SECTION 2.6.6—TOXICOLOGY WRITTEN SUMMARY

BICTEGRAVIR/EMTRICITABINE/TENOFOVIR ALAFENAMIDE FIXED-DOSE COMBINATION (B/F/TAF FDC)

Gilead Sciences

[redacted] **20**

CONFIDENTIAL AND PROPRIETARY INFORMATION

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LIST OF ABBREVIATIONS

^{3}HTdR	^{3}H-methyl thymidine
8-MOP	8-methoxypsoralen
ALP	alkaline phosphatase
ALT	alanine aminotransferase
API	active pharmaceutical ingredient
APPT	activated partial thromboplastin time
ARV	antiretroviral
AST	aspartate amino transferase
ATP	adenosine triphosphate
AUC	area under the concentration versus time curve
AUC_{x-xx}	partial area under the concentration versus time curve from time "x" to time "xx"
AUC_{inf}	area under concentration versus time curve extrapolated to infinite time, calculated as $AUC_{last} + (C_{last}/\lambda_z)$
AUC_{ss}	area under the concentration versus time curve at steady state
AUC_{tau}	area under the concentration versus time curve over the dosing interval
B/F/TAF	bictegravir/emtricitabine/tenofovir alafenamide (coformulated)
B-ALP	bone alkaline phosphatase
BCOP	bovine corneal opacity permeability
BIC	bictegravir (GS-9883)
GS-004*	[redacted]
BID	twice daily
BMD	bone mineral density
BUN	blood urea nitrogen
cAMP	cyclic adenosine monophosphate
CatA	cathepsin A
CD	cluster determinant
CHMP	Committee for Medicinal Products for Human Use
CHO	Chinese Hamster ovary
CK	creatinine kinase
CL	clearance
CL/F	apparent oral clearance after administration of the drug: $CL/F = Dose/AUC_{inf}$, where "Dose" is the dose of the drug
CL_{ss}/F	apparent oral clearance at steady state: $CL/F = Dose/AUC_{inf}$, where "Dose" is the dose of the drug
C_{max}	maximum observed concentration of drug
COBI	cobicistat (Tybost®)
CSF	cerebrospinal fluid
CYP	cytochrome P450 enzyme
DMSO	dimethylsulfoxide
DNA	deoxyribonucleic acid

* 新薬承認情報提供時に置き換え

Dpd	deoxypyridinoline
DTG	dolutegravir
DXA	dual-emission X-ray absorptiometry
ECG	electrocardiogram
ELISA	enzyme-linked immunosorbent assay
EMA	European Medicines Agency
EVG	elvitegravir (Vitekta®)
f.b.e.	free base equivalents
F/TAF	emtricitabine/tenofovir alafenamide (coformulated; Descovy®)
FDA	Food and Drug Administration
FDC	fixed-dose combination
FTC	emtricitabine (Emtriva®)
FTC/TDF	emtricitabine/tenofovir disoproxil fumarate (coformulated; Truvada®)
FTU	5-fluorouracil
GALT	gut-associated lymphoid tissue
GD	gestation day
GGT	gamma-glutamyltransferase
GHS	Globally Harmonized System of Classification and Labeling of Chemicals
GI	gastrointestinal
Gilead	Gilead Sciences
GLP	Good Laboratory Practice
GMP	Good Manufacturing Practice
Hb	hemoglobin
HCT	hematocrit
HIV	human immunodeficiency virus
HIV-1	human immunodeficiency virus type 1
HPMC	hydroxypropylmethylcellulose
ICH	International Council for Harmonisation (of Technical Requirements for Pharmaceuticals for Human Use)
IND	investigational new drug
INSTI	integrase strand-transfer inhibitor
IVIS	in vitro irritancy score
λ_z	terminal elimination rate constant, estimated by linear regression of the terminal elimination phase of the log plasma/serum concentration of drug versus time curve of the drug
LC/MS	liquid chromatography/mass spectrometry
LD	lactation day
LLNA	local lymph node assay
LNC	lymph node cell
MATE1	multidrug and toxin extrusion protein 1
MATE2-K	multidrug and toxin extrusion protein 2-K

MCH	mean corpuscular hemoglobin
MCV	mean corpuscular volume
NA	not applicable
NAG	N-acetyl-β-glucosaminidase
NC	not calculated (due to insufficient data)
NCE	normochromatic erythrocyte
NDA	new drug application
NK	natural killer
NNRTI	nonnucleoside reverse transcriptase inhibitor
NOAEL	no observed adverse effect level
NOEL	no observed effect level
NRTI	nucleoside reverse transcriptase inhibitor
NtRTI	nucleotide reverse transcriptase inhibitor
NZW	New Zealand White (rabbit)
OECD	Organization for Economic Cooperation and Development
PBMC	peripheral blood mononuclear cell
PBS	phosphate buffered saline
PCE	polychromatic erythrocyte
PDCO	Pediatric Development Committee
P-gp	P-glycoprotein
PIP	pediatric investigation plan
PK	pharmacokinetic(s)
PM	premating
PND	postnatal day
PP	postpairing
PR interval	electrocardiographic interval occurring between the onset of the P wave and the QRS complex representing time for atrial and ventricular depolarization, respectively
PRTD	proximal renal tubular dysfunction
PSP	pediatric study plan
PTH	parathyroid hormone
QT	interval between the start of the Q wave and the end of the T wave on ECG
RAL	raltegravir
RBC	red blood cell
RDW	red cell distribution width
sALP	skeletal alkaline phosphatase
SC	subcutaneous
SD	standard deviation
SI	test-to-control ratio
SIV	simian immunodeficiency virus
STB	elvitegravir/cobicistat/emtricitabine/tenofovir disoproxil fumarate (coformulated; Stribild®)

$t_{1/2}$	estimate of the terminal elimination half-life of the drug, calculated by dividing the natural log of 2 by the terminal elimination rate constant (λ_z)
T3	triiodothyronine
TAF	tenofovir alafenamide (Vemlidy®)
TDF	tenofovir disoproxil fumarate (Viread®)
TFV	tenofovir
TFV-DP	tenofovir diphosphate
TID	3 times daily
TK	toxicokinetic
T_{max}	time (observed time point) of C_{max}
TSH	thyroid-stimulating hormone
TVD	emtricitabine/tenofovir disoproxil fumarate (coformulated; Truvada®)
UN	United Nations
US	United States
USP	United States Pharmacopeia
UV	ultraviolet
UVA	ultraviolet A
UVB	ultraviolet B
WBC	white blood cell

NOTE TO REVIEWER

This application is being submitted for a fixed-dose combination (FDC) that contains the integrase strand-transfer inhibitor (INSTI) bictegravir (BIC, B), the nucleoside reverse transcriptase inhibitor (NRTI) emtricitabine (FTC, F, Emtriva®), and the nucleotide reverse transcriptase inhibitor (NtRTI) tenofovir alafenamide (TAF); the B/F/TAF FDC (50/200/25 mg) tablet. All nonclinical safety studies to support the B/F/TAF FDC application are included. This comprises all nonclinical studies utilizing BIC, FTC, or TAF, and includes relevant combination studies, including FTC/tenofovir disoproxil fumarate (TDF); and other studies necessary to support the proposed product labeling.

In the development of TAF, 3 forms of the active drug substance have been used: GS-7340, synonym for GS-7340 as the free base; GS-7340-02, synonym for GS-7340 as the monofumarate (1:1 molar ratio of free base to fumaric acid), and GS-7340-03, synonym for the hemifumarate (2:1 molar ratio of free base to fumaric acid). The hemifumarate, GS-7340-03 (TAF fumarate) has been selected as the form for final development. GS-7340-03 is considered comparable to GS-7340-02 based on physical/chemical properties and both exist as the free base in blood and biological fluids. Repeat-dose toxicity studies utilized GS-7340-02 but any potential effects of the hemifumarate have been evaluated by studies of the monofumarate. The hemifumarate, GS-7340-03 was used in the male and female fertility study, dermal and ocular irritation studies, the local lymph node assay, and a second impurity qualification study. Although all doses are provided as both the respective form and the free base equivalents (f.b.e.), the following conversion table is provided as a reference.

GS-7340 Equivalents		
mg GS-7340 as the free base (GS-7340)	**mg GS-7340 as the hemifumarate (GS-7340-03)**	**mg GS-7340 as the monofumarate (GS-7340-02)**
0.8	0.9	1
4	4.5	5
8	9	10
12	13	15
16	18	20
20	22	25
24	27	30
32	36	40
36	39	45
40	45	50
60	66	75
80	90	100
240	270	300
400	450	500
800	900	1000

The following conversions are also provided to aid the reviewer:

1 µM BIC (GS-9883) = 0.449 µg/mL

1 ng/mL BIC = 2.22 nM

1 µM FTC (GS-9019) = 0.247 µg/mL

1 ng/mL FTC = 4.05 nM

1 µM TFV (GS-1278) = 0.287 µg/mL

1 ng/mL TFV = 3.48 nM

1 µM TAF (GS-7340) = 0.477 µg/mL

1 ng/mL TAF = 2.10 nM

1. BRIEF SUMMARY

This dossier is being submitted in support of a marketing application for a fixed dose combination (FDC) of bictegravir (BIC, B, GS-9883), emtricitabine (FTC, F, GS-9019) and tenofovir alafenamide (TAF, GS-7340): the B/F/TAF (50/200/25 mg) FDC. Bictegravir is a low molecular weight HIV-1 integrase strand transfer inhibitor (INSTI) active against a broad panel of HIV-1 viral lab strains and clinical isolates and is fully active against a panel of mutant viruses with resistance to nucleoside reverse transcriptase inhibitors (NRTI), non-nucleoside reverse-transcriptase inhibitors (NNRTI) and protease inhibitors (PI). Emtricitabine is a nucleoside reverse transcriptase inhibitor (NRTI) and is approved for the treatment of HIV-1 infection as a single agent (Emtriva®) for use in combination with other ARVs for the treatment of HIV-1 infection, and in the FDC products Truvada® (FTC/tenofovir disoproxil fumarate [TDF]), Atripla® (efavirenz/FTC/TDF), Complera®/ Eviplera® (FTC/rilpivirine [RPV]/TDF), Stribild® (elvitegravir [EVG; E]/cobicistat [COBI; C]/ FTC/TDF), Genvoya® (E/C/F/TAF), Descovy® (F/TAF) and Odefsey® (FTC/RPV/TAF). Tenofovir alafenamide is a prodrug of tenofovir (TFV), a nucleotide reverse transcriptase inhibitor (NtRTI). Tenofovir alafenamide is approved for the treatment of HIV-1 infection in the FDC products Genvoya®, Descovy® and Odefsey®. Tenofovir alafenamide is also approved for the treatment of hepatitis B virus (HBV) infection as a single agent (Vemlidy®). Information from all nonclinical studies with FTC, and TAF/TFV should be considered in the context of their clinical experience within ARV combination therapy for the treatment of HIV-1 infection.

Comprehensive programs of nonclinical toxicology studies with BIC, FTC and TAF have been conducted (Table 1). All of the definitive toxicology and toxicokinetic (TK) studies reported in this summary were conducted in accordance with the Good Laboratory Practice (GLP) procedures promulgated by international health authorities. Pilot, exploratory, and mechanistic studies were not all conducted under strict GLP procedures but were conducted using appropriate protocols and documentation to assure data integrity. Based on the well-characterized toxicity profiles of BIC, FTC and TAF, the absence of overlapping toxicities with the individual agents, and the clinical safety data available for B/F/TAF, and other FTC and/or TDF- (TAF-) containing regimens (Emtriva, Truvada, Atripla, Complera/Eviplera, Stribild, Genvoya, Odefsey, Descovy, or Vemlidy), additional combination toxicity studies are not considered warranted.

The nonclinical data discussed within this document support the proposed use of the B/F/TAF FDC for the treatment of HIV-1 infection. All information from nonclinical toxicity studies that is of relevance to the prescriber and patient has been included in the proposed Summary of Product Characteristics and Package Leaflet.

BIC

Bictegravir is a low molecular weight HIV-1 INSTI active against a broad panel of HIV-1 viral lab strains and clinical isolates and is fully active against a panel of mutant viruses with resistance to NRTIs, NNRTIs, and protease inhibitors (PIs).

The nonclinical toxicologic profile of BIC has been well characterized in a variety of in vivo and in vitro studies. The rat and monkey were demonstrated to have in vivo metabolic profiles similar to human (m2.6.4, Section 5) and were, therefore, chosen as the rodent and non-rodent

nonclinical species. With the exception of the initial investigational new drug (IND)-enabling studies conducted with BIC as a free base form, a sodium salt was utilized for the toxicology program; all dose levels stated are represented as free base equivalents (f.b.e.) of BIC. BIC was evaluated in mouse, rat, and monkey repeat-dose toxicity studies. In addition, BIC was evaluated in rat for an in vivo genetic toxicity study, in rat and rabbit for embryo-fetal developmental, in rat for fertility and pre/postnatal toxicity and in a transgenic mouse model for carcinogenicity assessment. BIC was dosed via oral administration in all in vivo studies, the clinical route of administration, with the exception of studies to characterize the risk profile for workers, such as dermal sensitization and skin and eye irritation. As there were no major circulating Phase 1 metabolites (>10%) in humans requiring assessment in nonclinical species, BIC AUC exposure margins were calculated by comparing BIC exposures in nonclinical species to human exposure with 50 mg BIC in B/F/TAF FDC (BIC AUC_{tau} = 102 µg·h/mL; based upon HIV-1 infected subjects in clinical studies GS-US-380-1489, GS-US-380-1490, GS-US-380-1844, GS-US-380-1878). Bictegravir had similarly high protein binding across species (> 98% bound) and, therefore, exposure margins were based on total drug concentrations.

The vehicle used for pivotal toxicity studies was 0.5% [w/w] hydroxypropylmethylcellulose (HPMC) and 0.1% [w/w] polysorbate 20 [Tween® 20] in reverse osmosis water. For repeat dose toxicity studies, the high doses in transgenic mice (1000 mg/kg/day) and rats (300 mg/kg/day) were selected based on saturation of absorption in each species (m2.6.4, Section 3.2.1.2). The high dose in monkeys was selected based on the International Council for Harmonisation (ICH) recommended limit dose of 1000 mg/kg/day.

The toxicity of BIC following oral dosing was studied in rats and monkeys for treatment periods of up to 39 weeks. No target organs of toxicity were identified in the 26-week rat study at exposure margins ≥18-fold versus clinical exposures in HIV-1 patients with the B/F/TAF FDC. In the 39 week chronic toxicology study in monkeys, hepatobiliary toxicity was the only observed target organ toxicity at a 16-fold exposure margin; the no observed effect level (NOEL) in the chronic monkey study corresponded to a 7.0-fold exposure margin.

Bictegravir was negative for mutagenicity in the bacterial reverse mutation (Ames) assay, negative for the induction of chromosome damage in the chromosome aberration test in human peripheral blood lymphocytes, and negative in an in vivo rat micronucleus study. Bictegravir is considered not genotoxic. Bictegravir demonstrated no carcinogenic potential in a 6-month transgenic mouse study at dose levels up to 100/300 mg/kg/day (males/females), corresponding to exposure margins of ≥15-fold. A 2-year rat carcinogenicity study with BIC is ongoing.

The BIC oral rat fertility study data indicated no effects on male or female reproductive performance, sperm parameters, number of corpora lutea, implantation sites, early and late resorptions, and live fetuses at doses up to 300 mg/kg/day. The NOEL for reproductive and early embryonic toxicity was 300 mg/kg/day, corresponding to a 29-fold exposure margin.

There was no effect on fetal viability or fetal development in pregnant rats administered doses of BIC up to 300 mg/kg/day (NOEL), corresponding to a 36-fold exposure margin. In pregnant rabbits, the high dose of 1000 mg/kg/day showed significant maternal toxicity associated with abortion and decreased fetal weights. The NOEL for maternal and embryofetal toxicity in pregnant rabbits is 300 mg/kg/day, corresponding to a 0.59-fold exposure margin.

In a pre- and postnatal study with BIC, no BIC-related effects were noted and the NOEL for F_0 maternal systemic toxicity, and F_1 neonatal/developmental toxicity, parental systemic toxicity, and reproductive toxicity, and F_2 neonatal/early postnatal toxicity was 300 mg/kg/day, corresponding to a 30-fold exposure margin.

BIC was considered non-phototoxic. Local tolerance toxicity studies concluded that BIC was predicted to be a noncorrosive and nonirritating to skin, and moderately irritating to eyes. BIC did not show the potential to cause skin sensitization.

The impurities and degradation products in the BIC drug substance have been identified and qualified in toxicology studies.

FTC

Emtricitabine (Emtriva) is an NRTI that is marketed as a once-daily capsule (200 mg) and as an oral solution (10 mg/mL) for the treatment of HIV-1 infection. Emtricitabine is a synthetic analogue of the naturally occurring pyrimidine nucleoside, 2′-deoxycytidine that is structurally similar to lamivudine (Epivir®). Intracellularly, FTC is phosphorylated by cellular enzymes to form the active metabolite, emtricitabine triphosphate.

The general systemic (single and repeat dose) toxicity, genotoxicity, carcinogenicity, reproductive toxicity, and immunotoxicity of FTC have been characterized in a variety of in vitro and in vivo studies. The nonclinical toxicity studies demonstrated that FTC was well tolerated after oral dosing for up to a year at doses producing systemic exposure levels in animals much greater than those anticipated in patients treated with the recommended clinical dose.

Treatment-related effects were confined to high-dose groups only and included changes in red blood cell (RBC) parameters, interpreted as a mild, reversible anemia (mice 1 month, 6 months; rat 3 months; monkey 1 year); changes in various organ weights without any associated adverse histopathological effects in rodents (mouse 1 month, 6 months; rat 3 months), increased urine output (mice 6 months), and soft feces (monkeys 1 month, 3 months). No observed effect levels could be established for all treatment-related effects, and in several cases the minor effects observed were reversible after a recovery period.

Emtricitabine was not genotoxic or clastogenic. Long-term carcinogenicity studies in mice and rats showed no evidence of carcinogenic potential at exposures greater than 25 times those observed in humans at the therapeutic dose. Emtricitabine did not adversely affect reproduction or embryo-fetal development. It was relatively noncytotoxic to human cells in vitro, including bone marrow progenitor cells, and did not produce mitochondrial toxicity.

The impurities and degradation products in the FTC drug substance have been identified and qualified in toxicology studies.

TAF

Tenofovir alafenamide is a prodrug of TFV, an NRTI. Tenofovir alafenamide is more stable in plasma than TDF, allowing for it to circulate for a longer period of time and interact with target cells. Tenofovir alafenamide provides enhanced delivery of TFV to the liver and HIV-target cells and tissues, resulting in higher intracellular levels of the active phosphorylated metabolite TFV diphosphate (TFV-DP). In lymphocytes and macrophages, TAF is predominantly hydrolyzed intracellularly to TFV by cathepsin A (CatA) {Birkus 2008}. Once released intracellularly, TFV is metabolized to the active metabolite, TFV-DP {Robbins 1998}, a competitive inhibitor of HIV-1 reverse transcriptase that terminates the elongation of the viral DNA chain {Cherrington 1995, Yokota 1994}. The increased stability of TAF in plasma combined with the enhaced delivery of TFV to the target cells and tissues resulting in subsequent formation of 4-fold (3- to 7-fold at 90% confidence interval) higher intracellular levels of the active phosphorylated metabolite TFV-DP in peripheral blood mononuclear cells (PBMCs) and 90% lower circulating levels of TFV relative to TDF {Markowitz 2011}. These features have the potential to translate into less risk of nephrotoxicity and less decrease (or improvements) in bone mineral density (BMD), both of which are known risks with TDF administration {Department of Health and Human Services (DHHS) 2012, Gilead Sciences Inc 2012, Sax 2014}.

Blood was collected for plasma concentration analysis of TAF and/or TFV in repeat-dose and developmental and reproductive toxicity studies. Based on the pharmacokinetic (PK) profile of TAF in CD-1 mice, Sprague-Dawley rats, and TgRasH2 wild-type mice, TAF has been shown to be rapidly converted to TFV. In vitro plasma studies confirmed that TAF is rapidly hydrolyzed in rat ($t_{1/2}$ = 31 minutes) and mouse ($t_{1/2}$ < 3 minutes). Unlike TDF, TAF is relatively stable in human plasma ($t_{½}$ ~75 minutes), but rapidly converts to TFV inside cells. Shorter duration repeat-dose rodent toxicity studies had TFV but not TAF blood level determinations; repeat-dose nonrodent toxicity studies evaluated both TAF and TFV toxicokinetics. Both TAF and TFV toxicokinetics were evaluated in the rat and rabbit developmental and reproductive toxicity studies. Protein binding of GS-7340-02 was determined; the free fraction in human and beagle dog plasma was 46.8% and 48.0%, respectively (m2.6.4, Section 4.1.3).

The nonclinical toxicologic profile of TAF has been well characterized in a variety of in vivo and in vitro studies. The toxicology program for TAF is provided in Table 1. TAF was evaluated in mouse, rat, dog, and monkey single- and/or repeat-dose toxicity studies for up to 39 weeks in duration. In addition, the mouse was used for an in vivo genetic toxicity test and rat and rabbit were used for developmental and reproductive toxicity studies. All in vivo studies utilized oral administration, the clinical route of administration, with the exception of the sensitization and dermal irritation studies. The rat and dog were demonstrated to have similar in vivo metabolic profiles to humans (m2.6.4, Section 5). The vehicle used for toxicity studies was 1) 25 mM citric acid, 2) 0.5% polysorbate 20, 0.5% carboxymethylcellulose, 0.9% benzyl alcohol, or 3) 0.1% (volume concentration [v/v]) Tween 20 and 0.1% (v/v) HPMC.

Renal tubular karyomegaly was observed in rats and dogs orally administered TAF. Focal areas of minimal renal cortical tubular basophilia and associated minimal nuclear karyomegaly were present in rats administered 400 mg/kg/day for 4 weeks and 100 mg/kg/day for 26 weeks. Renal tubular karyomegaly and/or basophilia were observed in dogs administered 3 and 10 mg/kg/day

for 4 weeks and dogs administered 6 or 18/12 mg/kg/day for at least 13 weeks. Renal cortical tubular degeneration/regeneration (13 weeks) and karyomegaly (13 and 39 weeks) were present in dogs administered 6 or 18/12 mg/kg/day. Atrophy of metaphyseal cancellous bone was observed in rats administered TAF at 100 mg/kg/day for 26 weeks. TAF also increased biochemical markers of bone turnover and decreased serum 1,25-dihydroxy- and 25-hydroxyvitamin D_3 in rats (≥ 25 mg/kg/day) and dogs (≥ 37.5 mg/kg/day for 6 days). In the 39-week dog study, bone mineral density changes at 18/12 mg/kg/day may have been secondary to body weight loss, but these changes were accompanied by a slight but significant decrease in serum 1,25 dihydroxy vitamin D_3 in males only and a significant increase in 25-hydroxyvitamin D_3 in females only.

TAF administered by oral gavage for up to 13 weeks to mice at ≥10 mg/kg/day resulted in adverse degenerative (olfactory) and acute inflammatory (infiltrate neutrophil) changes in the nasal mucosa. Because these changes were not observed in rats, dogs, or monkeys for longer durations of administration, the relevance to humans is unknown and the risk of nasal inflammation in humans is very low.

TAF had no discernible electrocardiograph effect at the low dose of 2 mg/kg/day. There was some evidence at 6 and 18/12 mg/kg/day for an effect to slightly prolong PR intervals (approximately 13% to 24%), which was associated with significant decreases in triiodothyronine (T3) {Kienle 1994, Tribulova 2010}. After the 13-week recovery period, serum T3 values returned to levels similar to the control group animals at the end of the study.

At 18/12 mg/kg/day in dogs, the highest dose tested, a minimal infiltration of histiocytes was present in some organs (eye [choroid plexus, ciliary body], lung, and spleen) in some animals. In-life ophthalmologic examinations were normal. The histiocytic infiltration observed in multiple organs was most likely an indirect drug effect due to general debilitation and were not observed in other repeat-dose toxicity studies. There were no drug-related effects on ophthalmic exams or microscopic exams of ocular tissue observed in repeat-dose toxicity studies in mice (up to 13 weeks), rats (up to 26 weeks), nonhuman primates (4 weeks), or in the 4-week dog toxicology study. The posterior uveitis in dogs administered the highest dose of TAF occurred at 3.7- and 17-fold higher exposure to TAF and TFV, respectively, than that observed in human subjects administered a 25-mg dose of TAF. No difference in distribution was observed between Sprague-Dawley and Long Evans rats, including in the skin and eyes, suggesting no binding to melanin. Because TAF has poor penetration across the blood brain and blood retinal barrier in dogs, it is unlikely that TAF directly caused the observed histiocytic infiltration in the posterior uvea. Based on these data from tissue distribution and toxicology studies, Gilead concludes that the risk of posterior uveitis in humans is very low.

Tenofovir alafenamide was not genotoxic in a battery of in vitro and in vivo assays. The in vitro assays included gene mutation assays with bacterial strains (*Salmonella typhimurium, Escherichia coli*), and a L5178Y gene mutation assay in mouse lymphoma cells. The in vivo evaluation consisted of a mouse bone marrow micronucleus study (at oral doses of 500, 1000, and 2000 mg/kg).

Per separate agreements with the Food and Drug Administration (FDA) and the Committee for Medicinal Products for Human Use (CHMP), carcinogenicity studies were not conducted with TAF. Toxicokinetic data obtained following dosing of TAF to the transgenic mouse and rat clearly demonstrate an inability to measure plasma concentrations of the prodrug, TAF. Although significant plasma levels of the active drug, TFV, were obtained in the rat following chronic dosing with TAF, the TFV exposure was less than that already tested in the TDF carcinogenicity study. Lifetime daily administration of TDF at dosage levels of up to 300 mg/kg/day given for 104 weeks did not reveal any evidence of carcinogenicity in Swiss Crl:CDR-1(ICR)BR mice. At 600 mg/kg/day, a low incidence of duodenal tumors, possibly treatment-related, were observed. Lifetime daily administration of TDF at dosage levels up to 300 mg/kg/day did not reveal any evidence of carcinogenicity in Sprague-Dawley rats.

The TAF oral rat fertility study data indicated dose-related decreases in body weight gain in males and females, but no drug-related changes occurred in male or female fertility endpoints including mating index, fertility index, sperm motility, sperm concentration, number of corpora lutea, implantation sites, early and late resorptions, and live fetuses at doses up to 160 mg free base equivalents/kg/day. The NOAEL for reproductive and early embryonic toxicity was 160 mg/kg/day.

There was no effect on fetal viability or fetal development in pregnant rats administered doses of TAF up to 250 mg/kg/day or in pregnant rabbits administered TAF up to 100 mg/kg/day. The highest doses were maternally toxic. In the rat, decreased fetal body weight associated with some minor transitory delays in the rate of ossification was observed at 250 mg/kg/day, a maternally toxic dose. The NOAEL for embryo-fetal development was 100 mg/kg/day in rats and rabbits.

Per separate agreements with the FDA and CHMP, a perinatal and postnatal study is not planned. As with the carcinogenicity study rationale, there is an inability to measure plasma concentrations of TAF in rats, and TFV exposure after TAF administration was less than that already tested in the TDF peri/postnatal study. The peri/postnatal studies with TDF clearly characterized the potential postnatal toxicity of TFV.

Local toxicity studies concluded that TAF fumarate was predicted to be a noncorrosive/nonsevere eye irritant, and nonirritating/noncorrosive to rabbit skin under semi-occluded conditions. TAF fumarate did not show the potential to cause skin sensitization with repeated application to the dorsal surface of mouse ears.

The impurities and degradation products in the TAF drug substance have been identified and qualified in toxicology studies.

B/F/TAF

In accordance with the CHMP Guideline on the Non-Clinical Development of Fixed Combinations of Medicinal Products (EMEA/CHMP/SWP/258498/2005, January 2008), and Human Immunodeficiency Virus-1 Infection: Developing Antiretroviral Drugs for Treatment, Guidance for Industry (November 2015), nonclinical safety studies with the B/F/TAF combination were not conducted. Based on the well-characterized toxicity profiles of BIC, FTC and TAF, the clinical safety data available from Phase 2 and Phase 3 studies with the B/F/TAF

combination, and the well-established safety profile with the approved FTC- and/or TDF-(TAF-) containing regimens (Emtriva, Truvada, Atripla, Complera/Eviplera, Stribild, Genvoya, Odefsey, Descovy , and Vemlidy), there are no anticipated clinically relevant PK or toxicological interactions anticipated, and data demonstrate an acceptable benefit/risk profile for the proposed use of the B/F/TAF FDC and additional combination toxicity studies are not considered warranted.

Table 1. B/F/TAF GLP Toxicology Programs

Study Type & Duration	Route of Administration	Species	Compound Administered
SINGLE-DOSE TOXICITY			
FTC	Oral, Intravenous	Mouse, rat	FTC
TAF	Oral	Rat, dog	TAF
REPEAT-DOSE TOXICITY			
BIC			
2 Weeks	Oral	Rat, monkey	BIC
4 Weeks	Oral	Transgenic Mice	BIC
26 Weeks	Oral	Rat	BIC
13/39 Weeks	Oral	Monkey	BIC
FTC			
2 Weeks	Oral	Mouse	FTC
4 Weeks	Oral	Mouse, monkey	FTC
13 Weeks	Oral	Rat, monkey	FTC
26 Weeks	Oral	Mouse	FTC
1 Year	Oral	Monkey	FTC
TAF			
6-15 days	Oral	Mouse, rabbit	TAF
4 weeks	Oral	Rat, dog, monkey	TAF
13 weeks	Oral	Mouse	TAF
26 weeks	Oral	Rat	TAF
39 weeks	Oral	Dog	TAF
FTC/TDF			
14 Days	Oral	Rat	FTC/TDF
28 Days	Oral	Dog	FTC/TDF

Study Type & Duration	Route of Administration	Species	Compound Administered
GENOTOXICITY			
BIC			
In vitro nonmammalian system (reverse mutation assay)	In Vitro	Bacteria	BIC
In vitro mammalian cell system (chromosome aberration)	In Vitro	Human peripheral lymphocytes	BIC
In vivo micronucleus assay	Oral	Rat	BIC
FTC			
In vitro nonmammalian system (reverse mutation assay)	In Vitro	Bacteria	FTC
In vitro mammalian cell system (chromosome aberration)	In Vitro	Mouse lymphoma cells	FTC
In vivo micronucleus assay	Oral	Mouse	FTC
TAF			
In vitro nonmammalian system (reverse mutation assay)	In Vitro	Bacteria	TAF
In vitro mammalian cell system (forward mutation)	In Vitro	Mouse lymphoma cells	TAF
In vivo micronucleus assay	Oral	Mouse	TAF
FTC/TDF			
In vitro nonmammalian system (reverse mutation assay)	In Vitro	Bacteria	FTC/TDF
In vitro mammalian cell system (forward mutation)	In Vitro	Mouse lymphoma cells	FTC/TDF
CARCINOGENICITY			
BIC			
6 month	Oral	Transgenic mouse	BIC
2 Years (ongoing)	Oral	Rat	BIC
FTC			
2 Years	Oral	Mouse, rat	FTC
TDF			
2 Years	Oral	Mouse, rat	TDF

Study Type & Duration	Route of Administration	Species	Compound Administered
REPRODUCTIVE TOXICITY			
BIC			
Fertility and early embryonic development	Oral	Rat	BIC
Embryo-fetal development	Oral	Rat, rabbit	BIC
Prenatal and postnatal development	Oral	Rat	BIC
FTC			
Fertility and early embryonic development	Oral	Mouse, rat	FTC
Embryo-fetal development	Oral	Mouse, rabbit	FTC
Prenatal and postnatal development	Oral	Mouse	FTC
TAF			
Fertility and early embryonic development	Oral	Rat	TAF
Embryo-fetal development	Oral	Rat, rabbit	TAF
TDF			
Prenatal and postnatal development	Oral	Rat	TDF
LOCAL TOLERANCE			
BIC			
Eye irritation	Topical	Bovine ex vivo	BIC
Skin irritation, corrosion	Topical	In vitro skin model	BIC
In vitro phototoxicity	In Vitro	In vitro mouse fibroblasts	BIC
In vivo phototoxicity	Oral	Rat	BIC
TAF			
Eye irritation	Topical	Bovine ex vivo	TAF
Skin irritation	Topical	Rabbit	TAF
OTHER STUDIES			
BIC			
Antigenicity - local lymph node assay	Topical	Mouse	BIC
Impurities – qualification studies	Oral	Rat	BIC
Genotoxicity qualification assays	In Vitro	Bacteria	BIC
Investigative 2 week repeat dose	Oral	Rat	BIC
FTC			
Immunotoxicity	Oral	Rat	FTC
Impurities – qualification studies	Oral	Mouse	FTC
Genotoxicity qualification assays	In Vitro	Bacteria, Chinese hamster ovary cells	FTC
TAF			
Antigenicity - local lymph node assay	Topical	Mouse	TAF
Bone evaluation	Oral	Rat, dog	TAF
Renal effects	Oral	Rat	TAF
Impurity evaluation	Oral	Rat	TAF

2. SINGLE DOSE TOXICITY

2.1. BIC

No formal single dose toxicity studies were conducted with BIC. However, single dose PK studies in transgenic mice, rats and monkeys were performed (m2.6.4, Section 3.2.1).

Single oral doses of BIC up to 1500 mg/kg were well-tolerated in transgenic mice, with saturation of absorption observed at 1000 mg/kg (m2.6.5, Section 3.1.2, AD-141-2307). Single oral doses of BIC up to 1000 mg/kg were well-tolerated in Wistar-Han rats (m2.6.5, Section 3.1.4, AD-141-2286), with saturation observed at 300 mg/kg. In monkeys, single oral doses of BIC up to 1000 mg/kg were well-tolerated (m2.6.5, Section 3.1.11, AD-141-2297).

2.2. FTC

2.2.1. Single Oral Dose Toxicity Study in the Mouse

Five male and 5 female CD-1 mice were orally administered 4000-mg/kg FTC (m2.6.7, Section 5.2, TTEP/93/0020). The animals were observed daily for clinical symptoms of toxicity and weighed on Days 0, 7, 13, and 14. On Day 14, they were necropsied. There were no deaths or clinical findings. Emtricitabine was well tolerated and the NOEL was > 4000 mg/kg.

2.2.2. Single Intravenous Dose Toxicity Study in the Mouse

Five male and 5 female CD-1 mice were intravenously administered 200 mg/kg FTC (m2.6.7, Section 5.2, TTEP/93/0023). The animals were observed daily for clinical symptoms of toxicity and weighed on Days 0, 7, 13, and 14. On Day 14, they were necropsied. There were no deaths or clinical findings. Emtricitabine was well tolerated and the NOEL was > 200 mg/kg.

2.2.3. Single Oral Dose Toxicity Study in the Rat

Five male and 5 female CD rats were orally administered 4000 mg/kg FTC (m2.6.7, Section 5.2, TTEP/93/0021). The animals were observed daily for clinical symptoms of toxicity and weighed on Days 0, 7, 13, and 14. On Day 14, they were necropsied. There were no deaths or clinical findings. Emtricitabine was well tolerated and the NOEL was 4000 mg/kg.

2.2.4. Single Intravenous Dose Toxicity Study in the Rat

Five male and 5 female CD rats were intravenously administered 200 mg/kg FTC dissolved in 0.9% saline (m2.6.7, Section 5.2, TTEP/93/0024). The animals were observed daily for clinical symptoms of toxicity and weighed on Days 0, 7, 13, and 14. On Day 14, they were necropsied. There were no deaths or clinical findings and FTC was well tolerated. It was concluded that the approximate lethal dose in rats dosed intravenously was > 200 mg/kg.

2.3. TAF

2.3.1. Single Oral Dose Toxicity in Rats

In a non-GLP study, a single oral dose of 400 mg/kg of GS-7340-02 to rats was generally tolerated (m2.6.7, Section 5.3, R990133). In a GLP study, male and female Sprague-Dawley rats (5/sex/group) were administered single oral dose (15 mL/kg) of GS-7340-02 at doses of 0 (vehicle), 100, 300, or 1000 mg/kg (80, 240, 800 mg/kg f.b.e./kg) followed by a 14-day observation period (m2.6.7, Section 5.3, R990185). The NOAEL for single oral dose GS-7340-02 in the rat was determined to be >1000 mg/kg.

2.3.2. Single Oral Dose Toxicity in the Dog

Male and female beagle dogs (1/sex/group) were administered a single oral dose (15 mL/kg) of GS-7340-02 at doses of 0 (vehicle), 30, 90, or 270 mg/kg (24, 72, 216 mg f.b.e./kg) followed by a 14-day observation period (m2.6.7, Section 5.3, D990181). Once prior to the commencement of treatment and on Days 2 and 14, hematology, clinical biochemistry, and urinalysis were performed on all animals. Mild clinical signs (salivation, vomiting, reduced activity, tremors, incoordination) observed at 270 mg/kg resolved 2 days following dosing. There was a transient increase in blood urea nitrogen in animals administered 270 mg/kg (present on study Day 2, not study Day 14). There were no other significant changes in clinical chemistry, hematology, or urinary parameters 14 days after dosing. Thymus weights were reduced in all treated groups compared with controls, and thymic atrophy was present in males administered 90 and 270 mg/kg. Renal tubular changes characterized by basophilia and/or karyomegaly were present in the male administered 270 mg/kg and the females administered 270 mg/kg and 90 mg/kg. The NOAEL for single oral dose of TAF in the dog was determined to be 30 mg/kg.

2.4. B/F/TAF

No single-dose studies have been performed with the combination of BIC, FTC and TAF. BIC was well-tolerated at high doses in PK studies, and single-dose toxicity studies indicated that FTC and TAF had low acute toxicity. With no overlapping toxicities in longer term studies with the single agents and clinical safety data with the combination, co-administration of BIC, FTC, and TAF is unlikely to change the acute toxicity profile.

3. REPEAT DOSE TOXICITY

3.1. BIC

All repeat dose in vivo toxicity studies utilized oral BIC administration, the clinical route of administration. BIC was administered to transgenic mice, rats or cynomolgus monkeys suspended in an aqueous vehicle consisting of 0.5% HPMC [Methocel K100 LV] and 0.1% polysorbate 20 [Tween®20] in reverse osmosis water (%w/w). The high dose in transgenic mice was based on saturation of absorption at 1000 mg/kg (m2.6.5, Section 3.1.2, AD-141-2307). The high dose in rats was selected based on single dose oral pharmacokinetic studies with multiple formulations showing saturation of absorption at 300 mg/kg/day (m2.6.5, Section 3.1.4, AD-141-2286). The high dose in monkeys was selected based on PK studies and the ICH recommended limit dose of 1000 mg/kg/day (m2.6.5, Section 3.1.11, AD-141-2297). For toxicokinetic (TK) analyses, BIC was quantified in plasma. BIC AUC exposure margins were calculated by comparing BIC exposures in nonclinical species to human exposure with 50 mg BIC in B/F/TAF FDC (BIC AUC_{tau} = 102 µg·h/mL; based upon HIV-1 infected subjects in clinical studies GS-US-380-1489, GS-US-380-1490, GS-US-380-1844, GS-US-380-1878).

3.1.1. 4-Week Oral (Gavage) Toxicity Study in Transgenic Mice

This range-finding study evaluated the toxicity and TK of BIC when administered daily via oral gavage to 001178-W (wild type) RasH2 mice for 4 weeks (m2.6.7, Section 7.1.1, TX-141-2042) and served to determine dose levels for a 26-week carcinogenicity study in transgenic mice (m2.6.7, Section 10.1, TX-141-2047). Male and female mice (10 toxicity animals/sex/group) received BIC at 0, 30, 100, or 1000 mg/kg/day at a dose volume of 5 mL/kg. Blood samples were collected from satellite groups of animals for TK evaluation on Day 1 and Week 4. Assessment of toxicity was based on mortality, clinical observations, body weight, food consumption, and clinical and anatomic pathology.

No BIC-related mortality, clinical observations, food consumption changes or effects on hematology parameters were observed. No BIC-related organ weight or macroscopic changes were noted. No BIC-related effects were noted on body weight or clinical chemistry results for females. Nonadverse, minor, decreased mean body weight change (significant in the last week only) and slightly increased glucose (+16% compared to controls) were observed in males given 1000 mg/kg/day; these were considered of uncertain relationship to BIC. Nonadverse, minimally to slightly decreased centrilobular hepatocellular glycogen was observed in the livers of males administered 1000 mg/kg/day.

Plasma exposure to BIC generally increased with the increase in dose level from 30 to 1000 mg/kg/day (Table 2). Increases in C_{max} and AUC were less-than-dose proportional between 30 and 1000 mg/kg/day. Gender-based differences were less than 2-fold in C_{max} and AUC values. No accumulation of BIC was observed after multiple doses in mice.

Table 2. Combined Male/Female Toxicokinetic Parameters for BIC in 001178-W (Wild Type) RasH2 Mice – Day 1 and Week 4

BIC Dose Level (mg/kg/day)	Study Day/Week	C_{max} (µg/mL)	AUC_{0-24} (µg·h/mL)
30	Day 1	57.7	644
	Week 4	57.7	629
100	Day 1	109	1300
	Week 4	96.8	1180
1000	Day 1	149	2490
	Week 4	158	2330

In summary, BIC was well-tolerated up to 1000 mg/kg/day following daily oral gavage administration to male and female mice for 4 weeks with no adverse effects. The NOAEL is 1000 mg/kg/day, corresponding to Week 4 mean C_{max} and AUC values of 158 µg/mL and 2330 µg·h/mL, respectively, for males and females combined. At the NOAEL in this study, mean BIC AUC is 23-fold higher than BIC AUC following clinical administration of the B/F/TAF FDC.

3.1.2. 2-Week Oral (Gavage) Toxicity and Toxicokinetic Study in Rats

This study evaluated the toxicity and determined the TK of BIC when administered daily via oral gavage to rats for 2 weeks (m2.6.7, Section 7.1.2, TX-141-2029). Male and female Crl:WI(Han) rats (10/sex/group) received BIC at 0, 10, 30, 100 or 300 mg/kg/day at a dose volume of 5 mL/kg. Blood samples were collected from satellite groups of animals for TK evaluation on Days 1 and 14. Assessment of toxicity was based on mortality, clinical observations, body weight, food consumption, ophthalmic examinations, and clinical and anatomic pathology. Additionally, liver was collected for hepatic microsomal enzyme analysis and bone marrow was collected for micronucleus assay evaluation at necropsy on Day 15 (results summarized in Section 4.1.3).

No BIC-related mortality, clinical observations, body weight changes, food consumption changes, ophthalmic examination findings, or anatomic pathology findings, including organ weights were noted. BIC-related effects on hematology were limited to minimally lower red blood cell count (-5%) and hematocrit (-4%) for females administered 300 mg/kg/day and minimally higher phosphorus (+10%) for males administered 300 mg/kg/day compared to vehicle control values; these changes were not considered toxicologically important due to their small magnitude. No other BIC related clinical pathology findings were observed and there were no BIC-related changes in hepatic microsomal enzyme levels. BIC did not induce statistically significant increases in micronucleated PCEs.

Plasma exposure to BIC increased with increasing dose level from 10 to 100 mg/kg/day (Table 3). The increases in C_{max} and AUC were less than dose-proportional between 10 and 100 mg/kg/day. Both C_{max} and AUC were similar between the 100 and 300 mg/kg/day dose levels, indicative of saturation of exposure. Gender-based differences in BIC C_{max} and AUC values were less than 2-fold. No significant accumulation of BIC was observed after once daily dosing for 2 weeks.

Table 3. Toxicokinetic Parameters for BIC in Rat Plasma – Days 1 and 14

BIC Dose Level (mg/kg/day)	Study Day	C_{max} (µg/mL)			AUC_{0-24} (µg·h/mL)		
		Male	Female	Combined Male and Female	Male	Female	Combined Male and Female
10	1	42.2	41.6	41.9	712	867	790
	14	66.0	74.8	68.4	835	1490	1160
30	1	97.0	90.9	94.0	1670	1790	1730
	14	84.2	131	103	1470	2110	1790
100	1	138	180	159	2470	3310	2890
	14	115	185	150	1820	3290	2550
300	1	141	182	159	2860	3850	3360
	14	117	189	150	2270	3670	2970

In conclusion, oral administration of BIC for 2 weeks at doses up to 300 mg/kg/day to male and female rats was well-tolerated and the NOAEL for BIC in this study was 300 mg/kg/day (Day 14 mean C_{max} and AUC: 150 µg/mL and 2970 µg·h/mL, respectively; sexes combined). At the NOAEL in this study, mean BIC AUC is 29-fold higher than BIC AUC following clinical administration of the B/F/TAF FDC.

3.1.3. 26-Week Oral (Gavage) Toxicity and Toxicokinetic Study in Rats with a 4-Week Recovery Phase

This study evaluated the toxicity and determined the TK of BIC when administered daily via oral gavage to rats for 26 weeks and to assess the reversibility or persistence of any effects after a 4-week recovery (m2.6.7, Section 7.1.3, TX-141-2031). Male and female Crl:WI(Han) rats (20 toxicity animals/sex in the control and high-dose groups, 15 toxicity animals/sex in the low- and mid-dose groups) received BIC at 0, 5, 30, or 300 mg/kg/day at a dose volume of 5 mL/kg. Blood samples were collected from satellite groups of animals for TK evaluation on Day 1 and Weeks 13 and 26. Assessment of toxicity was based on mortality, clinical observations, body weight, food consumption, ophthalmic examinations, and clinical and anatomic pathology.

No BIC-related mortality, clinical observations, body weight changes, food consumption changes, ophthalmic examination findings, or anatomic pathology findings, including organ weights, were noted. Non-adverse, mildly higher phosphorus values (+15 to 33%) were observed in males administered 300 mg/kg/day (Week 26) and females administered ≥ 30 mg/kg/day (Weeks 13 and 26) compared to vehicle control values. This minor, reversible effect was not associated with correlative findings and, hence, was not considered toxicologically important. No other clinical pathology changes were noted.

Plasma exposure to BIC generally increased with the increase in BIC dose from 5 to 300 mg/kg/day (Table 4). The increases in C_{max} and AUC were less than dose proportional between 5 and 300 mg/kg/day. Females had higher C_{max} and AUC values than males, with

gender-based differences of 2-to 3-fold in Weeks 13 and 26 at 300 mg/kg/day. In general, with the exception of females administered 5 mg/kg/day, no accumulation of BIC was observed after multiple doses.

Table 4. Toxicokinetic Parameters for BIC in Rat Plasma - Day 1, Week 13 and Week 26

BIC Dose Level (mg/kg/day)	Study Day/Week	C_{max} (µg/mL)		AUC_{0-24} (µg·h/mL)	
		Male	Female	Male	Female
5	Day 1	19.4	22.9	341	436
	Week 13	33.5	72.4	460	1420
	Week 26	28.8	70.0	457	1290
30	Day 1	82.4	113	1420	1870
	Week 13	75.0	162	1040	2810
	Week 26	76.9	169	1010	2910
300	Day 1	143	185	2380	3530
	Week 13	92.7	212	1880	4570
	Week 26	100	213	1830	4680

In conclusion, daily oral gavage administration of BIC to male and female rats at doses up to 300 mg/kg/day for 26 weeks was well tolerated with no adverse effects. The NOAEL is 300 mg/kg/day (Week 26 mean C_{max} values of 100 and 213 µg/mL and AUC values of 1830 and 4680 µg·h/mL for males and females, respectively). At the NOAEL in this study, mean BIC AUC for males/females is 18/46-fold higher than BIC AUC following clinical administration of the B/F/TAF FDC.

3.1.4. 2-Week Oral (Gavage) Toxicity Study in Cynomolgous Monkeys

This study evaluated the toxicity and determined the TK of BIC when administered daily via oral gavage to monkeys for 2 weeks (m2.6.7, Section 7.1.4, TX-141-2030). Male and female cynomolgus monkeys (3/sex/group) received BIC at 0, 30, 100, or 1000 mg/kg/day at a dose volume of 5 mL/kg. Assessment of toxicity was based on mortality, clinical observations, body weights, body weight changes, food consumption, ophthalmic and electrocardiogram (ECG) examinations, and clinical and anatomic pathology. Liver was collected for hepatic microsomal enzyme analysis and blood samples were collected on Days 1 and 14 for TK evaluations.

No BIC-related mortality, clinical observations, body weight changes, food consumption changes, ophthalmic or ECG examination findings, notable hepatic microsomal enzyme changes, or clinical or anatomic pathology findings were noted.

Plasma exposure to BIC generally increased with the increase in dose level from 30 to 1000 mg/kg/day on Day 1 and Day 14 (Table 5). The increases in C_{max} and AUC were less than dose proportional between 30 and 1000 mg/kg/day. Gender-based differences were less than 2-fold in BIC C_{max} and AUC values. No significant accumulation of BIC was observed after once daily dosing for 2 weeks in monkeys.

Table 5. Toxicokinetic Parameters for BIC in Cynomolgus Monkey Plasma - Days 1 and 14

BIC Dose Level (mg/kg/day)	Study Day	C_{max} (µg/mL)			AUC_{0-24} (µg·h/mL)		
		Male	Female	Combined Male and Female	Male	Female	Combined Male and Female
30	1	26.9	22.5	25.1	306	247	282
	14	20.3	14.9	17.6	273	152	212
100	1	29.8	24.4	27.1	342	280	311
	14	27	21	24	413	236	324
1000[a]	1	109	91.6	100	1250	1060	1150
	14	87.5	70.8	79.1	1170	1020	1090

a Actual dose was 1145 mg/kg/day on Day 1.

In conclusion, oral administration of BIC for 2 weeks at doses up 1000 mg/kg/day to male and female monkeys was well tolerated, and the NOAEL for BIC in this study was 1000 mg/kg/day (Day 14 mean C_{max} and AUC of 79.1 µg/mL and 1090 µg·h/mL, respectively; sexes combined). At the NOAEL in this study, mean BIC AUC is 11-fold higher than BIC AUC following clinical administration of the B/F/TAF FDC.

3.1.5. 39-Week Oral (Gavage) Toxicity Study in Cynomolgus Monkeys with 13-Week Interim Sacrifice and a 4-Week Recovery Phase

This study evaluated the toxicity and determined the TK of BIC when administered daily via oral gavage to monkeys for 13 or 39 weeks (m2.6.7, Section 7.1.5, TX-141-2032). Male and female cynomolgus monkeys (7/sex in low and mid dose groups, 9/sex in control and high dose groups) received the vehicle control article or BIC at 30, 200, or 1000 mg/kg/day at a 5 mL/kg dose volume. Three animals/sex/group were necropsied after 13 weeks of dosing (interim sacrifice), 4/sex/group were necropsied after 39 weeks dosing (terminal sacrifice), and 2/sex/group in control and high dose groups were necropsied after a 4-week recovery period (recovery sacrifice). Assessment of toxicity was based on mortality, clinical observations, body weights, body weight changes, food consumption, ophthalmic and ECG examinations, and clinical and anatomic pathology. Blood samples were collected on Day 1 and Weeks 13 and 39 for TK evaluations.

No BIC-related mortality, body weight changes, food consumption changes, ophthalmic or ECG examination findings were noted. Potentially BIC-related observations of emesis and vomitus in males administered 1000 mg/kg/day were not considered adverse; no other abnormal clinical observations were noted.

Clinical pathology changes in 1000 mg/kg/day animals were limited to increases in alanine aminotransferase (ALT) and gamma glutamyltransferase (GGT). Slight increases in ALT activity were noted in 4 of 9 males at Week 4 and 7 of 9 males at Week 13, but were only observed in 2 females at Week 26 and Week 39 (Table 6). Increases in GGT were only observed in one male and one female during Week 26. These changes were possibly associated with microscopic liver changes observed at Week 39 in 1000 mg/kg/day animals, although they were poorly correlated on an individual animal basis. There were no clinical pathology changes noted at the 4-week recovery necropsy and there were no similar changes in 30 or 200 mg/kg/day animals.

Table 6. Incidence and Severity of Hepatobiliary Changes and ALT Activity at 1000 mg/kg/day in Cynomolgus Monkeys

1000 mg/kg/day BIC	Sacrifice Phase	Animal No.	Hepatobiliary Finding(s)	ALT Activity Fold-Change[a]				
				Wk 4	Wk 13	Wk 26	Wk 39	Rec
Males	Interim (13 weeks)	I02700	-	-	-			
		I02704	-	-	-			
		I02706	-	4.8	4.4			
	Terminal (39 weeks)	I02701	Bile duct hyperplasia (minimal)	2.0	4.3	4.1	4.5	
		I02702	Bile duct hyperplasia (minimal)	-	2.0	-	-	
		I02703[b]	Bile duct hyperplasia (marked) Hepatocyte hypertrophy (moderate) Regenerative hyperplasia (minimal) Neutrophil infiltrate (slight)	2.8	7.6	3.6	2.7	
		I02705	Bile duct hyperplasia (moderate) Neutrophil infiltrate (minimal)	-	2.1	-	2.3	
	Recovery	I02707	Bile duct hyperplasia (marked) Hepatocyte hypertrophy (slight) Regenerative hyperplasia (minimal) Lymphocyte infiltrate (slight)	-	4.6	-[c]	-	-
		I02708	-	3.7	2.5	2.3	2.3	-
Females	Interim (13 weeks)	I02732	-	-	-			
		I02739	-	-	-			
		I02740	-	-	-			
	Terminal (39 weeks)	I02733	Bile duct hyperplasia (minimal)	-	-	-	-	
		I02734	Bile duct hyperplasia (slight)	-	-	-	-	
		I02735	Bile duct hyperplasia (slight) Hepatocyte hypertrophy (minimal)	-	-	-	-	
		I02736	Bile duct hyperplasia (moderate)	-	-	6.6[c]	4.1	
	Recovery	I02737	-	-	-	2.0	2.4	-
		I02738	Bile duct hyperplasia (slight)	-	-	-	-	-

Note: Gray box indicates no data available due to 13 week interim or 39 week terminal animal sacrifice.
Wk – week; Rec – recovery phase
a Number indicates fold-change in ALT activity from the average of two predose values if ≥ 2-fold
b Animal with macroscopic observations in liver (discolored/rough surface) at 39 week terminal sacrifice
c Animal with increased GGT activity at Week 26
- Indicates no finding (< 2-fold ALT change from baseline)

BIC-related anatomic findings were only observed at the 39-week terminal and recovery phase necropsies. There were no changes noted in 30 or 200 mg/kg/day animals. BIC-related microscopic findings in the liver at the terminal sacrifice (Table 6) included minimal to marked bile duct hyperplasia (8/8 animals), minimal or moderate hepatocyte hypertrophy (2/8 animals), minimal regenerative hyperplasia (1/8 animals), and minimal or slight neutrophil infiltrate (2/8 animals) at 1000 mg/kg/day. The macroscopic finding of rough surface on the liver in one male administered 1000 mg/kg/day correlated with moderate hepatocyte hypertrophy and marked bile duct hyperplasia. At the recovery sacrifice, BIC-related microscopic liver findings showed partial reversibility with hepatobiliary changes noted in 1 of 2 males and 1 of 2 females (Table 6).

Bile duct hyperplasia was characterized by one or more of the following: increased bile duct profiles that sometimes extended between portal regions (bridging) and also surrounded and separated small groups and individual hepatocytes; increased portal connective tissue (fibrosis); and occasional cellular debris and pyknotic nuclei in affected areas. The hypertrophic hepatocytes were characterized by one or more of the following: increased amphophilic to eosinophilic cytoplasm; homogenous to vacuolated cytoplasm; enlarged sometimes vesicular nuclei; and prominent, sometimes multiple nucleoli. Regenerative hyperplasia was characterized by focal areas of increased numbers of small hepatocytes that sometimes compressed adjacent liver parenchyma and occurred in one terminal and one recovery male administered 1000 mg/kg/day. This was considered a response to the marked bile duct hyperplasia and hepatocyte hypertrophy. In three males, a neutrophil or lymphocyte infiltrate was observed predominantly in areas of bile duct hyperplasia.

Plasma exposure to BIC generally increased with the increase in dose level from 30 to 1000 mg/kg/day (Table 7). The increases in C_{max} and AUC were less than dose proportional between 30 and 1000 mg/kg/day. No gender differences or accumulation of BIC were observed after multiple dosing of BIC.

Table 7. Toxicokinetic Parameters for BIC in Cynomolgus Monkey Plasma - Day 1, Week 13 and Week 39

BIC Dose Level (mg/kg/day)	Study Day/Week	C_{max} (µg/mL)			AUC_{0-t} (µg·h/mL)		
		Male	Female	Combined Male and Female	Male	Female	Combined Male and Female
30	Day 1	40.1	24.6	32.3	280	243	262
	Week 13	30.2	25.1	27.7	288	254	271
	Week 39	28.3	24.4	26.4	248	253	251
200	Day 1	43.4	55.1	49.2	514	797	655
	Week 13	66.7	59.7	63.2	855	664	760
	Week 39	67.8	70.4	69.1	595	823	709
1000	Day 1	97.3	111	104	1450	1520	1480
	Week 13	112	89.8	101	1540	1180	1360
	Week 39	104	109	106	1450	1750	1600

In conclusion, daily oral gavage administration of BIC to cynomolgus monkeys at doses up to 1000 mg/kg/day for 39 weeks resulted in microscopic liver changes that showed partial reversibility following four weeks of a nondosing phase. The NOEL is 200 mg/kg/day (Week 39 mean C_{max} and AUC values of 69 µg/mL and 709 µg·h/mL, respectively, sexes combined). At the NOEL in this study, mean BIC AUC is 7.0-fold higher than BIC AUC following clinical administration of the B/F/TAF FDC.

3.2. FTC

3.2.1. 2-Week Oral (Gavage) Toxicity Study in Mice

Four groups of 15 male and 15 female CD-1 mice were administered FTC, suspended in 0.5% methyl cellulose, at doses of 0, 120, 600, and 3000 mg/kg/day (m2.6.7, Section 7.2.1, TOX001). The dose was administered by gavage and as a divided dose with 6 hours between the morning and afternoon dose. Ten mice/sex/group were necropsied at the end of the exposure period; 5/sex/group were necropsied at the end of a 2-week postdose recovery period. There were no important differences in levels of FTC measured when males were compared to females and Day 2 values were compared to Day 14 values. Mean concentrations for FTC were proportional to dose. At the high dose, mean concentrations on Day 2 were 318 and 358 µg/mL for males and females, respectively; and on Day 14 were 389 and 405 µg/mL, respectively. There were no signs of toxicity.

3.2.2. 4-Week Oral Toxicity Study in Mice

Groups of 14 male and female mice were orally administered (once daily) FTC suspended in 0.5% methylcellulose at 0, 120, 600, and 3000 mg/kg/day for 30 days, with 4 males and females from each group held for an additional 13-day recovery period (m2.6.7, Section 7.2.2, TOX599). Also exposed to the same dosing regimen were groups of 10 males and females, which were used for clinical pathology evaluations prestudy and on Days 31 to 32. Groups of 38 male and female satellite TK animals were dosed at 120, 600, and 3000 mg/kg/day and drug level determinations made from samples collected on Days 3 and 32. There were no clinical signs of toxicity that were thought to be treatment related. Two high dose male mice died of undetermined causes, and a relationship to treatment cannot be ruled out. No treatment-related changes in feed consumption or body weight were seen. At the end of the study, hemoglobin (Hb) and hematocrit (HCT) were reduced in high-dose females, and total erythrocyte count (RBC) was decreased in both sexes, whereas mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and red cell distribution width (RDW) were increased in both sexes. All the hematology changes were reversed in females following the recovery period, but high-dose males experienced a further decline in HCT and RBC, with some improvement in MCV and RDW. There were no changes in clinical chemistry values, urinalysis parameters, or ocular toxicity endpoints. Pituitary, heart, spleen, and ovary weights were increased in high-dose females and the spleen weight was increased in high-dose males, whereas the testes and thymus weights were decreased. There were no accompanying histopathologic correlates noted. A NOEL for hematological changes was established at 600 mg/kg/day. Day 32 TK AUC_{inf} values for high dose males and females were 7830 and 6130 μM·h (1934 and 1514 μg h/mL), respectively. Day 3 values were similar.

3.2.3. 6-Month Oral (Gavage) Toxicity Study in Mice with 3-Month Interim Sacrifice

Groups of 30 male and 30 female CD-1 mice were administered FTC at 0, 167, 500 and 1500 mg/kg/day, given as a single oral dose each day for 26 weeks (m2.6.7, Section 7.2.3, TOX022). After 13 weeks of treatment, 10 males and 10 females from each group were killed and necropsied. After 26 weeks of treatment, 15 males and 15 females from each group were killed and necropsied. The remaining 5 males and 5 females from each group went untreated for 1 month and were then necropsied. Satellite groups of 40 males and 40 females per dose group were used for TK blood collections after 13 and 26 weeks. Clinical observations were made daily and ophthalmoscopic exams at predose and Weeks 13 and 26. Unfasted blood samples were taken at each necropsy for clinical pathology evaluations. At necropsy, organs were examined grossly, weighed, and processed for histopathologic evaluations.

Compared to controls, there were no significant clinical observations, body weight changes, ophthalmoscopic findings, blood chemistry alterations, organ weight changes, or gross and histopathologic findings. Mean corpuscular volume and MCH were elevated in the high-dose group after 26 weeks of exposure, more so in females than males. These changes were still evident after the 1-month recovery period.

At 3 and 6 months of exposure, plasma samples were collected from 3 satellite animals/sex/group at predose, 0.5, 1, 2, 5, and 8 hours after the daily dose. With the exception of females in the low dose group, mice at all doses had measurable concentrations of FTC in the predose blood samples. Peak plasma concentrations (C_{max}) of FTC were reached by 30 to 60 minutes after dosing. Following 3 months exposure, C_{max} values at 167 mg/kg/day were 44.0 and 37.2 µg/mL; at 500 mg/kg/day were 128.0 and 88.8 µg/mL; and at 1500 mg/kg/day were 225.0 and 187.1 µg/mL for males and females, respectively. Following 6 months exposure, C_{max} values at 167 mg/kg/day were 41.7 and 48.6 µg/mL; at 500 mg/kg/day were 87.3 and 111.7 µg/mL; and at 1500 mg/kg/day were 310.9 and 306.0 µg/mL for males and females, respectively.

The systemic exposure to FTC over the steady state dosing interval ($AUC_{0–24h}$) increased proportionally as the dose increased. Following 3 months exposure, $AUC_{0–24h}$ increased from 63.8 and 67.9 µg·h/mL at 167 mg/kg/day to 513.4 and 569.5 µg·h/mL at 1500 mg/kg/day for males and females, respectively. Following 6 months exposure, $AUC_{0–24h}$ increased from 82.3 and 93.1 µg·h/mL at 167 mg/kg/day to 732.1 and 899.4 µg·h/mL at 1500 mg/kg/day for males and females, respectively. A NOEL of 500 mg/kg/day was established (equivalent to an $AUC_{0–24h}$ of 266.4 µg·h/mL; sexes combined).

3.2.4. 6-Month Oral Toxicity Study (with a 3 Month Interim Sacrifice) in Mice

Groups of 25 male and 25 female CD-1 mice were administered once daily doses of FTC at 0, 120, 600, or 3000 mg/kg for up to 6 months (m2.6.7, Section 7.2.4, TOX628).
Five mice/sex/dose were subjected to a 3-week recovery period following 6-months of dosing. An additional 28/sex/group were used to assess plasma concentrations of drug, and 10/sex/dose (20 in the control group) were used for clinical pathology evaluations. The following parameters were evaluated for evidence of toxicity: body weights, feed consumption, clinical signs, hematology, clinical chemistry, urinalysis, ophthalmoscopic examinations, organ weights, gross and histopathology, and electron microscopy.

After 6-months of exposure at the high dose, there were reversible decreases in RBCs, and reversible increases in MCV and urine quantity for both sexes. In high dose females, there was a reversible increase in absolute and relative thyroid weight, which was not associated with histopathological changes. Electron microscopy of liver, skeletal and cardiac muscle did not reveal any ultrastuctural changes. The $AUC_{0–24h}$ for sexes combined on Day 175 was 142.5, 538.5, and 1787.5 µg·h/mL for the low-, mid-, and high-dose groups, respectively. The no-effect dose was 600 mg/kg. Females had statistically significantly lower ($p < 0.05$) plasma AUC values than did males at all dose levels. At the NOEL (600 mg/kg/day), $AUC_{0–24h}$ values were 2369 µM·h (585 µg·h/mL) for males and 1985 µM·h (490 µg·h/mL) for females.

3.2.5. 3-Month Oral Gavage Study for Bioassay Dose Selection in CD Rats

To evaluate the repeat dose toxicity in rats of FTC and help select doses for a 2-year carcinogenicity bioassay, Charles River CD rats (10/sex/dose) were administered 0, 120, 600, or 3000 mg/kg/day for 3 months, euthanized, and necropsied (m2.6.7, Section 7.2.5, TOX097). An additional 18 rats/sex/dose were administered the same doses to evaluate systemic exposure after the second and ninetieth doses. During the dosing period, rats were evaluated for clinical signs of

toxicity, and body weight and food consumption were measured. Urine samples were collected for urinalysis at the end of the dosing period. At necropsy, blood was collected for clinical pathology studies; organs were weighed; rats were examined for gross pathologic lesions; and tissues were collected for light microscopic examination.

Rats tolerated daily FTC doses up to 3000 mg/kg/day for 3 months without clinical signs of toxicity or effects on body weight gain, food consumption, or clinical chemistry parameters. Mild anemia was noted at 3000 mg/kg/day. No gross pathologic or histopathologic lesions occurred. Pituitary and thyroid glands weighed less at 3000 mg/kg/day, but there were no histopathologic changes to correlate with the reduced weights.

Emtricitabine was rapidly absorbed, with females absorbing the compound more rapidly than males (T_{max} = 1–2 hours). Day 90 C_{max} was 20% to 50% greater in females than males. Day 90 mean C_{max} was greater than Day 2 C_{max} for both sexes (5% to 30% in males, 20% to 60% in females). Day 90 mean $AUC_{0–24h}$ was 20% to 40% greater than Day 2 $AUC_{0–24h}$ in females, but about the same as Day 2 $AUC_{0–24h}$ in males. Mean $t_{1/2}$ was 2.4 to 3.8 hours on Day 2 and 3.6 to 6.5 hours on Day 90. Dose-normalized C_{max} tended to decrease with increasing dose, but dose-normalized $AUC_{0–24h}$ remained relatively constant, suggesting a linear relationship between systemic exposure and daily dose of FTC in rats from 120 to 3000 mg/kg/day.

3.2.6. 4 Week Oral Toxicity Study in Cynomolgus Monkeys

In a 4 week oral toxicity study, cynomolgus monkeys (5/sex/dose) were administered FTC at 0, 80, 400, or 2000 mg/kg/day in 0.5% methylcellulose, as equal divided doses 6 hours apart via nasogastric intubation for 28 days, after which 3/sex/dose were necropsied (m2.6.7, Section 7.2.6, TOX600). Remaining monkeys (2/sex/dose) were held without dosing for 3 weeks, then necropsied to evaluate recovery from treatment-related effects. The following parameters were monitored for signs of toxicity: clinical signs, appetite, body weights, ophthalmology, electrocardiography, neurophysiology, hematology, clinical chemistry, urinalysis, plasma drug concentrations, cerebrospinal fluid (CSF) drug concentrations, organ weights, gross pathology and histopathology, and neurohistopathology. Samples of skeletal and cardiac muscle from control and high dose animals were examined by transmission electron microscopy.

There were no changes in any of the observed parameters suggestive of treatment-related effects. The only possibly treatment-related clinical sign was an increased incidence of soft feces in females at 2000 mg/kg/day. Systemic exposure to FTC was similar between sexes and between Days 3 and 27. The C_{max} and the AUC values increased in proportion to the dose. No significant drug accumulation occurred over the dosing period. Drug levels in CSF measured 1 hour after dosing in the third week of the study (together with a companion blood sample) demonstrated that CSF drug levels rose proportionately with plasma drug levels and were 4.3%, 3.7%, and 3.7% of plasma drug levels in the 80-, 400-, and 2000-mg/kg/day groups, respectively. A NOEL of 400 mg/kg/day was established; corresponding systemic exposure levels ($AUC_{0–6h}$) at 3 days were 687 to 688 μM·h (170 μg·h/mL) for males and females, respectively, and at Day 27 were 793 to 664 μM·h (196 to 164 μg·h/mL) for males and females, respectively.

3.2.7. 3-Month Oral Toxicity Study in Cynomolgus Monkeys

In a 3-month GLP oral toxicity study, cynomolgus monkeys (5/sex/dose) were administered FTC at 0, 40, 200, or 1000 mg/kg/day in 0.5% methylcellulose, as equal divided doses 6 hours apart for 3 months, then 3/sex/dose were killed (m2.6.7, Section 7.2.7, TOX627). The tissues were fixed by perfusion and the monkeys were necropsied. Remaining monkeys (2/sex/dose) were allowed to recover for 3 weeks, and evaluated for reversibility of treatment-related effects. The following parameters were observed for signs of toxicity: clinical signs, body weight, ophthalmology, electrocardiography, neurophysiology and neurohistopathology, hematology, clinical chemistry, urinalysis, and plasma drug concentrations. At terminal necropsy, organs were weighed and gross and histopathology were performed. Liver, cardiac, and skeletal muscle was examined by transmission electron microscopy.

There were no treatment-related changes in any of the observed parameters at any dose. A NOEL of 1000 mg/kg/day was established. C_{max} and AUC increased with dose and there was no evidence of accumulation. Systemic exposure to FTC was similar between sexes and between Days 3 and 87. In the 1000 mg/kg/day group, AUC_{0-6h} values on Day 3 for males and females were 1339 and 942 μM·h (331 and 233 μg·h/mL), respectively, and on Day 87 were 1630 and 1109 μM·h (403 and 274 μg·h/mL), respectively.

3.2.8. 52-Week Oral Toxicity Study in Cynomolgus Monkeys with a 4-Week Recovery Period

In a 1-year oral toxicity study with a 3-month interim necropsy, cynomolgus monkeys were administered FTC at 0, 50, 200, or 500 mg/kg/day in 0.5% methylcellulose (n = 8, 4, 4, and 8 males and females, respectively) as equal divided doses at least 5 hours apart, by nasogastric intubation, daily for 52 weeks (m2.6.7, Section 7.2.8, TOX032). After dosing for 3 months, 2 monkeys/sex at 0 and 500 mg/kg/day were necropsied. At the end of dosing, 4 monkeys/sex/dose were necropsied. Remaining monkeys (2/sex at 0 and 500 mg/kg/day) were allowed to recover for a month and evaluated for reversibility of treatment-related effects. Data collected were body weights, food consumption, clinical signs, ophthalmic examinations, ECG recordings (with measurement of QT interval), neurophysiology parameters, hematology, clinical chemistry, plasma FTC concentrations (Day 1 and Weeks 13, 26, and 52), gross pathology, organ weights, and histopathology findings.

There were no signs of toxicity after dosing for 3 months at any dose. At the end of 52 weeks of dosing, the only treatment-related effects were a slight (nonsignificant) decrease in RBC count and increased MCH at 500 mg/kg/day in females only, which were absent at the end of the recovery period. No other signs of toxicity occurred at any dose. Based on these findings, the NOEL was 200 mg/kg/day.

Emtricitabine was rapidly and well absorbed with peak plasma concentrations occurring between 0.5 to 2 hours postdosing. Plasma half-life was 2 to 4 hours for all dose levels. Half-life may have been underestimated because of the short (6 hour) sampling period. A longer half-life was expected, based on the measurable predose drug concentrations and the slightly higher plasma FTC concentrations after repeated dosing. No major differences were noted in plasma FTC

exposure between male and female monkeys. Plasma C_{max}, $AUC_{0–6h}$, and estimated steady-state $AUC_{0–24h}$ (Weeks 13, 26, and 52) increased linearly over the dose range of 50 to 500 mg/kg/day. Systemic exposure to FTC was similar between sexes and at Weeks 13, 26, and 52. Week 52 $AUC_{0–24h}$ values at the NOEL of 200 mg/kg/day were 97.2 and 97.8 µg·h/mL in males and females, respectively. Corresponding C_{max} values were 17.4 and 21.3 µg/mL, respectively.

3.3. TAF

3.3.1. 2-Week Oral Toxicity Study in Mice

Daily oral administration (10 mL/kg) of TAF (GS-7340-02) at 100, 500, or 1000 mg/kg/day (80, 400, 800 mg f.b.e./kg/day) to Crl:CD1(ICR) mice (10/sex/group) for up to 14 days resulted in early death for 10 and 20 mice administered 500 or 1000 mg/kg/day, respectively (m2.6.7, Section 6.2, TX-120-2006). Based on the number of confirmed gavage errors, the viscosity of the formulation, the number of abnormal lung findings noted at gross necropsy, and histopathologic changes consistent with nonfatal gavage injury and secondary inflammation, it is hypothesized that the early deaths for animals administered 500 or 1000 mg/kg/day were due in some undeterminable part to the character of the dose formulation concentrations of 50 and 100 mg/mL, respectively.

Minimal to moderate clinical pathology changes occurred in animals administered 500 or 1000 mg/kg/day and included mildly lower absolute reticulocyte counts, minimally to moderately higher total protein and albumin. Nonadverse microscopic changes in the rectal epithelium (minimal to slight vacuolation/apoptosis of the epithelium were observed in males administered ≥ 100 mg/kg/day and females administered 500 or 1000 mg/kg/day, which were not considered adverse as the epithelium remained intact and there was no resulting inflammation or necrosis). The NOAEL for TAF administered by oral gavage for 14 days to male and female mice was 100 mg/kg/day.

The TAF C_{max} at 100 mg/kg/day on Day 14 was 21.2 ng/mL for both sexes combined; AUC_{0-t} could not be calculated due to the lack of sufficient data points from which to estimate AUC (Table 8). TAF was rapidly converted to its metabolite TFV. The TFV C_{max} at this dose level on Day 14 was 1.24 µg/mL for both sexes combined, and AUC_{0-t} was 5.74 µg·h/mL for both sexes combined.

Table 8. Mean Repeat-Dose Toxicokinetics of TFV Following Oral Administration of TAF in Male and Female Mice for 2 Weeks

GS-7340-02 (mg/kg/day)	Study Day	Toxicokinetic Parameters			
		TFV		TAF (GS-7340)	
		C_{max} (µg/mL)	AUC_{0-t} (µg·h/mL)	C_{max} (µg/mL)	AUC_{0-t} (µg·h/mL)
100	Day 1	0.931	5.34	0.012	NC
	Day 14	1.24	5.74	0.021	NC
500	Day 1	4.13	25.9	6.14	13.02
	Day 14	3.93	29.4	2.76	1.91
1000	Day 1	4.46	48.2	8.77	22.5
	Day 8	6.28	5.67	23.3	17.8

NC = not calculated (due to insufficient data)

3.3.2. 13-Week Oral Mouse Toxicity Study in Mice

Crl:CD1(ICR) mice (15/sex/group) were administered daily oral doses (10 mL/kg) of GS-7340-02 at 10, 30 or 100 mg/kg/day (8, 24, 80 mg f.b.e./kg/day) for up to 13 weeks (m2.6.7, Section 7.3.1, TX-120-2007). Administration of TAF resulted in nonadverse minor effects on body weight gain for males at 100 mg/kg/day and females at all dose levels. A transient decrease in food consumption occurred at the start of dosing for animals administered 30 or 100 mg/kg/day. There were no test article-related clinical signs or effects on clinical pathology, organ weight parameters, or macroscopic observations. Data indicate increased incidence and severity of test article-related microscopic findings present in the nasal turbinates (minimal to slight infiltrates of neutrophils in respiratory and olfactory mucosa, and minimal to moderate [high dose only] degeneration of olfactory epithelium) of males and females at ≥ 10 mg/kg/day; an additional adverse finding in the nasal turbinates (exudate in the lumen) was present in males and females administered ≥ 30 mg/kg/day. Minimal infiltrates and minimal olfactory degeneration were observed at a lower incidence in control animals. A test article-related microscopic finding also occurred in the rectum (minimal increased apoptosis) of males and females at 100 mg/kg/day.

Based on the increased incidence and severity of the microscopic findings for the nasal turbinates, the NOAEL for TAF administered for 13 weeks to mice could not be determined in this study. Due to limited concentration data for TAF, AUC values could not be calculated. At week 13, the TFV AUC_{tau} (combined sexes) was 0.213 µg·h/mL at 10 mg/kg/day (Table 9).

Table 9. Mean Repeat-Dose Toxicokinetics of TFV Following Oral Administration of GS-7340-02 in Mice for 13 Weeks

GS-7340-02 (mg/kg/day)	Study Day/Week	TFV[a]	
		C_{max} (µg/mL)	AUC_{0-t} (µg·h/mL)
10	Day 1	0.060	0.171
	Week 13	0.069	0.213
30	Day 1	0.292	1.28
	Week 13	0.330	1.51
100	Day 1	1.01	6.53
	Week 13	0.863	7.40

a Toxicokinetic parameters for TAF could not be calculated due to limited concentration data.

3.3.3. 4-Week Oral Toxicity Study in Rats

Daily oral administration of GS-7340-02 (15 mL/kg) at 1.5, 6.25, 25, 100, and 400 mg/kg/day (1.2, 5, 20, 80, 320 mg f.b.e/kg/day) to CD (Crl: CD®(SD)BR) rats (10/sex/group) for 28 days resulted in treatment-related effects including (at 400 mg/kg/day) decreased body weight gain, reduced food consumption, decreases in white blood cell (WBC) and RBC parameters, calciuria, decreased BMD, decreased 1,25 dihydroxy vitamin D_3, renal karyomegaly, thymic atrophy, and atrophy of cancellous bone of the femur (m2.6.7, Section 7.3.2, R990182). Most effects were restricted to the 400-mg/kg/day group; however, selected changes were noted as low as 25 mg/kg/day with a nonsignificant decrease in 1,25 dihydroxy vitamin D_3 observed at 6.25 mg/kg/day. Based on changes in WBCs, the TAF NOAEL was considered to be 6.25 mg/kg/day (no change in WBC count was observed at doses up to 100 mg/kg/day in the subsequent 26-week rat toxicity study).

3.3.4. 26-Week Oral Toxicity Study in Rats

Daily oral administration of GS-7340-02 (10 mL/kg) at 5, 25, and 100 mg/kg/day (4, 20, 80 mg f.b.e./kg/day) to Crl: CD® (SD) BR rats (15/sex/group) for 26 weeks resulted in treatment-related effects that included minimal renal cortical tubular karyomegaly (100 mg/kg/day) and minimal to slight tibial cancellous bone atrophy (females, 100 mg/kg/day), changes in bone density measurements (100 mg/kg/day) and changes in biochemical markers of bone turnover (25 and 100 mg/kg/day) (m2.6.7, Section 7.3.3, TOX-120-001). These effects were not observed at 5 mg/kg/day. GS-7340-02 appeared to dose-dependently increase biochemical markers of bone turnover in males and dose-independently decrease serum 1,25-dihydroxy- and 25-hydroxyvitamin D_3 in males and females at the 25- and 100-mg/kg/day dose levels.

There were no treatment-related mortality or treatment-related clinical signs in any dose group during the 26-week treatment period. There were no significant effects on body weight, food consumption, ophthalmology, hematology, routine biochemistry, or urinalysis parameters. There were no treatment-related effects on organ weights or macroscopic findings. As the effects (increases in biochemical markers of bone turnover and changes in related hormones) seen at 25 mg/kg/day were minimal, it was concluded that the NOAEL was 25 mg/kg/day.

Toxicokinetic analysis of plasma samples demonstrated that TAF was rapidly absorbed after oral dosing and was rapidly converted to TFV (Table 10). No consistent differences in plasma pharmacokinetics were found between male and female rats. Tenofovir was eliminated from the plasma with half-lives ranging from 7 to 13 hours. Mean TFV C_{max} and AUC values for combined sex groups increased dose proportionally over the dose range of 5 mg/kg/day to 100 mg/kg/day at each study period.

Table 10. Mean Repeat-Dose Pharmacokinetics of TFV Following Oral Administration of GS-7340-02 in Male and Female Rats for 26 Weeks

GS-7340-02 (mg/kg/day)	Study Day/Week	TFV[a]	
		C_{max} (µg/mL)	AUC (µg·h/mL)
5	Day 1	0.309	0.604
	Week 13	0.344	0.727
	Week 26	0.267	0.670
25	Day 1	1.284	3.364
	Week 13	1.464	3.724
	Week 26	1.523	3.758
100	Day 1	4.944	12.415
	Week 13	5.514	15.044
	Week 26	4.911	15.534

a Toxicokinetic parameters for TAF could not be calculated due to limited concentration data

3.3.5. 1-Week Oral Toxicity Study in Rabbits

This non-GLP study evaluated the toxicity and determined the TK of TAF (GS-7340-02), when administered daily via oral gavage to female New Zealand White rabbits, for 7 consecutive days (m2.6.7, Section 6.2, TX-120-2003). The study provided information on dose selection for a subsequent developmental toxicity study.

Animals (3 per group) were administered vehicle control (0.1% [v/v] Tween 20, 0.1% [w/v] HPMC K100LV prepared in ultrapure water) or GS-7340-02 at doses of 20, 50 or 75 mg/kg/day for 7 days using a dose volume of 10 mL/kg/day.

Assessment of toxicity was based on mortality, clinical signs, body weight, body weight change, food consumption, and clinical pathology parameters. Toxicokinetic blood sampling was performed on Days 1 and 7.

All animals survived to their scheduled sacrifice. There were no test article-related effects on clinical condition or food consumption. There was a slight reduction in mean cumulative body weight gains at 50 and 75 mg/kg/day (74% and 57%, respectively compared to controls), but there was no decrease in body weight. There were no test article-related changes in clinical pathology parameters or macroscopic findings.

Plasma exposure to TAF and TFV generally increased with increase in dose level from 20 to 75 mg/kg/day. Values for mean C_{max} and AUC_{0-t} of TFV were generally higher on Day 7 than on Day 1. TAF was rapidly and extensively converted to TFV. The mean TAF AUC_{0-t} on day 7 was unable to be calculated for the 20 mg/kg/day dose (due to values below the lower limit of quantitation of 1.00 ng/mL) and was 0.252 and 1.174 µg·h/mL for the 50- and 75-mg/kg/day groups, respectively. The mean TFV AUC_{0-t} on day 7 was 2.256, 5.741 and 10.070 µg·h/mL for the 20-, 50-, and 75-mg/kg/day groups, respectively.

In conclusion, daily oral administration of GS-7340-02 at 20, 50 and 75 mg/kg/day to female rabbits, for 7 days, was generally well tolerated. There were no test article-related effects on food consumption, clinical pathology parameters or macroscopic findings. There was a slight decrease in cumulative body weight gain at 50 and 75 mg/kg/day, compared to controls, but there was no effect on body weight. The NOAEL was 75 mg/kg/day; the highest dose administered. Exposure to TAF and TFV generally increased with the increasing GS-7340 dose from 20 to 75 mg/kg/day. At 75 mg/kg/day, mean AUC_{0-t} values were 1.2 and 10.1 µg·h/mL for TAF and TFV, respectively.

3.3.6. 4-Week Oral Toxicity Study in Dogs

Daily oral administration of TAF (GS-7340-02; 10 mL/kg) at 0.1, 0.3, 1, 3, or 10 mg/kg/day (0.08, 0.24, 0.8, 2.4, 8 mg f.b.e./kg/day) (m2.6.7, Section 7.3.4, D990175) to male and female beagle dogs (4/sex/group) for 28 days resulted in a marginal rise in aspartate aminotransferase (AST) in high-dose females only (10 mg/kg/day) of questionable toxicologic significance, and renal tubular karyomegaly and/or basophilia in all males and females at 10 mg/kg/day and 1 male and 1 female at 3 mg/kg/day. There were no other adverse effects. Mean values for bone specific alkaline phosphatase, N telopeptide, parathyroid hormone, 1,25 dihydroxyvitamin D and 25 hydroxyvitamin D were generally similar among all groups on each occasion. There were no TAF effects on peripheral quantitative computed tomography-derived bone densitometry parameters (eg, bone mineral content and bone mineral density of the total slice and trabecular and cortical/subcortical compartments). The TAF NOEL was 1 mg/kg/day.

At the lower doses, only C_{max} and T_{max} values for TAF were determined as most values were below the lower limit of quantitation of the assay. TAF was rapidly absorbed on Day 1, with median peak values within 0.25 to 0.5 hours of 18.5, 38.7, and 0.582 µg/mL at 1.0, 3.0, and 10 mg/kg/day, respectively. Peak TFV concentrations occurred within 1 hour. For the 10-mg/kg/day dose group, Day 28 TFV C_{max} and AUC_{tau} were 0.44 µg/mL and 5.26 µg h/mL, respectively (males and females combined). Comparisons between Day 1 and Day 28 for the 10-mg/kg/day dose group showed potential accumulation upon repeat dosing. Tenofovir in PBMCs was measurable (18.6 µg/mL) after 28 days of 10 mg/kg/day TAF.

Table 11. Median Repeat-Dose Pharmacokinetics of TFV and TAF Following Oral Administration of GS-7340-02 in Beagle Dogs for 28 Days

GS-7340-02 (mg/kg/day)	Study Day	TFV		TAF (GS-7340)		TFV PBMC (µg/mL)
		C_{max} (µg/mL)	AUC_{tau} (µg·h/mL)	C_{max} (µg/mL)	AUC_{tau} (µg·h/mL)	
10	Day 1	0.385	NC	0.582	NC	NC
	Day 28	0.444	5.26	1.28	0.985	18.6

NC = not calculated (due to insufficient data); PBMC = peripheral blood mononuclear cell

3.3.7. 39-Week Oral Toxicity Study in Dogs

Male and female beagle dogs were administered daily oral doses (10 mL/kg) of TAF (GS-7340-02) at 2, 6, or 18/12 mg/kg/day (1.6, 4.8, 14.4/9.6 mg f.b.e./kg/day) for 13 weeks (2/sex/group) or 39 weeks (4/sex/group) with a 3-month post dose recovery phase (2/sex at 18/12 mg/kg/day) (m2.6.7, Section 7.3.5, TOX-120-002). Daily oral administration of GS-7340-02 for 39-weeks resulted in a dose-related decrease in body weight gain at 39 weeks for all male treated groups and for females receiving 18/12 mg/kg/day. The dose level in the high-dose group was reduced from 18 to 12 mg/kg/day on Days 45 and 51, for males and females, respectively, due to the occurrence of severe clinical signs and reduced body weight.

There were 2 unscheduled deaths of high-dose males. One of these was considered to be due to a gavage accident and not treatment related. A different high-dose male was euthanized on Day 45 due to its deteriorating clinical condition, considered to be treatment related. Prior to necropsy the animal had shown reduced body weight; reduced food consumption; increased AST, globulin levels, triglyceride, cholesterol, total bilirubin; and decreased monocyte and platelet counts. Macroscopically, there was bilateral enlargement of the submandibular lymph nodes, which histologically had slight inflammation and plasmacytosis. Histopathology findings of significance included a mild, mononuclear infiltrate in the ocular posterior uvea; renal cortical tubular degeneration; atrophy of gut-associated lymphoid tissue (GALT), mesenteric lymph node, and thymus accompanied by an infiltrate of macrophages; mucosal atrophy of the fundic gland; mucosal hyperplasia of the pyloric gland; and mucosal degeneration and/or regeneration in the cecum and colon.

Hematology, serum chemistry and urinalysis parameters remained within normal historical ranges, with the exception of increased mean AST (~2.6x compared to control) and total bilirubin (~1.6x compared to control) in dogs administered 18/12 mg/kg/day. No ECG changes occurred at 2 mg/kg/day. At Week 39, a dose-related prolongation of PR interval was observed in the 6- (~+13%) and 18/12-mg/kg/day (~ +24%) groups. GS-7340 appeared to reversibly reduce heart rate with an associated mild QT interval prolongation in the high-dose group only. These changes were associated with decreases in serum T3 {Kienle 1994, Tribulova 2010}. After the 13-week recovery period, serum T3 values returned to levels similar to the control group animals.

All bone markers showed age-related decreases. After 3 months of treatment, there were some small differences noted among mean values for bone formation (skeletal alkaline phosphatase [sALP]) and bone resorption markers (urinary free deoxypyridinoline and N telopeptide) for groups administered GS-7340-02 compared to controls. After 9 months of GS-7340-02 administration, statistically significant increases in mean values for the bone resorption marker urinary N telopeptide were noted for males and females treated with GS-7340-02 at 18/12 mg/kg/day ($p \leq 0.05$), compared to controls. A similar though not statistically significant trend was noted in animals treated with GS-7340-02 at 6 mg/kg/day, suggesting a dose-related response. No significant changes in free deoxypyridinoline were observed in animals treated with GS-7340-02 with no consistent effects (increases) among treated groups. For the formation marker, serum sALP values in treated animals were comparable to vehicle controls except for one high dose male value (Animal 401), which was outside the control ranges. At the end of the recovery period, bone marker values returned to below the control range consistent with an age effect and recovery from treatment.

Treatment with GS-7340-02 at 18/12 mg/kg/day administered once daily to young beagle dogs for 39 weeks resulted in changes in bone densitometry parameters (by dual-energy x-ray absorptiometry [DXA] analysis) considered to reflect primarily effects on bone growth. These changes were considered secondary to the effects on body weight.

Compound-related histopathology alterations were noted in the kidneys, eyes, lungs, and spleen after both 13-week interim and 39-week treatment phases. The liver and possibly the adrenal glands were additional target organs identified after the longest treatment duration. After 13-weeks of treatment, findings of renal cortical tubular degeneration/regeneration and karyomegaly were limited to animals administered either 6 or 18/12 mg/kg/day; findings after 39 weeks of treatment were similar. These changes were minimal to slight (Grade 1 to 2) in affected mid-dose (6 mg/kg/day) males and females. In high-dose males (18/12 mg/kg/day) severity ranged from mild to moderate (Grade 2 to 3). After 39-weeks of treatment, similar lesions (karyomegaly and tubular degeneration) but of only minimal (Grade 1) severity were also present in 2 low-dose males administered 2 mg/kg/day. A minimal to slight (Grade 1 to 2) infiltration of mononuclear cells in the ocular posterior uvea was noted in some animals administered 18/12 mg/kg/day after both treatment periods. Alveolar histiocytosis was the sole compound-related finding present in the lungs after 13-weeks of treatment in animals administered 18/12 mg/kg/day. Additional pulmonary findings were noted following 39-weeks of treatment and included a macrophage accumulation with pigment, which could be detected predominantly in high-dose (18/12 mg/kg/day) group animals and in few animals in 6- or 2-mg/kg/day dose groups. An infiltration of macrophages laden with pigment was very frequently seen in the splenic white pulp of animals administered 18/12 mg/kg/day after both treatment periods. After 39 weeks of treatment centrilobular hepatocellular cytoplasmic acidophilic inclusions were identified in dogs administered 18/12 mg/kg/day, whereas pigment deposits in hepatic macrophages and/or sinusoidal cells (Kupffer cells) could be found in animals treated with 6 or 18/12 mg/kg/day. Also, similar pigment deposits in the sinusoidal cells (tissue macrophages) of the adrenal glands were seen in a few dogs administered 18/12 mg/kg/day. The cause of the intracellular pigment in tissue macrophages in the lung, liver, spleen, and adrenal is not known but could represent accumulation of the test article and/or test article metabolite(s) in these cells of the mononuclear phagocyte system. After a 13-week recovery period, treatment-related histological changes were still observed in the kidneys, lungs, and liver but were of reduced incidence and severity.

Based on these findings, the NOAEL of GS-7340-02 after 39 weeks of treatment in this study was determined to be 2 mg/kg/day. Treatment-related findings were completely or partially reversible following a 13-week recovery period.

Toxicokinetic analysis showed that TAF was rapidly absorbed and converted to TFV following oral dose administration, with peak plasma concentrations of TAF and TFV occurring 0.5 and 1 hour after dosing, respectively. The systemic exposure of TAF was dose dependent. Plasma C_{max} and AUC values for GS-7340 increased more than proportionally over the dose range of 2 to 18/12 mg/kg/day. The systemic exposure of TFV, as measured by plasma C_{max} and AUC, increased roughly in proportion to the administered dose. There was some accumulation of TFV following repeat dosing (approximately 3-fold) as expected based on its terminal phase half-life. There was no effect of sex on plasma pharmacokinetics of TAF and TFV after oral administration.

Tenofovir concentrations in PBMCs were measurable at 24 hours after dosing for all dose groups. The median terminal phase half-life of total TFV in PBMCs was estimated to be 31 hours (similar to the TFV plasma estimate) from the recovery animals with PBMC concentrations measured up to 72 hours. Dose-normalized PBMC mean AUC values of total TFV increased more than dose proportionally during Week 39/40. The mean TK parameters for TAF and TFV are summarized in Table 12.

Table 12. Mean Pharmacokinetics of TFV and TAF Following Repeat-Dose Oral Administration of GS-7340-02 in Beagle Dogs for 39 Weeks

GS-7340-02 (mg/kg/day)	Study Week	TFV		TAF (GS-7340)		TFV PBMC AUC_{tau} (ng·h/10^6 cells)
		C_{max} (µg/mL)	AUC_{tau} (µg·h/mL)	C_{max} (µg/mL)	AUC_{tau} (µg·h/mL)	
2	Day 1	0.08	0.40	0.08	0.03	NA
	Week 13	0.16	1.21	0.13	0.07	NA
	Week 39/40	0.18	1.18	0.14	0.08	258.2
6	Day 1	0.25	1.31	0.72	0.37	NA
	Week 13	0.56	4.10	1.10	0.66	NA
	Week 39/40	0.54	4.45	1.42	1.03	1263.5
18	Day 1	0.80	3.80	3.60	2.07	NA
12	Week 13	0.54	12.48	3.24	2.23	NA
	Week 39/40	1.32	13.73	2.62	1.95	3118.2

NA = not applicable (only Week 39/40 samples were analyzed), PBMC = peripheral blood mononuclear cell

3.3.8. 4-Week Oral Rhesus Monkey Toxicity Study

Daily oral administration of GS-7340-02 at 3 or 30 mg/kg/day (2.4, 24 mg f.b.e./kg/day) or TFV at 15 mg/kg/day to male and female rhesus monkeys (3/sex/group, 10 mL/kg/day) for 28 days resulted in no adverse clinical signs and no clearly treatment-related effects on body weight, serum chemistry, plasma chemistry, hematology (including lymphocyte subsets determined by flow cytometry), standard urinalysis parameters, organ weights, and bone-related or histologic parameters. There was 1 death in the 30-mg/kg/day TAF group, which was not treatment related. Kidney, liver, and skeletal muscle samples assayed for indicators of mitochondrial integrity showed no effects (m2.6.7, Section 7.3.6, P2000114). The NOAEL for TAF administered orally to rhesus monkeys for 28 consecutive days was ≥ 30 mg/kg/day.

The TAF C_{max} values were nonlinear with dose, with greater than expected increases in C_{max} with dose (Table 13). The TAF AUC_{tau} could only be calculated for the 30-mg/kg/day group, with a mean value of 1.03 µg·h/mL and a terminal elimination half-life of 0.335 hours. No differences between sexes were observed.

Table 13. Mean Pharmacokinetics of TAF Following Repeat-Dose Oral Administration of GS-7340-02 in Male and Female Rhesus Monkeys, Day 28

GS-7340-02 (mg/kg/day)	C_{max} (µg/mL)	T_{max} (hours)	$t_{1/2}$ (hours)	AUC_{tau} (µg·h/mL)	CL_{ss}/F (mL/h/kg)
3	0.0188	0.917	NC	NC	NC
30	1.37	0.500	0.335	1.03	44600

NC = not calculated (due to insufficient data)

Day 28 TFV C_{max} and AUC exhibited slightly greater than proportional increases with increasing dose (Table 14). Comparison between Days 1 and 28 showed no statistical difference for C_{max} or AUC indicating no change in clearance over time. No differences between sexes were observed.

Table 14. Mean Pharmacokinetics of TFV Following Repeat-Dose Oral Administration of GS-7340-02 in Male and Female Rhesus Monkeys, Day 28

GS-7340-02 (mg/kg/day)	C_{max} (µg/mL)	T_{max} (hours)	$t_{1/2}$ (hours)	AUC_{tau} (µg·h/mL)	CLss/F (mL/h/kg)
3	0.0504	1.67	13.5	0.352	4250
30	0.963	0.700	16.1	5.87	2710

3.4. FTC/TDF

3.4.1. 14-Day Oral Gavage Toxicity Studies Comparing Non-Degraded and Degraded FTC/TDF in Rats

Two 14-day oral gavage GLP studies were conducted to investigate the potential toxicity of nondegraded and degraded FTC/TDF formulations following daily oral administration to rats for a minimum of 14 days (m2.6.7, Section 17.4, TX-164-2001 and TX-164-2005). There were no toxicologically significant differences between groups treated with nondegraded and degraded TDF/FTC, and no exacerbation of toxicity with the FTC/TDF combination compared to data with the individual agents.

3.4.2. 4-Week Oral Gavage Toxicity and Toxicokinetic Study with FTC/TDF in Male Dogs with a 4-Week Recovery Period

The purpose of this study was to evaluate the toxicity and to determine the TK of the test articles FTC and TDF, alone or in combination, when administered daily via oral gavage to dogs for at least 4 weeks and to assess the reversibility, persistence, or delayed occurrence of any effects after a 4-week recovery (m2.6.7, Section 7.4.1, TX-164-2004).

Male purebred beagles were assigned to 5 groups (n = 4/group) and administered vehicle (50 mM citrate buffer in reverse osmosis water), 20 mg/kg/day FTC, 30 mg/kg/day TDF, 2/3 mg/kg/day FTC/TDF, or 20/30 mg/kg/day FTC/TDF in combination. Two additional animals administered 20/30 mg/kg/day FTC/TDF were designated for recovery sacrifice and underwent 4 weeks of recovery following 29 days of treatment.

Assessment of toxicity was based on mortality, clinical observations, ophthalmic examinations, body weights, food consumption, and clinical and anatomic pathology. Samples were also collected for toxicokinetic evaluation. In addition, immunophenotyping and natural killer (NK) cell analysis were conducted.

All animals survived to scheduled sacrifice. No clear test article-related effects on clinical signs and ophthalmic examinations were observed. Decreases in body weight gain and food consumption were noted in animals administered 30 mg/kg/day of TDF and 20/30 mg/kg/day of FTC/TDF respectively; however, these were not statistically significant.

Between Days 1 and 27, no accumulation of FTC occurred when FTC was administered alone at 20 mg/kg/day or in combination with 30 mg/kg/day TDF. The mean AUC values for Days 1 and 27 were 20.85 and 25.28 μg·h/mL, respectively, when administered alone and 24.46 and 32.33 μg·h/mL, respectively, when administered in combination. During the same period, no unexpected accumulation of TFV occurred when TDF was administered alone at 30 mg/kg/day or in combination with 20 mg/kg/day FTC. The mean AUC values for Days 1 and 27 were 7.60 and 18.41 μg·h/mL, respectively, when administered alone and 8.85 and 20.87 μg·h/mL, respectively, when administered in combination.

TDF administration at 30 mg/kg/day, alone or in combination with FTC administration, was associated with minimally increased activated partial thromboplastin time (APPT) and creatinine. Both effects appeared to be reversible following recovery. Urinalysis test results appeared unaffected.

No remarkable changes were observed for immunophenotyping or NK cell assay values for test article-treated groups as compared with the vehicle control group.

No test article-related macroscopic findings or organ weight variations were present at either the terminal or recovery sacrifice.

Test article-related microscopic findings at the terminal sacrifice were seen in the kidneys of animals in 2 groups. Minimal tubular epithelial necrosis and an increased incidence and mean severity of tubular epithelial regeneration were seen in animals administered 30 mg/kg/day of TDF alone or in combination with 20 mg/kg/day of FTC. Kidney lesions were morphologically similar in the 2 affected groups, and there were no overall differences in incidences and mean severities of tubular necrosis and regeneration noted between the 2 groups.

No test article-related microscopic changes were observed at the recovery sacrifice, indicating that the kidney findings noted microscopically at dosing termination were reversible.

In conclusion, administration of FTC alone at a dose of 20 mg/kg/day, or FTC/ TDF combination at 2/3 mg/kg/day, was well tolerated by dogs for 4 weeks. TDF alone at 30 mg/kg/day and FTC/TDF at 20/30 mg/kg/day resulted in microscopic kidney lesions, which in the case of FTC/TDF at 20/30 mg/kg/day were reversible after a 4-week recovery period. Based on the clinical and anatomic pathology findings, the NOAEL was 20 mg/kg/day for FTC alone and 2/3 mg/kg/day for the FTC/TDF combination. A NOAEL for TDF could not be determined at the doses used in the study.

3.5. B/F/TAF

Administration of BIC in combination with FTC and TAF is unlikely to exacerbate known toxicities of the individual agents. Bictegravir, FTC and TAF exhibit different patterns of target organ toxicity. Hepatobiliary changes in monkeys at BIC AUC values 16-fold higher than clinical BIC AUC values was the only BIC-related toxicity observed in chronic rat and monkey studies; the NOEL in the 39-week monkey study provides a 7.0-fold exposure margin. In the 26-week rat study, the NOAEL provides 18/46-fold (males/females) exposure margins. The only toxicity observed in chronic animal studies with FTC was mild, reversible anemia in mice and minor decreases in erythrocyte counts/increases in MCH in monkeys at large multiples of clinical FTC AUC exposure values; therefore, these hematological findings are not considered relevant to clinical use. Extensive nonclinical investigations have shown that target organs for TAF are distinctly different (bone and kidney) from BIC or FTC. Exaggerated hepatobiliary, renal or bone toxicity is not anticipated to be a concern with the B/F/TAF combination product.

Comprehensive nonclinical safety databases on these individual drugs, including combination toxicity studies with FTC and TDF, strongly indicate that further toxicological investigations of the combination of agents are unlikely to yield new data relevant to humans. Based on the well-characterized toxicity profiles of the single agents, low potential for toxicologic interaction noted in combination toxicology studies with FTC and TDF, and clinical safety data available for B/F/TAF and other FTC- and/or TDF-/TAF-containing regimens (Emtriva, Truvada, Atripla, Complera/Eviplera, Stribild, Genvoya, Odefsey, Descovy, and/or Vemlidy), additional combination toxicity studies are not considered warranted.

4. GENOTOXICITY

4.1. BIC

4.1.1. In Vitro

4.1.1.1. Mutagenicity Test in the *Salmonella – Escherichia coli*/Mammalian-Microsome Reverse Mutation Assay with a Confirmatory Assay

BIC mutagenic potential was evaluated in a bacterial reverse mutation assay using histidine-dependent mutant strains of *Salmonella typhimurium* (*Salmonella*; TA98, TA100, TA1535, and TA1537), and the tryptophan-dependent *Escherichia coli* (*E. coli*) strain, WP2*uvr*A, in the presence or absence of an exogenous mammalian metabolic activation system (S9) using the plate incorporation methodology (m2.6.7, Section 8.1, TX-141-2026). BIC was formulated in dimethylsulfoxide (DMSO), and evaluated in all five tester strains at doses up to 5000 µg/plate with and without S9 metabolic activation. Bacteria were also incubated with standard positive control agents, and the response of the various bacterial strains to these agents confirmed the sensitivity of the test system and the activity of the S9 mix.

There were no increases in the numbers of revertant colonies observed with any of the tester strains at any dose level in the presence or absence of S9 metabolic activation. Precipitate was observed on all *Salmonella* strain plates at the 5000 µg/plate dose level. Toxicity was evident at dose levels ≥ 1600 µg/plate with TA98, TA100, TA1535, and TA1537 by reduced numbers of revertant colonies and by reduced or absent background lawn growth in tests with and without S9. Toxicity was also evident at 5000 µg/plate with WP2*uvr*A by reduced numbers of revertant colonies compared to vehicle control values. Thus, under the conditions of this study, BIC was considered negative in the bacterial reverse mutation assay.

4.1.1.2. In Vitro Mammalian Chromosome Aberration Test in Human Lymphocytes

The objective of this in vitro assay was to evaluate the ability of BIC to cause structural chromosomal aberrations in cultured human lymphocytes with and without metabolic activation (m2.6.7, Section 8.2, TX-141-2027). In the initial test, DMSO vehicle or BIC (3.39 - 500 µg/mL) were evaluated for 3-hour treatment periods with and without metabolic activation and for an approximate 24 hour continuous treatment without metabolic activation. In the 3-hour treatment assay without metabolic activation, chromosomal aberrations were analyzed from the cultures treated with 84.0, 172, and 245 µg/mL. In the 24-hour treatment assay without metabolic activation, chromosomal aberrations were analyzed from the cultures treated with 9.89, 20.2, and 28.8 µg/mL. In the 3-hour treatment assay with metabolic activation, chromosomal aberrations were analyzed from the cultures treated with 120, 172, and 245 µg/mL.

There were no statistically significant or biologically relevant increases in the number of cells with chromosomal aberrations or endoreduplication observed in the cultures analyzed under any of the test conditions. An increase in the number of polyploid cells was observed at all dose levels evaluated under all test conditions. The reason for the increase in polyploidy cells was not determined. Treatment of cultures with the vehicle (DMSO) and positive control articles

produced the expected response meeting acceptance criteria and validating test sensitivity. Thus, BIC was considered negative for inducing chromosomal aberrations in cultured human lymphocytes when tested with and without metabolic activation.

4.1.2. In Vivo

4.1.3. 2-Week Oral Gavage Toxicity and Toxicokinetic Study in Rat with Micronucleus Assessment

The objective of the micronucleus portion of the 2-week rat toxicity study was to evaluate BIC for in vivo clastogenic activity and/or disruption of the mitotic apparatus by counting micronuclei in polychromatic erythrocytes (PCEs) in male and female Crl:WI (Han) rat bone marrow following 2 weeks of oral gavage dosing of BIC at 0, 10, 30, 100, and 300 mg/kg/day (m2.6.7, Section 9.1, TX-141-2029). Femoral bone marrow was extracted and at least 2000 PCEs per animal were analyzed for the frequency of micronuclei. Cytotoxicity was assessed by scoring the number of PCEs and normochromatic erythrocytes (NCEs) in at least 1000 total erythrocytes for each animal.

BIC was evaluated as negative in the male and female rat bone marrow micronucleus assay due to the lack of statistically significant increases in micronucleated PCEs at any dose examined. Mean BIC $AUC_{0\text{-}24}$ on Day 14 rats administered 300 mg/kg/day was 2970 µg h/mL (sexes combined), corresponding to a 29-fold exposure margin.

4.2. FTC

4.2.1. In Vitro

4.2.1.1. Mutagenicity Test with FTC in the *Salmonella* – *Escherichia coli*/Mammalian-Microsome Reverse Mutation Assay with a Confirmatory Assay

FTC was dissolved in DMSO and tested for mutagenic activity in the *Salmonella-Escherichia coli*/Mammalian-Microsome Reverse Mutation Assay (m2.6.7, Section 8.3, 18637-0-409R). The concentrations tested, along with vehicle and positive control test substances, were 5000, 3300, 1000, 333, and 100 µg/plate in the presence and in the absence of metabolic activation. The tester strains were *Salmonella typhimurium* TA98, TA100, TA1535, TA1537, and *Escherichia coli* strain WP2*uvr*A. Emtricitabine did not cause a positive increase in the number of revertants per plate in any of the tester strains in either the presence or absence of metabolic activation. The confirmatory assay, at the concentrations of FTC listed above, also gave negative results for mutagenic activity. It was concluded that FTC was negative in the reverse mutation (Ames) assay.

4.2.1.2. *Salmonella*/Mammalian Microsome Assays with FTC

Emtricitabine was tested for its ability to induce reverse mutations in 2 Ames *Salmonella*/mammalian-microsome assays, the *Salmonella* plate incorporation assay and the *Salmonella* preincubation assay (m2.6.7, Section 8.4, MUT203). The tester strains used were TA1535, TA1537, TA1538, TA98, and TA100, both in the presence and absence of Arochlor-induced rat liver S9 metabolic action. The range of concentrations of FTC was 100 to 5000 μg/plate. There was no detectable mutagenic activity at any dose of FTC when tested by either Ames assay method.

4.2.1.3. Mutagenicity Test of FTC using Microorganisms

The ability of FTC to induce mutations was investigated by using *Salmonella typhimurium* strains TA100, TA1535, TA98, and TA1537, and *Escherichia coli* strain WP2 *uvrA* with a preincubation method in the presence and absence of a metabolic activation system (S9 mix) (m2.6.7, Section 8.5, K01-3154). The mutagenicity of the test substance was judged negative because the number of revertant colonies in the test substance groups was less than twice that of each negative control in all test strains. It is concluded that FTC has no ability to induce mutations under the conditions of the assay.

4.2.1.4. In Vitro Mammalian Cell Gene Mutation Test

The free base of FTC was dissolved in sterile distilled water and tested for its potential to induce forward mutations at the TK locus in the mouse lymphoma L5178Y cell line (m2.6.7, Section 8.6, TOX012). Exposures to FTC were in the presence and in the absence of Aroclor-induced rat liver S9 metabolic activation. A preliminary experiment was used to select FTC concentrations ranging from 0.5 to 5000 μg/mL for testing. Cells at test concentrations of 1000, 2000, 3000, 4000, and 5000 μg/mL were cloned and evaluated with and without S9. For FTC, no cloned cultures exhibited mutant frequencies that were at least 55 mutants/10^6 clonable cells over that of sterile distilled water. Consistent with the low order of cytotoxicity established for FTC, no toxicity in the cloned cultures, ie, total growth of ≤ 50% of the distilled water vehicle, was observed at any dose level. The colony sizing for the methyl methanesulfonate positive control yielded the expected increase in small colonies, verifying the adequacy of the methods used to detect small colony mutants. It was concluded that FTC was negative in the L5178Y/$TK^{+/-}$ mouse lymphoma mutagenesis assay at concentrations up to, and including, 5000 μg/mL.

4.2.2. In Vivo

4.2.2.1. Mammalian Erythrocyte Micronucleus Test

In this study, the free base of FTC was dissolved in sterile distilled water and administered by gavage to groups of 5 male ICR mice (m2.6.7, Section 9.2, TOX011). The doses administered were 0, 500, 1000, and 2000 mg/kg. Bone marrow was aspirated from the femur at 24 and 48 hours postdose. The slides of bone marrow were stained with May-Gruenwald-Giemsa stain. Light microscopy was used to count the number of micronuclei per 2000 PCEs. The data were analyzed using a 1-way analysis of variance and pairwise comparisons were made using

Dunnett's t-test. The positive control, cyclophosphamide (60 mg/kg by gavage), produced a marked and significant increase in micronuclei. No significant increase in micronucleated PCEs was observed, at either time point or at any dose, in mice administered FTC. It was concluded that FTC was negative in the mouse in vivo micronucleus assay.

4.3. TAF

4.3.1. In Vitro

4.3.1.1. Mutagenicity Test in the *Salmonella-Escherichia Coli/*Mammalian-Microsome Reverse Mutation Assay

The mutagenic potential of GS-7340-02 was evaluated in vitro in the Salmonella-Escherichia coli/mammalian microsome reverse mutation assay and GS-7340-02 up to 5000 µg/mL (4000 µg f.b.e./mL) did not cause a positive increase in the mean number of revertants per plate with any of the tester strains (m2.6.7, Section 8.7, V990212).

4.3.1.2. Mutagenicity Test in the L5178Y TK+/- Mouse Lymphoma forward Mutation Assay with a Confirmatory Assay

GS-7340-02 was evaluated for its ability to induce forward mutations at the tk locus in the mouse lymphoma L5178Y cell up to 5000 µg/mL (4000 µg f.b.e/mL) following exposure times of either 4 hours or 24 hours (m2.6.7, Section 8.8, V990213). All tests were performed either in the presence or in the absence of microsomal enzymes prepared from Aroclor™-induced rat liver. GS-7340-02 was evaluated as negative for inducing forward mutations at the tk locus in L5178Y mouse lymphoma cells under the nonactivating and activating conditions used.

4.3.2. In Vivo

4.3.2.1. Mouse Micronucleus Assay

Crl:CD-1®(CR) BR mice were administered GS-7340-02 to evaluate in vivo clastogenic activity and/or disruption of the mitotic apparatus in the bone marrow (m2.6.7, Section 9.3, M2000113). Five males administered 500 and 1000 mg/kg (400 and 800 mg f.b.e./kg) and 5 animals administered the positive control article were euthanized ~24 hours postdosing and the bone marrow harvested and evaluated for micronuclei. Five males administered 2000 mg/kg and 5 animals administered the vehicle control article were euthanized ~24 and 48 hours postdosing and the bone marrow harvested and evaluated for micronuclei. GS-7340-02 induced no signs of clinical toxicity. Cytotoxicity to the bone marrow (statistically significant decrease in the polychromatic erythrocyte:normochromatic erythrocyte [PCE:NCE] ratio) was noted at the 2000 mg/kg dose group at the 24- and 48-hour harvest time points. No statistically significant increase in micronucleated PCEs occurred. TAF was negative for genotoxicity in this study. Statistically significant increases were apparent in the positive control animals.

4.4. FTC/TDF

4.4.1. In Vitro

4.4.1.1. Bacterial Reverse Mutation Assay with FTC/TDF

A combination of FTC and TDF was tested in the Bacterial Reverse Mutation Assay using *Salmonella typhimurium* tester strains TA98, TA100, TA1535, and TA1537, and *Escherichia coli* tester strain WP2 *uvr*A in the presence and absence of Aroclor-induced rat liver S9 (m2.6.7, Section 8.9, TX-164-2002). The assay was performed in 2 phases using the plate incorporation method. The first phase, the initial toxicity-mutation assay, was used to establish the dose range for the confirmatory mutagenicity assay and to provide a preliminary mutagenicity evaluation. The second phase, the confirmatory mutagenicity assay, was used to evaluate and confirm the mutagenic potential of the test article. Dimethylsulfoxide was selected as the solvent of choice based on compatibility with the target cells. Dosing solutions of the 2 test article components were prepared in a fixed ratio of 2 parts of FTC to 3 parts of TDF. Each component was dissolved separately in DMSO and then combined. Subsequent dilutions of the combination were prepared by serial dilution in DMSO. The dose levels refer to the test article combination.

The dose levels tested in the initial toxicity-mutation assay were 1.5, 5.0, 15, 50, 150, 500, 1500, and 5000 µg per plate. No positive mutagenic responses were observed. Neither precipitate nor appreciable toxicity was observed. Based on the findings of the initial toxicity-mutation assays, the dose levels tested in the confirmatory mutagenicity assay were 50, 150, 500, 1500, and 5000 µg per plate. In the confirmatory mutagenicity assay, no positive mutagenic response was observed. Neither precipitate nor appreciable toxicity was observed. Under the conditions of this study, the combination of FTC and TDF were concluded to be negative in the Bacterial Reverse Mutation Assay.

4.4.1.2. In Vitro Mammalian Cell Gene Mutation Test (L5178Y/TK$^{+/-}$ Mouse Lymphoma Assay) with FTC/TDF

The test article, FTC and TDF at a fixed ratio of 2:3 (FTC/TDF), was tested in the L5178Y/TK$^{+/-}$ Mouse Lymphoma Mutagenesis Assay in the absence and presence of Aroclor-induced rat liver S9 (m2.6.7, Section 8.10, TX-164-2003). The preliminary toxicity assay was used to establish the concentration range for the mutagenesis assay. The mutagenesis assay was used to evaluate the mutagenic potential of the test article. Dimethylsulfoxide was selected as the solvent of choice based on solubility information and compatibility with the target cells.

In the preliminary toxicity assay, the maximum concentration of FTC/TDF was 3575 µg/mL. No visible precipitate was present at any concentration in treatment medium; however, substantial toxicity (ie, suspension growth of ≤ 50% of the solvent control) was observed at ≥ 500 µg/mL with and without S9 activation (4-hour exposure) and at ≥ 150 µg/mL without activation (24-hour exposure). Based on the results of the preliminary toxicity assay, the concentrations chosen for treatment in the mutagenesis assay ranged from 15 to 1000 µg/mL for the nonactivated cultures and 15 to 1500 µg/mL for the S9-activated cultures. No visible precipitate was present at any concentration in treatment medium. The concentrations chosen for cloning

were 15, 50, 100, 150, and 200 µg/mL without activation, and 50, 100, 200, 300, 400, and 500 µg/mL with S9 activation. There was a concentration-related increase in mutant frequency in both the nonactivated and activated assays with a 4-hour exposure (mutant frequencies ≥ 100 mutants per 10^6 clonable cells over that of the solvent control). Toxicity in the cloned cultures (total growth of ≤ 50% of the solvent control) was observed at concentrations ≥ 100 µg/mL without activation and ≥ 300 µg/mL with activation.

The trifluorothymidine-resistant colonies for the cloned cultures and for the positive and solvent control cultures from both assays were sized according to diameter over a range from approximately 0.2 to 1.1 mm. There was an increase in the frequency of small colonies when the treated cultures were compared to the solvent control cultures. An increase in the frequency of small colonies is consistent with damage to multiple loci on chromosome 11 in addition to functional loss of the TK locus. Under the conditions of this study, the test article FTC/TDF was concluded to be positive in the L5178Y/$TK^{+/-}$ Mouse Lymphoma Mutagenesis Assay.

4.5. B/F/TAF

No genotoxicity studies were conducted with the combination of B/F/TAF. BIC, FTC, and TAF as single agents and FTC/TDF in combination were negative in genotoxicity studies. The combination of BIC, FTC and TAF is therefore not expected to have an altered genotoxicity profile as compared with that of the individual agents.

5. CARCINOGENICITY

The 6-month oral carcinogenicity study of BIC in transgenic mice has been completed. The 2-year rat carcinogenicity study will be completed by November 2017, and the final carcinogenicity report will be submitted to specific health authorities during the marketing application review or after approval, in accordance with the agreed upon timelines.

Per separate agreements with FDA and CHMP, carcinogenicity studies were not required for TAF registration based on the lack of TAF exposure in rats and TgRasH2 mice, and lower TFV exposure in rats and mice compared to TDF. Summaries of the carcinogenicity studies with TDF are provided below. Two-year carcinogenicity studies were completed in mice and rats with FTC and provided below.

5.1. BIC

5.1.1. 6-Month Oral Carcinogenicity Study with BIC in Transgenic Mice

To evaluate the carcinogenicity of BIC, male/female 001178-T (hemizygous) rasH2 mice (25/sex/group) received BIC at 0/0, 5/10, 15/30, or 100/300 mg/kg/day by oral gavage for 26 weeks (m2.6.7, Section 10.1, TX-141-2047) at a dose volume of 5 mL/kg. Dose levels were agreed upon via communication with the FDA Carcinogenicity Assessment Committee (Ref ID 3860358). Animals were observed for clinical signs of toxicity, palpable masses, and changes in body weight and food consumption. At scheduled euthanasia, gross pathologic changes were recorded and tissues were examined microscopically. Histopathologic findings were peer-reviewed, and neoplastic findings were evaluated for statistically significant differences and trends. Blood samples were collected from satellite groups of rasH2 (wild type) animals for TK evaluation on Day 1 and Week 26.

No treatment-related effects on animal survival or clinical signs were noted. No BIC-related anatomic pathology findings, clinical observations, mass observations, or organ weight changes were noted. Minor nonadverse changes in mean body weight and mean body weight change observed were of uncertain relationship to BIC as they were not dose-dependent and were not consistently found between males and females.

Plasma exposure to BIC increased with the increase in dose level from 5 to 100 mg/kg/day for males and from 10 to 300 mg/kg/day for females. The increases in C_{max} and AUC were generally dose-proportional between 5 and 15 mg/kg/day for males and 10 and 30 mg/kg/day for females, while the increases were less than dose-proportional between 15 and 100 mg/kg/day for males and 30 and 300 mg/kg/day for females. Accumulation was observed after multiple doses in mice, approximately 4-fold in males and 2-fold in females.

Table 15. Toxicokinetic Parameters for BIC in 001178-T (hemizygous) RasH2 Mice – Day 1 and Week 26

BIC Dose Level (mg/kg/day)	Study Day/Week	C_{max} (µg/mL)		AUC_{0-24} (µg·h/mL)	
		Male	Female	Male	Female
5	Day 1	7.54	-	46.6	-
	Week 26	18.2	-	190	-
10	Day 1	-	13.8	-	150
	Week 26	-	25.8	-	341
15	Day 1	15.9	-	147	-
	Week 26	42.3	-	526	-
30	Day 1	-	28.0	-	382
	Week 26	-	69.2	-	861
100	Day 1	42.0	-	383	-
	Week 26	104	-	1560	-
300	Day 1	-	89.1	-	1240
	Week 26	-	143	-	2340

- Denotes no data available

In conclusion, daily administration of BIC at dosage levels of up to 100/300 mg/kg/day (males/females) administered for 26 weeks was not carcinogenic in rasH2 mice. At 100/300 mg/kg/day for males/females, mean BIC AUC values are 15/23-fold higher than BIC AUC following clinical administration of the B/F/TAF FDC.

5.1.2. 2-Year Oral Carcinogenicity Study with BIC in Rats

The 2-year rat carcinogenicity study with BIC was completed in November 2017 (m2.6.7, Section 1.1.1, TX-141-2040) at dose levels up to 300 mg/kg/day. Dose levels were agreed upon per Special Protocol Assessment by the FDA Carcinogenicity Assessment Committee (Ref ID 3733591). The first dose was administered 21 May 2015.

5.2. FTC

5.2.1. 2-Year Oral Carcinogenicity Study with FTC in CD-1 Mice

This study was conducted to evaluate the oncogenic potential of FTC and to characterize any chronic toxicities in Charles River CD-1 mice following 104 weeks of daily oral gavage administration (m2.6.7, Section 10.3, TOX109). The main study consisted of 3 groups of 60 male and 60 female mice that received the test article at a dose level of 80, 250, or 750 mg/kg/day, and an additional group of 60 male and 60 female mice that served as the control and were administered the vehicle (0.5% aqueous methylcellulose in deionized distilled water). Three additional treatment groups each containing 40 animals per sex per group were designated

for toxicokinetic evaluation at dose levels of 80, 250, and 750 mg/kg/day. In addition, a sentinel group (nondosed) containing 25 animals per sex were used to evaluate fecal parasites and serology during the course of the study. The dose volume used in all groups was 10 mL/kg.

All animals were observed at least twice daily for morbidity, mortality, injury, overt clinical signs, and availability of food and water. Body weight and food consumption measurements were recorded for all animals during the course of the study. Detailed observations for clinical signs were recorded weekly for main study animals during the course of the study. Various hematologic evaluations were conducted at study termination for main study animals. Blood was collected for toxicokinetic evaluations at various time points during Weeks 2, 26, and 104. At study termination, complete necropsy examinations were performed and selected tissues were microscopically examined.

No effect of treatment was seen in any parameter evaluated, including survival, clinical and mass findings, body weight, food consumption, hematology, and macroscopic and microscopic pathology. No treatment-related microscopic changes suggestive of a carcinogenic effect were observed.

Emtricitabine was rapidly absorbed following all doses with peak plasma concentrations occurring at 0.5 to 1 hour postdose (see m2.6.5, Section 4.2.1, TOX109). $AUC_{0–24h}$ and C_{max} increased proportionally with doses over the range of 80 to 750 mg/kg/day. In general, the exposures ($AUC_{0–24h}$) in male mice were similar to those in female mice at all doses. $AUC_{0–24h}$ and C_{max} values were higher at Week 26 compared to Week 2. At Week 104, FTC concentrations were below the limit of quantitation in most control animals. The average exposure in mice in this oncogenicity study ranged from 2.1- to 25-fold greater (at 80 and 750 mg/kg/day, respectively) than clinical exposure of FTC in the B/F/TAF FDC (based upon FTC AUC_{tau} = 12.3 µg h/mL in HIV-1 infected subjects in clinical studies GS-US-380-1489, GS-US-380-1490, GS-US-380-1844, and GS-US-380-1878).

In summary, the daily administration of FTC for 2 years to Charles River CD-1 mice at dose levels at 80, 250, and 750 mg/kg/day did not produce any evidence of a carcinogenic effect.

5.2.2. 2-Year Oral Oncogenicity Study with FTC in Rats

This study was conducted to evaluate the oncogenic potential of FTC and to characterize any chronic toxicities in CD (Crl: CD® [SD]IGS BR) rats following 104 weeks of daily oral gavage administration (m2.6.7, Section 10.4, TOX108). The main study consisted of 3 groups of 60 male and 60 female rats that received the test article at dose levels of 60, 200, or 600 mg/kg/day, and an additional group of 60 male and 60 female rats that served as the control and were administered the vehicle (0.5% aqueous methylcellulose in deionized distilled water). Three additional treatment groups each containing 20 animals per sex per group were designated for toxicokinetic evaluation at dose levels of 60, 200, or 600 mg/kg/day. The dose volume was 10 mL/kg.

All animals were observed at least twice daily for morbidity, mortality, injury, overt clinical signs, and availability of food and water. Body weight and food consumption measurements were recorded for all animals during the course of the study. Detailed observations for clinical

signs were recorded weekly for main study animals during the course of the study. Various hematologic evaluations were conducted at study termination for main study animals. Blood was collected for toxicokinetic evaluations at various time points during Weeks 2, 26, and 104. At study termination, complete necropsy examinations were performed and selected tissues were microscopically examined.

No effect of treatment was seen in any parameter evaluated including survival, clinical and mass findings, body weight, food consumption, hematology, and macroscopic and microscopic pathology. No treatment-related microscopic changes suggestive of a carcinogenic effect were observed.

Emtricitabine was rapidly absorbed following all doses, with peak plasma concentrations occurring at 0.5 hours postdose. AUC_{0-24h} and C_{max} increased with dose over the range of 60 to 600 mg/kg/day. In general, exposures (AUC_{0-24h}) in male rats were similar to those in female rats. AUC_{0-24h} and C_{max} values were higher at Week 26 compared to Week 2. The average exposure, based on Week 2 and Week 26 AUC_{0-24h} values, in rats in this oncogenicity study ranged from 3.9- to 30-fold greater (at 60 and 600 mg/kg/day, respectively) than clinical exposure of FTC in the B/F/TAF FDC (based upon FTC AUC_{tau} = 12.3 µg·h/mL as noted in above section).

In summary, the daily administration of FTC for 2 years to CD rats at dose levels of 60, 200, and 600 mg/kg/day did not produce any evidence of a carcinogenic effect.

5.3. TDF

5.3.1. 104-Week Oral Carcinogenicity Study with TDF in Mice

In a carcinogenicity study of TDF, Swiss Crl:CD1(ICR) BR mice (60/sex/group) received daily doses of 0, 100, 300, or 600 mg/kg/day by oral gavage for 104 weeks (m2.6.7, Section 10.5, M990205).

Treatment-related clinical signs were restricted to salivation. Salivation following dose administration was noted for all animals receiving 300 or 600 mg/kg/day, with a higher incidence being seen in animals receiving 600 mg/kg/day.

A reduced group mean body weight was recorded for animals receiving 300 (4.2% and 0.6% for males and females, respectively) or 600 mg/kg/day (4.6% and 3.3% for males and females, respectively) when compared with the concurrent controls over the 104-week treatment period. The differences from the controls were dosage related and on occasion attained statistical significance. There were no effects on body weight in animals receiving 100 mg/kg/day.

There were no treatment-related effects on food consumption, hematology parameters, or ophthalmoscopic findings.

Treatment-related effects were observed in the bone marrow, duodenum, kidneys, liver, and testes. The results of the histopathology examination revealed a low incidence of treatment-related changes in the duodenum (adenoma/adenocarcinoma) at 600 mg/kg/day. A

statistically significant increase in hepatocellular adenoma in females at 600 mg/kg/day was observed; however the incidence observed was within the spontaneous background range and were considered likely incidental. Nonneoplastic lesions were observed in duodenum (epithelial hyperplasia) at 100, 300, and 600 mg/kg/day; in liver (eosinophilic and basophilic cell foci, hepatocellular hypertrophy, nuclear inclusions, cyto/karyomegaly, single cell necrosis, proliferation of sinusoidal cells) and testes (vascular mineralization) at 300 or 600 mg/kg/day; and in kidneys (karyomegaly) and bone marrow (hypercellularity: myeloid) at 600 mg/kg/day. An NOEL for nonneoplastic lesions (by histological criteria) was not established in this study.

Concentrations of TFV in plasma were determined during the study (m2.6.7, Section 10.5, M990205). Blood samples were taken from mice (3 mice/sex/time point) on Day 1 and Day 180 following daily oral dosing of 600 mg/kg/day TDF. Time points included predose; 5 (Day 1 only), 15, and 30 minutes; and 1, 2, 4, 8 (on Day 180, 2 males and 2 females were used for sampling), 12 and 24 hours postdose. Samples were processed for plasma and concentrations of TFV in plasma were determined using a validated liquid chromatography/mass spectrometry (LC/MS) method.

Following the first oral dose in male and female mice, concentrations of TFV reached mean peak values of 11.5 and 17.6 μg/mL, respectively. The corresponding mean calculated AUC_{inf} values in male and female mice were 50.4 and 48.7 μg·h/mL, respectively. The extent of exposure (AUC_{0-24h}) of TFV appeared to be similar between the male and female mice on Days 1 and 180. AUC_{inf} on Day 1 appeared to be comparable to AUC_{0-24h} on Day 180 in both the male and female mice, suggesting TFV possesses linear toxicokinetic properties at a dose of 600 mg/kg/day.

In conclusion, lifetime daily administration of TDF at dosage levels of up to 300 mg/kg/day administered for 104 weeks did not reveal any evidence of carcinogenicity in Swiss Crl:CD^{R}-1(ICR)BR mice. At 600 mg/kg/day, a low incidence of duodenal tumors, possibly treatment-related, was observed.

5.3.2. 104-Week Oral Carcinogenicity Study with TDF in Rats

In a carcinogenicity study of TDF, Sprague-Dawley CD rats (60/sex/group) received daily doses of 0, 30, 100, or 300 mg/kg/day by oral gavage for 103/104 weeks (m2.6.7, Section 10.6, R990204). The high dose animals (300 mg/kg/day) were initially assigned as the low-dose group and received 10 mg/kg/day until Day 10 when the dose was increased to 300 mg/kg/day.

Survival ranged from 27% to 47% at termination, which was within the historical range for the laboratory. Treatment-related clinical signs included salivation in the 100 and 300 mg/kg/day animals. The number of rats with clinically observed masses was similar between control and treated groups. Lower mean body weights were recorded for males (100 and 300 mg/kg/day) and females (300 mg/kg/day) during the first 3 quarters of the study (with differences reaching statistical significance on occasion). Mean food consumption for all treated groups was comparable to controls. There were no changes in hematology (RBC, WBC) at Months 12, 18, or 24.

A dose-related increase in the incidence of renal tubular karyomegaly was observed in treated animals. This finding is consistent with previous observations in repeat-dose studies of TDF in rats.

Once daily oral administration of TDF to rats for 103/104 consecutive weeks did not result in any increase in the incidence of neoplastic lesions in various organ and tissue systems examined. The benign and malignant tumors observed followed no pattern, appeared to be spontaneous in nature, and were within the limits reported for aging CD Sprague-Dawley rats.

Concentrations of TFV in plasma were obtained during the study (m2.6.7, Section 10.6, R990204). Blood samples were collected from rats (3 rats/sex/time point) on Day 1 and Day 180 following daily oral dosing of 30, 100, and 300 mg/kg TDF. Time points included predose and 0.25, 0.5, 1, 2, 4, 8, and 24 hours postdose. Samples were processed for plasma and concentrations of TFV in plasma were determined using a validated LC/MS method.

Following the first oral dose in male rats, concentrations of TFV in plasma reached mean peak values of 0.54, 1.31, and 1.99 µg/mL at the 30, 100, and 300 mg/kg/day dose levels, respectively. The corresponding calculated mean AUC_{inf} values in male rats were 2.53, 5.82, and 19.36 µg·h/mL. For female rats, the C_{max} following the first dose was 0.77, 1.42, and 6.61 µg/mL at the 30, 100, and 300 mg/kg/day dose levels, respectively. The corresponding calculated mean AUC_{inf} values in female rats were 2.39, 5.27, and 14.18 µg·h/mL. On Day 180, mean plasma $AUC_{ss(0–24h)}$ at dose levels of 30, 100, and 300 mg/kg/day were 2.70, 6.60, and 16.69 µg h/mL, respectively, for male rats; and 3.06, 7.46, and 15.72 µg·h/mL, respectively, for female rats. The extent of exposure ($AUC_{0–24h}$) of TFV appeared to be similar between the male and female rats on Days 1 and 180. C_{max} values were generally comparable between males and females. Exposure was linear within each dose level, but did not increase in a dose-proportional fashion when doses were increased from 30 to 300 mg/kg/day.

In conclusion, lifetime daily administration of TDF at dosage levels up to 300 mg/kg/day did not reveal any evidence of carcinogenicity in Sprague-Dawley rats.

5.4. B/F/TAF

BIC was not carcinogenic in the 6-month transgenic mouse study. The 2 year rat carcinogenicity study for BIC is ongoing. No clinically relevant increases in tumor incidence were found in mice or rats administered FTC in long-term carcinogenicity studies. With TDF, a low incidence of duodenal tumors was noted at a very high dose in mice; there was no evidence of carcinogenic potential in rats. Exposure to TFV is lower after TAF than TDF administration, supporting the low carcinogenic potential of TAF. Combination dosing of B/F/TAF would not likely change these profiles as no exposure difference would be expected and no exacerbation of toxicity/genotoxicity is expected. Based on the overall lack of genotoxicity and/or the data from carcinogenicity studies with the individual agents, the conduct of carcinogenicity studies with the B/F/TAF combination are not considered necessary nor are such studies likely to add to the safety evaluation of the B/F/TAF FDC.

6. REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

6.1. BIC

6.1.1. Fertility and Early Embryonic Development

6.1.1.1. Oral Fertility and Early Embryonic Development Study in Male and Female Rats

The purpose of this study was to evaluate the effects of BIC on mating and fertility when administered daily via oral gavage to male and female Crl:CD(SD) rats prior to mating and until termination (males) or through early gestation (females) (m2.6.7, Section 12.1, TX-141-2039). This study included assessment of potential effects on gonadal function, mating behavior, implantation, female estrous cyclicity, and fertility.

Male and female Crl:CD(SD) rats (25/group/sex) received BIC at 0, 5, 30, or 300 mg/kg/day by once daily oral gavage at a dose volume of 5 mL/kg. Females were dosed for 14 days prior to mating, throughout the mating period and through Gestation Day (GD) 7 for a total of 22-35 doses. On GD 15, females were necropsied and the uterus of each was examined for the number of live and dead fetuses and resorptions, and ovaries were examined for the number of corpora lutea. Males were dosed for 28 days prior to mating, during the mating period and through the day prior to termination for a total of 61-62 doses, and evaluated for reproductive function. Assessment of toxicity was based on mortality, clinical signs, body weight, body weight gain, food consumption, reproductive performance, estrous cyclicity (females), macroscopic observations, organ weights, cesarean section findings (numbers of corpora lutea, implantation sites, resorptions, and live fetuses), and sperm analysis (sperm motility and counts).

There was no test article-related mortality. No BIC-related effects on clinical signs, body weight, food consumption, and no effects on parental toxicity were observed. There were no BIC-related macroscopic observations or changes in reproductive organ weights. No effects on male or female reproductive performance (male and female mating, fertility, and copulation/conception indices, estrous cyclicity, and the number of days between pairing and mating) were observed. Average numbers of corpora lutea, implantation sites, resorptions, and viable embryos were similar across groups, indicating that BIC had no effect on embryofetal viability. All sperm parameters evaluated were unaffected by BIC.

Significantly decreased mean cauda epididymal sperm concentrations were noted in the 30 and 300 mg/kg/day groups compared to the concurrent vehicle control group; however, the values were within the range of the historical control data values at the Test Facility. Additionally, as the weight of the cauda epididymis may correspond with the spermatid concentration stored in the cauda portion of the epididymis (cauda sperm concentration) {Coder 2010}, a significant test article-related reduction in epididymal sperm concentration would be expected to correlate with decreased cauda epididymal weights and decreased testicular sperm concentration. In the current study, there was no correlating effect on cauda epididymal weights on a mean or individual animal basis or a reduction in testicular sperm concentration at 30 and 300 mg/kg/day. In addition, all sperm parameters (testicular sperm concentration, sperm production rate, motility, and sperm morphology) in these groups were similar to the vehicle control group, and no effects

on male fertility were noted at 30 or 300 mg/kg/day. Based on the weight of the evidence, the decreased mean cauda epididymal sperm concentrations at 30 and 300 mg/kg/day relative to the vehicle control group were considered incidental and not test article-related.

In conclusion, oral administration of BIC to male and female rats prior to mating and until termination (males) or through early gestation (females) up to 300 mg/kg/day showed no effects on male or female reproductive performance or embryonic viability. The NOEL for male and female fertility and early embryonic development in rats was 300 mg/kg/day BIC. At the NOEL in this study, mean BIC AUC is 29-fold higher than BIC AUC following clinical administration of the B/F/TAF FDC (based on Day 14 values (sexes combined) of the 2-week rat study; m2.6.7, Section 7.1.2, TX-141-2029).

6.1.2. Embryofetal Development

6.1.2.1. Oral Dose Range-Finding Embryofetal Development Study in Rats

This range-finding study evaluated the potential for maternal and developmental toxicity and TK of BIC when administered via oral gavage to pregnant female rats during the period of organogenesis (GD 7 through GD 17) (m2.6.7, Section 11.1, TX-141-2034) for the purpose of setting dosage levels for a definitive developmental study (m2.6.7, Section 13.1, TX-141-2036). Time-mated pregnant female Crl:CD(SD) rats (6/group) received BIC at 0, 5, 30, and 300 mg/kg/day from GDs 7 to 17 at a dose volume of 5 mL/kg. On GD 21, rats were euthanized and laparohysterectomies were performed on all females and standard endpoints were measured. A satellite group of time-mated female rats were used for TK analyses on GDs 7 and 17. Assessment of toxicity was based on mortality, clinical signs, body weights (maternal and fetal), food consumption, maternal necropsy, cesarean section data, and fetal external and visceral evaluations.

All rats survived to scheduled euthanasia. There were no abnormal clinical signs and all rats appeared normal at necropsy. No BIC-related effects on body weights, body weight gain, or food consumption occurred. No BIC-related effects on Caesarean sectioning or litter parameters were noted. No dam had a litter consisting of only resorbed conceptuses, and there were no dead fetuses or late resorptions. All placentae appeared normal. There were no BIC-related fetal gross external or visceral alterations.

Plasma exposure to BIC generally increased with the increase in dose level from 5 to 300 mg/kg/day (Table 16). The increases in C_{max} and AUC were less than dose proportional between 5 and 300 mg/kg/day. No accumulation of BIC was observed at the 30 and 300 mg/kg/day dose levels, while accumulation was observed at the 5 mg/kg/day dose level between the first and last dose (AUC; approximately 3.5-fold higher).

Table 16. Mean Toxicokinetic Parameters of BIC in Pregnant Rats on Gestation Days (GD) 7 and 17

BIC Dose Level (mg/kg/day)	Gestation Day	C_{max} (µg/mL)	AUC_{0-24} (µg·h/mL)
5	7	27.2	464
	17	80.1	1630
30	7	94.8	1940
	17	164	3080
300	7	171	3360
	17	180	3650

Based on the lack of observed effects, BIC maternal and embryo-fetal development NOELs are 300 mg/kg/day (GD 17 mean C_{max} and AUC values of 180 µg/mL and 3650 µg·h/mL, respectively), the highest dose tested. At the maternal and developmental NOEL, mean BIC AUC is 36-fold higher than BIC AUC following clinical administration of B/F/TAF FDC. Doses of 5, 30, 300 mg/kg/day BIC were similarly selected for the definitive rat embryofetal development study (m2.6.7, Section 13.1, TX-141-2036).

6.1.2.2. Oral Embryofetal Development Study in Rats

The purpose of this study was to evaluate the maternal and embryo-fetal toxicity of BIC administered daily via oral gavage to pregnant rats during the period of organogenesis (m2.6.7, Section 13.1, TX-141-2036). Time-mated pregnant female Crl:CD(SD) rats (22/dosing group) received BIC at 0, 5, 30, and 300 mg/kg/day from GDs 7 to 17. On GD 21, rats were euthanized and laparohysterectomies were performed on all females and standard endpoints were measured. Assessment of toxicity was based on mortality, clinical signs, body weights (maternal and fetal), food consumption, maternal necropsy, cesarean section data, and fetal external and skeletal and visceral evaluations. No TK analyses were performed as dose levels reflected those in the dose range-finding study (m2.6.7, Section 11.1, TX-141-2034).

All rats survived to scheduled euthanasia. There were no abnormal clinical signs and all rats appeared normal at necropsy. No BIC-related effects on body weights, body weight gain, or food consumption occurred. No BIC-related effects on Caesarean sectioning or litter parameters were observed. Specifically, the litter averages for corpora lutea, implantations, percent preimplantation loss, litter sizes, live fetuses, early resorptions, percent postimplantation loss, fetal body weights, percent dead or resorbed conceptuses, and percent live male fetuses were comparable among the four dose groups. No dam had a litter consisting of only resorbed conceptuses, and there were no dead fetuses or late resorptions. All placentae appeared normal. There were no BIC-related fetal gross external, visceral, or skeletal alterations and ossification sites were comparable across all groups.

In conclusion, the BIC maternal and embryo-fetal developmental NOELs are 300 mg/kg/day. At the maternal and developmental NOEL in this study, mean BIC AUC (based upon TK values from dose range-finding study; m2.6.7, Section 11.1, TX-141-2034) is 36-fold higher than BIC AUC following clinical administration of B/F/TAF FDC.

6.1.2.3. Oral Dose Range-Finding Embryofetal Development Study in Rabbits

The objective of this exploratory study (m2.6.7, Section 11.1, TX-141-2035) was to determine the maternal and developmental toxicity and TK of BIC when administered daily via oral gavage to pregnant rabbits during the period of organogenesis (GDs 7 to 19) for the intended purpose of setting dosage levels for a definitive developmental study. Time-mated female New Zealand White [Crl:KBL(NZW)] rabbits (8/group) received vehicle (25% Solutol HS-15 and 75% propylene glycol) or BIC at 100, 300, or 1000 mg/kg/day at a dose volume of 3 mL/kg. On GD 29, all surviving rabbits were euthanized and laparohysterectomies were performed on all females and standard endpoints were measured. Assessment of toxicity was based on mortality, clinical signs, body weights (fetal and maternal), food consumption, maternal necropsy, Cesarean section data, and fetal external and visceral evaluations. In addition, blood for TK analysis was collected from selected animals on GD 7 and GD 19.

Significant maternal and developmental toxicity related to vehicle control article was exacerbated by BIC in a dose-dependent manner. In the vehicle control group, adverse clinical signs (including scant/absent/liquid feces, thin body condition, ungroomed coat), body weight loss after GD 13 (-2% from GD 7 to GD 20) and low food consumption were noted. Similar and more severe effects on clinical signs, body weight and food consumption were noted across treated groups; high dose animals (1000 mg/kg/day) were terminated at GD 14/16 due to excessive toxicity. One and 5 does in the 100 and 300 mg/kg/day dose groups, respectively, aborted and were euthanized, and one doe in the 300 mg/kg/day dose group was euthanized due to adverse clinical observations. Abortions were considered secondary to overt maternal toxicity. The average numbers of resorptions were increased in the vehicle and 100 mg/kg/day dose groups and the percentage of does with resorptions was increased in the 100 mg/kg/day dose group as compared to historical control data in New Zealand White rabbits. Average fetal weights were generally below the historical control averages in the vehicle and 100 and 300 mg/kg/day dose groups, although values in the 300 mg/kg/day dose group were based on only 1 litter. There were no test article-related fetal gross external or visceral alterations.

Plasma exposure to BIC generally increased with the increase in dose level from 100 to 1000 mg/kg/day on GD 7 and from 100 to 300 mg/kg/day on GD 19 (Table 17). The increases in C_{max} were less than dose proportional between 100 and 300 mg/kg/day on GDs 7 and 19 and between 300 and 1000 mg/kg/day on GD 7. The increases in AUC were roughly dose proportional between 100 and 300 mg/kg/day on GDs 7 and 19 and less than dose proportional between 100 and 1000 mg/kg/day on GD 7. Accumulation was observed after multiple dosing at the 100 (6.4-fold) and 300 (5.0-fold) mg/kg/day dose levels.

Table 17. Mean Toxicokinetic Parameters of BIC in an Organic Vehicle in Pregnant Rabbits on Gestation Days GD 7 and 19

BIC Dose Level (mg/kg/day)	Gestation Day	C_{max} (µg/mL)	AUC_{0-24} (µg·h/mL)
100	7	7.75	34.5
	19	11.7	145
300	7	8.69	84.8
	19	24.1	412
1000	7	10.4	168
	19	NC	NC

NC = not calculated (due to insufficient data)

Although maternal and developmental toxicity (i.e, early termination of 1000 mg/kg/day group, abortions at 100 and 300 mg/kg/day, adverse clinical signs, weight loss, reduced food consumption, and reduced fetal body weights) were observed in a dose-dependent manner in BIC-treated groups, there was also significant maternal and developmental toxicity in the vehicle control group; NOAELs for maternal and developmental toxicity could not be determined. Based on these data, the 100% organic (Solutol/propylene glycol) vehicle was not recommended for use in the definitive developmental toxicity study in rabbits.

6.1.2.4. Oral Dose Range-Finding Embryofetal Development Study in Rabbits

Due to the vehicle toxicity noted in an initial dose range-finding study (m2.6.7, Section 11.1, TX-141-2035), a second dose range-finding study was conducted with BIC formulated in an aqueous vehicle. This exploratory study (m2.6.7, Section 11.1, TX-141-2038) determined the maternal and developmental toxicity and TK of BIC when administered daily via oral gavage to pregnant rabbits during the period of organogenesis (GD 7 through GD 19) for the purpose of setting dosage levels for a definitive developmental study (m2.6.7, Section 13.2, TX-141-2037). Time-mated female New Zealand White [Hra:(NZW)SPF] rabbits (8/group) received vehicle [0.5% HPMC and 0.5% Tween® 20 in RO water] or BIC at 100, 300, or 1000 mg/kg/day at a dose volume of 5 mL/kg. On GD 29, all surviving rabbits were euthanized and laparohysterectomies were performed on all females and standard endpoints were measured. Assessment of toxicity was based on mortality, clinical signs, body weights (fetal and maternal), food consumption, maternal necropsy, Cesarean section data, and fetal external and visceral evaluations. In addition, blood for TK analysis was collected from selected animals on GD 7 and GD 19.

One female rabbit (1/8) in the 1000 mg/kg/day dose group aborted and was euthanized on GD 26. Clinical signs noted for this rabbit included pale or light brown feces, scant feces, and thin body condition; the animal lost 15% body weight between GD 7 and 26. Food consumption values in this animal were moderately to severely reduced throughout the study (ranging from 9 to 64 g/day). The abortion was considered to be secondary to maternal toxicity (i.e., adverse clinical signs, severely reduced food consumption, and body weight loss).

Increased numbers of does had pale or light brown feces at 1000 mg/kg/day and ungroomed coat at 300 and 1000 mg/kg/day. Body weight loss (–4% from GDs 7 to 9) and reduced food consumption (entire dosing period) were observed at 1000 mg/kg/day. Fetal body weights were slightly reduced (-7% to -8% of vehicle control; not statistically significant) at 1000 mg/kg/day; values were within the historical control ranges at the Testing Facility. No other BIC-related effects on Caesarean-sectioning or litter parameters were observed. No doe had a litter consisting of only resorbed conceptuses, and there were no dead fetuses. All placentae appeared normal. There were no BIC-related fetal gross external or visceral alterations.

Plasma exposure to BIC increased with the increase in dose level from 100 to 1000 mg/kg/day (Table 18). The increases in C_{max} and AUC were generally less than dose proportional between 100 to 1000 mg/kg/day. In general, no accumulation of BIC was observed after multiple dosing in rabbits.

Table 18. Mean Toxicokinetic Parameters of BIC in an Aqueous Vehicle in Pregnant Rabbits on Gestation Days GD 7 and 19

BIC Dose Level (mg/kg/day)	Gestation Day	C_{max} (µg/mL)	AUC_{0-24} (µg·h/mL)
100	7	3.41	17.0
	19	3.96	38.5
300	7	4.08	62.5
	19	4.05	60.3
1000	7	9.62	146
	19	11.0	138

In conclusion, based upon significant maternal toxicity at 1000 mg/kg/day, the maternal NOAEL and embryofetal development NOEL are 300 mg/kg/day (GD 19 mean C_{max} and AUC values of 4.05 µg/mL and 60.3 µg·h/mL, respectively). At the NOEL, mean BIC AUC is 0.59-fold of the BIC AUC following clinical administration of B/F/TAF FDC. Doses of 100, 300, and 1000 mg/kg/day BIC were similarly selected for the definitive rabbit embryofetal development study with the same aqueous vehicle.

6.1.2.5. Oral Embryofetal Development Study in Rabbits

The purpose of this study was to evaluate the maternal and embryo-fetal toxicity of BIC administered daily via oral gavage to pregnant rabbits during the period of organogenesis (GD 7 through GD 19; m2.6.7, Section 13.2, TX-141-2037). Time-mated female New Zealand White [Hra:(NZW)SPF] rabbits (22/group) received vehicle [0.5% HPMC and 0.5% Tween® 20 in RO water] or BIC at 100, 300, or 1000 mg/kg/day at a dose volume of 5 mL/kg. On GD 29, rabbits were euthanized and laparohysterectomies were performed on all females and standard endpoints were measured. Assessment of toxicity was based on mortality, clinical signs, body weights (maternal and fetal), food consumption, maternal necropsy, Cesarean section data, and fetal external and skeletal and visceral evaluations. No TK analyses were performed as dose levels and the vehicle reflected those in the dose range-finding study (m2.6.7, Section 11.1, TX-141-2038).

Two does (Rabbits 5075 and 5080) in the 1000 mg/kg/day dose group aborted and were euthanized on GD 25 or 26. Adverse clinical signs noted for these does included scant feces, light brown or yellow feces, thin body condition, cold to touch, and/or sparse hair coat with reduced food consumption and body weight loss. Rabbit 5075 was noted with low food consumption from GDs 8 to 10 (43 to 74 g/day) and severely reduced food consumption after GD 10 until abortion on GD 25 (0 to 16 g/day); body weight loss was observed after GD 8 (-13%, GD 7 to GD 25). Similarly, Rabbit 5080 showed low food consumption throughout the study, which was severely reduced from GDs 16 through 24 (1 to 4g/day) with body weight loss (-9% from GD 7 to 26). Both abortions at 1000 mg/kg/day were considered to be secondary to overt maternal toxicity (ie, clinical signs, severely reduced food consumption and body weight loss). All other rabbits survived until scheduled euthanasia.

Adverse clinical signs in surviving females administered 1000 mg/kg/day included abnormal fecal observations (scant, light brown or yellow), thin body condition, and cold to touch. Significant body weight loss from GDs 7 to 10 (-3%) and a subsequent reduction in mean body weight gain from GDs 10 to 13 (-57%) were observed. As a result of these changes, body weight gain for the entire dose period (calculated as GDs 7 to 20) was significantly reduced (-26%). Food consumption at 1000 mg/kg/day was significantly reduced from GDs 7 to 20 (-41%) compared to vehicle control group. No BIC-related abnormal clinical observations or effects on body weights or food consumption were noted at 100 or 300 mg/kg/day. There were no BIC-related maternal necropsy observations at any dose level. At 1000 mg/kg/day, fetal body weights were significantly decreased (up to -13%). No other BIC-related effects on Caesarean-sectioning or litter parameters were observed. No BIC-related fetal gross external, visceral, or skeletal fetal abnormalities were observed at any dose level.

In conclusion, based on significant maternal toxicity at 1000 mg/kg/day, the maternal NOAEL and embryofetal development NOEL are 300 mg/kg/day. Abortions (2/22 animals) and decreased mean fetal body weights at 1000 mg/kg/day were considered secondary to overt maternal toxicity as demonstrated by adverse clinical signs, body weight loss/decreased body weight gain, and decreased food consumption. At the NOEL, mean BIC AUC (based on TK values from dose range-finding study; m2.6.7, Section 11.1, TX-141-2038) is 0.59-fold of the BIC AUC following clinical administration of B/F/TAF FDC.

6.1.3. Prenatal and Postnatal Development Including Maternal Function

6.1.3.1. Oral Pre- and Postnatal Development Study in Rats

A pre- and postnatal development study in time-mated Crl:CD(SD) female rats (25/group) was conducted to evaluate the potential adverse effects of maternal BIC exposure from implantation to weaning on pregnancy, parturition, and lactation of the maternal animals and on the growth, viability, and development of the F_1 neonates. Reproductive performance of the F_1 generation was also assessed. Female rats were orally administered BIC at 0, 2, 10, and 300 mg/kg/day once daily from GD 6 through Lactation Day (LD) 20 (m2.6.7, Section 14.1, TX-141-2045) at a dose volume of 5 mL/kg. A satellite group of maternal rats were used for TK analyses on GD 7 and LD 10.

The F_0 dams raised their pups through weaning at postnatal day (PND) 21, at which time the F_1 weanlings were allowed to mature to at least 10 weeks of age. During maturation, attainment of sexual development (preputial separation and vaginal patency) and neurodevelopmental milestones (motor skills, learning, and memory) were monitored. Immediately prior to mating, estrous cyclicity of F_1 females was evaluated. F_1 animals were then cohabited within dose groups (sibling matings avoided) and the resulting F_2 litters were evaluated and the pups sacrificed.

There was no treatment-related toxicity observed in the F_0 dams during gestation or lactation, including mortality, body weight changes, or reduced feed consumed. The F_1 litters were normal at all doses tested with respect to number of pups, pup weights, and sex ratios. Maturation of F_1 pups through weaning proceeded normally. Attainment of neurodevelopmental and sexual development milestones were all normal.

Body weights of F_1 animals during the postweaning maturation phase were normal across all groups. Although a possible effect on F_1 generation male and female fertility was observed at 300 mg/kg/day, the decreases in fertility indices were not statistically significant and remained within historical control ranges at the Testing Facility (Table 19); therefore findings were not considered BIC-related. Survival, body weights, and sex ratios of F_2 litters were normal across all groups. Necropsy of F_1 animals yielded no treatment-related findings.

Table 19. Summary of Reproductive Parameters of BIC in F_1 Pups

Reproductive Performance Parameter	BIC Dose Levels (mg/kg/day)				Historical Control Data[a]
	0	2	10	300	Vehicle
Male Fertility Index[b,c]	100.0 (24)	100.0 (25)	100.0 (25)	82.6 (19)	90.2 (60.0-100.0)
Female Fertility Index[b,d]	100.0 (24)	100.0 (25)	100.0 (25)	83.3 (20)	92.9 (60.0-100.0)

a Test Facility historical control data shown as mean with ranges noted in parentheses.
b Presented as percentage with number of animals confirmed as siring a litter or pregnant in parentheses.
c Male fertility index (%) = No. of males siring a litter/Total no. of males used for mating (× 100)
d Female fertility index (%) = No. of females with confirmed pregnancy/Total no. of females used for mating (× 100)

Plasma exposure to BIC increased with the increase in maternal dose level from 2 to 300 mg/kg/day for maternal rats and pups (Table 20). The increases in C_{max} and AUC were slightly less than dose proportional between 2 and 10 mg/kg/day and less than dose proportional between 10 and 300 mg/kg/day for maternal rats and pups. No accumulation of BIC was observed after multiple doses in maternal rats. Gender-based differences were less than 2-fold in BIC C_{max} and AUC values for pups. Exposure to BIC in maternal rats was roughly similar to pups at 2 mg/kg/day, slightly higher (approximately 1.5-fold) in maternal rats than in pups at 10 mg/kg/day, and greater than 2-fold higher (approximately 2.8-fold) in maternal rats than in pups at 300 mg/kg/day.

Table 20. Mean Toxicokinetic Parameters of BIC in Pregnant and Lactating Rats (F_0) and F_1 Pups Following Daily Oral Administration of BIC from GD 6 to LD 10

Interval	BIC dose level (mg/kg/day)	Generation	Sex	C_{max} (µg/mL)	AUC_{0-24} (µg·h/mL)	M:O Ratio AUC_{0-24}
GD 6	2	F_0	Female	12.7	217	NA
	10		Female	52.7	840	
	300		Female	179	3350	
LD 10	2	F_0	Female	20.0	335	NA
	10		Female	74.7	1170	
	300		Female	156	3100	
PND 10	2	F_1	Male	16.3	326	1.03
			Female	16.9	343	0.977
			Combined	16.6	334	1.00
	10	F_1	Male	34.4	750	1.56
			Female	35.4	773	1.52
			Combined	33.5	762	1.54
	300	F_1	Male	48.6	1120	2.77
			Female	48.8	1130	2.75
			Combined	48.0	1120	2.76

M:O = maternal to offspring ratio; GD = gestation day; LD = lactation day; PND = postnatal day
NA = not applicable; data reflect maternal animal TK data only
Note: Combined (male/female) data is based on the analysis of the combined mean concentration data for both F_1 sexes.

In conclusion, no test article-related effects were noted at any dosage level during the study and the NOEL for F_0 maternal systemic toxicity, and F_1 neonatal/developmental toxicity, parental systemic toxicity, and reproductive toxicity, and F_2 neonatal/early postnatal toxicity was 300 mg/kg/day (mean F_0 female LD10 AUC = 3100 µg•h/mL; mean F_1 pup PND10 AUC = 1120 µg·h/mL). At 300 mg/kg/day, maternal and pup BIC AUC values were 30-fold and 11-fold higher, respectively, than BIC AUC following clinical administration of B/F/TAF FDC.

6.2. FTC

6.2.1. Fertility and Early Embryonic Development

6.2.1.1. Oral Fertility and Early Embryonic Development Study of FTC in Mice

Four groups of 20 to 21 male and 20 female mice received vehicle or 250, 500, or 1000 mg/kg/day of FTC by oral gavage (m2.6.7, Section 12.2, TOX036). The dose was administered as 2 equal installments given approximately 6 hours apart. Male mice were dosed 28 days prior to breeding, 21 days during mating, and 21 days postmating for a total of 70 days. Females were dosed 14 days

prior to breeding, 21 days during mating, and 7 days during gestation for a total of 42 days. There were no treatment-related deaths and all mice were observed to be normal during all observation periods. Body weight, body weight change, and food consumption were also unaffected by treatment. There was no indication of reproductive toxicity. Mating and fertility indices were unaffected by treatment, as was epididymal sperm number and motility. There were no gross pathological findings at necropsy. Embryonic development was also unaffected by treatment with FTC; there was no effect on the number of ovarian corpora lutea or uterine implantation sites per dam; the number or percent of resorptions and nonlive implants per litter; the number and percent of litters with nonlive implants; the number of live implants per litter; the number or percent of litters with resorptions; or the percent preimplantation or postimplantation loss per litter. In this study, the NOAEL of FTC for male and female mouse systemic, reproductive, and developmental toxicity was considered to exceed 1000 mg/kg/day.

6.2.1.2. Oral Fertility Study of FTC in Male Rats

The potential for adverse effects of orally administered FTC on the reproductive performance of male Sprague-Dawley rats was evaluated (m2.6.7, Section 12.3, TTEP/95/0028). Male rats were administered once daily doses at 0, 150, 750, or 3000 mg/kg/day for 73 days prior to mating and throughout the mating period. Body weight, feed consumption, organ weights, gross lesions, histopathology of reproductive organs, sperm parameters, and fertility were evaluated. Naive females were cohabited with treated males and monitored for evidence of mating. On GD 13, the females were inspected for pregnancy by cesarean section. The number of viable fetuses, number early and late resorptions, and number of deaths were then quantified. The only treatment-related effect was salivation immediately following dosing at 3000 mg/kg/day. There were no adverse effects on fertility, reproductive performance, sperm count or motility, and there were no pathological changes in the male reproductive organs. Based on these findings, the NOEL was 750 mg/kg/day.

6.2.2. Embryofetal Development

6.2.2.1. Oral Dose Range-Finding Embryofetal Development Study of FTC in Mice

A dose range-finding study was conducted to study the potential maternal and developmental toxicity of FTC in CD-1 mice for the purpose of selecting doses for the definitive study (m2.6.7, Section 11.2, TOX033). On GDs 6 to 15, FTC was orally administered at 0, 250, 500, and 1000 mg/kg/day as a divided dose with 6 hours between doses (0.5% methylcellulose vehicle). On GD 18, laparohysterectomies were performed on all females and standard endpoints were measured. One high-dose female delivered 2 pups of 13 total on GD 18, just prior to the scheduled necropsy. All other animals progressed normally to the GD 18 necropsy. There were no treatment-related internal findings at any dose level, including gravid uterine weight, numbers of fetuses, fetus weights, sex ratios, external developmental malformations and variations, early and late resorptions, total implantations, and corpora lutea numbers. Maternal body weight gain and feed consumption were unaffected at all dose levels. Based upon these results, doses of 250, 500, and 1000 mg/kg/day were selected for the definitive mouse teratology study.

6.2.2.2. Oral Embryofetal Development Study of FTC in Mice

The potential for FTC to cause developmental and maternal toxicity was evaluated in this GLP-compliant study (m2.6.7, Section 13.3, TOX037). Groups of 25 pregnant mice were orally administered FTC suspended in 0.5% aqueous methylcellulose or vehicle alone, given twice daily as a divided dose with 6 hours between doses, from GDs 6 through 15. Doses tested were 0, 250, 500, and 1000 mg/kg/day (5 mL/kg/dose; 10 mL/kg/day). Body weights and feed consumption were recorded at the specified intervals. On GD 18, laparohysterectomies were performed on all females and data for maternal and fetal indices collected. Dams in satellite groups for each dose level were treated similarly, except that on GD 15 following a single dose of 250, 500, or 1000 mg/kg, they were exsanguinated at 0.5 hour postdose and plasma was harvested for toxicokinetic evaluations. All maternal animals survived to the necropsy on GD 18; there were no treatment-related maternal findings at that time. Feed and body weight data were all normal. Intrauterine growth and survival of fetuses were not affected by FTC at any dose level, and there were no external, visceral, or skeletal malformations or developmental variations observed at any dose. Toxicokinetic evaluations demonstrated dose proportional exposures to the drug with mean plasma values at 0.5 hour postdose of 52 ± 15, 88 ± 14, and 186 ± 14 µg/mL for the low-, mid-, and high-dose groups, respectively. Based on the results of this study, a dose level of 1000 mg/kg/day was considered to be the NOAEL for maternal and developmental toxicity of FTC when administered twice daily to mice.

6.2.2.3. Oral Dose Range-Finding Embryofetal Development Study of FTC in Rabbits

A GLP-compliant dose range-finding study of TP-0006 (FTC) for a developmental toxicity study was conducted in New Zealand White rabbits (m2.6.7, Section 11.2, TOX034). Groups of 5 female artificially inseminated rabbits were dosed with FTC in 0.5% methylcellulose at 0, 250, 500, and 1000 mg/kg/day, administered in two equally divided doses with 6 hours between doses (GDs 7 to 19; GD 0 = day of insemination). Standard data parameters (body weights, feed consumption) were collected throughout the study. On GD 29, a laparohysterectomy was performed and the uteri and their contents examined. All females survived to the scheduled necropsy with no evidence of toxic or pharmacologic effects. Mean maternal body weight gain and feed consumption were normal. Growth and survival of the conceptuses were also normal. There were no external malformations or variations observed in fetuses at any dose level.

6.2.2.4. Oral Embryofetal Development Study of FTC in Rabbits

The potential for FTC to cause developmental and maternal toxicity were evaluated in rabbits (m2.6.7, Section 13.4, TOX038). Groups of 20 artificially inseminated rabbits were orally dosed with FTC in 0.5% aqueous methylcellulose from GDs 7 through 19. Dose levels were 0, 100, 300, and 1000 mg/kg/day. Dosing occurred twice daily, with approximately 6 hours between doses, at a dose volume of 5 mL/kg/dose (10 mL/kg/day). Clinical observations for signs of toxicity, body weights, and feed consumption were recorded. On GD 29, a laparohysterectomy was performed on all rabbits and the uteri and ovaries examined. Standard fetal evaluations were made to evaluate any effects on external, visceral, or skeletal development. Plasma concentrations of drug in maternal and fetal circulation was evaluated in a satellite group

of rabbits (n = 5/group) on GDs 19 (maternal exposure) and 20 (fetal/maternal exposure). There were no test article-related deaths or abortions. The only test article-related clinical observation was decreased defecation in the high-dose group. Body weight gain and feed consumption were reduced in the 300 and 1000 mg/kg/day groups during GDs 11 to 15 (high dose only), 15 to 18, and/or 18 to 20. Intrauterine growth and survival were unaffected by FTC, and there were no treatment-related developmental malformations or variations in fetuses at any dose.

Toxicokinetic evaluation demonstrated that FTC was rapidly absorbed with C_{max} occurring generally within 1 hour postdose. AUC and C_{max} increased linearly with dose. Plasma elimination $t_{1/2}$ was 3 to 4 hours at all dose levels. Mean maternal exposures ($AUC_{0–24h}$) on GD 19 were 87, 315, 1258 µg·h/mL for the low-, mid-, and high-dose groups, respectively. Fetal/maternal exposure ratios were around 0.4 to 0.5 at 1 hour after dosing (at T_{max}) for all dose levels.

Based on the results of this study, a dose level of 100 mg/kg/day was considered to be the NOAEL for maternal toxicity and a dose level of 1000 mg/kg/day was considered the NOAEL for developmental toxicity of FTC when administered twice daily to rabbits.

6.2.3. Prenatal and Postnatal Development Including Maternal Function

6.2.3.1. Oral Pre- and Postnatal Development Study of FTC in CD-1® Mice

Groups of 25 pregnant female CD-1 mice were administered FTC by oral gavage at 0, 250, 500, and 1000 mg/kg/day (5 mL/kg/dose; 10 mL/kg/day) (m2.6.7, Section 14.2, TOX039). Doses were administered to F_0 dams twice daily, with approximately 6 hours between equally divided doses, from GD 6 through PND 20. The F_0 dams raised their pups through weaning at PND 21, at which time the F_1 weanlings were allowed to mature to at least 10 weeks of age. During maturation, attainment of sexual development (preputial separation and vaginal patency) and neurodevelopmental milestones (motor skills, learning, and memory) were monitored. Immediately prior to mating, estrous cyclicity of F_1 females was evaluated. F_1 animals were then cohabited within dose groups (sibling matings avoided) and the resulting F_2 litters were evaluated and the pups sacrificed.

There was no treatment-related toxicity observed in the F_0 dams during gestation or lactation, including mortality, body weight changes, or reduced feed consumed. The F_1 litters were normal at all doses tested with respect to number of pups, pup weights, and sex ratios. Maturation of F_1 pups through weaning proceeded normally. Attainment of neurodevelopmental and sexual development milestones were all normal. Body weights of F_1 animals during the postweaning maturation phase were normal across all groups. F_1 dams in the 1000-mg/kg/day group had slightly longer estrous cycles (0.64 days) than controls. Survival, body weights, and sex ratios of F_2 litters were normal across all groups. Necropsy of F_1 animals yielded no treatment-related findings. In summary, the NOAEL for reproductive toxicity in the mouse under the conditions of this study was found to be > 1000 mg/kg/day.

Exposure following oral administration of FTC to pregnant mouse dams and the exposure of murine fetuses to FTC was examined in a separate study (m2.6.5, Section 7.2.1, TOX103). On GD 15 following approximately 10 days administration of 500 mg/kg of FTC twice daily, pregnant mice and their viable fetuses had measurable concentrations of FTC at 1 hour following administration of the first 500-mg/kg dose. The mean (± SD) plasma concentration in the pregnant mice was 137.1 ± 28.0 µg/mL. The mean concentration of FTC in pooled fetal homogenate was 55.7 ± 10.4 µg/g. The mean fetal/maternal concentration ratio was 0.41 ± 0.04.

6.3. TAF

6.3.1. Fertility and Early Embryonic Development

6.3.1.1. Oral Fertility and General Reproduction Toxicity Study of TAF in Rats

The purpose of this study was to evaluate effects of GS-7340-03 on mating and fertility when administered daily via oral gavage to male and female rats prior to mating and until termination (males) or through early gestation (females) (m2.6.7, Section 12.4, TX-120-2012). This included assessment of potential effects on gonadal function, mating behavior, implantation, female estrous cyclicity and general fertility of male and female rats. Male and female Crl:CD(SD) rats were assigned to 4 groups (22 animals/sex/group). Each group received dose preparations containing the vehicle control article or GS-7340-03 at 20, 80, or 160 mg f.b.e./kg/day (22, 90, 180 mg GS-7340-03/kg/day) at a dose volume of 10 mL/kg. Males were necropsied after at least 10 weeks of dosing, the reproductive organs were weighed followed by assessment of sperm motility and total concentration. Male and female reproductive performance was evaluated based on results of confirmation of mating and pregnancy.

No TAF-related mortality occurred. No adverse change in clinical condition was attributed to TAF. For males, dose-dependent decreased mean body weight was noted at 80 and 160 mg/kg/day throughout the study. At 160 mg/kg/day mean body weights were significantly less than the control group on Premating (PM) Day 28, Pairing Days 3, 7, and 10, and on postpairing (PP) Day 0 through the end of the study when the 160-mg/kg group mean body weight was 12% less than the control group. At 160 mg/kg/day, mean female body weight on PM Day 17 was slightly but significantly less (6%) than the control group. Mean gestational body weight gain was significantly reduced at 160 mg/kg/day between GD 0-7 (19%). Mean food consumption was significantly reduced in the male 160 mg/kg/day group during much of the premating phase. Female food consumption was slightly reduced premating at 80 and 160 mg/kg/day and significantly during gestation at 160 mg/kg/day.

There were no differences in premating estrous cycles. There were no test article-related differences in male or female reproductive parameters. Mating/fecundity/fertility indices were 100/95/95, 95/100/95, 91/95/86, and 95/95/91 in the control, 20, 80, and 160 mg/kg/day group males, respectively and 100/95/95, 100/100/100, 100/95/95, and 100/95/95 in the control, 20, 80, and 160 mg/kg/day group females. There were no test article-related effects on the uterine and fetal parameters and no significant differences in female reproductive organ weights.

In males, there was a slight increase in absolute testis weight (significant increase (9%) in the adjusted mean of the left testis only) at 160 mg/kg/day. This was considered test article-related but not adverse, as there were no other reproductive organ weight or functional reproductive effects. There were no test article-related effects observed on mean epididymal sperm motility or on sperm concentration. Mean sperm motility in the control, 20, 80, and 160 mg/kg/day groups was 91.8%, 95.2%, 95.5%, and 94.0%, respectively. Mean sperm concentration in the control, 20, 80, and 160 mg/kg/day groups was 942.2, 833.2, 966.1, and 928.5 millions/gram of caudal epididymal weight, respectively.

In conclusion, TAF was administered by oral gavage to male and female rats at 20, 80, or 160 mg f.b.e./kg/day prior to mating and until termination (males) or through early gestation (females). The TAF NOAEL for male and female toxicity was 80 mg/kg/day. The TAF NOAEL for reproductive and early embryonic toxicity was 160 mg/kg/day (the highest dose administered).

6.3.2. Embryofetal Development

6.3.2.1. Oral Range Finding Embryofetal Development Study of TAF in Rats

The purpose of this study was to provide information for selection of oral dosages to be used in a subsequent embryo-fetal development study in female rats and to provide a preliminary evaluation of the effects of GS-7340-02 on pregnancy and embryo-fetal development (m2.6.7, Section 11.3, TX-120-2001). In addition, the toxicokinetic characteristics of GS-7340-02 in pregnant rats were determined.

Sixty-two time-mated female Crl:CD(SD) rats were assigned to 4 study groups (n = 11, 17, 17, and 17) and received daily doses of either the vehicle control or 5, 100, or 200 mg/kg GS-7340-02 in the vehicle (4, 80, 160 mg f.b.e./kg/day). The dose volume was 5 mL/kg. Animals were dosed on Days 6 to 17 of gestation, inclusive.

All animals survived to scheduled necropsy, and there were no TAF-related clinical signs of toxicity. At 200 mg/kg/day, body weight gains were significantly lower between GDs 8 and 10 when compared to controls, with marked differences also seen from GDs 10 to 12 and 16 to 18. Corrected body weights (body weight gains minus gravid uterus weights) at 200 mg/kg/day on GD 21 were 5% lower than controls, and mean body weight gain was 18% lower from GDs 6 to 18, compared to the control group.

Food consumption was slightly decreased (-10%) in animals administered 200 mg/kg/day, during the treatment period. There were no maternal macroscopic findings attributed to TAF. There were no TAF-related effects noted on the number of corpora lutea, implantation sites, sex ratio, incidence of live/dead fetuses, resorptions, preimplantation and postimplantation losses, or gravid uterus weights.

The mean fetal weight was statistically significantly lower (-7.7%) in the 200 mg/kg/day group, compared to the control group. However, as these fetal weights were within historical control range, this was considered to be non-adverse. There were no major fetal malformations or minor anomalies noted.

Following oral gavage, toxicokinetic parameters for TAF could not be calculated as most concentration values of TAF were below the lower limit of quantitation (< 10.0 ng/mL). TAF was extensively converted to TFV. Concentrations of TFV readily appeared in plasma. Exposure to TFV increased with the increase in dose level from 5 to 200 mg/kg. The increases in TFV C_{max} and AUC_{0-t} were generally greater than dose proportional between the 5 to 200 mg/kg/day dose levels, with the exception of the increase in C_{max} from 5 to 200 mg/kg/day dose level on Day 17. Accumulation of TFV was observed after multiple dosing of TAF in pregnant rats.

In conclusion, treatment of female rats by oral gavage during GDs 6 to 17, inclusive, with TAF at dose levels of 5, 100, and 200 mg/kg/day resulted in decreased body weight gains (-18%) and food consumption (-10%) at 200 mg/kg/day. Based on these results, the TAF NOAEL for maternal toxicity was considered to be 100 mg/kg/day and the TAF NOAEL for embryo-fetal development was 200 mg/kg/day.

Table 21. Mean Toxicokinetic Parameters of TFV and TAF in Pregnant Rats on Gestation Days (GD) 6 and 17

GS-7340-02 (mg/kg/day)	Gestation Day	TFV		TAF (GS-7340)	
		C_{max} (µg/mL)	AUC_{0-t} µg·h/mL	C_{max} (µg/mL)	AUC_{0-t} µg·h/mL
5	6	0.109	0.17	NC	NC
	17	0.218	0.41	NC	NC
100	6	4.36	12.90	0.209	NC
	17	5.71	20.6	0.773	NC
200	6	6.94	26.9	0.215	NC
	17	5.92	35.7	0.397	0.649

NC = not calculated (due to insufficient data)

6.3.2.2. Oral Embryofetal Development Study of TAF in Rats

The purpose of this study was to provide an evaluation of the effects of GS-7340-02 on pregnancy and embryo-fetal development (m2.6.7, Section 13.5, TX-120-2002). This study evaluated ICH Harmonised Tripartite Guideline stages C to D of the reproductive process. In addition, the toxicokinetic characteristics of GS-7340-02 in pregnant rats were determined.

Four groups of 25 pregnant female Crl:CD(SD) rats were administered TAF once daily, by oral gavage, from GD 6 to 17, inclusive. Targeted dose levels were 0 (vehicle control), 25, 100 and 250 mg/kg/day (targeted formulation concentrations of 0, 5, 20 and 50 mg/mL, respectively) and the dose volume was 5 mL/kg. A satellite group of pregnant animals (3, 9, 9, and 9 respectively) were dosed and used for toxicokinetic sampling.

Dose formulation analysis showed that each 5 mg/kg/day animal was administered a GS-7340-02 concentration of 3.85 mg/mL instead of 5 mg/mL for 5 to 8 days between GDs 10 and 17, providing a daily dose of 19.3 mg/kg/day (77% of targeted dose) on these days. Additionally dose formulation analysis showed that each 20 mg/kg/day animal was administered a GS-7340-02 concentration of 12.9 mg/mL instead of 20 mg/mL for 4 to 7 days between GDs 6 and 12, providing a daily dose of 64.6 mg/kg/day (65% of targeted dose) on these days.

The only potentially TAF-related clinical sign was transient salivation, which was noted in 7 of 25 animals in the 250 mg/kg/day group. For the 250 mg/kg/day group body weights, body weight gains and food consumption were significantly decreased during the treatment period. On GD 21, the mean body weight of the 250 mg/kg/day group was 10 % lower than that of the control group. Mean corrected body weights (body weight on GD 21 minus gravid uterus weight) and mean corrected body weight gains (body weight gain on GDs 6 to 21 minus gravid uterus weight) were also lower in animals administered 250 mg/kg/day, with the corrected mean body weights also 10% lower than controls on GD 21.

No TAF-related gross findings were noted. No effects were observed on the total number of corpora lutea, implantation sites, male fetuses, female fetuses, live fetuses, dead fetuses, or resorptions. Pre and post implantation loss was considered comparable to controls.

Fetal weights (males, females and sexes combined) were decreased in a dose dependent manner and remained within the range of historical control data, however fetal weights at 250 mg/kg/day were at the lower extreme of this range. The incidences of fetal major malformations, minor external, visceral and skeletal anomalies and were not affected by GS-7340-02. Sternebrae variants (1 to 4 and 5 and 6) were increased at 250 mg/kg/day.

Plasma concentrations of TAF were all below the lower limit of quantitation at a dose level 25 mg/kg/day. Exposure to TAF increased with the increase in TAF dose level from 25 to 250 mg/kg/day. Exposure to TFV increased with the increase in TAF dose level from 25 to 250 mg/kg/day. No accumulation of TFV was observed after multiple dosing of GS-7340-02 in rats. GS-7340 was extensively converted to TFV in rats following oral administration of GS-7340-02.

In conclusion, administration of TAF by once daily oral gavage in rats at levels of 25 (mean overall achieved dose 22 mg/kg/day), 100 (mean overall achieved dose of 84 mg/kg/day), and 250 mg/kg/day resulted in decreased body weights and food consumption at 250 mg/kg/day (Table 22). At 250 mg/kg/day, there was decreased fetal body weight associated with some minor transitory delays in the rate of ossification. There was no evidence of embryolethality or teratogenicity attributed to TAF in this study. Based on these results, the maternal TAF NOAEL and the TAF NOAEL for embryo-fetal development were both considered to be the mid dose level, which resulted in GD 17 AUC_{0-t} values of 17.4 and 0.2 µg·h/mL for TFV and TAF, respectively.

Table 22. Mean Toxicokinetic Parameters of TFV and TAF in Pregnant Rats on Gestation Days (GD) 6 and 17

GS-7340-02 (mg/kg/day)	Gestation Day	TFV		TAF (GS-7340)	
		C_{max} (µg/mL)	AUC_{0-t} (µg·h/mL)	C_{max} (µg/mL)	AUC_{0-t} (µg·h/mL)
25	6	1.11	2.72	NC	NC
	17	0.870	2.80	NC	NC
100	6	2.77	9.45	0.038	NC
	17	4.13	17.4	0.149	0.242
250	6	9.86	54.2	0.756	1.38
	17	7.35	55.7	0.597	1.38

NC = not calculated (due to insufficient data)

6.3.2.3. Oral Range Finding Embryofetal Development Study of TAF in Rabbits

The objectives of this study were to provide information for selection of dosages to be used in subsequent embryo-fetal development studies in rabbits and to provide a preliminary evaluation of the effects of GS-7340-02 on pregnancy and embryo-fetal development (m2.6.7, Section 11.3, TX-120-2004). In addition, the toxicokinetic characteristics of TAF were determined. Thirty timed-mated New Zealand White rabbits were assigned to 5 groups (6 animals/group) and were dosed once daily, by oral gavage, from Day 7 to 20 of gestation. Dose levels were 0 (vehicle control), or GS-7340-02 at dose levels of 5, 25, 50, or 100 mg/kg/day at a dose volume of 10 mL/kg.

All animals survived to their scheduled termination. Decreased fecal output was noted on a few occasions throughout the study in all groups. At 100 mg/kg/day, 4 of 6 animals were noted as being thin. At 100 mg/kg/day, a slight mean (2.5%) weight loss was observed between GD 7 to 21. A decrease (8.5%) compared to controls for corrected mean body weight (body weight from GD 7 to 29 minus gravid uterus weight) was noted at 100 mg/kg/day. Decreased food intake was noted at 100 mg/kg/day during the treatment period, which persisted to termination.

There were no TAF-related gross pathology findings for the dams. The pregnancy rate was 100% in all groups. The numbers of corpora lutea, implantation sites, live and dead fetuses and resorptions, the sex ratio, and the preimplantation and postimplantation losses were unaffected by TAF administration. The fetal weights were comparable to controls at each dose level. There were no major external malformations or minor external anomalies attributed to the test article administration.

Exposure to TAF and TFV increased with the increase in dose level from 5 to 100 mg/kg/day (Table 23). Between the 5 to 100 mg/kg/day dose levels, the increases in C_{max} and AUC_{0-t} were greater than dose proportional for TAF and were approximately proportional for TFV. No accumulation of TFV was observed. TAF was extensively converted to TFV in rabbits following oral administration of GS-7340-02.

Table 23. Mean Toxicokinetic Parameters of TFV and TAF in Pregnant Rabbits on Gestation Days (GD) 7 and 20

GS-7340-02 (mg/kg/day)	Gestation Day	TFV		TAF (GS-7340)	
		C_{max} (µg/mL)	AUC_{0-t} (µg·h/mL)	C_{max} (µg/mL)	AUC_{0-t} (µg·h/mL)
5	7	0.227	1.23	0.009	NC
	20	0.187	1.56	0.109	0.173
25	7	0.922	5.23	0.104	0.125
	20	0.907	6.01	1.01	1.14
50	7	1.93	8.85	0.349	0.390
	20	1.95	11.8	4.81	5.66
100	7	3.92	20.9	2.04	2.15
	20	3.09	23.5	8.88	10.7

NC = not calculated (due to insufficient data)

In conclusion, administration of TAF to pregnant rabbits by oral gavage at dose levels of 5, 25, 50 and 100 mg/kg/day on GD 7 to 20, inclusive, resulted in maternal effects evidenced by slightly decreased body weights and food intake at 100 mg/kg/day. There was no evidence of embryolethality, fetotoxicity or teratogenicity at any dose level. Based on this study, the TAF NOEL for maternal toxicity was considered to be 50 mg/kg/day (AUC_{0-t} = 11.8 and 5.7 µg·h/mL for TFV and TAF, respectively) and the TAF NOEL for embryo-fetal development was 100 mg/kg/day (AUC_{0-t} = 23.5 and 10.7 µg·h/mL for TFV and TAF, respectively).

6.3.2.4. Oral Embryofetal Development Study of TAF in Rabbits

The objective of this study was to detect potential adverse effects of GS-7340-02 on pregnant New Zealand White female rabbits and development of the embryo and fetus from implantation to closure of the hard palate, GD 7 to 20 (m2.6.7, Section 13.6, TX-120-2005). This study evaluated ICH Harmonised Tripartite Guideline stages C to D of the reproductive process. In addition, the toxicokinetic characteristics of GS-7340-02 were determined. GS-7430-02 was administered by oral gavage to time-mated F_0 generation female rabbits (20 main study females per group and 3 toxicokinetic females per group) at 0 (vehicle control), 10, 30 and 100 mg/kg/day in a dose volume in a dose volume of 10 mL/kg.

There was no mortality during the study. An increased incidence of decreased fecal output was noted at 100 mg/kg/day, compared to the vehicle control group and was associated with decreased food intake. Yellow staining of the fur was also observed more often in animals administered 30 and 100 mg/kg/day.

Lower body weight gains were noted at 100 mg/kg/day for the first week following treatment initiation. Lower food intake was noted at 100 mg/kg/day from GD 8 to 24. Three animals at this dose consumed less than 30 g for at least 4 days during the dosing period. There were no TAF-related macroscopic changes. The number of corpora lutea, implantation sites, live fetuses, dead fetuses, resorptions, the sex ratio and the pre and post implantation losses were unaffected by treatment with TAF. There was no effect of TAF upon fetal weights. The incidence of major malformations, minor external, visceral, skeletal anomalies and common skeletal variants were unaffected by TAF.

Exposure to TAF increased with the increase in GS-7340-02 dose level from 10 to 100 mg/kg/day. The increases in C_{max} were greater than proportional between the 10 to 100 mg/kg/day dose levels and the increases in AUC_{0-t} were greater than proportional between the 30 to 100 mg/kg/day dose level on GD 20.

Exposure to TFV increased with the increase in TAF dose level from 10 to 100 mg/kg/day. The increases in C_{max} and AUC_{0-t} were roughly proportional between the 10 to 100 mg/kg/day dose levels. Accumulation of TFV was observed after multiple dosing of TAF in rabbits. Concentrations of TFV were much higher than concentrations of TAF, therefore indicating that TAF was extensively converted to TFV in rabbits following oral administration of TAF.

The TAF NOAEL for maternal toxicity was 30 mg/kg/day (AUC_{0-t} = 1.1 and 5.0 µg h/mL for TAF and TFV, respectively) and the TAF NOEL for embryo-fetal development was 100 mg/kg/day (AUC_{0-t} = 11.0 and 27.3 µg·h/mL for TAF and TFV, respectively), the highest dose tested (Table 24).

Table 24. Mean Toxicokinetic Parameters of TFV and TAF in Pregnant Rabbits on Gestation Days (GD) 7 and 20

GS-7340-02 (mg/kg/day)	Gestation Day	TFV		TAF (GS-7340)	
		C_{max} (µg/mL)	AUC_{0-t} (µg·h/mL)	C_{max} (µg/mL)	AUC_{0-t} (µg·h/mL)
10	7	0.261	1.94	0.008	NC
	20	0.260	2.02	0.155	NC
30	7	0.398	3.01	0.077	NC
	20	0.676	5.01	0.937	1.14
100	7	2.19	13.4	0.846	0.918
	20	2.97	27.3	9.19	11.0

NC = not calculated (due to insufficient data)

In conclusion, TAF was administered to pregnant rabbits by daily oral gavage at dose levels of 0 (vehicle control), 10, 30 and 100 mg/kg/day on GD 7 to 20, inclusive. Treatment resulted in reduced maternal body weights and food consumption at 100 mg/kg/day compared to the control group. There was no evidence of embryolethality, fetotoxicity, or teratogenicity at any dose level.

6.3.3. Prenatal and Postnatal Development

Per separate agreements with the FDA and CHMP, a perinatal and postnatal study was not required for TAF due to the lack of TAF exposure in rats and lower TFV exposure compared to TDF. The pre- and postnatal study conducted with TDF is provided below.

6.3.3.1. Oral Developmental and Perinatal/Postnatal Reproduction Toxicity Study of TDF in Rats, Including a Postnatal Behavioral/Functional Evaluation

One hundred twenty-five presumed pregnant Crl:CD®(SD)IGS BR VAF/Plus® rats were randomly assigned to 5 dose groups, 25 rats per group (F_0 generation rats; m2.6.7, Section 14.3, R990202). An additional 12 rats were assigned to each of Groups II through V for satellite toxicokinetic evaluations. Suspensions of the test article, TDF (GS-4331-05), or the suspension vehicle were administered orally once daily to these naturally-bred female rats from GD 7 through LD 20, or GD 24 (rats that did not deliver a litter), at dosages of 0 (vehicle), 50, 150, 450, and 600 mg/kg/day. The dosage volume was 10 mL/kg, adjusted daily on the basis of the individual body weights recorded immediately before administration. Rats were observed for viability at least twice each day of the study. Observations for clinical signs of effects of the test article, abortions, premature deliveries, and deaths were made daily before dosage and approximately 60 ± 10 minutes after dosage administration. Body weights were recorded GD 0, daily during the dosage period, and at sacrifice. Feed consumption values were recorded on GDs 0, 7, 10, 12, 15, 18, 20, and 25 (if necessary) and LDs 1, 4, 7, 10, and 14. Rats were evaluated for adverse clinical signs observed during parturition, duration of gestation, litter sizes, and pup viability at birth. Maternal behavior was evaluated on LDs 1, 4, 7, 14, and 21. On GD 7 and LD 20, blood samples were collected predose, at 15 and 40 minutes, and at 1, 2, 4, 6, 8, 12,

and 24 hours postdose. Milk samples were collected from rats assigned to the satellite portion of the study and from 1 vehicle control group rat approximately 1 hour postdose on LD 11. After completion of the 21-day postpartum period, female rats were sacrificed and a gross necropsy of the thoracic, abdominal, and pelvic viscera was performed. Kidneys were excised, weighed (paired weight), and retained for possible histologic examination. The number and distribution of implantation sites was recorded. Gross lesions were retained for possible future evaluation.

Each F_1 generation litter was evaluated for viability at least twice each day. The pups in each litter were counted once each day. Clinical observations were recorded once daily during the preweaning period. Pup body weights were recorded on LDs 1, 4, 7, 14, and 21. On LD 21, all pups not selected for continued evaluation were sacrificed and examined for gross lesions. The following tissues were excised and retained for possible histologic evaluation: kidneys (paired weight recorded); sternum (with costochondral junction); and femur. Necropsy of the pups included a single cut at the suture of the frontal and parietal bones of the skull, and the cross-sectioned brain was examined for hydrocephaly. Tissues with gross lesions were retained for possible future evaluation.

All F_1 generation male and female rats were observed for viability at least twice each day of the study. These rats were also examined for clinical observations and general appearance once weekly during the postweaning period. Body weights for male rats were recorded weekly during the postweaning period and at sacrifice. Body weights for female rats were recorded weekly during the postweaning period and on GDs 0, 7, 10, 14, 17, and 20. Feed consumption values for male rats were recorded weekly, except during cohabitation. Feed consumption values for female rats were recorded weekly during the postweaning period, except during cohabitation and on GDs 0, 7, 10, 14, 17, and 20.

Female rats were evaluated for the age of vaginal patency, beginning on Day 28 postpartum. Male rats were evaluated for the age of preputial separation, beginning on Day 39 postpartum. Beginning at 24 ± 1 day postpartum, 1 male rat and 1 female rat from each litter, where possible, were evaluated in a passive avoidance test for learning, short-term retention, and long-term retention. Beginning at approximately 70-days postpartum, 1 male rat and 1 female rat from each litter were evaluated in a water-filled M-maze for overt coordination, swimming ability, learning, and memory. At approximately 90 days of age, the F_1 generation rats were assigned to cohabitation.

Male rats were sacrificed after completion of the 21-day cohabitation period. A gross necropsy of the thoracic, abdominal, and pelvic viscera was performed. Testes and epididymides of male rats were excised and paired organ weights were recorded. The epididymides were retained in neutral buffered 10% formalin. The testes were fixed in Bouin's solution for 48 to 96 hours and then retained in neutral buffered 10% formalin. Female rats were sacrificed on GD 20, cesarean-sectioned, and a gross necropsy of the thoracic, abdominal, and pelvic viscera was performed. The rats were examined for number and distribution of corpora lutea, implantation sites, live and dead fetuses, and early and late resorptions. Each fetus was weighed and examined for sex and gross external alterations. All fetuses were retained in alcohol.

No deaths related to TDF occurred in the F_0 generation rats. One rat in the 150 mg/kg/day dosage group was found dead. One rat in each of the 0 (vehicle), 450, and 600 mg/kg/day dosage groups were moribund sacrificed. All of these deaths were considered related to intubation errors, with the exception of the vehicle control group rat, which appeared to have dystocia. All other rats survived until scheduled sacrifice. Clinical observations of red perioral substance and excess salivation occurred in an increased or statistically significantly ($p \leq 0.05$ to $p \leq 0.01$) increased number of rats in the 150, 450, and 600 mg/kg/day dosage groups during the gestation period. Excess salivation and perioral substance were not considered related to treatment and probably did not reflect systemic toxicity of the test article. Test article-related clinical observations during gestation and urine-stained abdominal fur was observed in statistically significantly ($p \leq 0.05$ to $p \leq 0.01$) increased numbers of rats in the 450 and 600 mg/kg/day dosage groups, and ungroomed coat occurred in a statistically significantly ($p \leq 0.01$) increased number of rats in the 600 mg/kg/day dosage group. Two rats in each of the 450 and 600 mg/kg/day dosage groups had soft or liquid feces during gestation. Observations of urine-stained abdominal fur, red perioral substance, and ungroomed coat continued in statistically significantly increased numbers of rats in the 450 and 600 mg/kg/day dosage groups during the lactation period. Additionally, dehydration and emaciation occurred in increased or statistically significantly increased numbers of rats in the 450 and 600 mg/kg/day dosage groups, and cold to touch occurred in a significant number of 600 mg/kg/day dosage group rats during lactation. Soft or liquid feces occurred in 4 rats in each of the 450 and 600 mg/kg/day dosage groups during lactation. All other clinical observations were considered unrelated to the test article.

All necropsy observations were considered unrelated to administration of the test article. Terminal body weights, paired kidney weights, and the ratios of the kidney weights to the terminal body weights were comparable among the 5 dosage groups and did not statistically significantly differ. Body weight gains for the entire gestation dosage period (GDs 7 to 20) and the entire gestation period (GDs 0 to 20) were statistically significantly reduced in the 150, 450, and 600 mg/kg/day dosage groups. Gestation body weights were statistically significantly reduced in the 150 mg/kg/day dosage group on GDs 19 and 20; in the 450 mg/kg/day dosage group on GDs 16 through 20; and in the 600 mg/kg/day dosage group on GDs 10 through 20. During the lactation period, maternal body weight gains tended to be increased over or comparable to the control group values in the 150, 450, and 600 mg/kg/day dosage groups and were statistically significantly increased in the 450 and 600 mg/kg/day dosage groups for the entire lactation period (LDs 1 to 21). Reflecting the reductions in body weight gain that occurred during the gestation period, lactation body weights continued to be statistically significantly reduced in the 450 mg/kg/day dosage group on LDs 1 through 6 and 8 and in the 600 mg/kg/day dosage group on LDs 1 through 5 and 7 through 9. Absolute (g/day) and relative (g/kg/day) feed consumption values during gestation were statistically significantly reduced in the 450 and 600 mg/kg/day dosage groups for the entire gestation dosage period (GDs 7 to 20) and the entire gestation period (GDs 0 to 20). During lactation, significant reductions in absolute and relative feed consumption values occurred in the 450 (absolute only) and 600 mg/kg/day dosage groups for the entire tabulated lactation period (LDs 1 to 14). Peripartum/postpartum pup mortality and stillbirths were increased in the 450 and 600 mg/kg/day dosage groups, and there was a concomitant significant reduction in the percentage of live born pups in the 600 mg/kg/day dosage group. The percentages of pups found dead or presumed cannibalized were increased or statistically significantly increased on LDs 1 and 2 to 4 in the 450 and 600 mg/kg/day dosage

groups; and a significant number of dams had no surviving pups on LDs 2, 3, or 4 in these 2 dosage groups (5 and 10, respectively). Additionally, one dam in the 600 mg/kg/day dosage group had no surviving pups on LD 5. As a result of these pup deaths, the Viability Index was reduced in the 450 mg/kg/day dosage group and statistically significantly reduced in the 600 mg/kg/day dosage group. A significant number of pups were found dead or presumed cannibalized on LDs 8 to 14 in the 450 mg/kg/day dosage group and there was a corresponding slight reduction in the Lactation Index. Pup weights were statistically significantly reduced on LDs 1, 4, 7, 14, and 21 in the 450 and 600 mg/kg/day dosage groups. Increased or statistically significant increased numbers (compared to the control group values) of F_1 generation litters had pups that were cold to touch, pale, and had umbilical hernias in the 450 and 600 mg/kg/day dosage groups. A statistically significant increased number of litters had dams and pups that were not nesting in the 600 mg/kg/day dosage group. An increased number of pups that were found dead and necropsied in the 450 and 600 mg/kg/day dosage groups had no milk in the stomach. Weights of the kidneys were statistically significantly reduced in the 450 and 600 mg/kg/day dosage groups, reflecting the statistically significantly reduced average body weights in these groups on LD 21. Ratios of the kidney weights to the terminal body weights were comparable among the 5 dosage groups. All other natural delivery and litter observations were unaffected by dosages of the test article as high as 600 mg/kg/day.

Of the F_1 generation rats, 1 male rat in the 450-mg/kg/day group and 2 male rats in the 600 mg/kg/day dosage group, respectively, were found dead. One male rat in the 600 mg/kg/day dosage group was moribund sacrificed. Two female rats in each of the 450 and 600 mg/kg/day dosage groups were found dead. One female rat in the 450 mg/kg/day dosage group was moribund sacrificed. The F_1 male death in the 150 mg/kg/day dosage group was not considered related to maternal test-article exposure. The deaths in the 450 and 600 mg/kg/day dosage groups were considered related to the test article because: (1) they occurred in F_1 generation rats at maternally toxic dosages of 450 and 600 mg/kg/day; and (2) the incidences were dosage-dependent. These rats are discussed below. All other F_1 generation rats survived until scheduled sacrifice. There were no adverse clinical observations in the F_1 generation male and female rats that were considered to be test article related. Necropsy observations of empty stomach, small and large intestines distended with gas, dark red area on the glandular mucosal surface of the stomach, and tan spleen were considered to be associated with dead or moribund sacrificed F_1 generation male and female rats. Dosage-dependent reduced to statistically significantly reduced terminal body weights of the F_1 generation male rats were observed at all dosage levels. Paired testes weights were reduced and statistically significantly reduced in the 450 and 600 mg/kg/day dosage groups, and the paired epididymides weight was statistically significantly reduced for the 600 mg/kg/day dosage group. Paired testes weights were also reduced, although not statistically significantly reduced in the 50 and 150 mg/kg/day dosage groups. The result was statistically significantly increased epididymides to body weight ratio for the 150, 450, and 600 mg/kg/day dosage groups. The significant reduction in the weight of the epididymides in the 600 mg/kg/day dosage group was not considered biologically important because the mating and fertility of the male rats in this dosage group were unaffected. Reflecting the reductions in pup body weights during the preweaning period, body weights and body weight gain of the F_1 generation rats in all treated groups were reduced or statistically significantly reduced during the postweaning period (Days 1 and 71 postweaning). Gestation body weights for the F_1 generation female rats were statistically significantly reduced in all treated groups.

Gestation body weight gains were comparable for the 5 groups of the F_1 generation female rats. Absolute feed consumption values during the postweaning period for the F_1 generation males were statistically significantly reduced in all treated groups. Absolute feed consumption values during the postweaning period for the F_1 generation females were statistically significantly reduced over the postweaning period Days 8 to 15 by maternal dosages of 600 mg/kg/day of the test article. Absolute feed consumption values during the postweaning period for the F_1 generation females were comparable for all 5 groups, with the exception noted above. Relative feed consumption values during the postweaning period for the F_1 generation females statistically significantly increased for the 50 mg/kg/day dosage group and above from Days 1 to 71 postweaning. These changes were not considered biologically significant for the 50 mg/kg/day dosage group. The average day on which preputial separation was evident in male rats was statistically significantly increased and was dosage-dependent for F_1 generation male rats whose dams were administered dosages of the test article of 450 and 600 mg/kg/day. The time to reach vaginal patency in female rats was statistically significantly prolonged (450 mg/kg/day group) or prolonged (600 mg/kg/day group) for F_1 generation females whose dams were administered 450 or 600 mg/kg/day, respectively.

In the passive avoidance paradigm, F_1 male and female rats the 450 mg/kg/day dosage group had first trial latencies that were statistically significant increased. This effect was not dosage-dependent, probably because of the few animals tested in the 600 mg/kg/day dosage group. No other biologically important differences occurred for short- or long-term memory or learning in this paradigm. There were no biologically important differences in the values for learning, short-term retention, long-term retention, or response inhibition in the F_1 generation male or female rats, as evaluated by performance in a water maze swim task. The biologically important differences in the values for learning, short-term retention, long-term retention, or response inhibition in the F_1 generation males and females from the 450 and 600 mg/kg/day groups were considered improved performance for the passive avoidance paradigm compared to the vehicle group. Mating performance of the F_1 generation male and female rats was unaffected by administration of the test article to the F_0 generation dams at dosages as high as 600 mg/kg/day. Cesarean-sectioning and litter parameters in the F_1 generation female rats were unaffected by administration of dosages of the test article as high as 600 mg/kg/day to the F_0 generation dams.

Tenofovir concentrations were determined in plasma and milk from pregnant female rats administered TDF by oral gavage for approximately 27 days at 0, 50, 150, 450, or 600 mg/kg/day. Blood samples were taken twice, once on Day 7 of presumed gestation (the first day of dosing) and once on Day 20 postpartum (the last day of dosing). Milk samples were collected at approximately 1 hour postdose on Day 11 postpartum.

Results for Day 7 median TFV plasma concentration verses time profiles showed rapid absorption of TFV followed by biexponential elimination for all dose groups. Plasma pharmacokinetic T_{max} values varied from 0.25 to 0.67 hours across the dose range. Median C_{max} and AUC_{inf} values showed a general increasing trend with dose that was not proportional, but this may be misleading due to the high intersubject variability and the short sampling time (12 to 24 hours) (Table 25).

Results for Day 20 median TFV plasma concentration verses time profiles showed rapid absorption of TFV followed by biexponential elimination for all dose groups. The 600-mg/kg/day dose group showed an almost linear decline from 0.67 to 12 hours. T_{max} values varied from 0.67 to 1.0 hour across the dose range.

Comparison of single dose Day 7 data with steady state Day 20 data revealed overall similar T_{max} and apparent terminal $t_{1/2}$ values. Also, both showed generally increasing values with dose for C_{max} and AUC and some apparent accumulation with multiple dosing, although this evaluation is confounded by the high variability and the limited data from the highest dose groups. Results for Day 11 median TFV concentrations from rat milk were 154, 249, 532, and 730 ng/mL for the 50-, 150-, 450-, and 600-mg/kg/day dose groups, respectively; representing 13.5%, 11.0%, 20.8%, and 23.5% of the TFV plasma concentration at similar times. Although there is a trend toward increasing percent in milk with increasing dose, the data is weak due to the comparison of data from different rats and the different numbers of observations between plasma data (n = 3 to 4) and milk (n = 6 to 11).

Therefore, all rats in dose groups that received TDF demonstrated exposure based on the measurable levels of TFV in plasma on Days 7 and 20. Both Day 7 and Day 20 postpartum pharmacokinetic rat plasma profiles demonstrated increased exposure of TFV with increasing oral doses of TDF. Day 11 postpartum data confirmed the presence of TFV in rat milk, with the results suggesting that TFV was not concentrated in milk.

Table 25. Mean Toxicokinetic Parameters of TFV in Rats on Gestation Day (GD) 7 and Lactation Day (LD) 20

		TFV	
TDF (mg/kg/day)	**Day**	**C_{max} (µg/mL)**	**$AUC_{0-\infty}$ (µg·h/mL)**
50	GD7	1.27	3.81
	LD20	1.46	5.88
150	GD7	2.53	7.84
	LD20	2.26	11.7
450	GD7	3.43	54.4
	LD20	4.93	26.8
600	GD7	4.71	29.0
	LD20	2.89	27.2

In conclusion, the maternal NOEL of TDF was 50 mg/kg/day. The 150, 450, and 600 mg/kg/day dosages caused adverse clinical observations, reductions in body weight gain during gestation, and increases in body weight gain during lactation; and the 450 and 600 mg/kg/day dosages reduced gestation and lactation feed consumption values. The developmental NOEL was 150 mg/kg/day. The 450 and 600 mg/kg/day dosages resulted in increased peri/postpartum pup mortality, reduced pup survival, and reduced pup body weights. The NOEL for general toxicity

of TDF in the F_1 generation was 50 mg/kg/day (mortality occurred or body weights and/or feed consumption were reduced in the 150-, 450-, and 600-mg/kg/day dosage groups). The F_1 generation male and female NOEL for behavioral, reproductive, and developmental toxicity of TDF was 150 mg/kg/day (effects on sexual maturation were observed at dosages of 450 and/or 600 mg/kg/day).

6.4. B/F/TAF

With no expected toxicologic interactions with the B/F/TAF combination, further reproductive or developmental toxicity studies with B/F/TAF combination are not considered necessary. There were no significant effects on embryofetal development in rats or rabbits in the absence of maternal toxicity when BIC, FTC or TAF were tested individually.

For BIC, there were no effects on fertility in rats at an exposure margin of 29-fold, and no effects on embryo-fetal development in rats at a 36-fold exposure margin. In rabbits, decreases in fetal weight were observed at a dose associated with overt maternal toxicity; no effects were observed in the absence of maternal toxicity at exposures 0.59-fold clinical BIC clinical exposures. There were no effects on F_1 generation or F_2 generation rats in the BIC rat pre-/postnatal study at a 30-fold exposure margin. For FTC, slightly longer estrous cycles were observed in F_1 generation rats after exposure to high doses. After exposure to maternally toxic high doses of TDF, a delay in sexual maturation was observed in F_1 generation rats, and reduced viability index and weight of pups in the pre- and post-natal toxicity studies were noted. No additive effects are anticipated with the three-drug combination; therefore, no specific studies were conducted with the BIC/F/TAF combination.

6.5. Juvenile Toxicity

6.5.1. BIC

Per agreement with FDA and EMA Pediatric Committee, pediatric study plan (PSP) (Ref ID 3952985) and pediatric investigation plan (PIP) (EMEA-001766-PIP01-15-M01) agreements, respectively, do not include the requirement to conduct juvenile toxicity studies with BIC.

6.5.2. FTC

Emtricitabine is approved for use in infants (aged 4 months of age or older), children, adolescents, and adults. No specific juvenile toxicity studies are considered warranted with FTC.

6.5.3. TAF/TFV

Although no specific juvenile toxicity studies have been conducted with TAF or TDF, data are available from efficacy studies of TFV in SIV-infected and non-infected rhesus macaques {Tarantal 1999, Van Rompay 2004, Van Rompay 2008}. Investigators from the California Regional Primate Research Center have reported results of antiviral efficacy studies of TFV in simian immunodeficiency virus (SIV)-infected and non-infected rhesus monkeys {Tarantal 1999, Van Rompay 2004, Van Rompay 2008}. These studies included administration of TFV to

12 gravid rhesus macaques, and to more than 85 infant and juvenile rhesus macaques from 1 day to 7.5 years of age at initiation of dosing. This age range covers the human equivalent of prenatal, infant, juvenile and adolescent phases of growth (see Table 26). The duration of treatment has ranged from 12 weeks to 13 years. Clinically relevant renal and bone pathology occurred only in rhesus monkeys in which TFV was chronically administered at 30 mg/kg/day by daily subcutaneous injection. Exposure levels (AUC_{ss} 140 μg·h/mL) at this dose were more than 30-fold higher than those of adult subjects (4.4 μg·h/mL) after a 300 mg/day dose of TDF. Effects in rhesus monkeys were reversible by decreasing or stopping exposure. Administration of lower doses of TFV did not cause renal dysfunction or abnormal bone density or growth.

The published studies (see Table 26) were conducted in collaboration with Gilead Sciences and published in peer review journals. All drug substance for these studies was provided by Gilead Sciences.

Table 26. Nonclinical Pharmacology/Toxicology Studies with TFV

API/Route/n	Age at Start of Treatment[a]	Dose (mg/kg/day)	Duration of Daily Treatment	Reference
Tenofovir/SC/n=12	Gestation Day 80; Infants Day 2	30	Gestation Days 80-157 (n=12); Infants: up to 9 months (n=9)	{Tarantal 1999}
Tenofovir/SC/n=54	1 day to 20 months	4, 10, 30	Up to 12 weeks (n=39) At least 8 months (n=13) At least 5 years (n=2)	{Van Rompay 2004}
Tenofovir/SC/n=32[b]	1 day to 33 months	1.25 to 30	1 year to 13 years	{Van Rompay 2008}

SC = subcutaneous

a A general comparison of growth phases between human and rhesus monkeys is as follows {Chellman 2009}:

Infant:	Human = 1-24 months;	Rhesus = 1-12 months
Child/Juvenile:	Human = 2-11 years;	Rhesus = 1-2 years
Adolescent:	Human = 12-16/18 years	Rhesus = 2-4 years

b 15 animals previously reported in Van Rompay 2004, but observations extended.

Studies were conducted to assess the safety, efficacy, and placental transfer of TFV when administered once daily subcutaneously to gravid rhesus monkeys during the second and third trimesters and to their offspring (30 mg/kg/day) {Tarantal 1999}. Fetuses (SIV-infected, N = 6; noninfected; N = 6) were monitored sonographically and maternal/fetal blood samples were collected at select time points for hematologic, clinical chemical, virologic, immunologic, and pharmacologic assessments. Newborns were delivered by cesarean section at term and nursery reared for postnatal studies. Infants were administered TFV once daily beginning on Day 2 of life until 9-months postnatal age. Results of these studies have shown significant placental transport of TFV, with peak fetal levels at 1 to 3 hours postmaternal administration; a significant and sustained reduction in viral load in SIV-infected fetuses and infants; and marked improvements in outcome (eg, survival, growth, health) in SIV-infected offspring. However, decreased infant body weights and alterations of select serum biochemical parameters (eg, decreased phosphorus levels, elevated alkaline phosphatase [ALP]) have been shown to occur in 67% of TFV-treated infants, with severe growth restriction and bone-related toxicity in approximately 25% of animals studied. These data suggest that there is the potential for bone-related toxicity at chronic, high dosages, particularly in infants.

Daily subcutaneous administration of TFV at 10 mg/kg/day to 2 newborn rhesus monkeys for more than 5 years had no observed adverse effect on their bone density or growth {Van Rompay 2004, Van Rompay 2008}. The steady state AUC for these 2 rhesus monkeys was approximately 19 µg·h/mL (~4.3-fold exposure in adult human populations). Although data were variable, no drug-related changes occurred in the urinary excretion of phosphorus, calcium, potassium, protein, magnesium, or chloride. Radiographs and DXA scans of the animals were collected regularly from birth to 5 years of age. There was no radiological evidence of decreased bone opacity, changes in growth plates, bone formation, or bone resorption compared to control data. Bone biopsies at 3.5 years of age showed no significant differences in bone structure measurements.

Subcutaneous administration of TFV from 4 to 30 mg/kg/day to 39 infant rhesus monkeys for up to 3 months had no observed adverse effect on their health or growth {Van Rompay 2004}. No proximal renal tubular dysfunction (PRTD) or growth restriction or bone abnormalities were observed after 12 weeks of dosing or after 3.5 months to 14 months after the end of treatment.

Subcutaneous administration of TFV at 30 mg/kg/day to 13 rhesus monkeys for at least 8 months resulted in PRTD with glucosuria, aminoaciduria, hypophosphatemia, growth restriction, osteomalacia and reduced clearance of TFV {Van Rompay 2004}. Bone lesions were only observed in animals that also had PRTD. The long bone metaphyses and the physes and metaphyses of the vertebral bodies were incompletely ossified and there was overall decreased bone opacity. In 4 of the more severely affected animals, there were bone deformations and/or pathological fractures in the long bones or ribs. Serum phosphorus levels were markedly decreased and bone-ALP isoenzymes levels were increased. Urine samples collected by cystocentesis generally showed increased glucose and amino acid levels. Pharmacokinetic data collected after 11 to 24 months of TFV administration from 4 of the rhesus monkeys with moderate to severe bone disease had a mean AUC of 140 µg·h/mL (range 101 to 222 µg h/mL), more than 30-fold that in adult subjects. The adverse effects were partially or totally reversible following either complete withdrawal of TFV or reduction of the daily regimen from 30 mg/kg/day to 2.5 or 10 mg/kg/day.

Disposition of the 13 rhesus monkeys chronically treated with TFV at 30 mg/kg/day included euthanasia (n = 6), a reduction in TFV dose (n = 6), or discontinuation of dosing (n = 1) {Biron 2012}. In the animals euthanized with severe skeletal lesions, the bone histology revealed irregular hyperplastic growth plates and trabecular hyperplasia with widened osteoid seams. In the animal with severe bone disease and in which administration of TFV was discontinued, the serum phosphorus and ALP concentrations returned to normal in about 6 and 8 months, and the metaphyseal osteopenia resolved by 18 months. For the other 6 animals whose TFV dosage was decreased to either 30 mg/kg twice weekly (n = 1) or 10 mg/kg once daily (n = 5), there was improvement in serum phosphorus levels and radiological bone opacity, and a resumption of growth within approximately 6 months.

Van Rompay et. al. extended their observations and reported on the longer-term safety and pharmacokinetics of TFV in 32 infant and juvenile rhesus monkeys {Van Rompay 2008}. As reported earlier, the primary toxicity of prolonged subcutaneous TFV administration was PRTD, characterized by glucosuria, decreased serum phosphorus, and increased serum ALP levels.

The effect of daily subcutaneous administration of TFV at 10 to 30 mg/kg/day for 7 weeks to 25 months was described for 29 animals. Seven animals dosed with 10 to 30 mg/kg/day TFV had minimal to no evidence of persistent PRTD, which was associated with a relatively high TFV clearance. Twenty two animals with relatively reduced TFV clearance had evidence of PRTD based on urine and serum markers. Five of these animals dosed at 10 to 30 mg/kg/day with PRTD had DXA scans performed. DXA confirmed low BMD in 3 of these animals with PRTD that had growth retardation and severe bone lesions. Two animals with PRTD that had their doses reduced and dietary supplements provided had normal BMD or reduced BMD. Animals that received low-dose regimens of TFV and had no persistent PRTD had relatively normal BMD.

7. LOCAL TOLERANCE

7.1. BIC

7.1.1. Ocular Irritation Study in Bovine Corneas with BIC

The bovine corneal opacity permeability (BCOP) assay was performed to assess the ocular irritancy potential of BIC in vitro (m2.6.7, Section 16.1, TX-141-2060). Imidazole was tested in parallel as a positive control. The assay uses isolated bovine corneas as a means of assessing the ocular corrosivity or severe irritancy potential of test substances in vitro. Two endpoints, corneal opacity and permeability, were measured and combined to give an in vitro irritancy score (IVIS), which may be used to classify and rank test substances as potential eye irritants according to Organization for Economic Cooperation and Development (OECD) Guideline 437 (OECD 437). A substance that induces an IVIS of > 55.1 is defined as a corrosive or severe irritant. A substance that induces an IVIS of ≤ 3 is defined as non-irritating. BIC elicited an IVIS of 27.58 with a 4-hour incubation and was predicted to be moderately irritating (United Nations [UN] Globally Harmonized System of Classification and Labeling of Chemicals [GHS] No Prediction).

7.1.2. Dermal Irritation and Corrosion Study in In Vitro Models with BIC

7.1.2.1. *In Vitro* Skin Corrosion Assessment of BIC

The skin model EpiDerm™ assay was conducted to determine whether BIC is considered corrosive in an in vitro dermal model (m2.6.7, Section 16.1, TX-141-2058). Duplicate EpiDerm™ inserts were treated with BIC, purified water (negative control) and 8M potassium hydroxide (positive control) for 3 minutes and 60 minutes. At the end of the treatment period, the tissues were washed with phosphate buffered saline (PBS) and cell viability was assessed. The skin corrosivity potential was classified according to the remaining cell viability obtained after test material treatment with either of the two treatment times. Acceptance criteria for negative and positive controls were met. According to the UN GHS classification system, mean skin viability value of ≥ 50% or ≥ 15% after 3 or 60 minute exposure, respectively are considered non-corrosive. Mean skin viability after a 3 minute or 60 minute exposure to BIC was 76% and 94%, respectively; therefore, BIC was considered to be non-corrosive in this model.

7.1.2.2. *In Vitro* Skin Irritation Assessment of BIC

The skin model EpiDerm™ assay was conducted to determine whether BIC is considered an irritant in an in vitro dermal model (m2.6.7, Section 16.1, TX-141-2059). EpiDerm™ SIT (EPI-200) inserts were treated with BIC, PBS (negative control) and 5% w/v sodium dodecyl sulphate (SDS; positive control) for 60 minutes. At the end of the treatment period, the tissues were washed with PBS and cell viability was assessed using the MTT assay. The skin irritation potential was classified according to the remaining cell viability obtained after test article treatment. Acceptance criteria for negative and positive controls were met. Test articles are considered to be non-irritant to skin in accordance with UN GHS No Category if the tissue viability after exposure and post-treatment incubation is >50%, relative to the negative control. The group mean viability for BIC was 91.6%; therefore, BIC was considered not to be an irritant in this model.

7.1.3. Phototoxicity Assessment of BIC

7.1.3.1. Neutral Red Uptake Phototoxicity Assay of BIC in Balb/c 3T3 Mouse Fibroblasts

This in vitro study evaluated the phototoxicity potential of BIC as measured by the relative reduction in viability of Balb/c 3T3 mouse fibroblasts exposed to BIC and ultraviolet (UV) radiation compared with the viability of fibroblasts exposed to BIC in the absence of UV radiation in two definitive assays (m2.6.7, Section 16.1, TX-141-2044). Promethazine was used as the positive control.

Bictegravir demonstrated phototoxicity in the assay by the Photoirritancy Factor >1, and mean photo effect values were < 0.15 in definitive assay 1 and >0.15 in definitive assay 2. Therefore, it was concluded that BIC demonstrated phototoxic potential in this assay. Bictegravir was not cytotoxic at the highest concentration tested. Promethazine cytotoxicity and phototoxicity criteria met acceptance criteria. In the in vitro assay, BIC demonstrated phototoxicity with no cytotoxic potential. As a result, a repeat dose in vivo study was conducted (m2.6.7, Section 16.1, TX-141-2046).

7.1.3.2. A Multiple Dose Phototoxicity Study to Determine the Effects of Oral Administration of BIC on Skin in Pigmented Rats

The objectives of this study were to determine the potential phototoxic effects of BIC on the skin of Crl:LE (Long-Evans) pigmented rats, when administered by oral gavage once daily for 3 consecutive days, followed by exposure to radiation from a xenon lamp (to simulate sunlight) (m2.6.7, Section 16.1, TX-141-2046). Bictegravir was compared with 8-methoxypsoralen (8-MOP), the comparator article. Bictegravir was administered by oral gavage at 0, 2, 10, or 300 mg/kg/day for 3 consecutive days to female LE rats (5 rats/group). On the day of UV radiation exposure, one group of 3 rats was administered 8-MOP. Approximately 4 hours after the final administration, rats were sedated and the eyes and lightly and darkly pigmented dorsal skin sites on the rats were exposed to a dose of 10.05 to 10.78 J/cm^2 of ultraviolet A (UVA) and 141 to 152 mJ/cm^2 of ultraviolet B (UVB), delivered to the rats over a period of 41 to 44 minutes. Rats were observed for clinical observations and skin reactions twice on the day of UV radiation exposure and once on the following 3 days. Body weights were recorded daily during administration, UV radiation exposure and postexposure observation. Ocular examinations were conducted prior to study start and three days post ultraviolet radiation exposure. Eyes were collected for animals assigned to the phototoxicity phase for histopathology examination. Blood samples were collected from satellite groups of animals on Day 3 prior to and approximately 4 hours postdose.

There were no remarkable clinical observations or effects on body weight and all rats survived to scheduled euthanasia. Three daily administrations of BIC at doses up to 300 mg/kg/day followed by a single exposure of UVA and UVB did not result in skin reactions (erythema or edema) indicative of phototoxicity. No light microscopic changes suggestive of ocular phototoxicity were observed in the eyes of the rats administered BIC at doses up to and including 300 mg/kg/day. Rats administered 8-MOP had skin findings consistent with phototoxicity, thereby validating the study. Bioanalysis demonstrated dose-related increases in BIC plasma levels at the time of UV radiation exposure with mean plasma levels 4 hours postdose on Day 3 of 16.2, 72.6, and 204 µg/mL at dose levels of 2, 10 and 300 mg/kg/day, respectively.

In conclusion, BIC was considered nonphototoxic when administered to rats at 3 oral doses up to 300 mg/kg/day, corresponding to an estimated C_{max} exposure margin of at least 33-fold higher than BIC C_{max} (6.2 µg/mL) following clinical administration of the B/F/TAF FDC.

7.2. FTC

No specific local tolerance studies have been conducted with FTC. In repeat dose oral toxicity studies, there were no FTC-related changes in the GI tract of mice, rats and monkeys.

7.3. TAF

7.3.1. Ocular Irritation Study in Bovine Corneas

The BCOP was performed to assess the ocular irritancy potential of GS-7340-03 in vitro (m2.6.7, Section 16.2, TX-120-2013). Imidazole was tested in parallel as a positive control. The assay uses isolated bovine corneas as a means of assessing the ocular corrosivity or severe irritancy potential of test substances in vitro. The isolated corneas were obtained as a by-product of the meat production industry. Two endpoints, corneal opacity and permeability, were measured and combined to give an IVIS, which can be used to classify and rank test substances as potential eye irritants according to OECD guideline 437. TAF elicited an IVIS of 21.0 ± 8.7 with a 4-hour incubation and was predicted to be a noncorrosive/nonsevere eye irritant.

7.3.2. Dermal Irritation Study in Rabbits

Three female New Zealand White rabbits received a single 4 hour, semi-occlusive, dermal administration of approximately 0.5 g of GS-7340-03 as supplied moistened with water and were observed for 4 days (m2.6.7, Section 16.2, TX-120-2011). No local dermal reaction was observed in any animal throughout the duration of the study. The Primary Irritation Index was calculated to be 0.0; TAF was classified as a ‘non-irritant’.

7.3.3. Phototoxicity Assessment

TAF fumarate does not absorb light within the range of natural sunlight; therefore, no phototoxicity studies are not considered necessary for TAF. The ultraviolet absorption spectrum was obtained with a TAF fumarate solution in 20 mM potassium phosphate buffer at pH 6.0. The absorption maxima (λ_{max}) were observed at 205 and 262 nm and the measured molar extinction coefficient at the characteristic peak at 262 nm was 1.5×10^4 $M^{-1}cm^{-1}$.

7.4. B/F/TAF

The B/F/TAF tablet is intended for oral use. No local tolerance studies were conducted for the B/F/TAF combination. No gastrointestinal (GI) toxicity was observed in the BIC, FTC or TAF toxicity studies. The potential for additive effects among the three agents in the B/F/TAF FDC is minimal.

8. OTHER TOXICITY STUDIES

8.1. Antigenicity

8.1.1. BIC: Assessment of Skin Sensitization Potential using the Local Lymph Node Assay (LLNA) in the Mouse

The study was performed to assess the skin sensitization potential of BIC using the local lymph node assay (LLNA) according to OECD Guideline 429 (m2.6.7, Section 17.1.2, TX-141-2061). Bictegravir was formulated in propylene glycol at 10, 25, or 50% (w/v). Twenty-five microliters of vehicle, positive control (25% v/v α-hexylcinnamaldehyde), and each concentration of BIC were applied for 3 consecutive days on the dorsal surface of both ears of 5 female CBA/CaCrl mice per group. The proliferative response of the lymph node cells (LNCs) from the draining auricular lymph nodes was assessed 6 days following the initial application, by measurement of the incorporation of ^{3}H-methyl thymidine by β-scintillation counting of LNC suspensions.

All animals survived to the terminal sacrifice. No BIC-related adverse clinical observations, dermal reactions, ear thickness or body weight changes were noted during the study. The test-to-control ratios (SIs) of radioactivity from thymidine incorporated in the draining lymph node obtained for 10%, 25%, and 50% w/v were 1.15, 1.47, and 1.25, respectively, which indicates that BIC did not show the potential to induce skin sensitization. The SI for the positive control was 13.16, demonstrating validity of the study. In conclusion, BIC is not regarded as a potential skin sensitizer.

8.1.2. FTC

In view of the absence of indications from repeat-dose toxicity studies that FTC might elicit allergic reactions, no specific antigenicity studies were conducted.

8.1.3. TAF: Assessment of Skin Sensitization Potential using the Local Lymph Node Assay in the Mouse (Individual animal approach)

Three groups, each comprising 5 female mice were administered GS-7340-03 at concentrations of 10, 25 or 50% w/v (m2.6.7, Section 17.3, TX-120-2014). Similarly constituted groups received the vehicle (acetone: olive oil (4:1 v/v)) or positive control substance (25% v/v hexyl cinnamic aldehyde). The mice were administered TAF by daily application of 25 µL of the appropriate concentration or control (vehicle or positive), to the dorsal surface of both ears for 3 consecutive days. The proliferative response of the LNCs from the draining auricular lymph nodes was assessed 5 days following the initial application, by measurement of the incorporation of ^{3}H-methyl thymidine (^{3}HTdR) by β-scintillation counting of LNC suspensions. The response was expressed as radioactive disintegrations per minute per lymph node (dpm/node) and as the ratio of ^{3}HTdR incorporation into LNC of test nodes relative to that recorded for control nodes (test/control ratio), termed as SI. The test substance is regarded as a sensitizer if at least one concentration of the chemical has a SI of 3 or more.

The SIs obtained for 10%, 25%, and 50% w/v were 0.9, 1.0, and 1.0, respectively, which indicates that TAF did not show the potential to induce skin sensitization. The EC3 value (the "estimated concentration of 3" is the concentration of test substance that would result in a SI of 3 in the LLNA) was determined to be higher than 50% w/v. The SI for the positive control substance hexyl cinnamic aldehyde was 6.3, which demonstrates the validity of this study.

8.1.4. B/F/TAF

Studies with BIC, FTC and TAF as individual agents have not indicated that antigenicity is of concern. No studies were deemed necessary with the B/F/TAF combination.

8.2. Immunotoxicity

8.2.1. BIC

No specific immunotoxicity studies were conducted with BIC. There were no findings in the repeat dose toxicity studies with BIC to indicate an immunological concern.

8.2.2. FTC: TP-0006 – 28 Day Oral (Gavage) Immunotoxicity Study in Rats

The immunotoxicity of FTC was evaluated in rats administered once daily oral doses of FTC for 28 consecutive days (m2.6.7, Section 17.2, TOX146). Emtricitabine was suspended in 0.5% methylcellulose and administered by gavage to groups of 5 male and 5 female Sprague-Dawley rats at 0, 60, 240, and 1000 mg/kg/day. Following the final dose, each rat was administered an intravenous dose of 2 × 108 sheep RBCs. Five days later, blood samples were collected and the serum was analyzed by enzyme-linked immunosorbent assay (ELISA) to detect immunoglobulin M (IgM) antibodies specific to sheep RBCs. There were no adverse effects of FTC during the dose period. Body weights were unaffected by treatment with FTC. Emtricitabine did not affect the titers to sheep RBCs at any of the doses administered. It was concluded that 1000 mg/kg/day FTC was a no-effect dose for immunotoxic potential.

8.2.3. TAF

No specific immunotoxicity studies were conducted with TAF. There were no findings in the repeat dose toxicity studies with TAF to indicate an immunological concern.

8.2.4. B/F/TAF

Data from repeat-dose toxicity studies (hematology, lymphoid organ weights, microscopy of lymphoid tissues, bone marrow cellularity) with BIC, FTC, or TAF and specialized immunotoxicity studies with FTC do not suggest immunotoxic potential for BIC, FTC, or TAF. There were no notable effects of the combination of FTC/TDF on immunophenotyping or natural killer cell assay values in a 4-week repeat dose dog study (m2.6.7, Section 7.4.1, TX-164-2004). No further studies were deemed necessary with the B/F/TAF combination.

8.3. Mechanistic Studies

8.3.1. BIC

No specific mechanistic studies with BIC were conducted.

8.3.2. FTC

For FTC, specific endpoints were included in repeat dose toxicity studies in mice and cynomolgus monkeys to evaluate the potential for mitochondrial toxicity.

8.3.3. TAF

8.3.3.1. Bone Turnover

Three non-GLP rat studies and one GLP dog study were conducted to evaluate the potential for TAF to alter bone metabolism.

8.3.3.1.1. Rats

A preliminary study in Sprague Dawley rats was conducted by oral daily dosing male and female rats 0 (vehicle), 6.25, 25, 100 or 400 mg/kg/day of GS-7340-02 for 6 days (m2.6.7, Section 6.2, R990139). However, the results are confounded due to administration of another compound in addition to GS-7340-02 on study day 3. Effects observed in all treatment groups included a dose dependent increase in PTH and a non-dose dependent reduction in 1,25-dihydroxycholecalciferol. The NOEL for this study was determined to be < 6.25 mg GS-7340-02/kg.

Changes in bone markers were assessed after daily oral administration of GS-7340-02 to Sprague-Dawley rats. Male rats were administered 0 (2 males; vehicle only) or 400 mg/kg GS-7430-02 (4 males) by oral gavage daily for 6 days (m2.6.7, Section 17.3, R990177). Urine was collected continuously over a 24-hour period while the animals were housed in the metabolic cages at the beginning and end of dosing. The treatment and control group animals were euthanized after 6 days of treatment. Blood samples were collected for clinical chemistry. No observable toxicities were observed over the duration of the study. One animal was euthanized due to non-drug related injury. Serum parathyroid hormone (PTH) concentrations were unchanged in animals administered TAF compared to controls on Day 6. Serum 1,25-dihydroxycholcalciferol was decreased by 80% in animals administered TAF compared to control rats. Urinary deoxypyridinoline (Dpd) was decreased by 38% in rats administered GS-7340-02 compared to control rats. The urinary calcium/creatinine and phosphorus/creatinine ratios of rats administered GS-7340-02 were increased 444% and 202%, respectively, compared to controls by Day 6. The results of this study demonstrated that TAF had effects on selected urinary and serum parameters. However, lack of strong statistical comparison to control animals and lack of correlation between data prevented any interpretation on the overall toxicity.

In the second study (m2.6.7, Section 17.3, R2000044), an additional dose was evaluated. This study examined changes in markers of calcium homeostasis in male Sprague-Dawley rats after once daily oral administration of TAF. Male Sprague-Dawley rats were administered

100 or 400 mg/kg GS-7340-02 by oral gavage on Days 0, 1, 2, 3, 4, and 5. The animals were housed in metabolic cages throughout the study. The animals were euthanized on Day 6. Blood samples were collected for clinical chemistry on Days 0, 1, and 6. Urine was collected on Days 0 to 6.

Rats administered 400 mg/kg/day had statistically significant decreases in rates of percent change of body weight from Day 0 on Days 2 through 6. No changes in plasma calcium, phosphorus, or ionized calcium were observed during the first day of the study in either treatment group. No changes in serum 25-hydroxyvitamin D_3, parathyroid hormone, or total carbon dioxide (CO_2) were observed in either treatment group. Significant dose-dependent reductions of 35% and 46% were observed in 1,25-dihydroxyvitamin D_3 on Day 6 from rats administered 100 or 400 mg/kg/day TAF, respectively. Significant non-dose-dependent reductions of 15% and 14% were observed in serum calcium on Day 6 from rats administered 100 or 400 mg/kg/day TAF, respectively. Significant dose-dependent decreases of 20% and 31% in serum phosphate were observed in rats administered 100 or 400 mg/kg/day TAF, respectively, on Day 6. Urinary calcium: creatinine ratio values were significantly decreased by approximately 71% and 53% on Days 1 and 2, respectively, in rats administered 100 mg/kg/day TAF. Urinary calcium: creatinine ratio values were significantly decreased by 42% (Day 1) then significantly increased by 213% (Day 6) in rats administered 400 mg/kg/day TAF. No changes in urinary phosphorus: creatinine ratio, N-acetyl-ß-glucosaminidase (NAG):creatinine, cyclic adenosine monophosphate (cAMP):creatinine, pH, protein, urobilinogen, or specific gravity were observed. Daily administration of TAF to rats for 6 days caused significant and dose-dependent reductions in 1,25-dihydroxyvitamin D_3 and serum phosphate. Slight, significant decreases in serum calcium were observed in animals administered 400 mg/kg/day TAF on Day 6. Urinary excretion of calcium was initially decreased on Day 1 for both treatment groups. By Day 6, a significant increase in urinary calcium was observed in rats administered 400 mg/kg/day TAF. These data indicated that daily administration of TAF to rats for 6 days altered plasma vitamin D, calcium and phosphate levels as well as urinary excretion of calcium. The mechanistic basis of these changes and their biological relationships in terms of cause and effect are unknown.

8.3.3.1.2. Dogs

Oral doses of 37.5 or 75 mg/kg/day of GS-7340-02 or vehicle were administered to male beagle dogs (3/group) (m2.6.7, Section 17.3, D990142). Doses were administered by oral gavage once each day for 5 days. Emesis, excessive salivation and diarrhea were observed and weight loss and reduced food intake occurred.

On day 6, dogs had decreased WBC, neutrophil, lymphocyte, monocyte, and eosinophil counts with a left shift in the myeloid series with under representation of mature segmented neutrophils as the predominant bone marrow finding. Dogs also had increased total protein, globulin, triglyceride, cholesterol, AST, ALP, and creatinine kinase (CK) values. A trend toward proteinuria was observed in the urinalysis data.

There were no biologically important changes observed in mean values for ionized calcium or N telopeptide. The lack of the expected age-related reduction in mean serum bone ALP (B-ALP) after GS-7340-02 administration suggested increased bone remodeling. The mean serum calcitriol (1, 25 dihydroxycholecalciferol) concentration was reduced by approximate 68% for both dose

groups administered TAF. The mean serum 25-hydroxyvitamin D (25 hydroxycholecalciferol) concentration was reduced approximately 35% and 33% for the 37.5 and 75 mg/kg/day dose groups.

Gastrointestinal lesions characterized by epithelial cell necrosis, regenerative hyperplasia, and cyst formation were most prevalent in dogs administered 5 daily doses of 75 mg/kg/day TAF. Lymphoid cell depletion was noted in the lymph nodes, thymus and tonsil, also observed only in the 75 mg/kg/day animals. Bile duct hyperplasia and periportal inflammation was observed after administration of TAF.

8.3.3.2. Renal Function

The objective of this GLP study was to evaluate the pharmacological effects of GS-7340-02 on the renal system after oral gavage of 0 (vehicle), 100 or 1000 mg/kg/day GS-7340-02 to male Sprague-Dawley CD (Crl:CD®(SD)BR) rats (m2.6.7, Section 17.3, R990186). Thirty minutes after volume loading by oral gavage with 10 mL/kg saline, rats were administered a single oral gavage dose of the test or vehicle article (10 mL/kg). No adverse effects on renal function were observed. Although urinary output of calcium was increased at 1000 mg/kg/day, this correlated with an increase in serum calcium concentration and indicated that the kidneys were functioning in order to reduce the serum calcium load.

8.4. Dependence

No specific studies have been conducted to evaluate dependency of BIC, FTC, or TAF. Integrase inhibitors or nucleoside analogs, as classes, have no known properties that would suggest development of dependence and there was no evidence of development of dependence in nonclinical studies. Low levels of [^{14}C]BIC radioactivity were detected in brain (< 2% relative to blood) suggesting low BIC permeability across the blood brain barrier (m2.6.5, Section 5.1.2, AD-141-2276; m2.6.5, Section 5.1.3, AD-141-2276). In the tissue distribution studies using [^{14}C]TAF, little or no radioactivity was observed in brain tissues from dog (m2.6.5, Section 5.3.4, D990173-BP) and rat (m2.6.5, Section 5.3.2, AD-120-2020), while low levels of radioactivity was detected in mouse brain tissues, suggesting low permeability across the blood brain barrier in mouse (m2.6.5, Section 5.3.1, AD-120-2011). Consequently, dependency studies were considered not warranted.

8.5. Metabolites

No specific toxicology studies with BIC metabolites were conducted as there were no unique human metabolites; all human metabolites were also found in nonclinical species (m2.6.4, Section 1).

No specific toxicology studies with TAF metabolites were conducted as there were no unique human metabolites. The hydrolytic pathway of TAF to TFV via TFV monoamidate is similar to that observed in vivo; monophenyl PMPA, phenol, and PMPA are known metabolites. Studies have shown that metabolite levels in nonclinical species used for toxicology studies exceed those observed clinically (m2.6.4, Section 5.3.3). The by-products formed during conversion of TAF to TFV are isopropanol, l-alanine, and phenol and are formed at nontoxic levels. The by-products formed during conversion of TDF to TFV are isopropanol, carbon dioxide and formaldehyde.

Clinical data (GS-US-120-0104) demonstrated that the mean intracellular concentrations of TFV-DP ranged 0.677-1.49 μM in PBMCs in the patients dosed once daily at 25 mg TAF. Since the predominant PBMC metabolite of TAF is TFV-DP (m2.6.5, Section 14.3.14, AD-120-2035), the total TAF-related intracellular metabolites in PBMCs should not exceed 5 μM (conservative estimate). Therefore, the intracellular concentrations of each by-product formed during conversion of TAF to TFV should not exceed 5 μM. The intracellular concentrations of Metabolite X or Metabolite Y should be significantly lower than 5 μM {Lee 2005}. No prodrug was found in PBMCs; Tenofovir alafenamide showed low cytotoxicity in resting and dividing PBMCs, T-lymphoblastoid cells, and hepatocellular carcinoma (HepG2) cells, and provided > 1997-fold selectivity or greater relative to antiviral activity in T-lymphoblastoid cell lines (m2.6.2, Section 3.1.3).

l-Alanine is a naturally occurring nonessential amino acid, which is found in a wide variety of foods. Isopropanol accounts for ~12.6% of the total molecular weight of GS-7340 (free base). Therefore the total amount of isopropanol being administered at a 10 mg dose is ~ 1.26 mg, 0.025 mg/kg for a 50 kg person. Doses of more than 5 g/kg have been reported to cause adverse effects (http://chem.sis.nlm.nih.gov/chemidplus/rn/67-63-0; accessed 13May2014)

Phenol accounts for ~ 20% of the total molecular weight of GS-7340. Therefore, the total amount of phenol being administered at a 10 mg dose is ~2 mg; 0.04 mg/kg for a 50 kg person. The probable oral lethal dose to humans is estimated at 50-500 mg/kg (http://toxnet.nlm.nih.gov/cgi-bin/sis/search/f?./temp/~CtJ8Sm:1; accessed 13May2014.) IARC has determined that phenol is not classifiable as to its carcinogenicity to humans (Group 3) {International Agency for Research on Cancer (IARC) 1999}. Phenol is an inactive ingredient in the US Food and Drug Administration (FDA) approved products delivered intravenously, intramuscularly or subcutaneously at levels up to 1.3%. (http://www.accessdata.fda.gov/scripts/cder/iig/getiigWEB.cfm; accessed 12 May 2014). Examples of approved prescription drugs used chronically (eg, glycemic control, acromegaly) that contain phenol are presented in Table 27.

Table 27. Examples of Approved Prescription Drugs Containing Phenol

Product	Phenol concentration	Estimated daily dose of drug and phenol
LEVEMIR® - insulin detemir [rDNA origin] injection: 3mL PenFill 100 U insulin detemir/mL	1.8 mg/mL SC	Assuming 1.2 U/kg BID use and 60 kg, 144 U/day is 1.44 mL, which would give 2.5 mg phenol/day
Victoza® - liraglutide [rDNA origin] injection (GLP-1); 6 mg/mL	1.8 mg/mL C	The max recommended dose is 1.8 mg/day (0.3 mL), which equals 0.54 mg phenol/day
Sandostatin® -octreotide acetate Injection, 200 and 1000 mcg/mL	5 mg/mL SC	The maximum recommended dose is 500 μg TID, which would be 0.5 mL TID or 7.5 mg phenol/day

BID = twice daily; TID = 3 times a day

In conclusion, the by-products formed with the conversion of TAF to TFV are at low levels, showed low cytotoxicity, were evaluated in the TAF toxicology studies and of little toxicological concern.

8.6. Impurities

8.6.1. BIC: Bacterial Reverse Mutation Assays with GS-001* , GS-002* and GS-003*

GS-001* (m2.6.7, Section 17.1.1, TX-141-2041) or GS-002* and GS-003* (m2.6.7, Section 17.1.1, TX-141-2048), potential impurities from starting material in the manufacturing process, were evaluated for the ability to induce reverse mutations at the histidine locus in *Salmonella typhimurium* tester strains TA98, TA100, TA1535, and TA1537, and at the tryptophan locus of *Escherichia coli* strain WP2*uvr*A. Evaluations were conducted in the presence or absence of an exogenous mammalian activation system (S9) containing microsomal enzymes. GS-001* , GS-002* and GS-003* were evaluated in the initial mutagenicity assay in all 5 tester strains at dose levels of 5.00 to 5000 µg/plate in the presence and absence of S9. All positive and vehicle control values were within the historical range, and all criteria for valid studies were met.

Due to contamination of TA1537 in the initial assay, GS-001* was re-evaluated in a repeat mutagenicity assay in the presence and absence of S9 at the same GS-001* dose levels used in the initial assay. No precipitate or toxicity and no positive increase in the mean number of the revertant colonies were observed at any tested dose level.

In the assay with GS-002* , precipitate was observed at 5000 µg/plate in all tester strains in the absence of S9. No toxicity was observed at any tested concentration in any tester strain in the presence or absence of S9. In the assay with GS-003* , precipitate was observed at 5000 µg/plate in all *Salmonella* tester strains in the presence and absence of S9. Toxicity was observed at 5000 µg/plate in all *Salmonella* tester strains in the absence of S9. No increase in the mean number of revertant colonies was observed at any tested concentrations in any tester strains with either GS-002* or GS-003* in the presence or absence of S9.

These results indicate that GS-001* , GS-002* and GS-003* were negative in the Bacterial Reverse Mutation Assay when tested up to 5000 µg/plate in the presence and absence of S9.

8.6.2. BIC: 4-Week Rat Oral Gavage Qualification Toxicity and Toxicokinetic Study with BIC in Female Rats

This study evaluated the toxicity and determine the TK of BIC and served to qualify the potential impurities between different lots of BIC when administered once daily via oral gavage to female rats for 4 weeks (m2.6.7, Section 17.1.2, TX-141-2043). Female Crl:WI(Han) rats received BIC at 300 mg/kg/day, or GS-004* (lot containing spiked impurities) at 100 or 300 mg/kg/day at a dose volume of 5 mL/kg. Assessment of toxicity was based on mortality, clinical observations, body weights, body weight change, food consumption, and clinical and anatomic pathology. Blood samples from satellite animals were collected on Days 1 and 28 for TK evaluations.

No BIC or GS-004*-related mortality, clinical observations, effects on body weight or food consumption, or anatomic pathology findings was noted. Administration of BIC or GS-004* had no obvious or toxicologically important effects on clinical pathology test results. The only finding

considered potentially test article-related was mildly non-adverse higher (15%) inorganic phosphorus concentration, relative to controls, for animals administered 300 mg/kg/day BIC, with no correlative findings.

Exposure to BIC increased with the increase in GS-004* dose level from 100 to 300 mg/kg/day. The increases in C_{max} and AUC_{0-24} were less-than-dose proportional between 100 and 300 mg/kg/day GS-004* dose levels. No accumulation of BIC was observed after multiple doses of BIC or GS-004* in female rats. Plasma exposure profiles were similar between BIC and GS-004* at 300 mg/kg/day.

In conclusion, oral administration of BIC or GS-004* up to 300 mg/kg/day to female rats for 4 weeks was well-tolerated. The NOAEL for BIC or GS-004* was 300 mg/kg/day, corresponding to a mean Week 4 C_{max} of 205 µg/mL and AUC_{0-24} of 4440 µg•h/mL for BIC and a mean C_{max} of 201 µg/mL and AUC_{0-24} of 4190 µg•h/mL for GS-004*. Based on the results, potential BIC-related process impurities were considered qualified, as findings were similar to the comparator lot in this study and those observed in previous studies.

8.6.3. FTC: 1-Month Mouse Oral Qualification Study of FTC and TP-0296 (Degradant)

The toxicity of FTC in combination with 2% (by weight) TP-0296 (5-flourouracil (FTU); FTC degradant) when administered orally by gavage to mice for 28 days was evaluated in order to qualify the degradant, TP-0296 (m2.6.7, Section 17.2, TOX153). Four groups of mice (10/sex/group) were administered 0.5% aqueous methylcellulose vehicle or 50/1, 150/3, or 450/9 mg/kg/day of the combination of FTC/TP-0296, respectively. Each dose level represented the desired FTC dosage in combination with 2% (by weight) dosage of TP-0296. The standard toxicological endpoints were recorded at regular intervals during the study.

All mice survived to the scheduled necropsy. There were no observations related to treatment with the combination of FTC and TP-0296 at any dose level. Body weight, food consumption, hematology, serum biochemistry values, and organ weights were unaffected by administration of the test article combination at any dose level. There were no gross or microscopic findings attributed to the test article combination. The NOEL for oral administration of FTC in combination with TP-0296 was 450/9 mg/kg/day FTC/TP-0296, the highest dose combination tested.

8.6.4. FTC: A 1-Month Oral Qualification Study of FTC Produced by the Menthol Process in CD-1 Mice

The objective of the study was to evaluate the toxicity of FTC produced by the Menthol Process when administered orally by gavage to mice for a minimum of 28 days to qualify potential impurities and/or degradants in FTC produced by this process (m2.6.7, Section 17.2, TX-162-2001). The test article, FTC (GS-9019) in the vehicle, 0.5% aqueous methylcellulose, was administered orally by gavage once daily for a minimum of 28 consecutive days to 3 groups (Groups 2 to 4) of Crl:CD-1®(ICR) BR mice. Dosage levels were 50, 150, and 450 mg/kg/day of the FTC test article. A concurrent control group (Group 1) received the vehicle on a comparable regimen. The dose volume was 10 mL/kg for all groups. Each group consisted of 10 animals/sex.

All animals were observed twice daily for mortality and moribundity. Clinical examinations were performed daily and detailed physical examinations were performed weekly. Individual body weights and food consumption were recorded weekly. Clinical pathology evaluations (hematology or serum chemistry) were performed for all mice at the scheduled necropsy (study Week 4). Complete necropsies were conducted on all animals, and selected organs were weighed at the scheduled necropsies. Selected tissues were examined microscopically from all animals in the control and 450-mg/kg/day groups; gross lesions were examined microscopically for all animals.

All animals survived to the scheduled necropsy. There were no test article-related clinical observations. No test article-related effects on body weights, food consumption, hematology, serum chemistry parameters, and organ weights were noted. There were no test article-related macroscopic or microscopic findings at any dose level of the FTC test article.

Based on the results of this study, the NOEL for oral (gavage) administration of the FTC test article (produced by the Menthol chemistry method of synthesis) to mice for 28 consecutive days was 450 mg/kg/day, the highest dose tested.

8.6.5. FTC: Bacterial Reverse Mutation Assay of FTC to Qualify Degradant TP-0296

Emtricitabine in combination with 1% (by weight) TP-0296 (FTU, degradant), was tested in the Bacterial Reverse Mutation Assay to qualify degradant TP-0296 using *Salmonella typhimurium* tester strains TA98, TA 100, TA 1535, and TA 1537 and *Escherichia coli* tester strain WP2*uvr*A in the presence and absence of S9 metabolic activation (m2.6.7, Section 17.2, TOX151). The dose solutions were prepared separately in distilled water and both test articles were soluble at all concentrations used in the study. The assay was performed in 2 phases, using the plate incorporation method. The first phase, an initial toxicity-mutation assay, was used to establish the dose-range for the confirmatory mutagenicity assay and to provide a preliminary mutagenicity evaluation. The second phase, the confirmatory mutagenicity assay, was used to evaluate and confirm the mutagenic potential of the test article combination.

In the initial toxicity-mutation assay, dose levels of FTC tested were 1.5, 5.0, 15, 50, 150, 500, 1500, and 5000 µg/plate, and the highest dose of TP-0296 tested was 50 µg/plate. No toxicity and no positive mutagenic response were observed at any dose. Based on these findings, the maximum doses plated in the confirmatory mutagenicity assay were 5000 µg/plate for FTC and 50 µg/plate for TP-0296.

In the confirmatory mutagenicity assay, no positive mutagenic response was observed. The dose levels of FTC tested were 50, 150, 500, 1500, and 5000 µg/plate. The dose levels of TP-0296 tested were 0.50, 1.5, 5.0, 15, and 50 µg/plate. Neither precipitate nor toxicity was observed. The combination of FTC and 1% TP-0296 was concluded to be negative.

8.6.6. FTC: In Vitro Mammalian Chromosome Aberration Study of FTC to Qualify Degradant TP-0296

The chromosome aberration assay was used to evaluate the clastogenic potential of the combination of FTC and 1% TP-0296 (FTU, degradant) (m2.6.7, Section 17.2, TOX152). This GLP-compliant study of FTC was conducted to qualify TP-0296 using Chinese Hamster ovary (CHO) cells in the absence and presence of S9 metabolic activation. Both FTC and TP-0296 were soluble in water at all concentrations used in the study.

Based on the lack of cytotoxicity in a preliminary study, doses of FTC chosen for the chromosome aberration assay ranged from 313 to 5000 µg/mL for both the activated and nonmetabolically activated treatment groups. The cells were treated for 4 and 20 hours in the nonactivated test system and for 4 hours in the S9 activated test system. All cells were harvested 20 hours after treatment initiation. The percentage of cells with structural or numerical aberrations in the FTC/TP-0296 groups was not significantly increased above that of the solvent control at any dose level ($p > 0.056$, Fisher's exact test).

Based on the results of this study, it was concluded that FTC with 1% TP-0296 was negative for the induction of structural and numerical chromosome aberrations in CHO cells.

8.6.7. TAF: 2-Week Oral Toxicity Impurity Qualification Study in Rats with GS-7340-02

The purpose of this study was to evaluate the toxicity and to qualify potential impurities of TAF when administered daily via oral gavage to male rats for at least 14 days (m2.6.7, Section 17.3, TX-120-2008). Male Hsd:Sprague Dawley®™SD®™ rats were assigned to 5 groups (10 animals/toxicity group) and received either the vehicle [0.1% (v/v) Tween 20 and 0.1% (w/v) hydroxypropylmethylcellulose (Dow Chemical K100LV) prepared in reverse osmosis water] or GS-7340-02 at 5 or 50 mg/kg/day (10 mL/kg) from 2 different purity lots (Lot No. 1 - 97.7% and Lot No. 2 - 83.1%)] at a volume of 10 mL/kg. The impurities, including 13% GS-7339, were added to the more pure lot. Additional animals were assigned for toxicokinetic evaluation on Days 1 and 14.

Administration of 97.7% pure and 83.1% pure GS-7340-02 by oral gavage for 14 days to male rats was well tolerated at dose levels of 5 and 50 mg/kg/day. No test article-related findings were noted, and no differences were found between the 2 lots tested. The NOAEL for both lots is 50 mg kg/day (40 mg f.b.e./kg/day). In males administered 50 mg/kg/day on Day 14, the TFV C_{max} was 218 ng/mL and the AUC_{0-t} was 1965 ng·h/mL for 97.7% pure GS-7340-02 (Lot No. 1: [redacted]) and the TFV C_{max} was 395 ng/mL and the AUC_{0-t} was 2481 ng h/mL for 83.1% pure GS-7340-02 (Lot No. 2: [redacted]).

8.6.8. TAF: 4-Week Oral Toxicity Impurity Qualification Study in Rats with GS-7340-03

The purpose of this study was to evaluate the toxicity and to qualify potential impurities of TAF when administered daily via oral gavage to male and female rats (10/sex/toxicity group) for at least 28 days (m2.6.7, Section 17.3, TX-120-2021). Three lots of GS-7340-03 were each administered at 25 and 50 mg/kg/day (f.b.e). Test article 1 was 99.3% pure GS-7340-03. Test article 2 was 98% pure GS-7340-03 containing 11 spiked impurities. Test article 3 was 97.8% pure GS-7340-03 containing 4 spiked impurities. Vehicle control animals were administered the vehicle control article (0.1% [w/v] hydroxypropylmethylcellulose K100LV and 0.1% [v/v] Tween® 20 in 99.8% [v/v]) reverse osmosis water). Doses were administered via oral gavage once daily for 29 days at a dose volume of 10 mL/kg. Additional animals were assigned for toxicokinetic evaluation on Days 1 and 28.

Administration of GS-7340-03 drug substance with 3 different impurity profiles by oral gavage for 28 days to rats was well tolerated at dose levels of 25 and 50 mg/kg/day. There were no significant clinical or histopathological differences between the 3 lots tested. The NOAEL for all 3 lots is 50 mg f.b.e./kg/day.

8.6.9. FTC/TDF: Degraded Tablets

Two qualification studies to investigate the potential toxicity of nondegraded and degraded FTC/TDF tablets following daily oral administration to the rat for a minimum of 14 days have been conducted. The second qualification study was conducted to verify the qualification of cFTU1 (cyclic FTU; GS-9237) and cFTU2 (GS-492127) due to the development of a new analytical assay for these two degradants.

8.6.9.1. FTC/TDF: 14-Day Oral Gavage Toxicity Study Comparing Non-Degraded and Degraded FTC/TDF in Sprague-Dawley Rats

The purpose of this study was to investigate the potential toxicity of non-degraded and degraded FTC/TDF following daily oral administration to the rat for a minimum of 14 days (m2.6.7, Section 17.4, TX-164-2001). Animals (10 males/group) were administered doses of 20/30, 67/100, or 200/300 mg/kg/day of non-degraded or degraded FTC/TDF in suspension vehicle at a dose volume of 10 mL/kg. The following parameters were measured during the study: mortality, clinical signs, detailed physical examination, body weights, food consumption, laboratory investigations (hematology, biochemistry, and urinalysis), gross pathology, organ weight analysis, and histopathology.

In this study there were no deaths related to treatment with FTC/TDF (degraded or nondegraded). Clinical signs were limited to dose-related postdosing salivation. There were no effects on body weight or food consumption considered to be related to treatment with FTC/TDF (non-degraded and degraded). There were no toxicologically significant treatment-related changes in hematology, biochemistry, or urine parameters. Organ weight evaluation revealed marginal increases in the weights of adrenal glands (noted in all treated groups but those receiving degraded FTC/TDF at the lowest dose). No gross or histological changes were identified that might account for this increased adrenal weight. No treatment-related gross changes were

observed at necropsy. Microscopic evaluation revealed hyperplasia of the anterior duodenal mucosa overlying Brunner's glands. It was seen in 7/10 animals treated at the high-dose level with nondegraded FTC/TDF and in 2/10 animals receiving degraded FTC/TDF and was considered to be treatment-related.

Daily oral administration of non-degraded and degraded FTC/TDF at dose levels of 20/30, 67/100, and 200/300 mg/kg/day for 14 consecutive days to male Sprague-Dawley rats was well tolerated. There were no toxicologically significant differences between groups treated with non-degraded and degraded FTC/TDF. A minor increase in adrenal weights at necropsy (all dose levels, except those receiving degraded FTC/TDF at the lowest dose) and a hyperplasia of the anterior duodenal mucosa overlying Brunner's glands was seen in animals treated at the high-dose level with non-degraded and degraded FTC/TDF. In conclusion, the NOAEL in this study was considered to be 67/100 mg/kg/day FTC/TDF.

8.6.9.2. FTC/TDF: 14-Day Study of Non-degraded and Degraded FTC/TDF by Oral Gavage in Rats

The objective of this study was to determine the potential toxicity of degraded FTC/TDF when compared to non-degraded material administered by daily oral gavage for 14 days to rats. In addition, the toxicokinetic characteristics of non-degraded and degraded FTC/TDF were compared (m2.6.7, Section 17.4, TX-164-2005).

Male Sprague-Dawley rats were assigned to seven toxicity groups (10 animals/group) and received dose preparations containing the vehicle control article [Suspension vehicle (carboxymethylcellulose, 0.5% (w/v), benzyl alcohol, 0.9% (w/v), polysorbate 20, 0.5% (w/v) and sterile water for injection, USP] or non-degraded or degraded FTC/TDF at 20/30, 67/100 or 200/300 mg/kg/day.

Assessment of toxicity was based on clinical signs, body weights, body weight changes, food consumption, clinical pathology parameters (hematology, coagulation, clinical chemistry, and urinalysis), toxicokinetic parameters, gross necropsy findings, organ weights, and histopathologic examinations. Blood samples were collected from satellite animals for toxicokinetic evaluations of non-degraded and degraded TDF/FTC on Days 1 and 14.

There were no preterminal deaths, or non-degraded or degraded TDF/FTC-related clinical observations or changes in body weights, food consumption, coagulation or urinalysis parameters. There were no gross necropsy findings or changes in organ weights.

Administration of non-degraded or degraded FTC/TDF at 200/300 mg/kg/day was associated with minimal, non-adverse, decreases in red cell mass parameters (Hb and HCT). These changes were associated with minimal, non-adverse, red cell indices (MCV, MCH and RDW) changes indicating microcytosis. Findings observed in animals administered non-degraded FTC/TDF or degraded FTC/TDF regarding red cell mass parameters or indices were considered to be of comparable magnitude and had no microscopic correlates. Non-adverse, minimal, dose level-dependent increases in ALT activity were observed in rats administered non-degraded FTC/TDF at ≥ 67/100 mg/kg/day and degraded FTC/TDF at 200/300 mg/kg/day. Increased ALT activity was not associated with histological findings.

In the duodenum, there was minimal cryptal epithelium hyperplasia and minimal single cell necrosis with both degraded and non-degraded formulations of FTC/TDF at 200/300 mg/kg/day.

In conclusion, administration of non-degraded or degraded FTC/TDF by once daily oral gavage was well tolerated in rats at levels of 20/30, 67/100 and 200/300 mg/kg/day. Microscopically, minimal cryptal epithelium hyperplasia and minimal single cell necrosis were observed in the duodenum of animals administered both FTC/TDF formulations at 200/300 mg/kg/day. Based on these minor findings, the NOAEL was considered to be 200/300 mg/kg/day for both non-degraded and degraded TDF/FTC. No differences in toxicity were observed between non-degraded and degraded material. At all dose levels tested, exposure of FTC and TFV was similar for both non-degraded and degraded material (Day 14 FTC AUC_{0-24} 163.3 and 132.6 µg h/mL; TFV AUC_{0-24} 13.6 and 13.7 µg·h/mL, for non-degraded and degraded materials, respectively).

8.7. Qualification of Impurities

8.7.1. BIC

The impurity profiles for batches of BIC active pharmaceutical ingredient (API) or drug product used in nonclinical toxicology studies are provided in m2.6.7, Section 4.1. A 4-week repeat dose oral toxicity study was conducted in rats to qualify impurities and potential impurities in the Good Manufacturing Practice (GMP) material (m2.6.7, Section 17.1.2, TX-141-2043). The impurities spiked into the drug substance did not alter the NOAEL in rats (300 mg/kg/day). Three potential impurities, GS-001* , GS-002* and GS-003* , were qualified in genotoxicity studies.

Based on their impurity profiles, the multiple GLP batches of BIC tested in the toxicology program are considered, in composite, to be representative of the GMP material and support the specified limits of impurities and degradation products proposed for commercial production (m3.2.P.5.5, Characterization of Impurities [B/F/TAF Tablets] and m3.2.P.5.6, Justification of Specifications [B/F/TAF Tablets]).

8.7.2. FTC

The impurity profiles for batches of API or drug product used in nonclinical toxicology studies are provided in m2.6.7, Section 4.2.

Two 4-week oral toxicity studies have been conducted in mice to qualify impurities and degradants in the GMP material (m2.6.7, Section 17.2, TOX153 and TX-162-2001). Further, the FTC degradant, FTU, has also been qualified in genotoxicity studies (m2.6.7, Section 17.2, TOX151 and TOX152).

Based on their impurity profiles, the multiple GLP batches of FTC tested in the toxicology program are considered, in composite, to be representative of the GMP material and support the specified limits of impurities and degradation products proposed for commercial production (m3.2.P.5.5, Characterization of Impurities [B/F/TAF Tablets] and m3.2.P.5.6, Justification of Specifications [B/F/TAF Tablets]).

* 新薬承認情報提供時に置き換え

8.7.3. TAF

The impurity profiles for batches of API or drug product used in nonclinical toxicology studies are provided in m2.6.7, Section 4.3.

Fourteen-day and 28-day oral toxicity studies were conducted in rats to qualify impurities and potential impurities in the GMP material (m2.6.7, Section 17.3, TX-120-2008, and m2.6.7, Section 17.3, TX-120-2021). The impurities spiked into the drug substance did not alter the NOAEL in rats (40 and 50 mg free base equivalents/kg/day).

Based on their impurity profiles, the multiple GLP batches of TAF tested in the toxicology program are considered, in composite, to be representative of the GMP material and support the specified limits of impurities and degradation products proposed for commercial production (m3.2.P.5.5, Characterization of Impurities [B/F/TAF Tablets] and m3.2.P.5.6, Justification of Specifications [B/F/TAF Tablets]).

8.7.4. Qualification of Degradation Products in B/F/TAF Tablets

The degradation products of BIC, FTC or TAF observed in the B/F/TAF tablets are consistent with those in the BIC, FTC or TAF drug substance, respectively. There are no unique impurities or degradants in the B/F/TAF tablets.

The impurities and degradation products present in BIC, FTC or TAF drug substance and in B/F/TAF tablets have been qualified through toxicology studies, which employed drug substance from normal productions batches, laboratory scale batches with enhanced levels of impurities, or samples subjected to forced degradation conditions (high heat and humidity) (m3.2.P.5.5, Characterization of Impurities [B/F/TAF Tablets] and m3.2.P.5.6, Justification of Specifications [B/F/TAF Tablets]).

8.8. Other Studies

8.8.1. 2-Week Oral Gavage Investigative Toxicity Study with BIC in Male Wistar Han Rats

An investigative study (m2.6.7, Section 17.1.2, TX-141-2033) was conducted to evaluate preliminary histopathology findings (vacuolation) in the brain and spinal cords of a subset of animals in the 2-week toxicity study (m2.6.7; Section 7.1.2, TX-141-2029). However, upon further review of laboratory records and additional slides from the study, the vacuolar changes were confirmed to be a processing artifact (laboratory error) and not related to BIC administration. The investigative study used additional fixation methods for tissue preservation, and confirmed the absence of treatment-related central nervous system changes in rats.

Male Crl:WI(Han) rats were assigned to two toxicity groups dosed once daily for two weeks via oral gavage with vehicle control article (13 males) or BIC at 300 mg/kg/day (15 males) at a dose volume of 5 mL/kg. Brain and spinal cord were harvested at necropsy from 10 rats/group and fixed routinely by immersion in excess 10% neutral buffered formalin. The remaining rats were exsanguinated under deep anesthesia and perfused with Karnovsky’s fixative prior to harvesting

brain and spinal cord for post-fixation by immersion in additional Karnovsky's fixative. All specimens were processed identically for routine microscopic examination. Blood samples were collected from animals on Day 14 at predose and 4 hours postdose to confirm exposure. Assessment of toxicity was based on mortality, clinical observations, body weight, food consumption, macroscopic (all tissues) and microscopic (brain and spinal cord only) examination.

All animals survived to their scheduled sacrifice on Day 15. There were no BIC-related effects on clinical signs, body weights, body weight change, or food consumption. No BIC related macroscopic or microscopic changes in brain or spinal cord were observed. Mean BIC exposure levels in males administered 300 mg/kg/day (119 µg/mL; 4 hours postdose) were similar to C_{max} levels in the 2-week toxicity study (117 µg/mL; m2.6.7, Section 7.1.2, TX-141-2029) on Day 14. In conclusion, there were no adverse effects in male rats administered 300 mg/kg/day BIC for 2 weeks.

8.8.2. Mutagenicity Test with TFV in the *Salmonella-Escherichia Coli*/ Mammalian-Microsome Reverse Mutation Assay

Tenofovir was tested at 4610, 3070, 922, 615, 307, and 92.2 µg per plate in both the presence and absence of S9 mix on Salmonella typhimurium tester strains TA98, TA100, TA1535, TA1537, and *Escherichia coli* tester strain WP2uvrA (m2.6.7, Section 17.3, 95-TOX-1278-006). The doses tested in an additional trial were 5000, 3330, 1000, 667, 333, and 100 µg per plate with tester strains TAl00 and TA1535 only in the presence of S9 mix.

Tenofovir caused positive (6.2- and 6.8-fold) increases in the mean number of revertants per plate with tester strain TA1535 only in the presence of S9 mix. No other increase in the mean number of revertants per plate was observed with any of the remaining tester strain/activation condition combinations either in the presence or absence of S9.

8.8.3. Mutagenicity Test on TFV in the L5178Y $TK^{+/-}$ Mouse Lymphoma Forward Mutation Assay

The ability of TFV to induce forward mutations at the TK locus in the mouse lymphoma L5178Y cell line was evaluated (m2.6.7, Section 17.3, 95-TOX-1278-007). Tenofovir was soluble in water at 50.0 mg/mL.

In the preliminary assay, the test article was shown to be nontoxic with and without S9 metabolic activation at concentrations of 9.77 to 5000 µg/mL. In the main assay, TFV was negative at concentrations of 156 to 5000 µg/mL without metabolic activation, and in the presence of S9 metabolic activation the 5000 µg/mL treatment induced a small (approximately 2.5-fold) increase in the mutant frequency that exceeded the minimum criterion. Since only a single small increase was observed, TFV was considered equivocal with activation in this trial. The test article, TFV, was therefore considered negative without activation and equivocal with activation at the TK locus in L5178Y mouse lymphoma cells under the conditions used in this study.

9. DISCUSSION AND CONCLUSIONS

Comprehensive programs of nonclinical studies with BIC, FTC, TAF and TDF have been conducted. The nonclinical data indicates administration of the BIC/F/TAF FDC is unlikely to exacerbate known toxicities of the individual agents. Exposure margin values provided below describe the calculation in which the plasma AUC values in nonclinical species are divided by human plasma AUC values following administration of B/F/TAF FDC (50/200/25 mg). For BIC (50 mg), the human plasma AUC value (AUC_{tau} = 102 ug·h/mL) in the FDC was based upon exposure in HIV-1 infected subjects in clinical studies (GS-US-380-1489, GS-US-380-1490, GS-US-380-1844, GS-US-380-1878).

9.1. Target Organ Toxicity

9.1.1. BIC

The oral toxicity of BIC has been studied in transgenic mice, rats, and monkeys for treatment periods up to 39 weeks. In a 39 week repeat dose chronic toxicology study in monkeys, hepatobiliary toxicity at the high dose of 1000 mg/kg/day was the only notable adverse finding. No target organs were identified following repeat oral administration of 1000 mg/kg/day BIC in cynomolgus monkeys for up to 13 weeks. No adverse findings were noted in transgenic mouse or rat repeat dose toxicology studies up to 26 weeks dosing duration with BIC plasma exposure at the NOAELs of ≥18-fold the BIC plasma exposure at the clinical dose of 50 mg in the B/F/TAF FDC. Bictegravir demonstrated no carcinogenic potential in a 6-month transgenic mouse study at an exposure margin of ≥15-fold.

9.1.1.1. Hepatobiliary system

The only significant effect identified in chronic animal studies with BIC was hepatobiliary toxicity in monkeys following 39 weeks of administration at a dose (1000 mg/kg/day) which produced plasma exposures that were 16-fold higher than those observed in patients at a 50 mg BIC dose in the B/F/TAF FDC. The effects were partially reversible after a four week period of non-exposure (recovery). No toxicity was observed at the mid-dose of 200 mg/kg/day (NOEL) which provided a margin of exposure of at least 7.0-fold.

At 39 weeks, livers from all high dose animals showed evidence of bile duct hyperplasia (Table 6). The most severely affected animals (one male in the terminal sacrifice and one male in the recovery group) also showed evidence of slight to moderate hepatocyte hypertrophy and minimal regenerative hyperplasia. A single female with slight biliary hyperplasia also exhibited minimal hepatocyte hypertrophy. Clinical pathology changes at this dose level were confined to elevations in ALT and GGT but a clear association with all individual histologic findings was not obvious in most animals. For example, ALT increases (≥2-fold) were consistently elevated in 4 out of 9 males throughout their dosing period. Of these, only 2 showed histologic evidence of injury at necropsy. In female animals, ALT was not elevated at 4 or 13 weeks in any of the 9 animals evaluated at that time, and was only elevated in 2 of 6 animals at 26 and 39 weeks. In those two animals, only one showed evidence of histologic injury at necropsy. GGT was only elevated in two animals (one male, one female). Additionally, there were animals with evidence of hepatic injury histologically for which there was no elevation in GGT and no (or no consistent) elevation in ALT. Mean bilirubin concentrations were not affected.

After a 4-week recovery period, hepatobiliary toxicity was partially reversible; findings were noted in one of two males and one of two females, while the other two animals in the 1000 mg/kg/day recovery group had no histologic evidence of hepatobiliary toxicity. Neither ALT nor GGT activity were increased following the 4 week recovery period in any animal. There were no other adverse findings in this chronic study in cynomolgous monkeys with BIC. The NOEL after 39-weeks administration was 200 mg/kg/day, corresponding to a BIC exposure margin of at least 7.0-fold.

9.1.2. FTC

The only significant effect of FTC identified at dose levels constituting large clinical multiples was a minor anemia.

9.1.3. TAF

The oral toxicity of TAF has been studied in mice, rats, dogs, and monkeys for treatment periods up to 39 weeks. In chronic studies, kidneys (karyomegaly, tubular degeneration), and bone (atrophy of metaphyseal cancellous bone) were the primary target organs. TAF also appeared to increase biochemical markers of bone turnover and decrease serum 1, 25-dihydroxy vitamin D_3 and 25-hydroxyvitamin D_3 at doses in rats and dogs.

9.1.3.1. Kidney

Renal tubular karyomegaly was observed in rats and dogs orally administered TAF. Focal areas of minimal renal cortical tubular basophilia and associated minimal nuclear karyomegaly were present in rats administered 400 mg/kg/day for 4 weeks and 100 mg/kg/day for 26 weeks. Renal tubular karyomegaly and/or basophilia were observed in dogs administered 3 and 10 mg/kg/day for 4 weeks and dogs administered 6 or 18/12 mg/kg/day for at least 13 weeks.

Renal cortical tubular degeneration/regeneration findings were limited to animals administered 6 or 18/12 mg/kg/day for at least 13 weeks in the 39-week dog toxicity study. Similar findings of renal cortical tubular degeneration/regeneration and karyomegaly were present in dogs administered either 6 or 18/12 mg/kg/day for 39 weeks. These changes were minimal to slight in affected males and females at 6 mg/kg/day. In high-dose males (18/12 mg/kg/day) the severity ranged from mild to moderate. Similar lesions (karyomegaly and tubular degeneration) but of only minimal severity were also present in 2 males administered 2 mg/kg/day of TAF for 39 weeks. After a 13-week recovery period, treatment-related histology changes were still observed in the kidney but were of reduced incidence and severity.

9.1.3.2. Bone

Atrophy of metaphyseal cancellous bone was observed in rats administered TAF at 100 mg/kg/day for 26 weeks. TAF also increased biochemical markers of bone turnover and decrease serum 1, 25 dihydroxy vitamin D_3 and 25-hydroxyvitamin D_3 in rats ($\geq$ 25 mg/kg/day) and dogs ($\geq$ 37.5 mg/kg/day for 6 days). In the 39-week dog study, bone mineral density changes at 18/12 mg/kg/day may have been secondary to body weight loss but these changes were accompanied by a slight but significant decrease in serum 1, 25 dihydroxy vitamin D_3 in males only and a significant increase in 25-hydroxyvitamin D_3 in females only.

9.1.3.3. Other

TAF administered by oral gavage for up to 13 weeks to mice at ≥ 10 mg/kg/day resulted in adverse degenerative (olfactory) and acute inflammatory (infiltrate neutrophil) changes in the nasal mucosa. Because these changes were not observed in rats, dogs or monkeys for longer durations of administration, the relevance to humans is unknown and the risk of nasal inflammation in humans is very low.

TAF had no discernible electrocardiograph effect at the low dose of 2 mg/kg/day. There was some evidence at 6 and 18/12 mg/kg/day for an effect to slightly prolong PR intervals (~13-24%), which was associated with significant decreases in T3 {Kienle 1994, Tribulova 2010}. After the 13-week recovery period, serum T3 values returned to levels similar to the control group animals at the end of the study.

At 18/12 mg/kg/day in dogs, the highest dose tested, a minimal infiltration of histiocytes was present in some organs (eye (choroid plexus, ciliary body), lung, and spleen) in some animals. In-life ophthalmologic examinations were normal. The histiocytic infiltration observed in multiple organs was most likely an indirect drug effect due to general debilitation and was not observed in other repeat-dose toxicity studies. There were no drug-related effects on ophthalmic exams or microscopic exams of ocular tissue observed in repeat-dose toxicity studies in mice (up to 13 weeks), rats (up to 26 weeks), and nonhuman primates (4 weeks) or in the 4-week dog toxicology study. The minimal to slight infiltration of mononuclear cells in the ocular posterior uvea occurred only in dogs administered the highest dose of TAF where compound-related toxicities occurred on clinical condition, weight gain, serum chemistry, BMD, and other organ histopathology. Distribution of ^{14}C-TAF to eyes has been assessed in mice, rats, and dogs. Melanin binding has specifically been assessed by comparing distribution in pigmented and non-pigmented mice (C57 black and CD-1, respectively) and rats (Long Evans and Sprague-Dawley, respectively). [^{14}C]-TAF-related radioactivity distributed poorly to the eyes of rats and dogs (C_{max} in eyes <8% that observed in plasma). Transient exposure to low levels of [^{14}C]-TAF-related radioactivity was observed in the eyes of rats decreasing to undetectable levels at 8 hours postdose. No difference in distribution was observed between Sprague-Dawley and Long Evans rats, including in the skin and eyes, suggesting no binding to melanin. The distribution of [^{14}C]-TAF to eyes in mice was higher than other species studied (C_{max} in eyes 15%-20% that observed in plasma). More persistent exposures in eye lens, eye uveal tract, and eyes were observed in C57 black mice compared to CD-1 mice. However, no difference in distribution between pigmented and nonpigmented skin was observed illustrating that [^{14}C]-TAF-related radioactivity was not selectively associated with melanin-containing tissues. The minimal infiltration of histiocytes observed in the eye of dogs administered the highest dose of TAF and does not correlate with the tissue distribution where TAF was found to poorly penetrate across the blood brain and blood retinal barrier in dogs. Because TAF has poor penetration across the blood brain and blood retinal barrier in dogs, it is unlikely that TAF directly caused the observed histiocytic infiltration in the posterior uvea. Based on the evidence from tissue distribution and toxicology studies, Gilead concludes that the risk of posterior uveitis in humans is very low.

TAF is unlikely to cause mitochondrial toxicity. TAF did not affect the amount of mitochondrial DNA levels in a 10-day assay at the highest tested concentrations of 1 μM TAF in HepG2 cells or 3.33 μM in MT-2 and Jurkat cells (≥ 2-fold higher than C_{max} after a 25 mg TAF dose). The active metabolite of TAF, tenofovir diphosphate is highly discriminated as a substrate by mitochondrial DNA polymerase γ relative to the natural substrate, adenosine triphosphate (ATP; > 10,000 fold) {Johnson 2001}. No toxicity indicative of mitochondrial toxicity was observed in nonclinical or clinical studies. Therefore, TAF is unlikely to inhibit mitochondrial DNA polymerase γ under clinically relevant conditions.

9.1.4. B/F/TAF

The toxicity profiles of BIC, FTC and TAF differ substantially with no clinically significant overlapping toxicity. Administration of BIC, FTC, and TAF in combination is unlikely to exacerbate known toxicities of the individual agents. Bictegravir, FTC, and TAF exhibit different patterns of target organ toxicity. Toxicity was noted in the hepatobiliary system of monkeys administered BIC following daily oral administration at 1000 mg/kg/day for 39 weeks at a 16-fold exposure margin. No target organs of toxicity were observed in rats following 26 weeks of daily oral administration of BIC; exposure margins at the highest dose administered are 18/46-fold (males/females). The only toxicities observed in chronic animal studies with FTC were mild, reversible anemia in mice and minor decreases in erythrocyte counts/increases in MCH in monkeys at large multiples of clinical exposure that are not considered clinically relevant. In chronic studies with TAF, kidneys (karyomegaly, tubular degeneration) and bone (atrophy of metaphyseal cancellous bone) were the primary target organs.

Comprehensive nonclinical safety databases on these individual drugs, including combination toxicity studies with FTC and TDF, strongly indicate that further toxicological investigations are unlikely to yield new data relevant to humans. Hepatobiliary toxicity has not been shown for FTC or TAF, and changes in the bone or kidney have not been observed with BIC or FTC. Based on the well-characterized single agent toxicity profiles of BIC, FTC and TAF, the low potential for overlapping toxicities, and clinical safety data available for B/F/TAF and other FTC- and/or TDF-/TAF- containing regimens, additional combination toxicology studies are considered unwarranted.

9.2. Genetic Toxicology

9.2.1. BIC

BIC is not genotoxic. There was no evidence of mutagenic potential in in vitro genotoxicity tests (Ames test and human lymphocyte assay) or in an in vivo evaluation that consisted of a 2-week repeat dose rat bone marrow micronucleus study.

9.2.2. FTC

Emtricitabine was negative in the standard genotoxicity battery. There was no evidence of exacerbation of mutagenic potential in in vitro genotoxicity tests (Ames test and mouse lymphoma assay) with the combination of FTC and TDF as compared to the agents alone.

9.2.3. TAF

Tenofovir alafenamide was not genotoxic in a battery of in vitro and in vivo assays. The in vitro assays included gene mutation assays with bacterial strains (*Salmonella typhimurium*, *Escherichia coli*), and a L5178Y gene mutation assay in mouse lymphoma cells. The in vivo evaluation consisted of a mouse bone marrow micronucleus study (at oral doses of 500, 1000, and 2000 mg/kg).

9.2.4. B/F/TAF

Bictegravir, FTC, and TAF were negative in genotoxicity studies. Therefore, the combination of the 3 components is not expected to have an altered genotoxicity profile as compared with that of the individual agents.

9.3. Carcinogenicity

9.3.1. BIC

Bictegravir demonstrated no carcinogenic potential in a 6-month transgenic mouse study at exposure margins that exceeded 15-fold. A 2-year carcinogenicity study with BIC in Wistar-Han rats is ongoing (the first dose was administered 21 May 2015).

9.3.2. FTC

Emtricitabine demonstrated low carcinogenic potential in conventional 2-year carcinogenicity studies in mice and rats at exposures that far exceeded (25-fold) human exposures at the therapeutic dose.

9.3.3. TAF

No carcinogenicity studies were conducted with TAF. Long-term oral carcinogenicity studies of TDF in mice and rats were carried out at TFV exposures up to approximately 16 times (mice) and 5 times (rats) those observed in humans at the therapeutic dose for HIV-1 infection. At the high dose in mice, liver adenomas (females only) and duodenal adenoma/adenocarcinomas (both sexes) were increased at TFV exposures 151 times those observed in humans. In rats, the study was negative for carcinogenic findings at TFV exposures up to 51 times those observed in humans at the therapeutic dose.

9.3.4. B/F/TAF

FTC and TDF have demonstrated low carcinogenic potential in conventional 2-year bioassays. Bictegravir demonstrated no carcinogenic potential in a 6-month transgenic mouse study and the 2-year rat bioassay is ongoing. Given the data available, it is considered unlikely that combination dosing would change these profiles, and no exacerbation of toxicity/genotoxicity is expected. Based on the overall lack of genotoxicity and the available carcinogenicity study data with the individual agents, the conduct of carcinogenicity studies with the B/F/TAF combination is not considered necessary nor would they add to the safety evaluation.

9.4. Reproductive Toxicity

9.4.1. BIC

There were no remarkable effects on reproductive performance or embryonic viability in the rat fertility study and the NOEL was the high dose of 300 mg/kg/day. At the NOEL, BIC exposures in the rat were approximately 29-fold. In the rat embryo-fetal development studies, there were no effects on embryofetal development and the high dose of 300 mg/kg/day was the NOEL with exposures corresponding to a 36-fold exposure margin. In rabbits, there were no effects on embryofetal development in the absence of maternal toxicity. Embryo-fetal effects (abortion, decrease fetal weights) were consistent with the findings of several published studies in rabbits and secondary to maternal toxicity {Cappon 2005, Danielsson 2013}. At the 300 mg/kg/day NOEL, the margin of exposure is 0.59-fold. In the pre- and postnatal development study in rats, there were no BIC-related effects at any dosage level, and the high dose of 300 mg/kg/day was the NOEL for maternal systemic toxicity, F_1 neonatal/developmental toxicity, F_1 parental systemic toxicity, F_1 reproductive toxicity, and F_2 neonatal/early postnatal toxicity of BIC. At the NOEL, maternal and pup BIC exposures were approximately 30-fold and 11-fold, respectively.

9.4.2. FTC

There were no effects on fertility or embryo-fetal development when FTC was tested. Slightly longer oestrous cycles were observed in F_1 generation rats after exposure to high doses of FTC. No other significant effects were noted and NOELs were at exposures above human exposures.

9.4.3. TAF

Reproductive tissues were examined in repeat-dose toxicology studies in the rat, dog, and monkey. There were no treatment-related histologic alterations or changes in organ weights in the rat and the dog following chronic daily dosing, or in the monkey following 28 days of daily oral administration.

The TAF fumarate (GS-7340-03) oral rat fertility study data indicate dose related decreases in body weight gain in males and females, but no drug related changes occurred in male or female fertility endpoints including mating index, fertility index, sperm motility, sperm concentration, number of corpora lutea, implantation sites, early and late resorptions and live fetuses at doses up to 160 mg free base equivalents/kg/day. The TAF NOAEL for reproductive and early embryonic toxicity was 160 mg/kg/day.

There was no effect on fetal viability or fetal development in pregnant rats administered doses of GS-7340-02 up to 250 mg/kg/day, or in pregnant rabbits administered GS-7340-02 up to 100 mg/kg/day. The highest doses were maternally toxic. In the rat, decreased fetal body weight associated with some minor transitory delays in the rate of ossification was observed at 250 mg/kg/day, a maternally toxic dose. At the NOAEL for embryo-fetal development of approximately 100 mg/kg/day in rats, $AUC_{0-\tau}$ values for TAF and TFV on Day 17 were 0.2 and 17.4 μg·h/kg, respectively. At the NOAEL for embryo-fetal development of 100 mg/kg/day in rabbits, $AUC_{0-\tau}$ values for TAF and TFV on Day 20 were 11.0 and 27.3 μg h/kg, respectively.

Tenofovir disoproxil fumarate, but not TAF, has been tested in a peri/postnatal study. A perinatal-postnatal study of TDF showed increased pup mortality at maternally toxic doses ≥ 450 mg/kg/day. No TDF-related deaths occurred. The maternal NOEL of TDF was 50 mg/kg/day. The 150, 450, and 600 mg/kg/day dosages caused adverse clinical observations, reductions in body weight gain during gestation and increases in body weight gain during lactation and the 450 and 600 mg/kg/day dosages reduced gestation and lactation feed consumption values. The developmental NOEL was 150 mg/kg/day. The 450- and 600-mg/kg/day dosages resulted in increased peri/postpartum pup mortality, reduced pup survival and reduced pup body weights. The NOEL for general toxicity of TDF in the F_1 generation was 50 mg/kg/day (mortality occurred or body weights and/or feed consumption were reduced in the 150-, 450-, and 600-mg/kg/day dosage groups). The F_1 generation male and female NOEL for behavioral, reproductive, and developmental toxicity of TDF was 150 mg/kg/day (effects on sexual maturation were observed at dosages of 450 and/or 600 mg/kg/day).

9.4.4. B/F/TAF

Bictegravir, FTC, TDF/TAF have not shown significant adverse effects in reproductive and developmental toxicity studies. The combination of BIC, FTC, and TAF is not expected to have an altered reproductive toxicity profile compared with that of the individual agents. For BIC, there were no effects on reproductive and developmental parameters in the absence of maternal toxicity. FTC has not shown any adverse effects in reproductive and developmental toxicity studies. Slightly longer estrous cycles were observed in F_1 generation rats after exposure to high doses of FTC. For TDF, a delay in sexual maturation was observed in F_1 generation rats after exposure to high (maternally toxic) doses. Notwithstanding the low exposures in the rabbit developmental toxicity study, the NOELs/NOAELs for the three single agents were at exposures well above human exposures. No additive effects are anticipated with the three-drug combination.

9.5. Local Tolerance

The B/F/TAF tablet is intended for oral use. No local tolerance studies were conducted for the B/F/TAF combination. Evaluation of local tolerance to the GI tract has been conducted during the repeat-dose oral toxicity studies with each of the individual agents. No GI toxicity was observed in the BIC, FTC or TAF toxicity studies. For TDF, GI toxicity is dose limiting in rodents and was due to high local concentrations. This TDF-related toxicity is not considered relevant for humans.

BIC was predicted to be non-phototoxic, noncorrosive and not irritating to skin, and a moderate eye irritant. No local tolerance studies have been conducted with FTC. TAF fumarate was predicted to be a noncorrosive/nonsevere eye irritant and nonirritating/noncorrosive to rabbit skin under semi-occluded conditions. BIC or TAF fumarate did not show the potential to cause skin sensitization.

9.6. Other Toxicity Studies

9.6.1. BIC

There are no major human metabolites of BIC that require independent study.

9.6.2. FTC

There are no major human metabolites of FTC that require independent study. The identified metabolites have been assessed as part of the routine toxicology and qualification (of impurities) studies.

9.6.3. TAF

Investigative toxicology studies were conducted in rats to examine the potential bone toxicity to TAF. The studies support the conclusion that TAF is not a direct bone toxin and that the bone loss observed may be secondary to treatment related perturbations in phosphate homeostasis.

No specific studies with TAF metabolites were conducted as there were no unique human metabolites in humans with TAF. The by-products formed during conversion of TAF to TFV are isopropanol, l-alanine, and phenol and are formed at nontoxic levels.

Two 1-month impurity qualification studies were conducted in rats to evaluate potential drug substance impurities. In the first study, administration of 97.7% pure and 83.1% pure GS-7340-02 by oral gavage for 14 days to male rats was well tolerated at dose levels of 5 and 50 mg/kg/day. No test article related findings were noted, and no differences were found in the 2 lots tested. The NOAEL was considered 50 mg/kg/day (40 mg f.b.e./kg/day). In the second study, administration of 98% pure GS-7340-03 containing 11 spiked impurities and 97.8% pure GS-7340-03 containing 4 spiked impurities by oral gavage for 29 days to male and female rats resulted in no significant clinical or histopathological differences compared to administration of 99.3% pure GS-7340-03. The NOAEL was 50 mg f.b.e./kg/day.

9.6.4. B/F/TAF

There are no new impurities or new degradants that require further qualification in the B/F/TAF tablet.

9.7. Comparative Exposures and Margins

Cross-species comparisons of exposure, relative margins, and the relevance of these effects for human health are discussed in m2.4, Section 4.

9.8. Conclusions

Comprehensive nonclinical toxicology programs were conducted for BIC, FTC or TAF. Bictegravir, FTC, and TAF exhibit different patterns of target organ toxicity with no clinically-significant overlapping toxicity. The data are adequate to support the safety of the B/F/TAF FDC based on the following considerations.

Administration of the combination of BIC, FTC, and TAF is unlikely to exacerbate the toxicities of the individual agents. The only target organ for BIC in monkeys is the hepatobiliary system, at approximately 16-fold exposure margins; the NOEL is at a 7.0-fold margin. The only toxicity observed in chronic animal studies with FTC was mild, reversible anemia at large multiples of clinical exposure. The primary target organs for TAF are the kidney and bone.

Both FTC and TAF have shown a low potential for mitochondrial toxicity in long term toxicity studies and there was no evidence of toxicity to mitochondria in vitro and in vivo. INSTIs have not been associated with mitochondrial toxicity. No toxicity indicative of mitochondrial toxicity was observed in nonclinical or clinical studies with BIC, FTC, or TAF. Therefore, the potential for mitochondrial toxicity with B/F/TAF FDC is low.

Bictegravir, FTC and TAF are not genotoxic. The B/F/TAF combination is not anticipated to alter the genotoxicity profile of the individual agents. BIC demonstrated no carcinogenic potential in a 6 month transgenic mouse bioassay, and FTC and TDF have all demonstrated low carcinogenic potential in conventional 2-year bioassays. The 2-year rat carcinogenicity study with BIC is currently ongoing. Combination dosing with the individual agents is unlikely to alter this profile.

Bictegravir, FTC, or TAF have not shown significant adverse effects in reproductive and developmental toxicity studies, and the combination of BIC, FTC and TAF is not expected to have an altered reproductive toxicity profile compared with that of the individual agents. For BIC, there were no effects on reproductive and developmental parameters in rabbits in the absence of maternal toxicity. There were no effects on reproduction or developmental effects with BIC in rats at exposure margins of at least 36-fold. FTC has not shown any adverse effects in reproductive and developmental toxicity studies. Slightly longer estrous cycles were observed in F_1 generation rats after exposure to high doses of FTC. For TDF, a delay in sexual maturation was observed in F_1 generation rats after exposure to high (maternally toxic) doses. Neither TDF nor TAF showed an effect on rabbit fetuses. No additive effects are anticipated with the three-drug combination.

The B/F/TAF combination is not anticipated to produce any new human metabolites. Identified impurities and degradants have been assessed as part of the routine toxicology or qualification studies with the individual agents.

The absence of nonclinical safety studies with the combination is in accordance with the CHMP Guideline on the Non-Clinical Development of Fixed Combinations of Medicinal Products (EMEA/CHMP/SWP/258498/2005, January 2008) and Human Immunodeficiency Virus-1 Infection: Developing Antiretroviral Drugs for Treatment, Guidance for Industry (November 2015). There are no anticipated clinically relevant pharmacokinetic or toxicological interactions expected in the B/F/TAF FDC. Extensive clinical safety data are available for the approved drugs, FTC (Emtriva) and TAF (Vemlidy), and the approved FTC/TAF- (TDF-) containing regimens (Truvada, Atripla, Complera/Eviplera, Stribild, Genvoya, Odefsey, and Descovy). Additionally, data from the Phase 2 and 3 trials with the B/F/TAF FDC supports the use of these agents. The clinical data, along with the lack of overlapping toxicity in animals, support the safety of the new combination product for the treatment of HIV-1 infection.

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SECTION 2.6.7—TOXICOLOGY TABULATED SUMMARY

BICTEGRAVIR/EMTRICITABINE/TENOFOVIR ALAFENAMIDE FIXED DOSE COMBINATION (BIC/FTC/TAF [B/F/TAF] FDC)

Gilead Sciences

[redacted] **20**

CONFIDENTIAL AND PROPRIETARY INFORMATION

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NOTE TO REVIEWER

This application is being submitted in support of a fixed dose combination (FDC) that contains the integrase strand-transfer inhibitor (INSTI) bictegravir (BIC, B), nucleoside reverse transcriptase inhibitor (NRTI) emtricitabine (FTC, F, Emtriva®) and the nucleotide reverse transcriptase inhibitor (NtRTI) tenofovir alafenamide (TAF, GS-7340) fumarate (GS-7340-03): the B/F/TAF FDC (50/200/25 mg) tablet.

All nonclinical studies to support the B/F/TAF FDC application are included with no cross-referencing to data previously submitted. This comprises all nonclinical tests utilizing BIC, FTC, or tenofovir disoproxil fumarate (TDF)/TAF, including relevant combination studies, eg FTC/TDF; and other studies necessary to support the proposed product labeling. Links to all study reports included in the dossier are highlighted in blue text.

Comprehensive programs of nonclinical studies have been conducted with BIC, FTC, and TDF/TAF as single agents. Information from all nonclinical studies with BIC, FTC, or TDF/TAF should be considered in the context of the substantial clinical experience with FTC and TDF/TAF within antiretroviral combination therapy for the treatment of human immunodeficiency virus-1 (HIV-1) infection, and experience in the Phase 2 and 3 studies in combination with BIC as BIC/FTC/TAF FDC.

The following conversions are provided to aid the reviewer:

- BIC (GS-9883) 1 μM = 0.449 μg/mL
- FTC (GS-9019) 1 μM = 0.247 μg/mL
- TAF (GS-7340) 1 μM = 0.477 μg/mL
- TFV (GS-1278; tenofovir) 1 μM = 0.287 μg/mL

1. TOXICOLOGY: OVERVIEW

Test Articles: BIC, FTC, TAF, TDF

Type of Study/Species/Strain	Method of Administration	Duration of Dosing	Dose (mg/kg)[a]	GLP[b]	Testing Facility	Study No. (CRO Study No.)
Single-Dose Toxicity of FTC						
Mice, Charles River CD-1	Oral Gavage	1 Day	4000	Yes	[redacted] USA	TTEP/93/0020 (ACU531)
Mice, Charles River CD-1	IV	1 Day	200	Yes	[redacted] USA	TTEP/93/0023 (ACU529)
Rat, Charles River CD	Oral Gavage	1 Day	4000	Yes	[redacted] USA	TTEP/93/0021 (ACU532)
Rat, Charles River CD	IV	1 Day	200	Yes	[redacted] USA	TTEP/93/0024 (ACU530)
Single-Dose Toxicity of TAF						
Rat/Sprague Dawley; 7340-02	Oral Gavage	1 day	0, 6.25, 25, 100[c], 400	No	Gilead Sciences, CO	R990133 (NA)
Rat/Sprague Dawley; 7340-02	Oral Gavage	1 day	0, 100, 300, 1000	Yes	[redacted], Canada	R990185 ([redacted] 89186)
Dog/Beagle;7340-02	Oral Gavage	1 day	0, 30[d], 90, 270	Yes	[redacted], Canada	D990181 ([redacted] 89187)

Test Articles: BIC, FTC, TAF, TDF

Type of Study/Species/Strain	Method of Administration	Duration of Dosing	Dose (mg/kg)[a]	GLP[b]	Testing Facility	Study No. (CRO Study No.)
Repeat-Dose Toxicity of BIC						
Mice/RasH2 (001178-W [wild type] CByB6F1-Tg[HRAS]2Jic)	Oral Gavage	4-Weeks	0, 30, 100, 1000	Yes	[redacted] USA	TX-141-2042 ([redacted] 8316726)
Rat/ Crl:WI(Han)	Oral Gavage	2-Weeks	0, 10, 30, 100, 300	Yes	[redacted] USA	TX-141-2029 ([redacted] 8292936)
Rat/ Crl:WI(Han)	Oral Gavage	26-Weeks with 4-week recovery	0, 5, 30, 300	Yes	[redacted] USA	TX-141-2031 ([redacted] 8298346)
Monkey/ Cynomolgus	Oral Gavage	2-Weeks	0, 30, 100, 1000	Yes	[redacted] USA	TX-141-2030 ([redacted] 8293153)
Monkey/ Cynomolgus	Oral Gavage	39-Weeks with 13-Week interim necropsy and 4-week recovery	0, 30, 200[d], 1000	Yes	[redacted] USA	TX-141-2032 ([redacted] 8298309)
Repeat-Dose Toxicity of FTC						
Mouse, Charles River CD-1	Oral Gavage	2 Weeks	0, 120, 600, 3000	Yes	[redacted] UK	TOX001 (IUW00701)
Mouse, Charles River CD-1	Oral Gavage	4 Weeks	0, 120, 600, 3000	Yes	[redacted] USA	TOX599
Mouse, Charles River CD-1	Oral Gavage	26 Weeks	0, 167, 500, 1500	Yes	[redacted] UK	TOX022 (IUW01001)
Mouse, Charles River CD-1	Oral Gavage	26 Weeks	0, 120, 600, 3000	Yes	[redacted] USA	TOX628
Rat, CD	Oral Gavage	3 Months	0, 120, 600, 3000	Yes	[redacted] USA	TOX097
Monkey, Cynomolgus	Oral Gavage	4 Weeks	0, 80, 400, 2000	Yes	[redacted] USA	TOX600 (HWA 309-227)
Monkey, Cynomolgus	Oral Gavage	13 Weeks	0, 40, 200, 1000	Yes	[redacted] USA	TOX627 (HWA 309-229)
Monkey, Cynomolgus	Oral Gavage	1 Year	0, 50, 200[d], 500	Yes	[redacted] USA	TOX032 (6758-114)

Test Articles: BIC, FTC, TAF, TDF

Type of Study/Species/Strain	Method of Administration	Duration of Dosing	Dose (mg/kg)[a]	GLP[b]	Testing Facility	Study No. (CRO Study No.)
Repeat-Dose Toxicity of TAF						
Mouse/CD:CD1(ICR); GS-7340-02	Oral Gavage	2 weeks	0, 100, 500, 1000	No	[redacted]	TX-120-2006 ([redacted] 8235665)
Mouse/CD:CD1(ICR); GS-7340-02	Oral Gavage	13 weeks	0[g], 10, 30, 100	Yes	[redacted]	TX-120-2007 ([redacted] 8241074)
Rat/Sprague Dawley; GS-7340-02	Oral Gavage	7 days	0, 6.25, 25, 100, 400	No	Gilead Sciences, Inc.	R990139 (NA)
Rat/(Crl:CD (SD) BR); GS-7340-02	Oral Gavage	4 weeks	0, 1.5[d], 6.25, 25, 100, 400	Yes	[redacted] Canada	R990182 ([redacted] 89176)
Rat/(Crl:CD (SD) BR); GS-7340-02	Oral Gavage	26 weeks	0, 5, 25, 100	Yes	[redacted] Canada	TOX-120-001 ([redacted] 89686)
Rabbit/NZW; GS-7340-02	Oral Gavage	7 days	0, 20, 50, 75	No	[redacted] Canada	TX-120-2003 ([redacted] 902246)
Dog/Beagle; GS-7340-02	Oral Gavage	4 weeks	0, 0.1, 0.3, 1[d], 3, 10	Yes	[redacted] Canada	D990175 ([redacted] 89128)
Dog/Beagle; GS-7340-02	Oral Gavage	39 weeks	0, 2, 6, 18/12	Yes	[redacted] Canada	TOX-120-002 ([redacted] 89647)
Monkey/cynomolgus; GS-7340-02	Nasogastric Gavage	4 weeks	0, 3, 30	Yes	[redacted]	P2000114 ([redacted] 1050-11)
Repeat-Dose Toxicity of FTC/TDF						
Dog, Beagle	Oral Gavage	4 Weeks	FTC/TDF: 20/0, 0/30, 2/3, 20/30	Yes	[redacted] USA	TX-164-2004 (6511-155)

Test Articles: BIC, FTC, TAF, TDF

Type of Study/Species/Strain	Method of Administration	Duration of Dosing	Dose (mg/kg)[a]	GLP[b]	Testing Facility	Study No. (CRO Study No.)
Genotoxicity of BIC						
Bacterial Mutation/ *Salmonella typhimurium* TA98, TA100, TA1535, and TA1537; *E. coli* WP2*uvr*A	In Vitro	*ca.* 52 hours	5 - 5000 µg/plate	Yes	[redacted] USA	TX-141-2026 ([redacted] 8292935)
Chromosome aberration/ Cultured human peripheral blood lymphocytes	In Vitro	3 hours (± S9) or 24 hours (-S9 only)	Without S9 3 h: 84.0, 172, and 245 µg/mL 24 h: 9.89, 20.2, and 28.8 µg/mL With S9 3 h: 120, 172, and 245 µg/mL	Yes	[redacted] USA	TX-141-2027 ([redacted] 8292931)
In Vivo Micronucleus/Rat/ Crl:WI(Han)	Oral Gavage	2-Weeks	0, 10, 30, 100, 300	Yes	[redacted] USA	TX-141-2029 ([redacted] 8292936)
Genotoxicity of FTC						
In Vitro/*S. typhimurium* and *E. coli*	In Vitro	NA	100 to 5000 µg/plate	Yes	[redacted] USA	([redacted] 18637-0-409R)
In Vitro/*S. typhimurium*	In Vitro	NA	6.67 - 5000 µg/plate	Yes	[redacted] USA	MUT203 (15437-0-401)
In Vitro/*S. typhimurium* and *E. coli*	In Vitro	NA	313 - 5000 µg/plate	Yes	[redacted] Japan	K01-3154
In Vitro/Mouse Lymphoma/ L5178Y TK$^{+/-}$ Cells	In Vitro	NA	1000 to 5000 µg/mL	Yes	[redacted] USA	TOX012 (G97BG07.702)
In Vivo/Micronucleus/ Mouse/ICR	Oral Gavage	1 Day	0, 500, 1000, 2000	Yes	[redacted] USA	TOX011 (G97BG07.123M)
Genotoxicity of TAF						
In Vitro/*S. typhimurium, E. coli:* GS-7340-02	In vitro	52h	100, 333, 1000, 3330, 5000 µg/plate	Yes	[redacted]	V990212 ([redacted] 20900-0-409OECD)
In Vitro/L5178Y mouse lymphoma forward mutation assay/GS-7340-02	In vitro	24h	125 to 1500 µg/mL	Yes	[redacted]	V990213 ([redacted] 20900-0-431 ICH)
In Vivo/Micronucleus/ Mouse/Crl:CD-1 (ICR) BR; GS-7340-02	Oral Gavage	1 day	500, 1000, 2000	Yes	[redacted]	M2000113 ([redacted] 21816-0-455OECD)

Test Articles: BIC, FTC, TAF, TDF

Type of Study/Species/Strain	Method of Administration	Duration of Dosing	Dose (mg/kg)[a]	GLP[b]	Testing Facility	Study No. (CRO Study No.)
Genotoxicity of FTC/TDF						
In Vitro/*S. typhimurium* and *E. coli*	In Vitro	NA	Fixed ratio of 2 parts FTC to 3 parts TDF up to 5000 µg/plate	Yes	[redacted] USA	TX-164-2002 (AA97YC-YD.503.BTL)
In Vitro/Mouse Lymphoma/ L5178Y $TK^{+/-}$ Cells	In Vitro	NA	Fixed ratio of 2 parts FTC to 3 parts TDF, 15 to 500 µg/mL	Yes	[redacted] USA	TX-164-2003 (AA97YC-YD.704.BTL)
Carcinogenicity of BIC						
26-week / Mice/ 001178-T (Hemizygous) RasH2	Oral Gavage	26-Weeks	0, 5, 15, 100 (Males) 0, 10, 30, 300 (Females)	Yes	[redacted] USA	TX-141-2047 ([redacted] 8328591)
104-week / Rat/ Crl:WI(Han)	Oral Gavage	In Progress (First Dose 21 May 2015)	Water control, vehicle control, 2, 10, 300	Yes	[redacted] USA	TX-141-2040 ([redacted] 8315273) In Progress
Carcinogenicity of FTC						
Mouse/CD-1 (ICR)	Oral Gavage	2 Years	0, 80, 250, 750	Yes	[redacted] USA	TOX109 (814-005)
Rat/CD (Sprague Dawley)	Oral Gavage	2 Years	0, 60, 200, 600	Yes	[redacted] USA	TOX108 (814-006)
Carcinogenicity of TDF						
Mouse/CD-1 (ICR)	Oral Gavage	2 Years	0, 100, 300 600	Yes	[redacted] Canada	M990205 (89284)
Rat/CD (Sprague Dawley)	Oral Gavage	2 Years	0, 30, 100, 300	Yes	[redacted] Canada	R990204 (89282)

Test Articles: BIC, FTC, TAF, TDF

Type of Study/Species/Strain	Method of Administration	Duration of Dosing	Dose (mg/kg)[a]	GLP[b]	Testing Facility	Study No. (CRO Study No.)
Reproductive and Developmental Toxicity of BIC						
Fertility/ Rat/ Crl:CD(SD)	Oral Gavage	Males: 28 days prior to mating through day prior to necropsy Females: 14 days prior to mating through day 7 of gestation	0, 5, 30, 300[d]	Yes	[redacted] USA	TX-141-2039 ([redacted]-604142)
Embryo-fetal development dose range Finding/ Rat/ Crl:CD(SD)	Oral Gavage	GD 7 to 17	0, 5, 30, 300[d]	Yes	[redacted] USA	TX-141-2034 (CRL 20055845)
Embryo-fetal development/ Rat/ Crl:CD(SD)	Oral Gavage	GD 7 to 17	0, 5, 30, 300[d]	Yes	[redacted] USA	TX-141-2036 (CRL 20055846)
Embryo-fetal development dose range finding/ Rabbit/ Crl:KBL(NZW)	Oral Gavage	GD 7 to 19	0, 100, 300, 1000[q]	Yes	[redacted] USA	TX-141-2035 (CRL 20055847)
Embryo-fetal development dose range finding/ Rabbit/ [Hra:(NZW)SPF]	Oral Gavage	GD 7 to 19	0, 100, 300[r], 1000	Yes	[redacted] USA	TX-141-2038 (CRL 20065265)
Embryo-fetal development/ Rabbit/ [Hra:(NZW)SPF]	Oral Gavage	GD 7 to 19	0, 100, 300[r], 1000	Yes	[redacted] USA	TX-141-2037 (CRL 20055848)
Prenatal and postnatal developmental toxicity/ Rat/ Crl:CD(SD)	Oral Gavage	GD 6 to LD 20	0, 2, 10, 300[d]	Yes	[redacted] USA	TX-141-2045 ([redacted]-604145)

Test Articles: BIC, FTC, TAF, TDF

Type of Study/Species/Strain	Method of Administration	Duration of Dosing	Dose (mg/kg)[a]	GLP[b]	Testing Facility	Study No. (CRO Study No.)
Reproductive and Developmental Toxicity of FTC						
Fertility and Early Embryonic Development/ Mouse/ CD-1	Oral Gavage	F: 2 wks prior to mating to GD 6 M: 4 wks prior to mating and throughout mating	0, 250, 500, 1000	Yes	[redacted] USA	TOX036 (65C-7204-100)
Fertility and Early Embryonic Development/ Rat/CD Sprague-Dawley (males only)	Oral Gavage	M: 73 days + throughout mating	0, 150, 750, 3000	Yes	[redacted] USA	TOX679 (TTEP/95/0028)
Embryo-fetal Development/ Mouse/CD-1	Oral Gavage	GD 6–15	0, 250, 500, 1000	Yes	[redacted] USA	TOX033 ([redacted]-345001)
Embryo-fetal Development\ Mouse/CD-1	Oral Gavage	GD 6–15	0, 250, 500, 1000	Yes	[redacted] USA	TOX037 ([redacted]-345003)
Embryo-fetal Development/ Rabbit/New Zealand White	Oral Gavage	GD 7–19	0, 250, 500, 1000	Yes	[redacted] USA	TOX034 ([redacted]-345002)
Embryo-fetal Development/ Rabbit/New Zealand White	Oral Gavage	GD 7–19	0, 100, 300, 1000	Yes	[redacted] USA	TOX038 ([redacted]-345004)
Pre-and Post-natal Development Including Maternal Function/ Mouse/CD-1	Oral Gavage	F_0 F dosed from GD 6 to PND 20	0, 250, 500, 1000	Yes	[redacted] USA	TOX039 (65C-7204-200)
Reproductive and Developmental Toxicity of TDF						
Pre-& Post-natal Development/ Rat/ Crl: CD (SD)	Oral Gavage	F_0 F dosed from GD 7–LD 20 or GD 7–24	0, 50, 150, 450, 600	Yes	[redacted] USA	R990202 (707-016)

Test Articles: BIC, FTC, TAF, TDF

Type of Study/Species/Strain	Method of Administration	Duration of Dosing	Dose (mg/kg)[a]	GLP[b]	Testing Facility	Study No. (CRO Study No.)
Reproductive and Developmental Toxicity of TAF						
Fertility/Rat, Crl:CD(SD)/ GS-7340-03	Oral Gavage	M: 4 weeks premating, 10 weeks total F: 2 weeks premating - GD 13	0, 20, 80[h], 160[i] base eq	Yes	[redacted]	TX-120-2012 ([redacted] 8264528)
Dose Range Finding Embryo-Fetal Development/ Rat, Crl:CD(SD); GS-7340-02	Oral Gavage	GD 6-17	0, 5, 100[j], 200[k]	Yes	[redacted] Canada	TX-120-2001 (CRL 902244)
Embryo-Fetal Development/ Rat, Crl:CD (SD); GS-7340-02	Oral Gavage	GD 6-17	0, 25, 100[jk], 250	Yes	[redacted] Canada	TX-120-2002 (CRL 902245)
Dose Range Finding Developmental DRF/Rabbit, Hra: (NZW) SPF; GS-7340-02	Oral Gavage	GD 7-20	0, 5, 25, 50[dj], 100[ch]	Yes	[redacted] Canada	TX-120-2004 (CRL 902247)
Embryo-Fetal Developmental/ Rabbit, Hra: (NZW) SPF; GS-7340-02	Oral Gavage	GD 7-20	0, 10, 30[j], 100[dk]	Yes	[redacted] Canada	TX-120-2005 (CRL 902248)
Local Tolerance of BIC						
Skin Corrosion/EpiDerm™	In Vitro	3 or 60 minute exposure	25 mg	Yes	[redacted] England	TX-141-2058 ([redacted] 8338975)
Skin Irritation/EpiDerm™ SIT	In Vitro	60 minute treatment	25 mg	Yes	[redacted] England	TX-141-2059 ([redacted] 8338976)
Bovine Corneal Opacity and Permeability (BCOP) Assay	In Vitro	Opacity assessment (4 hours) followed by permeability assessment (90 minutes)	750 µL of 20% w/v suspension	Yes	[redacted] England	TX-141-2060 ([redacted] 8338977)
Phototoxicity/ Fibroblasts / Mouse / BALB/c 3T3 cells (clone A31)	In Vitro	90 minutes	Assay #1 & 2: 0.178, 0.316, 0.562, 1.00, 1.78, 3.16, 5.62 and 10 µg/mL	Yes	[redacted] USA	TX-141-2044 ([redacted] 20077249)
Phototoxicity/ Pigmented rat/ Crl:LE (Long-Evans)	Oral Gavage	3 Days	0, 2, 10, 300	Yes	[redacted] USA	TX-141-2046 ([redacted] 20083296)

Test Articles: BIC, FTC, TAF, TDF

Type of Study/Species/Strain	Method of Administration	Duration of Dosing	Dose (mg/kg)[a]	GLP[b]	Testing Facility	Study No. (CRO Study No.)
Local Tolerance of TAF						
In Vitro Tests/Bovine Corneal Opacity and Permeability Assay; (BCOP); GS-7340-03	Topical	4 hr	20% (w/w) in 0.9% sodium chloride solution	Yes	[redacted] UK	TX-120-2013 ([redacted] UK EUN0017)
In Vivo Tests/Rabbit Dermal Irritation/Hra: (NZW)SPF; GS-7340-02	Topical	4 hr	0.5g/rabbit	Yes	[redacted]	TX-120-2011 ([redacted] 8253834)
Other Toxicity Studies with BIC						
Impurities/Bacterial Mutation Study/*Salmonella typhimurium* and *Escherichia coli*	In Vitro	52 ± 4 hours	5–5000 µg/plate	Yes	[redacted] USA	TX-141-2048 ([redacted] 8335231)
Impurities/ Bacterial Mutation Study/*Salmonella typhimurium* and *Escherichia coli*	In Vitro	52 ± 4 hours	5–5000 µg/plate	Yes	[redacted] USA	TX-141-2041 ([redacted] 8320816)
Other/ Antigenicity (LLNA)/ Mouse/ CBA/CaCrl	Topical	3 days	10, 25, 50% w/v	Yes	[redacted] England	TX-141-2061 ([redacted] 8338978)
Other/ Investigative Repeat Dose/ Rat/ Crl:WI(Han)	Oral Gavage	2 Weeks	0, 300[s]	Yes	[redacted] USA	TX-141-2033 ([redacted] 8298544)
Impurities/ Repeat Dose/ Rat/ Crl:WI(Han)	Oral Gavage	4 weeks	0 (vehicle), 300 (Lot No. [redacted]; 97.1% pure), 100 or 300 (Lot No. [redacted]; 95.4% pure)	Yes	[redacted] USA	TX-141-2043 ([redacted] 8329288)
Other Toxicity Studies with FTC						
Immunotoxicity/Rat/ Sprague Dawley	Oral Gavage	4 Weeks	0, 60, 240, 1000	Yes	[redacted] Scotland	TOX146 (503791)
Impurities/Mouse/CD-1	Oral Gavage	4 Weeks	50/1, 150/3 and 450/9	Yes	[redacted] USA	TOX153 ([redacted]-345018)
Impurities/Mouse/CD-1	Oral Gavage	4 Weeks	0, 50, 150, 450	Yes	[redacted] USA	TX-162-2001 ([redacted]-345019)
Other/Reverse Mutation/*S. typhimurium., E. coli*	In Vitro	NA	1.5 to 5000 µg/plate and 1% TP-0296, up to 50 µg/plate	Yes	[redacted], USA	TOX151 (AA74EM.503.BTL)
Other/Chromosome Aberration, CHO (±S9)	In Vitro	NA	313 to 5000 µg/mL, 1% TP-0296	Yes	[redacted], USA	TOX152 (AA74EM.331.BTL)

Test Articles: BIC, FTC, TAF, TDF

Type of Study/Species/Strain	Method of Administration	Duration of Dosing	Dose (mg/kg)[a]	GLP[b]	Testing Facility	Study No. (CRO Study No.)
Other Toxicity Studies with TAF						
LLNA/Mouse, CBA/Ca; GS-7340-03	Topical	3 days	10, 25, 50% (w/v)	Yes	[redacted] UK	TX-120-2014 ([redacted] EUN0018)
Impurity Qualification in Rat; GS-7340-02	Oral Gavage	2 weeks	0, 5, 50	Yes	[redacted]	TX-120-2008 ([redacted] 8246754)
Impurity Qualification in Rat; GS-7340-03	Oral Gavage	4 weeks	0, 25, 50	Yes	[redacted]	TX-120-2021 ([redacted] 8298810)
Bone Metabolism in Rat (Sprague Dawley); GS-7340-02	Oral Gavage	6 days	0, 400	No	Gilead Sciences, CO	R990177 (NA)
Bone Metabolism in Rat (Sprague Dawley); GS-7340-02	Oral Gavage	6 days	0, 100, 400	No	Gilead Sciences, CO	R2000044 (NA)
Renal Effect in Rat (Sprague Dawley); GS-7340-02	Oral Gavage	1 day	0, 100, 1000	Yes	[redacted] Canada	R990186 ([redacted] 56520)
Bone marker Assessment Dog (Beagle); GS-7340-02	Oral gavage	5 days	37.5, 75	Yes	[redacted]	D990142 ([redacted] 9529.3)
Other Toxicity Studies with FTC/TDF						
Impurities/Rat/SD	Oral Gavage	14 Days	TDF/FTC: 30/20, 100/67, 300/200	Yes	[redacted] Canada	TX-164-2001 (800392)
Impurities/Rat/SD	Oral Gavage	14 Days	TDF/FTC: 0, 30/20, 100/67, 300/200, 30/20, 100/67, 300/200	Yes	[redacted]	TX-164-2005 (805427)

Test Articles: BIC, FTC, TAF, TDF

Type of Study/Species/Strain	Method of Administration	Duration of Dosing	Dose (mg/kg)[a]	GLP[b]	Testing Facility	Study No. (CRO Study No.)
Other Toxicity Studies with TDF						
TDF: Interaction study/ mitochondrial toxicity/ Rat/ Sprague Dawley	Oral Gavage	28 days	0, 40 (adefovir dipivoxil), 300 (tenofovir DF) & 40/300 adefovir dipivoxil/ tenofovir DF	No	Gilead Sciences, CO USA	R2000096

GS-9883 = bictegravir (BIC); JTK-303 = GS-9137, elvitegravir (EVG); GS-9350 = cobicistat (COBI); GLP = Good Laboratory Practices; NA = not applicable; RTV = ritonavir; ATV = atazanavir; DRF = dose range-finding; GD = gestation day; LD = lactation day; PPD = post-partum day; PND = post natal day; w/w = weight/weight; w/v = weight/volume; CA = citric acid; SC = subcutaneous; IV = intravenous; F = female; M = male; GS-8374 = investigational HIV protease inhibitor; bid =twice daily; qd = every day; q2d = every two days; q7d = every 7 days

a Unless otherwise specified, the highest No-Observed-Adverse-Effect Level (NOAEL) is underlined.
b 'Yes', indicates study includes a GLP Compliance statement.
c NOAEL greater than
d NOEL (No-Observed-Effect Level)
e Due to animal deaths in the high dose group at 300 mg/kg/day, the high dose animals were not dosed on Dosing Phase Day 2. Beginning on Dosing Phase Day 3, the high dose level was reduced to 200 mg/kg/day
f NOAEL: < 50 mg/kg/day COBI
g NOAEL could not be determined
h Male and female
i Early embryonic toxicity
j Maternal
k Embryo-fetal
l For both lots (# [redacted] and # [redacted]).
m GS-9350, Lot # [redacted].
n GS-9350, Lot # [redacted] (spiked with impurities)
o GS-9350/GS-9137 non-degraded crushed tablets, Lot # [redacted].
p GS-9350/GS-9137 degraded crushed tablets, Lot # [redacted].
q No NOEL or NOAEL determined due to vehicle-related maternal and developmental toxicity observed.
r Maternal NOAEL, Developmental NOEL.
s No toxicity observed at 300 mg/kg/day; limited toxicity endpoints evaluated.

2. TOXICOKINETICS: OVERVIEW OF TOXICOKINETICS STUDIES

2.1. BIC

Test Article: BIC

Type of Study	Test System	Method of Administration	Dose (mg/kg)	GLP[a]	Gilead Study No. (CRO Study No.)
4-Week Toxicity	Mouse	Oral Gavage	0, 30, 100, 1000	Yes	TX-141-2042 ([redacted] 8316726)
26-Week Carcinogenicity	Mouse	Oral Gavage	0, 5, 15, 100 (Males) 0,10, 30, 300 (Females)	Yes	TX-141-2047 ([redacted] 8328591)
2-Week Toxicity	Rat	Oral Gavage	0, 10, 30, 100, 300	Yes	TX-141-2029 ([redacted] 8292936)
Rat Dose Range- Finding EFD	Rat	Oral Gavage	0, 5, 30, 300	Yes	TX-141-2034 (CRL 20055845)
Rat PPND	Rat	Oral Gavage	0, 2, 10, 300	Yes	TX-141-2045 ([redacted]-604145)
26-Week Toxicity	Rat	Oral Gavage	0, 5, 30, 300	Yes	TX-141-2031 ([redacted] 8298346)
Rabbit Dose Range-Finding EFD	Rabbit	Oral Gavage	0, 100, 300, 1000	Yes	TX-141-2038 (CRL 20065265)
2-Week Toxicity	Cynomolgus Monkey	Oral Gavage	0, 30, 100, 1000	Yes	TX-141-2030 ([redacted] 8293153)
39-Week Toxicity	Cynomolgus Monkey	Oral Gavage	0, 30, 200, 1000	Yes	TX-141-2032 ([redacted] 8298309)

EFD = Embryofetal Developmental; PPND = Pre- and Postnatal Development
a 'Yes', indicates study includes a GLP Compliance statement

2.2. FTC

Test Article: FTC

Type of Study	Test System	Method of Administration	Dose (mg/kg)	GLP[a]	Gilead Study No.
4-Week Repeat Dose	Mouse	Oral Gavage	0, 120, 600, 3000	Yes	TOX599, Appendix 5
26-Week Repeat Dose	Mouse	Oral Gavage	0, 167, 500, 1500	Yes	TOX022 Addendum
26-Week Repeat Dose	Mouse	Oral Gavage	0, 120, 600, 3000	Yes	TOX628, Appendix F
Carcinogenicity	Mouse	Oral Gavage	0, 80, 250, 750	Yes	TOX109
13-Week Repeat Dose	Rat	Oral Gavage	0, 120, 600, 3000	Yes	TOX097, Appendix N
Carcinogenicity	Rat	Oral Gavage	0, 60, 200, 600	Yes	TOX108
4-Week Repeat Dose	Monkey	Oral Gavage	0, 80, 400, 2000	Yes	TOX600, Appendix 12
13-Week Repeat Dose	Monkey	Oral Gavage	0, 40, 200, 1000	Yes	TOX627, Appendix 14
52-Week Repeat Dose	Monkey	Oral Gavage	0, 50, 200, 500	Yes	TOX032, Appendix 6
Embryo/Fetal Development	Rabbits	Oral Gavage	0, 100, 300, 1000	Yes	TOX038

a 'Yes', indicates study includes a GLP Compliance statement.

2.3. TAF

Test Article: TAF

Type of Study	Test System	Method of Administration	Dose (mg/kg) GS-7340-02	GLP[a]	Gilead Study No.
2-Week Repeat Dose	Mouse	Oral Gavage	0, 100, 500, 1000	No	TX-120-2006
13-Week Repeat Dose	Mouse	Oral Gavage	0, 10, 30, 100	Yes	TX-120-2007
4 -Week Repeat Dose	Rat	Oral Gavage	0, 1.5, 6.25, 25, 100, 400	Yes	R990182
26-Week Repeat Dose	Rat	Oral Gavage	0, 5, 25, 100	Yes	TOX-120-001
4-Week Repeat Dose	Dog	Oral Gavage	0, 0.1, 0.3, 1, 3, 10	Yes	D990175
39-Week Repeat Dose	Dog	Oral Gavage	0, 2, 6, 18/12	Yes	TOX-120-002
4-Week Repeat Dose	Monkey	Oral Gavage	0, 3, 30	Yes	P2000114
Dose Range Finding Embryo/Fetal Development	Rat	Oral Gavage	0, 5, 100, 200	Yes	TX-120-2001
Embryo/Fetal Development	Rat	Oral Gavage	0, 25, 100, 250	Yes	TX-120-2002
7-Day Tolerability Study	Rabbit	Oral Gavage	0, 20, 50, 75	No	TX-120-2003
Dose Range Finding Embryo/Fetal Development	Rabbit	Oral Gavage	0, 5, 25, 50, 100	Yes	TX-120-2004
Embryo/Fetal Development	Rabbit	Oral Gavage	0, 10, 30, 100	Yes	TX-120-2005
Qualification of Impurities	Rat	Oral Gavage	0, 5, 50	Yes	TX-120-2008
Qualification of Impurities	Rat	Oral Gavage	0, 25, 50	Yes	TX-120-2021

a ‘Yes’ indicates study includes a GLP Compliance statement.

2.4. FTC/TDF

Test Article: FTC/TDF

Type of Study	Test System	Method of Administration	Dose (mg/kg)	GLP[a]	Gilead Study No.
4-Week Repeat Dose with FTC/TDF	Dog	Oral Gavage	20/0, 0/30, 2/3, 20/30	Yes	TX-164-2004

a ‘Yes’, indicates study includes a GLP Compliance statement.

3. TOXICOKINETICS: OVERVIEW OF TOXICOKINETICS DATA

3.1. BIC

Test Article: BIC

	BIC Steady State AUC_{0-24} (µg•h/mL)						
Species	**Mouse**		**Rat**		**Rabbit**	**Cynomolgus Monkey**	
Method of Administration	**Oral Gavage**		**Oral Gavage**		**Oral Gavage**	**Oral Gavage**	
Gender	**M**	**F**	**M**	**F**	**F**	**M**	**F**
Dose (mg/kg/day)							
2				335[j], 334[k]			
5	190[g]		457[d]	1290[d], 1630[h]			
10		341[g]	835[a]	1490[a], 1170[j], 762[k]			
15	526[g]						
30	606[c]	652[c], 861[g]	1470[a], 1010[d]	2110[a], 2910[d], 3080[h]		273[b], 288[e], 248[f]	152[b], 254[e], 253[f]
100	1030[c], 1560[g]	1330[c]	1820[a]	3290[a]	38.5[i]	413[b]	236[b]
200						855[e], 595[f]	664[e], 823[f]
300		2340[g]	2270[a], 1830[d]	3670[a], 4680[d], 3650[h], 3100[j], 1120[k]	60.3[i]		
1000	2230[c]	2420[c]			138[i]	1170[b], 1540[e], 1450[f]	1020[b], 1180[e], 1750[f]

M = male; F = female; DG = Day of gestation; LD = Lactation day; PND = Postnatal day; AUC_{0-24} = partial area under the concentration versus time curve from 0 to 24 hours
a TX-141-2029 (Day 14)
b TX-141-2030 (Day 14)
c TX-141-2042 (Week 4)
d TX-141-2031 (Week 26)
e TX-141-2032 (Week 13 AUC_{0-t})
f TX-141-2032 (Week 39 AUC_{0-t})
g TX-141-2047 (Week 26)
h TX-141-2034 (DG 17 AUC_{0-t})
i TX-141-2038 (DG 19 AUC_{0-t})
j TX-141-2045 (F_0 LD 10)
k TX-141-2045 (F_1 PND 10 combined sex)

3.2. FTC

Test Article: FTC

	Steady State AUC (µg·h/mL)						
Species	**Mouse**		**Rat**		**Monkey**		**Rabbit**
Method of Administration	**Oral Gavage**		**Oral Gavage**		**Oral Gavage**		**Oral Gavage**
Gender (M/F)	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**	**Female**
Dose (mg/kg/day)							
40					13.1[n], 12.2[o]	14.4[n], 13.4[o]	
50					16.1[p], 17.0[q], 18.1[r]	18.1[p], 22.9[q], 33.2[r]	
60			29.9[j], 42.9[k]	30.9[j], 52.5[k]			
80	25.5[f], 27.5[g]	27.6[f], 23.7[g]			37.1[l], 31.6[m]	37.8[l], 33.6[m]	
100							87.3[s]
120	169.7[e], 103[a], 78[b]	115.4[e], 74[a], 83[b]	61.6[h], 65.6[i]	55.6[h], 69.3[i]			
167	63.8[c], 82.3[d]	67.9[c], 93.1[d]					
200			97.3[j], 137[k]	156[j], 171[k]	64.8[n], 71.0[o], 74.9[p], 102.9[q], 97.2[r]	54.6[n], 51.9[o], 99.9[p], 119.9[q], 97.8[r]	
250	91.0[f], 90.9[g]	65.0[f], 91.78[g]					
300							315[s]
400					170[l], 196[m]	170[l], 164[m]	
500	181.4[c], 248.4[d]	209.6[c], 284.4[d]			265.5[p], 341.7[q], 274[r]	195.7[p], 321.1[q], 238[r]	
600	586[e], 499[a], 410[b]	491[e], 304[a], 326[b]	296[h], 329[i] 280[j], 327[k]	260[h], 362[i] 260[j], 404[k]			
750	235[f], 287[g]	209[f], 323[g]					
1000					403[n], 331[o]	274[n], 233[o]	1258[s]

Test Article: FTC

	Steady State AUC (µg·h/mL)						
Species	**Mouse**		**Rat**		**Monkey**		**Rabbit**
Method of Administration	**Oral Gavage**		**Oral Gavage**		**Oral Gavage**		**Oral Gavage**
Gender (M/F)	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**	**Female**
Dose (mg/kg/day)							
1500	513[c], 732[d]	569[c], 899[d]					
2000					631[l], 730[m]	765[l], 732[m]	
3000	2026[e], 1936[a] 1800[b]	1549[e], 1516[a] 1711[b]	1413[h], 1276[i]	1357[h], 1646[i]			

Note: In a combination study (FTC + TDF) in dogs, AUCs for FTC were 25.3 and 32.3 µg*h/mL, respectively, for 20 mg/kg/day FTC alone or in combination with 30 mg/kg/day TDF (TX-164-2004).

a TOX599 (Week 4)
b TOX599 (Day 3)
c TOX022 (Week 13)
d TOX022 (Week 26)
e TOX628 (Week 26)
f TOX109 (Week 2)
g TOX109 (Week 26)
h TOX097 (Day 2)
i TOX097 (Day 90)
j TOX108 (Week 2)
k TOX108 (Week 26)
l TOX600 (Day 3)
m TOX600 (Week 4)
n TOX627 (Week 12)
o TOX627 (Day 3)
p TOX032 (Week 13)
q TOX032 (Week 26)
r TOX032 (Week 52)
s TOX038 (GD 19)

3.3. TAF

Test Article: TAF

	Steady State Tenofovir (R-PMPA) AUC (µg•h/mL)							
Species	**Mouse**		**Rat**		**Rabbit**	**Dog**		**Monkey**
Method of Administration	**Oral Gavage**		**Oral Gavage**		**Oral Gavage**	**Oral Gavage**		**Oral Gavage**
Gender (M/F)	**M**	**F**	**M**	**F**	**F**	**M**	**F**	**M & F**
Dose (mg/kg/day)								
0.1						NC[e]	NC[e]	
0.3						NC[e]	NC[e]	
1						NC[e]	NC[e]	
1.5			0.05[c@]	0.06[c@]				
2						1.30[f]*	1.07[f]*	
3						NC[e]	NC[e]	0.35[a]*
5			0.7[d]* 0.02[k$] 0.07[k§]	0.7[d]* 0.41[g]	1.6[i]			
6						3.89[f]*	5.01[f]*	
6.25			0.4 [c@]	0.3 [c@]				
10	0.19[b]	0.24[b]			2.0[j]	5.26[e*]	5.26[e]*	
18/12						14.90[f*]	12.57[f]*	
20					2.3[l]			
25			2.5[c@] 3.8[d]* 2.4[n]*[∞] 3.0[n]*[∂] 2.6[n]*[ß]	2.8[c@] 3.8[d]* 2.8[h]	6.0[i]			
30	1.56[b]	1.45[b]			5.0[j]			5.87[a]*
50			2.0[k$] 2.5[k§] 6.8[n]*[∞] 6.0[n]*[∂] 6.0[n]*[ß]		11.8[i] 5.7[l]			

Test Article: TAF

	Steady State Tenofovir (R-PMPA) AUC (µg•h/mL)							
Species	**Mouse**		**Rat**		**Rabbit**	**Dog**		**Monkey**
Method of Administration	**Oral Gavage**		**Oral Gavage**		**Oral Gavage**	**Oral Gavage**		**Oral Gavage**
Gender (M/F)	**M**	**F**	**M**	**F**	**F**	**M**	**F**	**M & F**
Dose (mg/kg/day)								
75					10.1[l]			
100	5.97[m] 7.80[b]	5.51[m] 6.55[b]	12.2[c@] 15.5[d]*	14.6[c@] 15.5[d]* 20.6[g] 17.4[h]	23.5[i] 27.3[j]			
200				35.7[g]				
250				55.7[h]				
270								
400			62.7[c@]	75.1[c@]				
500	27.2[m]	30.8[m]						

NA = not applicable; NC = not calculable; DR = dose reduced from 18 to 12 mg/kg/day on Days 45 and 51, for males and females respectively
Other than as indicated all AUC values are specified as AUC0-t; @ = AUC0-last; * = males and females combined; $ = 97.7% purity; § = 83.1% purity; ∞ = 99.3% purity; ∂ = 98.0% purity; ß = 97.7% purity

a P2000114 (Day 28)
b TX-120-2007 (Week 13)
c R990182 (Day 28)
d TOX-120-001 (Week 26)
e D990175 (Day 28)
f TOX-120-002 (Week 39) *(dose reduced from 18 to 12 mg/kg/day on Days 45 and 51 for males and females, respectively)*
g TX-120-2001 (Day 17)
h TX-120-2002 (Day 17)
i TX-120-2004 (Day 20)
j TX-120-2005 (Day 20)
k TOX-120-2008 (Day 14)
l TOX-120-2003 (Day 7)
m TX-120-2006 (Day 14)
n TX-120-2021 (Day 28)

4. TOXICOLOGY: DRUG SUBSTANCE

4.1. BIC

Batch Number	Purity (%)[b]	Individual Impurities (%)										Type of Toxicity Study
		[redacted]										
Proposed Specification Limit:	[redacted]	[redacted]										-
[redacted]	90.3	—	—	1.1	1.62	3.59	0.07	—	—	0.20	0.07	d
[redacted]	94.9	0.12	0.15	0.2	1.64	3.33	—	—	—	0.32	—	e
[redacted]	96.8	—	—	0.2	0.43	2.26	—	Tr	0.06	0.06	0.38	f
[redacted]	97.0	Tr	0.12	NT	0.20	3.36	0.06	—	—	0.05	—	g
[redacted]	95.4	0.16	—	NT	0.51	2.14	—	0.08	0.08	—	0.15	h

Batch Number	Purity (%)[b]	Individual Impurities (%)												Type of Toxicity Study
		[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	
Proposed Specification Limit:	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	-
[redacted]	90.3	—	—	0.07	0.18	Tr	0.07	—	Tr	Tr	0.12	Tr	Tr	d
[redacted]	94.9	—	—	—	—	—	—	0.06	—	—	—	—	—	e
[redacted]	96.8	—	—	—	—	—	—	—	—	—	—	—	—	f
[redacted]	97.0	—	—	—	—	—	—	—	—	—	—	—	—	g
[redacted]	95.4	0.07	Tr	—	—	—	—	—	—	—	—	—	—	h

—: None detected, < 0.03%
Tr: Trace, < 0.05%
NT: Not Tested

a Sample was retested to quantify the amount of impurities present including GS-626446.
b Purity is determined by external standard.
c Determined from the enantiomeric purity of BIC.
d Used for study numbers: PC-141-2046, PC-141-2047, PC-141-2048, PC-141-2049, TX-141-2026, TX-141-2027, TX-141-2029, TX-141-2030, TX-141-2033 (safety pharmacology, genetic toxicity, repeat dose toxicity)
e Used for study numbers: TX-141-2045, TX-141-2044, TX-141-2046, TX-141-2047, TX-141-2040 (carcinogenicity, local tolerance, reproductive toxicity)
f Used for study numbers: TX-141-2058, TX-141-2059, TX-141-2060, TX-141-2061(local tolerance, antigenicity)
g Used for study numbers: TX-141-2042, TX-141-2031, TX-141-2032, TX-141-2034, TX-141-2035, TX-141-2038, TX-141-2039, TX-141-2036, TX-141-2037, TX-141-2043 (repeat dose toxicity, reproductive toxicity, impurity qualification)
h Used for study numbers: TX-141-2043 (impurity qualification)

4.2. FTC

Lot No.	Purity (%)		Individual Impurities (%)				Total Impurities	Type of Toxicity Study
	[redacted]	HPLC (w/w)	[redacted]	[redacted]	[redacted]	Individual Unspecified Impurities		
SPECIFICATION	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	
[redacted]	1.1[b]	97.7	NA[b]	NA[c]	NA[c]	NR	NR	Genetic[i]
[redacted]	2.0[b]	95.6[e]	ND	0.33	0.36	0.08% TP-0270; <0.05% TP-0296; <0.05% RRT 0.67; 0.05% RRT 1.21; 0.1% RRT 1.27; 0.05% RRT 1.77; <0.05% RRT 1.99; 0.05% RRT 2.27; 0.08% RRT 2.78; 0.08% RRT 3.25	1.19	Repeat Dose[j]
[redacted]	1.0[b]	93.0[e]	ND	ND	0.26	0.07% TP-0296; 0.09% RRT 0.52; 0.12% RRT 0.53; 0.10% RRT 0.54; 0.06% RRT 0.58; <0.05% RRT 0.59; 0.05% RRT 0.72; 0.10% RRT 0.74; 0.16% RRT 0.92; <0.05% RRT 0.93; 0.83% RRT 1.01; 0.13% RRT 1.06; 0.06% RRT 1.13; 0.09% RRT 1.45; 0.10% RRT 1.49; 0.05% RRT 1.51; 0.24% RRT 1.99; 0.19% RRT 2.27; 0.06% RRT 2.29; 0.07% RRT 2.39; 0.09% RRT 2.42; 0.09% RRT 2.47	3.04[e]	Repeat Dose[j]
[redacted]	1.0[b]	96.6	ND	ND	0.12	0.05% TP-0270; 0.07% TP-0296; <0.05% RRT 0.92; <0.05% RRT 0.97; <0.05% RRT 1.06; 0.13% RRT 1.27; <0.05% RRT 1.45; 0.13% RRT 1.51; <0.05% RRT 1.57; 0.07% RRT 2.27	0.57	Repeat Dose[j]
[redacted]	1.0[b]	97.3	ND	ND	0.43	<0.05% TP-0270; 0.05% TP-0296; 0.06% RRT 0.92; <0.05% RRT 0.97; <0.05% RRT 1.06; <0.05% RRT 1.27; <0.05% RRT 1.52; 0.06% RRT 2.27	0.60	Repeat Dose[j]

Lot No.	Purity (%)		Individual Impurities (%)				Total Impurities	
	[redacted]	HPLC (w/w)	[redacted]	[redacted]	[redacted]	Individual Unspecified Impurities		
SPECIFICATION	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	**Type of Toxicity Study**
[redacted]	1.4[b]	94.3[e]	ND	ND	0.24	0.07% TP-0296; <0.05% RRT 0.51; 0.10% RRT 0.54; <0.05% RRT 0.72; 0.06% RRT 0.74; 0.16% RRT 0.92; <0.05% RRT 0.93; 0.96% RRT 1.02; 0.11% RRT 1.06; <0.05% RRT 1.45; 0.05% RRT 1.50; <0.05% RRT 1.51; 0.29% RRT 1.99; 0.14% RRT 2.27; 0.06% RRT 2.30; 0.09% RRT 2.42 <0.05% RRT 2.47	2.34[e]	Repeat Dose[k]
[redacted]	1.7[b]	92.9[e]	ND	ND	0.26	0.08% TP-0296; 0.09% RRT 0.52; 0.12% RRT 0.53; 0.10% RRT 0.54; 0.06% RRT 0.58; 0.05% RRT 0.59; 0.05% RRT 0.72; 0.11% RRT 0.74; 0.17% RRT 0.92; <0.05% RRT 0.93; 0.83% RRT 1.01; 0.14% RRT 1.06; 0.10% RRT 1.45; 0.10% RRT 1.50; 0.05% RRT 1.51; 0.25% RRT 1.99; 0.19% RRT 2.27; 0.06% RRT 2.29; 0.07% RRT 2.40; 0.10% RRT 2.42; 0.09% RRT 2.46	3.13[e]	Reproductive[l]
[redacted]	0.7[b]	98.1	NA[c]	NA[c]	0.2[f] (RRT 0.83)	0.1% RRT 0.58; 0.1% RRT 0.60; 0.1% RRT 1.17; 0.1% RRT 1.24; 0.2% RRT 1.27[g]; 0.1% RRT 1.63	0.9	Repeat Dose[m]
[redacted]	1.3[b]	95.2	ND	ND	0.21	0.07% RRT 2.00; 0.25% RRT 2.27[h]; 0.09% RRT 2.30; <0.05% RRT 2.42 <0.05% RRT 2.47	0.63	Repeat Dose[m]

Lot No.	Purity (%)		Individual Impurities (%)				Total Impurities	Type of Toxicity Study
	[redacted]	HPLC (w/w)	[redacted]	[redacted]	[redacted]	Individual Unspecified Impurities		
SPECIFICATION	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	
[redacted]	1.4[b]	97.0	ND	ND	0.29	0.06% RRT 1.99; 0.07% RRT 2.27 0.06% RRT 2.42	0.48	Repeat Dose[m]
[redacted]	1.8[b]	97.1	ND	ND	0.41	<0.05% RRT 0.74; <0.05% RRT 1.45; 0.05% RRT 1.50; 0.07% RRT 1.52; 0.10% RRT 1.99; 0.15% RRT 2.27[c]; 0.06% RRT 2.30; <0.05% RRT 2.42 0.05% RRT 2.47	0.89	Repeat Dose[m]
[redacted]	1.1[b]	98.0	ND	ND	0.19	0.10% RRT 2.27; <0.05% RRT 2.30 <0.05% RRT 2.42	0.29	Reproductive[n]
[redacted]	0.5[b]	99.0	ND	ND	0.21	0.06% RRT 0.67; <0.05% RRT 1.45 <0.05% RRT 1.50	0.28	Repeat Dose[o]
[redacted]	0.7	99.6	ND	ND	0.07	Trace	0.1	Impurity Qualification[p]
[redacted]	0.3	99.5	ND	ND	0.1	0.1%	0.2	Immunotoxicity[q]
[redacted]	0.5	99.8	ND	ND	0.1	ND	0.1	Other Toxicity[r]
[redacted]	NT	87.0	0.35	0.07	ND	0.09% 5-FC; 0.15% FTU; 0.88% trans-(+/-)-FTC; 0.08% RRT 1.50	1.6	Impurity Qualification[s]
[redacted]	NT	100.0	ND	ND	0.1	0.1% RRT 1.445	0.2	Carcinogenicity[t]
[redacted]	0.3	99.5	ND	ND	0.1	0.1%	0.2	Carcinogenicity[t]
[redacted]	0.0	100.1	0.17	ND	ND	ND	0.2	Repeat Dose[u]
[redacted]	0.0	99.6	0.05	ND	ND	ND	0.1	Genetic[v]

Lot No.	Purity (%)		Individual Impurities (%)				Total Impurities	Type of Toxicity Study
	[redacted]	HPLC (w/w)	[redacted]	[redacted]	[redacted]	Individual Unspecified Impurities		
SPECIFICATION	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	
[redacted]	0.0	99.7	0.10	ND	ND	ND	0.1	Genetic[w]
[redacted]	0.0	99.1	0.12	ND	ND	ND	0.1	Repeat Dose[u], Genetic[w]

NA = not applicable; NMT = not more than; ND = none detected (<0.05%); NR = not reported; NT = not tested; RRT = relative retention time

a Specification has limit of NLT 98.5% cis-(-)-FTC.
b Chiral purity reported on Certificate of Analysis as %(-)FTC; value reported in table is [100% - (value reported on Certificate of Analysis)].
c Not a "Specified Impurity" at time of release testing
d Assay and chromatographic purity data generated during re-analysis using current analytical method; data will differ from Certificate of Analysis for this batch.
e Value is w/w percent from re-analysis; batch met AUC specification in place for this attribute at time of release.
f Drug product impurity at RRT ~ 0.84 is TP-0297 (FTC-dioxolane) which is a drug substance manufacturing impurity, not a drug degradation product.
g Batch met Individual Unspecified Impurity Limit in place at time of release testing (NMT 0.50% for each).
h Batch met Individual Unspecified Impurity Limit in place at time of release testing (NMT 0.30% for each).
i Used for study number(s): TOX011; TOX012.
j Used for study number(s): TOX022
k Used for study number(s): TOX022, TOX032
l Used for study number(s): TOX036, TOX037, TOX033, TOX034, TOX038, TOX039, TOX022
m Used for study number(s): TOX032
n Used for study number(s): TOX038, TOX103
o Used for study number(s): TOX097
p Used for study number(s): TOX153
q Used for study number(s): TOX146
r Used for study number(s): TOX151, TOX152
s Used for study number(s): TX-162-2001
t Used for study number(s): TOX108, TOX109
u Used for study number(s): TX-164-2004
v Used for study number(s): TX-164-2002, TX-164-2003
w Used for study number(s): K01-3154; Note: material was a mixture of lots [redacted] and [redacted].

4.3. TAF

Batch No.	Purity* (%)	Impurities (%)										Type of Study
Specification												
	99.3	—	—	—	—	—	—	—	—	—	NT	a
	99.3	—	—	—	—	—	—	1.67	—	—	NT	b
	99.0	—	—	—	—	—	—	0.29	—	—	NT	c
	96.6	—	—	—	—	—	—	1.30	—	—	NT	d
	99.7	—	—	—	—	—	—	0.75	—	—	NT	e
	97.7	0.15	0.05	0.22	Tr	Tr	Tr	0.94	0.43	0.08	NT	f
	96.2	0.15	0.09	0.18	Tr	Tr	Tr	1.25	0.50	0.09	0.28	g
	83.1	0.22	0.41	0.28	—	0.12	0.08	13.1	0.34	0.21	0.15	h
	94.3	0.08	0.08	0.05	Tr	0.07	Tr	4.53	0.08	Tr	0.16	i
	98.6	0.17	0.19	Tr	Tr	—	—	0.48	Tr	—	0.06	j
	99.3	0.13	Tr	Tr	0.06	0.16	—	0.35	—	—	NT	k

Batch No.	Purity (%)	Observed Impurities (%)												Type of Study
	97.8	0.13	Tr	0.09	0.07	—	—	0.25	0.25	—	—	0.15	—	k
	98.0	0.13	Tr	Tr	0.06	0.24	0.22	—	—	0.23	0.22	0.15	0.18	k

Batch No.	Observed Impurities (%)													
[redacted]	Tr	—	—	0.35	—	—	—	—	0.25	0.22	0.25	—	0.05	k
[redacted]	—	0.21	0.25	0.53[@]	0.26	0.24	0.25	0.23	—	—	—	—	0.06	k

—: Not detected, < 0.02%
Tr: trace < 0.05 (quantitation limit (QL) = 0.05%)
NT: Not tested

* These two batches of drug substance were used to qualify known and/or potential impurities. They were prepared by spiking process impurities and therefore have a different impurity profile to other batches.
\# Not specified in 3.2.S.4.1
@ Coeluted with GS-645502
a Used for study numbers V990212, V990213(Genetic toxicity)
b Used for study numbers R990133, R990185, D990181, R990139, R990182, D990142, D990175, R990177, R990186 (Single Dose; Repeat Dose; Other)
c Used for study number TOX-120-001 (Repeat Dose)
d Used for study number TOX-120-002 (Repeat Dose)
e Used for study numbers P2000114, R2000044, M2000113 (Repeat Dose; Genetic toxicity)
f Used for study numbers TX-120-2001, TX-120-2002, TX-120-2004, TX-120-2005, TX-120-2006, TX-120-2007, TX-120-2008, TX-120-2003 (Reprotox; Repeat Dose; Impurity Qualification)
g Used for study number TX-120-2011 (Local Tolerability)
h Used for study number TX-120-2008 (Impurity Qualification)
i Used for study number TX-120-2012 (Reprotox)
j Used for study numbers TX-120-2013, TX-120-2014 (Worker Safety)
k Used for study number TX-120-2021 (Impurity Qualification)

4.4. Ground FTC/TDF Tablets

Lot No.	% Purity (HPLC)		Impurity Content TDF (%)										Impurity Content FTC (%)						Type of Toxicity Study
	TDF	FTC																	
SPECIFICATION																			
[redacted] (non-degraded)	95.1	95.9	1.3	0.35			0.10					See below[b]	0.1		0.06			1.06	Impurity Qual.[c]
[redacted] (degraded)	89.8	80.6	4.9	2.67	0.15	0.26	0.12	0.11	0.08	0.18	0.04	See below[a]	7.7	7.51	0.14	5.96	1.64	Diox-olane 0.04	Impurity Qual.[c]
[redacted] (non-degraded)	99.8	101.5	0.7	0.56	tr							iPr-POC PMPA 0.11	0.1			0.07	tr		Impurity Qual.[d]
[redacted] (degraded)	91.3	87.2	5.4	3.25	0.55	0.28	0.35	0.14	0.10	0.24	0.15	See below[e]	11.5	3.18	0.06	6.36	1.69	Trans -(+)-FTC 0.18	Impurity Qual.[d]

RRT = relative retention time; tr = trace (observed level < 0.05%)

a 0.09% RRT 0.18; 0.05% RRT 0.35; 0.09% RRT 0.36; 0.05% RRT 0.45; 0.06% RRT 0.47; 0.06% IPC-POC PMPA; 0.12% RRT 0.86; 0.11 N6-Carbamate; 0.06% RRT 1.21.

b 0.05 RRT 0.68; 0.11 N6-Carbamate; 0.04 RRT 1.21.

c Used for study number: TX-164-2001

d Used for study number: TX-164-2005

e 0.05% PMPA; 0.07% RRT 0.759; 0.10% iPr-POC PMPA; 0.05% N6-Carbamate; 0.06% RRT 0.628

5. TOXICOLOGY: SINGLE-DOSE TOXICITY

5.1. BIC

No single-dose toxicity studies have been conducted with BIC.

5.2. FTC

Test Article: FTC

Species / Strain	Method of Administration (Vehicle)	Dose (mg/kg)	Gender / No. per Group	NOAEL (mg/kg)	Noteworthy Findings	Gilead Study No.
Mice, Charles River CD-1	Oral Gavage	4000	5 M 5 F	4000	No clinical signs of toxicity were noted. No effects on body weight were noted. No gross pathology findings were noted.	TTEP/93/0020
Mice, Charles River CD-1	Intravenous	200	5 M 5 F	200	No clinical signs of toxicity were observed. No effects on body weight were noted. No gross pathology findings were noted.	TTEP/93/0023
Rat, Charles River CD	Oral Gavage	4000	5 M 5 F	4000	No clinical signs of toxicity were observed. No effects on body weight were noted. No gross pathology findings were noted.	TTEP/93/0021
Rat, Charles River CD	Intravenous	200	5 M 5 F	200	No clinical signs of toxicity were observed. No effects on body weight were noted. No gross pathology findings were noted. The approximate lethal dose in rats dosed intravenously was > 200 mg/kg.	TTEP/93/0024

NOAEL = No-Observed-Adverse-Effect Level; M = male; F = female

5.3. TAF

Test Article: TAF

Species / Strain	Method of Administration (Vehicle)	Duration of Dosing	Dose (mg/kg)	Gender and No./Group	NOAEL (mg/kg)	Noteworthy Findings	Gilead Study No. (CRO Study No.)
Rat/ Sprague-Dawley (Harlan)	Oral Gavage (50 mM citrate buffer)	1 day (with 13 day observation period)	0, 6.25, 25, 100, 400	4/sex/group	100	Slight reductions in lymphocyte counts and significantly decreased (14%) absolute and relative liver weights were observed in females at 400 mg/kg. Histologic evaluation of tissues including lymphoid tissues and liver revealed no treatment related gross or microscopic findings.	R990133 (NA)
Rat/ Sprague-Dawley (Crl)	Oral Gavage (25 mM citric acid)	1 day (with 14 day observation period)	0, 100, 300, 1000	5/sex/group	1000	Salivation was noted immediately following dose administration in both males and females at all dose levels, with the exception of females at a dose of 100 mg/kg, and was most severe in animals treated at 1000 mg/kg. Slight reduction in BW gain was seen at 1000 mg/kg, although BW gain in these animals was comparable to the controls over Days 8 to 14 of the observation period.	R990185[a] ([redacted] 89186)

Test Article: TAF

Species / Strain	Method of Administration (Vehicle)	Duration of Dosing	Dose (mg/kg)	Gender and No./Group	NOAEL (mg/kg)	Noteworthy Findings	Gilead Study No. (CRO Study No.)
Dog/Beagle	Oral Gavage (25 mM citric acid)	1 day (with 14 day observation)	0, 30, 90, 270	1/sex/group	30 (NOEL)	Vomiting, retching and salivation observed at 270 mg/kg. This was more pronounced in the male that also showed signs of reduced activity, weakness, tremors and incoordination. Salivation was also noted at 90 mg/kg. BW was variable between groups, with the most marked loss seen in the 270 mg/kg male. FC was also variable with markedly lower consumption in the male at 270 mg/kg. An increase in BUN was also noted in the male treated at 270 mg/kg, and in females at 30, 90 and 270 mg/kg on Day 2. Differences were no longer apparent by Day 14. Increases in kidney and spleen weight relative to BW were noted for males at 30, 90 and 270 mg/kg, with an increase in relative spleen weight also observed in females at 30 or 270 mg/kg. Treatment-related renal tubular changes characterized by basophilia and karyomegaly were seen in the male and female at 270 mg/kg, and also the female at 90 mg/kg. Moderate atrophy of the thymus was noted in the male treated at 270 mg/kg and minimal atrophy was noted in the male treated at 90 mg/kg. Etiology of the thymus change was uncertain.	D990181[a] ([redacted] 89187)

NOEL = No-Observed-Effect Level; NOAEL = No-Observed-Adverse-Effect Level; M = male; F = female; NA = not applicable; BW = body weight; FC = food consumption; BUN = blood nitrogen urea
a Indicates GLP compliant

5.4. TDF

Test Article: TDF

Species / Strain	Method of Administration (Vehicle)	Duration of Dosing	Dose (mg/kg)	Gender and No. / Group	NOAEL (mg/kg)	Noteworthy Findings	Gilead Study No.
Rat/ Sprague-Dawley CD (Crl:CD®[SD]IGSBR)	Oral Gavage (aqueous suspension vehicle)	1 day (with 14 day observation period)	0, 160, 500, 1500	5/sex/group	1500 mg/kg	NOAEL = 1500 mg/kg	R990200

NOAEL = No-Observed-Adverse-Effect Level

6. REPEAT DOSE TOXICITY (NONPIVOTAL STUDIES)

6.1. BIC

No repeat-dose nonpivotal studies have been conducted with BIC.

6.2. TAF

Test Article: TAF

Species / Strain	Method of Administration (Vehicle / Formulation)	Duration of Dosing	Dose (mg/kg/day)	Gender and No. per Group	NOAEL (mg/kg/day)	Noteworthy Findings	Gilead Study No. (CRO Name and Study No.)
Mouse/ Crl:CD1(ICR)	Oral Gavage (0.1% (v/v) Tween 20 and 0.1% (w/v) hydroxypropylmethylcellulose (HPMC) K100LV in reverse osmosis water)	2 weeks	0, 100, 500, 1000	10/sex/group	100	Early deaths occurred at 500 and 1000 mg/kg/day. Test article-related effects included minimal to moderate clinical pathology changes at 500 or 1000 mg/kg/day and anatomic pathology findings for all animals given GS-7340-02. NOAEL; 100 mg/kg/day	TX-120-2006 ([redacted] 8235665)
Rat/ Sprague Dawley	Oral Gavage	7 days	0, 6.25, 25, 100, 400	4/sex/group	NA	Significant effects at 400 mg/kg/day: decrease in: body weight gain, kidney weights WBC and splenic extramedullary hematopoiesis. Dose-dependent increase in PTH and a non-dose dependent reduction in 1,25-dihydroxycholecalciferol at all dose levels. The NOEL for this study was determined to be < 6.25 mg	R990139 (NA)
Rabbit/ NZW	Oral Gavage	7 days	0, 20, 50, 75	3 males/group	75	Slight decrease in cumulative body weight gain at 50 and 75 mg/kg/day	TX-120-2003 (902246)

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

7.1. BIC

7.1.1. 4-Week Study in Mice

Test Article: BIC

Report Title: 4-Week Oral Gavage Dose Range-Finding Toxicity and Toxicokinetic Study with GS-9883-01 in 001178-W (Wild Type) RasH2 Mice

Gilead Study No. TX-141-2042
[redacted] Study No. 8316726

Species / Strain: Mouse / RasH2 (001178-W [wild type], CByB6F1-Tg[HRAS]2Jic)

Duration of Dosing: 4 weeks

Initial Age: 7 to 9 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: 0.5% (w/w) hydroxypropyl methylcellulose (Methocel K100 LV) and 0.1% (w/w) Tween® 20 in reverse osmosis water

Special Features: None

No Observed Adverse Effect Level: 1000 mg/kg/day

Daily Dose (mg/kg/day)	0 (Control)		30		100		1000	
Gender:Number of Animals (TK)	**M: 6**	**F: 6**	**M: 41**	**F: 41**	**M: 41**	**F: 41**	**M: 41**	**F: 41**
Toxicokinetics: AUC_{0-24} (µg·h/mL)								
Day 1	NA[a]	NA[a]	679	610	1310	1280	2590	2390
Week 4	NA[a]	NA[a]	606	652	1030	1330	2230	2420
Toxicokinetics: C_{max} (µg/mL)								
Day 1	NA[a]	NA[a]	65.3	50.0	97.4	121	141	158
Week 4	NA[a]	NA[a]	62.5	55.1	90.0	108	146	175

Daily Dose (mg/kg/day)	0 (Control)		30		100		1000	
Gender: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	0	0	0
Clinical Observations	No Test Article-Related Findings							
Body Weight Change (g)								
Day 22 to 29	0.8	-	0.8	-	0.6	-	0.2*	-
Food Consumption	No Test Article-Related Findings							
Hematology	No Test Article-Related Findings							
Coagulation	No Test Article-Related Findings							
Clinical Chemistry	No Test Article-Related Findings							
Organ Weights	No Test Article-Related Findings							
Gross Pathology	No Test Article-Related Findings							
Histopathology								
Liver								
Glycogen, decreased, hepatocyte, centrilobular								
Minimal	0	0	0	0	0	0	7	0
Slight	0	0	0	0	0	0	1	0

AUC_{0-24} = Area under concentration-time curve from time 0 to 24 hours postdose; C_{max} = Maximum observed drug concentration; M = Male; F = Female; TK = Toxicokinetic; NA = Not Applicable; − Comparable to control animals or no test article-related findings; * = $p \leq 0.05$
a Values below the lower limit of quantitation

7.1.2. 2-Week Study in Rats

Test Article: BIC

Report Title: 2-Week Oral Gavage Toxicity and Toxicokinetic Study with GS-9883 in Han Wistar Rats with Bone Marrow Micronucleus Assay

Gilead Study No. TX-141-2029
[redacted] Study No. 8292936

Species / Strain: Rat / Crl:WI(Han)

Duration of Dosing: 2 weeks

Initial Age: 6 weeks old

Duration of Postdose Recovery: Not applicable

GLP Compliance: Yes

Date of First Dose: [redacted] 20[redacted]

Method of Administration: Oral gavage

Lot Number: [redacted]

Vehicle: 0.5% (w/w) hydroxypropyl methylcellulose (Methocel K100 LV) and 0.1% (w/w) Tween 20® in reverse osmosis water

Positive Control Article for Micronucleus Assay (Group 6): Cyclophosphamide

Special Features: Hepatic microsomal enzyme analysis and bone marrow micronucleus assay

No Observed Adverse Effect Level: 300 mg/kg/day

Daily Dose (mg/kg/day)	**0 (Control)**		**10**		**30**		**100**		**300**	
Gender:Number of Animals (TK)	**M: 3**	**F: 3**	**M: 9**	**F: 9**	**M: 9**	**F: 9**	**M: 9**	**F: 9**	**M: 9**	**F: 9**
Toxicokinetics: AUC_{0-24} (µg·hr/mL)										
Day 1	NA[a]	NA[a]	712	867	1670	1790	2470	3310	2860	3850
Day 14	NA[a]	NA[a]	835	1490	1470	2110	1820	3290	2270	3670
Toxicokinetics: C_{max} (µg/mL)										
Day 1	NA[a]	NA[a]	42.2	41.6	97.0	90.9	138	180	141	182
Day 14	NA[a]	NA[a]	66.0	74.8	84.2	131	115	185	117	189

Daily Dose (mg/kg/day)	0 (Control)		10		30		100		300	
Gender:Number of Animals (Main)	**M: 10**	**F: 10**	**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	0	0	0	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[b]										
Red blood cell count ($E^6/\mu L$)	-	8.52	-	-3.29	-	-6.22*	-	-1.88	-	-5.28*
Hematocrit (%)	-	47.7	-	-2.10	-	-2.94	-	+0.42	-	-3.56*
Serum Chemistry[b]										
Inorganic phosphorus (mg/dL)	8.3	-	-1.2	-	+6.0	-	+3.6	-	+9.6*	-
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 Enzyme Analysis	No Test Article-Related Findings									
Bone Marrow Micronucleus Assay	No Test Article-Related Findings									

AUC_{0-24} = Area under the concentration-time curve from hour 0 to hour 24; C_{max} = Maximum observed concentration; M = Male; F = Female; TK = Toxicokinetic;
NA = Not Applicable; − Comparable to control animals or no test article-related findings; * = $p \leq 0.05$ (ANOVA and Dunnett's t-test)
a Values below the lower limit of quantitation (<1000 ng/mL)
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.

7.1.3. 26-Week Study in Rats

Test Article: BIC

Report Title: 26-Week Oral Gavage Toxicity and Toxicokinetic Study with GS-9883-01 in Han Wistar Rats with a 4-Week Recovery Phase

Gilead Study No. TX-141-2031
[redacted] Study No. 8298346

Species / Strain: Rat / Crl:WI(Han)

Duration of Dosing: 26 weeks

Initial Age: 6 to 7 weeks

Duration of Postdose: 4 weeks

GLP Compliance: Yes

Date of First Dose: [redacted] 20[redacted]

Method of Administration: Oral gavage

Lot Number: [redacted]

Vehicle: 0.5% (w/w) hydroxypropyl methylcellulose (Methocel K100 LV) and 0.1% (w/w) Tween® 20 in reverse osmosis water

Special Features: Starting Week 11 of the dosing phase, and continuing throughout the remainder of the study, all dose formulations were pH-adjusted to 7.5 to 8.5.

No Observed Adverse Effect Level: 300 mg/kg/day

Daily Dose (mg/kg/day)	**0 (Control)**		**5**		**30**		**300**	
Gender:Number of Animals (TK)	**M: 3**	**F: 3**	**M: 9**	**F: 9**	**M: 9**	**F: 9**	**M: 9**	**F: 9**
Toxicokinetics: $AUC_{0\text{-}24}$ (µg·hr/mL)								
Day 1	NA[a]	NA[a]	341	436	1420	1870	2380	3530
Week 13 (Day 90)	NA[a]	NA[a]	460	1420	1040	2810	1880	4570
Week 26 (Day 181)	NA[a]	NA[a]	457	1290	1010	2910	1830	4680
Toxicokinetics: C_{max} (µg/mL)								
Day 1	NA[a]	NA[a]	19.4	22.9	82.4	113	143	185
Week 13 (Day 90)	NA[a]	NA[a]	33.5	72.4	75.0	162	92.7	212
Week 26 (Day 181)	NA[a]	NA[a]	28.8	70.0	76.9	169	100	213

Daily Dose (mg/kg/day)	**0 (Control)**		**5**		**30**		**300**	
Gender:Number of Animals (Main)	**M: 20**	**F: 20**	**M: 15**	**F: 15**	**M: 15**	**F: 15**	**M: 20**	**F: 20**
Noteworthy Findings								
Died or Sacrificed Moribund	0	1	0	2	1	0	1	1
Clinical Observations	No Test Article-Related Findings							
Body Weight	No Test Article-Related Findings							
Food Consumption	No Test Article-Related Findings							
Ophthalmoscopy	No Test Article-Related Findings							
Hematology	No Test Article-Related Findings							
Coagulation	No Test Article-Related Findings							
Clinical Chemistry								
Inorganic Phosphorus (mg/dL)								
Week 13 (Day 86)	7.3	6.2	7.4	6.8	8.2*	7.6*	7.8	7.6*
Week 26 (Day 184)	5.2	4.3	5.1	5.0	5.6	5.7*	6.0*	5.5*
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							
Postdose Evaluation	No Test Article-Related Findings							

AUC_{0-24} = Area under the concentration-time curve from hour 0 to hour 24; C_{max} = Maximum observed concentration; M = Male; F = Female; TK = Toxicokinetic;
NA = Not Applicable; − Comparable to control animals or no test article-related findings; * = $p \leq 0.05$
a Values below the lower limit of quantitation (<1000 ng/mL)

7.1.4. 2-Week Study in Monkeys

Test Article: BIC

Report Title: 2-Week Oral Gavage Toxicity and Toxicokinetic Study with GS-9883 in Cynomolgus Monkeys

Gilead Study No. TX-141-2030
[redacted] Study No. 8293153

Species / Strain: Primate / Cynomolgus (*Macaca fascicularis*)

Duration of Dosing: 2 weeks

Initial Age: 2 to 3 years old

Duration of Postdose: Not applicable

GLP Compliance: Yes

Date of First Dose: [redacted] 20[redacted]

Method of Administration: Oral gavage

Lot Number: [redacted]

Vehicle: 0.5% (w/w) hydroxypropyl methylcellulose (Methocel K100 LV) and 0.1% (w/w) Tween 20® in reverse osmosis water

Special Features: Hepatic microsomal enzyme analysis

No Observed Adverse Effect Level: 1000 mg/kg/day

Daily Dose (mg/kg/day)	**0 (Control)**		**30**		**100**		**1000**	
Gender:Number of Animals	**M: 3**	**F: 3**	**M: 3**	**F: 3**	**M: 3**	**F: 3**	**M: 3**	**F: 3**
Toxicokinetics: $AUC_{0\text{-}24}$ (µg·hr/mL)								
Day 1	NA[a]	NA[a]	306	247[b]	342	280	1250	1060
Day 14	NA[a]	NA[a]	273	152	413	236	1170	1020
Toxicokinetics: C_{max} (µg/mL)								
Day 1	NA[a]	NA[a]	26.9	22.5[b]	29.8	24.4	109	91.6
Day 14	NA[a]	NA[a]	20.3	14.9	27.0	21.0	87.5	70.8
Noteworthy Findings								
Died or sacrificed moribund	0	0	0	0	0	0	0	0
Clinical Observations	No Test Article-Related Findings							
Body Weight	No Test Article-Related Findings							
Food Consumption	No Test Article-Related Findings							
Ophthalmoscopy	No Test Article-Related Findings							

Daily Dose (mg/kg/day)	**0 (Control)**		**30**		**100**		**1000**	
Electrocardiogram Examinations	No Test Article-Related Findings							
Hematology	No Test Article-Related Findings							
Serum Chemistry	No Test Article-Related Findings							
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 Enzyme Analysis								
Ethoxyresorufin O-deethylase (CYP1A) Activity (pmol/min/mg protein)	233	-	281	-	272	-	96.4**	-

AUC_{0-24} = Area under the concentration-time curve from hour 0 to hour 24; C_{max} = Maximum observed concentration; M = Male; F = Female; TK = Toxicokinetic; NA = Not Applicable

− = Comparable to control animals or no test article-related findings; ** = Represents a notable change, which is less than half compared to the control value

a Values below the lower limit of quantitation (<1000 ng/mL)

b On Day 1, Animal No. I00413 (Group 2 female) vomited immediately after dosing; therefore, this animal was excluded from descriptive statistics and interpretation.

7.1.5. 39-Week Study in Monkeys

Test Article: BIC

Report Title: 39-Week Oral Gavage Toxicity and Toxicokinetic Study with GS-9883-01 in Cynomolgus Monkeys with a 13-Week Interim Sacrifice and a 4-Week Recovery Phase

Gilead Study No. TX-141-2032
[redacted] Study No. 8298309

Species / Strain: Primate / Cynomolgus (*Macaca fascicularis*)

Duration of Dosing: 39 weeks

Initial Age: 30 to 47 months

Duration of Postdose: 4 weeks

GLP Compliance: Yes

Date of First Dose: [redacted] 20[redacted] (Males)
[redacted] 20[redacted] (Females)

Method of Administration: Oral gavage

Lot Number: [redacted]

Vehicle: 0.5% (w/w) hydroxypropyl methylcellulose (Methocel K100 LV) and 0.1% (w/w) Tween® 20 in reverse osmosis water

Special Features: 13-week interim necropsy and a 4-week recovery period after 39-weeks of dosing

No Observed Effect Level: 200 mg/kg/day

Daily Dose (mg/kg/day)	**0 (Control)**		**30**		**200**		**1000**	
Gender:Number of Animals	**M: 9**	**F: 9**	**M: 7**	**F: 7**	**M: 7**	**F: 7**	**M: 9**	**F: 9**
Toxicokinetics: AUC_{0-t} (µg ·hr/mL)								
Day 1	NA[a]	NA[a]	280	243	514	797	1450	1520
Week 13	NA[a]	NA[a]	288	254	855	664	1540	1180
Week 39	NA[a]	NA[a]	248	253	595	823	1450	1750
Toxicokinetics: C_{max} (µg /mL)								
Day 1	NA[a]	NA[a]	40.1	24.6	43.4	55.1	97.3	111
Week 13	NA[a]	NA[a]	30.2	25.1	66.7	59.7	112	89.8
Week 39	NA[a]	NA[a]	28.3	24.4	67.8	70.4	104	109

Daily Dose (mg/kg/day)	**0 (Control)**		**30**		**200**		**1000**	
Noteworthy Findings								
Died or Sacrificed Moribund	0	0	0	0	0	0	0	0
Clincal Observations[b]								
Emesis	73 (6)	-	8 (2)	-	8 (4)	-	194 (9)	-
Discharge, vomitus	87 (7)	-	16 (6)	-	15 (5)	-	293 (9)	-
Body Weight	No Test-Article Related Findings							
Food Consumption	No Test-Article Related Findings							
Electrocardiographic Evaluations	No Test-Article Related Findings							
Ophthalmoscopy	No Test-Article Related Findings							
Hematology	No Test-Article Related Findings							
Coagulation	No Test-Article Related Findings							
Clinical Chemistry								
Alanine Aminotransferase (U/L)								
Week 4	32	-	39	-	32	-	86*	-
Week 13	40	-	49	-	53	-	157*	-
Week 26	35	35	43	31	44	47	98	118
Week 39	40	44	73	43	68	77	95*	95*
Urinalysis	No Test-Article Related Findings							
Organ Weights	No Test-Article Related Findings							
Gross Pathology								
Interim Sacrifice (Week 13) (n=3/sex/group)	No Test-Article Related Findings							
Terminal Sacrifice (Week 39)								
Gender:Number of Animals	**M:4**	**F:4**	**M:4**	**F:4**	**M:4**	**F:4**	**M:4**	**F:4**
Liver								
Discolored	0	0	0	0	0	0	1	0
Rough Surface	0	0	0	0	0	0	1	0

Daily Dose (mg/kg/day)	**0 (Control)**		**30**		**200**		**1000**	
Histopathology								
Interim Sacrifice (Week 13) (n=3/sex/group)	No Test-Article Related Findings							
Terminal Sacrifice								
Gender:Number of Animals	**M:4**	**F:4**	**M:4**	**F:4**	**M:4**	**F:4**	**M:4**	**F:4**
Liver								
Hyperplasia, bile duct								
Minimal	0	0	0	0	0	0	2	1
Slight	0	0	0	0	0	0	0	2
Moderate	0	0	0	0	0	0	1	1
Marked	0	0	0	0	0	0	1	0
Hypertrophy, hepatocyte								
Minimal	0	0	0	0	0	0	0	1
Moderate	0	0	0	0	0	0	1	0
Hyperplasia, regenerative								
Minimal	0	0	0	0	0	0	1	0
Infiltrate, neutrophils								
Minimal	0	0	0	0	0	0	1	0
Slight	0	0	0	0	0	0	1	0
Postdose Evaluation								
Gender:Number of Animals	**M: 2**	**F: 2**	**M: 0**	**F: 0**	**M: 0**	**F: 0**	**M: 2**	**F: 2**
Histopathology								
Recovery Sacrifice								
Liver								
Hyperplasia, bile duct								
Slight	0	0	NA	NA	NA	NA	0	1
Marked	0	0	NA	NA	NA	NA	1	0

Daily Dose (mg/kg/day)	0 (Control)		30		200		1000	
Hypertrophy, hepatocyte								
Slight	0	0	NA	NA	NA	NA	1	0
Hyperplasia, regenerative								
Minimal	0	0	NA	NA	NA	NA	1	0
Infiltrate, lympohcytes								
Slight	0	0	NA	NA	NA	NA	1	0

AUC_{0-t} = Area under the concentration-time curve from hour 0 to last measurable concentration; C_{max} = Maximum observed concentration; M = Male; F = Female;
TK = Toxicokinetic; NA = Not Applicable; − Comparable to control animals or no test article-related findings; * = $p \leq 0.05$
a Values below the lower limit of quantitation (<1000 ng/mL)
b Observations presented as Number of Occurances in Dosing phase (Number of of Animals Affected).

7.2. FTC

7.2.1. 2-Week Oral Toxicity Study in Mice

Test Article: FTC

Report Title: 14-Day Oral (Gavage) Toxicity Study in Mice Given FTC **Gilead Study No.:** TOX001 ([redacted] IUW00701)
Species/Strain: Mouse / Charles River CD-1 **Duration of Dosing:** 14 Days **Location in CTD:** 4.2.3.2
Initial Age: Approximately 3-4 Weeks Old **Duration of Postdose:** 14 Days **GLP Compliance:** Yes
Date of First Dose: [redacted] 19[redacted] **Vehicle/Formulation:** 0.5% methylcellulose **Lot No.:** [redacted]
No Observed Adverse-Effect Level: 3000 mg/kg **Method of Administration:** Oral, divided doses spaced by approximately six hours

Dose (mg/kg)	**0 (Vehicle)**		**120**		**600**		**3000**	
Number of Animals	**M: 15**	**F: 15**	**M: 15**	**F: 15**	**M: 15**	**F: 15**	**M: 15**	**F: 15**
Plasma Conc. 45 Min Post-Dose: (µg/mL; Mean ± SD)								
Day 2	NA	NA	12.2 ± 1.87	11.7 ± 1.39	62.7 ± 9.69	63.0 ± 2.83	318 ± 4.3	358 ± 108.2
Day 14	NA	NA	13.6 ± 3.37	14.7 ± 10.01	66.9 ± 2.48	55.4 ± 2.04	389 ± 85.4	405 ± 59.2
Noteworthy Findings								
Died or Sacrificed Moribund[a]	1	0	0	0	0	0	0	1
Clinical Observations	No treatment related findings.							
Body Weight (%)	No treatment related findings.							
Food consumption (%)	No treatment related findings.							
Ophthalmic Examinations	No treatment related findings.							
Hematology and Chemistry	No treatment related findings.							
Organ Weights (%)	No treatment related findings.							
Gross and Histopathology	No treatment related findings.							
Additional Examinations	Proof of Absorption – An additional 3 groups of 8 M/F mice received 120, 600, and 3000 mg/kg/day FTC, daily by oral gavage, split over two doses, for 2 or 14 days. Blood samples were obtained from 3 M/F from these groups approximately 45 minutes after the first dose on that day. An additional 5 M/F mice in groups 1-4 were used for a pre-study blood screen.							
Postdose Evaluation	5 M/F per group continued without dosing for 14 days to investigate recovery: No FTC-related findings were noted.							

NA = not applicable
a Two mice died during the study; both were considered to be a result of gavage error.

7.2.2. 4-Week Toxicity Study in Mice

Test Article: FTC

Report Title: A Thirty-Day Oral Toxicity Study in Mice Given 524W91 **Gilead Study No.: TOX599**

Species/Strain: Mouse / Charles River CD-1 **Duration of Dosing:** 30-32 Days **Location in CTD:** 4.2.3.2

Initial Age: Approximately 48 day males and 52 day females **Duration of Postdose:** 13 Days **GLP Compliance:** Yes

Date of First Dose: [redacted] 19[redacted] **Method of Administration:** Oral **Lot No.:** [redacted]

Special Features: Electron microscopy **Vehicle/Formulation:** Vehicle control: 0.5% aqueous methylcellulose

No Observed Adverse-Effect Level: 600 mg/kg

Dose (mg/kg)	**0 (Vehicle)**		**120**		**600**		**3000**	
Number of Animals	**M: 14**	**F: 14**	**M: 14**	**F: 14**	**M: 14**	**F: 14**	**M: 14**	**F: 14**
Toxicokinetics:								
C_{max} ss (µg/mL)								
Day 3	NA	NA	40.8	52.2	200	196	749	623
Day 32	NA	NA	52.2	38.8	219	211	742	675
AUCss (µg.h/mL)								
Day 3	NA	NA	78.1	83.1	410	326	1800	1711
Day 32	NA	NA	103	73.9	499	304	1680	1320
Noteworthy Findings								
Died or Sacrificed Moribund[a]	0	0	0	1	0	0	4	2
Clinical Observations	No treatment related findings.							
Body Weight (%)	No treatment related findings.							
Food Consumption (%)	No treatment related findings.							
Ophthalmic Examinations	No treatment related findings.							

Dose (mg/kg)	**0 (Vehicle)**		**120**		**600**		**3000**	
Number of Animals	**M: 14**	**F: 14**	**M: 14**	**F: 14**	**M: 14**	**F: 14**	**M: 14**	**F: 14**
Hematology	The once-daily administration of 3000 mg/kg for 30–32 days resulted in decreased total erythrocyte count, hemoglobin, and hematocrit and increased mean corpuscular volume, mean corpuscular hemoglobin, and red cell distribution width. The no-effect level for hematological changes was 600 mg/kg/day.							
Hematocrit								D* t
Hemoglobin								D t
Total erythrocyte count							D*	D* t
Mean corpuscular volume							I*	I t
Mean corpuscular hemoglobin							I*	I* t
Red cell distribution width							I*	I* t
Serum Chemistry	No treatment related findings.							
Urinalysis	No treatment related findings.							
Organ Weights (%)	In females, mean relative organ weight (for pituitary, heart, spleen, ovary) increased at 3000 mg/kg/day. In males, mean relative spleen weight increased at 3000 mg/kg/day and decreased for testes and thymus at 3000 mg/kg/day. The weight changes were unaccompanied by any histopathological alterations.							
Changes in organ weights (no histopathological changes)							D-Thymus	I-Pituitary
							D-Testes	I-Heart
							I-Spleen	I-Ovary
								I-Spleen
Gross and Histopathology	No treatment related findings.							
Electron Microscopy	No drug related ultrastructural changes noted in heart and skeletal muscle sampled from 5 mice/sex/group and from 5 males and 5 females from the control and 3000 mg/kg/day groups.							
Postdose Evaluation:	Four mice/sex/group held 13 days for post-dose recovery.							

NA = not applicable; D = decrease; I = increase; p = permanent; t = transitory; * = $p < 0.05$ or < 0.01

a Seven animals died, one animal in the 120 mg/kg/day group and 6 in the 3000 mg/kg/day group. Cause of death was either mis-dosing, or probable mis-dosing in 5 animals. The cause of death was undetermined in 2 high-dose mice (3000 mg/kg/day), and a relationship to drug treatment could not be ruled out.

7.2.3. 26-Week Toxicity Study in Mice

Test Article: FTC

Report Title: Six Month Oral (Gavage) Toxicity Study in Mice Given FTC with a 3-Month Interim Sacrifice

Gilead Study No. TOX022 ([redacted] IUW01001)

Species/Strain: Mouse / Charles River CD-1

Duration of Dosing: 13 Weeks

Location in CTD: 4.2.3.2

Initial Age: 4 – 5 Weeks

Duration of Postdose: 1 Month

GLP Compliance: Yes

Date of First Dose: [redacted] 19[redacted]

Method of Administration: Oral Gavage

Lot No.: [redacted]

No Observed Adverse-Effect Level: 500 mg/kg/day

Vehicle/Formulation: 0.5% aqueous methylcellulose

Dose (mg/kg)	**0 (Vehicle)**		**167**		**500**		**1500**	
Number of Animals	**M: 30**	**F: 30**	**M: 30**	**F: 30**	**M: 30**	**F: 30**	**M: 30**	**F: 30**
Toxicokinetics:								
C_{max} (µg/mL)								
3 months	NA	NA	44.0	37.2	128.0	88.8	225.0	187.1
6 months	NA	NA	41.7	48.6	87.3	111.7	310.9	306.0
AUC $_{0\text{-}24}$ (µg.h/mL)								
3 months	NA	NA	63.8	67.9	181.4	209.6	513.4	569.5
6 months	NA	NA	82.3	93.1	248.4	284.4	732.1	899.4
Noteworthy Findings								
Died or Sacrificed Moribund[a]	2	0	0	0	0	0	0	0
Clinical Observations	No treatment related findings.							
Body Weight (%)	No treatment related findings.							
Food consumption (%)	No treatment related findings.							
Ophthalmoscopy	No treatment related findings.							

Dose (mg/kg)	0 (Vehicle)		167		500		1500	
Number of Animals	**M: 30**	**F: 30**	**M: 30**	**F: 30**	**M: 30**	**F: 30**	**M: 30**	**F: 30**
Hematology	Mean corpuscular volume and mean cell hemoglobin showed a small increase at 1500 mg/kg/day at week 27. This was more notable in females and was still slightly elevated in females at the end of the recovery period.							
Serum Chemistry	No treatment related findings.							
Organ Weights (%)	No treatment related findings.							
Gross Pathology	No treatment related findings.							
Histopathology	No treatment related findings.							
Postdose Evaluation	Changes in mean cell volume and hemoglobin still evident at end of recovery period.							

NA = not applicable

a There were 4 deaths during the study. One control male was sacrificed on Day 7 (had shown convulsions, piloerection, and unsteady gait on day 6; no notable findings at necropsy); and a further control male was killed on Day 128 (animal was prostrate, subdued, had weight loss, and had lost the use of the hindlimbs; no notable findings at necropsy but at histopathological evaluation had evidence of a possible spinal injury). A group 6 male was found dead (following a convulsion and also showed weight loss) on day 85 and 1 group 7 male was found dead on day 25 (no clinical signs). Deaths were considered unrelated to FTC treatment.

7.2.4. 6-Month Oral Toxicity Study in Mice

Test Article: FTC

Report Title: A Six Month Oral Toxicity Study (With a Three Month Interim Sacrifice) in Mice Given 524W91 **Gilead Study No. TOX628**

Species/Strain: Mouse / Charles River CD-1 **Duration of Dosing:** 6 Months **Location in CTD:** 4.2.3.2

Initial Age: Approx. M: 62 days; **F:** 67 days **Duration of Postdose:** 3 Weeks **GLP Compliance:** Yes

Date of First Dose: [redacted] 19[redacted] **Method of Administration:** Oral Gavage

Special Features: Electron microscopy **Vehicle/Formulation:** 0.5% aqueous methylcellulose

No Observed Adverse-Effect Level: 600 mg/kg/day

Dose (mg/kg)	**0 (Vehicle)**		**120**		**600**		**3000**	
Number of Animals	**M: 25**	**F: 25**	**M: 25**	**F: 25**	**M: 25**	**F: 25**	**M: 25**	**F: 25**
Toxicokinetics:								
C_{max} (µg/mL)	NA	NA	69.0	44.7	151	188	634	502
AUC[a] (µg.h/mL)	NA	NA	170	115	585	490	2024	1548
Noteworthy Findings								
Died or Sacrificed Moribund[b]	1	0	0	0	0	0	1	1
Clinical Observations	No treatment related findings.							
Body Weight (%)	No treatment related findings.							
Food Consumption (%)	No treatment related findings.							
Ophthalmic Examinations	No treatment related findings.							
Hematology	Very slight and reversible changes in total erythrocyte count (RBC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and red cell distribution width (RDW) relative to control values were observed in male and female mice given 3000 mg/kg/day. These changes were present after approximately 3 months of dosing and were no greater in severity after 6 months of dosing.							
Total erythrocyte count							D t ns	D t ns
Mean corpuscular volume							I t ns	I t ns
Mean corpuscular hemoglobin							I t ns	I t ns
Red cell distribution width							I t ns	I t ns

Dose (mg/kg)	**0 (Vehicle)**		**120**		**600**		**3000**	
Number of Animals	**M: 25**	**F: 25**	**M: 25**	**F: 25**	**M: 25**	**F: 25**	**M: 25**	**F: 25**
Serum Chemistry	No treatment related findings.							
Urinalysis	A very slight reversible increase in urine quantity was noted on dose day 179 in male and female mice given 3000 mg/kg/day. However, no treatment-related microscopic changes were observed in the kidney to correlate with this finding.							
Organ Weights (%)	There was a reversible increase in absolute and relative thyroid weights in female mice given 3000 mg/kg/day. Values were within control ranges and there were no histological changes to correlate with this finding.							
Gross Pathology	No treatment related findings.							
Histopathology	No treatment related findings.							
Electron Microscopy	Liver, heart, and skeletal muscle (from terminal sacrifice control and high-dose mice; first 3/sex/group) – No treatment related changes.							
Postdose Evaluation	Findings were noted to be reversible during the recovery period.							

NA = not applicable; d = decrease; I = increase; P = permanent; t = transitory; ns = not significant

a Drug plasma concentrations were evaluated in 3 additional groups: 28 males, 28 females in each group. Three animals/group/sex were bled at 4 time points (0.5, 4, 12 and 24 hours) after dosing on days 175 and 176. Body weights and observations for clinical signs of toxicity were also evaluated in these animals. C_{max} and AUC increased with dose. Females had statistically significantly lower ($p < 0.05$) plasma AUC values than did males at all dose levels.

b 6 animals died during the study. Based on the clinical signs, gross and/or histopathological findings, the deaths were considered unrelated to treatment. Two additional deaths occurred in the toxicokinetic groups. The cause of death was undetermined in these 2 males (120 mg/kg/day, 600 mg/kg/day) and the deaths were considered unrelated to treatment since no treatment-related deaths occurred at 3000 mg/kg/day in the toxicokinetic groups.

7.2.5. 3-Month Oral Toxicity Study in Rats

Test Article: FTC

Report Title: A 3-Month Oral Gavage Study for Bioassay Dose Selection in CD Rats **Gilead Study No. TOX097**

Species/Strain: Rat / CD **Duration of Dosing:** 90 Days **Location in CTD:** 4.2.3.2

Initial Age: Approximately 6 Weeks Old **Duration of Postdose:** None **GLP Compliance:** Yes

Date of First Dose: [redacted] 20[redacted] **Method of Administration:** Oral **Lot No.:** [redacted]

No Observed Adverse-Effect Level: 600 mg/kg/day **Vehicle/Formulation:** 0.5% aqueous methylcellulose

Dose (mg/kg)	**0 (Vehicle)**		**120**		**600**		**3000**	
Number of Animals	**M: 10**	**F: 10**	**M: 10**	**F: 10**	**M: 10**	**F: 10**	**M: 10**	**F: 10**
Toxicokinetics:								
C_{max} (µg/mL)								
Day 2	NA	NA	15.3	18.1	59.2	73.2	211	225
Day 90	NA	NA	17.5	21.0	77.9	120	222	323
AUC_{0-24} (µg.h/mL)								
Day 2	NA	NA	61.6	55.6	296	260	1413	1357
Day 90	NA	NA	65.6	69.3	329	362	1275	1646
Died or Sacrificed Moribund	0	0	0	0	0	0	0	0
Clinical Observations	No treatment-related findings.							
Body Weight (%)	No treatment-related findings.							
Food Consumption (%)	No treatment-related findings.							
Hematology	Slightly decreased RBC counts and hemoglobin values were observed in both sexes at 3000 mg/kg/day, which reached statistical significance for females only.							
Serum Chemistry	No treatment-related findings.							
Urinalysis	No treatment-related findings.							
Organ Weights (%)	Pituitary and thyroid/parathyroid gland weights were decreased at 3000 mg/kg/day.							
Gross Pathology/Histopathology	No treatment-related findings.							

NA = not applicable; M = male; F = female

7.2.6. 1-Month Oral Toxicity Study in Monkeys

Test Article: FTC

Report Title: A 30-Day Oral Toxicity Study in Cynomolgus Monkeys Given 524W91 **Gilead Study No. TOX600**

Species/Strain: Monkey/Cynomolgus (Macaca fascicularis) **Duration of Dosing:** At least 28 Days **Location in CTD:** 4.2.3.2

Initial Age: Approximately 2.5–7 year males and 6–17 year females **Duration of Postdose:** 3 Weeks **GLP Compliance:** Yes

Date of First Dose: [redacted] 19[redacted] **Method of Administration:** Oral, divided doses spaced by approximately six hours **Lot No.:** [redacted]

Vehicle/Formulation: 0.5% aqueous methylcellulose

No Observed Adverse-Effect Level: 400 mg/kg/day **Special Features:** Electron microscopy, CSF, Neurophysiology

Dose (mg/kg)	**0 (Vehicle)**		**80**		**400**		**2000**	
Number of Animals	**M: 5**	**F: 5**	**M: 5**	**F: 5**	**M: 5**	**F: 5**	**M: 5**	**F: 5**
Toxicokinetics:								
C_{max} (µg/mL)								
Day 3	NA	NA	14.6	15.8	58.3	66.0	194	204
Day 27	NA	NA	12.4	12.9	63.8	63.0	202	191
AUC^{a}_{0-6} (µg.h/mL)								
Day 3	NA	NA	37.1	37.8	170	170	631	765
Day 27	NA	NA	31.6	33.6	196	164	730	732
Died or Sacrificed Moribund	0	0	0	0	0	0	0	0
Clinical Observations	Increased incidence of soft feces in females given 2000 mg/kg/day.							
Body Weight (%)	No treatment-related findings.							
Food Consumption (%)	No treatment-related findings.							
Ophthalmoscopy[b]	No treatment-related findings.							
Electrocardiography[c]	No treatment-related findings.							
Hematology/Serum Chemistry	No treatment-related findings.							

Dose (mg/kg)	**0 (Vehicle)**		**80**		**400**		**2000**	
Number of Animals	**M: 5**	**F: 5**	**M: 5**	**F: 5**	**M: 5**	**F: 5**	**M: 5**	**F: 5**
Urinalysis	No treatment-related findings.							
Organ Weights (%)[d]	No treatment-related findings.							
Gross Pathology Necropsy [d]	No treatment-related findings.							
Histopathology [d]	No treatment-related findings.							
Additional Examinations								
Electronic microscopy[e]	Focal areas of "moth-eaten" mitochondria were seen in control (Group 1) cardiac muscle specimens. Moth-eaten mitochondria were also seen in cardiac muscle in the 2000 mg/kg/day group (Group 4) but some of the Group 4 samples exhibited normal mitochondria. Since no drug-related ultrastructural changes were seen in cardiac muscle in a 90 day study in monkeys given FTC, these mitochondrial findings were not considered to be drug-related. No drug-related electron microscopic changes were observed in skeletal muscle at any dose level.							
CSF concentration	4.3% of plasma levels for 80 mg/kg/day; 3.7% of plasma levels for 400 mg/kg/day; 3.7% of plasma levels for 2000 mg/kg/day groups. CSF levels increased as plasma levels increased.							
Neurophysiology	No treatment-related findings.							
Neuropathology	No treatment-related findings.							
Postdose Evaluation	No treatment-related findings.							

NA = not analyzed

a Drug plasma concentrations – No gender-specific differences were noted in any of the pharmacokinetic parameters measured. Also, no significant differences in pharmacokinetic parameters were determined between Day 3 and Day 27. C_{max} and the AUC values increased in proportion to the dose. No significant drug accumulation occurred over the dosing period.

b Ophthalmic examination (pupillary dilation was achieved with 1% tropicamide ophthalmic solution and ketamine HCl for immobilization).

c Electrocardiography (unanesthetized monkeys).

d At the termination of dosing 3 monkeys/sex/group were sacrificed, examined grossly, selected organs were weighed, and tissues were taken for histopathological examination.

e Electronic microscopy – samples of skeletal and cardiac muscle from the control and 2000 mg/kg/day group were taken for ultrastructural examination.

7.2.7. 3-Month Oral Toxicity Study in Monkeys

Test Article: FTC

Report Title: A 3-Month Oral Toxicity Study in Cynomolgus Monkeys Given 524W91 **Gilead Study No. TOX627** (HWA 309-229)

Species/Strain: Monkey/Cynomolgus (Macaca fascicularis) **Duration of Dosing:** 3 Months **Location in CTD:** 4.2.3.2

Initial Age: Approximately 2–5.5 Years **Duration of Postdose:** 3 Weeks **GLP Compliance:** Yes

Date of First Dose: [redacted] 19[redacted] **Method of Administration:** Oral, divided doses spaced by approximately six hours **Lot No.:** [redacted]

Special Features: Electron microscopy, Neurophysiology

No Observed Adverse-Effect Level: 1000 mg/kg/day **Vehicle/Formulation:** Vehicle control: 0.5% aqueous methylcellulose

Dose (mg/kg)	**0 (Vehicle)**		**40**		**200**		**1000**	
Number of Animals	**M: 5**	**F: 5**	**M: 5**	**F: 5**	**M: 5**	**F: 5**	**M: 5**	**F: 5**
Toxicokinetics:								
C_{max} (µg/mL)								
Day 3	NA	NA	5.19	6.06	28.9	22.9	101	92.5
Day 87	NA	NA	5.27	5.96	26.0	23.1	117	94.4
AUC $_{0-6}$ (µg.h/mL)[a]								
Day 3	NA	NA	12.2	13.4	71.0	51.9	331	233
Day 87	NA	NA	13.1	14.5	64.8	54.6	403	274
Died or Sacrificed Moribund	0	0	0	0	0	0	0	0
Clinical Observations	No treatment-related findings.							
Body Weight (%)	No treatment-related findings.							
Food Consumption (%)	No treatment-related findings.							
Ophthalmic Examinations[b]	No treatment-related findings.							
Electrocardiography[c]	No treatment-related findings.							
Hematology/ Serum Chemistry	No treatment-related findings.							
Urinalysis	No treatment-related findings.							

Dose (mg/kg)	**0 (Vehicle)**		**40**		**200**		**1000**	
Number of Animals	**M: 5**	**F: 5**	**M: 5**	**F: 5**	**M: 5**	**F: 5**	**M: 5**	**F: 5**
Organ Weights (%)[d]	No treatment-related findings.							
Gross Pathology Necropsy[d]	No treatment-related findings.							
Histopathology[d]	No treatment-related findings.							
Additional Examinations								
Neurophysiology	No treatment-related findings.							
Neuropathology	No treatment-related findings.							
Electronmicroscopy[e]	No treatment-related findings.							
Postdose Evaluation	No treatment-related findings.							

NA = not applicable

a Drug plasma concentrations – C_{max} and AUC increased with the dose and there was no evidence of accumulation: Systemic exposure to FTC was similar between sexes and between days 3 and 87.

b Ophthalmic examination (pupillary dilation was achieved with 1% tropicamide ophthalmic solution and ketamine HCl for immobilization).

c Electrocardiography (unanesthetized monkeys)

d At the termination of dosing 3 monkeys/sex/group were sacrificed, examined grossly, selected organs were weighed and tissues were taken for histopathologic examination.

e Electronmicroscopy – samples of liver, skeletal and cardiac muscle for 3 monkeys/sex/group were taken for ultrastructural examination.

7.2.8. 52-Week Oral Toxicity Study in Monkeys

Test Article: FTC

Report Title: 52-Week Oral Toxicity Study of TP-0006 (FTC) in Cynomolgus Monkeys with a 4-Week Recovery Period

Gilead Study No. TOX032 ([redacted] 6758-114)

Species/Strain: Monkey / Cynomolgus (Macaca fascicularis)

Duration of Dosing: 52 Weeks

Location in CTD: 4.2.3.2

Initial Age: Approx. 3–7 Years

Duration of Postdose: 4 Weeks

GLP Compliance: Yes

Date of First Dose: [redacted] 19[redacted]

Method of Administration: Oral, divided doses spaced by approximately six hours

Lot No.: [redacted]

Vehicle/Formulation: 0.5% aqueous methylcellulose

No Observed -Effect Level: 200 mg/kg/day

Special Features: Neurophysiology

Dose (mg/kg)	0 (Vehicle)		50		200		500	
Number of Animals	**M: 8**	**F: 8**	**M: 4**	**F: 4**	**M: 4**	**F: 4**	**M: 8**	**F: 8**
Toxicokinetics:								
C_{max} (µg/mL)								
Week 13	NA	NA	3.7	2.9	11.1	16.9	41.5	26.9
Week 26	NA	NA	3.7	4.8	15.6	24.8	51.2	39.3
Week 52	NA	NA	4.3	7.3	17.4	21.3	50.6	37.6
AUC_{0-24} (µg.h/mL)[a]								
Week 13	NA	NA	16.1	18.1	74.9	100	266	196
Week 26	NA	NA	17.0	22.9	103	120	342	321
Week 52	NA	NA	18.1	33.3	97.2	97.8	274	238
Died or Sacrificed Moribund	0	0	0	0	0	0	0	0
Body Weight (%)	No treatment-related findings.							
Clinical Observations	No treatment-related findings.							

Dose (mg/kg)	**0 (Vehicle)**		**50**		**200**		**500**	
Number of Animals	**M: 8**	**F: 8**	**M: 4**	**F: 4**	**M: 4**	**F: 4**	**M: 8**	**F: 8**
Ophthalmic Examinations	No treatment-related findings.							
Electrocardiography	No treatment-related findings.							
Hematology	Slightly decreased mean erythrocyte counts, increased mean corpuscular volume and increased mean corpuscular hemoglobin in females treated with 500 mg/kg/day.							
Serum Chemistry	The activity of alanine aminotransferase was increased in several individuals (including controls). No treatment related patterns were identified.							
Urinalysis	No treatment-related findings.							
Organ Weights (%)	No treatment-related findings.							
Gross Pathology Necropsy	No treatment-related findings.							
Histopathology	No treatment-related findings.							
Additional Examinations								
Neurophysiology	No treatment-related findings.							
Postdose Evaluation	No treatment-related findings.							

a FTC was rapidly and well absorbed with peak plasma concentrations occurring between 0.5 to 2 hours post dosing. Plasma half-life was 2–4 hours for all dose levels. Half-life may have been underestimated because of the short (6 hour) sampling period. A longer half-life was expected, based on the measurable pre-dose drug concentrations and the slightly higher plasma FTC concentrations after repeated dosing. No major differences in plasma FTC exposure between male and female monkeys. Plasma C_{max}, AUC_{0-6}, and estimated steady-state AUC_{0-24} (Weeks 13, 26, and 52) increased linearly over the dose range of 50 to 500 mg/kg/day.

7.3. TAF

7.3.1. 13-Week Oral Gavage Toxicity and Toxicokinetic Study in Mice

Test Article: TAF

Report Title: 13-Week Oral Gavage Toxicity and Toxicokinetic Study with GS-7340-02 in Mice

Gilead Study No. TX-120-2007
[redacted] Study No. 8241074

Species / Strain: Mouse/Crl:CD1(ICR)

Duration of Dosing: 13 weeks

Location in CTD: 4.2.3.2

Initial Age: 5 to 6 weeks old

Duration of Postdose: NA

GLP Compliance: yes

Date of First Dose: [redacted] 20[redacted]

Method of Administration: Oral Gavage

Lot Number: [redacted]

Vehicle: 0.1% (v/v) Tween 20 and 0.1% (w/v) HPMC K100LV prepared in reverse osmosis water

Special Features: Insufficient GS-7340 data for TK analysis; Tenofovir TK analyses presented

No Observed Adverse Effect Level: Not determined

Daily Dose (mg/kg/day)	**0 (Control)**		**10**		**30**		**100**	
Gender: Number of Animals (TK)	**M: 6**	**F: 6**	**M: 42**	**F: 42**	**M: 42**	**F: 42**	**M: 42**	**F: 42**
Toxicokinetics (Tenofovir): $AUC_{(0\text{-}t)}$ (ng·hr/mL)								
Day 1	NA[a]	NA[a]	148	193	1140	1424	5829	7239
Week 13	NA[a]	NA[a]	191	239	1561	1453	7797	6545
Toxicokinetics (Tenofovir): C_{max} (ng/mL)								
Day 1	NA[a]	NA[a]	62.3	57.6	248	337	1024	999
Week 13	NA[a]	NA[a]	58.8	99.6	422	280	958	767

Daily Dose (mg/kg/day)	0 (Control)		10		30		100	
Gender: Number of Animals (Main)	**M: 15**	**F: 15**	**M: 15**	**F: 15**	**M: 15**	**F: 15**	**M: 15**	**F: 15**
Noteworthy Findings								
Died or sacrificed moribund	-	-	-	-	-	-	1[b]	-
Body Weight Change								
Dosing Days 1 – 92 (g)	9.3	8.0	9.4	7.1	9.3	6.1	7.9	7.3
Food Consumption (%)[c]								
Dosing Week 1	41.6 g	36.7 g	+3.4	-0.8	-13.9*	-12.8	-10.8*	-12.5*
Dosing Week 2	31.1 g	28.9 g	+2.9	+0.3	+11.6*	+8.3	+14.1*	+13.1*
Clinical Observations	-	-	-	-	-	-	-	-
Ophthalmoscopy	-	-	-	-	-	-	-	-
Hematology	-	-	-	-	-	-	-	-
Serum Chemistry	-	-	-	-	-	-	-	-
Dosing Phase – Final Phase Sacrifice								
Number Evaluated	15	15	15	15	15	15	14	15
Organ Weights	-	-	-	-	-	-	-	-
Dosing Phase – Final Phase Sacrifice (cont.)								
Number Evaluated	15	15	15	15	15	15	15	15
Macroscopic Observations	-	-	-	-	-	-	-	-
Histopathology								
Nasal turbinates								
Infiltrate, neutrophil, respiratory mucosa								
Minimal	1	1	3	3	4	6	6	10
Slight	0	0	2	0	2	0	5	4

Daily Dose (mg/kg/day)	0 (Control)		10		30		100	
Gender: Number of Animals (Main)	**M: 15**	**F: 15**	**M: 15**	**F: 15**	**M: 15**	**F: 15**	**M: 15**	**F: 15**
Infiltrate, neutrophil, olfactory mucosa								
Minimal	1	1	7	4	7	4	7	11
Slight	0	0	2	0	1	0	7	2
Degeneration, olfactory epithelium								
Minimal	2	2	5	4	5	7	3	8
Slight	0	0	3	0	2	0	8	4
Moderate	0	0	0	0	1	0	2	1
Exudate, lumen								
Minimal	0	0	0	0	2	2	2	2
Rectum								
Apoptosis, increased								
Minimal	0	0	0	0	0	0	5	2

M = Male; F = Female; TK = toxicokinetic; NA = Not Applicable

− Comparable to control animals or no treatment-related findings; * = $p \leq 0.05$ (ANOVA and Dunnett's t-test where applicable)

a Values below the lower limit of quantitation (with the exception of Animal No. A54618 (Group 5 female) for tenofovir on Day 1 of the dosing phase)

b On Day 54 of the dosing phase Animal No. A54419 (Group 4 male) was sacrificed in moribund condition due to gavage-related injury. Respectively on Days 15 and 45 of the dosing phase, Animal Nos. A54647 (Group 6 TK female) and A54746 (Group 8 TK female) were found dead. These deaths were not considered test article-related.

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

7.3.2. A 28-Day Oral Toxicity Study in the Albino Rat

Test Article: TAF

Report Title: A 28-Day Oral Toxicity Study of GS-7340-02 in the Albino Rat

Gilead Study No. R990182
[redacted] Study No. 89176

Species / Strain: Sprague-Dawley rat

Duration of Dosing: 28 days

Location in CTD: 4.2.3.2

Initial Age: 10 weeks old

Duration of Postdose: None

GLP Compliance: yes

Date of First Dose: [redacted] 19[redacted]

Method of Administration: Oral gavage

Lot Number: [redacted]

Vehicle: 50 mM Citric Acid

Special Features: Biochemical markers of bone turnover , Peripheral Quantitative Computed Tomography (pQCT)

No Observed Adverse Effect Level: 6.25 mg/kg/day

Daily Dose (mg/kg/day)	**0 (Control)**		**1.5**		**6.25**		**25**		**100**		**400**	
Gender: Number of Animals (Main)	**M: 10**	**F: 10**	**M: 10**	**F: 10**	**M: 10**	**F: 10**	**M: 10**	**F: 10**	**M: 10**	**F: 10**	**M: 10**	**F: 10**
Toxicokinetics (Tenofovir):												
AUC (hr·µg/mL)												
Day 1	NA[a]	NA[a]	0.05	0.05	0.3	0.3	2.4	2.8	12.7	13.6	61.8	78.4
Day 28	NA[a]	NA[a]	0.05	0.06	0.4	0.3	2.5	2.8	12.2	14.6	62.7	75.1
Toxicokinetics (Tenofovir):												
C_{max} (µg/mL)												
Day 1	NA[a]	NA[a]	0.04	0.04	0.2	0.3	1.3	2.0	7.0	7.0	12.9	20.1
Day 28	NA[a]	NA[a]	0.05	0.05	0.2	0.2	1.3	1.7	4.8	6.2	10.3	14.4

Daily Dose (mg/kg/day)	0 (Control)		1.5		6.25		25		100		400	
Gender: Number of Animals (Main)	**M: 10**	**F: 10**	**M: 10**	**F: 10**	**M: 10**	**F: 10**	**M: 10**	**F: 10**	**M: 10**	**F: 10**	**M: 10**	**F: 10**
Noteworthy Findings												
Died or sacrificed moribund	-	-	-	-	1	-	-	-	-	-	2	-
Body Weight Gain (%)[b]												
Week 1 to 2	49.9	30.9	-	-	-	-	-29% A	-35% B	-37% B	-24% A	-67% B	-56% B
Week 2 to 3	20.4	9.8	-	-	-	-	-	-	-	-	-68% B	-
Week 3 to 4	5.5	1.8	-	+49% B	-	-	-	-	-	-	-	-
Food Consumption (%)[b]												
Week 1 to 2	209.2	162.0	-	-	-	-	-	-	-	-	-17% B	-14% B
Week 2 to 3	203.4	159.5	-	-	-	-	-	-	-	-	-18% B	-14% A
Week 3 to 4	195.3	154.6	-	-	-	-	-	-	-	-	-14% B	-11% A
Clinical Observations												
Fur, staining, red, muzzle	1	1	1	-	1	-	1	-	2	-	4	2
Hematology[b]												
White blood cell ($x10^3/mm^3$)	7.9	-	-	-	-	-	-	-	-	-	-57% B	-
Monocytes (n/mm^3)	286.9	-	-53% A	-	-100%	-	-98% B	-	-91% B	-	-93% A	-
Lymphocytes (n/mm^3)	6590.3	-	-	-	-	-	-	-	-	-	-57% B	-
Red blood cell ($x10^6/mm^3$)	7.71	7.06	-	-	-	-	-	-	-	-	-11% B	-12% B
Hemoglobin (g/dL)	14.9	14.1	-	-	-	-	-	-	-	-	-6% B	-7% B
Hematocrit (%)	42.4	39.7	-	-	-	-	-	-	-	-	-8% B	-8% B
Mean Corpuscular hemoglobin (pg)	19.3	20.0	-	-	-	-	-	-	-	-	+6% B	+6%A
Red blood cell distribution width (%)	12.2	11.7	-	-	-	-	-	-	-	-	+20% B	+15% B
Clinical Chemistry[b]												
Urea nitrogen (mg/dL)	16.3		-	-	-	-	-	-	-	-	+28% B	-
Alkaline phosphatase (U/L)	110.3	71.0	-	-	-	-	-	-	-	-	-37% B	-44% B
Cholesterol (mg/dL)	54.8	70.3	-	-	-	-	-	-	-	-	+71%B	+30% A

Daily Dose (mg/kg/day)	0 (Control)		1.5		6.25		25		100		400	
Gender: Number of Animals (Main)	**M: 10**	**F: 10**	**M: 10**	**F: 10**	**M: 10**	**F: 10**	**M: 10**	**F: 10**	**M: 10**	**F: 10**	**M: 10**	**F: 10**
Organ Weights[b]												
Relative liver weight (%)	-	2.630	-	-	-	-	-	-	-	-	-	+11% B
Relative thymus weight (%)	0.071	-	-	-	-	-	-	-	-	-	-61% B	-
Gross Pathology												
Thymus - Number Evaluated	10	10	10	10	10	10	10	10	10	10	10	10
Discoloration dark	-	-	-	-	-	-	-	-	-	-	1	-
Small	-	-	-	-	-	-	-	-	-	-	7	1
Histopathology												
Femur - Number Evaluated	10	10	-	-	-	-	-	-	-	-	10	10
Atrophy – cancellous bone	-	-	-	-	-	-	-	-	-	-	4	9
Kidney – Number evaluated	10	10	10	10	10	10	10	10	10	10	10	10
Tubular basophilia	1	-	1	-	1	-	-	-	-		8	3
Tubular karyomegaly	-	-	-	-	-	-	-	-	-	-	7	5
Hyaline droplets	1	-	-	-	2	-	3	-	6	-	2	-
Thymus – Number examined	10	10	10	10	10	10	10	10	10	10	10	10
Atrophy	-	-	-	-	1	-	-	-	-	-	9	6
Bone parameters[b]												
Periosteal circumference (mm)	16.09	13.44	-	-	-	-	-	-	-	-	-4%	-1.5%
Total mean slice area – distal femur (mm^2)	20.65	14.41	-	-	-	-	-	-	-	-	-7%	-3%
Bone mineral density (mg/cm^3)	576.5	752.3	-	-	-	-	-	-	-	-	-7%	-14% B
1.25-Dihydroxy Vitamin D (pg/mL)	51.67		-		-		-		-51% A		-74% A	

M = Male; F = Female, NA = Not applicable, − Comparable to control animals or no treatment-related findings, Significantly different from control (group 1) value: A - $p<0.05$, B - $p<0.01$ (Dunnett's)

a Values below the lower limit of quantitation

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.

7.3.3. A 26-Week Oral Gavage Toxicity Study of GS-7340-02 in the Albino Rat

Test Article: TAF

Report Title: A 26-Week Oral Gavage Toxicity Study of GS-7340-02 in the Albino Rat

Gilead Study No. TOX-120-001
[redacted] Study No. 89686

Species / Strain: Sprague-Dawley rats (Crl: CD® (SD) BR) / *Rattus norvegicus*

Duration of Dosing: 26 weeks

Location in CTD: 4.2.3.2

Initial Age: 6 weeks at start of dosing

Duration of Postdose: N/A

GLP Compliance: yes

Date of First Dose: [redacted] 20[redacted]

Method of Administration: Oral gavage

Lot Number: [redacted]

Vehicle: 0.5% polysorbate 20, NF; 0.5% carboxymethylcellulose; 0.9% Benzyl Alcohol

Special Features: Bone parameters

No Observed Adverse Effect Level: 25 mg/kg/day

Daily Dose (mg/kg/day)	**0 (Control)**		**5**		**25**		**100**	
Gender: Number of Animals (TK)	**M: 0**	**F: 0**	**M: 8**	**F: 8**	**M: 8**	**F: 8**	**M: 8**	**F: 8**
Toxicokinetics (Tenofovir):								
AUC (hr·µg/mL)[e]								
Day 1	NA[a]	NA[a]	0.625	0.584	3.024	3.704	12.555	12.275
Week 13	NA[a]	NA[a]	0.798	0.656	3.639	3.809	15.844	14.245
Week 26	NA[a]	NA[a]	0.739	0.601	3.526	3.991	15.931	15.138
Toxicokinetics (Tenofovir):								
C_{max} (µg/mL)								
Day 1	NA[a]	NA[a]	0.326	0.292	1.214	1.354	5.071	4.817
Week 13	NA[a]	NA[a]	0.337	0.351	1.335	1.593	4.766	6.262
Week 26	NA[a]	NA[a]	0.282	0.252	1.249	1.798	4.499	5.323

Daily Dose (mg/kg/day)	**0 (Control)**		**5**		**25**		**100**	
Gender: Number of Animals (Main)	**M: 15**	**F: 15**	**M: 15**	**F: 15**	**M: 15**	**F: 15**	**M: 15**	**F: 15**
Died or sacrificed moribund[d]	-	1	-	2	-	-	-	3
Body Weights	-	-	-	-	-	-	-	-
Food Consumption	-	-	-	-	-	-	-	-
Clinical Observations	-	-	-	-	-	-	-	-
Ophthalmology	-	-	-	-	-	-	-	-
Hematology	-	-	-	-	-	-	-	-
Clinical Chemistry	-	-	-	-	-	-	-	-
Urinalysis	-	-	-	-	-	-	-	-
Organ Weights	-	-	-	-	-	-	-	-
Macroscopic Observations	-	-	-	-	-	-	-	-
Noteworthy Findings								
Histopathology								
Bone – Tibia								
Number Evaluated	15	14	-	13	-	15	15	13
Atrophy: cancellous bone	-	-	-	-	-	-	-	8
Kidney								
Number Evaluated	15	15	15	15	15	15	15	15
Basophilia: tubular	6	4	4	4	6	3	7	3
Karyomegaly: tubular	-	-	-	-	-	-	11	2
Gender: Number of Animals (Main)	M: 15	F: 15	M: 15	F: 15	M: 15	F: 15	M: 15	F: 15
Degeneration: tubular	-	-	-	-	1	-	-	-
Hyaline droplet: tubular	8	-	3	-	4	-	11	-
Pyelitis/pyelonephritis	-	1	-	4	-	1	1	1
Dilatation: pelvis	-	1	1	1	1	1	-	-
Cyst	-	1	-	1	1	1	-	-
Fibrosis	-	-	-	-	1	-	-	1

Daily Dose (mg/kg/day)	**0 (Control)**		**5**		**25**		**100**	
Gender: Number of Animals (Main)	**M: 15**	**F: 15**	**M: 15**	**F: 15**	**M: 15**	**F: 15**	**M: 15**	**F: 15**
Bone Densitometry (pQCT)[b]								
Right proximal tibia - metaphysis								
Trabecular bone mineral density (BMD), Week 26 (mg/cm^3)	221.9	280.79	-2.1%	-9.3%	-2.3%	-2.3%	-18.4% A	-21% A
Trabecular bone mineral content (BMC), Week 26 (mg/mm)	2.26	0.83	-6.2%	-12%	-4.4%	-2.4%	-22.6% B	-33.7% C
Right distal femur - metaphysis								
Trabecular (BMD), ex-vivo (mg/cm^3)	259.8	301.2	c	c	c	c	-15.4% B	-5.3%
Trabecular (BMC), ex-vivo (mg/mm)	3.5	1.9	c	c	c	c	-17.1% B	-5.3%
Urine Biochemical markers of bone turnover, Week 13[b]								
C-Telopeptide (µg/mmol creatinine)	3.673	-	-		+78% D	-	+135% F	-
Gender: Number of Animals (Main)	M: 15	F: 15	M: 15	F: 15	M: 15	F: 15	M: 15	F: 15
Deoxypyridinoline (nM/mM creatinine)	80.603	-	-	-	-	-	+25% A	-
C-Telopeptide (µg/mmol creatinine)	3.673	-	+55%	-	+78% D	-	+135% F	-
Serum Biochemical markers of bone turnover and hormones, Week 26 [b]								
1,25-Dihydroxy Vitamin D (pmol/L)	-	105.021	-	-10%	-	-45%	-	-59% F
25-Hydroxy Vitamin D (ng/mL)	-	22.358	-	+14%	-	-15%	-	-22% B

M = Male; F = Female; TK = toxicokinetic; NA = Not Applicable; − Comparable to control animals or no treatment-related findings; pQCT = peripheral quantitative computed tomography

A = $p \leq 0.05$; B = $p \leq 0.01$; C = $p \leq 0.001$ (Dunnett); D = $p \leq 0.05$; E = $p \leq 0.01$; F = $p \leq 0.001$ (Dunn's)

a Values below the lower limit of quantitation

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.

c Bone densitometry (pQCT) was only evaluated in the right distal femur (metaphysis) of animals in the control and 100 mg/kg/day groups.

d There were no unscheduled deaths recorded during the 26-week treatment period which were considered to treatment related. Two high dose female TK animals were euthanized on days 732 and 164; in both cases the deterioration of their condition could not be attributed to an accident during dose administration.

e $AUC_{0-\infty}$ on Day 1 and $AUC_{0-\tau}$ in Weeks 13 and 26

7.3.4. A 28-Day Oral Gavage Toxicity Study in the Beagle Dog

Test Article: TAF

Report Title: A 28-Day Oral Gavage Toxicity Study of GS-7340-02 in the Beagle Dog

Gilead Study No. D990175
[redacted] Study No. 89128

Species / Strain: Beagle dog

Duration of Dosing: 28 days

Location in CTD: 4.2.3.2

Initial Age: 7 to 8 months at dosing

Duration of Postdose: N/A

GLP Compliance: yes

Date of First Dose: [redacted] 19[redacted]

Method of Administration: Oral gavage

Lot Number: [redacted]

Vehicle: Citric Acid (formulated in Sterile Water for Irrigation, USP)

Special Features: bone parameters

No Observed Effect Level: 1 mg/kg/day

Daily Dose (mg/kg/day)	**0 (Control)**		**0.1**		**0.3**		**1**		**3**		**10**	
Gender: Number of Animals	**M: 4**	**F: 4**	**M: 4**	**F: 4**	**M: 4**	**F: 4**	**M: 4**	**F: 4**	**M: 4**	**F: 4**	**M: 4**	**F: 4**
Toxicokinetics (Tenofovir): AUC (hr·µg/mL)												
Day 1	NA[a]	NA[a]	NC	NC	NC	NC	NC	NC	NC	NC	NC	
Day 28	NA[a]	NA[a]	NC	NC	NC	NC	NC	NC	NC	NC	5.26 $_{(0\text{-}\tau)}$	
Toxicokinetics (Tenofovir): C_{max} (µg/mL)												
Day 1	NA[a]	NA[a]	NC	NC	NC	NC	NC	NC	NC	NC	0.385	
Day 28	NA[a]	NA[a]	NC	NC	NC	NC	NC	NC	NC	NC	0.444	
Toxicokinetics (GS-7340): AUC (hr·µg/mL)												
Day 1	NA[a]	NA[a]	NC	NC	NC	NC	NC	NC	NC	NC	NC	
Day 28	NA[a]	NA[a]	NC	NC	NC	NC	NC	NC	NC	NC	0.985 $_{(0\text{-}\tau)}$	
Toxicokinetics (GS-7340): C_{max} (µg/mL)												
Day 1	NA[a]	NA[a]	NC	NC	NC	NC	NC	NC	NC	NC	0.582	
Day 28	NA[a]	NA[a]	NC	NC	NC	NC	NC	NC	NC	NC	1.28	

Daily Dose (mg/kg/day)	0 (Control)		0.1		0.3		1		3		10	
Gender: Number of Animals	**M: 4**	**F: 4**	**M: 4**	**F: 4**	**M: 4**	**F: 4**	**M: 4**	**F: 4**	**M: 4**	**F: 4**	**M: 4**	**F: 4**
Noteworthy Findings												
Died or sacrificed moribund, adverse findings etc.	There were no unscheduled deaths or adverse findings for body weight, food consumption, clinical observations, ophthalmology examinations or hematology parameters.											
Serum Chemistry												
AST (U/L)												
Day 14	38.3	39.8	37.0	37.5	43.0	34.5	34.0	34.3	38.3	46.0	42.8	46.8
Day 28	34.8	35.3	33.8	36.0	39.8	33.8	34.5	35.8	37.5	36.0	47.0	52.5*
Urinalysis	-	-	-	-	-	-	-	-	-	-	-	-
Organ Weights	-	-	-	-	-	-	-	-	-	-	-	-
Gross Pathology												
Number Evaluated	4	4	4	4	4	4	4	4	4	4	4	4
Cecum, Area dark	1	1	-	1	-	-	1	1	2	-	2	-
Colon, Area dark	-	-	-	-	-	-	-	1	-	-	2	1
Duodenum, Area dark	-	-	-	-	-	-	-	-	-	-	1	-
Rectum, Area dark	-	-	-	-	-	-	-	-	-	-	-	2
Lung, Area depressed	-	-	-	-	1	-	-	-	-	1	-	-
Area dark	-	-	-	-	-	-	-	1	-	-	-	-
Area pale	-	-	-	-	-	-	1	-	1	1	-	-
Area raised	2	1	1	2	-	1	1	-	1	1	-	1
Discoloration pale	-	-	-	-	-	-	-	-	1	-	-	-
Spleen, Area dark	-	-	-	-	-	-	1	-	-	-	-	-
Area depressed	1	1	1	-	1	1	-	-	-	-	-	1
Area pale	1	-	1	1	-	-	-	-	-	-	1	-
Area raised	-	-	-	-	-	-	-	-	-	-	1	-
Adhesion	-	-	-	-	-	-	-	-	-	-	-	1

Daily Dose (mg/kg/day)	0 (Control)		0.1		0.3		1		3		10	
Gender: Number of Animals	**M: 4**	**F: 4**	**M: 4**	**F: 4**	**M: 4**	**F: 4**	**M: 4**	**F: 4**	**M: 4**	**F: 4**	**M: 4**	**F: 4**
Histopathology												
Kidney												
Karyomegaly: tubular	-	-	-	-	-	-	-	-	1	1	4	4
Basophilia: tubular	-	-	-	-	-	-	-	-	-	-	2	3
Bone Densitometry (pQCT)	-	-	-	-	-	-	-	-	-	-	-	-

M = Male; F = Female; TK = toxicokinetic; NA = Not Applicable; NC = Not calculated (insufficient data); − Comparable to control animals or no treatment-related findings,
* = $p \leq 0.01$ (Dunnett); pQCT = peripheral quantitative computed tomography
a not assayed

7.3.5. A 9 Month Oral Gavage Toxicity Study in the Beagle Dog with a 3 Month Recovery Period for Selected Animals

Test Item: TAF

Report Title: A 9 Month Oral Gavage Toxicity Study of GS-7340-02 in the Beagle Dog with a 3 Month Recovery Period For Selected Animals

Gilead Study No. TOX-120-002
[redacted] Study No. 89647

Species / Strain: beagle dog
Duration of Dosing: 9 months
Location in CTD: 4.2.3.2

Initial Age: 5-6 months
Duration of Postdose: 3 months
GLP Compliance: yes

Date of First Dose: [redacted] 20[redacted]
Method of Administration: oral gavage
Lot Number: [redacted]

Vehicle: 0.5% polysorbate 20, NF; 0.5% carboxymethylcellulose; 0.9% Benzyl Alcohol

Special Features: Bone densitometry, biochemical markers of bone turnover and associated parameters

No Observed Adverse Effect Level: 2 mg/kg/day

Daily Dose (mg/kg/day)	**0 (Control)**		**2**		**6**		**18/12[a]**	
Gender: Number of Animals	**M: 6**	**F: 6**	**M: 6**	**F: 6**	**M: 6**	**F: 6**	**M: 8**	**F: 8**
Toxicokinetics (GS-7340): AUC_{0-t} (hr·µg/mL)								
Day 1	NA [b]		0.03		0.37		2.07	
Week 13	NA [b]		0.07		0.66		2.23	
Week 39/40	NA [b]		0.08		1.03		1.95	
Toxicokinetics (GS-7340): C_{max} (µg/mL)								
Day 1	NA [b]		0.08		0.72		3.60	
Week 13	NA [b]		0.13		1.10		3.24	
Week 39/40	NA [b]		0.14		1.42		2.62	
Toxicokinetics (Tenofovir): AUC_{0-24} (hr·µg/mL)								
Day 1	NA [b]		0.40		1.31		3.80	
Week 13	NA [b]		1.21		4.10		12.48	
Week 39/40	NA [b]		1.18		4.45		13.73	
Toxicokinetics (Tenofovir): C_{max} (µg/mL)								
Day 1	NA [b]		0.08		0.25		0.80	
Week 13	NA [b]		0.16		0.56		1.25	
Week 39/40	NA [b]		0.18		0.54		1.32	

Daily Dose (mg/kg/day)	0 (Control)		2		6		18/12[a]	
Noteworthy Findings								
Died or sacrificed moribund[d]	-	-	-	-	-	-	2	-
Body Weight (%) [c]								
Week 13	9.95 kg	-	-	-	-	-	-22.4 [B]	-
Week 26	10.95 kg	-	-	-	-	-	-23.1 [B]	-
Week 39	11.50 kg	8.88	-6.7	-	-13.2	-	-25.7 [C]	-11.9
Food Consumption (%) [c]								
Week 13	256.72	-	-	-	-	-	-23.8	-
Week 26	319.40	-	-	-	-	-	-21.7	-
Week 39	329.48	-	-	-	-	-	-19.8	-
Clinical Observations	An increased incidence of salivation was recorded both pre-dose and following dose administration for animals from all treated groups including the controls. In addition, a red/brown fur staining was also observed on the paws of the treated animals with a higher incidence observed in animals administered 18/12 mg/kg/day. This change was most often distributed on the lower extremities, the hind paws and the forepaws, and was still apparent to a lesser degree during the three month recovery period.							
Ophthalmoscopy	-	-	-	-	-	-	-	-
Electrocardiography [c]								
Heart Rate (beats/min)								
Week 13	117.0	106.0	-	-	-	-	-32.5% [B]	-29.2% [B]
Week 39	111.0	-	-	-	-	-	-49.5% [C]	-
PR (msec)								
Week 13	-	86.7	-	-	-	-	-	+26.2% [B]
Week 39	83.8	87.5	-	-	+28.3% [B]	+25.7%	+31.3% [B]	+41.9% [D]
QT								
Week 13	189.2	200.8	-	-	-	-	+17.2% [B]	+10.2% [B]
Week 39	200.0	217.5	-	-	-	-	+26.3% [C]	+10.7% [A]
Hematology [c]								
Eosinophil (n)								
Week 13	0.175	0.257	-	-	-	-	-53.1%	-64.6% [E]
Week 26	0.273	0.218	-	-	-	-	-76.2% [A]	-48.6% [D]
Week 39	0.323	0.190	-	-	-	-	-82.3%	-60.5%

Daily Dose (mg/kg/day)	0 (Control)		2		6		18/12[a]	
Gender: Number of Animals	**M: 6**	**F: 6**	**M: 6**	**F: 6**	**M: 6**	**F: 6**	**M: 6**	**F: 6**
Hemoglobin (g/dL)								
Week 13	15.52	-	-	-	-	-	-12.5% [B]	-
Week 26	16.23	-	-	-	-	-	-11.5% [B]	-
Week 39	16.28	-	-	-	-	-	-14.6% [B]	-
Biochemistry[c]								
AST (U/L)								
Week 13	26.3	27.0	-	-	+48% [D]	-	+124% [F]	+96% [F]
Week 26	30.0	31.8	-	-	+42% [A]	-	+103% [C]	+48% [A]
Week 39	23.0	20.3	-	-	-	-	+162% [E]	+170% [B]
ALP (U/L)								
Week 13	-	80.2	-	-	-	-	-	+29% [A]
Week 26	65.5	-	-	-	-	-	+103% [A]	-
Week 39	56.3	-	-	-	-	-	+146% [E]	-
Total bilirubin (g/dL)								
Week 13	-	0.128	-	-	-	-	-	+39% [B]
Week 39	-	0.138	-	-	-	-	-	+74% [D]
Creatinine (mg/dL)								
Week 13	0.80	-	-	-	-	-	+19% [B]	-
Week 26	0.85	-	-	-	-	-	+27% [A]	-
Week 39	0.83	0.70	-	-	-	-	+17% [A]	+31% [A]
Triglycerides (mg/dL)								
Week 26	32.0	-	-	-	-	-	+34% [A]	-
Week 39	39.3	50.3	-	-	-	+53% [A]	+64% [B]	+67% [B]
A/G-P (ratio)								
Week 13	1.465	1.468	-	-	-	-	-30% [B]	-21% [B]
Week 26	1.325	-	-	-	-	-	-37% [B]	-
Week 39	1.565	1.578	-	-	+15% [A]	-	-37% C	+27% [A]

Daily Dose (mg/kg/day)	0 (Control)		2		6		18/12[a]	
T3 (ng/dL)								
Week 39	108.8	100.2	-	-	-	-	-32% [B]	-30% [D]
Phosphorus (mg/dL)								
Week 13	5.298	-	-	-	-	-	+18% [B]	-
Creatinine Kinase (U/L)								
Week 39	91.5	87.5	-	-	+76% [A]	-	+207% [C]	+127% [E]
Albumin (g/dL)								
Week 13	3.55	3.37	-	+6% [A]	-	+8% [B]	-10% [B]	-7% [A]
Week 26	3.55	-	-	-	-	-	-11% A	-
Week 39	4.03	3.80					-15% [C]	-8% [A]
Bone Parameters[c]								
1.25-dihydroxy Vitamin D (pmol/L)								
Week 13	539.15	-	-	-	-	-	-27% [A]	-
Week 39	440.06	-	-	-	-	-	-51% [A]	-
25-hydroxy Vitamin D (ng/mL)								
Week 13	-	28.87	-	-	-	-	-	+185% [E]
Week 39	56.00	-	-	-	-	-	-41% [A]	-
N-telopeptide; NTx (nM BCE/mM Creatinine)								
Week 39	253.7	229.3	-	-	-	-	-153% [E]	+60% [A]
Body mineral density (g/cm^2)								
Whole body								
Week 13	0.336	-	-	-	-	-	-13% [C]	-
Week 39	0.372	-	-	-	-	-	-19% [F]	-
Lumbar vertebrae								
Week 13	0.554	0.533	-	-	-	-	-15% [C]	-9% [A]
Week 39	0.588	0.596	-	-	-	-	-21% [C]	-13% [B]

Daily Dose (mg/kg/day)	0 (Control)		2		6		18/12[a]	
Organ weight relative to body weight (%)								
Lung								
Week 39	0.78400	0.83031	-	-	-	-	+58% [B]	+35% [B]
Spleen								
Week 39	0.66659	-	-	-	-	-	+119% [C]	-
Gross pathology								
No. examined Week 13	(2)	(2)	(2)	(2)	(2)	(2)	(2)	(2)
Kidney: discoloration pale	-	-	-	-	-	-	-	2
No. examined Week 39	(4)	(4)	(4)	(4)	(4)	(4)	(4)	(4)
Kidney: discoloration pale	-	-	-	-	1	-	4	4
Lung: area raised	1	-	-	-	1	2	3	2
Histopathology								
No. examined Week 39	(4)	(4)	(4)	(4)	(4)	(4)	(4)	(4)
Eye Infiltration: mononuclear cell	-	-	-	-	-	-	3	1
No. examined Week 39	(4)	(4)	(4)	(4)	(4)	(4)	(4)	(4)
Adrenal								
Deposits: pigment	-		-	-	-	-	1	1
Kidney								
Degeneration/regeneration: tubular	-	-	1	-	4	2	4	4
Karyomegaly: tubular	-	-	2	-	4	3	4	4
Infiltration: mononuclear cell	1	-	-	-	-	-	2	2
Liver								
Deposits: pigment	-	-	-	-	2	1	4	4
Hepatocellular cytoplasmic acidophilic inclusion	-	-	-	-	-	-	3	2

Daily Dose (mg/kg/day)	**0 (Control)**		**2**		**6**		**18/12[a]**	
Lung								
Infiltration: macrophage	-	-	2	1	1	1	4	4
Histiocytosis	-	-	-	-	-	1	3	2
Inflammation: interstitial	-	-	2	-	-	2	4	-
Spleen								
Infiltration: macrophage	-	-	-	-	-	-	4	3
Recovery Phase								
Gender:Number of Animals	**M: 0**	**F: 0**	**M: 0**	**F: 0**	**M: 0**	**F: 0**	**M: 2**	**F: 2**
Recovery phase	NA	NA	NA	NA	NA	NA	-	-

M = Male; F = Female; TK = toxicokinetic; NA = Not Applicable; − Comparable to control animals or no treatment-related findings

a Due to severe clinical signs and reduced body weights the dose level was decreased on Day 45 and 51 for males and females, respectively.

b Values below the lower limit of quantitation.

c 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.

d One death was related to gavage trauma. Significantly different from control group (group 1) value: A - $p \leq 0.05$ B - $p \leq 0.01$ C - $p \leq 0.001$ (Dunnett); D - $p \leq 0.05$ E - $P \leq 0.01$ F - $p \leq 0.001$ (Dunn).

7.3.6. A 28-Day Toxicity Study of GS-7340-02 and Tenofovir (GS-1278) Administered Orally to Rhesus Monkeys

Test Article: TAF

Report Title: A 28-Day Toxicity Study of GS-7340-02 and Tenofovir (GS-1278) Administered Orally to Rhesus Monkeys

Gilead Study No. P2000114
[redacted] Study No. [redacted] 1050-11

Species / Strain: Rhesus monkey

Duration of Dosing: 4 weeks

Location in CTD: 4.2.3.2

Initial Age: 2.5 -5.2 years old

Duration of Postdose: None

GLP Compliance: yes

Date of First Dose: [redacted] 20[redacted]

Method of Administration: Nasogastric gavage

Lot Number: [redacted]

Vehicle: 50mM Citric Acid

Special Features: Biochemical markers of bone marrow turnover, parathyroid serum levels, and ionized calcium and lactate levels in plasma

No Observed Adverse Effect Level: 30 mg/kg/day

Daily Dose (mg/kg/day)	**0 (Control)**		**3**		**30**	
Gender: Number of Animals (TK)	**M: 3**	**F: 3**	**M: 3**	**F: 3**	**M: 3**	**F: 3**
Toxicokinetics (Tenofovir): AUC (hr·µg/mL)[d]						
Day 1	NA[a]		0.472		6.67	
Day 28	NA[a]		0.352		5.87	
Toxicokinetics: (Tenofovir) C_{max} (µg/mL)						
Day 1	NA[a]		0.090		0.904	
Day 28	NA[a]		0.050		0.963	
Noteworthy Findings						
Died or sacrificed moribund	-	-	-	-	1[c]	-
Body Weight (%)[b]	4.1 kg		- 9.7%		+2.4%	

Daily Dose (mg/kg/day)	**0 (Control)**		**3**		**30**	
Gender: Number of Animals (Main)	**M: 3**	**F: 3**	**M: 3**	**F: 3**	**M: 3**	**F: 3**
Low Food Consumption (Number of animals effected)	3	2	3	3	2	3
Clinical Observations						
Unformed or watery stools (Number of animals effected)	3	2	3	2	3	3
Hematology[b]						
White blood cell count	15.1		- 43.7%*		- 22.5%	
Serum Chemistry						
Alkaline phosphatase (U/mL)	261		+ 43.3%		+ 54.4%*	
Urinalysis[b]						
Specific gravity [Day 7]	1.001		0%		-0.1%	
Specific gravity [Day 14]	1.007		- 0.3%*		-0.1%	
Specific gravity [Day 28]	1.011		+ 0.2%		-0.3%	
Creatine (mg/dL) [Day 7]	36.3		+ 25.3%		+ 4.4%	
Creatine (mg/dL) [Day 14]	28		- 35.4%*		-36.4%	
Creatine (mg/dL) [Day 28]	32.8		+ 48.2%		+58.8%	
Histopathology	No test article histopathological changes were observed					

M = Male; F = Female; TK = toxicokinetic; NA = Not Applicable; + Mild, ++ Moderate, +++ Marked

− Comparable to control animals or no treatment-related findings; * = $p \leq 0.05$ (Dunnett's)

a Values below the lower limit of quantitation

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

c Euthanized on Day 18 – this animal had been dosed previously with GS-7340-02 in a single dose PK study (SBI 1006-11). Although it had been determined healthy prior to assignment to that study, it developed chronic diarrhea during the 5-day period prior to dosing in and this continued throughout that study, and also into this study. The animal was administered Pepto-Bismol and additional fluids, but still developed signs of dehydration by Day 5. The animal also exhibited signs of malaise. By Day 14 body weight had decreased 17% from prestudy weight. Microscopic evaluation of the GI tract was suggestive of bacterial infection.

d $AUC_{0-\infty}$ on Day 1 and $AUC_{0-\tau}$ on Day 28

7.4. FTC/TDF

7.4.1. 4-Week Oral Toxicity Study in Dogs

Test Article: FTC + TDF

Report Title: 4-Week Oral Gavage Toxicity and Toxicokinetic Study in Male Dogs with a 4-Week Recovery Period

Gilead Study No. TX-164-2004 ([redacted] 6511-155)

Species/Strain: Dog/Beagle

Duration of Dosing: 4 Weeks (29 days)

Location in CTD: 4.2.3.2

Initial Age: Approximately 4.5 to 5 Months Old

Duration of Postdose: 4 Weeks

GLP Compliance: Yes

Date of First Dose: [redacted] 20[redacted]

Method of Administration: Oral gavage

Vehicle/Formulation: 50mM citrate buffer

Special Features: Immunophenotyping and Natural Killer Cell Assay

Lot No.: FTC = [redacted]; [redacted]
TDF = [redacted]; [redacted]

No observable Effect Level: 20 mg/kg/day (FTC), 2/3 mg/kg/day (FTC/TDF)

Dose (mg/kg)	**0 (Vehicle)**	**20 FTC**	**30 TDF**	**2 FTC/3 TDF**	**20 FTC/30 TDF**
Number of Animals	**M: 4**	**M: 4**	**M: 4**	**M: 4**	**M: 6[a]**
Toxicokinetics:					
C_{max} (Day 27)					
(µg/mL for FTC)	-	6.9		0.9 FTC	7.3 FTC
(ng/mL for TFV)			2996 TFV	154 TFV	3439 TFV
AUC_{tau} (Day 27)					
(µg.h/mL for FTC)	-	25.3		2.7 FTC	32.3 FTC
(ng.h/mL for TFV)			18413 TFV	909 TFV	20872 TFV
Noteworthy Findings					
Died or Sacrificed Moribund	0	0	0	0	0
Clinical Observations	No treatment-related findings.				
Body Weight (%)	No treatment-related findings.				
Food Consumption (%)	No treatment-related findings.				

Dose (mg/kg)	**0 (Vehicle)**	**20 FTC**	**30 TDF**	**2 FTC/3 TDF**	**20 FTC/30 TDF**
Number of Animals	**M: 4**	**M: 4**	**M: 4**	**M: 4**	**M: 6[a]**
Ophthalmic Examinations	No treatment-related findings.				
Hematology					
Increase APTT	-	-	+	-	+
Increase MCV	-	+	-	-	+
Serum Chemistry					
Creatinine	-	-	I	-	I
Urinalysis	No treatment-related findings.				
Organ Weights (%)	No treatment-related findings.				
Gross Pathology	No treatment-related findings.				
Histopathology					
+ Tubular epithelial necrosis	-	-	2/4		3/4
+ Tubular regeneration	1/4	1/4	4/4	-	4/4
Additional Examinations					
Immunophenotyping	No treatment-related findings.				
NK cell analysis	No treatment-related findings.				
Postdose Evaluation					
Number Evaluated	-	-	-	-	2
Findings	No treatment-related findings.				

- = no noteworthy findings; I = increased; + = mild; ++ = moderate; +++ = marked
a Two males designated for recovery. The hematological and kidney microscopic effects were reversed following recovery.

8. GENOTOXICITY: IN VITRO

8.1. BIC: Ames

Test Article: BIC

Report Title: Bacterial Reverse Mutation Assay Plate Incorporation with GS-9883

Gilead Study No. TX-141-2026
[redacted] Study No. 8292935

Test for Induction of: Reverse mutation in bacterial cells
Strains: *Salmonella typhimurium* and *Escherichia coli*
Metabolizing System: Aroclor™-induced rat liver S9
Vehicle: Dimethylsulfoxide (DMSO)

No. of Independent Assays: 1
No. of Replicate Cultures: 3
No. of Cells Analyzed/Culture: ~10^8
Vehicle for Positive Controls: Deionized (DI) Water (sodium azide only); DMSO (all other positive controls)

GLP Compliance: Yes
Lot Number: [redacted]

Treatment: Plate incorporation for 52 ± 4 hours
Cytotoxic Effects: Yes
Genotoxic Effects: No

Date of Treatment: [redacted] 20[redacted]

Treatment	Dose (µg/plate)	Revertant Colony Counts (Mean ± SD)				
		Strain				
		TA98	TA100	TA1535	TA1537	WP2*uvr*A
Without Metabolic Activation						
DMSO		13 ± 5 N	91 ± 13 N	10 ± 2 M N	5 ± 1 N	15 ± 3 N
BIC	5.00	9 ± 3 N	99 ± 15 N	16 ± 2 N	3 ± 2 N	14 ± 1 N
	16.0	10 ± 2 N	92 ± 9 N	15 ± 4 N	5 ± 2 N	16 ± 2 N
	50.0	10 ± 4 N	109 ± 11 N	14 ± 1 N	6 ± 2 N	15 ± 5 N
	160	11 ± 8 N	95 ± 5 N	18 ± 2 N	1 ± 1 N	15 ± 3 N M
	500	11 ± 4 N	80 ± 18 N	15 ± 2 N	5 ± 1 N	12 ± 4 N
	1600	2 ± 2 R M	7 ± 2 R M	5 ± 4 R M	2 ± 1 R M	11 ± 1 N
	5000	0 ± 0 P R M	1 ± 1 P R M	0 ± 0 P R M	0 ± 0 P A	6 ± 5 N M

Treatment	Dose (µg/plate)	Revertant Colony Counts (Mean ± SD)				
		Strain				
		TA98	TA100	TA1535	TA1537	WP2*uvr*A
2-nitrofluorene	1.0	152 ± 6 [N]				
sodium azide	2.0		738 ± 130 [N]	647 ± 76 [N]		
ICR-191	2.0				308 ± 42 [N]	
4-nitroquinoline-N-oxide	1.0					95 ± 6 N
With Metabolic Activation						
DMSO		16 ± 3 [N]	125 ± 16 [N]	11 ± 3 [N]	5 ± 4 [M N]	15 ± 3 [N]
GS-9883	5.00	16 ± 3 [N M]	118 ± 5 [N]	13 ± 4 [N]	10 ± 2 [N]	15 ± 3 [N]
	16.0	12 ± 3 [N]	107 ± 20 [N]	14 ± 3 [N]	6 ± 2 [N M]	19 ± 1 [N]
	50.0	19 ± 3 [N]	125 ± 5 [N]	5 ± 3 [N]	4 ± 2 [N]	14 ± 7 [N M R]
	160	18 ± 4 [N]	115 ± 3 [N]	14 ± 5 [N]	7 ± 1 [N]	15 ± 7 [N]
	500	17 ± 5 [N]	100 ± 15 [N]	12 ± 3 [N]	5 ± 2 [N]	21 ± 2 [N]
	1600	4 ± 1 [R M]	13 ± 3 [R M]	1 ± 1 [R M]	4 ± 2 [R M]	13 ± 4 [N]
	5000	0 ± 0 [P R M]	1 ± 1 [P R M]	0 ± 0 [P A]	0 ± 0 [P A]	8 ± 4 [N M]
benzo[a]pyrene	2.5	410 ± 20 [N]				
2-aminoanthracene	2.5		1602 ± 226 [N]	174 ± 23 [N]	65 ± 7 [N]	
2-aminoanthracene	25.0					371 ± 12 [N]

GLP = Good Laboratory Practice; SD = Standard Deviation; A = Absence of background bacterial lawn; M = Plate counted manually; N = Normal background bacterial lawn; P = Precipitation of test article observed; R = Reduced background bacterial lawn

8.2. BIC: Chromosome Aberrations

Test Article: BIC

Report Title: Chromosomal Aberrations in Cultured Human Peripheral Blood Lymphocytes with GS-9883

Gilead Study No. TX-141-2027
[redacted] Study No. 8292931

Test for Induction of: Chromosome aberrations

No. of Independent Assays: 1

Strains: Primary human lymphocytes

No. of Replicate Cultures: 2

GLP Compliance: Yes

Metabolizing System: Aroclor™-induced rat liver S9

No. of Cells Analyzed/Culture: 100 (or ≥25 if >25% cells with aberrations)

Lot Number: [redacted]

Vehicle for Test Article: Dimethylsulfoxide (DMSO)

Vehicle for Positive Controls: Water

Date of Treatment: [redacted] 20[redacted]

Treatment: Continuous 24 hour without S9; pulse treatment 3 hour and recovery time 21 hour with and without S9

Cytotoxic Effects: Yes

Genotoxic Effects: None

Metabolic Activation	Test Article	Concentration (µg/ml)	Cytotoxicity (% of Control)[a]	% Aberrant Cells (Mean -g)	% Aberrant Cells (Mean +g)	% Polyploid Cells	% Endoreduplicate Cells
Initial Assay							
3-Hour Treatment Without Activation	DMSO	10 µL/mL	100	0.5	0.5	0.0	0.0
	BIC	84.0	87	0.5	1.0	4.5	0.0
		172	75	0.5	1.0	3.5	0.0
		245[b]	58	2.0	3.5	14.0	0.0
	Mitomycin C	1.00	81	56.0	59.0	0.5	0.0
3-Hour Treatment With Activation	DMSO	10 µL/mL	100	0.0	1.0	0.0	0.0
	BIC	120	98	0.0	0.5	4.5	0.0
		172	97	0.0	0.0	5.0	0.0
		245[b]	95	0.0	1.5	5.0	0.0
	Cyclophosphamide	25.0	37	48.0	50.0	0.0	0.0

Metabolic Activation	Test Article	Concentration (µg/ml)	Cytotoxicity (% of Control)[a]	% Aberrant Cells (Mean -g)	% Aberrant Cells (Mean +g)	% Polyploid Cells	% Endoreduplicate Cells
Confirmatory Assay							
24-Hour Treatment Without Activation	DMSO	10 µL/mL	100	0.0	0.5	0.0	0.0
	GS-9883	9.89	78	2.0	4.0	18.5	0.0
		20.2	57	3.0	5.5	17.0	0.0
		28.8	36	1.0	4.5	10.5	0.0
	Mitomycin C	0.300	46	63.0	65.0	0.0	0.0

GLP = Good Laboratory Practice
a Based on mitotic index
b Precipitate present at dose and wash.
-g = % of cells with chromosome aberrations; +g = % of cells with chromosome aberrations + % of cells with gaps

8.3. FTC: Ames

Test Article: FTC

Report Title:	Mutagenicity Test with FTC in the Salmonella-Escherichia coli/Mammalian-Microsome Reverse Mutation Assay with a Confirmatory Assay	**Gilead Study No. 18637-0-409R**
Test for Induction of: Reverse mutations at the histidine locus in *S. typhimurium* & Reverse mutations at the tryptophan locus in *E. coli*		
Strains: *Salmonella typhimurium* (TA98, TA100, TA1535, TA1537) & *Escherichia coli* (WP2uvrA)	**No. of Independent Assays:** - S9: 4 experiments; + S9: 4 experiments	**Location in CTD:** 4.2.3.3.1 **GLP Compliance:** Yes
Metabolizing System: Aroclor™-induced rat liver (S9 mix)	No. of Cells Analyzed/Culture: 0.5 x 10^9 bacteria/mL	**Lot No.:** [redacted]
Vehicles: DMSO **For Test Articles:** FTC- lot [redacted] (± S9)	**For Positive Controls:** DMSO (± S9) 2-aminoanthracene or 2-nitrofluorene or sodium azide or ICR-191 or 4-nitroquinoline-N-oxide	**No. of Replicate Cultures:** [a] Rangefinding study a) single b) single Mutagenicity assays a) triplicate b) triplicate
Treatment[b]: After the overlay had solidified, the plates were inverted and incubated for 48 ± 8 hr at 37 ± 2°C.		**Date of Treatment:** [redacted], 19[redacted] – [redacted], 19[redacted]
Cytotoxic Effects:	No cytotoxicity was observed in the presence or absence of S9 mix as evidenced by a normal background lawn and no test article precipitation.	
Genotoxic Effects:	Range-finding study – No decrease in the number of revertants per plate. Mutagenicity assays – No positive increases in the number of revertants per plate were observed In the confirmatory assay - No positive increases in the number of revertants per plate were observed. The 2.1-fold, non-dose responsive increase was considered artifactual due to a lower than routinely observed vehicle control value. **Conclusion:** FTC did not cause a positive increase in the number of revertants per plate of any of the tester strains in either the presence or absence of microsomal enzymes prepared from Arochlor®-induced rat liver (S9).	

a Dose Range-finding study: a) - S9: 6.67, 10.0, 33.3, 66.7, 100, 333, 667, 1000, 3330, 5000 µg/plate. Vehicle control. b) + S9: (same)
Mutagenicity assays (initial, confirmatory, additional): a) - S9: 100, 333, 1000, 3330, 5000 µg/plate. Positive and vehicle controls. b) + S9: (same)

b When all the required components have been added (tester strain, vehicle or test article, ± S9), the mixture was vortexed and overlaid onto the surface of 25 mL of minimal bottom agar contained in a 15 × 100 mm petri dish.

Assay 1 Experiment ID 18637-B1 **Test Article: FTC**

Report Title: Mutagenicity Test with FTC in the Salmonella-Escherichia coli/Mammalian-Microsome Reverse Mutation Assay with a Confirmatory Assay **Gilead Study No. 18637-0-409R**

Metabolic Activation	Dose Level (µg/plate)	Mean revertants per plate with standard deviation									
		TA98		TA100		TA1535		TA1537		WP2uvrA	
		MEAN	S.D.	MEAN	S.D.	MEAN	S.D.	MEAN	S.D.	MEAN	S.D.
Microsomes: rat liver Vehicle Control		28	7	114	11	13	4	12	2	13	5
Test Article	100	27	5	116	10	10	2	7	3	15	1
	333	28	11	83	7	10	6	8	2	8	2
	1000	26	6	100	15	10	3	8	2	9	2
	3330	23	5	93	12	15	3	12	2	11	4
	5000	21	7	85	9	12	2	7	2	12	1
Positive Control**		1116	109	1103	128	133	8	150	21	160	28
Microsome: none Vehicle Control		10	4	87	8	11	5	6	1	8	4
Test Article	100	14	4	93	8	13	2	9	4	14	5
	333	14	3	88	9	12	1	6	1	9	2
	1000	12	2	90	5	13	3	7	2	11	4
	3330	11	1	73	9	12	2	3	3	9	1
	5000	12	4	74	16	11	3	5	2	7	3
Positive Control***		142	30	542	14	527	82	289	30	253	10

Assay 2: Experiment ID 18637-C1 **Test Article: FTC**

Report Title: Mutagenicity Test with FTC in the Salmonella-Escherichia coli/Mammalian-Microsome Reverse Mutation Assay with a Confirmatory Assay **Gilead Study No. 18637-0-409R**

Metabolic Activation	Dose Level (µg/plate)	Mean Revertants Per Plate With Standard Deviation									
		TA98		TA100		TA1535		TA1537		WP2uvrA	
		MEAN	S.D.	MEAN	S.D.	MEAN	S.D.	MEAN	S.D.	MEAN	S.D.
Microsomes: rat liver Vehicle Control		16	5	97	7	11	5	9	1	7	4
Test Article	100	17	5	94	8	10	1	7	4	12	4
	333	10	4	93	18	10	1	8	3	10	4
	1000	12	3	67	5	11	3	5	0	10	1
	3330	15	6	70	16	11	3	7	2	15	4
	5000	11	2	52	9	13	5	6	4	12	3
Positive Control**		1045	44	1051	37	109	6	165	27	268	27
Microsome: none Vehicle Control		6*	2	69	9	5	1	6	1	11	3
Test Article	100	NC	-	66	7	10	1	6	1	10	6
	333	NC	-	50	5	8	5	5	1	12	5
	1000	NC	-	79	13	8	2	4	2	12	3
	3330	NC	-	57	7	5	3	2	2	9	4
	5000	NC	-	56	7	2	3	3	1	14	9
Positive Control***		NC	-	411	46	431	38	236	34	203	35

Assay 3: Experiment ID 18637-D1 **Test Article: FTC**

Report Title: Mutagenicity Test with FTC in the Salmonella-Escherichia coli/Mammalian-Microsome Reverse Mutation Assay with a Confirmatory Assay **Gilead Study No. 18637-0-409R**

Metabolic Activation	Dose Level (µg/plate)	Mean Revertants Per Plate With Standard Deviation				
		WP2uvrA			WP2uvrA	
		1	2	3	MEAN	S.D.
Microsomes: rat liver Vehicle Control		18	24	7	16	9
Test Article	100	11	14	13	13	2
	333	15	12	15	14	2
	1000	9	12	17	13	4
	3330	13	8	12	11	3
	5000	13	10	9	11	2
Positive Control**		289	227	182	233	54

Metabolic Activation	Dose Level (µg/plate)	TA98			TA98	
		1	2	3	MEAN	S.D.
Microsomes: rat liver Vehicle Control		14	15	15	15	1
Test Article	100	10	12	11	11	1
	333	17	22	22	20	3
	1000	6	17	9	11	6
	3330	9	7	12	9	3
	5000	6	7	11	8	3
Positive Control***		137	129	141	136	6

* The mean vehicle control value for tester strain TA98 in the absence of S9 was not within the acceptable range. See retest data (experiment ID 18637-D1)

** TA98 2-aminoanthracene: 2.5 µg/plate
TA100 2-aminoanthracene: 2.5 µg/plate
TA1535 2-aminoanthracene: 2.5 µg/plate
TA1537 2-aminoanthracene: 2.5 µg/plate
WP2uvrA 2-aminoanthracene: 25.0 µg/plate

*** TA98 2-nitrofluorene: 1.0 µg/plate
TA100 sodium azide: 2.0 µg/plate
TA1535 sodium azide: 2.0 µg/plate
TA1537 ICR-191: 2.0 µg/plate
WP2uvrA 4-nitroquinoline-N-oxide: 1.0 µg/plate

8.4. FTC: Ames

Test Article: FTC

Report Title: Salmonella/Mammalian-Microsome Assays with 524W91		**Gilead Study No. MUT203**
Test for Induction of: Reverse mutations at histidine locus in *S. typhimurium*	**No. of Independent Assays:** - S9: 3 experiments; + S9: 3 experiments	**Location in CTD**: 4.2.3.3.1
Date of Treatment: [redacted], 19[redacted] – [redacted], 19[redacted]	**No. of Replicate Cultures:** [a] Range-finding study a) single b) single Mutagenicity assays a) triplicate b) triplicate	**GLP Compliance:** Yes **Lot No.:** [redacted]
Metabolizing System: Aroclor™-induced rat liver (S9 mix)	**No. of Cells Analyzed/Culture:** 0.5 x 10^9 bacteria/mL	
Vehicles For Test Articles: DMSO 524W91UO reference number; 92/0277-049-C and (± S9)	**Vehicles For Positive Controls:** DMSO + 2-aminoanthracene or 2-nitrofluorene or Sodium azide or ICR-191 or 2-nitrofluorene and (± S9)	
Treatment:[b] Incubation was at 37 ± 2°C for 48 ± 8 hours.	**Strains:** *Salmonella typhimurium* (TA98, TA100, TA1535, TA1537, TA1538)	
Cytotoxic Effects:	No cytotoxicity was observed in presence or absence of S9 mix as evidenced by a normal background lawn and no test article precipitation.	
Genotoxic Effects:	FTC exerted no detectable mutagenic activity in the Ames *Salmonella* assay, either by plate incorporation or by suspension preincubation modification.	

a Dose Range finding study: a) - S9: 6.67, 10.0, 33.3, 66.7, 100, 333, 667, 1000, 3330, 5000 µg/plate. Vehicle control. b) + S9: (same)
Mutagenic, confirmatory, additional: a) - S9: 100, 333, 1000, 3330, 5000 µg/plate. Positive and vehicle controls. b) +S9: (same)

b The test article, the tester strain, and the S9 mix (where appropriate) were combined in molten agar which was overlaid into a minimal agar.

Assay 1: Experiment ID 15437-B1 **Test Article: FTC**

Report Title: Salmonella/Mammalian-Microsome Assays with 524W91 **Gilead Study No. MUT203**

Metabolic Activation	Dose Level (µg/plate)	Mean Revertants Per Plate With Standard Deviation									
		TA98		TA100		TA1535		TA1537		TA1538	
		MEAN	S.D.	MEAN	S.D.	MEAN	S.D.	MEAN	S.D.	MEAN	S.D.
Microsomes: rat liver Vehicle Control		35	5	251	9	13	1	8	1	20	7
Test Article	100	28	7	NC	-	14	5	9	2	20	3
	333	35	3	NC	-	13	2	8	3	20	7
	667	30	6	NC	-	13	3	6	3	22	10
	1000	23	7	NC	-	10	1	6	1	20	3
	3330	40	9	NC	-	8	3	5	2	24	3
	5000	28	11	NC	-	14	8	6	1	19	7
Positive Control**		976	56	1182	59	181	17	128	7	1189	58
Microsome: none											
Vehicle Control		19	8	159	26	12	4	5	4	14	3
Test article	100	16	3	167	13	11	3	3	1	9	1
	333	16	4	165	20	8	4	9	2	12	4
	667	14	5	164	29	12	4	5	2	15	3
	1000	16	8	176	9	9	3	7	4	13	1
	3330	19	3	187	25	10	3	5	1	10	2
	5000	16	5	188	7	8	0	7	2	10	1
Positive Control***		193	73	719	54	321	24	267	32	327	52

NC = No count due to unacceptable mean vehicle control value. See retest data experiment ID 15437-B2

** = TA98 2-aminoanthracene 2.5 µg/plate
TA100 2-aminoanthracene 2.5 µg/plate
TA1535 2-aminoanthracene 2.5 µg/plate

*** = TA98 2-nitrofluorene 1.0 µg/plate
TA100 sodium azide 2.0 µg/plate
TA1535 sodium azide 2.0 µg/plate

8.5. FTC: Ames

Test Article: FTC

Report Title: Mutagenicity Test of FTC Using Microorganisms

Gilead Study No. K01-3154

Test for Induction of: Reverse mutation in bacterial cells

No. of Independent Assays: 2

Location in CTD: 4.2.3.3.1

Species/Strain: *S. typhimurium* (TA98, TA100, TA1535, TA1537), *E. coli(*WP2 *uvr A)*

No. of Replicate Cultures: 3 (all negative controls), 2 (#1), 2 (#2)

GLP Compliance: Yes

Lot No.: [redacted]

Metabolizing System: Phenobarbital and 5, 6-benzoflavone induced rat liver S9

Genotoxic Effects: None

Vehicles: Distilled water (FTC); DMSO, except distilled water for sodium azide (positive controls)

Treatments: Pre-incubation method

Cytotoxic Effects: No appreciable toxicity was observed

Date of Treatments: [redacted] 20[redacted], [redacted] 20[redacted]

Metabolic Activation	Test Article	Dose Level (µg/plate)	Assay No. 1 Initial Mutagenicity Assay Revertant Colony Counts (Mean)				
			TA100	TA1535	WP2 *uvr* A	TA98	TA1537
Without Activation	(Vehicle)	--	131	13	30	26	13
	Test Article	4.88	137	10	33	21	9
		19.5	127	9	35	24	14
		78.1	129	14	26	20	14
		313	118	8	32	23	15
		1250	123	10	35	23	11
		5000	125	12	33	19	13
	AF-2	0.01	523		284		
	Sodium azide	0.5		451			
	AF-2	0.1				509	
	ICR-191	0.5					1139

Metabolic Activation	Test Article	Dose Level (µg/plate)	Assay No. 1 Initial Mutagenicity Assay Revertant Colony Counts (Mean)				
			TA100	TA1535	WP2 *uvr* A	TA98	TA1537
With Activation	(Vehicle)	--	149	8	34	27	31
	Test Article	4.88	148	7	34	40	21
		19.5	140	11	40	37	23
		78.1	135	6	37	31	26
		313	135	9	32	29	23
		1250	128	5	41	37	29
		5000	137	10	40	33	22
	2AA	1	772				
	2AA	2		203			213
	2AA	10			565		
	2AA	0.5				312	

Metabolic Activation	Test Article	Dose Level (µg/plate)	Assay No. 2 Confirmatory Mutagenicity Assay Revertant Colony Counts (Mean)				
			TA100	TA1535	WP2 *uvr* A	TA98	TA1537
Without Activation	(Vehicle)	--	117	10	20	19	8
	Test Article	313	110	8	23	20	12
		625	118	9	26	25	10
		1250	112	12	15	17	12
		2500	115	7	22	16	12
		5000	111	9	26	18	10
	AF-2	0.01	471		216		
	Sodium azide	0.5		411			
	AF-2	0.1				484	
	ICR-191	0.5					1270
With Activation	(Vehicle)	--	121	8	23	25	19
	Test Article	313	132	9	24	32	20
		625	118	7	21	35	28
		1250	137	10	26	25	22
		2500	123	14	29	33	14
		5000	127	9	27	28	16
	2AA	1	650				
	2AA	2		178			172
	2AA	10			538		
	2AA	0.5				381	

AF2: 2-(2-Furyl)-3-(5-nitro-2-furyl) acrylamide
ICR-191: 2-Methoxy-6-chloro-9[3-(2-chloroethyl)-aminopropylamino]acridine-2HCl
2AA: 2-Aminoanthracene

8.6. FTC: Mouse Lymphoma Assay

Test Article: FTC

Report Title: In Vitro Mammalian Cell Gene Mutation Test (Mouse Lymphoma Assay)	**Gilead Study No. TOX012**
Test for Induction of: Forward mutations at the thymidine kinase locus in cultured L5178Y cells. Mutants are unable to metabolize the non-toxic thymidine metabolite, trifluorothymidine (TFT) to its lethal metabolite and hence are resistant to its lethal effect.	**Location in CTD**: 4.2.3.3.1 **Lot No.:** [redacted]
No. of Independent Assays: Preliminary Toxicity assay: 1 experiment; Mutagenicity assay: 1 experiment	**GLP Compliance:** Yes
Strains: L5178Y mouse lymphoma cells TK+/-	**No. of Replicate Cultures:**[a] Preliminary Toxicity assay a) single b) single Mutagenicity assay a) duplicate b) duplicate
Metabolizing System: Aroclor 1254-induced rat liver (S9 mix)	**No. of Cells Analyzed/Culture:** Adjusted to 3 x 10^5/mL at 24-48 hours after treatment, Cultures less than 3 x 10^5/mL were not adjusted
Vehicles: Sterile distilled water **For Test Article:** FTC (lot [redacted] + (± S9))	**For Positive Controls:** a) - S9: Methyl methanesulfonate (MMS) 10 and 20 µg/mL b) + S9: 7,12-Dimethyl-benz(a)anthracene (7,12-DMBA) 2.5 and 4.0 µg/mL
Treatment: Treatment period was for 4 hours at 37 ± 1°C. Recovery at approximately 24 and 48 hours after treatment (± S9).	**Date of Treatment:** [redacted], 19[redacted] – [redacted], 19[redacted]
Cytotoxic Effects:	Suspension growth relative to the solvent controls at 5000 µg/mL was 76% without activation and 99% with S9 activation.
Genotoxic Effects:	FTC was concluded to be negative in the L5178Y/TK+/- Mouse Lymphoma Mutagenesis Assay.

a Preliminary Toxicity assay: a) - S9: 0.5, 1.5, 5.0, 15, 50, 150, 500, 1500, 5000 µg/mL. Vehicle control. b) + S9: (same)
Mutagenicity assay: a) - S9: 1000, 2000, 3000, 4000, 5000 µg/mL. Positive and vehicle controls. b) +S9: (same)

Test Article: FTC

Report Title: In Vitro Mammalian Cell Gene Mutation Test (Mouse Lymphoma Assay) **Gilead Study No. TOX012**

Assay 1: Study No. G97BG07.702			**MUTATION ASSAY WITHOUT ACTIVATION**							
	Daily Cell Counts (conc.10^6cells)		**Mutant Colonies Avg.**	**Suspension Growth[a] Total[b]**	**Suspension Growth Control[c] (%)**	**Viable Colonies (VC) Avg.**	**Cloning Growth Avg. VC**	**Cloning Growth Control[d] (%)**	**Total Growth[e] (%)**	**Mutant Frequency[f] (10^6 cells)**
Test Condition	**Day 1**	**Day 2**								
Vehicle Control 1	1.475	1.514	48	24.8		173	173	-		55
Vehicle Control 2	1.398	1.502	51	23.3		172	172	-		60
Positive Control 1										
Methyl Methanesulfonate (µg/mL)										
10	1.029	1.356	224	15.5	64	105	105	61	39	426
20	0.885	0.910	218	8.9	37	50	50	29	11	865
FTC (µg/mL)										
1000 A	1.245	1.575	45	21.8	91	174	174	101	91	52
B	1.304	1.631	39	23.6	98	141	141	82	80	55
2000 A	1.180	1.611	55	21.1	88	165	165	96	84	66
B	1.221	1.467	58	19.9	83	146	146	85	70	79
3000 A	1.098	1.641	53	20.0	83	176	176	102	85	61
B	1.081	1.557	54	18.7	78	145	145	84	65	74
4000 A	1.064	1.502	55	17.7	74	143	143	83	61	77
B	0.945	1.505	59	15.8	66	142	142	82	54	83
5000 A	0.856	1.410	54	13.4	56	166	166	96	54	66
B	0.874	1.387	44	13.5	56	161	161	93	52	55

Assay 1: Study No. G97BG07.702			MUTATION ASSAY WITHOUT ACTIVATION							
Test Condition	Daily Cell Counts (conc.10^6cells) Day 1	Daily Cell Counts (conc.10^6cells) Day 2	Mutant Colonies Avg.	Suspension Growth[a] Total[b]	Suspension Growth Control[c] (%)	Viable Colonies (VC) Avg.	Cloning Growth Avg. VC	Cloning Growth Control[d] (%)	Total Growth[e] (%)	Mutant Frequency[f] (10^6 cells)
Vehicle Control 1	1.124	1.510	51	18.9		130	130			79
Vehicle Control 2	1.052	1.517	40	17.7		171	171			47
Positive Control 1										
7,12-Dimethyl-benz(a)anthracene (µg/mL)										
2.5	0.816	1.467	158	13.3	73	112	112	74	54	282
4	0.640	0.993	231	7.1	39	87	87	57	22	534
FTC (µg/mL)										
1000 A	1.178	1.530	42	20.0	109	159	159	106	116	53
B	1.163	1.525	42	19.7	108	144	144	96	103	58
2000 A	1.038	1.568	33	18.1	99	132	132	88	87	50
B	1.100	1.513	43	18.5	101	117	117	78	78	74
3000 A	1.106	1.475	50	18.1	99	166	166	110	109	61
B	1.103	1.390	53	17.0	93	138	138	91	85	77
4000 A	1.055	1.450	46	17.0	93	153	153	101	94	60
B	1.058	1.529	46	18.0	98	170	170	112	111	54
5000 A	1.072	1.338	52	15.9	87	171	171	113	99	61
B	1.001	1.571	52	17.5	96	129	129	86	82	81

a Cultures containing < 0.3×10^6 cells/mL on day 1 and 2 are considered as having 0% total suspension growth.
b Total suspension growth = (Day 1 cell conc./0.3×10^6 cells/mL) × (Day 2 cell conc./Day 1 adjusted cell conc.).
c % of control growth = (total treatment suspension growth / average solvent control total suspension growth) × 100.
d % control cloning growth = (average V.C. of treated culture/average V.C. of solvent control) × 100.
e % total growth = (% suspension growth × % cloning growth)/100.
f Mutant frequency (per 10^6 surviving cells) = (Average # TFT colonies / average # V.C. colonies) × 200.
Note: A and B or 1 and 2 are duplicate cultures

8.7. TAF: Ames

Test Article: TAF

Report Title: Salmonella – Escherichia coli/Mammalian-Microsome Reverse Mutation Assay with a Confirmation Assay with GS-7340-02

Gilead Study No. V990212
[redacted] Study No. 20900-0-409OECD

Test for Induction of: Reverse mutation in bacterial cells

No. of Independent Assays: 2

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

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 52 ± 4 hours

Date of Treatment: initial [redacted] 19[redacted] /confirmatory [redacted] 19[redacted]

Cytotoxic Effects: None

Genotoxic Effects: None

Metabolic Activation	Treatment	Dose Level (µg/plate)	Plate Incorporation – Initial Assay Revertants/Plate (Mean ± SD)				
			TA98	TA100	TA1535	TA1537	WP2*uvr*A
Without Activation	DMSO	50 µL/plate	17 ± 6 N	96 ± 12 N	9 ± 5 N	5 ± 4 N	16 ± 6 N
	GS-7340-02	100	14 ± 4 N	98 ± 10 N	8 ± 2 N	5 ± 1 N	21 ± 4 N
		333	21 ± 7 N	98 ± 13 N	12 ± 6 N	5 ± 2 N	21 ± 1 N
		1000	18 ± 3 N	90 ± 8 N	10 ± 3 N	7 ± 2 N	16 ± 2 N
		3330	14 ± 7 N	93 ± 2 N	16 ± 5 N	5 ± 2 N	18 ± 3 N
		5000	12 ± 2 R	84 ± 19 N	13 ± 6 N	8 ± 3 N	17 ± 1 N
	2-nitrofluorene	1.0	244 ± 32 N				
	sodium azide	2.0		796 ± 63 N	668 ± 60 N		
	ICR-191	2.0				564 ± 19 N	
	4-nitroquinoline-N-oxide	1.0					184 ± 24 N

Metabolic Activation	Treatment	Dose Level (µg/plate)	Plate Incorporation – Initial Assay Revertants/Plate (Mean ± SD)					
			TA98	TA100	TA1535	TA1537	WP2*uvr*A	
With Activation	DMSO	50 µL/plate	31 ± 12^{N}	146 ± 9^{N}	18 ± 1^{N}	9 ± 5	17 ± 3	
	GS-7340-02	100	30 ± 8^{N}	117 ± 9^{N}	12 ± 1^{N}	10 ± 4^{N}	16 ± 13^{N}	
		333	30 ± 2^{N}	117 ± 18^{N}	10 ± 3^{N}	5 ± 3^{N}	23 ± 2^{N}	
		1000	29 ± 4^{N}	129 ± 10^{N}	18 ± 6^{N}	11 ± 3^{N}	22 ± 4^{N}	
		3330	26 ± 3^{N}	116 ± 18^{N}	14 ± 2^{N}	9 ± 3^{N}	20 ± 2^{N}	
		5000	29 ± 3^{N}	114 ± 26^{N}	13 ± 7^{N}	4 ± 4^{N}	15 ± 3^{N}	
	benzo[a]pyrene	2.5	412 ± 35^{N}					
	2-aminoanthracene	2.5		862 ± 132^{N}	162 ± 22^{N}	174 ± 12^{N}		
	2-aminoanthracene	25.0					280 ± 36^{N}	

N = Normal
R = Slightly reduced
SD = Standard Deviation

Test Article: TAF

Report Title: Salmonella – Escherichia coli/Mammalian-Microsome Reverse Mutation Assay with a Confirmation Assay with GS-7340-02

Gilead Study No. V990212
[redacted] Study No. 20900-0-409OECD

Test for Induction of: Reverse mutation in bacterial cells

No. of Independent Assays: 2

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

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 52 ± 4 hours

Date of Treatment: initial [redacted] 19[redacted]/confirmatory [redacted] 19[redacted]

Cytotoxic Effects: None

Genotoxic Effects: None

Metabolic Activation	Treatment	Dose Level (µg/plate)	Plate Incorporation – Confirmatory Assay Revertants/Plate (Mean ± SD)					
			TA98	TA100	TA1535	TA1537	WP2*uvr*A	
Without Activation	DMSO	50 µL/plate	20 ± 4[N]	85 ± 7[N]	13 ± 7[N]	4 ± 3[N]	15 ± 2[N]	
	GS-7340-02	100	15 ± 8[N]	74 ± 3[N]	9 ± 5[N]	5 ± 2[N]	14 ± 2[N]	
		333	22 ± 3[N]	80 ± 10[N]	13 ± 6[N]	6 ± 3[N]	19 ± 4[N]	
		1000	23 ± 1[N]	92 ± 11[N]	9 ± 5[N]	4 ± 0[N]	16 ± 5[N]	
		3330	20 ± 4[N]	96 ± 7[N]	9 ± 4[N]	6 ± 5[N]	19 ± 3[N]	
		5000	15 ± 3[N]	83 ± 12[N]	11 ± 3[N]	6 ± 2[N]	15 ± 6[N]	
	2-nitrofluorene	1.0	234 ± 17[N]					
	sodium azide	2.0		752 ± 30[N]	615 ± 28[N]			
	ICR-191	2.0				614 ± 58[N]		
	4-nitroquinoline-N-oxide	1.0					196 ± 50[N]	

Metabolic Activation	Treatment	Dose Level (μg/plate)	Plate Incorporation – Confirmatory Assay Revertants/Plate (Mean ± SD)					
			TA98	TA100	TA1535	TA1537	WP2*uvr*A	
With Activation	DMSO	50 μL/plate	27 ± 4[N]	109 ± 23[N]	10 ± 2[N]	7 ± 3[N]	15 ± 6[N]	
	GS-7340-02	100	25 ± 2[N]	94 ± 14[N]	11 ± 4[N]	5 ± 2[N]	15 ± 2[N]	
		333	28 ± 3[N]	103 ± 11[N]	9 ± 4[N]	6 ± 2[N]	20 ± 9[N]	
		1000	27 ± 2[N]	89 ± 7[N]	13 ± 5[N]	10 ± 3[N]	23 ± 6[N]	
		3330	21 ± 4[N]	107 ± 18[N]	14 ± 3[N]	7 ± 1[N]	20 ± 2[N]	
		5000	26 ± 1[N]	90 ± 6[N]	11 ± 2[N]	7 ± 4[N]	21 ± 6[N]	
	benzo[a]pyrene	2.5	441 ± 10[N]					
	2-aminoanthracene	2.5		1225 ± 162[N]	158 ± 8[N]	193 ± 24[N]		
	2-aminoanthracene	25.0					242 ± 15[N]	

N = Normal
SD = Standard Deviation

8.8. TAF: Mouse Lymphoma Mutation Assay

Report Title: L5178Y TK+/- Mouse Lymphoma Forward Mutation Assay with a Confirmatory Assay with GS-7340-02

Gilead Study No. V990213
[redacted] Study No. 20900-0-431 ICH

Test for Induction of: Forward gene mutation at TK locus

No. of Independent Assays: 2

Location in CTD: 4.2.3.3.1

Cell Line: L5178Y/TK$^{+/-}$

No. of Replicate Cultures: 1

GLP Compliance: yes

Metabolizing System: Aroclor 1254-induced rat liver (S9)

No. of Cells Analyzed/Culture:
Treated: 6 x 10^6 Cloned: 3 x 10^6

Lot Number: [redacted]

Vehicle for Test Article: Dimethylsulfoxide (DMSO)

Vehicle for Positive Controls: Cell culture grade water for methyl methanesulfonate; DMSO for methylcholanthrene

Date of Treatment:
initial [redacted] 19[redacted] /
confirmatory [redacted] 19[redacted]

Treatment: 4 hours without and with S9 (Initial Assay)

Cytotoxic Effects: None

Genotoxic Effects: None

Metabolic Activation	Test Article	Concentration (µg/mL)	RSG (%)[a]	Cloning Efficiency[b]	Relative Growth[c]	Mutant Frequency (10^{-6})[d]
Without Activation 4 hour Treatment	DMSO	1% (µL/mL)	113.3	92.5	104.8	57.4
		1% (µL/mL)	103.1	93.9	96.8	57.5
		1% (µL/mL)	83.7	113.6	95.1	53.4
	GS-7340-02	39.3	77.1	92.9	71.6	53.6
		78.5	87.8	89.4	78.5	51.9
		157	74.1	112.5	83.3	53.9

Metabolic Activation	Test Article	Concentration (µg/mL)	RSG (%)[a]	Cloning Efficiency[b]	Relative Growth[c]	Mutant Frequency (10^{-6})[d]
Without Activation 4 hour Treatment	GS-7340-02	313	109.9	93.5	102.8	69.4
		625	88.5	105.2	93.1	70.0
		1250	78.8	94.1	74.2	67.1
		2500	55.1	106.1	58.4	77.3
		5000	28.4	88.3	25.1	106.6
	Methyl methanesulfonate	6.5	88.1	88.1	77.6	149.6
		13	67.7	61.2	41.4	252.8
With Activation 4 hour Treatment	DMSO	1% (µL/mL)	119.9	106.1	127.2	61.2
		1% (µL/mL)	92.5	96.9	89.6	64.3
		1% (µL/mL)	87.7	96.9	85.0	72.9
	GS-7340-02	1250	110.4	93.4	103.2	91.1
		1500	123.1	94.1	115.8	99.3
		2000	91.5	98.7	90.3	82.7
		2500	80.8	86.0	69.5	126.0
		3000	80.2	102.4	82.1	82.2
		3500	53.5	121.5	65.0	85.2
		4000	61.2	103.7	63.5	84.6
		4500	46.1	98.9	45.6	94.5
	Methycholanthrene	2	79.5	81.1	64.5	310.8
		4	75.6	113.6	85.9	272.2

a Cum. RSG = Cumulative Suspension Growth Relative to the Average Vehicle Control Suspension Growth
b Cloning Efficiency = Total Viable Colony Count/Number of Cells seeded * 100
c Relative Growth = (Relative Suspension Growth * Relative Cloning Efficiency) / 100
d Mutant Frequency = (Total Mutant Colonies/Total Viable Colonies) * 2x10E-4
Decimal is moved to express the frequency in units of 10E-6
Expressed as Total Mutant Frequency

Report Title: L5178Y TK+/- Mouse Lymphoma Forward Mutation Assay with a Confirmatory Assay with GS-7340-02

Gilead Study No. V990213
[redacted] Study No. 20900-0-431 ICH

Test for Induction of: Forward gene mutation at TK locus

No. of Independent Assays: 2

Location in CTD: 4.2.3.3.1

Cell Line: L5178Y/TK$^{+/-}$

No. of Replicate Cultures: 1

GLP Compliance: yes

Metabolizing System: Aroclor 1254-induced rat liver (S9)

No. of Cells Analyzed/Culture:
Treated: 6 x 10^6 Cloned: 3 x 10^6

Lot Number: [redacted]

Vehicle for Test Article: Dimethylsulfoxide (DMSO)

Vehicle for Positive Controls: Cell culture grade water for methyl methanesulfonate; DMSO for methylcholanthrene

Date of Treatment:
initial [redacted] 19[redacted] /
confirmatory [redacted] 19[redacted]

Treatment: 24 hours without S9 and 4 hours with S9 (Confirmatory Assay)

Cytotoxic Effects: None

Genotoxic Effects: None

Metabolic Activation	Test Article	Concentration (µg/mL)	RSG (%)[a]	Cloning Efficiency[b]	Relative Growth[c]	Mutant Frequency (10^{-6})[d]
Without Activation 24 hour Treatment	DMSO	1% (µL/mL)	99.9	86.5	86.4	62.4
		1% (µL/mL)	96.8	118.3	114.5	50.4
		1% (µL/mL)	103.3	95.2	98.4	70.4
	GS-7340-02	125	54.2	109.0	59.1	75.1
		250	49.0	107.5	52.7	75.4
		500	28.5	98.3	28.0	88.3
		750	17.9	96.3	17.2	111.8
		1000	17.5	102.6	17.9	110.2
		1250	14.3	88.9	12.7	109.7
		1500	14.5	90.5	13.1	119.5
	Methyl methanesulfonate	6.5	25.2	46.9	11.8	745.6
		6.5	27.9	54.7	15.2	732.3

Metabolic Activation	Test Article	Concentration (µg/mL)	RSG (%)[a]	Cloning Efficiency[b]	Relative Growth[c]	Mutant Frequency (10^{-6})[d]
With Activation 4 hour Treatment	DMSO	1% (µL/mL)	106.7	108.3	115.5	55.2
		1% (µL/mL)	75.5	100.8	76.1	60.9
		1% (µL/mL)	117.8	90.9	107.1	66.7
	GS-7340-02	250	113.8	81.2	92.4	72.6
		500	104.7	78.6	82.3	78.1
		1000	70.8	107.7	76.3	83.7
		2000	101.0	62.4	63.0	126.9
		3000	81.9	84.2	69.0	95.7
		4000	52.2	74.5	38.9	108.9
		4500	44.0	52.9	23.3	106.9
		4800	31.0	66.7	20.7	99.4
	Methycholanthrene	2	88.2	100.0	88.2	240.0
		4	85.9	67.1	57.6	303.6

a Cum. RSG = Cumulative Suspension Growth Relative to the Average Vehicle Control Suspension Growth
b Cloning Efficiency = Total Viable Colony Count/Number of Cells seeded * 100
c Relative Growth = (Relative Suspension Growth * Relative Cloning Efficiency) / 100
d Mutant Frequency = (Total Mutant Colonies/Total Viable Colonies) * 2x10E-4
Decimal is moved to express the frequency in units of 10E-6
Expressed as Total Mutant Frequency

8.9. FTC/TDF: Ames

Test Article: FTC + TDF

Report Title: Bacterial Reverse Mutation Assay
Gilead Study No. TX-164-2002
Test for Induction of: Reverse mutation in bacterial cells
No. of Independent Assays: 2
Location in CTD: 4.2.3.3.1
Species/Strain: *S. typhimurium, E. coli*
No. of Replicate Cultures: 2 (#1), 3 (#2)
GLP Compliance: Yes
Date of Treatments: [redacted] 20[redacted] and [redacted] 20[redacted]
Treatments: Plate incorporation
Lot No.: [redacted] (TDF); [redacted] (FTC)

Metabolizing System: Aroclor-induced rat liver S9
Vehicles: DMSO (FTC/TDF), DMSO, except water for sodium azide (positive controls)
Cytotoxic Effects: No appreciable toxicity was observed
Genotoxic Effects: None

Metabolic Activation	Test Article	Dose Level (µg/plate)	Assay No. 1 Initial Mutagenicity Assay Revertant Colony Counts (Mean ± SD)				
			TA98	TA100	TA1535	TA1537	WP2 *uvr*A
Without Activation	(Vehicle)	50 µL/plate	13 ± 4	112 ± 12	19 ± 6	5 ± 1	16 ± 4
	Test Article	1.5	13 ± 1	149 ± 16	21 ± 5	5 ± 1	13 ± 4
		5.0	12 ± 1	135 ± 14	17 ± 4	6 ± 1	14 ± 2
		15	15 ± 1	111 ± 8	15 ± 0	5 ± 2	12 ± 4
		50	17 ± 1	127 ± 5	16 ± 1	7 ± 3	13 ± 1
		150	13 ± 3	144 ± 16	16 ± 4	8 ± 1	19 ± 1
		500	15 ± 3	135 ± 16	16 ± 6	5 ± 1	14 ± 3
		1500	13 ± 3	157 ± 3	17 ± 3	6 ± 1	14 ± 2
		5000	11 ± 0	155 ± 6	16 ± 7	7 ± 1	12 ± 3
	2-nitrofluorene	1.0	170 ± 11				
	Sodium azide	1.0		546 ± 11	398 ± 6		
	9-aminoacridine	75				1424 ± 127	
	MMS	1000					128 ± 14

Metabolic Activation	Test Article	Dose Level (µg/plate)	Assay No. 1 Initial Mutagenicity Assay Revertant Colony Counts (Mean ± SD)				
			TA98	TA100	TA1535	TA1537	WP2 *uvr*A
With Activation	(Vehicle)	50 µL/plate	16 ± 1	143 ± 11	14 ± 8	9 ± 2	15 ± 0
	Test Article	1.5	20 ± 8	131 ± 25	14 ± 1	7 ± 1	13 ± 1
		5.0	17 ± 1	146 ± 5	10 ± 3	8 ± 4	15 ± 2
		15	18 ± 2	143 ± 21	11 ± 1	10 ± 2	16 ± 1
		50	22 ± 8	127 ± 6	15 ± 4	8 ± 1	22 ± 4
		150	18 ± 1	140 ± 1	15 ± 1	6 ± 1	18 ± 2
		500	19 ± 4	148 ± 31	12 ± 4	7 ± 1	14 ± 4
		1500	22 ± 4	148 ± 9	11 ± 1	8 ± 0	11 ± 3
		5000	20 ± 1	127 ± 4	13 ± 1	10 ± 1	17 ± 6
	2-aminoanthracene	1.0	689 ± 145	734 ± 0	107 ± 28	93 ± 33	
	2-aminoanthracene	10					608 ± 76

Metabolic Activation	Test Article	Dose Level (µg/plate)	Assay No. 2 Confirmatory Mutagenicity Assay Revertant Colony Counts (Mean ± SD)				
			TA98	TA100	TA1535	TA1537	WP2 *uvr*A
Without Activation	(Vehicle)	50µL/plate	22 ± 3	109 ± 22	16 ± 2	6 ± 2	12 ± 4
	Test Article	50	27 ± 2	147 ± 3	19 ± 4	6 ± 4	12 ± 2
		150	29 ± 6	104 ± 23	14 ± 2	5 ± 1	13 ± 4
		500	32 ± 6	142 ± 6	18 ± 3	6 ± 2	13 ± 2
		1500	29 ± 3	127 ± 3	17 ± 2	4 ± 1	14 ± 3
		5000	28 ± 4	132 ± 25	22 ± 4	5 ± 3	10 ± 4
	2-nitrofluorene	1.0	173 ± 23				
	Sodium azide	1.0		569 ± 17	357 ± 20		
	9-aminoacridine	75				1282 ± 126	
	MMS	1000					111 ± 13
With Activation	(Vehicle)	50µL/plate	26 ± 5	136 ± 5	15 ± 3	4 ± 1	11 ± 4
	Test Article	50	30 ± 4	139 ± 14	12 ± 4	6 ± 2	14 ± 1
		150	32 ± 2	143 ± 8	10 ± 3	5 ± 2	11 ± 1
		500	28 ± 3	159 ± 21	13 ± 5	7 ± 4	11 ± 4
		1500	30 ± 3	150 ± 9	13 ± 3	5 ± 3	14 ± 4
		5000	27 ± 3	130 ± 19	13 ± 1	10 ± 2	11 ± 3
	2-aminoanthracene	1.0	640 ± 75	917 ± 64	101 ± 13	79 ± 17	
	2-aminoanthracene	10					581 ± 82

8.10. FTC/TDF: Mouse Lymphoma Assay

Test Article: FTC + TDF

<table>
<tr><td colspan="2">Report Title: In Vitro Mammalian Cell Gene Mutation Test (L5178Y/TK+/-)</td><td colspan="2">Gilead Study No. TX-164-2003 ([redacted] AA97YC-YD.704.BTL)</td></tr>
<tr><td colspan="2">Test for induction of : Forward mutations at the thymidine kinase locus</td><td colspan="2">No of independent assays: Preliminary Toxicity assay: 1, Mutagenicity assay: 1</td><td>Location in CTD: 4.2.3.3.1
GLP compliance: Yes</td></tr>
<tr><td colspan="2">Strains: L5178Y mouse lymphoma cells</td><td colspan="2">Metabolizing system: Aroclor 1254-induced rat liver (S9 mix)</td><td rowspan="2">Lot No.: [redacted] (FTC); [redacted] (TDF)</td></tr>
<tr><td colspan="4">No. of replicate cultures[a]: Preliminary assay: a) single, b) single; Mutagenicity assay: a) duplicate, single for positive controls, b) duplicate, single for positive controls</td></tr>
<tr><td colspan="5">No. of cells analyzed / culture: Adjusted to 3 x 10^5/mL at 24 and 48 hours after treatment; cultures less than 3 x 10^5/mL were not adjusted</td></tr>
<tr><td>Test Article Vehicle: DMSO</td><td colspan="2">Positive control:
a) –S9: methyl methanesulfonate (MMS) 15 and 20 µg/mL,
b) +S9: 7,12-dimethyl-benz(a)anthracene) 2.5 and 4.0 µg/mL</td><td colspan="2">Dates of treatment:
Preliminary toxicity assay: [redacted] 20[redacted]
Mutagenicity assay: [redacted] 20[redacted]</td></tr>
<tr><td colspan="5">Treatment: Treatment period was 4 hours with and without S9 activation at 37±1ºC. Recovery at approximately 24 and 48 hours after treatment (+/-S9)</td></tr>
<tr><td colspan="5">Cytotoxic effects: Suspension growth relative to the solvent controls at 3575 µg/mL (10 mM) was 3% without activation and 0% with S9 activation</td></tr>
<tr><td colspan="5">Genotoxic effects: FTC/TDF was concluded to be positive in the L5178Y/TK$^{+/-}$ Mouse Lymphoma Mutagenesis Assay</td></tr>
</table>

a Preliminary toxicity assay a) –S9: 0.5, 1.5, 5, 15, 50, 150, 500, 1500, 3575 µg/mL. Vehicle control. b) +S9: (same)
Mutagenicity assay a) –S9: 15, 50, 100, 150, 200, 300 µg/mL. Positive and vehicle controls. b) +S9: 50, 100, 200, 300, 400, 500, µg/mL. Positive and vehicle controls.

Test Article: FTC + TDF

Report Title: In Vitro Mammalian Cell Gene Mutation Test (L5178Y/TK+/-) **Gilead Study No. TX-164-2003** ([redacted] AA97YC-YD.704.BTL)

Assay 1: MUTATION ASSAY WITHOUT ACTIVATION

Test Condition	Daily cell counts (Conc. 10^6 cells)[a]		Mutant colonies avg.	Suspension growth total[b]	Suspension growth control (%)[c]	Viable colonies (VC) avg.	Cloning growth control (%)[d]	Total growth (%)[e]	Mutant frequency (10^6 cells)[f]
	Day 1	Day 2							
Vehicle control									
1	1.366	1.688	78	25.6		221			71
2	1.386	1.654	68	25.5		173			78
Positive control: Methyl methanesulfonate (µg/mL)									
15	1.194	1.590	188	21.1	83	150	76	63	251
20	1.154	1.726	205	22.1	87	162	83	72	253
FTC/TDF (µg/mL)									
15 A	1.265	1.563	53	22.0	86	191	97	84	56
15 B	1.266	1.799	63	25.3	99	188	96	95	67
50 A	1.214	1.735	69	23.4	92	183	93	85	75
50 B	1.307	1.709	69	24.8	97	165	84	82	84
100 A	1.128	1.691	128	21.2	83	154	78	65	167
100 B	1.159	1.651	134	21.3	83	69	35	29	386
150 A	0.823	1.466	289	13.4	52	97	49	26	595
150 B	0.857	1.461	257	13.9	54	109	55	30	474
200 A	0.592	0.980	++	6.4	25	66	33	8	++
200 B	0.612	0.966	247	6.6	26	84	43	11	591

Test Condition	Daily cell counts (Conc. 10^6 cells)[a]		Mutant colonies avg.	Suspension growth total[b]	Suspension growth control (%)[c]	Viable colonies (VC) avg.	Cloning growth control (%)[d]	Total growth (%)[e]	Mutant frequency (10^6 cells)[f]
	Day 1	Day 2							
Vehicle control									
1	0.953	1.462	94	15.5		196			96
2	0.983	1.458	84	15.9		169			100
Positive control: 7,12 Dimethylbenz(a)anthracene (µg/mL)									
2.5	0.770	1.489	229	12.7	81	112	61	50	411
4.0	0.600	1.074	249	7.2	46	77	42	19	650
FTC/TDF (µg/mL)									
50 A	0.890	1.499	141	14.8	94	159	87	82	177
50 B	0.919	1.455	115	14.9	95	158	86	82	146
100 A	0.850	1.598	171	15.1	96	167	91	88	205
100 B	0.846	1.561	176	14.7	93	121	66	62	291
200 A	0.738	1.596	149	13.1	83	141	77	64	211
200 B	0.800	1.628	122	14.5	92	140	77	71	174
300 A	0.592	1.408	197	9.3	59	125	68	40	316
300 B	0.593	1.441	179	9.5	60	129	71	43	277
400 A	0.339	1.133	201	4.3	27	91	50	14	443
400 B	0.345	1.167	227	4.5	28	102	56	16	447

a Cultures containing < 0.3×10^6 cells/mL on day 1 and 2 are considered as having 0% total suspension growth.
b Total suspension growth = (Day 1 cell conc./0.3×10^6 cells/mL) × (Day 2 cell conc./Day 1 adjusted cell conc.)
c % of control growth = (total treatment suspension growth / average solvent control total suspension growth) × 100
d % control cloning growth = (average V.C. of treated culture/average V.C. of solvent control) × 100
e % total growth = (% suspension growth × % cloning growth)/100
f Mutant frequency (per 10^6 surviving cells) = (Average # TFT colonies / average # V.C. colonies) × 200
++ - Too toxic to count, total growth < 10%

9. GENOTOXICITY: IN VIVO

9.1. BIC: Micronucleus in Rat

Test Article: BIC

Report Title: 2-Week Oral Gavage Toxicity and Toxicokinetic Study with GS-9883 in Han Wistar Rats with Bone Marrow Micronucleus Assay

Gilead Study No. TX-141-2029
[redacted] Study No. 8292936

Test for Induction of: Bone-marrow micronuclei

Treatment Schedule: 14 days for GS-9883-treated; 1 day for positive control-treated

Location in CTD:

Species/Strain: Rat/ Crl:WI(Han)

Sampling Time: Approximately 24 hours after final dose

GLP Compliance: Yes

Age: 8 to 10 weeks

Method of Administration: Oral gavage

Lot Number: [redacted]

No. of Cells Evaluated/Animal: At least 2000

Vehicle: 0.5% (w/w) hydroxypropyl methylcellulose (Methocel K100 LV) and 0.1 % (w/w) Tween® 20 in reverse osmosis water

Date of Final Treatment: [redacted] 20[redacted]

Special Features: NA

Vehicle for Positive Control: Reverse Osmosis Water

Toxic/Cytotoxic Effects: No bone marrow related cytotoxicity

Genotoxic Effects: None

Daily Dose (mg/kg/day)	**0 (Control)**		**10**		**30**		**100**		**300**	
Gender:Number of Animals (TK)	M: 3	F: 3	M: 9	F: 9	M: 9	F: 9	M: 9	F: 9	M: 9	F: 9
Toxicokinetics: AUC_{0-24} (µg·hr/mL)										
Day 1	NA[a]	NA[a]	712	867	1670	1790	2470	3310	2860	3850
Day 14	NA[a]	NA[a]	835	1490	1470	2110	1820	3290	2270	3670
Toxicokinetics: C_{max} (µg/mL)										
Day 1	NA[a]	NA[a]	42.2	41.6	97.0	90.9	138	180	141	182
Day 14	NA[a]	NA[a]	66.0	74.8	84.2	131	115	185	117	189

Test Article	Dose (mg/kg/day)	No. of Animals/Sex	Sampling Time (Hours)	Mean % MN PCEs (±SD) Males	Mean % MN PCEs (±SD) Females	Mean Ratio PCE:NCE (±SD) Males	Mean Ratio PCE:NCE (±SD) Females
Vehicle	0	5	24	0.08 ± 0.03	0.12 ± 0.04	0.82 ± 0.11	0.88 ± 0.10
GS-9883	10	5	24	0.07 ± 0.04	0.09 ± 0.07	0.77 ± 0.07	0.81 ± 0.08
	30	5	24	0.06 ± 0.05	0.05 ± 0.04	0.80 ± 0.11	0.79 ± 0.13
	100	5	24	0.04 ± 0.04	0.06 ± 0.04	0.91 ± 0.04	0.79 ± 0.10
	300	5	24	0.01 ± 0.02	0.05 ± 0.00	0.87 ± 0.04	0.83 ± 0.06
Cyclophosphamide	60	5	24	0.71* ± 0.32	0.80* ± 0.24	0.85 ± 0.10	0.67* ± 0.11

PCE = Polychromatic erythrocyte; MN PCE = Micronucleated PCE; NCE = Normochromatic erythrocyte; * Significantly different than the corresponding vehicle control, $p \leq 0.05$ (Dunnet's T-test)
a Value below the lower limit of quantitation (<1000 ng/mL)

9.2. FTC: Micronucleus in Mice

Test Article: FTC

Report Title: Mammalian Erythrocyte Micronucleus Test **Gilead Study No. TOX011**

Test for Induction of: Micronucleus formation **Treatment Schedule:** Single dose 500, 1000 & 2000 mg/kg **Location in CTD:** 4.2.3.3.2

Strains: Mouse / ICR **Sampling Time:** 24 hours and 48 hours (vehicle control and high dose only) **GLP Compliance:** Yes

Age: 6 to 8 Weeks Old **Method of Administration:** Oral gavage **Lot No.:** [redacted]

Date of Dosing: [redacted], 19[redacted] – [redacted], 19[redacted] **Vehicle/Formulation:** Sterile distilled water 10 mL/kg + FTC TP-0006/96/M (free base)

Special Features: none **Cells Evaluated:** Polychromatic erythrocytes in bone marrow

No. of Cells Analyzed/Animal: Bone marrow was collected from femurs and 2 to 4 slides were prepared from each mouse. The number of micronucleated normochromic erythrocytes in the field of 2000 polychromatic erythrocytes was enumerated.

Toxic/Cytotoxic Effects: No mortality, clinical signs of toxicity, or evidence of bone marrow toxicity were observed in any mice in any FTC treated group during the course of the micronucleus study. All mice appeared normal throughout the observation period.

Genotoxic Effects: FTC did not induce a significant increase in the incidence of micronucleated polychromatic erythrocytes in bone marrow and was concluded to be negative in the micronucleus test using male ICR mice.

Test Article	Dose (mg/kg)	No. of Animals	Harvest Time (hour)	PCE/Total Erythrocytes (mean ± SD)	Number/1000 PCEs (mean ± SD)	Number/PCEs Scored
Vehicle control (water)	Vehicle	5	24	0.60 ± 0.01	0.8 ± 0.27	8/10,000
	Vehicle	5	48	0.59 ± 0.05	0.4 ± 0.42	4/10,000
Positive control (Cyclophosphamide)		5	24	0.57 ± 0.02	40.0 ± 6.62	400/10,000*
Test Article						
Low test dose	500	5	24	0.61 ± 0.02	0.5 ± 0.35	5/10,000
Mid test dose	1000	5	24	0.56 ± 0.06	1.1 ± 0.55	11/10,000
High test dose	2000	5	24	0.58 ± 0.03	1.2 ± 1.52	12/10,000
	2000	5	48	0.57 ± 0.05	0.2 ± 0.27	2/10,000

PCE = polychromatic erythrocytes; * = $p \leq 0.05$, ANOVA followed by Dunnett's t-test

9.3. TAF: Micronucleus in Mice

Test Article: TAF

Report Title: In Vivo Mouse Micronucleus Assay with GS-7340-02

Gilead Study No. M2000113
[redacted] Study No. 21816-0-445OECD

Test for Induction of: : Bone marrow micronuclei
Treatment Schedule: single dose
Location in CTD: 4.2.3.3.2
Species / Strain: Mouse/CD-1®(ICR) BR
Sampling Time: 24 and 48 hours after dosing
GLP Compliance: yes
Age: 9 weeks
Method of Administration: Oral Gavage
Date of Treatment: [redacted] 20[redacted]
No. of Cells Evaluated / Animal: 2000
Vehicle: 50mM citric acid
Lot No. [redacted]
Special Features: None
Positive Control Vehicle: Deionized (DI) Water

Toxic / Cytotoxic Effects: Cytotoxicity to the bone marrow was noted at the 2000 mg/kg dose group at the 24 and 48 hour harvest timepoints.

Genotoxic Effects: GS-7340-02 was evaluated as negative in the mouse bone marrow micronucleus assay under the conditions of the protocol.

Treatment	Dose (mg/kg)	Number of Males	Harvest Time (Hours)	Mean Ratio PCE:NCE (±SE)	Mean % MNPCE (±SE)
				Male	Male
50mM Citric Acid	20 mL/kg	5	24	0.76 ± 0.08	0.03 ± 0.01
		5	48	0.78 ± 0.06	0.04 ± 0.02
GS-7340-02	500	5	24	0.74 ± 0.02	0.08 ± 0.03
	1000	5	24	0.74 ± 0.07	0.10 ± 0.05
	2000	5	24	0.49 ± 0.07**	0.09 ± 0.02
		5	48	0.24 ± 0.01**	0.12 ± 0.04
Cyclophosphamide	80	5	24	0.65 ± 0.05	2.71 ± 0.39*

* Significantly greater than the corresponding vehicle control, $p<0.01$
** Significantly less than the corresponding vehicle control, $p\leq0.05$
GLP = Good Laboratory Practice
MNPCE = Micronucleated PCEs
NCE = Normochromatic erythrocyte
PCE = Polychromatic erythrocyte
SE = Standard Error

10. CARCINOGENICITY

10.1. BIC: Transgenic Mouse

Test Article: BIC

Report Title: 26-Week Oral Gavage Carcinogenicity and Toxicokinetic Study of GS-9883-01 in 001178-T (Hemizygous) rasH2 Transgenic Mice

Gilead Study No. TX-141-2047 ([redacted] Study No. 8328591)

Species / Strain: Mice/ rasH2 (001178-T [hemizygous], CByB6F1-Tg[HRAS]2Jic) and rasH2 (001178-W [wild type], CByB6F1-Tg[HRAS]2Jic)

Duration of Dosing: 26 weeks

Location in CTD:

Initial Age: 8 weeks

Method of Administration: Oral gavage

GLP Compliance: Yes

Date of First Dose: [redacted] 20[redacted] (males); [redacted] 20[redacted] (females)

Vehicle: 0.5% (w/w) hydroxypropyl methylcellulose (Methocel K100 LV) and 0.1% (w/w) Tween® 20 in reverse osmosis water

Lot Number: [redacted]

Treatment of Controls: N-methyl-N-nitrosourea (MNU)

Special Features: none

Daily Dose (mg/kg/day)	0 (Control)		5 (Low)	10 (Low)	15 (Mid)	30 (Mid)	100 (High)	300 (High)
Sex: Number of Animals (TK)	**M: 7**	**F: 7**	**M: 40**	**F: 40**	**M: 40**	**F: 40**	**M: 40**	**F: 40**
Toxicokinetics: AUC_{0-24} (µg·hr/mL)								
Day 1	BLQ	BLQ	46.6	150	147	382	383	1240
Week 26	BLQ	BLQ	190	341	526	861	1560	2340
Toxicokinetics:C_{max} (µg/mL)								
Day 1	BLQ	BLQ	7.54	13.8	15.9	28	42	89.1
Week 26	BLQ	BLQ	18.2	25.8	42.3	69.2	104	143

Daily Dose (mg/kg/day)	0 (Control)		5 (Low)	10 (Low)	15 (Mid)	30 (Mid)	100 (High)	300 (High)
Sex:Number of Animals (Main)	**M: 25**	**F: 25**	**M: 25**	**F: 25**	**M: 25**	**F: 25**	**M: 25**	**F: 25**
Noteworthy Findings								
Died/Sacrificed Moribund[a]	2	1	0	1	1	2	1	0
Terminal Sacrifice	23	24	25	24	24	23	24	25
Survival (%)								
Week 1	100	100	100	100	100	100	100	100
Week 13	100	100	100	100	100	100	100	100
Week 26	92	96	100	96	96	92	96	100
Clinical Observations	No test-article related findings							
Mass Observations	No test-article related findings							
Body Weight Change (g)	No test-article related findings							
Weeks 1-27	7.2	3.8	7.3	4.6*	7.0	3.9	5.7	4.9*
Food Consumption (g)	No test-article related findings							
Week 15-16	3.7	3.5	3.3	3.3	3.2*	2.9	3.1*	2.9
Week 19-20	3.7	2.6	3.6	2.9*	3.7	2.9	3.3*	2.7
Week 21-22	5.3	4.2	4.8	3.8	6.4	3.2*	3.6*	3.0*
Week 22-23	4.9	3.6	4.7	4.9*	5.1	3.7	3.5*	3.1
Week 23-24	3.6	3.0	3.7	4.0	3.7	2.9	3.3*	3.2
Organ Weights	No test-article related findings							
Macroscopic Observations	No test-article related findings							
Neoplastic Findings	No test-article related findings							
Primary Neoplastic Findings, Groups 1 through 4 – Scheduled and Unscheduled Sacrifices and Deaths								
Vascular neoplasms								
B-Hemangioma	0	0	0	1	1	3	3	0
M-Hemangiosarcoma	1	1	2	2	3	4	0	2
Hemolympho-reticular neoplasms								
M-Lymphoma	1	0	0	0	0	0	0	0

Daily Dose (mg/kg/day)	0 (Control)		5 (Low)	10 (Low)	15 (Mid)	30 (Mid)	100 (High)	300 (High)
Sex:Number of Animals (Main)	**M: 25**	**F: 25**	**M: 25**	**F: 25**	**M: 25**	**F: 25**	**M: 25**	**F: 25**
Lung								
B- Adenoma, Bronchiolo-alveolar	2	1	1	1	3	0	4	1
M- Carcinoma, Bronchiolo-alveolar	0	1	0	1	0	0	0	0
M-Mesothelioma	0	0	0	1	0	0	0	0
Thymus								
B-Thymoma	0	2	0	2	0	1	0	5
M-Thymoma	0	2	0	0	0	0	0	1
Harderian gland								
B-Adenoma	1	0	0	0	0	1	0	0
M-Carcinoma	0	0	1	0	0	0	0	0
Mammary Gland								
M-Adenocarcinoma	0	0	0	0	0	0	0	1
Prostate								
M-Carcinoma	1	NA	0	NA	0	NA	0	NA
Uterus								
B-Polyp, endrometrial stromal	NA	1	NA	0	NA	0	NA	0
Non-Neoplastic Findings	No test-article related findings							

B- = Benign, M- = Malignant, NA = Not applicable

10.2. BIC: Rat

104-Week Oral Gavage Carcinogenicity and Toxicokinetic Study with GS-9883-01 in Han Wistar Rats (Gilead Study No. TX-141-2040; Covance Study No. 8315273). This study is currently in progress. The first dose was administered 21 May 2015.

10.3. FTC: Mouse

Test Article: FTC

Report Title: TP-0006: Two-Year Oral Oncogenicity Study in CD-1 Mice **Gilead Study No. TOX109 ([redacted] 814-005)**

Test Article: FTC

Report Title: TP-0006: Two-Year Oral Oncogenicity Study in CD-1 Mice **Gilead Study No. TOX109 ([redacted] 814-005)**

Species/ Strain: Mouse/ CD-1® (ICR) BR **Duration of Dosing:** 104 Weeks **Location in CTD:** 4.2.3.4.1

Initial Age: 6 Weeks **Method of Administration:** Oral gavage **GLP Compliance:** Yes

Date of First Dose: [redacted] 20[redacted] **Vehicle/Formulation:** 0.5% Methylcellulose **Lot No.:** [redacted];[redacted]

Special Features: None **Basis for High-Dose Selection:** Results from 6 month mouse study; Toxicokinetic endpoint

Dose (mg/kg/day)		**0 (Vehicle)**		**80**		**250**		**750**	
Gender		**M: 60**	**F: 60**	**M: 60**	**F: 60**	**M: 60**	**F: 60**	**M: 60**	**F: 60**
Toxicokinetics									
AUC (µg.h/mL) Week 26		nd*	nd*	27.48	23.74	90.94	91.71	287.30	322.65
Number of Animals									
At Start		60	60	60	60	60	60	60	60
Died/Sacrificed Moribund		33	38	31	38	37	35	30	34
Terminal Sacrifice		27	22	29	22	23	25	30	26
Survival (%)		45	37	48	37	38	42	50	43
Body Weight[a] (%)		40.6 g	31.9 g	+1	-1	0	-3	-3	-3
Food Consumption[a] (%)		6.0 g/day	5.8 g/day	0	+3	+3	+7	-2	+7
Number of Animals with Neoplastic Lesions									
Adrenal Glands	Adenoma, subcapsular cell, benign, primary	0	2	0	1	0	0	0	0
	Pheochromocytoma, benign, primary	0	0	0	0	0	1	0	0

Dose (mg/kg/day)		0 (Vehicle)		80		250		750	
Number Evaluated		**M: 60**	**F: 60**	**M: 60**	**F: 60**	**M: 60**	**F: 60**	**M: 60**	**F: 60**
Number of Animals with Neoplastic Lesions, cont.									
Bone	Osteoma, benign, primary	0	1	0	0	0	0	0	0
Bone Marrow, Femur	Hemangiosarcoma, malignant, primary	0	0	0	0	0	0	0	1
Bone, Vertebra	Osteosarcoma, malignant, primary	0	0	0	0	0	0	0	1
Brain	Astrocytoma, malignant, primary	0	0	1	0	0	0	0	0
Cavity, Abdominal	Carcinoma, squamous cell, malignant, secondary	0	0	0	0	0	1	0	0
	Fibrosarcoma, malignant, secondary	0	0	0	2	0	0	0	0
	Hemangiosarcoma, malignant, primary	0	0	0	0	0	1	0	0
	Leiomyosarcoma, malignant, primary	0	0	0	0	0	1	0	0
	Liposarcoma, malignant, secondary	0	1	0	0	0	0	0	0
	Osteosarcoma, malignant, primary	0	1	0	0	0	0	0	0
Cavity, Oral	Carcinoma, squamous cell, malignant, primary	1	0	0	0	0	0	0	0
Cavity, Thoracic	Fibrosarcoma, malignant, primary	0	0	0	1	0	0	0	0
	Fibrosarcoma, malignant, secondary	0	0	0	1	0	0	0	0
	Liposarcoma, malignant, secondary	0	1	0	0	0	0	0	0

Dose (mg/kg/day)		0 (Vehicle)		80		250		750	
Number Evaluated		**M: 60**	**F: 60**	**M: 60**	**F: 60**	**M: 60**	**F: 60**	**M: 60**	**F: 60**
Number of Animals with Neoplastic Lesions, cont.									
Gall Bladder	Adenoma, benign, primary	0	0	1	0	0	0	1	1
Harderian Glands	Adenocarcinoma, malignant, primary	1	2	1	0	0	1	0	1
	Adenoma, benign, primary	4	1	4	3	6	5	4	5
Heart	Hemangiosarcoma, malignant, primary	0	0	0	0	0	1	0	0
Kidneys	Adenoma, tubular cell, benign, primary	1	0	0	0	2	0	0	0
Liver	Adenoma, hepatocellular, benign, primary	5	0	7	0	7	1	4	2
	Carcinoma, hepatocellular, malignant, primary	3	1	4	0	0	1	1	0
	Fibrosarcoma, malignant, secondary	0	0	0	1	0	0	0	0
	Hemangiosarcoma, malignant, primary	2	0	1	0	2	2	2	0
Lung	Adenocarcinoma, malignant, secondary	0	0	0	1	0	0	0	
	Adenoma, bronchiolar alveolar, benign, primary	7	9	14	15	15	8	13	6
	Carcinoma, bronchiolar alveolar, malignant, primary	1	1	2	3	0	1	1	1
	Fibrosarcoma, malignant, secondary	0	0	0	2	0	1	0	0
Lymph Node, Axillary	Fibrosarcoma, malignant, secondary	0	0	0	1	0	0	0	0
Lymph Node, Cervical	Lymphangioma, benign, primary	0	0	0	0	0	0	0	1

Dose (mg/kg/day)		0 (Vehicle)		80		250		750	
Number Evaluated		**M: 60**	**F: 60**	**M: 60**	**F: 60**	**M: 60**	**F: 60**	**M: 60**	**F: 60**
Number of Animals with Neoplastic Lesions, cont.									
Lymph Node, Hepatic	Leiomyosarcoma, malignant, secondary	0	0	0	0	0	1	0	0
Lymph Node, Mediastinal	Fibrosarcoma, malignant, secondary	0	0	0	1	0	0	0	0
	Liposarcoma, malignant, secondary	0	1	0	0	0	0	0	0
Lymph Node, Mesenteric	Leiomyosarcoma, malignant, secondary	0	0	0	0	0	1	0	0
Lymph Node, Renal	Leiomyosarcoma, malignant, secondary	0	0	0	0	0	1	0	0
Mammary Gland	Adenocarcinoma, malignant, primary	0	0	0	1	0	0	0	1
Mesentery/Peritoneum	Leiomyoma, benign, primary	0	1	0	0	0	0	0	0
Multicentric Neoplasm	Leukemia, granulocytic, malignant, multicentric	0	1	0	0	0	0	0	0
	Lymphoma, malignant, multicentric	4	5	0	7	3	6	4	4
	Sarcoma, histiocytic, malignant, multicentric	0	6	0	2	1	6	1	2
Ovaries	Cystadenoma, benign, primary	NA	0	NA	1	NA	2	NA	1
	Hemangioma, benign, primary	NA	1	NA	0	NA	0	NA	0
	Leiomyosarcoma, malignant, secondary	NA	0	NA	0	NA	1	NA	0
	Sex-cord/stromal tumor, benign, primary[b]	NA	0	NA	0	NA	0	NA	2
	Sex-cord/stromal tumor, malignant, primary	NA	1	NA	0	NA	1	NA	0

Dose (mg/kg/day)		0 (Vehicle)		80		250		750	
Number Evaluated		**M: 60**	**F: 60**	**M: 60**	**F: 60**	**M: 60**	**F: 60**	**M: 60**	**F: 60**
Number of Animals with Neoplastic Lesions, cont.									
Pancreas	Fibrosarcoma, malignant, secondary	0	0	0	1	0	0	0	0
	Leiomyosarcoma, malignant, secondary	0	0	0	0	0	1	0	0
	Liposarcoma, malignant, secondary	0	1	0	0	0	0	0	0
Pituitary Gland	Adenoma, pars distalis, benign, primary	0	1	0	2	0	0	0	0
Skeletal Muscle	Leiomyosarcoma, malignant, secondary	0	0	0	0	0	1	0	0
Skin	Carcinoma, squamous cell, malignant, primary	0	0	0	0	0	1	1	0
Skin, Subcutis	Fibrosarcoma, malignant, primary	1	1	0	2	1	5	1	1
	Liposarcoma, malignant, primary	0	1	0	0	0	0	0	0
Small Intestine, Ileum	Adenocarcinoma, malignant, primary	0	1	0	0	0	0	0	0
Spleen	Fibrosarcoma, malignant, secondary	0	0	0	1	0	0	0	0
	Hemangiosarcoma, malignant, primary[c]	0	0	1	1	2	0	3	1
Testes	Adenoma, interstitial cell, benign, primary	0	NA	0	NA	2	NA	1	NA
Thymus Gland	Fibrosarcoma, malignant, secondary	0	0	0	1	0	0	0	0
Thyroid Gland	Adenoma, c-cell, benign, primary	0	1	0	0	0	0	0	0
	Adenoma, follicular cell, benign, primary	0	1	0	0	0	0	0	0

Dose (mg/kg/day)		0 (Vehicle)		80		250		750	
Number Evaluated		**M: 60**	**F: 60**	**M: 60**	**F: 60**	**M: 60**	**F: 60**	**M: 60**	**F: 60**
Number of Animals with Neoplastic Lesions, cont.									
Tooth/Teeth	Odontoma, benign, primary	0	0	1	0	0	0	0	0
Urinary Bladder	Leiomyosarcoma, malignant, secondary	0	0	0	0	0	1	0	0
	Mesenchymal tumor, benign, primary	0	1	0	0	0	0	0	0
Uterus with Cervix	Adenocarcinoma, malignant, primary	NA	0	NA	3	NA	1	NA	0
	Carcinoma, squamous cell, malignant, primary	NA	0	NA	1	NA	0	NA	0
	Hemangiosarcoma, malignant, primary	NA	0	NA	1	NA	0	NA	1
	Leiomyoma, benign, primary	NA	4	NA	4	NA	1	NA	2
	Leiomyosarcoma, malignant, primary	NA	0	NA	0	NA	0	NA	1
	Leiomyosarcoma, malignant, secondary	NA	0	NA	0	NA	1	NA	0
	Polyp, glandular, benign, primary	NA	1	NA	1	NA	0	NA	0
	Polyp, stromal, benign, primary	NA	7	NA	5	NA	2	NA	3
	Sarcoma, stromal, malignant, primary	NA	0	NA	3	NA	1	NA	1
Vagina	Polyp, stromal, benign, primary	NA	1	NA	0	NA	0	NA	0

NA = not applicable; nd = not determined; * = sample taken at Week 104, concentrations were generally below level of quantitation.
Noteworthy Findings: Gross Pathology, None; Microscopic Pathology – ; Non-neoplastic lesions, None
a At 6 months; for controls group means are shown. For treated groups percent differences from control are shown.
b Peto Test; P-value 0.040.
c Peto Test; P-value 0.031.

10.4. FTC: Rat

Test Article: FTC

Report Title: TP-0006: Two-Year Oral Oncogenicity Study in CD Rats **Gilead Study No. TOX108 ([redacted] 814-006)**

Species/ Strain: Rat/ CD® [Crl:CD® (SD) IGS BR] **Duration of Dosing:** 104 Weeks **Location in CTD:** 4.2.3.4.1

Initial Age: 6 Weeks **Method of Administration:** Oral gavage **GLP Compliance:** Yes

Date of First Dose: [redacted] 20[redacted] **Vehicle/Formulation:** 0.5% Methylcellulose **Lot No.:** [redacted]; [redacted]

Special Features: None **Basis for High-Dose Selection:** Results from 90 day rat study; toxicokinetic endpoint

Dose (mg/kg/day)		**0 (Vehicle)**		**60**		**200**		**600**	
Gender:No. of Animals		**M: 60**	**F: 60**	**M: 60**	**F: 60**	**M: 60**	**F: 60**	**M: 60**	**F: 60**
Toxicokinetics									
AUC_{0-24} (µg.h/mL) (Week 26)		nd*	nd*	42.87	52.53	137.42	170.68	326.77	404.07
Number of animals									
At Start		60	60	60	60	60	60	60	60
Died/Sacrificed Moribund		31	36	40	28	34	31	32	40
Terminal Sacrifice		29	24	20	32	26	29	28	20
Survival (%)		48	40	33	53	43	48	47	33
Body Weight[a] (%)		584 g	339 g	0	-4	+2	0	+3	+1
Food Consumption[a] (%)		26 g/day	20 g/day	+4	0	+8	+5	+8	+10
Number of Animals with Neoplastic Lesions									
Adipose Tissue, Brown	Hibernoma, benign, primary	1	0	1	0	0	0	0	0
	Hibernoma, malignant, primary	0	1	0	0	0	1	0	1
Adrenal Glands	Adenoma, cortical, benign, primary	0	1	0	1	1	0	1	1
	Carcinoma, cortical, malignant, primary	0	3	1	1	0	0	0	0
	Pheochromocytoma, benign, primary	4	1	7	1	12	1	7	2
	Pheochromocytoma, malignant, primary	0	0	1	0	0	1	0	1

Dose (mg/kg/day)		0 (Vehicle)		60		200		600	
Gender:No. of Animals		**M: 60**	**F: 60**	**M: 60**	**F: 60**	**M: 60**	**F: 60**	**M: 60**	**F: 60**
Number of Animals with Neoplastic Lesions, cont.									
Bone, Tibia	Hemangiosarcoma, malignant, secondary	0	0	1	0	0	0	0	0
Brain	Astrocytoma, malignant, primary	2	0	0	0	1	1	0	0
	Carcinoma, pars distalis, malignant, secondary	0	0	0	0	1	0	0	0
	Carcinoma, zymbals gland, malignant, secondary	0	0	0	0	1	1	0	0
	Granular cell tumor, benign, primary	0	1	1	0	0	1	0	0
	Reticulosis, malignant, primary	0	1	0	0	0	0	0	0
Cavity, Abdominal	Carcinoma, malignant, primary	0	0	0	1	0	0	0	0
	Fibrosarcoma, malignant, primary	1	0	0	0	0	0	0	0
	Lipoma, benign, primary	0	0	0	0	0	0	1	1
	Neuroendocrine tumor, benign, primary	1	0	0	0	0	0	0	0
Cavity, Thoracic	Hibernoma, benign, primary	0	1	0	0	0	0	0	0
	Osteosarcoma, malignant, primary	0	0	0	0	1	0	0	0
Coagulating Glands	Adenoma, benign, primary	0	0	0	0	0	0	1	0
Ears	Fibroma, benign, primary	1	0	0	0	0	0	0	0
Hard Palate	Fibrosarcoma, malignant, primary	0	0	1	0	0	0	0	0
Harderian Glands	Carcinoma, zymbals gland, malignant, secondary	1	0	0	0	0	0	0	0
Implantation Site	Fibrosarcoma, malignant, primary	0	0	0	0	0	0	1	0

Dose (mg/kg/day)		0 (Vehicle)		60		200		600	
Gender:No. of Animals		**M: 60**	**F: 60**	**M: 60**	**F: 60**	**M: 60**	**F: 60**	**M: 60**	**F: 60**
Number of Animals with Neoplastic Lesions, cont.									
Heart	Adenocarcinoma, malignant, secondary	0	1	0	0	0	0	0	0
Kidneys	Carcinoma, tubular cell, malignant, primary	0	0	1	0	0	1	0	0
	Lipoma, benign, primary	0	1	0	0	0	0	0	0
	Liposarcoma, malignant, primary		1	1	0	0	0	1	0
Large Intestine, Cecum	Fibrosarcoma, malignant, secondary	1	0	0	0	0	0	0	0
Large Intestine, Rectum	Granular cell tumor, malignant, secondary	0	0	0	1	0	1	0	0
Liver	Adenoma, hepatocellular, benign, primary	1	3	0	0	0	1	1	2
	Carcinoma, hepatocellular, malignant, primary	0	0	0	1	0	0	0	0
Lung	Adenoma, bronchiolar alveolar, benign, primary	1	0	0	0	0	0	0	1
	Hibernoma, malignant, secondary	0	1	0	0	0	0	0	0
	Carcinoma, zymbals gland, malignant, secondary	1	0	0	0	0	0	0	0
Lymph Node, Cervical	Carcinoma, c-cell, malignant, secondary	0	0	0	0	0	0	1	0
Lymph Node, Mediastinal	Adenocarcinoma, malignant, secondary	0	1	0	0	0	0	0	0
Lymph Node, Mesenteric	Hemangiosarcoma, malignant, primary	0	0	0	1	0	0	0	0

Dose (mg/kg/day)		0 (Vehicle)		60		200		600	
Gender:No. of Animals		**M: 60**	**F: 60**	**M: 60**	**F: 60**	**M: 60**	**F: 60**	**M: 60**	**F: 60**
Number of Animals with Neoplastic Lesions, cont.									
Mammary Gland	Adenocarcinoma, malignant, primary	0	18	1	9	1	13	0	7
	Adenoma, benign, primary	0	3	0	2	0	3	0	1
	Fibroadenoma, benign, primary	0	22	2	17	1	18	0	16
Multicentric Neoplasm	Lymphoma, malignant, multicentric	1	1	2	0	1	2	1	0
	Sarcoma, histiocytic, malignant, multicentric	0	1	2	0	0	1	1	2
Nose, Level B	Carcinoma, zymbals gland, malignant, secondary	1	0	0	0	0	1	0	0
	Lymphangioma, benign, primary	0	0	0	0	1	0	0	0
Nose, Level C	Carcinoma, zymbals gland, malignant, secondary	1	0	0	0	0	1	0	0
	Papilloma, benign, primary	0	0	0	0	1	0	0	0
Nose, Level D	Carcinoma, zymbals gland, malignant, secondary	1	0	0	0	0	1	0	0
	Polyp, benign, primary	0	0	0	0	1	0	0	0
Ovaries	Sex-cord/stromal tumor, benign, primary	NA	1	NA	0	NA	1	NA	0
Pancreas	Adenoma, acinar cell, benign, primary	0	0	0	0	0	0	1	0
	Adenoma, islet cell, benign, primary	2	3	3	0	3	0	3	2
	Carcinoma, islet cell, malignant, primary	0	0	1	0	1	0	1	0
	Carcinoma, malignant, secondary	0	0	0	1	0	0	0	0
	Fibroma, benign, primary	0	0	0	0	0	0	0	1
	Fibrosarcoma, malignant, secondary	1	0	0	0	0	0	0	0

Dose (mg/kg/day)		0 (Vehicle)		60		200		600	
Gender:No. of Animals		**M: 60**	**F: 60**	**M: 60**	**F: 60**	**M: 60**	**F: 60**	**M: 60**	**F: 60**
Number of Animals with Neoplastic Lesions, cont.									
Parathyroid Glands	Adenoma, benign, primary	0	0	0	0	4	1	0	0
	Carcinoma, pars distalis, malignant, primary	0	0	0	0	1	1	0	1
Seminal Vesicles	Carcinoma, squamous cell, malignant, primary	0	NA	0	NA	1	NA	0	NA
	Fibrosarcoma, malignant, secondary	1	NA	0	NA	0	NA	0	NA
Skin	Carcinoma, squamous cell, malignant, primary	1	0	1	0	0	0	0	1
	Hemangioma, benign, primary	0	0	0	0	1	0	0	0
	Papilloma, squamous cell, benign, primary	0	0	2	0	1	0	0	0
	Trichoepithelioma, benign, primary	0	0	1	0	1	0	0	0
Skin, Subcutis	Fibroma, benign, primary	0	1	2	2	4	0	2	0
	Fibrosarcoma, malignant, primary	1	1	0	0	0	0	0	1
	Hemangiosarcoma, malignant, primary	0	0	1	0	0	0	0	0
	Hibernoma, benign, primary	0	1	0	0	0	0	0	0
	Lipoma, benign, primary	0	1	0	1	0	0	1	0
	Schwannoma, malignant, primary	1	0	0	0	1	0	0	0
Small Intestine, Duodenum	Carcinoma, malignant, secondary	0	0	0	1	0	0	0	0
Spleen	Carcinoma, malignant, secondary	0	0	0	1	0	0	0	0

Dose (mg/kg/day)		0 (Vehicle)		60		200		600	
Gender:No. of Animals		**M: 60**	**F: 60**	**M: 60**	**F: 60**	**M: 60**	**F: 60**	**M: 60**	**F: 60**
Number of Animals with Neoplastic Lesions, cont.									
Stomach, Glandular	Fibrosarcoma, malignant, secondary	1	0	0	0	0	0	0	0
	Osteosarcoma, malignant, primary	0	0	1	0	0	0	0	0
Testes	Adenoma, interstitial cell, benign, primary	0	NA	0	NA	1	NA	1	NA
Thymus gland	Lymphoma, malignant, primary	0	0	0	1	0	0	0	0
	Thymoma, benign, primary	0	0	1	0	0	0	0	0
	Thymoma, malignant, primary	1	0	0	0	1	0	0	0
Thyroid Gland	Adenoma, c-cell, benign, primary	3	5	2	2	7	3	0	1
	Adenoma, follicular cell, benign, primary	7	1	0	0	4	0	1	3
	Carcinoma, follicular cell, malignant, primary	0	1	0	0	0	0	0	0
	Carcinoma, c-cell, malignant, primary	1	0	1	0	0	0	1	0
Tongue	Carcinoma, squamous cell, malignant, primary	0	0	0	1	0	0	0	0
Urinary Bladder	Carcinoma, squamous cell, malignant, primary	0	0	1	0	0	0	0	0
	Carcinoma, squamous cell, malignant, secondary	0	0	0	0	1	0	0	0
	Carcinoma, transitional cell, malignant, primary	0	0	0	0	1	0	0	0

Dose (mg/kg/day)		0 (Vehicle)		60		200		600	
Gender:No. of Animals		**M: 60**	**F: 60**	**M: 60**	**F: 60**	**M: 60**	**F: 60**	**M: 60**	**F: 60**
Number of Animals with Neoplastic Lesions, cont.									
Uterus with Cervix	Adenocarcinoma, malignant, primary	NA	0	NA	1	NA	0	NA	1
	Granular cell tumor, benign, primary	NA	6	NA	6	NA	4	NA	2
	Granular cell tumor, benign, secondary	NA	1	NA	0	NA	0	NA	0
	Granular cell tumor, malignant, secondary	NA	0	NA	0	NA	1	NA	0
	Leiomyoma, benign, primary	NA	0	NA	0	NA	1	NA	0
	Polyp, stromal, benign, primary	NA	6	NA	3	NA	4	NA	6
	Sarcoma, stromal, malignant, primary	NA	0	NA	0	NA	1	NA	1
	Schwannoma, malignant, primary	NA	0	NA	0	NA	0	NA	1
Vagina	Granular cell tumor, benign, primary	NA	1	NA	2	NA	4	NA	1
	Granular cell tumor, malignant, primary	NA	0	NA	0	NA	1	NA	0
	Polyp, stromal, benign, secondary	NA	0	NA	0	NA	1	NA	0
	Sarcoma, stromal, malignant, secondary	NA	0	NA	0	NA	0	NA	1
Zymbal's Gland	Carcinoma, zymbals gland, malignant, primary	1	0	1	0	1	1	0	0

NA = not applicable; nd = not determined; * = sample taken at Week 104, concentrations were below level of quantitation
a At 6 months; for controls group means are shown. For treated groups percent differences from control are shown.

10.5. TDF: Mouse

Test Article: TDF

Report Title: An Oral Carcinogenicity Study of Tenofovir Disoproxil Fumarate (TDF) in the Albino Mouse **Gilead Study No. M990205**

Species/ Strain: Mouse, Swiss Crl:CD^{R}-1(ICR)BR **Duration of Dosing:** 104 Weeks **Location in CTD:** 4.2.3.4.1

Initial Age: 6 Weeks (approx) **Method of Administration:** Oral gavage **GLP Compliance:** Yes

Date of First Dose: [redacted] 20[redacted] (Main) **Vehicle/ Formulation:** Suspension vehicle **Lot No.:** [redacted]

Basis for High-Dose Selection: The dose levels were selected according to the potential human exposure, existing toxicity data and limitations imposed by the test article. A previous 13 week oral toxicity study indicated that 600 mg/kg/day (60 mg/mL) is the highest dose level of TDF that may be practically administered to albino mice.

Special Features: Separate populations for toxicokinetic evaluations were included in the study: Groups of 30 males and 30 females were sampled on Days 1 and 180 for the high dose only. Animals were euthanized on completion of the blood collection & subjected to necropsy. Before assignment of animals to a treatment group, 10 males & 10 females animals, selected randomly, were subjected to a health screen.

Dose (mg/kg/day)		**0 (Vehicle)**		**100**		**300**		**600**	
Number Evaluated		**M: 60**	**F: 60**	**M: 60**	**F: 60**	**M: 60**	**F: 60**	**M: 60**	**F: 60**
Toxicokinetics									
AUCss $_{0\text{-}24}$ (µg.h/mL) Day 1		-	-	-	-	-	-	30.9	35.1
AUCss $_{0\text{-}24}$ (µg.h/mL) Day 180		-	-	-	-	-	-	44.3	50.9
Number of Animals									
At Start		60	60	60	60	60	60	60	60
Died/Sacrificed Moribund		33	39	37	32	31	29	40	43
Terminal Sacrifice		27	21	23	28	29	31	20	17
Survival (%)		45	35	38	47	48	52	33	28
Body Weight (%)[a]		38.65g	30.79g	-4.4*	+1.5%	-3.4	-1.6%	-3.7%	-0.07%
Food Consumption (%)[a]		37.4g	39.4g	+8.6%	+2.2%	+5.1%	+0.3%	+9.9%	-0.3%
Abdomen	Leiomyosarcoma	-	-	1	-	-	-	-	1
	Osteosarcoma	-	-	-	1	-	-	-	-
	Rhabdomyosarcoma (metastasis)	-	-	1	-	-	-	-	-
	Sarcoma	-	-	-	1	-	-	-	-

Dose (mg/kg/day)		0 (Vehicle)		100		300		600	
Number Evaluated		**M: 60**	**F: 60**	**M: 60**	**F: 60**	**M: 60**	**F: 60**	**M: 60**	**F: 60**
Number of Animals with Neoplastic Lesions									
Adrenal	Adenoma: cortical	-	1	-	1	1	1	-	-
	Sarcoma: fusiform cell	1		-	1	-	-	-	-
	Benign pheochromocytoma	-	-	-	2	-	-	-	-
	Sarcoma (metastasis)	-	-	-	1	-	-	-	-
Bone - Femur	Hemangiosarcoma	-	-	1	-	-	-	-	-
Bone - Misc	Hemangiosarcoma	1	-	-	-	-	-	-	-
	Osteosarcoma	-	-	-	1	-	-	-	-
	Rhabdomyosarcoma	-	-	-	-	-	1	-	-
Brain	Malignant meningioma	1	-	-	-	-	-	-	-
Cecum	Leiomyoma	-	-	-	-	-	1	-	-
Diaphragm	Rhabdomyosarcoma (metastasis)	-	-	1	-	-	-	-	-
	Sarcoma(metastasis)	-	-	-	1	-	-	-	-
Duodenum	Adenoma	-	-	-	-	-	-	-	1
	Adenocarcinoma	-	-	-	-	-	-	1	1
Epididmydis	Rhabdomyosarcoma (metastasis)	-	-	1	-	-	-	-	-
Eye	Meningioma	1	-	-	-	-	-	-	-
	Carcinoma (metastasis)	1	-	-	-	-	-	-	-
Fat	Leiomysarcoma (metastasis)	-	1	-	-	-	-	-	-
Harderian Gland	Adenoma	1	2	5	-	3	3	7	3
Head	Hemangiosarcoma	-	-	1	-	-	-	-	-
Hemolym Tissue	Malignant lymphoma	3	10	1	17	3	4	2	5
	Histiocytic sarcoma	-	9	1	4	-	5	1	1
Jejunum	Adenoma	-	-	-	-	1	-	-	-
	Adenocarcinoma	-	-	-	1	1	-	-	1
Kidney	Carcinoma: tubular cell	-	-	1	-	-	-	-	-
	Sarcoma (metastasia)	-	-	-	1	-	-	-	-

Dose (mg/kg/day)		0 (Vehicle)		100		300		600	
Number Evaluated		**M: 60**	**F: 60**	**M: 60**	**F: 60**	**M: 60**	**F: 60**	**M: 60**	**F: 60**
Number of Animals with Neoplastic Lesions, cont.									
Liver	Adenoma: hepatocellular	5	1	1	1	2	-	4	9
	Carcinoma: hepatocellular	6	-	2	-	-	-	-	-
	Hemangioma	1	-	-	-	-	-	2	-
	Hemangiosarcoma	2	-	1	-	-	2	-	1
	Leiomyosarcoma (metastasis)	-	1	1	-	-	-	-	-
	Rhabdomyosarcoma (metastasis)	-	-	1	-	-	-	-	-
	Carcinoma: metastasis	1	-	-	-	-	-	-	-
Lung	Carcinoma: metastasis	1	1	-	-	-	1	-	-
	Adenoma: alveolar/bronchiolar	10	10	8	9	11	5	10	7
	Adenocarcinoma: Alveolar/bronchiolar	9	5	7	2	9	2	9	1
	Mammary carcinoma (metastasis)	-	1	-	-	-	-	-	-
	Rhabdomyosarcoma (metastasis)	-	-	1	-	-	-	-	-
	Sarcoma (metastasis)	-	-	-	1	-	-	-	-
	Carcinosarcoma (metastasis)	1	-	-	-	-	-	-	1
	Fibroma	-	-	-	-	-	1	-	-
Lymph Node	Hemangioma	1	-	-	-	-	-	-	-
	Carcinoma (metastasis)	1	-	-	-	-	-	-	-
	Mammary Carcinoma (metastasis)	-	1	-	-	-	-	-	-
Lymph Node Mesenteric	Hemangioma	1	1	-	-	-	-	-	-
	Rhabdomyosarcoma	-	-	1	-	-	-	-	-
Mammary Gland	Adenocarcinoma	-	3	-	-	-	-	-	-
	Adenoacanthoma	-	1	-	-	-	1	-	-
Muscle Skeletal	Hemangioma	-	1	-	-	-	-	-	-
Muscle Skeletal Misc.	Rhabdomyosarcoma	-	-	1	-	-	-	-	-
	Sarcoma (Metastasis)	-	-	-	1	-	-	-	-

Dose (mg/kg/day)		0 (Vehicle)		100		300		600	
Number Evaluated		**M: 60**	**F: 60**	**M: 60**	**F: 60**	**M: 60**	**F: 60**	**M: 60**	**F: 60**
Number of Animals with Neoplastic Lesions, cont.									
Ovary	Cystadenoma	-	1	-	1	-	-	-	-
	Carcinoma: tubulostromal	-	-	-	1	-	-	-	-
	Malignant granulosa-theca cell tumor	-	1	-	-	-	-	-	-
	Leiomyosarcoma (metastasis)	-	1	-	-	-	-	-	-
	Carcinoma (metastasis)	-	-	-	-	-	1	-	-
Pancreas	Adenocarcinoma (metastasis)	-	-	-	-	1	-	-	-
	Carcinoma (metastasis)	-	-	-	-	-	1	-	-
	Leiomyosarcoma (metastasis)	-	1	-	-	-	-	-	-
	Rhabdomyosarcoma (metastasis)	-	-	1	-	-	-	-	-
Pituitary	Adenoma: pars distalis	1	1	-	3	-	3	-	-
	Adenoma: pars intermedia	-	-	-	-	-	1	-	-
Skin Misc.	Keratoacanthoma	-	-	-	-	-	1	-	-
Spleen	Hemangioma	-	-	1	-	-	-	-	-
	Hemangiosarcoma	1	-	3	-	1	1	-	-
	Sarcoma (metastasis)	-	-	1	1	-	-	-	-
Stomach	Adenoma	1	-	-	-	-	-	-	-
	Carcinoma (metastasis)	-	-	-	-	-	1	-	-
	Papilloma	-	1	-	-	-	1	-	-
	Leiomyosarcoma (metastasis)	-	1	-	-	-	-	-	-
	Rhabdomyosarcoma (metastasis)	-	-	1	-	-	-	-	-
	Sarcoma (metastasis)	-	-	-	1	-	-	-	-
Subcutaneous Tissue	Hemangiosarcoma	1	-	-	-	-	-	-	-
	Fibrosarcoma	1	-	-	-	-	-	1	-
	Rhabdomyosarcoma (metastasis)	-	-	1	-	-	1		

Dose (mg/kg/day)		0 (Vehicle)		100		300		600	
Number Evaluated		**M: 60**	**F: 60**	**M: 60**	**F: 60**	**M: 60**	**F: 60**	**M: 60**	**F: 60**
Number of Animals with Neoplastic Lesions, cont.									
Tail	Hemangioma	1	-	-	-	-	1	-	-
Testis	Adenoma: interstitial cell	1	-	1	-	2	-	-	-
	Adenoma: rete testis	2	-	-	-	-	-	-	-
Thyroid	Adenoma: follicular cell	1	-	-	-	-	-	-	-
	Carcinoma: follicular cell	-	1	-	-	-	1	-	-
Urinary Bladder	Carcinoma: transitional cell	1	-	-	-	-	-	-	-
Uterus	Adenocarcinoma: endometrial	-	-	-	-	-	1	-	1
	Carcinosarcoma	-	-	-	-	-	-	-	1
	Sarcoma: endometrial stromal	-	1	-	-	-	2	-	1
	Leiomyoma	-	-	-	1	-	-	-	2
	Leiomysarcoma	-	2	-	2	-	-	-	-
	Fibroma	-	-	-	1	-	-	-	-
	Carcinoma: squamous cell	-	-	-	-	-	1	-	-
	Benign granular cell Tumor	-	-	-	-	-	1	-	-
	Hemangioma	-	-	-	1	-	-	-	-
	Hemangiosarcoma	-	1	-	2	-	2	-	1
	Sarcoma (metastasis)	-	-	-	1	-	-	-	-
	Polyp: endometrial stromal	-	6	-	5	-	3	-	4
Vagina	Osteosarcoma (metastasis)	-	-	-	1	-	-	-	-
Gross Pathology									
Duodenum	Dilation	3	-	4	3	1	6	9	11
	Thickening	4	1	2	2	3	2	5	9
	Mass	-	-	-	-	-	-	1	2
Jejunum	Dilatation	2	2	5	3	2	7	8	9
Ileum	Dilatation	2	1	5	3	3	5	6	6
Cecum	Dilatation	1	1	3	2	1	2	4	5
Liver	Surface irregular	-	2	1	2	-	5	4	9
	Mass	13	2	4	3	-	2	5	10

Dose (mg/kg/day)		0 (Vehicle)		100		300		600	
Number Evaluated		**M: 60**	**F: 60**	**M: 60**	**F: 60**	**M: 60**	**F: 60**	**M: 60**	**F: 60**
Non-Neoplastic Lesions									
Bone Marrow	Hypercellularity: myeloid	6	11	4	7	8	12	21	20
Duodenum	Hyperplasia: Villous/mucosal	-	2	7	6	26	23	32	27
Kidney	Karyomegaly: tubular	-	-	-	-	-	1	3	8
Liver	Basophilic cell focus	3	1	-	1	-	3	-	4
	Eosinophillic cell focus	2	-	1	2	3	7	8	8
	Hypertrophy; Hepatocellular	9	6	14	6	22	27	39	46
	Cyto/karyomegaly	16	2	9	4	21	27	35	36
	Inclusions: nuclear	4	1	8	-	12	13	33	26
	Necrosis: single cell	7	5	1	2	11	7	22	18
	Proliferation: sinusoidal lining cells	3	2	3	3	7	15	20	25
Testis	Mineralization: vascular	-	-	1	-	6	-	17	-
Tumor analysis									
Liver-hepatocellular adenomata								-	P=0.0037[b]
Harderian gland								P=0.0098[b]	-

* = $p < 0.05$, Dunnetts "t" test

a At 6 months, for controls group means are shown. For treated groups percent differences from controls are shown. Statistical differences are based upon the actual data not percent differences.

b Pair wise test.

10.6. TDF: Rat

Test Article: TDF

Report Title: An Oral Carcinogenicity Study of Tenofovir Disoproxil Fumarate (TDF) in the Albino Rat **Gilead Study No. R990204**

Species/ Strain: Rat, Sprague Dawley CD (Crl: CD^{R} (SD) BR)- VAF **Duration of Dosing:** 104 Weeks **Location in CTD:** 4.2.3.4.1

Initial Age: 6 Weeks (approx) **Method of Administration:** Oral gavage **GLP Compliance:** Yes

Date of First Dose: [redacted] 20[redacted] (Main) **Vehicle/ Formulation:** Suspension vehicle **Lot No.:** [redacted]

Basis for High-Dose Selection: Toxicity-based endpoint. The high dose was based on toxicity (GI tract toxicity, kidney tubular karyomegaly and bone toxicity) observed in a 13/42 week rat study. On day 10 the dose level for group 2 was increased to 300 mg/kg/day.

Special Features: Separate populations for toxicokinetic evaluation were included in the study: Initial TK group (Set 1) comprised of 16 males and 16 females. Blood samples were taken on days 1 & 30. Animals euthanized on day 30 & subjected to necropsy. An additional group of TK animals were added to the study (Group 6, Set 2) to obtain plasma data from Day 1, 30, 60, and 90 at the revised dose level of 300 mg/kg/day. Animals euthanised on day 91 & subjected to necropsy. Additional populations of animals of 24 males and 24 females were added to Groups 1 to 4 to evaluate TK response for a period of 26 Weeks (Set 3). Animals euthanized on day 182 & subjected to necropsy. Before assignment of animals to a treatment group, 10 males & 10 females animals, selected randomly, were subjected to a health screen.

Dose (mg/kg/day)		**0 (Vehicle)**		**10/300[a]**		**30**		**100**	
Number evaluated		**M: 60**	**F: 60**	**M: 60**	**F: 60**	**M: 60**	**F: 60**	**M: 60**	**F: 60**
Toxicokinetics									
AUCss 0-24 (µg.h/mL) Day 180		-	-	16.69	15.72	2.70	3.06	6.60	7.46
Number of Animals									
At Start		60	60	60	60	60	60	60	60
Died/Sacrificed Moribund		42	44	42	32	42	45[b]	38	43
Terminal Sacrifice		18	16	18	28	18	15	22	17
Survival (%)		30	27	30	47	30	25	37	28
Body Weight (%)		644.8g	363.2g	-5.5%*	-3.7%	+2.0%	+0.6%	-2.9%	-1.1%
Food Consumption (%)		206.4	144.0	-1.5%	+5.6%	-0.2%	+2.8%	-3.5%	+3.9%
Number of Animals with Neoplastic Lesions									
Abdomen	Sarcoma	-	-	-	-	-	-	1	-

Dose (mg/kg/day)		0 (Vehicle)		10/300[a]		30		100	
Number evaluated		**M: 60**	**F: 60**	**M: 60**	**F: 60**	**M: 60**	**F: 60**	**M: 60**	**F: 60**
Number of Animals with Neoplastic Lesions, cont.									
Adrenal	Adenoma: cortical	-	2	-	-	1	-	-	-
	Malignant Pheochromocytoma	1	-	-	2	1	-	-	-
	Benign pheochromocytoma	-	-	3	1	1	1	3	-
	Metastasis	-	1	-	-	-	-	-	-
Brain	Malignant astrocytoma	-	1	1	1	3	-	2	1
	Malignant mixed glioma	-	-	-	-	-	-	1	-
	Metastasis	2	2	-	1	-	2	-	1
	Benign granular cell Tumor	-	1	-	-	-	-	-	-
Cavity Cranial	Metastasis	-	-	-	1	-	-	-	-
Clitoral Gland	Carcinoma: squamous cell	-	-	-	-	-	-	-	1
Eye	Malignant schwannoma	-	-	-	1	-	-	-	-
Harderian Gland	Metastasis	-	-	-	1	-	-	-	-
Heart	Metastasis	-	1	-	-	-	-	-	-
	Benign schwannoma: Endocardial	-	1	-	-	-	-	2	-
Hemolym Tissue	Malignant lymphoma	-	2	1	-	-	2	-	1
	Histiocytic sarcoma	2	0	1	1	2	2	2	-
Ileum	Adenocarcinoma	1	-	-	-	1	-	-	-
Jejunum	Adenocarcinoma	-	-	-	-	1	-	-	-
Kidney	Carcinoma: tubular cell	-	-	-	-	1	-	-	-
	Adenoma: tubular cell	1	-	-	-	-	-	-	-
	Renal mesenchymal tumor	-	-	-	-	1	-	-	-

Dose (mg/kg/day)		**0 (Vehicle)**		**10/300[a]**		**30**		**100**	
Number evaluated		**M: 60**	**F: 60**	**M: 60**	**F: 60**	**M: 60**	**F: 60**	**M: 60**	**F: 60**
Number of Animals with Neoplastic Lesions									
Liver	Carcinoma: hepatocellular	3	-	1	-	1	-	1	-
	Adenoma: hepatocellular	1	1	2	-	-	-	-	-
	Cholangioma	-	-	-	-	-	-	1	-
	Cholangiocarcinoma	1	-	1	-	-	-	-	-
	Metastasis	1	-	-	-	-	-	-	-
Lung	Carcinoma: metastasis	-	-	1	1	1	2	-	2
	Adenoma: Alveolar/bronchiolar	-	-	2	-	2	-	3	-
	Metastasis	-	1	-	-	-	-	-	-
Lymph node	Metastasis	1	-	-	-	2	-	-	-
Lymph Node Mandibular	Metastasis	-	-	-	1	-	-	-	-
Mammary Gland	Fibroadenoma	1	23	2	25	1	23	-	20
	Adenoma	-	3	-	2	-	1	-	2
	Adenocarcinoma	-	14	-	11	-	13	-	10
Nerve sciatic	Metastasis	-	-	-	1	-	-	-	
Ovary	Benign luteoma	-	1	-	-	-	-	-	-
	Benign granulosa-theca cell tumor	-	-	-	2	-	2	-	2
	Malignant schwannoma								

Dose (mg/kg/day)		0 (Vehicle)		10/300[a]		30		100	
Number evaluated		**M: 60**	**F: 60**	**M: 60**	**F: 60**	**M: 60**	**F: 60**	**M: 60**	**F: 60**
Number of Animals with Neoplastic Lesions									
Pancreas	Adenoma-acinar cell	-	-	-	-	1	-	-	-
	Carcinoma: islet cell	8	3	7	1	10	2	12	6
	Adenoma: islet cell	4	-	6	2	5	2	2	1
Parathyroid Gland	Adenoma	-	-	-	-	1	-	-	-
Pituitary	Carcinoma: pars distalis	3	2	-	2	-	2	-	1
	Adenoma: pars distalis	35	52	25	39	30	47	28	42
	Adenoma: pars intermedia	3	-	-	1	-	-	-	-
Skin Misc.	Papilloma: squamous cell	1	-	1	-	3	-	-	-
	Carcinoma: squamous cell	1	2	1	-	1	-	1	1
	Keratoacanthoma	-	-	1	1	1	-	4	-
	Adenoma: sebaceous	-	-	-	-	-	-	1	-
	Carcinoma: basal cell	-	1	1	-	-	-	2	-
	Adenoma: basal cell	-	-	-	-	-	-	1	-
	Fibrosarcoma	-	-	-	-	1	-	-	-
	Histiocytoma	-	1	-	-	-	-	-	-
Spleen	Hemangioma	-	-	-	-	1	-	-	-
Spinal Cord, Cervical	Benign astrocytoma	-	-	-	1	-	-	-	-
Subcutaneous Tissue	Sarcoma	-	-	1	-	-	-	-	-
	Fibroma	2	1	2	-	-	-	2	-

Dose (mg/kg/day)		0 (Vehicle)		10/300[a]		30		100	
Number evaluated		**M: 60**	**F: 60**	**M: 60**	**F: 60**	**M: 60**	**F: 60**	**M: 60**	**F: 60**
Number of Animals with Neoplastic Lesions									
Subcutaneous Tissue	Fibrosarcoma	1	1	1	-	-	1	-	-
	Lipoma	-	-	3	2	-	2	-	1
Tail	Fibrosarcoma	1	-	-	-	-	-	1	-
	Osteosarcoma	-	-	-	-	-	1	-	-
Testis	Adenoma: intestitial cell	2	-	2	-	1	-	-	-
	Carcinoma: interstitial cell	-	-	-	-	1	-	-	-
	Adenoma: rete testis	-	-	-	-	1	-	-	-
Thorax	Liposarcoma	-	-	-	-	1	-	-	-
Thyroid	Adenoma: follicular cell	2	-	3	-	-	1	1	1
	Carcinoma: follicular cell	-	-	-	-	2	1	-	-
	Adenoma: C-cell	4	4	4	6	2	3	3	2
	Carcinoma: C-cell	2	1	1	-	-	-	1	-
Tongue	Papilloma: squamous cell	-	-	-	-	1	-	1	-
	Sarcoma	-	-	-	-	-	-	-	1
Urinary Bladder	Papilloma: transitional cell	-	-	-	-	-	1	1	-
Uterus	Polyp: endometrial stromal	-	2	-	11	-	4	-	3
	Benign granular cell tumor	-	3	-	-	-	-	-	-
Vagina	Polyp	-	-	-	1	-	-	-	1
	Benign granular cell tumor	-	-	-	-	-	1	-	-
	Papilloma: squamous cell	-	-	-	1	-	-	-	-

Dose (mg/kg/day)		0 (Vehicle)		10/300[a]		30		100	
Number evaluated		**M: 60**	**F: 60**	**M: 60**	**F: 60**	**M: 60**	**F: 60**	**M: 60**	**F: 60**
Gross Pathology		-	-	-	-	-	-	-	-
Histopathology- Non-Neoplastic Findings									
Kidney	Karyomegaly, tubular	-	-	29	17	1	-	5	-
	Chronic progressive nephropathy	46	23	29	11	47	16	38	13
Tumor Analysis									
Uterus – Polyp:endometrial Stromal Data			P= 0.0053**		P= 0.0065**				

a Dose increased on Day 10 from 10 to 300 mg/kg/day.
b Terminated at Week 103.
* = Pair wise test; ** = P < 0.05, Dunnett's

10.7. TAF

No carcinogenicity studies have been conducted with TAF, but carcinogenicity studies with TDF are included in Sections 10.3 and 10.4.

11. REPRODUCTIVE AND DEVELOPMENTAL TOXICITY (NONPIVOTAL STUDIES)

11.1. BIC

Test Article: BIC

Species / Strain	Method of Administration (Vehicle / Formulation)	Dosing Period	Doses (mg/kg/day)	No. Per Group	Noteworthy Findings	Gilead Study No. (CRO Name and Study No.)
Rat/ Crl:CD(SD)	Once daily oral gavage [0.5% (w/w) HPMC and 0.1% (w/w) Tween® 20 in R.O. deionized water]	GD 7-17	0, 5, 30, 300	Main: 6F TK:3F control; 9F treated	No GS-9883-related effects on clinical observations, body weights, body weight gain, or absolute or relative food consumption. No GS-9883-related effects on Caesarean sectioning or litter parameters and no GS-9883-related fetal gross external alterations. 300 mg/kg/day: Maternal and Fetal NOEL (GD 17 mean C_{max} = 180 µg/mL and AUC_{0-24} = 3650 µg·hr/mL)	TX-141-2034[a] CRL 20055845
Rabbit/ Crl:KBL(NZW)	Once daily oral gavage (25% Solutol HS-15 and 75% propylene glycol)	GD 7-19	0, 100, 300, 1000	8F	No NOEL or NOAEL for maternal or developmental toxicity was determined due to vehicle-related toxicity.	TX-141-2035[a] CRL 20055847
Rabbit/ Hra:(NZW)SPF	Once daily oral gavage [0.5% (w/w) HPMC and 0.5% (w/w) Tween® 20 in R.O. deionized water, pH 8.0 ± 0.5],	GD 7-19	0, 100, 300, 1000	8F	100 mg/kg/day: no maternal or developmental effects 300 mg/kg/day: pale or light brown feces, ungroomed coat. Maternal NOAEL and developmental NOEL (GD 19 C_{max} = 4050 ng/mL and AUC_{0-24} = 60300 ng·hr/mL) 1000 mg/kg/day: pale or light brown feces, ungroomed coat, transient BW loss, reduced food consumption in does and abortion in one female. Slightly decreased fetal BW from vehicle control but within Test Facility historical control ranges.	TX-141-2038[a] CRL 20065265

GD = Day of presumed gestation; fbe = free base equivalents; AUC_{0-24h} = Area under the curve (0-24 hours); NOEL = No-Observed-Effect-Level; NOAEL = No-Observed-Adverse-Effect-Level; TK = toxicokinetics; BW = body weight; HPMC = hydroxypropylmethylcellulose; R.O. = reverse osmosis membrane-processed
a Indicates GLP compliant

11.2. FTC

Test Article: FTC

Species/Strain	Method of Administration (Vehicle/Formulation)	Dosing Period	Dose (mg/kg/day)	No. per Group	Noteworthy Findings	Study Number
Mouse / CD-1	Oral Gavage	GD 6-15	0, 250, 500, 1000, divided doses spaced by approximately six hours	5 F	One fetus in the control group had exencephaly without open eyelids (bilateral). No other external malformations and no developmental variations were observed in fetuses in this study. No other significant effects were reported. NOAEL = 1000 mg/kg/day for maternal and developmental toxicity of FTC administered twice daily.	TOX033[a]
Rabbit / New Zealand White	Oral Gavage	GD 7-19	0, 250, 500, 1000, divided doses spaced by approximately six hours	5 F	All animals survived to the scheduled necropsy on gestation day 29. No treatment-related clinical signs were observed at any dose level. Mean maternal body weights, body weight changes, net body weights, net body weight changes, gravid uterine weights, and food consumption were unaffected by treatment at all dose levels.Intrauterine growth and survival were also unaffected by treatment. Parameters evaluated included postimplantation loss, mean fetal body weights, viable litter size, and mean numbers of corpora lutea and implantation sites. No external malformations or developmental variations were observed in fetuses. No maternal toxicity or developmental toxicity was noted at dosage levels up to 1000 mg/kg/day.	TOX034[a]

GD = gestation day; F = female; NOAEL = No-Observed-Adverse-Effect-Level
a Indicates GLP compliant.

11.3. TAF

Test Article: TAF

Species/Strain	Method of Administration (Vehicle/Formulation)	Dosing Period	Doses (mg/kg)	No. Per Group	Noteworthy Findings	Gilead Study No. (CRO Name and Study No.)
Rats/ Sprague-Dawley Crl:CD(SD)	Oral Gavage (0.1% (v/v) Tween 20, 0.1% (w/v) (HPMC) K100LV prepared in ultra-pure water)	12 days (GD 6-17)	5, 100, 200	8F/group	Decreased body weight gain (-18%) and food consumption at 200 mg/kg/day. NOAEL for maternal toxicity: 100 mg/kg/day NOAEL for embryo-fetal development:200 mg/kg/day (AUC0-t = 35.7µg•hr/mL)	TX-120-2001[a] (CRL 902244)
Rabbits/New Zealand White	Oral Gavage (0.1% (v/v) Tween 20, 0.1% (w/v) (HPMC) K100LV prepared in ultra pure water)	13 days (GD 7-20)	0, 5, 25, 50, 100	6F/group	Maternal effects: decreased body weights and food intake at 100 mg/kg/day. No evidence of embryolethality, fetotoxicity or teratogenicity at any dose level. NOEL for maternal toxicity : 50 mg/kg/day (AUC0-t = 11.8 and 5.7 µg•hr/mL for R-PMPA and GS-7340, respectively) NOEL for embryo-fetal development: 100 mg/kg/day (AUC0-t = 23.5 and 10.7 µg•hr/mL for R-PMPA and GS-7340, respectively).	TX-120-2004[a] (CRL 902247)

GD= gestation day; NOEL = No-Observed- Effect Level; NOAEL = No-Observed-Adverse-Effect Level; F = female; R-PMPA = tenofovir
a Indicates GLP compliant

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

12.1. BIC: Fertility and Early Embryonic Development in Rats

Test Article: BIC

Report Title: An Oral (Gavage) Study of Fertility and Early Embryonic Development to Implantation of GS-9883-01 in Sprague Dawley Rats

Gilead Study No. TX-141-2039 [redacted] Study No. 604142

Species / Strain: Rat / Crl:CD(SD)

Duration of Dosing: Male: once daily for 28 days prior to mating through day prior to necropsy

Location in CTD:

Initial Age: Males: 9 weeks
Females: 10 weeks

Female: once daily for 14 days prior to mating through day 7 of gestation

GLP Compliance: yes

Date of First Dose: [redacted] 20[redacted] (Males), [redacted] 20[redacted] (Females)

Day of Mating: study day 27

Lot Number: [redacted]

No-Observed-Effect Level

Day of C-Section: gestation day 15

Design Similar to ICH 4.1.1? yes

F_0 Males: 300 mg/kg/day

Method of Administration: Oral (gavage)

F_0 Females: 300 mg/kg/day

Vehicle: 0.5% (w/w) hydroxypropyl methylcellulose and 0.1% Tween® 20 in reverse osmosis water, pH adjusted to 7.5-8.5

F_1 Litters: 300 mg/kg/day

Special Features: None

Daily Dose (mg/kg/day)		**0 (Control)**	**5**	**30**	**300**
Males	No. Evaluated	25	25	25	25
	No. Died or Sacrificed Moribund	0	0	0	0
	Clinical Observations	No Test-Article Related Findings			
	Body Weight	No Test-Article Related Findings			
	Food Consumption	No Test-Article Related Findings			
	Necropsy Observations	No Test-Article Related Findings			
	Organ Weights	No Test-Article Related Findings			
	Male Reproductive Performance	No Test-Article Related Findings			
	Sperm Motility	No Test-Article Related Findings			

Daily Dose (mg/kg/day)		0 (Control)	5	30	300
Females	No. Evaluated	25	25	25	25
	No. Died or Sacrificed Moribund	0	0	0	0
	Clinical Observations	No Test-Article Related Findings			
	Premating Body Weight	No Test-Article Related Findings			
	Gestation Body Weight	No Test-Article Related Findings			
	Premating Food Consumption	No Test-Article Related Findings			
	Gestation Food Consumption	No Test-Article Related Findings			
	Necropsy Observations	No Test-Article Related Findings			
	Organ Weights	No Test-Article Related Findings			
	Mean Length of Estrous Cycle (days)	4.3	4.1	4.2	4.3
	Mean No. Days Prior to Mating	3.5	2.5	2.8	2.4
	Female Reproductive Performance	No Test-Article Related Findings			
	Laparohysterectomy	No Test-Article Related Findings			

12.2. FTC: Fertility and Early Embryonic Development in Mice

Test Article: FTC

Report Title: Study of Fertility and Early Embryonic Development of TP-0006 Administered by Gavage to CD-1 Mice **Gilead Study No. TOX036**

Duration of Dosing: M: received 4 Weeks pre-breeding exposure, 21 days during mating and 21 days post-mating, for a total of 70 days; **F:** 14 days prebreed, 21 days mating, 7 days post coitum (gestational day 0-6) for total maximum of 42 days.

Location in CTD: 4.2.3.5.1

GLP Compliance: Yes

Species/Strain: Mouse / CD-1

Day of Mating: 21 Days

Design similar to ICH 4.1.1: Yes

Initial Age: 51 days old upon arrival

Day of C-Section: GD 13

Vehicle/Formulation: 0.5% methylcellulose

Date of First Dose: M: [redacted] or [redacted], 19[redacted] **F:** [redacted], 19[redacted]

Lot No.: [redacted]; [redacted]

Special Features: none

Method of Administration: Oral gavage, divided doses spaced by approximately six hours

No Observed Adverse-Effect Level: F_0 M/F =1000 mg/kg/day; F_1 Litters =1000 mg/kg/day

	0 (Vehicle)	250	500	1000
Toxicokinetics: AUC (males)	NA	NM	NM	NM
No. Evaluated	21	20	21	21
Mortality				
No. Died	0/20	0/20	0/20	0/20
Sacrificed Moribund[a]	0	1	1	1
Clinical Observations	N	N	N	N
Necropsy Observations				
Paired testes weight (g)	0.247	0.248	0.252	0.231
Paired epididymides weight (g)	0.116	0.119	0.120	0.111
Body Weight (%)	N	N	N	N
Food Consumption (%)	N	N	N	N
Reproductive Toxicity				
Mating index (%)	100	100	100	100
Fertility index (%)	96	90	100	90
Pregnancy index (%)	100	94.7	100	94.7
Epididymal sperm count (10^6/g)	620.1	692.2	667.3	757.2
Sperm motility (% motile)	69.3	68.9	69.7	71.1

	0 (Vehicle)	250	500	1000
Toxicokinetics: AUC (females)	NA	NM	NM	NM
No. Evaluated	20	20	20	20
Mortality				
No. Died[b]	0/20	0/20	0/20	0/20
Sacrificed moribund	0	0	0	0
Clinical Observations	N	N	N	N
Necropsy Observations				
Gravid uterine weight (g)	6.65	6.92	6.30	6.59
Paired ovary weight (g)	0.049	0.055	0.064	0.058
Body Weight (%)	N	N	N	N
Food Consumption (%)	N	N	N	N
Reproductive Toxicity				
Mating index (%)	100	100	100	100
Fertility index (%)	96.0	90.0	100	90.0
Gestational index (%)	100	100	100	100
Preimplantation loss/litter (%)	5.74	4.08	11.46	9.04
Postimplantation loss/litter (%)	4.10	4.98	13.42	2.56
Parents				
Females that mated (Mating Index, %)	20 (100.0)	20 (100.0)	20 (100.0)	20 (100.0)
Fertility Index (No. females pregnant/ No. females mated, %)	96.0	90.0	100.0	90.0
Evaluated pregnant females	20	18	20	18

	0 (Vehicle)	250	500	1000
Litter Means (mean per litter ± standard deviation)				
Corpora lutea	13.25 ± 0.42	13.22 ± 0.46	14.10 ± 0.69	14.06 ± 0.89
Implantations (sites per litter)	12.50 ± 0.34	12.89 ± 0.42	12.35 ± 0.50	12.61 ± 0.52
Live fetuses (live implants per litter)	12.00 ± 0.36	12.22 ± 0.39	10.90 ± 0.73	12.28 ± 0.52
Dead fetuses (nonlive implants per litter)	0.50 ± 0.14	0.67 ± 0.18	1.45 ± 0.45	0.33 ± 0.11
Resorptions	0.45 ± 0.14	0.61 ± 0.16	1.35 ± 0.45	0.33 ± 0.11
Weight of fetuses (g)	NA	NA	NA	NA
Sex ratio of fetuses	NA	NA	NA	NA

Conclusion: Exposure of F_0 male and female mice by oral gavage during prebreeding and mating for both sexes and through implantation (on gestation day 6) for females, resulted in no toxicity for body weight or feed consumption. There were no effects of treatment on F_0 male or female reproduction or on development of the F_1 offspring through gestation day 13.

a Three males were euthanized moribund due to injury or infection.
b No females died, were euthanized moribund, or were removed from study.
N = Normal or no change from control group value; NM = Not measured; NA = not applicable

12.3. FTC: Male Fertility in Rats

Test Article: FTC

Report Title: A Fertility Study in Male Rats Given 524W91 by Gavage **Gilead Study No. TTEP/95/0028 (TOX679)**

Duration of Dosing: M: 73 days (prior to mating and throughout mating, dose days 1–95); **F:** untreated **Location in CTD:** 4.2.3.5.1

Species/Strain: Rat / CD (Sprague-Dawley) **Day of Mating:** Bred-dams day 0 (date of insemination) **GLP Compliance:** Yes

Initial Age: M: F_0 males approx. 17 Weeks of age at time of pairing, **F:** F_0 approx. 12 Weeks of age at time of pairing **Day of C-Section:** GD 13 **Design similar to ICH 4.1.1:** Yes

Method of Administration: Oral gavage **Lot No.:** [redacted]

Date of First Dose: M: [redacted], 19[redacted] **F:** [redacted], 19[redacted] **Vehicle/Formulation:** 524W91 + 0.5% methylcellulose, 20 mL/kg/dose **Special Features:** none

No Observed Adverse-Effect Level: F_0 Males: NOEL = 750 mg/kg/day, NOAEL = 3000 mg/kg/day; **F_0 Females:** 3000 mg/kg/day; **F_1 Litters:** 3000 mg/kg/day

Dose (mg/kg/day)	0 (Vehicle)	150	750	3000
Results for F_0 Generation Males:				
No. Evaluated	25	25	25	25
Mortality	0	0	0	1 (dosing accident)
Clinical Observations	None	None	Transient postdose salivation	Postdose salivation
Body weight (%)	-	No change	No change	No change
Food consumption (%)	-	Normal	Normal	Normal
Necropsy Observations				
Histopathology	1/10 nephritis 2/10 prostatitis	-	-	3/10 prostatitis
Gross lesions	1[a]	0	0	0
Organ weight (%)	-	No change	No change	No change
Sperm count and motility	-	Normal	Normal	Normal
Results for F_0 Generation Untreated Females:				
No. Evaluated	25	25	25	25
Body weight change (gestation)	-	No change	No change	No change
Food consumption (gestation)	-	No change	No change	No change
Maternal gross lesions[a]	None	None	None	None

Dose (mg/kg/day)	0 (Vehicle)	150	750	3000
Reproductive performance				
Fertility index, %	87.5	96.0	92.0	100.0
Pregnancy rate, %	88.0	96.0	92.0	100.0
Number of pregnant females	22	24	23	25
Total resorption	0	1	0	0
Implantation sites/dam	15.6	15.6	17.3	17.0
Viable fetuses/dam	14.4	14.8	15.7	16.2
Post-implantation loss/dam	1.2	0.8	1.6	0.8
Parents				
Females with sperm (Fertility index, %)	24 (87.5)	25 (96.0)	25 (92.0)	25 (100.0)
Pregnant females (Pregnancy rate, %)	21 (88.0)	24 (96.0)	23 (92.0)	25 (100.0)
Evaluated pregnant females	21	24	23	25
Litter Means (mean per litter ± standard deviation)				
Corpora lutea	18.2 ± 3.4	17.4 ± 3.9	18.3 ± 1.8	19.0 ± 2.4
Implantations	15.6 ± 3.5	15.6 ± 5.2	17.3 ± 1.7	17.0 ± 1.5
Live fetuses	14.4 ± 3.4	14.8 ± 5.3	15.7 ± 2.9	16.2 ± 1.6
Dead fetuses	NA	NA	NA	NA
Resorptions	1.2 ± 1.3	0.8 ± 1.2	1.6 ± 2.0	0.8 ± 0.9
Weight of fetuses (g)	NA	NA	NA	NA
Sex ratio of fetuses	NA	NA	NA	NA

NA = not applicable; NM = not measured; N = normal or no change from control group value
a Raised white areas on the kidneys

Conclusion: Male rats given 150, 750 or 3000 mg 524W91/kg/day orally for 73 days prior and throughout the period of mating to untreated female rats exhibited postdose salivation primarily at the high dose, but did not show any other evidence of treatment-related toxicity. No adverse effects on fertility, reproductive performance, sperm count, and sperm motility or pathological changes in the reproductive organs were observed.

12.4. TAF: Fertility and General Reproduction in Rats

Test Article: TAF

Report Title: Oral Gavage Study of Fertility and Early Embryonic Development to Implantation with GS 7340-03 in Rats

Gilead Study No. TX-120-2012
[redacted] Study No.: 8264528

Species / Strain: Rat/Sprague-Dawley

Duration of Dosing:
M: at least 28 days prior to mating and throughout the mating period through the day prior to termination (at least 10 weeks prior to sacrifice)
F: at least 14 days prior to mating, throughout mating through Day 7 of gestation (confirmed mated) or gestation Day 13 (confirmed non-mated)

Location in CTD: 4.2.3.5.1

Initial Age:
Males: Approximately 10 weeks
Females: Approximately 12 weeks

GLP Compliance: yes

Date of First Dose:
Males: [redacted] 20[redacted]
Females: [redacted] 20[redacted]

Day of Mating: GD 0

Lot Number: [redacted]
(hemifumarate salt)

No-Observed-Adverse-Effect Level
F_0 Males: 80 mg/kg/day
F_0 Females: 80 mg/kg/day
F_1 Litters: 160 mg/kg/day

Day of C-Section: GD 13
Method of Administration: Oral Gavage
Vehicle: 0.1% hydroxypropylmethylcellulose K100LV (HPMC),0.1% Tween 20 in reverse osmosis (RO) water
Special Features: early embryonic toxicity

Design Similar to ICH 4.1.1? yes

Dose Volume: 10 mL/kg

Daily Dose (mg/kg/day)		0 (Control)	20	80	160
Males	No. Evaluated	22	22	22	22
	No. Died or Sacrificed Moribund	0	0	0	0
	Clinical Observations				
	Discolored haircoat – left periorbital brown, perineal brown, front legs brown, and/or periorbital red	0	1	1	0
	Rough haircoat	0	2	2	2
	Body Weight (%)[a]	608	-0.5	-4.8	-12.3[#H]
	Food Consumption (%)[a]	225.1	-0.2	-2.0	-9.6[+H]
	Necropsy Observations	-	-	-	-
	Mean No. Days Prior to Mating	3.2	3.0	3.2	3.6
	No. of Males that Mated	22	21	20	21
	No. of Fertile Males	21	21	19	20
	Sperm Motility (Motlile %)	91.8 (± 8.43)	95.2 (± 3.08)	95.5 (± 4.01)	94.0 (± 6.10)

Daily Dose (mg/kg/day)		0 (Control)	20	80	160
Females	No. Evaluated	22	22	22	22
	No. Died or Sacrificed Moribund	0	0	0	0
	Clinical Observations				
	Discolored haircoat (perineal yellow, shoulder(s) – yellow or brown, midline abdomen yellow, or front legs orange)	1	1	2	4
	Premating Body Weight (%)[a]	305	-2.0	-3.6	-5.9*H
	Gestation Body Weight (%)[a]	380	-0.5	-1.6	-5.3
	Premating Food Consumption (%)[a]	169.2	0.2	-2.4	-10.0+H
	Gestation Food Consumption (%)[a]	95.1	-0.1	0.7	-2.6
	Necropsy Observations	-	-	-	-
	Mean No. Estrous Cycles/14 days	2.8	2.6	2.3	2.5
	Mean No. Days Prior to Mating	3.2	3.0	3.2	3.6
	No. of Pregnant Females	21	22	21	21
	No. Aborted or with Total Resorption of Litter	0	1	0	0
	Mean No. Corpora Lutea	16.3	16.0	15.8	16.0
	Mean No. Implantations	16.2	15.0	15.1	15.7
	Mean % Preimplantation Loss	0.5	7.1	4.3	1.8
	Mean No. Live Conceptuses	15.3	14.5	13.9	14.5
	Mean No. Early Resorptions	0.9	0.5	1.3	1.0
	Mean % Postimplantation Loss	5.8	7.9	8.6	7.3

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

- No noteworthy findings; + Mild, ++ Moderate, +++ Marked; * = $p \leq 0.05$ (<site statistical method used here>)
+H = Dunnett Exact Homogeneous Test Significant: 0.01 level
#H = Dunnett Exact Homogeneous Test Significant: 0.001 level
*H = Dunnett Exact Homogeneous Test Significant: 0.05 level

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

13.1. BIC: Embryo/Fetal Development in Rat

Test Article: BIC

Report Title: An Oral Gavage Embryo-Fetal Development Study with GS-9883-01 in Sprague Dawley Rats

Gilead Study No. TX-141-2036
[redacted] Study No. 20055846

Species / Strain: Rat / Crl:CD(SD)
Initial Age: Approximately 56 to 70 days at receipt
Date of First Dose: [redacted] 20[redacted]
No-Observed-Effect Level
F_0 Females: 300 mg/kg/day
F_1 Litters: 300 mg/kg/day

Duration of Dosing: DG 7 through 17
Day of Mating: DG 0
Day of C-Section: DG 21
Method of Administration: **Oral Gavage**
Vehicle: 0.5% (w/w) hydroxypropylmethylcellulose (HPMC) and 0.1% (w/w) Tween® 20 in reverse osmosis membrane-processed deionized water (R.O. deionized water)
Special Features: **None**

GLP Compliance: Yes
Lot Number: [redacted]
Design Similar to ICH 4.1.3? yes

Daily Dose (mg/kg/day)		**0 (Control)**	**5**	**30**	**300**
Dams/Does:	No. Pregnant	22	21	21	22
	No. Died or Sacrificed Moribund	0	0	0	0
	No. Aborted or with Total Resorption of Litter	0	0	0	0
	Clinical Observations	No Test Article-Related Findings			
	Body Weight, DG 18 (g)	322.0	310.8	321.1	313.5
	Body Weight Change DG 7 to 18 (g)	97.4	86.8**	94.4	87.3**
	Food Consumption DG 7 to 18 (g/day)	21.7	21.1	22.0	20.9
	Necropsy Observations	No Test Article-Related Findings			
	Mean No. Corpora Lutea	12.8	12.7	13.3	13.3
	Mean No. Implantations	12.6	12.0	12.6	12.3
	Mean % Preimplantation Loss	1.3	4.9	4.3	6.7

Daily Dose (mg/kg/day)		0 (Control)	5	30	300
Litters:	Litters Evaluated	22	21	21	22
	Live Fetuses	272	245	252	256
	Dead Fetuses	0	0	0	0
	Mean No. Resorptions	0.2	0.3	0.6	0.7
	Mean % Postimplantation Loss	1.8	2.8	4.8	5.8
	Mean Fetal Body Weight (g)	5.87	5.79	5.78	5.75
	Fetal Sex Ratios (Live No. of M:F)	136:136	128:117	120:132	111:145
	Fetal Anomalies	No Test Article-Related Findings			
	Gross External Anomalies	No Test Article-Related Findings			
	Visceral Anomalies	No Test Article-Related Findings			
	Skeletal Anomalies	No Test Article-Related Findings			
	Total Affected Fetuses (Litters)	13 (9)	16 (10)	11 (8)	$27^{\#\#}$ (12)

** = $p \leq 0.01$ (Dunnett's test); $^{\#\#}$ = $p \leq 0.01$ (Variance Test for Homogeneity of the Binomial Distribution); DG = Day of Gestation

13.2. BIC: Embryo/Fetal Development in Rabbit

Test Article: BIC

Report Title: An Embryo-Fetal Development Study of GS-9883-01 by Oral Gavage in Rabbits

Gilead Study No. TX-141-2037
[redacted] Study No. 20055848

Species / Strain: Rabbit / Hra:(NZW)SPF
Initial Age: Approximately 5 to 6 months of age at receipt
Date of First Dose: [redacted] 20[redacted]

Duration of Dosing: DG 7 to 19
Day of Mating: DG 0
Day of C-Section: DG 29
Method of Administration: Oral Gavage

GLP Compliance: Yes
Lot Number: [redacted]
Design Similar to ICH 4.1.3? Yes

No-Observed-Adverse-Effect Level

F_0 Females: 300 mg/kg/day
F_1 Litters: 300 mg/kg/day (NOEL)

Vehicle: 0.5% (w/w) hydroxypropylmethylcellulose (HPMC) and 0.5% (w/w) Tween® 20 in reverse osmosis membrane-processed deionized water (R.O. deionized water), pH 8.0 ± 0.5

Special Features: None

Daily Dose (mg/kg/day)		0 (Control)	100	300	1000
Does:	No. Pregnant	20	19	20	21
	No. Died or Sacrificed Moribund	0	0	0	0
	No. Aborted or with Total Resorption of Litter	0	0	0	2
	Clinical Observations (No. Affected)				
	scant feces	1	2	0	8##
	light brown and/or yellow feces	0	0	0	7##
	thin body condition	0	0	0	6##
	cold to touch	0	0	0	5##
	Body Weight DG 20 (kg)	3.50	3.59	3.50	3.25**
	Food Consumption DG 7 to 20 (g/day)	162.4	166.9	159.5	95.4**
	Necropsy Observations	No Test Article-Related Findings			
	Mean No. Corpora Lutea	9.4	10.0	9.2	10.4
	Mean No. Implantations	9.2	9.8	9.0	10.0
	Mean % Preimplantation Loss	2.0	2.1	0.9	2.8

Daily Dose (mg/kg/day)		0 (Control)	100	300	1000
Litters:	Litters Evaluated	20	19	20	19
	Live Fetuses	179	181	173	177
	Dead Fetuses	0	0	0	0
	Mean No. Resorptions	0.2	0.3	0.4	0.7
	Mean % Postimplantation Loss	2.3	3.4	4.8	5.5
	Mean Fetal Body Weight (g)	44.04	43.21	43.62	39.03**
	Fetal Sex Ratios (Live No. of M:F)	90:89	103:78	83:90	92:85
	Fetal Anomalies	No Test Article-Related Findings			
	Gross External Anomalies	No Test Article-Related Findings			
	Visceral Anomalies	No Test Article-Related Findings			
	Skeletal Anomalies	No Test Article-Related Findings			
	Total Affected Fetuses (Litters)	5 (4)	13 (9)	10 (7)	8 (6)

** = $p \leq 0.01$ (Dunnett's test); ## = $p \leq 0.01$ (Variance Test for Homogeneity of the Binomial Distribution); DG = gestation day; NOEL = No-Observed-Effect-Level

13.3. FTC: Embryo/Fetal Development in Mice

Test Article: FTC

Report Title: A Study of the Effects of TP-0006 (FTC) on Embryo/Fetal Development in Mice **Gilead Study No. TOX037**

Species/Strain: Mouse/CD-1 **Duration of Dosing:** GD 6–15 **Location in CTD:** 4.2.3.5.2

Initial Age: 72 day old upon receipt **Day of Mating:** Day 0 **GLP Compliance:** Yes

Date of First Dose: [redacted] 19[redacted] **Day of C-Section:** GD 18 **Lot No.:** [redacted]

Method of Administration: Oral, divided doses spaced by approximately six hours **Design similar to ICH 4.1.3:** Yes

Special Features: none **Vehicle/Formulation:** 0.5% methylcellulose

No Observed Adverse-Effect Level: 1000 mg/kg/day for maternal and developmental toxicity

Dose (mg/kg/day)	0 (Vehicle)	250	500	1000
F_0 Toxicokinetics[a]: Conc. mean ± SD (µg/mL)	NA	52 ± 15	88 ± 14	186 ± 14
Females				
Females with Sperm	ND	ND	ND	ND
Pregnant Females	23	25	23	22
Evaluated Pregnant Females	23	25	23	22
Litters				
No. Corpora Lutea	15.3 ± 3.59	15.3 ± 2.69	14.3 ± 2.37	14.9 ± 3.00
No. Implantations	12.3 ± 2.42	12.6 ± 2.58	11.9 ± 3.23	12.1 ± 2.47
Live Fetuses	11.4 ± 2.68	11.7 ± 2.84	11.0 ± 3.20	11.7 ± 2.36
Dead Fetuses	0	0	0.0 ± 0.21	0.0 ± 0.21
Resorptions (early)	0.8 ± 1.17	0.8 ± 1.09	0.7 ± 0.70	0.4 ± 0.58
Resorptions (late)	0.1 ± 0.29	0.2 ± 0.37	0.1 ± 0.34	0.0 ± 0.21
Weight of Fetuses (g)	1.34 ± 0.088	1.34 ± 0.113	1.34 ± 0.117	1.26 ± 0.102
Sex of Fetuses (% of live fetuses, m|f)	48.0 52.0	51.8 48.2	47.6 52.4	49.8 50.2
Litters with any Fetal Alteration (%)	1 (1)	4 (3)	1 (1)	3 (3)
Fetuses available for Morphological Evaluation (%)	263 (23)	292 (25)	254 (23)	257 (22)

NA = not applicable; ND = not determined; Conc = concentration; SD = standard deviation

a A toxicokinetic phase was conducted with 5 female mice/group administered 250, 500, and 1000 mg/kg/day as a single dose on gestation day 15. The mean ± standard deviation for plasma concentrations were determined. The increase in plasma levels was roughly in proportion to the increase in dose administered at 0.5 hr.

13.4. FTC: Embryo/Fetal Development in Rabbits

Test Article: FTC

Report Title: A Study of the Effects of TP-0006 on Embryo/Fetal Development in Rabbits **Gilead Study No. TOX038**
Species/Strain: Rabbit/New Zealand White **Duration of Dosing:** GD 7–19 **Location in CTD:** 4.2.3.5.2
Initial Age: approximately 4.5 months old **Day of Mating:** Day 0 **GLP Compliance:** Yes
Date of First Dose: [redacted], 19[redacted] **Day of C-Section:** GD 29 **Lot No.:** [redacted]
Method of Administration: Oral, divided doses spaced by approximately six hours **Design similar to ICH 4.1.3:** Yes
NOAEL: F_0 Fem. =100 mg/kg/day for maternal toxicity; F_1 Litters =1000 mg/kg/day for developmental toxicity **Vehicle/Formulation:** 0.5% methylcellulose

Dose (mg/kg/day)	0 (Vehicle)	100	300	1000
F_0 Toxicokinetics[a]:				
AUC_{0-12} (µg.h/mL) GD 19	NA	43.6	157.6	628.9
AUC_{0-24} (µg.h/mL) GD 19	NA	87.3	315	1258
Females				
Females with Sperm	ND	ND	ND	ND
Pregnant Females	18	19	19	17
Evaluated Pregnant Females	17	18	18	16
Litters (means per litter ± standard deviation)				
No. Corpora Lutea	9.9 ± 1.73	10.4 ± 2.31	10.2 ± 2.29	12.1 ± 3.86
No. Implantations	8.1 ± 2.37	7.6 ± 2.52	7.2 ± 2.04	7.0 ± 2.90
Live Fetuses	7.6 ± 2.37	6.8 ± 3.01	6.2 ± 2.60	6.9 ± 2.70
Dead Fetuses	0	0	0	0
Resorptions (early)	0.4 ± 0.70	0.7 ± 1.28	0.8 ± 1.76	0.1 ± 0.25
Resorptions (late)	0.2 ± 0.39	0.1 ± 0.32	0.1 ± 0.32	0.1 ± 0.25
Weight of Fetuses (g)	44.4 ± 4.86	45.7 ± 6.44	47.0 ± 5.72	42.4 ± 5.77
Sex of Fetuses (% of live fetuses, m|f)	ND	ND	ND	ND
Litters with Any Fetal Alteration (%)	0	0	0	0
Fetuses Available for Morphological Evaluation (%)	129 (17)	123 (17)	112 (18)	110 (16)

ND = not determined; NA = not applicable: NOAEL = no-observed-adverse-effect level; GD = gestation day
a Additional evaluations: On gestation day 19, satellite groups of 5 rabbits per dose were used to provide plasma samples to evaluate drug absorption. Animals were bled pre-dose, 1, 3, 7, and 12 hours after the first dose (the second dose was withheld until after plasma collection).

13.5. TAF: Developmental Toxicity in Rat

Test Article: TAF

Report Title: An Embryo-fetal Development Study of GS-7340-02 by Oral Gavage in Rats

Gilead Study No. TX-120-2002
[redacted] Study No. 902245

Species / Strain: rats / Sprague-Dawley Crl:CD(SD)

Duration of Dosing: 12 days (GD 6 to 17)

Location in CTD: 4.2.3.5.2

Initial Age: 11 to 12 weeks at treatment start

Day of Mating: Time-mated

GLP Compliance: yes

Date of First Dose: [redacted] 20[redacted] (GD 6)

Day of C-Section: GD 21

Special Features: Toxicokinetic evaluation

Method of Administration: Oral Gavage

Batch Number: [redacted]

No Observed Adverse Effect Level:
F_0 Females: 100 mg/kg/day (Targeted dose level)
F_1 Litters: 100 mg/kg/day (Targeted dose level)

Vehicle: 0.1% (v/v) Tween 20, 0.1% (w/v) hydroxypropylmethylcellulose (HPMC) K100LV prepared in ultra pure water

Daily Dose (mg/kg/day)		0 (Control)	25[b]	100[b]	250
Dams	**Number of Animals (toxicokinetic)**	**3**	**9**	**9**	**9**
	Toxicokinetics (GS-7340): AUC_{0-t} (µg*hr/mL)				
	Gestation Day 6	-	NC	NC	1.376
	Gestation Day 17	-	NC	0.242	1.382
	Toxicokinetics (GS-7340): C_{max} (µg/mL)				
	Gestation Day 6	-	NC	0.038	0.756
	Gestation Day 17	-	NC	0.149	0.597
	Toxicokinetics (R-PMPA): AUC_{0-t} (µg*hr/mL)				
	Gestation Day 6	-	2.715	9.453	54.2
	Gestation Day 17	-	2.803	17.392	55.728

Daily Dose (mg/kg/day)		0 (Control)	25[b]	100[b]	250
Dams	**Number of Animals (toxicokinetic)**	**3**	**9**	**9**	**9**
	Toxicokinetics (R-PMPA): C_{max} (µg/mL)				
	Gestation Day 6	-	1.110	2.767	9.860
	Gestation Day 17	-	0.870	4.130	7.350
	Number of Animals (main study)	**25**	**25**	**25**	**25**
	Noteworthy Findings				
	No. Pregnant (excluding animal with ammonium sulfide)	25	22	24	24 (23)
	No. Died or Sacrificed Moribund	0	0	0	0
	No. Aborted or with Total Resorption of Litter	0	0	0	1
	Clinical Observations	-	-	-	Transient salivation (7/25)
	Body Weight Change GD 6 to 18 (%)[a]	103.4	0	-2	-41***
	Corrected Body Weight, GD 21 (%)[a]	339.4	-3	-1	-10***
	Corrected Body Weight Gain, GD 6 to 21 (%)[a]	51.8	-11	-8	-56***
	Overall Food Consumption GD 6 to 18 (%)[a]	371.4	-2	-1	-17***
	Necropsy Observations	-	-	-	-
	Mean No. Corpora Lutea	17.4	17.9	18.6	17.3
	Mean No. Implantations	15.0	16.2	16.0	14.5
	Mean % Preimplantation Loss	13.2	9.8	13.4	15.3

Daily Dose (mg/kg/day)		0 (Control)	25[b]	100[b]	250
Litters	**Number of Animals (main study)**	**25**	**25**	**25**	**25**
	Noteworthy Findings				
	Litters Evaluated	25	22	24	23
	Live Fetuses	14.3	15.7	15.6	14.0
	Dead Fetuses	0	0	0	0
	Mean No. Resorptions	0.7	0.5	0.3	0.5
	Mean % Postimplantation Loss	4.70	2.99	2.01	7.12
	Mean Fetal Body Weight (total) (g)	5.83	5.62	5.53**	4.94***
	Fetal Sex Ratios (% Males)	49.9	49.3	48.8	44.0
	Overall Major Malformations	No treatment related findings.			
	Overall Minor External and Visceral Anomalies	No treatment related findings.			
	Overall Minor Skeletal Anomalies: litters (fetuses)	20 (48)	14 (35)	20 (49)	13 (19)***
	Interparietal Bone Incomplete Ossification: litters (fetuses)	11 (20)	7 (16)	9 (19)	2** (2)***
	Hyoid Bone Incomplete Ossification: litters (fetuses)	9 (16)	7 (15)	7 (20)	0** (0)***
	Common Skeletal Variants Sternebra 5 and Xiphisternum (unossified/incomplete/irregular/semi-bitartite/bi-partite)	14.74	17.33	18.90	31.43 **

GD = gestation day; - = no noteworthy findings; NC = not calculated
a 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.
b From GD 6 to 9, all Group 3 animals (100 mg/kg/day) received 65% of the target dose (65 mg/kg/day), and from GD 13 to 17, all Group 2 animals (25 mg/kg/day) received 77% of the target dose (19.3 mg/kg/day).
** = $p \leq 0.01$; *** = $p \leq 0.001$

13.6. TAF: Developmental Toxicity in Rabbits

Test Article: GS-7340-02

Report Title: An Embryo-fetal Development Study of GS-7340-02 by Oral Gavage in Rabbits

Gilead Study No. TX-120-2005
[redacted] Study No. 902248

Species / Strain: New Zealand White Rabbits (Oryctolagus cuniculus)

Duration of Dosing: Days 7 to 20 postcoitum (i.e., gestation)

Location in CTD: 4.2.3.5.2

Initial Age: 5 months

Day of Mating: Time-mated

GLP Compliance: Yes

Date of First Dose: [redacted] 20[redacted]

Day of C-Section: Day 29 postcoitum (i.e., gestation)

Batch Number: [redacted]

Special Features: Toxicokinetics

Method of Administration: Oral Gavage

Vehicle: 0.1% (v/v) Tween 20, 0.1% (w/v) hydroxypropylmethylcellulose (HPMC) K100LV prepared in ultra pure water

Daily Dose (mg/kg/day)		**0 (Control)**	**10**	**30**	**100**
Number of Animals		**3**	**3**	**3**	**3**
Does:	Toxicokinetics:				
	GS-7430 - C_{max} (µg/mL) – GD 7	BLLQ	0.008	0.077	0.846
	GS-7430 - C_{max} (µg/mL) – GD 20	BLLQ	0.155	0.937	9.190
	R-PMPA - C_{max} (µg/mL) – GD 7	BLLQ	0.261	0.398	2.193
	R-PMPA - C_{max} (µg/mL) – GD 20	BLLQ	0.260	0.676	2.970
	GS-7430 - AUC_{0-t} (µg•h/mL) – GD 7	BLLQ	NC	NC	0.918
	GS-7430 - AUC_{0-t} (µg•h/mL) – GD 20	BLLQ	NC	1.135	11.04
	R-PMPA - AUC_{0-t} (µg•h/mL) – GD 7	BLLQ	1941	3.013	13.44
	R-PMPA - AUC_{0-t} (µg•h/mL) – GD 20	BLLQ	2018	5.005	27.25

Daily Dose (mg/kg/day)		0 (Control)	10	30	100
Number of Animals		**3**	**3**	**3**	**3**
Does:	No. Pregnant	18	17	19	20
	No. Died or Sacrificed Moribund	0	0	0	0
	No. with Total Resorption of Litter	0	1	0	0
	Clinical Observations				
	Feces Output Decreased	2	3	2	8
	Fur staining yellow	1	2	6	7
	Body Weight Gain (total per interval) (%)[a]				
	GD 7 to 21	0.28 g	-17.9	-14.3	-60.7***
	GD 7 to 29	0.36 g	-8.3	13.9	-16.7
	GD 21 to 29	0.08 g	25.0	112.5*	137.5**
	Food Consumption (total per interval) (%)[a]				
	GD 7 to 21	2341 g	-4.1	-5.5	-17.6***
	GD 7 to 29	3332 g	-3.1	-1.2	-11.6
	GD 21 to 29	991 g	-0.6	7.8	1.9
	Necropsy Observations	No treatment-related findings			
	Mean No. Corpora Lutea[b]	9.3	8.6	9.0	9.4
	Mean No. Implantations[b]	8.8	8.2	8.4	8.9
	Mean % Pre-implantation Loss[b]	5.6	6.9	6.5	5.2
Litters:	Litters Evaluated[b]	18	17	19	20
	Live Fetuses[b]	8.6	8.1	8.0	8.5
	Dead Fetuses[b]	0.0	0.0	0.0	0.0
	Mean No. Resorptions[b]	0.2	0.2	0.4	0.4
	Mean % Postimplantation Loss[b]	2.6	7.2	4.5	3.8
	Mean Fetal Body Weight (total) (g)	41.2	42.7	43.6	40.5
	Fetal Sex Ratios (% Males)	52.0	49.9	56.4	47.5
	Fetal Abnormalities (external, visceral, skeletal)	No treatment-related findings			

- No noteworthy findings; * = p ≤ 0.05 (Dunnett) & = p ≤ 0.05 (Wilcoxon); ** = p ≤0.01 (Dunnett), *** = p ≤0.001 (Dunnett), GD = gestation day; NC = Not calculable;
BLLQ = Below lower limit of quantification;
R-PMPA = Tenofovir

a 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.

b Including animal with total resorption

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

14.1. BIC: Pre- and Post-natal Development in Rats

Test Article: BIC

Report Title: An Oral (Gavage) Study of the Effects of GS-9883-01 on Pre- and Postnatal Development, Including Maternal Function in Sprague Dawley Rats

Gilead Study No. TX-141-2045
[redacted] Study No. 604145

Species / Strain: Rat / Crl:CD(SD)
Initial Age: 14 Weeks
Date of First Dose: [redacted] 20[redacted]
No-Observed-Effect Level
F_0 Females: 300 mg/kg/day
F_1 developmental: 300 mg/kg/day
F_1 systemic/reproductive: 300 mg/kg/day
F_2 developmental: 300 mg/kg/day

Duration of Dosing: GD 6 through LD 20
Day of Mating: GD 0
Method of Administration: Oral (gavage)
Vehicle: 0.5% (w/w) hydroxypropyl methylcellulose and 0.1% (w/w) Tween® 20 in deionized water, pH 7.5-8.5
Litters Culled / Not Culled: Culled
Special Features: Toxicokinetics

Location in CTD:
GLP Compliance: Yes
Lot Number: [redacted]
Design Similar to ICH 4.1.3? Yes

Daily Dose (mg/kg/day)		**0 (Control)**	**2**	**10**	**300**
Number of Animals		**3**	**9**	**9**	**9**
F_0 Females	Toxicokinetics				
	GD 6				
	C_{max} (ng/mL)	NA[a]	12,700	52,700	179,000
	T_{max} (hr)	NA[a]	4.00	2.00	2.00
	AUC_{0-24} (ng·h/mL)	NA[a]	217,000	840,000	3,350,000
	LD 10				
	C_{max} (ng/mL)	NA[a]	20,000	74,700	156,000
	T_{max} (hr)	NA[a]	1.00	4.00	4.00
	AUC_{0-24} (ng·h/mL)	NA[a]	335,000	1,170,000	3,100,000

Daily Dose (mg/kg/day)		**0 (Control)**	**2**	**10**	**300**
Number of Animals		**3**	**9**	**9**	**9**
F_1 (combined)	Toxicokinetics				
	PND 10				
	C_{max} (ng/mL)	NA[a]	16,600	33,500	48,000
	T_{max} (hr)	NA[a]	12.0	4.00	8.00
	AUC_{0-24} (ng·h/mL)	NA[a]	334,000	762,000	1,120,000
F_0 Females	No. of Animals	25	25	25	25
	No. Pregnant	25	25	25	25
	No. Died or Euthanized *in Extremis*	0	0	0	0
	No. Aborted or with Total Resorption of Litter	0	0	0	0
	Clinical Observations	No Test Article-related findings.			
	Gestation Body Weight	No Test Article-related findings.			
	Lactation Body Weight	No Test Article-related findings.			
	Gestation Food Consumption	No Test Article-related findings.			
	Lactation Food Consumption	No Test Article-related findings.			
	Mean Duration of Gestation (days)	21.9	21.8	21.8	22.0
	Abnormal Parturition	No Test Article-related findings.			
	Necropsy Observations	No Test Article-related findings.			

Daily Dose (mg/kg/day)		**0 (Control)**	**2**	**10**	**300**
Number of Animals		**3**	**9**	**9**	**9**
F_1 Litters (Pre-weaning)	No. Litters Evaluated	25	25	25	25
	Mean No. of Implantations	14.8	15.4	15.2	14.8
	Mean No. of Pups Born/Litter	13.8	14.5	14.4	14.0
	Mean No. Liveborn Pups/Litter	13.7	14.5	14.2	13.9
	Postnatal Survival to PND 4 (% per Litter)	97.1	99.0	98.0	91.2
	Postnatal Survival to Weaning (PND 4 to 21; % per Litter)	100.0	99.0	100.0	98.4
	Pup Body Weight Change (g)	No Test Article-related findings.			
	Pup Sex Ratio (% Males per Litter)	50.1	53.0	50.1	45.1
	Pup Clinical Observations	No Test Article-related findings.			
	Pup Necropsy Observations	No Test Article-related findings.			
F_1 Males (Post-weaning)	No. Evaluated Postweaning	50	50	49	47
	No. Died or Euthanized *in Extremis*	0	0	0	1
	Clinical Observations	No Test Article-related findings.			
	Body Weight Change	No Test Article-related findings.			
	Mean Age at Balanopreputial Separation (days)	43.1	43.0	43.3	43.1
	Startle Response	No Test Article-related findings.			
	Motor Activity	No Test Article-related findings.			
	Learning and Memory	No Test Article-related findings.			
	Mean No. of Days Prior to Mating	2.4	3.2	2.7	2.5
	Mating Index (%)	100.0	100.0	100.0	95.7
	Fertility Index (%)	100.0	100.0	100.0	82.6[b]
	Copulation Index (%)	100.0	100.0	100.0	86.4[b]
	Necropsy Observations	No Test Article-related findings.			

Daily Dose (mg/kg/day)		**0 (Control)**	**2**	**10**	**300**
Number of Animals		**3**	**9**	**9**	**9**
F_1 Females (Post-weaning)	No. Evaluated Postweaning	50	50	50	48
	No. Died or Euthanized *in Extremis*	1	0	0	0
	Clinical Observations	No Test Article-related findings.			
	Premating Body Weight Change	No Test Article-related findings.			
	Gestation Body Weight Change	No Test Article-related findings.			
	Lactation Body Weight Change	No Test Article-related findings.			
F_1 Females (Post-weaning)	Mean Age at Vaginal Patency (days)	34.1	34.0	33.8	34.3
	Startle Response	No Test Article-related findings.			
	Motor Activity	No Test Article-related findings.			
	Learning and Memory	No Test Article-related findings.			
	Mean No. of Days Prior to Mating	2.4	3.2	2.7	2.5
	Mating Index (%)	100.0	100.0	100.0	95.8
	Fertility Index (%)	100.0	100.0	100.0	83.3[b]
	Conception Index (%)	100.0	100.0	100.0	87.0[b]
	No. Pregnant	24	25	25	20
	Mean Duration of Gestation (days)	21.9	21.8	21.8	21.4**
	Abnormal Parturition	No Test Article-related findings.			
	Necropsy Observations	No Test Article-related findings.			

Daily Dose (mg/kg/day)		**0 (Control)**	**2**	**10**	**300**
Number of Animals		**3**	**9**	**9**	**9**
F_2 Litters	No. of Litters Evaluated	23	25	25	20
	Mean No. of Implantations	16.0	16.1	15.5	16.0
	Mean No. of Pups Born/Litter	15.1	15.0	14.0	14.6
	Mean No. Liveborn Pups/Litter	15.1	14.7	13.8	14.6
	Postnatal Survival to Day 4 (% per Litter)	96.6	97.1	95.6	97.1
	Pup Body Weight Change	No Test Article-related findings.			
	Pup Sex Ratio (% Males per Litter)	52.9	52.2	50.1	48.0
	Pup Clinical Observations[a]	No Test Article-related findings.			
	Pup Necropsy Observations	No Test Article-related findings.			

GD = gestation day; LD = lactation day; PND = postnatal day
** = p ≤0.01 using Dunnett's test
a Values below the lower limit of quantitation (<1000 ng/mL)
b Values within historical control value database at Test Facility

14.2. FTC: Pre- and Post-natal Development in Mice

Test Article: FTC

Report Title: Pre-and Postnatal Development Study in CD-1 Mice Given TP-0006 by Gavage **Gilead Study No. TOX039**
Species/Strain: Mouse/CD-1 **Duration of Dosing: F:** GD 6 to PND 20 **Location in CTD:** 4.2.3.5.3
Initial Age: 58 days old upon arrival **Day of Mating:** F_0 GD 0; F_1 GD 0 **GLP Compliance:** Yes
Date of First Dose: [redacted], 19[redacted] **Vehicle/Formulation:** 0.5% methylcellulose **Lot No.:** [redacted]; [redacted]
Method of Administration: Oral, divided doses spaced by approximately six hours **Design similar to ICH 4.1.2:** Yes
Special Features: none
No Observed Adverse-Effect Level: F_0 Females = 1000 mg/kg/day for maternal and reproductive toxicity; F_1 M/F = 1000 mg/kg/day

Dose (mg/kg/day)	0 (Vehicle)	250	500	1000
F_0 Toxicokinetics: AUC	ND	ND	ND	ND
Females				
Females with Sperm	25/25	25/25	24/25[a]	25/25
Pregnant Females	24	24	24	25
Females with Delivery	24	24	22[b]	25
F_0 Maternal Toxicity				
Mortality (number): Gestation	0	0	0	0
Lactation[c]	0	2	0	2
Gestational Body Weights	No treatment-related findings.			
Gestational Weights Changes	No treatment-related findings.			
Gestational Feed Consumption	No treatment-related findings.			
Gestational Clinical Observations	No treatment-related findings.			
Lactational Body Weights	No treatment-related findings.			
Lactation Weight Changes	No treatment-related findings.			
Lactational Feed Consumption	No treatment-related findings.			
Lactational Clinical Observations	No treatment-related findings.			
F_0 Reproductive Indices	No treatment-related findings.			
Parental Gross Necropsy				
F_0 Female Terminal Body Weights and Findings	No treatment-related findings.			

Dose (mg/kg/day)	**0 (Vehicle)**		**250**		**500**		**1000**	
Litters								
Implantation Sites per Litter	12.54 ± 0.41		12.83 ± 0.52		12.25 ± 0.37		12.12 ± 0.54	
Live Births	12.54 ± 0.48		12.63 ± 0.47		12.91± 0.41		12.76 ± 0.29	
Stillbirths	0.04 ± 0.04		0		0.05 ± 0.05		0	
Survivors Index pnd 4[d]	94.93 ± 4.16		94.66 ± 4.15		98.67 ± 0.62		97.92 ± 0.79	
Lactation Index pnd 21[e]	97.75 ± 1.05		98.21 ± 1.30		99.43 ± 0.57		98.91 ± 0.75	
Weight at Birth (g) – Male	1.60 ± 0.03		1.62 ± 0.03		1.56 ± 0.02		1.63 ± 0.02	
Weight at Birth (g) – Female	1.56 ± 0.03		1.55 ± 0.03		1.52 ± 0.02		1.55 ± 0.02	
Weight at Weaning (g) – Male	13.97 ± 0.32		14.21 ± 0.29		13.22 ± 0.21		14.01 ± 0.29	
Weight at Weaning (g) – Female	13.51 ± 0.29		13.50 ± 0.27		12.70 ± 0.22		13.33 ± 0.28	
Sex ratio of Live Newborns (% of live newborns m|f)	47.0	53.0	48.1	51.9	49.6	50.4	49.2	50.8
F_1 Offspring Toxicity								
Lactational Survival (number of litters died)	1		1		0		0	
Postwean Developmental Landmarks:								
Estrous Cyclicity	N		N		N		↑**	
Postwean Body Weights	N		N		↓**[f]		N	
Postwean Body Weight Changes	N		N		↓**[g]		↓*[h]	
Lactational Body Weights/Litter	No treatment-related findings.							
Lactational Sex Ratio/Litter	No treatment-related findings.							
Lactational Clinical Observations	No treatment-related findings.							
Postwean Developmental Landmarks (vaginal patency, pupillary reflex, motor activity, learning and memory, postwean clinical observations)	No treatment-related findings.							

Dose (mg/kg/day)	0 (Vehicle)	250	500	1000
F_1 Reproductive Toxicity				
Reproductive Indices	No treatment-related findings.			
Gestational Body Weights	No treatment-related findings.			
Gestational Body Weight Changes	No treatment-related findings.			
Gestational clinical observations	No treatment-related findings.			
Lactational body weights (PND 0 and 1)	No treatment-related findings.			
Lactation body weight changes	No treatment-related findings.			
Lactational clinical observations	No treatment-related findings.			
Parental Gross Necropsy				
F_0 male terminal body weights	N	N	↓*	N
F_0 female terminal body weights and findings	No treatment-related findings.			
F_0 male findings	No treatment-related findings.			
F_2 Offspring Toxicity				
Lactational survival (PND 0–1)	No treatment-related findings.			
Lactational body weight/litter (PND 0)	No treatment-related findings.			
Lactational sex ratio/litter	No treatment-related findings.			

GD = gestation day; PND = postnatal day; ND = not determined; N = normal; no statistically significant or biologically relevant change; ↓ or ↑ = increase or decrease; * = $p < 0.05$ versus control; ** = $p < 0.01$ versus control

a Female 3 was removed due to a dosing error (misdirected dose) on gestational day 12.
b Female 6 had implant sites only and was included as a pregnant female that did not have a live litter. Female 14 had started to deliver but had no pups the next morning. She was noted as a pregnant female but was not included in the live litter since it was not observed if live pups were present when she was in the process of delivering.
c The causes of 4 deaths were not determined; the lack of a dose-response relationship indicates that they were not treatment-related.
d Number surviving 4 days/number live on PND 0.
e Number surviving 21 days/number live on pnd 4.
f On study days 63 and 70 and at sacrifice in males, but not at 1000 mg/kg/day.
g For study days 56–63 only in males, but not at 1000 mg/kg/day.
h For study days 14–21 only in males.

14.3. TDF: Developmental and Perinatal/Postnatal Reproduction in Rats

Test Article: TDF

Report Title: Oral (Gavage) Developmental and Perinatal/Postnatal Reproduction Toxicity Study of GS-4331-05 (bis-POC PMPA) in Rats Including a Postnatal Behavioral/Functional Evaluation

Gilead Study No. R990202

Species/Strain: Rat/Crl:CD(SD)IGS BR VAF/Plus

Duration of Dosing: F: From day 7 of presumed gestation to LD 20 or day 24 of presumed gestation (in rats that did not deliver a litter)

Location in CTD: 4.2.3.5.3

Initial age: 59 Days

Day of mating: Day 0

GLP Compliance: Yes

Date of First Dose: [redacted] 20[redacted]

Method of Administration: Oral gavage

Lot No.: [redacted]

Litters Culled/Not Culled: At weaning (day 21 lactation) 25 pups per sex, per group, where possible, were randomly chosen for continued evaluation. All other pups were sacrificed.

Design similar to ICH 4.1.2: Yes

NOEL: F_o Females = 50mg/kg/day; F_1 General Toxicity = 50 mg/kg/day; F_1 Behavioral, reproductive & developmental = 150 mg/kg/day

Special Features: None

Dose (mg/kg/day)		0 (Vehicle)	50	150	450	600
F_0 Females	Toxicokinetics (From study R990202-PK)					
	C_{max} (µg/mL) Day 20 post partum	-	1.46	2.26	4.93	2.89
	$AUC_{0-\tau}$ (µg.h/mL) Day 20 post partum	-	5.88	11.7	26.8	27.3
	No. Evaluated	25	25	25	25	25
	Mortality[a]					
	Number died	0	0	1	0	0
	Moribund sacrificed	1	0	0	1	1
	Presumed Gestation					
	Maximal Possible Incidence (days x rats)/No. of Rats/Group	561/25	556/25	559/25	562/25	569/25
	Red Perioral Substance	0/0	1/1	37/14*	123/24**	124/25**
	Excess Salivation	0/0	0/0	7/5	12/10**	28/13**
	Urine Stained Abdominal Fur	0/0	0/0	0/0	9/4*	13/8**
	Ungroomed Coat	0/0	0/0	0/0	6/1	17/5**

Dose (mg/kg/day)		0 (Vehicle)	50	150	450	600
F_0 Females, cont.	Lactation					
	Maximal Possible Incidence (days x rats)/No. of Rats/Group	504/24	525/25	494/24	382/24	260/22
	Dehydration	4/4	1/1	2/2	10/6	16/8**
	Emaciation	0/0	6/1	0/0	5/4*	12/7**
	Urine Stained Abdominal Fur	4/2	6/3	8/3	45/9**	28/11**
	Red of Dried Perioral Substance	0/0	0/0	3/3	9/8**	12/7**
	Ungroomed Coat	0/0	1/1	0/0	6/4*	11/7**
	Cold to Touch	0/0	0/0	0/0	0/0	9/4**
	Necropsy Observations					
	Gestation/Lactation Body Weight (%)[b]	327.8g	0	0	0	0
	Gestation/Food Consumption (%)[c]	23.8g/day	0	0	-12**	-18**
	Lactation/Food Consumption (%)[c]	54.2g/day	0	0	-7%*	-15**
	Delivery Observations					
	Females with Sperm	25	25	25	25	25
	Pregnant Females (%)	25 (100)	25 (100)	24 (96)	24 (96)	23 (92)
	Females with Delivery (%)	24 (100)[d]	25 (100)	24 (100)	24 (100)	22 (100)[e]
	Mean Duration of Gestation Days (SD)	23.0 (0.2)	23.0 (0.2)	23.2 (0.4)	23.3 (0.5)*[f]	23.3 (0.5)
F_1 Litters	Implantations per Delivered Litter (SD)	16.3 (2.1)	15.5 (2.6)	15.2 (1.6)	15.4 (1.7)	15.5 (1.6)
	Live Births per Litter (SD)	14.7(2.2)	14.4 (2.9)	14.2 (1.7)	13.8 (3.0)	12.8 (2.9)
	Stillbirths (SD)	0.2 (0.6)	0.2(0.4)	0 (0.2)	0.7 (2.3)	2.0 (2.8)*
	Survivors Day 4 pp (%) Viability Index	99.4 351/353	99.2 357/360	99.1 338/341	80.8 256/317	53.9 152/282**

Dose (mg/kg/day)		0 (Vehicle)		50		150		450		600	
F_1 Litters Means											
F_1 Litters	Survivors at Weaning (%) Lactation Index	98.6 346/351		98.0 350/357		98.1 319/325		95.0 230/242		97.4 148/152	
	Weight at Birth (g)	6.7 (0.5)		6.5 (0.5)		6.4 (0.4)		5.5 (0.7)**		5.1 (0.6)**	
	Weight at Weaning g (SD)	37.4 (5.4)		35.9 (7.1)		35.4 (5.8)		27.1(3.6) **		25.7 (2.5) **	
	Sex Ratio of Fetuses	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
		49.9	50.1	46.5	53.5	52.4	47.6	48.0	52.0	47.0	53.0
	Clinical Observations (Total Frequency: Days x Pups)										
	Cold to Touch	3/1		1/1		0/0		64/6		80/11**	
	Pale	3/1		0/0		0/0		32/3		80/11**	
	Not Nesting	0/0		0/0		0/0		0/0		31/4**	
	Umbilical Hernia	0/0		0/0		0/0		28/4**		3/1	
	Necropsy Observations										
	Incidence Pup Appearance Normal (%)	295 (99.7)		298 (99.3)		269 (100)		169 (94.4) **		97 (99)	
	Incidence Pup Liver Appearance Pale (%)	0 (0)		0 (0)		0 (0)		10 (5.6)*		0 (0)	
	Incidence Pup Testes Small & Flaccid (%)	0 (0)		0 (0)		0 (0)		0 (0)		1 (1.0)**	
	Kidney Weights g (mean (SD)	0.54 (0.08)		0.52 (0.09)		0.51(0.09)		0.40 (0.04) **		0.36 (0.05)**	

a No deaths were related to test article. All deaths were related to intubation errors with the exception of the rat in control group, which had dystocia.
b Terminal weights for control groups (means, at day 21, postpartum) are shown. For treatment groups, % differences from controls are shown.
c Maternal absolute food consumptions for control groups (during gestation & lactation) are shown. For treatment groups, % differences from controls are shown.
d Excludes 1 rat which was scarified moribund day 22 gestation.
e Excludes 1 rat which was scarified moribund day 19 gestation.
f Not considered test article related.
* = $p \leq 0.05$; ** = $p \leq 0.01$

14.4. TAF

No pre- and post-natal development toxicity studies have been conducted with TAF, but a pre- and post-natal development toxicity study with TDF is included in Section 14.2.

15. STUDIES ON JUVENILE ANIMALS

No juvenile toxicity studies have been conducted with BIC, FTC or TAF.

16. LOCAL TOLERANCE

16.1. BIC

Test Article: BIC

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.)
Skin Corrosion Assay	EpiDerm™ inserts	In vitro (moistened with 25 µL water)	3 or 60 minute exposure	25 mg	2 tissues per test article per exposure duration	Mean skin viability after a 3 minute or 60 minutes exposure to GS-9883-01 was 76% and 94%, respectively. GS-9883-01, was considered to be non-corrosive according to the UN GHS classification system.	TX-141-2058[a] ([redacted] 8338975)
Skin Irritation Assay	EpiDerm™ SIT (EPI-200) inserts	In vitro	60 minute treatment	25 mg	3 tissues per test article	Mean viability for GS-9883-01 was 91.6%. GS-9883-01 was considered not to be an irritant in the *in vitro* skin model EpiDerm™ SIT (EPI-200).	TX-141-2059[a] ([redacted] 8338976)
Bovine Corneal Opacity and Permeability (BCOP) Assay	Bovine Cornea	In vitro (0.9% saline)	Opacity assessment (4 hours) followed by permeability assessment (90 minutes)	750 µL of 20% w/v suspension	3 Corneas	In Vitro Irritancy Score was 27.58; classified as moderately irritating (UN GHS No Prediction)	TX-141-2060[a] ([redacted] 8338977)

Test Article: BIC

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.)
Phototoxicity (in vitro)	Fibroblasts/ Mouse/ BALB/c 3T3 cells (clone A31)	In Vitro (Vehicle: 1% DMSO/DPBS, pH of 7.0-7.3)	90 minutes	Assay #1 & 2: 0.178, 0.316, 0.562, 1.00, 1.78, 3.16, 5.62 and 10 µg/mL		A GS-9883 cytotoxicity IC_{50} was not achieved in the assay. The GS-9883 phototoxicity IC_{50} values were 5.407 µg/mL and 5.221 µg/mL in Definitive Assays 1 and 2, respectively. The Photoirritancy Factor[b] (PIF) values were >1.853 and >1.916 and the Mean Photo Effect[c] (MPE) values were 0.124 and 0.184 in Definitive Assays 1 and 2, respectively. With > PIF values >1, GS-9883 is predicted to have phototoxic potential. However, MPE values less than 0.15 are of questionable toxicological relevance for systemic drugs (Note 4 of the ICH S10 guidance), as seen in definitive assay 1. While the MPE value for GS-9883 was below 0.15 in definitive assay 1, it was above 0.15 in definitive assay 2 and the PIF values did achieve the phototoxicity threshold in both assays. Therefore, it was concluded that GS-9883 demonstrated phototoxic potential in this assay.	TX-141-2044[a] ([redacted] 20077249)
Phototoxicity (in vivo)	Pigmented rat/ Crl:LE (Long-Evans)	Oral Gavage (0.5% [w/w] hydroxypropyl methylcellulose and 0.1% [w/w] Tween® 20 in reverse osmosis water pH adjusted to 7.5-8.5)	3 Days	0, 2, 10, 300	Females 5/group in vehicle control and test article groups and 3 in the positive control (8-MOP) group	No skin reactions or ocular reactions indicative of phototoxicity occurred at doses up to 300 mg/kg GS-9883 followed by UVR exposure approximately 4 hours after dosing. The rats administered the positive control article (8-MOP) had skin and ocular findings consistent with phototoxicity, validating the assay. Mean GS-9883 concentration at 4 hours post dose on Day 3= 204333 ng/mL (300 mg/kg)	TX-141-2046[a] ([redacted] 20083296)

DMSO/DPBS = Dimethylsulfoxide/Dulbecco's phosphate buffered saline supplemented with Ca^{2+} and Mg^{2+} ions
a Indicates GLP compliant; UN GHS = United Nations Globally Harmonized System of Classification and Labelling of Chemicals
b Photoirritancy Factor: the criterion for "phototoxic" is PIF >5.
c Mean Photo Effect: the criterion for "phototoxic" is MPE >0.15.

16.2. TAF

Test Article: TAF

Type of Study	Species / Strain	Method of Administration (Vehicle/Formulation)	Duration of Dosing	Dose (mg/kg)	Gender and No. per Group	Noteworthy Findings	Gilead Study No. (CRO Name & Study No.)
BCOP	Bovine Cornea	In Vitro(prepared in 0.9% saline)	Opacity = 4 ± 5 hr.; Permeability = 90 ± 5 min.	20% (w/w) in 0.9% sodium chloride solution	3 Corneas	GS-7340-03 elicited an in vitro irritancy score of 21.0 ± 8.7 and was predicted to be a non-corrosive/non-severe eye irritant.	TX-120-2013[a] ([redacted] EUN0017)
Dermal Irritation	Rabbit/NZW	Dermal(moistened in 0.5 – 0.6 mL distilled water)	4 hours	0.5 g/rabbit	3M	No signs of erythema or edema were noted. Test article was considered nonirritating/ noncorrosive.	TX-120-2011[a] ([redacted] 1000078)

BCOP = bovine corneal opacity and permeability; NA = not applicable; RO = reverse osmosis; M = male; F = female; w/w = weight/weight
a Indicates GLP compliant

17. OTHER TOXICITY STUDIES

17.1. BIC

17.1.1. In Vitro

Type of Study	Species / Strain	Method of Administration	Duration of Dosing	Dose (mg/kg)	Noteworthy Findings	Gilead Study No. (CRO Name and Study No.)
Impurities/ Bacterial Mutation Study	Salmonella typhimurium and Escherichia coli	In Vitro (plate incorporation method)	52 ± 4 hours	5-5000 µg/plate	GS-001* and GS-002* are negative in all strains up to 5000 µg/plate in the absence and presence of S9.	TX-141-2048[a] ([redacted] 8335231)
Impurities/ Bacterial Mutation Study	Salmonella typhimurium and Escherichia coli	In Vitro (plate incorporation method)	52 ± 4 hours	5-5000 µg/plate	GS-003* is negative in all strains up to 5000 µg/plate in the absence and presence of S9.	TX-141-2041[a] ([redacted] 8320816)

a Indicates GLP compliant.

* 新薬承認情報提供時に置き換え

17.1.2. In Vivo

Test Article:

Type of Study	Species / Strain	Method of Administration	Duration of Dosing	Dose (mg/kg)	Gender and No. per Group	Noteworthy Findings	Gilead Study No. (CRO Name and Study No.)
Other/ Antigenicity (LLNA)	Mouse/ CBA/CaCrl	Topical (Propylene Glycol)	3 days	10, 25, 50% w/v (0.025 mL/pinna)	5 F	GS-9883-01 is not regarded as a potential skin sensitizer (UN GHS No Category).	TX-141-2061[a] ([redacted] 8338978)
Other/ Investigative Repeat Dose	Rat/ Crl:WI(Han)	Oral Gavage	2Weeks	0, 300	13M/Control 15M/Treated	Histopathological evaluation limited to the brain and spinal cord (cervical, thoracic, and lumbar). No toxicity observed at 300 mg/kg/day. Mean Day 14 BIC concentration 4 hours post dose at 300 mg/kg/day was 119 µg/mL	TX-141-2033[a] ([redacted] 8298544)
Impurities/ Repeat Dose	Rat/ Crl:WI(Han)	Oral Gavage (0.5% [w/w] hydroxypropyl methylcellulose [Methocel™ K100 LV] and 0.1% [w/w] Tween® 20 in reverse osmosis water pH adjusted to 8.0 ± 0.5)	4 weeks	0 (vehicle), 300 (Lot No. [redacted]; 97.1% pure), 100 or 300 (Lot No. [redacted]; 95.4% pure)	10F/group toxicology 9F/group (3F controls) TK	No adverse effects for either lot were noted. No discernible differences for mortality, clinical observations, ophthalmic examinations, and clinical and anatomic pathology were apparent between lots administered at 300 mg/kg/day. NOAEL is 300 mg/kg/day (both lots) Week 4 AUC_{0-24}: Lot No. [redacted] = 4440 µg•hr/mL. Lot No. [redacted] = 4190 µg•hr/mL	TX-141-2043[a] ([redacted] 8329288)

TK = toxicokinetics; F = female; M = male; UN GHS = United Nations Globally Harmonized System of Classification and Labelling of Chemicals;
LLNA = Local Lymph Node Assay; NOAEL = No-Observed-Adverse-Effect-Level
a Indicates GLP compliant.

17.2. FTC

Test Article: FTC

Type of Study	Species/Strain	Method of Administration	Duration of Dosing	Dose (mg/kg/day)	Gender and No. per Group	Noteworthy Findings	Gilead Study Number
Immuno-toxicity	Rat/ Sprague-Dawley	Oral Gavage	28 Days	0, 60, 240, 1000	5 M, 5 F	The objective of the study was to assess the immunotoxicity of TP-0006 (FTC) in the rat following once daily oral gavage administration for 28 days. No adverse clinical signs were noted during the dosing period. Body weight was unaffected by treatment with TP-0006. There was no effect of FTC at dose levels up to 1000 mg/kg/day on the immune response to sheep red blood cells.	TOX146[a]
Impurities	Mouse/ CD-1 (ICR)	Oral Gavage	28 Days	133, 400, 1200	10 M, 10 F	The objective of the study was to assess the oral toxicity of FTC in CD-1 mice containing a degradation product and two potential process impruities. The only finidngs were increased kidney weight in males and increased hemoglobin, hematocrit, and MCV (mean corpuscular volume) in both sexes at 1200 mg/kg/day. There were no histopathologic findings associated with increased male kidney weights.	TOX118

Test Article: FTC

Type of Study	Species/Strain	Method of Administration	Duration of Dosing	Dose (mg/kg/day)	Gender and No. per Group	Noteworthy Findings	Gilead Study Number
Impurities	Mouse/ CD-1 (ICR)	Oral Gavage	28 Days (13 day post dose recovery)	FTC/FTU: 0, 50/1, 150/3, 450/9	20 (10M, 10F)	All mice survived to the scheduled necropsy. There were no observations related to treatment with the combination of TP-0006 (FTC) and TP-0296 (FTU) at any dose level. Body weight, food consumption, hematology, serum biochemistry values and organ weights were unaffected by administration of the test article combination at any dose level. There were no gross or microscopic findings attributed to the test article combination. The no-observed-effect level (NOEL) for oral administration of TP-0006 in combination with TP-0296 was 450/9 mg/kg/day TP-0006/TP-0296, the highest dose combination tested.	TOX153[a]
Other	Mouse/ CD-1 (ICR)	Oral Gavage	28 Days	0, 50, 150, 450	20 (10M, 10F)	The NOEL for oral gavage administration of TP-0006 test article (FTC produced by the menthol chemistry method of synthesis) to mice for 28 consecutive days was 450 mg/kg/day, the highest dose tested.	TX-162-2001[a]
Other/ Reverse Mutation Assay	*S. typhimurium* & *E. Coli*	In Vitro	NA	For TP-0006: 1.5 to 5000 µg/plate. For TP-0296: 0.015 to 50 µg/plate. Mutagenicity assay: For TP-0006: 50 to 5000 µg/plate. For TP-0296: 0.50 to 50 µg/plate	NA	**Cytotoxic Effects:** no positive mutagenic responses were observed with any of the tester strains in either the presence or absence of S9 activation. The maximum dose of TP-0006 (FTC) and TP-0296 tested were 5000 and 50 µg per plate respectively. Neither precipitate nor appreciable toxicity was observed. **Genotoxic Effects:** No positive mutagenic responses were observed with any of the tester strains in either presence or absence of S9 activation. Mutagenic responses were observed with the positive control articles.	TOX151[a]

Test Article: FTC

Type of Study	Species/Strain	Method of Administration	Duration of Dosing	Dose (mg/kg/day)	Gender and No. per Group	Noteworthy Findings	Gilead Study Number
Other/ Chromosome Aberration	CHO-K_1 cells	In Vitro	NA	0.5 to 5000 µg/mL with 1% TP-0296	NA	**Cytotoxic Effects:** Substantial toxicity (i.e., at least 50% cell growth inhibition, relative to the solvent control) was not observed at any dose level in both the non-activated and S9 activated 4 hour exposure groups, or in the non-activate 20 hour continuous exposure group. Based upon the results of the toxicity study, the dose levels selected for testing in the chromosome aberration assay were ranging from 313 to 5000 µg/mL for TP-0006 (FTC) and from 3.13 to 50 µg/mL for TP-0296. **Genotoxic Effects:** Based on the result of this study, it was concluded that TP-0006 with 1% TP-0296 was negative for the induction of structural and numerical chromosome aberrations in CHO cells.	TOX152[a]

TP-0006 = FTC produced by the menthol chemistry method of synthesis; TP-0296 = FTU
a Indicates GLP Study.

17.3. TAF/TVF

Test Article: TAF

Type of Study	Species / Strain	Method of Administration (Vehicle / Formulation)	Duration of Dosing	Dose (mg/kg/day)	Gender and No. per Group	Noteworthy Findings	Gilead Study No. (CRO Name & Study No.)
Other/ Reverse Mutation Assay	*S. typhimurium* & *E. Coli*	In Vitro Tenofovir	NA	Up to 5000 µg/plate with and without S9 activation	NA	Increase in revertants in TA1535 only in presence of S9	95-TOX-1278-006 ([redacted] 17444-0-409)
Other/ Mouse lymphoma L5178Y	Mouse lymphoma L5178Y cells	In Vitro Tenofovir	NA	Up to 5000 µg/mL with and without S9 activation	NA	Negative with activation; equivocal with activation at 5000 µg/mL	95-TOX-1278-007 ([redacted] 17444-0-431)
LLNA	Mouse	Topical GS-7340-03 (acetone:olive oil, 4:1 v/v)	3 days	25 µL application of 10, 25, 50% (w/v)	5F	TAF not regarded as a potential skin sensitizer.	TX-120-2014[a] ([redacted] UK EUN0018)
Impurity Qualification	Rat/Crl:CD (SD)	Oral Gavage (0.1% (v/v) Tween 20 and 0.1% (w/v) HPMC in RO water	2 weeks	0, 5, 50 Lot-1: [redacted] (97.7% purity) Lot-2: [redacted] (83.1% purity)	10M	No GS-7340-02-related effects on clinical signs, body weights, food consumption, clinical or anatomic pathology for either lot. At 50 mg/kg/day the Day 14 tenofovir C_{max} was 0.2 µg/mL and the AUC_{0-t} was 2.0 µg·hr/mL for Lot No.1, and for Lot No. 2 the respective C_{max} was 0.4 µg/mL and the AUC_{0-t} was 2.5 µg·hr/mL.	TX-120-2008[a] ([redacted] 8246754)

Test Article: TAF

Type of Study	Species / Strain	Method of Administration (Vehicle / Formulation)	Duration of Dosing	Dose (mg/kg/day)	Gender and No. per Group	Noteworthy Findings	Gilead Study No. (CRO Name & Study No.)
Impurity Qualification	Rat/Crl:CD (SD)	Oral Gavage (1% (w/v) HPMC cellulose K100LV and 0.1% (v/v) Tween® 20 in 99.8% (v/v) reverse osmosis (RO) water)	4 weeks	0, 25, 50 (free base equivalents) Lot-1: [redacted] (99.3% purity) Lot-2: [redacted] (98.0% purity) Lot-3: [redacted] (97.8% purity)	10M; 10F	No GS-7340-03-related clinical observations, ophthalmic observations, or effects on clinical pathology parameters, bone biomarker parameters, or macroscopic and microscopic observations were noted. NOAEL = 50 mg/kg/day (free base equivalents) At 50 mg/kg/day the Day 28 R-PMPA combined male/female C_{max} was 1.79 µg/mL and the combined male/female AUC_{0-t} was 6.76 µg·hr/mL for Lot No.1, for Lot No. 2 the respective C_{max} was 1.38 µg/mL and the AUC_{0-t} was 6.03 µg·hr/mL, and for Lot 3 the respective C_{max} was 1.95 µg/mL and the AUC_{0-t} was 6.04 µg·hr/mL.	TX-120-2021[a] ([redacted] 8298810)
Bone Metabolism	Rat/Harlan:CD (SD)	Oral Gavage GS-7340-02 (50mM citrate buffer)	6 days	0, 400	4M	Serum 1,25-dihydroxycholcalciferol was decreased by 80% and urinary calcium/creatinine and phosphorus/ creatinine ratios were increased 444% and 202%, respectively, compared to controls.	R990177 (NA)
Bone Metabolism	Rat/Crl:CD (SD)	Oral Gavage GS-7340-02 (50 mM citric acid)	6 days	0, 100, 400	8M	Statistically significant decreases in serum 1,25-dihydroxyvitamin, calcium and phosphate were observed at 100 and 400 mg/kg/day. Urinary calcium:creatinine ratios were significantly decreased on Days 1 and 2, at 100 and on Day 1 at 400mg/kg/day, but were then significantly increased by Day 6, at 400 mg/kg/day.	R2000044 (NA)

Test Article: TAF

Type of Study	Species / Strain	Method of Administration (Vehicle / Formulation)	Duration of Dosing	Dose (mg/kg/day)	Gender and No. per Group	Noteworthy Findings	Gilead Study No. (CRO Name & Study No.)
Renal Effect	Rat/Crl:CD (SD)	Oral Gavage GS-7340-02 (50 mM citric acid)	1 day	0, 100, 1000[b]	10M	No adverse effect on renal function. Urinary output of calcium was increased at 1000 mg/kg. This correlated with an increase in serum calcium concentration and indicated that the kidneys were functioning in order to reduce the serum calcium load.	R990186[a] ([redacted] 56520)
Bone Marker Assessment	Dog, Beagle	Oral Gavage GS-7340-02 (50 mM citric acid)	5 days	0, 37.5, 75	3M	Emesis, excessive salivation and diarrhea at 37.5 and 75 mg/kg/day. Decreased body weight and food consumption at both dose levels. Marked decreases in neutrophils, lymphocytes, monocytes and eosinophils were observed at both levels. Increases were noted in total protein, globulin, triglyceride, cholesterol, ALP, AST and CK at both dose levels. Test-article related findings were noted in the GI, lymph nodes, thymus, tonsil and bone marrow at both dose levels	D990142[a] ([redacted] 9529.3)

M = male; F = female; NA = not applicable; w/w = weight/weight; w/v = weight/volume; v/v = volume/volume
ALP = alkaline phosphatase, AST = serum aspartate aminotransferase, CK = creatine kinase
a Indicates GLP compliant
b No effect level

17.4. FTC/TDF

Test Article: FTC/TDF

Type of Study	Species/Strain	Method of Administration	Duration of Dosing	Dose (mg/kg)	Gender and No. per Group	Noteworthy Findings	Gilead Study No. (CRO Name and Study No.)
Impurities	Rat/ Sprague-Dawley	Oral Gavage	14 Days	TDF/FTC: 30/20, 100/67, 300/200 (each dose level with non-degraded and degraded)	10 M	There were no toxicologically significant differences between groups treated with non-degraded and degraded TDF/FTC. A minor increase in adrenal weights at necropsy (all dose levels, except those receiving degraded TDF/FTC at the lowest dose) and a hyperplasia of the anterior duodenal mucosa overlying Brunner's glands was seen in animals treated at the high-dose level with non-degraded and degraded TDF/FTC. The NOAEL in this study was considered to be 100/67 mg/kg/day.	TX-164-2001[c] ([redacted] 800392)
Impurities	Rat/ Sprague-Dawley	Oral Gavage	14 Days	TDF/FTC: 0, 30/20[a], 100/67[a], 300/200[a], 30/20[b], 100/67[b], 300/200[b]	10 M	Minimal, non-adverse changes in red cell parameters noted in animals given non-degraded or degraded TDF/FTC at 300/200 mg/kg/day. Non-adverse, minimal, dose level-dependent increases in ALT activity were observed in rats given non-degraded TDF/FTC at ≥ 100/67 mg/kg/day and degraded TDF/FTC at 300/200 mg/kg/day. Minimal cryptal epithelium hyperplasia and minimal single cell necrosis in the duodenum with both degraded and non-degraded TDF/FTC at 300/200 mg/kg/day. NOAEL = 300/200 mg/kg/day for both non-degraded and degraded TDF/FTC, with no difference in toxicity between the non-degraded and degraded material. TDF Day 14 C_{max} 1.4 and 1.2 µg/mL, AUC_{0-24} 13.6 and 13.7 µg•h/mL; FTC Day 14 C_{max} 15.8 and 13.4 µg/mL, AUC_{0-24} 163 and 133 µg•h/mL for non-degraded and degraded tablets, respectively.	TX-164-2005[c] ([redacted] 805427)

a Non-degraded TDF/FTC.
b Degraded TDF/FTC.
c Indicates GLP compliant.

17.5. TDF

Test Article: TDF

Type of Study	Species/Strain	Method of Administration	Duration of Dosing	Dose (mg/kg)	Gender and No. per Group	Noteworthy Findings	Gilead Study No.
Interaction Study	Rat/ Sprague-Dawley	Oral Gavage	28 Days	0, 40 (adefovir dipivoxil ADV, alone), 300 TDF alone & 300 TDF + 40 ADV combination group	6 Male	For both ADV alone & combination treated groups mean body weights ↓, BUN & creatinine ↑ & glucosuria, proteinuria, calciuria & renal tubular nephrosis observed; severity > in ADV alone group. Hypophosphaturia seen in TDF alone & combination groups. Combination & ADV alone groups has proliferative & degenerative changes in the intestine; while group with TDF alone had slight proliferative changes & thickening of intestinal mucosa. All groups had AST & ALT ↑ through day 14, however there were no histologic correlated to these changes. Combination group mean serum lactate ↑, however because there wereno changes in cytochrome c oxidase, citrate synthase, or mtDNA content in liver, kidney or skeletal muscle these changes were most likely not caused by mitochondrial dysfunction.	R2000096

SECTION 2.6.7—TOXICOLOGY TABULATED SUMMARY

BICTEGRAVIR
(GS-9883-01)

Gilead Sciences

[redacted] **20**

CONFIDENTIAL AND PROPRIETARY INFORMATION

2.6.7.10 Carcinogenicity (Continued)

1.1.1. Rat

Test Article: GS-9883-01

Report Title: **104-Week Oral Gavage Carcinogenicity and Toxicokinetic Study with GS-9883-01 in Han Wistar Rats**		**Gilead Study No. TX-141-2040 [redacted] 8315273**
Species / Strain: Crl:WI(Han) rats	**Duration of Dosing:** 104 weeks	**Location in CTD:** 4.2.3.4.1
Initial Age: 6 to 7 weeks	**Method of Administration:** Oral Gavage	**GLP Compliance:** Yes
Date of First Dose: [redacted] 20[redacted] (Males) [redacted] 20[redacted] (Females)	**Vehicle Control Article:** 0.5% (w/w) hydroxypropyl methylcellulose (Methocel™ K100 LV) and 0.1% (w/w) Tween® 20 in reverse osmosis water, pH adjusted to 7.5 to 8.5	**Lot Number:** [redacted]
Water Control Article: reverse osmosis water	**Basis for High Dose Selection:** In the 26-week toxicity study, the no-observable-adverse-effect level (NOAEL) for GS-9883 was 300 mg/kg/day, the highest dose administered.	

Special Features: None

Daily Dose (mg/kg/day)	**0 (Water)**		**0 (Vehicle)**		**2 (Low)**		**10 (Mid)**		**300 (High)**	
Sex: Number of Animals (TK)	M: 6	F: 6	M: 6	F: 6	M: 12	F: 12	M: 12	F: 12	M: 12	F: 12
Toxicokinetics										
AUC_{0-24} (ug·h/mL) Day 1	BLQ	BLQ	BLQ	BLQ	205	235	711	982	2550	3560
AUC_{0-24} (ug·h/mL) Week 52	BLQ	BLQ	BLQ	BLQ	439	750	829	1900	2170	4200
Number of Animals (Main)										
At Study Start	55	55	55	55	55	55	55	55	55	55
Died/Sacrificed Moribund	16	25	21	26	20	25	19	19	19	27
Terminal Sacrifice	39	30	34	29	35	30	36	36	36	28
Daily Dose (mg/kg/day)	**0 (Water)**		**0 (Vehicle)**		**2 (Low)**		**10 (Mid)**		**300 (High)**	
Survival (%)	71	56	62	53	64	56	67	65	65	53
Body Weight	No Test-Article Related Findings									

Test Article: GS-9883-01

Report Title: **104-Week Oral Gavage Carcinogenicity and Toxicokinetic Study with GS-9883-01 in Han Wistar Rats**										**Gilead Study No. TX-141-2040 [redacted] 8315273**
Food Consumption	No Test-Article Related Findings									
Histopathology	No Test-Article Related Findings									
Neoplastic Lesions	No Test-Article Related Findings									
Primary Neoplastic Findings, Groups 1 through 5 – Scheduled and Unscheduled Sacrifices and Deaths										
Adipose, Other										
B-Lipoma	0	1	1	2	0	2	1	2	1	0
N-Carcinoma, metastatic	0	0	0	1	0	0	0	2	0	0
Adrenal, Cortex										
B-Adenoma	4	2	0	2	2	1	3	3	3	5
B-Lipoma	0	0	0	0	0	1	1	0	0	0
F-Hemolymphoreticular tumor	0	0	0	0	0	1	0	0	0	0
M-Carcinoma	0	0	0	0	0	0	1	1	0	0
M-Malignant schwannoma	0	0	0	0	1	0	0	0	0	0
N-Carcinoma, metastatic	0	1	0	0	0	0	0	0	0	0
Adrenal, Medulla										
B-Pheochromocytoma	4	0	1	0	1	1	0	0	2	1
M-Complex malignant pheochromocytoma	1	0	0	0	0	0	0	0	0	0
M-Malignant pheochromocytoma	1	0	0	0	0	0	0	0	0	0
N-Carcinoma, metastatic	0	0	0	1	0	0	0	0	0	0
Aorta										
F-Hemolymphoreticular tumor	0	0	1	0	0	0	0	0	0	0
N-Schwannoma, metastatic	0	0	1	0	0	0	0	0	0	0
Brain										

Test Article: GS-9883-01

Report Title: **104-Week Oral Gavage Carcinogenicity and Toxicokinetic Study with GS-9883-01 in Han Wistar Rats**							**Gilead Study No. TX-141-2040 [redacted] 8315273**			
B-Glioma	0	0	1	0	0	0	0	0	0	0
B-Granular cell tumor	3	0	3	1	2	1	1	0	0	0
F-Hemolymphoreticular tumor	0	0	0	0	0	1	0	0	0	0
M-Glioma	1	0	0	0	1	1	0	0	0	0
M-Malignant oligodendroglioma	0	0	0	0	1	1	0	0	0	0
N-Carcinoma, metastatic	0	0	1	0	0	0	0	0	0	0
Cecum										
F-Hemolymphoreticular tumor	0	0	1	0	0	0	0	0	0	0
Cervix										
B-Leiomyoma	NA	1	NA	0	NA	1	NA	0	NA	0
B-Papilloma	NA	0	NA	0	NA	0	NA	0	NA	1
F-Hemolymphoreticular tumor	NA	0	NA	0	NA	1	NA	0	NA	0
M-Carcinoma	NA	0	NA	1	NA	0	NA	1	NA	0
M-Carcinoma, squamous cell	NA	1	NA	1	NA	0	NA	0	NA	0
M-Malignant schwannoma	NA	0	NA	0	NA	0	NA	0	NA	1
M-Sarcoma, stromal	NA	0	NA	0	NA	0	NA	1	NA	0
N-Carcinoma, metastatic	NA	1	NA	1	NA	0	NA	0	NA	0
Clitoral Gland										
F-Hemolymphoreticular tumor	NA	0	NA	0	NA	1	NA	0	NA	0
M-Carcinoma, squamous cell	NA	0	NA	0	NA	1	NA	0	NA	0
Colon										
N-Carcinoma, metastatic	0	1	0	0	0	0	0	0	0	0
Duodenum										

Test Article: GS-9883-01

Report Title:	**104-Week Oral Gavage Carcinogenicity and Toxicokinetic Study with GS-9883-01 in Han Wistar Rats**						**Gilead Study No. TX-141-2040 [redacted] 8315273**			
N-Carcinoma, metastatic	0	0	0	0	0	0	0	0	1	0
N-Sarcoma, metastatic	0	0	0	0	1	0	0	0	0	0
Epididymis										
F-Hemolymphoreticular tumor	0	NA	0	NA	1	NA	0	NA	0	NA
I-Malignant mesothelioma, locally invasive	1	NA	0	NA	0	NA	1	NA	0	NA
M-Malignant mesothelioma	1	NA	0	NA	0	NA	0	NA	0	NA
N-Carcinoma, metastatic	0	NA	0	NA	0	NA	0	NA	1	NA
Esophagus										
F-Hemolymphoreticular tumor	0	0	1	0	0	0	0	0	0	0
N-Carcinoma, metastatic	0	0	1	0	0	0	0	0	0	0
Eye										
F-Hemolymphoreticular tumor	0	0	1	0	0	1	0	0	0	0
GALT/Peyer's Patch										
F-Hemolymphoreticular tumor	0	0	0	0	2	1	0	0	0	0
HarderiaN-Gland										
F-Hemolymphoreticular tumor	0	0	1	0	0	1	0	0	0	0
Heart										
F-Hemolymphoreticular tumor	0	1	1	0	0	1	0	0	0	1
M-Endocardial schwannoma	0	0	1	0	0	0	0	1	1	0
M-Malignant schwannoma	0	0	0	0	0	0	0	0	1	0
Hemolympho-reticular System										
M-Granulocytic sarcoma	1	0	0	0	0	0	0	0	0	0
M-Histiocytic Sarcoma	0	1	0	0	0	0	0	0	0	0

Test Article: GS-9883-01

Report Title:	**104-Week Oral Gavage Carcinogenicity and Toxicokinetic Study with GS-9883-01 in Han Wistar Rats**						**Gilead Study No. TX-141-2040 [redacted] 8315273**			
M-Malignant lymphoma	1	3	3	2	4	4	3	5	0	4
Ileum										
M-Fibrosarcoma	0	0	0	0	1	0	0	0	0	0
N-Carcinoma, metastatic	0	0	0	0	0	0	0	0	1	0
Jejunum										
M-Fibrosarcoma	0	0	0	0	0	0	1	0	0	0
N-Sarcoma, metastatic	0	0	0	0	1	0	0	0	0	0
Kidney										
B-Lipoma	0	0	1	0	0	0	0	1	0	0
F-Hemolymphoreticular tumor	0	1	1	0	2	1	0	0	0	0
M-Carcinoma, transitional cell	0	0	0	0	0	0	1	0	0	0
M-Carcinoma, tubule cell	0	0	0	1	0	0	0	0	0	0
N-Carcinoma, metastatic	0	1	0	0	0	0	0	1	1	0
Lacrimal Gland										
F-Hemolymphoreticular tumor	0	0	1	0	1	1	0	0	0	0
N-Carcinoma, metastatic	0	0	0	0	0	1	0	0	0	0
Larynx										
F-Hemolymphoreticular tumor	0	0	1	0	1	1	0	0	0	0
Liver										
B-Adenoma, hepatocellular	2	1	1	2	0	1	1	3	0	1
F-Hemolymphoreticular tumor	0	0	0	0	3	1	0	0	0	1
N-Carcinoma, metastatic	0	0	0	0	0	0	0	0	1	0
N-Schwannoma, metastatic	0	0	1	0	0	0	0	0	0	0

Test Article: GS-9883-01

Report Title: **104-Week Oral Gavage Carcinogenicity and Toxicokinetic Study with GS-9883-01 in Han Wistar Rats**							**Gilead Study No. TX-141-2040 [redacted] 8315273**			
Lung										
B-Adenoma, bronchiolo alveolar	0	1	0	1	0	0	0	1	1	0
F-Hemolymphoreticular tumor	0	1	1	0	2	1	0	0	0	0
N-Carcinoma, metastatic	1	2	0	2	0	0	1	2	1	0
N-Sarcoma, metastatic	0	0	1	0	1	0	1	0	0	0
X-Sarcoma, primary unknown	0	0	0	0	0	0	0	0	1	0
Lymph Node, Hepatic										
I-Malignant schwannoma, locally invasive	0	0	0	0	0	0	1	0	0	0
Lymph Node, Inguinal										
F-Hemolymphoreticular tumor	0	0	0	0	1	0	0	0	0	0
Lymph Node, Mandibular										
F-Hemolymphoreticular tumor	1	0	1	0	3	1	0	0	0	0
M-Hemangiosarcoma	0	0	0	0	0	0	1	0	0	0
Lymph Node, Mediastinal										
F-Hemolymphoreticular tumor	0	0	0	0	0	1	0	0	0	0
N-Carcinoma, metastatic	0	0	0	0	0	0	0	1	0	0
Lymph Node, Mesenteric										
B-Hemangioma	1	1	1	0	1	0	2	0	1	0
B-Lymphangioma	3	0	0	1	0	0	0	1	0	0
F-Hemolymphoreticular tumor	1	1	1	0	3	1	0	0	0	0
N-Carcinoma, metastatic	0	1	0	0	0	0	0	0	0	0
Lymph Node, Pancreatic										
F-Hemolymphoreticular tumor	0	0	0	0	1	0	0	0	0	0

Test Article: GS-9883-01

Report Title: **104-Week Oral Gavage Carcinogenicity and Toxicokinetic Study with GS-9883-01 in Han Wistar Rats**							**Gilead Study No. TX-141-2040 [redacted] 8315273**			
Lymph Node, Renal										
F-Hemolymphoreticular tumor	0	0	0	0	0	1	0	0	0	0
Mammary Gland										
B-Adenoma	0	1	0	0	0	0	0	1	0	0
B-Fibroadenoma	1	19	1	16	1	18	1	12	1	16
F-Hemolymphoreticular tumor	0	0	0	0	0	1	0	0	0	0
M-Carcinoma	0	4	0	4	0	3	0	6	0	6
Mandibular Salivary Gland										
F-Hemolymphoreticular tumor	0	0	1	0	1	0	0	0	0	0
I-Sarcoma, locally invasive	0	0	0	1	0	0	0	0	0	0
M-Fibrosarcoma	0	0	0	0	0	0	0	0	1	0
M-Malignant schwannoma	1	0	0	0	0	0	1	0	0	0
Marrow, Femur										
F-Hemolymphoreticular tumor	0	0	0	0	1	0	0	0	0	0
Marrow, Sternum										
F-Hemolymphoreticular tumor	0	0	0	0	1	0	0	0	0	0
Mesentery										
F-Hemolymphoreticular tumor	0	0	0	0	0	0	0	0	0	1
N-Carcinoma, metastatic	1	0	0	0	0	0	0	0	1	0
Muscle, Biceps Femoris										
F-Hemolymphoreticular tumor	0	0	1	0	0	1	0	0	0	0
M-Hemangiosarcoma	0	0	0	0	1	0	0	0	1	0
Muscle, Diaphragm										

Test Article: GS-9883-01

Report Title:	**104-Week Oral Gavage Carcinogenicity and Toxicokinetic Study with GS-9883-01 in Han Wistar Rats**						**Gilead Study No. TX-141-2040 [redacted] 8315273**			
N-Carcinoma, metastatic	0	0	0	0	0	0	0	0	1	0
Muscle, Latissimus dorsi										
M-Fibrosarcoma	0	0	0	0	0	0	1	0	0	0
Ovary										
B-Granulosa/theca cell tumor	NA	1	NA	1	NA	0	NA	1	NA	0
B-Leiomyoma	NA	0	NA	0	NA	0	NA	0	NA	1
F-Hemolymphoreticular tumor	NA	0	NA	0	NA	1	NA	0	NA	0
I-Malignant schwannoma, locally invasive	NA	0	NA	0	NA	0	NA	1	NA	0
M-Carcinoma	NA	0	NA	0	NA	0	NA	1	NA	0
N-Carcinoma, metastatic	NA	1	NA	1	NA	0	NA	0	NA	0
Pancreas										
B-Adenoma, islet cell	3	0	2	1	3	2	2	0	4	2
F-Hemolymphoreticular tumor	0	0	0	0	0	1	0	0	0	0
I-Malignant schwannoma, locally invasive	0	0	0	0	0	0	1	0	0	0
M-Carcinoma, acinar cell	0	1	0	0	0	0	0	0	0	0
M-Carcinoma, islet cell	1	0	1	0	0	0	0	0	1	0
M-Fibrosarcoma	1	0	0	0	0	0	0	0	0	0
N-Carcinoma, metastatic	1	0	0	1	0	0	0	1	1	0
Parathyroid										
B-Adenoma	0	2	1	1	1	1	2	0	0	0
F-Hemolymphoreticular tumor	0	0	1	0	0	0	0	0	0	0
Pituitary										

Test Article: GS-9883-01

Report Title: **104-Week Oral Gavage Carcinogenicity and Toxicokinetic Study with GS-9883-01 in Han Wistar Rats**							**Gilead Study No. TX-141-2040 [redacted] 8315273**			
B-Adenoma	27	32	20	35	23	33	21	38	15	38
B-Craniopharyngioma	0	0	0	1	0	0	0	0	0	0
F-Hemolymphoreticular tumor	0	0	0	0	0	1	0	0	0	0
I-Oligodendroglioma, locally invasive	0	0	0	0	1	0	0	0	0	0
M-Carcinoma	0	0	1	0	0	0	0	0	0	0
Preputial Gland										
F-Hemolymphoreticular tumor	0	NA	1	NA	0	NA	0	NA	0	NA
M-Carcinoma, squamous cell	1	NA	0	NA	0	NA	0	NA	0	NA
N-Sarcoma, metastatic	1	NA	0	NA	0	NA	0	NA	0	NA
Prostate										
B-Adenoma	0	NA	1	NA	0	NA	1	NA	0	NA
F-Hemolymphoreticular tumor	0	NA	1	NA	1	NA	0	NA	0	NA
I-Malignant schwannoma, locally invasive	0	NA	0	NA	0	NA	1	NA	0	NA
M-Carcinoma	0	NA	0	NA	0	NA	1	NA	2	NA
N-Carcinoma, metastatic	1	NA	0	NA	0	NA	0	NA	1	NA
N-Sarcoma, metastatic	1	NA	0	NA	0	NA	0	NA	0	NA
Rectum										
I-Carcinoma, locally invasive	0	0	0	1	0	0	0	0	0	0
Seminal Vesicle										
F-Hemolymphoreticular tumor	0	NA	1	NA	1	NA	0	NA	0	NA
M-Carcinoma	1	NA	0	NA	0	NA	0	NA	0	NA
N-Carcinoma, metastatic	0	NA	0	NA	0	NA	1	NA	0	NA
Skin/Subcutis										

Test Article: GS-9883-01

Report Title:	**104-Week Oral Gavage Carcinogenicity and Toxicokinetic Study with GS-9883-01 in Han Wistar Rats**						**Gilead Study No. TX-141-2040 [redacted] 8315273**			
B-Adenoma, sebaceous	1	0	1	0	0	1	1	0	1	0
B-Basal cell tumor	1	0	0	0	3	0	0	0	1	0
B-Fibroma	3	0	1	0	2	0	1	0	4	0
B-Hemangioma	0	0	0	0	1	0	2	0	1	0
B-Hemangiopericytoma	0	0	0	0	0	0	0	1	0	0
B-Keratoacanthoma	4	0	6	1	6	0	3	0	5	0
B-Lipoma	1	0	2	0	0	1	1	0	0	0
B-Papilloma, squamous cell	2	0	1	0	0	0	2	0	0	0
F-Hemolymphoreticular tumor	0	0	1	0	0	0	0	0	0	0
M-Carcinoma, squamous cell	0	0	2	0	0	0	0	0	0	0
M-Carcinoma, undifferentiated	0	1	0	0	0	0	2	0	0	0
M-Fibrosarcoma	0	1	2	2	3	1	1	0	2	1
M-Fibrous histiocytoma	0	0	0	0	0	0	1	0	0	0
M-Malignant basal cell tumor	0	0	1	0	0	1	0	0	1	0
M-Malignant schwannoma	1	0	0	0	0	0	0	0	2	0
Spleen										
F-Hemolymphoreticular tumor	0	0	0	0	2	1	0	1	0	0
I-Malignant schwannoma, locally invasive	0	0	0	0	0	0	1	0	0	0
M-Hemangiosarcoma	2	0	0	0	0	1	0	0	0	0
N-Carcinoma, metastatic	0	1	1	0	0	0	0	0	0	0
Sternum										
F-Hemolymphoreticular tumor	0	1	0	0	0	0	0	0	0	0
N-Schwannoma, metastatic	0	0	1	0	0	0	0	0	0	0

Test Article: GS-9883-01

Report Title: **104-Week Oral Gavage Carcinogenicity and Toxicokinetic Study with GS-9883-01 in Han Wistar Rats**							**Gilead Study No. TX-141-2040 [redacted] 8315273**			
Stomach, Glandular										
F-Hemolymphoreticular tumor	0	0	1	0	1	1	0	0	0	0
I-Malignant schwannoma, locally invasive	0	0	0	0	0	0	1	0	0	0
I-Sarcoma, locally invasive	0	0	0	0	1	0	0	0	0	0
M-Carcinoma	0	0	1	0	0	0	0	0	0	0
N-Carcinoma, metastatic	0	1	0	1	0	0	0	0	1	0
N-Sarcoma, metastatic	1	0	0	0	0	0	0	0	0	0
Stomach, Nonglandular										
B-Papilloma, squamous cell	0	0	0	0	0	0	0	1	0	0
F-Hemolymphoreticular tumor	0	0	0	0	0	1	0	0	0	0
M-Carcinoma, squamous cell	0	0	1	0	0	0	0	0	1	0
M-Fibrosarcoma	0	0	0	0	1	0	0	0	0	0
M-Malignant schwannoma	0	0	0	0	0	0	1	0	0	0
Testis										
B-Hemangioma	0	NA	0	NA	1	NA	0	NA	0	NA
B-Interstitial cell tumor	0	NA	1	NA	3	NA	0	NA	3	NA
M-Interstitial cell tumor	1	NA	2	NA	2	NA	0	NA	0	NA
M-Malignant mesothelioma	1	NA	0	NA	0	NA	1	NA	0	NA
N-Carcinoma, metastatic	0	NA	0	NA	0	NA	0	NA	1	NA
N-Mesothelioma, metastatic	1	NA	0	NA	0	NA	0	NA	0	NA
Thymus										
B-Lipoma	0	0	1	0	0	0	0	0	0	0
B-Thymoma	0	0	0	1	0	1	0	0	0	0

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F-Hemolymphoreticular tumor	2	3	3	2	4	4	3	4	0	3
M-Malignant thymoma	0	0	0	1	0	0	0	0	0	0
N-Carcinoma, metastatic	0	1	0	0	0	0	0	0	0	0
Thyroid										
B-Adenoma, C cell	6	8	5	7	5	4	5	7	8	4
B-Adenoma, follicular cell	12	2	6	4	10	2	5	4	11	2
F-Hemolymphoreticular tumor	0	0	1	0	1	1	0	0	0	0
I-Sarcoma, locally invasive	0	0	0	1	0	0	0	0	0	0
M-Carcinoma, C cell	0	0	1	1	0	0	0	0	0	0
M-Carcinoma, follicular cell	0	1	4	0	1	0	0	0	2	0
Tongue										
F-Hemolymphoreticular tumor	0	0	1	0	0	0	0	0	0	0
Trachea										
F-Hemolymphoreticular tumor	0	0	1	0	0	1	0	0	0	0
Ureter										
F-Hemolymphoreticular tumor	0	0	0	0	2	1	0	0	0	0
Urinary Bladder										
F-Hemolymphoreticular tumor	0	0	1	0	0	1	0	0	0	0
N-Carcinoma, metastatic	1	0	0	0	0	0	1	0	0	0
N-Sarcoma, metastatic	1	0	0	0	0	0	0	0	0	0
Uterus										
B-Adenoma	NA	1	NA	0	NA	0	NA	0	NA	0
B-Polyp, endometrial stromal	NA	9	NA	1	NA	3	NA	5	NA	1

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F-Hemolymphoreticular tumor	NA	0	NA	0	NA	1	NA	0	NA	0
M-Carcinoma	NA	0	NA	1	NA	2	NA	0	NA	1
M-Malignant Schwannoma	NA	0	NA	0	NA	1	NA	1	NA	0
N-Carcinoma, metastatic	NA	0	NA	1	NA	0	NA	1	NA	0
N-Sarcoma, metastatic	NA	0	NA	0	NA	0	NA	1	NA	0
Vagina										
B-Papilloma, squamous cell	NA	1	NA	0	NA	0	NA	0	NA	0
F-Hemolymphoreticular tumor	NA	0	NA	0	NA	1	NA	0	NA	0
M-Malignant Schwannoma	NA	0	NA	0	NA	0	NA	0	NA	1
N-Carcinoma, metastatic	NA	1	NA	0	NA	0	NA	0	NA	0
Zymbal Gland										
F-Hemolymphoreticular tumor	0	0	1	0	0	0	0	0	0	0
M-Carcinoma, squamous cell	0	1	0	0	2	1	0	0	1	0
Clinical Observations	No Test-Article Related Findings									
Gross Pathology	No Test-Article Related Findings									

AUC_{0-24} = Area under the concentration time curve from 0 to 24 hours postdose; BLQ = below the lower limit of quantitation (<1000 ng/mL), C_{max} = maximum observed dose; M = Male; F = Female; B- = Benign; F- = Infiltrating; I- = Locally invasive; M- = Malignant; N- = Metastatic; X- = Other NA = Not applicable