on the measured parameters between the animals given LEN (pure lot)-, LEN-A, or LEN-B at 100 mg/kg. The NOAEL is 100 mg/kg, and this dose level corresponded to sex-combined mean C_{max} and $AUC_{0\text{-}672h}$ values of 2230 ng/mL and 638000 ng•h/mL LEN, 1980 ng/mL and 599,900 ng•h/mL LEN-A, and 1900 ng/mL and 533000 ng•h/mL LEN-B, respectively.

8.4.3.2. A Single-Dose Subcutaneous Injection Impurity Qualification Study with LEN in Wistar Han Rats With a 3-Month Observation Period

The purpose of this study was to qualify new impurities as a single dose of 100 mg/kg when administered via subcutaneous injection to rats; after dosing, animals were observed for at least 13 weeks (Day 92 terminal necropsy) to assess the reversibility, persistence, or delayed occurrences of effects (m2.6.7, Section 17, TX-200-2050).

Male and female Wistar Han (Crl:WI[Han]) rats (10 animals/sex/group) were administered vehicle control article (PEG-300/Sterile Water for Injection, USP/Poloxamer 188) or 100 mg/kg of LEN (pure lot, sodium salt form of GS-6207 and hereafter referred to as LEN), LEN-A (with impurities, free acid form), or LEN-B (with impurities, sodium salt form) on Day 1 via subcutaneous injection into the dorsal region at a dose volume of 1.34 mL/kg. A subgroup of animals (3 animals/sex in the control group, 6 animals/sex in LEN-treated groups) were included for toxicokinetic evaluation.

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

All animals survived to their scheduled sacrifice.

No LEN (pure lot), LEN-A, or LEN-B-related alterations in body weight or food consumption; ophthalmic observations; or effects in hematology, coagulation, clinical chemistry, or urinalysis test results; or effects on organ weights or macroscopic observations were noted.

Nonadverse LEN-related clinical observations in animals administered LEN-A were limited to a single occurrence of swollen area at the injection site on Day 22 in 2 males. No clinical observations were noted in animals administered LEN (pure lot) or LEN-B or females administered LEN-A.

Nonadverse LEN (pure lot), LEN-A-, or LEN-B-related microscopic findings were present at the subcutaneous injection site of males and females and consisted of minimal to slight infiltrates of macrophages in males administered any of the test articles and females administered LEN (pure lot) or LEN-B. Other microscopic findings noted at the subcutaneous injection sites of 1 or more females, but not males, included minimal granulomatous inflammation in 1 female administered LEN (pure lot) and pseudocyst formation in 2 females administered LEN (pure lot) or LEN-A. There were no notable difference in the incidence and severities of microscopic findings between groups treated with LEN (pure lot) alone or with added impurities.

LEN (pure lot), LEN-A, and LEN-B did not induce micronuclei in the peripheral blood reticulocytes of rats administered 100 mg/kg.

No significant differences occurred in the mean LEN (pure lot) C_{max} and $AUC_{0\text{-}2184}$ values among all groups. No difference in exposure occurred between males and females.

In conclusion, administration of a single dose of 100 mg/kg LEN (pure lot), LEN-A, or LEN-B via subcutaneous injection to rats was well tolerated. There were no notable differences in the incidence and severities of microscopic findings at the subcutaneous injection sites. The no-observed-adverse-effect-level (NOAEL) is 100 mg/kg and corresponded to sex combined mean C_{max} and $AUC_{0\text{-}2184}$ values of 2350 ng/mL and 640,000 ng•h/mL for LEN (pure lot), 1470 ng/mL and 987,000 ng•h/mL for LEN-A, and 1580 ng/mL and 865,000 ng•h/mL for LEN-B.

8.5. Other Toxicity Studies

8.5.1. Transgenic Mouse Dose Range Finding Toxicity Study

The purpose of this study was to evaluate the toxicity and determine the TK of LEN sodium when administered once via SC injection to rasH2 transgenic mice with a 13-week observation period (m2.6.7, Section 17, TX-200-2045).

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

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

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

No LEN-related adverse effect on mortality, body weights, food consumption, or ophthalmic observations were noted.

Lenacapavir-related clinical observations noted in animals administered 300 mg/kg included palpable masses and abnormal skin color at the dose site which correlated with macroscopic observations of white raised areas at the dose site and microscopic findings of minimal to marked granulomatous inflammation. LEN-related microscopic findings were noted at the SC injection site in both sexes administered ≥ 30 mg/kg and consisted of minimal to marked granulomatous inflammation in both sexes administered ≥ 30 mg/kg and increased minimal to slight mixed cell infiltrates in females administered ≥ 99 mg/kg. The marked granulomatous inflammation noted in females administered 300 mg/kg was considered locally adverse.

Lenacapavir-related changes in clinical chemistry parameters consisted of minimal to mild increases in alanine aminotransferase (ALT) activities, in the absence of microscopic correlates in the liver, in one female each administered 30, 99, or 300 mg/kg LEN.

Lenacapavir-related slight increases in mean liver weight values (12 to 17%) were noted in both sexes administered 300 mg/kg. Increased liver weights in males administered 300 mg/kg correlated with the microscopic finding of slight centrilobular hepatocyte vacuolation; no microscopic correlate was noted in females.

In conclusion, male and female rasH2 transgenic mice were administered 0, 30, 99, or 300 mg/kg LEN via a single SC injection followed by a 13-week observation period. Lenacapavir-related clinical observations included palpable masses and abnormal skin color at the dose site for animals administered 300 mg/kg, which correlated with microscopic observations of minimal to marked granulomatous inflammation at the SC injection site. Minimal to moderate granulomatous inflammation was also observed at the SC injection site in animals administered 30 or 99 mg/kg. Lenacapavir-related increases in ALT activities were noted in one female each administered 30, 99, or 300 mg/kg. Lenacapavir-related slight increases in mean liver weight values were noted in both sexes administered 300 mg/kg. Increased liver weights in males administered 300 mg/kg correlated with the microscopic finding of slight centrilobular hepatocyte vacuolation. Thus, the LEN systemic NOAEL in rasH2 transgenic mice is 300 mg/kg. The local tolerance/toxicity NOAEL is 300 mg/kg for males and 99 mg/kg for females.

Table 21. Mean Toxicokinetic Parameters for LEN in Mouse Plasma After Subcutaneous Injection

Dose (mg/kg)	Sex	C_{max} (ng/mL)	AUC_{0-336h} (ng•h/mL)	$AUC_{0-2184h}$ (ng•h/mL)
30	M	3200	416,000	1,030,000
	F	3920	485,000	1,180,000
	MF	3440	449,000	1,100000
99	M	5450	739,000	3,470,000
	F	5460	865,000	3,160,000
	MF	5310	802,000	3,310,000
300	M	8190	1,300,000	7,250,000
	F	9180	1,870,000	9,400,000
	MF	8690	1,570,000	8,310,000

Notes: MF data are based on the analysis of the combined concentration data for both males and females.
$AUC_{0-2184h}$ is equivalent to AUC_{0-t}.

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

The purpose of this study was to investigate the pharmacokinetics of LEN solution in Capmul® when administered as a single dose via oral gavage to male and female Wistar Han rats. Three groups (n = 6 animals/sex/group) of Wistar Han (Crl:WI[Han]) rats were administered a single oral dose of LEN solution in 100% Capmul MCM at dose levels of 3, 30, or 30 mg/kg, respectively. A dose volume of 2 mL/kg was used to administer 3 mg/kg; a dose volume of 2 or 0.6 mL/kg was used to administer 30 mg/kg. Blood samples for bioanalytical and pharmacokinetic analyses were collected from the first or second 3 animals/sex/group at predose and at approximately 1, 3, 8, 12, 24, 48, 72, 96, and 120 hours postdose. Following the final pharmacokinetic blood collection all animals were euthanized (m2.6.7, Section 17, TX-200-2026).

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

8.5.3. 7-Day Once Daily Oral (Gavage) Tolerability and Toxicokinetic Study of LEN in Female Nonpregnant New Zealand White Rabbits

The purpose of this nonGLP study was to determine the tolerability of LEN in nonpregnant female New Zealand White rabbits (m2.6.7, Section 17, TX-200-2035). LEN solution was administered at doses of 0 (100% Capmul MCM), 100, 300, and 1000 mg/kg/day once daily via oral gavage to nonpregnant female New Zealand White rabbits (3/group) for 7 days at dose volumes of 1.3, 0.2, 0.6, and 2 mL/kg, respectively. Assessment of toxicity was based on mortality, clinical signs, body weights, body weight gains, food consumption, toxicokinetic parameters, and gross necropsy findings.

In the 1000 mg/kg/day group, 1 female was found dead on Study Day 4, and 1 female was euthanized in extremis on Study Day 5. Both females were noted with a perforated esophagus at necropsy, and therefore the mortality and moribundity noted at 1000 mg/kg/day were attributed to intubation errors.

All other females in the 100, 300, and 1000 mg/kg/day groups survived to the scheduled necropsy on Study Day 10. There were no LEN-related clinical observations noted at the daily examinations or approximately 4 hours following dose administration.

Body weights, body weight changes, and food consumption values among surviving females in the 100, 300, and 1000 mg/kg/day groups were generally comparable to the vehicle control group values for the overall treatment period (Study Days 0 to 7) and during the post-treatment period (Study Days 7 to 10).

No remarkable macroscopic findings were noted at the scheduled necropsy at any dose level.

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

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

Table 22. Mean Toxicokinetic Parameters for LEN in NonPregnant Rabbits After Daily Oral Administration

Dose (mg/kg/day)	$AUC_{(0-24h)}$ (ng•hr/mL)		$AUC_{(0-96h)}$ (ng•hr/mL)		C_{max} (ng/mL)	
	Day 0	Day 6	Day 0	Day 6	Day 0	Day 6
100	491	1510	NA	2780	34.9	145
300	1540	4490	NA	9760	128	257
1000 (n=1)	1030	2150	NA	5270	96.4	103

NA = Not applicable.

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

The purpose of this non-GLP study was to determine the physical characteristics of a subcutaneous (SC) depot when delivered to rats over various infusion durations (m2.6.7, Section 17, TX-200-2027).

Male Wistar-Han rats were assigned to 6, 30 or 240-mL/hour infusion rate groups (7 groups total, 3 rats/group). Rats were administered a single dose of either vehicle 1 (77:10:13 w/w/w PEG300:ethanol:SWI), Vehicle 2 (85:15 w/w PEG300:SWI) or LEN in Vehicle 1 or 2 at a dose concentration of 300 mg/mL and a fixed volume of 0.25 mL. A 21-gauge needle was used for administration of all groups excluding the group administered LEN in Vehicle 1 at 6 mL/hour for which a 25-gauge needle was used.

Body weights were collected once prestudy and on Day 1 prior to dosing. Dose administration occurred in three replicates over three days, and each replicate consisted of 1 animal/group. The test and vehicle formulations, described as clear liquids, were administered to anesthetized rats in the lumbar/sacral region using a butterfly and syringe pump once on Day 1.

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

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

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

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

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

9. DISCUSSION AND CONCLUSIONS

Two dosage forms of LEN are currently in development, a solution for SC injection, for administration once every 6 months and a film-coated tablet formulation for oral administration used for initial loading. The toxicology studies generally used the SC route of administration. To achieve multiples over a clinically relevant plasma concentration of LEN, the rats were dosed SC every 3 months; the dogs were dosed SC monthly. Rats and dogs in repeat toxicity studies via the oral route were dosed daily. Large exposure margins were achieved in the SC and/or oral studies (Section 4.9, m2.4). These studies, therefore, have adequately assessed the potential systemic toxicity and local oral tolerance of LEN and support the clinical regimen for the treatment of HIV-1 infection.

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

9.1. Target Organ Toxicity

9.1.1. Effects on the Liver

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

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

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

The transient acute toxicity and the presence of icteric clinical signs, bile pigment, increased bile acids and increased conjugated bilirubin in the chronic dog study are consistent with disruption in secretion of bilirubin into bile. Inhibition of the BSEP transporter induces hepatotoxicity by causing intracellular concentrations of bile acids to rise above a toxic threshold. Lenacapavir is a much more potent inhibitor of the dog BSEP (dBsep) transporter (IC_{50} = 0.124 µg/mL) than the human BSEP transporter (IC_{50} =1.15 µg/mL) (m 2.6.4, Section 7.4, AD-200-2053). Table 23 summarizes the dog and human plasma exposure data.

The mean LEN plasma C_{max} achieved at 10 mg/kg in the single dose IV dog study of 15.4 µg/mL is 124-fold and 13-fold higher than the dBsep and hBSEP IC_{50}s, respectively.

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

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

Table 23. Systemic Exposure of Dogs and Humans to LEN compared to BSEP IC_{50}

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

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

9.2. Genetic Toxicology

LEN is considered not genotoxic in in vitro (TX-200-2007, TX-200-2008) and in vivo (TX-200-2005) assays.

9.3. Reproductive Toxicology

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

Nonclinical reproductive toxicity studies with LEN have demonstrated no adverse effect on fertility, embryo-fetal development or growth, viability, and development of F_1 neonates.

No adverse effects occurred in a male and female fertility study in rats that evaluated potential effects in the reproductive process resulting from SC administration of a long-acting LEN formulation. Endpoints included evaluation of estrous cycling, tubal transport, implantation, development of the preimplantation stages of the embryo in the female, and functional reproductive effects (alterations in libido and epididymal sperm maturation) in the male. No adverse effects occurred upon external, visceral, and skeletal examination.

In the rat SC peri- and postnatal toxicity study, parameters evaluated included pregnancy, parturition, and lactation of the maternal (F_0) animals and on the growth, viability, and development of the F_1 neonates. Reproductive performance of the F_1 generation was also assessed. No LEN-related systemic effects were noted at any dose level in the F_0 or F_1 generation. The mean maternal concentration on LD 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 10.

9.4. Other Toxicology

9.4.1. Injection Site reactions

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

9.5. Conclusions

A comprehensive nonclinical safety program has been completed for LEN. Dose-related, reversible hepatobiliary toxicity consistent with BSEP transporter inhibition was observed in dogs at exposure levels more than 51-fold higher than human exposure.

All information from the nonclinical toxicology studies that is of relevance to the prescriber and patient has been included in the proposed prescribing information. The nonclinical toxicological assessment of LEN supports its safe and effective use in the treatment of HIV-1 infection.

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SECTION 2.6.7—TOXICOLOGY TABULATED SUMMARY

LENACAPAVIR

Gilead Sciences Inc.,

[redacted] **20**[redacted]

CONFIDENTIAL AND PROPRIETARY INFORMATION

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1. TOXICOLOGY: OVERVIEW

Test Article: LEN

Type of Study/Species/Strain	Method of Administration	Doses/Duration of Dosing[a]	Dose (mg/kg)	GLP[b]	Testing Facility	Gilead Study No. (CRO Study No.)
Single-Dose Toxicity						
Rat/Crl:WI(Han)	Intravenous infusion	30-minute infusion	0 (vehicle)[c],3, 10, 30	Yes	[redacted] USA	TX-200-2029 (8391711)
Dog/Beagle	Intravenous infusion	30-minute infusion	0 (vehicle)[d],3, 10, 30	Yes	[redacted] USA	TX-200-2030 (8391725)
Repeat-Dose Toxicity						
Rat/Crl:WI(Han)	Subcutaneous/Intramuscular	2 doses/7 days	Subcutaneous: 0 (vehicle)[e], 5, 30, 100 Intramuscular: 0 (vehicle)[e], 5	No	[redacted] USA	TX-200-2002 (8338788)
Rat/Crl:WI(Han)	Subcutaneous	4 Doses/ EOW	0 (vehicle)[f], 10, 30, 100	Yes	[redacted] USA	TX-200-2005 (8353952)
Rat/Crl:WI(Han)	Subcutaneous	1 or 2 Doses/ Once every 13 weeks	0 (vehicle)[l], 100	Yes	[redacted] USA	TX-200-2014 (8375766)
Rat/Crl:WI(Han)	Oral	4 Weeks	0 (vehicle)[h], 3, 10, 30	Yes	[redacted] USA	TX-200-2011 (8367905)
Dog/Beagle	Subcutaneous	2 Doses/7 days	0 (vehicle)[e], 3, 10, 30	No	[redacted] Canada	TX-200-2004 (5001864)
Dog/Beagle	Subcutaneous	4 Doses/ EOW	0 (vehicle)[g], 10, 30, 100	Yes	[redacted] USA	TX-200-2006 (8353953)
Dog/Beagle	Subcutaneous	10 Doses/ Once monthly	0 (vehicle)[k], 20, 40	Yes	[redacted] USA	TX-200-2017 (8375767)
Dog/Beagle	Subcutaneous	10 Doses/ Once monthly	0 (vehicle)[i], 130, 411	Yes	[redacted] USA	TX-200-2047 (8419595)
Dog/Beagle	Oral	4 Weeks	0 (vehicle)[h], 1, 5, 30	Yes	[redacted] USA	TX-200-2012 (8367906)

Test Article: LEN

Type of Study/Species/Strain	Method of Administration	Doses/Duration of Dosing[a]	Dose (mg/kg)	GLP[b]	Testing Facility	Gilead Study No. (CRO Study No.)
Genotoxicity						
Bacterial Mutation/ Salmonella typhimurium TA98, TA100, TA1535, and TA1537; E. coli WP2uvrA	In vitro	48 hours (± S9)	5-5000 µg/mL	Yes	[redacted] USA	TX-200-2007 (8353954)
Chromosome Aberration/ Cultured Human Peripheral Blood Lymphocytes	In vitro	3 hours (± S9) or 24 hours (-S9 only)	3 h without S9: 84, 120, and 172 µg/mL 24 h without S9: 41.2, 120, and 172 µg/mL 3 h with S9: 120, 172, and 245 µg/mL	Yes	[redacted] USA	TX-200-2008 (8353955)
In Vivo Micronucleus Rat/Crl:WI(Han)	Subcutaneous	4 Doses (EOW)	0 (vehicle)[g], 10, 30, 100	Yes	[redacted] USA	TX-200-2005 (8369701)
Reproductive and Developmental Toxicity						
Fertility/Rat/Crl:CD Sprague-Dawley	Subcutaneous	Males: 6 weeks prior to mating through day prior to necropsy Females: 4 weeks prior to mating through day 7 of gestation	0 (vehicle)[k], 20, 100	Yes	[redacted] USA	TX-200-2043 (00604466)
Embryo-Fetal development Dose-Range Finding/Rat/Crl:CD Sprague-Dawley	Oral	GD 6 to 17	0 (vehicle)[h], 3, 30, 300	Yes	[redacted] USA	TX-200-2031 (00604390)
Embryo-Fetal Development/Rat/Crl:CD Sprague-Dawley	Oral	GD 6 to 17	0 (vehicle)[h], 3, 10, 30	Yes	[redacted] USA	TX-200-2036 (00604416)
Embryo-fetal development Dose-Range Finding/Rabbit/ New Zealand White Hra:(NZW)SPF	Intravenous infusion	GD 7 to 19	0 (vehicle)[d], 5, 10, 20	Yes	[redacted] USA	TX-200-2032 (00604391)
Embryo-Fetal Development/Rabbit/ New Zealand White Hra:(NZW)SPF	Intravenous infusion	GD 7 to 19	0 (vehicle)[d], 5, 10, 20	Yes	[redacted] USA	TX-200-2037 (00604417)

Test Article: LEN

Type of Study/Species/Strain	Method of Administration	Doses/Duration of Dosing[a]	Dose (mg/kg)	GLP[b]	Testing Facility	Gilead Study No. (CRO Study No.)
Prenatal and Postnatal Development/Rat/ Crl:CD Sprague-Dawley	Subcutaneous	GD 6 to LD 21	0 (vehicle)[l], 30, 300	Yes	[redacted] USA	TX-200-2049 (00604474)
Local Tolerance						
Phototoxicity/Fibroblasts/ Mouse / BALB/c 3T3 cells (clone A31)	In vitro	90 minutes	Assay #1 & 2: 1.78, 3.16, 5.62, 10.0, 17.8, 31.6, 56.2 and 100 µg/mL	Yes	[redacted] USA	TX-200-2013 (20129744)
Ocular Irritation Assay/Bovine Cornea	In vitro	Opacity assessment: 4 hours Permeability assessment: 90 minutes	750 mg	Yes	[redacted] England	TX-200-2051 (8426789)
Skin Corrosion Assay/ EpiDerm™ human skin	In vitro	60 minutes	25 mg	Yes	[redacted] England	TX-200-2052 (8426790)
In Vitro Hemolytic Potential and Plasma Compatibility/Rat, Dog, Human/Blood and Plasma	In vitro	40 to 45 minutes	Vehicle 1[c]: 0.2, 2 (rat and dog), 3 (human) mg/mL Vehicle 2[d]: 30 (dog, human) mg/mL	Yes	[redacted] USA	TX-200-2034 (8392255)
Rabbit/Hra:(NZW)SPF	Subcutaneous	Single dose/ Up to 1-month observation	0 (vehicle)[j], 50 mg, 100 mg	Yes	[redacted] USA	TX-200-2010 (8366190)
Rabbit/Hra:(NZW)SPF	Subcutaneous	Single dose/ Up to 1-month observation	0 (vehicle)[c], 150 mg, 300 mg	Yes	[redacted] USA	TX-200-2033 (8390752)
Rabbit/Hra:(NZW)SPF	Subcutaneous	Single dose/ Up to 1-month observation	0 (vehicle)[m], 300 mg	Yes	[redacted] USA	TX-200-2048 (8420104)
Rabbit/Hra:(NZW)SPF	Subcutaneous	Single dose/ Up to 3-month observation	0 (vehicle)[n], 300 mg	Yes	[redacted] USA	TX-200-2038 (8397773)
Rabbit / Hra:(NZW)SPF	Subcutaneous	Single dose/ Up to 9 -month observation	0 (vehicle)[k], 100 mg, 200 mg, 400 mg	Yes	[redacted] USA	TX-200-2019 (8379568)

Test Article: LEN

Type of Study/Species/Strain	Method of Administration	Doses/Duration of Dosing[a]	Dose (mg/kg)	GLP[b]	Testing Facility	Gilead Study No. (CRO Study No.)
Other Studies						
In Chemico Skin Sensitization	In vitro	24±2 hours	GS-6207 dissolved in 1:1 mixture of water and acetonitrile; these solutions were incubated at 1:10 and 1:50 ratios with the cysteine and lysine peptides	Yes	[redacted] England	TX-200-2071 (8426791)
Skin Sensitization/HaCaT human keratinocytes	In vitro	48±1 hours	GS-6207 in dimethylformamide	Yes	[redacted] England	TX-200-2072 (8426792)
Skin Sensitization/h-CLAT	In vitro	30 minutes	GS-6207 in dimethylformamide	Yes	[redacted] England	TX-200-2073 (8426793)
Skin Sensitization Local Lymph Node Assay/ Mouse/CBA: CaCrl	Topical	3 days	10%, 25% 50% GS-6207 in dimethylformamide	Yes	[redacted] England	TX-200-2053 (8456125)
Impurity (*不純物1)/Bacterial Mutation/ Salmonella typhimurium TA98, TA100, TA1535, and TA1537; E. coli WP2uvrA	In vitro	3 days	5-5000 µg/mL	Yes	[redacted] England	TX-200-2025 (8382042)
Impurity *不純物2 /Bacterial Mutation/ Salmonella typhimurium TA98, TA100, TA1535, and TA1537; E. coli WP2uvrA	In vitro	48-72 hours	25-5000 µg/mL	Yes	[redacted] USA	TX-200-2041 (AF65BC.502005I CH.BTL)

＊新薬承認情報提供時に置き換え

Test Article: LEN

Type of Study/Species/Strain	Method of Administration	Doses/Duration of Dosing[a]	Dose (mg/kg)	GLP[b]	Testing Facility	Gilead Study No. (CRO Study No.)
Impurity *不純物3 /Bacterial Mutation/ Salmonella typhimurium TA98, TA100, TA1535, and TA1537; E. coli WP2uvrA	In vitro	3 days	5-5000 µg/mL	Yes	[redacted] England	TX-200-2023 (8381836)
Impurity *不純物4 /Bacterial Mutation/ Salmonella typhimurium TA98, TA100, TA1535, and TA1537; E. coli WP2uvrA	In vitro	3 days	5-5000 µg/mL	Yes	[redacted] England	TX-200-2024 (8382041)
Impurity (*不純物1)/Chromosome Aberration/Cultured Human Peripheral Blood Lymphocytes	In vitro	4 hours (± S9) or 20 hours (-S9 only)	4 h without S9: 10, 25 and 70 µg/mL; 20 h without S9; 5, 15, 25 µg/mL; 4 h with S9: 5, 25, 50 µg/mL	Yes	[redacted]	TX-200-2075 (AG57PA.341ICH .BTL)
Impurity (*不純物5)/ Chromosome Aberration/Cultured Human Peripheral Blood Lymphocytes	In vitro	4 hours (± S9) or 20 hours (-S9 only)	4 hours (± S9) or 20 hours (-S9 only): 21.9, 43.9 and 87.8 µg/mL	Yes	[redacted]	TX-200-2076 (AG57PB.341ICH .BTL)
Impurity Qualification/Single Dose//Rat/Crl:WI(Han)	Subcutaneous	Single dose/ Up to 4-week observation	0 (vehicle)[o], 100 mg/kg LEN-pure; 100 mg/kg LEN-A (impurity); 100 mg/kg LEN-B (impurity)	Yes	[redacted] USA	TX-200-2042 (8407444)
Impurity Qualification/ Single Dose/Rat/Crl:WI(Han)	Subcutaneous	Single dose/ Up to 13-week observation	0 (vehicle)[o], 100 mg/kg LEN-pure; 100 mg/kg LEN-A (impurity); 100 mg/kg LEN-B (impurity)	Yes	[redacted] USA	TX-200-2050 (8423935)

Test Article: LEN

Type of Study/Species/Strain	Method of Administration	Doses/Duration of Dosing[a]	Dose (mg/kg)	GLP[b]	Testing Facility	Gilead Study No. (CRO Study No.)
Transgenic Mice/ RasH2 [001178-W (wild type), CByB6F1-Tg(HRAS)2Jic]	Subcutaneous	Single dose/ Up to 13-week observation	0 (vehicle)[p], 30, 99, 300	Yes	[redacted] USA	TX-200-2045 (8411930)
Single Dose PK/Rat/Crl:WI(Han)	Oral	Single dose	0 (vehicle)[q], 3, 30, 30	Yes	[redacted] USA	TX-200-2026 (8384035)
Tolerability/Rabbit/ Hra:[NZW]SPF	Oral	7-day	0 (vehicle)[r], 100, 300, <u>1000</u>	No	[redacted] USA	TX-200-2035 (00604415)
Assessment of Subcutaneous Depot/ Rat/Crl:WI(IGS)	Subcutaneous	Single dose	0 (vehicle)[s], 6, 30, 240 mL/infusion rate	No	[redacted] Canada	TX-200-2027 (6800022)

EOW = every other week; HaCaT = Cultured Human Keratinocyte (cells); LEN = lenacapavir (GS-6207); NOAEL = no observed adverse effect level; NOEL = no observed effect level

a Unless otherwise specified, the repeat-dose toxicity, the highest NOAEL is underlined, for embryo/fetal development studies the NOEL for the maternal and fetal effects is underlined; for pre and postnatal development the NOEL for F0 and F1 is underlined.
b 'Yes', indicates study includes a GLP Compliance statement.
c 67.0 % (w/w) polyethylene glycol 300 (PEG 300) and 33.0% (w/w) sterile water for injection.
d 6.12% (w/w) PEG 300 and 93.88% (w/w) sterile water for injection.
e 2% (w/v) poloxamer 388 and 98% (w/v) sterile water for injection.
f 2% (w/v) poloxamer 188 prepared in 0.9% (w/v) sodium chloride in sterile water for injection and 400 mM phosphate buffer.
g 2% (w/v) poloxamer 188 prepared in 0.9% (w/v) sodium chloride, in sterile water for injection, USP
h 10% v/v Dimethyl sulfoxide (DMSO), 10% v/v vitamin E TPGS (TPGS = D-α-Tocopherol polyethylene glycol 1000 succinate), and 80% v/v propylene glycol (PG)
i 68.17% (w/w) PEG 300/31.83% (w/w) sterile water for injection.
j 100 mg/mL LEN was formulated in one of two vehicles: 2% (w/v) poloxamer 188 prepared in 0.76% (w/v) sodium chloride in 20 mM phosphate buffer or 2% (w/v) poloxamer 188 and 4.74% mannitol (w/v) prepared in 20 mM phosphate buffer.
k 77% (w/w) PEG 200, 10% (w/w) ethanol, 13% (w/w) in sterile water for injection.
l 68.2% (w/w) PEG 300, 31.8% (w/w) in sterile water for injection
m 52.81% (w/w) PEG 300, 24.66% (w/w) in sterile water for injection, 8.78% (w/w) ethanol, and 13.75% (w/w) poloxamer 188
n 63.0% w/w PEG 300, 30.8% w/w in sterile water for injection, and 6.2% w/w poloxamer 188
o 67.24% w/w PEG 300, 31.40% w/w in sterile water for injection, and 1.36% w/w poloxamer 188
p 63.7% w/w PEG 300, 29.7% w/w in sterile water for injection, and 6.6% w/w poloxamer 188
q Capmul MCM, EP/NF solution
r 100% Capmul MCM, NF/solution
s 300 mg/mL LEN was formulated in one of two vehicles: 77:10:13 w/w/w/ PEG 300: ethanol:sterile water for injection OR 85:15 w/w/ PEG 300: Sterile water for injection

2. TOXICOKINETICS: OVERVIEW OF TOXICOKINETICS STUDIES

Test Article: LEN

Type of Study	Test System	Method of Administration	Dose (mg/kg)	GLP[a]	Gilead Study No. (CRO Study No.)
2 doses/7 days	Rat	Subcutaneous/Intramuscular	Subcutaneous: 0 (vehicle)[e], 5, 30, 100 Intramuscular: 0 (vehicle)[b], 5	No	TX-200-2002 (8338788)
4-dose EOW[d]	Rat	Subcutaneous	0 (vehicle)[e], 10, 30, 100	Yes	TX-200-2005 (8353952)
1 or 2 Doses	Rat	Subcutaneous	0 (vehicle)[f], 100	Yes	TX-200-2014 (8375766)
4-Weeks	Rat	Oral	0 (vehicle)[g], 3, 10, 30	Yes	TX-200-2011 (8367905)
Single dose	Rat	Intravenous infusion	0 (vehicle)[b], 3, 10, 30	Yes	TX-200-2029 (8391711)
2 Doses/7 days	Dog	Subcutaneous	0 (vehicle)[b], 3, 10, 30	No	TX-200-2004 (5001864)
4-dose EOW[d]	Dog	Subcutaneous	0 (vehicle)[e], 10, 30, 100	Yes	TX-200-2006 (8353953)
10 Dose (9-month)	Dog	Subcutaneous	0 (vehicle)[f], 20, 40	Yes	TX-200-2017 (8375767)
10 Dose (9-month)	Dog	Subcutaneous	0 (vehicle)[h], 130, 411	Yes	TX-200-2047 (8419595)
4-Weeks	Dog	Oral	0 (vehicle)[g], 1, 5, 30	Yes	TX-200-2012 (8367906)
Single dose	Dog	Intravenous infusion	0 (vehicle)[c], 3, 10, 30	Yes	TX-200-2030 (8391725)
Embro-fetal Dose Range-Finding	Rat	Oral	0 (vehicle)[g], 3, 30, 300	Yes	TX-200-2031 (00604390)
Embryo-fetal Development	Rat	Oral	0 (vehicle)[g], 3, 10, 30	Yes	TX-200-2036 (00604416)

Test Article: LEN

Type of Study	Test System	Method of Administration	Dose (mg/kg)	GLP[a]	Gilead Study No. (CRO Study No.)
Embryo-fetal Dose Range-Finding	Rabbit	Intravenous infusion	0 (vehicle)[i], 5, 10, 20	Yes	TX-200-2032 (00604391)
Prenatal and Postnatal Development	Rat	Subcutaneous	0 (vehicle)[j], 30, 300	Yes	TX-200-2049 (00604474)
Single Dose	Rat	Subcutaneous	0 (vehicle)[k], 20, 100	Yes	TX-200-2043 (00604466)
Single Dose Impurity Qualification	Rat	Subcutaneous	0 (vehicle)[l], 100 mg/kg LEN-pure; 100 mg/kg LEN-A (impurity); 100 mg/kg LEN-B (impurity)	Yes	TX-200-2042 (8407444)
Single Dose Impurity Qualification	Rat	Subcutaneous	0 (vehicle)[l], 100 mg/kg LEN-pure; 100 mg/kg LEN-A (impurity); 100 mg/kg LEN-B (impurity)	Yes	TX-200-2050 (8423935)
Tolerability	Rabbit	Oral	0 (vehicle)[m], 100, 300, 1000	No	TX-200-2035 (00604415)

EOW: every other week; LEN = lenacapavir (GS-6207)

a 'Yes', indicates study includes a GLP Compliance statement.
b 67.0 % (w/w) polyethylene glycol 300 (PEG 300) and 33.0% (w/w) sterile water for injection.
c 6.12% (w/w) PEG 300 and 93.88% (w/w) sterile water for injection.
d Subcutaneous doses of 100 mg/mL LEN were administered once every other week for 4 doses. In the dog study, a single dose of 100 mg/kg was administered to 6 males and 2 females.
e 2% (w/v) poloxamer 188 prepared in 0.9% (w/v) sodium chloride in sterile water for injection and 400 mM phosphate buffer.
f 77% (w/w) PEG 200, 10% (w/w) ethanol, 13% (w/w) in sterile water for injection.
g 10% v/v dimethyl sulfoxide, 10% v/v vitamin E TPGS (TPGS = D-α-Tocopherol polyethylene glycol 1000 succinate) and 80% v/v propylene glycol.
h 68.17% (w/w) PEG 300/31.83% (w/w) sterile water for injection
i 6.12% (w/w) PEG 300 and 93.88% (w/w) sterile water for injection
j 68.2% (w/w) PEG 300, 31.8% (w/w) in sterile water for injection
k 77:10:13 w/w/w PEG 200: ethanol:sterile water for injection
l 67.24% w/w PEG 300, 31.40% w/w in sterile water for injection, and 1.36% w/w poloxamer 188
m 100% Capmul MCM, NF/solution

3. TOXICOKINETICS: OVERVIEW OF TOXICOKINETICS DATA

3.1. Subcutaneous

Test Article: LEN

	AUC (ng•h/mL) following the last dose			
Species	**Rat**		**Dog**	
Method of Administration	**Subcutaneous**			
Gender (M/F)	**M**	**F**	**M**	**F**
Dose (mg/kg)				
3	N/A	N/A	9200[k]	N/A
5	11,900[l], 10,400[o]	N/A	N/A	N/A
10	134,000[a]	106,000[a]	127,000[b], 23,400[k]	110,000[b]
20	103,000[n]	91,100[n]	143,000[c] 138,000[d]	102,000[c] 107,000[d]
30	314,000[a], 16,400[o]	244,000[a], 10,900[m]	391,000[b], 73,100[k]	481,000[b]
40	N/A	N/A	159,000[e]	170,000[e]
100	608,000[a] 338,000[f] 276,000[g] 265,000[h] 270,000[n] 31,700[o] 743,000[p] 594,000[q] 516,000[r] 684,000[s] 1,070,000[t] 1,100,000[u]	559,000[a] 276,000[f] 293,000[g] 156,000[h] 192,000[n] 533,000[p] 604,000[q] 551,000[r] 605,000[s] 902,000[t] 636,000[u]	—	—
130	N/A	N/A	2850000[i]	3540000[i]
300	N/A	54,800[m]	N/A	N/A
411	N/A	N/A	2490000[j]	2030000[j]

$AUC_{0\text{-}336h}$ = area under the plasma concentration-time curve from 0 to 336 hours postdose; $AUC_{0\text{-}672h}$ = area under the plasma concentration-time curve from 0 to 672 hours postdose; — = species received one dose at this level, exposure was not determined on Day 43; F= Female; LEN = lenacapavir (GS-6207); M = Male; N/A = not applicable

a $AUC_{0\text{-}336h,}$ TX-200-2005 (Day 43) Suspension formulation
b $AUC_{0\text{-}336h,}$ TX-200-2006 (Day 43) Suspension formulation
c $AUC_{0\text{-}336h,}$ TX-200-2017 200 mg/mL (Day 225)
d $AUC_{0\text{-}336h,}$ TX-200-2017 200 mg/mL 1 mol-eq NaOH (Day 225)
e $AUC_{0\text{-}336h,}$ TX-200-2017 400 mg/mL (Day 225)
f $AUC_{0\text{-}672h,}$ TX-200-2014 200 mg/mL (Day 92)
g $AUC_{0\text{-}672h,}$ TX-200-2014 200 mg/mL 1 mol-eq NaOH (Day 92)
h $AUC_{0\text{-}672h,}$ TX-200-2014 400 mg/mL (Day 92)
i $AUC_{0\text{-}672h,}$ TX-200-2047 309 mg/mL (Day 225)
j $AUC_{0\text{-}336h,}$ TX-200-2047 309 mg/mL (Day 57)
k $AUC_{0\text{-}24h,}$ TX-200-2004 4, 13.3 and 40 mg/mL (Day 7)
l $AUC_{0\text{-}24h,}$ TX-200-2002 5 mg/mL via intramuscular injection (Day 7)
m $AUC_{0\text{-}192h,}$ TX-200-2049 0.1 and 1 mg/mL (Gestation Day 6)
n $AUC_{0\text{-}672h,}$ TX-200-2043 0.1 and 0.5 mg/mL (Day 28)
o $AUC_{0\text{-}24h,}$ TX-200-2002 5, 30, 100 mg/mL via subcutaneous injection (Day 7)
p $AUC_{0\text{-}672h,}$ TX-200-2042 75 mg/mL LEN-Pure (Day 1)
q $AUC_{0\text{-}672h,}$ TX-200-2042 75 mg/mL LEN-A (Day 1)
r $AUC_{0\text{-}672h,}$ TX-200-2042 75 mg/mL LEN-B (Day 1)
s $AUC_{0\text{-}2184h,}$ TX-200-2050 75 mg/mL LEN-Pure (Day 1)
t $AUC_{0\text{-}2184h,}$ TX-200-2050 75 mg/mL LEN-A (Day 1)
u $AUC_{0\text{-}2184h,}$ TX-200-2050 75 mg/mL LEN-B (Day 1)

3.2. Oral

Test Article: LEN

	AUC (ng•h/mL) following the last dose				
Species	**Rat**		**Dog**		**Rabbit**
Method of Administration	**Oral**				
Gender (M/F)	**M**	**F**	**M**	**F**	**F**
Dose (mg/kg)					
1	ND	ND	4,610[b]	2,270[b]	
3	16,400[a]	14,200[a], 11,600[c], 9,200[d]	ND	ND	
5	ND	ND	20,400[b]	18,200[b]	
10	27,800[a]	27,800[a], 17,800[d]	ND	ND	
30	33,700[a]	35,400[a], 29,300[c], 22,000[d]	82,900[b]	70,600[b]	
100	N/A	N/A	N/A	N/A	2780[e]
300	N/A	26,300[c]	N/A	N/A	9760[e]
1000	N/A	N/A	N/A	N/A	5270[e]

$AUC_{0\text{-}24h}$ = area under the plasma concentration-time curve from 0 to 24 hours postdose; F=Female; GD= gestation day; LEN = lenacapavir (GS-6207); M = Male; N/A = not applicable; ND = not dosed at this level

a $AUC_{0\text{-}24h,}$ TX-200-2011 1.5, 5 and 15 mg/mL (Day 28)
b $AUC_{0\text{-}24h,}$ TX-200-2012 0.5. 2.5 and 15 mg/mL (Day 28)
c $AUC_{0\text{-}24h,}$ TX-200-2031 1.5, 15 and 150 mg/mL (GD 17)
d $AUC_{0\text{-}24h,}$ TX-200-2036 1.5, 5 and 15 mg/mL (GD 17)
e $AUC_{0\text{-}96h,}$ TX-200-2035 500 mg/mL (Day 6)[1000 mg/kg/day was based on N=1

3.3. Intravenous

Test Article: LEN

	AUC (ng•h/mL)				
Species	**Rat**		**Dog**		**Rabbit**
Method of Administration	**Intravenous Infusion**				
Gender (M/F)	M	F	M	F	F
Dose (mg/kg)					
3	33,600[a]	31,400[a]	39,000[b]	31,400[b]	
5					26,600[c]
10	99,000[a]	98,700[a]	265,000[b]	124,000[b]	85,500[c]
20					178,000[c]
30	235,000[a]	210,000[a]	460,000[b]	762,000[b]	

F= Female; LEN = lenacapavir (GS-6207); M = Male

a $AUC_{0\text{-}168.5h,}$ TX-200-2029 (Day 1) Single intravenous infusion administered over 30 minutes with 14-day observation.
b $AUC_{0\text{-}168.5h,}$ TX-200-2030 (Day 1) Single intravenous infusion administered over 30 minutes with 14-day observation.
c $AUC_{0\text{-}24.5h,}$ TX-200-2032 (Gestation Day 19) 30 mg/mL intravenous infusion administered daily over 30 minutes

4. TOXICOLOGY: DRUG SUBSTANCE

Test Article: LEN

Batch No.	Strength (%)[a]	Observed Impurities (%)										Type of Study
[redacted] (aqueous suspension)	99.9	0.19	0.26	0.16	0.06	0.30	0.22	0.45	0.59	0.12	0.12	0.07	Repeat Dose Toxicity, In Vivo Safety Pharmacology[b]

Batch No.	Strength (%)[a]	Observed Impurities (%)										Type of Study
[redacted] (aqueous suspension)	99.9	0.21	0.26	0.23	0.08	0.06	0.25	0.15	0.06	0.10	0.06	Repeat Dose Toxicity, In Vivo Safety Pharmacology[b]

LEN = lenacapavir (GS-6207); RRT= relative retention time; trace level impurities (<0.05%) not tabulated.

a Strength (purity) is determined by external standard.
b Used for study numbers TX-200-2005, TX-200-2006, PC-200-2014

Test Article: LEN

Batch No.	Purity (%)[a]	Observed Impurities (%)												Type of Study
[redacted]	98.3	0.19	0.08								0.05	0.18		Genotoxicity[b]
[redacted]	95.5				0.06		0.07		0.24	0.13			0.12	0.05	Repeat Dose Toxicity, Local Tolerance[c]
[redacted]	99.0														Single Dose Toxicity, Repeat Dose Toxicity, Local Tolerance, Reproductive Toxicity[d]
[redacted][h]	100.2			0.11											Local Tolerance[e]
[redacted][h]	102.5			0.12											Local Tolerance[e]
[redacted][h]	92.1														Local Tolerance[f]

LEN = lenacapavir (GS-6207); RRT= relative retention time; trace level impurities (<0.05%) not tabulated, unless otherwise noted

a Purity is determined by external standard.
b Used for study numbers TX-200-2007, TX-200-2008
c Used for study numbers TX-200-2014, TX-200-2017, TX-200-2019
d Used for study numbers TX-200-2017, TX-200-2026, TX-200-2029, TX-200-2030, TX-200-2031, TX-200-2034
e Used for study number TX-200-2033
f Used for study number TX-200-2038
g Reported as [redacted] in the analytical report based on the observed major atropisomer peak of [redacted].
h Reporting threshold is 0.10%. Impurities below 0.10% are reported as trace and not tabulated

Test Article: LEN

Batch No.	Purity (%)[a]	Observed Impurities (%)							Type of Study
[redacted]	99.3		0.05		0.06		0.05		Repeat Dose Toxicity[b]
[redacted]	98.7	0.05		0.06		0.07		0.05	Reproductive Toxicity[c], Impurity Qualification[d]

LEN = lenacapavir (GS-6207); RRT= relative retention time; trace level impurities (<0.05%) not tabulated, unless otherwise noted

a Strength (purity) is determined by external standard.
b Used for study numbers TX-200-2011, TX-200-2012
c Used for study number TX-200-2036
d Used for study number TX-200-2042

Test Article: LEN

Batch No.	Purity (%)[a]	Observed Impurities (%)									Type of Study
[redacted]	99.7	0.08	0.07		0.10				0.06	0.05	Local Tolerance[b]
[redacted]	101.8		0.09		0.10				0.06	0.05	Local Tolerance[b]
[redacted][f]	99.0		0.04	0.04			0.08	0.05			Reproductive Toxicity[c]
[redacted]	99.7					0.16					Repeat Dose Toxicity, Reproductive Toxicity, Carcinogenicity, Impurity Qualification, Worker Safety[d]
[redacted]	100.0					0.10					Local Tolerance[e]

LEN = lenacapavir (GS-6207); RRT= relative retention time; trace level impurities (<0.05%) not tabulated, unless otherwise noted

a Purity is determined by external standard.
b Used for study number TX-200-2010
c Used for study numbers TX-200-2032, TX-200-2037, TX-200-2043
d Used for study numbers TX-200-2045, TX-200-2047, TX-200-2049, TX-200-2050, TX-200-2051, TX-200-2052, TX-200-2053, TX-200-2071, TX-200-2072, TX-200-2073
e Used for study number TX-200-2048
f Reporting threshold is 0.03%. Impurities below 0.03% are reported as trace and not tabulated.

Test Article: LEN

Batch No.	Purity (%)[a]	Observed Impurities (%)											Type of Study
[redacted][f]	94.6			0.14	0.12	0.15	0.16						Genotoxicity[b]
[redacted]	99.3								0.08	0.06	0.54	0.05	Genotoxicity[c]
[redacted]	97.6	2.29	0.15										Genotoxicity[d]
[redacted]	99.8							0.09					Genotoxicity[e]

LEN = lenacapavir (GS-6207): RRT= relative retention time; trace level impurities (<0.05%) not tabulated, unless otherwise noted

a Purity is determined by external standard.
b Used for study number TX-200-2041
c Used for study number TX-200-2023
d Used for study number TX-200-2024
e Used for study number TX-200-2025
f Reporting threshold is 0.10%. Impurities below 0.10% are reported as trace and not tabulated

Test Article: LEN

Batch No.	Purity (%)[a]	Observed Impurities (%)																Type of Study
[redacted]	89.0	0.66			1.16	2.32	2.07	1.08	0.75	0.81	0.58						0.03	Impurity Qualification[b]
[redacted]	90.8		0.75	3.35	0.03	0.14						1.21	0.04	1.31	0.42	0.75	2.61	Impurity Qualification[b]

LEN = lenacapavir (GS-6207): RRT= relative retention time; trace level impurities (<0.03%) not tabulated

a Purity is determined by external standard.
b Used for study number TX-200-2050

Test Article: LEN

Batch No.	Purity (%)[a]	Observed Impurities (%)											Type of Study
[redacted]	91.3	0.48	0.23	0.47	0.50	0.93	0.55	0.10	1.04	1.00	1.03	0.19	Impurity Qualification[b]

LEN = lenacapavir (GS-6207): RRT= relative retention time; trace level impurities (<0.05%) not tabulated, unless otherwise noted
a Purity is determined by external standard.
b Used for study number TX-200-2042

Test Article: LEN

Batch No.	Purity (%)[a]	Observed Impurities (%)													Type of Study
[redacted]	93.0	0.36	0.07	0.67	0.39	0.61	1.67	0.75	0.11	0.44	0.48	0.47	0.06	0.11	Impurity Qualification[b]

LEN = lenacapavir (GS-6207): RRT= relative retention time; trace level impurities (<0.05%) not tabulated, unless otherwise noted
a Purity is determined by external standard.
b Used for study number TX-200-2042
c $CyP(O)Ph_2$ = Cyclohexyldiphenylphosphine Oxide

Test Article: LEN

Batch No.	Purity (%)[a]	Observed Impurities (%)							Type of Study
		LEN	[redacted][b]						
[redacted]	98.7	0.13	0.35	0.08	0.05	0.09	0.08	0.09	Impurity Genotoxicity[c]
[redacted]	93.7[d]								Impurity Genotoxicity[e]

LEN = lenacapavir (GS-6207); RRT= relative retention time; trace level impurities (<0.05%) not tabulated, unless otherwise noted

a Purity is determined by external standard
b Reported as [redacted] in the analytical report based on the observed major atropisomer peak of [redacted]
c Used for study number TX-200-2075
d Purity by external standard is determined by QNMR.
e Used for study number TX-200-2076

5. TOXICOLOGY: SINGLE-DOSE TOXICITY

5.1. Single Dose Rat Intravenous Infusion

Test Article: LEN

Species / Strain	Method of Administration (Vehicle)	Dose (mg/kg)	Gender and No. per Group	NOAEL (mg/kg)	Noteworthy Findings	Gilead Study No. (CRO Study No.)
Rat / Crl:WI(Han)	Intravenous infusion (67.0 % [w/w] polyethylene glycol 300 [PEG 300] and 33.0% [w/w] sterile water for injection)	0 3 10 30	Main: 15M/15F TK: 3M/3F control; 9M/9F treated	30	No LEN-related mortality, or changes in hematology, coagulation, clinical chemistry or urinalysis. Vehicle related mortality and transient clinical findings consistent with hemolysis were observed in animals in all groups (including controls). **30 mg/kg/day:** reversible ↑ in liver weight parameters (mean absolute weight and mean relative to body weight) observed **NOAEL: 30 mg/kg (C_{max} =12,700 ng/mL; $AUC_{0\text{-}168.5}$ = 22,4000 ng·h/mL)**	TX-200-2029 ([redacted] 8391711)

F = Female; LEN = lenacapavir (GS-6207); M = Male; NOAEL = No-Observed-Adverse-Effect Level; TK = Toxicokinetic; w/w = weight/weight; ↑ = increase

5.2. Single Dose Dog Intravenous Infusion

Test Article: LEN

Species / Strain	Method of Administration (Vehicle)	Dose (mg/kg)	Gender and No. per Group	NOAEL (mg/kg)	Noteworthy Findings	Gilead Study No. (CRO Name and Study No.)
Dog/ Beagle	Intravenous infusion (6.12% (w/w) polyethylene glycol 300 (PEG 300) and 93.88% (w/w) sterile water for injection[a])	0 3 10 30	5M/5F	3	**3 mg/kg:** ↓ BW and mean BW gains; ↓ mean food consumption in F. Hepatocyte degeneration minimal to moderate in M; minimal to slight hemorrhage around central vein and connective tissue between liver and gall bladder present (Day 2 but not Day 15). **10 mg/kg:** ↓ BW and BW gains; ↓ mean food consumption in F. Minimally prolonged PT and minimally ↓ fibrinogen concentration on Day 2, reversed by Day 15 in 1 F. Day 2 moderately to markedly ↑ ALT (still noted on Day 15), and mildly to moderately ↑ AST (all M and 1 F, returned to predose values at Day 15); mildly ↑ GGT (1F, Day 2, returned to predose values at Day 15). Minimal to moderate hepatocyte degeneration (M), and minimal to slight (F); individual hepatocyte necrosis minimal (1 F); minimal mixed cell infiltrates (M/F); minimal to slight hemorrhage (F); all microscopic findings were reversible. Clinical pathology liver changes were considered adverse due to large magnitude and were not fully reversible by Day 15.	TX-200-2030 ([redacted] 8391725)

Test Article: LEN

Species / Strain	Method of Administration (Vehicle)	Dose (mg/kg)	Gender and No. per Group	NOAEL (mg/kg)	Noteworthy Findings	Gilead Study No. (CRO Name and Study No.)
					30 mg/kg: ↓ BW, BW gains; ↓ mean food consumption in F. Minimally ↑ red cell mass (red blood cell count [+8.7%], hemoglobin [+8.4%], and hematocrit [+9.0%] (M)); minimally prolonged PT, aPTT (2 M, 1 F); minimally ↓ fibrinogen concentration (M and 1 F); reversible. Moderately to markedly ↑ ALT, AST and GGT activities; mildly ↑ ALP activity; mildly to moderately ↑ total bilirubin (direct and/or indirect; M, 1 F). AST, ALP and total bilirubin reversible. Mildly to moderately ↑ ALT activity in animals and mildly to moderately ↑ GGT activity still noted on Day 15. Minimally ↑cholesterol concentration (M), reversible. Hepatocyte degeneration was minimal to moderate in M and minimal to slight in F; individual hepatocyte necrosis was minimal to slight (M); minimal mixed cell infiltrates (M/F); minimal to slight hemorrhage (M/F) all microscopic findings were reversible. **NOAEL – 3 mg/kg (C_{max}=5140 ng/mL; $AUC_{0\text{-}168.5}$ 35,200 ng·h/mL)**	

alanine aminotransferase = ALT; alkaline phosphatase = ALP; aPTT= activated prolonged prothrombin time; aspartate aminotransferase= AST BW= Body weight; $AUC_{0\text{-}168.5}$ = Area under the concentration-time curve from 0 to 168.5 hours postdose; C_{max} = Maximum observed concentration; F = Female; gamma glutamyl transferase= GGT; LEN = lenacapavir (GS-6207); M = Male; NOAEL = No-Observed-Adverse-Effect Level; PT= Prothrombin Time; Sac= sacrifice; w/w = weight/weight, ↑ = increased; ↓ = decreased

a For LEN dosed animals, vehicle was comprised of 5.74% [w/w] polyethylene glycol 300 [PEG 300], 88.14% [w/w] Sterile water for injection, and 3.17% [w/w] 1N sodium hydroxide [NaOH]

6. REPEAT DOSE TOXICITY (NONPIVOTAL STUDIES)

6.1. Rat

Test Article: LEN

Species / Strain	Method of Administration (Vehicle)	Duration of Dosing	Dose (mg/kg/day)	Gender and No. per Group	NOAEL (mg/kg/day)	Noteworthy Findings	Gilead Study No. (CRO Study No.)
Rat / Crl:WI(Han)	Subcutaneous injection and Intramuscular injection (Vehicle: 2% poloxamer 388 and 98% sterile water for both routes)	2 Doses/7 days	Subcutaneous: 0, 5, 30, 100 Intramuscular: 0, 5	Main: 5M TK: Group 6:3M Group 7-10: 6M	Not achieved for SC injection 5 for Intramuscular injection	**Subcutaneous (SC) injection:** LEN-related coagulation and serum chemistry findings consisted of dose/concentration related changes consistent with inflammation in animals administered ≥30 mg/kg. Minimally to slightly ↑ fibrinogen and globulin concentrations at ≥30 mg/kg. Minimally ↓ albumin concentrations at 100 mg/kg and were correlated with the subcutaneous injection site reactions. Minimally ↑ creatinine, blood urea nitrogen (BUN) concentrations occurred in animals administered 100 mg/kg and were consistent with dehydration; no microscopic LEN-related kidney changes were observed. LEN-related microscopic changes were limited to local injection site reactions; pockets of minimal to marked necrosis, surrounded by mixed cellular infiltrates (primarily neutrophils, lymphocytes, and macrophages), were noted in the subcutis, occasionally with hemorrhage and secondary fibroplasia at doses of ≥5 mg/kg,	TX-200-2002 ([redacted] 8338788)

Test Article: LEN

Species / Strain	Method of Administration (Vehicle)	Duration of Dosing	Dose (mg/kg/day)	Gender and No. per Group	NOAEL (mg/kg/day)	Noteworthy Findings	Gilead Study No. (CRO Study No.)
						and were considered adverse. Severity was dose/concentration related. Microscopic injection site reactions correlated with macroscopic observations of thickened and discolored skin/subcutis and clinical pathology findings consistent with inflammation. Due to these injection site reactions the NOAEL for LEN when administered via SC injection was not achieved **Intramuscular (IM) Injection:** LEN-related clinical observations included injection site reactions (muscle twitching) and vocalization, on Day 1 but not on Day 7. LEN-related coagulation and serum chemistry changes included minimally to slightly ↑ fibrinogen, minimally ↑ globulin concentrations, and were consistent with inflammation. **NOAEL – 5 mg/kg for IM (C_{max} = 580 ng/mL; AUC_{0-24} 11,900 ng·h/mL)**	

AUC_{0-24} = Area under the concentration-time curve from 0 to 24 hours postdose; C_{max} = Maximum observed concentration; LEN = lenacapavir (GS-6207); M = Male; NOAEL = No Observed Adverse Effect Level; TK = Toxicokinetic, ↑ = increased; ↓= decreased

6.2. Dog

Test Article: LEN

Species / Strain	Method of Administration (Vehicle)	Duration of Dosing	Dose (mg/kg/day)	Gender and No. per Group	NOAEL (mg/kg/day)	Noteworthy Findings	Gilead Study No. (CRO Study No.)
Dog/ Beagle	Subcutaneous injection (2% Poloxamer 338 and 98% Sterile Water)	2 Doses/7 days	0, 3, 10, 30	M: 3	3	At the injection site, a dose-dependent increased severity of microscopic findings of mixed cell infiltration (correlating with macroscopic findings of swelling, thickening and mass) and hemorrhage as well as presence of fibrin exudates and vascular inflammation was observed at all dose levels. ↓ cellularity in the thymus correlated with small size, and ↓ thymus weight observed at all dose levels. **10 mg/kg:** Clinical pathology changes included minimal ↑ fibrinogen, cholesterol, globulin; mildly ↓ A/G ratio. Microscopic changes included moderate to marked mixed cell infiltration, evidence of abscess formation characterized by a center of necrotic debris and fibrin that was surrounded by granulation tissue at the site of the first injection; **30 mg/kg:** LEN-related clinical signs at the injection site consisted of firm swelling (soft to firm). Clinical pathology changes included minimal ↑ fibrinogen, neutrophil monocyte and white blood cell count, ALP, cholesterol, globlin,; ↓ A/G ratio . Microscopic changes same as above. **NOAEL – 3 mg/kg (C_{max} = 630 ng/mL; AUC_{0-72} = 32,200 ng·h/mL)**	TX-200-2004 ([redacted] 5001864)

ALP = alkaline phosphatase; A/G ratio = albumin/globulin ratio; AUC_{0-72} = Area under the concentration-time curve from 0 to 72 hours postdose; C_{max} = Maximum observed concentration; LEN = lenacapavir (GS-6207); M = Male; NOAEL = No-Observed-Adverse-Effect Level

7. TOXICOLOGY: REPEAT-DOSE TOXICITY (PIVOTAL STUDIES)

7.1. 4-Dose (Twice Monthly) Rat

Test Article: LEN

Report Title:	**A Four-Dose (Twice Monthly) Subcutaneous Injection Toxicity and Toxicokinetic Study of GS-6207 in Wistar Han Rats**	**Gilead Study No. TX-200-2005 [redacted] 8353952**
Species / Strain: Rat/Crl:WI(Han)	**Duration of Dosing:** Four doses[a]	**Location in CTD:** 4.2.3.2
Initial Age: 6 to 7 weeks old	**Duration of Postdose:** NA[a]	**GLP Compliance:** Yes
Date of First Dose: [redacted] 20[redacted]	**Method of Administration:** Subcutaneous injection (test article), oral gavage (positive control article)	**Lot Number:** [redacted]

Vehicle: 2% (w/v) poloxamer 188 prepared in 0.9% (w/v) sodium chloride in sterile water for injection, and 400 mM phosphate buffer.

Positive Control: Cyclophosphamide (cyclophosphamide monohydrate; CAS No. 6055-19-2)

Special Features: bone marrow micronucleus assay, functional observational battery, locomotor activity assessment, hepatic enzyme analysis

No Observed Adverse Effect Level: 100 mg/kg/dose for systemic effects, NOAEL for local injection site effects was not established [b]

Biweekly Dose (mg/kg/dose)	**0 (Control)**		**10 (Low)**		**30 (Mid)**		**100 (High)**		**60 (Positive Control)**	
Sex:Number of Animals (TK)	M: 3	F: 3	M: 12	F: 12	M: 12	F: 12	M: 12	F: 12	M: NA	F: NA
Toxicokinetics: GS-6207 AUC_{0-336} (ng·h/mL)										
Study Day 1	NC	NC	64100	55700	146000	124000	338000	338000	NA	NA
Study Day 43	NC	NC	134000	106000	314000	244000	608000	559000	NA	NA
Toxicokinetics: GS-6207 C_{max} (ng/mL)										
Study Day 1	BLQ	BLQ	241	215	586	445	1230	1290	NA	NA
Study Day 43	BLQ	BLQ	410	451	1060	843	2500	2130	NA	NA

Test Article: LEN

Biweekly Dose (mg/kg/dose)	**0 (Control)**		**10 (Low)**		**30 (Mid)**		**100 (High)**		**60 (Positive Control)**	
Sex:Number of Animals (Main)	M: 20	F: 20	M: 20	F: 20	M: 20	F: 20	M: 20	F: 20	M: 5	F: 5
Died or sacrificed moribund	1[c]	0	0	1[d]	0	0	0	0	0	0
Body Weight Change g (%)[e]										
Study Day 1 - 8	42	24	-14.3*	-4.2	-16.7*	-20.8*	-16.7*	-16.7*	NA	NA
Food Consumption	No GS-6207-related findings									
Clinical Observations	No GS-6207-related findings									
Ophthalmoscopy	No GS-6207-related findings									
Functional Observational Battery	No GS-6207-related findings									
Locomotor Activity	No GS-6207-related findings									
Hematology	No GS-6207-related findings									
Coagulation	No GS-6207-related findings									
Serum Chemistry										
Cholesterol (mg/dL), Study Day 57	74	61	77	70	79	66	96*	81	NA	NA
Cholesterol (mg/dL), Study Day 85	83	-	83	-	89	-	98	-	NA	NA
Urinalysis	No GS-6207-related findings									
Organ Weights	No GS-6207-related findings									
Gross Pathology – Terminal Sacrifice 1										

Test Article: LEN

Biweekly Dose (mg/kg/dose)	**0 (Control)**		**10 (Low)**		**30 (Mid)**		**100 (High)**		**60 (Positive Control)**	
Sex:Number of Animals (Main)	M: 20	F: 20	M: 20	F: 20	M: 20	F: 20	M: 20	F: 20	M: 5	F: 5
Number Evaluated	9	10	10	10	10	10	10	10	NA	NA
Subcutaneous Injection Site A, Thickened	0	0	0	0	1	1	1	0	NA	NA
Subcutaneous Injection Site B, Thickened	0	0	1	0	0	0	0	0	NA	NA
Subcutaneous Injection Site C, Thickened	0	0	0	0	0	0	1	1	NA	NA
Subcutaneous Injection Site D, Thickened	0	0	0	0	1	0	5	2	NA	NA
Gross Pathology – Terminal Sacrifice 2										
Number Evaluated	10	10	10	10	10	10	10	10	NA	NA
Subcutaneous Injection Site A, Thickened	0	0	0	0	0	0	3	3	NA	NA
Subcutaneous Injection Site B, Thickened	0	0	1	0	1	0	2	2	NA	NA
Subcutaneous Injection Site C, Thickened	0	0	0	0	1	0	3	1	NA	NA
Subcutaneous Injection Site D, Thickened	0	0	1	1	2	2	7	7	NA	NA
Histopathology - Terminal Sacrifice 1										
Number Evaluated	9	10	10	10	10	10	10	10	NA	NA
Subcutaneous Injection Site A, Inflammation, granulomatous										
Minimal	0	0	3	2	1	1	0	2	NA	NA
Slight	0	0	2	1	4	5	2	1	NA	NA
Moderate	0	0	0	0	0	3	3	3	NA	NA
Marked	0	0	0	0	0	0	0	1	NA	NA

Test Article: LEN

Biweekly Dose (mg/kg/dose)	**0 (Control)**		**10 (Low)**		**30 (Mid)**		**100 (High)**		**60 (Positive Control)**	
Sex:Number of Animals (Main)	M: 20	F: 20	M: 20	F: 20	M: 20	F: 20	M: 20	F: 20	M: 5	F: 5
Subcutaneous Injection Site B, Inflammation, granulomatous										
Minimal	0	0	1	0	0	0	4	0	NA	NA
Slight	0	0	5	3	5	9	1	4	NA	NA
Moderate	0	0	0	1	3	0	1	1	NA	NA
Subcutaneous Injection Site C, Inflammation, granulomatous										
Minimal	0	0	1	0	0	0	0	0	NA	NA
Slight	0	0	2	0	0	0	0	0	NA	NA
Moderate	0	0	0	0	2	0	1	2	NA	NA
Subcutaneous Injection Site D, Inflammation, granulomatous										
Minimal	0	0	0	0	0	1	0	0	NA	NA
Slight	0	0	0	0	0	0	0	2	NA	NA
Moderate	0	0	0	1	4	1	3	4	NA	NA
Marked	0	0	0	0	0	0	2	1	NA	NA
Histopathology – Terminal Sacrifice 2										
Number Evaluated	10	10	10	10	10	10	10	10	NA	NA
Subcutaneous Injection Site A, Inflammation, granulomatous										
Minimal	0	0	2	0	2	1	1	4	NA	NA
Slight	0	0	1	0	0	3	3	2	NA	NA
Moderate	0	0	0	0	0	1	2	1	NA	NA

Test Article: LEN

Biweekly Dose (mg/kg/dose)	0 (Control)		10 (Low)		30 (Mid)		100 (High)		60 (Positive Control)	
Sex:Number of Animals (Main)	M: 20	F: 20	M: 20	F: 20	M: 20	F: 20	M: 20	F: 20	M: 5	F: 5
Subcutaneous Injection Site B, Inflammation, granulomatous										
Minimal	0	0	0	2	0	4	3	2	NA	NA
Slight	0	0	2	0	2	1	3	2	NA	NA
Moderate	0	0	0	0	0	0	2	0	NA	NA
Subcutaneous Injection Site C, Inflammation, granulomatous										
Minimal	0	0	0	0	0	0	0	1	NA	NA
Slight	0	0	1	0	1	1	0	0	NA	NA
Moderate	0	0	0	0	0	0	3	1	NA	NA
Subcutaneous Injection Site D, Inflammation, granulomatous										
Minimal	0	0	2	0	1	0	1	0	NA	NA
Slight	0	0	2	0	0	4	3	3	NA	NA
Moderate	0	0	0	1	2	0	3	5	NA	NA
Marked	0	0	0	0	0	0	1	0	NA	NA
Hepatic Microsomal Enzymes										
CYP3A (6β-OH) (pmol/min/mg protein)	-	87.7	-	93.3	-	103	-	200	NA	NA

AUC_{0-336} = Area under the concentration-time curve from hour 0 to hour 336; BLQ = below the lower limit of quantitation (< 2.00 ng/mL); C_{max} = Maximum observed concentration; F = Female; LEN = lenacapavir (GS-6207); M = Male; NA = Not Applicable; NC = Not Calculated; TK = toxicokinetic; w/v = weight/volume.

− Comparable to control animals or no test article-related findings; + = increased; - = decreased; * = $p \leq 0.05$ (ANOVA and Dunnett's)

a Animals in Groups 1 through 4 were dosed once every two weeks for a total of four doses and were monitored for at least 6 weeks after the last dose.

b The NOAEL for systemic effects is 100 mg/kg/dose. A NOAEL for local injection site effects was not established due to microscopic granulomatous inflammation at all dose levels.

c One toxicity male administered the vehicle control article was found dead on Study Day 21 soon after it was shaved for dosing. The death was considered to be associated with the shaving procedure and was not vehicle-related.

d One toxicokinetic female administered 10 mg/kg/dose was found dead on Study Day 15. No necropsy occurred since this was a toxicokinetic animal, but it had no prior abnormal clinical observations or effects on body weight. This death was considered unrelated to the test article.

e For controls, group means are shown. For treated groups, percent differences from controls are shown. Statistical significance is based on actual data not on the percent.

7.2. 6-Month Rat –Single or Two Dose

Test Article: LEN

Report Title:	**A Single- and Two-Dose Subcutaneous Injection Toxicity and Toxicokinetic Study with 3 Formulations of GS-6207 in Wistar Han Rats with a 13-Week Observation Period after each Dose**	**Gilead Study No. TX-200-2014 ([redacted] 8375766)**
Species / Strain: Rat / Crl:WI(Han)	**Duration of Dosing:** One (Groups 1 through 4) or two doses (Days 1 and 92; Groups 5 through 8)	**Location in CTD:** 4.2.3.2
Initial Age: 6 to 8 weeks	**Duration of Postdose:** Not applicable	**GLP Compliance**: Yes
Date of First Dose : [redacted] 20[redacted] (Groups 1 through 4) [redacted] 20[redacted] (Groups 5 through 8)	**Method of Administration:** Subcutaneous injection	**Lot Number:** [redacted]
Vehicle Control Article: 77:10:13 w/w/w polyethylene glycol 200 (PEG 200):ethanol:water (sterile water for injection)		
Special Features: Dermal Observations		
No Observed Adverse Effect Level: Not determined		

Daily Dose (mg/kg/dose)	**0 (Vehicle Control, 0 mol-eq NaOH) [a]**		**100 (200 mg/mL, 0 mol-eq NaOH) [a]**		**100 (200 mg/mL, 1 mol-eq NaOH) [a]**		**100 (400 mg/mL, 0 mol-eq NaOH) [b]**	
Sex:Number of Animals/Group (TK)	M: 3	F: 3	M: 4	F: 4	M: 4	F: 4	M: 4	F: 4
Toxicokinetics: $AUC_{0\text{-}672h}$ (ng·h/mL)								
Day 1	BLQ	BLQ	155000	98500	184000	166000	133000	102000
Day 92	BLQ	BLQ	338000	276000	276000	293000	265000	156000
Toxicokinetics: C_{max} (ng/mL)								
Day 1	BLQ	BLQ	427	331	544	539	318	254
Day 92	BLQ	BLQ	846	637	856	703	570	332

Test Article: LEN

Daily Dose (mg/kg/dose)	0 (Vehicle Control, 0 mol-eq NaOH) [a]		100 (200 mg/mL, 0 mol-eq NaOH) [a]		100 (200 mg/mL, 1 mol-eq NaOH) [a]		100 (400 mg/mL, 0 mol-eq NaOH) [b]	
Sex:Number of Animals/Group (Main)	M: 10 [c]	F: 10 [c]	M: 10 [c]	F: 10 [c]	M: 10 [c]	F: 10 [c]	M: 10 [c]	F: 10 [c]
Noteworthy Findings								
Died or sacrificed moribund	0	0	0	0	0	0	0	0
Body Weight	No GS-6207-related findings							
Food Consumption	No GS-6207-related findings							
Clinical Observations	No GS-6207-related findings							
Ophthalmoscopy	No GS-6207-related findings							
Dermal Observations	GS-6207-related nonadverse dermal observations of very slight to moderate edema were noted for animals administered 200 mg/ml, with or without NaOH, or 400 mg/mL GS-6207. Edema was present 24 hours after the Days 1 and/or 92 doses and at an increased incidence starting 72 hours after the doses for all groups administered GS-6207. Edema persisted throughout the observation periods (either 13 or 26 weeks) in animals administered GS-6207, with the highest incidence in animals administered 200 mg/mL GS-6207 with NaOH or 400 mg/mL GS-6207 without NaOH, but exhibited evidence of recovery by Days 92 and 176.							
Hematology and Coagulation	No GS-6207-related findings							
Clinical Chemistry	No GS-6207-related findings							
Urinalysis	No GS-6207-related findings							
Organ Weights	No GS-6207-related findings							
Gross Pathology								
Number Evaluated at Terminal Sacrifice 1 (Week 13)	M:10	F:10	M:10	F:10	M:10	F:10	M:10	F:10
Subcutaneous Injection Site								
Thickened	0	0	1	1	3	2	8	7
Discolored	0	0	0	0	0	0	2	1

Test Article: LEN

Daily Dose (mg/kg/dose)	0 (Vehicle Control, 0 mol-eq NaOH) [a]		100 (200 mg/mL, 0 mol-eq NaOH) [a]		100 (200 mg/mL, 1 mol-eq NaOH) [a]		100 (400 mg/mL, 0 mol-eq NaOH) [b]	
Sex:Number of Animals/Group (Main)	M: 10 [c]	F: 10 [c]	M: 10 [c]	F: 10 [c]	M: 10 [c]	F: 10 [c]	M: 10 [c]	F: 10 [c]
Number Evaluated at Terminal Sacrifice 2 (Week 26)	M:10	F:10	M:10	F:10	M:10	F:10	M:10	F:10
Subcutaneous Injection Site A								
Thickened	0	0	7	5	8	7	8	9
Discolored	0	0	1	1	2	1	0	0
Subcutaneous Injection Site B								
Thickened	0	0	8	8	10	9	9	9
Histopathology								
Number Evaluated at Terminal Sacrifice 1	M:10	F:10	M:10	F:10	M:10	F:10	M:10	F:10
Subcutaneous Injection Site, Inflammation, Granulomatous								
Minimal	0	0	0	1	1	0	0	0
Slight	0	0	1	1	1	1	3	2
Moderate	0	0	0	0	1	2	6	2
Number Evaluated at Terminal Sacrifice 2	M:10	F:10	M:10	F:10	M:10	F:10	M:10	F:10

Test Article: LEN

Daily Dose (mg/kg/dose)	0 (Vehicle Control, 0 mol-eq NaOH) [a]		100 (200 mg/mL, 0 mol-eq NaOH) [a]		100 (200 mg/mL, 1 mol-eq NaOH) [a]		100 (400 mg/mL, 0 mol-eq NaOH) [b]	
Sex:Number of Animals/Group (Main)	M: 10 [c]	F: 10 [c]	M: 10 [c]	F: 10 [c]	M: 10 [c]	F: 10 [c]	M: 10 [c]	F: 10 [c]
Subcutaneous Injection Site A Inflammation, Granulomatous								
Minimal	0	0	5	4	5	3	2	1
Slight	0	0	2	2	0	3	4	7
Moderate	0	0	1	1	0	1	1	0
Infiltrate, Macrophage								
Minimal	0	0	0	1	1	1	0	0
Necrosis								
Minimal	0	0	1	0	0	0	0	0
Subcutaneous Injection Site B Inflammation, Granulomatous								
Minimal	0	0	2	0	1	0	0	2
Slight	0	0	0	4	2	6	2	2
Moderate	0	0	7	4	7	2	7	5
Necrosis								
Slight	0	0	0	0	1	0	0	0

BLQ = below the lower limit of quantitation (< 2.00 ng/mL); F = Female; GLP = Good Laboratory Practices; LEN = lenacapavir (GS-6207); M = Male; TK = toxicokinetic
a dose formulations were administered at a volume of 0.5
b dose formulations were administered at a volume of 0.25 mL/kg
c 10/sex/group for Groups 1-8; Groups 1-4 received a single subcutaneous injection on Day 1 and Groups 5-8 received single subcutaneous injections on Days 1 and 92.

7.3. 4-Week Oral Rat

Test Article: LEN

Report Title: A 4-Week Oral Gavage Toxicity and Toxicokinetic Study of GS-6207 in Wistar Han Rats		**Gilead Study No. TX-200-2011 ([redacted] 8367905)**
Species / Strain: Crl:WI(Han) rats	**Duration of Dosing:** 4 weeks	**Location in CTD:** 4.2.3.2
Initial Age: 7 weeks	**Duration of Postdose:** Not Applicable	**GLP Compliance:** Yes
Date of First Dose: [redacted] 20[redacted]	**Method of Administration:** Oral Gavage	**Lot Number:** [redacted]
Vehicle Control Article: 10% v/v Dimethyl sulfoxide (DMSO), 10% v/v vitamin E TPGS, and 80% v/v propylene glycol (PG)		
Special Features: Hepatic Microsomal Enzyme Analysis		
No Observed Adverse Effect Level: 30 mg/kg/day		

Daily Dose (mg/kg/day)	**0 (Vehicle Control)**		**3 (Low)**		**10 (Mid)**		**30 (High)**	
Sex:Number of Animals (TK)	**M: 3**	**F: 3**	**M: 9**	**F: 9**	**M: 9**	**F: 9**	**M: 9**	**F: 9**
Toxicokinetics: $AUC_{0\text{-}24h}$ (ng·h/mL)								
Day 1	BLQ	BLQ	2890	2700	12000	8560	16200	14700
Day 28	BLQ	BLQ	16400	14200	27800	27800	33700	35400
Toxicokinetics: C_{max} (ng/mL)								
Day 1	BLQ	BLQ	178	149	727	439	792	889
Day 28	BLQ	BLQ	829	658	1500	1460	1650	2110

Test Article: GS-6207

Daily Dose (mg/kg/day)	**0 (Vehicle Control)**		**3 (Low)**		**10 (Mid)**		**30 (High)**	
Sex:Number of Animals (Main)	**M: 10**	**F: 10**	**M: 10**	**F: 10**	**M: 10**	**F: 10**	**M: 10**	**F: 10**
Noteworthy Findings								
Died or sacrificed moribund	0	0	0	0	0	1[a]	0	0
Body Weight	No test-article related findings							
Food Consumption	No test-article related findings							
Clinical Observations	No test-article related findings							
Ophthalmoscopy	No test-article related findings							
Hematology	No test-article related findings							
Coagulation								
Day 29								
Activated Partial Prothrombin Time (sec)	14.7	-	14.9	-	15.5*	-	15.5*	-
Clinical Chemsitry								
Cholesterol (mg/dL)								
Day 7	-	56	-	69*	-	68*	-	76*
Day 29	-	55	-	68	-	72*	-	74*
Urinalysis	No test-article related findings							
Sex:Number of Animals (Main)	M: 10	F: 10	M: 10	F: 10	M: 10	F: 10	M: 10	F: 10
Organ Weights	No test-article related findings							
Gross Pathology	No test-article related findings							
Histopathology	No test-article related findings							
Hepatic Microsomal Fractions								
CYP3A (pmol/min/mg protein)	-	89.9 ± 28.9	-	131 ± 28.7	-	135 ± 23.2	-	241 ± 61.9

$AUC_{0\text{-}24h}$ = Area under the concentration time curve from 0 to 24 hours postdose; BLQ = below the lower limit of quantitation (< 2.00 ng/mL); C_{max} = Maximum observed concentration; F = Female; LEN = lenacapavir (GS-6207); M = Male; TK = toxicokinetic; TPGS = D-α-Tocopherol polyethylene glycol 1000 succinate (Vitamin E); * = Statistically significant from Group 1 where p≤0.05.; - = No noteworthy observations.

a One toxicity female administered 10 mg/kg/day was euthanized on Day 6 after falling and with clinical observations of bent tail, red discharge on the tail, and broken skin on the tail. This death was considered accidental and not GS-6207 related due to the clinical observations

7.4. 4-Dose (Twice Monthly) Dog

Test Article: LEN

Report Title:	**A 4-Dose (Twice Monthly) Subcutaneous Toxicity and Toxicokinetic Study with GS-6207 in Beagle Dogs**	**Gilead Study No. TX-200-2006 ([redacted] 8353953)**
Species / Strain: Dog/purebred Beagle	**Duration of Dosing:** 4 doses (Study Days 1, 15, 29, and 43)	**Location in CTD:** 4.2.3.2
Initial Age: 9 to 10 months	**Duration of Postdose:** Not applicable	**GLP Compliance:** Yes
Date of First Dose: [redacted] 20[redacted] (males) [redacted] 20[redacted] (females)	**Method of Administration:** Subcutaneous injection	**Lot Number:** [redacted]

Vehicle Control Article: 2% (w/v) Poloxamer 188 prepared in 0.9% (w/v) sodium chloride, in sterile water for injection

Special Features: Blood pressure measurements, electrocardiograms using jacketed external telemetry, hepatic microsomal enzyme analysis

No Observed Adverse Effect Level: Not established

Dose (mg/kg/dose)	**0 (Control)**		**10 (Low)**		**30 (Mid)**		**100 (High)**	
Sex:Number of Animals	M: 6	F: 6	M: 6	F: 6	M: 6	F: 6	M: 6[a]	F: 6[a]
Toxicokinetics: GS-6207 AUC_{0-336} (ng·h/mL)								
Study Day 1	BLQ	BLQ	112000	79900	311000	339000	925000	1350000[b]
Study Day 43	BLQ	BLQ	127000	110000	391000	481000	NA	NA
Toxicokinetics: GS-6207 C_{max} (ng/mL)								
Study Day 1	BLQ	BLQ	632	507	1730	2040	4260	6020
Study Day 43	BLQ	BLQ	634	648	2210	2240	NA	NA

Test Article: LEN

Dose (mg/kg/dose)	**0 (Control)**		**10 (Low)**		**30 (Mid)**		**100 (High)**	
Sex:Number of Animals	M: 6	F: 6	M: 6	F: 6	M: 6	F: 6	M: 6[a]	F: 6[a]
Noteworthy Findings								
Died or sacrificed moribund	0	0	0	0	0	0	6[a]	2[a]
Body Weight	No Test Article-Related Findings							
Clinical Observations[c]								
Vocalization During Dosing	0 (0)	0 (0)	1 (1)	6 (4)	2 (2)	6 (5)	8 (6)	2 (2)
Struggling During Dosing	0 (0)	0 (0)	0 (0)	1 (1)	1 (1)	0 (0)	3 (3)	1 (1)
Barrel Rolling	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (1)	2 (2)	0 (0)
Ophthalmoscopy	No Test Article-Related Findings							
Electrocardiography	No Test Article-Related Findings							
Blood Pressure Determination	No Test Article-Related Findings							
Hematology and Coagulation	No Test Article-Related Findings							
Clinical Chemistry	No Test Article-Related Findings							
Urinalysis	No Test Article-Related Findings							
Organ Weights	No Test Article-Related Findings							
Gross Pathology								
Terminal Sacrifice 1								
Number Evaluated	3	3	3	3	3	3	0	0

Test Article: LEN

Dose (mg/kg/dose)	**0 (Control)**		**10 (Low)**		**30 (Mid)**		**100 (High)**	
Sex:Number of Animals	M: 6	F: 6	M: 6	F: 6	M: 6	F: 6	M: 6[a]	F: 6[a]
Subcutaneous Injection Site B, Raised Area	0	0	0	0	0	1	NA	NA
Subcutaneous Injection Site C, Raised Area	0	0	1	0	1	2	NA	NA
Subcutaneous Injection Site D, Raised Area	0	0	1	0	3	2	NA	NA
Terminal Sacrifice 2								
Number Evaluated	3	3	3	3	3	3	0	0
Subcutaneous Injection Site B, Raised Area	0	0	0	0	2	0	NA	NA
Subcutaneous Injection Site C, Raised Area	0	0	1	0	1	0	NA	NA
Subcutaneous Injection Site D, Raised Area	0	0	1	0	0	2	NA	NA
Histopathology								
Terminal Sacrifice 1								
Number Evaluated	3	3	3	3	3	3	0	0
Subcutaneous Injection Site A, Inflammation, Granulomatous								

Test Article: LEN

Dose (mg/kg/dose)	**0 (Control)**		**10 (Low)**		**30 (Mid)**		**100 (High)**	
Sex:Number of Animals	M: 6	F: 6	M: 6	F: 6	M: 6	F: 6	M: 6[a]	F: 6[a]
Minimal	0	0	0	1	1	0	NA	NA
Slight	0	0	1	1	0	1	NA	NA
Moderate	0	0	0	0	1	0	NA	NA
Subcutaneous Injection Site B, Inflammation, Granulomatous								
Minimal	0	0	0	1	0	1	NA	NA
Slight	0	0	1	2	0	0	NA	NA
Moderate	0	0	0	0	1	1	NA	NA
Subcutaneous Injection Site C, Inflammation, Granulomatous								
Minimal	0	0	0	1	0	0	NA	NA
Slight	0	0	1	1	1	0	NA	NA
Moderate	0	0	1	0	0	3	NA	NA
Marked	0	0	0	0	1	0	NA	NA
Subcutaneous Injection Site D, Inflammation, Granulomatous								
Minimal	0	0	0	0	0	1	NA	NA
Moderate	0	0	2	0	0	0	NA	NA
Marked	0	0	1	0	3	2	NA	NA

Test Article: LEN

Dose (mg/kg/dose)	**0 (Control)**		**10 (Low)**		**30 (Mid)**		**100 (High)**	
Sex:Number of Animals	M: 6	F: 6	M: 6	F: 6	M: 6	F: 6	M: 6[a]	F: 6[a]
Terminal Sacrifice 2								
Number Evaluated	3	3	3	3	3	3	0	0
Subcutaneous Injection Site A, Inflammation, Granulomatous								
Minimal	0	0	0	0	0	1	NA	NA
Slight	0	0	1	0	0	0	NA	NA
Subcutaneous Injection Site B, Inflammation, Granulomatous								
Minimal	0	0	0	1	0	0	NA	NA
Slight	0	0	1	0	0	1	NA	NA
Moderate	0	0	1	0	2	0	NA	NA
Subcutaneous Injection Site C, Inflammation, Granulomatous								
Minimal	0	0	0	1	0	0	NA	NA
Slight	0	0	0	1	0	1	NA	NA
Moderate	0	0	0	0	1	1	NA	NA
Marked	0	0	0	0	1	0	NA	NA

Test Article: LEN

Dose (mg/kg/dose)	**0 (Control)**		**10 (Low)**		**30 (Mid)**		**100 (High)**	
Sex:Number of Animals	M: 6	F: 6	M: 6	F: 6	M: 6	F: 6	M: 6[a]	F: 6[a]
Subcutaneous Injection Site D, Inflammation, Granulomatous								
Slight	0	0	0	1	0	0	NA	NA
Marked	0	0	0	0	1	0	NA	NA
Hepatic Microsomal Enzyme Analysis	No Test Article-Related Findings							

AUC_{0-336} = Area under the concentration-time curve from 0 to 336 hours postdose; BLQ = Below the lower limit of quantitation (<2.00 ng/mL); C_{max} = Maximum observed concentration; F = Female; GLP = Good Laboratory Practices; LEN = lenacapavir (GS-6207); M = Male; NA = Not applicable; w/v = weight/volume

a All males and 2/6 females in Group 4 received the full dose on Study Day 1. Two males in Group 4 received a partial dose on Study Day 15, and no females in Group 4 were dosed after Study Day 1. All males and 2/6 females in Group 4 (the only Group 4 females that were dosed) underwent an early sacrifice on Study Day 22 (males) or Study Day 15 (females) because the high dose exceeded the maximum tolerable dose volume. The remaining four females in Group 4 were returned to the stock colony.

b Females were sacrificed on Study Day 15; therefore, the 336-hour samples are from the unscheduled collection (approximately 333 hours).

c Data presented as number of observations (number of animals affected)· If observation was observed multiple times on a given day on the same animal it was only counted once.

7.5. 9-Month Dog

Test Article: LEN

Report Title: A 9 Month (Once Monthly) Subcutaneous Injection Toxicity and Toxicokinetic Study with 3 Formulations of GS-6207 in Beagle Dogs		**Gilead Study No. TX-200-2017** **CRO Study No. 8375767**
Species / Strain: Dog / Beagle	**Duration of Dosing:** 9 months[a]	**Location in CTD:** 4.2.3.2
Initial Age: 9 to 11 months	**Duration of Postdose:** NA	**GLP Compliance:** Yes
Date of First Dose: [redacted] 20[redacted] (males); [redacted] 20[redacted] (females)	**Method of Administration:** subcutaneous injection in the dorsal thoracic region[b]	**Lot Number:** [redacted]; [redacted][c]
Vehicle: 77:10:13 w/w/w polyethylene glycol 200 (PEG 200):ethanol:water (sterile water for injection)		
Special Features: dermal observations		
NOAEL: Not evaluated[d]		

Monthly Dose (mg/kg/dose)	**0 (Vehicle Control, 0 mol-eq NaOH)**		**20 (200 mg/mL, 0 mol-eq NaOH)**		**20 (200 mg/mL, 1 mol-eq NaOH)**		**40 (400 mg/mL, 0 mol-eq NaOH)**	
Gender: Number of Animals	**M: 9**	**F: 9**	**M: 9**	**F: 9**	**M: 9**	**F: 9**	**M: 9**	**F: 9**
Toxicokinetics: $AUC_{0\text{-}672}$ (ng·h/mL)								
Day 1	NC	NC	50300	64100	108000	143000	67800	65100
Day 29	NC	NC	122000	129000	262000	232000	176000	187000
Day 58[f]	NC	NC	150000	148000	217000	226000	185000	224000
Day 85	NC	NC	215000	175000	266000	292000	246000	263000
Day 113	NC	NC	219000	185000	223000	185000	269000	332000
Day 141	NC	NC	246000	191000	280000	314000	330000	367000
Day 169	NC	NC	265000	183000	274000	300000	367000	417000
Day 197	NC	NC	311000	167000	276000	309000	355000	335000
Day 225	NC	NC	312000	221000	301000	254000	363000	340000

Test Article: LEN

Monthly Dose (mg/kg/dose)	**0 (Vehicle Control, 0 mol-eq NaOH)**		**20 (200 mg/mL, 0 mol-eq NaOH)**		**20 (200 mg/mL, 1 mol-eq NaOH)**		**40 (400 mg/mL, 0 mol-eq NaOH)**	
Gender: Number of Animals	**M: 9**	**F: 9**	**M: 9**	**F: 9**	**M: 9**	**F: 9**	**M: 9**	**F: 9**
Toxicokinetics: C_{max} (ng/mL)								
Day 1	BLQ	BLQ	110	149	294	357	180	171
Day 29	BLQ	BLQ	218	257	494	445	322	362
Day 58	BLQ	BLQ	295	282	408	464	319	452
Day 85	BLQ	BLQ	383	311	479	558	434	458
Day 113	BLQ	BLQ	404	332	456	390	487	662
Day 141	BLQ	BLQ	413	327	467	577	624	634
Day 169	BLQ	BLQ	416	316	492	581	607	719
Day 197	BLQ	BLQ	511	294	472	625	612	589
Day 225	BLQ	BLQ	571	416	580	530	723	592

Test Article: LEN

Test Article: GS-6207

Monthly Dose (mg/kg/dose)	**0 (Vehicle Control, 0 mol-eq NaOH)**		**20 (200 mg/mL, 0 mol-eq NaOH)**		**20 (200 mg/mL, 1 mol-eq NaOH)**		**40 (400 mg/mL, 0 mol-eq NaOH)**	
Gender: Number of Animals	**M: 9**	**F: 9**	**M: 9**	**F: 9**	**M: 9**	**F: 9**	**M: 9**	**F: 9**
Died or sacrificed moribund	0	0	0	0	0	0	0	0
Body Weight	No GS-6207 related findings.							
Food Consumption	No GS-6207 related findings.							
Dermal Observations	GS-6207-related dose site observations of edema and erythema were noted for animals administered GS-6207[e]. Dose site observations of very slight to severe edema were noted at the dose sites starting 24 hours postdose. Animals administered 40 mg/kg/dose had observations of edema longer after dosing than those animals administered 20 mg/kg/dose. Dose site observations of very slight to severe erythema were noted with a lower incidence than edema, and they tended to resolve sooner than edema.							
Clinical Observations								
Vocalization after dosing	0	1	0	1	1	2	0	0
Vocalization during dosing	2	0	1	1	2	3	4	0
Struggling during dosing	0	0	0	0	0	1	1	0
Barrel rolling	1	0	2	1	2	3	1	2
Circling, left	2	0	0	0	0	0	0	0
Excessive licking	1	0	1	0	1	0	0	0
Excessive licking at the dose site	2	2	2	3	3	3	2	2
Scratching at the dose site	9	6	7	5	5	8	4	4
Snapping/biting at the dose site	3	8	6	6	7	8	4	6
Raised area at the dose site	1	6	2	7	5	3	9	6
Ophthalmoscopy	No GS-6207 related findings.							
Hematology	No GS-6207 related findings.							
Coagulation	No GS-6207 related findings.							

Test Article: LEN

Monthly Dose (mg/kg/dose)	**0 (Vehicle Control, 0 mol-eq NaOH)**		**20 (200 mg/mL, 0 mol-eq NaOH)**		**20 (200 mg/mL, 1 mol-eq NaOH)**		**40 (400 mg/mL, 0 mol-eq NaOH)**	
Gender: Number of Animals	**M: 9**	**F: 9**	**M: 9**	**F: 9**	**M: 9**	**F: 9**	**M: 9**	**F: 9**
Serum Chemistry	No GS-6207 related findings.							
Urinalysis	No GS-6207 related findings.							
Organ Weights	No GS-6207 related findings.							
Gross Pathology - Interim Sacrifice 1								
No. Evaluated	3	3	3	3	3	3	3	3
Subcutaneous Injection Site A								
Thickened	0	0	2	1	2	0	1	2
Discolored	0	0	0	1	1	0	0	2
Subcutaneous Injection Site B								
Thickened	0	0	2	0	1	1	1	3
Discolored	0	0	1	1	0	2	0	2
Scab	0	0	0	0	0	1	0	0
Subcutaneous Injection Site C								
Thickened	0	0	3	1	2	2	3	2
Discolored	0	0	1	2	1	2	1	2
Subcutaneous Injection Site D								
Thickened	0	0	3	1	3	2	3	2
Discolored	0	0	1	2	1	2	1	2
Gross Pathology - Interim Sacrifice 2								
No. Evaluated	3	3	3	3	3	3	3	3
Subcutaneous Injection Site A								
Thickened	0	0	3	0	1	0	1	0

Test Article: LEN

Monthly Dose (mg/kg/dose)	0 (Vehicle Control, 0 mol-eq NaOH)		20 (200 mg/mL, 0 mol-eq NaOH)		20 (200 mg/mL, 1 mol-eq NaOH)		40 (400 mg/mL, 0 mol-eq NaOH)	
Gender: Number of Animals	**M: 9**	**F: 9**	**M: 9**	**F: 9**	**M: 9**	**F: 9**	**M: 9**	**F: 9**
Subcutaneous Injection Site B								
Thickened	0	0	3	3	0	3	3	3
Subcutaneous Injection Site C								
Thickened	0	0	3	3	2	3	3	3
Subcutaneous Injection Site D								
Thickened	0	0	2	3	2	3	3	3
Subcutaneous Injection Site E								
Thickened	0	0	3	3	1	3	3	3
Subcutaneous Injection Site F								
Thickened	0	0	3	3	3	3	3	3
Subcutaneous Injection Site G								
Thickened	0	0	3	3	3	3	3	3
Gross Pathology - Terminal Sacrifice								
No. Evaluated	3	3	3	3	3	3	3	3
Subcutaneous Injection Site A								
Thickened	0	0	2	1	1	0	0	3
Subcutaneous Injection Site B								
Thickened	0	0	3	3	2	3	3	3
Subcutaneous Injection Site C								
Thickened	0	0	3	1	3	3	3	3
Subcutaneous Injection Site D								
Thickened	0	0	3	2	3	3	3	3
Subcutaneous Injection Site E								
Thickened	0	0	3	2	3	2	3	3

Test Article: LEN

Monthly Dose (mg/kg/dose)	0 (Vehicle Control, 0 mol-eq NaOH)		20 (200 mg/mL, 0 mol-eq NaOH)		20 (200 mg/mL, 1 mol-eq NaOH)		40 (400 mg/mL, 0 mol-eq NaOH)	
Gender: Number of Animals	**M: 9**	**F: 9**	**M: 9**	**F: 9**	**M: 9**	**F: 9**	**M: 9**	**F: 9**
Subcutaneous Injection Site F								
Thickened	0	0	1	2	3	2	3	3
Subcutaneous Injection Site G								
Thickened	0	0	2	2	3	2	3	3
Subcutaneous Injection Site H								
Thickened	0	0	3	2	3	3	3	3
Subcutaneous Injection Site I								
Thickened	0	0	3	3	3	3	3	3
Subcutaneous Injection Site J								
Thickened	0	0	3	3	3	3	3	3
Histopathology - Interim Sacrifice 1								
No. Evaluated	3	3	3	3	3	3	3	3
Subcutaneous Injection Site, A								
Inflammation, granulomatous								
Minimal	0	0	1	1	0	0	0	0
Slight	0	0	1	0	1	0	0	0
Moderate	0	0	1	1	0	0	2	1
Marked	0	0	0	0	0	0	0	1

Test Article: LEN

Monthly Dose (mg/kg/dose)	0 (Vehicle Control, 0 mol-eq NaOH)		20 (200 mg/mL, 0 mol-eq NaOH)		20 (200 mg/mL, 1 mol-eq NaOH)		40 (400 mg/mL, 0 mol-eq NaOH)	
Gender: Number of Animals	**M: 9**	**F: 9**	**M: 9**	**F: 9**	**M: 9**	**F: 9**	**M: 9**	**F: 9**
Subcutaneous Injection Site, B								
Inflammation, granulomatous								
Slight	0	0	1	0	1	1	0	0
Moderate	0	0	1	0	0	0	0	1
Marked	0	0	0	0	0	0	2	1
Necrosis								
Minimal	0	0	0	0	0	0	1	1
Slight	0	0	0	0	0	0	1	0
Subcutaneous Injection Site, C								
Inflammation, granulomatous								
Minimal	0	0	0	0	1	0	0	0
Slight	0	0	1	2	0	0	0	0
Moderate	0	0	2	0	0	1	1	0
Marked	0	0	0	0	1	1	1	2
Necrosis								
Minimal	0	0	2	0	0	1	0	0
Slight	0	0	0	0	1	1	0	1
Moderate	0	0	0	0	0	0	1	0
Erosion								
Minimal	0	0	0	0	0	1	0	0
Hemorrhage								
Minimal	0	0	0	0	1	0	0	0

Test Article: LEN

Monthly Dose (mg/kg/dose)	0 (Vehicle Control, 0 mol-eq NaOH)		20 (200 mg/mL, 0 mol-eq NaOH)		20 (200 mg/mL, 1 mol-eq NaOH)		40 (400 mg/mL, 0 mol-eq NaOH)	
Gender: Number of Animals	**M: 9**	**F: 9**	**M: 9**	**F: 9**	**M: 9**	**F: 9**	**M: 9**	**F: 9**
Subcutaneous Injection Site, D								
Necrosis								
Slight	0	0	1	1	1	0	0	0
Moderate	0	0	2	1	2	1	0	1
Marked	0	0	0	0	0	2	2	1
Inflammation, mixed cell								
Minimal	0	1	0	1	0	0	0	1
Slight	0	0	3	1	3	1	2	0
Moderate	0	0	0	0	0	2	0	1
Ulcer								
Slight	0	0	0	0	0	1	0	0
Moderate	0	0	0	0	0	0	1	0
Hemorrhage								
Minimal	0	0	0	1	0	1	1	1
Slight	0	0	0	0	0	1	0	0
Erosion								
Minimal	0	0	0	0	0	1	0	0

Test Article: LEN

Monthly Dose (mg/kg/dose)	0 (Vehicle Control, 0 mol-eq NaOH)		20 (200 mg/mL, 0 mol-eq NaOH)		20 (200 mg/mL, 1 mol-eq NaOH)		40 (400 mg/mL, 0 mol-eq NaOH)	
Gender: Number of Animals	**M: 9**	**F: 9**	**M: 9**	**F: 9**	**M: 9**	**F: 9**	**M: 9**	**F: 9**
Histopathology - Interim Sacrifice 2								
No. Evaluated	3	3	3	3	3	3	3	3
Subcutaneous Injection Site, A								
Inflammation, granulomatous								
Minimal	0	0	2	0	0	0	0	0
Slight	0	0	1	0	1	0	1	0
Infiltrate, macrophage								
Minimal	0	0	0	0	0	0	1	0
Subcutaneous Injection Site, B								
Inflammation, granulomatous								
Minimal	0	0	2	3	0	2	0	0
Slight	0	0	1	0	0	0	0	2
Moderate	0	0	0	0	0	0	2	0
Marked	0	0	0	0	0	0	1	1
Necrosis								
Minimal	0	0	0	0	0	0	0	1
Slight	0	0	0	0	0	0	1	0
Infiltrate, macrophage								
Minimal	0	0	0	0	1	0	0	0
Slight	0	0	0	0	0	1	0	0

Test Article: LEN

Monthly Dose (mg/kg/dose)	0 (Vehicle Control, 0 mol-eq NaOH)		20 (200 mg/mL, 0 mol-eq NaOH)		20 (200 mg/mL, 1 mol-eq NaOH)		40 (400 mg/mL, 0 mol-eq NaOH)	
Gender: Number of Animals	**M: 9**	**F: 9**	**M: 9**	**F: 9**	**M: 9**	**F: 9**	**M: 9**	**F: 9**
Subcutaneous Injection Site, C								
Inflammation, granulomatous								
Minimal	0	0	2	1	2	3	0	0
Slight	0	0	1	1	1	0	1	2
Moderate	0	0	0	1	0	0	2	1
Subcutaneous Injection Site, D								
Inflammation, granulomatous								
Minimal	0	0	1	1	2	3	1	0
Slight	0	0	1	2	1	0	0	1
Moderate	0	0	0	0	0	0	2	2
Subcutaneous Injection Site, E								
Inflammation, granulomatous								
Slight	0	0	3	2	1	3	0	2
Moderate	0	0	0	1	1	0	2	1
Marked	0	0	0	0	0	0	1	0
Necrosis								
Minimal	0	0	1	2	1	1	0	0
Slight	0	0	0	0	0	0	1	0
Inflammation, mixed cell								
Minimal	0	0	1	0	0	0	0	0

Test Article: LEN

Monthly Dose (mg/kg/dose)	0 (Vehicle Control, 0 mol-eq NaOH)		20 (200 mg/mL, 0 mol-eq NaOH)		20 (200 mg/mL, 1 mol-eq NaOH)		40 (400 mg/mL, 0 mol-eq NaOH)	
Gender: Number of Animals	**M: 9**	**F: 9**	**M: 9**	**F: 9**	**M: 9**	**F: 9**	**M: 9**	**F: 9**
Subcutaneous Injection Site, F								
Inflammation, granulomatous								
Slight	0	0	2	2	0	1	0	1
Moderate	0	0	1	1	2	2	1	1
Marked	0	0	0	0	1	0	2	1
Necrosis								
Minimal	0	0	1	1	1	2	1	1
Slight	0	0	0	1	0	0	0	1
Inflammation, mixed cell								
Minimal	0	0	0	1	0	0	0	0
Subcutaneous Injection Site, G								
Necrosis								
Minimal	1	0	1	0	1	0	0	0
Slight	0	0	0	3	1	2	1	1
Moderate	0	0	2	0	1	1	2	2
Inflammation, mixed cell								
Minimal	1	1	1	3	3	3	2	1
Slight	0	0	1	0	0	0	1	2
Moderate	1	0	1	0	0	0	0	0

Test Article: LEN

Monthly Dose (mg/kg/dose)	0 (Vehicle Control, 0 mol-eq NaOH)		20 (200 mg/mL, 0 mol-eq NaOH)		20 (200 mg/mL, 1 mol-eq NaOH)		40 (400 mg/mL, 0 mol-eq NaOH)	
Gender: Number of Animals	**M: 9**	**F: 9**	**M: 9**	**F: 9**	**M: 9**	**F: 9**	**M: 9**	**F: 9**
Histopathology - Terminal Sacrifice								
No. Evaluated	3	3	3	3	3	3	3	3
Subcutaneous Injection Site, A								
Inflammation, granulomatous								
Minimal	0	0	1	0	0	0	0	0
Slight	0	0	0	1	0	0	0	2
Moderate	0	0	0	0	0	0	0	1
Necrosis								
Minimal	0	0	0	1	0	0	0	0
Infiltrate, macrophage								
Minimal	0	0	0	0	1	0	0	0
Subcutaneous Injection Site, B								
Inflammation, granulomatous								
Minimal	0	0	1	1	1	0	0	0
Slight	0	0	1	2	0	0	1	1
Moderate	0	0	0	0	0	0	2	0
Necrosis								
Minimal	0	0	0	0	0	0	1	0
Slight	0	0	0	1	0	1	0	0
Moderate	0	0	0	0	0	0	1	0
Infiltrate, macrophage								
Minimal	0	0	0	0	1	1	0	0
Slight	0	0	0	0	0	1	0	0

Test Article: LEN

Monthly Dose (mg/kg/dose)	0 (Vehicle Control, 0 mol-eq NaOH)		20 (200 mg/mL, 0 mol-eq NaOH)		20 (200 mg/mL, 1 mol-eq NaOH)		40 (400 mg/mL, 0 mol-eq NaOH)	
Gender: Number of Animals	**M: 9**	**F: 9**	**M: 9**	**F: 9**	**M: 9**	**F: 9**	**M: 9**	**F: 9**
Subcutaneous Injection Site, C								
Inflammation, granulomatous								
Minimal	0	0	2	0	1	2	0	0
Slight	0	0	1	1	0	0	0	2
Moderate	0	0	0	0	0	0	2	1
Marked	0	0	0	0	0	0	1	0
Infiltrate, macrophage								
Minimal	0	0	0	0	0	1	0	0
Subcutaneous Injection Site, D								
Inflammation, granulomatous								
Minimal	0	0	0	1	1	1	1	1
Slight	0	0	2	1	0	0	0	2
Moderate	0	0	0	0	0	0	2	0
Necrosis								
Minimal	0	0	0	0	0	0	1	1
Slight	0	0	0	0	0	0	1	0
Infiltrate, macrophage								
Minimal	0	0	0	0	0	2	0	0
Subcutaneous Injection Site, E								
Inflammation, granulomatous								
Minimal	0	0	2	0	3	1	0	0
Slight	0	0	0	1	0	0	0	1
Moderate	0	0	1	1	0	0	2	2
Marked	0	0	0	0	0	0	1	0

Test Article: LEN

Monthly Dose (mg/kg/dose)	0 (Vehicle Control, 0 mol-eq NaOH)		20 (200 mg/mL, 0 mol-eq NaOH)		20 (200 mg/mL, 1 mol-eq NaOH)		40 (400 mg/mL, 0 mol-eq NaOH)	
Gender: Number of Animals	**M: 9**	**F: 9**	**M: 9**	**F: 9**	**M: 9**	**F: 9**	**M: 9**	**F: 9**
Necrosis								
Minimal	0	0	0	0	1	0	0	1
Slight	0	0	0	1	0	0	2	0
Subcutaneous Injection Site, F								
Inflammation, granulomatous								
Minimal	0	0	1	0	1	1	0	1
Slight	0	0	0	2	1	0	1	0
Moderate	0	0	1	0	0	0	2	2
Necrosis								
Minimal	0	0	0	1	1	0	0	1
Slight	0	0	0	0	0	0	1	0
Infiltrate, macrophage								
Minimal	0	0	0	0	1	0	0	0
Subcutaneous Injection Site, G								
Inflammation, granulomatous								
Minimal	0	0	0	0	0	1	0	0
Slight	0	0	1	2	2	2	0	1
Moderate	0	0	1	0	1	0	2	1
Marked	0	0	0	0	0	0	1	1
Necrosis								
Minimal	0	0	0	0	1	0	1	1
Slight	0	0	0	1	0	0	0	1

Test Article: LEN

Monthly Dose (mg/kg/dose)	0 (Vehicle Control, 0 mol-eq NaOH)		20 (200 mg/mL, 0 mol-eq NaOH)		20 (200 mg/mL, 1 mol-eq NaOH)		40 (400 mg/mL, 0 mol-eq NaOH)	
Gender: Number of Animals	**M: 9**	**F: 9**	**M: 9**	**F: 9**	**M: 9**	**F: 9**	**M: 9**	**F: 9**
Subcutaneous Injection Site, H								
Inflammation, granulomatous								
Minimal	0	0	1	0	2	0	0	0
Slight	0	0	0	0	0	3	0	0
Moderate	0	0	1	2	0	0	2	2
Marked	0	0	0	0	0	0	1	1
Necrosis								
Minimal	0	0	0	0	0	0	1	1
Slight	0	0	0	1	0	0	0	0
Infiltrate, macrophage								
Minimal	0	1	0	0	0	0	0	0
Subcutaneous Injection Site, I								
Inflammation, granulomatous								
Minimal	0	0	0	1	1	0	0	0
Moderate	0	0	3	2	2	2	0	2
Marked	0	0	0	0	0	1	3	1
Necrosis								
Minimal	0	0	0	1	1	1	1	0
Slight	0	0	0	1	0	0	1	0
Inflammation, mixed cell								
Minimal	0	1	1	1	0	0	0	0

Test Article: LEN

Monthly Dose (mg/kg/dose)	0 (Vehicle Control, 0 mol-eq NaOH)		20 (200 mg/mL, 0 mol-eq NaOH)		20 (200 mg/mL, 1 mol-eq NaOH)		40 (400 mg/mL, 0 mol-eq NaOH)	
Gender: Number of Animals	**M: 9**	**F: 9**	**M: 9**	**F: 9**	**M: 9**	**F: 9**	**M: 9**	**F: 9**
Subcutaneous Injection Site, J								
Necrosis								
Minimal	0	0	1	0	0	0	0	0
Slight	0	0	2	2	2	0	2	1
Moderate	0	0	0	1	1	3	1	2
Inflammation, mixed cell								
Minimal	0	2	3	0	2	1	2	2
Slight	0	0	0	3	0	2	1	1
Moderate	0	0	0	0	1	0	0	0

$AUC0\text{-}_{672}$ = Area under the concentration-time curve from hour 0 to hour 672; BLQ = Below the lower limit of quantitation (< 2.00 ng/mL); C_{max} = Maximum observed concentration; F = Female; LEN = lenacapavir (GS-6207); M = Male; NA = Not Applicable; NC = Not Calculated; NOAEL = No-Observed-Adverse-Effect Level; TK = Toxicokinetic.

a Dose formulations were administered as a single dose on Days 1, 29, 58, 85, 113, 141, 169, 197, 225, and 253.

b Dose formulations were administered by subcutaneous injection in the dorsal thoracic region at a dose volume of 0.1 mL/kg. Starting on Day 29, all remaining subcutaneous injections were administered in a unique site for each injection (10 total dose sites).

c Lot [redacted] was used for dosing on Days 1 through 58. Lot [redacted] was used for dosing on Days 85 through 253.

d No systemic toxicity was noted and GS-6207 was well tolerated in male and female beagle dogs when administered as 10 monthly subcutaneous injections of 20 mg/kg/dose with or without NaOH or 40 mg/kg/dose without NaOH in the vehicle.

e Edema and erythema were considered not adverse because they did not impact the health and wellbeing of animals.

f $AUC_{0\text{-}672}$ values are equivalent to $AUC_{0\text{-}648}$ due to administration time of next dose.

7.6. 9-Month Dog

Test Article: LEN

Report Title: A 9 month (Once Monthly) Subcutaneous Injection Toxicity and Toxicokinetic Study of GS-6207-02 in Beagle Dogs with a 2 and 13-Week Observation Period		**Gilead Study No.** TX-200-2047 **CRO Study No.** 8419595
Species / Strain: Dog/ Beagle	**Duration of Dosing:** 37 weeks[a]	**Location in CTD:** 4.2.3.2
Initial Age: 9 to 10 months	**Duration of Postdose:** 2 or 13 weeks	**GLP Compliance:** Yes
Date of First Dose: [redacted] 20[redacted]	**Method of Administration:** Subcutaneous Injection	**Lot Number:** [redacted]
Vehicle Control Article: 68.17% (w/w) polyethylene glycol 300 (PEG 300)/31.83% (w/w) sterile water for injection		
Special Features: Dermal observations		
NOAEL: Not established		

Dose (mg/kg/dose)[a]	**0 (Control)**		**130 (Low)**		**411 (High)**	
Gender: Number of Animals	**M: 6**	**F: 6**	**M: 4**	**F: 4**	**M: 6**	**F: 6**
Toxicokinetics: $AUC_{0\text{-}336h}$ (h*ng/mL)						
Day 1	BLQ	BLQ	279000	395000	1110000	882000
Day 57	BLQ	BLQ	792000	1240000	2490000	2030000
Day 253	BLQ	BLQ	NA	NA	NA	NA
Toxicokinetics: $AUC_{0\text{-}672h}$ (h*ng/mL)						
Day 1	BLQ	BLQ	814000	1210000	2480000	2290000
Day 57	BLQ	BLQ	1630000	2440000	NA	NA
Day 225	BLQ	BLQ	2850000	3540000	NA	NA
Day 253	BLQ	BLQ	NA	NA	NA	NA
Toxicokinetics: C_{max} (ng/mL)						
Day 1	BLQ	BLQ	1910	3240	5090	4760

Test Article: LEN

Report Title: A 9 month (Once Monthly) Subcutaneous Injection Toxicity and Toxicokinetic Study of GS-6207-02 in Beagle Dogs with a 2 and 13-Week Observation Period		**Gilead Study No.** TX-200-2047 **CRO Study No.** 8419595
Species / Strain: Dog/ Beagle	**Duration of Dosing:** 37 weeks[a]	**Location in CTD:** 4.2.3.2
Initial Age: 9 to 10 months	**Duration of Postdose:** 2 or 13 weeks	**GLP Compliance:** Yes
Date of First Dose: [redacted] 20[redacted]	**Method of Administration:** Subcutaneous Injection	**Lot Number:** [redacted]
Vehicle Control Article: 68.17% (w/w) polyethylene glycol 300 (PEG 300)/31.83% (w/w) sterile water for injection		
Special Features: Dermal observations		
NOAEL: Not established		

Test Article: LEN

Dose (mg/kg/dose)[a]	**0 (Control)**		**130 (Low)**		**411 (High)**	
Gender: Number of Animals	**M: 6**	**F: 6**	**M: 4**	**F: 4**	**M: 6**	**F: 6**
Day 57	BLQ	BLQ	2670	4120	9950	8620
Day 225	BLQ	BLQ	4640	6320	NA	NA
Day 253	BLQ	BLQ	4520	5210	NA	NA
Noteworthy Findings						
Died or sacrificed moribund	0	0	0	0	1[b]	2[b]
Body Weight	No-GS-6207-02-related findings					
Food Consumption	No-GS-6207-02-related findings					
Clinical Observations Immediately Postdose						
Dosing Day 1						
Aggression to handler	0	0	1	0	0	0
Vocalization	0	0	2	0	0	0
Writhing	0	0	0	0	0	0

Test Article: LEN

Report Title: A 9 month (Once Monthly) Subcutaneous Injection Toxicity and Toxicokinetic Study of GS-6207-02 in Beagle Dogs with a 2 and 13-Week Observation Period					**Gilead Study No.** TX-200-2047 **CRO Study No.** 8419595
Species / Strain: Dog/ Beagle		**Duration of Dosing:** 37 weeks[a]			**Location in CTD:** 4.2.3.2
Initial Age: 9 to 10 months		**Duration of Postdose:** 2 or 13 weeks			**GLP Compliance:** Yes
Date of First Dose: [redacted] 20[redacted]		**Method of Administration:** Subcutaneous Injection			**Lot Number:** [redacted]
Vehicle Control Article: 68.17% (w/w) polyethylene glycol 300 (PEG 300)/31.83% (w/w) sterile water for injection					
Special Features: Dermal observations					
NOAEL: Not established					
Circling	0	0	1	0	0	0
Excessive biting	0	0	0	0	0	0
Dosing Day 29						
Aggression to handler	1	0	1	NA	NA	NA
Vocalization	0	2	1	NA	NA	NA
Writhing	0	0	1	NA	NA	NA
Excessive biting	0	0	1	NA	NA	NA
Dosing Day 32						
Vocalization	NA	NA	0	0	2	0
Clinical Observations						
Swollen Area, Abdomen, Right	0	0	2	1	0	0
Swollen Area, Dorsal Cervical	0	0	0	0	3	1
Swollen Area, Dorsal Cervical, Left	0	0	0	0	2	1
Swollen Area, Dorsal Thoracic, Left	0	0	0	1	0	0

Test Article: LEN

Report Title: A 9 month (Once Monthly) Subcutaneous Injection Toxicity and Toxicokinetic Study of GS-6207-02 in Beagle Dogs with a 2 and 13-Week Observation Period		**Gilead Study No.** TX-200-2047 **CRO Study No.** 8419595
Species / Strain: Dog/ Beagle	**Duration of Dosing:** 37 weeks[a]	**Location in CTD:** 4.2.3.2
Initial Age: 9 to 10 months	**Duration of Postdose:** 2 or 13 weeks	**GLP Compliance:** Yes
Date of First Dose: [redacted] 20[redacted]	**Method of Administration:** Subcutaneous Injection	**Lot Number:** [redacted]
Vehicle Control Article: 68.17% (w/w) polyethylene glycol 300 (PEG 300)/31.83% (w/w) sterile water for injection		
Special Features: Dermal observations		
NOAEL: Not established		

Swollen Area, Dose Site A	0	0	1	1	4	4
Swollen Area, Dose Site B	0	0	0	3	3	5
Swollen Area, Dose Site C	0	0	1	3	1	2
Swollen Area, Dose Site D	0	0	2	1	0	1
Swollen Area, Dose Site E	0	0	2	3	0	0
Swollen Area, Dose Site F	0	0	0	1	0	0
Swollen Area, Shoulder, Left	0	0	0	0	2	1
Palpable Mass, Medium, Dorsal Cervical, Left	0	0	0	0	1	3
Palpable Mass, Medium, Dorsal Thoracic, Left	0	0	0	0	1	0
Palpable Mass, Small, Dorsal Thoracic, Left	0	0	0	0	1	1

Test Article: LEN

Report Title: A 9 month (Once Monthly) Subcutaneous Injection Toxicity and Toxicokinetic Study of GS-6207-02 in Beagle Dogs with a 2 and 13-Week Observation Period		**Gilead Study No.** TX-200-2047 **CRO Study No.** 8419595
Species / Strain: Dog/ Beagle	**Duration of Dosing:** 37 weeks[a]	**Location in CTD:** 4.2.3.2
Initial Age: 9 to 10 months	**Duration of Postdose:** 2 or 13 weeks	**GLP Compliance:** Yes
Date of First Dose: [redacted] 20[redacted]	**Method of Administration:** Subcutaneous Injection	**Lot Number:** [redacted]
Vehicle Control Article: 68.17% (w/w) polyethylene glycol 300 (PEG 300)/31.83% (w/w) sterile water for injection		
Special Features: Dermal observations		
NOAEL: Not established		

Dermal Observations	GS-6207-02-related dermal observations of edema and erythema were noted for animals administered 130 or 411 mg/kg/dose GS-6207-02. Dermal observations of very slight to severe edema were noted at the dose sites starting 24 hours postdose. The incidences of edema at Dose Sites A and B were substantially higher compared to the incidence noted at dose sites dosed post-anesthetization. Dermal observations of very slight to severe erythema were noted at an incidence that was less than that noted for edema, and the erythema observations tended to resolve sooner than edema. Edema and erythema were considered not adverse because they did not impact the health and wellbeing of animals.					
Ophthalmic Observations	No-GS-6207-02-related findings					
Hematology						
White Blood Cell Count (E3/uL)						
Day 60	9.09	9.06	8.77	9.88	8.95	7.98
Day 73	9.03	8.07	9.31	7.68	10.30	11.50
Neutrophil Count (E3/uL)						
Day 60	5.16	5.62	5.71	6.31	5.36	4.81
Day 73	5.02	4.76	6.07	4.55	6.74	7.93

Test Article: LEN

Report Title: A 9 month (Once Monthly) Subcutaneous Injection Toxicity and Toxicokinetic Study of GS-6207-02 in Beagle Dogs with a 2 and 13-Week Observation Period		**Gilead Study No.** TX-200-2047 **CRO Study No.** 8419595
Species / Strain: Dog/ Beagle	**Duration of Dosing:** 37 weeks[a]	**Location in CTD:** 4.2.3.2
Initial Age: 9 to 10 months	**Duration of Postdose:** 2 or 13 weeks	**GLP Compliance:** Yes
Date of First Dose: [redacted] 20[redacted]	**Method of Administration:** Subcutaneous Injection	**Lot Number:** [redacted]
Vehicle Control Article: 68.17% (w/w) polyethylene glycol 300 (PEG 300)/31.83% (w/w) sterile water for injection		
Special Features: Dermal observations		
NOAEL: Not established		

Coagulation						
Fibrinogen (mg/dL)						
Day 60	232	204	286	324	325*	283
Day 73	251	203	194	212	275	283
Day 87	266	209	352	338*	285	275
Day 115	254	205	344*	286	205	254
Clinical Chemistry						
Total Bilirubin (mg/dL)						
Day 60	<0.1	<0.1	<0.1	<0.1	1.5	0.3
Day 73	<0.1	<0.1	<0.1	<0.1	0.4	0.6
Day 87	<0.1	<0.1	<0.1	<0.1	<0.3	0.2
Day 115	<0.1	<0.1	<0.1	<0.1	<0.1	0.1

Test Article: LEN

Report Title: A 9 month (Once Monthly) Subcutaneous Injection Toxicity and Toxicokinetic Study of GS-6207-02 in Beagle Dogs with a 2 and 13-Week Observation Period		**Gilead Study No.** TX-200-2047 **CRO Study No.** 8419595
Species / Strain: Dog/ Beagle	**Duration of Dosing:** 37 weeks[a]	**Location in CTD:** 4.2.3.2
Initial Age: 9 to 10 months	**Duration of Postdose:** 2 or 13 weeks	**GLP Compliance:** Yes
Date of First Dose: [redacted] 20[redacted]	**Method of Administration:** Subcutaneous Injection	**Lot Number:** [redacted]
Vehicle Control Article: 68.17% (w/w) polyethylene glycol 300 (PEG 300)/31.83% (w/w) sterile water for injection		
Special Features: Dermal observations		
NOAEL: Not established		

GGT (U/L)						
Day 60	<3	<3	<3	<3	35	<8
Day 73	<3	<3	<3	<3	<10	<10
Day 87	<3	<3	<3	<3	<7	<6
Day 115	<3	<3	<4	<3	<3	5
Day 268	<3	<3	<3	<3	NA	NA
ALP (U/L)						
Day 60	50	47	53	75*	1059*	288*
Day 73	50	42	50	61*	582*	276*
Day 87	57	51	75	94	341*	353
Day 115	53	54	94	119	73	265*
Day 268	35	48	123	96	NA	NA

Test Article: LEN

Report Title: A 9 month (Once Monthly) Subcutaneous Injection Toxicity and Toxicokinetic Study of GS-6207-02 in Beagle Dogs with a 2 and 13-Week Observation Period		**Gilead Study No.** TX-200-2047 **CRO Study No.** 8419595
Species / Strain: Dog/ Beagle	**Duration of Dosing:** 37 weeks[a]	**Location in CTD:** 4.2.3.2
Initial Age: 9 to 10 months	**Duration of Postdose:** 2 or 13 weeks	**GLP Compliance:** Yes
Date of First Dose: [redacted] 20[redacted]	**Method of Administration:** Subcutaneous Injection	**Lot Number:** [redacted]
Vehicle Control Article: 68.17% (w/w) polyethylene glycol 300 (PEG 300)/31.83% (w/w) sterile water for injection		
Special Features: Dermal observations		
NOAEL: Not established		

Alanine Aminotransferase (U/L)						
Day 60	42	28	35	30	494*	168*
Day 73	34	25	32	35*	249*	153*
Day 87	41	33	41	38	133	168*
Day 115	51	44	81	63	36	96
Day 268	30	30	86*	85*	NA	NA
Total Bile Acids (umol/L)						
Day 60	<1	<1	<2	<2	78	8
Day 73	<1	<2	3	<1	23	34
Day 87	<1	<1	3	<3	<3	2
Day 115	<1	<5	3	3	2	3
Day 268	2	<1	6	3	NA	NA
Albumin (g/dL)						
Day 60	3.5	3.4	3.7	3.6	2.6*	3.1*
Day 73	3.6	3.4	3.6	3.7*	2.7*	3.0*

Test Article: LEN

Report Title: A 9 month (Once Monthly) Subcutaneous Injection Toxicity and Toxicokinetic Study of GS-6207-02 in Beagle Dogs with a 2 and 13-Week Observation Period		**Gilead Study No.** TX-200-2047 **CRO Study No.** 8419595
Species / Strain: Dog/ Beagle	**Duration of Dosing:** 37 weeks[a]	**Location in CTD:** 4.2.3.2
Initial Age: 9 to 10 months	**Duration of Postdose:** 2 or 13 weeks	**GLP Compliance:** Yes
Date of First Dose: [redacted] 20[redacted]	**Method of Administration:** Subcutaneous Injection	**Lot Number:** [redacted]
Vehicle Control Article: 68.17% (w/w) polyethylene glycol 300 (PEG 300)/31.83% (w/w) sterile water for injection		
Special Features: Dermal observations		
NOAEL: Not established		

Total Protein (g/dL)						
Day 60	5.5	5.5	5.9	5.5	4.8*	5.0*
Day 73	5.4	5.4	5.7	5.5	4.8*	4.9*
Albumin: Globulin						
Day 60	1.8	1.7	1.7	2.0	1.2*	1.7
Day 73	1.9	1.8	1.9	2.1	1.3*	1.6
Urinalysis						
Day 60						
Discolored Urine, Orange	0	0	0	0	2	0
Urine Bilirubin						
Trace	0	1	0	0	0	0
1+ (30 mg/dL)	0	0	1	0	0	0
2+ (100 mg/dL)	0	1	0	0	1	0
Organ Weights	No-GS-6207-02-related findings					
Gross Pathology						

Test Article: LEN

Report Title: A 9 month (Once Monthly) Subcutaneous Injection Toxicity and Toxicokinetic Study of GS-6207-02 in Beagle Dogs with a 2 and 13-Week Observation Period		**Gilead Study No.** TX-200-2047 **CRO Study No.** 8419595
Species / Strain: Dog/ Beagle	**Duration of Dosing:** 37 weeks[a]	**Location in CTD:** 4.2.3.2
Initial Age: 9 to 10 months	**Duration of Postdose:** 2 or 13 weeks	**GLP Compliance:** Yes
Date of First Dose: [redacted] 20[redacted]	**Method of Administration:** Subcutaneous Injection	**Lot Number:** [redacted]
Vehicle Control Article: 68.17% (w/w) polyethylene glycol 300 (PEG 300)/31.83% (w/w) sterile water for injection		
Special Features: Dermal observations		
NOAEL: Not established		

No. Evaluated	6	6	4	4	6	6
Subcutaneous Site A						
Discolored	0	0	0	0	0	1
Thickened	0	0	1	2	6	4
Subcutaneous Site B						
Discolored	0	0	0	0	1	3
Thickened	0	0	0	1	5	3
Subcutaneous Site C						
Discolored	0	0	0	1	0	2
Thickened	0	0	0	0	5	4
No. Evaluated	6	6	4	4	1	0
Subcutaneous Site D						
Discolored	0	1	0	1	0	NA
Subcutaneous Site E						
Discolored	0	0	1	0	0	NA

Test Article: LEN

Report Title: A 9 month (Once Monthly) Subcutaneous Injection Toxicity and Toxicokinetic Study of GS-6207-02 in Beagle Dogs with a 2 and 13-Week Observation Period		**Gilead Study No.** TX-200-2047 **CRO Study No.** 8419595
Species / Strain: Dog/ Beagle	**Duration of Dosing:** 37 weeks[a]	**Location in CTD:** 4.2.3.2
Initial Age: 9 to 10 months	**Duration of Postdose:** 2 or 13 weeks	**GLP Compliance:** Yes
Date of First Dose: [redacted] 20[redacted]	**Method of Administration:** Subcutaneous Injection	**Lot Number:** [redacted]
Vehicle Control Article: 68.17% (w/w) polyethylene glycol 300 (PEG 300)/31.83% (w/w) sterile water for injection		
Special Features: Dermal observations		
NOAEL: Not established		

Thickened	0	0	1	1	0	NA
Subcutaneous Site F						
Discolored	2	2	0	2	0	NA
No. Evaluated	4	4	4	4	1	0
Subcutaneous Site G						
Thickened	0	0	1	1	0	NA
Subcutaneous Site H						
Thickened	0	0	2	0	0	NA
Subcutaneous Site I						
Discolored	0	1	0	0	0	NA
Thickened	0	0	2	1	0	NA
Subcutaneous Site J						
Discolored	1	0	0	0	0	NA
Thickened	0	1	2	1	0	NA
Histopathology						

Test Article: LEN

Report Title: A 9 month (Once Monthly) Subcutaneous Injection Toxicity and Toxicokinetic Study of GS-6207-02 in Beagle Dogs with a 2 and 13-Week Observation Period					**Gilead Study No.** TX-200-2047 **CRO Study No.** 8419595	
Species / Strain: Dog/ Beagle		**Duration of Dosing:** 37 weeks[a]			**Location in CTD:** 4.2.3.2	
Initial Age: 9 to 10 months		**Duration of Postdose:** 2 or 13 weeks			**GLP Compliance:** Yes	
Date of First Dose: [redacted] 20[redacted]		**Method of Administration:** Subcutaneous Injection			**Lot Number:** [redacted]	
Vehicle Control Article: 68.17% (w/w) polyethylene glycol 300 (PEG 300)/31.83% (w/w) sterile water for injection						
Special Features: Dermal observations						
NOAEL: Not established						
No Evaluated: Interim Sacrifice 1 and Unscheduled – Days 51 to 82	0	0	0	0	4	4
Liver						
Degeneration, Vacuolar, Hepatocyte						
Slight	NA	NA	NA	NA	1	0
Degeneration, Vacuolar, Bile Duct Epthelium						
Minimal	NA	NA	NA	NA	2	0
Pigment Bile						
Minimal	NA	NA	NA	NA	0	3
Slight	NA	NA	NA	NA	1	0
Infiltrate, Mixed Cell						
Minimal	NA	NA	NA	NA	3	3

Test Article: LEN

Report Title: A 9 month (Once Monthly) Subcutaneous Injection Toxicity and Toxicokinetic Study of GS-6207-02 in Beagle Dogs with a 2 and 13-Week Observation Period					**Gilead Study No.** TX-200-2047 **CRO Study No.** 8419595	
Species / Strain: Dog/ Beagle			**Duration of Dosing:** 37 weeks[a]		**Location in CTD:** 4.2.3.2	
Initial Age: 9 to 10 months			**Duration of Postdose:** 2 or 13 weeks		**GLP Compliance:** Yes	
Date of First Dose: [redacted] 20[redacted]			**Method of Administration:** Subcutaneous Injection		**Lot Number:** [redacted]	
Vehicle Control Article: 68.17% (w/w) polyethylene glycol 300 (PEG 300)/31.83% (w/w) sterile water for injection						
Special Features: Dermal observations						
NOAEL: Not established						
Hyperplasia, Oval Cell/Bile Ductule						
Minimal	NA	NA	NA	NA	0	0
Sight	NA	NA	NA	NA	1	4
Moderate	NA	NA	NA	NA	2	0
Gallbladder						
Hyperplasia, Epithelium						
Minimal	NA	NA	NA	NA	3	1
Slight	NA	NA	NA	NA	0	2
Edema, Mucosa						
Minimal	NA	NA	NA	NA	1	3
Increased Secretion, Lumen						
Minimal	NA	NA	NA	NA	1	2
Moderate	NA	NA	NA	NA	1	0

Test Article: LEN

Report Title: A 9 month (Once Monthly) Subcutaneous Injection Toxicity and Toxicokinetic Study of GS-6207-02 in Beagle Dogs with a 2 and 13-Week Observation Period		**Gilead Study No.** TX-200-2047 **CRO Study No.** 8419595
Species / Strain: Dog/ Beagle	**Duration of Dosing:** 37 weeks[a]	**Location in CTD:** 4.2.3.2
Initial Age: 9 to 10 months	**Duration of Postdose:** 2 or 13 weeks	**GLP Compliance:** Yes
Date of First Dose: [redacted] 20[redacted]	**Method of Administration:** Subcutaneous Injection	**Lot Number:** [redacted]
Vehicle Control Article: 68.17% (w/w) polyethylene glycol 300 (PEG 300)/31.83% (w/w) sterile water for injection		
Special Features: Dermal observations		
NOAEL: Not established		

Pigment, Lumen						
Minimal	NA	NA	NA	NA	2	3
Slight	NA	NA	NA	NA	0	0
Moderate	NA	NA	NA	NA	1	0
Kidney						
Degeneration, Tubule						
Minimal	NA	NA	NA	NA	2	0
Dilation, Tubule						
Minimal					1	0
Pigment						
Minimal	NA	NA	NA	NA	3	3

Test Article: LEN

Report Title: A 9 month (Once Monthly) Subcutaneous Injection Toxicity and Toxicokinetic Study of GS-6207-02 in Beagle Dogs with a 2 and 13-Week Observation Period		**Gilead Study No.** TX-200-2047 **CRO Study No.** 8419595
Species / Strain: Dog/ Beagle	**Duration of Dosing:** 37 weeks[a]	**Location in CTD:** 4.2.3.2
Initial Age: 9 to 10 months	**Duration of Postdose:** 2 or 13 weeks	**GLP Compliance:** Yes
Date of First Dose: [redacted] 20[redacted]	**Method of Administration:** Subcutaneous Injection	**Lot Number:** [redacted]
Vehicle Control Article: 68.17% (w/w) polyethylene glycol 300 (PEG 300)/31.83% (w/w) sterile water for injection		
Special Features: Dermal observations		
NOAEL: Not established		

Stomach						
Degeneration/Atrophy, Mucosa						
Minimal	NA	NA	NA	NA	1	1
Slight	NA	NA	NA	NA	0	1
Moderate	NA	NA	NA	NA	1	0
Subcutaneous Site A						
Inflammation, Granulomatous						
Minimal	NA	NA	NA	NA	0	0
Slight	NA	NA	NA	NA	0	1
Moderate	NA	NA	NA	NA	3	1
Marked	NA	NA	NA	NA	1	2
Necrosis, Adipose Tissue						
Minimal	NA	NA	NA	NA	0	2
Slight	NA	NA	NA	NA	1	1
Moderate	NA	NA	NA	NA	1	1

Test Article: LEN

Report Title: A 9 month (Once Monthly) Subcutaneous Injection Toxicity and Toxicokinetic Study of GS-6207-02 in Beagle Dogs with a 2 and 13-Week Observation Period		**Gilead Study No.** TX-200-2047 **CRO Study No.** 8419595
Species / Strain: Dog/ Beagle	**Duration of Dosing:** 37 weeks[a]	**Location in CTD:** 4.2.3.2
Initial Age: 9 to 10 months	**Duration of Postdose:** 2 or 13 weeks	**GLP Compliance:** Yes
Date of First Dose: [redacted] 20[redacted]	**Method of Administration:** Subcutaneous Injection	**Lot Number:** [redacted]
Vehicle Control Article: 68.17% (w/w) polyethylene glycol 300 (PEG 300)/31.83% (w/w) sterile water for injection		
Special Features: Dermal observations		
NOAEL: Not established		

Fibrosis						
Minimal	NA	NA	NA	NA	1	2
Slight	NA	NA	NA	NA	1	2
Subcutaneous Site B						
Inflammation, Granulomatous						
Slight	NA	NA	NA	NA	0	1
Moderate	NA	NA	NA	NA	2	1
Marked	NA	NA	NA	NA	2	2
Necrosis, Adipose Tissue						
Minimal	NA	NA	NA	NA	1	1
Slight	NA	NA	NA	NA	2	0
Moderate	NA	NA	NA	NA	0	2
Fibrosis						
Minimal	NA	NA	NA	NA	3	1
Slight	NA	NA	NA	NA	1	2

Test Article: LEN

Report Title: A 9 month (Once Monthly) Subcutaneous Injection Toxicity and Toxicokinetic Study of GS-6207-02 in Beagle Dogs with a 2 and 13-Week Observation Period		**Gilead Study No.** TX-200-2047 **CRO Study No.** 8419595
Species / Strain: Dog/ Beagle	**Duration of Dosing:** 37 weeks[a]	**Location in CTD:** 4.2.3.2
Initial Age: 9 to 10 months	**Duration of Postdose:** 2 or 13 weeks	**GLP Compliance:** Yes
Date of First Dose: [redacted] 20[redacted]	**Method of Administration:** Subcutaneous Injection	**Lot Number:** [redacted]
Vehicle Control Article: 68.17% (w/w) polyethylene glycol 300 (PEG 300)/31.83% (w/w) sterile water for injection		
Special Features: Dermal observations		
NOAEL: Not established		

No. Evaluated	0	0	0	0	3	4
Subcutaneous Site C						
Inflammation, Granulomatous						
Slight	NA	NA	NA	NA	2	4
Moderate	NA	NA	NA	NA	1	0
Inflammation, Mixed Cell						
Minimal	NA	NA	NA	NA	1	1
Necrosis, Adipose Tissue						
Minimal	NA	NA	NA	NA	0	1
Slight	NA	NA	NA	NA	0	1
Moderate	NA	NA	NA	NA	3	2
Fibrosis						
Minimal	NA	NA	NA	NA	3	4
No. Evaluated: Terminal Sacrifice 1 – Day 143	0	0	0	0	2	2

Test Article: LEN

Report Title: A 9 month (Once Monthly) Subcutaneous Injection Toxicity and Toxicokinetic Study of GS-6207-02 in Beagle Dogs with a 2 and 13-Week Observation Period		**Gilead Study No.** TX-200-2047 **CRO Study No.** 8419595
Species / Strain: Dog/ Beagle	**Duration of Dosing:** 37 weeks[a]	**Location in CTD:** 4.2.3.2
Initial Age: 9 to 10 months	**Duration of Postdose:** 2 or 13 weeks	**GLP Compliance:** Yes
Date of First Dose: [redacted] 20[redacted]	**Method of Administration:** Subcutaneous Injection	**Lot Number:** [redacted]
Vehicle Control Article: 68.17% (w/w) polyethylene glycol 300 (PEG 300)/31.83% (w/w) sterile water for injection		
Special Features: Dermal observations		
NOAEL: Not established		

Liver						
Hyperplasia, Oval Cell/Bile Ductule						
Minimal	NA	NA	NA	NA	2	1
Sight	NA	NA	NA	NA	0	1
Gallbladder						
Hyperplasia, Epithelium						
Minimal	NA	NA	NA	NA	1	0
Increased Secretion, Lumen						
Minimal	NA	NA	NA	NA	1	1
Pigment Lumen						
Minimal	NA	NA	NA	NA	1	0
Slight	NA	NA	NA	NA	0	1
Subcutaneous Site A						
Inflammation, Granulomatous						
Minimal	NA	NA	NA	NA	1	0

Test Article: LEN

Report Title: A 9 month (Once Monthly) Subcutaneous Injection Toxicity and Toxicokinetic Study of GS-6207-02 in Beagle Dogs with a 2 and 13-Week Observation Period		**Gilead Study No.** TX-200-2047 **CRO Study No.** 8419595
Species / Strain: Dog/ Beagle	**Duration of Dosing:** 37 weeks[a]	**Location in CTD:** 4.2.3.2
Initial Age: 9 to 10 months	**Duration of Postdose:** 2 or 13 weeks	**GLP Compliance:** Yes
Date of First Dose: [redacted] 20[redacted]	**Method of Administration:** Subcutaneous Injection	**Lot Number:** [redacted]
Vehicle Control Article: 68.17% (w/w) polyethylene glycol 300 (PEG 300)/31.83% (w/w) sterile water for injection		
Special Features: Dermal observations		
NOAEL: Not established		

Slight	NA	NA	NA	NA	1	0
Moderate	NA	NA	NA	NA	0	1
Marked	NA	NA	NA	NA	0	1
Necrosis, Adipose Tissue						
Slight	NA	NA	NA	NA	1	0
Fibrosis						
Minimal	NA	NA	NA	NA	2	2
Subcutaneous Site C						
Inflammation, Granulomatous						
Minimal	NA	NA	NA	NA	0	1
Slight	NA	NA	NA	NA	1	0
Moderate	NA	NA	NA	NA	1	0
Necrosis, Adipose Tissue						
Slight	NA	NA	NA	NA	1	0

Test Article: LEN

Report Title: A 9 month (Once Monthly) Subcutaneous Injection Toxicity and Toxicokinetic Study of GS-6207-02 in Beagle Dogs with a 2 and 13-Week Observation Period		**Gilead Study No.** TX-200-2047 **CRO Study No.** 8419595
Species / Strain: Dog/ Beagle	**Duration of Dosing:** 37 weeks[a]	**Location in CTD:** 4.2.3.2
Initial Age: 9 to 10 months	**Duration of Postdose:** 2 or 13 weeks	**GLP Compliance:** Yes
Date of First Dose: [redacted] 20[redacted]	**Method of Administration:** Subcutaneous Injection	**Lot Number:** [redacted]
Vehicle Control Article: 68.17% (w/w) polyethylene glycol 300 (PEG 300)/31.83% (w/w) sterile water for injection		
Special Features: Dermal observations		
NOAEL: Not established		

Fibrosis						
Minimal	NA	NA	NA	NA	1	0
No. Evaluated: Terminal Sacrifice 2—Day 268	4	4	4	4	0	0
Liver						
Hyperplasia, Oval Cell, Bile Ductule						
Minimal	0	0	2	2	NA	NA
Slight	0	0	2	2	NA	NA
Fibrosis, Periductular						
Minimal	0	0	1	0	NA	NA
Pigment Macrophages, Increased						
Minimal	0	0	1	1	NA	NA

Test Article: LEN

Report Title: A 9 month (Once Monthly) Subcutaneous Injection Toxicity and Toxicokinetic Study of GS-6207-02 in Beagle Dogs with a 2 and 13-Week Observation Period		**Gilead Study No.** TX-200-2047 **CRO Study No.** 8419595
Species / Strain: Dog/ Beagle	**Duration of Dosing:** 37 weeks[a]	**Location in CTD:** 4.2.3.2
Initial Age: 9 to 10 months	**Duration of Postdose:** 2 or 13 weeks	**GLP Compliance:** Yes
Date of First Dose: [redacted] 20[redacted]	**Method of Administration:** Subcutaneous Injection	**Lot Number:** [redacted]
Vehicle Control Article: 68.17% (w/w) polyethylene glycol 300 (PEG 300)/31.83% (w/w) sterile water for injection		
Special Features: Dermal observations		
NOAEL: Not established		

Gallbladder						
Hyperplasia, Epithelium						
Minimal	0	0	1	1	NA	NA
Increased Secretion, Lumen						
Minimal	0	0	2	1	NA	NA
Slight	0	0	1	1	NA	NA
Pigment, Lumen						
Minimal	0	0	2	2	NA	NA
Slight	0	0	1	0	NA	NA
Subcutaneous Site A						
Inflammation, Granulomatous						
Minimal	0	0	1	2	NA	NA
Moderate	0	0	1	0	NA	NA
Necrosis, Adipose Tissue						
Minimal	0	0	1	0	NA	NA

Test Article: LEN

Report Title: A 9 month (Once Monthly) Subcutaneous Injection Toxicity and Toxicokinetic Study of GS-6207-02 in Beagle Dogs with a 2 and 13-Week Observation Period		**Gilead Study No.** TX-200-2047 **CRO Study No.** 8419595
Species / Strain: Dog/ Beagle	**Duration of Dosing:** 37 weeks[a]	**Location in CTD:** 4.2.3.2
Initial Age: 9 to 10 months	**Duration of Postdose:** 2 or 13 weeks	**GLP Compliance:** Yes
Date of First Dose: [redacted] 20[redacted]	**Method of Administration:** Subcutaneous Injection	**Lot Number:** [redacted]
Vehicle Control Article: 68.17% (w/w) polyethylene glycol 300 (PEG 300)/31.83% (w/w) sterile water for injection		
Special Features: Dermal observations		
NOAEL: Not established		

Fibrosis						
Minimal	0	0	1	0	NA	NA
Pseudocyst						
Present	0	0	1	0	NA	NA
Subcutaneous Site B						
Inflammation, Granulomatous						
Minimal	0	0	1	1	NA	NA
Subcutaneous Site C						
Inflammation, Granulomatous						
Minimal	0	0	1	0	NA	NA
Subcutaneous Site D						
Inflammation, Granulomatous						
Minimal	0	0	1	0	NA	NA

Test Article: LEN

Report Title: A 9 month (Once Monthly) Subcutaneous Injection Toxicity and Toxicokinetic Study of GS-6207-02 in Beagle Dogs with a 2 and 13-Week Observation Period		**Gilead Study No.** TX-200-2047 **CRO Study No.** 8419595
Species / Strain: Dog/ Beagle	**Duration of Dosing:** 37 weeks[a]	**Location in CTD:** 4.2.3.2
Initial Age: 9 to 10 months	**Duration of Postdose:** 2 or 13 weeks	**GLP Compliance:** Yes
Date of First Dose: [redacted] 20[redacted]	**Method of Administration:** Subcutaneous Injection	**Lot Number:** [redacted]
Vehicle Control Article: 68.17% (w/w) polyethylene glycol 300 (PEG 300)/31.83% (w/w) sterile water for injection		
Special Features: Dermal observations		
NOAEL: Not established		

Subcutaneous Site E						
Inflammation, Granulomatous						
Minimal	0	0	0	1	NA	NA
Slight	0	0	0	1	NA	NA
Moderate	0	0	1	0	NA	NA
Necrosis, Adipose Tissue						
Slight	0	0	1	0	NA	NA
Fibrosis						
Minimal	0	0	1	0	NA	NA
Pseudocyst						
Present	0	0	1	0	NA	NA

Test Article: LEN

Report Title: A 9 month (Once Monthly) Subcutaneous Injection Toxicity and Toxicokinetic Study of GS-6207-02 in Beagle Dogs with a 2 and 13-Week Observation Period		**Gilead Study No.** TX-200-2047 **CRO Study No.** 8419595
Species / Strain: Dog/ Beagle	**Duration of Dosing:** 37 weeks[a]	**Location in CTD:** 4.2.3.2
Initial Age: 9 to 10 months	**Duration of Postdose:** 2 or 13 weeks	**GLP Compliance:** Yes
Date of First Dose: [redacted] 20[redacted]	**Method of Administration:** Subcutaneous Injection	**Lot Number:** [redacted]
Vehicle Control Article: 68.17% (w/w) polyethylene glycol 300 (PEG 300)/31.83% (w/w) sterile water for injection		
Special Features: Dermal observations		
NOAEL: Not established		

Subcutaneous Site F						
Inflammation, Granulomatous						
Minimal	0	0	0	1	NA	NA
Slight	0	0	0	1	NA	NA
Moderate	0	0	1	0	NA	NA
Necrosis, Adipose Tissue						
Slight	0	0	1	0	NA	NA
Pseudocyst						
Present	0	0	1	0	NA	NA
Subcutaneous Site G						
Inflammation, Granulomatous						
Minimal	0	0	2	1	NA	NA
Slight	0	0	0	1	NA	NA
Moderate	0	0	1	1	NA	NA

Test Article: LEN

Report Title: A 9 month (Once Monthly) Subcutaneous Injection Toxicity and Toxicokinetic Study of GS-6207-02 in Beagle Dogs with a 2 and 13-Week Observation Period		**Gilead Study No.** TX-200-2047 **CRO Study No.** 8419595
Species / Strain: Dog/ Beagle	**Duration of Dosing:** 37 weeks[a]	**Location in CTD:** 4.2.3.2
Initial Age: 9 to 10 months	**Duration of Postdose:** 2 or 13 weeks	**GLP Compliance:** Yes
Date of First Dose: [redacted] 20[redacted]	**Method of Administration:** Subcutaneous Injection	**Lot Number:** [redacted]
Vehicle Control Article: 68.17% (w/w) polyethylene glycol 300 (PEG 300)/31.83% (w/w) sterile water for injection		
Special Features: Dermal observations		
NOAEL: Not established		

Necrosis, Adipose Tissue						
Slight	0	0	1	0	NA	NA
Fibrosis						
Minimal	0	0	1	2	NA	NA
Pseudocyst						
Present	0	0	1	0	NA	NA
Subcutaneous Site H						
Inflammation, Granulomatous						
Minimal	0	0	0	1	NA	NA
Marked	0	0	1	0	NA	NA
Necrosis, Adipose Tissue						
Slight	0	0	1	0	NA	NA

Test Article: LEN

Report Title: A 9 month (Once Monthly) Subcutaneous Injection Toxicity and Toxicokinetic Study of GS-6207-02 in Beagle Dogs with a 2 and 13-Week Observation Period					**Gilead Study No.** TX-200-2047 **CRO Study No.** 8419595	
Species / Strain: Dog/ Beagle			**Duration of Dosing:** 37 weeks[a]		**Location in CTD:** 4.2.3.2	
Initial Age: 9 to 10 months			**Duration of Postdose:** 2 or 13 weeks		**GLP Compliance:** Yes	
Date of First Dose: [redacted] 20[redacted]			**Method of Administration:** Subcutaneous Injection		**Lot Number:** [redacted]	
Vehicle Control Article: 68.17% (w/w) polyethylene glycol 300 (PEG 300)/31.83% (w/w) sterile water for injection						
Special Features: Dermal observations						
NOAEL: Not established						
Subcutaneous Site I						
Inflammation, Granulomatous						
Slight	0	0	1	0	NA	NA
Moderate	0	0	2	0	NA	NA
Marked	0	0	0	1	NA	NA
Necrosis, Adipose Tissue						
Slight	0	0	1	0	NA	NA
Fibrosis						
Minimal	0	0	0	1	NA	NA
Subcutaneous Site H						
Inflammation, Granulomatous						
Slight	0	0	1	1	NA	NA
Moderate	0	0	3	0	NA	NA

Test Article: LEN

Report Title: A 9 month (Once Monthly) Subcutaneous Injection Toxicity and Toxicokinetic Study of GS-6207-02 in Beagle Dogs with a 2 and 13-Week Observation Period		**Gilead Study No.** TX-200-2047 **CRO Study No.** 8419595
Species / Strain: Dog/ Beagle	**Duration of Dosing:** 37 weeks[a]	**Location in CTD:** 4.2.3.2
Initial Age: 9 to 10 months	**Duration of Postdose:** 2 or 13 weeks	**GLP Compliance:** Yes
Date of First Dose: [redacted] 20[redacted]	**Method of Administration:** Subcutaneous Injection	**Lot Number:** [redacted]
Vehicle Control Article: 68.17% (w/w) polyethylene glycol 300 (PEG 300)/31.83% (w/w) sterile water for injection		
Special Features: Dermal observations		
NOAEL: Not established		

Inflammation, Mixed Cell						
Slight	0	1	0	0	NA	NA
Moderate	0	0	0	1	NA	NA
Marked	0	0	1	0	NA	NA
Necrosis, Adipose Tissue						
Minimal	0	0	1	0	NA	NA
Slight	0	0	2	1	NA	NA
Moderate	0	0	1	0	NA	NA
Fibrosis						
Minimal	0	1	1	1	NA	NA
Slight	0	1	1	0	NA	NA
Pseudocyst						
Present	0	0	1	1	NA	NA

ALP = Alanin Phosphatate; $AUC_{0\text{-}336h}$ = Area under the concentration time curve 0 to 336 hours postdose; $AUC_{0\text{-}672h}$ = Area under the concentration time curve 0 to 672 hours postdose; BLQ = Below the lower limit of quantitation (2.00 ng/mL); C_{max} = Maximum observed concentration; F = Female; GS-6207-02 = sodium salt of GS-6207; LEN = lenacapavir (GS-6207); M = Male; NA = Not Applicable; NOAEL = No-Observed-Adverse-Effect Level; TK = Toxicokinetic; − = Comparable to control animals or no treatment-related findings; * = $p \leq 0.05$

a Dose formulations were administered once on Days 1, 29, 57, 85, 113, 141, 169, 197, 225, and 253 by subcutaneous injection in the dorsal region at a dose volume of 1.33 mL/kg (Groups 1 and 3) or 0.42 mL/kg (Group 2). In addition, all Group 2 animals (excluding one Group 2 male) and all Group 3 animals were dosed once on Day 32 as they were unable to be dosed on Day 29. Group 3 animals were not dosed after Day 57.

b GS-6207-02-related mortality occurred for 1 male and 2 females administered 411 mg/kg/dose. The male was necropsied on Day 51 in a GS-6207-02-related moribund condition (yellow color of oral mucosa, severely thin physical appearance, swollen dose site, 20% drop from peak body weight). Two females were necropsied in a GS-6207-02-related moribund condition on Day 71 or 82, respectively. One female was hunched and thin, had yellow color of conjunctiva (as noted in veterinary observations), and suffered a 19% drop from peak body weight. The other female was also thin and suffered a 28% drop from its peak body weight. GS-6207-02-related decreased food consumption, secondary to moribund condition, was noted for the three animals. GS-6207-02-related clinical pathology changes for these animals included moderately to markedly increased total bilirubin concentration, predominantly composed of direct (conjugated) bilirubin concentration; alanine aminotransferase, alkaline phophatase, and gamma glutamyltransferase activities; total bile acid concentration; and mildly increased aspartate aminotransferase activity and decreased albumin (with secondary decreases in protein-bound calcium concentrations) and total protein concentrations with decreased albumin:globulin (A:G) ratio. GS-6207-02-related macroscopic findings were limited to 2 animals administered 411 mg/kg/dose and necropsied in a moribund condition on Day 51 or 71; these included bilateral yellow eye sclera discoloration for 1 animal and yellow discoloration of liver, kidney, aorta, and oral mucosa for the other, and thickened gall bladder.

7.7. 4-Week Oral Dog

Test Article: LEN

Report Title: A 4-Week Oral Gavage Toxicity and Toxicokinetic Study of GS-6207 in Dogs		**Gilead Study No. TX-200-2012 ([redacted] 8367906)**
Species / Strain: Dog / Beagle	**Duration of Dosing:** 4 weeks	**Location in CTD:** 4.2.3.2
Initial Age: 9 to 10 months	**Duration of Postdose:** Not Applicable	**GLP Compliance:** Yes
Date of First Dose: [redacted] 20[redacted]	**Method of Administration:** Oral Gavage	**Lot Number:** [redacted]
Vehicle Control Article: 10% v/v Dimethyl sulfoxide (DMSO), 10% v/v vitamin E TPGS, and 80% v/v propylene glycol (PG)		
Special Features: Hepatic Microsomal Enzyme Analysis		
No Observed Adverse Effect Level: 30 mg/kg/day		

Daily Dose (mg/kg/day)	**0 (Vehicle Control)**		**1 (Low)**		**5 (Mid)**		**30 (High)**	
Sex:Number of Animals	M: 4	F: 4	M: 4	F: 4	M: 4	F: 4	M: 4	F: 4
Toxicokinetics: $AUC_{0\text{-}24h}$ (ng·h/mL)								
Day 1	BLQ	BLQ	1920	1400	9510	6500	54700	45700
Day 28	BLQ	BLQ	4610	2270	20400	18200	82900[a]	70600
Toxicokinetics: C_{max} (ng/mL)								
Day 1	BLQ	BLQ	129	92.7	721	467	3110	2470
Day 28	BLQ	BLQ	263	146	1310	1160	4520[a]	4290
Daily Dose (mg/kg/day)	**0 (Vehicle Control)**		**1 (Low)**		**5 (Mid)**		**30 (High)**	
Sex:Number of Animals (Main)	M: 4	F: 4	M: 4	F: 4	M: 4	F: 4	M: 4	F: 4
Noteworthy Findings								
Died or sacrificed moribund	0	0	0	0	0	0	1[a]	0
Body Weight	No test-article related findings							
Food Consumption	No test-article related findings							

Test Article: LEN

Report Title: **A 4-Week Oral Gavage Toxicity and Toxicokinetic Study of GS-6207 in Dogs**							**Gilead Study No. TX-200-2012 ([redacted]8367906)**	
Clinical Observations								
Struggling during dosing[b]								
Week 1	0 (0)	0 (0)	6 (2)	0 (0)	1 (1)	2 (2)	6 (2)	5 (3)
Week 2	0 (0)	0 (0)	1 (1)	0 (0)	0 (0)	0 (0)	3 (3)	1 (1)
Week 3	0 (0)	0 (0)	0 (0)	0 (0)	1 (1)	2 (1)	3 (2)	4 (3)
Week 4	0 (0)	0 (0)	0 (0)	1 (1)	0 (0)	0 (0)	0 (0)	0 (0)
Ophthalmoscopy	No test-article related findings							
Hematology and Coagulation	No test-article related findings							
Clinical Chemistry								
Day 29								
Alanine Aminotransferase (U/L)	32	39	33	37	32	29	56	104
Aspartate Aminotransferase (U/L)	31	30	28	34	29	34	32	37
Alkaline Phosphatase (U/L)	38	52	50	47	49	49	78	98
Gamma Glutamyl Transferase (U/L)	<3	<3	<3	<3	<3	<4	<3	5
Daily Dose (mg/kg/day)	**0 (Vehicle Control)**		**1 (Low)**		**5 (Mid)**		**30 (High)**	
Sex:Number of Animals (Main)	M: 4	F: 4	M: 4	F: 4	M: 4	F: 4	M: 4	F: 4
Urinalysis	No test-article related findings							
Organ Weights	No test-article related findings							
Gross Pathology	No test-article related findings							
Histopathology	No test-article related findings							
Hepatic Microsomal Enzymes								
CYP1A (pmol/min/mg protein)	-	44.8 ± 13.0	-	117 ± 61.7	-	40.9 ± 22.6	-	41.8 ± 12.3
CYP2B (pmol/min/mg protein)	-	54.6 ± 22.2	-	62.0 ± 10.2	-	55.4 ± 18.9	-	110 ± 25.5

$AUC_{0\text{-}24h}$ = Area under the concentration time curve from 0 to 24 hours postdose; BLQ = below the lower limit of quantitation (< 2.00 ng/mL); C_{max} = Maximum observed concentration; F = Female; LEN = lenacapavir (GS-6207); M = Male; TPGS = D-α-Tocopherol polyethylene glycol 1000 succinate (Vitamin E)

a One male administered 30 mg/kg/day was sacrificed on Day 19 shortly after dosing due to gavage error; n=3 for Day 28 toxicokinetics.
b Data is presented as Number of observations (Number of animals affected).

8. GENOTOXICITY: IN VITRO

8.1. Ames

Test Article: LEN

Report Title:	**GS-6207: Bacterial Reverse Mutation Assay Using the Plate Incorporation Method**	**Gilead Study No. TX-200-2007 [redacted] Study No. 8353954**
Test for Induction of: Reverse mutation in bacterial cells	**No. of Independent Assays:** 1	**Location in CTD:** 4.2.3.3.1
Strains: *Salmonella typhimurium* and *Escherichia coli*	**No. of Replicate Cultures:** 3	**GLP Compliance:** Yes
Metabolizing System: Aroclor™-induced rat liver S9 fraction	**No. of Cells Analyzed/Cultured:** ~10^8	**Lot Number:** [redacted]
Vehicle: Dimethylsulfoxide (DMSO)	**Vehicle for Positive Controls:** Deionized (DI) Water (sodium azide only); DMSO (all other positive controls)	
Treatment: Plate incorporation for 2-3 days (minimum 48 hours)		**Date of Treatment:** [redacted] 20[redacted]
Cytotoxic Effects: None		
Genotoxic Effects: None		

Test Article: LEN

Treatment	Dose Level (μg/plate)	Plate Incorporation – Initial Assay Revertants/Plate (Mean ± SD)											
		TA98			TA100			TA1535			TA1537			WP2*uvr*A		
Without Activation																
DMSO		43	±	5N	133	±	16N	13	±	7N	22	±	8N	23	±	4N
GS-6207	5.00	40	±	12N	136	±	10N	14	±	3N	25	±	8N	31	±	6N
	16.0	39	±	7N	126	±	6N	10	±	9N	15	±	2N	19	±	3N
	50.0	43	±	6N	132	±	21N	8	±	3N	20	±	2NM	29	±	7N
	160	42	±	4N	138	±	16N	15	±	1N	16	±	6N	22	±	2N
	500	38	±	6PN	158	±	6PN	11	±	3PN	21	±	5PN	27	±	4N
	1600	37	±	5PNM	137	±	9PNM	13	±	4PNM	17	±	2PNM	27	±	2POM
	5000	46	±	9POM	127	±	12POM	12	±	2POM	22	±	6POM	26	±	7POM
Positive Controls																
2-Nitrofluorene	1.0	207	±	16N												
Sodium azide	2.0				1026	±	25N	643	±	36N						
ICR-191 acridine	2.0										280	±	19N			
4-Nitroquinoline-N-oxide	1.0													406	±	39N

Test Article: LEN

Treatment	Dose Level (µg/plate)	Plate Incorporation – Initial Assay Revertants/Plate (Mean ± SD)											
		TA98			TA100			TA1535			TA1537			WP2*uvr*A		
With Activation																
DMSO		44	±	13N	158	±	5N	13	±	3N	25	±	2N	27	±	5N
GS-6207	5.00	43	±	5N	150	±	8N	17	±	2N	17	±	5N	25	±	1N
	16.0	45	±	10N	145	±	17N	12	±	3N	20	±	3N	27	±	4N
	50.0	43	±	6N	136	±	13N	15	±	7N	26	±	6N	25	±	3N
	160	61	±	10N	148	±	8N	16	±	6N	20	±	5N	23	±	5N
	500	51	±	17PNM	156	±	16PN	19	±	7PN	19	±	8PN	32	±	10PNM
	1600	33	±	11PNM	153	±	6PNM	13	±	6PNM	18	±	6PN	26	±	8POM
	5000	34	±	4POM	156	±	9POM	11	±	4POM	17	±	3POM	23	±	8POM
Positive Controls																
Benzo{a}pyrene	2.5	365	±	45N												
2-Aminoanthracene	2.5				1292	±	22N	156	±	9N	156	±	17N			
2-Aminoanthracene	25.0													343	±	53N

GLP = Good Laboratory Practice, LEN = lenacapavir (GS-6207); M = Plate counted manually, N = Normal background bacterial lawn, O = Obscured background bacterial lawn, P = Precipitation of test article observed, SD = Standard Deviation
Criteria for significance: ≥ 2-fold (TA98, TA100, WP2*uvr*A) and ≥3-fold (TA1535 and TA1537)

8.2. Chromosome Aberrations

Test Article: LEN

Report Title: GS-6207: *In Vitro* Human Lymphocyte Chromosome Aberration Assay		**Gilead Study No.:** TX-200-2008 [redacted] **Study No.:** 8353955
Test for Induction of: Chromosome Aberrations	**No. of Independent Assays:** 2	**Location in CTD:** 4.2.3.3.1
Strains: Human Peripheral Blood Lymphocytes	**No. of Replicate Cultures:** 2	**GLP Compliance:** Yes
Metabolizing System: Aroclor™-induced rat liver S9	**No. of Cells Analyzed/Culture:** 150 (or ≥ 15 if >20% cells with aberrations)	**Test Article Lot Number:** [redacted]
Vehicle for Test Article: Dimethyl sulfoxide (DMSO)	**Vehicle for Positive Controls:** Deionized (DI) Water	**Date of Treatment:** [redacted] 20[redacted] and [redacted] 20[redacted]
Treatment: Continuous 24 hours without S9; pulse treatment 3 hours and recovery time 21 hours with and without S9. All cultures harvested at 24-hr.		
Cytotoxic Effects: Yes		
Genotoxic Effects: None		

Initial Assay

Metabolic Activation	Test Article	Concentration (µg/mL)	Total Cells Analyzed	MIH (%)[a]	Cells with Aberrations Excluding Gaps	% Cells with Aberrations Excluding Gaps	Significance	% P + E
3hr Treatment, Without Activation (-S9)	DMSO	10.0 µL/mL	300	-	0	0.00	-	0.00
	GS-6207	84.0	300	1P	1	0.33	NS	0.00
		120	300	5P	2	0.67	NS	0.00
		172	300	3PE	1	0.33	NS	0.00
	Mitomycin C	1.00	84	38	60	71.4	$p \leq 0.001$	0.00
24hr Treatment, Without Activation (-S9)	DMSO	10.0 µL/mL	300	-	0	0.00	-	0.00
	GS-6207	41.2	300	21	0	0.00	NS	0.00
		120	300	35P	1	0.33	NS	0.00
		172	300	49P	0	0.00	NS	0.00
	Mitomycin C	0.300	109	36	60	55.0	$p \leq 0.001$	0.00
3hr Treatment, With Activation (+S9)	DMSO	10.0 µL/mL	300	-	0	0.00	-	0.00
	GS-6207	120	300	25P	1	0.33	NS	0.00
		172	300	19P	2	0.67	NS	0.00
		245	300	31PE	3	1.00	NS	0.00
	Cyclophosphamide	15.0	114	46	60	52.6	$p \leq 0.001$	0.00

** $p \leq 0.01$, significantly different from control based on Fisher's Exact Test; LEN = lenacapavir (GS-6207)
a Based on mitotic index
DMSO = Dimethylsulfoxide; GLP = Good Laboratory Practice; hr = hour; No. = number, NS=Not significant, - = not evaluated, MIH = Mitotic Index Inhibition,
P + E = polyploidy and endoreduplication
P = Precipitates observed at start of test article treatment.
E = Precipitates observed at end of test article treatment.

9. GENOTOXICITY: IN VIVO

Test Article: LEN

Report Title:	**A Four-Dose (Twice Monthly) Subcutaneous Injection Toxicity and Toxicokinetic Study of GS-6207 in Wistar Han Rats**	**Gilead Study No. TX-200-2005 [redacted] 8353952**
Test for Induction of: Micronucleated PCEs	**Treatment Schedule:** Biweekly dose, four doses	**Location in CTD:** 4.2.3.3.2
Species / Strain: Rat/Crl:WI(Han)	**Sampling Time:** 24 hours (±1.5 hours) post last dose	**Test Article Lot Number:** [redacted]
Age: 14 to 16 weeks old	**Method of Administration:** Subcutaneous injection[a]	**GLP Compliance:** Yes
No. of Cells Evaluated / Animal: 20000[c]	**Vehicle:** 2% (w/v) poloxamer 188 prepared in 0.9% (w/v) sodium chloride in sterile water for injection	**Date of Treatment:** [redacted] 20[redacted]
Special Features: Bone marrow micronucleus assay	**Positive Control Article:** Cyclophosphamide	
Toxic / Cytotoxic Effects: None		
Genotoxic Effects: None		

Biweekly Dose (mg/kg/dose)	**0 (Control)**		**10 (Low)**		**30 (Mid)**		**100 (High)**	
Sex: Number of Animals (TK)	**M: 3**	**F: 3**	**M: 12**	**F: 12**	**M: 12**	**F: 12**	**M: 12**	**F: 12**
GS-6207 Toxicokinetics								
$AUC_{0\text{-}336}$ (ng·h/mL)								
Study Day 1	NC	NC	64100	55700	146000	124000	338000	338000
Study Day 43	NC	NC	134000	106000	314000	244000	608000	559000
C_{max} (ng/mL)								
Study Day 1	BLQ	BLQ	241	215	586	445	1230	1290
Study Day 43	BLQ	BLQ	410	451	1060	843	2500	2130

Test Article: GS-6207

Report Title:	**A Four-Dose (Twice Monthly) Subcutaneous Injection Toxicity and Toxicokinetic Study of GS-6207 in Wistar Han Rats**	**Gilead Study No. TX-200-2005 [redacted] 8353952**

Test Article	**Biweekly Dose (mg/kg/dose)**	**No. of Animals/Sex**	**Sampling Time (Hours)**	**Mean Ratio PCE:NCE Males**	**Mean Ratio PCE:NCE Females**	**Mean % MN PCEs (±SD) Males**	**Mean % MN PCEs (±SD) Females**
Vehicle	0	5	24 hours (±1.5 hours)	50.26	54.42	0.05 ± 0.02	0.09 ± 0.05
GS-6207	10	5	24 hours (±1.5 hours)	50.42	53.18	0.07 ± 0.05	0.11 ± 0.10
GS-6207	30	5	24 hours (±1.5 hours)	49.22	52.30	0.04 ± 0.02	0.12 ± 0.02
GS-6207	100	5	24 hours (±1.5 hours)	51.90	52.70	0.05 ± 0.04	0.05 ± 0.05
Cyclophosphamide[d]	60	5	24 hours (±1.5 hours)	40.38	45.28	1.15 ± 0.16	1.51 ± 0.47

$AUC_{0\text{-}336}$ = Area under the concentration-time curve from hour 0 to hour 336; BLQ = below the lower limit of quantitation (<2.00 ng/mL); C_{max} = Maximum observed concentration; F = Female; LEN = lenacapavir (GS-6207); M = male; MN PCE = Micronucleated PCE; NC = Not calculated; NCE = Normochromatic erythrocyte; PCE = Polychromatic erythrocyte; TK = toxicokinetic; w/v = weight/volume

a Animals in Groups 1 through 4 were dosed once every two weeks for a total of four doses and were monitored for at least 6 weeks after the last dose.
b Age at time of bone marrow collection.
c Males in Group 5 had 16000 cells/animal evaluated.
d The positive control article formulation was administered via oral gavage once on Study Day 56 at a dose volume of 10 mL/kg to animals in Group 5, approximately 24 hours (±1.5 hours) prior to bone marrow collections.

10. CARCINOGENICITY

Not applicable.

11. REPRODUCTIVE AND DEVELOPMENTAL TOXICITY (NONPIVOTAL STUDIES)

Test Article: LEN

Species/ Strain	Method of Administration (Vehicle)	Dosing Period	Doses (mg/kg/day)	Gender and No. Per Group	Noteworthy Findings	Gilead Study No. (CRO Name and Study No.)
Rat/Crl:CD(SD)	Oral gavage (10% (v/v) Dimethyl sulfoxide, 10% (v/v) vitamin E TPGS, and 80% (v/v) propylene glycol)	GD 6-17	0, 3, 30, 300	Main: 8 F TK: Control - 3 F Treated -6 F	No LEN-related effects on maternal or embryofetal developmental parameters. NOEL- 300 mg/kg/day (C_{max} = 1240 ng/mL, AUC_{0-24} = 26,300 ng·hr/mL)	TX-200-2031 ([redacted]; 00604390)
Rabbit/ Hra(NZW) SPF	Intravenous infusion (6.12% [w/w] polyethylene glycol 300 [PEG 300] and 93.88% [w/w] sterile water for injection)	GD 7-19	0, 5, 10, 20	6 F	No LEN-related effects on maternal or embryofetal developmental parameters. Maternal NOAEL and Fetal NOEL- 20 mg/kg/day (C_{max} = 45,700 ng/mL, $AUC_{0-24.5}$ = 178,800 ng·hr/mL)	TX-200-2032 ([redacted]; 00604391)

F = Female, w/w = weight/weight, AUC, Cmax and v/v = volume/volume/; NOAEL = No Observed Effect Level; NOEL,= No Observed Effect Level; GD = Gestation Day; LEN = lenacapavir (GS-6207); TPGS = D-α-Tocopherol polyethylene glycol 1000 succinate (Vitamin E)

12. REPRODUCTIVE AND DEVELOPMENTAL TOXICITY: FERTILITY AND EARLY EMBRYONIC DEVELOPMENT TO IMPLANTATION

Test Article: LEN

Report Title: **A Subcutaneous Injection Study of the Effects of GS-6207 on Fertility and Early Embryonic Development to Implantation in Sprague Dawley Rats**		**Gilead Study No. TX-200-2043** [redacted]**: 00604466**
Species / Strain: Rat/Crl:CD(SD)	**Duration of Dosing:** **M:** once 6 weeks prior to mating	**Location in CTD:** 4.2.3.5.1
Initial Age: M: 7 weeks **F:** 10 weeks	**F:** once 4 weeks prior to mating	**GLP Compliance:** yes
Date of First Dose: [redacted] 20[redacted]	**Day of Mating:** SD 27	**Lot Number:** [redacted]
No-Observed-Effect Level	**Day of C-Section:** GD 15	**Design Similar to ICH 4.1.1?** yes
Male Systemic: 100 mg/kg	**Method of Administration:** Subcutaneous injection	
Male Reproductive: 100 mg/kg		
Female Systemic: 100 mg/kg	**Vehicle:** 77% (w/w) polyethylene glycol 200, 10% (w/w) ethanol, 13% (w/w) sterile water for injection (with 1 M equivalent NaOH [200 mg/mL] for GS-6207 groups only)	
Female Reproductive: 100 mg/kg		
Embryos: 100 mg/kg	**Special Features:** Toxicokinetics (n=3/sex control; n=6/sex treated)	

Daily Dose (mg/kg)		**0 (Control)**	**20**	**100**
Males	Toxicokinetics – GS-6207			
	C_{max} (ng/mL) – SD 0	NA	188	577
	$AUC_{0\text{-}672h}$ (ng•h/mL) – SD 0	NA	103,000	270,000
	$AUC_{0\text{-}1008h}$ (ng•h/mL) – SD 0	NA	161,000	441,000
	No. Evaluated	25	25	25

Test Article: LEN

Report Title:	**A Subcutaneous Injection Study of the Effects of GS-6207 on Fertility and Early Embryonic Development to Implantation in Sprague Dawley Rats**		**Gilead Study No. TX-200-2043 [redacted]: 00604466**	
Daily Dose (mg/kg)		**0 (Control)**	**20**	**100**
	No. Died or Sacrificed Moribund	0	0	0
	Clinical Observations	-	-	-
	Body Weight	-	-	-
	Body Weight Change	-	-	-
	Food Consumption			
	Pre-Mating Period	-	-	-
	Post-Mating Period	-	-	-
	Necropsy Observations	-	-	-
	Organ Weights	-	-	-
	No. of Males that Mated	25	24	24
	No. of Fertile Males	23	23	24
	Spermatogenic Evaluation	-	-	-
Females	Toxicokinetics – GS-6207			
	C_{max} (ng/mL) – SD 0	NA	153	597
	$AUC_{0\text{-}672h}$ (ng•h/mL) – SD 0	NA	91,100	192,000
	No. Evaluated	25	25	25
	No. Died or Sacrificed Moribund	0	0	0
	Clinical Observations	-	-	-
	Premating Body Weight	-	-	-

Test Article: LEN

Report Title:	**A Subcutaneous Injection Study of the Effects of GS-6207 on Fertility and Early Embryonic Development to Implantation in Sprague Dawley Rats**		**Gilead Study No. TX-200-2043 [redacted]: 00604466**	
Daily Dose (mg/kg)		**0 (Control)**	**20**	**100**
	Gestation Body Weight	-	-	-
	Pre-Mating Food Consumption	-	-	-
	Gestation Food Consumption	-	-	-
	Necropsy Observations	-	-	-
	Organ Weights	-	-	-
	Mean Length of Estrous Cycle (days)	4.12	4.05	4.06
	Mean No. Days Prior to Mating	2.2	2.7	2.7
	No. of Females Sperm-Positive	25	25	25
	No. of Pregnant Females	23	24	25
	Mean No. of Corpora Lutea	18.2	17.8	17.5
	Mean No. of Implantation Sites	15.6	15.1	15.3
	Mean No. of Live Embryos	15.0	13.9	14.2
	Mean Litter Proportion of Pre-implantation Loss (% per Litter)	13.27	14.81	12.03
	Mean Litter Proportion of Postimplantation Loss (% per Litter)	3.68	7.81	7.02

F = Female GD = Gestation Day; LEN = lenacapavir (GS-6207); M = Male; NaOH = sodium chloride; SD = Study Day; - = No noteworthy findings; NA = Not applicable

13. REPRODUCTIVE AND DEVELOPMENTAL TOXICITY: EFFECTS ON EMBRYO-FETAL DEVELOPMENT

13.1. Rat

Test Article: LEN

Report Title: An Oral (Gavage) Study of the Effects of GS-6207 on Embryo/Fetal Development in Sprague Dawley Rats				**Gilead Study No. TX-200-2036** **CRO Study No.** 00604416
Species / Strain: Rat/Crl:CD(SD)		**Duration of Dosing:** GD 6 to 17		**Location in CTD:** 4.2.3.5.2
Initial Age: 10-12 weeks		**Day of Mating:** GD 0		**GLP Compliance:** Yes
Date of First Dose: [redacted] 20[redacted]		**Day of C-Section:** GD 21		**Lot Number:** [redacted]
Vehicle: 10% (v/v) dimethyl sulfoxide (DMSO), 10% (v/v) vitamin E TPGS, and 80% (v/v) propylene glycol (PG)		**Method of Administration:** Oral (Gavage)		**Design Similar to ICH 4.1.3?** Yes
Special Features: None				
NOEL: F_0 Females: 30 mg/kg/day; F_1 Litters: 30 mg/kg/day				

Daily Dose (mg/kg/day)		**0 (Control)**	**3**	**10**	**30**
Dams:	Toxicokinetics: $AUC_{0\text{-}24h}$ (h*ng/mL)				
	Gestation Day 6	BLQ	4410	10,700	13,300
	Gestation Day 17	BLQ	9200	17,800	22,000
	Toxicokinetics: C_{max} (ng/mL)				
	Gestation Day 6	BLQ	209	529	692
	Gestation Day 17	BLQ	435	1050	1210

Test Article: LEN

Report Title: An Oral (Gavage) Study of the Effects of GS-6207 on Embryo/Fetal Development in Sprague Dawley Rats			**Gilead Study No. TX-200-2036** **CRO Study No.** 00604416	
Daily Dose (mg/kg/day)	**0 (Control)**	**3**	**10**	**30**
Dams/Does: Noteworthy Findings				
No. Pregnant	24	25	25	25
No. Died or Sacrificed Moribund	0	1	0	0
No. Aborted or with Total Resorption of Litter	0	0	0	0
Clinical Observations	No GS-6207-related findings			
Body Weight	No GS-6207-related findings			
Food Consumption	No GS-6207-related findings			
Necropsy Observations	No GS-6207-related findings			
Mean No. Corpora Lutea	16.4	15.2	16.4	15.3
Mean No. Implantations	13.8	13.4	14.0	12.6
Mean % Preimplantation Loss	14.93	11.48	13.79	17.02
Litters: Noteworthy Findings				
No. Litters Evaluated	24	24	25	25
No. Live Fetuses	12.6	12.0	13.0	11.2
No. Dead Fetuses	0.0	0.0	0.0	0.0
Mean No. Resorptions	1.3	1.4	1.0	1.4
Early	1.2	1.4	1.0	1.4
Late	0.0	0.0	0.0	0.0
Mean % Postimplantation Loss	9.04	12.82	7.28	11.37
Mean Fetal Body Weight (g)	5.71	5.91	5.85	5.75
Fetal Sex Ratios (Live No. of M:F)	149:153	152:136	151:175	136:145
Fetal Anomalies	No GS-6207-related findings			

AUC_{0-24} = Area under the concentration-time curve from 0 to 24 hours postdose; BLQ = Below the lower limit of quantitation (2.00 ng/mL); C_{max} = Maximum observed concentration; GD = gestation day; F = Female; LEN = lenacapavir (GS-6207); M = Male;; NOEL = No-Observed-Effect level; TPGS = D-α-Tocopherol polyethylene glycol 1000 succinate (Vitamin E); v/v = volume/volume

13.2. Rabbit

Test Article: LEN

Report Title: A 30-Minute IV Infusion Study of the Effects of GS-6207 on Embryo/Fetal Development in New Zealand White Rabbits		**Gilead Study No. TX-200-2037** **CRO Study No.** 00604417
Species / Strain: Rabbit / Hra: (NZW) SPF New Zealand White	**Duration of Dosing:** GD 7 to 19	**Location in CTD:** 4.2.3.5.2
Initial Age: 6 to 7 months	**Day of Mating:** GD 0	**GLP Compliance:** Y
Date of First Dose: [redacted] 20[redacted]	**Day of C-Section:** GD 29	**Lot Number:** [redacted]
Vehicle: Group 1: 6.12% (w/w) polyethylene glycol 300 (PEG 300) and 93.88% (w/w) sterile water for injection. Groups 2, 3 and 4: 5.74% (w/w) polyethylene glycol 300 (PEG 300) and 88.14% (w/w) sterile water for injection, and 3.17% (w/w) 1N sodium hydroxide	**Method of Administration:** Intravenous infusion	**Design Similar to ICH 4.1.3?** Y
Special Features: none		
NOAEL (maternal toxicity): 20 mg/kg/day; NOEL (embryo/fetal development): 20 mg/kg/day		

Daily Dose (mg/kg/day)	**0 (Control)**	**5 mg/kg/day**	**10 mg/kg/day**	**20 mg/kg/day**
Dams/Does: Noteworthy Findings				
No. Pregnant	22	21	19	22
No. Died or Sacrificed Moribund	0	0	0	1
No. Aborted or with Total Resorption of Litter	0	0	0	0
Clinical Observations				
Skin discoloration	1	15	16	20
Skin bruising	2	11	21	22
Scabbing	2	6	6	10

Test Article: LEN

Report Title: A 30-Minute IV Infusion Study of the Effects of GS-6207 on Embryo/Fetal Development in New Zealand White Rabbits			**Gilead Study No. TX-200-2037** **CRO Study No.** 00604417	
Daily Dose (mg/kg/day)	**0 (Control)**	**5 mg/kg/day**	**10 mg/kg/day**	**20 mg/kg/day**
Dams/Does: Noteworthy Findings				
Body Weight (%)[a]				
GD 7 to 10	65.9	39.8	-7.0**	-10.2**
GD 10 to 13	58.0	14.5**	16.1**	22.4*
GD 13 to 20	167.9	139.8	122.4	82.2**
GD 7 to 20	291.8	194.1*	131.5**	92.9**
GD 20 to 29	198.7	197.7	206.8	244.1
Food Consumption (%)[a]				
GD 7 to 10	160.21	157.79	153.58	137.48**
GD 10 to 13	160.62	133.38**	133.02**	115.42**
GD 13 to 20	156.99	127.19**	124.50**	96.36**
Food Consumption (%)[a]				
GD 7 to 20	158.57	135.68**	133.17**	110.20**
GD 20 to 29	130.15	122.84	130.09	134.95
Necropsy Observations	No GS-6207-related findings			
Mean No. Corpora Lutea[a]	10.6	11.0	10.7	10.4
Mean No. Implantations[a]	9.1	9.7	8.7	9.5
Mean % Preimplantation Loss[a]	13.90	11.06	18.05	8.54

Test Article: LEN

Report Title: A 30-Minute IV Infusion Study of the Effects of GS-6207 on Embryo/Fetal Development in New Zealand White Rabbits			**Gilead Study No. TX-200-2037** **CRO Study No.** 00604417	
Litters: Noteworthy Findings				
Daily Dose (mg/kg/day)	**0 (Control)**	**5 mg/kg/day**	**10 mg/kg/day**	**20 mg/kg/day**
No. Litters Evaluated	22	21	19	21
Mean No. Live Fetuses	8.4	9.5	8.5	9.0
Mean No. Dead Fetuses	0.0	0.0	0.0	0.0
Mean No. Resorptions	0.6	0.2	0.2	0.5
Mean % Postimplantation Loss	7.43	2.59	4.61	5.52
Mean Fetal Body Weight (g)	44.28	42.49	41.23	41.11
Fetal Sex Ratios (Live No. of M:F)	4.6 : 3.8	4.6 : 4.9	4.4 : 4.1	4.7 : 4.2
Fetal Anomalies				
Gross External Anomalies	0	0	0	1
Visceral Anomalies	2	0	0	0
Skeletal Anomalies	0	0	4	0
Total Affected Fetuses (Litters)	2 (2)	0 (0)	4 (3)	1 (1)

F = Female; LEN = lenacapavir (GS-6207); Male = Male; * = $p \leq 0.05$; ** = $p \leq 0.01$ (Anova & Dunnett)
a Group means are shown. Statistical significance is based on actual data.

14. REPRODUCTIVE AND DEVELOPMENTAL TOXICITY: EFFECTS ON PRE- AND POST-NATAL DEVELOPMENT, INCLUDING MATERNAL FUNCTION

Test Article: LEN

Report Title: A Subcutaneous Injection Study of the Effects of GS-6207 on Pre- and Postnatal Development, Including Maternal Function in Sprague Dawley Rats		**Gilead Study No. TX-200-2049** **CRO Study No.** 00604474
Species / Strain: Rat/Crl:CD(SD)	**Duration of Dosing:** GD 6 (main and TK)	**Location in CTD:** 4.2.3.5.3
Initial Age: 11 weeks	**Day of Mating:** GD 0	**GLP Compliance:** Yes
Date of First Dose: [redacted] 20[redacted]	**Method of Administration:** Subcutaneous injection	**Lot Number:** [redacted]
Vehicle: Vehicle Control Group: 68.2% (w/w) Polyethylene glycol 300 (PEG 300), 31.8% (w/w) sterile water for injection (SWFI) **Test Article Groups**: 50.1% (w/w) PEG 300, 23.4% (w/w) SWFI.	**Litters:** Culled	**Design Similar to ICH 4.1.2?** Yes
Special Features: toxicokinetics		
NOAEL: F_0 systemic: 300 mg/kg; NOEL: F_0 Dev/neonatal: 300 mg/kg; F_1 systemic: 300 mg/kg; F_1 reproductive: 300 mg/kg; F_2 intrauterine survival: 300 mg/kg		

Dose (mg/kg)		**0 (Control)**	**30**	**300**
F_0 Females	Gestation Day 6			
	$AUC_{0–192h}$ (h*ng/mL)	BLQ	10,900	54,800
	C_{max} (ng/mL)	BLQ	87.6	412
	Maternal and Fetal (LD 10/PND 10)			
	Maternal Concentration (ng/mL)	BLQ	36.7	196
	Pup Concentration (ng/mL)	BLQ	7.12	32.8
	Maternal : Pup Ratio	NA	5.15	5.96

Test Article: LEN

Report Title: A Subcutaneous Injection Study of the Effects of GS-6207 on Pre- and Postnatal Development, Including Maternal Function in Sprague Dawley Rats			**Gilead Study No. TX-200-2049** **CRO Study No.** 00604474
Dose (mg/kg)	**0 (Control)**	**30**	**300**
F_0 Females: Noteworthy Findings			
No. Pregnant	24	24	23
No. Died or Sacrificed Moribund	0	0	0
No. Aborted or with Total Resorption of Litter	0	0	0
Clinical Observations[a]			
Swollen Dorsal Trunk	0/0	0/0	335/21
Scabbing Dorsal Trunk	0/0	3/1	29/5
Necropsy Observations			
Injection Site – Dorsal Scapula – Thickened	0	15	23
Gestation Body Weight - GD 20 (%)[b]	No GS-6207-related changes		
Lactation Body Weight - LD 21 (%)[b]	No GS-6207-related changes		
Gestation Food Consumption - GD 6–20 (g/animal/day)	No GS-6207-related changes		
Lactation Food Consumption – LD 1–21 (g/animal/day)	No GS-6207-related changes		
Mean Duration of Gestation (days)	21.7	21.8	21.6
Abnormal Parturition	0	0	0
F_1 Litters (Preweaning): Noteworthy Findings			
No. Litters Evaluated	24	24	23
Mean No. of Implantations	13.5	13.7	14.5
Mean No. Pups/Litter	12.5	12.6	13.2
Mean No. Liveborn Pups/Litter	12.3	12.6	12.8

Test Article: LEN

Report Title: A Subcutaneous Injection Study of the Effects of GS-6207 on Pre- and Postnatal Development, Including Maternal Function in Sprague Dawley Rats			**Gilead Study No. TX-200-2049** **CRO Study No.** 00604474
Dose (mg/kg)	**0 (Control)**	**30**	**300**
F_1 Litters (Preweaning): Noteworthy Findings			
Postnatal Survival to Day 4 (% per litter)	97.0	97.9	95.4
Postnatal Survival PND 4 (post-selection) to Weaning (% per litter)	100.0	99.5	99.5
No. of Total Litter Losses	0	0	0
Pup Body Weights - PND 21 (g)[b]			
Males	55.4	+4.2	+0.4
Females	52.8	+6.1*	+1.9
Pup Sex Ratios (% males per litter at birth)	47.4	50.2	50.0
Pup Clinical Signs	No GS-6207-related findings		
Developmental Parameters (PND of attainment; sexes combined)			
Surface Righting Response	2.8	2.8	3.0
Pinnal Detachment	3.3	3.3	3.3
Incisor Eruption	8.5	8.1	8.6
Hair Growth	11.8	11.3**	12.0
Eye Opening	14.0	13.8	14.1
Pupillary Reflex	21.0	21.0	21.0
Dose (mg/kg)	**0 (Control)**	**30**	**300**
F_1 Litters (Preweaning): Noteworthy Findings			
Pup Necropsy Observations	No GS-6207-related findings		

Test Article: LEN

Report Title: A Subcutaneous Injection Study of the Effects of GS-6207 on Pre- and Postnatal Development, Including Maternal Function in Sprague Dawley Rats			**Gilead Study No. TX-200-2049** **CRO Study No.** 00604474
F_1 Males (Postweaning): Noteworthy Findings			
No. Evaluated Postweaning	24	24	23
No. Died or Sacrificed Moribund	0	0	1
Clinical Observations	No GS-6207-related findings		
Body Weight - PND 112(g)[b]	597	+3.7	-3.4
Necropsy Observations	No GS-6207-related findings		
Mean Age of Preputial Separation (days)	45.5	45.9	46.0
Auditory Startle Response	No GS-6207-related findings		
Motor Activity	No GS-6207-related findings		
Learning and Memory	No GS-6207-related findings		
No. of Males that Mated	24	24	22
No. of Fertile Males	21	23	22
F_1 Females (Postweaning): Noteworthy Findings			
No. Evaluated Postweaning	24	24	23
No. Died or Sacrificed Moribund	0	0	0
Clinical Observations	No GS-6207-related findings		
Premating Body Weight - PND 84 (g)[b]	270	+3.0	+3.7
F_1 Females (Postweaning): Noteworthy Findings			
Gestating Body Weight - GD 15 (g)[b]	358	+0.8	+0.8
Necropsy Observations	No GS-6207-related findings		
Mean Age of Vaginal Patency (days)	32.1	32.1	32.8
Auditory Startle Response	No GS-6207-related findings		

Test Article: LEN

Report Title: A Subcutaneous Injection Study of the Effects of GS-6207 on Pre- and Postnatal Development, Including Maternal Function in Sprague Dawley Rats			**Gilead Study No. TX-200-2049** **CRO Study No.** 00604474
Motor Activity	No GS-6207-related findings		
Learning and Memory	No GS-6207-related findings		
Mean No. Days Prior to Mating	2.8	2.4	2.6
No. of Females with Evidence of Mating	22	21	22
No. of Pregnant Females	20	20	22
Mean No. Corpora Lutea	17.4	18.2	16.7
Mean No. Implantations	16.0	16.2	15.0
Mean % Preimplantation Loss	7.5	10.4	11.0
F_2 Intrauterine Survival	No GS-6207-related findings		

BLQ = Below the lower limit of quantitation (<state limit here> ng/mL); LEN = lenacapavir (GS-6207); NA = Not Applicable; NOAEL = No Observed Adverse Effect Level; * = $p \leq 0.05$ (Dunnett's test); ** = $p \leq 0.01$ (Dunnett's test)

a Presented as total occurrence/number of animals.
b For controls, group means are shown. For treated groups, percent differences from controls are shown. Statistical significance is based on actual data not on the percent.

15. STUDIES ON JUVENILE ANIMALS

Not applicable.

16. LOCAL TOLERANCE

Test Article: LEN

Type of Study	Species / Strain	Method of Administration (Vehicle)	Duration of Dosing	Dose (mg)	Gender and No. Per Group	Noteworthy Findings	Gilead Study No. (CRO Study No.)
Phototoxicity (in vitro)[a]	Fibroblasts/ Mouse/ BALB/c 3T3 cells (clone A31)	In vitro (DMSO/DPBS)	90 minutes	1.78, 3.16, 5.62, 10.0, 17.8, 31.6, 56.2, 100 µg/mL	NA	A LEN cytotoxicity IC_{50} was not achieved in the assay. The LEN phototoxicity IC_{50} values were not achieved and 89.079 µg/mL in Definitive Assays 1 and 2, respectively. The Photoirritancy Factor[b] (PIF) values were *1 and 1.123 but the Mean Photo Effect[c] (MPE) values were 0.112 and 0.111 in Definitive Assays 1 and 2, respectively. The mean PIF value was >1 but with the mean MPE value of less than 0.15 is of questionable toxicological relevance for systemic drugs (Note 4 of the ICH S10 guidance). However, the MPE value in Definitive Assay 2 is below 0.15. Therefore, LEN is not predicted to have phototoxic potential.	TX-200-2013 ([redacted] 20129744)
Ocular Irritation Assay/Bovine Cornea	Bovine cornea	In vitro (undiluted test article)	Opacity assessment: 4 hours Permeability assessment: 90 minutes	750 mg	3 Corneas	Nonirritant	TX-200-2051 ([redacted] 8426789)

Test Article: LEN

Type of Study	Species / Strain	Method of Administration (Vehicle)	Duration of Dosing	Dose (mg)	Gender and No. Per Group	Noteworthy Findings	Gilead Study No. (CRO Study No.)
Skin Corrosion Assay/ EpiDerm™ human skin	EpiDerm™ human skin	In vitro (PBS)	3 or 60 minutes	25 mg	2 tissues per test article per exposure duration	Noncorrosive	TX-200-2052 ([redacted] 8426790)
Hemolytic Potential and Plasma Compatibility (in vitro)[a]	Rat, Dog, Human/Blood and Plasma	In vitro (vehicles[d]: PEG 300, water and NaOH)	40 minutes	Vehicle 1: 0.2, 2 mg/mL (rat and dog), 3 mg/mL (human) Vehicle 2: 30 mg/mL (dog, human)	NA	LEN in vehicle 1 and vehicle 1 alone resulted in minimal to moderate hemolysis of rat, dog and human whole blood, and macroscopic and microscopic observations of cloudy appearance/ flocculation in plasma LEN in vehicle and vehicle 2 alone resulted in moderate and minimal, respectively, hemolysis in dog whole blood but not human whole blood. LEN in vehicle 2 resulted in microscopic and macroscopic observations of cloudy appearance and flocculation with dog and human plasma but vehicle 2 alone did not.	TX-200-2034 ([redacted] 20129744)
Single-Dose Local Tolerance[a]	Rabbits/Hra:(NZW)SPF	Subcutaneous injection/ (vehicle)[e]	72 hours and 1 month	0, 50 and 100 mg	M:6	Clinically tolerated. Slight to marked granulomatous inflammation, considered adverse when noted as moderate or marked although showed evidence of reversibility.	TX-200-2010 ([redacted] 8366190)

Test Article: LEN

Type of Study	Species / Strain	Method of Administration (Vehicle)	Duration of Dosing	Dose (mg)	Gender and No. Per Group	Noteworthy Findings	Gilead Study No. (CRO Study No.)
Single-Dose Local Tolerance[a]	Rabbits/Hra:(NZW)SPF	Subcutaneous injection/ (vehicle)[f]	72 hours and 1 month	0, 150 and 300 mg	M:6	Clinically tolerated. Nonadverse edema correlated with thickening and mixed cell or granulomatous inflammation, necrosis, and mononuclear cell infiltrates at the subcutaneous injection site of animals administered LEN.	TX-200-2033 ([redacted] 8390752)
Single-Dose Local Tolerance[a]	Rabbits/Hra:(NZW)SPF	Subcutaneous injection/ (vehicle)[g]	72 hours and 1 month	0, 300 mg	M:6	Clinically tolerated. Dermal findings included very slight edema and very slight erythema at Dose site B which resolved by Day 15. Based on the resolution of dermal observations by Day 15, transient clinical observations, and the minimal severity of microscopic findings from Day 29, the dose level of 500 mg/mL (0.6 mL) LEN was considered tolerated.	TX-200-2048 ([redacted] 8420104)
Single-Dose Local Tolerance[a]	Rabbits/Hra:(NZW)SPF	Subcutaneous injection/ (vehicle)[h]	72 hours and 1 month and 3-months	0, 300 mg	F:9	Clinically tolerated. **300 mg:** Edema[i]; tan discoloration at injection site (Terminal Sacrifice 1); thickened injection site (Terminal Sacrifice 2 and 3); necrosis at injection site (Terminal Sacrifice 1); mixed cell inflammation at injection site (Terminal Sacrifice 1); granulomatous inflammation at injection site (Terminal Sacrifice 2 and 3)[j].	TX-200-2038 ([redacted] 8397773)

Test Article: LEN

Type of Study	Species / Strain	Method of Administration (Vehicle)	Duration of Dosing	Dose (mg)	Gender and No. Per Group	Noteworthy Findings	Gilead Study No. (CRO Study No.)
Single-Dose Local Tolerance[a]	Rabbits/Hra:(NZW)SPF	Subcutaneous injection/ (vehicle)[k]	72 hours and 3, 6 and 9-months	0, 100, 200, 400 mg/kg	M:12	Clinically tolerated. LEN related nonadverse edema (day 4-141) and microscopic observations of necrosis and mixed cell inflammation (at 72 hours postdose). Necrosis resolved by 3 months and was replaced by granulomatous inflammation, which ↓ in incidence and/or severity at 6 and 9 months and indicative of the maturation/recovery process.	TX-200-2019 ([redacted] 8379568)

DMSO/DPBS = Dimethylsulfoxide/Dulbecco's phosphate buffered saline supplemented with Ca^{2+} and Mg^{2+} ions; F = Female; LEN = lenacapavir (GS-6207); M = Male; NA =-not applicable; w/v = weight/volume; PBS = phosphate buffered saline

a Indicates GLP compliant.
b Photoirritancy Factor: the criterion for "phototoxic" is PIF >5.
c Mean Photo Effect: the criterion for "phototoxic" is MPE >0.15.
d Vehicle 1: 67.0% polyethylene glycol 300 (PEG 300) and 33.0% (w/w) sterile water for injection with 1.0 molar equivalent NaOH for each mole of active LEN, Vehicle 2: 6.12% (w/w) PEG 300 and 93.88% (w/w) sterile water for injection with 1.0 molar equivalent NaOH for each mole of active LEN
e Vehicle Control Article 1 for Test Article 1**:** 2% (w/v) poloxamer 188, 0.76% (w/v) sodium chloride in 20 mM phosphate buffer; Vehicle Control Article 2 for Test Article 2**:** 2% (w/v) poloxamer 188, 4.74% mannitol (w/v) in 20 mM Phosphate buffer; 0.5 mL (50 mg) or 1.0 mL (100 mg) of LEN in Vehicle Control Article 1 is TA 1; 0.5 mL (50 mg) or 1.0 mL (100 mg) of GS-6207 in Vehicle Control Article 2 is TA 2.
f 67.0 % (w/w) polyethylene glycol 300 (PEG 300) and 33.0% (w/w) sterile water for injection.
g 52.81% (w/w) polyethylene glycol 300, 24.66% (w/w) sterile water for injection, 8.78% (w/w) ethanol, and 13.75% (w/w) poloxamer 188.
h 63.0% w/w polyethylene glycol 300, 30.8% w/w water for injection, and 6.2% w/w poloxamer 188.
i Edema (very slight to severe) was noted on Day 4 (72 hours postdose) and on Days 15, 29, 36, 43, 50, and 57 in animals administered 300 mg/mL. This was considered not adverse as it had no impact on the health of the animals.
j At Terminal Sacrifice 2, a LEN-related finding of marked or severe granulomatous inflammation was observed. At Terminal Sacrifice 3, LEN-related granulomatous inflammation was still evident, but the severity had reduced to slight or moderate, which indicated partial reversal.
k 200 mg/mL LEN was formulated in one of two vehicles: 77:10:13 w/w/w/ PEG 200:ethanol:sterile water for injection, with or without NaOH, to achieve 1.0 molar equivalent NaOH for each mole of active LEN; 400 mg/mL LEN was formulated in 77:10:13 w/w/w/ PEG 200:ethanol:sterile water for injection. LEN formulated TA= solution

17. OTHER TOXICITY STUDIES

Test Article: LEN

Type of Study	Species / Strain	Method of Administration (Vehicle)	Duration of Dosing	Dose (mg/kg)	Gender and No. Per Group	Noteworthy Findings	Gilead Study No. (CRO Name and Study No.)
In Chemico Skin Sensitization-DPRA	synthetic peptides containing either lysine or cysteine	In vitro (GS-6207 dissolved in 1:1 mixture of water and acetonitrile)	24±2 hours	1:10 and 1:50 ratios with the cysteine and lysine peptides	N/A	LEN, was considered to be positive with low reactivity in the Direct Peptide Reactivity Assay	TX-200-2071 ([redacted] 8426791)
Skin Sensitization-ARE-Nrf2 Luciferase	HaCaT human keratinocytes	In vitro (GS-6207 in dimethyl sulphoxide)	48±1 hours	Concentrations ranging from 0.98 to 2000 µM	3 plates	Nonsensitizer	TX-200-2072 ([redacted] 8426792)
Skin Sensitization/ h-CLAT	Human monocytic leukemia cell line	In vitro (GS-6207 in dimethyl sulphoxide)	30 minutes	Concentration ranging from 17.53-62.80 µg/mL	N/A	LEN was considered to be positive in the human Cell Line Activation Test, at the maximum attainable concentration.	TX-200-2073 ([redacted] 8426793)
Skin Sensitization local lymph node assay	Mouse/CBA/Ca Crl	Topical (in dimethylformamide)	3 days	10%, 25% 50%	Females 5/group	Nonsensitizer	TX-200-2053 ([redacted] 8456125)

Test Article: LEN

Type of Study	Species / Strain	Method of Administration (Vehicle)	Duration of Dosing	Dose (mg/kg)	Gender and No. Per Group	Noteworthy Findings	Gilead Study No. (CRO Name and Study No.)
Impurity (*不純物1)/Bacterial Mutation	Salmonella typhimurium TA98, TA100, TA1535, and TA1537; E. coli WP2uvrA Study	In vitro (in dimethyl sulphoxide)	3 days	5-5000 µg/mL	N/A	*不純物1 did not induce mutation in four histidine-requiring strains of *Salmonella typhimurium* (TA98, TA100, TA1535 and TA1537) and one tryptophan-requiring strain of *Escherichia coli* (WP2 *uvrA*)	TX-200-2025 ([redacted] 8382042)
Impurity *不純物2 /Bacterial Mutation	Salmonella typhimurium TA98, TA100, TA1535, and TA1537; E. coli WP2uvrA Study	In vitro (in dimethyl sulphoxide)	48-72 hours	25-5000 µg/mL	N/A	*不純物2 did not induce mutation in four histidine-requiring strains of *Salmonella typhimurium* (TA98, TA100, TA1535 and TA1537) and one tryptophan-requiring strain of *Escherichia coli* (WP2 *uvrA*)	TX-200-2041 ([redacted] AF65BC.502005ICH.BTL)

Test Article: LEN

Type of Study	Species / Strain	Method of Administration (Vehicle)	Duration of Dosing	Dose (mg/kg)	Gender and No. Per Group	Noteworthy Findings	Gilead Study No. (CRO Name and Study No.)
Impurity *不純物3 /Bacterial Mutation	Salmonella typhimurium TA98, TA100, TA1535, and TA1537; E. coli WP2uvrA Study	In vitro (in dimethyl sulphoxide)	3 days	5-5000 µg/mL	N/A	*不純物3 induced mutation in three histidine-requiring strains TA98, TA100 and TA1537, and one tryptophan-requiring strain WP2 *uvrA* of *Escherichia coli* in the presence of S-9 when tested under the conditions of this study	TX-200-2023 ([redacted] 8381836)
Impurity *不純物4 /Bacterial Mutation	Salmonella typhimurium TA98, TA100, TA1535, and TA1537; E. coli WP2uvrA Study	In vitro (in dimethyl sulphoxide)	3 days	5-5000 µg/mL	N/A	*不純物4 did induce mutation in histidine-requiring strain *Salmonella typhimurium* TA100 when tested in the presence of a rat liver metabolic activation system (S-9) under the conditions of this study.	TX-200-2024 ([redacted] 8382041)

Test Article: LEN

Type of Study	Species / Strain	Method of Administration (Vehicle)	Duration of Dosing	Dose (mg/kg)	Gender and No. Per Group	Noteworthy Findings	Gilead Study No. (CRO Name and Study No.)
Impurity (*不純物1)/ Chromosome aberration / Cultured human peripheral blood lymphocytes	Human Peripheral Blood Lymphocytes	In vitro (in dimethyl sulphoxide)	4 hours (± S9) or 20 hours (-S9 only)	4 h without S9: 10, 25 and 70 µg/mL; 20 h without S9; 5, 15, 25 µg/mL; 4 h with S9: 5, 25, 50 µg/mL	N/A	LEN is considered negative for inducing chromosomal aberrations in cultured human lymphocytes without and with metabolic activation when tested	TX-200-2075 ([redacted] (AG57PA.341ICH.BTL)
Impurity (*不純物5)/ Chromosome aberration /Cultured human peripheral blood lymphocytes	Human Peripheral Blood Lymphocytes	In vitro (in dimethyl sulphoxide)	4 hours (± S9) or 20 hours (-S9 only)	4 hours (± S9) or 20 hours (-S9 only): 21.9, 43.9 and 87.8 µg/mL	N/A	LEN is considered negative for inducing chromosomal aberrations in cultured human lymphocytes without and with metabolic activation when tested	TX-200-2076 ([redacted] AG57PB.341ICH.BTL)
Impurity Qualification	Rat/Crl:WI(Han)	Subcutaneous (Vehicle)[a]	Single dose	0 (vehicle), 100 mg/kg GS-6207-pure; 100 mg/kg GS-6207-A (impurity); 100 mg/kg GS-6207-B (impurity)	**Main-** M:10 F:10 **TK-** Control: M:3 F:3 Treated: M:6 F:6	**NOAEL = 100 mg/kg** No test article-related findings	TX-200-2042 ([redacted] 8407444)

Test Article: LEN

Type of Study	Species / Strain	Method of Administration (Vehicle)	Duration of Dosing	Dose (mg/kg)	Gender and No. Per Group	Noteworthy Findings	Gilead Study No. (CRO Name and Study No.)
Impurity Qualification	Rat/Crl:WI(Han)	Subcutaneous (Vehicle)[a]	Single dose	0 (vehicle), 100 mg/kg GS-6207-pure; 100 mg/kg GS-6207-A (impurity); 100 mg/kg GS-6207-B (impurity)	**Main-** M:10 F:10 Control: M:3 F:3 Treated: M:27 F:27	**NOAEL = 100 mg/kg** No test article-related findings	TX-200-2050 ([redacted] 8423935)
Transgenic Mice	Mouse/ RasH2 [001178-W (wild type), CByB6F1-Tg(HRAS)2Jic]	Subcutaneous (Vehicle)[a]	Single dose	0, 30, 99, 300	**Main-** M:10 F:10 TK-Control: M:3 F:3 Treated: M:27 F:27	LEN-related clinical observations included palpable masses and abnormal skin color at the dose site for animals administered 300 mg/kg, which correlated with microscopic observations of minimal to marked granulomatous inflammation at the subcutaneous injection site. Minimal to moderate granulomatous	TX-200-2045 ([redacted] 8411930)

Test Article: LEN

Type of Study	Species / Strain	Method of Administration (Vehicle)	Duration of Dosing	Dose (mg/kg)	Gender and No. Per Group	Noteworthy Findings	Gilead Study No. (CRO Name and Study No.)
						inflammation was also observed at the subcutaneous injection site in animals administered 30 or 99 mg/kg. ↑ALT in one female each at ≥30 mg/kg; ↑liver weights for males at 300 mg/kg correlated with microscopic findings of slight centrilobular hepatocyte vacuolation. LEN systemic NOAEL is 300 mg/kg. The local NOAEL is 300 mg/kg for males and 99 mg/kg for females	

Test Article: LEN

Type of Study	Species / Strain	Method of Administration (Vehicle)	Duration of Dosing	Dose (mg/kg)	Gender and No. Per Group	Noteworthy Findings	Gilead Study No. (CRO Name and Study No.)
Single dose PK	Rat/Crl:WI(Han)	Oral (Vehicle: Capmul MCM EP/NF)	Single dose	0, 3, 30	M:6 F:6	The mean concentration-time profiles for males, females, and combined sexes showed that exposure to LEN increased with the increase in dose level from 3 to 30 mg/kg. Exposure was generally similar in animals administered 30 mg/kg at a dose volume of either 2 or 0.6 mL/kg.	TX-200-2026 ([redacted] 8384035)
Tolerability	Rabbit/ Hra:[NZW]SPF	Oral (100% Capmul MCM, NF/solution)	7-day	0, 100, 300, 1000	F:3	**NOAEL = 1000 mg/kg** No treatment-related findings	TX-200-2035 ([redacted] 00604415)
Assessment of Subcutaneous Depot	Rat/Crl:WI(IGS)	Subcutaneous infusion	Single dose	0, 6, 30, 240 mL/infusion rate (300 mg/mL)	M:3	Both vehicles and all LEN dosed groups presented similar depot volume immediately following	TX-200-2027 ([redacted] 6800022)

Test Article: LEN

Type of Study	Species / Strain	Method of Administration (Vehicle)	Duration of Dosing	Dose (mg/kg)	Gender and No. Per Group	Noteworthy Findings	Gilead Study No. (CRO Name and Study No.)
						dosing. Based on results obtained, the infusion rate and needle gauge didn't seem to impact depot size or heterogeneity.	

ALT = alanine aminotransferase; ARE = antioxidant response element; EOW = every other week; F = female; DPRA = Direct Peptide Reactivity Assay; HaCaT = Cultured Human Keratinocyte (cells); h-CLAT=human Cell Line Activation Test; LEN = lenacapavir (GS-6207); M = male; N/A = non-applicable NOAEL = no observed adverse effect level; NOEL = no observed effect level; Nrf2 = nuclear factor erythroid 2-related factor 2; PK= pharmacokinetics; ↑ = increase

a 67.24% w/w polyethylene glycol 300, 31.40% w/w water for injection, and 1.36% w/w poloxamer 188
b 63.7% w/w polyethylene glycol 300, 29.7% w/w water for injection, and 6.6% w/w poloxamer 188