CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

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PHARMACOLOGY REVIEW(S)

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

PHARMACOLOGY/TOXICOLOGY NDA/BLA REVIEW AND EVALUATION

Application number:	207561
Supporting document/s:	0000
Applicant's letter date:	5 November 2014
CDER stamp date:	5 November 2014
Product:	Genvoya, consisting of: Elvitegravir (150 mg), Cobicistat (150 mg), Emtricitabine (200 mg), Tenofovir Alafenamide (10 mg)
Indication:	Treatment of HIV-1 infection in adults and pediatric patients 12 years of age and older
Applicant:	Gilead
Review Division:	DAVP
Reviewer:	Claudia Wrzesinski, DVM, Ph.D.
Supervisor/Team Leader:	Hanan Ghantous, Ph.D., DABT
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1 Executive Summary

1.1 Introduction

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) fumarate. The E/C/F/TAF FDC (150/150/200/10 mg) tablet , Genvoya, 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. The E/C/F/TAF FDC tablet contains the same dosages of EVG, COBI, and FTC that are currently approved within Vitekta, Tybost, Emtriva, Truvada (FTC/TDF), and Stribild (E/C/F/TDF, STB) for use in adults (150 mg of EVG, 150 mg COBI, 200 mg of FTC).

TAF is the only new chemical entity in this submission, it is a prodrug of tenofovir (TFV) and is rapidly converted into TFV. A comprehensive nonclinical toxicology program was undertaken and is reviewed in Appendix A. These studies have characterized the acute, subchronic/chronic toxicity, mutagenicity, and reproductive toxicity. Per separate agreements with the FDA, carcinogenicity studies and a perinatal and postnatal study have not been conducted for TAF registration due to the rapid conversion of TAF to TFV resulting in a lack of TAF exposure in rats and TgRasH2 mice. Nonclinical studies on impurities of EVG, COBI, FTC and TAF are reviewed in Appendix B.

1.2 Brief Discussion of Nonclinical Findings

Ample nonclinical safety information is available on EVG, COBI, FTC as well as combination toxicity studies (EVG+COBI and FTC+TDF) (from previous NDAs) and TAF (current NDA). The four drugs exhibit different patterns of main target organ toxicity; therefore, administration of TAF in combination with EVG, COBI and FTC is unlikely to exacerbate known toxicities of the individual agents. EVG caused changes in the cecum and upper small intestine in rats and dogs due to high local concentrations which were not considered adverse, COBI had dose-dependent effects on the liver (mouse, rat and dog) and thyroid (rat). These effects were considered adaptive and secondary to hepatic microsomal enzyme induction and thyroid hormone imbalance. The only notable effect of FTC was a minor anemia identified at dose levels constituting large clinical multiples. The main target organs for TAF were kidney, bone, and eye. Nonclinical toxicities for TAF as well as potential overlapping toxicities with EVG, COBI, FTC are discussed below.

Renal toxicity: Chronic administration of TAF led to a dose-dependent, slight to moderate renal cortical tubular degeneration/regeneration and karyomegaly in the dog as well as renal karyomegaly in the rat. In the dog, partial recovery was observed after three months. COBI caused serum creatinine and BUN changes with no morphological evidence of kidney damage in dogs. COBI seems to reversibly block secretion of creatinine in humans. Given the absence of renal pathology, the lack of inhibition by COBI of major renal transporters of TFV at clinically relevant concentrations, and different routes of excretion for TFV and COBI, **it is not anticipated that the combination of E/C/F/TAF could exacerbate the renal toxicity of TAF.**

Bone toxicity: After long term toxicity studies with TAF, dose dependent reductions in bone mineral density and mineral content, as well as changes in bone turnover markers and in related hormones, was observed in rats and dogs. Partial recovery was observed after three months in dogs. Mechanistic toxicity studies suggest that TAF might directly inhibit 1, 25-dihydroxyvitamin D_3 production, thus resulting in decreased gastrointestinal absorption of calcium and phosphate and decreased renal reabsorption of calcium. **COBI, EVG, and FTC have not shown bone toxicity; therefore, exacerbation of TAF effects on bone is not expected.**

The TAF exposure levels at the NOAEL for bone and kidney toxicity in the dog were lower than the human TAF exposure after Genvoya administration. Since TAF has a very short $T_{1/2}$ in rat plasma, no plasma exposure for TAF could be measured. However, the bone and kidney toxicities have also been seen with another TFV-prodrug (TDF) and are believed to be due to TFV exposure. The TFV exposures at the NOAEL was 13 (rats) and 4 (dogs) times the human TFV exposure after Genvoya administration. **In clinical trials, reductions in bone density, changes in serum bone markers as well as reduction in renal function (reduced CK clearance) were seen after administration of Genvoya. However, these observed changes were less severe in patients receiving Genvoya compared to patients receiving the previously approved Stribild (E/C/F/TDF), which contains TDF.**

Posterior uveitis: In dogs, a minimal to slight infiltration of mononuclear cells of the posterior uvea was seen in the high dose group with similar severity after three and nine month administration of TAF. Reversibility was seen after a three months recovery period. At the NOAEL for eye toxicity the systemic TAF/TFV exposure in dogs was 5 (TAF) and 15 (TFV) times the exposure seen in humans at the recommended daily Genvoya dosage. **In clinical trials, monitoring for ocular symptoms was included and if necessary, followed by an ophthalmological examination. No safety signals were reported.**

Cardiac toxicity: COBI showed the potential for cardiotoxicity (decreases in left ventricular function and prolonged PR interval) in rabbit (in vitro) and dog (in vivo) studies. Follow up data from clinical trials did not reveal clinically-significant changes in these parameters at the proposed dosage of COBI. Also, TAF showed a PR prolongation at the mid and high dose, and a reversible reduction in heart rate associated with mild QT prolongation in the high dose animals at week 39 in the chronic dog study. These changes were associated with decreases in serum T3. Recovery was observed after 13-weeks. At the NOAEL, the systemic TAF exposure was lower in dogs than in humans; therefore, no safety margins were established. The systemic exposure in dogs for TFV was 4 times higher than the exposures seen in humans. No PR prolongation or any change in ECG results occurred in the single dose safety pharmacology study as well as in the one month clinical QT study with TAF. **The potential for cardiac toxicity in the combination product Genvoya after chronic use is probably low, but cannot be excluded.**

No specific cause for concern has been identified in genotoxicity, carcinogenicity, and reproductive toxicity studies with the individual agents. The lack of overlapping toxicity in animals, along with clinical data with FTC, EVG and COBI, and the E/C/F/TAF tablet, support the approval of E/C/F/TAF tablet.

1.3 Recommendations

1.3.1 Approvability

There are no nonclinical pharmacology and toxicology issues which would preclude the approval of E/C/F/TAF NDA.

1.3.2 Additional Non Clinical Recommendations

None.

1.3.3 Labeling

The following label is suggested by the reviewer. Additionally, the sponsor was asked to verify if the exposure multiples for elvitegravir, cobicistat and emtricitabine were calculated by using the clinical AUC levels for Genvoya and not Stribild administration. Also, the label might be converted to the PLLR format before approval.

8 USE IN SPECIFIC POPULATIONS

8.1 Pregnancy

Pregnancy Category B

There are no adequate and well-controlled studies in pregnant women. Because animal reproduction studies are not always predictive of human response, Genvoya should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus.

Antiretroviral Pregnancy Registry: To monitor fetal outcomes of pregnant women exposed to Genvoya, an Antiretroviral Pregnancy Registry has been established. Healthcare providers are encouraged to register patients by calling 1-800-258-4263.

Animal Data

Elvitegravir: Studies in animals have shown no evidence of teratogenicity or an effect on reproductive function. In offspring from rat and rabbit dams treated with elvitegravir during pregnancy, there were no toxicologically significant effects on developmental endpoints. The exposures (AUC) at the embryo-fetal No Observed Adverse Effects Levels (NOAELs) in rats and rabbits were respectively 23 and 0.2 times higher than the exposure in humans at the recommended daily dose of 150 mg.

Cobicistat: Studies in animals have shown no evidence of teratogenicity or an effect on reproductive function. In offspring from rat and rabbit dams treated with cobicistat during pregnancy, there were no toxicologically significant effects on developmental endpoints. The exposures (AUC) at the embryo-fetal NOAELs in rats and rabbits were respectively (b) (4) and (b) (4) times higher than the exposure in humans at the recommended daily dose of 150 mg.

Emtricitabine: The incidence of fetal variations and malformations was not increased in embryo-fetal toxicity studies performed with emtricitabine in mice at exposures (AUC) approximately 60

times higher and in rabbits at approximately (b) (4) times higher than human exposures at the recommended daily dose.

Tenofovir Alafenamide: Embryonic fetal development studies have been performed in rats and rabbits revealed no evidence of impaired fertility or harm to the fetus due to tenofovir alafenamide. The embryo-fetal NOAELs in rats and rabbits occurred at tenofovir alfenamide exposures similar to and 53 times higher than, respectively, the exposure in humans at the recommended daily dose. Tenoforvir alfenamide is rapidly converted to tenofovir, the observed tenofovir exposure in these studies was 59 (rat) and 93 (rabbit) times higher than human tenofovir exposures at the recommended daily dose.

8.3 Nursing Mothers

The Centers for Disease Control and Prevention recommend that HIV-infected mothers not breastfeed their infants to avoid risking postnatal transmission of HIV. Studies in rats have demonstrated that elvitegravir, cobicistat, and tenofovir are secreted in milk. It is not known whether elvitegravir, cobicistat, or tenofovir alafenamide is excreted in human milk.

In humans, samples of breast milk obtained from five HIV-1 infected mothers show that emtricitabine is secreted in human milk. Breastfeeding infants whose mothers are being treated with emtricitabine may be at risk for developing viral resistance to emtricitabine. Other emtricitabine-associated risks in infants breastfed by mothers being treated with emtricitabine are unknown.

Because of both the potential for HIV transmission and the potential for serious adverse reactions in nursing infants, mothers should be instructed not to breastfeed if they are receiving Genvoya.

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

Elvitegravir: Long-term carcinogenicity studies of elvitegravir were carried out in mice (104 weeks) and in rats for up to 88 weeks (males) and 90 weeks (females). No drug-related increases in tumor incidence were found in mice at doses up to 2000 mg/kg/day alone or in combination with 25 mg/kg/day RTV at exposures 3- and 14-fold, respectively, the human systemic exposure at the recommended daily dose of 150 mg. No drug-related increases in tumor incidence were found in rats at doses up to 2000 mg/kg/day at exposures 12- to 27-fold, respectively in male and female, the human systemic exposure.

Elvitegravir was not genotoxic in the reverse mutation bacterial test (Ames test) and the rat micronucleus assay. In an in vitro chromosomal aberration test, elvitegravir was negative with metabolic activation; however, an equivocal response was observed without activation.

Elvitegravir did not affect fertility in male and female rats at approximately 16- and 30-fold higher exposures (AUC), respectively, than in humans at the therapeutic 150 mg daily dose.

Fertility was normal in the offspring of rats exposed daily from before birth (in utero) through sexual maturity at daily exposures (AUC) of approximately 18-fold higher than human exposures at the recommended 150 mg daily dose.

Cobicistat: In a long-term carcinogenicity study in mice, no drug-related increases in tumor incidence were observed at doses up to 50 and 100 mg/kg/day (males and females, respectively). Cobicistat exposures at these doses were approximately 7 (male) and 16 (females) times, respectively, the human systemic exposure at the therapeutic daily dose. 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 at doses of 25 and 50 mg/kg/day in males, and at 30 mg/kg/day in females. The follicular cell findings are considered to be rat-specific, secondary to hepatic microsomal enzyme induction and thyroid hormone imbalance, and are not relevant for humans. At the highest doses tested in the rat carcinogenicity study, systemic exposures were approximately 2 times the human systemic exposure at the therapeutic daily dose.

Cobicistat was not genotoxic in the reverse mutation bacterial test (Ames test), mouse lymphoma or rat micronucleus assays.

Cobicistat did not affect fertility in male or female rats at daily exposures (AUC) approximately 4-fold higher than human exposures at the recommended 150 mg daily dose.

Fertility was normal in the offspring of rats exposed daily from before birth (in utero) through sexual maturity at daily exposures (AUC) of approximately 1.2-fold higher than human exposures at the recommended 150 mg daily dose.

Emtricitabine: In long-term carcinogenicity studies of emtricitabine, no drug-related increases in tumor incidence were found in mice at doses up to 750 mg/kg/day (23 times the human systemic exposure at the therapeutic dose of 200 mg/kg/day) or in rats at doses up to 600 mg/kg/day (28 times the human systemic exposure at the therapeutic dose).

Emtricitabine was not genotoxic in the reverse mutation bacterial test (Ames test), mouse lymphoma or mouse micronucleus assays.

Emtricitabine did not affect fertility in male rats at approximately 140-fold or in male and female mice at approximately 60 fold higher exposures (AUC) than in humans given the recommended 200 mg daily dose. Fertility was normal in the offspring of mice exposed daily from before birth (in utero) through sexual maturity at daily exposures (AUC) of approximately 60-fold higher than human exposures at the recommended 200 mg daily dose.

Tenofovir Alafenamide: Since tenofovir alafenamide is rapidly converted to tenofovir and a lower tenofovir exposure in rats and mice is observed after tenofovir alafenamide administration compared to tenofovir disoproxil fumarate administration, carcinogenicity studies were conducted only with tenofovir disoproxil fumarate. Long-term oral carcinogenicity studies of tenofovir disoproxil fumarate in mice and rats resulted in TFV exposures up to approximately (b) (4) times (mice) and (b) (4) times (rats) higher than TFV exposure in humans reached after administration of Genvoya. In female mice, liver adenomas were increased at TFV exposures (b) (4) times those observed in humans. In rats, the study was negative for carcinogenic findings (b) (4)

Tenofovir alafenamide was not genotoxic in the reverse mutation bacterial test (Ames test), mouse lymphoma or rat micronucleus assays.

There were no effects on fertility, mating performance or early embryonic development when tenofovir alafenamide was administered to male rats at a dose equivalent to 155 times the human dose based on body surface area comparisons for 28 days prior to mating and to female rats for 14 days prior to mating through day seven of gestation.

2 Drug Information

2.1 Drug

Elvitegravir (EVG)

CAS Registry Number: 697761-98-1

Generic Name: Elvitegravir

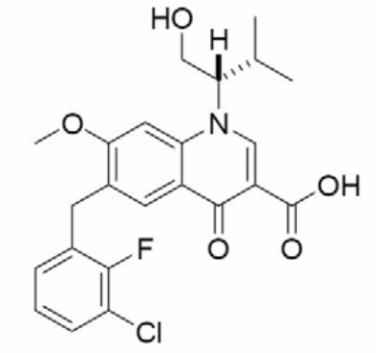
Code Name: GS-9137; JTK-303; EVG

Chemical Name: 3-Quinolinecarboxylic acid, 6-[(3-chloro-2-fluorophenyl)methyl]-1,4-dihydro-1-[(1S)-1-(hydroxymethyl)-2-methylpropyl]-7-methoxy-4-oxo-

Molecular Formula/Molecular Weight: $C_{23}H_{23}ClFNO_5$/447.88

Structure or Biochemical Description:

Figure 1: EVG Structure



Pharmacologic Class: HIV-integrase inhibitor

Cobicistat (COBI)

CAS Registry Number: 1004316-88-4

Generic Name: Cobicistat

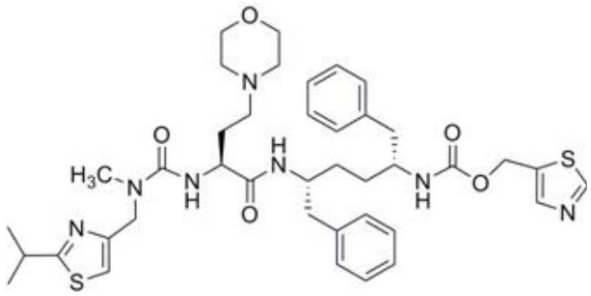
Code Name: COBI, GS-9350

Chemical Name: 1,3-thiazol-5-ylmethyl [(2*R*,5*R*)-5-{[(2*S*)-2-[(methyl{[2-(propan-2-yl)-1,3-thiazol-4-yl]methyl}carbamoyl)amino]-4-(morpholin-4-yl)butanoyl]amino}-1,6-diphenylhexan-2-yl]carbamate

Molecular Formula/Molecular Weight: $C_{40}H_{53}N_7O_5S_2$/776.0

Structure or Biochemical Description:

Figure 2: COBI Structure



Pharmacologic Class: Inhibition of CYP3A-mediated metabolism

Emtricitabine (FTC)

CAS Registry Number: 143491-57-0

Generic Name: Emtricitabine

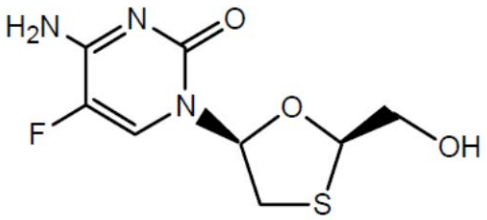
Code Name: GS-9019**,** FTC

Chemical Name: 5-fluoro-1-[(2*R*,5*S*)-2-(hydroxymethyl)-1,3-oxathiolan-5-yl]cytosine

Molecular Formula/Molecular Weight: $C_8H_{10}FN_3O_3$/247.24

Structure or Biochemical Description:

Figure 3: FTC Structure



Pharmacologic Class: NRTI (Nucleoside Reverse Transcriptase Inhibitor)

Tenofovir Alafenamide (TAF)

CAS Registry Number: 379270-37-8

Generic Name: Tenofovir alafenamide fumarate

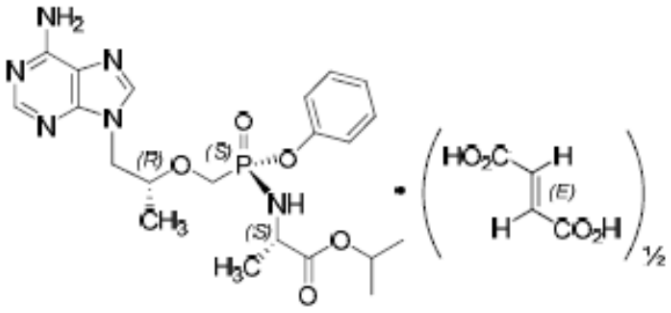
Code Name: GS-7340-03, TAF

Chemical Name: Propan-2-yl *N*-[(*S*)-({[(2*R*)-1-(6-amino-9*H*-purin-9-yl)propan-2-yl]-oxy}methyl)(phenoxy) phosphoryl]-lalaninate,(2*E*)-but-2-enedioate (2:1)

Molecular Formula/Molecular Weight: $C_{23}H_{31}O_7N_6P$/534.50

Structure or Biochemical Description:

Figure 4: TAF Structure



Pharmacologic Class: NtRTI (Nucleotide reverse transcriptase inhibitors)

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.

2.2 Relevant INDs, NDAs, BLAs and DMFs

NDA 021500, NDA 021896, and IND 053971 for FTC (Emtriva); NDA 021356 and IND 052849 for TDF (Viread); NDA 021752 and IND 067671 for TVD (Truvada); NDA 203100 and IND 103093 for STB (Stribild); NDA 203093, and IND 072177 for EVG (Vitekta); and NDA 203094 and IND 101 283 for COBI (Tybost).

2.3 Drug Formulation

E/C/F/TAF tablets are an immediate-release tablet dosage form containing 150 mg of elvitegravir (EVG), 150 mg of cobicistat (COBI), 200 mg of emtricitabine (FTC), and 10 mg of tenofovir alafenamide (TAF). E/C/F/TAF tablets are green, capsule-shaped, film-coated tablets with “GSI” debossed on one side and “510” on the other side

Table 1: Quantitative Composition of E/C/F/TAF Tablets *(excerpted from the sponsor)*

Component	Composition (% w/w)	Unit Formula (mg/tablet)	Reference Quality Standards	Function
(b) (4)				

2.4 Comments on Novel Excipients

No novel excipients are used in the manufacture of E/C/F/TAF tablets

2.5 Comments on Impurities/Degradants of Concern

The proposed specifications for impurities in the EVG, COBI, FTC and TAF drug substance were deemed acceptable based on results from general toxicology studies, experimental genotoxicity data, and/or assessments of potential mutagenicity using (Q)SAR. For further information, please see Appendix B of the E/C/F/TAF review by Dr. Mark Powley.

2.6 Proposed Clinical Population and Dosing Regimen

The E/C/F/TAF FDC (150/150/200/10 mg) tablet 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 a once-a-day oral pill.

2.7 Regulatory Background

This application is being submitted in support of a NDA for a film-coated single tablet regimen that contains the active substances elvitegravir (EVG), cobicistat (COBI), emtricitabine (FTC), and tenofovir alafenamide fumarate (TAF). The EVG/COBI/FTC/TAF tablet is referred as E/C/F/TAF. TAF is a new chemical entity and this NDA contains full nonclinical data set (except for carcinogenicity studies and peri/postnatal development studies) to support TAFs approval. The E/C/F/TAF FDC tablet contains the same dosages of EVG, COBI, and FTC that are currently approved within Vitekta, Tybost, Emtriva, Truvada (FTC/TDF), and Stribild (E/C/F/TDF, STB) for use in adults (150 mg of EVG, 150 mg COBI, 200 mg of FTC). Per the agreement reached between Gilead and the Food and Drug, this NDA is supported by right of reference to applicable sections of Gilead's applications: NDA 021500, NDA 021896, and IND 053971 for FTC (Emtriva); NDA 021356 and IND 052849 for TDF (Viread); NDA 021752 and IND 067671 for TVD (Truvada); NDA 203100 and IND 103093 for STB (Stribild); NDA 203093, and IND 072177 for EVG (Vitekta); and NDA 203094 and IND 101 283 for COBI (Tybost).

3 Studies Submitted

A comprehensive nonclinical toxicology program was undertaken in support of TAF and is reviewed in Appendix A. These studies have characterized the acute, subchronic/chronic toxicity, mutagenicity, and reproductive toxicity. Per separate agreements with the FDA carcinogenicity studies and a perinatal and postnatal study are not conducted for TAF registration due to the lack of TAF exposure in rats and TgRasH2 mice. Studies with EVG, COBI and FTC are approved drug products and have been reviewed under their respective NDAs.

The toxicity profiles of EVG, COBI, FTC, and TAF have been well-characterized. 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. Combination toxicology studies with EVG+COBI and FTC+TDF have been reviewed under the STB NDA

and identified a low potential for toxicologic interaction. Further toxicological investigations are unlikely to yield new data relevant to humans. 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). Extensive clinical safety data are available for the approved drugs FTC (Emtriva), TDF (Viread), the FTC/TDF FDC product (Truvada), and the E/C/F/TDF FDC product (STB, Stribild) and support the overall risk/benefit of this new E/C/F/TAF FDC product for HIV-1 infection.

3.1 Studies Reviewed

Effects of HIV Protease Inhibitors and Pharmacokinetic Enhancers on TAF Metabolism In Vitro (AD-120-2027)

In Vitro Assessment of Cobicistat as a Substrate for Human OCT2 Transporter (AD-216-2112)

Metabolism of JTK-303 by Recombinant Human CYP Isoforms (JTK-303-AD-017)

3.3 Previous Reviews Referenced

Appendix A (TAF), Appendix B (Impurities of TAF) contain the relevant review of the NME drug product and impurities. Carcinogenicty study and perinatal/postnatal reproduction toxicity study for TFV-DF has been reviewed under Viread NDA. EVG (Stribild), COBI (Srtibild) and FTC (Truvada) are reviewed in their respective NDAs.

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

Title: AD-120-2027: Effects of HIV Protease Inhibitors and Pharmacokinetic Enhancers on TAF Metabolism In Vitro

This study evaluated in vitro the effects of the HIV protease inhibitors, atazanavir and darunavir, and the pharmacokinetic enhancers, ritonavir and cobicistat on the metabolic stability of the phoshonoamidate prodrug of TFV in pooled human intestinal postmitochondrial supernatant (S9 subcellular fraction). In the absence of inhibitors, GS-7340 was moderately stable in intestinal S9 fractions from human (T½ 24.5 min). The HIV-PIs and pharmacokinetic enhancers, at concentrations up to 100 μM, had little effect on the intestinal S9 stability of GS-7340. Therefore atazanavir, darunavir, ritonavir or cobicistat at concentrations up to 100 μM did not critically affect the intestinal metabolism of GS-7340 by human intestinal subcellular.

Title: In Vitro Assessment of Cobicistat as a Substrate for Human OCT2 Transporter (AD-216-2112)

This study assess whether cobicistat is a substrate of the organic cation transporter 2 (OCT2; SLC22A2) which is primarily localized on the basolateral membrane of the renal proximal tubule. The rate of uptake of COBI in OCT2 transfected cells was up to 3-fold higher compared to non-transfected cells. The rate of uptake of COBI in transfected cells was decreased in the presence of the OCT2 inhibitor cimetidine (600μM). These results show that COBI was transported by OCT2 into the cells.

Title: Metabolism of JTK-303 by Recombinant Human CYP Isoforms (JTK-303-AD-017)

This study is designed to identify the cytochrome P450(CYP) isoforms responsible for the metabolism of JTK-303 (elvitegravir) in humans. The main metabolite of the human liver microsomes M1 was produced by CYP1A1, CYP3A4 and CYP3A5. Its formation rate was the highest by CYP 3A4. The miner metabolite M2 was produced by CYP 1A1, where M5 and M8 were only produced by CYP3A4. C-JTK-303 was not metabolized by other CYP isoforms.

11 Integrated Summary and Safety Evaluation

The Genvoya (E/C/F/TAF FDC) tablet contains the same dosages of elvitegravir (EVG, E), cobicistat (COBI, C) and emtricitabine (FTC, F) that are currently approved within Vitekta, Tybost, Emtriva, Truvada (FTC/TDF), and Stribild (E/C/F/TDF, STB) for use in adults (150 mg of EVG, 150 mg COBI, 200 mg of FTC). Comprehensive nonclinical pharmacology/virology, pharmacokinetic, and toxicology programs were conducted for EVG, COBI and FTC and have been reviewed under their respective NDAs. A comprehensive nonclinical toxicology program was undertaken in support of tenofovir alfenamid fumarate (TAF) and is reviewed in Appendix A. These studies have characterized the acute, subchronic/chronic toxicity, mutagenicity, and reproductive toxicity. Per separate agreements with the FDA, carcinogenicity studies and a perinatal and postnatal study have not been conducted for TAF registration due to the rapid conversion of TAF to TFV resulting in a lack of TAF exposure in rats and TgRasH2 mice and (TAF). Nonclinical studies on impurities of EVG, COBI, FTC and TAF are reviewed in Appendix B.

In general, the toxicity profiles of the four agents were different target organs and no significant overlapping toxicities were identified. 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. Additionally, the extensive clinical safety data available from the clinical trials with Striblid (E/C/F/TDF) and with Genvoya (E/C/F/TAF) support the safety of this combination product for the treatment of HIV-1 infection.

The four drug combination showed an increase in the bioavailability and a decrease in the rate of elimination of EVG due to inhibition of CYP3A activity by COBI, and a consequent profound reduction in the formation of M1 EVG metabolite. This interaction has been well characterized in vitro. This has not been observed in the animal models due to the lack of mechanism-based inhibition by COBI in nonhuman species. The other potential drug interactions among the four components include inhibition of intestinal efflux of TAF by COBI and inhibition of OATP-mediated hepatic uptake of TAF by COBI and EVG. The increase in TAF exposure due to inhibition of intestinal efflux by COBI has been taken into account during the TAF clinical dose selection for the E/C/F/TAF FDC.

Although NRTIs carry a class labeling for mitochondrial toxicity, FTC, EVG and TAF have been shown to have a low potential for mitochondrial toxicity. No mitochondrial toxicity is expected for COBI based on its structure and its mechanism of action as a CYP3A inhibitor. In conclusion, the potential for mitochondrial toxicity is low for Genvoya.

Administration of TAF in combination with EVG, COBI and FTC is unlikely to exacerbate known toxicities of the individual agents. EVG related changes in the cecum and upper small intestine in rats and dogs were due to high local concentrations and were not considered adverse or relevant to clinical use. Potential toxicities related to COBI observed in nonclinical toxicology studies (hematology, clinical chemistry, and urinalysis changes; lower IgG antibody titers; and adaptive liver and thyroid changes) have not been observed in clinical studies with E/C/F/TAF. The only toxicity observed in chronic animal studies with FTC was mild, reversible anemia at large multiples of clinical exposure; therefore, these hematological findings are not considered relevant to clinical use. The principal target organs of toxicity in animals following oral administration of TAF were the kidney (karyomegaly, tubular degeneration/regeneration), bone (reduction in bone mineral density and mineral content, changes in bone turnover markers and in related hormones), and eye (posterior uveitis in dogs). Renal and bone toxicity findings correlate with the known clinical toxicities for TFV. Combination toxicity studies with EVG+COBI or FTC+TDF did not reveal any new or additive toxicities (reviewed under STB).

After long term toxicity studies with TAF, dose dependent reduction in bone mineral density and mineral content as well as changes in bone turnover markers and in related hormones was observed in rats and dogs. Partial recovery was observed after 3 months in dogs. Mechanistic toxicity studies suggest that TAF might directly inhibit 1, 25-dihydroxyvitamin D_3 production thus resulting in decreased gastrointestinal absorption of calcium and phosphate and decreased renal reabsorption of calcium. Cobicistat, EVG, and FTC have not shown any potential for bone toxicity. Thus, exacerbation of any TAF effects on bone is not expected.

Chronic administration of TAF led to a dose-dependent, slight to moderate renal cortical tubular degeneration/regeneration and karyomegaly in the dog as well as renal karyomegaly in the rat, partial recovery was observed in the dog. COBI alone was associated with non adverse urinalysis and urine chemistry changes (diuretic effects) at high doses in rats and dogs, which were reversible. COBI also caused serum creatinine and BUN changes, but without morphological evidence of kidney damage. Nonclinical and clinical data, suggest that COBI reversibly blocks secretion of creatinine in humans. Since no pathological changes were observed in the kidney due to COBI, the routes of excretion differ for TFV and COBI, and that COBI would not be

expected to inhibit the major renal transporters of TFV at clinically relevant concentrations, it is not anticipated that the combination of E/C/F/TAF could exacerbate the renal toxicity of TAF.

The TAF exposure levels at the NOAEL for bone and kidney toxicity in the dog were lower than the human TAF exposure after Genvoya administration. Since TAF has a very short $T_{1/2}$ in rats, no exposure for TAF could be measured. However, the bone and kidney toxicities have also been seen with another TFV-prodrug (TDF) and are due to TFV exposure. The TFV exposures at the NOAEL was 13 (rats) and 4 (dogs) times the human TFV exposure after Genvoya administration. In clinical trials, reductions in bone density, changes in serum bone markers, as well as reduction in renal function (reduced CK clearance) were seen after administration of Genvoya (E/C/F/TAF). However, these observed changes were less severe in patients receiving Genvoya compared to patients receiving the previously approved Stribild (E/C/F/T).

Minimal to slight infiltration of mononuclear cells of the posterior uvea of dogs was seen in the high dose group, with similar severity after three and nine month administration of TAF. Reversibility was seen after a three months recovery period. Ocular findings were not seen with TAF in any other animal model (mouse, rat, monkey) and were not seen with Viread (TDF, prodrug of TFV). At the NOAEL for eye toxicity, the systemic TAF/TFV exposure in dogs was 5 (TAF) and 15 (TFV) times the exposure seen in humans at the recommended daily Genvoya dosage. No ocular toxicities were described for EVG, COBI and FTC. In clinical trials, monitoring for ocular symptoms was included and, if necessary, followed by an ophthalmological exam. No safety signals were reported.

COBI showed the potential for cardiotoxicity (decreases in left ventricular function and prolonged PR interval) in isolated rabbit hearts, follow up data from clinical trials did not reveal clinically-significant changes in these parameters at the proposed dosage of COBI. Also, TAF showed some potential to prolong the PR interval. In the long term dog study, a dose-related prolongation of PR interval was observed. Further, TAF reversibly reduced the heart rate with an associated mild QT interval prolongation in the week 39 chronic dog study (high dose group). These changes were associated with decreases in serum T3, and recovery was observed after the 13-week recovery period. However, 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, and no safety signal was reported in a clinical one month QT study. Taken together, the potential for cardiovascular effects with the E/C/F/T tablet is considered low, but cannot be excluded. COBI also showed some effects on the thyroid glands in the chronic rat study, characterized by decreases in T4, and increases in TSH, thyroid weights, with thyroid follicular cell hypertrophy. These findings were reversible, and were not considered adverse. An increased incidence of follicular cell adenomas and/or carcinomas in the thyroid gland was observed in the rat carcinogenicity study. However, these thyroid effects were considered rodent specific. No clinically relevant adverse effects on thyroid function have been observed in clinical studies conducted to date with COBI, or with the E/C/F/TAF.

Of the E/C/F/TAF products, none had positive findings in genotoxicity studies. The E/C/F/TAF combination is not anticipated to alter the genotoxicity profiles of the individual agents. EVG, FTC, and TDF demonstrated low carcinogenic potential in the 2-year bioassays. In long-term carcinogenicity studies of EVG and FTC, no drug-related increases in tumor incidence were

found in mice and rats. COBI did not show a drug-related increase in tumor incidence in a carcinogenicity study in mice. However, in a long-term carcinogenicity study of COBI in rats, an increased incidence of follicular cell adenomas and/or carcinomas in the thyroid gland was observed at systemic exposures approximately 2 times the human systemic exposure at the therapeutic daily dose. The follicular cell findings are considered to be rat-specific, secondary to hepatic microsomal enzyme induction and thyroid hormone imbalance, and are not relevant for humans. Since TAF is rapidly converted to TFV, no carcinogenicity studies were conducted with TAF. However, carcinogenicity studies were conducted with TDF which is also converted to TFV. Long-term oral carcinogenicity studies of TDF in mice and rats resulted in TFV exposures up to approximately 163 times (mice) and 55 times (rats) higher than TFV exposure in humans reached after administration of Genvoya. In female mice, liver adenomas were increased at TFV exposures 163 times those observed in humans. In rats, the study was negative for carcinogenic findings at TFV exposures up to 55 times those observed in humans at the therapeutic dose. No exacerbation of carcinogenic potential is expected. EVG, COBI, FTC, and TAF alone did not have significant adverse effects in reproductive and developmental toxicity studies. The E/C/F/TAF combination is not expected to have an altered reproductive toxicity profile compared with that of the individual agents. The proposed specifications for impurities in the EVG, COBI, FTC and TAF drug substance were deemed acceptable based on results from general toxicology studies, experimental genotoxicity data, and/or assessments of potential mutagenicity using (Q)SAR. The lack of overlapping toxicity in animals, along with clinical data with, FTC, EVG and COBI, and the E/C/F/TAF tablet support the overall risk/benefit of E/C/F/TAF tablet.

12 Appendix/Attachments

Appendix A: TAF review
Appendix C: EVG, COBI, FTC and TAF Impurities Review

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

PHARMACOLOGY/TOXICOLOGY NDA/BLA REVIEW AND EVALUATION

Application number:	207-561 (Appendix A)
Supporting document/s:	0000
Applicant's letter date:	5 November 2014
CDER stamp date:	5 November 2014
Product:	Genvoya, consisting of: Elvitegravir (150 mg), Cobicistat (150 mg), Emtricitabine (200 mg), Tenofovir Alafenamide (10 mg) Appendix A: Tenofovir Alafenamide (10 mg)
Indication:	Treatment of HIV-1 infection in adults and pediatric patients 12 years of age and older.
Applicant:	Gilead
Review Division:	DAVP
Reviewer:	Claudia Wrzesinski, DVM, Ph.D.
Supervisor/Team Leader:	Hanan Ghantous, Ph.D., DABT
Division Director:	Debra Birnkrant, MD
Project Manager:	Myung-Joo, Hong, M.S.

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1 Executive Summary

1.1 Introduction

Tenofovir Alafenamide (TAF, GS-7340) is a new chemical entity that belongs to the class of human immunodeficiency virus-1 (HIV-1) nucleotide reverse transcriptase inhibitors (NtRTI). TAF is a prodrug of tenofovir (TFV) and is more stable in plasma than the TFV prodrug tenofovir disoproxil fumarate (TDF, Viread). TAF is taken up into peripheral mononuclear cells and intracellular metabolized to TFV and phosphorylated to the active metabolite Tenofovir Diphosphate (TFV-DP). TAF administration leads to higher TFV-DP levels in HIV target cells as well as lower circulating level of TFV which is expected to result in reduced off-target effects of TFV and an improved safety profile as compared to TDF.

Gilead has co-formulated TAF with the integrase strand transfer inhibitor (INSTI) EVG, the pharmacokinetic enhancer COBI, and the nucleoside reverse transcriptase inhibitor FTC into an FDC. The Elvitegravir/cobicistat/emtricitabine/tenofovir alafenamide (E/C/F/TAF) FDC (150/150/200/10 mg) tablet 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.

1.2 Brief Discussion of Nonclinical Findings

Long term toxicology studies in two species (rat and dog) were conducted to support chronic use of tenofovir alafenamide (TAF, prodrug of tenofovir). Bones and kidney were identified as the main target organs. After long term toxicity studies with TAF, dose-dependent reductions in bone mineral density and mineral content, as well as changes in bone turnover markers and in related hormones, was observed in rats and dogs. Partial recovery was observed after three months in the dog. Mechanistic toxicity studies suggest that TAF might directly inhibit 1, 25-dihydroxyvitamin D_3 production, thus resulting in decreased gastrointestinal absorption of calcium and phosphate, and decreased renal reabsorption of calcium. Chronic administration of TAF also lead to a dose-dependent slight to moderate renal cortical tubular degeneration/regeneration and karyomegaly in the dog, as well as renal karyomegaly in the rat. Partial recovery was observed in the dog. These findings occurred in the dog at TAF exposure levels lower than the human exposure. In the rat, plasma TAF exposures could not be measured because of the short $T_{1/2}$. However, the same bone and kidney toxicities have also been seen after the administration of another prodrug of TFV, TDF, and are believed to be due to TFV exposure. The measured TFV exposures after TAF administration at the NOAEL for bone and kidney toxicity was 13 (rats) and 4 (dogs) times the human exposure at the recommended daily Genvoya dose. In clinical trials, reductions in bone density, changes in serum bone markers as well as reduction in renal function (reduced CK clearance) were seen after administration of Genvoya (E/C/F/TAF). However, these observed changes were less severe in patients receiving Genvoya compared to patients receiving the previously approved Stribild (E/C/F/TDF) which contains TDF.

Histopathological, a minimal to slight infiltration of mononuclear cells of the posterior uvea of dogs was seen in the high dose group, with similar severity after three and nine month administration of TAF. Reversibility was seen after a three months recovery period. In-life fundoscopic and biomicroscopic exams of the dog eye did not detect posterior uveitis. At the NOAEL for eye toxicity, the systemic TAF/TFV exposure in dogs was 5 (TAF) and 15 (TFV) times the exposure seen in humans at the recommended daily Genvoya dosage. Ocular findings were not seen with TAF in any other animal model (mouse, rat, monkey) and were not seen with Viread (TDF, prodrug of TFV). In rats and dogs, radioactive labeled TAF distributed poorly to the eye. TAF was not selectively associated with melanin-containing tissue in studies with Sprague Dawley and Long Evans rats, as well as C57 black mice and CD-1 mice. In clinical trials, monitoring for ocular symptoms was included and, if necessary followed, by an ophthalmological exam. No safety signals were reported.

In the chronic dog study, a PR prolongation occurred at the mid and high dose and a reversible reduction in heart rate associated with mild QT prolongation occurred in the high dose animals at week 39. This toxicity has not been observed after TDF administration. At the NOAEL, the systemic TAF exposure was lower in dogs and rats than in humans; therefore no safety margins were established.The systemic TFV exposure in dogs was 4 times higher than the exposures seen in humans after the recommended daily dose of Genvoya. No PR prolongation or any change in ECG results occurred in the single dose safety pharmacology study, or in the one month clinical QT study.

TAF was not genotoxic. Carcinogenicity studies have not been performed with TAF since TAF is rapidly metabolized to TFV in mice and rats. However, carcinogenicity studies have been carried out with TDF in mice and rats which resulted in TFV exposures up to approximately 163 times (mice) and 55 times (rats) higher than TFV exposure in humans reached after Genvoya administration. In female mice, liver adenomas were increased at TFV exposures 163 times those observed in humans.

In a rat fertility study, no drug related changes occurred at dose equivalent to 155 times the human dose based on body surface area comparison. The reproductive developmental toxicity was evaluated in pregnant rats and rabbits. The measured TAF exposures at the NOAEL (100 mg/kg/day) were similar to (rats) or 53 times higher (rabbits) than the exposure in humans at the recommended daily dose. The observed TFV exposure at the NOAEL was 59 (rat) and 93 (rabbit) times higher than human TFV exposures at the recommended daily doses in rats and rabbits, respectively. TAF has not been tested in a peri/postnatal study since toxicokinetic data showed that no meaningful TAF but only TFV exposure was seen in rats. TFV, delivered via TDF, has previously been assessed for peri/postnatal effects at exposures 100-times human exposure of TFV via Genvoya. The peri/postnatal study with TDF characterized the potential postnatal toxicity of TFV. The measured TFV exposures in the dams at the NOEL for developmental toxicity (150 mg/kg/day) and F1 toxicity (50 mg/kg/day) were 27 and 14 times higher than the exposure in humans at the recommended daily dose.

1.3 Recommendations

1.3.1 Approvability

There are no nonclinical pharmacology and toxicology issues which would preclude the approval of TAF 10 mg as part of the E/C/F/TAF FDC.

1.3.2 Additional Non Clinical Recommendations

None

1.3.3 Labeling

See E/C/F/TAF NDA

2 Drug Information

2.1 Drug

Tenofovir Alafenamide (TAF)

CAS Registry Number: 379270-37-8

Generic Name: Tenofovir Alafenamide Fumarate

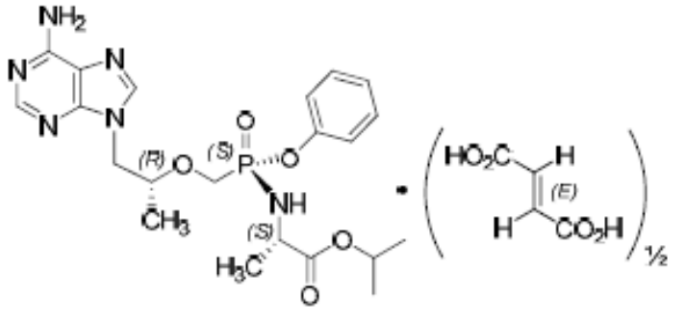
Code Name: GS-7340-03, TAF

Chemical Name: Propan-2-yl *N*-[(*S*)-[[(2*R*)-1-(6-amino-9*H*-purin-9-yl)propan-2-yl]-oxy}methyl)(phenoxy) phosphoryl]-L-alaninate,(2*E*)-but-2-enedioate (2:1)

Molecular Formula/Molecular Weight: $C_{23}H_{31}O_7N_6P/534.50$

Structure or Biochemical Description:

Figure 1: TAF Structure



Pharmacologic Class: Nucleotide reverse transcriptase inhibitors

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.

2.2 Relevant INDs, NDAs, BLAs and DMFs

NDA 021356 and IND 052849 for TDF (Viread); (Truvada); NDA 203100 and IND 103093 for STB (Stribild)

2.3 Drug Formulation

E/C/F/TAF tablets are an immediate-release tablet dosage form containing 150 mg of elvitegravir (EVG, E), 150 mg of cobicistat (COBI, C), 200 mg of emtricitabine (FTC, F), and 10 mg of tenofovir alafenamide (TAF). E/C/F/TAF tablets are green, capsule-shaped, film-coated tablets with “GSI” debossed on one side and “510” on the other side. See table 1 for details.

2.4 Comments on Novel Excipients

No novel excipients are used in the manufacture of E/C/F/TAF tablets.

2.5 Comments on Impurities/Degradants of Concern

The proposed specifications for impurities in the TAF drug substance were deemed acceptable based on results from general toxicology studies, experimental genotoxicity data, and/or assessments of potential mutagenicity using (Q)SAR. For further information, please see Appendix B of the E/C/F/TAF review by Dr. Mark Powley.

2.6 Proposed Clinical Population and Dosing Regimen

The E/C/F/TAF FDC (150/150/200/10 mg) tablet 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 a once-a-day oral pill.

2.7 Regulatory Background

TAF is a new chemical entity and is reviewed in this review. EVG, COBI and FTC are approved products and are reviewed under their respective NDAs.

3 Studies Submitted

3.1 Studies Reviewed

Secondary Pharmacodynamics:

PC-120-2016 Evaluation of Tenofovir Alafenamide (GS-7340) Effects on Human Myeloid and Erythroid Progenitors
PC-120-2018 Tenofovir Alafenamide is Not a Substrate for Renal Organic Anion Transporters (OATs) and Does Not Exhibit OAT-Dependent Cytotoxicity
PC-120-2006 In Vitro Evaluation of GS-7340 Effects on Mitochondrial DNA Content

Absorption:

99-DDM-1278 Analysis of Data from (b) (4) Oral Bioavailability Study M059-98 or GS-7340 in dogs
001-PK
D990175 Toxicokinetics of a 28-Day Oral Gavage Toxicity Study of GS-7340-02 in Beagle Dogs
AD-120-2014 Collection of Samples for Determination of the Pharmacokinetics of GS-7340-02 and GS-7340-03 After a Single Oral Dose to Mice
AD-120-2015 Collection of Samples for Determination of the Pharmacokinetics of GS-7340-02 and GS-7340-03 After a Single Oral Dose to Rats
AD-120-2016 Collection of Samples for Determination of the Pharmacokinetics of GS-7340-03 After a Single Oral Gavage Dose to Male and Female 001178-W (wild type) Mice
AD-120-2033 Plasma and Liver Pharmacokinetics of Tenofovir Alafenamide (GS-7340) Following 7-Day Oral Administration in Male Beagle Dogs
AD-120-2034 Plasma and Liver Pharmacokinetics of Tenofovir Alafenamide (TAF) Following Single Oral Administration in Male Beagle Dogs
P2000114-PK 28-Day Toxicity Study of TAF in Monkeys
R2000065 Comparison of Plasma Pharmacokinetics in Rats of Tenofovir Following Oral Administration of GS-7340-02 or Tenofovir DF as Either a suspension in CMC or a Solution in Citric Acid
P2000087 A Single Dose Pharmacokinetic and Oral Bioavailability Study of GS-7340-02 in Rhesus Monkeys (non-GLP)
R990130 Tenofovir plasma pharmacokinetics following a single oral dose of GS-7340-2 in Sprague-Dawley rats
D990175-PK A 28-Day Oral Gavage Toxicity Study of GS-7340-02 in the Beagle Dog

Distribution:

AD-120-2009 Absorption and Distribution of [^{14}C]GS-7340 Following Single and Multiple Oral Doses to Dogs
AD-120-2011 Pharmacokinetics, Absorption, Distribution, and Excretion of [^{14}C]GS-7340 Following Oral Administration to Mice
AD-120-2020 Pharmacokinetics, Distribution, Metabolism, and Excretion of [^{14}C]GS-7340 Following Single Oral Administration to Rats
AD-120-2026 Plasma Protein Binding of GS-7340
D990173-BP Tissue distribution of ^{14}C-GS-7340-2 in beagle dogs following oral administration

Metabolism:

AD-120-2008 Profiling and Identification of Metabolites in Selected Plasma, Urine, Bile, Feces, Bone, and Liver Samples from Dogs after Oral Administration of [^{14}C]GS-7340
AD-120-2012 Profiling and Identification of Metabolites in Selected Plasma, Urine, Feces, Kidney, Liver, and Nasal Turbinate Samples from Mice after Oral Administration of [^{14}C]GS-7340 and Stability of [^{14}C]GS-7340 in vitro using CD-1 Mouse Hepatic Microsomes and Plasma
AD-120-2017 In Vitro Activation of GS-1278, GS-4331 and GS-7340 in Primary Human Hepatocytes
AD-120-2021 Profiling and Identification of Metabolites in Selected Plasma, Urine, Bile, and Feces Samples from Rats after Oral Administration of [^{14}C]GS-7340
AD-120-2023 In Vitro Metabolism of GS-7340 in Hepatic Subcellular Fractions from Dog and Human
AD-120-2024 Vitro Metabolism of GS-7340 in Intestinal Subcellular Fractions from Dog and Human
AD-120-2025 In Vitro Metabolism of GS-7340 in Plasma from Dog and Human

Pharmacokinetic Drug Interactions:
AD-120-2003 In In Vitro Assessment of Human Liver Cytochrome P450 Inhibition Potential of GS-7340
AD-120-2004 Cytochrome P450 Metabolic Reaction Phenotyping of GS-7340
AD-120-2006 In Vitro Assessment of Human UGT1A1 Inhibition Potential of GS-7340
AD-120-2005 In Vitro Assessment of Induction Potential of GS-7340 in Humans
AD-120-2018 Bi-Directional Permeability of GS-7340 Through Monolayers of P-glycoprotein and BCRP Over-expressing Cells
AD-120-2040 Human CYP Mechanism-Based Inhibition of TAF
AD-120-2027 Effects of HIV Protease Inhibitors and Pharmacokinetic Enhancers on TAF Metabolism In Vitro
AD-120-2019 In Vitro Assessment of GS-7340 Inhibition of Human OATP1B1, OATP1B3, Pgp and BCRP
AD-120-2022 In Vitro Assessment of GS-7340 as a Substrate for Human OATP1B1 and OATP1B3
AD-120-2037 Concentration Dependent Permeability of Tenofovir Alafenamide through Caco-2 Cell Monolayers
AD-120-2013 Effect of GS-9350 on the Bi-Directional Permeability of GS-7340 through Caco 2
AD-120-2031 Effect of Inhibitors of Cathepsin A, Carboxylesterase1, and CYP3A4 on Metabolism of Tenofovir Alafenamide Fumarate (GS-7340) in Primary Human Hepatocytes
AD-120-2032 Evaluation of Induction Potential of GS-7340 in Cultured Human Hepatocytes
AD-120-2035 Effect of Cyclosporin a Pretreatment on Pharmacokinetics of Tenofovir Alafenamide in Dogs
AD-120-2036 Studies to Determine if Tenofovir Alafenamide (GS-7340) is an Inhibitor of OAT1, OAT3, OCT1, OCT2, MATE1, and BSEP or a Substrate for OCT1
AD-216-2112 In Vitro Assessment of Cobicistat as a Substrate for Human OCT2 Transporter

Excretion:

AD-120-2007 Pharmacokinetics, Absorption, and Excretion of ^{14}C-GS-7340 Following Oral Administration to Intact and Bile Duct-Cannulated Dogs

Safety Pharmacology:

PC-120-2005 Effect of GS-7340-03 on cloned hERG Potassium Channels Expressed in Human Embryonic Kidney Cells

R990187 A Pharmacological Assessment of the Effect of GS-7340-2 on Gastrointestinal Motility in the Rat

R990188 A Pharmacological Assessment of the Effect of GS-7340-2 on the Central Nervous System of the Rat

D2000006 A Pharmacological Assessment of the Effect of GS-7340-2 on the Cardiovascular System of the Beagle Dog

Single and repeated-dose Toxicology:

D990181 An Acute Oral Gavage Toxicity Study of GS-7340-02 in Beagle Dog

R990133 An Acute Oral Gavage Toxicity Study of GS-7340-02 in Spargue-Dawley Rats

R990185 An Acute Oral Gavage Toxicity Study of GS-7340-02 in Albino Rat

TX-120-2006 2-Week non-GLP Oral Gavage Range-Finding Toxicity and Toxicokinetic Study of GS-7340-02 in CD-1 Mice

TX-120-2007 13-Week Oral Gavage Toxicity and Toxicokinetic Study with GS-7340-02 in Mice

R990139 A 7-day Repeat Dose Oral Gavage Toxicity Study of GS-7340-2 in the Albino Rat, Lot # 1504-187-19, Gilead Sciences, Boulder, CO, September 8, 2000

R990182 A 28-Day Oral Gavage Toxicity Study of GS-7340-02 in the Albino rat

TOX-120-001: A 26-Week Oral Gavage Toxicity Study of GS-7340-02 in the Albino rat

TX-120-2003: A Tolerability Study of GS-7340-2 by Oral Gavage in Non-Pregnant Rabbit

D990175 A 28-Day Oral Gavage Toxicity Study of GS-7340-02 in the Beagle dog

TOX-120-002 Nine-Month Oral Toxicity Study of GS-7340-02 in Dogs (with a 3-Month interim sacrifice and a 3-Month recovery period)

P2000114 A 28-Day toxicity study of GS-7340-02 and Tenofovir (GS-1278) Administered Orally to Rhesus monkey

Genotoxicity:

V990212 Salmonella - Escherichia coli/Mammalian-Microsome Reverse Mutation Assay with a Confirmatory Assay

V990213 L5178Y TK+/- mouse Lymphoma Forward Mutation Assay with a Confirmatory Assay with GS-7340-02

M2000113 In Vivo Mouse Micronucleus Assay of GS-7340-2

Reproductive and developmental Toxicology:

TX-120-2012	Oral Gavage Study of Fertility and Early Embryonic Development to Implantation with GS-7340-03 in Rats
TX-120-2002	An Embryo-fetal Development Study of GS-7340-02 by Oral Gavage in Rats
TX-120-2005	An Embryo-fetal Development Study of GS-7340-02 by Oral Gavage in Rabbits

Other Toxicology Studies:

TX-120-2011	Primary Dermal Irritation/Corrosion Study with GS-7340 in Rabbits (8253834)
TX-120-2014	GS-7340-03 Assessment of Skin Sensitization Potential using the Local Lymph Node Assay in Mouse
TX-120-2013	The Bovine Corneal Opacity and Permeability Assay (BCOP) (EUN0017)
R990177	7-Day Repeat Dose Toxicity Study of GS-7304-02 and GS-7503 in Male Sprague-Dawley Rats
R2000044	A 6-day Repeat Dose Oral Gavage Exploratory Toxicity Study of GS-7340-2 in the Albino Rat
D990142	Five-Day Oral Toxicity Study of GS-7340-02 and GS-7503 in Beagle Dogs
R990186	A pharmacological assessment of the effects of GS-7340-02 on the renal system of the rat

Impurity Studies: are reviewed under Appendix B by Dr. Mark Powley

TX-120-2008	2-Week Oral Gavage Qualification Toxicity and Toxicokinetic Study of GS-7340 in Male Rat
TX-120-2021	4-Week Oral Gavage Toxicity and Toxicokinetic Bridging Study with GS-7340-03 in Sprague-Dawley Rats

3.2 Studies Not Reviewed

Dose range-finding studies have not been reviewed.

3.3 Previous Reviews Referenced

Appendix A (TAF), Appendix B (Impurities of TAF) contain the relevant review of the NME drug product and impurities. Carcinogenicity study and perinatal/postnatal reproduction toxicity study for TFV-DF has been reviewed under Viread NDA. EVG (Stribild), COBI (Stribild) and FTC (Truvada) are reviewed in their respective NDAs.

4 Pharmacology

4.1 Primary Pharmacology

TAF is predominantly hydrolyzed to TFV in target lymphoid cells. Intracellular, TFV is metabolized to the active metabolite TFV-DP which is a competitive inhibitor of HIV-1 RT and terminates the elongation of the viral DNA chain. TAF exhibits potent anti-HIV activity in lymphoid T-cells, primary human PBMCs, and macrophages with EC_{50} values ranging from 3 to 14 nM. Refer to clinical virology reviews for a detailed description and review of the primary pharmacology data for TAF.

4.2 Secondary Pharmacology

Evaluation of Tenofovir Alafenamide (GS-7340) Effects on Human Myeloid and Erythroid Progenitors (PC-120-2016)

TAF effects on human myeloid and erythroid progenitor cells were evaluated using bone marrow from 3 human donors. The effects of continuous TAF incubation for 14 days as well as a 12 hour pulse incubation with 12 hour washout for 14 days on human erythroid and myeloid progenitor proliferation was evaluated using three different pre-qualified frozen bone marrow lots. In the continuous incubation, TAF was more potent against erythroid (IC_{50} range: 3.30 μM – >3 μM) than myeloid (IC_{50} values > 3μM) progenitor proliferation. 5-Fluorouracil demonstrated similar potencies on erythroid (IC_{50} range: 3.20 – 4.09 μM) and myeloid (IC_{50} range: 1.06 – 3.97 μM) progenitor proliferation. In the 12 hour pulse incubation, GS-7340 did not show potency against erythroid or myeloid progenitor proliferation at concentrations tested in any of the bone marrow lots tested (IC_{50} values > 3μM). 5-Fluorouracil demonstrated similar potencies on erythroid (IC50 range: 20.34 μM to extrapolated value of 118.80 μM) and myeloid (IC50 range: 51.41 – 91.15 μM) progenitor proliferation. The IC_{50} values for TAF under continuous and pulsed exposure conditions were at least 7 fold higher than the clinical C_{max} supporting a favorable profile in myeloid and erythroid progenitor cells.

Tenofovir Alafenamide is Not a Substrate for Renal Organic Anion Transporters (OATs) and Does Not Exhibit OAT-Dependent Cytotoxicity (PC-120-2018)

This assay evaluated the interaction of TAF and TFV with human OAT1 and OAT3 transporters and the potential for OAT-mediated cytotoxicity of TAF and TFV in OAT-expressing cells. While cellular expression of OAT1 and OAT3 increased intracellular accumulation of TFV by > 70-fold and 8.2-fold, respectively, OAT expression did not significantly change TAF intracellular accumulation. The results indicate that TAF does not undergo active transport in human kidney cells expressing OAT1 or OAT3 renal transporters. TFV was more cytotoxic in OAT1- and OAT3-expressing cells (> 21- and > 3.6-fold changes in CC_{50} values, respectively), TAF *in vitro* cytotoxicity showed little to no change upon overexpression of the OAT1 transporter (0.5-fold change in CC_{50}), some increased toxicity was seen after the overexpression of OAT3 (3.5-fold change in CC_{50}). These studies indicate that, unlike TFV, TAF does not interact with renal transporters OAT1 or OAT3 and exhibits no OAT-dependent cytotoxicity. Therefore, TAF is unlikely to actively accumulate in renal proximal tubules in an OAT-dependent manner, supporting the potential for an improved renal safety profile.

In Vitro Evaluation of GS-7340 Effects on Mitochondrial DNA Content (PC-120-2006)

This study evaluated the potential of GS-7340 to induce mtDNA depletion. Relative levels of mtDNA in HepG2 cells treated with with 0.1, 0.3, or 1.0 μ M of GS-7340 for 10 days were measured by quantitative real-time PC. No statistical significant reduction in mtDNA compared to untreated cells was observed. It has been shown previously that TFV-DP does not show inhibition of the mitochondrial DNA polymerase γ. In conclusion, this suggests that GS-7340 has

a low potential for inhibiting mtDNA synthesis and inducing NRTI-related mitochondrial toxicities.

Table 2: Effects of TAF on mitochondrial DNA levels in HepG2 Cells

Drug	Concentration (µM)	Relative Amount of mtDNA (% mtDNA)[a]	p-value compared with DMSO (Control)[b]
DMSO (control)	-	100.0 ± 15.3	-
TAF	0.1	86.4 ± 30.5	0.190
	0.3	88.1 ± 35.5	0.294
	1.0	94.6 ± 17.3	0.318
ddC	0.2	86.7 ± 24.2	0.127
	2.0	11.5 ± 6.2	< 0.0001
	20.0	6.6 ± 1.5	< 0.0001

ddC = zalcitabine; DMSO = dimethylsulfoxide; mtDNA = mitochondrial DNA; TAF = tenofovir alafenamide
a Data represent the mean ± SD of 3 independent experiments performed in triplicate.
b Paired, 2-tailed Student's t-test
Source: Report PC-120-2006

4.3 Safety Pharmacology

Note: The following TAF studies were reviewed by Dr. Pritam Verma under IND-111-007.

Effect of GS-7340-03 on Cloned hERG Potassium Channels Expressed in Human Embryonic Kidney Cells (PC-120-2005/8259094)

This study examined the *in vitro* effects of GS-7340-03 on hERG at 0, 1, 10 µM concentrations. The stability of the test article formulation was confirmed during the method validation study. Test article concentrations were applied to the test system and dose solution analysis was conducted within the validated stability timeframe. The results from the sample analysis indicated that the measured concentrations of GS-7340-03 at both test concentrations were within ±10% of the nominal concentrations thereby meeting the acceptance criteria. GS-7340-03 inhibited hERG current by (Mean ± SEM) 0.9 ± 0.3% at 1 M (n = 3) and 0.3 ± 0.2% at 10 µM (n = 3) versus 0.8 ± 0.1% (n = 3) in control. hERG inhibition at 1 and 10 µM was not statistically significant ($P < 0.05$) when compared to vehicle control values. The IC_{50} for the inhibitory effect of GS-7340-03 on hERG potassium current was not calculated but was estimated to be greater than 10 µM. Under identical conditions, the positive control (60 nM terfenadine) inhibited hERG potassium current by (Mean ± SD) 73.9 ± 5.8% (n = 2). This result confirms the sensitivity of the test system to hERG inhibition.

A pharmacological assessment of the effect of GS-7340-2 on the cardiovascular system of the beagle dog (D2000006/Project No. 93205)

Two groups of male beagle dogs under isoflurane/oxygen anesthesia (3 beagle dogs/group) were administered a single oral gavage dose of GS-7340-2 at dose levels of 30 (LD) or 100 mg/kg (HD) to determine effects on the cardiovascular/hemodynamic system over a period of 24 hrs. Results: There were no deaths. Transient clinical signs related to treatment included vomitus (HD) and one animal (LD) following dosing. There were no drug related effects on heart rate,

systemic blood pressure or electrocardiograms at either dose level. In this study, a dose level of 100 mg/kg may be considered the NOAEL.

A pharmacological assessment of the effect of GS-7340-2 on gastrointestinal motility in the rat (R990187)

Groups of male rats (strain: CD (Crl:CD(SD)BR; 9 rats/group) were administered a single dose of GS-7340-2 via oral gavage at dose levels of 0 (vehicle control), 100 (LD) or 1000 mg/kg (HD) to observe the effects on the passage of activated charcoal along the GI tract; animals were sacrificed at 2, 4 and 6 hr after the treatment. Results: The increased weight of the stomach, and content and observation of charcoal in the stomachs (HD) at all time points indicated that drug reduced the rate of gastric emptying and intestinal motility. There were no changes at the low dose. In this study, a dose level of 100 mg/kg may be considered the NOEL.

A pharmacological assessment of the effect of GS-7340-2 on the central nervous system of the rat (R990188)

Groups of male rats (strain: CD (Crl:CD(SD)BR; 10 rats/group) were administered a single dose of GS-7340-2 via oral gavage at dose levels of 0 (vehicle control), 100 (LD) or 1000 mg/kg (HD) to observe for apparent neuropharmacological signs following dosing. Results: There were no statistically or biologically significant differences from controls for any of the qualitative or quantitative functional assessments. In this study, a dose level of 1000 mg/kg may be considered the NOAEL.

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

Absorption:

Mice:

Plasma pharmacokinetic studies following a single oral administration of TAF in CD-1and 001178-W mice showed that TAF was rapidly absorbed and metabolized to TFV, the plasma T_{max} for TFV was less than 15 minutes. A greater than dose proportional pharmacokinetic response was observed for TFV exposure (C_{max} and AUC_{0-t}) after GS-7340-02 and GS-7340-03 administration. No significant differences in pharmacokinetic profiles were observed between the two salt forms of GS-7340.

Title: Collection of Samples for Determination of the Pharmacokinetics of GS-7340-02 and GS-7340-03 After a Single Oral Dose to Mice (AD-120-2014)

The objective of this study was to determine the pharmacokinetics of GS-7340 and its metabolite TFV following single dose oral gavage administration of GS-7340-02 and GS-7340-03 to male CD-1 mice. GS-7340-02 and GS-7340-03 were each administered at dose levels of 10, 30, and 100 mg/kg to 36 animals/group.

TAF: Exposure to the prodrug GS-7340 was low in mice following oral gavage administration of GS-7340-02 or GS-7340-03 due to rapid conversion to TFV. GS-7340 was generally not detected after administration of 10 and 30 mg/kg and was detected only for 1.5 hours after a dose of 100 mg/kg (Table 2).

TFV: The increases in TFV C_{max} and AUC_{0-t} were greater than dose proportional between the 10 to 100 mg/kg/day for GS-7340-02 and GS-7340-03. Values for C_{max} and AUC_{0-t} for TFV were generally similar between GS-7340-02 and GS-7340-03.

Table 3: Plasma pharmacokinetic parameters following a single dose for GS 7340-02 and GS-7340-03 in male CD-1 mice *(excerpted from sponsor)*

Test Article	GS-7340-02						GS-7340-03					
Dose (mg/kg)	10		30		100		10		30		100	
Analyte	TAF	TFV	TAF	TFV	TAF	TFV	TAF	TFV	TAF	TFV	TAF	TFV
C_{max} (µg/mL)	5.53	106	NA	440	37.1	1827	NA	85.4	10.3	383	34.7	2152
t_{max} (h)	0.083	0.50	NA	0.25	0.083	0.75	NA	0.50	4.00	0.50	0.25	1.50
$t_{½}$ (h)	NA	NA	NA	NA	NA	NA	NA	5.16	NA	10.1	NA	NA
AUC_{0-t} (ng·h/mL)	NA	455	NA	2005	26.0	10643	NA	493	NA	2477	11.3	10866

NA = not applicable

Title: Collection of Samples for Determination of the Pharmacokinetics of GS-7340-03 After a Single Oral Gavage Dose to Male and Female 001178-W (wild type) Mice (AD-120-2016)

This study evaluated the pharmacokinetics of GS-7340 and its metabolite TFV following single dose oral gavage administration of GS-7340-03 to male and female 001178-W (wild type) mice. GS-7340-03 was administered at dose levels of 0 (vehicle control), 10, 30, and 100 mg/kg to 44 animals/sex/group. Plasma samples were assayed for the prodrug GS-7340 and the metabolite TFV.

GS-7340: Exposure to GS-7340 increased with the increase in GS-7340-03 dose level from 30 to 100 mg/kg. The increases in C_{max} were not consistently dose proportional between the 30 to 100

mg/kg dose levels. No consistent gender-based differences were observed in GS-7340 C_{max} and AUC0-t values.

TFV: Exposure to TFV increased with the increase in GS-7340-03 dose level from 10 to 100 mg/kg. The increases in C_{max} and AUC_{0-t} were greater than dose proportional between the 10 to 100 mg/kg. Gender differences in plasma concentration of TFV were less than 2-fold in C_{max} and AUC0-t values. GS-7340 was extensively converted to TFV in mice following oral gavage administration of GS-7340-03.

Table 4: Plasma pharmacokinetic parameters following a single dose of GS-7340-03 to wild type mice *(excerpted from sponsor)*

Dose (mg/kg)	10				30				100			
Analyte	TAF		TFV		TAF		TFV		TAF		TFV	
Sex	M	F	M	F	M	F	M	F	M	F	M	F
C_{max} (ng/mL)	NA	NA	175	100	8.80	117	615	421	648	280	1988	1733
t_{max} (h)	NA	NA	0.25	0.50	0.083	0.5	0.25	0.25	0.25	0.50	0.50	0.50
$t_{½}$ (h)	NA	NA	9.78	8.20	NA	NA	9.51	10.9	NA	NA	8.04	11.0
AUC_{0-t} (ng·h/mL)	NA	NA	735	354	NA	NA	2639	2053	194	104	10026	7131

NA = not applicable

Rats:

Plasma pharmacokinetic studies following a single oral administration of TAF in rats showed that TAF was rapidly absorbed and metabolized to TFV, the plasma T_{max} for TFV was less than 1 hour. A greater than dose proportional pharmacokinetic response was observed for TFV exposure (C_{max} and AUC_{0-t}) after GS-7340-2 dose and GS-7340-93 administration. No significant differences in pharmacokinetic profiles were observed between the two salt forms of GS-7340. Oral administration of 400 mg/kg TAF resulted in a 2 to 3 times higher plasma TFV C_{max} and AUC compared to 400 mg/kg TDF administration.

Title: Collection of Samples for Determination of the Pharmacokinetics of GS-7340-02 and GS-7340-03 after a Single Oral Dose to Rats (AD-120-2015)

The objective of this study was to determine the pharmacokinetics of GS-7340 and its metabolite TFV following single dose oral gavage administration of GS-7340-02 and GS-7340-03 (two salt forms of GS-7340) to male Sprague Dawley (SD) rats. GS-7340-02 and GS-7340-03 were each administered at dose levels of 5, 25, and 100 mg/kg.

GS-7340: All plasma concentration values of GS-7340 were below the lower limit of quantitation (10 ng/mL) for all dose groups following oral gavage administration of GS-7340-02 and GS-7340-03.

TFV: Exposure of TFV increased with the increase in dose level from 5 to 100 mg/kg following administration of GS-7340-02 and GS-7340-03. The increases in C_{max} and AUC_{0-t} were greater than dose proportional between the 5 to 100 mg/kg dose levels for both salt forms. GS-7340 was extensively converted to TFV in rats and the mean C_{max} and AUC_{0-t} for TFV were generally similar at each dose group following administration of GS-7340-02 and GS-7340-03. The differences in the mean AUC_{0-t} were less than 30% between the two salt forms of GS-7340.

Table 5: Plasma Pharmacokinetic parameters following a single dose of GS-7340-02 and GS-7340-03 in male SD rats *(excerpted from sponsor)*

Test Article	GS-7340-02			GS-7340-03		
Dose (mg/kg)	5	25	100	5	25	100
Analyte	TFV	TFV	TFV	TFV	TFV	TFV
C_{max} (µg/mL)	32.5	199	1240	39.3	364	1670
t_{max} (h)	0.667	0.583	0.833	0.583	0.833	0.667
$t_{½}$ (h)	NA	11.2	10.3	NA	7.89	7.85
AUC_{0-t} (ng·h/mL)	122	1395	7771	88.5	1810	9759

Title: Tenofovir plasma pharmacokinetics following a single oral dose of GS-7340-2 in Sprague-Dawley rats (R990130)

Groups of male Sprague-Dawley rats (2-3 animals/group) were administered a single oral dose of GS-7340-2 via gavage at dose levels of 6.25, 25, 100 or 400 mg/kg. Plasma samples were obtained over the course of 24 hr and concentrations of TFV were determined. Following the oral administration, GS-7340-2 was rapidly absorbed and converted to TFV (T_{max} 30 min or less). A proportional pharmacokinetic response was observed for total TFV exposure versus GS-7340-2 dose (25-400 mg/kg doses).

Title: Comparison of Plasma Pharmacokinetics in Rats of Tenofovir Following Oral Administration of GS-7340-02 or Tenofovir DF as Either a suspension in CMC or a Solution in Citric Acid (R2000065**), non-GLP**

This study was designed to determine the possible influence of the drug delivery vehicle Carboxymethylcellulose (CMC) and citric acid on the pharmacokinetic parameters of GS-7340-02 (TAF monofumerate) and TDF. Further this study compared the systemic TVR exposure after oral administration of TAF and TDF. Oral administration of 400 mg/kg TAF resulted in a 2 to 3 times higher plasma C_{max} and AUC of TFV compared to 400 mg/kg TDF administration.

Table 6: Plasma pharmacokinetic parameters following a single dose of GS-7340-02 and TDF to rats *(excerpted from the sponsor)*

Assay	LC/MS/MS			
Test Article	GS-7340-02		TDF	
Sex (M/F) / N of Animals	M/3			
Vehicle Formulation	CMC suspension	50 mM citric acid	CMC suspension	50 mM citric acid
Analyte	TFV			
PK Parameters				
t_{max} (h)	0.50	0.25	0.25	0.50
C_{max} (ng/mL)	14229	8418	8101	2699
$t_{½}$ (h)	11.3	11.4	7.21	8.31
AUC_{0-t} (ng•h/mL)	36288	33067	15774	11403
$AUC_{0-\infty}$ (ng•h/mL)	36795	33638	15848	11581
t_{last} (h)	55.0	55.0	55.0	48.0

CMC = carboxymethylcellulose; F = female; M = male; TAF = tenofovir alafenamide; TFV = tenofovir

Dogs:

After oral administration TAF was rapidly absorbed and eliminated with a T_{max} of less than 0.5 h and $T_{½}$ ranging below 1h. The rapid disappearance of TAF was accompanied by an increase in TFV. Levels of TFV in PBMCs 2 hr after dosing were 10 to 50-fold greater than those observed in plasma and remained elevated until 24 hr while the plasma levels declined significantly. Plasma exposures to TAF and TFV as well as PBMC exposure to TFV were approximately 2.5-fold higher in fasted dogs than in fed dogs.

Title: Analysis of data from (b) (4) oral bioavailability of GS-7340-2 in beagle dogs (99-DDM-1278-001-PK)

This study assed the effects of the stereo configuration, fumarate form, food, and the route of administration. Plasma and PBMC pharmacokinetic profiles were determined in Beagle dogs following intravenous (IV) bolus (GS-7340-02 [6.3 mg/kg], TAF monofumarate), or oral administration (TAF as free base [18.0 mg/kg], its diastereomer GS-7339 [18.0 mg/kg], the mixture GS-7171 [16.0 mg/kg], or GS-7340-02 [4,8, 5.0, and 20 mg/kg under fasted and 5.0 mg/kg under fed conditions]). After oral administration, TAF was rapidly absorbed and eliminated with a T_{max} of less than 0.5 h and $t_{½}$ ranging from 0.2-0.9 h. Levels of TFV in PBMCs 2 hr after dosing were 10 to 50-fold greater than those observed in plasma and remained elevated until 24 hr while the plasma levels declined significantly. The TFV exposure in PBMCs was approximately 4-fold higher when animals were dosed with TAF than GS-7339. All the pharmacokinetic parameters were similar for the free base and fumarate form. Plasma exposures to TAF and TFV and PBMC exposure to TFV were approximately 2.5-fold higher in fasted dogs than in fed dogs.

Title: Plasma and Liver Pharmacokinetics of Tenofovir Alafenamide (TAF) Following Single Oral Administration in Male Beagle Dogs (AD-120-2034)

Male Beagle dogs received a single dose of 10 mg/kg TAF. Plasma and liver samples were collected from all animals. Plasma concentrations of TAF and its metabolite, TFV, and the liver concentrations of TFV and its mono- and diphosphate metabolites were determined by LC-MS/MS analysis. Following oral administration, TAF was rapidly absorbed and exhibited a short terminal half-life ($T_{1/2}$) of 0.24 h in plasma. The rapid disappearance of TAF was accompanied by an increase in TFV. TFV was the major metabolite detected in plasma achieving a maximal plasma concentration (C_{max}) of 2.23 μM. The pharmacologically active diphosphate metabolite, TFV-DP, was efficiently formed in dog liver achieving a maximal concentration of 126 μM at 4.0 h post-dose.

Title: Plasma and Liver Pharmacokinetics of Tenofovir Alafenamide (GS-7340) Following 7-Day Oral Administration in Male Beagle Dogs (AD-120-2033)

Male Beagle dogs received 8.29 mg/kg daily for 7 days. Plasma pharmacokinetic profiles for TAF and its metabolite, TFV were determined and the liver concentrations of TFV and its mono- and diphosphate metabolites were determined. Following oral administration, TAF was rapidly absorbed and exhibited a short terminal half-life ($T_{1/2}$) of 0.3 h in plasma on both Day 1 and 7. The rapid disappearance of TAF was accompanied by an increase in TFV. TFV was the major metabolite detected in plasma achieving a maximal plasma concentration (C_{max}) of 1.47 and 2.12 μM on Day 1 and 7, respectively. The pharmacologically active diphosphate metabolite, TFV-DP, was efficiently formed in dog livers achieving concentrations of 242 and 153 μM at 4.0 and 24 h post-dose on Day 7, respectively.

Monkey:

Title: A Single Dose Pharmacokinetic and Oral Bioavailability Study of GS-7340-02 in Rhesus Monkeys (non-GLP) (P2000087)

This study evaluated the pharmacokinetic profiles for TAF and TFV in the plasma as well as TFV concentrations in PBMCs in rhesus monkeys following a single oral dose of GS-7340-02 at 0.5, 5.0, and 50 mg/kg. TAF and TFV were rapidly absorbed with T_{max} of approximately 0.5 and 1 hour, respectively. TAF plasma concentrations declined rapidly while TFV declined at a slower rate. TFV was also measured in PBMCs and showed a slower decline than in plasma. TFV persisted in PBMCs up to 96 hours. When PBMCs were treated with acid phosphatase which converts all phosphorylated metabolites to TFV significantly higher TFV levels were observed in PBMCs suggesting that significant proportion of TFV-related material in PBMCs was in phosphorylated forms.

Table 7: Plasma pharmacokinetic parameters for TAF and TFV following a single dose of GS-7340-02 in Rhesus Monkeys *(excerpted from sponsor)*

GS-7340-02 Dose (mg/kg)	0.5	5	50	0.5	5	50
Analyte	TAF			TFV		
C_{max} (ng/mL)	2.79	125	4143	7.72	161	1326
t_{max} (h)	0.38	0.8	0.5	1	1.33	1.0
$t_{½}$ (h)	0.61	0.23	0.40	4.62	9.92	17.33
AUC_{0-last} (ng·h/mL)	1.22	95.1	3811	39.9	1037	9934
$AUC_{0-\infty}$ (ng·h/mL)	2.47	80.0	3846	52.7	1069	10250

Table 8: Concentrations of TFV in PBMCs from Rhesus Monkeys dosed with GS-7340-02 *(excerpted from sponsor)*

GS-7340-02 Dose (mg/kg)	TFV PBMC Levels (ng/10^6 Cells)			
	Without Phosphatase Treatment		With Phosphatase Treatment	
	5	50	5	50
2 h	0.47	17.0	0.73	34.2
24 h	0.06	6.82	0.62	20.1
96 h	BLQ	3.03	0.18	8.68

Distribution:

Protein binding of TAF was around 48.0% and 46.8% in dog and human in vitro plasma studies, respectively. In human ex vivo studies with the mean percent unbound TAF ranged from 14% to 23%. In rodent plasma TAF is highly unstable because of high levels of plasma esterases expressed in some rodent species which lead to hydrolytic cleavage. TFV showed very low protein binding in human plasma, 99.3 ± 3.3% of TFV was unbound in human plasma.

Following oral administration of [^{14}C] TAF Tissue distribution was evaluated in mice, rats and dogs. TAF was extensively distributed to most tissues. The highest accumulation was found in kidneys, PBMCs, liver, large intestine and bile. In mice low levels of radioactivity were detected in brain and testis suggesting that ^{14}C-TAF derived radioactivity poorly crossed the blood:brain and blood:testis barrier. CD57 black mice showed a more persistent exposures in eye lens, eye uveal tract, and eyes 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.

Metabolism:

The metabolic profiles of TAF were determined in mice (plasma, urine, feces, kidney, liver, and nasal turbinate), rats (plasma, urine, bile, and feces) and dogs (plasma, urine, bile, feces, bone, and liver). In humans the metabolite profiles were also determined in plasma, urine, and feces following administration of a single oral dose of ^{14}C-TAF.

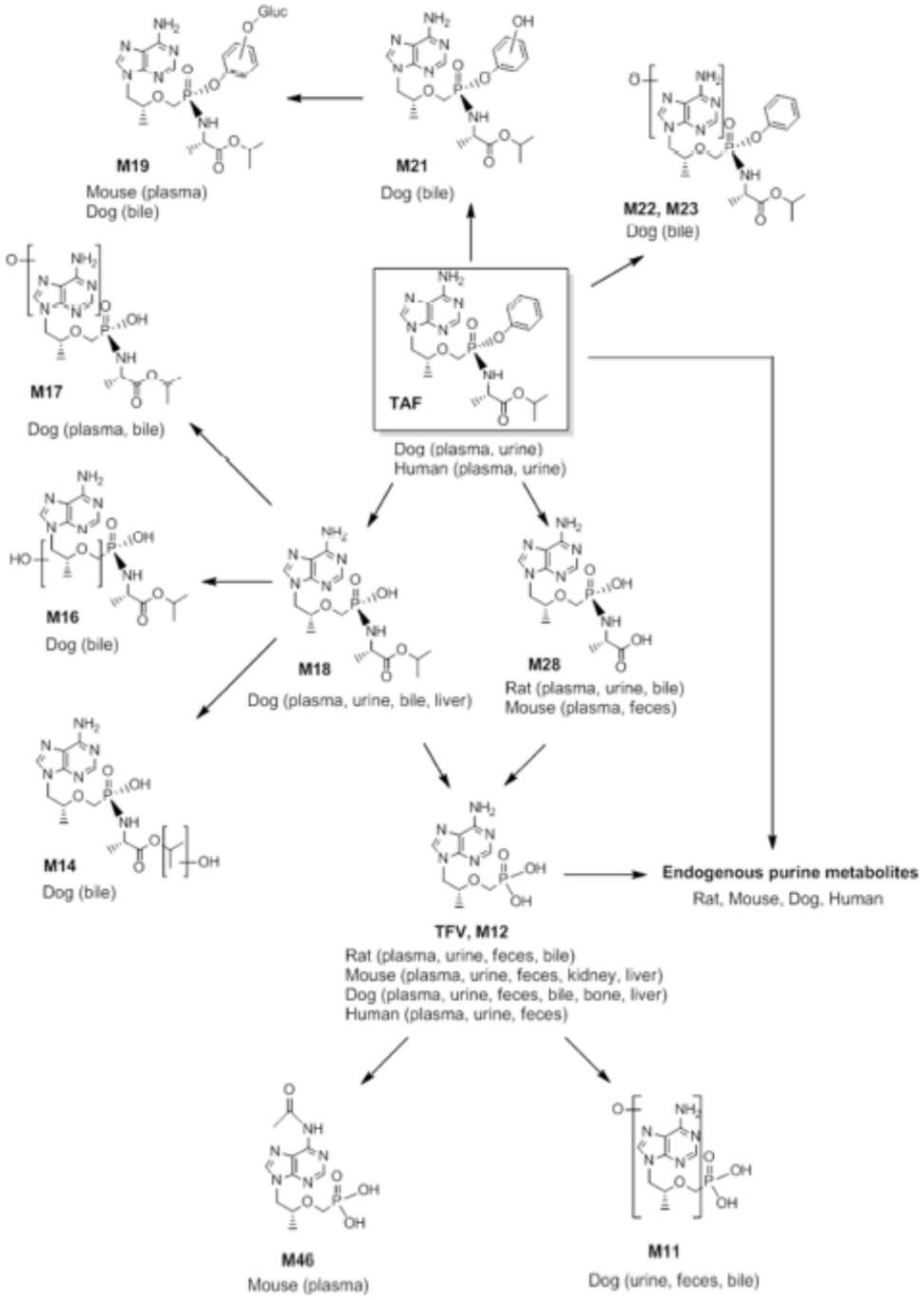
TAF is hydrolized intracellular to TFV by CatA cleavage in lymphoid cells. TFV is metabolized to the active metabolite, TFV-DP which is a competitive inhibitor of HIV-1 RT and terminates the elongation of the viral DNA chain. The in vivo metabolism of TAF was analyzed in mouse, rat, dog and humans. Endogenous purine metabolites including hypoxanthine, xanthine, allantoin, and uric acid were observed in all species. No metabolites unique to human were observed. TFV accounted for a majority of drug related material in plasma, urine, and feces from all species except for human plasma, in which uric acid was the predominant metabolite accounting for 73.9% of the total AUC over 96 hours. M18 was the major metabolite in rat bile (63% of total radioactivity recovered in bile). M18 and its oxidized metabolite, M16 were the major metabolites in dog bile accounted for 29 and 38% of total radioactivity recovered in bile. Various oxidative metabolites were found in dog bile.

In mice TFV was the main TAF metabolite in kidney and liver, M7 was the major identified metabolite in nasal turbinates. In dogs TFV was the main TAF metabolite in bone and liver.

M18 (isopropylalaninyl TFV) and M28 (alaninyl TFV) are considered to be intermediate metabolites during intracellular conversion of TAF to TFV. Relatively high levels of M18 were observed in bile. Low levels of M28 were observed in rat and mouse plasma with relatively high levels in rat bile.

In monkeys the kinetics of intracellular TFV anabolism in PBMCs, red blood cells (RBCs), and lymph was studied after single dose of TFV. TFV was efficiently taken up by PBMCs and the half-life of TFV-DP in this experiment was > 50 hours. Similar concentrations of TFV anabolites were observed in RBCs. Significant intracellular concentrations of TFV and its anabolites were observed in axillary, inguinal, and mesenteric lymph nodes.

Figure 5: Metabolites of TAF



Pharmacokinetic Drug Interactions:

The inhibitory activity of TAF with human liver microsomal CYP isozymes, CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, and CYP3A were assessed at concentrations up to 25 μM.. GS-7340 was identified as a substrate for CYP3A4 but the rate of metabolism was slow. Since COBI is a specific and potent inhibitor of CYP3A, TAF-mediated CYP3A inhibition is unlikely to play any role. The induction of CYP, P-gp and UGT1A1 mRNA and CYP activity by TAF was evaluated in cultured human hepatocytes. TAF showed little or no potential for CYP induction at clinically relevant concentration. Further, no significant induction (less than 2-fold) of P-gp and UGT1A1 mRNA was observed. The potential for TAF to induce human drug metabolizing enzymes and drug transporters through the activation of human AhR or human PXR was further evaluated and TAF was not identified as an activator of either the aryl hydrocarbon receptor (AhR) or human pregnane-X-receptor (PXR). This suggests that TAF is unlikely to induce human CYP isoforms or transport proteins whose expression is controlled by these xenobiotic receptors. Data from these studies, taken in combination with the relatively low plasma exposures of TAF in humans suggest that the potential of TAF to cause or be affected by clinically relevant drug-drug interactions is low.

Different from TFV, TAF does not interact with renal transporters OAT1 or OAT3 and exhibits no OAT-dependent cytotoxicity. Therefore, TAF is unlikely to actively accumulate in renal proximal tubules in an OAT-dependent manner. TAF has been shown to be a substrate for the hepatic uptake transporters OATP1B1 and OAPTP1B3 and may be affected by inhibitors of these transporters. Further, TAF was identified to be a substrate for efflux transporters, P-gp and BCRP. In vitro studies showed an increase of TAF absorption in the presence of efflux transport inhibitors CsA and COBI. In vivo, oral administration of TAF following pretreatment with CsA lead to an increase of the TAF plasma exposure and oral bioavailability by approximately 10-fold, the exposure to TFV-DP in PBMCs in CdA pretreated dogs as also around 2-fold higher compared to untreated dogs. Therefore the coadministration of efflux transport inhibitors might increase TAF absorption.

Table 9: Transporter inhibition assessment of E/C/F/TAF components *(excerpted from the sponsor)*

	IC_{50} (µM)				
Transporter	EVG	COBI	FTC	TAF	TFV
P-gp	69.7	36	>100	>100	>1000
BCRP	88.9	59	>100	>100	>100
BSEP	>20	6.5	>100	>100	>100
OATP1B1	>2	3.50	>100	>100	>100
OATP1B3	0.44	1.88	>100	>100	>100
MATE1	2.0	1.87	>100	>100	>300
MATE2-K	ND	33.5	ND	ND	ND
OAT1	>20	>100	>100	>100	33.8[a]
OAT3	>20	>100	>100	>100	>1000
OCT1	>20	14.7	>100	>100	>100
OCT2	>20	14.4	>100	>100	>300
OCTN1	ND	2.49	ND	ND	ND
MRP1	ND	45-90	ND	ND	>500
MRP2	>20	45-90	>100	ND	>100
MRP4	>20	20.7	>100	ND	>1000[b]

BCRP = breast cancer resistance protein; BSEP = bile salt excretory pump; MATE1 or 2-K = multidrug and toxin extrusion protein 1 or 2-K; MRP1 2, 3, or 4 = multidrug resistance associated protein 1, 2, or 4; ND = not determined; OAT1 or 3 = organic anion transporter 1 or 3; OATP1B1 or B3 = organic anion transporting polypeptide 1B1 or B3; OCT1 or 2 = Organic cation transporter 1; OCTN1 = organic cation transporter novel, type 1; P-gp = permeability glycoprotein
[a] Binding constant for uptake into CHO cells reported by Cihlar et al, 2009, [b] Imaoka et al 2007

Excretion:

Excretion of TAF after oral administration was evaluated in mice, rats and dogs using ^{14}C-TAF. Most of the radiolabeled material was excreted in feces or urine in all species. In BDC rats, 72.6%, 23.2%, and 2.11% of the administered radioactivity were excreted in feces, urine, and bile, respectively, by 168 hours postdose. In BDC dogs, 42.7%, 26.5%, and 14.0% of the administered radioactivity were excreted in feces, urine, and bile, respectively, through 168 hours postdose. For TFV renal excretion was determined to be the major route of excretion in rats and dogs.

TFV was mainly excreted by the kidney in the tested animal species. After IV administration of ^{14}C-TFV rats and dogs excreted 85.2% by 24 hours (rats) and 70.03% by 48 hours (dogs) of the radioactivity in the urine.

Title: Pharmacokinetics, Absorption, and Excretion of 14C-GS-7340 Following Oral Administration to Intact and Bile Duct-Cannulated Dogs (AD-120-2007)

This study evaluated the excretion of TAF after administration of a single 15-mg/kg oral dose of ^{14}C-TAF. The radioactivity was mostly excreted after 48 hours. The mean values of 37.4% and 35.9% of the administered radioactivity were excreted in feces and urine, respectively, by 168 hours postdose. Overall mean recovery of radioactivity was 80.4%. The excretion of TFV was evaluated following IV administration of ^{14}C-TFV. TFV was mainly excreted via the kidney, 70.03% of the radioactive dose was recovered in urine within the first 48 hours after dosing.

Toxicokinetics

Title: Toxicokinetics of a 28-Day Oral Gavage Toxicity Study of GS-7340-02 in Beagle dogs (D990175)

The plasma PK of TAF and TFV and TFV levels in PBMCs were determined in a 28-day oral gavage toxicity study in adult male and female beagle dogs following daily administration of either vehicle, 0.1, 0.3, 1.0, 3.0, or 10 mg/kg/day of GS-7340-02. Only the 10 mg/kg/day dose cohort provided adequate data over time for a complete pharmacokinetic analysis. Repeat dosing at 10 mg/kg/day resulted in nonlinear pharmacokinetics between Days 1 and 28 with TAF median AUC values of 0.454 and 0.985 μg·h/mL, C_{max} values of 582 and 1280 ng/mL, and $t_{½}$ values of 18 and 23 minutes, respectively. The TFV C_{max} values appeared to be linear with increasing dose as well as repeat dosing. The TFV $t_{½}$ was estimated to be 37 hours and substantial accumulation of TFV was observed after repeat dosing [AUC_{∞} on Day 1 (1.68 μg*hr/mL) to Day 28 (5.26 μg*hr/mL)]. TFV concentrations in PBMCs were approximately 100-fold higher than corresponding plasma concentrations.

Multidose TK studies are reviewed under their respective toxicity studies.

6 General Toxicology

6.1 Single-Dose Toxicity

Note: The following TAF studies were summarized from the review done by Dr. Pritam Verma under IND-111-007.

Single dose in rats:

Title: An acute oral gavage toxicity study of GS-7340-2 in the albino rat, Lot # 1504-187-19, (b) (4) August 31, 2001, (R990185)*

Groups of male and female Sprague-Dawley rats were orally gavaged with single doses of GS-7340-2 at dose levels of 0 (vehicle control), 100 (low), 300 (mid) or 1000 mg/kg (high) followed by a 14-day observation period. In this study, a single dose of GS-7340-2 was well tolerated at doses up to 1000 mg/kg. NOAEL was considered to be 1000 mg/kg.

Title: An acute oral gavage toxicity study of GS-7340-2 in the albino rat, Lot # 1504-187-19, Gilead Sciences, Boulder, CO, August 2, 2000, (R990133)

Groups of male and female Sprague-Dawley rats were orally gavaged with single dose of GS-7340-2 at dose levels of 0, 6.25, 25, 100 or 400 mg/kg followed by a 14-day observation period. In this study, a single dose of GS-7340-2 was well tolerated at doses up to 400 mg/kg. A dose level of 400 mg/kg may be considered the NOAEL.

Single dose in dogs:

Title: An Acute Oral Gavage Toxicity Study of GS-7340-02 in the Beagle Dog (Project No.: 89187/Gilead Study No.: D990181) GLP

Groups of male and female beagle dogs (1 animal/sex/group) received a single oral gavage of GS-7340-02 at dose levels of 0, 30, 90 or 270 mg/kg and animals were observed for 14 days (euthanized on Day 15).There were no deaths during the 14-day observation period. Clinical signs, including vomiting, retching and salivation following dosing, were observed in animals treated with GS-7340-02 at a dose of 270 mg/kg, most marked in the male. The male animals treated at 270 mg/kg was also observed to show signs of reduced activity, weakness, reduced food consumption, tremors and incoordination, generally no longer apparent on Day 2. In addition, salivation was noted in animals treated at a dose of 90 mg/kg.

An increase in blood urea nitrogen was noted in the male treated at a dose level of 270 mg/kg and in females treated at dose levels of 30, 90 or 270 mg/kg on Day 2 of the observation period, when compared to both the control and pre-treatment values. The differences were no longer apparent on Day 14.

Increases in kidney and increases in kidney and spleen weight relative to body weight were noted for the males treated with GS- 7340-02 at dose levels of 30, 90 or 270 mg/kg, with an increase in relative spleen weight also observed in females at a dose level of 30 or 270 mg/kg. Treatment-related renal tubular changes characterized by basophilia and karyomegaly were seen in the male and female treated with GS-7340-02 at a dose level of 270 mg/kg and the female at a dose level of 90 mg/kg. Treatment-related clinical signs, slight weight loss, and reduced food consumption were noted in a number of animals, with microscopic renal changes in animals treated at dose levels of 90 or 270 mg/kg, most marked at a dose level of 270 mg/kg.

A moderate atrophy of the thymus was noted in one male treated at a dose level of 270 mg/kg and a minimal atrophy was noted in one male treated at a dose level of 90 mg/kg. The etiology of the thymus change was not clear and the possibility of an effect of treatment could not be discounted however, the change is frequently associated with stress.

In view of the treatment-related effects at mid and high doses, the NOAEL was considered to be 30 mg/kg.

6.2 Repeat-Dose Toxicity

Note: The following TAF studies were reviewed by Dr. Pritam Verma under IND-111-007 and either summarized from his review or his original review was used.

Repeated-Dose Toxicity Studies in Mice

Study title: 2-Week non-GLP Oral Gavage Range-Finding Toxicity and Toxicokinetic Study of GS-7340-02 in CD-1 Mice (TX-120-2006)

Thirty toxicity and toxicokinetic animals (5 males and 5 females given 500 mg/kg/day and 7 males and 13 females given 1000 mg/kg/day) were found dead or were sacrificed in moribund condition. The cause of death of six animals was determined to be gavage-related. Based on histopathological changes and observation during the dose preparation, the most likely cause of death for all other animals was due to nonfatal gavage injury and secondary inflammation or problems with the dose formulation, respectively.

Test article-related effects included minimal to moderate reduced absolute reticulocyte count, increased total protein and albumin, increased ALP, and increased inorganic phosphorus. Microscopically, test article-related findings were observed in the rectum. Minimal to slight vacuolation/apoptosis of the epithelium was observed in males given >100 mg/kg/day and females given 500 or 1000 mg/kg/day. This finding was not considered adverse as the epithelium remained intact and there was no resulting inflammation or necrosis.

Findings with an unclear relationship to the test article were observed in the bone marrow, and may correlate with alterations in clinical pathology. Minimal to slightly expanded blood sinuses were observed in the bone marrow of the femur of males given 500 mg/kg/day and females given >100 mg/kg/day and were also present in the bone marrow of the sternum of one control male, males given 500 or 1000 mg/kg/day and females given 100 or 500 mg/kg/day. Relative increases

in granulocytes were observed in the bone marrow of the femur of males given 1000 mg/kg/day and females given 100 mg/kg/day and bone marrow of the sternum of females given >100 mg/kg/day. Adrenal gland cortices were hypertrophied in males given >100 mg/kg/day and atrophied (reduced vacuolation/cortical thickness) in one male given 100 mg/kg/day and females given 500 mg/kg/day. These findings correlated with an mean adrenal gland weight increase in high dose males (+54% relative to body weight) and decreased in high dose females (-25% relative to body weight) compared with nonage-matched controls. Thymic atrophy and lymphocyte necrosis were observed in males given 500 or 1000 mg/kg/day, and lymphocyte necrosis was observed in the thymus of males given >100 mg/kg/day and females given 500 or 1000 mg/kg/day. These changes correlated with a decrease in mean thymus weights in middle and high dose males (-19% and -37% relative to body weight, respectively) and may have been stress related. Lymphocyte necrosis was observed in the mesenteric lymph node of males and females given 1000 mg/kg/day.

The NOAEL was defined as 100 mg/kg/day. For GS-7340 this dose corresponded to a Day 14 C_{max} of 27.1 and 2.89 ng/mL for males and females, respectively; the AUC_{0-24} could not be calculated due to the lack of a distinct elimination phase. GS-7340 rapidly converted to its metabolite, TFV. For TFV, a dose of 100 mg/kg/day corresponded to Day 14 AUC_{0-24} values of 5973 and 5506 ng•hr/mL for males and females, respectively.

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

Study no.:	TX-120-2007
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	25 January 2011
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	GS-7340-02, 7340-02-AC-1EA, 97.7%

Key Study Findings: GS-7340-02 was administered by oral gavage for up to 13 weeks to male and female Crl: CD1 (ICR) mice at a dose of 10, 30, or 100 mg/kg/day. Findings included histopathology in nasal mucosa at all doses. Specifically, degenerative changes in olfactory cells and acute inflammatory changes (neutrophil infiltration) were seen in the nasal mucosa at all dose levels. Decreases in body weight gain for animals administered GS-7340-02 at all dose levels, a transient decrease in food consumption for animals administered 30 or 100 mg/kg/day, and microscopic findings in the rectum (increased apoptosis) for animals administered 100 mg/kg/day were not considered adverse. Due to the microscopic findings in the nasal turbinates at all doses, a NOAEL was not defined.

Methods

Doses:	10, 30, and 100 mg/kg/day
Frequency of dosing:	Once per day
Route of administration:	Oral gavage
Dose volume:	10 mL/kg
Formulation/Vehicle:	0.1% (v/v) Tween 20 and 0.1% (w/v) hydroxypropylmethylcellulose (HPMC) K100LV prepared in reverse osmosis water
Species/Strain:	Mice/CD-1
Number/Sex/Group:	See sponsor's table below
Age:	5-6 weeks
Weight:	25.1 to 37.1 g for males and 21.7 to 33.7 g for females
Satellite groups:	See sponsor's table below
Unique study design:	no
Deviation from study protocol:	The reported deviations are not expected to affect study conclusions.

Table 10: Study design of 13-week toxicity study in mice *(excerpted form sponsor)*

Group[a]	No. of Animals Male	No. of Animals Female	Dose Level (mg/kg/day)	Dose Concentration[b] (mg/mL)
Toxicity Animals				
1 (Control)	15	15	0	0
2 (Low)	15	15	10	1
3 (Mid)	15	15	30	3
4 (High)	15	15	100	10
Toxicokinetic Animals				
5 (Control)	6	6	0	0
6 (Low)	42	42	10	1
7 (Mid)	42	42	30	3
8 (High)	42	42	100	10

a Groups 1 and 5 received vehicle control article only.
b Doses and dose concentrations were based on fumarate salt.

Observations and Results

Mortality: Mortality/morbidity checks were performed twice daily. No test article-related mortality occurred. A high dose (100 mg/kg/day) toxicity group male was sacrificed in moribund condition on Day 54. This animal was first observed as hunched on Day 52, and subsequently became hypoactive and debilitated with rough haircoat, pale eyes, and/or red oral discharge. The microscopic finding of inflammation in the thoracic cavity (affecting multiple organs in the thoracic cavity) was consistent with a gavage-related injury. Two toxicokinetic animals were found dead on Days 15 and 45. Per protocol, the cause of death was not determined.

Clinical Signs: Cageside observations were recorded daily during dosing, 1-2 and 4-6 hours post dose. Detailed clinical observations were performed predose and weekly during dosing. There were no clinical signs associated with test article administration.

Body Weights: Body weights were recorded weekly during dosing. Although not statistically significant, mean body weight change relative to the controls was decreased during the overall dosing phase (Days 1 through 92); 15% for males administered 100 mg/kg/day, and 11, 24, and 9% for females administered 10, 30, or 100 mg/kg/day, respectively.

Feed Consumption: Feed consumption was determined weekly during dosing. Mean food consumption relative to the controls was significantly decreased for the males and females administered 30 or 100 mg/kg/day for up to two weeks at the start of dosing.

Ophthalmoscopy: Ophthalmoscopic examinations were conducted pre-dose and on Day 88 of dosing. No visible lesions were noted.

Clinical Pathology: Blood samples were collected for hematology and clinical chemistry on the day of scheduled sacrifice.

Hematology: A minimal but statistically significant decrease (-37.7%) in the lymphocyte count in high dose females (Day 93) was not considered toxicologically significant. Though decreased, lymphocyte counts were within the range of control values.

Clinical Chemistry: There were no remarkable findings.

Gross Pathology: There were no significant macroscopic observations.

Organ Weights: The following organs were weighed: adrenal, brain, epididymis, heart, kidney, liver with gallbladder (drained), lung, ovary, pituitary gland, prostate, salivary gland (mandibular), seminal vesicle, spleen, testis, thymus, thyroid with parathyroid, uterus. There were no remarkable findings.

Histopathology: Adequate Battery Yes. Peer Review No.

Histological Findings: Adverse test article-related microscopic findings were present in the nasal turbinates [minimal to slight infiltrates of neutrophils in respiratory and olfactory mucosa, and minimal to moderate degeneration of olfactory epithelium] of males and females administered >10 mg/kg/day; an additional finding in the nasal turbinates (minimal exudate in the lumen) was present in males and females administered >30 mg/kg/day. A test article-related microscopic finding also occurred in the rectum (minimal increased apoptosis) of males and females administered 100 mg/kg/day.

Table 11: Incidence and severity of test article-related nasal turbinate findings *(excerpted from sponsor)*

	GS-7340-02							
Sex	Males				Females			
Dose Level (mg/kg/day)	0	10	30	100	0	10	30	100
Nasal Turbinates								
Number Examined/Group	15	15	15	15	15	15	15	15
Infiltrate, Neutrophil, Respiratory Mucosa								
Minimal	1	3	4	6	1	3	6	10
Slight	0	2	2	5	0	0	0	4
Total Incidence	1	5	6	11	1	3	6	14
Infiltrate, Neutrophil, Olfactory Mucosa								
Minimal	1	7	7	7	1	4	4	11
Slight	0	2	1	7	0	0	0	2
Total Incidence	1	9	8	14	1	4	4	13
Degeneration, Olfactory Epithelium								
Minimal	2	5	5	3	2	4	7	8
Slight	0	3	2	8	0	0	0	4
Moderate	0	0	1	2	0	0	0	1
Total Incidence	2	8	8	13	2	4	7	13
Exudate, Lumen								
Minimal	0	0	2	2	0	0	2	2
Total Incidence	0	0	2	2	0	0	2	2

Test article-related microscopic finding also occurred in the rectum (minimal increased apoptosis) of males and females given 100 mg/kg/day (see table below for incidence, *excerpted from sponsor*).

Table 12: Incidence and severity of test article-related rectum findings

	GS-7340-02							
Sex	Males				Females			
Dose Level (mg/kg/day)	0	10	30	100	0	10	30	100
Rectum								
Number Examined	15	15	15	15	15	15	15	15
Apoptosis, Increased								
Minimal	0	0	0	5	0	0	0	2
Total Incidence	0	0	0	5	0	0	0	2

Toxicokinetics: The increases in C_{max} and AUC_{0-t} were generally greater than proportional between the 10 to 100 mg/kg/day dose levels. Gender-based differences were less than 2-fold in TFV C_{max} and AUC_{0-t} values. No unexpected accumulation of TFV was observed after multiple dosing of GS-7340-02 in mice. GS-7340 was rapidly and extensively converted to TFV after oral administration in mice. Mean toxicokinetic parameters, for males and females combined, are presented (Table 13, *excerpted from sponsor*).

Table 13: Mean toxicokinetic parameters for TFV in mouse plasma on Day1 and Week 13

GS-7340-02 (mg/kg/day)	Day 1		Week 13	
	C_{max} (ng/mL)	$AUC_{(0-t)}$ (ng·h/mL)	C_{max} (ng/mL)	$AUC_{(0-t)}$ (ng·h/mL)
10	59.5	171	69.2	213
30	292	1282	330	1507
100	1011	6534	863	7397

Note: Data is based on the analysis of the combined mean concentration data of both sexes.

Stability and Homogeneity: The stability of samples prepared at 0.05 and 240 mg/mL GS-7340 (free base) and at 0.06 mg/mL, 77 mg/mL, and 307 ng/mL GS-7340-02 was confirmed for 15 days protected from light at room temperature (15 to 30°C) and refrigerated conditions. Replicates of the out-of-specification location were 78.9 and 98.2% of the nominal concentration, resulting in a mean of 88.6% of theoretical for this location, an overall mean of 92.3%, and a relative standard deviation of 7.9% for the 1 mg/mL preparation.

Repeated-Dose Toxicity Studies in Rats

A 7-day repeat dose oral gavage toxicity study of GS-7340-2 in the albino rat, Lot # 1504-187-19, Gilead Sciences, Boulder, CO, September 8, 2000, (R990139)

Groups of male and female Sprague-Dawley rats [weight: 184-219 g; strain: Crl:CD(SD)BR; 4 animals/sex/group] were orally gavaged with GS-7340-2 at dose levels of 0 (vehicle control), 6.25, 25, 100 or 400 mg/kg/day for 7 consecutive days to asses toxicity in rats. No clinical abnormalities or mortalities were observed. The body weights of male and female (high) were 8% less than controls by the end of the study. The following effects were seen at the high dose unless otherwise stated: Hematology: the mean total white blood cell count was reduced 19 to 27% (high). The decrease was predominantly due to a 35 to 43% reduction in the absolute neutrophil counts and an 18 to 24% reduction in the absolute lymphocyte count. The absolute reticulocyte count was reduced 35 to 58% without a noteworthy concomitant reduction of the RBC count. Clinical chemistry: the mean plasma blood urea nitrogen value was raised approximately 22 to 37% (high). The mean plasma creatinine value was raised approximately 9-22%. The mean AST value was raised approximately 18-24%. The mean LDH value was raised to 63-98% (mid and high). The mean ALP value was reduced to 26-25% (high). The mean plasma sodium, potassium and chloride values were raised 5-11% while the mean plasma total CO_2 was reduced 9-13%. The mean plasma phosphorus value was reduced 24-25%. Hormones: there was a dose-dependent rise of the mean plasma parathyroid hormone value of approximately 31-85% (high). There was a 28-48% dose-dependent reduction of the mean plasma 1,25-dihydroxycholecalciferol (vitamin D_3) in low, mid and high groups. There were no treatment-related gross alterations. Histopathology: treatment-related microscopic abnormalities in male and female rats (high) consisted of mildly decreased splenic extramedullary hematopoiesis (EMH). Organ weights: significant drug related deceases in absolute liver (10%), spleen (16%), kidney (16%) and heart (12%) weights were seen in male and female rats (high).

A NOAEL could not be identified because effects on PTH and vitamin D_3 were seen at all treated animals; it could be considered less than 6.25 mg/kg/day. Based on the body surface area factor, an equivalent dose in humans would be less than 1.0 mg/kg/day (60 mg/day for a 60 kg person).

Study title: A 28-Day Oral Gavage Toxicity Study Of GS-7340-02 In The Albino Rat

Study no.:	R990182
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	December 13, 1999
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	GS-7340-02: 1504-187-19; 97% pure

Key Study Findings: The changes included a reduced body weight gain in animals treated at 25, 100 and 400 mg/kg/day, reduced food consumption in animals treated at 400 mg/kg/day, changes in hematology and clinical chemistry parameters at dose levels of 25, 100 and 400 mg/kg/day, dose-related decreases in $1,25(OH)_2D_3$ at dose levels of 6.25 mg/kg/day and higher, a decrease in metaphyseal bone mineral density and bone mineral content and gross and microscopic changes in the thymus and kidney, mainly observed at a dose level of 400 mg/kg/day, although kidney changes were noted microscopically at 100 mg/kg/day in the males. In addition, atrophy of the cancellous bone of the femur was noted at a dose level of 400 mg/kg/day. Based on bone effect, the NOAEL of GS7340-02 was 1.5 mg/kg/day. Based on changes in WBCs the NOAEL was 6.25 mg/kg/day. The TFV exposure (AUC and C_{max}) at the NOAEL (6.25 mg/kg/day) was 0.34 µg*hr/ml and 0.19 µg/ml, respectively.

Methods

Doses:	1.5, 6.25, 25, 100 and 400 mg/kg/day
Frequency of dosing:	Once per day
Route of administration:	Oral gavage
Dose volume:	15 mL/kg
Formulation/Vehicle:	50 mM citric acid
Species/Strain:	Sprague-Dawley CD rats (Crl: CD (SD) BR)
Number/Sex/Group:	10/sex/group + 8/sex/group for toxicokinetics
Age:	10 weeks
Weight:	Males: 337 to 399 g, Females: 206 to 246g.
Satellite groups:	none
Unique study design:	no
Deviation from study protocol:	none

Observations and Results

Mortality: Mortality/morbidity checks were performed twice daily. There were five deaths during the treatment period, 2 males (400 mg/kg/day) and 1 female (25 mg/kg/day) and two animals assigned to the toxicokinetic portion of the study. It was considered that all three main study animals died as a consequence of esophageal puncture caused by the dosing procedure and

not related to the action of the test article. Animals in the toxicokinetic portion of the study were not necropsied as specified by the protocol; no cause of death was established.

Clinical signs: All animals were examined twice daily for signs of ill health or reaction to treatment. A number of animals were noted to have red fur staining around the mouth and/or muzzle. The staining was most marked in the males and females of Group 6, treated at a dose level of 400 mg/ kg/day.

Body weights: Individual body weights were recorded weekly. Mean body weight gains at a dose level of 400 mg/kg/day were lower than the controls over the course of the study. The effect was most marked in the males and attained statistical significance in the males over Weeks 0 to 1, 1 to 2 and 2 to 3. In addition, mean body weight gains of males and females treated at dose levels of 25, 100 and 400 mg/kg/day were statistically significantly lower than the controls over Weeks 1 to 2.

Food consumption: Individual food consumption was recorded weekly. Mean food consumption was markedly reduced in males and females at 400 mg/kg/day compared to controls.

Ophthalmology: Once prior to the start of treatment and again during week 4. There were no treatment related findings.

Blood samples for laboratory investigations (hematology, clinical biochemistry and biochemical markers of bone turnover) were collected from all main study animals at necropsy.

Hematology: When compared to the controls, dose related decreases in mean white blood cell parameters were noted in males treated at dose levels of 25, 100 and 400 mg/kg/day, most marked in the males at 400 mg/kg/day. Decreases were observed in mean total white blood cell count, mean absolute and relative monocytes and mean absolute lymphocyte values, with a slight increase noted in mean segmented neutrophils. Slight decreases in mean white blood cell count were also observed in the females treated at 400 mg/kg/day, with a decreased mean monocyte count noted in females treated at 25, 100 and 400 mg/kg/day. Slight but significant changes were also noted in a number of red blood cell parameters, in males and females treated at a dose level of 400 mg/kg/day. The changes included a decrease in mean red blood cell count, mean hemoglobin concentration and mean hematocrit. Increases were seen in mean corpuscular hemoglobin and mean red cell distribution width.

Chemistry: A slight increase in mean blood urea nitrogen was noted in males and females at a dose level of 400 mg/kg/day. Mean creatinine kinase tended to decrease in males and females with increasing concentrations of GS-7340-02. Mean ALP phosphatase was significantly decreased in males and females at 400 mg/kg/day. In addition, males and females treated at a dose level of 400 mg/kg/day had an increased mean cholesterol level and a decreased mean triglyceride concentration, when compared to the controls.

Urinalysis: Urine samples were collected from all animals on Day 7 and in Week 4. There were no effects.

Biochemical markers of bone turnover: A significant loss of calcium in the urine was noted on Days 7 and 29 in animals at dose level of 400 mg/kg/day, possibly a consequence of renal changes observed the in animals. There was no effect of treatment on urinary phosphorus levels. PTH was increased in animals treated at a dose level of 400 mg/kg/day relative to controls but the effect was not statistically significant and not dose-related. There was a dose-related decrease in 1, $25(OH)_2D_3$ in both males and females dosed at 6.25 mg/kg/day or higher. Mean values attained significance for combined data for both sexes at dose levels of 100 and 400 mg/kg/day, compared to controls. Combined mean values were decreased approximately 19% for animals dosed at 6.25 mg/kg/day and 74% at the highest dose. There were no meaningful differences among mean values for $25(OH)D_3$.

Peripheral Quantitative Computed Tomography pQCT: Treatment at 400 mg/kg/day produced a treatment-related effect on bone growth consistent with the observed effects on body weight, for both males and females, compared to controls. Effects were determined by decreases in the total bone slice area and periosteal circumference in the metaphyseal region for males and females, as well as the diaphysis region for males. The effects were seemingly more marked for the males than the females and were similar to the magnitude of the effect on body weight.

Additionally, there was also an effect on bone mineral density (BMD) for high dose animals. In the metaphyseal region for males, decreases in trabecular area (8%), cortical/subcortical area (6%) and endosteal circumference (6%) were consistent with the decrease in total slice area (7%). A marginal decrease in cortical/subcortical BMC (bone mineral content) (2%) resulted in a slight increase in BMD (5%). In the metaphyseal region for females, the trabecular area expanded (+35%) at the expense of the cortical/subcortical area (-20%) with a corresponding increase in endosteal circumference (+8%). The overall effect of these compartmental changes in males and females on the total slice were decreases in total BMC of 13% and 17%, and BMD of 7% and 14%, for males and females, respectively. There was notably more trabecular bone in the slices evaluated for females, than the males, which explains differences in the pattern of bone loss between the sexes. There were no meaningful effects of treatment at 1.5, 6.25, 25 or 100 mg/kg/ day on pQCT-derived parameters for males and females.

Organ weights: The following organs were weighed: adrenal gland, brain, heart, kidneys, liver, lungs, ovaries, testes, pituitary, prostate, spleen, thymus, thyroid lobes and parathyroid glands, uterus. A marked, statistically significant decrease in mean thymus weight relative to body weight was noted in males and females treated at dose levels of 100 and 400 mg/kg/day. Thymic atrophy was observed histologically and in the absence of other associated findings, the changes were considered most likely related to stress due to response of the animals to treatment, although the possibility of a direct effect of the test article could not be discounted. A statistically significant increase in mean kidney weight relative to body weight was also observed in the females at a dose level of 400 mg/kg/day. An increase in mean liver weight relative to body weight was also observed in animals treated at a dose level of 400 mg/kg/day, attaining statistical significance in the females only. No associated changes were noted during the histological evaluation of the liver.

Gross pathology: An increased incidence of gross thymic changes (dark discoloration and

small size) when compared to the controls, were recorded in at dose level of 400 mg/kg/day.

Histopathology: Adequate Battery: Yes Peer Review: No**.**

Histopathology: Treatment related changes were observed in the renal cortex and thymus. Foci and occasional contiguous areas of cortical tubular basophilia were seen in animals treated at 400 mg/kg/day. Generally associated with this change was a minimal to slight focal nuclear karyomegaly. Hyaline droplets present in the cortical tubules were observed in a number of treated males, most marked at a dose level of 100 mg/kg/day. Thymic atrophy was noted in a number of treated males and females, most marked in animals treated at a dose level of 400 mg/kg/day. It was considered that the change could be associated with stress due to the response of the animals to treatment as no changes were observed in the spleen or lymph nodes, however, as the changes were noted mainly in the high dose animals, the possibility of a direct effect of the test article on the thymus cannot be discounted. Examination of sections of femur obtained from both control animals and animals treated at a dose level of 400 mg/kg/day, revealed slight to moderate atrophy of the cancellous bone region in several males (4/10) and the majority of females (9/10) in the treated group. Minimal cecal epithelial hyperplasia was noted in two males treated at a dose level of 400 mg/kg/day, with a mild inflammation also noted in one of the males. In the absence of any other related findings, the change was not considered to be related to treatment.

Toxicokinetic: The analysis of plasma samples is shown in Table 14 (*excerpted from sponsor).* The apparent T_{max} for TFV following oral administration of GS-7340-02 was 30 minutes or less suggesting rapid absorption of GS-7340-02 and conversion to TFV. Following peak plasma concentrations, TFV plasma levels declined in an apparent bi-exponential manner. Plasma terminal half-lives ranged from 5.41 to 7.98 hours on Day 1 and from 6.70 to 11.10 hours on Day 28. A non-linear pharmacokinetic response was observed for total plasma exposure versus dose for both sexes on both Day 1 and Day 28. A greater than linear increase in plasma exposure was observed as the dose was increased. However, there was no observed plasma accumulation over the 28 day study for any dose group.

Table 14: Mean toxicokinetic parameters for TFV in rat plasma on Days 1 and 28

GS-7340-02 (mg/kg/day)	Day 1		Day 28	
	C_{max} (ng/mL)	$AUC_{(0-t)}$ (ng·h/mL)	C_{max} (ng/mL)	$AUC_{(0-t)}$ (ng·h/mL)
Males				
1.5	35.5	45.5	49.7	52.7
6.25	167	288	169	358
25	1340	2430	1300	2450
100	6970	12700	4780	12200
400	12900	61800	10300	62700
Females				
1.5	35.6	49.2	52.8	55.1
6.25	229	296	211	321
25	1970	2770	1690	2820
100	6990	13600	6160	14600
400	20100	78400	14400	75100

Stability and Homogeneity: Overall, the dose formulations analyzed were within the acceptable criteria of 85% to 115% of the nominal concentration, except for high dose, week 4 (72%). The acceptable criteria with respect to system stability were met.

Study title: A 26-Week Oral Gavage Toxicity Study Of GS-7340-02 In The Albino Rat

Study no.:	TOX-120-001
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	9 April 2002
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	GS-7340-02;1603-167-36, 98.1%

Key Study Findings: There were no treatment-related effects on organ weights or macroscopic findings. Microscopic findings associated with treatment were limited to the kidneys and tibia. Tibial cancellous bone atrophy and renal cortical tubular karyomegaly were recorded for animals that had received 100 mg/kg/day for 26 weeks. Treatment of rats at 100 mg/kg/day resulted in significant decreases in pQCT-derived trabecular bone mineral density (BMD) and bone mineral content (BMC) at the proximal tibia metaphysis and distal femur metaphysis, with no effects on diaphyseal bone parameters. This effect was consistent with significant increases in biochemical markers of bone turnover (deoxypyridine-DPD, C telopeptide-CTx,) and changes in related hormones (1,25 dihydroxyvitamin D_3 and 25 hydroxyvitamin D_3) observed for animals treated at 25 or 100 mg/kg/day. No effects of treatment were observed at 5 or 25 mg/kg/day on pQCT-derived parameters and at 5 mg/kg/day on biochemical markers of bone turnover and related parameters. 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 for this study was 25 mg/kg/day.** The TFV exposure (AUC and C_{max}) at the NOAEL was 3.758 µg*hr/ml and 1.523 µg/ml, respectively.

Methods

Doses:	0, 5, 25, and 100 mg/kg/day
Frequency of dosing:	Once per day
Route of administration:	Oral gavage
Dose volume:	10 mL/kg
Formulation/Vehicle:	Suspension vehicle. (0.5% polysorbate 20, NF; 0.5% carboxymethylcellulose ; 0.9%Benzyl Alcohol)
Species/Strain:	Sprague-Dawley CD rats (Crl: CD (SD) BR)
Number/Sex/Group:	15/sex/group + 8/sex/group for toxicokinetics
Age:	6 weeks
Weight:	161g to 209g for males and 132g to 171g for females
Satellite groups:	Health screen 10/sex/group
Unique study design:	No
Deviation from study protocol:	The reported deviations are not expected to affect study conclusions.

Observations and Results

Mortality: Mortality/morbidity checks were performed twice daily. There were no unscheduled deaths recorded during the 26-week treatment period, which were considered to be related to treatment. In the main study, six female rats were found dead prior to study completion. The cause of death of 5 of these animals was attributed to a localized hemorrhage, either in the axillary musculature or in one organ of the thoracic cavity. The jugular blood collection was regarded as the most likely cause of these preterminal events since all but one coincided with blood sampling, occurring approximately in weeks, 13 or 26.

Clinical signs: All animals were examined twice daily for signs of ill health or reaction to treatment. There were no drug related clinical signs recorded during the treatment period. Incidental findings occurred in all groups but were restricted to fur staining (brown, red and yellow), oily fur, thin fur and skin scabs.

Body weights: Individual body weights were recorded weekly. There was a slightly reduced body weight gain of approximately 6-8% recorded for the male and female rats (high dose), respectively during the study. There was no other effect on body weight during the treatment period. Generally group mean body weight and body weight gains for all treated groups were comparable to the concurrent controls.

Food consumption: Individual food consumption was recorded weekly. Food consumption for animals in all treatment groups was generally comparable to the controls and therefore unaffected by treatment.

Ophthalmology: Once prior to the start of treatment and again during Weeks 12 and 26 of treatment. There were no treatment related findings.

Hematology: During Week 13 clinical pathology (hematology & biochemistry) was performed on at least eight surviving main study animals. At study termination laboratory investigations

(hematology & chemistry) were performed on all main study animals. Statistical analysis revealed a significantly lower mean white blood cell count for males receiving 100 mg/kg/day during Week 13 and for males receiving 5 mg/kg/day at termination. However, these reductions were due to isolated animals, were not dose related, and as all values were within historical ranges

Chemistry: The mean total serum calcium was slightly significantly increased for the high dose males at week 26 although no increased loss of calcium in the urine was noted for treated animals at week 13 or 26. There were no effects on serum or urinary phosphorus levels.

Urinalysis: During Weeks 13 and 26 urinalysis was performed on all surviving main study animals. There were no effects of treatment.

Biochemical markers of bone turnover and associated parameters: Evaluation of bone turnover was done by assessment of biochemical and hormone markers and direct examination of bone mineral density by peripheral quantitative computed tomography (pQCT). Treatment of rats with GS-7340-02 at 100 mg/kg/day for 26 weeks resulted in significant decreases in pQCT-derived trabecular BMD and bone mineral content (BMC) at the proximal tibia metaphysis and distal femur metaphysis, with no effects on diaphyseal bone parameters. In proximal tibial metaphysis, BMD was reduced 18% for males and 21% for females, and BMC was reduced 23% for males and 34% for females. No drug effect was observed at 5 or 25 mg/kg/day on pQCT-derived parameters.

Table 15: Biomedical markers of bone turnover *(excerpted from sponsor)*

	MALES				FEMALES			
	0	5 mg/kg/d	25 mg/kg/d	100 mg/kg/d	0	5 mg/kg/d	25 mg/kg/d	100 mg/kg/d
Group Mean (% change from control) Serum Biochemical Markers of Bone Turnover and Hormones – Week 26 (n=14-15)								
Osteocalcin	24.62	24.25 (-1.5)	25.08 (1.9)	27.08 (13.2)	19.11	19.51 (2.1)	22.15 (15.9)	24.97 (30.7)
Bone AP	2.6	1.7 (-34.6)	1.6 (-38.5)	1.4 (-46.2)	5.9	7.2 (22.0)	8.5 (44.1)	6.5 (10.2)
PTH	319.23	345.66 (8.3)	475.76 (49.0)	461.86 (44.7)	162.11	169.38 (4.5)	187.85 (15.9)	256.97 (57.9)
diOHVitD	112.774	142.100 (26.0)	89.087 (-21.0)	80.989 (-31.8)	105.021	94.446 (-10.1)	57.891 (-44.9)	42.649** (-59.4)
OHVitD	19.064	19.575 (2.7)	19.626 (2.9)	18.151 (-4.8)	22.358	19.303 (-13.7)	18.925 (-15.4)	17.525* (-21.6)

AP = alkaline phosphatase; diOHVitD = 1,25 dihydroxy Vitamin D; OHVit = 25 hydroxy Vitamin D;
* p≤ 0.01 ** p≤ 0.001

Bone mineral density (BMD) measurements, peripheral quantitative computed tomography (pQCT): Treatment with GS-7340-02 at 100 mg/kg/day for 26 weeks resulted in significant decreases in trabecular BMD for males (18%) and females (21%) compared to controls at the tibial metaphysis, with no effect on cortical/subcortical BMD. A slight decrease in total slice BMD was noted for the males at 100 mg/kg/day (33%). Decreases in BMC were

also observed, significant for trabecular BMC for males (23%) and females (34%) and total slice BMC for males (11%), with a non-significant decrease for females (6%). The effects on total slice BMC reflects the response in trabecular bone, but also reflects slight decreases in total slice area and therefore possibly bone size, an effect possibly related to the slight treatment-related reduction in body weight gain observed for high dose animals relative to controls.

Ex vivo pQCT results obtained at the distal femur were generally consistent with the in vivo observations. In the distal femur metaphysis, significant decreases were noted in trabecular BMC (17%) and trabecular BMD (15%) in high dose males. A slight decrease was observed for high dose females for trabecular BMD (5%) compared to controls. The femur metaphysis total area was significantly decreased (7%) for high dose males compared to controls, an effect possibly related to bone size. Cortical/subcortical BMD was noted to be significantly increased compared to controls for high dose males, an effect considered incidental and unrelated to treatment

There were no meaningful effects of treatment on pQCT-derived parameters at the mid-diaphysis site of the tibia and femur for both males and females. There were no meaningful effects of treatment with GS-7340-02 at 5 or 25 mg/kg/day on pQCT-derived parameters for males or females.

Table 16: Urine markers of bone turnover *(excerpted from sponsor)*

	MALES				FEMALES			
	0	5 mg/kg/d	25 mg/kg/d	100 mg/kg/d	0	5 mg/kg/d	25 mg/kg/d	100 mg/kg/d
Group Mean (% change from control) Urine Biochemical Markers of Bone Turnover – Week 26 (n=15)								
C-Telopeptide	1.625	1.950 (20.0)	2.422 (49.0)	3.968 (144)	0.933	1.144 (22.6)	0.986 (5.7)	1.151 (23.4)
Group Mean (% change from control) Urinalysis – Week 26 (n=15)								
Phosphorus	236.761	206.011 (-13.0)	208.297 (-12.0)	178.089 (-24.8)	260.997	192.985 (-26.1)	228.712 (-12.4)	325.770 (24.8)
Calcium	8.33	7.50 (-10.0)	7.95 (-4.6)	9.23 (10.8)	30.09	27.67 (-8.0)	30.81 (2.4)	41.93 (39.3)

Organ weights: The following organs were weighed: adrenal gland, brain, heart, kidneys, liver, lungs, ovaries, testes, pituitary, prostate, spleen, thymus, thyroid lobes and parathyroid glands, uterus.

Statistically significant inter group differences were seen for the relative weight of the thyroid gland (relative to body weight) in males of mid and high dose animals.

Gross pathology: There were no changes.

Histopathology: Adequate Battery: Yes. Peer Review: No.

Histopathological Findings: Treatment related findings were observed in the kidneys and the tibia. Renal cortical tubular karyomegaly, minimal in severity, was only seen in the high dose animals. The incidence of this change in males and females (high) was 73% and 13%, respectively. The nuclear enlargement of the tubular epithelium was scattered in a small number

of tubules, mainly in the inner cortex, and was not associated with any other treatment-related tubular alterations at the light microscopic level. Atrophy of the cancellous bone compartment in the tibia, graded minimal to slight in severity, was noted in eight of fifteen females (high dose). This change was characterized by a loss of the osseous trabecular network in the proximal metaphysis, mainly in the median region of the bone. The bone atrophy in females (high dose) was corroborated by the decreased trabecular BMD and BMC at the same tibial site as measured by peripheral quantitative computer tomography at the end of the treatment period. The trabecular network of control males was much less dense compared to that of control females. This anatomical variation in control males may have masked detection of atrophy of the cancellous bone among male rats treated with GS-7340-02.

Toxicokinetic: GS-7340-02 was rapidly absorbed after oral dosing and was rapidly converted to TFV. Peak plasma TFV concentrations occurred at 0.25-0.5 hour post dose. Plasma TFV concentrations were measurable at 24 hour post dose except in the 5 mg/kg/day dose group where concentrations were measurable up to 12 hours post dose. No consistent differences in plasma pharmacokinetics were found between male and female rats. TFV 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. As study progressed from Day 1 to Week 26, mean TFV C_{max} was comparable between study periods, while mean TFV AUC obtained on Day 1 was slightly lower than those measured during Weeks 13 and 26, which suggested that there was a slight accumulation of TFV with repeat dosing.

Table 17: Mean pharmacokinetic parameters of TFV *(excerpted from sponsor)*

GS-7340-02 (mg/kg/day)	Tenofovir (mg-eq/kg/day)	Study Day	C_{max} (µg/mL)	AUC (hr*µg/mL)	$t_{1/2}$ (hr)
5	2.4	Day 1	0.309	0.604	9.82
		Week 13	0.344	0.727	9.77
		Week 26	0.267	0.670	13.45
25	12	Day 1	1.284	3.364	10.88
		Week 13	1.464	3.724	8.11
		Week 26	1.523	3.758	7.06
100	48	Day 1	4.944	12.415	7.25
		Week 13	5.514	15.044	8.22
		Week 26	4.911	15.534	8.11

Abbreviations: AUC, area under the concentration-time curve, $AUC_{0-\infty}$ on Day1 and AUC_τ in Weeks 13 and 26; C_{max}, maximum plasma tenofovir concentration; $t_{1/2}$, terminal phase half-life.

Stability and Homogeneity: TFV is stable at nominal temperature of -22°C and -80°C for 93 days in rat plasma (heparin). TFV is also stable in rat plasma (sodium citrate) at a nominal temperature of -22°C for 114 days. The results of the analyses indicated that the formulations were prepared as per protocol as all results were within +/-10% of nominal.

Repeated-Dose Toxicity Studies in Dogs

Study title: A 28-Day Oral Gavage Toxicity Study of GS-7340-02 in the Beagle Dog

Study no.:	D990175
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	November 19, 1999
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	GS-7340-02: 1504-187-19; >97% (assumed 100%)

Key Study findings: There were no treatment-related ocular changes. On Day 28, a marginal increase in AST was observed at 10 mg/kg/day, attaining statistical significance (females only) when compared with vehicle control values. There were no meaningful changes in biochemical markers of bone turnover and related parameters considered related to treatment with GS-7340-02 at dose levels up to 10 mg/kg/day, or peripheral quantitative computed tomography (pQCT)-derived bone densitometry parameters at a dose level of 10 mg/kg/day. Macroscopic findings most frequently observed were dark areas and/or foci in the gastrointestinal tract and pale and/or raised or depressed area(s) in the lungs and spleen in treated animals. Histopathological, treatment-related lesions were observed in the kidneys of all males and females at 10 mg/kg/day and in one male and one female at 3 mg/kg/day. Lesions were minimal and characterized by tubular karyomegaly and/or basophilia. The karyomegaly was usually diffuse in the cortex and affected the epithelial cells of the proximal convoluted tubules. The NOAEL of GS-7340-02 in this study was considered to be 1 mg/kg/day. Exposure of GS-7340-02 at the NOAEL could not be measured.

Methods

Doses:	0, 0.1, 0.3, 1, 3 or 10 mg/kg/day
Frequency of dosing:	Once daily
Route of administration:	Oral gavage
Dose volume:	10 mL/kg
Formulation/Vehicle:	50 mM citric acid
Species/Strain:	Beagle dog
Number/Sex/Group:	4 animals/sex/group
Age:	7-8 months
Weight:	7.5 to 10.5 kg for the males and 6.7 to 8.8 kg for the females.
Satellite groups:	None
Unique study design:	None
Deviation from study protocol:	No

Observations and Results

Mortality: All animals were checked twice daily. All dogs survived to schedule necropsy.

Clinical Signs: All animals were checked twice daily. There were no clinical signs considered to be related to treatment with GS-7340-02.

Body Weights: Individual body weights were measured weekly. There were no effects on body weight or body weight gain considered to be related to treatment with GS-7340-02.

Feed Consumption: Individual food consumption was measured daily. There were no effects on food consumption considered to be related to treatment

Ophthalmology: There were no ocular changes considered to be related to the treatment.

Clinical Pathology: Once during the pretreatment period and on Days 14 and 28 of treatment. There were no effects on hematology parameters. On Day 28, a marginal increase in AST was observed at 10 mg/kg/day, attaining statistical significance (females only) when compared with vehicle control values.

Urinalysis: There were no effects on urinalysis parameters considered to be related to the treatment.

Biochemical Markers of Bone Turnover and Associated Parameters: Mean values for bone specific ALP (bone formation marker), N-telopeptide (NTx) (bone resorption marker), parathyroid hormone, 1,25 dihydroxyvitamin D_3 ($1,25(OH)_2D_3$) and 25 hydroxyvitamin D ($25(OH)D_3$) were essentially similar among all groups on each occasion. Mean PTH values on Days 14 and 28 were marginally increased and $1,25(OH)_2D_3$ values Day 28 were marginally decreased for animals treated with GS-7340-02 at 10 mg/kg/day compared to controls, although these differences did not attain statistical significance.

Bone Mineral Density Measurements: There were no meaningful differences between group mean values for any peripheral quantitative computed tomography (pQCT)-derived parameters for animals treated with GS-7340-02 at 10 mg/ kg/day, compared with controls. Mean values for area and bone mineral content (BMC) of the total slice and trabecular and cortical/subcortical compartments were slightly decreased at 10 mg/ kg/day when compared to controls. Differences in mean values attained statistical significance only for the cortical/ subcortical area for males euthanized at the end of the treatment period. Total slice, trabecular and cortical/subcortical bone mineral density (BMC) values were comparable among groups. BMC was likely affected in treated groups consistent with the noted slight differences in area, an effect considered unrelated to treatment. Slight differences in mean values for periosteal and endosteal circumferences were also consistent with the slight differences in area. Mean values for parameters evaluated at the mid-diaphysis were similar to those in the metaphysis region. Diaphyseal cortical thickness measurements were essentially comparable for treated and control animals.

Gross Pathology: Macroscopic findings most frequently observed were dark areas and/or foci in the gastrointestinal tract and pale and/or raised or depressed area(s) in the lungs and spleen.

Organ Weights: The liver, kidney (left and right weighed separately), heart, spleen, adrenals (paired weight), brain, and testes (paired weight) from each dog were trimmed of fat and other contiguous tissues and weighed before samples were taken for histology. There were no differences in organ weights considered to be related to the treatment.

Histopathology: Adequate Battery: yes Peer Review: no

Histological Findings Histopathological, treatment-related lesions were observed in the kidneys of all males and females at 10 mg/kg/day and in one male and one female at 3 mg/kg/day. Lesions were minimal and characterized by tubular karyomegaly and/or basophilia. The karyomegaly was usually diffuse in the cortex and affected the epithelial cells of the proximal convoluted tubules.

Toxicokinetics: GS-7340 was rapidly absorbed following Day 1 oral administration with GS-7340 median peak values attained within 0.25 to 0.5 hours of 18.5, 38.7, and 582 ng/mL for the 1.0, 3.0, and 10 mg/kg/day dose cohorts, respectively (Tables 18 & 19, *excerpts from sponsor*). GS-7340 C_{max} values were not linear with dose. Only the 10 mg/kg/day dose cohort provided adequate data over time for complete pharmacokinetic analysis. Following the peak plasma level the concentration of GS-7340 decreased rapidly with a median elimination half-life of 18 minutes. Repeat dosing resulted in nonlinear pharmacokinetics between Days 1 and 28 with GS-7340 median AUC values of 0.454 and 0.985 μg*hr/mL, C_{max} values of 582 and 1280 ng/mL, and $T_{1/2}$ values of 18 and 23 minutes, respectively.

T_{max} of TFV occurred within 1 hour following oral administration of GS-7340-02 suggesting rapid hydrolysis of the prodrug following absorption, with median peak plasma concentrations of 124 and 385 ng/mL for the 3.0 and 10 mg/kg/day dose cohorts. TFV C_{max} values appeared to be linear with increasing dose as well as repeat dosing. The 10 mg/kg/day GS-7340-02 dose cohort was the only dose group with adequate TFV plasma concentrations over time to evaluate pharmacokinetic parameters. However, Day 1 TFV terminal half-life (14.2 hours) and $AUC_{0-\infty}$ (2.52 μg*hr/mL) were probably underestimated since only data through 24 hours were obtained and other studies of TFV (IV) and TFV prodrug (TDF, PO) have shown long terminal half-life values of approximately 42 and 64 hours, respectively.

Comparison of TFV AUC on Day 1 (1.68 μg*hr/mL) to Day 28 (5.26 μg*hr/mL) demonstrated substantial accumulation with an estimated terminal half-life of 37 hours consistent with the terminal half-life observed in other TFV studies in dogs.

Quantitative intracellular PBMC concentrations of TFV were obtained for some of the 3.0 and all of the 10 mg/kg/day dose groups prior to dose administration on Days 8, 22 and 28. PBMC concentrations appeared to be non-linear with increasing dose. A linear correlation was observed between intracellular PBMC concentrations of TFV and corresponding trough plasma concentrations with intracellular PBMC values approximately 100-fold greater than corresponding plasma concentrations.

Table 18: Non-compartmental pharmacokinetic parameters of GS-7340 following Day 1 and Day 28 oral administration of GS-7340-02 in Beagle Dogs (10 mg/kg/day)

Parameter	Day 1			Day 28		
	Median	Min	Max	Median	Min	Max
C_{max} (µg/mL)	0.582	0.299	1.721	1.28	0.464	4.48
T_{max} (hr)	0.500	0.250	0.50	0.500	0.250	0.500
$AUC_{0\rightarrow\infty}$ (µg*hr/mL)	0.454	0.218	1.37	-	-	-
$AUC_{0\rightarrow\tau}$ (µg*hr/mL)	-	-	-	0.985	0.734	1.89
$t_{1/2}\lambda_z$ (hr)	0.293	0.201	0.501	0.472	0.226	1.51
CL/F (mL/hr/kg)	22000	7300	45800	10150	5300	13600
MRT (hr)	0.654	0.491	1.03	1.48	1.15	2.64

Table 19: Non-compartmental pharmacokinetic parameters of TFV following Day 1 and Day 28 oral administration of GS-7340-02 in Beagle Dogs (10 mg/kg/day)

Parameter	Day 1			Day 28		
	Median	Min	Max	Median	Min	Max
C_{max} (µg/mL)	0.385	0.275	0.498	0.444	0.409	0.755
T_{max} (hr)	1.00	0.500	2.00	1.00	0.250	1.00
$AUC_{0\rightarrow\infty}$ (µg*hr/mL)	2.52	1.86	3.96	-	-	-
$AUC_{0\rightarrow\tau}$ (µg*hr/mL)	-	-	-	5.26	4.51	6.18
$t_{1/2}\lambda z$ (hr)	14.2	8.7	29.4	34.6	22.5	79.3
CL/F (mL/hr/kg)	1893	1226	2610	922	784	1075
MRT (hr)	18.2	8.4	35.7	44.2	28.1	98.8

Study title: Nine-Month Oral Toxicity Study of GS-7340-02 in Dogs (With a 3-Month Interim Sacrifice and a 3-Month Recovery Period)

Study no.: TOX-120-002
Study report location: Electronic
Conducting laboratory and location: (b) (4)
Date of study initiation: April 4, 2003
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: GS-7340-02: 1504-187-19; >97% (assumed 100%)

Key Study findings: There was evidence at the middle and high dose for an effect to slightly prolong PR intervals. The highest dose appeared to reversibly reduce heart rate

and with an associated mild QT prolonging effect but only at Week 39. Macroscopic changes were seen in the kidney (pale kidneys) at both the interim and main sacrifice. A brown dark discoloration of the fur, mainly on the extremities, at 6 or 18/12 mg/kg/day and various pulmonary findings seen at all tested dose levels. The pulmonary changes correlated with an increase in lung weights relative to body weight in animals given 18/12 mg/kg/day. Compound-related histopathological alterations were noted in the kidneys, eyes, lungs and spleen after both 3-month interim and 9-month treatment phases. The liver and possibly the adrenal glands were additional target organs identified after the longest treatment duration. After 3 months of treatment, renal cortical tubular degeneration/regeneration and karyomegaly were findings limited to animals dosed with 18/12 or 6 mg/kg/day. After 9 months of treatment, these renal findings were present in animals dosed with 18/12 or 6 mg/kg and were additionally also present, but of only minimal severity, in a few male animals treated with 2 mg/kg/day. A minimal to mild infiltration of mononuclear cells in the ocular posterior uvea was noted in 3 out of 4 animals after 3 months of treatment and in 4 out of 8 animals after 9 month of treatment in the high dose group (18/12 mg/kg/day). A significant increase in the bone resorption marker NTx was observed for all high dose animals after 9 months of treatment compared to controls. A significant decrease in 1,25-dihydroxyvitamin D_3 and in 25-hydroxyvitamin D_3 occurred in high dose males compared to controls at both the 3-month interim evaluation after 9 months of treatment. It was concluded that the NOAEL for GS-7340-02 was 2 mg/kg/day. The exposure (AUC) at the NOAEL for GS-7340-02 and TFV was 0.08 and 1.18 μg*hr/ml, respectively.

Methods

Doses:	0 (vehicle control), 2 (low), 6 (mid) or 18/12* mg/kg/day (high) for a period of 9 months. *=Due to severe clinical signs and reduced body weight and food consumption the dose level was decreased on day 45 and 51 for males and females, respectively.
Frequency of dosing:	Once daily
Route of administration:	Oral gavage
Dose volume:	10 mL/kg
Formulation/Vehicle:	0.5% polysorbate and 0.5% carboxymethylcellulose
Species/Strain:	Beagle dog
Number/Sex/Group:	2 animals/sex/group (interim 3-month), 4 animals/sex/group (main 9-month) and 2 animals/sex/high dose group only (recovery period)
Age:	5-6 months
Weight:	6.6 to 7.9 kg for males and 5.3 to 6.8 kg for females
Satellite groups:	None
Unique study design:	None
Deviation from study protocol:	No

Observations and Results

Mortality: All animals were checked twice daily. There were two unscheduled deaths. One male (high dose) was euthanized on day 45 due his critical condition. Prior to necropsy, this male had shown a reduced body weight associated with notably reduced food consumption. The results of preterminal laboratory results showed notable increases/decreases in AST, globulin levels, triglyceride, cholesterol, total bilirubin and monocyte counts when compared to the results of the predose investigations. Macroscopic finding present in this animal consisted of a single finding: bilateral enlargement of submandibular lymph nodes which histologically had slight inflammation and plasmacytosis. Histopathological finding of significance were present in the kidneys, lymphoid tissues, spleen and the gastrointestinal tract (stomach, cecum and colon) and the eye. Renal changes consisted of cortical tubular degeneration, similar to changes identified in all high dose animals at both the interim and terminal evaluations. Lymphoid tissues including GALT, mesenteric lymph node and thymus had atrophy accompanied by an infiltrate of macrophages. Changes present in the GI included mucosal atrophy of the fundic gland, mucosal hyperplasia of the pyloric gland and mucosal degeneration/and/or regeneration in the cecum and colon. Changes in the eyes consisted of a mild mononuclear infiltrate in the ocular posterior uvea, similar to changes seen in high dose dogs at the interim and terminal evaluation.

One high dose dog was euthanized on day 65 due to gavage accident; the diagnosis was supported by pulmonary hemorrhage with alveolar edema and fibrinous bronchoalveolar inflammation.

Clinical Signs: All animals were checked twice daily. An increased incidence of salivation was recorded both predose and following dose administration for animals for all treated groups. In addition, during the later stages of the study a red/brown fur staining was also observed on the paws of the treated animals with a higher incidence being seen for the high dose animals. This change was most often distributed on the lower extremities, the hind paws and the forepaws and was still apparent although to a lesser degree during the recovery period.

Body Weights: Individual body weights were measured weekly. During the initial seven weeks of dose administration notably reduced group mean body weight gains were recorded for animals receiving 18 mg/kg/day when compared to the controls. This finding was more apparent for males with the differences in group mean body weights from the controls attaining statistical significance from Weeks 3 which was maintained throughout the treatment period. This reduction in group mean body weight was associated with reduced food consumption. Following the reduction in dose level, some recovery in body weight was apparent. However, a dosage related decrease in body weight was observed on completion of the 39-week treatment period for all male treated groups (6.7, 13.2 and 25.7%, respectively) when compared to the concurrent controls and for females receiving 18/12 mg/kg/day (11.9%). As the recovery period progressed animals showed an increase in body weight, although the gain over the later stages of the recovery period was not as large as that recorded during the initial 4 weeks.

Body weight changes for animals receiving 2 mg/kg/day were generally comparable to the controls and therefore unaffected by treatment.

Feed Consumption: once weekly, daily last week of the study. During the initial seven weeks of dose administration a notable reduction in food consumption was recorded for animals receiving 18 mg/kg/day. Following the reduction to 12 mg/kg/day, some recovery in consumption was apparent although the food consumption remained below that of the controls. Significant reductions were recorded on several occasions up to an including Week 17, most notably for males.

Food consumption for animals receiving 2 or 6 mg/kg/day was generally comparable to the controls and therefore unaffected by treatment.

Ophthalmology: Weeks 13 and 39. There were no ocular changes considered to be related to treatment with GS-7340-02.

Electrocardiography: GS-7340-02 had no discernable electrocardiographic effect at the low dose (2 mg/kg/day). There was some evidence at the middle and high dose (6 and 18/12 mg/kg/day) for an effect to slightly prolong PR intervals. At the highest dose tested (18/12 mg/kg/day) GS-7340-02 appeared to reversibly reduce heart rate and with an associated mild QT prolonging effect but only at Week 39. The heart rate changes were reversible following a three-month recovery period and recovered to rates comparable to the control group. A reduction in T_3 levels in high dose animals at Week 39 may have been associated with a reduction in heart rates and prolonged QT intervals and could represent a plausible mechanism for some of the observed findings.

Hematology: Once during the pretreatment period and weeks 13, 26 and 39. Slight increases in group mean absolute monocyte counts, percent reticulocyte, red cell distribution width and/or MPV/MCV volume were recorded frequently for high dose animals (18/12 mg/kg/day) when compared to the controls at weeks 13, 26 and 39 of the treatment period. In addition, slight decreases in eosinophils and red cell parameters including red blood cell count, hemoglobin and hematocrit, and increased APTT were also recorded for high dose (18/12 mg/kg/day) animals when compared to the concurrent controls at weeks 13, 26 and 39 of the treatment period. With the exception of the eosinophil counts and the hemoglobin levels, the values in the high dose group were within normal historical ranges. Following the 3-month recovery period, the above hematological parameters, with the exception of APTT, returned to recover to values comparable to those obtained during the pre-treatment period.

Clinical Chemistry: Once during the pretreatment period and weeks 13, 26 and 39. Increases in group mean AST, ALP, total bilirubin, triglycerides and/or phosphorus (males only) were recorded for animals receiving 18/12 mg/kg/day when compared to the controls at weeks 13, 26 and/or 39 of the treatment period. During Week 26, slight decrease in triglycerides was recorded for females at 18/12 mg/kg/day. Compared to the control group, serum creatinine kinase was statistically significantly increased in the mid-dose (6 mg/kg/day) males at week 39 and in high dose (18/12 mg/kg/day) males at weeks 26 and 39. Similarly, creatinine kinase was statistically significantly elevated in high dose (18/12 mg/kg/day) females at week 39. Compared to the control group, animals receiving 18/12 mg/kg/day had significant increases in creatinine levels (males at weeks 13, 26 and 39, and females at week 39) and slight decreases in albumin and A/G ratios (males at weeks 13, 26 and 39 and females at weeks 13 and 39) were also recorded. A

slight increase in AST levels was also noted for males receiving 6 mg/kg/day during weeks 13 and 26. During the study, nearly exclusively in high dose animals (18/12 mg/kg/day), there were occasional, slight increases in serum sodium and chloride levels. Slight but statistically significant decreases in serum potassium were present in high dose (18/12 mg/kg/day) animals on weeks 26 and 39. The above noted changes resulted in mean values within normal historical ranges with the exception of the group mean AST and total bilirubin (females only) in high dose (18/12 mg/kg/day) animals. During Week 39 statistically significant decreases in serum T_3 levels were recorded for high dose animals. The percent decrease from the controls was 31.5% and 29.8% for males and females, respectively. At week 39, T_4 and TSH values for treated animals were comparable to the respective control group. Serum T_3 values returned to levels comparable to the control group during recovery.

There were no treatment related changes recorded for animals receiving 2 mg/kg/day.

Urinalysis: Weeks 13, 26 and 39. There were no clear effects on standard urinalysis parameters considered to be related to treatment with GS-7340-02.

Biochemical Markers of Bone Turnover and Related Parameters: All bone markers showed age-related decreases. After 3 months of treatment, there were small differences noted among mean values for bone formation (bone specific ALP) and bone resorption markers (urinary free deoxypyridinoline (DPD) and N-telopeptide (NTx) for treated groups compared to controls. After 9 months of treatment, statistically significant increases in mean values for the bone resorption marker urinary NTx 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 6 mg/kg/day, suggesting a dose-related response. For the formation marker, serum sALP values in treated animals were comparable to vehicle controls. 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.

Serum PTH levels were variable among animals in the treated groups after 3-months and 9-months of treatment. No meaningful difference among serum PTH values was observed in males or females treated with GS-7340-02. Values were normally within the range of control animals except for males, which were increased. At the end of the recovery period, PTH values of males and females treated with GS-7340-02 at 18/12 mg/kg/day were within the range of control animals at the end of treatment.

A significant treatment-related decrease in 1,25-dihydroxyvitamin D_3 of 51% ($p \leq 0.05$) was observed in high dose (18/12 mg/kg/day) males after 9 months of treatment compared to controls. This decrease was considerably greater than the 27% decrease observed at the 3-month interim phase evaluation ($p \leq 0.05$). Slight, non-significant decreases in 1,25-dihydroxyvitamin D_3 were observed for the females in the 6 and 18/12 mg/kg/day dose groups compared to the control group (decreases of 22 and 17%, respectively). At the end of the recovery period, serum 1,25 dihydroxyvitamin D_3 levels of high dose returned to normal and were within the range for control group animals at the end of treatment. There were no meaningful effects of treatment on total or ionized serum calcium or serum phosphorus.

Peripheral Quantitative Computed Tomography (pQCT): There were no effects of test article on bone growth in the distal radial diaphysis in female animals. However in mid (6 mg/kg/day) and high dose (18/12 mg/kg/day) males, minimal bone growth at the distal radius diaphysis was observed after 9 months of treatment. Relative to control group animals, which had normal bone growth at the 9-month endpoint, mean values for total slice area and periosteal circumference in high dose (18/12 mg/kg/day) males were reduced but these changes did not attain statistical significance. However, relative to control group animals, statistically significant decreases in mean values for cortical area, cortical BMC and cortical thickness did occur in high dose (18/12 mg/kg/day) males at both the 3-month interim phase evaluation and after 9 months of treatment. These changes were consistent with test article-related effects on bone size and body weight in these groups. Males in the mid-dose group (6 mg/kg/day) showed a tendency towards similar effects on bone geometry to the high dose males, suggesting a dose-response effect (statistically different for cortical BMC and cortical thickness). There was no test article effect on distal radial diaphyseal parameters for low dose (2 mg/kg/day) males or females at any dose level at both the 3-month interim phase evaluation and the 9-month endpoint. Importantly, distal radius diaphyseal cortical BMD was not affected by treatment with test article for either males or females at any dose level.

At the distal radius metaphysis, relative to the control group, total slice BMC and total slice BMD were significantly reduced in high dose (18/12 mg/kg/day) males after 9 months of treatment. The changes in total slice BMD were contributed to primarily by decreases observed in trabecular BMD (30% decrease), while cortical/subcortical BMD was only marginally and non-significantly decreased (7%). Relative to the control group, in both high dose (18/12 mg/kg/day) males and females, the distal radial metaphyseal trabecular area was non-significantly increased but the trabecular BMC was unchanged, resulting in a significant decrease in trabecular BMD ($P \leq 0.05$). Similar changes in the distal radius metaphysis trabecular area and BMD were seen in the mid dose (6 mg/kg/day) males and females (statistically significant only in females $p \leq 0.05$) and suggests a dose-response relationship.

Distal radius metaphyseal cortical/subcortical area was decreased in high dose (18/12 mg/kg/day) males after 9 months of treatment compared to controls ($p \leq 0.01$) resulting in significant decreases in BMC in high dose males at the 3-month interim evaluation and after 9 months of treatment. A similar trend was noted in high dose (18/12 mg/kg/day) females at the 3-month interim evaluation and after 9 months of treatment, with the differences from the control not attaining significance during Month 9. The BMD values for the distal radius metaphysis were slightly decreased in high dose (18/12 mg/kg/day) males (approximately 7%), but were unchanged in high dose females compared to the controls after 9 months of treatment. Cortical/subcortical BMC in the distal radius metaphysis was significantly reduced in both mid (6 mg/kg/day) and high dose (18/12 mg/kg/day) compared to controls after 9 months of treatment and suggested a dose-response relationship. There were no consistent effects of treatment with GS-7340-02 on pQCT-derived parameters at the distal radius metaphysis for males and females treated at 2 mg/kg/day.

At the end of the recovery period, pQCT parameters were unchanged or slightly increased in high dose (18/12 mg/kg/day) males and females indicating an effect of the growth and a partial recovery.

Dual Energy X-Ray Absorptiometry (DXA): High dose (18/12 mg/kg/day) males essentially failed to mature skeletally to the same extent as controls and after 9 months of treatment had mean BMD values for the whole body, femur (whole, proximal, central and distal femur) and lumbar spine (total L1-L4 and individual vertebrae) generally comparable to values attained at pretreatment (baseline) and at the 3-month interim phase evaluation. Females were similarly affected but showed some gains in mean BMD values between months 3 and 9. When compared to concurrent vehicle controls at the 3-month interim phase evaluation and after 9 months of treatment, aerial BMD measurements by DXA were generally significantly decreased at all regions evaluated for high dose (18/12 mg/kg/day) male animals. Statistically significant changes were not seen in high dose females for the whole body, central femur and L4 at the 3-month interim evaluation or for the whole body, distal femur and L1 after 9 months of treatment.

At the end of the recovery period, slight increases in most DXA values were noted in high dose (18/12 mg/kg/day) males and females indicating a partial recovery.

Organ weights:

Interim phase: With the exception of the increased kidney weight for one animal (high), all organ weights were within normal ranges and were not affected by the treatment.

Main phase: The weight of the lungs relative to body weight was significantly increased in both male (+58%) and female (+35%) in the high dose group.

Recovery phase: Organ weights returned to normal.

Gross pathology:

Interim phase: Pale discoloration of the kidneys was noted for some high dose animals.

Main phase: Pale discoloration of the kidneys was noted for all high dose animals and in a single male dog at the mid. Brown, dark discoloration of the fur was another finding noted in most animals (high) and two males (mid). In the lungs, the incidence of raises subpleural area(s) was substantially increased in dogs at the high dose level. An enlargement of the spleen was noted in two males (high)

Recovery phase: Pale discoloration of the kidneys was seen in one male and one female at the high dose level.

Bone Marrow report:

Interim phase: One single female (high) had a mildly decreased M: E ratio due to an increase in proportion of erythroid cells. One single male (high) had an increased M: E ratio due to an increase in proportion of granulocytic cells.

Main phase: There was a slight decreased in the M: E ratio in a single female (high) and this was attributed to an increase in the erythroid component. There were no findings in the maturation of the hematopoietic cell lineages.

Recovery phase: There were no treatment-related findings.

Histopathology: Adequate Battery: yes Peer Review: no
Histopathological Findings:

3-Month Interim phase: Drug related histopathological findings were identified in the kidneys, eyes, lungs and spleen. In the kidneys (all animals, mid or high), cortical tubular degeneration/regeneration and/or karyomegaly were seen. The cortical tubular degeneration/regeneration was multifocal to diffuse and was seen primarily in the proximal convoluted tubules as attenuation and increased basophilia of the tubular epithelium. In the eyes, an infiltration of mononuclear cells was found in the ocular posterior uvea (ciliary bodies and/or choroid) of both sexes (high). The cellular infiltrate was multifocal in distribution, graded minimal to slight in severity and consisted of a mixture of macrophages and lymphocytes. In the lungs, histiocytosis characterizes as a focal or multifocal accumulation of foamy macrophages in the alveolar lumen was seen in two females (high). In the spleen, all dogs (high) had a minimal infiltration of macrophages in the splenic white pulp.

9-Month treatment phase: Drug related histopathological findings were identified in the kidneys, eyes, lungs, spleen, liver and adrenal glands (Table 20). Most of the changes were of the same nature as described in the interim phase. After 9-month of treatment, in the kidney, similar finding of renal cortical tubular degeneration/regeneration and karyomegaly were present in mid and high dose animals. Similar kidney changes were also seen in two low dose males; females were not affected. In the lungs, an infiltration of macrophages was noted in all animals (high) and one or two dogs in each of the low and mid dose groups. In the liver, pigment deposits were noted in few (mid) or all dogs (high). These deposits were fine, brownish to pale green and accumulated perivascularly in macrophages and/or in the sinusoidal cells (Kupffer cells). The accumulation of hepatocellular cytoplasmic acidophilic inclusions was another finding that was limited to the high dose animals. In the adrenal glands, one dog in each sex (high) presented pigment deposits in the sinusoidal cells of the adrenal cortices and/or medullae cortical vacuolation.

Compound-related ocular findings were limited to some dogs given 18/12 mg/kg/day and were of the same nature and severity as discussed after 3 months of treatment.

Table 20: GS-7340-02 related histopathological findings noted after 9 months of treatment
(excerpts from sponsor)

Sex		Male				Female			
Dose level (mg/kg/day)		0	2	6	18/12	0	2	6	18/12
Number of animals examined		4	4	4	4	4	4	4	4
EYE									
Infiltration:	Minimal	—	—	—	1	—	—	—	1
mononuclear cell	Slight	—	—	—	2	—	—	—	—
	Total affected	—	—	—	3	—	—	—	1
KIDNEY									
Degeneration/	Minimal	—	1	3	—	—	—	1	—
regeneration:	Slight	—	—	1	1	—	—	1	3
tubular	Moderate	—	—	—	3	—	—	—	1
	Total affected	—	1	4	4	—	—	2	4
Karyomegaly:	Minimal	—	2	2	—	—	—	3	4
tubular	Slight	—	—	2	2	—	—	—	—
	Moderate	—	—	—	2	—	—	—	—
	Total affected	—	2	4	4	—	—	3	4
Infiltration:	Minimal	—	—	—	—	—	—	—	1
mononuclear cell	Slight	1	—	—	2	—	—	—	1
	Total affected	1	—	—	2	—	—	—	2
LIVER									
Deposits: pigment	Minimal	—	—	2	2	—	—	—	2
	Slight	—	—	—	1	—	—	1	2
	Moderate	—	—	—	1	—	—	—	—
	Total affected	—	—	2	4	—	—	1	4
Hepatocellular	Minimal	—	—	—	3	—	—	—	—
cytoplasmic acido.#	Slight	—	—	—	—	—	—	—	2
inclusion	Total affected	—	—	—	3	—	—	—	2
LUNG									
Infiltration:	Minimal	—	2	—	—	—	1	1	2
macrophage	Slight	—	—	1	4	—	—	—	2
	Total affected	—	2	1	4	—	1	1	4
Histiocytosis:	Minimal	—	—	—	3	—	—	1	1
	Slight	—	—	—	—	—	—	—	1
	Total affected	—	—	—	3	—	—	1	2
Inflammation:	Minimal	—	2	—	3	—	—	—	—
interstitial	Slight	—	—	—	1	—	—	2	—
	Total affected	—	2	—	4	—	—	2	—
SPLEEN									
Infiltration:	Minimal	—	—	—	3	—	—	—	3
macrophage	Slight	—	—	—	1	—	—	—	—
	Total affected	—	—	—	4	—	—	—	3

Acidophilic

Recovery phase: Drug related changes were still observed in the kidneys, lungs and liver (high) but were of a lesser incidences and severity than those seen the main phase. Microscopic changes were no longer seen in the eyes, adrenal glands and spleen.

Electron microscopy: Electron microscopy was performed on sections of liver from two males and two females (controls and high) dogs. In the high dose dogs, in sinusoidal cells (Kupffer cells), there was evidence of deposition of an irregular shaped, granular, electron dense material possessing features possibly consistent with the lipofuscin and that may represent the pigmented intracellular inclusions.

Toxicokinetics: The analysis of plasma samples is shown in Table 21. GS-7340-02 was rapidly absorbed after oral dosing and was rapidly converted to TFV. Peak plasma concentrations occurred at 0.5 and 1 hr postdose for GS-7340-02 and TFV, respectively. No consistent differences in plasma pharmacokinetics were found between male and female dogs. The systemic exposure was dose dependent, for GS-7340-02 the exposure increased more than proportionally for the dose range of 2 to 18/12 mg/kg/day whereas the systemic exposure of TFV was dose proportional to dose administration. In additional, there was some accumulation (approximately 3-fold) of TFV following the repeated dosing.

TFV concentrations in PBMCs were measurable at 24-hr post-dose for all dose groups. The median terminal phase half-life of total TFV in PBMCs was estimated to be 31 hrs. (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.

Table 21: Mean repeated-dose pharmacokinetics of GS-7340 and TFV following oral administration of GS-7340-02 in Beagle Dogs *(excerpts from sponsor)*

Study Period	GS-7340 (mg/kg/day)	C_{max} (µg/mL)		AUC_{tau} (µg·h/mL)		PBMC AUC_{tau} ng·h/10^6 cells
		GS-7340	TFV	GS-7340	TFV	
Day 1	2 (n = 12)	0.08	0.08	0.03	0.40	NA
	6 (n = 12)	0.72	0.25	0.37	1.31	NA
	18 (n = 16)	3.60	0.80	2.07	3.80	NA
Week 13	2 (n = 12)	0.13	0.16	0.07	1.21	NA
	6 (n = 12)	1.10	0.56	0.66	4.10	NA
	12 (n = 14)	3.24	1.25	2.23	12.48	NA
Week 39/40	2 (n = 8)	0.14	0.18	0.08	1.18	258.2
	6 (n = 8)	1.42	0.54	1.03	4.45	1263.5
	12 (n = 12)	2.62	1.32	1.95	13.73	3118.2

NA = not applicable (only Week 39/40 samples were analyzed), PBMC = peripheral blood mononuclear cell, TFV = tenofovir
Source: Report No. TOX-120-002*

Repeated-Dose Toxicity Study in Monkeys

Study title: A 28-Day toxicity study of GS-7340-02 and Tenofovir (GS-1278) administered orally to Rhesus Monkey

Study no.:	P2000114
Study report location:	Electronic
Conducting laboratory and location:	(b) (4)
Date of study initiation:	January 8, 2001
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	GS-7340-02: 7340-02-B-1, Tenofovir:H901; purity not known

Key Study findings: The NOAEL of GS-7340-02 in this study was considered to be 30 mg/kg/day. AUC at the NOAEL for GS-7340-02 and TFV were 1.03 µg*hr/ml and 5.87 µg*hr/ml, respectively.

Methods

Doses:	GS-7340-02: 0, 0.3 (low), 3 (mid), 30 mg/kg/day (high); tenofovir=15 mg/kg/day. Experimental design is shown below
Frequency of dosing:	Once daily
Route of administration:	Oral gavage
Dose volume:	10 mL/kg
Formulation/Vehicle:	50 mM citric acid
Species/Strain:	Rhesus Monkey
Number/Sex/Group:	3 animals/sex/group
Age:	2.2 to 5.2 years
Weight:	2.6 to 5.3 kg
Satellite groups:	None
Unique study design:	None
Deviation from study protocol:	No

Observations and Results

Mortality: All animals were checked twice daily. All dogs survived to schedule necropsy.

Clinical Signs: All animals were checked twice daily. There were no clinical signs considered to be related to treatment with GS-7340-02.

Body Weights: Individual body weights were measured weekly. There were no effects on body weight or body weight gain considered to be related to treatment with GS-7340-02.

Chemistry: Once during the pretreatment period and on Days 14 and 28 of treatment. No test article related changes were seen.

Hematology: No test article related changes were seen. Lymphocyte subsets determined by flow cytometry were not affected.

Urinalysis: No test article related changes were seen.

Bone toxicity parameters: Analysis of PTH levels, 1,25- $(OH)_2$ vitamin D_3, bone-derived ALP values, as well as urinary N-telopeptide concentration did not reveal any effects of the test article.

Mitochondrial toxicity: The analysis of kidney, liver and skeletal muscle samples indicator of mitochondrial integrity (ie, levels of cytochrome C oxidase and citrate synthase enzymes, and mitochondrial DNA content) did not reveal any changes. Hence there was no evidence of mitochondrial injury induced by the test article.

Toxicokinetics: Parameters are shown in Tables 22 to 24. Toxicokinetic analysis revealed rapid conversion of GS-7340-02 to TFV. TFV levels were approximately dose-proportional on days 1 and 28. There was no gender difference in the pharmacokinetic parameters of TFV.

Table 22: Mean plasma GS-7340-02 pharmacokinetic parameters of Rhesus Monkeys following oral gavage dose on Day 28 *(excerpt from sponsor)*

Parameter	GS-7340-02 3 mg/kg/day	GS-7340-02 30 mg/kg/day
N	6	5**
C_{max} (µg/mL)	0.0188 ± 0.0116	1.37 ± 0.919
T_{max} (hr)	0.917 ± 0.585	0.500 ± 0.00
$AUC_{0\rightarrow\tau}$ (µg*hr/mL)	NC	1.03 ± 0.649
$t_{1/2}\lambda_z$ (hr)	NC	0.335 ± 0.231
CL_{ss}/F (mL/hr*kg)	NC	44600 ± 32000
$MRT_{0\rightarrow\infty}$ (hr)	NC	0.806 ± 0.125
V_z/F (mL/kg)	NC	26900 ± 33900

NC: not calculated due to insufficient data
** N = 5; Animal # R14692M was sacrificed on Day 18.

Table 23: Mean plasma TFV pharmacokinetic parameters for Rhesus Monkeys following oral gavage dose on Day 1 *(excerpt from sponsor)*

Parameter	GS-7340-02 3 mg/kg/day	GS-7340-02 30 mg/kg/day	GS-1278 15 mg/kg/day
N	6	6	6
C_{max} (µg/mL)	0.0903 ± 0.0299	0.904 ± 0.312	0.139 ± 0.0613
T_{max} (hr)	1.00 ± 0.00	1.00 ± 0.00	1.50 ± 1.22
$AUC_{0\rightarrow\infty}$ (µg*hr/mL)	0.472 ± 0.137	6.67 ± 4.06	1.01 ± 0.518
AUC % Extrapolated	11.2 ± 4.09	22.4 ± 15.0	14.1 ± 15.8
$t_{1/2}\lambda_z$ (hr)	8.09 ± 1.37	12.5 ± 6.67	8.98 ± 5.98
CL/F (mL/hr*kg)	3310 ± 931	2700 ± 1100	18700 ± 9390
$MRT_{0\rightarrow\infty}$ (hr)	10.1 ± 1.92	16.4 ± 9.84	12.4 ± 9.08
V_z/F (mL/kg)	38000 ± 10200	43600 + 18200	247000 + 212000

Table 24: Mean plasma TFV pharmacokinetic parameters of Rhesus Monkeys following oral gavage dose on Day 28 *(excerpt from sponsor)*

Parameter	GS-7340-02 3 mg/kg/day	GS-7340-02 30 mg/kg/day	GS-1278 15 mg/kg/day
N	6	5**	6
C_{max} (µg/mL)	0.0504 ± 0.0235	0.963 ± 0.293	0.165 ± 0.0910
T_{max} (hr)	1.67 ± 1.21	0.700 ± 0.274	1.83 ± 1.29
$AUC_{0\rightarrow\tau}$ (µg*hr/mL)	0.352 ± 0.0618	5.87 ± 2.02	1.33 ± 0.655
$t_{1/2}\lambda_z$ (hr)	13.5 ± 4.87	16.1 ± 2.47	15.1 ± 11.4
CL_{ss}/F (mL/hr*kg)	4250 ± 827	2710 ± 884	17200 ± 15800
$MRT_{0\rightarrow\infty}$ (hr)	17.0 ± 5.07	19.2 ± 2.33	19.3 ± 14.0
V_z/F (mL/kg)	81800 ± 28600	62100 ± 21400	343000 ± 274000

** n = 5; Animal #R14692M was sacrificed on Day 18

Gross Pathology: No changes were seen.

Organ Weights: No changes were seen.

Histopathology: Adequate Battery: yes Peer Review: no

Histological Findings: The only noteworthy alteration was testicular degeneration in one animal at the mid dose.

7 Genetic Toxicology

Note: The following TAF studies were reviewed by Dr. Pritam Verma under IND-111-007.

1.Mutagenicity test with GS-7340-2 in Salmonella - Escherichia coli/mammalian microsome reverse mutation assay with a confirmatory assay, Lot # 1309-96-32, (b) (4) December 16, 1999, (20900-0-409ECD)*

GS-7340-2 was evaluated for mutagenic activity in the Bacterial Reverse Mutation Assay using Salmonella typhimurium strains TA98, TA100, TA1535 and TA1537, and Escherichia coli strain WP2uvrA. The assay was conducted in the presence and absence of a metabolic activation system using an S9 fraction prepared from the livers of Aroclor 1254-induced rats. The test compound was studied at concentrations ranging from 100 to 5000 µg/plate. Results: GS-7340-2 did not cause a positive increase in the mean number of revertants per plate with any of the tester strains either in the presence or absence of S9 mix. Conclusions: under the conditions of this study, GS-7340-2 was found to be non-mutagenic.

2. Mutagenicity test with GS-7340-2 in the L5178Y TK+/- mouse lymphoma forward mutation assay, Lot # 1309-96-32, (b) (4), January 3, 2000, (20900-0-431 ICH)*

This study was designed to evaluate the potential of GS to induce mutations at the thymidine kinase (TK) locus in cultured L5178Y cells in the presence and absence of an exogenous metabolic activation system (S9). The S9 homogenate was prepared from male Sprague-Dawley rats that had been injected (i.p.) with Aroclor™-1250 at a dose level of 500 mg/kg. GS-7340-2 was evaluated at concentrations ranging from 125 to 4800 µg/ml with and without S9. Results: GS-7340-2 was evaluated as negative for inducing forward mutations at the TK locus in L5178Y mouse lymphoma cells. Conclusions: under the conditions of the study, GS-7340-2 was found to be negative in inducing gene mutations.

3. In vivo mouse micronucleus assay of GS-7340-2, Lot # 1309-96-32, (b) (4) January 12, 2001, (21816-0-455OECD)*

Groups of male Crl:CD-1 mice (6 animal/dose level/harvest time point) were administered a single dose GS-7340-2 via oral gavage at dose levels of 0 (vehicle control), 50 (low), 100 (mid) or 2000 mg/kg (high) to evaluate the test article for in vivo clastogenic activity and/or disruption of the mitotic apparatus by detecting micronuclei in polychromatic erythrocyte (PCE) cells in the bone marrow. The dose levels were selected based upon the results of a dose-range finding study of GS-7340-2. Results: GS-7340-2 induced no signs of clinical toxicity in any of the treated animals and was not cytotoxic to the bone marrow (ie, no statistically significant decrease in the PCE:NCE ratio). The test compound did not induce a statistically significant increase in micronuclei in bone marrow PCEs. Conclusions: GS-7340-2 is considered negative in the mouse bone marrow micronucleus test under the conditions of exposure in this assay.

8 Carcinogenicity

The sponsor submitted two carcinogenicity studies evaluating TFV, titled "An Oral Carcinogenicity Study of TFV DISOPROXIL Fumarate (TFV-DF) in the Albino Mouse" (M990205) and "An Oral Carcinogenicity Study of TFV DISOPROXIL Fumarate (Tenofovir DF) in The Albino Rat" (990204). Both of these studies have been reviewed by Dr. Verma under NDA 21-356 (Viread). Since TAF is rapidly converted to TFV and no substantial TAF exposure is seen in mice and rats the sponsor did not have to perform a carcinogenicity study for TAF. Long-term oral carcinogenicity studies of tenofovir disoproxil fumarate in mice and rats were carried out at exposures up to approximately 10 times (mice) and 4 times (rats) those observed in humans at the 300 mg therapeutic dose of tenofovir disoproxil fumarate for HIV-1 infection. These TDF carcinogenicity in mice and rats resulted in TFV exposures up to approximately 163 times (mice, TFV mouse AUC = 16.2 µg*hr/ml) and 55 times (rats, rat AUC = 48.7µg*hr/ml) higher than TFV exposure in humans (human AUCss = 0.293 µg*hr/ml) reached after administration of Genvoya. In female mice, liver adenomas were increased at TFV exposures 163 times those observed in humans. In rats, the study was negative for carcinogenic findings at TFV exposures up to 55 times those observed in humans at the therapeutic dose.

9 Reproductive and Developmental Toxicology

Note: The following TAF studies were reviewed by Dr. Pritam Verma under IND-111-007.

9.1 Fertility and Early Embryonic Development

Study title: Oral Gavage Study of Fertility and Early Embryonic Development to Implantation with GS-7340-03 in Rats

Study no.:	TX-120-2012
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	04 June 2012
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	7340-03-AC-1P, 94.1% pure

Key Study Findings: At the end of the study high dose male animals (160 mg/kg/day) showed significantly reduced mean body weights compared to the control group (12% less than the control group). Also the mean body weight of female high dose animals (160 mg/kg/day) was slightly but significantly reduced (6% less than the control group) on Premating Day 17. Mean gestational body weight gain was significantly reduced in the high dose group between GD 0-7 (19%) compared to the control group. 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. A slight (9%) increase in adjusted mean weight of the testis in the 160 mg/kg/day group was seen but not considered adverse because no other test article-related effects were observed on male and female reproductive performance or intrauterine parameters. The NOAEL

for male and female toxicity was 80 mg/kg/day. The NOAEL for reproductive and early embryonic toxicity was 160 mg/kg/day.

Methods

Doses:	20, 80, or 160 mg/kg/day
Frequency of dosing:	Once daily
Dose volume:	10 ml/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	0.1% hydroxypropylmethylcellulose K100LV (HPMC), 0.1% Tween 20 in water
Species/Strain:	Crl:CD(SD) rats
Number/Sex/Group:	22 animals/sex/group
Satellite groups:	None
Study design:	Dose levels were selected on the basis of data from an embryo-fetal study in rats (Gilead TX-120-2002
Deviation from study protocol:	None

Observations and Results:

Mortality: Each animal was observed twice daily (a.m. and p.m.) for mortality, abnormalities, and signs of pain or distress. All animals survived to scheduled euthanasia in this study.

Clinical Signs: In males, minor nonadverse observations of discolored haircoat – left periorbital brown, perineal brown, front legs brown, and/or periorbital red were seen in 1 male each in the 20 and 80 mg/kg/day groups, as well as rough haircoat seen in 2 animals in each of the 20, 80, and 160 mg/kg/day groups. These were the only observations considered treatment-related and were likely associated with the slight decrease in body weight gain or decreased food consumption observed.

Body Weight: Female body weights were recorded on GD 0, 3, 7, 10, and 13. Decreased mean body weight was noted in the 80 and 160 mg/kg/day groups throughout the study. In the 80 mg/kg/day group, no body weights were significantly reduced, while in the 160 mg/kg/day group body weights were statistically significantly reduced on Premating (PM) Day 28, Pairing Days 3, 7, and 10, and on Postpairing (PP) Days 0 through the end of the study when compared to the control. Mean PP Day 35 body weights were 0.5%, 4.8% and 12% lower compare to the controls at 20, 80, and 160 mg/kg/day groups, respectively. Mean female PM body weight was slightly decreased in a dose-dependent manner in all GS-7340-03 treated groups throughout the study. A statistically significant decrease in body weight was seen on PM Day 14, only in the 160 mg/kg/day group.

During Gestation: Mean gestation body weight was reduced in a dosage dependant manner with statistically significant reductions noted on GD 3, 7, and 10 in the 160 mg/kg/day group. Average GD 13 body weights were 380, 378, 374, and 360 g in the control, 20, 80, and 160 mg/kg/day groups, respectively.

Feed Consumption: Beginning on GD 0, food consumption was measured at gestation body weight intervals. Premating average male food consumption was slightly reduced in a dose-dependent manner in all test article-treated groups. In the 20 and 80 mg/kg/day group, there were no statistically significant reductions, while in the 160 mg/kg/day group food consumption was statistically significantly reduced across the Premating (PM) interval of 14-21. Postpairing food consumption was comparable to the control in the 20 mg/kg/day group while the 80 and 160 mg/kg/day groups were slightly reduced. Statistically significant reductions were noted in the 160 mg/kg/day group on the PP intervals of 0-7, 7-14, 14- 21, 21-28, and 28-35.

Female premating food consumption was slightly reduced in the 80 and 160 mg/kg/day groups with statistical significance achieved in the 160 mg/kg/day group on the PM intervals of 0-7 and 7-14.

During gestation, female food consumption was comparable to the control in the 20 and 80 mg/kg/day groups. In the 160 mg/kg/day group food consumption was slightly reduced throughout gestation, but only statistically significantly during dose administration (gestation intervals of 0-3 and 3-7).

Toxicokinetics: Not reported.

Dosing Solution Analysis: Homogeneity analyses (low- and high-dose levels) indicated that the test article was homogeneously mixed. All replicates were within 5% of their respective means. Results of routine concentration analyses indicated that all dose preparations were within 10% of target.

Reproductive Performance

Estrous Cycle: There were no differences in the premating estrous cycles observed. Premating, the mean number of estrous cycles/mean cycle length was 2.8/4.2, 2.6/4.1, 2.3/4.5, and 2.5/4.3 in the control, 20, 80, and 160 mg/kg/day groups, respectively.

Reproductive Indices: No test article-related differences in any of the male reproductive parameters were determined. Mating/fecundity/fertilities 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 groups, respectively.

No test article-related differences in any of the female reproductive parameters were determined. Mating/fecundity/fertilities indices were 100/95/95, 100/100/100, 100/95/95, and 100/95/95 in the control, 20, 80, and 160 mg/kg/day groups, respectively.

Necropsy: There were no treatment-related macroscopic observations. Singular observations of epididymal mass (caudal end, left, tan) in a high dose male (B02705) and kidney discolored, right - tan and large with raised tan area (B02769) in a 80 mg/kg/day female are considered to be spontaneous and unrelated to treatment.

Cesarean Section: There were no test article-related effects on any of the cesarean section parameters.

Organ Weights: A slight increase in absolute weight of the testis (statistically significant increase in the adjusted mean of the left testis only) in the 160 mg/kg/day group was considered to be test article-related, but not adverse since there were no other reproductive organ weight effects or functional reproductive effects. There were no statistically significant differences in organ weights for the epididymis, prostate, seminal vesicles or pituitary. For the treated females there were no statistically significant differences in reproductive organ weights in any group.

Male Reproductive Assessment:

Sperm Motility: No test article-related effects were observed on mean epididymal sperm motility. 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.

Sperm Concentration: No test article-related effects were observed on sperm concentration. Mean sperm concentration in the control, 20, 80, and 160 mg/kg/day groups was 942.2, 833.2, 966.1, and 928.5 millions/gram of caudal epididymal weight, respectively

9.2 Embryonic Fetal Development

An Embryo-fetal Development Study of GS-7340-02 by Oral Gavage in Rats

Study no.:	TX-120-2002
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	01 February, 2011
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	7340-02-AC-1EA, 97.7% pure

Key study findings: 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 given 250 mg/kg/day, with the corrected mean body weights also 10% lower than controls on GD 21. The incidences of fetal major malformations, minor external, visceral and skeletal anomalies and were not affected by GS-7340-02. Sternebral variants (1 to 4 and 5 and 6) were increased at 250 mg/kg/day. There was no evidence of embryolethality or teratogenicity attributed to GS-7340-02 in this study. Based on these results, the maternal NOAEL and the NOAEL for embryo-fetal development were both considered to be the 100 mg/kg/day dose level, which resulted in GD17 AUC_{0-t} values of 17.4 and 0.2 µg·hr/mL for R-PMPA and GS-7340, respectively.

Methods

Doses:	0 (vehicle control), 25 (low), 100 (mid) or 250 mg/kg/day (high)
Frequency of dosing:	Once daily
Dose volume:	5 ml/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	0.1% hydroxypropylmethylcellulose K100LV (HPMC), 0.1% Tween 20 in water
Species/Strain:	Crl:CD(SD) rats
Number/Sex/Group:	25 animals/sex/group
Satellite groups:	A satellite group of pregnant animals (9/group) were dosed and used for toxicokinetic sampling
Study design:	rats were dosed from Gestation Days (GD) 6 to 17, inclusive. These doses were selected based on the results of a dose range study (doses: 5, 100, or 200 mg/kg)
Deviation from study protocol:	None

Observations and Results

Mortality (dams): Mortality/moribundity checks were conducted twice daily. There were no deaths.

Clinical signs (dams): Detailed clinical observations were performed on days of body weight assessment. The only potentially GS-7340-02-related clinical sign was transient salivation, which was noted in 7 out of 25 animals given 250 mg/kg/day.

Body weight (dams): Body weights were conducted on GD 0, 3, 4, 6, 8, 10, 12, 14, 16, 18 and 21. Body weight gains were significantly decreased ($P \leq 0.01$, $P \leq 0.001$) at all intervals during the treatment period in animals given 250 mg/kg/day. This resulted in lower body weights from GD 10 to the end of the study. On GD 21, the mean body weight of the 250 mg/kg/day group was 10 % lower than the control group mean body weight. Overall mean corrected body weights and mean corrected body weight changes were also significantly decreased at the same dose level, with corrected mean body weights -10% lower than controls on GD 21, and mean corrected body weight changes from GD 6 to 21 were noted to be -56% lower than controls.

Food consumption (dams): Food consumption was measured on GD 4 to 6, 6 to 8, 8 to 10, 10 to 12, 12 to 14, 14 to 16, 16 to 18 and 18 to 21. Food consumption was significantly ($P \leq 0.01$, $P \leq 0.001$) decreased in animals given 250 mg/kg/day throughout the treatment period.

Necropsy at Caesarean Section: No GS-7340-02-related gross findings were noted. The gross findings observed were considered incidental, of a nature commonly observed in this strain and age of rats, and/or were of similar incidence in control and treated animals, and therefore were considered unrelated to administration of GS-7340-02.

Ovarian and Uterine Findings: The pregnancy rate on this study was 100, 88, 96 and 96% for Groups 1, 2, 3 and 4, respectively. 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 losses were comparable to controls.

Toxicokinetics: After oral gavage administration of GS-7340-02, GS-7340 readily appeared in plasma, with T_{max} values of 0.500 hours on both GD 6 and 17. Values for C_{max} and AUC_{0-t} were generally similar on GD 6 and 17, indicating no accumulation of GS-7340 after multiple dosing of GS-7340-02 in rats at the 250 mg/kg/day dose level.

Concentrations of GS-7340 were all below the lower limit of quantitation at dose level 25 mg/kg/day. The increases in C_{max} and AUC_{0-t} were greater than proportional between the 100 and 250 mg/kg/day dose level, although on GD 6 the large difference may be due to the out of specification results observed in the dose analysis where concentrations were 35.4% less than expected at the 100 mg/kg/day dose level.

Table 25: Toxicokinetic parameters for GS-7340 in rat plasma on gestation Days 6 and 17
(excerpt from sponsor)

Interval	Dose Group	Dose Level (mg/kg/day)	C_{max} (ng/mL)	T_{max} (hr)	AUC_{0-t} (ng•hr/mL)	T_{last} (hr)	C_{last} (ng/mL)
6	2	25	NC	NC	NC	NC	NC
	3	100	38.4	0.500	NC[a]	NC[a]	NC[a]
	4	250	756	0.500	1376	4.00	84.1
17	2	25	NC	NC	NC	NC	NC
	3	100	149	0.500	242	4.00	32.4
	4	250	597	0.500	1382	4.00	153

Note: Group 2 concentrations were all below the lower limit of quantitation.
NC Not calculated.
a Only one measurable mean concentration in the profile.

Table 26: Toxicokinetic parameters for TFV in rat plasma on gestation Days 6 and 17
(excerpt from sponsor)

Interval	Dose Group	Dose Level (mg/kg/day)	C_{max} (ng/mL)	T_{max} (hr)	AUC_{0-t} (ng•hr/mL)	T_{last} (hr)	C_{last} (ng/mL)
6	2	25	1110	0.500	2715	24.0	12.4
	3	100	2767	0.500	9453	24.0	29.3
	4	250	9860	0.500	54200	24.0	314
17	2	25	870	0.500	2803	24.0	15.7
	3	100	4130	0.500	17392	24.0	73.5
	4	250	7350	0.500	55728	24.0	406

The increases in C_{max} and AUC_{0-t} were inconsistently proportional between the 25 to 250 mg/kg/day dose levels, which may be due to the out of specification results observed in the dose analysis where concentrations were 35.4% less than expected at the 100 mg/kg/day dose level.

After oral gavage administration of GS-7340-02, TFV readily appeared in plasma, with a T_{max} value of 0.500 on both GD 6 and 17. Values for C_{max} and AUC_{0-t} were generally similar on GD 6 and 17, indicating no accumulation of TFV after multiple dosing of GS-7340-02 in rats. Concentrations of TFV were much higher than concentrations of GS-7340, therefore indicating that GS-7340 was extensively converted to TFV in rats following oral administration of GS-7340-02.

Fetuses:

Fetal weights: Fetal weights were decreased in a dose dependent manner and noted with statistical significance ($P \leq 0.05$, $P \leq 0.01$ and $P \leq 0.001$) at 100 (-5%) and 250 (-15%) mg/kg/day. Values for males, females and total fetal weights were found to be within the historical control ranges, however, given the extent of the difference from controls, this was considered as an adverse effect at 250 mg/kg/day.

Major Malformations: The incidence of major malformations was not affected by GS-7340-02 administration. One fetus (No. 4509-8, 250 mg/kg/day) was noted to have multiple abnormalities (anascara, an absent philtrum, cleft palate, anophthalmia, gastroschisis, anal atresia, digits of fore and hind paws were absent and/or fused/shortened with the fore and hind limbs/limb bones shorter/smaller than normal). One other fetus (No. 2514-8, 25 mg/kg/day) was noted to have anal atresia and athread-like tail, and a control fetus (No. 1517-7) had a supernumerary lung lobe.

Minor External and Visceral Anomalies: GS-7340-02 administration did not affect the incidence of minor external and visceral anomalies. Those noted on this study were a kinked tail (No. 4509-8), small renal papillae (Nos. 1508-4, 3512-8) and dilated ureters (Nos. 1508-4, 3512-8, 4513-1).

Minor Skeletal Anomalies: Skeletal anomalies were not affected by GS-7340-02. A statistically significant decrease in the number of animals noted with incomplete ossification of the interparietal and hyoid bones was noted at 250 mg/kg/day. Other minor skeletal anomalies were comparable in incidence to controls.

Examinations at Caesarean Section: No treatment-related changes were seen in the number of corpora lutea, the number of implantations, the implantation index, the number of live fetuses, the fetal viability, the number of dead embryos and fetuses, post implantation deaths, the fetal sex distribution, the fetal body weights, the placental weights or the placental findings in any treated group.

Common Skeletal Variants: Sternebral variants (1 to 4 and 5 and 6) were noted to be increased at 250 mg/kg/day. However, the numbers were within historical control ranges (1.5 to 52.1%), and this was considered likely to be a minor transitory delay in the ossification rates, probably related to the lower fetal weights.

Conclusions: Effects on Embryo-Fetal Development: The administration of GS-7340-02 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 GS-7340-02 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 GS-7340, respectively

An Embryo-fetal Development Study of GS-7340-02 by Oral Gavage in Rabbits

Study no.:	TX-120-2005
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	October 21, 2011
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	7340-02-AC-1EA, 97.7% pure

Key study findings: 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 NOAEL for maternal toxicity was considered to be 30 mg/kg/day (AUC_{0-t} = 1.1 and 5.0 µg·h/mL for GS-7340 and TFV, respectively) and the NAOEL for embryo-fetal development was 100 mg/kg/day (AUC_{0-t} = 11.0 and 27.3 µg·h/mL for GS-7340 and TFV, respectively).

Methods

Doses:	0 (vehicle control), 10 (low), 30 (mid) or 100 mg/kg/day (high)
Frequency of dosing:	Once daily
Dose volume:	10 ml/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	0.1% hydroxypropylmethylcellulose K100LV (HPMC), 0.1% Tween 20 in water
Species/Strain:	New Zealand White rabbits
Number/Sex/Group:	20 animals/sex/group
Satellite groups:	A satellite group of 3 pregnant animals/group were dosed and used for toxicokinetic sampling.
Study design:	GS-7430-02 was administered by oral gavage to time-mated F_0 generation females. Parameters and endpoints evaluated: maternal gross necropsy, Cesarean-sectioning and fetal evaluation.
Deviation from study protocol:	None

Observations and Results

Mortality (dams): Animals were observed twice daily. There were no deaths.

Clinical signs (dams): Animals were observed twice daily. A complete detailed examination was performed on days of body weight assessments. A higher incidence of decreased fecal output was noted at 100 mg/kg/day compared to the vehicle control group during the treatment period and was associated with decreased food intake. Following the end of the treatment period, yellow staining of the fur (tail, pinna, muzzle, hindpaws, forepaws and cranium regions) was also observed at a higher incidence at 30 and 100 mg/kg/day.

Body weight (dams): Animals were individually weighed on GD 0 (at supplier), 4, 7, 9, 11, 13, 15, 18, 21, 24, 27 and 29. Mean body weights were significantly ($p \leq 0.05$) lower compared to controls on GD 21 and 24 (-5%). When compared to controls, there were significantly ($p \leq 0.05$) lower body weight gains between GD 11 and 13 at 100 mg/kg/day. Overall (i.e., GD 7 to 21) body weight gains significantly ($p \leq 0.001$) lower than controls at 100 mg/kg/day. Body weight gains were significantly ($p \leq 0.05$ or $p \leq 0.01$) greater than controls between GD 24 and 27 and between GD 21 and 29 at doses ≥ 30 mg/kg/day.

Body weight and body weight gain values were similar to controls at 10 mg/kg/day.

Food consumption (dams): Individual food consumption was quantitatively measured daily starting on GD 5. After 1 to 3 days of treatment, food intake was lower than controls for the 100 mg/kg/day dose group, with statistically significantly ($p \leq 0.05$ or $p \leq 0.01$) lower values at daily intervals between GD 13 and 21. This resulted in significantly ($p \leq 0.001$) lower (-18%) overall food intake at 100 mg/kg/day during the treatment period (i.e., GD 7 to 21). Also at 100 mg/kg/day 3 animals (Nos. 4503, 4508 and 4509) showed 4 or more days with food intake of less than 30 g during the dosing period. Food consumption at each dose level was comparable to controls thereafter and until termination on GD 29.

Gross Pathology: There were no treatment-related gross pathology findings.

Necropsy at Caesarean Section:

Ovarian and Uterine Findings: The pregnancy rate was at least 85% in each group. The pregnancy rates, numbers of corpora lutea, implantation sites, live and dead fetuses and resorptions, and the sex ratio and the pre and post implantation losses (%) were comparable to the controls in each GS-7430 treated group.

Toxicokinetics: data are shown in Table 27. All concentration values of GS-7340 and its metabolite, TFV in the control group were below the lower limit of quantitation. Exposure to GS-7340 increased with the increase in dose level from 10 to 100 mg/kg/day. The increases in C_{max} were greater than proportional between 10 and 100 mg/kg/day and the increases in AUC_{0-t} were greater than proportional between 30 and 100 mg/kg/day dose level on GD 20. Exposure to TFV increased with the increase in GS-7340-02 dose level from 10 to 100 mg/kg/day. The

increases in C_{max} and AUC_{0-t} were roughly proportional between 10 and 100 mg/kg/day. Potential accumulation of TFV was observed after multiple dosing of GS-7340-02. Concentrations of TFV were much higher than concentrations of GS-7340, indicating that GS-7340 was extensively converted to TFV.

Table 27: Toxicokinetic parameters for GS-7340 in rabbits *(excerpt from sponsor)*

GD	Group	Dose (mg/kg/day)	Toxicokinetic Parameters			
			GS-7430		R-PMPA	
			AUC_{0-t} (ng•h/mL)	C_{max} (ng/mL)	AUC_{0-t} (ng•h/mL)	C_{max} (ng/mL)
7	2	10	NA	8.46	1941	261
	3	30	NA	77.3	3013	398
	4	100	918	846	13443	2193
20	2	10	NA	155	2018	260
	3	30	1135	937	5005	676
	4	100	11043	9190	27251	2970

NA = Not applicable

Fetuses:

Fetal weights: The fetal weights (males, females and sex combined) were comparable to controls at each dose level.

Major Malformations: There were no malformations attributed to the administration of GS-7430. The number of litters with major malformations (i.e., 13% at 10 mg/kg/day and 15% at 100 mg/kg/day) was within the testing facility's historical control range (maximum of 27.8% of litters affected). Also, in view of the low incidence and the lack of a clear dose-dependent response, these abnormalities were considered to have no teratological significance.

Minor External and Visceral Anomalies: There were no treatment-related minor external and visceral anomalies.

Minor Skeletal Anomalies: The overall incidence of litters and fetuses with minor skeletal anomalies and of common skeletal variants were unaffected by treatment. The incidence of fetuses, but not litters, with incomplete ossification of the frontal bones was statistically significantly ($p \leq 0.05$) increased at 10 and 100 mg/kg/day, however, due to the lack of dose dependency, this was not considered treatment-related.

9.3 Prenatal and Postnatal Development

The sponsor submitted "A Oral (gavage) Developmental and Perinatal/Postnatal Reproduction Toxicity Study of GS-4331-05 (bis-POCPMPA) Including Postnatal Behavior/Functional Evaluation in Rats, (R990202)" evaluating systemic TFV exposure which was evaluated by Dr. Verma under NDA 21-356 (Viread). Since TAF is rapidly converted to TFV and no substantial TAF exposure is seen in mice and rats the sponsor did not have to perform a perinatal and postnatal study for TAF. The above mentioned study evaluated TFV exposure, delivered via TDF, 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 evaluated the potential postnatal toxicity of TFV, increased pup mortality reduced pup survival and reduced pup body weights at maternally toxic doses. The maternal NOEL of TDF was 50 mg/kg/day leading to a TFV exposure of 7.84 µg·h/mL. The developmental NOEL was 150 mg/kg/day. The NOEL for general toxicity in the F1 generation was 50 mg/kg/day. The F1 generation male and female NOEL for behavior, reproductive and developmental toxicity was 50 mg/kg/day. The measured TFV exposures in the dams at the NOEL for developmental toxicity (150 mg/kg/day) and F1 toxicity (50 mg/kg/day) were 27 and 14 times (using AUC at GD7, or 43 and 21 times when using the AUC at LD20) higher than the exposure in humans at the recommended daily dose.

Table 28: Mean toxicokinetic parameters of TFV in rats on Gestation Day 7 and lactation Day 20 *(excerpted from sponsor)*

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

10 Special Toxicology Studies

Local toxicity studies

The following TAF studies were reviewed by Dr. Pritam Verma under IND-111-007.

The Bovine Corneal Opacity and Permeability Assay (BCOP) (EUN0017/TX-120-2013)

The Bovine Corneal Opacity and Permeability Assay (BCOP) was performed to assess the ocular irritancy potential *in vitro* of the test substance. Imidazole was tested in parallel as a positive control. Isolated bovine corneas were used as a means of assessing the ocular corrosivity or severe irritancy potential of test substances in vitro. Two endpoints, corneal opacity and permeability, were measured and combined to give an In Vitro Irritancy Score which can be used to classify and rank test substances as potential eye irritants according to OECD guideline 437. The results of the BCOP assay are summarized in the table below.

Table 29: Results of the BCOP assay

Sample	Opacity ± SD	Permeability ± SD	In vitro irritancy Score ± SD	In vitro classification
GS-7340-03	20.667 ± 8.718	0.025 ± 0.017	21.0 ± 8.7	Non-Corrosive/ Non-Severe irritant
Imidazole	120.667 ± 10.149	2.879 ± 0.150	163.8 ± 8.9	Corrosive/Severe irritant
0.9% Saline	4.333 ± 2.887	0.061 ± 0.005	Not applicable	Not applicable

GS-7340-03 elicited an In Vitro Irritancy Score (IVIS) of 21.0 ± 8.7 with a 4 hour incubation and was predicted to be a non-corrosive/non-severe eye irritant. A substance that induces an IVIS of ≥ 55.1 is defined as a corrosive or severe irritant.

Primary Dermal Irritation/Corrosion Study with GS-7340 in Rabbits (TX-120-2011/8253834)

The purpose of this study was to assess the level of primary skin irritation/corrosion of GS-7340-02 on New Zealand White (Hra:(NZW)SPF) rabbits under semi-occluded conditions. GS-7340-02 was evenly applied to the intact test area (approximately 6 cm^2) on each rabbit, 0.5 g in the case of solids on Day 1. The test article was moistened with distilled water (0.5 to 0.6 mL), and the area of application was covered with a 4-ply 5 x 5 cm gauze patch secured with paper tape and over-wrapped with Saran Wrap and Tensoplast tape to provide a semi-occlusive dressing. Animals were not collared during the 4-hour application period. Approximately 4 hours after application, patches were removed, and residual test article was removed from the skin (as thoroughly and as gently as possible) using water and paper towels. The untreated skin of each animal served as its own control. The degree of erythema and edema was evaluated (Draize technique) approximately 60 minutes and 24, 48, and 72 hours after patch removal. Mortality, clinical observations, and body weight were also assessed. All animals survived to study termination. No test article-related clinical observations or effect on body weight were noted. No signs of erythema or edema were noted at any observation interval. Because no erythema or edema was present at the examination 72 hours after patch removal, no additional observations

were conducted, and the study was terminated. The primary dermal irritation index of GS-7340 was 0.0; therefore it was concluded that the test article was nonirritating/noncorrosive to the skin of the rabbits under the conditions of this study.

GS-7340-03: Assessment of Skin Sensitization Potential using the Local Lymph Node Assay in the Mouse (Individual animal approach) (EUN0018/TX-120-2014)

This study assessed the skin sensitization potential of GS-7340-03 using the local lymph node assay (LLNA). Preliminary investigations were performed at 25% and 50% w/v with 2 mice per concentration to establish the highest concentration of test substance which did not lead to systemic toxicity or excessive local irritation. The results of preliminary investigations indicated that 50% w/v would be a suitable high concentration for use on the main study.

The main study comprised three treated groups, each comprising five female mice receiving GS-7340-03 at concentrations of 10, 25 or 50% w/v. 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 treated by daily application of 25 µL of the appropriate concentration or control (vehicle or positive), to the dorsal surface of both ears for three consecutive days.

The proliferative response of the lymph node cells (LNC) from the draining auricular lymph nodes was assessed five days following the initial application, by measurement of the incorporation of ^{3}H-methyl Thymidine (^{3}HTdR) by ß-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 three or more.

The SI obtained for 10, 25 and 50% w/v were 0.9, 1.0 and 1.0 respectively which indicates that GS-7340-03 did not show the potential to induce skin sensitization. The SI for the positive control substance hexyl cinnamic aldehyde was 6.3, which demonstrates the validity of this study. GS-7340-03 is not regarded as a potential skin sensitizer.

Mechanistic studies

Bone turnover:

7-Day Repeat Dose Toxicity Study of GS-7304-02 and GS-7503 in Male Sprague-Dawley Rats (R990177)

This study evaluated changes in bone markers 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. Urine was collected continuously over a 24-hour period while the animals 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 clinical signs of toxicity were observed over the duration of the study. Serum PTH concentrations were unchanged in animals administered GS-7340-02 compared to controls on Day 6. Serum 1,25-dihydroxycholcalciferol (Vit D) was decreased by 80% in animals administered GS-7340-02 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 study could not conclude if these changes were due to bone turnover, altered renal function or both.

A 6-day repeat dose oral gavage exploratory toxicity study of GS-7340-2 in the albino rat, Lot # 7340-02-B-1, Gilead Sciences, Boulder, CO, September 25, 2000, (R2000044)

Groups of male Sprague-Dawley rats were orally gavaged with GS-7340-2 at dose levels of 100 (LD) or 400 mg/kg/day (HD) for 6 consecutive days to examined changes in marker of calcium homeostasis. There were no deaths in this study. Remarkable changes observed included a large increase in urinary output of calcium (HD). This calciuria continued to the end of the study and was accompanied by a marked decreased in 1,25 vitamin D_3. A slight non-dose dependent decreased in serum calcium concentrations, and a dose dependent decrease in serum phosphorous concentrations were observed (LD/HD) after 6 days of treatment. No effects were observed in urinary phosphorus, urinary cyclic AMP, serum or plasma ionized calcium and parathyroid hormone. The mechanistic basis of these changes and their biological relationships in terms of cause and effect are unknown. However, the data suggest that GS-7340-2 might directly inhibit 1, 25-dihydroxyvitamin D_3 production thus resulting in decreased gastrointestinal absorption of calcium and phosphate and decreased renal reabsorption of calcium.

Five-Day Oral Toxicity Study of GS-7340-02 and GS-7503 in Beagle Dogs

GS-7340-02 and GS-7503 are both prodrugs of TFV. Oral doses of 37.5 or 75 mg/kg/day GS-7340-02, 36 or 72 mg/kg/day GS-7503, or vehicle were each administered to groups of male beagle dogs for 5 days. Emesis and excessive salivation were noted in dogs given GS-7340-02 of GS-7503, diarrhea, was only noted in dogs given GS-7340-02. Weight loss was noted between Days 1 and 7 in dogs given 37.5 or 75 mg/kg/day GS-7340-02 or 72 mg/kg/day GS-7503. Reduced food consumption was noted between Days 3 and 6 for dogs in these same three dose groups. Renal lesions were noted in only one dog that received 5 daily doses of 72 mg/kg/day GS-7503. These lesions, which consist of tubular dilatation, tubular degeneration, tubular regeneration, and interstitial lymphocytic cell infiltration, were consistent with those seen previously in dogs treated with TFV, the parent drug of GS-7503. Drug-induced lesions in the gastrointestinal tract (epithelial cell necrosis, regenerative hyperplasia, and cyst formation) were only noted in dogs given GS-7340-02; while drug-induced lymphodepletion in the lymph nodes, thymus and tonsil, as well as hypocellularity in the bone marrow were detected in dogs treated with GS-7340-02 or GS-7503, the lesions occurred at higher incidence and greater severity in dogs treated with GS-7340-02. The incidence of hepatic alterations, primarily mixed inflammatory

cell infiltration, was greatest in animals given GS-7503. Bile duct hyperplasia and periportal inflammation were similar in severity in dogs treated with GS- 7340-02 or GS-7503.Since both GS-7340-02 and GS-7503 are prodrugs of TFV, the differences in drug-related toxicity are probably related to differences in the oral bioavailability of these two prodrugs.

Renal function:

A pharmacological assessment of the effects of GS-7340-02 on the renal system of the rat (R990186)

This study evaluates the pharmacological effects of GS-7340-02 on the renal system following oral administration of 0, 100 or 1000 mg/kg GS-7340-02 to albino rats. There were no treatment-related differences between creatinine clearance values for the control and treated groups observed. A slight, statistically significant, increase in group mean serum calcium and a marked, statistically significant, increase in total amount of calcium excreted in the urine were noted at 1000 mg/kg. At 100 mg/kg, slight increase in serum calcium was also noted but individual values were within historical ranges. The urinary increase of calcium output at 1000 mg/kg correlated with an increase in serum calcium concentration. This indicated that the kidneys were adapting in order to reduce the serum calcium load. Therefore, a dose of 1000 mg/kg was considered to be a no effect level on renal function.

A slight, statistically significant, increase in group mean serum creatinine concentration was noted at 1000 mg/kg when compared with the control group; however, individual values were within historical ranges and this difference was therefore considered to be of no biological significance. A slight, statistically significant, increase in group mean serum chloride at 100 and 1000 mg/kg when compared with control group. However, this was considered to be of no biological significance, since individual values were within historical ranges. A marked, statistically significant, increase in the total amount of calcium excreted was observed at 1000 mg/kg when compared with the control group.

11 Integrated Summary and Safety Evaluation

Tenofovir Alafenamide Fumarate (TAF), a new chemical entity that belongs to the class of Nucleoside Reverse Transcriptase Inhibitors (NRTI), is being evaluated as a component of the E/C/F/TAF (Elvitegravir 150 mg/Cobicistat 150 mg/Emtricitabine 200 mg/ Tenofovir Alafenamide Fumarate 10 mg) Fixed Dose Combination (FDC) tablet (Genvoya) for the treatment of HIV-1 infection. TAF is a prodrug of tenofovir (TFV) and is more stable in plasma than the TFV prodrug tenofovir disoproxil fumarate (TDF, Viread). TAF is taken up into peripheral mononuclear cells and intracellular metabolized to TFV and phosphorylated to the active metabolite Tenofovir Diphosphate (TFV-DP). TAF leads to higher TFV-DP levels in HIV target cells as well as lower circulating level of TFV which is expected to result in reduced off-target effects of TFV and an improved safety profile as compared to TDF.

TAF demonstrated no significant effect on vital organ systems in rats and dogs in the safety pharmacology studies, except for a reduction in gastric emptying in rats dosed at 1000 mg/kg which was not seen at 100 mg/kg. The IC_{50} for the inhibitory effect of GS-7340-03 on hERG potassium current was greater than 10 μM which was above the human exposure. 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. There were no test-article related changes detected in the renal system or in the CNS in rats administered 1000 mg/kg.

TAF is rapidly absorbed after oral administration and largely taken up by lymphocytes and macrophages; intracellular TAF gets metabolized to TFV and phosphorylated to the active form tenofovir diphosphate (TFV-DP) which is a competitive inhibitor of HIV-1 reverse transcriptase and terminates the elongation of the viral DNA chain. Compared with TDF, another prodrug of TFV, TAF has a longer plasma half-life and can deliver higher concentrations of TFV into target cells with lower plasma exposures of TFV. Consequently, the clinically efficacious dose of TAF is expected to be lower than that of TDF and a lower systemic TFV exposure is expected. Following oral administration in dogs and monkeys, TAF demonstrated rapid absorption, with peak plasma concentrations between 0.25 and 0.5 hours. Thereafter, TAF plasma concentrations declined rapidly with a terminal half-life of less than 1 hour. The TAF exposure (C_{max} and AUC values) was nonlinear with dose and greater than expected with increasing dose. In dogs, TAF demonstrated increased exposure of approximately 3-fold between Day 1 and Week 39, indicating nonlinear pharmacokinetics. The half-life of TAF was determined to be 3 minutes in mice and 31 minutes in rats, a meaningful systemic TAF exposure could not be established in these species. Dogs showed a $T_{1/2}$ of 25 to 30 minutes for systemic TAF exposure. Peak TFV plasma concentrations occurred following TAF absorption, with TFV T_{max} values between 0.25 to 1.7 hours in rats, dogs, and monkeys. Following C_{max}, TFV plasma concentrations declined with a terminal half-life of 11.2 to 16.4 hours in rats (fasted), > 24 hours in dogs (fasted) and 8.1 to 12.5 hours in rhesus monkeys. The in vivo metabolism of TAF was analyzed in mouse, rat, dog and humans. No metabolites unique to human were observed. TFV accounted for a majority of drug related material in plasma, urine, and feces from all species except for human plasma, in which uric acid was the predominant metabolite.

Although NRTIs carry a class labeling for mitochondrial toxicity, FTC, EVG and

TAF have been shown to have a low potential for mitochondrial toxicity.

Tissue distribution was evaluated in mice, rats and dogs after oral administration. TAF was extensively distributed to most tissues. The highest accumulation was found in kidneys, PBMCs, liver, large intestine and bile.

The toxicity profile of TAF was evaluated in repeated dose toxicity studies in mice, rats, dogs and monkeys. In the chronic studies, bones and kidneys were identified as the two main target organs TAF.

Bone:
TAF is a prodrug for TFV which has been shown to lead to bone toxicity in animal models as well as in clinical trials. In distribution studies TAF has been shown to extensively accumulate in the bone. After long term toxicity studies with GS-7340-02 reduction in bone mineral density (BMD) and mineral content (BMC) as well as changes in bone turnover markers in related hormones was observed in rats and dogs. In the dogs, partial recovery was observed after a 3 months. Mechanistic studies suggest that orally administered GS-7340-2 might directly inhibit 1, 25-dihydroxyvitamin D_3 production and result in decreased gastrointestinal absorption of calcium and phosphate as well as decreased renal reabsorption of calcium. In the dog, TAF exposure levels at the NOAEL were lower than the human TAF exposure after Genvoya administration; therefore no safety margins could be established. In rats TAF exposure was not measurable. However, bone toxicities are due to TFV exposure and these findings occurred at TFV exposures 13 (rats) and 4 (dogs) times the human exposure after daily Genvoya administration. Bone toxicity was monitored in the clinical trial, reductions in bone density as well as changes in serum bone markers were seen after administration of Genvoya (E/C/F/TAF). However, these observed changes were less severe in patients receiving Genvoya compared to patients receiving the previously approved Stribild (E/C/F/TDF).

Rat: Oral administration up to 100 mg/kg/day to rats for 26 weeks resulted in significant decreases in peripheral quantitative computed tomography (pQCT)-derived trabecular bone mineral density (BMD) and bone mineral content (BMC) at the proximal tibia metaphysis and distal femur metaphysis, with no effects on diaphyseal bone parameters. Atrophy of metaphyseal cancellous bone was observed in rats administered TAF at 100 mg/kg/day for 26 weeks. These effects were associated with significant increases in biochemical markers of bone turnover (deoxypyridine-DPD, C telopeptide-CTx,) and changes in related hormones (1,25 dihydroxyvitamin D_3 and 25 hydroxyvitamin D_3). No treatment effects were observed at the low dose (5 mg/kg/day) group, in the mid dose group (25 mg/kg/day) only biochemical markers of bone turnover and related parameters were affected, but not the BMD and BMC.

Dogs: In dogs, a significant increase in the bone resorption marker NTx was observed for all animals receiving the high dose (18/12 mg/kg/day) for 9 months compared to controls, a similar though not statistically significant trend was noted in mid dose animals (6 mg/kg/day). A significant reversible decrease in 1,25-dihydroxyvitamin D_3 and in 25-hydroxyvitamin D_3 occurred in male high dose animals. Statistical significant bone density changes were seen in

high dose animals, similar changes were also seen in mid dose animals but were not statistical significant. High dose and mid dose animals showed slight but not statistical significant reductions in bone growth. Taken together these data show that high dose dogs showed a reduced bone maturation compared to control animals. Partial recovery in bone mass and bone geometry was observed after a 3 months recovery phase.

Kidney:
In tissue distribution studies in mice, rats and dogs TAF has been shown to extensively accumulate in the kidney. Like TFV, also TAF has been shown to induce kidney toxicity in the rat and dog. In rats, minimal renal tubular karyomegaly was seen in animals recieving 100 mg/kg/day for 26 weeks. In dogs, renal tubular karyomegaly and/or basophilia with renal cortical tubular degeneration/regeneration were observed in dogs receiving TAF. The severity (minimal to moderate) of the findings depended on the length as well as dose and could be detected as early as 4 weeks after TAF administration. Even in the low dose group of the 39 week dog study karyomegaly and tubular degeneration were detected in 2 males administered but were of minimal severity. After a 13-week recovery period, treatment-related histology changes showed partial recovery. The TAF exposure levels at the NOAEL for renal toxicity in the dog were lower than the human TAF exposure after Genvoya administration. Since TAF has a very short $T_{1/2}$ in rats, no exposure for TAF could be measured. However, kidney toxicities are due to TFV exposure and these findings occurred at TFV exposures 13 (rats) and 4 (dogs) time the human exposure. In clinical trial, monitoring for kidney toxicity was included. A reduction in renal function (reduced CK clearance) was seen after administration of Genvoya (E/C/F/TAF). However, these observed changes were less severe in patients receiving Genvoya compared to patients receiving the previously approved Stribild (E/C/F/TDF)

Eye:

In the nine month dog study, a minimal to slight infiltration of mononuclear cells (macrophages and lymphocytes) in the ocular posterior uvea (ciliary bodies and/or choroid) of both sexes was seen in the high dose group. The posterior uveitis was seen in dogs dosed at 18/12 mg/kg/day at both the 3 months interim sacrifice and at the 9 month sacrifice. The severity of the ocular lesions did not increase/worsen between 3 and 9 months and was reversible after a t months recovery period. In-life fundoscopic and biomicroscopic exams of the eye in dogs did not detect posterior uveitis. At the NOAEL for eye toxicity the systemic TAF/TFV exposure in dogs was 5 (TAF) and 15 (TFV) times the exposure seen in humans at the recommended daily Genvoya dosage. Ocular toxicities have not been described with other TFV produgs (Viread). Ocular findings were not seen with TAF in other animal models. Radioactive labeled TAF distributed poorly to the eye, and TAF was not selectively associated with melanin-containing tissue in studies with Sprague Dawley and Long Evans rats as well as C57 black mice and CD-1 mice. In clinical trials, monitoring for ocular symptoms was included and if necessary followed by an ophthalmologist. No safety signals were reported.

Cardiovascular:

In the long term dog study, a dose-related prolongation of PR interval was observed in the 6- (~ +13%) and 18/12-mg/kg/day (~ +24%) dose. Further, GS-7340 reversibly reduced the heart rate with an associated mild QT interval prolongation in the high-dose group at week 39. These changes were associated with decreases in serum T3 which returned to levels similar to the control group animals after the 13-week recovery period. At the NOAEL the systemic TAF exposure was lower in dogs and rats than in humans, therefore no safety margins were established. The systemic exposure in dogs for TFV was 4 times higher than the exposures seen in humans after daily Genvoya administration. No safety signal was reported in the safety pharmacology study. The effect of TAF on the QT interval was evaluated in clinical trial in which 4 single-dose treatment days separated by 11 days of washout between doses were administered. No clinically meaningful changes were identified.

Less concerning toxicities were described in the following organ systems:

Lung, liver, spleen and adrenal gland:
In the chronic 9 month dog study, an infiltration of macrophages with pigment in the lungs was noted in all high dose (18/12 mg/kg/day) animals and one or two dogs in each of the low (2mg/kg/day) and mid (6 mg/kg/day) dose groups. These pulmonary changes correlated with an increase in lung weights relative to body weight in the high dose animals (18/12 mg/kg/day). The liver and possibly the adrenal glands were additional target organs identified after the longest treatment duration. In the liver, pigment deposits (fine, brownish to pale green) was seen accumulated in perivascular macrophages and/or in the sinusoidal Kupffer cells in a few mid dose and all high dose dogs. This was accompanied by increases in AST, ALT, total bilirubin. In the adrenal glands pigment deposits was observed in the sinusoidal cells of the adrenal cortex and/or medullae in one high dose male and female. In the spleen minimal infiltration of macrophages with pigment was seen in all high dose animals. The content of the intracellular pigment in tissue macrophages in the lung, liver, spleen, and adrenal is unknown, it could represent accumulation of the test article and/or test article metabolite(s) that have been phagocytized by the mononuclear cells. After the 13-week recovery period, a partial recovery was observed.

Nasal mucosa:
Nasal mucosal degeneration and acute inflammatory (infiltrate neutrophil) changes in the nasal mucosa were seen in mice receiving at ≥ 10 mg/kg/day for 13 weeks. M7 was the major identified metabolite in nasal turbinates. These changes have not been described in rats, dogs or monkeys. Although the relevance of these findings to humans is unknown, it is very likely that this finding is species specific and the risk of nasal inflammation in humans was determined to be low.

Genotoxicity and carcinogenicity:
TAF was not genotoxic in the Ames assay, the L5178Y gene mutation assay in mouse lymphoma cells and the mouse bone marrow micronucleus assay. Carcinogenicity studies have not been performed with TAF since TAF is rapidly metabolized to TFV in the rodent model, and

carcinogenicity studies have been carried out with TDF in mice and rats which resulted in TFV exposures up to approximately 163 times (mice, TFV mouse AUC = 16.2 µg*hr/ml) and 55 times (rats, rat AUC = 48.7µg*hr/ml) higher than TFV exposure in humans (human AUCss = 0.293 µg*hr/ml) reached after administration of Genvoya. In female mice, liver adenomas were increased at TFV exposures 163 times those observed in humans. In rats, the study was negative for carcinogenic findings at TFV exposures up to 55 times those observed in humans at the therapeutic dose.

Reproductive toxicology:
In an oral rat fertility study dose related decreases in body weight gain were observed in males and females. However, no drug related changes occurred in male or female fertility endpoints and the NAOEL for reproductive and early toxicity was determined to be 160 mg/kg/day a dose equivalent to 155 times the human dose based on body surface area comparison. The reproductive developmental toxicity was evaluated in pregnant rats and rabbits at doses up to 250 mg/kg/day or 100 mg/kg/day, respectively. At the highest doses maternal toxicity was observed. 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. Therefore the NOAEL for embryo-fetal development was determined to be 100 mg/kg/day in rats, leading to $AUC_{0-\tau}$ values for TAF and TFV of 200 and 17,400 ng·h/kg, respectively. The NOAEL for embryo-fetal development was determined to be 100 mg/kg/day in rabbits with $AUC_{0-\tau}$ values for TAF and TFV of 11,000 and 27,300 ng·h/kg, respectively. The TAF exposures in rats and rabbits were similar to and 53 times higher, respectively, than the exposure in humans at the recommended daily dose. The observed TFV exposure in these studies was 59 and 93 times higher than human tenofovir exposures at the recommended daily doses in rats and rabbits, respectively.

TAF has not been tested in a peri/postnatal study since toxicokinetic data showed that no meaningful TAF but only TFV exposure was seen in rats. TFV, delivered via TDF, has previously been assessed for peri/postnatal effects at exposures 100-times human exposure of TFV via Genvoya. The peri/postnatal study with TDF characterized the potential postnatal toxicity of TFV. The measured TFV exposures in the dams at the NOEL for developmental toxicity (150 mg/kg/day) and F1 toxicity (50 mg/kg/day) were 27 and 14 times higher than the exposure in humans at the recommended daily dose.

The table below summarizes the calculated exposure multiples for the respective toxicity studies comparing the plasma TAF and TVR exposure in animals to the human exposure after Genvoya administration.

Table 30: Estimated safety margins of TAF based on AUCss when comparing animal NOAELs to human exposure *(modified from original table included in NDA 207-561)*

Toxicity	Species	Study/Dose Duration	TAF NOAEL (mg/kg/day)	AUCss (µg·h/mL) NOAEL	Margin Relative to Human AUCss
				TFV/ TAF	TFV[a]/TAF[b]
Overall NOAEL	Rat	26 Weeks	25	3.8/NC	13/NA
	Dog	39 Weeks	2	1.2/0.08	4/0.4
	Monkey	4 Weeks	≥30	≥5.9/1.0	>20/5
Renal Toxicity	Rat	26 Weeks	25	3.8/NC	13/NA
	Dog	39 Weeks	2	1.2/0.08	4/0.4
	Monkey	4 Weeks	≥30	≥5.9/1.0	>20/5
Bone Mineral Loss	Rat	26 Weeks	25	3.8/NC	13/NA
	Dog	39 Weeks	2	1.2/0.08	4/0.4
	Monkey	4 Weeks	≥30	≥5.9/1.0	>20/5
Ocular toxicity	Dog	39 Weeks	6	4.45/1.03	15/5
Nasal Turbinate Toxicity	Mouse	13 Weeks	<10	<0.213/NC	<0.7/NA
Fertility[c]	Rat	Up to 10 weeks	160	NA	NA
Embryo fetal[c] Development	Rat	12 days	100	17.4/0.2	59/1
	Rabbit	14 days	100	27.3/11	93/53
Perinatal/postnatal[c]	Rat	27 days (Gestation day 7 to Lactation day 20)	Developmental NOEL: 150 (TDF)	7.84 (GD7), 11.7(LD20)/NA	27(GD7), 43 (LD20)/NA
			F1 NOEL: 50	3.81(GD7), 5.88(LD20)/NA	14(GD7), 21(LD20)/NA

NA = not applicable; NC = insufficient data to calculate

a) Predicted safety margin for TFV human exposure is based on pooled PK data from E/C/F/TAF Phase 3 pivotal studies GS-US-292-104 and GS-US-292-111 where the mean TFV AUCss = 0.293 µ g.h/mL;

b) Predicted safety margin for TAF human exposure is based on pooled PK data from E/C/F/TAF Phase 3 pivotal studies GS-US-292-104 and GS-US-292-111 where the mean TAF AUCss = 0.206 µ g.h/mL;

c) NOAEL for reproductive endpoints provided; AUC data is for maternal exposure, TFV AUC= 0.273 µ g.h/mL; the peri/postnatal study was conducted with TDF not TAF

12 Appendix/Attachments

Appendix B: Review of impurities by Dr. Mark Powley.

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

PHARMACOLOGY/TOXICOLOGY NDA REVIEW AND EVALUATION

Application number:	207-561 (Appendix B)
Supporting document/s:	

Supporting Document	Sponsor Submission Date	CDER Received Date
1	11/05/14	11/05/14

Product:	Elvitegravir/Cobicistat/Emtricitabine/Tenofovir Alafenamide
	Appendix B: Impurities, Degradants, and Excipients
Indication:	treatment of HIV-1 infection
Applicant:	Gilead Sciences Inc.
Review Division:	Division of Antiviral Products
Reviewer:	Mark W. Powley, Ph.D.
Supervisor/Team Leader:	Hanan Ghantous, Ph.D., DABT
Division Director:	Debra B. Birnkrant, M.D.
Project Manager:	Myong-Joo Patricia Hong, M.S.

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1 Executive Summary

1.1 Introduction

Gilead Science Inc. has submitted an NDA to support the fixed dose combination therapy of elvitegravir (EVG), cobicistat (COBI), emtricitabine (FTC), and tenofovir alafenamide (TAF) for treating HIV-1 infection in adults and pediatric patients ≥ 12 years of age without any known resistance to the individual components. The proposed dosing regimen includes 150 mg/day EVG + 150 mg/day COBI + 200 mg/day FTC + 10 mg TAF.

This review focuses on qualification of impurities, residual solvents, and heavy metals. Regulatory decisions utilize recommendations from ICH M7 "Assessment and Control of DNA Reactive (Mutagenic) Impurities in Pharmaceuticals to Limit Potential Carcinogenic Risk", ICH Q3A(R2) "Impurities in New Drug Substances", ICH Q3B(R2) "Impurities in New Drug Products", ICH Q3C(R5) "Impurities: Guideline for Residual Solvents", and ICH Q3D "Guideline for Elemental Impurities".

Overall, proposed specifications are considered acceptable from a pharmacology/toxicology perspective based on the results of general toxicology studies, experimental Ames assays, and/or (quantitative) structure-activity relationship [(Q)SAR] predictions of mutagenicity. Impurities that are also metabolites are considered qualified per ICH Q3A(R2) and ICH Q3B (R2).

2 Qualification of Elvitegravir Drug Substance

2.1 Organic Impurities

Proposed specifications for organic impurities in the EVG drug substance are within those previously accepted under NDA#203-100. To remain consistent with current guidelines, qualified levels have been recalculated using 50 kg body weight. Summary information is provided below.

Table 31. Elvitegravir drug substance organic impurity specifications

Organic Impurities	Toxicology Study Content	NOAEL	Qualified Levels[a]	Proposed Specification
				(b) (4)

(b) (4)

2.2 Residual Solvents

With the exception of (b) (4) *proposed residual solvent specifications in the EVG drug substance are identical to those previously accepted under NDA#203-100. While the* (b) (4) *specification exceeds the ICH Q3C(R5)* (b) (4) *%, the potential clinical exposure is well below the* (b) (4) *mg/day. Therefore, there is minimal toxicological concern. Summary information is provided below.*

Table 32. Elvitegravir drug substance residual solvent specifications

	ICH Q3C(R5) Limit		Proposed Specification	
Residual Solvents	Concentration Limit (Option 1)	PDE (Option 2)	Concentration	Clinical Exposure[a]
(b) (4)				

2.3 Genotoxic Impurities

A proposed specification is included for (b) (4) *(i.e.,* (b) (4) *pm or* (b) (4) *µg/day). Although the genotoxic potential of* (b) (4) *is not fully understood, this impurity is a rodent carcinogen and considered possibly carcinogenic to humans by the International Agency for Research on Cancer (IARC, 1999). Because there is rodent carcinogenicity data for* (b) (4) *a chemical specific risk assessment can be performed. Using the most conservative TD_{50} of* (b) (4) *mg/kg/day for the formation of hematopoietic system tumors in male rats* (b) (4) *the threshold of toxicological concern (TTC; exposure yielding an excess cancer risk of 1 in 1x10^5) for* (b) (4) *is* (b) (4) *µg/day (see calculation in Appendix). Therefore, the proposed specification of* (b) (4) *µg/day is overly conservative.*

References

(b) (4)

2.4 Heavy Metals

The proposed specification for (b) (4) (i.e., (b) (4) ppm or (b) (4) µg/day) in the EVG drug substance is identical to the specification previously accepted under NDA#203-100. This specification is also less than the PDE of (b) (4) µg/day for oral drugs as described in the recently finalized ICH Q3D guideline.

3 Qualification of Cobicistat Drug Substance

3.1 Organic Impurities

Proposed specifications for organic impurities in the COBI drug substance are within those previously accepted under NDA#203-100. To remain consistent with current guidelines, qualified levels have been recalculated using 50 kg body weight. Summary information is provided below.

Table 13. Cobicistat drug substance organic impurity specifications

Organic Impurities	Toxicology Study Content	NOAEL	Qualified Levels[a]	Proposed Specification
(b) (4)				

The specification for (b) (4) was previously accepted but the rationale was not described. Qualification data is derived from an acute toxicology study in rats ((b) (4) . Severe clinical signs (e.g., lethargy, limpness, hunched posture, etc.) and/or mortality occurred at doses (b) (4) mg/kg. The authors report an NOAEL of (b) (4) mg/kg for this study. (b) (4) was not mutagenic in Salmonella strains TA98, TA100, TA1535,

and TA1537 at doses (b) (4) µg/plate with and without metabolic activation (b) (4)). Overall, the available data do not suggest substantial toxicological concern for the proposed specification.

References

(b) (4)

(b) (4)

3.2 Residual Solvents

The proposed residual solvent specifications for (b) (4) and (b) (4) in the COBI drug substance are identical to those previously accepted under NDA#203-100. The proposed specification for (b) (4) are acceptable per ICH Q3C(R5) (b) (4) limits. Summary information is provided below.

Table 34. Cobicistat drug substance residual solvent specifications

	ICH Q3C(R5) Limit		Proposed Specification	
Residual Solvents	Concentration Limit (Option 1)	PDE (Option 2)	Concentration	Clinical Exposure[a]
(b) (4)				

3.3 Heavy Metals

The proposed specification for total heavy metals (i.e., (b) (4) ppm) in the COBI drug substance is identical to the specification previously accepted under NDA#203-100.

4 Qualification of Emtricitabine Drug Substance

4.1 Organic Impurities

Proposed specifications for organic impurities in the FTC drug substance are identical to those previously accepted under NDA#203-100. The qualification of these impurities was assessed under NDA# 21-752. Summary information is provided below.

Table 35. Emtricitabine drug substance organic impurity specifications

Organic Impurities	Proposed Specification
(b) (4)	

(b) (4)

4.2 Residual Solvents

All proposed residual solvent specifications in the FTC drug substance are identical to those previously accepted under NDA#203-100. Summary information is provided below.

Table 36. Emtricitabine drug substance residual solvent specifications

	ICH Q3C(R5) Limit		Proposed Specification	
Residual Solvents	Concentration Limit (Option 1)	PDE (Option 2)	Concentration	Clinical Exposure[a]
(b) (4)				

4.3 Heavy Metals

The proposed specification for (b) (4) in the FTC drug substance (i.e., (b) (4) ppm or (b) (4) µg/day) is identical to the specification previously accepted under NDA#203-100. This specification is also less than the PDE of (b) (4) µg/day for oral drugs as described in the recently finalized ICH Q3D guideline.

5 Qualification of Tenofovir Alafenamide Drug Substance

5.1 Organic Impurities

5.1.1 Specified Impurities

The qualification of specified impurities in the drug substance is based on results from general toxicology studies, experimental genotoxicity data, and/or assessments of potential mutagenicity using (Q)SAR.

General Toxicology – Specified impurities were present in the drug lots used in studies of (b) (4) weeks in rats (TX-120-2008 and TX-120-2021; reviewed in Appendix). Using the NOAELs established in these general toxicology studies, qualified levels of the impurities summarized below are deemed adequate to support the proposed specifications. (b) (4)

Table 37. Tenofovir alafenamide drug substance organic impurity specifications

Organic Impurity	Toxicology Study Content	NOAEL[a]	Qualified Level[b]	Proposed Specification
(b) (4)				

(b) (4)

Genotoxicity – (b) (4) *are covered by experimental testing with the parent drug (i.e., Ames negative, MLA negative, in vivo MN negative – see detailed review by Dr. Claudia Wrzesinski).*

Experimental data exists for the (b) (4) *was positive in the MLA assay but equivocal in the Ames assay (see pharm/tox review for NDA#21-356).Although results are mixed,* (b) (4) *appears to have some degree of genotoxic potential based on studies most commonly conducted in support of drug development (*(b) (4)

(b) (4) *are predicted to be negative for bacterial mutagenicity by Derek Nexus (v4.1.0), Leadscope Model Applier (v1.8.6-1), and Case Ultra (v1.4.6.6). Summary data is provided in the Appendix.*

Reference

(b) (4)

5.1.2 Unspecified Impurities

The qualification of unspecified impurities is limited to the Sponsor's assessment of mutagenic potential. Because development activities predate publication of ICH M7, Gilead's structural assessment is based solely on consideration of Ashby-Tennant alerts. (b) (4) *are identified as potentially genotoxic impurities. Through the manufacturing process, these potential impurities are controlled to levels below the appropriate TTC (i.e.,*

(b) (4)

. Therefore, these impurities are not included in the drug substance specifications.

5.2 Residual Solvents

With the exception of (b) (4)*, residual solvents are listed in the ICH Q3C(R5) guideline. While all proposed specifications for listed solvents exceed the* (b) (4) *specifications are well below the* (b) (4) *and there is minimal toxicological concern. Summary information is provided below.*

Table 38. Tenofovir alafenamide drug substance residual solvent specifications

	ICH Q3C(R5) Limit		Proposed Specification	
Residual Solvents	Concentration Limit (Option 1)	PDE (Option 2)	Concentration	Clinical Exposure[a]
(b) (4)				

(b) (4) *is not listed in ICH Q3C(R5). The solvent is non-mutagenic based on testing in Salmonella strains TA98, TA100, TA1535, TA1537 at doses* (b) (4) *µg/plate with and without metabolic activation* (b) (4) *The Sponsor cites a PDE of* (b) (4) *mg/day from the* (b) (4) *This reference mentions the PDE is derived from repeat dose toxicity and reproductive toxicity data; however, specific sources of data are not provided. A more appropriate reference is an NTP summary* (b) (4)

Although limited data is available for oral administration, results from a sub-chronic study in rats (3 doses/week x 6 weeks) are summarized (b) (4) *Mortality and convulsions were reported for rats administered* (b) (4) *mg/kg/day while "slight toxicity" occurred in the* (b) (4) *mg/kg/day group. Based on an NOEL of* (b) (4) *mg/kg/day, a PDE of* (b) (4) *mg/day is derived (see calculation in Appendix). This PDE supports the proposed specification.*

References

(b) (4)

5.3 Heavy Metals

All proposed specifications for heavy metals in the TAF drug substance are well below the oral PDE values listed in the recently finalized ICH Q3D guideline. Summary information is provided below.

Table 39. Tenofovir alafenamide drug substance heavy metal specifications

Heavy Metals	ICH Q3D PDE (oral)	Proposed Specification	
		Concentration	Clinical Exposure[a]
(b) (4)			

5 Qualification of the Elvitegravir/Cobicistat/Emtricitabine/ Tenofovir Alafenamide Drug Product

5.1 Degradants

Proposed specifications for degradants in the EVG, COBI, and FTC drug product are within those previously accepted under NDA#203-100. To remain consistent with current guidelines, qualified levels for COBI (Section 3.1) and FTC (below) have been recalculated using 50 kg body weight. TAF specifications are supported by data described in Section 5.1 above. Summary information is provided below.

Table 40. Drug product impurity proposed specifications (EVG, COBI, TAF)

Degradant	Qualified Levels	Proposed Specification	
		Release	Shelf Life
(b) (4)			

(b) (4)

Table 41. Drug product impurity proposed specifications (FTC)

Degradant	Toxicology Study Content	NOAEL	Qualified Levels[a]	Proposed Specification Release	Shelf Life
(b) (4)					

Appendix

General Toxicology Study Reviews

Title: 2-Week Oral Gavage Qualification Toxicity and Toxicokinetic Study of GS-7340 in Male Rats (Study no. TX-120-2008)

Summary – *Mortality, clinical signs/physical examinations, body weights, food consumption, hematology, coagulation, clinical chemistry, biomarkers of bone effects (1,25 dihydroxy vitamin D and parathyroid hormone), urinalysis, gross pathology, organ weights, histopathology, and toxicokinetics were evaluated in male Sprague-Dawley rats after oral administration of 4 and 40 mg free base equivalents/kg/day from 2 separate lots of TAF (i.e., purity = 97.7% and 83.1%) for 2 weeks. The Sponsor reported maximum impurity content included* (b) (4) *The vehicle/control article was 0.1% (v/v) Tween 20 and 0.1% (w/v) hydroxypropylmethylcellulose. There were no drug-related effects observed. Toxicokinetic analysis verified exposure to the active metabolite (R-PMPA; tenofovir).*

Based on the absence of adverse effects at any dose level, the NOAEL was (b) (4) *mg free base equivalents /kg/day of TAF.*

Title: 4-Week Oral Gavage Toxicity and Toxicokinetic Bridging Study with GS-7340-03 in Sprague-Dawley Rats (Study no. TX-120-2021)

Summary – *Mortality, clinical signs/physical examinations, body weights, food consumption, ophthalmic examinations, hematology, coagulation, clinical chemistry, biomarker of bone effects (parathyroid hormone), urinalysis, gross pathology, organ weights, histopathology, and toxicokinetics were evaluated in Sprague-Dawley rats after oral administration of 25 and 50 mg free base equivalents/kg/day from 3 separate lots of TAF (i.e., purity = 99.3%, 98.0%, and 97.8%) for 4 weeks. The Sponsor reported maximum impurity content included* (b) (4) *The vehicle/control article was 0.1% (v/v) Tween 20 and 0.1% (w/v) hydroxypropylmethylcellulose. Drug-related effects were limited to a non-adverse increase in body-weight gain at the high-dose and a correlating increase in food consumption. Toxicokinetic analysis verified exposure to the active metabolite (R-PMPA; tenofovir).*

Based on the absence of adverse effects at any dose level, the NOAEL was (b) (4) *mg free base equivalents/kg/day of TAF.*

Calculations

(Q)SAR Report

To: Mark Powley
cc: Hanan Ghantous
From: CDER/OTS/OCP/DARS: The Chemical Informatics Group
Re: NDA 207561
Date: February 26, 2015

Five impurities of tenofovir alafenamide fumarate have been evaluated by CDER/OTS/OCP/DARS for bacterial mutagenicity using (quantitative) structure-activity relationship [(Q)SAR] models. Three software programs were used: *Derek Nexus* 4.1.0 (*DX*), *Leadscope Model Applier* 1.8.6-1 (*LMA*), and *CASE Ultra* 1.4.6.6 (*CU*). To maximize sensitivity and negative predictivity, a positive prediction from any one software program was used to justify a positive study call.

The (Q)SAR assessment of mutagenic potential is consistent with recommendations described in the ICH M7 guideline (i.e., prediction of bacterial mutagenicity using multiple complementary methodologies). All (Q)SAR model outputs were reviewed with the use of expert knowledge in order to provide additional supportive evidence on the relevance of any positive, negative, conflicting or inconclusive prediction and provide a rationale to support the final conclusion.

Overall, all 5 impurities are predicted to be non-mutagenic.

Chemical 1: (b) (4)

Bacterial Mutagenicity[1]

(b) (4)	Software	*Salmonella* Mutagenicity	*E. coli/ TA102* Mutagenicity
	Derek Nexus	-*	-*
	Leadscope Model Applier	-	-
	CASE Ultra	-	NC
	Overall Software Prediction	-	-
	Overall Expert Prediction	-	-

*Structure contains unclassified features

(b) (4) is predicted to be negative for bacterial mutagenicity (i.e., both *Salmonella* and *E.coli/TA102* mutagenicity). The *DX* prediction is negative but indicates that the chemical contains an unclassified structural feature (highlighted above in red) that is not present in the *Lhasa* Ames test reference set. A portion of this same feature is also an unknown fragment to the *CU* bacterial mutagenicity models. Note that experimental data for (b) (4) support a lack of reactivity for the unclassified feature. These structural analogs are included in the Appendix.

Chemical 2: (b) (4)

Bacterial Mutagenicity[1]

(b) (4)	Software	*Salmonella* Mutagenicity	*E. coli/ TA102* Mutagenicity
	Derek Nexus	-*	-*
	Leadscope Model Applier	-	-
	CASE Ultra	NC	NC
	Overall Software Prediction	-	-
	Overall Expert Prediction	-	-

*Structure contains unclassified features

(b) (4) is predicted to be negative for bacterial mutagenicity (i.e., both *Salmonella* and *E.coli/TA102* mutagenicity). The *DX* prediction is negative but indicates that the chemical contains an unclassified structural feature (highlighted above in red) that is not present in the *Lhasa* Ames test reference set. A portion of this same feature is also an unknown fragment to the *CU* bacterial mutagenicity models leading to the no call prediction.

Chemical 3: (b) (4)

Bacterial Mutagenicity[1]

(b) (4)	Software	*Salmonella* Mutagenicity	*E. coli/ TA102* Mutagenicity
	Derek Nexus	-*	-*
	Leadscope Model Applier	-	-
	CASE Ultra	-	-
	Overall Software Prediction	-	-
	Overall Expert Prediction	-	-

*Structure contains unclassified features

(b) (4) is predicted to be negative for bacterial mutagenicity (i.e., both *Salmonella* and *E.coli/TA102* mutagenicity). The *DX* prediction is negative but indicates that the chemical contains an unclassified structural feature (highlighted above in red) that is not present in the *Lhasa* Ames test reference set. A portion of this same feature is also an unknown fragment to the *CU E.coli*/TA102 mutagenicity model.

Chemical 4: (b) (4)

Bacterial Mutagenicity[1]

(b) (4) Software	*Salmonella* Mutagenicity	*E. coli/ TA102* Mutagenicity
Derek Nexus	-	-
Leadscope Model Applier	-	-
CASE Ultra	-	-
Overall Software Prediction	-	-
Overall Expert Prediction	-	-

(b) (4) is predicted to be negative for bacterial mutagenicity (i.e., both *Salmonella* and *E.coli/TA102* mutagenicity).

Chemical 5: (b) (4)

Bacterial Mutagenicity[1]

(b) (4) Software	*Salmonella* Mutagenicity	*E. coli/ TA102* Mutagenicity
Derek Nexus	-	-
Leadscope Model Applier	-	-
CASE Ultra	-	-
Overall Software Prediction	-	-
Overall Expert Prediction	-	-

(b) (4) is predicted to be negative for bacterial mutagenicity (i.e., both *Salmonella* and *E.coli/TA102* mutagenicity).

Appendix

(b) (4)

This report has been reviewed and approved by CDER/OTS/OCP/DARS.

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

CLAUDIA WRZESINSKI
07/09/2015

HANAN N GHANTOUS
07/10/2015

I concur with the conclusion of Dr. Claudia Wrzesinski, there are no nonclinical Pharmacology and/or Toxicology issues which would preclude the approval of Genvoya.

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA

NDA/BLA Number: 207,561 **Applicant: Gilead** **Stamp Date:** November 5, 2014

Drug Name: Genvoya **NDA Type: Original**

On **initial** overview of the NDA/BLA application for filing:

	Content Parameter	Yes	No	Comment
1	Is the pharmacology/toxicology section organized in accord with current regulations and guidelines for format and content in a manner to allow substantive review to begin?	x		
2	Is the pharmacology/toxicology section indexed and paginated in a manner allowing substantive review to begin?	x		
3	Is the pharmacology/toxicology section legible so that substantive review can begin?	x		
4	Are all required (*) and requested IND studies (in accord with 505 b1 and b2 including referenced literature) completed and submitted (carcinogenicity, mutagenicity, teratogenicity, effects on fertility, juvenile studies, acute and repeat dose adult animal studies, animal ADME studies, safety pharmacology, etc)?	x		Per agreement with the FDA carcinogenicity, perinatal and postnatal toxicology studies were 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.
5	If the formulation to be marketed is different from the formulation used in the toxicology studies, have studies by the appropriate route been conducted with appropriate formulations? (For other than the oral route, some studies may be by routes different from the clinical route intentionally and by desire of the FDA).	x		
6	Does the route of administration used in the animal studies appear to be the same as the intended human exposure route? If not, has the applicant submitted a rationale to justify the alternative route?	x		
7	Has the applicant submitted a statement(s) that all of the pivotal pharm/tox studies have been performed in accordance with the GLP regulations (21 CFR 58) or an explanation for any significant deviations?	x		
8	Has the applicant submitted all special studies/data requested by the Division during pre-submission discussions?	x		

File name: 5_Pharmacology_Toxicology Filing Checklist for NDA_BLA or Supplement 010908

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA

	Content Parameter	Yes	No	Comment
9	Are the proposed labeling sections relative to pharmacology/toxicology appropriate (including human dose multiples expressed in either mg/m2 or comparative serum/plasma levels) and in accordance with 201.57?	x		Appropriateness of the content will be determined upon review and discussion at the labeling meeting.
10	Have any impurity – etc. issues been addressed? (New toxicity studies may not be needed.)	x		Evaluation of the sponsors analyses is a review issue.
11	Has the applicant addressed any abuse potential issues in the submission?	x		Not applicable
12	If this NDA/BLA is to support a Rx to OTC switch, have all relevant studies been submitted?	x		Not applicable

IS THE PHARMACOLOGY/TOXICOLOGY SECTION OF THE APPLICATION FILEABLE? Yes.

If the NDA/BLA is not fileable from the pharmacology/toxicology perspective, state the reasons and provide comments to be sent to the Applicant.

Please identify and list any potential review issues to be forwarded to the Applicant for the 74-day letter.

The following P/T comment was sent to the sponsor in the filing letter:

> Please provide your final study report for study TX-120-2021 "4-Week Oral Gavage Toxicity and Toxicokinetic Bridging Study with GS-7340-03 in Sprague Dawley Rats" submitted under impurity studies as soon as possible.

Claudia Wrzesinski 01/09/2014

Reviewing Pharmacologist Date

Team Leader/Supervisor Date

File name: 5_Pharmacology_Toxicology Filing Checklist for NDA_BLA or Supplement 010908

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

CLAUDIA WRZESINSKI
01/09/2015

HANAN N GHANTOUS
01/09/2015