**2.6—NONCLINICAL SUMMARY**

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**SECTION 2.6.6—TOXICOLOGY WRITTEN SUMMARY**

# ELVITEGRAVIR/COBICISTAT/EMTRICITABINE/TENOFOVIR ALAFENAMIDE FUMARATE FIXED-DOSED COMBINATION (EVG/COBI/FTC/TAF [E/C/F/TAF])

**Gilead Sciences**

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**CONFIDENTIAL AND PROPRIETARY INFORMATION**

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# LIST OF ABBREVIATIONS

| | |
|---|---|
| $\lambda_{max}$ | absorption maxima |
| ALP | alkaline phosphatase |
| AST | aspartate amino transferase |
| B-ALP | bone alkaline phosphatase |
| BCOP | bovine corneal opacity permeability |
| BID | twice daily |
| BMD | bone mineral density |
| CK | creatinine kinase |
| COBI | cobicistat |
| $CO_2$ | carbon dioxide |
| cm | centimeter |
| DNA | deoxyribonucleic acid |
| Dpd | deoxypyridinoline |
| dpm | disintegrations per minute |
| DRV | darunavir |
| DXA | dual-emission X-ray absorptiometry |
| EC3 | estimated concentration of 3is the concentration of test substance which would result in a SI of 3 in the LLNA |
| $EC_{50}$ | estimated concentration of drug for a half maximal response |
| ECG | electrocardiogram |
| EMA | European Medicines Agency |
| EU | European Union |
| EVG | elvitegravir (JTK-303, GS-9137) |
| E/C/F/TAF | elvitegravir/cobicistat/emtricitabine/tenofovir alafenamide (coformulated); EVG/COBI/FTC/TAF |
| $F_0$ | parental generation |
| $F_1$ | first generation |
| f.b.e. | free base equivalents |
| FDC | fixed-dose combination |
| FDA | United States Food and Drug Administration |
| FTC | Emtricitabine, Emtriva® (Gilead), FTC, TP-006, GS-9019, 524W91 |
| FTC/TDF | emtricitabine/tenofovir DF (Truvada®, Gilead Sciences), TVD |
| GALT | gut-associated lymphoid tissue |
| GD | gestation day |
| GI | gastrointestinal |
| GLP | Good Laboratory Practice |
| h | hours |
| $^3$HTdR | $^3$H-methyl Thymidine |
| HIV | human immunodeficiency virus |
| HPMC | hydroxypropylmethylcellulose |
| ICH | International Conference on Harmonization |
| IND | Investigational New Drug (application) |
| INSTI | integrase strand-transfer inhibitors |
| kg | kilogram |
| LC/MS | liquid chromatography/mass spectrometry |

## LIST OF ABBREVIATIONS (CONTINUED)

| | |
|---|---|
| LD | lactation day |
| LNC | lymph node cell |
| LLNA | local lymph node assay |
| M | molar |
| MATE1 | multidrug and toxin extrusion protein 1 |
| MATE2-K | multidrug and toxin extrusion protein 2-K |
| µg | microgram |
| µL | microliter |
| mg | milligram |
| MRP4 | multi-drug resistance-associated protein-4 |
| mL | milliliter |
| MTD | maximum tolerated dose |
| N | number |
| NAG | n-acetyl-β-glucosaminidase |
| NCE | normochromatic erythrocyte |
| NA | not applicable |
| NDA | New drug application |
| NNRTI | nonnucleoside reverse transcriptase inhibitor |
| NOAEL | no observed adverse effect level |
| NOEL | no observed effect level |
| NRTI | nucleoside reverse transcriptase inhibitors (NRTIs) |
| OECD | Organization for Economic Cooperation and Development |
| OCT2 | organic cation transporter 2 |
| OCTN1 | organic cation transporter novel, type 1 |
| PBMC | peripheral blood mononuclear cell |
| PCE | polychromatic erythrocyte |
| PGP | p-glycoprotein |
| PK | pharmacokinetic |
| PI | protease inhibitor |
| PM | premating |
| PP | postpairing |
| PTH | parathyroid hormone |
| PR | interval between the peak of the P wave and the peak of the R wave on ECG |
| QT | interval between the start of the Q wave and the end of the T wave on ECG |
| RBC | red blood cell |
| RO | reverse osmosis |
| sALP | skeletal alkaline phosphatase |
| SI | stimulation index |
| STB | Stribild® |
| T3 | triiodthyronine |
| TAF | tenofovir alafenamide, GS-7340 |
| TDF | tenofovir disoproxil fumarate (TDF), bis-POC PMPA fumarate, GS-4331-05, PMPA prodrug (Viread®, Gilead) |
| TFV | tenofovir, GS-1278, PMPA |

| | |
|---|---|
| TFV-DP | tenofovir diphosphate |
| TID | three times daily |
| tk | thymidine kinase |
| TK | toxicokinetic |
| TVD | Truvada® |
| US | United States |
| v/v | volume concentration |
| WBC | white blood cell |
| Y | yes |

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

## PHARMACOKINETIC ABBREVIATIONS

| | |
|---|---|
| $\lambda_z$ | Terminal elimination rate constant, estimated by linear regression of the terminal elimination phase of the serum, plasma, or peripheral blood mononuclear cells (PBMCs) concentration of drug versus time curve |
| $AUC_{inf}$ | The area under the serum, plasma, or PBMC concentration versus time curve extrapolated to infinite time, calculated as $AUC_{0-last} + (C_{last}/\lambda_z)$ |
| $AUC_{x-xx}$ | Partial area under the serum, plasma, or PBMC concentration versus time curve from time "x" to time "xx" |
| $AUC_{ss}$ | The area under the serum, plasma, or PBMC concentration versus time curve at steady state |
| $AUC_{tau}$ | The area under the serum, plasma, or PBMC concentration versus time curve over the dosing interval |
| $C_{1h}$ | The concentration determined 1 hour after dosing. |
| CL | The systemic clearance of the drug after intravenous administration |
| CL/F | The apparent oral clearance after administration of the drug: $CL/F = Dose/AUC_{inf}$, where "Dose" is the dose of the drug |
| $C_{max}$ | The maximum observed concentration of drug in serum, plasma, or PMBC |
| $T_{½}$ | An estimate of the terminal elimination half-life of the drug in serum, plasma, or PBMCs, calculated by dividing the natural log of 2 by the terminal elimination rate constant ($\lambda_z$) |
| $T_{max}$ | The time (observed time point) of $C_{max}$ |
| $\lambda_z$ | Terminal elimination rate constant, estimated by linear regression of the terminal elimination phase of the serum, plasma, or peripheral blood mononuclear cells (PBMCs) concentration of drug versus time curve |

## NOTE TO REVIEWER

This document contains a summary of nonclinical toxicology studies conducted in support of a fixed-dose combination (FDC) that contains the active substances elvitegravir (EVG, E), cobicistat (COBI, C), emtricitabine (FTC, F), and tenofovir alafenamide (TAF, GS-7340) fumarate (GS-7340-03); the E/C/F/TAF FDC (150/150/200/10 mg) tablet.

As TAF is a new chemical entity, this nonclinical summary contains all available data on this new component. Per the agreement reached between Gilead Sciences, Inc. (Gilead) and the Food and Drug Administration (FDA; ), this NDA is supported by .

. To assist the reviewer, Links to all study reports included in the application are highlighted in blue text.

Given the lack of remarkable effects in the nonclinical toxicology studies with the individual agents, no additional toxicology studies have been conducted for the E/C/F/TAF combination.

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

| GS-7340 Equivalents | | |
|---|---|---|
| **mg GS-7340 as the free base (GS-7340)** | **mg GS-7340 as the hemi fumarate (GS-7340-03)** | **mg GS-7340 as the mono fumarate (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 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 application is being submitted in support of a new drug application (NDA) for a fixed-dose combination (FDC) of elvitegravir (EVG, E, Vitekta®), cobicistat (COBI, C, Tybost®), emtricitabine (FTC, F, Emtriva®), and tenofovir alafenamide (TAF); the E/C/F/TAF (150/150/200/10 mg) FDC. The E/C/F/TAF FDC is indicated for the treatment of human immunodeficiency virus-1 (HIV-1) infection in adult and pediatric patients 12 years of age and older without any known mutations associated with resistance to the individual components of E/C/F/TAF.

Tenofovir alafenamide is a prodrug of TFV that, compared to tenofovir disoproxil fumarate (TDF), has a longer plasma half-life and delivers higher levels of TFV into target cells at lower exposure of circulating TFV. TAF has a unique metabolism that provides enhanced delivery of TFV to HIV-target cells including lymphocytes, resulting in higher intracellular levels of the active phosphorylated metabolite tenofovir diphosphate (TFV-DP), and lower circulating levels of TFV when compared with TDF {7415}, {13119}, {22029}. These features may improve the profile of tenofovir disoproxil fumarate (TDF), which is associated with nephrotoxicity and decreased bone mineral density {21762}, {22031}.

The EVG/COBI/FTC/TAF FDC contains the same dosages of EVG, COBI and FTC that are currently approved within Emtriva, Truvada, and Stribild for use in adults (150 mg EVG, 150 mg COBI, 200 mg of FTC). Information from all nonclinical studies with EVG, COBI, FTC, and TDF/tenofovir (TFV) should be considered in the context of their substantial clinical experience within antiretroviral combination therapy for the treatment of HIV-1 infection.

Comprehensive programs of nonclinical toxicology studies with EVG, COBI, FTC, and TAF have been conducted. The toxicology program for TAF, the only unapproved component, is provided in Table 1. TAF was evaluated in mouse, rat, dog, and monkey single- and/or repeat-dose toxicity studies up to 39 weeks in duration. In addition, the mouse was used for an in vivo genetic toxicity test, the rat was used for fertility and developmental toxicity studies and the rabbit was used for developmental and reproductive toxicity studies and local irritation. 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 vitro and in vivo metabolic profiles to humans (m2.6.4, Section 5.1.1). The vehicle for toxicity studies used was 1) 25mM citric acid or 2) 0.5% polysorbate 20, 0.5% carboxymethylcellulose, 0.9% benzyl alcohol or 3) 0.1% (v/v) Tween 20 and 0.1% (v/v) hydroxypropylmethylcellulose (HPMC).

Per agreement with the FDA and EMA, carcinogenicity studies (FDA Reference ID: 3161161, EMA FAL 2410-1-2012), and a perinatal and postnatal study (FDA Reference ID: 3244145, EMA FAL_2410-1-FU-1-2013) are not required for TAF registration due to the lack of TAF exposure in rats and TgRasH2 mice and lower TFV exposure in rats and mice compared to TDF.

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 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 ($t_{½}$ = 31 minutes) and mouse ($t_{½}$ = < 3 minutes). Shorter duration repeat-dose rodent toxicity studies had TFV but not TAF bioanalysis; repeat-dose nonrodent toxicity studies evaluated both TAF and TFV toxicokinetics. A sensitive TAF assay was developed later in the development program so that 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 4.1.1).

The absence of nonclinical safety studies with the E/C/F/TAF combination is in accordance with the United States Food and Drug Administration (FDA) Guidance for Industry, *Nonclinical Safety Evaluation of Drug or Biologic Combinations, March 2006* and the Committee for Medicinal Products for Human Use (CHMP) Guideline on the Non-Clinical Development of Fixed Combinations of Medicinal Products (EMEA/CHMP/SWP/258498/2005, January 2008). There are no anticipated clinically relevant pharmacokinetic or toxicological interactions expected in the E/C/F/TAF combination beyond the anticipated PK boosting of EVG by COBI.

## Table 1. Toxicology Program

| Study Type and Duration | GLP Y/N | Route of Administration | Species |
|---|---|---|---|
| **Single-Dose Toxicity** | N (Rat)<br>Y (Dog) | Oral | Rat, Dog |
| **Repeat-Dose Toxicity** | | | |
| 6-15 days | N | Oral | Mouse, Rat |
| 4 weeks | Y | Oral | Rat, Dog, Monkey |
| 13 weeks | Y | Oral | Mouse |
| 26 weeks | Y | Oral | Rat |
| 39 weeks | Y | Oral | Dog |
| **Genotoxicity** | | | |
| In vitro reverse mutation assay | Y | In vitro | *Salmonella typhimurium*, *Escherichia coli* |
| In vitro mutation assay | Y | In vitro | L5178 mouse lymphoma cells |
| In vivo micronucleus assay | Y | Oral | Mouse |
| **Carcinogenicity** | NA | NA | NA |
| **Reproductive and Developmental Toxicity** | | | |
| Fertility and early embryonic development | Y | Oral | Rat |
| Embryo-fetal development | Y | Oral | Rat, Rabbit |
| Prenatal and postnatal development, including maternal function | NA | NA | NA |
| **Local Tolerance** | | | |
| Dermal | Y | Topical | Rabbit |
| Ocular | Y | Topical (ex vivo) | Bovine |
| **Other Studies** | | | |
| Antigenicity - LLNA | Y | Topical | Mouse |
| Bone evaluation | N (Rat)<br>Y (Dog) | Oral | Rat, Dog |
| Renal effects | Y | Oral | Rat |
| Impurity evaluation | Y | Oral | Rat |

# 2. SINGLE DOSE TOXICITY

## 2.1. Single Oral Dose Toxicity in Rats

Male and female Sprague-Dawley rats (5/sex/group) were administered a 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 f.b.e./kg) followed by a 14-day observation period (m2.6.7, Section 5, R990185). The NOAEL for a single oral dose GS-7340-02 in the rat was determined to be > 1000 mg/kg.

## 2.2. Single Oral Dose Toxicity in 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, 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 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 to 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 TAF NOAEL for a single oral dose in the dog was determined to be 30 mg/kg.

## 2.3. EVG/COBI/FTC/TAF

No single-dose studies have been performed with the combination of EVG, COBI, FTC, and TAF. Single-dose toxicity studies indicated that EVG, FTC, and TAF had low acute toxicity. With no overlapping toxicities, coadministration is unlikely to change the acute toxicity profile.

# 3. REPEAT-DOSE TOXICITY

## 3.1. Mouse

### 3.1.1. 2-Week Oral Mouse Toxicity Study

Daily oral administration (10 mL/kg) of 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, 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 2). 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 2. Mean Repeat-Dose Toxicokinetics of TFV Following Oral Administration of GS-7340-02 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 = insufficient data to calculate

### 3.1.2. 13-Week Oral Mouse Toxicity Study

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 7.1, 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 3).

**Table 3. 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.

## 3.2. Rat

### 3.2.1. 4-Week Oral Rat Toxicity Study

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 red blood cell (RBC) parameters, calciuria, decreased BMD) decreased 1,25 vitamin $D_3$, renal karyomegaly, thymic atrophy, and atrophy of cancellous bone of the femur (m2.6.7, Section 7.2, 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 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).

### 3.2.2. 26-Week Oral Rat Toxicity Study

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, 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, with no TAF detected systemically (Table 4). 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 4. 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. 1-Week Oral Rabbit Toxicity Study

A 1-week nonGLP study evaluated the toxicity and determined the toxicokinetics of TAF (GS-7340-02), when administered daily via oral gavage to female New Zealand White rabbits, for 7 consecutive days. 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] hydroxypropylmethylcellulose [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·hr/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·hr/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·hr/mL for TAF and TFV respectively.

## 3.4. Dog

### 3.4.1. 4-Week Oral Dog Toxicity Study

Daily oral administration of 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 7.4, D990175) to male and female beagle dogs (4/sex/group) for 28 days resulted in a marginal rise in 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 NOAEL 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 (m2.6.5, Section 4.2, AD-D990175-PK).

**Table 5. 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 = insufficient data to calculate
PBMC = peripheral blood mononuclear cell

### 3.4.2. 39-Week Oral Dog Toxicity Study

Male and female beagle dogs were administered daily oral doses (10 mL/kg) of 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 7.5, 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 {29101}, {29104}. 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 toxicokinetic parameters for TAF and TFV are summarized in Table 6.

**Table 6. Mean Repeat-Dose Pharmacokinetics of TFV and TAF Following 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 | 3.60 | 2.07 | 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 | 2.23 | 1263.5 |
| 18 | Day 1 | 0.80 | 3.80 | 3.60 | 2.07 | NA |
| 12 | Week 13 | 0.54 | 12.48 | 3.24 | 1.03 | 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

## 3.5. Monkey

### 3.5.1. 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 7.6, 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 7) (P2000114-PK). 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 7. Mean Repeat-Dose Pharmacokinetics of TAF Following 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 = insufficient data to calculate

Day 28 TFV $C_{max}$ and AUC exhibited slightly greater than proportional increases with increasing dose (Table 8). 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 8. Mean Repeat-Dose Pharmacokinetics of TFV Following 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 |

## 3.6. EVG/COBI/FTC/TAF

Administration of TAF in combination with EVG, COBI and FTC is unlikely to exacerbate known toxicities of the individual agents. The 4 drugs, EVG, COBI, FTC, and TAF, exhibit different patterns of target organ toxicity (see also Section 9.8). For EVG, increases in cecal weights and/or its contents, likely due to weak antibacterial activity, and lipid-like vacuoles in the upper small intestine were associated with high local concentrations and are not considered relevant to clinical use. 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 exposure (137-fold in mice; 20-fold in monkeys); therefore, these hematological findings are not considered relevant to clinical use.

Cobicistat has the potential to prolong the PR interval; a modest, dosing-related increase in PR interval observed in the thorough QT study was not considered to be clinically significant (m2.7.2, Section 2.3.2.2.1 [Study GS-US-216-0107]). Although TAF showed some potential to prolong the PR interval (~13-24%) in the 39-week dog study at 18/12 mg/kg/day, the slight change was associated with bone and renal toxicity and included significant decreases in T3. No PR prolongation or any change in ECG results occurred in the safety pharmacology study that evaluated a TAF dose up to 100 mg/kg (m2.6.3, Section 4.2, D2000006) or in the thorough QT study (m2.7.2, Section 2.3.2.2.1 [Study GS-US-120-0107]).

Extensive nonclinical investigations of the toxicity of TAF have shown that unlike FTC, the bone marrow is not a target for this agent, and that the target organs for TAF are distinctly different (bone and kidney). Cobicistat, EVG, and FTC have not shown any potential for bone toxicity; thus, exacerbation of any TAF effects on bone is not expected. As pathological changes in the kidney have not been observed with EVG, COBI, or FTC, exaggerated renal toxicity is not anticipated to be an issue with the E/C/F/TAF combination product.

From in vitro data and clinical experience (m2.7.2), the anticipated drug-drug interaction upon administration of the 4-drug combination is 1) the intended inhibition of CYP3A activity by COBI and the consequent increase in EVG exposure and 2) the inhibition of Pgp-mediated intestinal secretion by TAF which increases the bioavailability of TAF. The inhibition of Pgp has minimal impact because of the comparatively low plasma concentrations of TAF and TFV.

Comprehensive nonclinical safety databases on these drugs, including combination toxicity studies with EVG and COBI, and with FTC and TDF, strongly indicate that further toxicological investigations are unlikely to yield new data relevant to humans. Further studies of longer duration with the combinations are not warranted because of the lack of significant overlapping toxicities, and the use of additional animals that would be required to obtain such information.

Extensive clinical safety data are available for each of the approved drugs. In addition the data for the FTC/TDF FDC product (Truvada), the Phase 2 and 3 trials with the EVG/COBI/FTC/TDF FDC approved product (Stribild) and the phase 2 and 3 trials with E/C/F/TAF FDC supports the use of these agents (see m2.7.4). The clinical data, along with the lack of overlapping toxicity in animals, support the safety of the new combination product for HIV-1 infection.

# 4. GENOTOXICITY

## 4.1. In Vitro

### 4.1.1. Mutagenicity Evaluation in the Ames *Salmonella*/Microsome Plate Assay

The mutagenic potential of GS-7340-02 was evaluated in vitro in the Salmonella-Escherichia coli/mammalian microsome reverse mutation assay either in the presence or absence of microsomal enzymes prepared from Aroclor™-induced rat liver. GS-7340-02 up to 5000 µg/plate (4000 µg f.b.e./plate) did not cause a positive increase in the mean number of revertants per plate with any of the tester strains (m 2.6.7, Section 8.1, V990212).

### 4.1.2. 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 thymidine kinase (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 (m 2.6.7, Section 8.2, 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.

## 4.2. In Vivo

### 4.2.1. 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 (m 2.6.7, Section 9, 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 at any dose level or harvest time point. TAF was negative for genotoxicity in this study. Statistically significant increases were apparent in the positive control animals.

## 4.3. EVG/COBI/FTC/TAF

Cobicistat, FTC, and TAF were negative in genotoxicity studies. Elvitegravir showed an equivocal effect in an in vitro chromosome aberration study, was negative in the reverse mutation study and in two separate in vivo micronucleus studies, and is unlikely to have

genotoxic potential in vivo. The combination of the 4 components is therefore not expected to have an altered genotoxicity profile as compared with that of the individual agents.

# 5. CARCINOGENICITY

Per agreement with the FDA (Reference ID: 3161161) and based on the scientific advice adopted by the CHMP, (EMA/CHMP/SAWP/629722/2012; EMEA/H/SA/2410/1/2012/1), carcinogenicity studies are not required for TAF registration due to 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.

## 5.1. 104-Week Oral Carcinogenicity Study in Mice with TDF

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 18.1, 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. 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. A no observed effect level (NOEL) for nonneoplastic lesions (by histological criteria) was not established in this study.

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 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.

### 5.2. 104-Week Oral Carcinogenicity Study in Rats with TDF

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 18.2, 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 ophthalmoscopic findings or 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.

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.

## 5.3. EVG/COBI/FTC/TAF

No clinically relevant increases in tumor incidence were found in mice or rats administered EVG, COBI, or FTC in long-term carcinogenicity studies. In a long-term carcinogenicity study of cobicistat in rats, an increased incidence of follicular cell adenomas and/or carcinomas in the thyroid gland was observed; these findings are considered to be rat-specific, secondary to hepatic microsomal enzyme induction and thyroid hormone imbalance and not relevant for humans.

Therefore, EVG, COBI, FTC and TDF have all demonstrated low carcinogenic potential in conventional 2-year bioassays at exposures that exceeded (TDF, COBI) or far exceeded (EVG, FTC) human exposures at the therapeutic dose (m2.4, Section 4.9.2). It is considered unlikely that combination dosing would 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 the data from the 2-year carcinogenicity studies with the individual agents, the conduct of carcinogenicity studies with the E/C/F/TAF combination are not considered necessary nor would they add to the safety evaluation.

# 6. REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

## 6.1. Fertility and Early Embryonic Development

### 6.1.1. Oral Gavage Study of Fertility and Early Embryonic Development to Implantation with GS-7340-03 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, 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 million/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).

## 6.2. Embryo-Fetal Development

### 6.2.1. Oral Range Finding Embryo-Fetal Development Study 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, 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 GD 8 and 10 when compared to controls, with marked differences also seen from GD10 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 GD 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 GD 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 9. 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

### 6.2.2. Oral Embryo-Fetal Development Study 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 13.1, 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 doses of 20, 80, 200 mg f.b.e./kg, 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 GD 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 GD 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 GD 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 GS-7340-02 dose level from 25 to 250 mg/kg/day. Exposure to TFV increased with the increase in GS-7340-02 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. 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 NOAEL and the NOAEL for embryo-fetal development were both considered to be the mid dose level, which resulted in GD17 $AUC_{0-t}$ values of 17.4 and 0.2 µg·hr/mL for TFV and TAF, respectively.

**Table 10. 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

### 6.2.3. Oral Range Finding Embryo-Fetal Development Study 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, 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 (4, 20, 40, 80 mg f.b.e./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. 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.

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·hr/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·hr/mL for TFV and TAF, respectively).

**Table 11. 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

### 6.2.4. Oral Embryo-Fetal Development Study 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 13.2, 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 (8, 24, 80 mg f.b.e./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 GS-7430-02-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 TAF 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.

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.

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 12. 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

## 6.3. Prenatal and Postnatal Development

Per agreement with the FDA (ID 3244145) and the EMEA (FAL_2410-1-FU-1-2013) a peri/postnatal study in rats is not required for TAF registration due to the lack of TAF exposure in rats and lower TFV exposure compared to TDF. The peri/postnatal study conducted with TDF is provided below.

### 6.3.1. 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 18.3,

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 reduced to 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 were increased to 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.

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).

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).

In conclusion, 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 13. Mean Toxicokinetic Parameters of TFV in Rats on Gestation Day (GD) 7 and Lactation Day (LD) 20**

| TDF (mg/kg/day) | Day | TFV | |
|---|---|---|---|
| | | $C_{max}$ (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 |

On the basis of these data, 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. EVG/COBI/FTC/TAF

With no expected toxicologic interactions with the E/C/F/TAF combination, further studies with E/C/F/TAF combination are not considered necessary.

There were no significant effects on embryo-fetal development in rats or rabbits when EVG, COBI, FTC, and TAF were tested individually, and no effects when EVG was administered in combination with RTV. No cause for concern has been identified and studies with the E/C/F/TAF combination are unlikely to show new effects.

Slightly longer estrous cycles were observed in $F_1$ generation rats after exposure to high doses of FTC and a delay in sexual maturation was observed in $F_1$ generation rats after exposure to high (maternally toxic) doses of TDF. No significant effects were noted for EVG, or COBI. For all 4 individual agents, NOELs/NOAELs were at exposures above human exposures.

Although COBI increased postimplantation loss and decreased fetal weights and TDF reduced the viability index and weight of pups in the peri- and post-natal toxicity studies at maternally toxic doses, the NOEL and NOAEL have been clearly identified. The TFV and COBI exposures were 29-fold and 1.8-fold above human exposures in the E/C/F/TAF FDC. COBI, TDF nor TAF showed an effect on rabbit fetuses. Elvitegravir and FTC have not shown any adverse effects in reproductive and developmental toxicity studies. No additive effects are anticipated with the four-drug combination.

There were no notable findings in the juvenile toxicity studies with EVG or with COBI. No specific studies were conducted with the E/C/F/TAF combination. The E/C/F/TAF FDC will initially be registered for the treatment of HIV-1 infection in patients ≥ 12 years of age.

# 7. LOCAL TOLERANCE

## 7.1. Ocular Irritation Study in Bovine Corneas

The Bovine Corneal Opacity and Permeability Assay (BCOP) was performed to assess the ocular irritancy potential of GS-7340-03 in vitro (m2.6.7, Section 16, 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 In Vitro Irritancy Score which can be used to classify and rank test substances as potential eye irritants according to OECD guideline 437. TAF elicited an In Vitro Irritancy Score of 21.0 ± 8.7 with a 4-hour incubation and was predicted to be a noncorrosive/nonsevere eye irritant.

## 7.2. 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, TX-120-2011). No dermal erythema or edema 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-irritating/noncorrosive to rabbit skin.

## 7.3. Phototoxicity Assessment

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 ($\lambda_{max}$) were observed at 205 and 262 nm and the measured molar extinction coefficient at the characteristic peak at 262nm was $1.5 \times 10^4\ M^{-1}cm^{-1}$. Because TAF fumarate does not absorb light within the range of natural sunlight, no phototoxicity evaluation is deemed appropriate.

## 7.4. EVG/COBI/FTC/TAF

The E/C/F/TAF tablet is intended for oral use. No local tolerance studies were conducted for the E/C/F/TAF combination.

# 8. OTHER TOXICITY STUDIES

## 8.1. Antigenicity

### 8.1.1. 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, 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 lymph node cells (LNC) 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 Stimulation Index (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 SI 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 which 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.

### 8.1.2. EVG/COBI/FTC/TAF

Studies with EVG, COBI and TAF have not indicated that antigenicity is of concern. No studies were deemed necessary with the E/C/F/TAF combination.

## 8.2. Immunotoxicity – EVG/COBI/FTC/TAF

Data from repeat-dose toxicity studies with EVG, FTC, or TAF (hematology, lymphoid organ weights, microscopy of lymphoid tissues, bone marrow cellularity) and specialized immunotoxicity studies with EVG and FTC do not suggest immunotoxic potential for any of these agents. There were no notable effects of the combination of FTC/TDF on immunophenotyping or natural killer cell assay values in a 4-week dog study. For COBI, decreased IgG levels were noted in female rats at dose levels above the NOAEL for systemic toxicity in the immunotoxicity study. However, in standard toxicity studies with COBI in mice, rats and dogs, and at higher dose levels and exposures and longer treatment periods, no signs of immune function changes have been observed. No further studies were deemed necessary with the E/C/F/TAF combination.

## 8.3. Mechanistic Studies

### 8.3.1. Bone Turnover

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

#### 8.3.1.1. 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 17, R990139). However, the results are confounded due to administration of another compound in addition to GS-7340-02 on 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, 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 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, 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 $CO_2$ 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 AMP (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.

8.3.1.2. 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, 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 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 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.

### 8.3.2. 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, 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 TAF. Nucleoside analogs, as a class, have no known properties that would suggest development of dependence. There was no evidence of development of dependence in preclinical studies with TAF. In the tissue distribution studies using [$^{14}$C]TAF, little or no radioactivity was observed in brain tissues from dog (m2.6.5, Sections 5.3 and 5.4, D990173-BP) and rat (m2.6.5, Section 5.2, 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 5.1, AD-120-2011). Consequently, dependency studies were not considered warranted.

## 8.5. Metabolites

In view of the absence of unique human metabolites of TAF (see m2.6.4, Section 5.1), no specific safety studies on TAF metabolites have been conducted.

No specific studies with TAF metabolites were conducted as there were no unique human metabolites in humans with TAF. 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 (m2.6.4). Isopropanol, l-alanine, and phenol are formed at nontoxic levels. The by-products formed during conversion of TDF to TFV are isopropanol, carbon dioxide and formaldehyde.

Clinical data from Study GS-US-120-0104 demonstrated that the mean intracellular concentrations of tenofovir diphosphate (TFV-DP) ranged 0.677-1.49 µM in PBMCs in the patients dosed once daily at 25 mg TAF (PC-120-2008). Since the predominant PBMC metabolite of TAF is TFV-DP (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 {7415}. No prodrug was found in PBMCs; overall, TAF showed low cytotoxicity in PBMC (6.8 µM in dividing cells) and T-cell lines (≥ 23.2 µM) compared to other clinically relevant antiretroviral inhibitors. Based on observed $CC_{50}$ and $EC_{50}$ values, TAF exhibited Selectivity Indexes relative to antiviral activity of > 1900 in dividing PBMCs and of 1997 to > 3607 in T-lymphoblastoid cell lines (m2.6.2, Section 3.4.3.1).

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 5g/kg have been reported to cause adverse effects (http://chem.sis.nlm.nih.gov/chemidplus/rn/67-63-0; accessed [redacted])

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 [redacted].) IARC has determined that phenol is not classifiable as to its carcinogenicity to humans (Group 3) {29103}. Phenol is an inactive ingredient in 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 [redacted]). Examples of approved prescription drugs used chronically (eg, glycemic control, acromegaly) that contain phenol are presented in Table 14.

**Table 14. 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<br>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 t.i.d. which would be 0.5 mL TID or 7.5 mg phenol/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. Oral Rat Toxicity Studies

There was no indication of any unexpected toxicity in any of these studies.

#### 8.6.1.1. 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, 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 (K100LV) prepared in reverse osmosis water] or GS-7340-02 at 5 or 50 mg/kg/day 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·hr/mL for 97.7% pure GS-7340-02 (Lot No. 1: 7340-02-AC-1EA) and the TFV $C_{max}$ was 395 ng/mL and the $AUC_{0-t}$ was 2481 ng·hr/mL for 83.1% pure GS-7340-02 (Lot No. 2: 7339-AZS-27-1094-41).

8.6.1.2. 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 for at least 28 days (m2.6.7, Section 17, TX-120-2021). Three lots of GS-7340-03 were each administered at 25 and 50 mg/kg/day (free base equivalents). 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.

## 8.7. EVG/COBI/FTC TAF Tablets

The E/C/F/TAF tablets are monolayer tablets. No degradation products related to EVG have been identified in batches of E/C/F/TAF tablets. Several degradation products related to COBI and FTC have been identified in batches of E/C/F/TAF tablets and all of them have been observed in batches of EVG/COBI/FTC/TDF tablets. Some of these products were tested in a 28-day toxicity study in rats using non-degraded and degraded EVG/COBI tablets (m2.6.7, Section 17.3, TX-236-2002). The impurity profile for these batches used in the toxicology study is provided in Tabulated Summary 2.6.7.4.3. The degradation products of TAF observed in the E/C/F/TAF tablets is consistent with those in the TAF drug substance. There are no unique impurities or degradants in the E/C/F/TAF tablets.

The impurities and degradation products present in EVG, COBI, FTC, and TAF and in E/C/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 [EVG/COBI/FTC/TAF Tablets] and m3.2.P.5.6, Justification of Specifications [EVG/COBI/FTC/TAF Tablets]).

# 9. DISCUSSION AND CONCLUSIONS

An extensive evaluation of the toxicologic profile of TAF has been conducted.

## 9.1. Target Organ Toxicity

The oral toxicity of TAF has been studied in mice, (TX-120-2006, TX-120-2007), rats (R990185, R990182, TOX-120-001) dogs (D990181, D990142, D990175, TOX-120-002) and monkeys (P2000114) 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- and 25-hydroxyvitamin $D_3$ at doses in rats and dogs.

### 9.1.1. 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.

### 9.1.2. 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- 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 vitamin $D_3$ in males only and a significant increase in 25-hydroxyvitamin $D_3$ in females only.

### 9.1.3. 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{29101}, {29104}. 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), 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 (AD-120-2011, AD-120-2020, and D990173-BP). 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 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 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 up to 1μM (approximately 2-fold higher than $C_{max}$ after a 25 mg TAF dose), the highest concentration tested, in HepG2 cells in a 10-day assay (PC-120-2006). The active metabolite of TAF, tenofovir diphosphate is highly discriminated as a substrate by mitochondrial DNA polymerase γ relative to the natural substrate, ATP (>10,000 fold) {4923}. No toxicity

indicative of mitochondrial toxicity was observed in nonclinical or clinical studies. Therefore, TAF is unlikely to inhibit mitochondrial DNA polymerase γ under clinical relevant conditions. Therefore, TAF is unlikely to inhibit mitochondrial DNA polymerase γ under clinical relevant conditions.

## 9.2. Genetic Toxicology

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).

Cobicistat, FTC and TAF were negative in genotoxicity studies. Elvitegravir showed an equivocal effect in an in vitro chromosome aberration study, was negative in the reverse mutation study and in 2 in vivo micronucleus studies, and is unlikely to have genotoxic potential in vivo. Therefore, the combination of the 4 components is not expected to have an altered genotoxicity profile as compared with that of the individual agents.

## 9.3. Carcinogenicity

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 female mice, liver adenomas were increased at exposures 16 times those observed in humans. In rats, the study was negative for carcinogenic findings at exposures up to 5 times those observed in humans at the therapeutic dose.

Therefore, EVG, COBI, FTC, and TDF have all demonstrated low carcinogenic potential exposures that exceeded (COBI, TDF/TFV) or far exceeded (EVG, FTC) human exposures at the therapeutic dose. It is considered unlikely that combination dosing would change these profiles as no exposure difference is expected and no exacerbation of toxicity/genotoxicity is expected.

Based on the overall lack of genotoxicity and the results of the 2-year carcinogenicity studies with the individual agents, the conduct of carcinogenicity studies with the E/C/F/TAF combination is not considered necessary nor would they add to the safety evaluation.

## 9.4. Reproductive Toxicity

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 (TX-120-2012) 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 (TX-120-2002), or in pregnant rabbits administered GS-7340-02 up to 100 mg/kg/day (TX-120-2005). 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 DF, 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 (R990202). No TDF-related deaths occurred. The maternal NOEL of TDFwas 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/postparturn pup mortality, reduced pup survival and reduced pup body weights. The NOEL for general toxicity of TDF in the F1 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 F1 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).

No peri/postnatal study with TAF in rats is planned. Toxicokinetic data obtained following dosing of TAF to the 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 peri/postnatal study (up to 15534 ng·h/mL via TAF compared to 27.3 µg h/mL via TDF). As summarized above, TFV, delivered via TDF, has previously been assessed for peri/postnatal effects at exposures 100-times human exposure of TFV via TAF (0.273 µg·h/mL). The peri/postnatal study with TDF clearly characterized the potential postnatal toxicity of TFV.

Elvitegravir, COBI, FTC, TAF and TDF have not shown significant adverse effects in reproductive and developmental toxicity studies. The combination of the 4 components is not expected to have an altered reproductive toxicity profile compared with that of the individual agents.

## 9.5. Local Tolerance

Evaluation of local tolerance to the GI tract was conducted during the repeat-dose oral toxicity studies. No clinical or histopathological changes occurred in the GI tract in the TAF repeat-dose toxicity studies in mice, rats or monkeys. After 39 weeks of dosing dogs at 18/12 mg/kg/day, observations included atrophy of gut-associated lymphoid tissue (GALT), 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. GI toxicity was not dose limiting.

Local toxicity studies concluded that TAF was predicted to be a noncorrosive/nonsevere eye irritant (TX-120-2013), and nonirriating/noncorrosive to rabbit skin under semi-occluded conditions (TX-120-2011). TAF did not show the potential to cause skin sensitization with repeated application to the dorsal surface of mouse ears (TX-120-2014).

The E/C/F/TAF tablet is intended for oral use. No local tolerance studies were conducted for the E/C/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. Although EVG and COBI have shown changes in the stomach, they have occurred at high dose levels associated with high local concentrations, or have been isolated occurrences that have not been observed in other long term studies and are not considered clinically relevant to the E/C/F/TAF FDC. In the pivotal registration clinical studies (m2.5), E/C/F/TAF was generally well tolerated; the incidences of GI disorders were consistent with those expected in the subject population and the known safety profiles of the study drugs.

## 9.6. Mechanistic Studies

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.

## 9.7. Metabolites/Impurities/Degradation Products

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 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.

## 9.8. Comparative Exposures and Safety Margins

Cross-species comparisons of exposure, relative safety margins, and the relevance of these effects for human health are discussed in m2.4, Section 4.9.2.

## 9.9. Conclusions

Comprehensive nonclinical toxicology programs were conducted with each of the 4 agents in support of the registration of EVG, COBI, FTC and TAF. Two-drug combination toxicity studies were also conducted. The data is adequate to support the safety of the E/C/F/TAF FDC based on the following considerations.

Administration of TAF in combination with EVG, COBI and FTC is unlikely to exacerbate known toxicities of the individual agents. The 4 drugs, EVG, COBI, FTC, and TAF, exhibit different patterns of target organ toxicity with no clinically-significant overlapping toxicity. For EVG, increases in cecal weights and/or its contents, likely due to weak antibacterial activity, and lipid-like vacuoles in the upper small intestine were associated with high local concentrations and are not considered relevant to clinical use. 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 exposure (137-fold in mice; 20-fold in monkeys). Slight but statistically significant decreases (-11%) in several red blood cell parameters were also noted in male and female rats administered TAF at a dose level of 400 mg/kg/day for 28 days. Anemia was not observed with EVG or COBI. Because hematological changes were slight and observed at high multiples of the clinical dose, effects are not considered relevant to clinical use.

Cobicistat was associated with urinalysis and urine chemistry changes (increased urine volume; decreased urine specific gravity; increased electrolyte excretions) at high doses in rats and dogs. These changes showed no progression after long-term dosing, were reversible, were not associated with remarkable clinical chemistry changes (including serum creatinine and BUN), and were without morphological evidence of kidney damage. COBI is associated with weak inhibition of human renal transporters OCT2, MRP4, and MATE2-K, and is a more potent inhibitor of OCTN1 and MATE1, with similar potencies being found for RTV (m2.6.4, Section 7.2.4.1). Clinical data with COBI show that COBI produces a small increase in serum creatinine, but that COBI does not affect actual glomerular filtration rate (GFR) when measured with iohexol or cysytin C (m2.7.4, Section 2.1.5). Given that there is no apparent pathological change in the kidney due to COBI and that the routes of excretion differ for TFV and COBI, and that COBI would not be expected to inhibit the renal transporters of TFV at clinically relevant concentrations, it is not anticipated that the combination of EVG/COBI/FTC/TAF would exacerbate the renal toxicity of TAF. The small increase in serum creatinine levels may be associated with inhibition of MATE 1 mediated creatinine secretion by COBI {20331}. In fact, the E/C/F/TAF FDC produces a smaller increase in serum creatinine than the EVG/COBI/FTC/TDF FDC.

Cobicistat has the potential to prolong the PR interval; a modest, dosing-related increase in PR interval observed in the thorough QT study was not considered to be clinically significant (m2.7.2, Section 2.3.2.2.1 [Study GS-US-216-0107]). Although TAF showed some potential to prolong the PR interval in the 39-week dog study at 18/12 mg/kg/day, the slight change was associated with bone and renal toxicity and included significant decreases in T3 {29101}, {29104}. No PR prolongation or any change in ECG results occurred in the safety pharmacology

study that evaluated a TAF dose up to 100 mg/kg (m2.6.3,Section 4.2 D2000006) or in the thorough QT study (m2.7.2, Section 2.3.2.2.1 [Study GS-US-120-0107]).

After COBI administration, the slight hepatocellular hypertrophy (in mice and rats) and follicular cell hyperplasia/hypertrophy in the thyroid observed in rats, and follicular cell adenomas/carcinomas in the thyroid in rats, are considered rodent-specific changes that are commonly seen with microsomal enzyme inducers. Because of the species specificity of the effects, these findings are not of concern for humans. Minimal to mild increases in liver enzyme activities (ALT, bilirubin, and ALP) were observed in dogs after 4 weeks of dosing, and hepatocyte vacuolation and/or hypertrophy were observed in the liver of dogs administered COBI in 4-week and 39-week studies. A 13-week combination toxicity study in rats with EVG and COBI showed no evidence of new toxicities or no additive toxicity with the combination.

The primary target organs for TAF are the kidney and bone. Cobicistat, EVG, and FTC have not shown any potential for pathology changes in the kidney or bone in chronic rat and dog toxicity studies; thus, exacerbation of any TAF effects on kidney and bone are not anticipated to be an issue with the E/C/F/TAF combination product. Adverse degenerative (olfactory) and acute inflammatory (infiltrate neutrophil) changes in the nasal mucosa was observed after administration of TAF to mice. 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 considered low. A minimal infiltration of histiocytes was present in some organs (eye [choroid plexus, ciliary body], lung, and spleen) in dogs administered the highest dose of TAF in the 39-week dog toxicity study. 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 and was most likely due to general debilitation. Based on the evidence from tissue distribution and toxicology studies, Gilead concludes that the risk of posterior uveitis in humans is very low.

The primary anticipated significant drug-drug interaction upon administration of the 4-drug combination is the intended inhibition of CYP3A activity by cobicistat and the consequent increase in EVG exposure. Cobicistat is also anticipated to increase the bioavailability of TAF through inhibition of intestinal Pgp.

NRTIs currently carry a class labeling for mitochondrial toxicity; however, both FTC and TAF have shown a low potential for mitochondrial toxicity in in vitro studies and in repeat-dose toxicity studies up to 39 weeks in duration. In an in vitro study, EVG showed no inhibitory effects on mitochondrial DNA suggesting a low potential for mitochondrial toxicity. No toxicity indicative of mitochondrial toxicity was observed in nonclinical or clinical studies. Therefore, TAF is unlikely to inhibit mitochondrial DNA polymerase γ under clinical relevant conditions. The potential for mitochondrial toxicity with the FDC is low.

None of the 4 compounds had positive findings in genotoxicity. Although EVG showed an equivocal effect in an in vitro chromosome aberration study, it was negative in 2 in vivo micronucleus studies and is unlikely to have the potential to induce chromosome aberrations in vivo. The E/C/F/TAF combination is not anticipated to alter the genotoxicity profile of the individual agents.

Elvitegravir, COBI, FTC, and TDF have all demonstrated low carcinogenic potential in conventional 2-year bioassays. Combination dosing with E/C/F/TAF is unlikely to alter this profile.

Elvitegravir, COBI, FTC, and TAF have not shown significant adverse effects in reproductive and developmental toxicity studies, and the combination of the 4 components is not expected to have an altered reproductive toxicity profile compared with that of the individual agents.

The E/C/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 FDA Guidance for Industry, *Nonclinical Safety Evaluation of Drug or Biologic Combinations, March 2006* and the CHMP Guideline on the Non-Clinical Development of Fixed Combinations of Medicinal Products (EMEA/CHMP/SWP/258498/2005, January 2008). The primary anticipated significant drug-drug interaction upon administration of the 4-drug combination is the intended inhibition of CYP3A activity by COBI and the consequent increase in EVG exposure. Cobicistat is also anticipated to increase the bioavailability of TAF through inhibition of intestinal Pgp. Because the target organ profiles are different, and there is no evidence of genotoxicity, carcinogenicity, or reproductive toxicity, administration of the E/C/F/TAF combination product is unlikely to introduce new toxicities or to exacerbate known toxicities of the individual agents. The ample nonclinical safety databases on these drugs, including combination toxicity studies with EVG and COBI, and with FTC and TDF, strongly indicates further toxicological investigations are unlikely to yield new data relevant to humans. Additionally, the extensive clinical safety data available from the clinical trials with the EVG/COBI/FTC/TDF FDC and with the E/C/F/TAF FDC support the safety of the new combination product for the treatment of HIV-1 infection in adult and pediatric patients 12 years of age and older without any known mutations associated with resistance to the individual components of E/C/F/TAF.

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**2.6 NONCLINICAL SUMMARY**

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**SECTION 2.6.7—TOXICOLOGY TABULATED SUMMARY**

# ELVITEGRAVIR/COBICISTAT/EMTRICITABINE/ TENOFOVIR ALAFENAMIDE FIXED-DOSE COMBINATION (EVG/COBI/FTC/TAF [E/C/F/TAF] FDC)

Gilead Sciences

[redacted] **20**[redacted]

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**CONFIDENTIAL AND PROPRIETARY INFORMATION**

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## NOTE TO REVIEWER

This application is being submitted in support of a new drug application (NDA) for a fixed dose combination (FDC) that contains the integrase strand transfer inhibitor (INSTI) elvitegravir (EVG, E, Vitekta®), the pharmacokinetic enhancer cobicistat (COBI, C, Tybost®), the nucleoside reverse transcriptase inhibitor (NRTI) emtricitabine (FTC, F, Emtriva®), and the nucleotide reverse transcriptase inhibitor (NtRTI) tenofovir alafenamide (TAF, GS-7340): the E/C/F/TAF FDC (150/150/200/10 mg) tablet. E/C/F/TAF is indicated for the treatment of human immunodeficiency virus, type 1 (HIV-1) infection in adult and pediatric patients 12 years of age and older without any known mutations associated with resistance to the individual components of E/C/F/TAF.

As TAF is a new chemical entity, this nonclinical summary contains all available data on this new component. Per the agreement reached between Gilead and the Food and Drug Administration (FDA; [redacted]), this NDA is supported by [redacted]. To assist the reviewer, [redacted]

[redacted]

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 ratio of free base to fumarate), and GS-7340-03, synonym for the hemifumarate (2:1 ratio of free base to fumarate). The hemifumarate, GS-7340-03 (TAF fumarate) was selected as the form for final development. GS-7340-03 is considered comparable to GS-7340-02 based on physical/chemical properties and pharmacokinetic data.

The following conversions are provided to aid the reviewer:

- EVG (GS-9137, JTK-303) 1 µM = 0.448 µg/mL
- COBI (GS-9350) 1 µM = 0.776 µg/mL
- FTC 1 µM = 0.247 µg/mL
- TAF (GS-7340) 1 µM = 0.477 µg/mL
- TFV 1µM = 0.287 µg/mL

# 1. TOXICOLOGY: OVERVIEW

**Test Article: TAF**

| Type of Study/Species/Strain/Form | Method of Administration | Duration of Dosing | Dose (mg/kg)[a] | GLP[b] | Testing Facility | Study No. (CRO Study No.) |
|---|---|---|---|---|---|---|
| **Single-Dose Toxicity** | | | | | | |
| Rat/Sprague Dawley; 7340-02 | Oral Gavage | 1 day | 0, 6.25, 25, 100[d], 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[c], 90, 270 | Yes | [redacted], Canada | D990181 ([redacted] 89187) |
| **Repeat-Dose Toxicity** | | | | | | |
| Mouse/CD:CD1(ICR); 7340-02 | Oral Gavage | 2 weeks | 0, 100, 500, 1000 | No | [redacted] | TX-120-2006 ([redacted] 8235665) |
| Mouse/CD:CD1(ICR); 7340-02 | Oral Gavage | 13 weeks | 0[e], 10, 30, 100 | Yes | [redacted] | TX-120-2007 ([redacted] 8241074) |
| Rat/Sprague-Dawley (Crl): 7340-02 | Oral Gavage | 6 days | 0, 100, 400 | No | Gilead Sciences, Inc. | R2000044 (NA) |
| Rat/Sprague Dawley; 7340-02 | Oral Gavage | 7 days | 0, 6.25, 25, 100, 400 | No | Gilead Sciences, Inc. | R990139 (NA) |
| Rat/(Crl:CD (SD) BR); 7340-02 | Oral Gavage | 4 weeks | 0, 1.5, 6.25, 25, 100, 400 | Yes | [redacted] Canada | R990182 ([redacted] 89176) |
| Rat/(Crl:CD (SD) BR); 7340-02 | Oral Gavage | 26 weeks | 0, 5, 25, 100 | Yes | [redacted], Canada | TOX-120-001 ([redacted] 89686) |

**Test Article: TAF**

| Type of Study/Species/Strain/Form | Method of Administration | Duration of Dosing | Dose (mg/kg)[a] | GLP[b] | Testing Facility | Study No. (CRO Study No.) |
|---|---|---|---|---|---|---|
| Rabbit/NZW; 7340-02 | Oral Gavage | 7 days | 0, 20, 50, 75 | No | [redacted], Canada | TX-120-2003 ([redacted] 902246) |
| Dog/Beagle; 7340-02 | Oral Gavage | 4 weeks | 0, 0.1, 0.3, 1[c], 3, 10 | Yes | [redacted], Canada | D990175 ([redacted] 89128) |
| Dog/Beagle; 7340-02 | Oral Gavage | 39 weeks | 0, 2, 6. 18/12 | Yes | [redacted], Canada | TOX-120-002 ([redacted] 89647) |
| Monkey/cynomolgus; 7340-02 | Nasogastric Gavage | 4 weeks | 0, 3, 30 | Yes | [redacted] | P2000114 ([redacted] 1050-11) |
| **Genotoxicity** | | | | | | |
| 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; 7340-02 | Oral Gavage | 1 day | 500, 1000, 2000 | Yes | [redacted] | M2000113 ([redacted] 21816-0-455OECD) |

**Test Article: TAF**

| Type of Study/Species/Strain/Form | Method of Administration | Duration of Dosing | Dose (mg/kg)[a] | GLP[b] | Testing Facility | Study No. (CRO Study No.) |
|---|---|---|---|---|---|---|
| **Reproductive and Developmental Toxicity** | | | | | | |
| Fertility/Rat, Crl:CD(SD); 7340-03 | Oral Gavage | M: 4 weeks premating, 10 weeks total<br>F: 2 weeks premating - GD 13 | 0, 20, 80[i], 160[j] base eq | Yes | [redacted] | TX-120-2012 ([redacted] 8264528) |
| Dose Range Finding Embryo-Fetal Development/ Rat, Crl:CD(SD); 7340-02 | Oral Gavage | GD 6-17 | 0, 5, 100[g], 200[h] | Yes | [redacted], Canada | TX-120-2001 ([redacted] 902244) |
| Embryo-Fetal Development/ Rat, Crl:CD (SD); 7340-02 | Oral Gavage | GD 6-17 | 0, 25, 100[gh], 250 | Yes | [redacted], Canada | TX-120-2002 ([redacted] 902245) |
| Dose Range Finding Developmental DRF/Rabbit, Hra: (NZW) SPF; 7340-02 | Oral Gavage | GD 7-20 | 0, 5, 25, 50[cg], 100[ch] | Yes | [redacted], Canada | TX-120-2004 ([redacted] 902247) |
| Developmental/Rabbit, Hra: (NZW) SPF; 7340-02 | Oral Gavage | GD 7-20 | 0, 10, 30[g], 100[ch] | Yes | [redacted], Canada | TX-120-2005 ([redacted] 902248) |
| **Local Tolerance** | | | | | | |
| In Vitro Tests/Bovine Corneal Opacity and Permeability Assay; (BCOP); 7340-03 | Topical | 4 hr | 20% (w/w) in 0.9% sodium chloride solution | Yes | [redacted], UK | TX-120-2013 ([redacted] 0017) |
| In Vivo Tests/Rabbit Dermal Irritation/Hra: (NZW)SPF; 7340-02 | Topical | 4 hr | 0.5g/rabbit | Yes | [redacted] | TX-120-2011 ([redacted] 8253834) |

**Test Article: TAF**

| Type of Study/Species/Strain/Form | Method of Administration | Duration of Dosing | Dose (mg/kg)[a] | GLP[b] | Testing Facility | Study No. (CRO Study No.) |
|---|---|---|---|---|---|---|
| **Other Toxicity Studies** | | | | | | |
| LLNA/Mouse, CBA/Ca; 7340-03 | Topical | 3 days | 10, 25, 50% (w/v) | Yes | [redacted], UK | TX-120-2014 ([redacted] 0018) |
| Impurity Qualification in Rat; 7340-02 | Oral Gavage | 2 weeks | 0, 5, <u>50</u> | Yes | [redacted] | TX-120-2008 ([redacted] 8246754) |
| Impurity Qualification in Rat; 7340-03 | Oral Gavage | 4 weeks | 0, 25, <u>50</u> | Yes | [redacted] | TX-120-2021 ([redacted] 8298810) |
| Bone Metabolism in Rat (Sprague Dawley); 7340-02 | Oral Gavage | 6 days | 0, 400 | No | Gilead Sciences, CO | R990177 (NA) |
| Bone Metabolism in Rat (Sprague Dawley); 7340-02 | Oral Gavage | 6 days | 100, 400 | No | Gilead Sciences, CO | R2000044 (NA) |
| Renal Effect in Rat (Sprague Dawley); 7340-02 | Oral Gavage | 1 day | 0, 100, 1000 | Yes | [redacted], Canada | R990186 ([redacted] 56520) |
| Bone marker Assessment Dog (Beagle); 7340-02 | Oral gavage | 5 days | 37.5, 75 | Yes | [redacted] | D990142 ([redacted] 9529.3) |

a Unless otherwise specified, the highest No-Observed-Adverse-Effect Level (NOAEL) is underlined.
b 'Yes' indicates study includes a GLP Compliance statement
c NOEL
d NOAEL greater than
e NOAEL could not be determined
f NOEL could not be determined
g Maternal
h Embryo-fetal
i Male and female
j Early embryonic toxicity
TAF = GS-7340; (GS-)7340-02 = monofumarate form; (GS-)7340-03 = hemifumarate form; NA = not applicable; DRF = dose range-finding; GD = gestation day; F = female; M = male;

# 2. TOXICOKINETICS: OVERVIEW OF TOXICOKINETICS STUDIES

**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-120-2001 |
| 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

# 3. TOXICOKINETICS: OVERVIEW OF TOXICOKINETICS DATA

**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] | |
| 0.15 | | | 0.05[c@] | 0.06 [c@] | | | | |
| 0.3 | | | | | | NC[e] | NC[e] | |
| 1 | | | | | | NC[e] | NC[e] | |
| 2 | | | | | | 1.30[f*] | 1.07[f*] | |
| 3 | | | | | | NC[e] | NC[e] | 0.35[a*] |
| 5 | | | 0.7 [d*]<br>0.02[k§]<br>0.07[k§] | 0.7 [d*]<br>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[*] | 12.57[f*] | |
| 20 | | | | | 2.3[l] | | | |
| 25 | | | 2.5[c@]<br>3.8[d*]<br>2.4[n*∞]<br>3.0[n*∂]<br>2.6[n*ß] | 2.8[c@]<br>3.8[d*]<br>2.8[h] | 6.0[i] | | | |

**Test Article: TAF**

| Species | Steady State Tenofovir (R-PMPA) AUC (µg•h/mL) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Mouse | | Rat | | Rabbit | Dog | | Monkey |
| 30 | 1.56[b] | 1.45[b] | | | 5.0[j] | | | 5.87[a*] |
| 50 | | | 2.0[k$]<br>2.5[k§]<br>6.8[n*∞]<br>6.0[n*∂]<br>6.0[n*ß] | | 11.8[i]<br>5.7[l] | | | |
| 75 | | | | | 10.1[l] | | | |
| 100 | 5.97[m]<br>7.80[b] | 5.51[m]<br>6.55[b] | 12.2[c@]<br>15.5[d*] | 14.6[c@]<br>15.5[d*]<br>20.6[g]<br>17.4[h] | 23.5[i]<br>27.3[j] | | | |
| 200 | | | | 35.7[g] | | | | |
| 250 | | | | 55.7[h] | | | | |
| 400 | | | 62.7[c@] | 75.1[c@] | | | | |
| 500 | 27.2[m] | 30.8[m] | | | | | | |

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)

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 $AUC_{0-t}$; [@] = $AUC_{0-last}$; [*] = males and females combined; [$] = 97.7% purity; [§] = 83.1% purity; [∞] = 99.3% purity; [∂] = 98.0% purity; [ß] = 987.9% purity

# 4. TOXICOLOGY: DRUG SUBSTANCE

| Batch No. | Purity (%) | TAF Impurities (%) | | | | | | | | | | Type of Study |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | *related substance A | *related substance B | [redacted] | [redacted] | *related substance C | [redacted] | GS-7339 | [redacted] | [redacted]# | [redacted] | |
| **Specification** | [redacted] | ≤ [redacted] | ≤ [redacted] | ≤ [redacted] | ≤ [redacted] | ≤ [redacted] | ≤ [redacted] | ≤ [redacted] | ≤ [redacted] | ≤ [redacted] | ≤ [redacted] | |
| 1309-96-32 | [redacted] | — | — | — | — | — | — | — | — | — | NT | a |
| 1504-187-19 | [redacted] | — | — | — | — | — | — | [redacted] | — | — | NT | b |
| 1603-167-36 | [redacted] | — | — | [redacted] | — | — | — | [redacted] | [redacted] | — | NT | c |
| 1864-16-30 | [redacted] | [redacted] | — | [redacted] | — | — | — | [redacted] | — | [redacted] | NT | d |
| 7340-02-B-1 | [redacted] | — | — | — | — | — | — | [redacted] | — | — | NT | e |
| 7340-02-AC-1EA | [redacted] | [redacted] | [redacted] | [redacted] | [redacted] | [redacted] | [redacted] | [redacted] | [redacted] | [redacted] | NT | f |
| 7340-02-AC-1EB | [redacted] | [redacted] | [redacted] | [redacted] | [redacted] | [redacted] | [redacted] | [redacted] | [redacted] | [redacted] | [redacted] | g |
| 7339-AZS-27 -1094-41 | [redacted] | [redacted] | [redacted] | [redacted] | — | [redacted] | [redacted] | [redacted] | [redacted] | [redacted] | [redacted] | h |
| 7340-03-AC-1P | [redacted] | [redacted] | [redacted] | [redacted] | [redacted] | [redacted] | [redacted] | [redacted] | [redacted] | [redacted] | [redacted] | i |
| 7340-03-DC-4P | [redacted] | [redacted] | [redacted] | [redacted] | [redacted] | — | — | [redacted] | [redacted] | — | [redacted] | j |
| 7340-03-DC-3PS | [redacted] | [redacted] | [redacted] | [redacted] | [redacted] | [redacted] | — | [redacted] | — | — | NT | k |

| Batch No. | Purity (%) | TAF Impurities (%) | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | *related substance A | *related substance B | [redacted] | [redacted] | GS-695144 # | GS-695139 # | GS-645374 # | GS-645376 # | GS-648417 # | GS-648418 # | *related substance C | GS-645380 # | Type of Study |
| 7340-03-AZS-446-1595-75* | [redacted] | [redacted] | [redacted] | [redacted] | [redacted] | — | — | [redacted] | [redacted] | — | — | [redacted] | — | k |
| 7340-03-AZS-448-1595-84* | [redacted] | [redacted] | [redacted] | [redacted] | [redacted] | [redacted] | [redacted] | — | — | [redacted] | [redacted] | [redacted] | [redacted] | k |

| Batch No. | TAF Impurities (%) | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | [redacted] | GS-645502 | GS-645503 | GS-7339 | GS-645504 | GS-645506 | GS-645505 | GS-645507 | GS-648419 # | GS-695142 Isomer 1 # | GS-695142 Isomer 2 # | [redacted] # | [redacted] | Type of Study |
| 7340-03-AZS-446-1595-75* | [redacted] | — | — | [redacted] | — | — | — | — | [redacted] | [redacted] | [redacted] | — | [redacted] | k |
| 7340-03-AZS-448-1595-84* | — | [redacted] | [redacted] | [redacted] | [redacted] | [redacted] | [redacted] | [redacted] | — | — | — | — | [redacted] | k |

* 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
—: < DL
Detection limit (DL) = [redacted]% for all impurities other than [redacted].
DL = [redacted]% for [redacted].
Tr: trace < [redacted] (quantitation limit (QL) = [redacted]%) NT = Not tested
a Used for study numbers V990212, V990213(Genetox)
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; Genetox)

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)

# 5. TOXICOLOGY: SINGLE-DOSE TOXICITY

**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.<br>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) |

a Indicates GLP compliant
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

# 6. REPEAT DOSE TOXICITY (NONPIVOTAL STUDIES)

**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<br>(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.<br>NOAEL; 100 mg/kg/day | TX-120-2006 ([redacted] 8235665) |

**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.) |
|---|---|---|---|---|---|---|---|
| Rat/Sprague-Dawley (Crl) | Oral Gavage (50 mM citric acid) | 6 days | 100, 400 | 8 males/group | NA | Reduced body weight gain at 400 mg/kg/day. Significant, dose-dependent reductions in 1,25-dihydroxyvitamin D3 and serum phosphate. Significant, nondose-dependent reductions in serum calcium were also observed.<br>Urinary excretion of calcium was decreased on Day 1 for both treatment groups; however by Day 6, urinary excretion of calcium was increased ~ 213% at 400 mg/kg. | R2000044 (NA) |

**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.) |
|---|---|---|---|---|---|---|---|
| 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) TOXICOLOGY:

## 7.1. 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:** 7340-02-AC-1EA |
| **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): Cmax (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 |

**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**

| **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 | - | - | - | - | - | - | - | - |

**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**

| **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 |
| 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 |
| 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 |

**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**

| **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 |
| Dosing Phase – Final Phase Sacrifice (cont.) | | | | | | | | |
| Exudate, lumen | | | | | | | | |
| Minimal | 0 | 0 | 0 | 0 | 2 | 2 | 2 | 2 |
| Rectum | | | | | | | | |
| Apoptosis, increased | | | | | | | | |
| Minimal | 0 | 0 | 0 | 0 | 0 | 0 | 5 | 2 |

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.

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)

## 7.2. 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:** 1504-187-19 |
| **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] | 45.5 | 49.2 | 288 | 296 | 2430 | 2770 | 12700 | 13600 | 61800 | 78400 |
| Day 28 | NA[a] | NA[a] | 52.7 | 55.1 | 358 | 321 | 2450 | 2820 | 12200 | 14600 | 62700 | 75100 |
| Toxicokinetics (Tenofovir): $C_{max}$ (µg/mL) | | | | | | | | | | | | |
| Day 1 | NA[a] | NA[a] | 35.5 | 35.6 | 167 | 229 | 1340 | 1970 | 6970 | 6990 | 12900 | 20100 |
| Day 28 | NA[a] | NA[a] | 49.7 | 52.8 | 169 | 211 | 1300 | 1690 | 4780 | 6160 | 10300 | 14400 |

**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** | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **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 |

**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** | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **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 |
| 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 |

**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** | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **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 |

**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** | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **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 |
| Bone parameters[b] | | | | | | | | | | | | |
| Periosteal circumference (mm) | 16.09 | 13.44 | - | - | - | - | - | - | - | - | -4% | -1.5% |
| Total mean slice area – distal ($mm^2$) | 20.65 | 14.41 | - | - | - | - | - | - | - | - | -7% | -3% |
| Bone mineral density ($mg/cm^3$) | 576.5 | 752.3 | - | - | - | - | - | - | - | - | -7% | -14% B |
| 1.25-Dihydroxyvitamin D (pg/mL) | 51.67 | | - | | - | | - | | -51% A | | -74% A | |

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.
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)

## 7.3. 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:** 1603-167-36 |
| **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 |

**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** | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Daily Dose: GS-7340-02 (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 |
| Non-adverse Findings | There were no adverse findings for body weight, food consumption, clinical observations, ophthalmology examinations, hematology, clinical chemistry and urinalysis parameters, organ weights or macroscopic observations. | | | | | | | | |
| **Noteworthy Findings** | | | | | | | | | |
| Died or sacrificed moribund[d] | - | 1 | - | 2 | - | | - | - | 3 |
| 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 |
| 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 |
| Bone Densitometry (pQCT)[b] | | | | | | | | | |
| Right proximal tibia - metaphysis | | | | | | | | | |

**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** | | | |
|---|---|---|---|---|---|---|---|---|
| **Daily Dose: GS-7340-02 (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 |
| 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% |
| 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 | - |
| 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 |

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

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)

## 7.4. 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<br>[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:** 1504-187-19 |
| **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 $_{(0\text{-}\tau)}$ | |

**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**

| **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 (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 | |
| **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 | - | - | - | - |

**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**

| **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 |
| 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 |
| 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

## 7.5. 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:** 1864-16-30 |
| **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 | |

**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** | |
|---|---|---|---|---|---|---|---|---|
| **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 (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 | |
| **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 | - | -3.2 | - | -25.7 [C] | -11.9 |

**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** | |
|---|---|---|---|---|---|---|---|---|
| **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 |
| 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] |

**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** | |
|---|---|---|---|---|---|---|---|---|
| **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 |
| 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% |
| 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] | - |

**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** | | |
|---|---|---|---|---|---|---|---|---|
| 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] |
| T3 (ng/dL) | | | | | | | | |
| Week 39 | 108.8 | 100.2 | - | - | - | - | -32% [B] | -30% [D] |
| Phosphorus (mg/dL) | | | | | | | | |
| Week 13 | 5.298 | - | - | - | - | - | +18% [B] | - |
| Creatine Kinase (U/L) | | | | | | | | |
| Week 39 | 91.5 | 87.5 | - | - | +76% [A] | - | +207% [C] | +127% [E] |

**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** | |
|---|---|---|---|---|---|---|---|---|
| **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 |
| 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] |

**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** | |
|---|---|---|---|---|---|---|---|---|
| **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 |
| 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] |
| 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) |

**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** | |
|---|---|---|---|---|---|---|---|---|
| **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 |
| 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<br>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 |

**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** | |
|---|---|---|---|---|---|---|---|---|
| **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 |
| 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 | - | - |

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)

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

## 7.6. 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. 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:** 7340-02-B-1 |
| **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% | |

**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. 1050-11** |
|---|---|---|

| **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% | |

**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. 1050-11** | |
|---|---|---|---|---|---|---|
| **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 |
| Histopathology | No test article histopathological changes were observed | | | | | |

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 determined healthy prior to assignment to that study, it developed chronic diarrhea during the 5-day period prior to dosing 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
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)

# 8. GENOTOXICITY: IN VITRO

## 8.1. 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**<br>**[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:** 1309-96-32 |
| **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] |

**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** |
|---|---|---|

| **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:** 1309-96-32 |
| **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 ± $50^N$ | |

**Test Article: TAF**

| 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. GENOTOXICITY: IN VITRO (CONTINUED)

## 8.2. Mouse Lymphoma

| | | |
|---|---|---|
| **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:** 1309-96-32 |
| **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 |

| **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** |
|---|---|---|

| **Metabolic Activation** | **Test Article** | **Concentration (µg/mL)** | **RSG (%)[a]** | **Cloning Efficiency[b]** | **Relative Growth[c]** | **Mutant Frequency ($10^{-6}$)[d]** |
|---|---|---|---|---|---|---|
| Without Activation | GS-7340-02 | 313 | 109.9 | 93.5 | 102.8 | 69.4 |
| 4 hour Treatment | | 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 |

| **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** |
|---|---|---|

| **Metabolic Activation** | **Test Article** | **Concentration (µg/mL)** | **RSG (%)[a]** | **Cloning Efficiency[b]** | **Relative Growth[c]** | **Mutant Frequency ($10^{-6}$)[d]** |
|---|---|---|---|---|---|---|
| With Activation<br><br>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:** 1309-96-32 |
| **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 | DMSO | 1% (µL/mL) | 99.9 | 86.5 | 86.4 | 62.4 |
| 24 hour Treatment | | 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 |

| 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 |
|---|---|---|

| Metabolic Activation | Test Article | Concentration (µg/mL) | RSG (%)[a] | Cloning Efficiency[b] | Relative Growth[c] | Mutant Frequency ($10^{-6}$)[d] |
|---|---|---|---|---|---|---|
| With Activation | DMSO | 1% (µL/mL) | 106.7 | 108.3 | 115.5 | 55.2 |
| 4 hour Treatment | | 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

# 9. GENOTOXICITY: IN VIVO

**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**. 7340-02-B-1 |
| **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

No carcinogenicity studies have been conducted with TAF. Carcinogenicity studies with tenofovir disoproxil fumarate (TDF) are included in Section 18

# 11. REPRODUCTIVE AND DEVELOPMENTAL TOXICITY (NONPIVOTAL STUDIES)

**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<br>NOAEL for embryo-fetal development:200 mg/kg/day (AUC0-t = 35.7µg•hr/mL) | TX-120-2001[a] ([redacted] 902244) |
| Rabbits/New Zealand White | Oral Gavage (0.1% (v/v) Tween 20, 0.1% (w/v) (HPMC) K100LV prepared in ultrapure 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)<br>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] ([redacted] 902247) |

a Indicates GLP compliant
GD= gestation day; NOEL = No-Observed- Effect Level; NOAEL = No-Observed-Adverse-Effect Level; F = female; R-PMPA = tenofovir

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

**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) | **Location in CTD:** 4.2.3.5.1 |
| **Initial Age:**<br>Males: Approximately 10 weeks<br>Females: Approximately 12 weeks | F: at least 14 days prior to mating, throughout mating through Day 7 of gestation (confirmed mated) or gestation Day 13 (confirmed non-mated) | **GLP Compliance:** yes |
| **Date of First Dose:**<br>Males: [redacted] 20[redacted]<br>Females: [redacted] 20[redacted] | **Day of Mating:** GD 0 | **Lot Number:** 7340-03-AC-1P (hemifumarate) |
| **No-Observed-Adverse-Effect Level** | **Day of C-Section:** GD 13 | **Design Similar to ICH 4.1.1?** yes |
| **$F_0$ Males:** 80 mg/kg/day | **Method of Administration:** Oral Gavage | |
| **$F_0$ Females:** 80 mg/kg/day | **Vehicle:** 0.1% hydroxypropylmethylcellulose K100LV (HPMC),0.1% Tween 20 in reverse osmosis (RO) water | **Dose Volume:** 10 mL/kg |
| **$F_1$ Litters:** 160 mg/kg/day | **Special Features:** early embryonic toxicity | |

| **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 |

**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** | | | |
|---|---|---|---|---|---|
| **Daily Dose (mg/kg/day)** | | **0 (Control)** | **20** | **80** | **160** |
| | 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 (Motile %) | 91.8 (± 8.43) | 95.2 (± 3.08) | 95.5 (± 4.01) | 94.0 (± 6.10) |
| **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] |

**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** | | | |
|---|---|---|---|---|---|
| **Daily Dose (mg/kg/day)** | | **0 (Control)** | **20** | **80** | **160** |
| | 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. 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:** 7340-02-AC-1EA |
| **No Observed Adverse Effect Level:**<br>**$F_0$ Females:** 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 | |
| **$F_1$ Litters:** 100 mg/kg/day (Targeted dose level) | | |

| Dams | Daily Dose (mg/kg/day) | 0 (Control) | 25 [b] | 100[b] | 250 |
|---|---|---|---|---|---|
| | **Number of Animals (toxicokinetic)** | 3 | 9 | 9 | 9 |
| | Toxicokinetics (GS-7340): $AUC_{0-t}$ (µg*hr/mL) | | | | |
| | Gestation Day 6 | - | NC | NC | 137.6 |
| | Gestation Day 17 | - | NC | 0.242 | 138.2 |
| | Toxicokinetics (GS-7340): $C_{max}$ (µg/mL) | | | | |
| | Gestation Day 6 | - | NC | 0.038 | 0.756 |
| | Gestation Day 17 | - | NC | 0.149 | 0.597 |

**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** | |
|---|---|---|---|---|---|
| | **Daily Dose (mg/kg/day)** | **0 (Control)** | **25[b]** | **100[b]** | **250** |
| Dams (cont'd) | Toxicokinetics (R-PMPA): $AUC_{0-t}$ (µg*hr/mL) | | | | |
| | Gestation Day 6 | - | 2.715 | 0.009 | 0.054 |
| | Gestation Day 17 | - | 2.803 | 0.017 | 0.056 |
| | Toxicokinetics (R-PMPA): $C_{max}$ (µg/mL) | | | | |
| | Gestation Day 6 | - | 1.110 | 0.003 | 0.010 |
| | Gestation Day 17 | - | 0.870 | 0.004 | 0.007 |
| | **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 |

**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** | |
|---|---|---|---|---|---|
| | **Daily Dose (mg/kg/day)** | **0 (Control)** | **25** | **100** | **250** |
| | Mean No. Implantations | 15.0 | 16.2 | 16.0 | 14.5 |
| | Mean % Preimplantation Loss | 13.2 | 9.8 | 13.4 | 15.3 |
| **Litters** | 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**<br>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).

** = $p \leq 0.01$; ***= $p \leq 0.001$

## 13.2. Rabbit

**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:** 7340-02-AC-1EA |
| **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 |

**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** | |
|---|---|---|---|---|---|
| **Daily Dose (mg/kg/day)** | | **0 (Control)** | **10** | **30** | **100** |
| **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 |

**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** | |
|---|---|---|---|---|---|
| **Daily Dose (mg/kg/day)** | | **0 (Control)** | **10** | **30** | **100** |
| **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 | | | |

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

- = No noteworthy findings; * = $p \leq 0.05$ (Dunnett) & = $p \leq 0.05$ (Wilcoxon); ** = $p \leq 0.01$ (Dunnett), *** = $p \leq 0.001$ (Dunnett), GD = gestation day; NC = Not calculable; BLLQ = Below lower limit of quantification;

R-PMPA = Tenofovir

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

No pre- and post-natal development toxicity studies have been conducted with TAF. A pre- and post-natal development toxicity study with tenofovir disoproxil fumarate (TDF) is included in Section 18

# 15. STUDIES ON JUVENILE ANIMALS

No juvenile toxicity studies have been conducted.

# 16. LOCAL TOLERANCE

**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]0017) |
| 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) |

a Indicates GLP compliant
BCOP = bovine corneal opacity and permeability; NA = not applicable; RO = reverse osmosis; M = male; F = female; w/w = weight/weight

# 17. OTHER TOXICITY STUDIES

**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.) |
|---|---|---|---|---|---|---|---|
| LLNA | Mouse | Topical (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] 0018) |
| 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<br>Lot-1: 7340-02-AC-1EA (97.7% purity)<br>Lot-2: 7339-AZS-27-1094-41 (83.1% purity) | 10M | No article-related effects on clinical signs, body weights, food consumption, clinical or anatomic pathology for either lot.<br>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<br>(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)<br><br>Lot-1: 7340-03-DC-3PS (99.3% purity)<br><br>Lot-2: 7340-03-AZS-448-1595-84 (98.0% purity)<br><br>Lot-3: 7340-03-AZS-446-1595-75 (97.8% purity) | 10M; 10F | No GS-7340-03-related clinical observations, ophthalmic observations, or effects on clinical pathology parameters, or macroscopic and microscopic observations were noted.<br>NOAEL = 50 mg/kg/day (free base equivalents)<br><br>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 (50 mM 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) |

**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.) |
|---|---|---|---|---|---|---|---|
| Bone Metabolism | Rat/Crl:CD (SD) | Oral Gavage (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.<br>Urinary calcium:creatinine ratios were significantly decreased on Days 1 and 2, at 100 and on Day 1 at 400 mg/kg/day, but were then significantly increased by Day 6, at 400 mg/kg/day. | R2000044 (NA) |
| Renal Effect | Rat/Crl:CD (SD) | Oral Gavage (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) |

**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.) |
|---|---|---|---|---|---|---|---|
| Bone Marker Assessment | Dog, Beagle | Oral Gavage (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) |

a Indicates GLP compliant
b No effect level
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

# 18. TENOFOVIR DISOPROXIL FUMARATE (TDF) TOXICITY STUDIES

## 18.1. TDF: Mouse Carcinogenicity Study

**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.:** 2454-A-10P |

**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.

| | | 0 (Vehicle) | | 100 | | 300 | | 600 | |
|---|---|---|---|---|---|---|---|---|---|
| **Dose (mg/kg/day)** | | **M: 60** | **F: 60** | **M: 60** | **F: 60** | **M: 60** | **F: 60** | **M: 60** | **F: 60** |
| Toxicokinetics | | | | | | | | | |
| AUCss 0-24 (µg.h/mL) Day 1 | | - | - | - | - | - | - | 30.9 | 35.1 |
| AUCss 0-24 (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 | - | - | - | - |
| Adrenal | Adenoma: cortical | - | 1 | - | 1 | 1 | 1 | - | - |
| | Sarcoma: fusiform cell | 1 | | - | 1 | - | - | - | - |

**Test Article: TDF**

| **Report Title:** An Oral Carcinogenicity Study of Tenofovir Disoproxil Fumarate (TDF) in the Albino Mouse | | | | | | | **Gilead Study No. M990205** | |
|---|---|---|---|---|---|---|---|---|
| **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, cont. | 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 | - | - | - | - |

**Test Article: TDF**

| **Report Title:** An Oral Carcinogenicity Study of Tenofovir Disoproxil Fumarate (TDF) in the Albino Mouse | | | | | | | **Gilead Study No. M990205** | | |
|---|---|---|---|---|---|---|---|---|---|
| **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 | - | - | - | - |

**Test Article: TDF**

| **Report Title:** An Oral Carcinogenicity Study of Tenofovir Disoproxil Fumarate (TDF) in the Albino Mouse | | | | | | **Gilead Study No. M990205** | | | |
|---|---|---|---|---|---|---|---|---|---|
| **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 | | |

**Test Article: TDF**

| **Report Title:** An Oral Carcinogenicity Study of Tenofovir Disoproxil Fumarate (TDF) in the Albino Mouse | | | | | | | **Gilead Study No. M990205** | |
|---|---|---|---|---|---|---|---|---|
| **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: endomertrial 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 |

**Test Article: TDF**

| **Report Title:** An Oral Carcinogenicity Study of Tenofovir Disoproxil Fumarate (TDF) in the Albino Mouse | | | | | | | **Gilead Study No. M990205** | |
|---|---|---|---|---|---|---|---|---|
| **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] | - |

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
* = $p < 0.05$, Dunnetts "t" test

## 18.2. TDF: Rat Carcinogenicity Study

**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.:** 2454-A-10P |

**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** | |
|---|---|---|---|---|---|---|---|---|---|
| | | **M: 60** | **F: 60** | **M: 60** | **F: 60** | **M: 60** | **F: 60** | **M: 60** | **F: 60** |
| Toxicokinetics | | | | | | | | | |
| $AUC_{ss}$ 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 | - |

**Test Article: TDF**

**Report Title:** An Oral Carcinogenicity Study of Tenofovir Disoproxil Fumarate (TDF) in the Albino Rat

**Gilead Study No. R990204**

| **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 | Malignant lymphoma | - | 2 | 1 | - | - | 2 | - | 1 |
| Tissue | Histiocytic sarcoma | 2 | 0 | 1 | 1 | 2 | 2 | 2 | - |
| Ileum | Adenocarcinoma | 1 | - | - | - | 1 | - | - | - |
| Jejunum | Adenocarcinoma | - | - | - | - | 1 | - | - | - |
| Kidney | Carcinoma: tubular cell | - | - | - | - | 1 | - | - | - |

**Test Article: TDF**

**Report Title:** An Oral Carcinogenicity Study of Tenofovir Disoproxil Fumarate (TDF) in the Albino Rat | **Gilead Study No. R990204**

| **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 | | | | | | | | | |
| Kidney, cont. | Adenoma: tubular cell | 1 | - | - | - | - | - | - | - |
| | Renal mesenchymal tumor | - | - | - | - | 1 | - | - | - |
| 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 |

**Test Article: TDF**

**Report Title:** An Oral Carcinogenicity Study of Tenofovir Disoproxil Fumarate (TDF) in the Albino Rat

**Gilead Study No. R990204**

| **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 | | | | | | | | | |
| Ovary, cont. | Malignant schwannoma | - | - | - | 1 | - | - | - | - |
| 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 | - |

**Test Article: TDF**

**Report Title:** An Oral Carcinogenicity Study of Tenofovir Disoproxil Fumarate (TDF) in the Albino Rat

**Gilead Study No. R990204**

| **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 | Fibrosarcoma | 1 | 1 | 1 | - | - | 1 | - | - |
| Tissue | 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 | - | - | - | - |

**Test Article: TDF**

| **Report Title:** An Oral Carcinogenicity Study of Tenofovir Disoproxil Fumarate (TDF) in the Albino Rat | | | | | | | | **Gilead Study No. R990204** | |
|---|---|---|---|---|---|---|---|---|---|
| **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

## 18.3. 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.:** 2454-A-2P |
| **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 = 50 mg/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** |

**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** | |
|---|---|---|---|---|---|---|
| **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 post partum (%)<br>Viability Index | 99.4<br>351/353 | 99.2<br>357/360 | 99.1<br>338/341 | 80.8<br>256/317 | 53.9<br>152/282** |

**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** | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Dose (mg/kg/day)** | | **0 (Vehicle)** | | **50** | | **150** | | **450** | | **600** | |
| **$F_1$ Litters Means** | | | | | | | | | | | |
| $F_1$ Litters | Survivors at Weaning (%)<br>Lactation Index | 98.6<br>346/351 | | 98.0<br>350/357 | | 98.1<br>319/325 | | 95.0<br>230/242 | | 97.4<br>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) ** | |
| | | **M** | **F** | **M** | **F** | **M** | **F** | **M** | **F** | **M** | **F** |
| | Sex Ratio of Fetuses | 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)/number of rats with observation | | | | | | | | | | |
| | 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
* = $p \leq 0.05$; ** = $p \leq 0.01$

# 19. REFERENCES

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