EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

24 September 2015
EMA/688326/2015
Committee for Medicinal Products for Human Use (CHMP)

Assessment report

Genvoya

International non-proprietary name: elvitegravir / cobicistat / emtricitabine / tenofovir alafenamide

Procedure No. EMEA/H/C/004042/0000

Note

Assessment report as adopted by the CHMP with all information of a commercially confidential nature deleted.

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List of abbreviations

3TC	lamivudine
ABC	abacavir
ADME	absorption, distribution, metabolism, and elimination
aGFR	actual glomerular filtration rate
AN(C)OVA	analysis of (co) variance
ATR	efavirenz/emtricitabine/tenofovir disoproxil fumarate (coformulated; Atripla)
ATV/co	cobicistat-boosted atazanavir
ATV/r	ritonavir-boosted atazanavir
BCS	Biopharmaceutics Classification System
BMD	bone mineral density
BMI	body mass index
COBI, C	cobicistat (Tybost)
C telopeptide	type I collagen C telopeptide
CQA	Critical Quality Attribute
ddI	didanosine
dNTP	2' deoxynucleoside triphosphate
DoE	Design of experiments
DRV, D	darunavir
DSC	Differential Scanning Calorimetry
DTG	dolutegravir
DXA	dual-energy x ray absorptiometry
E/C/F/TAF	elvitegravir/cobicistat/emtricitabine/tenofovir alafenamide (coformulated)
EFV	efavirenz
eGFR	estimated glomerular filtration rate
eGFRCG	estimated glomerular filtration rate calculated using the Cockcroft Gault equation
ESRD	end-stage renal disease
EU	European Union
EVG, E	elvitegravir (Vitekta)
FAS	Full Analysis Set
FTC, F	emtricitabine (Emtriva)
FTC-DP	emtricitabine diphosphate

GC	Gas Chromatography
HDL	high density lipoprotein
HDPE	High Density Polyethylene
HPLC	High performance liquid chromatography
ICH	International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
ICP-MS	Inductively coupled plasma mass spectrometry
INSTI	integrase strand transfer inhibitor
IR	Infrared
IUPAC	International Union of Pure and Applied Chemistry
KF	Karl Fischer titration
LDL	low-density lipoprotein
LOCF	last observation carried forward
LSM	least-squares mean
M = F	missing = failure
MA	Marketing Authorisation
mg	milligram
MS	Mass Spectrometry
mtDNA	mitochondrial DNA
N or n	number of subjects in a population (N) or subset (n)
N/A	Not applicable
NAS	New Active Substance
NCEP	National Cholesterol Education Program
NMR	Nuclear Magnetic Resonance
NNRTI	nonnucleoside reverse transcriptase inhibitor
NRTI	nucleoside reverse transcriptase inhibitor
NtRTI	nucleotide reverse transcriptase inhibitor
OATP	organic anion transporting polypeptide
P1NP	procollagen type 1 N-terminal propeptide
PAR	Proven Acceptable Range
PBMC	peripheral blood mononuclear cell
PD	pharmacodynamic(s)
P-gp	P-glycoprotein
Ph. Eur.	European Pharmacopoeia

PI	protease inhibitor
PRT	proximal renal tubulopathy
PIP	Paediatric Investigational Plan
PTH	parathyroid hormone
Q1, Q3	first quartile, third quartile
QbD	Quality by design
RBP	retinol binding protein
RH	Relative humidity
rNTP	ribonucleoside triphosphate
RPV	rilpivirine
RT	reverse transcriptase
RTV	ritonavir
SI	selectivity index (ratio of CC50 to IC50)
SmPC	Summary of Product Characteristics
STB	elvitegravir/cobicistat/emtricitabine/ tenofovir disoproxil fumarate (coformulated; Stribild)
TAF	tenofovir alafenamide
TAF fumarate	tenofovir alafenamide fumarate
TAM	thymidine analogue mutation
TDF	tenofovir disoproxil fumarate (Viread)
TFV	tenofovir
TFV DP	tenofovir diphosphate
TSE	Transmissible Spongiform Encephalopathy
TVD	emtricitabine/tenofovir disoproxil fumarate (coformulated; Truvada)
UACR	urine albumin to creatinine ratio
UGT	uridine diphosphate glucuronosyltransferase
UPCR	urine protein to creatinine ratio
UPLC	Ultra-high performance liquid chromatography
USP/NF	United States Pharmacopoeia/National Formulary
UV	Ultraviolet
XRPD	X-Ray (Powder) Diffraction

1. Background information on the procedure

1.1. Submission of the dossier

The applicant Gilead Sciences International Ltd submitted on 28 November 2014 an application for Marketing Authorisation to the European Medicines Agency (EMA) for Genvoya, through the centralised procedure falling within the Article 3(1) and point 3 of Annex of Regulation (EC) No 726/2004. The eligibility to the centralised procedure was agreed upon by the EMA/CHMP on 26 June 2014.

The applicant applied for the following indication: treatment of human immunodeficiency virus type 1 (HIV 1) infection in adults and adolescents 12 years of age and older, without any known mutations associated with resistance to the individual components of Genvoya (see sections 4.2 and 5.1).

The legal basis for this application refers to:

Article 8.3 of Directive 2001/83/EC - complete and independent application. The applicant indicated that tenofovir alafenamide was considered to be a new active substance.

The application submitted is composed of administrative information, complete quality data, non-clinical and clinical data based on applicants' own tests and studies and/or bibliographic literature substituting/supporting certain tests or studies.

Information on Paediatric requirements

Pursuant to Article 7 off Regulation (EC) No 1901/2006, the application included an EMA Decision P/0026/2014 on the agreement of a paediatric investigation plan (PIP).

At the time of submission of the application, the PIP P/0026/2014 was not yet completed as some measures were deferred.

Information relating to orphan market exclusivity

Similarity

Pursuant to Article 8 of Regulation (EC) No. 141/2000 and Article 3 of Commission Regulation (EC) No 847/2000, the applicant did not submit a critical report addressing the possible similarity with authorised orphan medicinal products because there is no authorised orphan medicinal product for a condition related to the proposed indication.

New Active Substance Status

The applicant requested the active substance tenofovir alafenamide contained in the above medicinal product to be considered as a new active substance in comparison to the known derivative tenofovir disoproxil previously authorised in the Union as active substance in Atripla, Eviplera, Stribild, Truvada, and Viread, and claimed that tenofovir alafenamide differs significantly in properties with regard to safety and efficacy from the already authorised substance.

Scientific Advice

The applicant received Scientific Advice from the CHMP on 18-10-2012 and 25-04-2013. The Scientific Advice pertained to non-clinical aspects of the dossier.

Licensing status

The product was not licensed in any country at the time of submission of the application.

1.2. Steps taken for the assessment of the product

The Rapporteur and Co-Rapporteur appointed by the CHMP were:

Rapporteur: Robert James Hemmings Co-Rapporteur: Pierre Demolis

- The application was received by the EMA on 28 November 2014.
- The procedure started on 24 December 2014.
- The Rapporteur's first Assessment Report was circulated to all CHMP members on 6 March 2015 . The Co-Rapporteur's first Assessment Report was circulated to all CHMP members on 17 March 2015 .
- During the PRAC meeting on 10 April 2015, the PRAC adopted an RMP Advice and assessment overview .
- During the meeting on 23 April 2015 the CHMP agreed on the consolidated List of Questions to be sent to the applicant .
- The applicant submitted the responses to the CHMP consolidated List of Questions on 22 May 2015
- The Rapporteurs circulated the Joint Assessment Report on the applicant's responses to the List of Questions to all CHMP members on 25 June 2015 .
- During the PRAC meeting on 9 July 2015, the PRAC adopted an RMP Advice and assessment overview .
- During the CHMP meeting on 23 July 2015, the CHMP agreed on a list of outstanding issues to be addressed in writing by the applicant .
- The applicant submitted the responses to the CHMP List of Outstanding Issues on 14 August 2015.
- The Rapporteurs circulated the Joint Assessment Report on the applicant's responses to the List of Questions to all CHMP members on 1 September 2015 .
- During the PRAC meeting on 10 September 2015, the PRAC adopted an RMP Advice and assessment overview .
- During the meeting on 24 September 2015, the CHMP, in the light of the overall data submitted and the scientific discussion within the Committee, issued a positive opinion for granting a Marketing Authorisation to Genvoya.

2. Scientific discussion

2.1. Introduction

HIV-1 infection is a life threatening and serious disease of major public health significance, with approximately 35 million people infected worldwide.

Standard of care for the treatment of HIV-1 infection uses combination antiretroviral therapy (ART) to suppress viral replication to below detectable limits, increase CD4 cell counts, and stop disease progression. For ART-naive HIV infected patients, current treatment guidelines suggest that initial therapy consist of two nucleos(t)ide reverse transcriptase inhibitors (N[t]RTIs) and either a non-nucleoside reverse transcriptase inhibitor (NNRTI), a boosted protease inhibitor (PI) or an integrase strand-transfer inhibitor (INSTI). Virologically suppressed, HIV infected patients may switch from their current regimen because of safety or tolerability concerns or for regimen simplification. All patient populations may benefit from once daily fixed dose combination (FDC) regimens as these have been shown to provide increased adherence and improved clinical and virologic outcomes.

The success of ART means that morbidity and mortality in the HIV infected population is increasingly driven by non AIDS–associated comorbidities. Clinical attention has become more focused on the optimization of tolerability, long term safety, and adherence to potent ART regimens. There remains a significant medical need for new, effective therapies that take into consideration the non-HIV comorbidities, demographics of the aging HIV infected population, antiretroviral (ARV) resistance and regimen simplification.

Chronic kidney disease is important, since observational studies have demonstrated a relationship between kidney disease and progression to AIDS and death. Moreover, HIV associated nephropathy present in up to 30% of patients and this is a common cause of end-stage renal disease (ESRD) requiring dialysis.

ART with proven efficacy and safety in the both elderly and young patients is important; limited data and treatment options are available in both populations. The elderly have increased risks for comorbidities, including those related to renal and bone. There are specific and complex challenges for the treatment of adolescents, who also represent the population that will require ART for the longest time.

Tenofovir (TFV) is a nucleotide analogue with limited oral bioavailability that inhibits HIV-1 reverse transcription. Tenofovir disoproxil fumarate (TDF), an oral prodrug of TFV, has improved bioavailability, and delivers high systemic exposures of TFV with favourable efficacy and safety data. TDF is a preferred NtRTI for use in combination with other antiretroviral agents for the treatment of HIV-1 infection.

While TDF is used broadly in the treatment of HIV-1 infection, an important identified risk with its use is nephrotoxicity, which is associated with increased creatinine in some patients, increased protein loss (particularly tubular), and occasional cases of proximal renal tubulopathy (PRT) (including Fanconi syndrome). These risks necessitate increased renal monitoring with use of TDF-containing products, placing burden on the patient and healthcare provider. Reductions in bone mineral density (BMD) have also been seen after the initiation of ART, with larger decreases in BMD observed with TDF than with other NRTIs.

Tenofovir alafenamide (TAF) is an investigational oral prodrug of TFV. TAF is more stable in plasma than TDF, provides higher intracellular levels of the active phosphorylated metabolite tenofovir diphosphate (TFV-DP), and approximately 90% lower circulating levels of TFV relative to TDF. The distinct metabolism of TAF offers the potential for an improved safety profile compared with TDF.

2.2. Quality aspects

2.2.1. Introduction

Genvoya is a medicinal product containing tenofovir alafenamide (as fumarate), a new active substance previously not authorised within the Union and three known active substances: elvitegravir, cobicistat, and emtricitabine.

The finished product is presented as film-coated tablets containing the following active substances: 150 mg of elvitegravir, 150 mg of cobicistat, 200 mg of emtricitabine, and 10 mg of tenofovir alafenamide (as 11.2 mg of the fumarate).

Other ingredients of the tablet core are: lactose (as monohydrate), microcrystalline cellulose, croscarmellose sodium, hydroxypropyl cellulose, silicon dioxide, sodium lauryl sulphate, and magnesium stearate. Ingredients of the film-coating are: polyvinyl alcohol (E1203), titanium dioxide (E171), polyethylene glycol (E1521), talc (E553b), indigo carmine aluminium lake (E132), and iron oxide yellow (E172).

The product is packaged in high density polyethylene (HDPE) bottle with a polypropylene continuous-thread, child-resistant cap, lined with an induction activated aluminium foil liner containing 30 film-coated tablets. Each bottle contains silica gel desiccant and polyester coil.

2.2.2. Active Substance

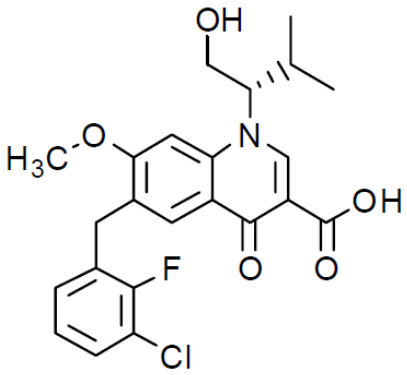
Elvitegravir

The information on chemistry, manufacturing and control of elvitegravir active substance has been previously assessed through centralised procedure and approved in the EU as part of the marketing authorisation applications for Vitekta (EU/1/13/883/001-2) and Stribild (EU/1/13/830/001-2).

The Module 3.2.S sections of the dossier for elvitegravir provided by the Applicant are identical to the 3.2.S sections submitted and approved with the aforementioned marketing authorisations.

General information

The chemical name of elvitegravir is 6-(3-chloro-2-fluorobenzyl)-1-[(2S)-1-hydroxy-3-methylbutan-2-yl]-7-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid and has the following structure:



Elvitegravir appears as a white to pale yellow crystalline non-hygroscopic powder, sparingly soluble in methanol and ethanol and practically insoluble in water and aqueous solutions at pH 2.0 to 8.3. Its pKa is 6.6 and the distribution coefficient Log D is 4.5 (at pH 6.8).

Elvitegravir exhibits polymorphism and appears in three polymorphs. The most thermodynamically stable polymorphic form has been determined and the crystallisation process is designed to consistently deliver this form. It contains a single asymmetric centre at C-11. The absolute configuration was established by single crystal X-ray crystallography and has been determined to be of "S" configuration. Enantiomeric purity is controlled routinely by chiral HPLC.

Manufacture, characterisation and process controls

Elvitegravir is manufactured in six well-defined synthetic steps using commercially available starting materials. The active substance is obtained from three manufacturers. The route of synthesis has been described in sufficient detail and adequate in-process controls are applied during the synthesis. The specifications and control methods for intermediate products, starting materials and reagents have been presented. Information about the formation, presence, origin and fate of impurities during manufacture has been satisfactorily discussed.

Representative batch analysis data provided for all three proposed manufacturing sites produced with the proposed synthetic route show that the active substance can be manufactured reproducibly.

Specification

The elvitegravir active substance specification includes tests and limits for appearance (visual inspection), identification (IR, HPLC, UV), water content (Ph. Eur.), assay (HPLC), enantiomeric purity (chiral HPLC), impurities (HPLC), residual solvents (GC), residue on ignition (Ph. Eur.), heavy metals (Ph. Eur.), polymorphic form (DSC-Ph. Eur.), and particle size (laser light scattering).

Impurities, including genotoxic impurities, have been evaluated and qualified where necessary. The proposed limits are found to be acceptable from a safety point of view and therefore they are considered justified.

A microbial limit test for the active substance is not required in accordance with ICH Q6A because the latter steps of the active substance manufacturing process conducted in aqueous organic solvent mixtures and are expected to limit microbial content. In addition, confirmatory testing demonstrated that elvitegravir is moderately to completely inhibitory to microbial growth. Furthermore, data presented in the dossier indicate that no significant bioburden is present.

The analytical methods have been well described and validated according to ICH Q2 (R1) and are suitable to control the quality of the active substance.

Batch analysis data on commercial scale batches of the active substance manufactured by all three proposed manufacturers have been provided. The results comply with the specifications and confirm consistency and uniformity of the manufacturing process regardless of the manufacturing site.

Stability

Stability studies have been conducted for four commercial scale batches from the first manufacturer and one batch from the second under ICH long term (25 °C±2 °C/60±5% RH) and accelerated conditions (40 °C±2 °C / 75%±5% RH) in the proposed packaging. Results were submitted for up 36 months at long term conditions and for up to 9 months at accelerated.

Long term and accelerated stability samples were tested for appearance, assay, impurity content, and water content. The enantiomeric purity and polymorphic form were tested annually during the long term studies. Enantiomeric purity was determined for 1 batch, at the beginning and end of the accelerated study and polymorphic form was tested at the end of the accelerated study. The analytical methods used are stability indicating.

All parameters remained within the specification limits under both conditions over the duration of study for all four batches. The data show no discernible trends for assay, total impurity content, individual specified impurities, degradation products or any other tested parameter.

In addition, a photostability study of elvitegravir has been assessed as per the ICH Q1B Guideline on one batch from the second manufacturer. No significant difference was observed between the control sample and exposed sample in appearance, assay, impurity content, polymorphic form and enantiomeric purity. The data indicate that elvitegravir is not sensitive to light.

Based on the presented stability data, the proposed re-test period and storage when the active substance is packed in the proposed packaging materials is acceptable.

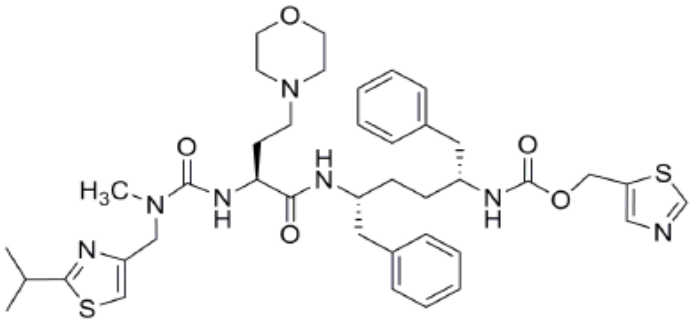
Cobicistat

The information on chemistry, manufacturing and control of cobicistat active substance has been previously assessed through centralised procedure and approved in the EU as part of the marketing authorisation applications for Tybost (EU/1/13/872/001-2) and Stribild (EU/1/13/830/001 2).

The Module 3.2.S sections of the dossier for cobicistat provided by the Applicant are identical to the 3.2.S sections submitted and approved with the aforementioned marketing authorisations.

General information

The chemical name of cobicistat is 1,3-thiazol-5-ylmethyl [(2R,5R)-5-{[(2S)-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 and has the following structure:



Cobicistat is an amorphous solid with a low glass transition temperature of 35 ºC. Because of the low glass transition temperature, cobicistat under ambient conditions undergoes moisture and temperature induced phase transition from a foam into a rubber-like material. To increase physical stability of cobicistat it is adsorbed on silicon dioxide. Cobicistat on silicon dioxide is a white to pale yellow amorphous powder and as cobicistat is also hygroscopic as determined by dynamic vapour sorption at

room temperature. The relatively higher water uptake of cobicistat on silicon dioxide compared to cobicistat is due to the hygroscopic nature of the silicon dioxide carrier. Importantly however and contrary to cobicistat itself, moisture uptake of cobicistat on silicon dioxide is reversible and therefore cobicistat is isolated by adsorption on silicon dioxide to provide a stable solid form, which is suitable for further finished product manufacture.

It shows three pKa; 1.8 (thiazole group), 2.5 (alkylthiazole group) and 6.4 (morpholino group). The partition coefficient Log P is 4.3 (at pH 8.5 buffer). No crystal forms have been found. It has three chiral centres and is produced as a single isomer. The stereochemical configuration is defined through the synthetic process and the use of starting material with suitable chirality. Appropriate specifications for these starting materials ensure consistent quality during manufacture of cobicistat.

Manufacture, characterisation and process controls

Three sites are proposed for the manufacture of the active substance. Cobicistat on silicon dioxide is manufactured in four well-defined synthetic steps. Details about possible reprocessing have been provided. The route of synthesis has been described in sufficient detail and adequate in-process controls are applied during the synthesis. The specifications and control methods for intermediate products, starting materials and reagents have been presented. Because the actual loading value of cobicistat on silicon dioxide is subject to manufacturing variability, a target loading with an appropriate range was adopted to ensure a robust down-stream manufacturing process.

All three sites have manufactured commercial scale batches of cobicistat on silicon dioxide and many smaller scale batches during the development of the product. Batch analysis data show that the active substance produced by both manufacturers is of similar quality and can be manufactured reproducibly.

Specification

Cobicistat on silicon dioxide specification includes tests and limits for appearance (visual), identification (cobicistat: IR, HPLC, UV; silicon dioxide: USP/NF), water content (Ph. Eur.), assay (HPLC), enantiomeric purity (chiral HPLC), impurities (HPLC), residual solvents (GC) and heavy metals (Ph. Eur.).

No crystal forms have been identified and since the active substance is produced as an amorphous solid adsorbed onto silicon dioxide, a test for polymorphism is not required as per ICH Q6A.

Cobicistat genotoxic potential has been evaluated in accordance with the recommendations in ICH Q3A. All the identified impurities are of low concern for genotoxicity, and therefore no further qualification studies were considered necessary. The proposed test and limits are acceptable.

A microbial limit test for the active substance is not required in accordance with ICH Q6A because the latter steps of the active substance manufacturing process are non-aqueous and been shown to limit microbial content. In addition data presented on several batches during development indicate no significant bioburden is present.

All in-house analytical methods have been validated according to ICH Q2A principles.

Batch analysis data on commercial scale batches of the active substance manufactured by all three proposed manufacturers have been provided. The results comply with the specifications and confirm consistency and uniformity of the manufacturing process regardless of the manufacturing site.

Stability

Two pilot scale and one production scale batch from the first manufacturer and on one full scale batch from the second manufacturer packaged in the proposed container were put on stability testing in accordance with the ICH Q1A (R2) Guideline under long-term conditions at 5 °C for up to 36 months and accelerated at 25 °C/60% RH for up to 36 months. Appearance, water content, assay, impurities and chiral purity have been monitored. The analytical methods used are stability indicating. All physicochemical attributes of cobicistat on silicon dioxide remained within the specification acceptance limits following 36 months of long-term storage at 5 °C and no apparent trend has been observed. A statistical analysis performed for assay, total impurities and the major chiral impurity also demonstrate that there is little change over time. The physicochemical attributes of cobicistat on silicon dioxide remained also within the specification acceptance limits following 36 months of storage at 25 °C/60% RH.

Furthermore, two of the above batches were also tested under 30 °C/75% RH for up to 12 months to evaluate the stability of cobicistat on silicon dioxide at elevated temperatures that may be encountered during shipping and handling. The duration of temperature and humidity excursions is limited to 3 months.

In addition, a photostability study was conducted on cobicistat on silicon dioxide according to ICH Q1B Guideline. The results showed no significant change in appearance, assay, and impurity content following exposure to light. The data indicate that cobicistat on silicon dioxide is not sensitive to light.

Based on the presented stability data, the proposed re-test period and storage when the active substance is packed in the proposed packaging materials is acceptable.

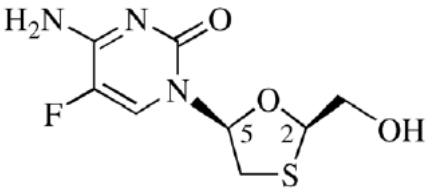
Emtricitabine

The information on chemistry, manufacturing and control of emtricitabine active substance has been previously assessed through centralised procedure and approved in the EU as part of the marketing authorisation applications for Emtriva (EU/1/03/261/001-3), Truvada (EU/1/04/305/001-2), Atripla (EU/1/07/430/001-2), Eviplera (EU/1/11/737/001-2) and Stribild (EU/1/13/830/001-2).

The Module 3.2.S sections of the dossier for emtricitabine provided by the Applicant are identical to the 3.2.S sections submitted and approved with the aforementioned marketing authorisations.

General information

The chemical name of emtricitabine is 4-amino-5-fluoro-1-[(2R,5S)-2-(hydroxymethyl)-1,3-oxathiolan-5-yl]-1,2-dihydropyrimidin-2-one and has the following structure:



Emtricitabine appears as a white to off-white non-hygroscopic crystalline powder, freely soluble in methanol and water. Its pKa is 2.65 and the partition coefficient Log P is -0.43. It has 2 chiral centres at carbons 2 and 5 of the oxathiolane ring. Two enantiomeric pairs of diastereomers can exist; cis-(-)-FTC and cis-(+)-FTC, trans-(-)-FTC and trans-(+)-FTC. The synthetic route has been chosen to be stereoselective for the formation of the desired cis-(-) enantiomer, emtricitabine. Three polymorphs of

emtricitabine have been observed. However, the most stable thermodynamically form at room temperature, is consistently produced.

Manufacture, characterisation and process controls

Emtricitabine is manufactured by two possible synthetic routes sharing a common first step and followed by two options comprising either one or two extra steps. The synthesis was described in sufficient detail. The synthetic process results in the stereoselective formation of an intermediate and thus the formation of the desired emtricitabine enantiomer. Five manufacturing sites are involved. Adequate in-process controls are applied during the synthesis. The specifications and control methods for intermediate products, starting materials and reagents have been presented. The process has been shown to consistently produce emtricitabine that meets the required quality standards.

Specification

Emtricitabine specification includes tests and limits for appearance (visual), identification (IR, HPLC), clarity of solution (visual), water content (Ph. Eur.), enantiomeric purity (chiral HPLC), assay (HPLC), impurities (HPLC), heavy metals (Ph. Eur.), residue on ignition (Ph. Eur.), residual solvents (GC), and particle size (Laser Light Scattering). Analytical methods have been validated in accordance with ICH guidelines. The testing and the proposed limits applied, conform to current ICH guidelines and are acceptable from a toxicological and clinical perspective.

Extended testing during development has demonstrated that only a single polymorphic form results from the synthetic process of emtricitabine. Therefore as per ICH Q6A, testing for polymorphic form at release is not necessary. Development data demonstrate the absence of indicator organisms and therefore as per ICH Q6 indicate that microbial testing of the active substance is not required.

Batch analysis data on 22 commercial scale batches of the active substance from all proposed manufacturers were provided. The results comply with the specifications and confirm consistency and uniformity of the manufacturing process.

Stability

Thirteen commercial scale and additional pilot scale batches of emtricitabine manufactured using both synthetic routes and packaged in the proposed container were put on stability testing in accordance with the ICH Q1A (R2) Guideline under long-term conditions at 25 ºC/60% RH for up to 36 months. Of the above batches, eight commercial scale and five pilot batches were put under accelerated at 40 ºC/75% RH for up to 6 months. In addition another three batches were put under intermediate 30 ºC/65% RH conditions for up to 12 months. Samples are tested for appearance, impurities and degradation products, assay, water content and for enantiomeric purity by validated stability indicating methods. Stability data for emtricitabine manufactured by both synthetic routes were comparable. All tested parameters remained within the specification limits throughout the tested period for all three stability conditions. In one isolated batch one degradation product exceeded the specification limit at the last time point (36 months). The same degradation product, is observed in emtricitabine stored at accelerated storage. Four batches exceeded the specification limit at 6 months. These data indicate that emtricitabine should not be exposed to elevated temperatures for extended periods of time but poses no quality issue for the active substance.

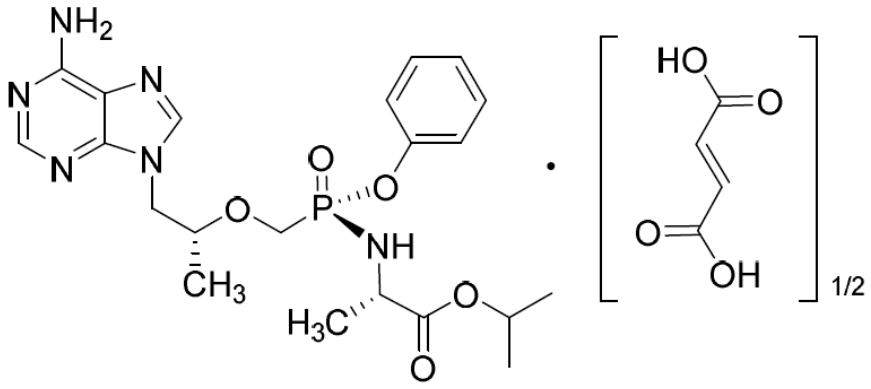
A photostability study was conducted on one batch of emtricitabine. The results showed no significant changes in appearance, purity, and impurity content and indicate that emtricitabine is not sensitive to light.

Based on the long-term stability data, the proposed re-test period and storage when the active substance is packed in the proposed packaging materials is considered acceptable.

Tenofovir alafenamide

General information

The chemical name of tenofovir alafenamide fumarate is propan-2-yl N-[(S)-({[(2R)-1-(6-amino-9H-purin-9-yl)propan-2-yl]-oxy}methyl)(phenoxy)phosphoryl]-L-alaninate, (2E)-but-2-enedioate (2:1) and has the following structure:



The chemical structure of tenofovir alafenamide fumarate has been adequately demonstrated by infrared spectroscopy, nuclear magnetic resonance spectroscopy (1H, 13C, and 31P), mass spectrometry, elemental analysis, ultraviolet absorption spectroscopy, and X-ray crystallography.

The active substance is a white to off-white or tan, slightly hygroscopic powder. Tenofovir alafenamide fumarate is a BCS Class 3 compound, with high aqueous solubility throughout the physiological pH range. The solubility of the active substance in aqueous media slightly decreases with increasing pH values. It is soluble at low pH (pH 2.0), sparingly soluble at pH 3.8, and slightly soluble at pH values up to 8.0. Tenofovir alafenamide fumarate is freely soluble in methanol, soluble in ethanol, sparingly soluble in isopropanol and slightly soluble in acetone.

Tenofovir alafenamide exhibits stereoisomerism due to the presence of three chiral centres. The chiral centre at the propyloxy side chain is in the R-configuration. The absolute stereoconfiguration of the carbonylethylamino substituent is derived from the amino acid L-alanine, which has the S-configuration at the alpha-carbon. The remaining stereocentre is located at the phosphorus atom and is in the Sp configuration. Enantiomeric purity is controlled routinely by chiral HPLC.

Polymorphism has been observed for tenofovir alafenamide fumarate. A single polymorphic form is consistently generated through the manufacturing process and this form has been adequately characterized.

The applicant has provided justification for TAF to be considered as a NAS on the basis of its unique chemical structure. However, both TAF and TDF which is a known active substance are prodrugs being metabolised to the same major active metabolite (TFV) in vivo. Therefore, both active substances share the same therapeutic moiety and as such, TAF is not considered a NAS on quality grounds.

Manufacture, characterisation and process controls

Tenofovir alafenamide fumarate is obtained from two manufacturers using the same synthetic route.

The active substance is synthesized in multiple steps. During the evaluation procedure, the active substance starting materials were redefined to be in line with ICH Q11. Commercially available well-defined starting materials with acceptable specifications are used.

Adequate in-process controls are applied during the synthesis. The specifications and control methods for intermediate products, starting materials and reagents have been presented. Critical process parameters were identified using a risk assessment approach.

Potential and actual impurities were well discussed with regards to their origin and characterised.

The commercial manufacturing process for the active substance was developed in parallel with the clinical development program. Changes introduced have been presented in sufficient detail and have been justified. The quality of the active substance used in the various phases of the development is considered to be comparable with that produced by the proposed commercial process.

The active substance packaging is in compliance with the EC directive 2002/72/EC and EC 10/2011 as amended.

The characterisation of the active substance and its impurities are in accordance with the EU guideline on chemistry of new active substances.

Specification

The active substance specification includes tests for: appearance (visual examination), identity (IR, HPLC), identity of fumaric acid (HPLC), clarity of solution (visual examination), water content (Ph. Eur.), assay (HPLC), impurities (HPLC, HPLC-MS, GC), residual solvents (GC), elemental impurities (ICP MS), and melting point (Ph. Eur.).

Impurities present at higher than the qualification threshold according to ICH Q3A were qualified by toxicological and clinical studies and appropriate specifications have been set.

The analytical methods used have been adequately described and non-compendial methods appropriately validated in accordance with the ICH guidelines.

Batch analysis data (n=16 using the proposed commercial process; 13 of which were commercial scale and 3 pilot scale) of the active substance, manufactured at both proposed manufacturing sites are provided. Additional batch analysis data for development batches used in pre-clinical pharmacokinetics and toxicological studies are provided. The results are within the specifications and consistent from batch to batch.

The active substance specifications are based on the active substance critical quality attributes (CQA).

Stability

Stability data on 6 commercial scale batches of active substance from the both proposed manufacturers stored in the intended commercial package for up to 36 months under long term conditions at 5 ºC and for up to 24 months under accelerated conditions at 25 ºC / 60% RH according to the ICH guidelines were provided. Photostability testing following the ICH guideline Q1B was performed on one batch. Results on stress conditions for up to 6 months at 40 ºC / 75% RH on 5 batches were provided. Additionally, results for 4 days at 60 ºC / ambient RH; for 4 days at 50 ºC / ambient RH; and for 4 days at -20 ºC were also provided on one batch.

The parameters tested are the same as for release. The analytical methods used were the same as for release and were stability indicating.

Degradation products increased under accelerated conditions but remained within the specification.

Photostability testing following the ICH guideline Q1B was performed on one batch, indication that the active substance is not photosensitive.

The stability results indicate that the active substance manufactured by the both proposed suppliers is sufficiently stable. The stability results justify the proposed retest period at the recommended long term storage conditions in the proposed container.

2.2.3. Finished Medicinal Product

Description of the product and pharmaceutical development

The proposed commercial finished product formulation is a green, capsule shaped, film-coated tablet with the dimensions of 19 mm x 8.5 mm, debossed with “GSI” on one side of the tablet and “510” on the other side of the tablet, containing 150 mg of elvitegravir, 150 mg of cobicistat, 200 mg of emtricitabine, and 10 mg of tenofovir alafenamide (as fumarate) as active substances.

The overall goal of Genvoya film coated tablet development was to create a tablet containing the same amounts of elvitegravir, cobicistat and emtricitabine active substances as currently combined in Stribild fixed dose combination film-coated tablet and to replace tenofovir disoproxil contained in Stribild with tenofovir alafenamide, a second-generation prodrug of tenofovir. The commercial formulation accommodates the required formulation and manufacturing process changes to incorporate tenofovir alafenamide fumarate (TAF fumarate) at a much lower dose than tenofovir disoproxil fumarate (TDF) while maintaining the established doses and biopharmaceutical performance of the other active substances, as well as ensuring satisfactory TAF fumarate chemical stability and content uniformity.

The aim of pharmaceutical development was to develop a stable immediate release solid dosage form for oral use, providing high bioavailability of the four active substances. The critical quality attributes (CQA) of the finished product are: size and colour, strength, content uniformity, % dissolved, degradation product content, water content, and microbiological quality.

Elvitegravir (EVG) and cobicistat (COBI) are BCS Class 2 compounds with low aqueous solubility and high permeability, and emtricitabine (FTC) is a BCS Class 1 compound with high aqueous solubility and high permeability. TAF fumarate is a BCS Class 3 compound with high aqueous solubility and low permeability. Co-formulation of EVG, COBI on silicon dioxide, FTC and TAF fumarate was enabled by the choice of the solid oral tablet as a dosage form and avoiding physical and chemical interactions between the four active substances. This was achieved through selection of appropriate intra- and extragranular excipients in amounts that achieve: chemical and physical stability, rapid and complete dissolution, consistent pharmacokinetic performance, satisfactory powder properties to ensure acceptable tablet content uniformity, and adequate tablet tensile strength to ensure physical tablet integrity.

Formulation composition, manufacturing process design and selection of excipients were intended to ensure content uniformity of all active substances within specifications.

All excipients are well known pharmaceutical ingredients and their quality is compliant with Ph. Eur. standards, except the coating material which is commercially available and tested according to an in house standard. All the components of the coating material comply with the standards in EU Regulation 231/2012 or compendial monographs. There are no novel excipients used in the finished product formulation. The list of excipients is included in section 6.1 of the SmPC and in paragraph 2.1.1 of this report.

The physical and chemical compatibility between EVG, COBI, FTC, and excipients have been established previously for Stribild film-coated tablets. The compatibility between TAF fumarate and the other ingredients was established with stability studies.

The formulations used during clinical studies following the definition of the desired tablet composition and TAF fumarate strength were differing only in the type of non-functional coating material used and therefore no bioequivalence study comparing early development formulations to the one proposed for marketing was required.

The discriminatory power of the dissolution method for routine release and stability testing of the finished medicinal product has been demonstrated.

Pharmaceutical development of the finished product contains QbD elements.

The manufacturing development has been evaluated through the use of risk assessment and design of experiments to identify the critical product quality attributes and critical process parameters. The risk identification was based on the prior knowledge of products with similar formulations and manufacturing processes as well as on the experience from formulation development and process design. The critical process parameters have been adequately identified.

The primary packaging is high density polyethylene (HDPE) bottle with a polypropylene continuous-thread, child-resistant cap, lined with an induction activated aluminium foil liner. Each bottle contains silica gel desiccant and polyester coil. The material complies with Ph.Eur. and EC requirements. The choice of the container closure system has been validated by stability data and is adequate for the intended use of the product.

Manufacture of the product and process controls

The manufacturing process consists of multiple main steps: blending of the active substance with intragranular/extragranular excipients, fluid-bed granulation/dry granulation and milling, compression, tablet film-coating and packaging. Critical process parameters have been identified. The process is considered to be a standard manufacturing process.

Major steps of the manufacturing process have been validated by a number of studies. The complete process will be validated at commercial scale prior to commercialisation; which was considered acceptable based on the enhanced development results presented and on experience gained in several commercial scale batches already manufactured. It has been demonstrated that the manufacturing process is capable of producing the finished product of intended quality in a reproducible manner. The in-process controls are adequate for this type of manufacturing process and pharmaceutical form.

Proven acceptable ranges have been defined for several steps. The available development data, the proposed control strategy and batch analysis data from commercial scale batches fully support the proposed PARs.

Product specification

The finished product release specifications include appropriate tests for this kind of dosage form: appearance (visual examination), identification (HPLC, UPLC, UV), water content (Ph. Eur.), assay (UPLC), degradation products (UPLC), uniformity of dosage units (HPLC), dissolution (UPLC), and microbiological examination (Ph. Eur.).

The analytical methods used have been adequately described and appropriately validated in accordance with the ICH guidelines.

Batch analysis results are provided for 16 commercial scale batches confirming the consistency of the manufacturing process and its ability to manufacture to the intended product specification. Additional batch results of 3 commercial scale batches used in clinical studies were provided as supporting data.

The finished product is released on the market based on the above release specifications, through traditional final product release testing.

Stability of the product

Stability data of 7 commercial scale batches of finished product stored under long term conditions for up to 24 months at both 25 ºC / 60% RH and 30 ºC / 75% RH and for up to 6 months under accelerated conditions at 40 ºC / 75% RH according to the ICH guidelines were provided. Results on stress conditions for up to 4 days at 60 ºC / ambient RH; at 50 ºC / ambient RH; and at -20 ºC were also provided on one batch. The batches of medicinal product are representative to those proposed for marketing and were packed in the primary packaging proposed for marketing.

In addition, one batch was exposed to light as defined in the ICH Guideline on Photostability Testing of New Drug Substances and Products.

It was demonstrated that stability data of the finished product manufactured using active ingredients from different suppliers is representative and there are no differences in stability.

Samples were tested for the same parameters as for release. The analytical methods used were the same as for release and were stability indicating. The specification acceptance limits were met for all quality attributes at long-term storage conditions. It was shown that the finished product is not photosensitive; however it should be stored in the original packaging in order to protect it from moisture.

Based on available stability data, the shelf-life of 24 months and storage conditions as stated in the SmPC are acceptable.

Adventitious agents

It is confirmed that the lactose is produced from milk from healthy animals in the same condition as those used to collect milk for human consumption and that the lactose has been prepared without the use of ruminant material other than calf rennet according to the Note for Guidance on Minimising the Risk of Transmitting Animal Spongiform Encephalopathy Agents Via Human and veterinary medicinal products.

2.2.4. Discussion on chemical, pharmaceutical and biological aspects

Information on development, manufacture and control of the active substances and finished product has been presented in a satisfactory manner. The results of tests carried out indicate consistency and uniformity of important product quality characteristics, and these in turn lead to the conclusion that the product should have a satisfactory and uniform performance in clinical use.

The applicant has applied QbD principles in the development of the active substance and finished product and their manufacturing process. However, no design spaces were claimed for the manufacturing process of the active substance, nor for the finished product.

2.2.5. Conclusions on the chemical, pharmaceutical and biological aspects

The quality of this product is considered to be acceptable when used in accordance with the conditions defined in the SmPC. Physicochemical and biological aspects relevant to the uniform clinical performance of the product have been investigated and are controlled in a satisfactory way. Data has been presented to give reassurance on viral/TSE safety.

2.2.6. Recommendation(s) for future quality development

N/A

2.3. Non-clinical aspects

2.3.1. Introduction

Elvitegravir (EVG) is an integrase strand transfer inhibitor (INSTI) that is licensed in the EU to be co-administered with a ritonavir (RTV)-boosted protease inhibitor and with other antiretroviral (ARV) agents for the treatment of HIV-1 infection in adults. Elvitegravir prevents integration of the HIV-1 genetic material into the host-cell genome.

Cobicistat (COBI) is a strong mechanism-based cytochrome P450 (CYP) 3A inhibitor (a pharmacokinetic enhancer) that increases the systemic levels of co-administered agents metabolised by CYP3A enzymes, including EVG and the HIV protease inhibitors (PIs) atazanavir (ATV) and darunavir (DRV).

Emtricitabine (FTC) is a nucleoside reverse transcriptase inhibitor (NRTI), and intracellularly it is phosphorylated to the active metabolite, emtricitabine triphosphate which has activity against HIV and hepatitis B virus.

Tenofovir alafenamide (TAF) is a prodrug of tenofovir (TFV), and after absorption TAF is converted to TFV intracellularly, which is phosphorylated to the active metabolite, tenofovir diphosphate (TFV-DP), that competes with natural 2'-deoxyadenosine triphosphate (dATP) for incorporation by the HIV-1 or HBV reverse transcriptase (RT) and, once incorporated, results in chain-termination. TAF differs from tenofovir disoproxil fumarate (TDF) as it is more stable in human plasma than TDF despite rapidly undergoing intracellular conversion to TFV. Treatment with TAF results in higher levels of TFV-DP in PBMCs and 90% lower circulating levels of TFV relative to TDF.

2.3.2. Pharmacology

Summary

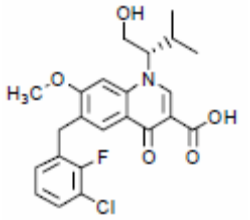
Pharmacology programmes have been completed for each of the four components of E/C/F/TAF, comprising of primary pharmacology, secondary pharmacology and safety pharmacology studies.

A small number of pharmacology studies have been completed with combinations of EVG and COBI, FTC and TDF, and EVG/COBI/FTC/TFV. No additional pharmacology studies have been conducted for the E/C/F/TAF combination. The proposed FDC is based on the complimentary pharmacological mechanisms of action of EVG, FTC, and TAF and clinical evidence is presented to support the use of nucleoside/nucleotide reverse transcriptase inhibitors (NtRTIs) and INSTIs in HIV-infected patients. In

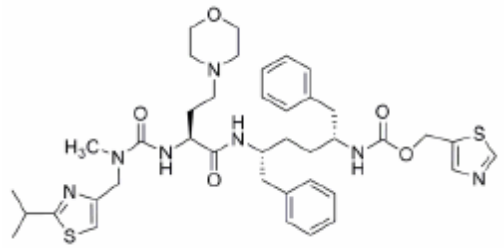
vitro cell based assays have established that combinations of these agents are not antagonistic and are in fact synergistic.

Physical chemistry

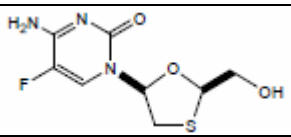
Elvitegravir (EVG, E)

Structure of the active substance Site of labelling (see structure).	 $C_{23}H_{23}ClFNO_5$
Molecular weight.	447.9
Solubility in water.	0.0003 mg/mL
Pka.	6.6
Distribution coefficient.	4.5
Solubility in other solvents.	33 mg/mL in acetonitrile, 647 mg/mL in N,N-dimethylformamide, 693 mg/mL in dimethyl sulfoxide 14 mg/mL in ethanol 16 mg/mL in methanol 7 mg/mL in 2-propanol 260 mg/mL in tetrahydrofuran
Possible chirality and its consequences.	Single asymmetric centre at C-11

Cobicistat (COBI, C)

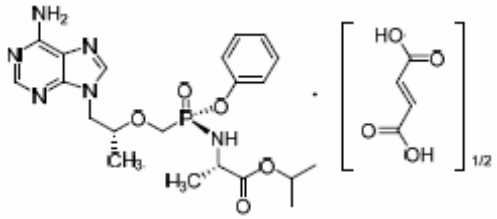
Structure of the active substance Site of labelling (see structure).	 $C_{40}H_{53}N_7O_5S_2$
Molecular weight.	776.0
Solubility in water.	0.1 mg/mL
Pka.	pKa1 = 1.8 (thiazole group) pKa2 = 2.5 (alkylthiazole group) pKa3 = 6.4 (morpholino group)
Distribution coefficient.	4.3
Solubility in other solvents.	>200 mg/mL in acetonitrile, dichloromethane, dimethyl sulfoxide and methanol. 0.005 mg/mL in *n*-Heptane
Possible chirality and its consequences.	Three chiral centres as a single isomer

Emtricitabine (FTC, F)

Structure of the active substance Site of labelling (see structure).	

	$C_8H_{10}FN_3O_3S$
Molecular weight.	247.24
Solubility in water.	112 mg/mL
Pka.	2.65
Distribution coefficient.	-0.43
Solubility in other solvents.	4 mg/mL in acetonitrile, 113 mg/mL in methanol 170 mg/mL in 0.1 N HCl 115 mg/mL in 0.1 N NaOH 0.3 mg/mL in isopropyl acetate
Possible chirality and its consequences.	The cis-(-)-enantiomer has a specific rotation of –137.9º when a 1% (w/v) solution in methanol is measured at 25 ºC.

Tenofovir alafenamide (TAF) fumarate

Structure of the active substance Site of labelling (see structure).	 $C_{23}H_{31}O_7N_6P$ ($C_{21}H_{29}O_5N_6P$ as free base)
Molecular weight.	534.6 (476.5 free base)
Solubility in water.	4.70 mg/mL (pH 6.8) 4.86 mg/mL (pH 8.0 85.4 mg/mL (pH 2.0 in HCl)
Pka.	3.96
Partition coefficient.	1.6
Solubility in other solvents.	2.30 mg/mL in acetonitrile, 189 mg/mL in methanol 69.6 mg/mL in ethanol 27.7 in isopropanol 9.16 in acetone 0.14 mg/mL in toluene
Possible chirality and its consequences.	Three chiral centres.

Primary pharmacodynamic studies

EVG:

Elvitegravir (EVG) has been shown to inhibit viral replication in a number of laboratory strains and isolates of HIV-1. EC_{50} values vary dependent on the cell line in question, but EVG has been shown to be effective against wild type HIV-1 in T-cell lines (EC_{50} = 0.38 nM), EC_{50} is 0.35 nM against HIV-1 macrophage-tropic virus in monocyte/macrophage cells, and 0.62 nM against clinical HIV-1 isolates in PBMCs. Activity of EVG has been shown against HIV-2. A calculated EC_{95} is 1.25 nM (0.56 ng/mL) in the absence of human serum (HS) components and 100 nM (44.8 ng/mL) in the presence of the HS components HSA and AAG in human PBMC cultures infected with HIV-1.

No *in vivo* animal studies have been conducted with EVG and evidence of pharmacodynamics has been obtained from clinical study.

COBI:

The primary pharmacological effect of cobicistat is to act as a potent inhibitor of human CYP3A. CYP3A inhibition studies, using an established clinical CYP3A inhibitor, ritonavir (RTV), as a comparator, were performed to test the specificity of CYP3A inhibition, mechanism of inhibition, and to determine the enzyme inactivation parameters of COBI.

The potency of COBI as an inhibitor of CYP3A was compared with RTV by using markers of activity for CYP3A enzymes (midazolam [MDZ] 1'-hydroxylase, testosterone 6β-hydroxylase, and terfenadine hydroxylase. Formation of the oxidative metabolite (M1) of EVG was measured as this indicated the extent of CYP3A enzyme activity. In addition, the effects of COBI and RTV on the human hepatic microsomal metabolism of atazanavir (ATV) and telaprevir (VX-950), were determined by monitoring the loss of the parent molecule.

COBI has been shown to be an effective inhibitor of human CYP3A enzymes, demonstrating similar activity against both MDZ 1'-hydroxylase and testosterone 6β-hydroxylase as RTV (Study No. AD-216-2028). In addition Cobicistat was found to be an efficient inactivator of human hepatic microsomal CYP3A activity, with kinetic parameters (kinact = 0.47 min^{-1}, KI = 1.1 μM) similar to those of RTV (kinact = 0.23 min^{-1}, KI = 0.26 μM. Clinical activity of COBI has been further established in human pharmacodynamic studies.

FTC:

Emtricitabine (FTC) is a synthetic analogue of the naturally occurring pyrimidine nucleoside, 2′-deoxycytidine. Intracellularly, FTC is converted through 3 phosphorylation reactions to its active tri-phosphorylated anabolite FTC-TP.

Emtricitabine triphosphate inhibits viral polymerases by direct binding competition with the natural deoxyribonucleotide substrate (deoxycytidine triphosphate; dCTP), and after incorporation into DNA, by DNA chain termination. Published literature describes that the EC_{50} of FTC against laboratory adapted strains of HIV-1 ranged from 0.001 to 0.62 μM depending on cell type and virus strain used in the assay. With clinical isolates of HIV-1, EC_{50} values ranged from 0.002 to 0.028 μM.

In vivo animal studies have been completed with FTC or FTC in combination to demonstrate its activity against immune deficiency in the macaque monkey. Macaques were infected with the simian immunodeficiency virus (SIV) and 50 days post inoculation animals were treated subcutaneously with either TFV (20 mg/kg) and FTC (50 mg/kg) or were not given any drugs. The treated macaques achieved SIV levels that were below the limit of detection (ie, < 100 copies/mL of viral RNA), whereas only 1 of the non-treated macaques showed a decrease in SIV RNA. SIV levels remained low in all treated animals for up to 6 months.

In another study, monkeys were exposed to 14 weekly doses of SHIV (SIV/HIV chimeric virus). Rhesus macaques were injected subcutaneously with TFV/FTC daily, at 2 hours before and 24 hours after the first virus exposure or at 2 hours before first virus exposure only. Twenty of the 21 control animals became infected, however all 6 of animals treated with TFV/FTC daily or before and 24 hours after the first challenge were fully protected after 14 challenges. In the single-dose group, 1 of 6 animals was infected, confirming that multiple dose therapy was highly effective at protecting these animals against rectal transmission of HIV.

TAF:

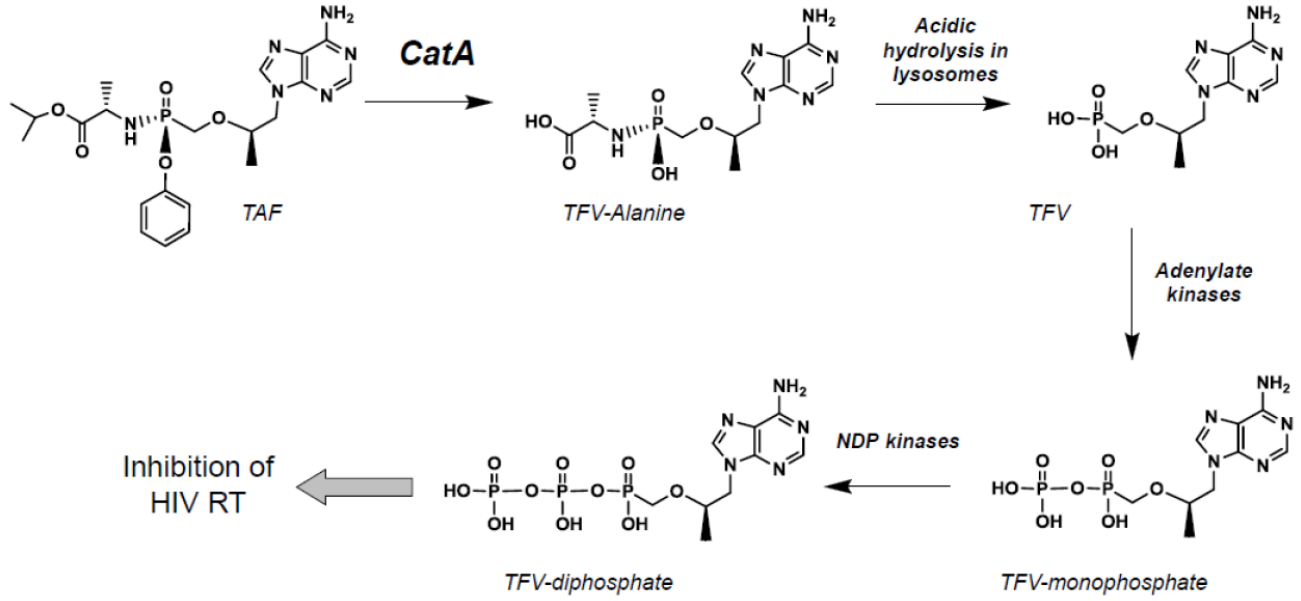
Tenofovir alafenamide is hydrolysed to tenofovir (TFV) by the lysosomal carboxypeptidase, Cathepsin A (CatA). To investigate the intracellular activity of TAF in lymphoid cells and tissues, $CD4^+$ T lymphocytes and monocyte-derived macrophage (MDMs) were isolated from PBMCs from viable donors (Study No. PC-120-2017) and extent of CatA activity determined by measuring the rate of conversion of TAF to TFV-alanine. Level of active CatA was similar across all donors, as was the rate of conversion from TAF to TFV-alanine in $CD4^+$ cells. Cathepsin A activity was approximately 2-fold greater in MDMs compared with $CD4^+$ T cells. There was conversion of TAF to TFV-DP across both cell types in all donor extracts (Figure 2).

As shown in Figure 1, TAF is hydrolysed to TFV, and is then metabolised to the active metabolite, TFV-DP, which is an inhibitor of HIV-1, HIV-2 and HBV polymerases, and is an inhibitor of HIV-1 RT that competes with deoxyadenosine triphosphate (dATP) for incorporation into nascent DNA and terminates the elongation of the viral DNA chain during the process of retroviral reverse transcription, thereby effectively blocking the replication and spread of infectious HIV.

In vitro studies have shown that TAF has effective anti-HIV activity in lymphoid T-cells, primary human PBMCs, and macrophages with EC_{50} values ranging from 3 to 14 nM. The *in vitro* activity of TAF against HIV-1 is 100- to 600-fold greater than TFV and 4- to 6-fold greater than TDF (Robbins *et al.* 1998).

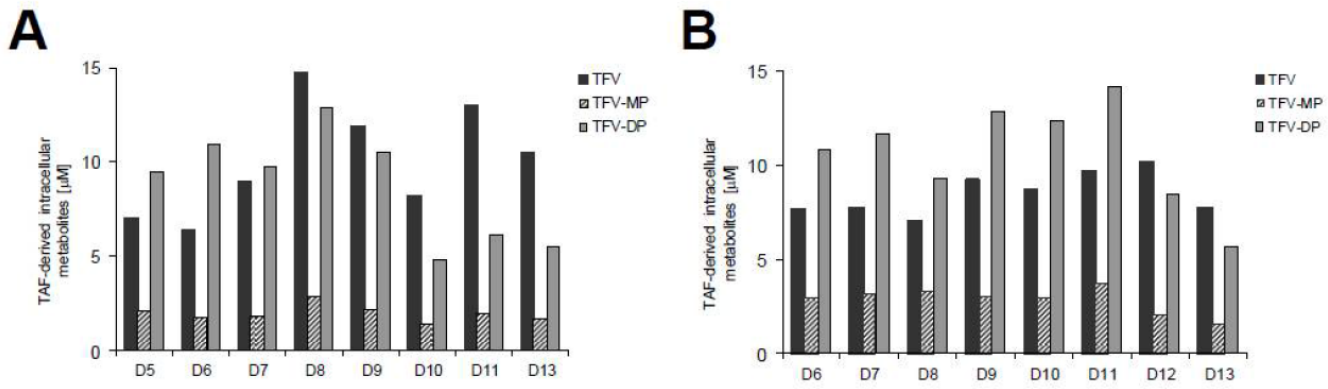
In MT-2 cells, TAF shows low cytotoxicity with a selectivity index (SI) of > 10,000). Based on data generated with the parent nucleotide TFV, TAF is expected to be active against a wide range of HIV-1 subtypes and also against HIV-2. In addition, TAF is a potent inhibitor of HBV replication, exhibiting *in vitro* activity comparable to that of TDF with an EC_{50} value of 18 nM.

Figure 1. Intracellular Activation of TAF in Lymphoid Cells and Tissues



Cat A = cathepsin A; NDP = nucleoside diphosphate; RT = reverse transcriptase; TAF = tenofovir alafenamide; TFV = tenofovir

Figure 2. Intracellular TAF Metabolites in CD4$^+$ T cells and Monocyte-derived Macrophages from Different Donors



TAF = tenofovir alafenamide; TFV = tenofovir; TFV-MP = tenofovir monophosphate; TFV-DP = tenofovir disphosphate Following incubation for 4 hours with 1µM TAF, the formation and quantity of intracellular TAF metabolites in CD4+ T cells (A) and MDMs (B) were determined by HPLC combined with mass spectrometry.

Further work evaluated the interaction potential of TAF with other CatA inhibitors such as HIV protease inhibitors (PIs) (Study No. PC-120-2001). The HIV PIs DRV, ATV, lopinavir (LPV), and RTV, as well as the pharmacoenhancing agent COBI, did not inhibit CatA-mediated hydrolysis of TAF up to a concentration of 50 µM, well above the clinical C_{max} of each drug. In a similar manner the potential for interaction with HCV PIs - TMC-435, BI-201355, MK-5172, GS-9256, and GS-9451 showed little-to-no inhibition of CatA, with IC_{50} values ranging from 25 to > 50 µM. 2 irreversible inhibitors of the HCV protease, telaprevir and boceprevir, were identified as potent inhibitors of CatA-mediated hydrolysis of TAF, with IC_{50} values of 0.3 and 0.2 µM, respectively. When adjusted for plasma binding, these IC_{50} values are 6- to 8-fold below the clinical maximum concentration (C_{max}) levels observed in patients.

No primary PD studies in animals were conducted; however studies were completed with the active component of TAF, TFV. Monkeys received a single dose of 30 mg/kg radiolabelled TFV subcutaneously, and the extent of TFV in plasma and levels of intracellular TFV and TFV metabolite concentrations were determined. The TFV concentration in plasma reached a maximum of approximately 50 µM and declined with a t1/2 of 5 to 7 hours. As was seen in the *in vitro* studies, TFV is efficiently taken up by PBMCs and is metabolised to TFV-DP, with the intracellular concentrations of the active metabolite TFV-DP reaching 0.9 µM.

E/C/F/TAF:

The anti-HIV-1 activity of TAF in combination with a broad panel of representatives from the major classes of approved anti-HIV agents (NtRTIs, NNRTIs, INSTIs, and PIs) was evaluated in HIV-1IIIB infected MT-2 cells.

The combination of TAF with TFV resulted in an additive effect as both will deliver TFV-DP to cells.

TAF exhibited moderate to high synergistic effects when combined with any of the NtRTIs or NNRTIs (Table 1). The combination of TAF with INSTIs resulted in a high level of synergy. The combination of TAF with PIs resulted in moderate synergy. Finally combining TAF with COBI, a PK enhancer co-formulated with TAF in E/C/F/TAF FDC and with no antiviral activity, resulted in an overall additive effect.

Table 1. TAF Anti-HIV-1 Activity in Combination

Drug combination	Class	Volume (µM^2 %)	Net Effect

Drug combination	Class	Volume (μM^2 %)		Net Effect
		Synergy[a]	Antagonism[a]	
TAF + TFV	NRTI	24	-14	Additive
TAF + FTC	NRTI	131	-9	Strong synergy
TAF + EFV	NNRTI	100	-7	Strong synergy
TAF + NVP	NNRTI	41	-14	Minor synergy
TAF + EVG	INSTI	271	-9	Strong synergy
TAF + RAL	INSTI	205	-10	Strong synergy
TAF + DTG	INSTI	179	-10	Strong synergy
TAF + ATV	PI	96	-10	Moderate synergy
TAF + DRV	PI	56	-12	Moderate synergy
TAF + COBI	PK enhancer	17	-22	Additive
TAF + TAF	Control	20	-17	Additive
ddi + RBV	Control	302	-20	Strong synergy
d4T + RVB	Control	20	-340	Strong antagonism

ATV=atazanavir, d4T=stavudine,ddI=didanosine, DRV=darunavir, DTG= dolutegravir, EFV=efavirienz, NVP= nevirapine, RAL=raltegravir, RBV= ribavirin

[a] Data shown represent the mean from > 3 independent experiments performed by triplicate. Volumes of $\geq$ 25 to < 25 μM^2 % indicate an additive effect, $\geq$ 25 – < 50 µM2 % indicate minor synergy, $\geq$ 50- < 100 µM2 % indicate moderate synergy, $\geq$ 100 µM2 % indicate strong synergy, $\geq$ -50 to < -25 indicate minor antagonism, $\geq$ -100 to < -50 indicate moderate antagonism and < -100 indicates strong antagonism.

Secondary pharmacodynamic studies

The ability EVG, COBI, FTC or TAF (TFV) to affect anti-viral activity of other antiretrovirals has been explored in some detail, reviewing their *in vitro* cytotoxicity, mitochondrial toxicity, off target activity and potential metabolic toxicities.

EVG:

The cytotoxicity of EVG was evaluated in a number of human cell lines and primary cells. EVG showed weak cytotoxicity in primary PBMCs (drug concentration that results in a 50% reduction in cell viability [CC_{50}] > 100 µM) (Study No. JTK303-PH-010, non-GLP), primary T-lymphocytes (CC_{50} 40 µM), primary monocytes/macrophages (CC_{50} >500 µM), and macrophages (CC_{50} 26 µM) (Study No. PC-186-2004, non-GLP).

Using a [^{3}H]thymidine incorporation assay, EVG cytotoxicity was observed in a dose-dependent manner after 7 days of culture with PBMCs, with a CC_{50} value of 9.7 µM (SI of > 48,000) in the absence of HS and 170 µM in the presence of 50% human serum (SI of > 100,000; Study No. JTK303-PH-006, non-GLP)). No difference in the cytotoxicity of EVG was detected in unstimulated versus stimulated PBMCs in the absence of human serum (mean CC_{50} values of 10.8 and 16.6 µM, respectively; Study No. PC-183-2001, non-GLP).

A variety of clinical symptoms observed in patients with HIV treated with prolonged NRTI therapy may be linked to mitochondrial toxicity. As a result EVG was tested *in vitro* for its effect on mitochondrial DNA levels in HepG2 liver cells. EVG produced no measurable changes in mitochondrial DNA levels at a dose of 10 µM (Study No. TX-183-2009, non-GLP).

Elvitegravir showed no significant inhibition of or increased binding to a series of 22 receptors, 7 enzymes, and 3 cell-based assay systems, including the immune cell functions of cell adhesion (ICAM-1/VCAM-1 mediated), interleukin-2 (IL-2) secretion, and mixed lymphocyte reaction (splenic lymphocytes; Study No. JTK303-PH-008, non-GLP).

In addition EVG did not inhibit the activity of human topoisomerase I and II enzymes at concentrations up to 50 and 150 µM, respectively (Study No. JTK303-PH-004).

COBI:

Cobicistat *in vitro* cytotoxicity has been evaluated in MT-2 lymphoblastoid T-cells following 5-day incubation and in HepG2 hepatoma cells following 3-day incubation (Study No. PC-216-2003, non-GLP). Cobicistat did not show significant cytotoxicity in MT-2 and HepG2 cells (CC_{50} of 88 and 44 µM, respectively).

Potential molecular targets for COBI were screened, using radioligand binding assays against a panel of 67 mammalian ion channels and receptors in two studies, Study No. TX-168-2007 and TX-168-2011. COBI demonstrated significant binding at calcium, potassium, and sodium ion channels at 10 µM.

Chronic treatment of HIV-infected patients with RTV is known to induce changes in body fat distribution (lipodystrophy), elevate blood levels of cholesterol (hypercholesterolemia) and triglycerides (hypertriglyceridemia), and cause insulin resistance. As COBI is an analogue of RTV the ability of COBI to affect metabolic toxicity was examined on proteasomes, adipocytes and proteases. COBI showed lower potential for metabolism-related toxicities compared to RTV, it had no effect on lipid accumulation and had reduced inhibitory effects on glucose uptake and proteasome activity (Study Nos. PC-216-2001 and PC-216-2004, non-GLP).

FTC:

For FTC, no cytotoxicity was observed *in vitro* in human PBMC, MT-2, HepG2, CEM, MOLT-4, and Vero cells at concentrations up to 100 µM. FTC was also found to be nontoxic to human bone marrow progenitor cells *in vitro*.

The potential for mitochondrial toxicity with FTC was evaluated. FTC was incubated with HepG2 cells at concentrations ranging between 0.1 and 10 µM for 2 weeks, and MT-2 cells were incubated with FTC at concentrations up to 100 µM for up to 8 weeks (Study No. TPI 11963, non-GLP). FTC had no adverse effects on cell growth, mitochondrial DNA synthesis, or lactic acid production. Further studies confirmed this finding.

The inhibition of mitochondrial DNA synthesis was also assessed in an *in vitro* cell culture assay using Molt-4 cells (a T-lymphoblast cell line) (Study No. TGZZ/93/0016 and TGZZ/93/0023, non-GLP). FTC did not reduce the ratio of mitochondrial to cellular DNA when tested at concentrations of up to 100 µM after 7 days of continuous cell exposure.

FTC had no significant binding affinity at 19 different receptors (Study No. TPZZ/93/0002, non-GLP), has shown little or no direct effect on various isolated muscle preparations (cholinergic, adrenergic, histaminergic, and serotonergic), and had no major inhibitory effects on the contractile responses to

acetylcholine, norepinephrine, serotonin, isoproterenol, arachidonic acid, histamine, bradykinin, and angiotensin II (Study No. TPZZ/92/0055, non-GLP).

TAF/TFV:

A range of studies have been completed to examine the potential secondary effects of TAF, utilising studies already completed for TFV.

The cytotoxicity profiles (CC_{50} values) of TAF, its stereoisomer GS-7339, TDF, and TFV were investigated in resting and dividing human PBMCs following 5 days of continuous drug incubation (Study No. PC-120-2009, non-GLP). The maximum concentrations of drugs used were 100, 100, 50, and 2000 µM, for TAF, GS-7339, TDF, and TFV, respectively. TAF doses used in this *in vitro* study were supra-therapeutic in concentration and duration. CC50 values for TAF ranged from 6.8 µM in dividing PBMCs to 25.1 µM in resting PBMCs. TAF showed low cytotoxicity in resting and in dividing PBMCs.

The cytotoxicity profiles (CC_{50} values) of TAF, TDF, TFV, and a panel of clinically relevant antiretroviral inhibitors were also evaluated in 2 T-lymphoblastoid cell lines (MT-2 and MT-4) following 5 days of exposure (Study No. PC-120-2007, non-GLP), TAF showed low cytotoxicity in T-lymphoblastoid cells providing ≥1997-fold increased selectivity relative to antiviral activity in T-lymphoblastoid cell lines. . Similarly TAF demonstrated low cytotoxicity to hepatic cells (Study No. PC-120-2007, non-GLP).

Tenofovir alafenamide also showed little to no effect on erythroid and myeloid progenitor proliferation *in vitro* (Study No. PC-120-2016, non-GLP).

TFV exhibited low levels of cytotoxicity in resting and activated human PBMCs, and in an established T-lymphocyte cell line, with CC_{50} values >1 mM. Similar findings for TFV were observed in HepG2 cells, skeletal muscle cells of human origin, and in human renal proximal tubule epithelial cells (RPTECs). Similarly, TFV has shown no effect on haematopoietic progenitor cells *in vitro* (Study No. PC-120-2016, non-GLP). Overall TFV has a low order of cytotoxicity *in vitro*.

The cytotoxicity of TAF and TFV was assessed in human HEK293T cells transiently expressing OAT1 and OAT3 (Study No. PC-120-2018, non-GLP). Cells were incubated with serial dilutions of TFV or TAF for 4 days. TAF did not interact with the renal organic anion transporters 1 or 3 (OAT1 or OAT3), and TAF exhibited no OAT-dependent cytotoxicity in human epithelial kidney cells transiently expressing these transporters. In addition, the selectivity index (considering CC_{50} in renal HEK293 cells expressing OAT1 or OAT3 relative to EC_{50} in primary $CD4^+$ T lymphocytes) for TAF (29,000 and 4270, respectively) was much higher than for TFV (14 and 82, respectively). As a result TAF is unlikely to accumulate in renal proximal tubules in an OAT-dependent manner, supporting the hypothesis that it has the potential for an improved renal safety profile.

When primary osteoblasts and PBMCs were treated with TAF doses consistent with human therapeutic exposure, comparable TFV-DP levels were achieved (Study No. PC-120-2008). At these therapeutically relevant doses of TAF, there were no *in vitro* effects on cell viability with primary osteoblasts or PBMCs.

Similar to the other components in E/C/F/TAF FDC, the impact of TAF on mitochondrial toxicity was assessed. Previous studies have demonstrated a minimal effect of TFV on the mitochondrial DNA synthesis *in vitro*. The potential for TAF to induce mitochondrial DNA depletion was evaluated in HepG2 cells (Study No. PC-120-2006, non-GLP). HepG2 cells treated with TAF (0.1, 0.3, or 1.0 µM) for 10 days exhibited no significant reduction in mitochondrial DNA compared with untreated cells.

No effect of TFV was seen on the synthesis of mitochondrial DNA or lactic acid production in HepG2 human liver cells or in normal human skeletal muscle cells (SkMCs) (Study No. P1278-00042, non-GLP). The results confirm the low potential for TFV to interfere with mitochondrial functions.

Combinations:

The combination of TFV and FTC was studied for cytotoxicity in MT-2 cells. No cytotoxicity was observed at the highest concentrations tested, up to 50 µM TFV and 5 µM FTC (Study No. PC-164-2002, non-GLP). Cytotoxicity studies were also conducted on the combination of TFV and FTC in HepG2 cells to evaluate their potential combined mitochondrial toxicity; no cytotoxicity was observed (Study No. TX-104-2001, non-GLP).

Given the lack of effects in the *in vitro* studies with the individual agents (EVG, COBI, FTC, TFV, or TAF) or with the combination of EVG+COBI+FTC+TFV, no additional secondary pharmacodynamic studies have been conducted for the E/C/F/TAF combination.

Safety pharmacology programme

Both *in vitro* and *in vivo* safety pharmacology studies have been completed for EVG, COBI, FTC, and TAF. No studies were conducted to examine the E/C/F/TAF combination.

EVG:

Cardiovascular System:

Elvitegravir at concentrations of 0.1 and 1 µM had no effect on the hERG tail current *in vitro* (Study No. JTK303-SP-003, GLP). A slight reduction (24.3%) in the hERG tail current was observed at the highest feasible concentration of 10 µM.

In isolated guinea pig papillary muscle, EVG at concentrations up to and including 3 µM had no effect on action potential parameters, including resting membrane potential, action potential amplitude, action potential duration at 50% and 90% of repolarisation, and maximal upstroke velocity (Study No. JTK303-SP-004, GLP).

Elvitegravir was orally administered to 4 male conscious, telemetered beagle dogs at 0 (vehicle; corn oil), 10, 30, and 100 mg/kg, in a dose volume of 2.5 mL/kg, in ascending order with an interval of 7 days between each of the doses. At doses up to 100 mg/kg, EVG did not produce adverse effects on blood pressure, heart rate (HR), or the ECG up to 24 hours after dose administration (Study No. JTK303-SP-002, GLP).

Central Nervous System:

Male SD rats were treated with single oral doses of EVG (100, 300 and 2000 mg/kg) or vehicle control (Study No. JTK303-SP-001, GLP) using gavage at a dose volume of 10 ml/kg. No effects were observed in terms of behaviour and general central nervous system effects at any dose.

Respiratory System

Elvitegravir was orally administered to 4 male beagle dogs at 0 (vehicle; corn oil), 10, 30, and 100 mg/kg in ascending order with an interval of 7 days between each of the doses. At doses up to 100

mg/kg, EVG did not produce any adverse effects on respiratory rate or oxygen saturation (Study No. JTK303-SP-002, GLP).

Gastrointestinal:

The effect of EVG on the autonomic nervous system and smooth muscle was examined using isolated guinea pig ileum (Study No. JTK303-SP-005, non-GLP). EVG at a high concentration (30 µM) inhibited single contractions by each of the contraction inducers, but the inhibition was slight, indicating that EVG has no definite effect on the autonomic nervous system or smooth muscle.

In vivo, EVG did not affect the intestinal transport of a charcoal meal in male SD rats at doses up to 2000 mg/kg (Study No. JTK303-SP-006, non-GLP).

Metabolites:

The most prominent metabolites of EVG, GS-9200 (M4) and GS-9202 (M1) were characterised to determine their ability to inhibit the hERG channel in CHO cells (Study No. TX-183-2005, non-GLP). GS-9200 and GS-9202 had IC_{50} values of > 100 µM and 81 µM, respectively. These concentrations are at least 250-fold above clinical exposures.

COBI:

Cardiovascular System:

Cardiovascular effects with COBI have been extensively examined during its development. *In vitro* studies included evaluation of hERG tail current. COBI inhibited hERG (IC_{50} 1.8 µM) and the hCav1.2 L-type calcium channel (IC_{50} 6 µM), but was a weak inhibitor of the hNav1.5 sodium channel (IC_{50} 86.5 µM) (Study Nos. TX-216-2009, GLP and TX-216-2015, non-GLP). Further *in vitro* work looking at action potential duration (APD) using rabbit Purkinje fibres revealed that COBI shortened APD at ≥1 µM but there was no indication that this could lead QT prolongation (Study No. TX-168-2012, non-GLP).

Two Langendorff studies using rabbit hearts were completed to further examine the effects of COBI. In a study completed with COBI alone, COBI was associated with negative inotropic effects and shortening of the APD on the isolated rabbit heart at concentrations ≥1 µM. At ≥3 µM, decreases in the QT interval and increases in the PR and RR intervals were noted (Study No. PC-216-2007, non-GLP). In a second Langendorff study in rabbit hearts, COBI produced similar negative inotropic effects (PR interval prolongation, and produced decreases in left ventricular [LV] function) at concentrations ≥1.5 µM. When COBI was combined with ATV, the changes to PR interval and left ventricular function were similar to that seen with COBI on its own (1.5 µM COBI coadministered with 1.5 µM ATV). QRS, QT interval was unaffected by COBI alone, or when in combination with ATV (Study No. PC-216-2009, non-GLP).

An *in vivo* cardiovascular study in telemetered conscious dogs was completed. In this study male dogs received escalating doses of COBI by oral gavage at 5, 15, and 45 mg/kg, at a dose volume of 2 mL/kg (Study No. TX-216-2008, GLP). A vehicle control group was also studied. There were no adverse effects on hemodynamic and electrocardiograph (ECG) parameters up to 45 mg/kg. Mild prolongation of PR interval was noted predominantly following the high dose (45 mg/kg) and sporadically at the mid dose (15 mg/kg). There was a slight increase in QTc interval (<4%) only at the highest dose tested, low enough to not be considered clinically significant.

Overall the results of these studies suggest that COBI has low potential for QT prolongation, and a likely capacity to prolong the PR interval and decrease LV function. No cardiac changes of note were observed in the 39 week toxicity study in dogs with COBI, and in a thorough QT clinical study there was no prolongation of QTc interval therapeutic and supratherapeutic exposures. A non-significant increase in PR interval was observed in the QT/QTc clinical study.

Central Nervous System:

In the rat CNS study, changes were limited to salivation, decreases in arousal, locomotor and motor activities, and decreases in body temperature at doses of 150 mg/kg and above (Study No. TX-216-2006, GLP). The NOAEL was 50 mg/kg. Tissue distribution studies in rats showed low levels of COBI-derived radioactivity in brain suggesting minimal transport across the blood:brain barrier.

Respiratory System:

No adverse effects were observed in the rat respiratory study (NOAEL 500 mg/kg) (Study No. TX-216-2007, GLP).

FTC:

Cardiovascular System:

In vitro the effects of FTC on cardiac preparations was evaluated using isolated cardiac muscle from rat, guinea pigs and cats (Study No. TPZZ/92/0056, non-GLP). Results from these *in vitro* studies suggested that FTC was free of negative cardiac effects at 1 µM.

No effects on the cardiovascular system were reported in anesthetised dogs administered a cumulative dose of 38.5 mg/kg of FTC intravenously over a 1-hour period (Study No. TPZZ/92/0076, non-GLP). Rats given oral doses of up to 250 mg/kg FTC showed no effect on heart rate or blood pressure (Study No. TPZZ/92/0057, non-GLP). In addition, there were no abnormalities reported on the ECG data obtained from the repeated-dose toxicity studies in monkeys, where AUC exposures were up to 26-fold higher than in humans administered the 200 mg dose.

Central Nervous System:

A range of central nervous system effects were examined in a single study conducted with male ICR rats (Study No. 477, non-GLP). Mice (10/dose) were given FTC orally at 0 (distilled water), 10, 30, or 100 mg/kg as a single dose. FTC did not affect reflex, spontaneous locomotion, motor coordination, anticonvulsant activity, proconvulsant activity or analgesic activity at any dose tested.

A further two studies in rats examined the effects of FTC on reflexes, analgesic activity and conditioned avoidance (Study Nos. TPZZ/93/0001 and TPZZ/93/0119, non-GLP). FTC had no effect on these parameters.

Respiratory System:

Effects of FTC on the respiratory system have been examined in mice, rats and dogs. In mice (Study No. TPZZ/93/0001, GLP) and rats (Study No. TPZZ/93/0001, GLP), animals were exposed to up to 1000 mg/kg of oral FTC with no effect on respiratory rate at any dose. In dogs (Study No. TPZZ/92/0076, GLP), male beagle dogs were intravenously administered FTC at 1, 2.5, 5, 10, and 20

mg/kg (cumulative dose = 38.5 mg/kg) over an hour. No changes were observed on respiratory function at any dose.

Gastrointestinal:

Male ICR mice (10/dose) were given FTC orally at 0 (distilled water), 10, 30, or 100 mg/kg, then given a charcoal suspension orally at 1 hour postdose. Emtricitabine did not affect GI motility at any dose (Study No. 477, non-GLP).

Renal:

Male Long Evans-derived rats were given FTC orally at 0 (distilled water), 10, 30, or 100 mg/kg and urine was collected for 6 hours postdose. FTC did not affect urine output, pH, or electrolyte excretion at any dose (Study No. 477, non-GLP).

TAF:

In vivo safety pharmacology experiments were conducted using TAF as the monofumarate form (GS-7340-02) in 50 mM citric acid. In the *in vitro* hERG assay, TAF as GS-7340-03 was dissolved in DMSO and diluted with HEPES-buffered physiological saline to a final concentration of 0.3% DMSO.

Cardiovascular System:

TAF (as GS-7340-03) was evaluated at concentrations of 1 and 10 µM (free base equivalents [fbe]), and hERG inhibition was not significant. The IC_{50} for the inhibitory effect of TAF on hERG was estimated to be greater than 10 µM (Study No. PC-120-2005, GLP).

Oral administration of TAF (as GS-7340-02) to conscious instrumented male beagle dogs at dose levels of 30 or 100 mg/kg (24 and 80 mg fbe/kg) did not induce pharmacologic effects on heart rate, systemic blood pressure, or ECGs (Study No. D2000006, GLP).

Central Nervous System:

The effect of TAF on the central nervous system has been examined in GLP Study No. R990188 using male SD rats. Animals were treated with single oral doses of TAF (as the monofumarate form) with doses of 0, 100 or 1000 mg/kg (80 or 800 mg free base equivalents [fbe]/kg). There was no evidence of any effect on the CNS at any dose tested up to 1000 mg/kg.

Gastrointestinal:

SD rats were administered TAF (as GS-7340-02) by oral gavage at doses of 0, 100 or 1000 mg/kg (0, 80 or 800 mg fbe/kg). At the highest dose the rate of gastric emptying was reduced, although this was not observed at 100 mg/kg (80 mg fbe/kg). A dose of 100 mg/kg was considered to have had no effect on gastric emptying or intestinal motility. (Study No. R990187, GLP).

Renal:

The effect of TAF (as GS-7340-02) on the renal system was evaluated in male SD rats following administration of single oral doses of 0, 100, or 1000 mg/kg (80 or 800 mg free base equivalents [fbe]/kg) (Study No. R990186, GLP). Urinary output of calcium was increased at 1000 mg/kg,

however this was correlated with an increase in serum calcium concentration and indicated that the kidneys were functioning well in order to reduce the serum calcium load. The no-effect dose for a pharmacological effect on the renal system was 1000 mg/kg.

E/C/F/TAF:

COBI has shown the potential for PR prolongation and to decrease LV function, no clinically-relevant cardiovascular changes have been observed with COBI administered as an individual agent, within the E/C/F/TDF FDC or within the E/C/F/TAF FDC.

From the cardiovascular studies with TAF, there is potential for PR interval to be prolonged, as seen in the 39 week dog study at 18/12 mg/kg/day. This change was observed to be associated with decreased weight gain, bone and renal toxicity, and significant decreases in triiodothyronine (T3). In the safety pharmacology study in dogs however there was no evidence of PR prolongation or any change in ECG results in doses of up to 1000 mg/kg. Results from the clinical thorough QT study also have revealed no cardiac signal.

As there has been a comprehensive program for each of the four components in respect of safety pharmacology, no studies have been conducted with the combination in accordance with CHMP guidance (EMEA/CHMP/SWP/258498/2005). There is sufficient knowledge of the individual components to assess potential overlaps in safety risks, and the results do not warrant additional investigation of the combination.

2.3.3. Pharmacokinetics

Pharmacokinetic studies

The absorption, distribution, metabolism, and excretion of EVG, COBI, FTC, and TFV/TAF were evaluated *in vitro* and in a variety of animal models *in vivo*. In addition, the drug-drug interaction profile was also evaluated. The pharmacokinetics of the E/C/F/TAF FDC is discussed based on the results of non-clinical studies completed with the individual agents, no additional pharmacokinetic studies have been conducted for the E/C/F/TAF combination.

Methods of analysis

EVG: The *in vivo* pharmacokinetic, toxicokinetics, distribution, and excretion of EVG were assessed in the mouse, rat, rabbit, and dog. Analysis of EVG in plasma from mouse, rat, and dog used validated methods based on high performance liquid chromatography coupled to tandem mass spectrometry (LC/MS/MS). Similar methods were utilised to detect levels of EVG metabolites: GS-9204, GS-9200, GS-9202 and GS-9203. For quantification of radiolabelled EVG, liquid scintillation counting (LSC), and radio-profiling was performed by LC with flow radio-detection. Validation of each *in vitro* assay to detect levels of EVG and its metabolites has also been extensively undertaken.

COBI: Analysis of COBI in plasma from mice, rats, rabbits, and dogs utilised fully validated methods based upon LC/MS/MS. Similar methods were used for the analysis of the COBI metabolite, GS-9612, in plasma from mice, rats, and dogs. LSC and radio-profiling were also utilised to detect radioactivity of [^{14}C]COBI in mice, rats and dogs.

FTC: The *in vivo* pharmacokinetic, toxicokinetics, distribution, and excretion of FTC were assessed in mouse, rat, and monkey. Analytical methods used to quantify FTC in mouse, rat, and monkey plasma from the early preclinical absorption, distribution, metabolism, and excretion studies employed

reverse-phase high performance liquid chromatography (HPLC), and HPLC-MS (plasma and urine). LC-radio-profiling has been utilised to detect [^{3}H]FTC in mice and monkeys, and to detect [^{14}C]FTC in rats and monkeys.

TAF: The *in vivo* pharmacokinetic, toxicokinetics, distribution, and excretion of TAF were assessed in mouse, rat, dog, and monkey. The *in vitro* absorption, metabolism, and drug interaction characteristics of TAF were studied in appropriate model systems. Levels of TAF and TFV in rats and dog plasma and PBMCs were determined using fluorescence derivitization/HPLC. Additional methods to detect levels of TAF and TFV in mouse, rat, rabbit and dog plasma/PMBCs included validated LC/MS/MS methods, and HPLC detection methods. The absorption, distribution, metabolism, and excretion of TAF were assessed in various species following a single oral administration of [^{14}C]TAF, and levels of TAF and its metabolites were measured using LSC, HPLC or LC/MS/MS coupled with flow-through detector (RFD). *In vitro* determination of TAF levels were in the main determined by LC/MS/MS, with some LC-radio-profiling. Induction potential of TAF on CYP activity measured mRNA levels using qRT PCR methods.

E/C/F/TAF: *In vitro* transporter methods were developed to measure the impact of EVG, COBI, FTC and TFV on various transporter assays. Validation reports are supplied to support the use of MDCKII and CHO cells, as well as assays using vesicular preparations.

Absorption

EVG:

The *in vitro* permeability of [^{14}C]EVG was studied using monolayers of LLC-PK1 porcine kidney cells transfected with an expression vector for human P-gp or with the empty control expression vector (Study No. JTK303-AD-026). EVG underwent significant efflux across these cells, suggesting that it is a substrate for human P-gp.

Single-dose pharmacokinetics of EVG and [^{14}C]EVG were studied in rats and dogs. Absorption was rapid following oral dosing to rats (Study Nos. JTK303-AD-005 and JTK303-AD-007) and dogs (Study Nos. JTK303-AD-006 and JTK303-AD-008). Bioavailability was also in rats (30-35%) and in dogs (26-33%), clearance also varied from low in rats to a more moderate level seen in dogs.

COBI:

Cellular permeability of COBI was studied using monolayers of Caco-2 cells (Study No. AD-216-2023). There was little evidence to suggest that COBI underwent significant efflux across these cells.

Single-dose pharmacokinetics were studied in a number of species including rats (Study No. AD-216-2020), dogs (Study No. AD-216-2021), and monkeys (Study No. AD-216-2022). oral bioavailability of COBI was moderate in the rat (33%) and low in the dog and monkey (11% and 7%, respectively). High clearance values indicated that there is potential for high hepatic metabolic first-pass extraction following oral absorption in these species.

The multiple-dose pharmacokinetic parameters for COBI were derived as part of the repeat-dose toxicity studies in mice (10 to 100 mg/kg/day; Study Nos. TX-216-2032, TX-216-2041, TX-216-2026), in rats (10 to 100 mg/kg/day; Study Nos. TX-216-2004,TX-216-2017), and in dogs (5 to 45 mg/kg/day; Study Nos. TX-216-2005,TX-216-2016) dosed for periods of 4 weeks to 39 weeks. There were species differences in autoinduction during these studies, with hepatic microsomal fractions from treated mice and rats showing higher levels of CYP3A, but with no increases in treated dogs. COBI has been found to activate rat PXR, but not in humans.

Multiple-dose toxicology studies in rats were also performed with COBI in combination with EVG and ATV. In general, COBI only modestly increased EVG and ATV steady state exposures compared to EVG and ATV when dosed alone, consistent with its dual action as a reversible CYP3A inhibitor and a P450 inducer in rodents.

FTC:

No *in vitro* permeability studies have been carried out with FTC, as FTC shows high, dose-independent bioavailability *in vivo* in mice and monkeys. Single-dose pharmacokinetics of FTC have been studied in mice, rats and cynomolgus monkeys. FTC was rapidly and well absorbed with oral bioavailability ranging from 58% to 97% over the dose range of 10 to 600 mg/kg.

The multiple-dose pharmacokinetic parameters for FTC were derived as part of the repeat-dose toxicity studies in mice (80 to 3000 mg/kg/day; Study Nos. TOX109; IUW00701; TOX599; TOX628), in rats (60 to 3000 mg/kg/day; Study Nos. TOX108; TOX097), and monkeys (40 to 2000 mg/kg/day; Study Nos. TOX600; TOX627; TOX032) dosed for periods of 3 days to 104 weeks. There were no significant differences in pharmacokinetics following single and multiple dosing. Exposure to FTC (Cmax and AUC) increased approximately proportionally with dose and was similar between males and females.

TAF:

In vitro: Permeability of TAF was examined using Caco-2 cells (Study No. AD-120-2037). TAF was applied to monolayers of these cells at 10, 100, and 1000 µM, and TAF showed a dose dependent increase in forward permeability and a decrease in efflux ratio indicating saturable efflux transport. Addition of the efflux transport inhibitor, cyclosporine A (CsA) diminished the efflux ratio and increased the permeability.

Mouse: Both single and repeat dose studies were completed in mice.

In the single dose pharmacokinetic study in mice, TAF/TFV were evaluated following administration of TAF by dosing either GS-7340-02 or GS-7340-03 to male CD-1 mice or GS-7340-03 to both male and female 001178-W mice via oral gavage (Study Nos. AD-120-2014 and AD-120-2016).

Tenofovir exposure increased with the increase in dose and was greater than dose proportional between 10 to 100 mg/kg. Gender differences in plasma TFV levels were less than 2-fold in Cmax and AUC0-t values. The pharmacokinetic profiles for the 2 different fumarate forms of TAF were observed to be generally similar.

Table 2. Plasma Pharmacokinetic Parameters Following a Single Dose of GS-7340-02 and GS-7340-03 to Male CD-1 Mice

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
Source: AD-120-2014

Table 3. Plasma Pharmacokinetic Parameters Following a Single Dose of GS-7340-03 to 001178-W Wild type Mice

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
Source: AD-120-2016

GS-7340-02 was administered by oral gavage for up to 14 days to male and female mice at a dose of 100, 500, or 1000 mg/kg/day (Study No. TX-120-2006). Due to early death for animals given 500 or 1000 mg/kg/day, only the 100 mg/kg/day dose group was evaluated. GS-7340 at 100 mg/kg/day corresponded to a Day 14 C_{max} of 27.1 and 2.89 ng/mL for males and females, respectively; the AUC0-24 could not be calculated due to the lack of a distinct elimination phase. GS-7340 rapidly converted to its metabolite, TFV. There were no significant differences in TFV pharmacokinetic profiles between males and females.

Following daily administration of GS-7340-02 to mice via oral gavage for at least 13 weeks at doses of 0, 10, 30, and 100 mg/kg/day, the pharmacokinetic parameters for TAF and TFV were determined (Study No. TX-120-2007). Exposure to TFV increased with the increase in GS-7340-02 dose from 10 to 100 mg/kg/day. The increases in Cmax 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. There was no sign of accumulation of TFV after multiple dosing, and there is rapid and extensive conversion of TAF to TFV after oral administration in mice.

Rat: Both single and repeat dose studies were completed in rats.

In the single dose pharmacokinetic study in rats, the two forms of TAF (GS-7340-02 and GS-7340-03) were again compared, as was the exposure to TFV between TAF and TDF (Study Nos. R990130, AD-120-2015, and R2000065). TAF was rapidly absorbed and generation of the major metabolite TFV was observed with a T_{max} of less than 1 hour. TFV exposure increased in a greater than dose proportional manner. There no significant difference in pharmacokinetic parameters between the two forms of TAF, GS-7340-02 and GS-7340-03.

Table 4. Plasma Pharmacokinetic Parameters following a Single Dose of GS-7340-02 and GS-7340-03 to Male Sprague-Dawley Rats

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

In a comparison between exposure of TFV generated due to TAF or TDF (Study No. R20000065), rats were treated orally with a single dose of 400 mg/kg of TAF (GS-7340-02) or TDF. The plasma Cmax and AUC for TFV were 2- to 3-fold higher with 400 mg/kg TAF compared to 400 mg/kg TDF.

The plasma pharmacokinetic profile of TFV was determined during the course of a 28 day oral gavage toxicity study in adult male and female albino rats following daily administration of either 1.5, 6.25, 25, 100 or 400 mg/kg/day GS-7340-02 (Study No. R990182). A greater than dose proportional increase in exposure was observed. There was no evidence of accumulation.

In a 26-week toxicology study, GS-7340-02 was administered once daily at doses of 0 (vehicle only), 5, 25 and 100 mg/kg/day by oral gavage and plasma pharmacokinetic parameters of TFV were determined on Day 1 and during Weeks 13 and 26 (Study No. TOX-120-001). No consistent differences in plasma pharmacokinetic parameters were found between male and female rats. Mean tenofovir Cmax and AUC values increased dose proportionally over the dose range of 5 to 100 mg/kg/day. Mean TFV AUC obtained on Day 1 was slightly lower than that measured during Weeks 13 and 26, which suggested that there was a slight accumulation of tenofovir with repeat dosing.

Dog: Both single and repeat dose studies were completed in dogs.

In Study No. 99-DDM-1278-001-PK the effect on pharmacokinetic parameters due to changes in the stereo configuration, fumarate form, food, and the route of administration was examined. In this study Beagle dogs were administered TAF as a single IV bolus (GS-7340-02 [6.3 mg/kg]), 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]). Following oral administration, TAF and its diasteroisomer were rapidly absorbed and eliminated with a tmax of less than 0.5 h and t½ ranging from 0.2-0.9 h. The plasma exposures to the intact prodrugs were similar when TAF or GS-7339 were dosed separately, however, when the isomeric mixture, GS-7171, was dosed, the exposure to GS-7339 was approximately 3-fold higher than TAF. TFV exposure was similar for both diasteroisomers, although exposure in PBMCs was higher following dosing with TAF than with GS-7339. The effect of food led to a decrease in overall plasma exposure of TFV and TAF (2.5 fold).

When male Beagle dogs were given a single oral dose of 10 mg/kg TAF, there was rapid absorption and elimination, tmax was less than 0.5 h and t½ ranged from 0.2-0.9 h. The pharmacologically active metabolite, TFV-DP was the major metabolite in liver achieving a Cmax of 126 µM at 4.0 hours postdose.

Following daily oral administration of 8.29 mg/kg TAF for 7 days to male Beagle dogs, the plasma and liver pharmacokinetic profiles were determined on day 1 and 7 (Study No. AD-120-2033). TAF was rapidly absorbed and exhibited a short terminal half-life (t½) of 0.3 hours in plasma on both Day 1 and 7. The rapid disappearance of TAF was accompanied by an increase in TFV. Tenofovir was the major metabolite detected in plasma achieving a maximal plasma concentration (Cmax) 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 hours postdose on Day 7, respectively.

The plasma PK of TAF and TFV and TFV levels in PBMCs were determined during the course of 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 GS-7340-02 (Study No. D990175-PK). 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, Cmax values of 582 and 1280 ng/mL, and t½ λz values of 18 and 23 minutes, respectively. The TFV Cmax values appeared to be linear with increasing dose as well as repeat dosing. The TFV t½ was estimated to be 37 h and substantial accumulation of TFV was

observed after repeat dosing. The TFV levels in PBMCs were not linear with increasing dose; however, a linear correlation was observed between TFV levels in PBMCs and corresponding trough plasma concentrations. PBMC concentrations were approximately 100-fold higher than corresponding plasma concentrations.

In a 9-month toxicology study in dog, GS-7340-02 was administered once daily at doses of 0, 2, 6, and 18 mg/kg/day (Study No. TOX-120-002). The dose of 18 mg/kg/day was decreased to 12 mg/kg/day on Day 2 of Week 7 for males and Day 2 of Week 8 for females due to severe clinical signs and reduced body weight and food consumption. The concentrations of GS-7340 and tenofovir in plasma samples and total TFV in Week 39/40 PBMC samples were determined. GS-7340 was rapidly absorbed and converted to tenofovir following oral dose administration, with peak plasma concentrations of GS-7340 and tenofovir occurring at 0.5 and 1 hour post dose, respectively. GS-7340 was eliminated rapidly from the plasma with a terminal phase half-life of less than 1 hour. The median t½ of tenofovir was estimated to be in the range of 25 to 31 hours on Day 1. The plasma pharmacokinetics of GS-7340 and tenofovir were comparable between male and female dogs after oral administration. Plasma Cmax and AUC values for TAF increased more than proportionally over the dose range of 2 to 18/12 mg/kg/day. The plasma TFV Cmax and AUC increased roughly dose proportional. There was some accumulation of tenofovir following repeat dosing (~3-fold). Tenofovir concentrations in PBMCs were measurable at 24-hour post dose for all dose groups. The median terminal phase half-life of total tenofovir in PBMCs was estimated to be 31 hours (similar to the tenofovir plasma estimate) from the recovery animals with PBMC concentrations measured up to 72 hours. Dose-normalized PBMC mean AUC values of total tenofovir increased more than dose proportionally during Week 39/40.

Monkey:

Single dose pharmacokinetics for TAF and TFV, and TFV in PBMCs was determined using rhesus monkeys administered single oral doses of GS-7340-02 at 0.5, 5.0, and 50 mg/kg (Study No. P2000087). Tenofovir alafenamide and TFV levels increased rapidly with tmax values of approximately 0.5 and 1 hour, respectively (Table 5). Levels of TFV in PBMCs were also detected, levels of TFV persisted in PBMCs for up to 96 h and persisted to a higher extent to samples treated with acid phosphatase suggesting that a significant proportion of TFV-related material in PBMCs was in phosphorylated forms (Table 6).

Table 5. Plasma Pharmacokinetic Parameters for TAF And TFV Following a Single Dose of GS-7340-02 to Rhesus Monkeys

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
$AUC_{0\text{-last}}$ (ng·h/mL)	1.22	95.1	3811	39.9	1037	9934
$AUC_{0\text{-}\infty}$ (ng·h/mL)	2.47	80.0	3846	52.7	1069	10250

Table 6. Concentrations of TFV in PBMCs from Monkeys Dosed with GS-7340-02

	TFV PBMC Levels (ng/10^6 Cells)			
	Without Phosphatase Treatment		With Phosphatase Treatment	
GS-7340-02 Dose (mg/kg)	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

Following daily oral administration of GS-7340-02 at 0, 3, and 30 mg/kg/day or TFV at 15 mg/kg/day for 28 days, pharmacokinetic profiles of TAF and/or TFV were determined on Day 1, Day 14 and Day 28 (Study No. P2000114-PK). No significant differences in pharmacokinetic parameters were found between males and females. The pharmacokinetic parameters for TFV were dose linear on Day 1 but were greater than dose-linear on Day 28 after oral administration of GS-7340-02. There was no statistically significant accumulation of TFV following repeat dosing of either GS-7340-02 or TFV. The intracellular TFV concentrations in PBMCs were determined from the 30 mg/kg/day GS-7340-02 dose group where 72.3 and 27.2 µg/mL were detected on Day 14 and Day 28, respectively.

E/C/F/TAF:

Potential drug interactions within the combination could affect absorption, FTC shows high passive permeability and is unlikely to be affected when administered with EVG, COBI, or TAF. Tenofovir alafenamide and EVG are both efflux substrates in the intestine; therefore, absorption is increased in the presence of COBI due to inhibition of intestinal efflux transport.

In vitro mechanistic studies on the potential for COBI to affect the absorption of TAF are described under drug interactions.

Non-clinical studies of the absorption kinetics of the E/C/F/TAF FDC have not been conducted, however this has been assessed in appropriate clinical studies with the combination.

Distribution

EVG:

Plasma protein binding of EVG is high and is concentration independent in rats, dogs, monkeys and humans (Study No. JTK303-AD-014). The fraction unbound varied from 0.1% in rats to 1.2% in monkey. The fraction unbound in human plasma, or in a physiological concentration of HSA, averaged 0.7%. EVG does not distribute to blood well from samples taken from rats, dogs, monkeys and humans (Study No. JTK303-AD-013).

After oral administration of [^{14}C]EVG to male or female albino rats, there was rapid distribution of radioactivity to highly perfused organs (liver, adrenal gland, kidney, heart, lung, and pancreas), with relative exclusion from the eye and brain (Study No. JTK303-AD-005). Exposure in pregnant rats was generally similar to that in non- pregnant animals.

COBI:

Binding of COBI in plasma was moderately high, yielding a fraction unbound of 6.3% in human plasma at 1 µM COBI (Study No. AD-216-2026; AD-216-2076). The extent of COBI binding to mouse, rat, and monkey plasma was similar, and the fraction of unbound COBI was similar acroos all species, including humans (4.75-6.54%). COBI does not distribute well into the cellular fraction of blood from mouse, rat, dog, or human.

After oral administration of [^{14}C]COBI to albino and pigmented rats (Study Nos. AD-216-2034 and AD-216-2060, respectively), radioactivity was rapidly and widely distributed to most tissues. Distribution was extensively to glandular and elimination organs - GI tract, included liver, adrenal, kidney, and pituitary. The tissues with the lowest Cmax values were eye, spinal cord, and brain, bone, and secondary sex organs. There was no difference in distribution patterns between pigmented and non-pigmented animals, except for higher concentrations to the uveal tract of the eye and to pigmented skin, suggesting an association between COBI and melanin. Similar to EVG, exposure was no different in pregnant animals as compared to non-pregnant animals.

FTC:

The protein binding of FTC was very low (< 5%) in mouse, rabbit, monkey, and human plasma (Study No. TBZZ/93/0025). The tissue distribution of [^{14}C]FTC was characterised in rats and cynomolgus monkeys after a single oral dose of 200 mg/kg (Study Nos. TOX092 and TOX063, respectively). Distribution was extensive and rapid; levels were detected within 1 h post oral administration. There was no sign of FTC accumulation and elimination was rapid, no radioactivity was observed after 72 hours post-dose.

Pharmacokinetic parameters for FTC in pregnant animals appeared to be generally similar to those reported for non-pregnant animals

TAF/TFV:

The extent of binding of TAF to plasma protein was determined using dog and human plasma only (Study No. AD-120-2026). Rat plasma was not included as TAF is highly unstable in rat plasma due to the presence of a high number of esterases. Protein binding of TAF was moderate in dog and human plasma with the percent unbound values of 48.0% and 46.8%, respectively. The *in vitro* values are slightly higher than those observed using *ex vivo* samples from TAF treated humans which ranged from 14 – 23%. For the use in the interaction studies, the percentage of unbound TAF was round up to be 20%.

The protein binding of TFV has been determined in human plasma and serum using centrifugal ultrafiltration (Study No. P0504-00039.1). Percent of unbound TFV was 99.3 ± 3.3% in human plasma, and 92.8 ± 3.6% in human serum. Tenofovir therefore showed very low protein binding in either human plasma or serum.

Extensive tissue distribution studies with TAF were completed using mice, rats and dogs.

Male CD-1 mice were treated with a single oral dose of 100 mg/kg [^{14}C]TAF (Study No. AD-120-2011). Most tissues reached maximum concentration by 1 hour post-dose. The tissues showing the highest maximum concentrations of radioactivity, excluding GI tract, included liver, gall bladder, urinary bladder, diaphragm, kidney cortex, kidneys, and kidney medulla. The tissues with the lowest Cmax values were testis, brain cerebrum, fat (abdominal), spinal cord, and brain medulla. Similar distribution profiles were seen in male C57 Black (pigmented) mice. More persistent exposures in eye lens, eye uveal tract, and eyes were observed in CD57 black mice compared to CD-1 mice, although there was no indication that there was a difference in distribution between pigmented and non-pigmented skin, or that TAF was more preferentially distributed to melanin-containing tissues.

Male SD or Long Evans rats were administered oral 5 mg/kg [^{14}C]TAF (Study AD-120-2020). There was rapid distribution to most tissues, both to pigmented and non-pigmented rats. The tissues showing the highest maximum concentrations of radioactivity included kidney cortex, kidney(s), kidney medulla, and liver. The tissues with the lowest Cmax values were brain olfactory lobe, seminal vesicle(s), eye vitreous humour, thymus, eyes, testis(es), and harderian gland for Sprague-Dawley rats and bone, brain olfactory lobe, seminal vesicle(s), fat (abdominal), muscle, eye vitreous humour,

and eye(s) for Long Evans rats. There was no indication that there was any difference in distribution between pigmented and non-pigmented animals, binding to melanin was unlikely.

The distribution of TAF and TFV in pregnant and lactating animals has been evaluated. In pregnant rats, rabbits and monkeys the extent of placental transfer of TAF and TFV was measured during the embryo-fetal developmental studies. In rats there was a clear increase in TFV exposure with increasing dose of TAF (Study Nos. TX-120-2001 and TX-120-2002). Multiple dosing in the dose-range finding study showed signs of accumulation of TFV, however this was not seen in the definitive study.

In rabbits, there was an increase in exposure to TAF and TFV with increasing dose, with no evidence of accumulation (Study Nos TX-120-2004 and TX-120-2005).

In the monkey the extent of placental transfer of TFV following subcutaneous administration was determined in pregnant rhesus monkeys (Study No. 96-DDM-1278-005). Placental transfer of TFV appeared to be significant with a foetal/maternal serum concentration ratio of 0.17 ± 0.07 (mean ± SD) at approximately 30 minutes post-dose.

E/C/F/TAF:

Drug interactions, within the 4-drug combination, that affect distribution would not be expected from the data available. Although plasma protein binding is high for EVG and moderate for COBI and TAF, the binding is very low for FTC and TFV. As a result, interactions through binding displacement would not be anticipated. An *in vivo* study with [^{14}C]EVG and co-dosed RTV revealed no change in the tissue distribution of EVG. As COBI shows similar transporter properties as RTV, albeit weaker, it is likely that COBI would not affect the overall distribution profiles for any of the other components in the FDC.

Metabolism

EVG:

The primary metabolic pathways for EVG are hydroxylation to M1 (GS-9202), catalysed by CYP3A (Study No. JTK303-AD-017), and glucuronidation at the carboxylic acid moiety, catalysed by UGT1A1 and UGT1A3 in humans (Study No. AD-183-2034), yielding M4 (GS-9200). Minor pathways detected include benzylic hydroxylation (M2), generation of the direct ether glucuronide (M3) and combinations of the primary pathways (M7 and M8).

After oral or intravenous administration of [^{14}C]EVG to rats (Study No. JTK303-AD-019) and dogs (Study No. JTK303-AD-020), parent EVG was the most abundant analyte in plasma, with the glucuronide, M4, being the most abundant circulating metabolite. The glucuronides, M4 and M7, were the most abundant analytes in rat bile, but in faeces these were apparently subject to deconjugation as EVG and M1 were the most abundant analytes. A similar pattern was seen in dog faeces with EVG and oxidative metabolites being the most abundant analytes.

COBI:

The primary metabolic pathways for COBI are methine oxidation of the isopropyl moiety (M31, GS-9612), cleavage adjacent to the methylurea (M26, GS-341842), cleavage of the carbamate (M21, GS-9454), and cleavage and deethylation of the morpholine (M39). Oxidation is primarily catalysed by CYP3A, which can generate all metabolites, with a minor role for CYP2D6 (which contributes to the generation of M31) (Study No. AD-216-2025).

After oral administration of [^{14}C]COBI to mice (Study No. AD-216-2073), rats (Study No. AD-216-2082), and dogs (Study No. AD-216-2101), COBI was the most abundant analyte in plasma. Parent COBI, M21, and M31 were the most abundant analytes in faeces, with M39 also being significant in dog faeces. Profiles in bile from rats and dogs were complex, with many small peaks being detected (each accounting for ≤5.3% of the dose).

FTC:

FTC is not subject to significant metabolism by CYP enzymes. Generation of a minor (~1%) sulfoxide metabolite (M1 and/or M2) was catalysed by CYP3A4, and inhibitor studies suggested that at least one other enzyme, possibly flavin-containing monooxygenase, may play a role (Study No. 15396 v1). A minor direct glucuronide metabolite, M3, was also detected.

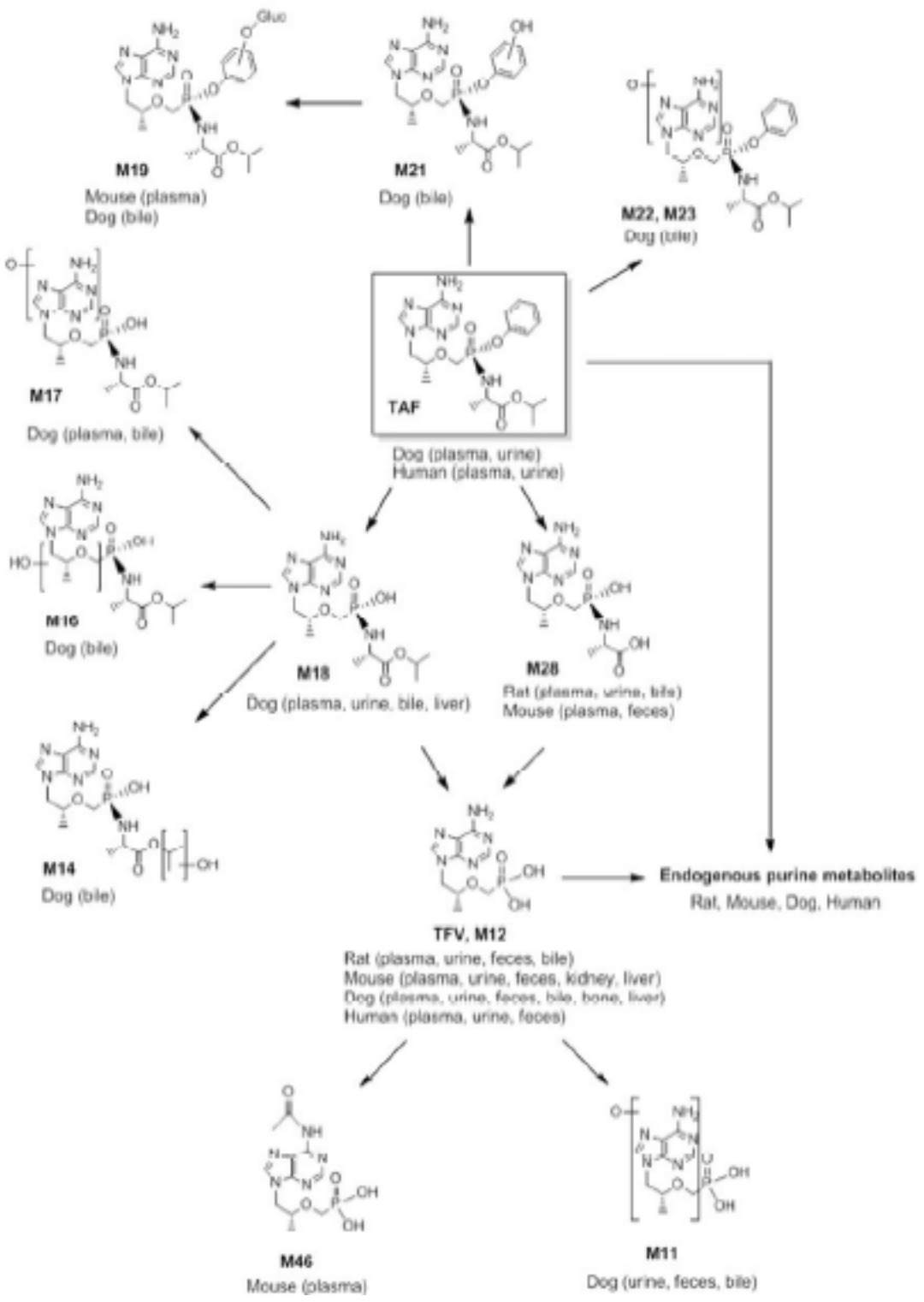
FTC was not extensively metabolised and is eliminated primarily as unchanged drug by renal excretion in mice, rats, and cynomolgus monkeys. Over 90% of the radioactivity in mouse and rat urine and 64% of the radioactivity in monkey urine was unchanged drug. Only trace levels of metabolites were found in faeces (Study Nos. TEIN/93/0015, TEIN/93/0016, TOX063). In all 3 species, metabolism accounted for only a minor percentage of FTC elimination. FTC is subject to Phase I metabolism (oxidation to a diastereomeric sulfoxide) and to some direct conjugation (glucuronidation of hydroxymethyl group) as minor metabolic routes.

TAF:

Tenofovir alafenamide is subject to intracellular metabolism to TFV, which is further phosphorylated to the anabolites, TFV-MP and TFV-DP with TFV-DP being the pharmacologically active form.

The applicant has proposed a possible metabolism pathway based upon the findings from mice, rats, dogs and humans (Figure 3.4.1). TAF is also subject to intracellular metabolism to TFV, which is further phosphorylated to the anabolites, tenofovir-monophosphate and TFV-DP with TFV-DP being the pharmacologically active form (Figure 3).

Figure 3. Proposed metabolism pathway for TAF



In vitro Metabolism: The potential for CYP enzymes to metabolise TAF was assessed by incubating TAF with 6 individual bacterially expressed human CYP enzyme preparations co-expressed with human NADPH CYP reductase (Study No. AD-120-2004). Metabolism of TAF was not detected by CYP1A2, CYP2C8, CYP2C9, CYP2C19 or CYP 2D6. Tenofovir alafenamide was slowly metabolised by CYP3A4 at a rate of 1.9 min^{-1} which was 26.6% of the positive control, testosterone.

Intracellular metabolic activation of TAF in PBMCs or other lymphatic tissues involves conversion to TFV by cathepsin A. In contrast to PBMCs, TAF was primarily hydrolysed by carboxylesterase 1 (CES1) in primary hepatocytes. Tenofovir is then further phosphorylated to TFV-DP by cellular nucleotide kinases.

The *in vitro* activation of TAF in human primary hepatocytes was evaluated and compared with that of TDF and TFV (Study No. AD-120-2017). Following a 24-hour continuous incubation of primary hepatocytes with 5 µM TAF, TDF, or TFV, the levels of TFV-Diphosphophosphonate (GS-77938) increased to 1,470, 302, and 12.1 pmol/million cells illustrating that incubation with TAF resulted in 5- and 120-fold higher intracellular levels of GS-77938 compared to TDF and TFV, respectively.

The *in vitro* metabolism of [^{14}C]TFV was studied in dog plasma, in control and induced (Aroclor 1254) rat liver microsomes, and also in dog liver and intestinal S9 fractions (Study No. 96-DDM-1278-003). Tenofovir was recovered unchanged under all conditions: no metabolites were detected in either rat microsomal preparation, with or without the addition of NADPH cofactor. There was no evidence of chiral inversion either.

In vivo Metabolism:

The metabolic profiles of TAF were determined in plasma, urine, faeces, kidney, liver, and nasal turbinate from mice (Study No. AD-120-2012); in plasma, urine, bile, and faeces from rats (Study No. AD-120-2021); and in plasma, urine, bile, faeces, bone, and liver from dogs (Study No. AD-120-2008). The metabolite profiles were also determined in human plasma, urine, and faeces following administration of a single oral dose of [^{14}C]TAF (Study No. GS-US-120-0109). The findings from these studies are summarised in Table 7.

TFV accounted for a majority of drug related material in plasma, urine, and faeces from all species except for human plasma, in which uric acid (M27B) was the predominant metabolite accounting for 73.9% of the total AUC over 96 hours. Uric acid is also detected to a large extent in mouse plasma (19.4%). M18 was the major metabolite in rat bile accounted for 63% of total radioactivity recovered in bile. M18 and its oxidised 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. No metabolites unique to human were observed.

The extent of TFV transformation to TFV-DP was examined in PBMCs, red blood cells (RBCs) and lymph nodes from monkeys (Study No. P2001025). Animals were administered a single dose of 15, 30, or 60 mg/kg of [^{14}C]TFV subcutaneously. TFV was taken up by PBMCs and anabolised to TFV-DP, with intracellular concentrations of the active antiviral anabolite reaching 1.6 µM (60 mg/kg dose group). The half-life of TFV-DP in this experiment was >50 hours. A similar pattern developed in RBCs and lymph nodes. The long intracellular half-life in this respect supports the proposed once daily clinical dosing regimen.

Table 7. Relative quantification of TAF Metabolites in Plasma, Urine, Faeces, and Bile as % Total Dose

		Plasma[a]	Urine	Feces	Bile
Mouse	TFV (M12)	54.8	18.1	30.7	NA[b]
	M28	1.02	0	0.7	NA
	Allantoin (M27A)	12.2	2.6	0.4	NA
	Uric acid (M27B)	19.4	0	0	NA
	Unknown metabolites	12.3	3.2	0	NA
	Total	100	23.9	31.8	NA
Rat	TFV (M12)	66.7	17.1	63.6	NA
	M28	5.8	0	0	NA
	Allantoin (M27A)	23.2	0.1	0	NA
	Unknown metabolites	4.3	1.6	0	NA
	Total	100	18.8	63.3	NA
Rat (BDC[c])	TFV (M12)	NA	17.1	61.7	0.66
	M28	NA	0.4	0	1.17
	Allantoin (M27A)	NA	0.2	0	0

	Uric acid (M27B)	NA	0	0	0.02
	Unknown metabolites	NA	1.7	0	0
	Total	NA	19.4	61.7	1.85
Dog	TAF	1.3	1.3	0	NA
	TFV (M12)	68.3	24.2	20.8	NA
	M11	0	0.4	0.4	NA
	M17	0.44	0	0	NA
	M18	17.6	0.2	0	NA
	M20	0.2	0	0	NA
	Unknown metabolites	12.2	3.0	0.2	NA
	Total	100	29.1	21.4	NA
Dog (BDC)	TAF	NA	1.3	0	0.2
	TFV (M12)	NA	16.8	26.4	1.0
	M11	NA	0.4	0.7	0.1
	M14	NA	0	0	0.2
	M16	NA	0	0	4.4
	M17	NA	0	0	0.5
	M18	NA	0	0	3.4
	M19	NA	0	0	0.1
	M21	NA	0	0	0.4
	M22	NA	0	0	0.2
	M23	NA	0.2	0	0.2
	Unknown metabolites	NA	2.9	0	0.9
	Total	NA	21.6	27.1	11.6
Human	TAF	1.8	1.41	0	NA
	TFV (M12)	1.5	22.2	31.4	NA
	Uric acid (M27B)	73.9	1.93	0	NA
	Xanthine/hypoxantine (M7.M8)	0	0.26	0	NA
	Unknown metabolites	22.8	0	0.29	NA
	Total	100	25.8	31.7	NA

a Plasma data represent % of total AUC.

b NA = not applicable

c BDC = bile duct cannulated

E/C/F/TAF:

EVG does not interact with drug metabolising enzymes as a substrate, inhibitor, or inducer while TAF is a weak substrate and inhibitor of CYP3A. The effect of CYP3A inhibition by TAF should be minimal as

COBI is already a potent inhibitor of CYP3A. The potential consequences of inhibition of CYP3A metabolism is further discussed in the section on pharmacokinetic interactions.

FTC and TAF are analogues of different nucleosides, cytidine and adenosine, respectively and so have no overlapping metabolism pathways, and in fact in experiments in which both drugs are incubated together at doses higher than that expected to be achieved in the clinic, there was no influence on conversion of TFV to TFV-DP with the presence of FTC. Conversion of FTC to FTC-triphosphate was also unaffected. *In vitro* studies also confirm that there exists a synergism between FTC and TAF (Study No. PC-183-2004).

Excretion

EVG:

Recovery of administered [^{14}C]EVG to rats and dogs was high, ≥97.7% following both oral and IV administration. Recovery was almost complete by 48 hours post-dose, and the majority was found in faeces, although approximately 1% was also seen in urine following IV dosing. In BDC rats 25% of radioactivity was recovered in bile and 69.2% in faeces.

Excretion of EVG in rat milk was studied as part of a prenatal and postnatal developmental toxicology study in rats (Study No. TX-183-2006). There is limited but detectable excretion of EVG from plasma to milk.

COBI:

After oral administration of [^{14}C]COBI to mice (Study No. AD-216-2073), rats (Study No. AD-216-2034), and dogs (Study No. AD-216-2067), recovery of radioactivity was high (≥86.1% in all groups) with the majority being found in faeces (≤2.06% in urine). Recovery was largely complete by 48 hours post- dose. In BDC animals, approximately 69.3% was recovered in bile in rats, and this was 63.9% in dogs.

Excretion of COBI in rat milk was studied as part of a prenatal and postnatal developmental toxicology study in rats (Study No. TX-216-2033). COBI was present in milk samples 2 hours post-dose.

FTC:

The primary route of elimination of [^{3}H]FTC and [^{14}C]FTC was via renal excretion of parent drug after oral and IV administration in mice, rats, and cynomolgus monkeys. The majority of the FTC recovered in the faeces after oral administration most likely represents unabsorbed drug, rather than biliary excretion. Although FTC is metabolised to only a minor extent, its metabolites are also excreted via the kidneys.

Excretion into milk has not been evaluated for FTC.

TAF/TFV:

Excretion of oral radiolabelled TAF has been reviewed across mice, rats and dogs.

Mice were administered a single oral dose of 100 mg/kg [^{14}C]TAF (Study No. AD-120-2011). Recovery of radioactivity was 61% from urine and faeces 48 hours post-dose. An average of 41.3 and 27.7% of the administered radioactivity were excreted in faeces and urine, respectively, by 168 hours post-dose.

Male bile duct-intact and BDC male SD rats were given a single 5 mg/kg oral dose of ^{14}C]TAF (Study No. AD-120-2020). [^{14}C]TAF was rapidly excreted within 24 hours after oral dosing. The mean values of 71.9 and 22.2% of the administered radioactivity were excreted in faeces and urine, respectively, by 168 hours post-dose. The mean overall recovery of radioactivity was 96.7%.

Excretion of radiolabelled TFV was examined following IV administration at doses of 10 or 50 mg/kg to SD rats. Excretion was 85.2 ± 7.63% at 24 hrs, and 92.7% ± 6.77 % by 7 days post dose in urine. Faecal elimination was 3.18% ± 1.85% by 24 hours, and 4.48% ± 1.89% by 7 days post dose.

In dogs the excretion of [^{14}C]TAF was determined after administration of a single 15-mg/kg oral dose of ^{14}C-TAF to bile duct-intact and BDC male dogs (Study No. AD-120-2007). [^{14}C]TAF was readily excreted mostly within 48 hours after oral dosing. The mean values of 37.4% and 35.9% of the administered radioactivity were excreted in faeces and urine, respectively, by 168 hours post-dose. Overall mean recovery of radioactivity was 80.4%.

Excretion of radiolabelled TFV was evaluated in dogs following a single IV dose of [^{14}C]TFV (Study No. 96-DDM-1278-002). The primary route of elimination was via urine, where 70.03% of total radioactivity was recovered. Total faecal recovery of radioactivity was 0.42% of the total dose.

Bile excretion: Bile excretion has been examined in both rat and dog studies following oral administration with radiolabelled TAF. 3.2%, and 2.11% of the administered radioactivity were excreted in faeces, urine, and bile, respectively, by 168 hours post-dose. The mean overall recovery of radioactivity after oral dosing to BDC rats was 99.9%.

The excretion of [^{14}C]TAF was determined following oral administration of a single 15-mg/kg dose of [^{14}C]TAF to male dogs (Study No. AD-120-2007). Mean values of 42.7%, 26.5%, and 14.0% of the administered radioactivity were excreted in faeces, urine, and bile, respectively, through 168 hours post-dose. Based on the radioactivity excreted in urine and bile, a minimum of approximately 41% of the orally administered dose was absorbed. Elimination via biliary excretion appears to be the major route of elimination of [^{14}C]TAF in dogs. The overall recovery of radioactivity in BDC dogs was 86.2%.

Excretion to milk: The extent of TFV excretion in lactating monkeys was evaluated. Milk was obtained from 2 lactating adult female rhesus monkeys following a single 30 mg/kg subcutaneous dose of TFV (Study No. P2000116). TFV was detected in the milk, the AUC in milk was between 18.6-21.5% of that seen in plasma.

E/C/F/TAF:

FTC and TFV are almost exclusively eliminated by renal excretion, while very little EVG or COBI is excreted in the urine, as a result any interaction between the compounds during excretion are thought to be unlikely. In addition COBI has also been shown to have no inhibitory effect on OAT1 and only weak inhibition of MRP4, which are the transporters responsible for renal excretion of TFV.

Pharmacokinetic drug interactions

EVG:

EVG showed no detectable inhibition of activities catalysed by CYP1A2, 2A6, 2C9, 2C19, 2D6, or 2E1 in human hepatic microsomal fraction and was not a clinically meaningful inhibitor of UGT 1A1, UGT1A3, and UGT2B7 while it was a weak inhibitor of UGT1A1 and UGT1A3 *in vitro*.

In studies in human hepatocytes EVG showed no significant ability to activate human aryl hydrocarbon receptor (AhR) at concentrations up to 10 µg/mL (22 µM). EVG does have potential to induce enzymes and transporters controlled by PXR, subsequently CYP3A activity is increased.

EVG affected human P-gp-dependent transport only at a concentration (30 µM) above its aqueous solubility, and is also a moderate inhibitor of human organic anion transporting polypeptide 1 (OATP1) but a more potent inhibitor of human OATP1B3 (IC_{50} 0.44 µM).

COBI:

COBI inhibits human CYP3A enzymes, its intended pharmacological action. Cobicistat is a potent mechanism-based inhibitor of human CYP3A with inactivation kinetics (kinact 0.47 min^{-1}, KI 1.1 µM), similar to those of RTV. COBI does not inhibit human CYP1A2, CYP2C9, or CYP2C19, is a very weak inhibitor of CYP2C8 (IC_{50} 30.1 µM), a weak inhibitor of CYP2D6 (IC_{50} 9.2 µM), and a modest inhibitor of CYP2B6 (IC_{50} 2.8 µM). COBI is also identified as a weak inhibitor of human hepatic microsomal UGT1A1 activity (IC_{50} 16.3 µM).

COBI showed no ability to activate human AhR and was a very weak activator of human PXR. Other targets for induction (uridine diphosphate glucuronosyltransferase 1A1 [UGT1A1] mRNA, P-gp mRNA, and CYP2B6 mRNA and protein) were all unaffected or weakly affected by COBI treatment.

At systemic concentrations achieved in plasma at the 150 mg COBI dose, COBI does not inhibit the drug transporters P-gp, MRP1, MRP2, breast cancer resistance protein (BCRP), organic anion transporter 1 (OAT1), or OAT3. However, at concentrations achievable briefly in the intestinal lumen during absorption ([I]2 = 770 µM) COBI can inhibit intestinal efflux transporters such as P-gp and BCRP. COBI is a moderate inhibitor of OATP1B1 and OATP1B3 (hepatic uptake transporters).

With respect to renal transporters, COBI is a weak inhibitor of MRP4, multidrug and toxin extrusion protein 2-K (MATE2-K), and organic cation transporter 2 (OCT2), and a more potent inhibitor of MATE1 and organic cation transporter novel, type 1 (OCTN1).

FTC:

FTC was not an inhibitor of activities catalysed by CYP1A2, 2A6, 2B6, 2C9, 2C19, 2D6, 2E1, or 3A in human hepatic microsomal fractions. FTC also did not show inhibition of the glucuronidation of 7-hydroxycoumarin, a general UGT substrate. FTC did not activate human AhR or PXR at concentrations up to 50 µM. *In vitro* studies indicate that FTC is not a substrate or an inhibitor of any of the transporters tested except for being a substrate of OAT3.

TAF/TFV:

The potential for TAF to be involved in drug-drug interactions has been assessed in a range of *in vitro* test systems. The potential of TAF or its metabolites to inhibit or induce CYP enzymes and serve as substrates or inhibitors of xenobiotic transporters was assessed. The effect of other drugs, including other antiviral agents that may be co-administered with TAF, on intestinal stability and the absorption potential was also determined. Considering the data generated using *ex vivo* human tissue the extent of unbound TAF was estimated to be 20% of total exposure.

Inhibition of Cytochrome P450 enzymes and UGT1A1:

The potential for TAF and TFV to inhibit human CYP-mediated drug metabolism was examined *in vitro* using hepatic microsomal fractions and enzyme-selective activities (Study Nos. AD-120-2003 and V990172-104). Inhibition of the following CYP450 enzymes was evaluated, CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, and CYP3A. TAF at a concentration of 25 µM was shown to weakly inhibit CYP3A with an IC_{50} ranging from 7.4 to 7.6 µM. TFV did not inhibit CYP1A2, CYP2C9, CYP2D6, CYP2E1, and CYP3A.

In Study No. AD-120-2040, the potential for TAF to be a mechanism based inhibitor of human CYP enzmes was investigated. TAF at a concentration of 50 µM had no effect on inhibition to any of the tested isoenzymes (CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19 and CYP2D6). Tenofovir alafenamide did not inhibit UGT1A1 up to 50 µM (IC_{50} > 50 µM) (Study No. AD-120-2006).

Enzymology of Metabolism:

To examine whether TAF may be metabolised by intestinal esterases and/or CYP enzymes following intestinal absorption, the effects of other HIV PIs and CYP inhibitors was explored in Study No. AD-120-2027. TAF was incubated with HIV-1 PIs (atazanivir or darunavir) or CYP inhibitors (ritonavir or COBI) at concentration of up to 100 µM. The stability of TAF was unaffected by the presence of these CYP inhibitors or PIs.

In order to investigate which enzymes are involved in activation of TAF in human hepatocytes, TAF was incubated alongside known CatA inhibitors (approved hepatitis C virus NS3 inhibitors, telaprevir and boceprevir), CES1 inhibitor (bis-p-nitrophenyl phosphate, BNPP), CYP3A4 and P-gp inhibitor (COBI), or telaprevir and BNPP together (Study No. AD-120-2031). BNPP inhibited the metabolism of TAF in a dose dependent manner. Formation of the active constituent of TAF, TFV-DP was unaffected on co-incubation with telaprevir, boceprevir, or COBI. Combining BNPP and telaprevir resulted in an enhanced level of inhibition. From the results of this study it is implied that TAF is primarily hydrolysed by CES1 and CatA.

Induction Liability:

The ability of TAF to induce CYP enzymes/activity, P-gp or UGT1A1 was examined using cultured human hepatocytes treated with 1, 10, and 100 µM TAF once daily for 3 consecutive days (Study No. AD-120-2032). Increases in CYP mRNA levels and increased CYP activity is represented in Table 8.

Table 8. Effect of TAF Treatment on CYP mRNA Levels and Activity in Cultured Human Hepatocytes

	Mean Fold Increase (% Positive Control)					
	mRNA			Activity[c]		
Concentration	CYP1A2	CYP2B6	CYP3A	CYP1A2	CYP2B6	CYP3A
1 µM TAF	1.2 (<1%)	0.95 (<1%)	0.92 (<1%)	1.0 (<1%)	1.1 (<1%)	0.97 (<1%)
10 µM TAF	3.0 (3%)	1.6 (4%)	8.3 (6%)	1.4 (1%)	0.85 (<1%)	0.99 (<1%)
100 µM TAF[a]	6.9 (8%)	2.5 (10%)	44 (36%)	0.84 (<1%)	0.42 (<1%)	0.37 (<1%)
Positive control[b]	72	16	120	28	13	29

a The viability of the hepatocytes was affected at this concentration of TAF and therefore caution should be taken when interpreting the corresponding induction data.
b Positive controls 50 µM omeprazole, 1000 µM phenobarbital, and 10 µM rifampin for CYP1A2, CYP2B6, and CYP3A, respectively.
c Phenacetin, bupropion, and testosterone were used as probe substrates for CYP1A2, 2B6, and 3A, respectively.

There was evidence of cytotoxicity following dosing with 100 µM TAF with reduced CYP activity but increased mRNA levels. After treatment with 10 µM TAF, the mRNA levels of CYP1A2 and CYP3A4 increased by 3.0- and 8.3-fold which correspond to 3% and 6% of control levels. This demonstrates that TAF has a potential to induce CYP isoenzymes at 10 µM but this was reduced to little or no induction potential at 1 µM. There was no evidence of a change in induction potential for P-gp or UGT1A1 mRNA.

The potential for TAF to induce human drug metabolising enzymes and drug transporters through the activation of human AhR or human PXR was further evaluated in cell-based systems (Study No. AD-120-2005). At a concentration of 50 µM TAF was only able to activate PXR at 23% of a positive control, rifampicin. This effect reduced to less than 5% with a dose of 15 µM TAF. Activation of AhR was not observed following dosing with 50 µM TAF. TAF is unlikely to activate either PXR or AhR xenobiotic receptors.

Potential for Transporter-Mediated Drug Interactions with TAF and TFV:

The ability of TAF and/TFV to affect to action of drug transporters has been explored in a number of *in vitro* studies. A run-down of the relevant transporters affected by TAF (and EVG, COBI and FTC) is listed in Table 9 (transporter substrates) and Table 10 (transport inhibition).

TAF:

In terms of inhibiting drug transporters, TAF was unable to inhibit P-gp, BCRP, OAT1, OAT3, and OCT2 (Study No.s. AD-120-2019 and AD-120-2036). Inhibition to OATP1B1, OATP1B3, BSEP, OCT1, and MATE1 was observed but only to a small extent, i.e. at doses that were 200-fold in excess to clinical meaningful exposures. TAF is unlikely to mediate the role of transporter-mediated drug interactions.

In terms of TAF acting as a substrate to drug transporters, TAF has been shown to be a substrate for intestinal efflux transporters, P-gp and BCRP. There is an increase in TAF absorption in the presence of cyclosporine A (CsA) and COBI (inhibitors of P-gp and BCRP) (Study Nos. AD-120-2037 and AD-120-2013). In a study completed in which dogs were orally administered TAF at 2 mg/kg following untreated or pretreated animals with 75 mg/kg CsA, there was increased exposure to TAF in the CsA pretreated animals, although this had no effect on the overall level of TFV present. The increased TAF plasma exposure led to an increase in levels of TFV-DP detected in PBMCs, suggesting that co-administration of TAF with an efflux transport inhibitor (i.e. COBI) would increase absorption and also result in higher levels of the active anti-viral substance, TFV-DP.

TAF was found to be a substrate for hepatic uptake transporters, OATP1B1 and OATP1B3. Exposure to TAF may be affected by inhibitors of these transporters or genetic polymorphisms that affect the transport activities. Unlike TFV, TAF was not a substrate for renal transporters, OAT1 and OAT3.

TFV:

The route of elimination of TFV is renal excretion by a combination of glomerular filtration and tubular secretion. In order to understand the role of transporters in the renal secretion of TFV and to explore potential drug interactions based on these transport systems, the interactions of TFV with a variety of both uptake and efflux transporters were studied *in vitro*.

Results of *in vitro* transport studies indicate that the active tubular secretion of TFV is mediated by human OAT1 (basolateral uptake) and MRP4 (apical efflux) transporters acting in series in proximal tubules (Study Nos. PC-103-2001, AD-104-2001, AD-104-2002). Human OAT3 may play a secondary role in the tubular uptake of TFV. Neither P-gp nor MRP2 appear to be involved in the efflux of TFV.

As the primary transporter handling the uptake of TFV, OAT1 has been assessed for its potential role in drug interactions between TFV and other renally secreted therapeutics including antibiotics, anti-inflammatory agents, and other antivirals (including PIs). Under physiologically relevant conditions, none of the tested drugs affected OAT1-mediated transport of TFV, indicating a low potential for renal interactions with TFV due to inhibition of this pathway (Study No. PC-104-2010 and Study No. PC-104-2011).

COBI also shows no detectable inhibition of human OAT1 or OAT3. Similarly, PIs ATV, LPV, and RTV did not exhibit any effect on the active cellular elimination of TFV mediated by the MRP4 efflux pump, and COBI is a very weak inhibitor of MRP4. The results of *in vitro* drug interaction studies indicate that PIs or COBI are unlikely to exert any substantial effect on the renal elimination of TFV in general or result in the accumulation of TFV in renal proximal tubules.

The results from *in vitro* studies investigating the contribution from MRP1 in tubular reabsorption of TFV (Study No. PC-104-2014) indicated that MRP1 is not involved in the reabsorption of TFV at the basolateral membrane of proximal tubule cells.

TFV did not inhibit the activity of human OCT2 or MATE1 (IC_{50} >300 µM) so TFV is unlikely to cause drug interactions through inhibition of these transporters (Study No. AD-104-2012).

E/C/F/TAF:

To complement the studies described for the individual components of E/C/F/TAF FDC, a number of transporter studies were conducted with the STB components, EVG, COBI, FTC, and TFV. The results from these studies have relevant impact on the TAF component as TFV is the major circulating metabolite of both TAF and TDF. The ability of each of the components in E/C/F/TAF FDC have also been summarised in Tables 9 and 10.

EVG, TAF, and TFV do not inhibit any of the transporters tested at clinically relevant concentrations *in vitro*, so are unlikely to pose any concern in this aspect of transporter-mediated drug interactions.

Renal excretion of TFV is facilitated by basolateral uptake by OAT1 and OAT3 and apical efflux by the MRP4 efflux transporter. There is no evidence for inhibition of TFV renal excretion by FTC, EVG, or COBI as they show weak or undetectable inhibition of OAT1, OAT3, and MRP4 *in vitro*. Transport of TFV by OAT1, OAT, and MRP4 was not inhibited by COBI under physiological conditions and clinically relevant concentrations (Study Nos. PC-236-2008 and PC-236-2009). In addition, COBI had no effect on the accumulation of TFV in human renal tissue slices at clinically relevant concentrations (Study No. PC-236-2007). Cobicistat is a weak to moderate inhibitor of OCT2, MATE2-K, and OCTN1, while COBI and EVG are more potent inhibitors of MATE1.

However, TAF is not an inhibitor of any of these transporters (Study No. AD-104-2012); therefore, there is low potential to further decrease the activity of the transporters relative to STB. The inhibition of BSEP by COBI is unlikely to be clinically meaningful as the IC_{50} (6.5 µM) is in excess of the total plasma Cmax of COBI.

COBI is a weak inhibitor of intestinal efflux transporters, but high concentrations of COBI in the intestinal lumen, achievable briefly during absorption, may inhibit P-gp and result in increase in TAF exposure. In the presence of 90 µM COBI in the Caco-2 permeability assay, TAF permeability increased 4.6-fold and the efflux ratio was significantly decreased, suggesting a P-gp mediated drug interaction (Study No. AD-120-2013).

Since both EVG and COBI are inhibitors and TAF is a substrate of OATP transporters *in vitro*, the exposure to TAF may be affected by EVG and COBI via inhibition of hepatic uptake. However, only a modest increase in exposure (not considered clinically relevant) of the OATP substrate, rosuvastatin, was observed when it was co-dosed with both EVG and COBI.

Table 9. Transporter Substrate Assessment of E/C/F/TAF Components

	Substrate Assessment (y/n)				
Transporter	EVG	COBI	FTC	TAF	TFV
P-gp	y	y	n	y	n
BCRP	y	y	n	y	n
OATP1B1	y	y	ND	y	ND
OATP1B3	y	y	ND	y	ND
OAT1	ND	ND	n	n	y
OAT3	ND	ND	y	n	y
OCT1	n	n	ND	n	n
OCT2	ND	y	n	ND	n
MRP1	ND	ND	ND	ND	n
MRP2	ND	ND	ND	ND	n
MRP3	ND	ND	n	ND	ND
MRP4	ND	ND	ND	ND	y

BCRP = breast cancer resistance protein; MDR1 = multidrug resistance protein 1; MRP1 2, 3, or 4 = multidrug resistance associated protein 1, 2, or 4; n = no; 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; y = yes

Table 10. Transporter Inhibition Assessment of E/C/F/TAF Components

Transporter	IC_{50} (μM)				
	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; MDR1 = multidrug resistance protein 1; 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

a Binding constant for uptake into CHO cells reported by Cihlar et al, 2009.

b Imaoka et al 2007

2.3.4. Toxicology

Tenofovir alafenamide (TAF) is a prodrug of Tenofovir (TFV). A comprehensive program of nonclinical studies has been conducted with EVG, COBI, and FTC in support of marketing authorisations for these medicinal products. Only new data relating to TAF will be discussed in this report. Tenofovir alafenamide was evaluated in mouse, rat, dog, and non-human primate repeat-dose toxicity studies up

to 39 weeks in duration. *In vitro* and *in vivo* genotoxicity studies were conducted. Rat fertility and developmental toxicity studies were conducted, along with developmental and reproductive toxicity studies and a local irritation study in the rabbit. The vehicle for toxicity studies used was 1) 25mM citric acid or 2) 0.5% polysorbate 20, 0.5% carboxymethylcellulose, 0.9% benzyl alcohol or 3) 0.1% (v/v) Tween 20 and 0.1% (v/v) hydroxypropylmethylcellulose (HPMC).

With agreement from Committee for Medicinal Products for Human Use (CHMP) (EMA/CHMP/SAWP/629722/2012; EMEA/H/SA/2410/1/2012/1), no carcinogenicity studies were conducted due to the lack of TAF exposure in rats and TgRasH2 mice and lower TFV exposure in rats and mice compared to the same studies in which TDF was administered. No peri/postnatal study was conducted based on scientific advice adopted by CHMP (EMA/CHMP/SAWP/ 214541/2013; EMEA/H/SA/2410/1/FU/1/2013/1). As with the carcinogenicity study rationale, there is an inability to measure plasma concentrations of TAF in rats, and TFV exposure after TAF administration was less than that already tested in the TDF peri/postnatal study.

The absence of nonclinical safety studies with the combination is in accordance with the CHMP Guideline on the Non-Clinical Development of Fixed Combinations of Medicinal Products (EMEA/CHMP/SWP/258498/2005, January 2008). 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.

In the development of TAF, 3 forms of the active drug substance were used: GS-7340, synonym for GS-7340 as the free base; GS-7340-02, synonym for GS-7340 as the monofumarate (1:1 molar ratio of free base to fumaric acid), and GS-7340-03, synonym for the hemifumarate (2:1 molar ratio of free base to fumaric acid). The hemifumarate, GS-7340-03 (TAF fumarate) is the selected for final development. It is stated that GS-7340-03 is considered comparable to GS-7340-02 based on physical/chemical properties and both exist as the free base in blood and biological fluids.

Repeat-dose toxicity studies used GS-7340-02 however the applicant states that any potential effects of the hemifumarate have been evaluated by studies of the monofumarate. The hemifumarate, GS-7340-03 was used in the male and female fertility study, dermal and ocular irritation studies, the local lymph node assay, and a second impurity qualification study.

1 µM TFV (GS-1278) = 0.287 µg/mL

1 ng/mL TFV = 3.48 nM

1 µM TAF (GS-7340) = 0.477 µg/mL

1 ng/mL TAF = 2.10 nM

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

Single dose toxicity

In male and female Sprague-Dawley rats (5/sex/group) given an oral dose (15 mL/kg) of TAF at 100, 300, or 1000 mg/kg (80, 240, 800 mg/kg free base equivalents [f.b.e.]/kg) followed by a 14-day observation period (study number: R990185) the NOAEL was considered to be 1000 mg/kg.

Male and female beagle dogs (1/sex/group) were given a single oral dose (15 mL/kg) of 30, 90, or 270 mg/kg (24, 72, 216 mg f.b.e./kg) TAF followed by a 14-day observation period (Study number: D990181). In-life observations of salivation, vomiting, reduced activity, tremors, incoordination seen at 270 mg/kg which resolved 2 days following dosing. There was an increase in blood urea nitrogen at 270 mg/kg (present on study Day 2, not study Day 14. Thymus weights were at all doses compared with controls, and thymic atrophy was present in males at 90 and 270 mg/kg. Renal tubular changes characterised by basophilia and/or karyomegaly were present in the male at 270 mg/kg and females at 270 mg/kg and 90 mg/kg. The NOAEL as considered to be 30 mg/kg.

Repeat dose toxicity

Mouse

A two week mouse study was conducted; however the results were not interpretable due to a large number of confirmed gavage errors and the viscosity of the formulation.

13-Week GLP Oral Mouse Toxicity Study (study number TX-120-2007)

Crl:CD1(ICR) mice (15/sex/group) were given 10, 30 or 100 mg/kg/day (8, 24, 80 mg f.b.e./kg/day). The vehicle used was 0.1% (v/v) Tween 20 and 0.1% (v/v) hydroxypropylmethylcellulose (HPMC). Reduced body weight gain was seen at 100 mg/kg/day in males and at all doses in females. Reduced

food consumption was noted at 30 and 100 mg/kg/day. In the nasal turbinates an increased incidence and severity of minimal to slight infiltrates of neutrophils in respiratory and olfactory mucosa, and minimal to moderate (100 mg/kg/day only) degeneration of olfactory epithelium was seen in both sexes at all doses. In addition adverse findings were noted in the nasal turbinates (exudate in the lumen) of both sexes at 30 mg/kg/day and 100 mg/kg/day. Minimal infiltrates and minimal olfactory degeneration were observed at a lower incidence in control animals. Minimal increased apoptosis of the rectum was seen in males and females at 100 mg/kg/day. No NOAEL could be determined. Due to limited concentration data for TAF, AUC values could not be calculated. At week 13, the TFV AUCtau (combined sexes) was 0.213 µgh/mL at 10 mg/kg/day.

Table 11. Mean Repeat-Dose Toxicokinetics of TFV Following Oral Administration of GS-7340-02 in Mice for 13 Weeks

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

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

Rat

4-Week Oral Rat Toxicity Study (Study number R990182)

Daily oral administration of GS-7340-02 (15 mL/kg) at 1.5, 6.25, 25, 100, and 400 mg/kg/day (1.2, 5, 20, 80, 320 mg free base equivalents (f.b.e.)/kg/day) to SD rats (10/sex/group) for 28 days resulted in decreased body weight gain, reduced food consumption, decreases in white blood cell (WBC) and RBC parameters, calciuria, decreased bone mineral density (BMD), decreased 1,25 dihydroxy vitamin D3, renal karyomegaly, thymic atrophy, and atrophy of cancellous bone of the femur. Most effects were seen at 400-mg/kg/day group; however, some changes were noted at 25 mg/kg/day with a non-significant decrease in 1,25 dihydroxy vitamin D3 observed at 6.25 mg/kg/day. Based on changes in WBCs, the NOAEL was considered to be 6.25 mg/kg/day (no change in WBC count was observed at doses up to 100 mg/kg/day in the subsequent 26-week rat toxicity study – see below).

26-Week Oral Rat Toxicity Study (TOX-120-001)

Daily oral administration of TAF (GS-7340-02) at 5, 25, and 100 mg/kg/day (4, 20, 80 mg f.b.e./kg/day) to SD rats (15/sex/group) for 26 weeks resulted in minimal renal cortical tubular karyomegaly (100 mg/kg/day) and minimal to slight tibial cancellous bone atrophy (females, 100 mg/kg/day), changes in bone density measurements (100 mg/kg/day) and changes in biochemical markers of bone turnover (25 and 100 mg/kg/day) were also noted. These effects were not observed at 5 mg/kg/day. TAF (GS-7340-02) dose-dependently increase biochemical markers of bone turnover in males and dose-independently decrease serum 1,25-dihydroxy- and 25-hydroxyvitamin D3 in both sexes at 25 and 100 mg/kg/day. It is stated that as the effects (increases in biochemical markers of bone turnover and changes in related hormones) seen at 25 mg/kg/day were minimal, it was concluded that the NOAEL was 25 mg/kg/day.

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

Table 12. Mean repeat-dose pharmacokinetics of TFV following oral administration of GS-7340-02 in male and female rats for 26 weeks

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

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

Rabbits (Study number TX-120-2003)

Daily oral administration of TAF (GS-7340-02) at 20, 50 and 75 mg/kg/day to female rabbits, for 7 days, was generally well tolerated. Plasma exposure to TAF and TFV generally increased with increase in dose level from 20 to 75 mg/kg/day. Values for mean Cmax and AUC0-t of TFV were generally higher on Day 7 than on Day 1. TAF was rapidly and extensively converted to TFV. The mean TAF AUC0-t on day 7 was unable to be calculated at 20 mg/kg/day (due to values below the lower limit of quantitation of 1.00 ng/mL) and was 0.252 and 1.174 µg·hr/mL at 50 and 75 mg/kg/day, respectively. The mean TFV AUC0-t on day 7 was 2.256, 5.741 and 10.070 µg·hr/mL at 20, 50, and 75 mg/kg/day, respectively.

Dog

4-Week Oral Toxicity Study (Study number: D990175)

Daily oral administration of TAF (GS-7340-02) at 0.1, 0.3, 1, 3, or 10 mg/kg/day (0.08, 0.24, 0.8, 2.4, 8 mg f.b.e./kg/day) to male and female beagle dogs (4/sex/group) for 28 days resulted in increased AST in females at 10 mg/kg/day and renal tubular karyomegaly and/or basophilia in both sexes at 10 mg/kg/day and 1 male and 1 female at 3 mg/kg/day. Mean values for bone specific alkaline phosphatase, N telopeptide, parathyroid hormone, 1,25 dihydroxyvitamin D and 25 hydroxyvitamin D were generally similar across all groups. There were no effects on peripheral quantitative computed tomography-derived bone densitometry parameters (eg, bone mineral content and bone mineral density of the total slice and trabecular and cortical/subcortical compartments). The NOAEL was considered to be 1 mg/kg/day.

At the lower doses, only Cmax and Tmax values for TAF were determined as most values were below the lower limit of quantitation of the assay. TAF was rapidly absorbed on Day 1, with median peak values within 0.25 to 0.5 hours of 18.5, 38.7, and 0.582 µg/mL at 1.0, 3.0, and 10 mg/kg/day,

respectively. Peak TFV concentrations occurred within 1 hour. At 10 mg/kg/day, Day 28 TFV Cmax and AUCtau were 0.44 µg/mL and 5.26 µgµh/mL, respectively (males and females combined). Comparisons between Day 1 and Day 28 at 10 mg/kg/day showed potential accumulation upon repeat dosing. Tenofovir in PBMCs was measurable (18.6 µg/mL) after 28 days of 10 mg/kg/day TAF.

Table 13. Median repeat-dose pharmacokinetics of TFV and TAF

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

NC= insufficient data to calculate

PBMC = peripheral blood mononuclear cell

39-Week Oral Toxicity Study with a 3 month recovery period (Study number: TOX-120-002). Male and female beagle dogs were administered daily oral doses (10 mL/kg) of TAF (GS-7340-02) at 2, 6, or 18/12 mg/kg/day (1.6, 4.8, 14.4/9.6 mg f.b.e./kg/day) for 13 weeks (2/sex/group) or 39 weeks (4/sex/group). A dose-related decrease in body weight gain at 39 weeks was seen in all males at all doses and for females at 18/12 mg/kg/day. The dose for the high dose group was reduced from 18 to 12 mg/kg/day on Days 45 and 51, for males and females, respectively, due to the occurrence of severe clinical signs and reduced body weight.

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

Increased mean AST (~2.6x compared to control) and total bilirubin (~1.6x compared to control) in dogs administered 18/12 mg/kg/day. No ECG changes occurred at 2 mg/kg/day. At Week 39, a dose-related prolongation of PR interval was observed at 6 (~ +13%) and 18/12-mg/kg/day (~ +24%) groups. TAF reduce heart rate with an associated QT interval prolongation was seen at 18/12 mg/kg/day. According to the Applicant these changes were associated with decreases in serum triiodothyronine (T3). After the 13-week recovery period, serum T3 values returned to levels similar to the controls.

The applicant states that all bone markers showed age-related decreases. After 3 months, there were some differences noted among mean values for bone formation (skeletal alkaline phosphatase [sALP]) and bone resorption markers (urinary free deoxypyridinoline and N telopeptide) at all doses compared to controls. After 9 months, statistically significant increases in mean values for the bone resorption marker urinary N telopeptide were noted for both sexes at 18/12 mg/kg/day ($p \leq 0.05$), compared to controls. A similar though not statistically significant trend was noted in animals at 6 mg/kg/day, suggesting a dose-related response. No significant changes in free deoxypyridinoline were observed,

with no consistent effects (increases) among treated groups. For the formation marker, serum sALP values at all doses were comparable controls except for one male at 18/12 mg/kg/day, which was outside the control ranges. At the end of the recovery period, bone marker values returned to below the control range consistent with an age effect and recovery from treatment.

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

Histopathology changes were noted in the kidneys, eyes, lungs, and spleen after both 13 and 39 weeks. The liver and possibly the adrenal glands were additional target organs identified after 39 weeks. After 13-weeks of treatment, findings of renal cortical tubular degeneration/regeneration and karyomegaly were seen at 6 or 18/12 mg/kg/day; findings after 39 weeks of treatment were similar. These changes were minimal to slight (Grade 1 to 2) at 6 mg/kg/day in both sexes. At 18/12 mg/kg/day severity ranged from mild to moderate (Grade 2 to 3). After 39-weeks of treatment, similar lesions (minimal (Grade 1 karyomegaly and tubular degeneration) were seen in 2 males at 2 mg/kg/day.

A minimal to slight (Grade 1 to 2) infiltration of mononuclear cells in the ocular posterior uvea was noted in some animals at 18/12 mg/kg/day after both treatment periods. Alveolar histiocytosis was present in the lungs after 13-weeks at 18/12 mg/kg/day. Additional pulmonary findings noted following 39-weeks of treatment and consisted of macrophage accumulation with pigment, which was detected predominantly at18/12 mg/kg/day and in few animals at 6- or 2-mg/kg/day. An infiltration of macrophages laden with pigment was very frequently seen in the splenic white pulp at 18/12 mg/kg/day after both treatment periods. After 39 weeks of treatment centrilobular hepatocellular cytoplasmic acidophilic inclusions were seen at 18/12 mg/kg/day, pigment deposits in hepatic macrophages and/or sinusoidal cells (Kupffer cells) was seen at 18/12 mg/kg/day. Also, similar pigment deposits in the sinusoidal cells (tissue macrophages) of the adrenal glands were seen in a few animals at 18/12 mg/kg/day. The cause of the intracellular pigment in tissue macrophages in the lung, liver, spleen, and adrenal is not known but could represent accumulation of the test article and/or test article metabolite(s) in these cells of the mononuclear phagocyte system. After the 13-week recovery period test article-related histological changes were still present in the kidneys, lungs, and liver however were reduced in incidence and severity.

At 18/12 mg/kg/day, a minimal infiltration of histiocytes was present in some organs (eye [choroid plexus, ciliary body], lung, and spleen) in some animals. In-life ophthalmologic examinations were normal.

The NOAEL after 39 weeks of treatment was considered to be 2 mg/kg/day. Treatment-related findings were completely or partially reversible following a 13-week recovery period.

Table 14. Mean pharmacokinetics of TFV and TAF

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

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

Non-human primate

4-Week Oral Rhesus Monkey Toxicity Study (Study number P2000114)
Animals were given TAF (GS-7340-02) at 3 or 30 mg/kg/day (2.4, 24 mg f.b.e./kg/day) or TFV at 15 mg/kg/day. According to the company there were no adverse in-life effects and no clear test article-related effects on body weight, serum chemistry, plasma chemistry, haematology (including lymphocyte subsets determined by flow cytometry), standard urinalysis parameters, organ weights, and bone-related or histologic parameters. There was 1 death at 30-mg/kg/day TAF, which was not considered test article-related (no further details are provided on the toxicology summary). Kidney, liver, and skeletal muscle samples assayed for indicators of mitochondrial integrity showed no effects. The NOAEL for TAF was considered to be 30 mg/kg/day.

The TAF C_{max} values were nonlinear with dose, with greater than expected increases in C_{max} with dose. The TAF AUC_{tau} could only be calculated at 30-mg/kg/day group, with a mean value of 1.03 µg·h/mL and a terminal elimination half-life of 0.335 hours. There was no sex difference.

Table 15. Mean Pharmacokinetics vales for TAF

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

NC = insufficient data to calculate

Day 28 TFV C_{max} and AUC exhibited slightly greater than proportional increases with increasing dose. Comparison between Days 1 and 28 showed no statistical difference for Cmax or AUC indicating no change in clearance over time. There was no sex difference.

Table 16. Mean Pharmacokinetics of TFV

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

Genotoxicity

A summary of genotoxicity studies and their results is illustrated in Table 17.

Table 17. Genotoxicity study results

Study	**Test system**	**Concentrations/ Concentration range/ Metabolising system**	**Results**
Gene mutations in bacteria – GLP Study number: V990212	TA98, TA100, TA1535, TA1537 & WP2uvrA	100, 333, 1000,3330, 5000 µg/plate +/- S9	Negative
Mouse Lymphoma – GLP Study number: V990213	L5178Y/TK	Up to 5000 µg/mL (4000 µg f.b.e/mL), +/- S9	Negative
Mouse Micronucleus – GLP Study number: M2000113	Male Mouse/CD-1(ICR) BR	500 and 1000 mg/kg (400 and 800 mg f.b.e./kg) & 2000 mg/kg	Negative

Carcinogenicity

Elvitegravir, FTC and TDF have all demonstrated low carcinogenic potential in conventional 2-year studies.

COBI was not carcinogenic at exposures that were 7-to 16-fold higher than those observed clinically. In the rat, following repeated oral administration for a minimum of 97 weeks at 10, 25, and 50 mg/kg/day (males) and 5, 15, and 30 mg/kg/day (females), COBI caused an increased incidence of combined thyroid follicular cell adenoma and carcinomas at exposures (AUC) that were lower than that observed clinically. It is acknowledged that the thyroid and liver changes are considered adaptive changes, secondary to hepatic microsomal enzyme induction due to activation of PXR. Given that this extent of activation of PXR and CYP3A does not occur at clinically relevant concentrations in humans, COBI is not considered to pose a carcinogenic risk in man.

Based on the scientific advice adopted by the CHMP (EMEA/H/SA/2410/1/2012/1), carcinogenicity studies are not required for TAF. Indeed, TAF is not genotoxic, the lack pre-neoplastic lesions in repeat-dose studies especially in dog where higher exposure to this prodrug was reached (8-fold the human recommended dose based on AUC), the low exposure to TAF in rodents make long term carcinogenicity studies with sufficient exposure unfeasible, the irrelevant TgRasH2 mouse model for carcinogenicity testing with TAF (limiting pharmacokinetic factor), the lack special hazard for humans based on carcinogenicity studies with tenofovir disoproxil fumarate and by the fact that TAF is rapidly converted to tenofovir and therefore disappears relatively quickly from plasma in humans ($_{Tmax}$ 1 h).

Given the lack of genotoxicity for each compound (EVG/COBI/FTC/TAF), the low carcinogenic potential for EVG, FTC, COBI and TDF, it is considered unlikely that new combination dosing would change this profile. Therefore the conduct of carcinogenicity study with the EVG/COBI/FTC/TAF combination is not considered necessary.

Reproduction Toxicity

Oral Fertility and General Reproduction Toxicity Study of TAF in Sprague-Dawley Rats (Study number TX-120-2012).

Male and female Crl:CD(SD) rats 20, 80, or 160 mg free base equivalent (f.b.e.)/kg/day (22, 90, 180 mg GS-7340-03/kg/day) TFV (GS-7340-03). Males were necropsied after at least 10 weeks of dosing, the reproductive organs were weighed followed by assessment of sperm motility and total concentration. Male and female reproductive performance was evaluated based on results of confirmation of mating and pregnancy. There were some effects on male body weight at 80 and 160 mg/kg/day and female at 160 mg/kg/day throughout the study.

There were no differences in premating estrous cycles. There were no test article-related differences in male or female reproductive parameters. There were no test article-related effects on the uterine and fetal parameters and no significant differences in female reproductive organ weights. There was a slight increase in absolute testis weight (significant increase (9%) in the adjusted mean of the left testis only) at 160 mg/kg/day. This was considered by the Applicant to be test article-related but not adverse, as there were no other reproductive organ weight or functional reproductive effects. There were no test article-related effects observed on mean epididymal sperm motility or on sperm concentration. The NOAEL for male and female toxicity was 80 mg/kg/day. The TAF NOAEL for reproductive and early embryonic toxicity was 160 mg/kg/day.

Oral Embryo-Fetal Development Study of TAF in Rats (Study number: TX-120-2002)

Four groups of 25 pregnant female Crl:CD(SD) rats were given daily doses of TAF (GS-7340-02), by oral gavage, from GD 6 to 17, inclusive. Targeted dose levels were 0 (vehicle control), 25, 100 and 250 mg/kg/day. Dose formulation analysis showed that each 5 mg/kg/day animal was administered a GS-7340-02 concentration of 3.85 mg/mL instead of 5 mg/mL for 5 to 8 days between GD 10 and 17, providing a daily dose of 19.3 mg/kg/day (77% of targeted dose) on these days. Dose formulation analysis showed that each 20 mg/kg/day animal was administered a GS-7340-02 concentration of 12.9 mg/mL instead of 20 mg/mL for 4 to 7 days between GD 6 and 12, providing a daily dose of 64.6 mg/kg/day (65% of targeted dose) on these days.

At 250 mg/kg/day 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. At this dose 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 controls. 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 at 250 mg/kg/day, with the corrected mean body weights also 10% lower than controls on GD 21. Fetal weights (males, females and sexes combined) were decreased dose dependently and remained within the range of historical control data, however fetal weights at 250 mg/kg/day were at the lower extreme of this range. The incidences of fetal major malformations, minor external, visceral and skeletal anomalies and were not affected by TAF. Sternebrae variants (1 to 4 and 5 and 6) were increased at 250 mg/kg/day.

In summary, at 250 mg/kg/day, there was decreased fetal body weight associated with some delays in the rate of ossification. There was no evidence of embryolethality or teratogenicity attributed to TAF in this study. The maternal TAF NOAEL and the TAF NOAEL for embryo-fetal development were both considered to be 100 mg/kg/day, which resulted in GD17 AUC0-t values of 17.4 and 0.2 µg·hr/mL for TFV and TAF, respectively.

Plasma concentrations of TAF were all below the lower limit of quantitation at 25 mg/kg/day. Exposure to TAF increased with the increasing dose from 25 to 250 mg/kg/day. Exposure to TFV increased with the increase in TAF dose from 25 to 250 mg/kg/day.

Table 18. Mean Toxicokinetic Parameters of TFV and TAF in Pregnant Rats on Gestation Days (GD) 6 and 17

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

NC: not calculated due to insufficient data

Oral Embryo-Fetal Development Study of TAF in Rabbits (TX-120-2005)

TAF (GS-7430-02) was administered by oral gavage to time-mated F0 generation female rabbits (20 main study females per group and 3 toxicokinetic females per group) at 0 (vehicle control), 10, 30 and 100 mg/kg/day. Lower body weight gains were noted at 100 mg/kg/day for the first week following treatment initiation. Lower food intake was noted at 100 mg/kg/day from GD 8 to 24. Three animals at this dose consumed less than 30 g for at least 4 days during the dosing period. There were no TAF-related macroscopic changes. The number of corpora lutea, implantation sites, live fetuses, dead fetuses, resorptions, the sex ratio and the pre and post implantation losses were not affected. There was no effect of TAF on fetal weights. The incidence of major malformations, minor external, visceral, skeletal anomalies and common skeletal variants were not affected by TAF.

Exposure to TAF increased increasing dose (10 to 100 mg/kg/day). The increases in Cmax were greater than proportional between 10 to 100 mg/kg/day and the increases in AUC0-t were greater than proportional between 30 to 100 mg/kg/day on GD 20. Exposure to TFV increased with increasing TAF doses from 10 to 100 mg/kg/day. The increases in Cmax and AUC0-t were roughly proportional between the 10 to 100 mg/kg/day. Accumulation of TFV was observed after multiple dosing.

Concentrations of TFV were higher than concentrations of TAF, indicating that TAF was extensively converted to TFV. The TAF NOAEL for maternal toxicity was 30 mg/kg/day (AUC0-t = 1.1 and 5.0 µg·h/mL for TAF and TFV, respectively) and the TAF NOEL for embryo-fetal development was 100 mg/kg/day (AUC0-t = 11.0 and 27.3 µg·h/mL for TAF and TFV, respectively.

Table 19. Mean toxicokinetic parameters of TFV and TAF in pregnant rabbits on gestation days (GD) 7 and 20

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

NC= not calculated due to insufficient data

Prenatal and postnatal development, including maternal function

Based on the scientific advice adopted by CHMP(EMA/CHMP/SAWP/214541/2013; EMEA/H/SA/2410/1/FU/1/2013/1), a peri/postnatal study in rats is not required for TAF registration due to the lack of TAF exposure in rats and lower TFV exposure compared to TDF.

Table 20. Estimated safety margins of TAF based on AUCss when comparing animal no-adverse-effect-level (NOAEL)

Study	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]
Fertility[c]	Rat	Up to 10 weeks	160	N/A	N/A
Embryo fetal Development[c]	Rat	12 days	84	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)	150 (TDF)	150 (TDF)	27/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 µg.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 µg.h/mL

c NOAEL for reproductive endpoints provided; AUC data is for maternal exposure; the peri/postnatal study was conducted with TDF not TAF

Local Tolerance

In a bovine corneal opacity and permeability assay (BCOP) TAF (GS-7340-03) elicited an *in vitro* irritancy score of 21.0 ± 8.7 with a 4-hour incubation and was predicted to be a noncorrosive/non-severe eye irritant.

In a dermal irritation study in rabbits animals were given a single 4 hour, semi-occlusive, dermal administration of approximately 0.5 g of TAF (GS-7340-03 and were observed for 4 days (Study

number: TX-120-2011). No local dermal reaction was observed in any animal throughout the duration of the study. The Primary Irritation Index was calculated to be 0.0; TAF was classified as a 'non-irritant'.

Other toxicity studies

Antigenicity

Female mice were given TAF (GS-7340-03) at of 10, 25 or 50% w/v. The animals were administered TAF by daily application of 25 µL of the appropriate concentration or control (vehicle or positive), to the dorsal surface of both ears for 3 consecutive days. The proliferative response of the lymph node cells (LNCs) from the draining auricular lymph nodes was assessed 5 days following the initial application, by measurement of the incorporation of 3H-methyl thymidine (3HTdR) by β-scintillation counting of LNC suspensions. The response was expressed as radioactive disintegrations per minute per lymph node (dpm/node) and as the ratio of 3HTdR incorporation into LNC of test nodes relative to that recorded for control nodes (test/control ratio), termed as SI. The test substance is regarded as a sensitizer if at least one concentration of the chemical has a SI of 3 or more. The SI obtained for 10%, 25%, and 50% w/v were 0.9, 1.0, and 1.0, respectively, which indicates that TAF did not show the potential to induce skin sensitisation. The EC3 value (the "estimated concentration of 3" is the concentration of test substance which would result in a SI of 3 in the LLNA) was determined to be higher than 50% w/v. The SI for the positive control substance hexyl cinnamic aldehyde was 6.3, which demonstrates the validity of this study.

Studies on impurities

Two studies were conducted to evaluate the toxicity and to qualify potential impurities of TAF. In the first male SD rats were given TAF (GS-7340-02) at 5 or 50 mg/kg/day from 2 different purity lots (Lot No. 1 - 97.7% and Lot No. 2 - 83.1%) for 14 days. The impurities, including 13% GS-7339, were added to the more pure lot. Administration of 97.7% pure and 83.1% pure GS-7340-02 by oral gavage for 14 days to male rats was well tolerated. No test article-related findings were noted, and no differences were found between the 2 lots tested. At 50 mg/kg/day on Day 14, the TFV Cmax was 218 ng/mL and the AUC0-t was 1965 ng·hr/mL for 97.7% pure GS-7340-02 (Lot No. 1:7340-02-AC-1EA) and the TFV Cmax was 395 ng/mL and the AUC0-t was 2481 ng·hr/mL for 83.1% pure GS-7340-02 (Lot No. 2: 7339-AZS-27-1094-41).

In the second study male and female rats were given oral doses of 25 and 50 mg/kg/day (free base equivalents) for 29 days. Test article 1 was 99.3% pure GS-7340-03. Test article 2 was 98% pure GS-7340-03 containing 11 spiked impurities. Test article 3 was 97.8% pure GS-7340-03 containing 4 spiked impurities. Administration of GS-7340-03 drug substance with 3 different impurity profiles was well tolerated. There were no significant in-life or histopathological differences between the 3 lots tested.

Several degradation products related to COBI and FTC have been identified in batches of EVG/COBI/FTC/TAF tablets and all of them have been observed in batches of EVG/COBI/FTC/TDF tablets. Some of these products were tested in a 28-day toxicity study in rats using non-degraded and degraded EVG/COBI tablets. The degradation products of TAF observed in the EVG/COBI/FTC/TAF tablets is consistent with those in the TAF drug substance. There are no unique impurities or degradants in the EVG/COBI/FTC/TAF tablets.

The impurities and degradation products present in EVG, COBI, FTC, and TAF and in EVG/COBI/FTC/TAF tablets have been qualified through toxicology studies which employed drug substance from normal productions batches, laboratory scale batches with enhanced levels of impurities, or samples subjected to forced degradation conditions (high heat and humidity).

2.3.5. Ecotoxicity / environmental risk assessment

In accordance with the Guideline on the Environmental Risk Assessment of Medicinal Products for Human use [EMEA/CHMP/SWP/4447/00], a full environmental risk assessment (ERA) has been performed.

Summary of main study results

Study / Active Substance	EVG	COBI	FTC	TAF (as environmentally relevant TFV)
n-octanol/water partition coefficient (Log Kow)	3.39 – 4.33 (OECD 117) <4.5 Not bioaccumulative	3.05 – 4.10 (OECD 117) <4.5 Not bioaccumulative	-0.693 – -0.670 (OECD 107) <4.5 Not bioaccumulative	-3.8 - -4.3 (OECD 107) <4.5 Not bioaccumulative
Ready Biodegradation (OECD 301)	Not readily biodegradable (28d: 0-2.5% mineralisation)	Not readily biodegradable	Not readily biodegradable	Not readily biodegradable
Transformation in Sediment/Water Systems (OECD 308 & & OCSPP 835.1110)	>10% AR associated with sediment from Day 7; System DT_{50} 6-53 days Water DT_{50} 2-3 days Sediment DT_{50} (degradation) >100 days No significant metabolites formed.	>10% AR associated with sediment from Day 7; DT_{50} (dissipation) 171-241 days; Water DT_{50} 5.6-12 days Sediment DT_{50} (deg) >100 days No significant metabolites formed.	>10% AR associated with sediment from Day 7; DT_{50} (dissipation) 36-151 days; DT_{50} (degradation) > 100 days; No significant metabolites formed.	>10% AR associated with sediment from Day 7; DT_{50} (degradation) 10.4-32.7 days; Water DT_{50} (dissipation) 2.0-3.5 days; Three significant metabolites formed.
Adsorption / desorption (OECD 106)	K_{oc} soil 25,500 – 104,000 L/kg K_d sludge 10,400 L/kg	K_{oc} soil 3,624 – 9,012 L/kg K_d sludge 830 – 1,287 L/kg	K_d sludge 12.9 L/kg	K_{oc} ads soil 351 - 1091 L/kg K_{oc} des soil 968 - 2791 L/kg K_F ads sludge 6.0 - 21 L/kg K_F des sludge 16 - 62 L/kg
Sewage microorganisms (OECD 209)	NOEC ≥ 500 mg/L	NOEC ≥ 1000 mg/L	NOEC ≥ 1000 mg/L	NOEC ≥1000 mg/L
Pimephales promelas (OECD 210)	NOEC 206 µg/L	NOEC 4.84 mg/L	NOEC 6.10 mg/L	NOEC ≥10 mg/L
Daphnia magna (OECD 211)	NOEC 390 µg/L	NOEC 17.5 mg/L	NOEC 110 mg/L	NOEC 100 mg/L
Pseudokirchneriella subcapitata (OECD 201)	NOEC 162 µg/L	NOEC 29.3 mg/L	NOEC 110 mg/L	NOEC 32 mg/L
Bioconcentration in Fish (OECD 305)	Not bioaccumulative	Not bioaccumulative	Not required	Not required
Toxicity to sediment dwellers (OECD 218/225)	*Lumbriculus* (OECD 225) NOEC ≥1000 mg/kg_{dwt} (normalised for 10% OC ≥4167 mg/kg_{dwt})	*Chironomus.sp* (OECD 218): NOEC 100 mg/kg_{dwt} (normalised for 10% OC 1250 mg/kg_{dwt})	*Chironomus sp* (OECD 218) NOEC 38 mg/kg_{dwt} (normalised for 10% OC 200 mg/kg_{dwt})	*Chironomus.sp* (OECD 218) NOEC 290 mg/kg_{dwt} (normalised for 10% OC 17.06 mg/kg_{dwt})

Acute Toxicity to Earthworms (OECD 207)	LC_{50} > 1000 mg/kg_{dwt}	Not required	Not required	Not required
Collembolan Reproduction Test (OECD 232)	NOEC ≥ 1000 mg/kg_{dwt}	Not required	Not required	Not required
Soil Microbes: Nitrogen Transformation Test (OECD 218)	NOEC ≥ 1000 mg/kg_{dwt}	Not required	Not required	Not required
Seedling Emergence and Growth Test (OECD 208)	NOEC ≥ 5 mg/kg_{dwt}	Not required	Not required	Not required
Soil Transformation Test (OECD 307)	DT_{50} > 1000 days. One significant transformation product	Not required	Not required	Not required

AR = applied radioactivity;

OC = organic carbon;

NOEC = no observed effect concentration;

DT50 = dissipation (dis.) /degradation (deg.) half life;

Koc = organic carbon normalised adsorption coefficient;

kd = adsorption coefficient

2.3.6. Discussion on non-clinical aspects

Detailed nonclinical data have been submitted in support of this application for E/C/F/TAF, including study reports for each of the individual components EVG, COBI, FTC and TAF. Combination studies are also provided for the EVG/COBI combination, FTC/TDF combination and the E/C/F/TAF combination. Nonclinical virology studies of EVG, COBI, FTC and TFV/TAF/TDF were also provided.

The pharmacodynamics of TAF has been reviewed in both in-vitro and ex-vivo studies, demonstrating its uptake into PBMCs, conversion to tenofovir and metabolism to its active form of tenofovir disphosphate (TFV-DP). TAF has demonstrated anti-HIV activity in studies using lymphoid T-cells, primary human PBMCs, and macrophages. There would be limited impact of substituting TAF for TDF in the combination product based on studies examining cytotoxicity, off target toxicity, changes in mitochondrial function or of metabolism toxicity. Safety pharmacology studies revealed no significant concerns for TAF over and above that already established for other components in the combination product.

The absorption, distribution, metabolism and excretion of EVG, COBI, FTC and TFV/TAF were evaluated *in vitro* and in a variety of animal models. In addition, the drug-drug interaction profile was evaluated. The pharmacokinetics of the E/C/F/TAF FDC is discussed based on the results of nonclinical studies completed with the individual agents; no additional pharmacokinetic studies have been conducted for the E/C/F/TAF combination.

The kidney and bone findings seen in the rat and dog toxicology studies are known toxicities of TFV. This has been adequately addressed in the product literature.

Prolonged PR intervals (approximately 13% to 24%) with associated QT interval prolongation were noted in the 39-week dog study. 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 or in the clinical thorough QT study.

At 18/12 mg/kg/day in dogs (39-week dog study), a minimal infiltration of histiocytes was present in some organs (eye [choroid plexus, ciliary body], lung, and spleen) in some animals. The posterior uveitis seen at the high dose (18/12 mg/kg) corresponds to 9 and 40-fold the human recommended dose based on TAF and TFV AUC respectively. In-life ophthalmologic examinations were normal in this study. There were no test article-related effects on ophthalmic exams or microscopic exams of ocular tissue observed in repeat-dose toxicity studies in mice (up to 13 weeks), rats (up to 26 weeks), nonhuman primates (4 weeks, or in the 4-week dog toxicology study. Although clinical data do not identify any cases of posterior uveitis, these data are limited and a potential clinical risk for ocular effects is reflected in the RMP.

Adverse degenerative (olfactory) and acute inflammatory (infiltrate neutrophil) changes in the nasal mucosa were noted in mice given TAF for 13 weeks. Given that these findings were not seen in any other species or in rats for longer durations of administration it can be agreed that they probably do not pose a clinical risk.

In the rat fertility and reproductive toxicology study an increase in absolute testis weight (significant increase [9%] in the adjusted mean of the left testis only) was seen at 160 mg/kg/day. This was considered by the applicant to be test article-related but not adverse, as there were no other reproductive organ weight or functional reproductive effects. Sternebrae variants (1 to 4 and 5 and 6) were increased at 250 mg/kg/day in the rat embryo-fetal development study (the NOEL was

considered to be 100 mg/kg/day [84 mg/kg/day achieved]). There were no effects seen the embryo-foetal development study in rabbits.

The product information states that both sexes should use contraception and female patients of childbearing potential should use either a hormonal contraceptive containing at least 30 µg ethinyloestradiol and containing norgestimate as the progestagen or should use an alternative reliable method of contraception. The effect of co-administration of the combination with oral contraceptives containing progestagens other than norgestimate is not known and, therefore, should be avoided. This is considered acceptable.

As has been demonstrated for other products containing EVG and COBI, EVG has been shown to be persistent in soil, although has demonstrated low toxicity to terrestrial and sediment dwelling species, and should not pose a risk to the environment. COBI does not bioaccumulate in aquatic organisms, and given the low toxicity of COBI to aquatic organisms, there should be no significant risk to the environment. FTC does not bioaccumulate and is of low risk to the aquatic or terrestrial environments.

TAF is a next generation pro-drug of tenofovir (TFV) and TFV has been shown to have low toxicity to aquatic organisms, no bioaccumulation and should pose no significant risk to the environment.

2.3.7. Conclusion on the non-clinical aspects

No major concerns have been identified from the nonclinical data. The applicant has submitted data to characterise TAF, which showed a similar toxicity profile to TDF which is overall qualitatively similar than TDF since mainly characterized by kidney (karyomegaly, tubular degeneration), bone. These correlate with the known clinical toxicities of TDF. Safety margins are higher for TAF in comparison to TDF.

2.4. Clinical aspects

2.4.1. Introduction

E/C/F/TAF is essentially Stribild (STB) in which TDF is substituted with tenofovir alafenamide [TAF; GS-7340]) that allows for ingestion of a very much lower oral dose compared to TD (10 mg vs. 245 mg). TAF is presented for clinical use as the fumarate (11 mg GS-7340-03 vs. 300 mg TDF in STB) but all doses reported below refer to the TAF content of the various formulations. The focus of the evaluation is on the following studies conducted with TAF alone or in FDCs with other agents in healthy subjects and HIV-infected patients:

Table 21. E/C/F/TAF Studies

Study	Dosage Form	Dose	n	Control or Co-administered
GS-US-292-0103 Phase 1 selection of formulation study	E/C/F/TAF FDC 150/150/200/10-mg tablet	150/150/ 200/10 mg	33	EVG 150-mg tablet, COBI 150-mg tablet, FTC 200-mg capsule TAF 25-mg tablet
GS-US-292-0101 Phase 1 selection of formulation study	E/C/F/TAF FDC 150/150/200/25-mg tablet E/C/F/TAF FDC 150/150/200/40-mg tablet (2 formulations each)	150/150/ 200/25 mg 150/150/ 200/40 mg	38	Stribild (EVG/COBI/FTC/TDF) 150/150/200/300-mg tablet TAF 25-mg tablet
GS-US-292-0102 Phase 2; ART-naive	E/C/F/TAF FDC 150/150/200/10-mg tablet	150/150/ 200/10 mg	112	E/C/F/TAF placebo tablet STB and matching placebo tablet
GS-US-292-0104 Phase 3; ART-naive	E/C/F/TAF FDC 150/150/200/10-mg tablet	150/150/ 200/10 mg	435	E/C/F/TAF placebo tablet STB and matching placebo tablet
GS-US-292-0111 Phase 3; ART-naive	E/C/F/TAF FDC 150/150/200/10-mg tablet	150/150/ 200/10 mg	431	E/C/F/TAF placebo tablet STB and matching placebo tablet
GS-US-292-0106 Phase 2/3; ART-naive	E/C/F/TAF FDC 150/150/200/10-mg tablet	150/150/ 200/10 mg	48	Not applicable
GS-US-292-0112 Phase 3; renally impaired	E/C/F/TAF FDC 150/150/200/10-mg tablet	150/150/ 200/10 mg	248	Not applicable
GS-US-292-0109 Phase 3; virologically suppressed	E/C/F/TAF FDC 150/150/200/10-mg tablet	150/150/ 200/10 mg	959	FTC/TDF+3rd Agent regimen (STB, ATR, ATV/co+TVD or ATV+RTV+TVD)
GS-US-292-0108 Phase 1 Japanese vs. Caucasian	E/C/F/TAF FDC 150/150/200/10-mg tablet	150/150/ 200/10 mg	20	Not applicable
GS-US-292-0110 Phase 1 food effect study	E/C/F/TAF FDC 150/150/200/10-mg tablet	150/150/ 200/10 mg	43	Not applicable
GS-US-292-1316 Phase 1 DDI	E/C/F/TAF FDC 150/150/200/10-mg tablet	150/150/ 200/10 mg	20	Sertraline 50-mg tablet
GS-US-342-1167 Phase 1 DDI	E/C/F/TAF FDC 150/150/200/10-mg tablet	150/150/ 200/10 mg	24	SOF/GS-5816 400/100-mg tablet ATR 600/200/300-mg tablet FTC/RPV/TDF 200/25/300-mg tablet, DTG 50-mg tablet

Table 22. F/TAF study

GS-US-311-0101 Phase 1 DDI	F/TAF 200/25-mg tablet F/TAF 200/40-mg tablet	200/25 mg 200/40 mg	50	COBI 150-mg tablet TAF 8-mg tablet EFV 600-mg tablet DRV 400-mg tablet

Table 23. TAF Studies*

Study	Dosage Form	Dose	n	
GS-US-120-0109 Phase 1 Metabolite profiling study	TAF [^{14}C]-labeled, 25-mg capsule (each containing a mixture of unlabeled TAF and 100 µCi [^{14}C]TAF)	25 mg	8	Not applicable
GS-US-120-0107 Phase 1 TQT study	TAF 25-mg tablet	25, 125 mg	58	TAF placebo tablet Moxifloxacin 400-mg tablet
GS-US-120-0104 Phase 1 10 days monotherapy in ARV-naive	TAF 8-mg tablet TAF 25-mg tablet TAF 40-mg tablet	8, 25, 40 mg	25	TDF 300-mg tablet TAF placebo tablet
GS-US-120-0108 Phase 1 renal impairment	TAF 25-mg tablet	25 mg	27	Not applicable
GS-US-120-0114 Phase 1 hepatic impairment	TAF 25-mg tablet	25 mg	40	Not applicable

*In addition GS-120-1101 was an early study with 50 mg and 150 mg doses of TAF as the monofumarate that assessed PK and antiviral efficacy during 14 days monotherapy

GCP

The Clinical trials were performed in accordance with GCP as claimed by the applicant.

The applicant has provided a statement to the effect that clinical trials conducted outside the community were carried out in accordance with the ethical standards of Directive 2001/20/EC.

2.4.2. Pharmacokinetics

Two forms of TAF were used during clinical development:

- TAF monofumarate (GS-7340-02) with 1:1 ratio of GS-7340 to fumarate
- TAF fumarate (GS-7340-03), which is the hemifumarate form, with a 2:1 ratio of GS-7340 to fumarate

TAF fumarate (GS-7340-03) was selected for Phase 2/3 studies and the commercial presentation.

The validated bioanalytical methods for TAF in human plasma and urine involved protein precipitation extraction of TAF and its internal standard (TAF-d7) followed by LC MS/MS with positive ionization. The linear ranges were 1 to 1000 ng/mL in plasma and 2-1000 ng/mL in urine.

Absorption

GS-US-292-0101 was an open-label crossover study that compared two formulations (monolayer and bilayer) of E/C/F/TAF (each containing either 25 or 40 mg TAF as the monofumarate) with Stribild and with TAF 25 mg alone. Healthy subjects were assigned to 1 of 2 cohorts and randomised to 1 of 4 treatment sequences within each cohort as follows.

Cohort 1 (10 M and 10 F) received:

- A: STR Formulation 1 containing 150 mg EVG/150 mg COBI/200 mg FTC/25 mg TAF
- B: STR Formulation 1 containing 150 mg EVG/150 mg COBI/200 mg FTC/40 mg TAF
- C: Stribild
- D: 25 mg TAF

Cohort 2 (10 M and 10 F) received:

- E: STR Formulation 2 containing 150 mg EVG/150 mg COBI/200 mg FTC/25 mg TAF
- F: STR Formulation 2 containing 150 mg EVG/150 mg COBI/200 mg FTC/40 mg TAF
- C and D as above

Each treatment was administered with food for 12 days with 2-day washout periods between treatments.

Following administration of E/C/F/TAF 25 mg as Formulations 1 and 2 the exposures to TAF and TFV were substantially higher vs. TAF 25 mg administered alone. TAF AUC_{last} and C_{max} were ~2.2 and 2.3-fold higher, respectively, while TFV AUC_{tau} and C_{max} were ~3.1 and 3.7-fold higher, respectively.

Table 24. GS-US-292-0101: Statistical Comparison of GS-7340 Pharmacokinetic Parameters for Test Versus Reference Treatments (Analysis Set: GS-7340 PK)

GS-7340 PK Parameter	GLSM		GLSM Ratio (%)	90% CI
	Test Treatment	Reference Treatment		
Cohort 1				
EVG/COBI/FTC/GS-7340 Formulation 1 (25 mg) (Test) vs. GS-7340 (25 mg) (Reference)	N=19	N=19		
AUC_{last} (ng•h/mL)	495.23	223.30	221.78	199.99, 245.95
C_{max} (ng/mL)	422.85	189.94	222.62	187.11, 264.87
EVG/COBI/FTC/GS-7340 Formulation 1 (40 mg) (Test) vs. EVG/COBI/FTC/GS-7340 Formulation 1 (25 mg)	N=19	N=19		
AUC_{last} (ng•h/mL)	888.51	495.23	179.41	161.78, 198.97
C_{max} (ng/mL)	708.87	422.85	167.64	140.90, 199.45
EVG/COBI/FTC/GS-7340 Formulation 1 (40 mg) (Test) vs. GS-7340 (25 mg) (Reference)	N=19	N=19		
AUC_{last} (ng•h/mL)	888.51	223.30	397.91	358.80, 441.27
C_{max} (ng/mL)	708.87	189.94	373.20	313.68, 444.02
Cohort 2				
EVG/COBI/FTC/GS-7340 Formulation 2 (25 mg) (Test) vs. GS-7340 (25 mg) (Reference)	N=18	N=18		
AUC_{last} (ng•h/mL)	534.80	231.58	230.93	205.52, 259.50
C_{max} (ng/mL)	429.55	192.62	223.01	188.40, 263.97
EVG/COBI/FTC/GS-7340 Formulation 2 (40 mg) (Test) vs. EVG/COBI/FTC/GS-7340 Formulation 1 (25 mg)	N=17	N=18		
AUC_{last} (ng•h/mL)	732.12	534.80	136.90	121.37, 154.41
C_{max} (ng/mL)	550.19	429.55	128.09	107.63, 152.43
EVG/COBI/FTC/GS-7340 Formulation 2 (40 mg) (Test) vs. GS-7340 (25 mg) (Reference)	N=17	N=18		
AUC_{last} (ng•h/mL)	732.12	231.58	316.14	280.28, 356.59
C_{max} (ng/mL)	550.19	192.62	285.64	240.02, 339.92

Table 25. GS-US-292-0101: Statistical Comparison of TFV Pharmacokinetic Parameters for Test Versus Reference Treatments (Analysis Set: Tenofovir PK)

TFV PK Parameter	GLSM		GLSM Ratio (%)	90% CI
	Test Treatment	Reference Treatment		
Cohort 1				
TFV, EVG/COBI/FTC/GS-7340 Formulation 1 (25 mg) (Test) vs. EVG/COBI/FTC/TDF (Reference)	N=19	N=19		
AUC_{tau} (ng•h/mL)	820.13	3626.35	22.62	21.39, 23.91
C_{max} (ng/mL)	58.81	419.50	14.02	12.20, 16.11
C_{tau} (ng/mL)	27.52	70.70	38.93	36.54, 41.47
EVG/COBI/FTC/GS-7340 Formulation 1 (25 mg) (Test) vs. GS-7340 (25 mg) (Reference)	N=19	N=19		
AUC_{tau} (ng•h/mL)	820.13	267.21	306.92	290.34, 324.45
C_{max} (ng/mL)	58.81	15.99	367.68	319.98, 422.50
C_{tau} (ng/mL)	27.52	9.13	301.52	283.03, 321.22
Cohort 2				
TFV, EVG/COBI/FTC/GS-7340 Formulation 2 (25 mg) (Test) vs. EVG/COBI/FTC/TDF (Reference)	N=18	N=18		
AUC_{tau} (ng•h/mL)	888.33	3953.76	22.47	21.11, 23.91
C_{max} (ng/mL)	64.96	479.74	13.54	11.62, 15.77
C_{tau} (ng/mL)	30.82	78.77	39.13	36.46, 41.99
EVG/COBI/FTC/GS-7340 Formulation 2 (25 mg) (Test) vs. GS-7340 (25 mg) (Reference)	N=18	N=18		
AUC_{tau} (ng•h/mL)	888.33	296.87	299.23	281.25, 318.37
C_{max} (ng/mL)	64.96	17.54	370.45	318.17, 431.34
C_{tau} (ng/mL)	30.82	10.26	300.33	279.91, 322.24

TFV plasma exposures were lower following administration of FDC Formulations 1 and 2 (25 and 40 mg) and TAF alone compared to Stribild. Mean TFV exposures (AUC_{tau} and C_{max}) following TAF alone were ~90% lower compared with those achieved after Stribild.

TAF and TFV exposures after STRs containing 25 or 40 mg TAF were generally dose-proportional.

A comparable increase in TAF and TFV exposures was observed in GS-US-311-0101 (see below) when TAF (8 mg) was given with and without COBI (150 mg) such that the TAF AUC_{last} and C_{max} were ~2.7 and 2.8 fold higher, respectively, while TFV AUC_{tau} and C_{max} were both ~3.3-fold higher. On this basis it was considered that the difference in exposures between Formulations 1 and 2 vs. TAF alone was COBI-mediated, likely due to inhibition of P-glycoprotein–mediated intestinal secretion of TAF.

On comparing STR Formulations 1 and 2 containing TAF 25 mg or 40 mg with Stribild the GMRs and 90% CIs for AUC_{tau}, C_{max} and C_{tau} of EVG, COBI and FTC were contained within the protocol-defined lack of interaction boundary of 70% to 143%. The stricter 80% to 125% boundary criterion for bioequivalence was also met for EVG, COBI and FTC exposures. The actual exposures of EVG, COBI and FTC were consistent with historical data from previous studies.

GS-US-292-0103 – Study Title: A Phase 1, Multiple Dose Study Evaluating the Relative Bioavailability of Elvitegravir/Cobicistat/Emtricitabine/ GS-7340 STR Relative to the Administration of Individual Components Cobicistat-Boosted Elvitegravir, Emtricitabine, and GS-7340

GS-US-292-0103 was an open-label crossover study in similar numbers of healthy male and female subjects aged 18 to 45 years per Cohort.

Cohort 1 assessed the effects of the STR on EVG and COBI and subjects received in randomised order:

- A: STR containing EVG 150 mg/COBI 150 mg/FTC 200 mg/TAF 10 mg
- B: EVG 150 mg + COBI 150 mg

Cohort 2 assessed the effects of the STR on FTC and TAF and subjects received in randomised order:

- A: as above
- C: FTC 200 mg + TAF 25 mg

Each treatment was administered once daily with food for 12 days with no washout periods.

There were 34 healthy subjects enrolled (Cohort 1-14; Cohort 2-20) with median ages of 26 years and 36 years, respectively, and overall mean eGFRCG at baseline of 126.9 mL/min.

The TAF and TFV exposures following administration of the 10 mg TAF as a constituent of the STR were comparable to those observed following administration of TAF 25 mg co-administered with FTC. The result confirmed the 2 to 3-fold increase in exposures observed when TAF was dosed with COBI. Specifically, the 90% CIs around the GLSM ratios for TAF and TFV exposures relative to FTC + TAF 25 mg were within 70% to 143%.

The mean (%CV) TFV AUC and C_{max} after multiple doses were > 90% lower than previously observed following administration of Stribild in GS-US-236-0110.

Table 26. GS-US-292-0103 Pharmacokinetic Results

GS-7340 PK Parameter	Test Mean (%CV)	Reference Mean (%CV)	Geometric Least-Squares Means Ratio (%) (90% CI)
Cohort 2 EVG/COBI/FTC/GS-7340 10 mg (Test) vs FTC + GS-7340 25 mg (Reference) (N = 19)			
AUC_{last} (ng•h/mL)	250.2 (24.7)	278.2 (28.8)	91.42 (84.12, 99.35)
C_{max} (ng/mL)	176.9 (35.1)	179.5 (33.9)	98.68 (84.57, 115.13)

TFV PK Parameter	Test Mean (%CV)	Reference Mean (%CV)	Geometric Least-Squares Means Ratio (%) (90% CI)
Cohort 2 EVG/COBI/FTC/GS-7340 10 mg (Test) vs FTC + GS-7340 25 mg (Reference) (N = 19)			
AUC_{tau} (ng•h/mL)	324.2 (15.4)	265.9 (22.2)	123.63 (116.97, 130.67)
C_{max} (ng/mL)	19.6 (13.9)	19.2 (76.0)	114.16 (97.52, 133.64)
C_{tau} (ng/mL)	11.4 (17.8)	9.2 (23.5)	125.37 (117.72, 133.51)

The data supported the selection of 10 mg TAF in the STR for further clinical development.

Following the administration of E/C/F/TAF 10 mg, the GLSM ratios and 90% CIs for EVG, COBI, and FTC were all contained within the protocol-defined 70% to 143% lack-of-effect boundary relative to EVG + COBI or FTC + TAF 25 mg, indicating no clinically relevant differences. The stricter 80% to 125% boundary was also met for the relevant EVG, COBI, and FTC exposure parameters, consistent with previous findings from GS-US-292-0101.

EVG PK Parameter	**Test Mean (%CV)**	**Reference Mean (%CV)**	**Geometric Least-Squares Means Ratio (%) (90% CI)**
Cohort 1 EVG/COBI/FTC/GS-7340 10 mg (Test) vs EVG + COBI (Reference) (N = 14)			
AUC_{tau} (ng•h/mL)	22067.1 (26.3)	23099.2 (22.7)	94.87 (91.51, 98.36)
C_{max} (ng/mL)	1943.5 (23.9)	2161.0 (27.0)	90.32 (85.07, 95.89)
C_{tau} (ng/mL)	422.2 (54.4)	418.6 (42.2)	97.83 (88.39, 108.27)

COBI PK Parameter	**Test Mean (%CV)**	**Reference Mean (%CV)**	**Geometric Least-Squares Means Ratio (%) (90% CI)**
Cohort 1 EVG/COBI/FTC/GS-7340 10 mg (Test) vs EVG + COBI (Reference) (N = 14)			
AUC_{tau}(ng•h/mL)	11209.8 (27.4)	10931.2 (25.5)	102.00 (98.10, 106.06)
C_{max} (ng/mL)	1560.7 (26.1)	1489.4 (23.2)	104.07 (99.41, 108.94)
C_{tau} (ng/mL)	34.6 (85.5)	26.7 (62.1)	116.43 (102.05, 132.83)

FTC PK Parameter	**Test Mean (%CV)**	**Reference Mean (%CV)**	**Geometric Least-Squares Means Ratio (%) (90% CI)**
Cohort 2 EVG/COBI/FTC/GS-7340 10 mg (Test) vs FTC + GS-7340 25 mg (Reference) (N = 19)			
AUC_{tau}(ng•h/mL)	12352.6 (13.5)	10520.9 (13.8)	117.57 (113.72, 121.55)
C_{max} (ng/mL)	1947.0 (21.2)	1788.8 (19.2)	108.99 (102.81, 115.55)
C_{tau} (ng/mL)	107.4 (25.8)	87.5 (20.6)	121.26 (114.66, 128.24)

Influence of food

GS-US-292-0110 – Study Title: A Phase 1, Randomized, Open-Label Study to Determine the Effect of Food on the Pharmacokinetics of Tenofovir Alafenamide When Administered as a Single Tablet Regimen Containing Elvitegravir/Cobicistat/Emtricitabine/Tenofovir Alafenamide in Healthy Volunteers

GS-US-292-0110 was an open-label crossover study that evaluated PK of TAF and TFV only when given as part of the E/C/F/TAF STR under fasted and fed conditions. Dosing was on days 1, 8 and 15 with E/C/F/TAF 10 mg in the fasted state (reference) or after:

- A light/low-fat meal of approximately 400 kcal and 20% fat
- A high-calorie/high-fat meal of approximately 800 kcal and 50% fat

Administration of E/C/F/TAF under fed conditions resulted in a lower TAF C_{max} and delayed T_{max} with a magnitude of effect that was similar between meal types. In contrast the GLSM ratios and 90% CI for TAF AUC_{last} after each meal type all exceeded 100% and the upper 90% boundaries were 124% and 126%.

Table 27. GS-US-292-0110: Summary of Tenofovir Alafenamide Pharmacokinetic Parameters by Treatment (TAF PK Analysis Set)

TAF PK Parameter (N = 42)	E/C/F/TAF Light/LF Meal	E/C/F/TAF HC/HF Meal	E/C/F/TAF Fasted
AUC_{inf} (ng•h/mL)	252.2 (32.3)	254.5 (29.6)	223.9 (44.9)
AUC_{last}(ng•h/mL)	250.3 (32.7)	251.4 (30.4)	222.5 (45.2)
C_{max} (ng/mL)	219.5 (43.7)	210.7 (47.0)	329.1 (47.0)
T_{max} (h)	1.00 (0.75, 1.50)	1.00 (0.75, 1.50)	0.50 (0.50, 0.75)
$T_{½}$ (h)	0.39 (0.37, 0.45)	0.50 (0.40, 0.56)	0.42 (0.34, 0.47)
Vz/F (L)	26.5 (43.4)	33.2 (61.9)	30.3 (33.1)
CL/F (L/h)	44.4 (37.2)	43.0 (33.7)	51.5 (35.3)

HC/HF = high-calorie, high-fat; LF = low-fat

Data are presented as mean (%CV), except for Tmax and T½, which are presented as median (Q1, Q3).

Table 28. Study GS-US-292-0110: Statistical Comparison of Selected TAF Pharmacokinetic Parameters (PK Analysis Set)

Treatment Condition (N = 42)	TAF PK Parameter		
	AUC_{inf} (ng•h/mL)	AUC_{last} (ng•h/mL)	C_{max} (ng/mL)
Test Treatment: Light/LF Meal GLSM	238.94	236.73	200.65
Test Treatment: HC/HF Meal GLSM	243.71	240.22	186.36
Reference Treatment: Fasted GLSM	207.37	205.94	294.90
Light/LF Meal vs. Fasted GLSM ratio (90% CI), %	115.22 (107.14, 123.91)	114.95 (106.82, 123.69)	68.04 (58.96, 78.52)
HC/HF Meal vs. Fasted GLSM ratio (90% CI), %	117.53 (109.28, 126.39)	116.65 (108.40, 125.52)	63.20 (54.76, 72.93)
HC/HF Meal vs. Light/LF Meal GLSM ratio (90% CI), %	102.00 (94.85, 109.69)	101.48 (94.30, 109.20)	92.88 (80.49, 107.18)

CI = confidence interval, GLSM = geometric least-squares mean, HC/HF = high-calorie/high-fat; LF = low-fat

GLSMs were obtained using a mixed-effects model.

Distribution

In the TAF metabolite profiling study GS-US-120-0109 (see below) the mean whole blood-to-plasma concentration ratio of [14C]radioactivity increased from 0.6 at 0.25 h post-dose to 2.4 at 216 h post-

dose suggesting slower clearance of radioactivity from blood cells relative to plasma. Radioactivity was not detectable in blood for 6/8 subjects at 504 h post-dose and the others had low radioactivity close to the LLOQ.

In AD-120-2026 the in-vitro binding of TAF to human plasma proteins was evaluated using equilibrium dialysis conducted over 3 h at 37°C. Human plasma was spiked with TAF at a final concentration of 2 µM and LC/MS/MS was used with LLOQ 0.3 nM. The free fraction in human plasma was similar to that in dogs.

Matrix	Free Fraction of GS-7340 (%)
Human	46.8 ± 6.2
Beagle Dog	48.0 ± 2.3

However, in several human ex vivo studies it seems that the estimated unbound fraction was ~20%, i.e. lower than reported above (e.g. see the control groups in the renal and hepatic impairment studies).

Based on POPPK analyses for each of TAF and TFV (see section 2.1.8):

- For TAF the apparent volume of the central compartment (Vc/F) was 10.3 L and the apparent volume of peripheral compartment (Vp/F) was 447 L.
- For TFV the Vc/F was 1600 L and the Vp/F was 1670 L.
- TFV Vc/F and Vp/F were lower in moderate renal impairment and higher in those with supra-normal eGFR compared to normals. Both were also lower in in healthy subjects vs. HIV-infected patients.

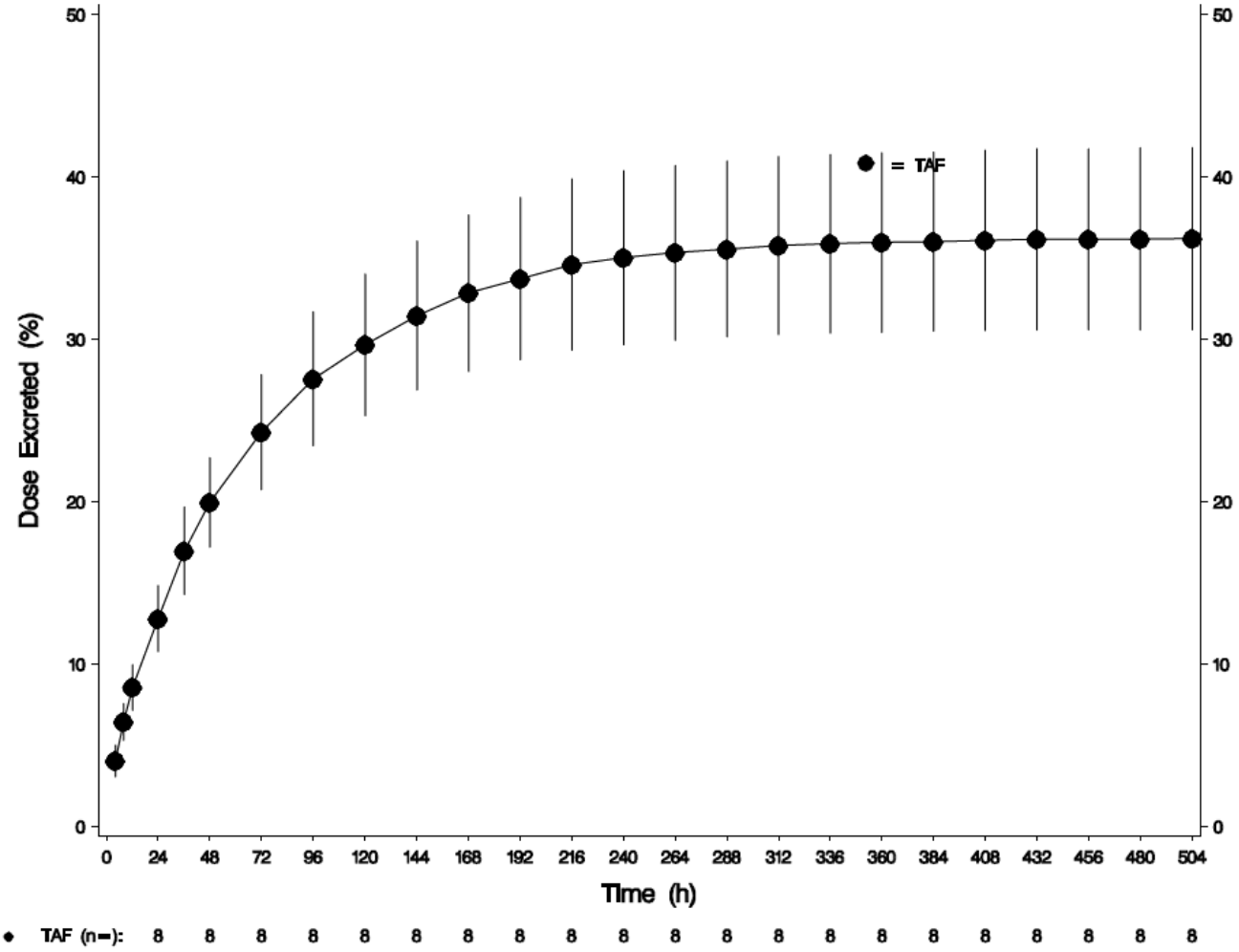
Elimination

GS-US-120-0109 – Study Title: A Phase 1 Study to Evaluate the Pharmacokinetics, Metabolism and Excretion of GS-7340

Excretion

In the TAF metabolite profiling study GS-US-120-0109 the total mean (± SD) recovery of [14C] radioactivity in faeces plus urine (n=7) was 84.4% (± 2.45%). The percent of radioactive dose recovered from faeces was 47.2% (± 4.62%) and the percent recovered from urine was 36.2% (± 5.62%).

Figure 4. GS-US-120-0109: Mean (SD) Cumulative Urinary Recovery of Total [14C]-Radioactivity (% of Radioactive Dose Excreted) Versus Time (PK Analysis Set)a,b,c

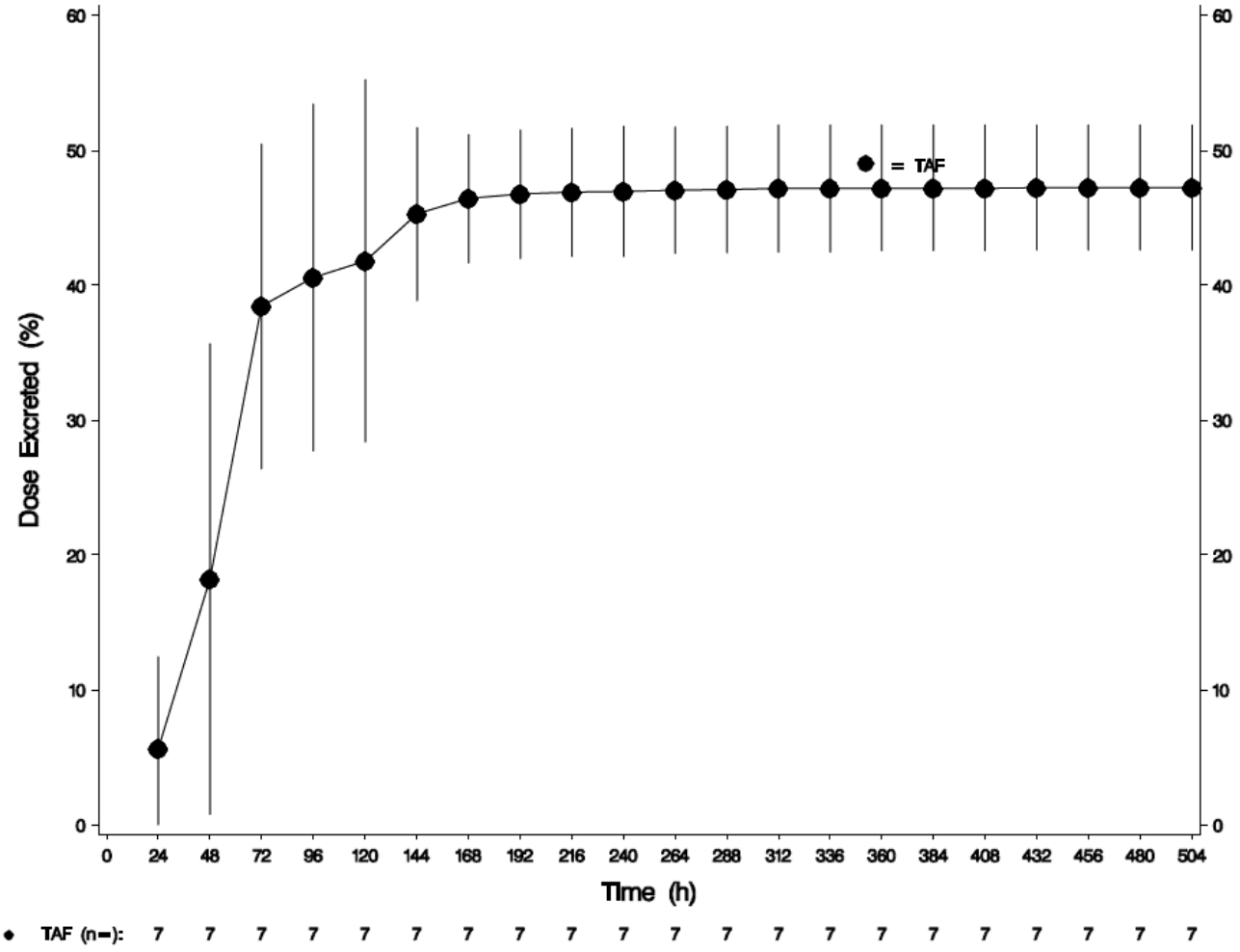


a Values below the lower limit of quantitation (BLQ) were treated as 0 for summary statistics and missing for log-normalized data.

b Values where no sample was available (NS) were treated as missing for summary statistics and log-normalized data.

c Values presented as mean ± standard deviation.

Figure 5. GS-US-120-0109: Mean (SD) Cumulative Fecal Recovery of Total [14C]-Radioactivity (% of Radioactive Dose Excreted) versus Time, Excluding Subject PPD (PK Analysis Set)a,b,c,d



a Values below the lower limit of quantitation (BLQ) were treated as 0 for summary statistics and missing for log-normalized data.

b Values where no sample was available (NS) were treated as missing for summary statistics and log-normalized data.

c Values presented as mean ± standard deviation.

d One subject was excluded from this summary because he did not provide sufficient stool samples.

Metabolism

In the TAF metabolite profiling study GS-US-120-0109 healthy male volunteers (median eGFRCG 117.5 mL/min; range 87.7 to 198.2 mL/min) received a single TAF 25 mg capsule containing 24.15 mg TAF plus 100 µCi [0.85 mg] radiolabeled [14C]TAF. Dosing was with water within 5 minutes of completing a standardised breakfast.

Quantifiable levels of [14C] radioactivity were observed in whole blood for up to 360 h post-dose in most subjects but radioactivity was undetectable in plasma after 192 h post-dose.

Quantifiable levels of TAF were observed in plasma for up to 6 h post-dose and then remained BLQ. TAF was extensively metabolised with only 1.41% (± 0.561%) of the total radioactive dose appearing in urine as TAF and no radioactive TAF was detected in faeces.

Table 29. GS-US-120-0109: Summary of TAF PK Parameters in Plasma by LC/MS/MS (PK Analysis Set)

PK Parameter	Mean (%CV) (N = 8)
C_{max} (ng/mL)	78.1 (34.6)
AUC_{last} (ng•h/mL)	157.3 (23.1)
AUC_{inf} (ng•h/mL)	161.8 (22.4)
T_{max} (h)[a]	2.00 (1.50, 2.76)
$T_{½}$ (h)[a]	0.51 (0.45, 0.62)

a Median (Q1, Q3)

TFV was quantifiable in plasma for up to 96 h post-dose. TFV accounted for 99% of the radioactivity recovered in faeces and 86% of the radioactivity recovered in urine.

Table 30. GS-US-120-0109: Summary of TFV PK Parameters in Plasma by LC/MS/MS (PK Analysis Set)

PK Parameter	Mean (%CV) (N = 8)
C_{max} (ng/mL)	7.2 (16.3)
AUC_{last} (ng•h/mL)	192.9 (24.0)
AUC_{inf} (ng•h/mL)[a]	224.6 (24.6)
T_{max} (h)[a]	3.25 (2.25, 4.00)
$T_{½}$ (h)[a]	32.37 (31.11, 36.19)

a Median (Q1, Q3)

Metabolite profiling (pooled samples) showed two concentration peaks in the plasma $^{[14C]}$radioactivity profile:

- At the first maximal plasma radioactivity concentration around 2 h post-dose the predominant species was TAF, accounting for 72.6% of the total $^{[14C]}$radioactivity quantified.
- At the second maximal plasma radioactivity concentration around 24 to 48 h post-dose the predominant species was uric acid, accounting for 97.6% of the total $^{[14C]}$radioactivity quantified.

Over 96 h post-dose the predominant species circulating in plasma was uric acid, which accounted for 73.9% of the total $^{[14C]}$radioactivity AUC. The TAF and TFV AUCs represented 1.8% and 1.5% of the total $^{[14C]}$radioactivity AUC, respectively. Low quantities of other metabolites were formed including xanthine, hypoxanthine and adenine (identical to the endogenous products of purine metabolism).

Table 31. GS-US-120-0109: Composite Estimates of Total [14C]-Radioactivity and [14C]-uric acid, [14C]-TAF, and [14C]-TFV Pharmacokinetic Parameters in Pooled Plasma using HPLC (PK Analysis Set)

PK Parameter	Total [^{14}C]-Radioactivity[a]	[^{14}C]-Uric Acid[a]	[^{14}C]-TAF[a]	[^{14}C]-TFV[a]
C_{max} (ng eq/g)	56.6	42.8	41.9	11.7
AUC_{1-96} (h•[ng eq /g])	4822	3565	86.2	74.0
T_{max} (h)	2	72	2	2

a Parameter estimates are based on pooled data; mean values are presented

For pooled urine a mean of 25.8% (± 5.50%) of the radioactive dose was quantified, within which the predominant species was TFV (M12), accounting for 22.2% (± 4.47%). All other metabolites appeared in trace amounts and none exceeded 2% of the administered dose of radioactivity.

Table 32. GS-US-120-0109: Percent of Total [14C]-Radioactivity Present as [14C] Metabolites in Pooled Urine From All Sampling Intervals by HPLC (PK Analysis Set)

[^{14}C]-TAF Metabolite	Mean (SD) Percent of Total [^{14}C]-Radioactivity
M27B	1.93 (1.72)
M7/M8	0.258 (0.372)
M12	22.2 (4.47)
TAF	1.41 (0.561)

Note: M27B = uric acid; M7 = xanthine; M8 = hypoxanthine; and M12 = TFV.

For pooled faeces a mean of 31.7% (± 10.5%) of the radioactive dose was quantified, within which the predominant species was TFV (M12), accounting for 31.4% (± 10.4%). Two unidentified metabolites appeared in trace amounts.

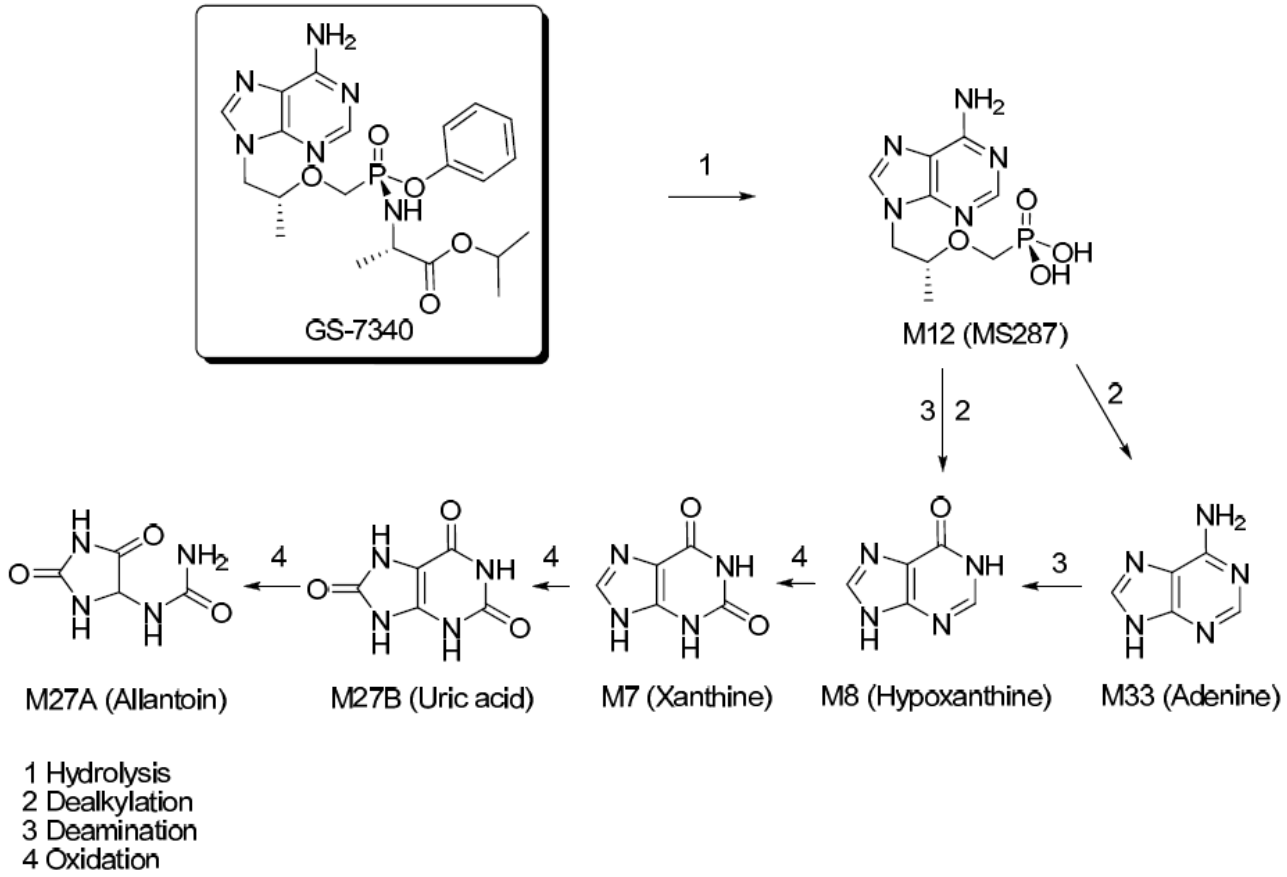
Table 33. GS-US-120-0109: Percent of Total [14C]-Radioactivity Present as [14C] Metabolites in Pooled Feces From All Sampling Intervals by HPLC (PK Analysis Set)

[^{14}C]-TAF Metabolite	Mean (SD) Percent of Total [^{14}C]-Radioactivity
M29	0.224 (0.328)
M12	31.4 (10.4)
M43	0.0628 (0.178)

Note: M29 = unknown; M12 = TFV; and M43 = unknown

The proposed biotransformation pathway of TAF is shown in Figure 6 (M12 = TFV).

Figure 6. GS-US-120-0109: Tentative Pathways for Metabolism of TAF by Humans



Note: M12 = TFV.

Note: Pathways are proposed based on general knowledge of metabolism and do not imply definitive pathways. Direct experimentation was not performed.

Additional information comes from in-vitro studies that assessed the stability of TAF in plasma, intestinal S9 and hepatic S9 fractions from humans. TAF was moderately stable in human plasma and intestinal S9 with half-lives of 74.7 and 58.3 minutes, respectively. TAF stability in human intestinal S9 fractions was also determined in the presence of HIV PIs resulting in a lower half-life of 24.5 minutes. TAF was relatively less stable in human hepatic S9 fractions with a half-life of 20.6 minutes. The predicted human hepatic extraction ratio was calculated to be 76.2%.

Using bacterially expressed human CYP enzyme preparations (Bactosomes), co-expressed with human NADPH CYP reductase, metabolism of TAF was not detected by CYP1A2, CYP2C8, CYP2C9, CYP2C19 or CYP 2D6. TAF was slowly metabolised by CYP3A4 (1.9 min-1), which was 26.6% of that for testosterone (7.2 min-1).

Consequences of possible genetic polymorphism

It was concluded that TAF is primarily hydrolysed by CatA in PBMCs. Cathepsin A is a ubiquitously expressed multifunctional enzyme with deamidase, esterase and carboxypeptidase activities and is encoded by the CTSA gene. Genetic polymorphisms have been described, some of which can result in depressed enzymic activity.

In primary human hepatocytes it has been reported in the literature that CES1 is the major enzyme that converts TAF to TFV and that CatA makes a small contribution. Several CES1 genetic variants that are associated with enzyme activity have been identified at very low frequency (G143E [heterozygous:

2%-4% and homozygous: 0.05%] and D260fs [very rare]). In a study of HBV-infected subjects all of the patients genotyped for the rs71647871 (G143E) variant of CES1 were found to carry the reference homozygous genotype (i.e. there were no carriers of the minor allele of this variant genotype). In addition, since TAF can be activated by both CES1 and CatA in liver, the genetic polymorphisms causing a marked effect on TAF activation are expected to be extremely rare.

Dose proportionality and time dependencies

Dose proportionality

In studies in which a range of TAF doses were used, including the monotherapy studies and the TQT study that used 25 mg and 125 mg doses, there was very approximate dose proportionality for TAF and TFV.

Time dependency

In GS-US-120-0109 plasma radioactivity showed two peaks. There was a time-dependent profile with TAF as the most abundant species in the first few hours and uric acid predominating in the remaining period.

Intra- and inter-individual variability

- The inter-subject variability in TAF plasma exposure varied across studies depending on whether intensive or sparse sampling was employed (% CV: 26% - 34% across Phase 1 studies and 58% - 118% across Phase 2 and Phase 3 studies).
- The inter-subject variability in TFV plasma exposure was generally modest/low and comparable across studies (% CV: 18% - 41% across Phase 2 and Phase 3 studies).

Inter-subject variability in intracellular concentrations of TFV-DP is greater than observed for parent drug in plasma.

Pharmacokinetics in target population

POPPK analyses were performed for TAF and TFV using PK data collected as shown in Table 34.

Table 34. Studies contributing to the POPPK analysis

Study	Phase	Population	Sampling (sparse/intensive)
GS-US-292-103	1	Healthy	Intensive
GS-US-292-108	1	Healthy	Intensive
GS-US-292-110	1	Healthy	Intensive
GS-US-292-102	2	HIV-infected	Intensive[a]/sparse
GS-US-292-106	2/3	HIV-infected	Intensive[a]/sparse
GS-US-292-104	3	HIV-infected	Intensive[a]/sparse
GS-US-292-111	3	HIV-infected	Intensive[a]/sparse
GS-US-292-109	3	HIV-infected	Sparse
GS-US-292-112	3	HIV-infected	Intensive[a]/sparse

a Intensive PK was conducted in PK substudies

The analysis was conducted via nonlinear mixed-effects modelling with NONMEM 7.3.0 software. Due to expected differences in characteristic time scales of TAF and TFV concentrations (rapid elimination of TAF with no accumulation and much longer half-life of TFV leading to significant TFV accumulation) PK modelling was first performed separately for TAF and TFV. The final models for TAF and TFV were then combined into a joint model.

For each of the separate models, the base model was first established followed by the development of the covariate model. One- and two compartment PK models with various oral absorption models were tried during base model development. Structural model refinement was based on various goodness-of-fit indicators, including diagnostic plots, plausibility and precision of the parameter estimates, the minimum objective function value and the number of estimated parameters.

Covariate modelling used a combination of the full model approach and backward elimination procedure. The covariates investigated were body size measures (weight, BSA or BMI), age, sex, race, creatinine clearance at baseline and population (healthy subjects, ARV-naive and ARV-experienced patients). Influence of identified covariates on exposure and a degree to which the covariate explained variability of exposure was determined by comparing 90% prediction intervals of estimated individual steady-state exposure parameters in the data set with the corresponding parameters computed from the models for typical subjects, where values of one covariate were altered from its reference value. For continuous covariates, 5th and 95th percentiles of the values in the data set were used; for categorical covariates, levels other than reference were used in computations.

The available data for analysis are summarised in Table 35.

Table 35. Summary of Available Quantifiable Concentration Values

Study	Number of subjects			Number of samples		Number of excluded samples	
	Total	With TAF	With TFV	TAF	TFV	TAF	TFV
102	111	65	111	269 [a]	1126	23 [a]	2
103	17	17	17	225	373	0	0
104	426	298	426	447	1646	19	1
106	23	23	23	133	383	0	0
108	20	20	20	318	688	0	0
109	327	272	262	431	981	8	2
110	42	42	42	976	1657	0	52
111	415	243 [b]	415	422 [b]	1469	21 [b]	0
112	241	213	241	745 [c]	1864 [d]	40 [c]	22 [d]
Total	**1622**	**1193**	**1557**	**3966**	**10187**	**111**	**79**

a. One additional TAF sample excluded from the covariate analysis and the final and joint models;

b. Three additional TAF samples excluded from the covariate analysis and the final and joint models, resulting in 419 TAF samples from 241 subjects in the analysis;

c. Two additional TAF samples excluded from the covariate analysis and the final and joint models;

d. One additional TFV sample excluded from the covariate analysis and the final and joint models

Visual predictive check simulations for the final models, for all data and stratified by covariates, indicated an agreement between the observed and simulated data. Model parameters obtained by bootstrap agreed with the parameters in the final models.

TAF pharmacokinetics

The two-compartment population PK model with absorption lag time and sequential zero- and first-order absorption described TAF concentrations. Extensive covariate analyses did not identify any covariates that had a statistically significant influence on TAF PK, and no covariates were retained in the final model.

The predicted steady-state AUC and C_{max} for a typical subject were 188.1 ng/mL*h and 165.6 ng/mL, respectively. The mean (95% CI; % CV) predicted steady-state AUC and Cmax in the pivotal studies (GS-US-292-0104 and GS-US-292-0111) were 206.4 (55.6 - 526.1; 71.8%) ng/mL*h and 162.2 (46.5 – 340; 51.1%) ng/mL, respectively. Elimination of TAF was rapid, and plasma concentrations were not detectable by 8 hours post dose in most patients. Tables 36 and 37 show predicted C_{max} and AUC by covariates.

Table 36. Summary of Individual Steady-State Predictions of TAF C_{max} by Covariates: All Subjects (Model 412)

Covariate	Level	N	Mean	SD	Geomean	CV	Median	Min	Max	Percentile			
										2.5th	25th	75th	97.5th
Sex	Males	987	165.6	90.4	147.1	0.546	152	19.2	1281.9	49.2	115.7	197	357.7
	Females	204	176.1	94.9	150.6	0.539	160.5	21.1	569.9	30.8	117.3	221.9	383.6
Race	White	698	167.8	94.8	148.6	0.565	153.2	19.2	1281.9	47.7	116.5	198.3	361
	Black	289	162.1	81.5	143	0.503	145.4	21.3	569.9	40.9	112.1	200.9	364
	Other	204	173.7	92	151.3	0.53	161.9	21.1	589.3	47.9	115.8	205.7	382.4
Population[a]	Healthy	79	178	82.6	158.2	0.464	162.5	30.9	422.9	55.9	122.1	220	368.3
	Treatment Naive	604	163.8	95.9	144.7	0.586	147.3	19.7	1281.9	46.4	115.3	194.8	347.8
	Treatment experienced	272	154	65.7	139.6	0.427	147.5	19.5	424	48.3	114.3	182.3	320.1
Weight	WT<=66 kg	322	177.7	88.8	156.9	0.499	159.7	21.1	569.9	49.6	121.5	212.3	406.9
	66<WT<=75 kg	281	172	87.3	151.4	0.507	162.5	25.6	662.8	42.9	116.4	211.2	374.3
	75<WT<= 86 kg	290	162.7	73.9	147.2	0.454	151.5	19.2	696.3	47.3	117.4	195.9	333.9
	WT>86 kg	298	156.5	110	135.4	0.703	139.5	19.5	1281.9	44.6	107.3	184	344.9
AGE	Age<55 years	994	165.3	91	146.1	0.551	151	19.5	1281.9	47	115.3	196.6	362
	Age>=55 years	197	178.2	92.2	155.8	0.518	161.5	19.2	647.4	41.6	120.6	218.2	414.7
	Age<65 years	1129	166.9	91.5	147.3	0.548	152.2	19.2	1281.9	46.5	115.7	198.6	366.6
	Age>=65 years	62	176.7	88	154.1	0.498	163	25.6	476.4	37.5	122.9	220.8	365.9
Renal impairment	eGFR<60 mL/min	139	210.3	110.1	181.8	0.524	199.5	19.2	696.3	43.1	130.5	267.8	455.6
	60<=eGFR<90 mL/min	212	168.9	75.1	153.1	0.445	156.7	26.9	477.5	53.6	120.6	202.3	381.2
	eGFR>=90 mL/min	840	159.9	89.7	141.4	0.561	146	19.5	1281.9	44.4	113.4	189.6	345.5

aSubjects from studies 106 (adolescents) and 112 (renal impairment) were excluded.

Table 37. Summary of Individual Steady-State Predictions of TAF AUC by Covariates: All Subjects (Model 412)

Covariate	Level	N	Mean	SD	Geomean	CV	Median	Min	Max	Percentile			
										2.5th	25th	75th	97.5th
Sex	Males	987	219.7	198.2	185.4	0.902	186	24.5	2978.2	57.4	146.3	240.7	585.2
	Females	204	258.4	280.8	209.4	1.09	206.3	22.6	3337.4	63.2	159	278.8	695.1
Race	White	698	227.4	217.6	190.3	0.957	188.5	24.6	2978.2	61.6	148.5	244.9	706.3
	Black	289	232.1	245.3	191.2	1.06	188.1	24.5	3337.4	56.8	148	250.5	570.2
	Other	204	214.5	151.2	183.5	0.705	187.2	22.6	1678.1	55.6	145	246.9	513.5
Population[a]	Healthy	79	250.4	79.1	237.7	0.316	246.5	93.9	471.7	109.3	203	298.5	415.2
	Treatment Naive	604	214.9	179.4	182.2	0.835	186.2	24.5	2683.3	55.1	141.7	239.3	595.8
	Treatment experienced	272	199.4	220.2	170.3	1.1	177.2	47.2	3337.4	60.9	142.1	211.3	405.7
Weight	WT<=66 kg	322	240.7	160.2	207.6	0.666	200	22.6	1678.1	63.1	161.1	272.7	667.8
	66<WT<=75 kg	281	248.8	296.9	197	1.19	189.2	24.5	3337.4	54.6	152.7	259.7	722.5
	75<WT<= 86 kg	290	210	171.8	184.4	0.818	189.2	24.6	2408.4	74.2	147.6	234.7	406.3
	WT>86 kg	298	205.6	210.2	169.3	1.02	177	47.2	2683.3	53.9	123.4	220.8	528.7
AGE	Age<55 years	994	223.8	222.2	186.9	0.993	187.3	22.6	3337.4	56.8	147	243.3	555
	Age>=55 years	197	239.2	174.1	201.9	0.728	196.5	24.6	1392.3	68	152.7	270.6	753.4
	Age<65 years	1129	225.2	215.6	188.4	0.957	187.6	22.6	3337.4	57.5	147	246.7	590.3
	Age>=65 years	62	247.3	204.7	206.6	0.828	212.2	51.7	1392.3	66.3	164.9	267.5	867.8
Renal impairment	eGFR<60 mL/min	139	304.7	336.6	239.4	1.1	239.7	24.6	2978.2	81	170.1	322.3	920.1
	60<=eGFR<90 mL/min	212	226.6	191.7	188.9	0.846	186.7	22.6	1678.1	64.4	144.6	242.6	572.5
	eGFR>=90 mL/min	840	213.3	191	182.2	0.895	186.1	24.5	3337.4	56.3	144.3	239.1	471.9

aSubjects from studies 106 (adolescents) and 112 (renal impairment) were excluded.

TFV pharmacokinetics

The two-compartment population PK model with sequential zero- and first-order absorption described TFV concentrations. Covariates retained in the final model were estimated CrCL, HIV status, sex and Black race.

The predicted steady-state AUC, C_{max}, and C_{min} values for a typical reference subject (not Black, male, HIV patient, CrCL=100 mL/min) were 331.2 ng/mL*h, 16.9 ng/mL, and 12.1 ng/mL for AUC, C_{max}, and C_{min} respectively. Corresponding mean (95% CI; % CV) predicted individual steady-state parameters in the pivotal studies were 292.6 ng/mL*h (179.8 - 445.7 ng/mL*h; 27.4%), 15.2 ng/mL (9.3 - 23.1 ng/mL; 26.1%) and 10.6 ng/mL (6.4 -16.6 ng/mL; 28.5%).

Table 38. Summary of Individual Steady-State Predictions of TFV C_{max} by Covariates: All Subjects (Model 540)

Covariate	Level	N	Mean	SD	Geomean	CV	Median	Min	Max	Percentile			
										2.5th	25th	75th	97.5th
Sex	Males	1301	17.7	7.5	16.6	0.423	15.7	6.7	84.8	9.7	13.5	19.4	37.7
	Females	256	21.2	10.5	19.4	0.494	18.6	7.5	84	10.2	14.6	24.2	46.3
Race	White	914	18.4	8.1	17.2	0.442	16	7.2	84.8	9.8	13.9	20.5	41.2
	Black	392	16.5	5.7	15.6	0.347	15	6.7	42.1	8.9	12.9	18.7	32.8
	Other	251	20.7	10.5	18.9	0.508	17.3	8.8	79.8	10.5	14.5	22.6	47.7
Population[a]	Healthy	79	21.2	5.1	20.6	0.239	20.2	11	37.9	12.7	18.1	23.9	33.1
	Treatment Naive	952	15.4	3.9	14.9	0.255	14.9	6.7	50.3	9.4	13	17.2	23
	Treatment experienced	262	16.8	6.5	16.1	0.386	15.9	7.7	84.8	9.8	13.8	18.8	24.7
Weight	WT<=66 kg	405	21.8	10.3	20	0.472	18.4	7.4	84	10.8	15.4	24.8	50.2
	66<WT<=75 kg	376	18.5	7.4	17.5	0.398	16.5	8.7	84.8	10.5	14.1	20.4	35.8
	75<WT<= 86 kg	393	17.3	5.8	16.5	0.336	15.6	7.6	44.6	10.1	13.7	19.7	34.8
	WT>86 kg	383	15.5	7.1	14.6	0.46	14	6.7	68.4	8.8	11.9	16.5	33.3
AGE	Age<55 years	1330	17	7.1	16	0.421	15.4	6.7	84.8	9.7	13.4	18.5	36.7
	Age>=55 years	227	26.2	9.3	24.7	0.356	24.8	9.7	84	13	19.8	30.8	47.8
	Age<65 years	1486	17.8	7.6	16.7	0.428	15.8	6.7	84.8	9.7	13.5	19.6	38.1
	Age>=65 years	71	29.5	11	27.8	0.372	28.1	13.4	84	15.4	21.9	34.1	50.7
Renal impairment	eGFR<60 mL/min	161	33.9	10.6	32.5	0.313	31.6	17.3	84	20.1	26.8	37.8	62.6
	60<=eGFR<90 mL/min	272	21.7	7.1	20.9	0.329	20.9	7.7	84.8	13.7	18.1	23.6	33.6
	eGFR>=90 mL/min	1124	15.3	4.2	14.8	0.273	14.8	6.7	68.4	9.5	12.9	17	23.7

aSubjects from studies 106 (adolescents) and 112 (renal impairment) were excluded.

Table 39. Summary of Individual Steady-State Predictions of TFV AUC by Covariates: All Subjects (Model 540)

Covariate	Level	N	Mean	SD	Geomean	CV	Median	Min	Max	Percentile			
										2.5th	25th	75th	97.5th
Sex	Males	1301	338.5	149.6	316.7	0.442	298.3	129	1665.2	184.3	257.2	361.5	751.2
	Females	256	412.7	216.1	373.7	0.524	348.3	151	1739.6	194.6	281.6	449.4	959.9
Race	White	914	356.6	169.2	331.2	0.474	306	151	1739.6	190.4	266.5	386.8	807
	Black	392	308.7	114.6	292.3	0.371	278.8	129	897.4	173.9	241.6	338.1	636.3
	Other	251	394.9	196.9	360.9	0.499	332	159.1	1408	203.3	278.4	429.4	982.8
Population[a]	Healthy	79	306.6	69.5	300.2	0.227	305.2	188.1	713.6	212.4	264.1	331.4	409.8
	Treatment Naive	952	295.2	78.8	286.6	0.267	285.9	129	1088.7	180.9	248.9	331.8	446.3
	Treatment experienced	262	324.7	133.5	310.4	0.411	305.9	131.4	1665.2	188	265.6	364.2	475.2
Weight	WT<=66 kg	405	418.1	200.9	383.8	0.481	347.6	136.7	1739.6	202.2	298	469.9	990.7
	66<WT<=75 kg	376	353.9	147.3	333.9	0.416	309.7	153.1	1665.2	212.3	274.6	380.2	718.3
	75<WT<= 86 kg	393	328.2	120.3	311.8	0.366	293.3	131.4	936.4	196.7	258	356.2	674.5
	WT>86 kg	383	299.5	153.7	278.5	0.513	266.7	129	1531.2	171.4	227.3	318	702.4
AGE	Age<55 years	1330	321.3	139.3	303.3	0.433	291.9	129	1665.2	184	253.4	344.2	707.8
	Age>=55 years	227	522.9	194.2	491.6	0.371	501.4	180.4	1739.6	251.4	383.1	620.6	989.9
	Age<65 years	1486	338.9	151.5	317	0.447	299.4	129	1665.2	185.6	258	364.2	788.6
	Age>=65 years	71	597.2	226.6	562.6	0.38	575.6	251.2	1739.6	323.7	454.1	686.5	1060.9
Renal impairment	eGFR<60 mL/min	161	683.1	217	654.2	0.318	632.8	361.5	1739.6	388.3	524.1	785.7	1178.4
	60<=eGFR<90 mL/min	272	418.8	126.5	404.9	0.302	401.7	131.4	1665.2	268.5	346.6	463.9	660.6
	eGFR>=90 mL/min	1124	286.6	76.9	279.3	0.268	281.8	129	1531.2	180.8	246.7	316.9	408.7

aSubjects from studies 106 (adolescents) and 112 (renal impairment) were excluded.

The main covariate that influenced the PK of TFV was CrCL.

- For those with CrCL 50 mL/min, apparent clearance (CLM/F), apparent central volume (VcM/F) and apparent peripheral volume (VpM/F) were respectively 44%, 44% and 65% lower vs. reference subjects.
- For those with CrCL 150 L/min the respective values were 40%, 40% and 85% higher vs. reference subjects.
- Differences in eGFR explained the main part of variability in AUC, C_{max} and C_{min}. These parameters were 73-78% higher in subjects with CrCL 50 mL/min (5th percentile of eGFR values in the analysis population) compared to 100 mL/min and they were 28-29% lower in subjects with CrCL of 150 mL/min (95^{th} percentile of eGFR values in the analysis population) compared to 100 mL/min.

In addition:

- CLM/F was 11% lower in females and 12% higher in Blacks
- VcM/F and VpM/F were 67% and 36% lower in healthy subjects compared to HIV patients

Of the other covariates, only being healthy contributed to explaining variability in Cmax, with 29% higher Cmax in healthy subjects. Apparent central and peripheral volumes of distributions were respectively 67% (95% CI: 61-74%) and 36% (95% CI: 26 - 46%) lower in healthy subjects compared to HIV patients.

The effects of the rest of the covariates on AUC, C_{max} and C_{min} were minor (respectively 13%, 10.6%, and 14.7% higher in females; 10.7%, 8.7%, and 12.1% lower in Blacks; 12% lower C_{min} in healthy subjects).

Differences in TFV exposure between subjects due to statistically significant covariates were less than 2-fold. Since the systemic TFV exposure following E/C/F/TAF STR is approximately 90% lower compared to E/C/F/TDF regimen, these differences were not considered clinically meaningful.

Intensive PK sub-study data

The Phase 2 and Phase 3 studies in the ART-naïve included intensive PK sub-studies that reported the TAF and TFV plasma concentrations. Thus, the data provide a direct comparison of plasma TFV levels between E/C/F/TAF and STB in HIV-infected patients rather than an historical comparison. The Phase 3 studies GS-US-292-0104 and 0111 showed that TFV plasma AUC_{tau} after TAF was 10.4% and 7.5% of the value observed after TDF, consistent with TAF 25 mg vs. TDF 300 mg given alone in GS-US-120-0104.

Table 40. GS-US-292-0104: Statistical Comparisons of TFV Pharmacokinetic Parameter Estimates Between Test and Reference Treatments (PK Substudy Analysis Set)

TFV PK Parameter	GLSMs by Treatment		GLSM Ratio (%)	90% CI (%)
	E/C/F/TAF (N = 15)	STB (N = 15)		
AUC_{tau} (ng•h/mL)	307.17	2948.64[a]	10.42	(9.13, 11.89)
C_{max} (ng/mL)	17.05	357.14[a]	4.77	(4.10, 5.56)
C_{tau} (ng/mL)	10.27[a]	57.62	17.83	(15.07, 21.08)

GLSM = geometric least-squares mean

a N = 14

Table 41. GS-US-292-0111: Statistical Comparisons of TFV Pharmacokinetic Parameter Estimates Between Test and Reference Treatments (PK Substudy Analysis Set)

TFV PK Parameter	GLSMs by Treatment		GLSM Ratio (%)	90% CI (%)
	E/C/F/TAF (N = 21)	STB (N = 15)		
AUC_{tau} (ng•h/mL)	278.73	3682.91	7.57	(6.62, 8.65)
C_{max} (ng/mL)	16.14	442.74	3.65	(3.12, 4.26)
C_{tau} (ng/mL)	9.34	74.69	12.51	(10.59, 14.78)

GLSM = geometric least-squares mean

The data also demonstrated that the intracellular TFV-DP AUC_{tau} was higher with TAF although there was clearly considerable variability (study 0104: 7.13 µmol.h/L vs. STB 2.11 µmol.h/L; study 0111: 21.02 µmol.h/L vs. STB 7.76 µmol.h/L).

The Phase 2 study GS-US-292-0102 reported similar differences for TFV between E/C/F/TAF and STB. This study also reported EVG, COBI and FTC plasma levels but only from the E/C/F/TAF group. Therefore the comparison was made with other E/C/F/TAF studies and with historical data for STB.

Table 42. GS-US-292-0102: Summary of EVG, COBI, and FTC Pharmacokinetic Parameters (PK Substudy Analysis Set, N = 19)

	AUC_{tau} (ng•h/mL) Mean (%CV)	C_{max} (ng/mL) Mean (%CV)	C_{tau} (ng/mL) Mean (%CV)	T_{max} (h) Median (Q1, Q3)	$t_{½}$ (h) Median (Q1, Q3)
EVG	22,797.0 (34.7)	2113.1 (33.7)	287.3 (61.7)	3.92 (2.00, 4.00)	6.59 (6.18, 7.63)
COBI	9459.1 (33.9)	1450.3 (28.4)	20.6 (85.2)	3.00 (2.00, 3.98)	3.00 (2.81, 3.36)
FTC	11,714.1 (16.6)	2056.3 (20.2)	95.2 (46.7)	1.50 (1.08, 2.00)	6.41 (5.82, 6.97)

Table 43. PK of EVG, COBI, FTC and TFV following STRIBILD in HIV-Infected Subjects

Parameter Mean ± SD [range: min:max]	Elvitegravir	Cobicistat	Emtricitabine	Tenofovir
Cmax (microgram per mL)	1.7 ± 0. 4 [0.4:3.7]	1.1 ± 0.4 [0.1:2.1]	1.9 ± 0.5 [0.6:3.6]	0.45 ± 0.2 [0.2:1.2]
AUCtau (microgram•hour per mL)	23.0 ± 7.5 [4.4:69.8]	8.3 ± 3.8 [0.5:18.3]	12.7 ± 4.5 [5.2:34.1]	4.4 ± 2.2 [2.1:18.2]
Ctrough (microgram per mL)	0.45 ± 0.26 [0.05:2.34]	0.05 ± 0.13 [0.01:0.92]	0.14 ± 0.25 [0.04:1.94]	0.10 ± 0.08 [0.04:0.58]

Special populations

Impaired renal function

1) TAF study GS-US-120-0108

GS-US 120-108 Study Title: A Phase 1, Open-Label, Parallel-Design Study to Evaluate the Pharmacokinetics of GS-7340 in Subjects with Severe Renal Impairment

A single 25 mg dose of TAF was administered with water and at 5 min after a standard meal to 14 subjects with severe renal impairment (15 ≤ CrCL ≤ 29 mL/min) and then to 13 matched controls.

Table 44. Pharmacokinetic Parameters for TAF and TFV after a Single Dose of TAF 25 mg in Subjects with Severe Renal Impairment or Normal Renal Function

Mean (%CV)	Severe Renal Impairment (n=14)	Normal Renal Function (n=13)
TAF		
AUC_{inf} (ng.h/mL)	513.2 (47.3)	267.3 (49.2)
AUC_{last} (ng.h/mL)	510.6 (47.4)	265.9 (49.5)
C_{max} (ng/mL)	363.7 (65.7)	198.8 (62.1)
$t_{1/2}$ (h)	0.75 (51.8)	0.53 (22.8)
CL/F (mL/h)	61,717.8 (56.8)	117,633.1 (53.9)
CL_{renal} (mL/min)	4.2 (77.6)	35.8 (51.7)
Percent of dose recovered in urine (%)	0.47 (95.6)	2.00 (34.6)
Ae (ng)	117,230.4 (95.6)	500,408.6 (34.6)
TFV		
AUC_{inf} (ng.h/mL)	2073.8 (47.1)	342.6 (27.2)
AUC_{last} (ng.h/mL)	1694.9 (43.1)	298.0 (26.1)
C_{max} (ng/mL)	26.4 (32.4)	9.5 (36.5)
$t_{1/2}$ (h)	56.53 (19.6)	51.28 (12.2)
CL/F (mL/h)	8531.4 (36.4)	47,013.8 (26.3)
CL_{renal} (mL/min)	51.4 (40.1)	209.4 (24.6)
Percent of dose recovered in urine (%)	30.12 (24.6)	24.17 (23.3)
Ae (ng)	4,548,490 (24.6)	3,650,168 (23.3)

TAF - In severe renal impairment there was a 92% (< 2-fold) higher mean plasma AUC_{inf}, 92% higher AUC_{last} and 79% higher C_{max}. Correspondingly the mean plasma TAF CL/F was lower (61,717.8 mL/h vs. 117,633.1 mL/h, respectively, p = 0.003) but the half-life was not statistically significantly different. At 1 and 4 h the mean percent unbound TAF was not different between those with severe renal impairment (20.0% and 14.2%) vs. controls (20.1% and 13.6%). Approximately 0.117 mg TAF (0.47% of the dose) was excreted in urine in renally impaired subjects vs. ~0.500 mg (2.00%) in controls with renal clearance of 4.2 mL/min and 35.8 mL/min, respectively (but renal clearance is not a relevant pathway for TAF elimination).

TFV – In severe renal impairment there was much higher (about 5-6-fold) plasma exposure to TFV vs. controls with lower plasma and renal clearance but no significant difference in half-life. TFV plasma protein binding at 2 and 24 h was not different between groups (e.g. 99.2% vs. 98.9% at 24 h). Approximately 30% vs. 24% of the dose was excreted in urine.

The plasma TFV exposures (mean TFV AUC_{inf} 2073.8 ng•h/mL vs. 342.6 ng•h/mL for controls) were within or below the TFV exposure ranges of subjects with normal renal function taking TDF 300 mg once daily.

2) E/C/F/TAF study GS-US-292-0112

GS-US-292-01112 Study Title: A Phase 3 Open-label Safety Study of Elvitegravir/Cobicistat/ Emtricitabine/Tenofovir Alafenamide Single-Tablet Regimen in HIV-1 Positive Patients with Mild to Moderate Renal Impairment

Details of the design of this open-label study in HIV-infected patients with eGFRCG 30-69 mL/min are provided in the efficacy and safety sections. There was an intensive PK/PD sub-study and sparse sampling. TAF PK parameters were consistent with data obtained from HIV-infected patients with normal renal function in other Phase 2/3 studies (mean AUC_{last} 227.5 ng*h/mL, 229.8 ng*h/mL and 259.0 ng*h/mL) and in healthy subjects (AUC_{last} 244.8 ng*h/mL). Exposure to TAF was numerically higher in those with baseline eGFR < 50 vs. ≥50 mL/min but lower than the mean AUC_{last} of 510.6 ng*h/mL in subjects with eGFRCG 15 to 29 mL/min observed in GS-US-120-0108 (see Table 45).

Table 45. GS-US-292-0112: Mean (%CV) TAF Plasma Pharmacokinetic Parameters by Baseline eGFRCG (< 50 or ≥ 50 mL/min) in Cohort 1 (PK Substudy Analysis Set)

TAF PK Parameter	AUC_{last}		C_{max}	
Mean (%CV)	N	(ng*h/mL)	N	(ng/mL)
$eGFR_{CG}$ <50 mL/min (Excluding Subject 1598-8021)[a]	7	340.5 (60.1)	7	308.9 (82.2)
$eGFR_{CG}$ ≥ 50 mL/min	22	226.7 (44.1)	22	251.3 (62.9)

a One subject was excluded for sensitivity analysis as TAF PK parameter estimates are unreliable due to an unusual PK profile

TFV PK was higher when $eGFR_{CG}$ was 30 to 69 mL/min compared to HIV-infected patients in Phase 2/3 studies (mean TFV AUC_{tau} 326.2, 311.8 and 286.2 ng*h/mL) but lower than that observed in those with $eGFR_{CG}$ 15-29 mL/min in GS-US-120-0108 (mean AUC_{inf} 2073.8 ng*h/mL) and lower than observed with TDF-containing regimens in patients with normal renal function. Exposure to TFV was higher in the subgroup with $eGFR_{CG}$ < 50 vs. those with values ≥ 50 mL/min but was still <30% of plasma levels observed with TDF.

Table 46. GS-US-292-0112: Mean (%CV) TFV Plasma Pharmacokinetic Parameters by Baseline eGFRCG (< 50 or ≥ 50 mL/min) in Cohort 1 (PK Substudy Analysis Set)

TFV PK Parameter	AUC_{tau}		C_{max}		C_{tau}	
Mean (%CV)	N	(ng*h/mL)	N	(ng/mL)	N	(ng/mL)
$eGFR_{CG}$ <50 mL/min	8	680.4 (28.7)	8	36.1 (27.9)	7	21.7 (27.8)
$eGFR_{CG}$ ≥ 50 mL/min	21	504.0 (29.1)	22	26.9 (24.7)	18	18.2 (31.9)

EVG and COBI - The EVG and COBI PK parameters were in the range of data following administration of E/C/F/TAF in the Phase 2 study (mean EVG AUC_{tau} 22,797.0 ng*h/mL; mean COBI AUC_{tau} 9459.1 ng*h/mL. Exposures were comparable across eGFR groups and consistent with non-renal elimination pathways.

FTC - FTC plasma levels were higher in those with screening $eGFR_{CG}$ 30 to 69 mL/min compared to the Phase 2 study patients (mean AUC_{tau} 20,968 ng*h/mL vs. 11,714.1 ng*h/mL).

Table 47. GS-US-292-0112: FTC Plasma Pharmacokinetic Parameters (PK Substudy Analysis Set)

	AUC_{tau} Mean (%CV)		C_{max} Mean (%CV)		C_{tau} Mean (%CV)		T_{max} Median (Q1, Q3)		$t_{1/2}$ Median (Q1, Q3)	
	N	(ng*h/mL)	N	(ng/mL)	N	(ng/mL)	N	(h)	N	(h)
FTC	29	20,968.6 (25.5)	30	2645.3 (24.7)	26	194.2 (33.8)	30	1.98 (1.50, 3.00)	29	6.39 (5.70, 6.77)

Exposures were higher in those with baseline eGFR < 50 vs. ≥ 50 mL/min. These data are consistent with the FTC PK parameters observed in subjects with mild (CrCL = 50 to 80 mL/min) or moderate renal impairment (CrCL = 30 to 49 mL/min).

Table 48. GS-US-292-0112: Mean (%CV) FTC Plasma Pharmacokinetic Parameters by Baseline eGFRCG (< 50 or ≥ 50 mL/min) in Cohort 1 (PK Substudy Analysis Set)

FTC PK Parameter	AUC_{tau}		C_{max}		C_{tau}	
Mean (%CV)	N	(ng*h/mL)	N	(ng/mL)	N	(ng/mL)
$eGFR_{CG}$ <50 mL/min	8	25,139.5 (21.8)	8	3042.5 (13.4)	7	203.3 (17.7)
$eGFR_{CG}$ ≥ 50 mL/min	21	19,379.7 (23.1)	22	2500.9 (26.9)	19	190.8 (38.9)

The AUC reported for the 29 patients with data (~21,000 ng*h/mL) was similar to that in adults with mild renal impairment not requiring dose adjustment (mean AUC 19,900 ng*h/mL). This comparison is based on the following data for Emtriva as per US Product Information (Table 49 is reproduced from the Table 7 of the US PI):

Table 49. Mean ± SD Pharmacokinetic Parameters in Adult Subjects with Varying Degrees of Renal Function

Creatinine Clearance (mL/min)	>80 (N=6)	50–80 (N=6)	30–49 (N=6)	<30 (N=5)	ESRD[a] <30 (N=5)
Baseline creatinine clearance (mL/min)	107 ± 21	59.8 ± 6.5	40.9 ± 5.1	22.9 ± 5.3	8.8 ± 1.4
C_{max} (μg/mL)	2.2 ± 0.6	3.8 ± 0.9	3.2 ± 0.6	2.8 ± 0.7	2.8 ± 0.5
AUC (μg·hr/mL)	11.8 ± 2.9	19.9 ± 1.2	25.1 ± 5.7	33.7± 2.1	53.2 ± 9.9
CL/F (mL/min)	302 ± 94	168 ± 10	138 ± 28	99 ± 6	64 ± 12
CLr (mL/min)	213 ± 89	121 ± 39	69 ± 32	30 ± 11	NA[b]

a. ESRD subjects requiring dialysis

b. NA = Not Applicable

The AUC observed after daily dosing with E/C/F/TAF in the subset with $eGFR_{CG}$ 30 - < 50 mL/min (25,139 ng*h/mL) is identical to that shown in Table 48.

Impaired hepatic function

TAF study GS-US-120-0114

This was an open-label study in which single doses of 25 mg TAF were administered with water immediately after completion of a moderate-fat meal (600 calories, 27% fat). Subjects were enrolled in:

Cohort 1: Group 1: Subjects with mild hepatic impairment (CPT Class A score of 5-6) (n = 10)

Group 2: Subjects with normal hepatic function (n = 10)

Cohort 2: Group 1: Subjects with moderate hepatic impairment (CPT Class B score of 7–9) (n = 10)

Group 2: Subjects with normal hepatic function (n = 10)

Geometric least squares means (GLSM) and the GLSM ratios (%; 90% CI) of TAF and TFV plasma exposure parameters following a single dose of TAF to subjects with hepatic impairment and to normal matched control subjects are shown in Tables 50 and 51.

Table 50. GS-US-120-0114: Statistical Comparison of Tenofovir Alafenamide and Tenofovir Pharmacokinetic Parameters for Test (Mild Hepatic Impairment) versus Reference (Matched Normal Hepatic Function) Group (TAF PK Analysis Set)

PK Parameter	GLSMs		GLSM Ratio (%) (90% CI)
	Reference Treatment: Normal Matched Control Group (N=10)	Test Treatment: Mild Hepatic Impairment Group (N=10)	
TAF			
AUC_{inf} (ng•h/mL)	223.08	206.30	92.48 (66.25, 129.09)
AUC_{last} (ng•h/mL)	218.93	201.04	91.83 (65.15, 129.43)
C_{max} (ng/mL)	162.51	144.65	89.01 (57.69, 137.33)
TFV			
AUC_{inf} (ng•h/mL)	289.73	258.32	89.16 (67.20, 118.30)
AUC_{last} (ng•h/mL)	256.74	229.30	89.31 (67.30, 118.53)
C_{max} (ng/mL)	8.06	7.82	97.03 (75.93, 124.00)

GLSMs were obtained using a mixed-effects model.

Plasma exposures to TAF and TFV were comparable between subjects with mild hepatic impairment and matched controls. The lower exposures vs. controls were not considered to be clinically relevant.

Table 51. GS-US-120-0114: Statistical Comparison of Tenofovir Alafenamide and Tenofovir Pharmacokinetic Parameters for Test (Moderate Hepatic Impairment) versus Reference (Matched Normal Hepatic Function) Group (TAF PK Analysis Set)

PK Parameter	GLSMs		GLSM Ratio (%) (90% CI)
	Reference Treatment: Normal Matched Control Group (N=10)	Test Treatment: Moderate Hepatic Impairment Group (N=10)	
TAF			
AUC_{inf} (ng•h/mL)	173.14	195.10	112.69 (87.29, 145.47)
AUC_{last} (ng•h/mL)	167.71	192.96	115.06 (88.50, 149.57)
C_{max} (ng/mL)	104.07	123.53	118.70 (78.94, 178.47)
TFV			
AUC_{inf} (ng•h/mL)	238.16	231.53	97.22 (77.03, 122.70)
AUC_{last} (ng•h/mL)	212.39	202.95	95.55 (75.20, 121.42)
C_{max} (ng/mL)	8.10	7.09	87.56 (70.49, 108.76)

GLSMs were obtained using a mixed-effects model.

In moderate hepatic impairment plasma exposures for TAF were slightly higher and TFV exposures were slightly lower vs. matched controls. The differences were not considered to be clinically relevant.

At 1 and 4 h post-dose the mean unbound TAF ranged from 16% to 19% in mild hepatic impairment and from 14% to 23% in moderate hepatic impairment. At 2 and 24 h post-dose the mean unbound TFV was > 99% in mild or moderate hepatic impairment. For TAF and TFV binding was similar to controls.

Elderly

The POPPK analysis did not detect an effect of increasing age on PK within the limits of the database (i.e. with 80 treated aged > 65 years).

Children

The data for TAF come from 24 adolescents enrolled into GS-US-292-0106, which suggested no difference between ART-naïve adolescents and adults (data from GS-US-292-0102) for TAF or TFV. The POPPK predicted values were also comparable between adolescents and adults for TAF and TFV. The COBI exposures were lower in adolescents vs. adults in studies 0102 and 0103 (based on ratio and 90% CI within 80, 125%) but the EVG Cmax and AUC were comparable and only the EVG C_{trough} was lower (69.3; CI 52.8, 91). FTC exposures were also comparable between age groups.

Table 52. Multiple-Dose TAF exposure in ARV-naive adolescents and adults

TAF PK Parameter	Adolescents E/C/F/TAF (N = 24)	Adults E/C/F/TAF (N = 19)
AUC_{last} (ng•h/mL)	188.9 (55.8)	227.5 (47.3)
C_{max} (ng/mL)	166.8 (64.4)	232.8 (64.6)
Multiple-Dose TFV exposure in ARV-naive adolescents and adults		
TFV PK Parameter	**Adolescents E/C/F/TAF (N = 24)**	**Adults E/C/F/TAF (N = 19)**
AUC_{tau} (ng•h/mL)	287.6 (18.8)	326.2 (14.8)
C_{max} (ng/mL)	17.6 (23.7)	18.2 (12.4)
C_{tau} (ng/mL)	10.0 (21.4)	11.4 (17.9)

Race

The POPPK analysis did not detect an effect of racial group on TAF PK. There was a statistically significant effect of race on TFV PK but the range of exposures was comparable and >80% lower than observed with STB so that the difference was not considered to be clinically relevant.

In **GS-US-292-0108** single and multiple QD dosing with E/C/F/TAF (10 mg) was compared between healthy Japanese (10) and Caucasian (10) subjects. After multiple dosing:

- The AUCs of each analyte were lower in Japanese vs. Caucasian subjects.
- The C_{max} values were more variable but were lower in Japanese subjects for EVG and COBI.
- The C_{tau} values for EVG, COBI, FTC and TFV were all lower in Japanese subjects.

The lower bounds of the 90% CI were well below 80% for:

- EVG and COBI Cmax, AUC_{tau} and C_{tau}.
- FTC AUC_{tau} and C_{tau}.
- TAF AUC_{last}.
- TFV AUC_{tau} and C_{tau}.

Nevertheless, the differences observed are not expected to affect the overall antiviral effect of the FDC.

Interactions - In vitro data

Cytochrome P450 inhibition

- Using human liver microsomal CYP isoenzymes (CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6 and CYP3A) **TAF** IC_{50} values were > 25 µM except that TAF showed weak inhibition of CYP3A-mediated oxidation of midazolam or testosterone with IC_{50} values of 7.6 or 7.4 µM, respectively.
- **TAF** was not a mechanism-based inhibitor of human CYP enzymes CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19 and CYP2D6 50 µM. The maximum change in activity was 17.4% with CYP2C8.
- **TFV** at 100 µM did not inhibit CYP1A2, CYP2C9, CYP2D6, CYP2E1 or CYP3A.

Cytochrome P450 induction

Due to cytotoxicity cell viability was significantly affected at 100 µM TAF and mixed responses to TAF with increased mRNA levels and reduced CYP activities were observed. At 1 and 10 µM TAF there were no significant increases in mRNA levels or CYP activities. At 10 µM TAF the mRNA levels of CYP1A2 and CYP3A4 increased by 3.0- and 8.3-fold, or 3% and 6% of the induction with positive controls.

UGT1A1 inhibition

TAF was assessed for inhibition of oestradiol 3-glucuronide formation in insect cell microsomal fractions containing baculovirus-expressed human UGT1A1. TAF did not inhibit UGT1A1 up to 50 µM.

UGT1A1 and P-gp induction

There was no significant induction of P-gp and UGT1A1 mRNA by **TAF** (less than 2-fold increases).

Other transporter studies

TAF

- TAF showed little or no inhibition of the transport of model substrates by P-gp, BCRP, OAT1, OAT3 and OCT2. Weak inhibition of OATP1B1, OATP1B3, BSEP, OCT1 and MATE1 was observed but none was inhibited by 50% at 100 µM TAF, which is approximately 200-fold Cmax in plasma.
- TAF is a substrate for the intestinal efflux transporters P-gp and BCRP. An increase in TAF absorption was observed in the presence of efflux transport inhibitors CsA or COBI. The applicant has started that co-administration of TAF with efflux inhibitors may potentiate antiviral efficacy by increasing TFV-DP levels in PBMCs.
- TAF is also a substrate for hepatic uptake transporters OATP1B1 and OATP1B3. Exposure to TAF may be affected by inhibitors of these transporters or genetic polymorphisms that affect transport activities.
- TAF was not a substrate for renal transporters OAT1 and OAT3 or for OCT1.

TFV

The active tubular secretion of TFV is mediated by human OAT1 (basolateral uptake) and MRP4 (apical efflux) transporters acting in series in proximal tubules. Human OAT3 may play a secondary role in the tubular uptake of TFV.

OAT1 has been assessed for its potential role in drug interactions between TFV and other renally secreted agents. Under physiologically relevant conditions, none of the tested drugs affected OAT1-mediated transport of TFV.

MRP1 is not involved in reabsorption of TFV at the basolateral membrane of proximal tubule cells.

Neither P-gp nor MRP2 appear to be involved in the tubular efflux of TFV.

TFV did not inhibit the activity of human OCT2 or MATE1 (IC50 > 300 µM).

Other relevant information

- There is no evidence for inhibition of TFV renal excretion by FTC, EVG or COBI.
- Transport of TFV by OAT1, OAT and MRP4 was not meaningfully inhibited by COBI at clinically relevant concentrations. In addition, COBI had no effect on the accumulation of TFV in human renal tissue slices at clinically relevant concentrations.
- COBI is a weak to moderate inhibitor of OCT2, MATE2-K and OCTN1 while COBI and EVG are more potent inhibitors of MATE1 but TAF is not an inhibitor of any of these transporters.

- Inhibition of BSEP by COBI is unlikely to be clinically meaningful as the IC50 (6.5 µM) is in excess of the total plasma Cmax.
- COBI is a weak inhibitor of intestinal efflux transporters but high concentrations in the intestinal lumen may inhibit P-gp and increase TAF exposure. In the presence of 90 µM COBI in the Caco-2 bidirectional permeability assay TAF forward permeability increased 4.6-fold and the efflux ratio significantly decreased, suggesting P-gp mediated drug interaction.
- COBI is a specific and potent inhibitor of CYP3A so that the weak inhibition effected by TAF is not expected to add to the DDI potential of the STR.
- TFV and FTC do not inhibit each other's pharmacological activation through phosphorylation.

Activation of human AhR or human PXR

- At 50 µM **TAF** the extent of activation of PXR was only 23% of the maximal effect of rifampicin and 15 µM TAF demonstrated activation of < 5% of the maximal induction elicited by rifampicin.
- **TAF** did not activate AhR up to 50 µM, the highest concentration tested.

Interactions - In vivo data

GS-US-311-0101 - F/TAF with Efavirenz [EFV] and Darunavir [DRV]; TAF with COBI

GS-US-311-0101 Study Title: A Phase 1 Study Evaluating the Drug Interaction Potential Between Once-Daily FTC/GS-7340 Fixed Dose Combination and Efavirenz or Cobicistat-Boosted Darunavir

Treatments were as follows:

Cohort 1	Days 1 to 12: Treatment A, FDC oral tablet containing FTC 200 mg and GS-7340 40 mg once daily in the morning, fasted	Days 13 to 26: Treatment B, FDC oral tablet containing FTC 200 mg and GS-7340 40 mg plus EFV 600-mg oral tablet once daily in the morning, fasted
Cohort 2	Days 1 to 12: Treatment C, FDC oral tablet containing FTC 200 mg and GS-7340 25 mg once daily in the morning, fed	Days 13 to 22: Treatment D, FDC oral tablet containing FTC 200 mg and GS-7340 25 mg plus DRV/co 2 × 400/1 × 150-mg oral tablets once daily in the morning, fed
Cohort 3	Days 1 to 10: Treatment E, DRV/co 2 × 400/1 × 150-mg oral tablets once daily in the morning, fed	Days 11 to 22: Treatment F, FDC oral tablet containing FTC 200 mg and GS-7340 25 mg plus DRV/co 2 × 400/1 × 150-mg oral tablets once daily in the morning, fed
Cohort 4	Days 1 to 12: Treatment G, Oral tablet containing single-agent GS-7340 8 mg once daily in the morning, fed	Days 13 to 22: Treatment H, Oral tablet containing single-agent GS-7340 8 mg plus COBI 150-mg oral tablet once daily in the morning, fed

In Cohort 1 FTC/TAF (40 mg) plus EFV resulted in no clinically relevant changes in FTC, TFV or TAF AUCs compared with FTC/TAF (40 mg) dosed alone based on the pre-defined acceptance criteria.

The TAF and TFV C_{max} values were lower on co-administration but the differences were not considered to be clinically meaningful. EFV exposures were comparable to historical data.

In Cohorts 2 and 3 co-administration of FTC/TAF (25 mg) with DRV/co (800/150 mg) for 10-12 days resulted in comparable exposures to TAF and FTC but substantially higher TFV exposures vs. FTC/TAF (25 mg) dosed alone in Cohort 2.

- TAF $AUCl_{ast}$ and Cmax increased following co-administration of single doses, suggesting an inhibitory drug interaction resulting in increased availability of TAF that abated following multiple dosing.
- The increase in TFV but not TAF exposures after multiple-dose co-administration was considered to be due to a mixed inhibitory/inductive effect of COBI on P-gp, influencing TAF absorption.
- COBI and FTC exposures were consistent with historical data (e.g. GS-US-236-0101 and 0110).
- There was no effect of co-administration with FTC/TAF on DRV PK.

In Cohort 4 co-administration of TAF 8 mg plus COBI 150 mg gave substantially higher TAF and TFV exposures vs. TAF 8 mg dosed alone. COBI exposures were in the range of historical data. The effect was ascribed to COBI-mediated inhibition of P-gp-mediated intestinal secretion of TAF.

Pharmacokinetic results for the primary exposure parameters of GS-7340, TFV, COBI, FTC, and DRV following administration of the test and reference treatments are summarized by cohort in Tables 53 and 54.

Table 53. Pharmacokinetic results GS-7340

GS-7340 PK Parameter	Test Mean (%CV)	Reference Mean (%CV)	GLSM Ratio (Test/Reference) (%)	90% Confidence Interval
Cohort 1: FTC/GS-7340 200/40 mg + EFV (Test) vs FTC/GS-7340 200/40 mg (Reference), (N = 11)				
AUC_{last} (ng·h/mL)	285.8 (46.4)	344.0 (60.9)	85.54	(72.08, 101.52)
C_{max} (ng/mL)	390.8 (62.2)	499.4 (82.8)	77.92	(57.68, 105.25)
Cohort 2: FTC/GS-7340 200/25 + DRV/co (Test) vs FTC/GS-7340 200/25 mg (Reference), (N = 11)				
AUC_{last} (ng·h/mL)	239.3 (41.0)	245.6 (41.9)	97.64	(80.38, 118.62)
C_{max} (ng/mL)	215.0 (59.2)	208.3 (40.2)	93.43	(72.16, 120.98)
Cohort 4: GS-7340 8 mg + COBI (Test) vs GS-7340 8 mg (Reference), (N = 12)				
AUC_{last} (ng·h/mL)	213.3 (37.7)	81.2 (43.9)	265.06	(229.00, 306.80)
C_{max} (ng/mL)	189.9 (45.6)	71.0 (72.9)	283.31	(219.65, 365.43)

Table 54. Pharmacokinetic results TFV

TFV PK Parameter	Test Mean (%CV)	Reference Mean (%CV)	GLSM Ratio (Test/Reference) (%)	90% Confidence Interval
Cohort 1: FTC/GS-7340 200/40 mg + EFV (Test) vs FTC/GS-7340 200/40 mg (Reference), (N = 11)				
AUC_{tau} (ng·h/mL)	350.2 (31.7)	430.9 (24.0)	79.72	(73.34, 86.65)
C_{max} (ng·h/mL)	24.0 (34.7)	31.1 (26.2)	75.49	(66.65, 85.50)
C_{tau} (ng/mL)	11.4 (32.4)	13.6 (22.5)	81.61	(74.74, 89.10)
Cohort 2: FTC/GS-7340 200/25 + DRV/co (Test) vs FTC/GS-7340 200/25 mg (Reference), (N = 11)				
AUC_{tau} (ng·h/mL)	953.4 (20.0)	299.3 (29.3)	323.88	(302.11, 347.21)
C_{max} (ng/mL)	57.4 (23.2)	18.3 (27.8)	316.03	(300.13, 332.76)
C_{tau} (ng/mL)	33.7 (19.7)	10.8 (33.2)	320.56	(290.05, 354.27)
Cohort 4: GS-7340 8 mg + COBI (Test) vs GS-7340 8 mg (Reference), (N = 12)				
AUC_{tau} (ng·h/mL)	286.9 (21.9)	86.1 (19.4)	330.88	(310.20, 352.93)
C_{max} (ng/mL)	19.3 (20.5)	5.8 (19.5)	334.09	(301.98, 369.62)
C_{tau} (ng/mL)	10.3 (24.4)	3.0 (19.9)	334.86	(312.43, 358.91)

GS-US-292-1316 – E/C/F/TAF with Sertraline [SER]

Co-administration of E/C/F/TAF (10 mg) with SER was evaluated because SER is eliminated by CYP2D6, CYP2C9, CYP2B6, CYP2C19 and CYP3A4 with percentage contributions estimated to be ~35%, 29%, 14%, 13% and 9%, respectively. COBI is an inhibitor of CYP3A (major) and CYP2D6 (minor).

Based on the pre-defined acceptance criteria co-administration had no clinically relevant effect on the PK of EVG, COBI, FTC, TAF, TFV or SER. Exposures to all analytes were consistent with historical data. All except two comparisons gave 90% CI that fell within 80, 125% and in the two exceptions (COBI C_{tau} and SER AUC0-∞) the lower boundary was only just below 80%.

GS-US-342-1167 – E/C/F/TAF with Sofosbuvir [SOF] and GS-5816

GS-5816 is an inhibitor of HCV NS5A which is being developed for use with SOF 400 mg daily. Cohorts 2, 3 and 4 were dosed within 5 minutes of completing breakfast (~ 600 kcal, 25-30% fat) with 4-day washouts.

Cohort 1 (dosed in the fasted state with no washout periods):

A: SOF/GS-5816 once daily for 14 days; B: EFV/FTC/TDF once daily for 14 days

C: SOF/GS-5816 plus EFV/FTC/TDF once daily for 14 days

Cohort 2:

D: SOF/GS-5816 once daily for 8 days; E: FTC/RPV/TDF once daily for 8 days

F: SOF/GS-5816 plus FTC/RPV/TDF once daily for 8 days

Cohort 3:

G: SOF/GS-5816 once daily for 8 days; H: DTG (50 mg) once daily for 8 days

I: SOF/GS-5816 plus DTG (50 mg) once daily for 8 days

Cohort 4:

J: SOF/GS-5816 once daily for 8 days; K: E/C/F/TAF once daily for 8 days

Treatment L: SOF/GS-5816 plus E/C/F/TAF once daily for 8 days

Co-administration of SOF/GS-5816 with E/C/F/TAF resulted in an increase in SOF, GS-331007 and GS-5816 exposures, which were attributed to COBI.

- The increase in SOF exposure was attributed to inhibition of drug transporters (e.g. P-gp) by COBI.
- The relative increase in GS-331007 exposure was similar to that observed when ledipasvir (LDV)/SOF was administered with EVG/COBI and does not require SOF dose adjustment.
- The ~50% increase in GS-5816 exposure was attributed to inhibition of drug transporters (e.g. P-gp) and CYP enzymes by COBI.
- Exposures to EVG and FTC were not altered following co-administration with SOF/GS-5816.
- There was ~100% increase in COBI Ctau when E/C/F/TAF was given with SOF/GS-5816. Due to the relatively short COBI t1/2 (~3.5 h) there was no impact on AUC COBI.
- TFV was unchanged when E/C/F/TAF was given with SOF/GS-5816. GS-5816 inhibits P-gp but there was no effect of co-administration on TAF (P-gp substrate) or TFV since COBI already effected P-gp inhibition.

It was concluded that SOF/GS-5816 may be administered with E/C/F/TAF without dose adjustment.

2.4.3. Pharmacodynamics

Mechanism of action

TAF is predominantly hydrolysed to TFV by Cathepsin A (CatA) cleavage in target lymphoid cells. TFV (a monophosphate; i.e. nucleotide analogue) is then metabolised to TFV diphosphate (TFV-DP, i.e. a triphosphate molecule), which is a competitive inhibitor of HIV-1 RT. In HIV-infected T-cells, macrophages and PBMCs TAF EC_{50} values ranged from 3 to 14 nM. The in-vitro activity of TAF against HIV-1 is 100- to 600-fold greater than TFV and 4- to 6-fold greater than TDF.

In primary CD4+ T lymphocytes and MDMs isolated from PBMCs from 13 donors the levels of active CatA were comparable across donors and the mean rate of TFV-alanine formation was similar between quiescent and activated CD4+T cells (2.7 vs. 3.0 pmol/min•µg) with ≤ 3-fold differences between

donors. In both primary cell types, the intracellular accumulation of TAF metabolites and conversion of TAF to TFV-DP were consistent across the 8 demographically diverse donors.

Certain viral PIs are inhibitors of CatA. Up to a concentration of 50 µM, which is well above the clinical Cmax of each of DRV, ATV, LPV, RTV and COBI, there was no significant inhibition of the CatA-mediated hydrolysis of TAF. In contrast, telaprevir and boceprevir (irreversible inhibitors) inhibited CatA-mediated hydrolysis of TAF with IC50 values of 0.3 and 0.2 µM, respectively, and use with E/C/F/TAF is not recommended.

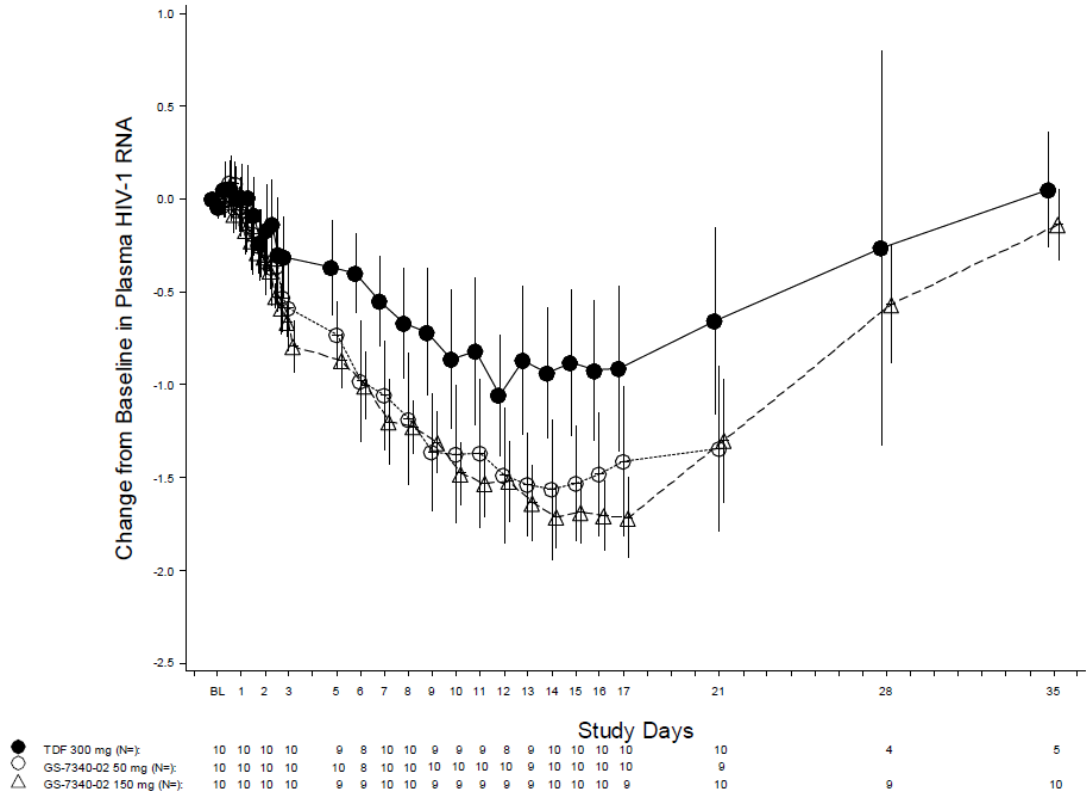
Primary and Secondary pharmacology

TAF monotherapy

GS-120-1101 was an early study (2003) that compared the antiviral activity of TAF (50 mg and 150 mg QD as the monofumarate) vs. TDF during 14 days monotherapy in 30 ART-naïve patients (27 male) with plasma HIV-1 RNA ≥ 15,000 copies/mL and CD4 cell count ≥ 200 cells/mm^3 at screening. All dosing used a 2 h pre- and post-dose fasting window. TAF appeared rapidly in plasma but had a half-life of 20 to 40 minutes. Repeat dosing showed a longer elimination half-life and greater TFV accumulation in the TAF groups. TFV was detectable within PBMCs earlier, more consistently, and at greater concentrations after TAF vs. TDF.

DAVG1, 2 and 3 were significantly greater in the TAF vs. TDF groups but there was no significant difference between the TAF doses. No resistance mutations to TAF or TDF were detected in any groups and there was no consistent effect on CD4 counts although there was an increase in each group.

Figure 7. Mean and 95% Confidence Interval of Change From Baseline in Plasma HIV-1 RNA Over Time



GS-US-120-0104 compared TAF monotherapy (8, 25 or 40 mg) to TDF and placebo over 10 days with all dosing once daily in the fasting state based on DAVG11. HIV-1 infected patients with HIV-1 RNA > 2000 copies/mL, CD4 counts ≥ 200 cells/mm^3 and without use of ARV within 90 days were eligible. TAF was rapidly absorbed with a short plasma t½ of ~0.40 hours and no evidence of accumulation on multiple dosing. TFV plasma concentrations were substantially higher when it was given as TDF 300 mg. At steady state, the mean TFV AUCtau after TAF doses were 97%, 86% and 79% lower, respectively, vs. the mean after TDF dosing while Cmax was 98%, 94% and 89% lower, respectively.

Table 55. GS-US-120-104: Individual estimates and summary statistics for plasma PK parameters by treatment (Analysis set: TFV PK, Analyte TFV)

TFV PK Parameter	TFV Single Dose PK[a] Day 1				TFV Multiple Dose PK Day 10			
	GS-7340 8 mg (n = 9)	GS-7340 25 mg (n = 8)	GS-7340 40 mg (n = 8)	TDF 300 mg (n = 6)	GS-7340 8 mg (n = 9)	GS-7340 25 mg (n = 8)	GS-7340 40 mg (n = 8)	TDF 300 mg (n = 6)
AUC_{inf} for single dose or AUC_{tau} for multiple dose (ng•h/mL), Mean (%CV)	49.4 (30.3)	195.9 (27.2)	287.3 (33.7)	1719.2 (57.9)	65.5 (23.5)	267.7 (26.7)	405.8 (12.7)	1918.0 (39.4)
C_{max} (ng/mL), Mean (%CV)	2.0 (31.1)	6.5 (40.1)	14.0 (20.3)	181.2 (50.5)	4.2 (24.7)	15.7 (22.1)	28.3 (8.7)	252.1 (36.6)
C_{tau} (ng/mL), Mean (%CV)	0.69 (19.8)	2.4 (23.5)	4.0 (27.2)	23.9 (57.5)	2.1 (33.8)	9.21 (26.1)	13.3 (16.0)[b]	38.7 (44.7)
T_{max} (h), Median (Q1, Q3)	1.00 (1.00, 2.00)	1.50 (1.03, 1.75)	1.00 (0.75, 1.00)	1.25 (0.53, 1.50)	1.50 (1.00, 1.98)	1.50 (1.25, 1.75)	1.29 (1.04, 1.50)	1.25 (0.58, 2.00)
$t_{½}$ (h), Median (Q1, Q3)	23.85 (18.32, 37.17)[b]	29.83 (26.87, 44.00)[b]	24.55 (20.33, 28.25)	15.56 (14.17, 17.07)	30.77 (26.90, 55.61)[c]	40.19 (28.98, 44.84)	35.95 (26.38, 42.90)[b]	14.86 (12.18, 16.81)

%CV, percent of coefficient of variation; Q1, first quartile; Q3, third quartile

a AUCinf and C24h are presented for single dose PK

b n = 7

c n = 8

The PBMC TFV-DP concentrations were highly variable across dose groups and time points. However, the mean AUCtau was similar between TAF 8 mg and TDF 300 mg and was ~ 7-fold and ~ 25-fold higher after 25 mg and 40 mg doses of TAF, respectively. After the last dose 3/6 TAF patients still had detectable TFV-DP on day 21 compared to 1/4 TDF patients studied. The antiviral effect of TAF 8 mg was similar to that of TDF 300 mg. There were statistically greater decreases in viral load with 25 mg and 40 mg TAF vs. TDF 300 mg and first phase decay slopes for plasma HIV-1 RNA were significantly steeper with TAF.

Table 56. Time-Weighted average change from baseline up to Day 11 (DAVG11) in plasma HIV-1 RNA (Full analysis set)

	GS-7340 (8 mg) (N=9)	GS-7340 (25 mg) (N=8)	GS-7340 (40 mg) (N=8)	TDF (300 mg) (N=6)	Placebo (N=7)
Baseline					
Mean (SD)	4.51 (0.369)	4.52 (0.377)	4.34 90.4770	4.96 (0.308)	4.24 (0.780)
95% CI	(4.23,4.79)	(4.21,4.84)	(3.95,4.74)	(4.63,5.28)	(3.52,4.96
Median	4.50	4.64	4.44	4.90	4.32
Q1-Q3	4.41,4.79	4.45,4.73	4.08,4.73	4.76,5.13	3.79,4.77
Min, Max	3.78,4.96	3.69,4.87	3.42,4.83	4.60,5.45	2.81,5.27
Pairwise p-values					
p-value vs placebo	0.46	0.45	0.77	0.054	
p-value vs TDF	0.052	0.061	0.033		
p-value vs GS-7340 (40mg)	0.53	0.49			
p-value vs GS-7340 (25mg)	0.96				
DAVG11					
Mean (SD)	-0.67 (0.265)	-0.94 (0.254)	-1.14 (0.226)	-0.45 (0.340)	0.13 (0.391)
95% CI	(-0.88,-0.47)	(-1.15,-0.72)	(-1.33,-0.95)	(-0.81,-0.09)	(-0.23,0.49)
Median	-0.76	-0.94	-1.08	-0.48	-0.01
Q1,Q3	-0.86,-0.57	-1.12,-0.76	-1.35,-0.97	-0.57,-0.34	-0.03,0.01
Min, Max	-0.97,-0.24	-1.13,-0.54	-1.46,-0.84	-0.94,0.11	-0.08,1.01
Pairwise p-values					
p-value vs placebo	0.001	0.001	0.001	0.038	
p-value vs TDF	0.22	0.017	0.006		
p-value vs GS-7340 (40mg)	0.003	0.13			
p-value vs GS-7340 (25mg)	0.075				

The TFV-DP in PBMCs and DAVG11 showed correlation coefficients as shown below.

Table 57. GS-US-120-0104: Correlation between PBMC Concentrations and Time-Weighted Average Change from Baseline up to Day 11 (DAVG11) (PK/PD Analysis Set)

TFV-DP PK Parameter	Treatment Group	Number of Data Pairs	Pearson Correlation Coefficient	p-value
AUCtau of TFV-DP in PBMC vs $DAVG_{11}$	GS-7340 (8 mg)	6	−0.063	0.906
	GS-7340 (25 mg)	4	−0.701	0.299
	GS-7340 (40 mg)	6	−0.338	0.512
	TDF (300 mg)	3	−0.933	0.234
	All	19	−0.535	0.018

Note: DAVG11 is the time-weighted average change from baseline to study Day 11.

Note: Correlation coefficients and p-values are from the Pearson correlation analysis

There was no statistically significant difference in changes from baseline in CD4 cell counts for any treatment group. Viral resistance to TAF or TDF did not develop based on data from 37/38 patients.

Resistance studies

Since TAF is a pro-drug of TFV it is affected by the same resistance associated mutations (RAMs). In-vitro studies indicated that TAF and TFV have similar propensities to select for mutational resistance and that EC_{50} values are affected to a similar extent by various mutations and combinations of mutations (in terms of fold differences vs. wild-type viruses).

Viral breakthrough experiments were conducted using known TDF-resistant HIV-1 isolates in MT-2 cells at a higher multiplicity of infection (MOI) compared to typical EC_{50} assays; the EC_{50} values for TAF and TFV were 0.02 and 5 µM, respectively. The cells were incubated in the presence of TAF or TFV (at the EC_{95}) followed by HIV-1 infection. After 4 or 5 days of incubation cultures were scored for viral breakthrough (i.e., CPE) and the procedure was repeated every 4 to 5 days for up to 4 weeks. Physiologically relevant concentrations of TAF inhibited viral breakthrough for the duration of the experiment for 9/11 viruses but viral breakthrough was only inhibited for 2/11 viruses in the presence of TFV. However, breakthrough of viruses with 5 TAMs was not prevented by TAF or TFV.

Secondary pharmacology

GS-US-120-0107 was a TQT study using single doses of 25 mg and 125 mg TAF taken within 5 minutes of consuming a standard breakfast. Pharmacokinetic parameters for TAF and TFV following single oral doses of 25 or 125 mg TAF were approximately proportional to dose. The lower bound of the 2-sided 90% CI for the mean difference between moxifloxacin and placebo was greater than 5 ms at 2 post-dose time points (3 and 4 h) establishing assay sensitivity. For the primary analysis, TAF was concluded to have no QTcF prolongation effect as the upper bounds of the 2-sided 90% CIs for the mean difference between 25 mg and 125 mg TAF and placebo were below 10 ms at each time point after dosing. Small negative changes in QTcF were observed at both doses.

Table 58. GS-US-120-0107: Statistical Analysis of Dose Proportionality (TFV PK Analysis Set)

	Geometric Least Squares Means			
TFV PK Parameter	Test Treatment TAF 125 mg (n = 48)	Reference Treatment TAF 25 mg (n = 48)	Geometric Least Squares Mean Ratio (%)	90% Confidence Interval (%)
AUC_{last} (ng·h/mL)	1121.29	190.91	587.35	(566.51, 608.96)
AUC_{inf} (ng·h/mL)	1505.60	250.08	602.04	(578.15, 626.92)
C_{max} (ng/mL)	49.17	8.40	585.28	(556.34, 615.71)

Table 59. GS-US-120-0107: Mixed-Model Analysis of Change from Predose Baseline in QTcF (msec) for Noninferiority Evaluation (PD Analysis Set)

	LS Means			Treatment Difference		90% Confidence Intervals	
Scheduled Time	TAF 25 mg (N=48)	TAF 125 mg (N=48)	Placebo (N=48)	TAF 25 mg - Placebo	TAF 125 mg - Placebo	TAF 25 mg - Placebo	TAF 125 mg - Placebo
20 mins	-3.6	-4.5	-3.5	-0.1	-1.0	(-2.1, 1.8)	(-2.9, 1.0)
40 mins	-9.8	-9.8	-8.8	-1.0	-1.0	(-2.9, 1.0)	(-2.9, 1.0)
1 hour	-9.2	-8.4	-8.8	-0.4	0.4	(-2.3, 1.5)	(-1.5, 2.4)
1.5 hours	-6.8	-7.4	-7.7	0.9	0.3	(-1.1, 2.9)	(-1.7, 2.2)
2 hours	-10.8	-8.9	-9.2	-1.6	0.3	(-3.5, 0.4)	(-1.7, 2.2)
3 hours	-12.1	-12.3	-12.9	0.8	0.6	(-1.1, 2.7)	(-1.3, 2.5)
4 hours	-6.6	-6.5	-7.1	0.5	0.5	(-1.4, 2.5)	(-1.4, 2.5)
12 hours	-6.5	-5.7	-6.6	0.1	0.9	(-1.8, 2.1)	(-1.0, 2.8)
24 hours	-3.5	-3.2	-2.5	-1.0	-0.7	(-3.0, 0.9)	(-2.7, 1.2)
48 hours	-7.7	-9.6	-8.2	0.5	-1.4	(-1.4, 2.5)	(-3.3, 0.6)
72 hours	-7.8	-7.4	-7.5	-0.4	0.1	(-2.3, 1.6)	(-1.9, 2.0)

LS Means = Least Squares Means

Note: LS means and CIs were based on the mixed-effect model including sequence, period, treatment, time point, treatment by time point interaction, and gender as fixed effects; subject within sequence as a random effect; and the predose baseline QTcF as a continuous covariate.

Analyses of secondary endpoints (QTcB, QTcN and QTcI) were consistent with the primary analysis. No-one had a treatment-emergent absolute QTc interval > 500 ms or a change from pre-dose baseline QTc > 60 ms with any correction factor. No subject had a treatment-emergent absolute QTc interval > 480 ms or a change from pre-dose baseline > 30 ms following either dose of TAF. There was no consistent, pharmacologically meaningful association between time-matched, baseline-adjusted and placebo-corrected QTc and TAF or TFV plasma concentrations. There was a weak negative association between TFV plasma concentrations and QTcF that was maintained after adjustment for gender.

Relationship between plasma concentration and effect

In **GS-US-120-0104** mean viral load declines for TAF 25 mg and 40 mg doses were statistically greater than for the 8 mg dose. The PK/PD relationships between TAF and TFV plasma exposures and antiviral activity were explored using a maximum (PD) effect (E_{max}) model, where Effect = (E_{max} • $\log_{10}$ PK parameter)/($\log_{10}$ PK parameter 50 + log PK parameter). The TAF AUC fitted well with an E_{max} model, with an E_{max} of ~1.7 to 1.8 $\log_{10}$ decline from baseline and the EC_{50} for AUC of ~32 ng•h/mL. A similar fit/E_{max} estimate was obtained using TAF C_{max}.

Upon comparison of antiviral activity with 40 mg and previous data with 120 mg (GS-120-1101), TAF 25 mg was expected to provide near-maximal activity. Plasma TFV AUC, which was substantially lower with TAF vs. TDF, did not correlate with antiviral activity.

In Phase 3 studies in ART-naïve patients **GS-US-292-0104 and 0111** the PK/PD analysis used available PK data and the primary efficacy endpoint (< 50 copies/mL at week 48; FDA snapshot algorithm). There were no trends in exposure-response relationship observed, which supported the

selection of TAF 10 mg for the E/C/F/TAF FDC based on equivalent exposure to unboosted TAF 25 mg, which was concluded to provide near maximal antiviral efficacy in the monotherapy study (see above). The results were also in accordance with the lack of statistically significant covariates on PK in the POPK analyses and the comparable efficacy observed across patient subgroups according to demographics. Evaluations of exposure-safety relationships in these two studies did not find an association between PK and diarrhoea, nausea, vomiting, GI/abdominal pain, percentage change from baseline at Week 48 in BMD or maximum increase from baseline in serum creatinine.

Pharmacodynamic interactions with other medicinal products or substances

The cellular enzymes responsible for TFV phosphorylation are adenylate kinase (AK) and nucleotide diphosphate kinase, which are highly active and ubiquitous. Adenylate kinase exists as 2 isozymes, AK1 and AK2, and phosphorylation of TFV is mediated more efficiently by AK2. The effects of combination of TFV with other agents that require intracellular phosphorylation has been investigated *in vitro*. Combination with ABC did not change the rates of phosphorylation of either compound to the triphosphate (active) forms.

The anti-HIV-1 activity of TAF in combination with a broad panel of representatives from the major classes of approved anti-HIV agents (N[t]RTIs, NNRTIs, INSTIs, and PIs) was evaluated in HIV-1_{IIIB} infected MT-2 cells. Viral growth/inhibition was evaluated by measuring virus-induced CPE. The ddI+ribavirin (RBV), d4T+RBV and TAF+TAF combinations were controls for synergy, antagonism and addition, respectively.

TAF exhibited moderate to high synergistic effects (synergy scores from 41 to 131) when combined with any of the N(t)RTIs or NNRTIs. The combination of TAF with INSTIs resulted in the highest level of synergy (synergy scores of 271, 205, and 179 for EVG, RAL and DTG, respectively. The combination of TAF with PIs resulted in moderate synergy (synergy scores of 96 and 56 for ATV and DRV, respectively). Combination of TAF with COBI resulted in an additive effect. The synergy values observed for TAF with all the drugs tested were comparable with those of TFV performed in parallel and to values previously reported for TFV. None of the drug combinations containing TAF exhibited antagonistic antiviral effects.

2.4.4. Discussion on clinical pharmacology

Selection of the formulation and dose of TAF

Stribild is marketed as a bilayer tablet whereas the E/C/F/TAF FDC is a monolayer tablet. After daily dosing with food in GS-US-292-0101 both the mono- and bilayer formulations containing 25 mg TAF (monofumarate) gave TAF and TFV plasma concentrations that were substantially higher (2- to 3-fold increase in GS-7340 and TFV) vs. 25 mg TAF given alone. In addition, both formulations containing 25 mg TAF gave very much lower TFV plasma levels compared to STB along with comparable exposures to EVG, COBI and FTC. These results indicated that mono- and bilayer tablets performed similarly and supported the monolayer presentation.

Co-administration of 8 mg TAF with 150 mg COBI in GS-US-311-0101 increased the C_{max} and AUC of TAF and TFV vs. 8 mg TAF alone to the same extent as observed with the test formulations in GS-US-292-0101 vs. 25 mg TAF alone. On this basis it was reasonable to conclude that the effect was mainly due to inhibition of P-gp-mediated intestinal secretion of TAF by COBI.

Based on the TAF monotherapy study GS-US-120-0104 it was calculated that the final FDC formulation should contain ~10 mg TAF in order to provide steady state plasma TAF and TFV exposures and antiviral effects similar to those achieved with ~25 mg TAF given alone. To confirm this calculation,

GS-US-292-0103 compared an FDC containing 10 mg TAF (as 11.2 mg [hemi]-fumarate; the final presentation) with FTC 200 mg and TAF 25 mg, there being no known interaction between the latter. After multiple daily dosing with food, the plasma TAF Cmax and AUC after the FDC containing 10 mg TAF vs. TAF 25 mg met the bioequivalence criteria. The plasma TFV $_{Cmax}$ and AUC were ~1.14 and 1.24-fold higher with the FDC but still very substantially lower than occurs after STB. Also, bioequivalence between groups was observed for EVG, COBI and FTC.

Dosing conditions

Oral dosing with the FDC containing 10 mg TAF in GS-US-292-0110 indicated that both meal types slowed the rate of absorption and increased the extent of absorption of TAF and TFV to a very similar extent vs. the fasted state. Nevertheless, FDC dosing was with food in efficacy studies because of the EVG and COBI content and this is recommended in the SmPC.

TAF distribution and fate

There does not appear to be a potential for in-vivo isomerisation.

TAF was selected for potential clinical use based on its rapid disappearance from plasma, efficient loading into PBMCs, intracellular conversion to TFV (via CES1 and CatA to form TFV-alanine and then hydrolysis in lysosomes to form TFV, which is then phosphorylated to the active diphosphate form exactly as happens after dosing with TDF. This hypothesis fits with the fact that <2% of an oral radioactive dose appeared in urine as intact TAF and none in faeces whereas TFV accounted for almost all of the radioactive dose recovered from urine and faeces. In contrast, although TAF accounted for most of the plasma radioactivity in the first few h, most of the radioactivity in plasma (74%) was associated with uric acid over 96 h post-dose. After conversion to TFV, TAF is mainly metabolised via the purine catabolic pathway. This includes formation of uric acid, which does not appear to be a significant safety issue for TAF (see the safety section).

It cannot be ruled out that there could be a clinically important effect on safety if E/C/F/TAF is co-administered with agents that could interfere with the purine catabolic pathway. Consequently this is appropriately described in the SmPC. In addition, the applicant committed to perform an in vitro study on the potential for significant effects on plasma TFV concentrations upon co-administration of TAF and xanthine oxidase inhibitors.

Based on nonclinical studies the applicant has concluded that the conversion of TAF to TFV in PBMCs is via lysosomal carboxypeptidase A (cathepsin A; CatA). In-vitro studies did not suggest significant inhibition of the conversion step by HIV protease inhibitors (known to inhibit CatA) but the HCV PIs telaprevir and boceprevir could have an effect intracellularly and co-administration of E/C/F/TAF with these agents is not recommended in the SmPC. It cannot be ruled out that future drugs likely to be co-administered with E/C/F/TAF could have an effect on CatA activity and significantly affect the conversion of TAF to TFV intracellularly. As a recommendation, the applicant is requested to review any new viral protease inhibitors that may be approved in future for their ability to inhibit Cat A.

Special populations

The SmPC recommends no dose adjustment in adult patients with estimated creatinine clearance ≥ 30 mL/min or with Class A or B hepatic impairment. This advice conflicted with the existing SmPC for Emtriva but it was agreed on the basis of the safety data in patients with baseline CrCL 30-50 mL/min in study GS-US-292-0112, suggesting that although FTC plasma levels are higher when CrCL falls into this range there are no important effects on safety. Plasma levels of TFV in subjects with severe renal impairment after a 25 mg dose of TAF did not exceed those observed after dosing subjects with normal renal function with 300 mg TDF. In addition, the safety data support a benign profile for TAF vs. TDF. Therefore, relaxation of the minimum CrCL applied to TDF was accepted for E/C/F/TAF.

Each of Stribild, EVG and COBI are indicated only for use in adults. FTC and TDF are both approved for use in adolescents at the same dose as in adults although for TDF this use is restricted for reasons of safety to adolescents *with NRTI resistance or toxicities precluding the use of first line agents*. In light of the substitution of TDF with TAF and the low plasma exposures, the PK, safety and efficacy data supported use of E/C/F/TAF in adolescents at the same dose as in adults.

Drug interactions
Changing TDF to TAF has no major effect on the risk for clinically significant DDIs to occur since most of the issues are due to the EVG and COBI content.

Pharmacodynamic considerations

The evidence to support selection of 25 mg TAF once daily comes from the monotherapy studies. In the second study 25 mg and 40 mg achieved effects on viral load that were similar to that of 50 mg and 150 mg in a prior study, suggesting a plateau effect from ~25 mg upwards and superiority over TDF 300 mg. It was clear that 8 mg TAF was not an appropriate dose. Doses between 8 and 25 mg were not studied.

The peripheral blood mononuclear cell TFV-DP concentrations were highly variable across dose groups and time points. This fact may or may not explain why the intracellular PBMC concentrations of TFV-DP were ~ 7-fold and ~ 25-fold higher after 25 mg and 40 mg doses of TAF, respectively, vs. TDF. Thus there was a lack of linearity with dose. Despite this apparent difference in intracellular TFV-DP the 40 mg dose of TAF did not have a superior antiviral effect to 25 mg. Since the antiviral effect should carry more weight, the TAF monotherapy studies support a conclusion that doses lower than 25 mg should not be pursued and that increasing the dose beyond 25 mg is unlikely to achieve a greater antiviral effect.

To further support the selection of the 25 mg TAF dose the applicant provided the results of Emax modelling. It is important to note that there was no relationship detected between plasma TFV and antiviral activity.

The models gave an Emax of ~1.7 to 1.8 $\log_{10}$ decline in viral load from baseline. The EC50 approximated to a TAF AUC of ~32 ng•h/mL. It was concluded that 25 mg TAF will provide near maximal activity.

In this regard it should be noted that after 10 days dosing with TAF 25 mg alone the mean and median TAF AUC_{last} values were 115 and 109 ng.h/mL, respectively. In addition, the POPPK analysis gave a mean (95% CI; % CV) predicted steady-state AUC from Phase 3 studies in the ART-naïve of 206.4 (55.6 - 526.1; 71.8%) ng/mL*h. The predicted range across various subgroups was from ~170-250 ng.h/mL. On this basis, the 10 mg TAF dose in the FDC is predicted to give AUC values that comfortably exceed the EC_{50} in the majority.

It is also relevant to note that the slight deviation from dose proportionality in TAF and TFV plasma levels observed in the negative TQT study for 25 vs. 125 mg TAF over the 5-fold range tested suggests that uptake of TAF into cells and conversion to TFV was not easily saturable.

2.4.5. Conclusions on clinical pharmacology

TAF is a prodrug of TFV with a distinct metabolic profile to TDF that results in > 90% lower circulating levels of TFV and > 4-fold higher intracellular levels of the active phosphorylated metabolite TFV-DP. Co-administration of 8 mg TAF with 150 mg COBI increased the C_{max} and AUC of TAF and TFV vs. 8 mg TAF alone to the same extent as observed with the test formulations vs 25 mg TAF alone. It was concluded that the effect was mainly due to inhibition of P-gp-mediated intestinal secretion of TAF by

COBI. Oral dosing with the FDC containing 10 mg TAF indicated that meals slowed the rate of absorption and increased the extent of absorption of TAF and TFV to a very similar extent vs. the fasted state. Nevertheless, FDC dosing was with food in efficacy studies because of the EVG and COBI content and therefore administration with food is recommended in the SmPC. Changing TDF to TAF has no major effect on the risk for clinically significant DDIs to occur since most of the issues are due to the EVG and COBI content.

2.5. Clinical efficacy

E/C/F/TAF (10 mg) was evaluated for efficacy in 6 clinical studies in the populations described below:

Table 60. Studies supporting clinical efficacy for E/C/F/TAF

Study	Study Design	Numbers by Treatment Regimen	Data
GS-US-292-0104 **ART-naive**	Phase 3, randomized, double-blind, multicenter, active-controlled study to evaluate the safety and efficacy of E/C/F/TAF FDC vs STB	E/C/F/TAF FDC + placebo-to-match STB (N = 435) STB + placebo-to-match E/C/F/TAF FDC (N = 432)	Week 48 efficacy, PK, and safety
GS-US-292-0111 **ART-naive**	Phase 3, randomized, double-blind, multicenter, active-controlled study to evaluate the safety and efficacy of E/C/F/TAF FDC vs STB	E/C/F/TAF FDC + placebo-to-match STB (N = 431) STB + placebo-to-match E/C/F/TAF FDC (N = 435)	Week 48 efficacy, PK, and safety
GS-US-292-0102 **ART-naive**	Phase 2, randomized, double-blind, multicenter, active-controlled study to evaluate the safety and efficacy of E/C/F/TAF FDC vs STB Open-label extension phase allowed crossover from STB to E/C/F/TAF after the Week 48 visit and enrollment of virologically suppressed adult subjects who had received a DRV+COBI-containing regimen in Study GS-US-299-0102	Randomized phase: E/C/F/TAF FDC + placebo-to-match STB (N = 112) STB + placebo-to-match E/C/F/TAF FDC (N = 58) Open-label extension phase: Continued on E/C/F/TAF FDC (N = 105) Switch to E/C/F/TAF FDC (N = 161) from STB to E/C/F/TAF FDC (N = 53) from D/C/F/TAF to E/C/F/TAF FDC (N = 70) from DRV+COBI+TVD to E/C/F/TAF FDC (N = 38)	Week 48[b] efficacy, PK, and safety
GS-US-292-0109 **Virologically suppressed**	Phase 3, open-label study to evaluate the efficacy, safety, and tolerability of switching from a TDF-containing combination regimen to E/C/F/TAF FDC	Switch to E/C/F/TAF FDC (N = 959) Stay on FTC/TDF+3rd Agent (N = 477)	Week 48 efficacy and safety
GS-US-292-0112 **Renal impairment (mild/moderate)**	Phase 3, open-label, multicenter, multiple cohort study evaluated the safety, efficacy, and tolerability of E/C/F/TAF FDC	E/C/F/TAF FDC (N = 248)	Week 24 efficacy and safety
GS-US-292-0106 **ART-naïve Adolescents**	Phase 2/3, open-label, multicenter, 2-part, single-arm study to evaluate the PK, safety, tolerability, and antiviral activity of E/C/F/TAF FDC	E/C/F/TAF FDC (N = 48) PK sub-study: N = 24	Week 24 efficacy, PK, and safety

a Subjects included in the Safety Analysis Set received at least 1 dose of study drug

b Randomised phase 48 weeks; most ART-naive who continued E/C/F/TAF in the extension completed 96 weeks

2.5.1. Dose response study

In support use of 10 mg TAF, a Phase 2 study was conducted in ART-naïve adults (GS-US-292-0102) with an open-label (OL) extension that also enrolled a cohort of virologically suppressed adults. In the double-blind phase patients were randomised (2:1) to E/C/F/TAF or STB once daily with food. After Week 48, patients continued on blinded study drug until treatment assignments were unblinded. At the unblinding visit they had the option to receive E/C/F/TAF in the OL extension.

In the double-blind phase

Of the 170 treated, study drug discontinuation occurred in 7 E/C/F/TAF (6.3%; 4 due to AEs) and 5 STB (8.6%; 0 due to AEs) patients. There was no difference in the Week 24 virologic success rates. An additional analysis of virologic success based on < 20 copies/mL using the FDA snapshot algorithm gave rates of 77.7% for E/C/F/TAF and 72.4% for STB (difference 2%; 95% CI -11.8, 15.9%).

Table 61. GS-US-292-0102: Virologic Outcome at Week 24 Using Snapshot Algorithm and HIV-1 RNA < 50 copies/mL (FAS)

	E/C/F/TAF (N=112)	STB (N=58)	E/C/F/TAF vs. STB	
			p-value[a]	Difference in Percentages (95% CI)[b]
Virologic Success at Week 24[c]				
HIV-1 RNA < 50 copies/mL	99 (88.4%)	52 (89.7%)	0.58	-2.9% (-13.5% to 7.7%)
Virologic Failure at Week 24	8 (7.1%)	6 (10.3%)		
HIV-1 RNA ≥ 50 copies/mL	7 (6.3%)	6 (10.3%)		
Discontinued Study Drug Due to Other Reasons and Last Available HIV-1 RNA ≥ 50 copies/mL[d]	1 (0.9%)	0		
No Virologic Data in Week 24 Window	5 (4.5%)	0		
Discontinued Study Drug Due to AE	4 (3.6%)	0		
Discontinued Study Drug Due to Other Reasons and Last Available HIV-1 RNA < 50 copies/mL[d]	1 (0.9%)	0		

a p-value for the superiority test comparing the percentages of virologic success was from the CMH test stratified by baseline HIV-1 RNA stratum.
b Difference in percentages of virologic success and its 95% CI were calculated based on baseline HIV 1 RNA stratum-adjusted MH proportion.
c Week 24 window was between Day 140 and 195 (inclusive).
d Discontinuation due to other reasons included subjects who discontinued study drug due to investigator's discretion, withdrew consent, lost to follow-up, subject noncompliance, protocol violation, pregnancy, and study discontinued by sponsor.

Similar rates for sustained virologic success were achieved through Week 48 (days 308-377).

Table 62. GS-US-292-0102: Virologic Outcome at Week 48 Using Snapshot Algorithm and HIV-1 RNA < 50 copies/mL (FAS)

	E/C/F/TAF (N=112)	STB (N=58)	E/C/F/TAF vs. STB	
			p-value[a]	Difference in Percentages (95% CI)[b]
Virologic Success at Week 48[c]				
HIV-1 RNA < 50 copies/mL	99 (88.4%)	51 (87.9%)	0.84	-1.0% (-12.1% to 10.0%)
Virologic Failure at Week 48 [c]	7 (6.3%)	6 (10.3%)		
HIV-1 RNA ≥ 50 copies/mL	6 (5.4%)	4 (6.9%)		
Discontinued Study Drug Due to Lack of Efficacy	0	1 (1.7%)		
Discontinued Study Drug Due to Other Reasons and Last Available HIV-1 RNA ≥ 50 copies/mL[d]	1 (0.9%)	1 (1.7%)		
No Virologic Data in Week 48 Window[c]	6 (5.4%)	1 (1.7%)		
Discontinued Study Drug Due to AE	4 (3.6%)	0		
Discontinued Study Drug Due to Other Reasons and Last Available HIV-1 RNA < 50 copies/mL[d]	2 (1.8%)	1 (1.7%)		

a p-value for the superiority test comparing the percentages of virologic success was from the CMH test stratified by baseline HIV-1 RNA stratum.
b Difference in percentages of virologic success and its 95% CI were calculated based on baseline HIV-1 RNA stratum-adjusted MH proportion.
c Week 48 window was between Day 308 and 377 (inclusive).
d Discontinuation due to other reasons included subjects who discontinued study drug due to investigator's discretion, withdrew consent, lost to follow-up, subject noncompliance, protocol violation, pregnancy, and study discontinued by sponsor.

High rates of virologic suppression (< 50 copies/mL) were maintained through Week 96 (M = F at Week 96: 87.5%; M = E at Week 96: 96.1%) and there were no cases of rebound between Weeks 48 and 96.

In the OL extension phase

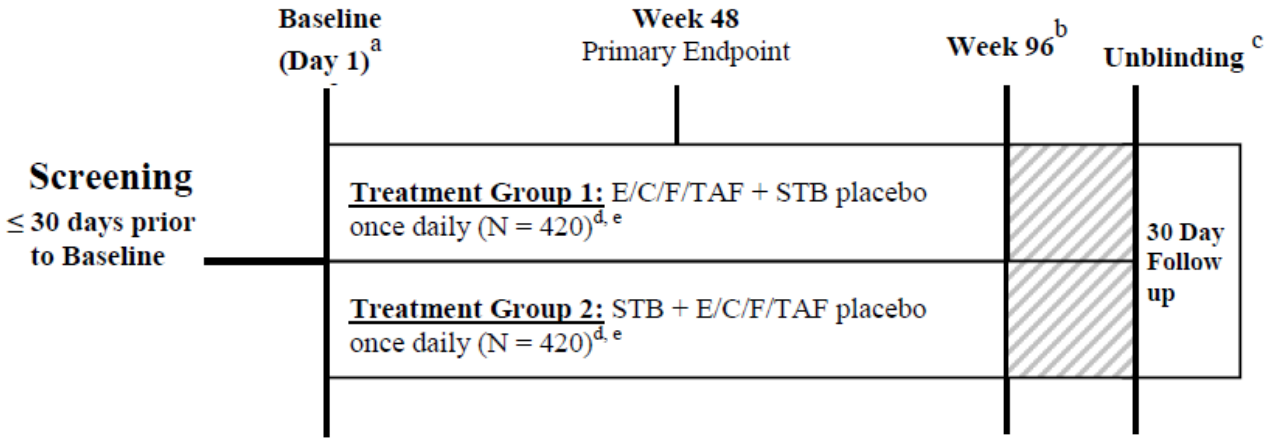
In the E/C/F/TAF group virologic suppression through Week 96 was 87.5% (M = F) and 96.1% (M = E). Virologic suppression was maintained and CD4 cell count increased in the 91 who switched to E/C/F/TAF in the extension phase so that at Week 24 98.9% in the all TDF to TAF group had < 50 copies/mL (M = E) and mean (SD) change from open-label baseline in CD4 cell count was 61 (159.1) cells/µL. Week 144 data for those randomised to the E/C/F/TAF group and Week 48 following switch to E/C/F/TAF from D/C/F/TAF (n = 70) or from a TDF-containing regimen showed that in the original E/C/F/TAF group 84.8% (95/112) maintained < 50 copies/mL at Week 144. Of those who switched 98.6% and 100% were still suppressed at Week 48.

2.5.2. Main studies

Two studies were conducted in ART-naïve patients (**GS-US-292-0104 and 0111**) and one in virologically suppressed patients who switched to E/C/F/TAF or continued the prior ART regimen (**GS-US-292-0109**).

GS-US-292-0104 and 0111

The two double-blind studies in ART-naïve patients were of the same design as summarised in the figure.



a Following the baseline visit, subjects returned for study visits at Weeks 2, 4, 8, 12, 16, and 24 and then every 12 weeks through Week 96.
b Subjects will continue to attend visits every 12 weeks following Week 96 until treatment assignments are unblinded.
c Once Gilead provides unblinded treatment assignments to the investigators, all subjects will return to the clinic (preferably within 30 days) for an unblinding visit. At the unblinding visit all subjects will discontinue their blinded study drugs and will be given an option to participate in an OL rollover study. Subjects who do not wish to participate in the OL rollover study will discontinue their blinded study drugs and will return for a 30 Day Follow-up visit following the unblinding visit.
d Subjects who have discontinued study drugs prior to the unblinding visit will not be eligible for the OL rollover study; these subjects will be asked to continue attending the scheduled study visits through the unblinding visit and discontinue the study after the unblinding visit.
e E/C/F/TAF and matching placebo were administered orally, 1 tablet, once daily with food at approximately the same time each day. STB and matching placebo were administered orally, 1 tablet, once daily, with food at approximately the same time each day

Study Titles: Phase 3, Randomized, Double-Blind Study to Evaluate the Safety and Efficacy of Elvitegravir/Cobicistat/Emtricitabine/ Tenofovir Alafenamide Versus Elvitegravir/Cobicistat/ Emtricitabine/Tenofovir Disoproxil Fumarate in HIV-1 Positive, Antiretroviral Treatment- Naive Adults

The main inclusion criteria were:

- Aged ≥ 18 years with plasma HIV-1 RNA ≥ 1000 copies/mL at screening
- No prior use of any approved or investigational ARV except the use for PrEP or PEP up to 6 months prior to screening
- Screening genotype report must have shown sensitivity to EVG, FTC and TDF
- Normal ECG or no clinically significant abnormalities
- $eGFR_{CG}$ ≥ 50 mL/min
- AST and ALT ≤ 5 × ULN and total bilirubin ≤ 1.5 mg/dL or normal direct bilirubin
- Absolute neutrophil count ≥ 1000/mm3; platelets ≥ 50,000/mm3; haemoglobin ≥ 8.5 g/dL
- Serum amylase ≤ 5 × ULN (or > 5 × ULN but with lipase ≤ 5 × ULN)

Randomisation using IVRS or IWRS was 1:1 to E/C/F/TAF or STB and was stratified by:

- HIV-1 RNA level (≤ 100,000, > 100,000 to ≤ 400,000 copies/mL, > 400,000 copies/mL)
- CD4 count (< 50 cells/µL, 50 to 199 cells/µL, ≥ 200 cells/µL)

- Region (US vs. ex-US)

Virologic outcome was categorised as follows:

- Virologic success - last available HIV-1 RNA < 50 copies/mL in the Week 48 analysis window while on assigned treatment
- Virologic failure - any of:

  a) Last available HIV-1 RNA ≥ 50 copies/mL in the Week 48 analysis window

  b) No on-treatment HIV-1 RNA data in the Week 48 analysis window and last available on-treatment HIV-1 RNA ≥ 50 copies/mL

  c) Non-study ARV added between the first dose and last on-treatment HIV-1 RNA within the
- Week 48 analysis window
- No virologic data in the Week 48 analysis window
- Suboptimal virologic response = < 1 $\log_{10}$ reduction in HIV-1 RNA from baseline and ≥ 50 copies/mL at the Week 8 visit, confirmed at a scheduled or unscheduled visit following Week 8.
- Virologic rebound = after achieving HIV-1 RNA < 50 copies/mL, there is ≥ 50 copies/mL confirmed at any scheduled or unscheduled visit.

Objectives

The primary objective was to evaluate the efficacy of E/C/F/TAF vs. STB in HIV-infected, ART naive adults based on HIV-1 RNA < 50 copies/mL at Week 48. The details of the assay(s) used to measure HIV RNA are not reported but virological responses are reported at the < 50 and < 20 copies/mL level.

A sample size of 840 patients randomised 1:1 (420 per group) was planned to achieve at least 95% power to assess a non-inferiority margin of 12% applied to the difference in Week 48 response rate (HIV-1 RNA < 50 copies/mL as defined by the FDA snapshot algorithm) between 2 groups. It was assumed that each treatment would elicit a response rate of 0.85. The significance level was 1-sided alpha of 0.025.

Two interim IDMC analyses were conducted prior to the primary analysis. An alpha penalty of 0.00001 was applied for each interim analysis so the primary efficacy hypothesis of non-inferiority of E/C/F/TAF vs. STB was tested with a 1-sided, 0.02499 alpha level. The Week 48 primary efficacy analysis used the FAS. The baseline stratum weighted difference in the response rate and its 95.002% CI were calculated based on stratum-adjusted Mantel-Haenszel (MH) proportion.

Based on planned Week 48 interim analyses conducted after all randomised patients had completed the Week 48 study visit or had prematurely discontinued. It was planned that data would be combined across studies to assess efficacy, including an assessment of superiority of E/C/F/TAF over STB. After Week 96, patients will continue to take their blinded study drugs and attend visits every 12 weeks until treatment assignments are unblinded, at which point all patients will return for an unblinding visit and will be given the option to participate in an open-label (OL) rollover study to receive E/C/F/TAF.

Results - GS-US-292-0104

Overall 867/872 randomised patients received a dose of study drug and 813 (E/C/F/TAF 94.9%; STB 92.6%) were still on treatment at the Week 48 cut-off date while 54 had discontinued study drugs (E/C/F/TAF 5.1%, 22; STB 7.4%, 32) mainly due to withdrawal of consent (8 vs. 7). Demographic and

disease characteristics were similar between treatments. The majority was male (85.4%), the median age was 33 years (18 to 76) and most were white (58.2%). Most had acquired HIV-1 via homosexual sex. The majority (93.4%) was asymptomatic. The baseline median $eGFR_{CG}$ value was slightly higher in the E/C/F/TAF group (118.5 mL/min vs. STB 112.8 mL/min) whilst 79 (9.1%) had dipstick proteinuria.

Table 63. GS-US-292-0104: Baseline Disease Characteristics (Safety Analysis Set)

	E/C/F/TAF (N = 435)	STB (N = 432)	Total (N = 867)	E/C/F/TAF vs STB p-value[a]
HIV-1 RNA (log10 copies/mL)				
N	435	432	867	0.81
Mean (SD)	4.55 (0.682)	4.55 (0.674)	4.55 (0.678)	
Median	4.59	4.62	4.61	
Q1, Q3	4.15, 4.98	4.20, 4.96	4.16, 4.97	
Min, Max	2.57, 6.89	2.13, 6.98	2.13, 6.98	
HIV-1 RNA Categories (copies/mL)[b]				
≤ 100,000	331 (76.1%)	336 (77.8%)	667 (76.9%)	0.62
> 100,000 to ≤ 400,000	79 (18.2%)	72 (16.7%)	151 (17.4%)	
> 400,000	25 (5.7%)	24 (5.6%)	49 (5.7%)	
CD4 Cell Count Categories (/uL)[b]				
< 50	10 (2.3%)	12 (2.8%)	22 (2.5%)	0.54
≥ 50 to < 200	48 (11.0%)	41 (9.5%)	89 (10.3%)	
≥ 200 to < 350	103 (23.7%)	111 (25.7%)	214 (24.7%)	
≥ 350 to < 500	122 (28.0%)	135 (31.3%)	257 (29.6%)	
≥ 500	152 (34.9%)	133 (30.8%)	285 (32.9%)	

The median rate of adherence to study drug up to the Week 48 visit was 98.8% in both treatment groups and most (84.6% vs. 80.9%) had adherence rates of ≥ 95%. At Week 48 the virologic success rates were very high and E/C/F/TAF was non-inferior to STB. Similar findings applied in the Week 48 PP Analysis Set (97.8% vs. 98.0%; 95.002% CI: -2.2% to 2.1%).

Table 64. GS-US-292-0104: Virologic Outcome at Week 48 Using FDA Snapshot Algorithm and HIV-1 RNA < 50 copies/mL (FAS)

	E/C/F/TAF (N = 435)	STB (N = 432)	E/C/F/TAF vs. STB	
			p-value[a]	Difference in Percentages (95.002% CI)[b]
Virologic Success at Week 48[c]				
HIV-1 RNA < 50 copies/mL	405 (93.1%)	399 (92.4%)	0.58	1.0% (-2.6% to 4.5%)
Virologic Failure at Week 48[c]	13 (3.0%)	11 (2.5%)		
HIV-1 RNA ≥ 50 copies/mL	9 (2.1%)	6 (1.4%)		
Discontinued Study Drug Due to Lack of Efficacy	0	2 (0.5%)		
Discontinued Study Drug Due to Other Reasons and Last Available HIV-1 RNA ≥ 50 copies/mL[d]	3 (0.7%)	3 (0.7%)		
Added New ARV	1 (0.2%)	0		
No Virologic Data in Week 48 Window[c]	17 (3.9%)	22 (5.1%)		
Discontinued Study Drug Due to AE/Death	4 (0.9%)	5 (1.2%)		
Discontinued Study Drug Due to Other Reasons and Last Available HIV-1 RNA < 50 copies/mL[d]	11 (2.5%)	15 (3.5%)		
Missing Data During Window but on Study Drug	2 (0.5%)	2 (0.5%)		

a P-value for the superiority test comparing the percentages of virologic success was from the CMH test stratified by baseline HIV-1 RNA (< = 100,000 or > 100,000 copies/mL) and region (US vs ex-US) stratum.

b Difference in percentages of virologic success between treatment groups and its 95.002% CI were calculated based on the MH proportions adjusted by baseline HIV-1 RNA and region stratum.

c Week 48 window is between Day 294 and 377 (inclusive).

d Discontinuation due to other reasons included subjects who prematurely discontinued study drug due to investigator's discretion, withdrew consent, lost to follow-up, noncompliance with study drug, protocol violation, pregnancy, and study termination by sponsor.

Virologic outcomes at Week 48 were also similar between treatments when assessed at HIV-1 RNA < 20 copies/mL. Similar findings applied to the PP population (91.1% vs. 92.4%; 95% CI -5.2, 2.4%). A pure virologic response through Week 48 occurred in 95.4% per treatment group.

Table 65. GS-US-292-0104: Virologic Outcome at Week 48 Using FDA Snapshot Algorithm and HIV-1 RNA < 20 copies/mL (FAS)

	E/C/F/TAF (N = 435)	STB (N = 432)	E/C/F/TAF vs. STB	
			p-value[a]	Difference in Percentages (95 % CI)[b]
Virologic Success at Week 48[c]				
HIV-1 RNA < 20 copies/mL	376 (86.4%)	377 (87.3%)	0.78	-0.6% (-5.1% to 3.8%)
Virologic Failure at Week 48	43 (9.9%)	34 (7.9%)		
HIV-1 RNA ≥ 20 copies/mL	38 (8.7%)	28 (6.5%)		
Discontinued Study Drug Due to Lack of Efficacy	0	2 (0.5%)		
Discontinued Study Drug Due to Other Reasons and Last Available HIV-1 RNA ≥ 20 copies/mL	4 (0.9%)	4 (0.9%)		
Added New ARV	1 (0.2%)	0		
No Virologic Data in Week 48 Window	16 (3.7%)	21 (4.9%)		
Discontinued Study Drug Due to AE/Death	4 (0.9%)	5 (1.2%)		
Discontinued Study Drug Due to Other Reasons and Last Available HIV-1 RNA < 20 copies/mL[d]	10 (2.3%)	14 (3.2%)		
Missing Data During Window but on Study Drug	2 (0.5%)	2 (0.5%)		

a P-value for the superiority test comparing the percentages of virologic success was from the CMH test stratified by baseline HIV-1 RNA (< = 100,000 or > 100,000 copies/mL) and region (US vs ex-US) stratum.

b Difference in percentages of virologic success between treatment groups and its 95% CI were calculated based on the MH proportions adjusted by baseline HIV-1 RNA and region stratum.

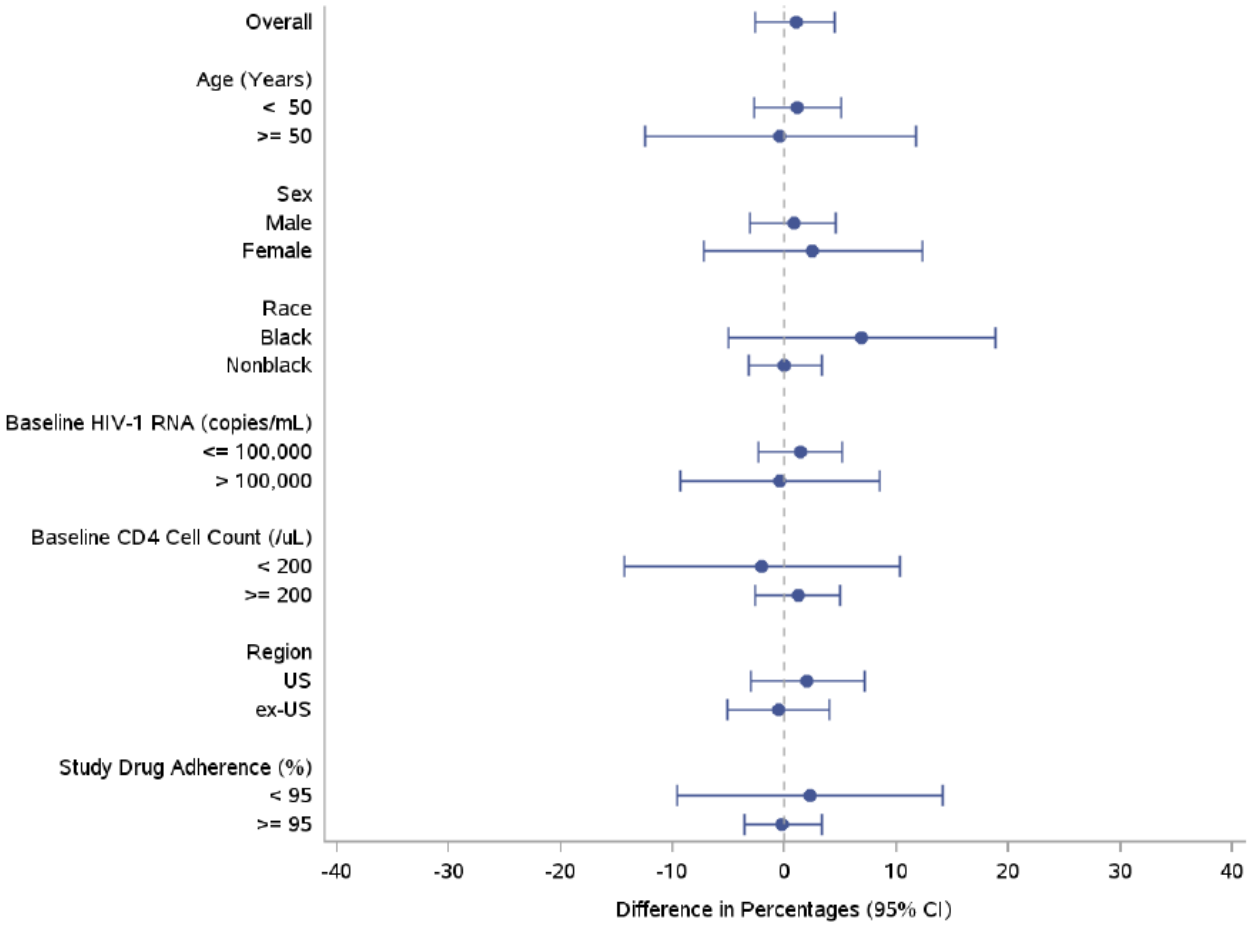
c Week 48 window is between Day 294 and 377 (inclusive).

d Discontinuation due to other reasons included subjects who prematurely discontinued study drug due to investigator's discretion, withdrew consent, lost to follow-up, noncompliance with study drug, protocol violation, pregnancy, and study termination by sponsor

HIV-1 RNA levels decreased rapidly in the first 2 weeks and were stable after Week 8 through Week 48, when mean decreases were E/C/F/TAF -3.24 $\log_{10}$ copies/mL and STB -3.27 $\log_{10}$ copies/mL.

The Week 48 virologic success rates for the pre-defined subgroups of interest were similar between treatments. Based on an additional analysis evaluating the interaction between regions and treatment effect, the differences between the E/C/F/TAF and STB groups in the rate of virologic success across 11 pre-defined regions were similar between E/C/F/TAF and STB groups.

Figure 8. GS-US-292-0104: Forest Plot of Treatment Difference in Virologic Success by Subgroup at Week 48 Using FDA Snapshot Algorithm and HIV-1 RNA < 50 copies/mL (FAS)



CD4 cell counts increased in each treatment group with mean increases at Week 48 (observed data) in the FAS of E/C/F/TAF 235 cells/µL and STB 222 cells/µL. Changes using the LOCF approach were also similar between treatments as were the results for the PP Analysis Set. The CD4% increased to Week 48 by E/C/F/TAF 8.9% and STB 9.1%.

Results – GS-US-292-0111

The study was conducted at 121 sites in 10 countries. Overall 866/872 patients randomised received a dose of study drug and 804 (E/C/F/TAF 94.7%, STB 91.0%) were still on study drug at the Week 48 cut-off date. The most common reasons for premature discontinuation of study drug were LTFU (2.3% vs. 2.1%). Three patients died (alcohol intoxication E/C/F/TAF; alcohol and multiple drug toxicity and myocardial infarction STB).

Demographic and disease baseline characteristics were similar between treatment groups. The majority was male (84.6%), the median age was 33 years (range 18 to 71) and most were white (55.2%). The majority had acquired HIV-1 via homosexual sex (74.9%). Most (89.9%) were asymptomatic. The median $eGFR_{CG}$ value was E/C/F/TAF 115.9 mL/min vs. STB 114.7 mL/min and 95 (11.0%) had proteinuria by dipstick.

The median rate of adherence to study drug up to Week 48 was high and similar between treatment groups (E/C/F/TAF 98.5%; STB 98.8%). Most patients (78.5% and 80.8%) had ≥ 95% adherence.

Table 66. GS-US-292-0111: Baseline Disease Characteristics (Safety Analysis Set)

	E/C/F/TAF (N=431)	STB (N=435)	Total (N=866)	E/C/F/TAF vs STB p-value[a]
HIV-1 RNA (log_{10} copies/mL)				
N	431	435	866	0.82
Mean (SD)	4.53 (0.647)	4.50 (0.690)	4.52 (0.669)	
Median	4.55	4.54	4.55	
Q1, Q3	4.12, 4.94	4.11, 4.96	4.12, 4.94	
Min, Max	2.85, 6.35	1.28, 6.61	1.28, 6.61	
HIV-1 RNA Categories (copies/mL)[b]				
≤ 100,000	339 (78.7%)	336 (77.2%)	675 (77.9%)	0.95
> 100,000 to ≤ 400,000	68 (15.8%)	82 (18.9%)	150 (17.3%)	
> 400,000	24 (5.6%)	17 (3.9%)	41 (4.7%)	
CD4 Cell Count Categories (/uL)[b]				
< 50	14 (3.3%)	15 (3.4%)	29 (3.4%)	0.70
≥ 50 to < 200	40 (9.3%)	49 (11.3%)	89 (10.3%)	
≥ 200 to < 350	115 (26.7%)	89 (20.5%)	204 (23.6%)	
≥ 350 to < 500	134 (31.2%)	149 (34.3%)	283 (32.7%)	
≥ 500	127 (29.5%)	133 (30.6%)	260 (30.1%)	
- Missing -	1	0	1	

Virologic success rates were high and non-inferiority was demonstrated for E/C/F/TAF vs. STB in the primary analysis. Similar findings applied in the PP Analysis Set (E/C/F/TAF 97.2%; STB 95.4%; 95.002% CI: -1.1% to 4.4%).

Table 67. GS-US-292-0111: Virologic Outcome at Week 48 Using FDA Snapshot Algorithm and HIV-1 RNA < 50 copies/mL (FAS)

	E/C/F/TAF (N=431)	STB (N=435)	E/C/F/TAF vs. STB	
			p-value[a]	Difference in Percentages (95.002% CI)[b]
Virologic Success at Week 48[c]				
HIV-1 RNA < 50 copies/mL	395 (91.6%)	385 (88.5%)	0.13	3.1% (-1.0% to 7.1%)
Virologic Failure at Week 48[c]	18 (4.2%)	24 (5.5%)		
HIV-1 RNA ≥ 50 copies/mL	11 (2.6%)	17 (3.9%)		
Discontinued Study Drug Due to Lack of Efficacy	2 (0.5%)	1 (0.2%)		
Discontinued Study Drug Due to Other Reasons and Last Available HIV-1 RNA ≥ 50 copies/mL[d]	5 (1.2%)	5 (1.1%)		
Added New ARV	0	1 (0.2%)		
No Virologic Data in Week 48 Window[c]	18 (4.2%)	26 (6.0%)		
Discontinued Study Drug Due to AE/Death	4 (0.9%)	9 (2.1%)		
Discontinued Study Drug Due to Other Reasons and Last Available HIV-1 RNA < 50 copies/mL[d]	10 (2.3%)	16 (3.7%)		
Missing Data During Window but on Study Drug	4 (0.9%	1 (0.2%)		

a P-value for the superiority test comparing the percentages of virologic success was from the CMH test stratified by baseline HIV-1 RNA (≤ 100,000 or > 100,000 copies/mL) and region (US vs ex-US) stratum.

b Difference in percentages of virologic success between treatment groups and its 95.002% CI were calculated based on the MH proportions adjusted by baseline HIV-1 RNA and region stratum.

c Week 48 window is between Day 294 and 377 (inclusive).

d Discontinuation due to other reasons included subjects who prematurely discontinued study drug due to investigator's discretion, withdrew consent, lost to follow-up, noncompliance with study drug, protocol violation, pregnancy, and study termination by sponsor.

Table 68. GS-US-292-0111: Virologic Outcome at Week 48 Using FDA Snapshot Algorithm and HIV-1 RNA < 20 copies/mL (FAS)

	E/C/F/TAF (N=431)	STB (N=435)	E/C/F/TAF vs. STB	
			p-value[a]	Difference in Percentages (95 % CI)[b]
Virologic Success at Week 48[c]				
HIV-1 RNA < 20 copies/mL	355 (82.4%)	351 (80.7%)	0.60	1.4% (-3.7% to 6.5%)
Virologic Failure at Week 48	60 (13.9%)	60 (13.8%)		
HIV-1 RNA ≥ 20 copies/mL	51 (11.8%)	51 (11.7%)		
Discontinued Study Drug Due to Lack of Efficacy	2 (0.5%)	1 (0.2%)		
Discontinued Study Drug Due to Other Reasons and Last Available HIV-1 RNA ≥ 20 copies/mL	7 (1.6%)	7 (1.6%)		
Added New ARV	0	1 (0.2%)		
No Virologic Data in Week 48 Window	16 (3.7%)	24 (5.5%)		
Discontinued Study Drug Due to AE/Death	4 (0.9%)	9 (2.1%)		
Discontinued Study Drug Due to Other Reasons and Last Available HIV-1 RNA < 20 copies/mL[d]	8 (1.9%)	14 (3.2%)		
Missing Data During Window but on Study Drug	4 (0.9%)	1 (0.2%)		

a P-value for the superiority test comparing the percentages of virologic success was from the CMH test stratified by baseline HIV-1 RNA (⩽ 100,000 or > 100,000 copies/mL) and region (US vs ex-US) stratum.

b Difference in percentages of virologic success between treatment groups and its 95% CI were calculated based on the MH proportions adjusted by baseline HIV-1 RNA and region stratum.

c Week 48 window is between Day 294 and 377 (inclusive).

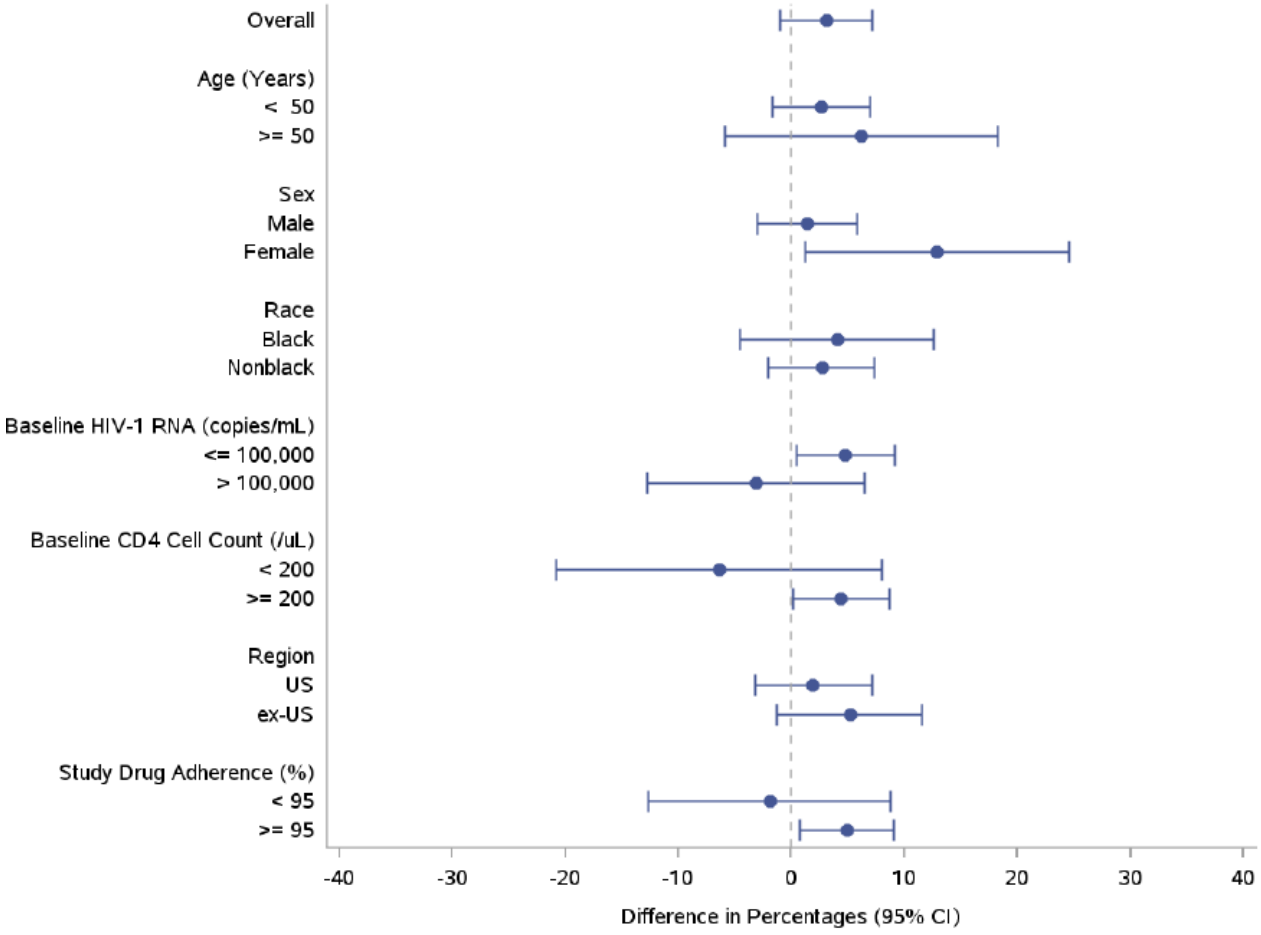
d Discontinuation due to other reasons included subjects who prematurely discontinued study drug due to investigator's discretion, withdrew consent, lost to follow-up, noncompliance with study drug, protocol violation, pregnancy, and study termination by sponsor.

Virologic outcomes at Week 48 were also similar between treatments when assessed using the FDA-defined snapshot algorithm applied to HIV-1 RNA < 20 copies/mL. Rates of virologic failure in this analysis were the same for the two treatments (13.9% vs. 13.8%) and similar findings applied in the PP Analysis Set (E/C/F/TAF 87.7%; STB 87.2%; 95% CI: -4.7% to 4.3%). The pure virologic response rates through Week 48 were E/C/F/TAF 93.7% vs. STB 93.1%.

HIV-1 RNA levels decreased rapidly in the first 2 weeks on study drugs and were then stable from Week 8 through Week 48. The mean decreases at Week 48 were E/C/F/TAF 3.19 $\log_{10}$ copies/mL and STB 3.14 $\log_{10}$ copies/mL.

The subgroup analyses based on the FDA-defined snapshot algorithm applied to Week 48 data showed that in four subgroups (females, baseline HIV-1 RNA ≤ 100,000 copies/mL, baseline CD4 count ≥ 200 cells/μL and adherence rate ≥ 95%) the virologic success rates favoured E/C/F/TAF vs. STB. The differences between the E/C/F/TAF and STB groups across the 8 predefined regions were similar.

Figure 9. GS-US-292-0111: Forest Plot of Treatment Difference in Virologic Success by Subgroup at Week 48 Using FDA Snapshot Algorithm and HIV-1 RNA < 50 copies/mL (FAS)



The CD4 cell counts increased from baseline to Week 48 (observed data) by E/C/F/TAF 225 cells/µL and STB 200 cells/µL with a pattern similar to that in 0104. Similarly, using LOCF to impute missing values the mean increases from baseline at Week 48 were E/C/F/TAF 224 cells/µL and STB 195 cells/µL. The mean CD4% increased from baseline to Week 48 by E/C/F/TAF 9.1% and STB 8.7%.

GS-US-292-0106

Study Title: A Phase 2/3, Open-Label Study of the Pharmacokinetics, Safety, and Antiviral Activity of the Elvitegravir/Cobicistat/Emtricitabine/Tenofovir Alafenamide (E/C/F/TAF) Single Tablet Regimen (STR) in HIV-1 Infected Antiretroviral Treatment-Naive Adolescents

This open-label study was conducted in ARV-naïve HIV-infected adolescents (aged 12 to < 18 years) with body weight ≥ 35 kg, plasma HIV-1 RNA ≥ 1000 copies/mL, CD4 cell counts > 100 cells/µL and eGFR ≥ 90 mL/min/1.73 m2 (Schwartz formula) at screening. Viruses were to be sensitive to TFV, EVG and FTC based on genotyping at screening. All patients received E/C/F/TAF QD with food.

In Part A: 18 to 24 patients (at least 6 aged 12 to < 15 years and 6 aged 15 to < 18 years) were to be enrolled to evaluate steady-state intensive PK at Week 4 (see Pharmacokinetics).

In Part B: The remaining patients (up to the planned total of 50 across Parts A and B) were enrolled to evaluate the safety and antiviral activity of E/C/F/TAF.

The completed Week 24 analyses showed that 90% (45/50) had < 50 copies/mL. There was no virologic resistance to E/C/F/TAF detected.

The mean (SD) increase from baseline in CD4 cell count at Week 24 was 191 (175.2) cells/µL

Table 69. GS-US-292-0106: Virologic Outcome at Week 24 Using FDA Snapshot Algorithm and HIV-1 RNA < 50 copies/mL (FAS)

	E/C/F/TAF (N=50)
Virologic Success at Week 24[a]; n (%)	
HIV-1 RNA < 50 copies/mL	45 (90.0)
Virologic Failure at Week 24[a]; n (%)	4 (8.0)
HIV-1 RNA >= 50 copies/mL	3 (6.0)
Discontinued Study Drug Due to Lack of Efficacy	0
Discontinued Study Drug Due to Other Reasons and Last Available HIV-1 RNA >= 50 copies/mL[b]	1 (2.0)
Added New ARV	0
No Virologic Data in Week 24 Window[a]; n (%)	1 (2.0)
Discontinued Study Drug Due to AE/Death	0
Discontinued Study Drug Due to Other Reasons and Last Available HIV-1 RNA < 50 copies/mL[b]	1 (2.0)
Missing Data During Window but on Study Drug	0

a Week 24 window is between Day 140 and 195 (inclusive).

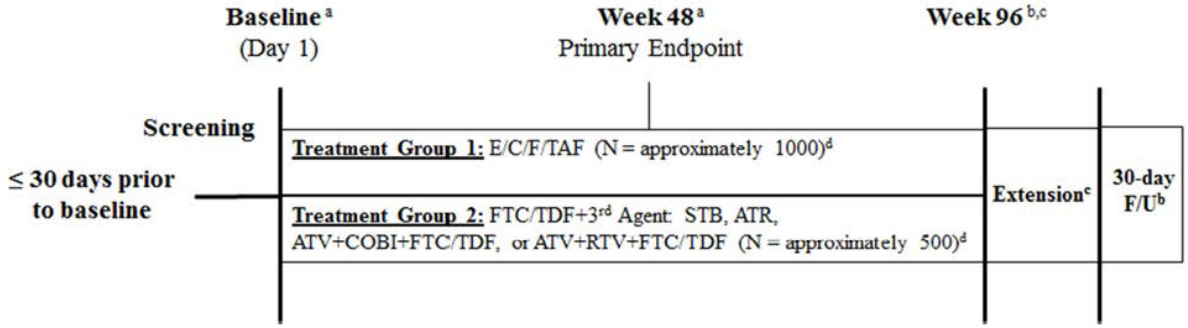
b Discontinuation due to other reasons includes subjects who discontinued study drug due to investigator's discretion, withdrew consent, lost to follow-up, noncompliance with study drug, protocol violation, pregnancy, and study terminated by sponsor.

Supportive studies

GS-US-292-0109

Study Title: A Phase 3, Open-Label Study to Evaluate Switching from a TDF-Containing Combination Regimen to a TAF-Containing Combination Single Tablet Regimen (STR) in Virologically-Suppressed, HIV-1 Positive Subjects

The study was open-label and had the following overall design:



F/U = follow-up

a Following the baseline visit, subjects were scheduled to return for study visits at Weeks 2, 4, 8, and 12, and then every 12 weeks through Week 96.

b Subjects who complete the study through Week 96 and do not wish to continue to participate will be required to return to the clinic 30 days after the completion of the study drug for a 30-day follow-up visit.

c After Week 96, all subjects will be given the option to receive open-label E/C/F/TAF and attend visits every 12 weeks until it becomes commercially available, or until Gilead terminates clinical development of E/C/F/TAF.

d The study drugs E/C/F/TAF, STB, ATR, ATV/r+TVD, and ATV/co+TVD were administered orally, once daily with food, at approximately the same time each day. Those randomized to continue with ATR were instructed to take the study drug once daily an empty stomach, preferably at bedtime.

HIV-infected patients aged ≥ 18 years were eligible if they had participated in pre-defined clinical studies and had achieved virological suppression on any of the following FTC/TDF regimens:

- STB
- Atripla (EFV/FTC/TDF; ATR)
- COBI-boosted atazanavir (ATV/co) + FTC/TDF (Truvada; TVD)
- Ritonavir (RTV)-boosted atazanavir (ATV/r) + TVD

Plasma HIV-1 RNA was to have been undetectable for at least 6 consecutive months. Other inclusion criteria were the same as for the two studies in ARV-naïve patients. Patients were randomised (2:1) to:

- **Switch** to E/C/F/TAF (n = 1000)
- **Maintain** existing ARV (n = 500)

Randomisation was stratified by prior treatment regimen (i.e. STB, ATR, ATV/r or ATV/co+TVD). All treatments were to be administered once daily with food at approximately the same time each day except for ATR, which was to be administered on an empty stomach, preferably at bedtime (due to EFV). After completing 96 weeks of randomised treatment, all patients will be offered open-label E/C/F/TAF.

Objective

The primary objective was to evaluate the non-inferiority of switching to a TAF-containing FDC relative to maintaining TDF-containing regimens in virologically suppressed patients based on HIV-1 RNA < 50 copies/mL at Week 48 (US FDA-defined snapshot algorithm). The definitions of virologic outcomes were as for the Phase 3 studies in ARV-naïve.

The study planned to enrol 1000 in the switch group and 500 in the continuation group. It was assumed that both treatment groups would have a response rate of 90% (HIV-1 RNA < 50 copies/mL at Week 48; FDA snapshot algorithm). There was an interim IDMC analysis prior to analysis for the primary endpoint. An alpha penalty of 0.0001 was applied for the IDMC meeting so the alpha level for the primary endpoint at Week 48 was adjusted to 0.0499. The difference in the response rate (P1 – P2) and its 95.01% CI was calculated using Mantel-Haenszel (MH) proportion stratified by prior treatment (STB, ATR or ATV/boosted+TVD). It was concluded that the E/C/F/TAF group was non-inferior to the FTC/TDF+3rd Agent group if the lower bound of the 2-sided 95.01% CI of the difference in response rates was greater than -12%.

Results – GS-US-292-0109

The study was conducted at 168 study sites across 20 countries. Overall 1436/1443 randomised patients received a dose of study drug. Demographic and disease baseline characteristics were similar between treatments except for E/C/F/TAF 25.9% vs. FTC/TDF+3rd Agent 17.2% of Hispanic or Latino ethnicity. The majority was male (89.3%) with a median age of 41 years (21 to 77 years) and most were white (67.2%). Most had acquired HIV via homosexual sex (78.6%). In accordance with the protocol, 1409/1436 (98.1%) had baseline HIV-1 RNA < 50 copies/mL. The median baseline CD4 count was 669 cells/µL and median baseline CD4% was 35.9%. The majority (90.8%) had no proteinuria on dipstick urinalysis and eGFR values measured by CG or by either of the CKD-EPI methods were similar between treatment groups. The distributions of patients by prior treatment regimens were as follows:

Prior Treatment Regimen, n (%)[a]	E/C/F/TAF (N=969)	FTC/TDF+3rd Agent (N=477)
STB	306 (31.9)	153 (32.1)
ATR	251 (26.2)	125 (26.2)
ATV/boosted+TVD	402 (41.9)	199 (41.7)
ATV/co+ TVD	147 (15.2)	69 (14.5)
ATV/r+ TVD	255 (26.3)	130 (27.3)

Following the completion of all patients through Week 48, comparable percentages maintained virologic suppression at < 50 c/mL. Rates in the PP Analysis were 99.1% for E/C/F/TAF and 98.9% for FTC/TDF+3rd Agent. In addition, percentages with HIV-1 RNA < 20 copies/mL at Week 48 were E/C/F/TAF 93.5% and FTC/TDF+3rd Agent 90.4% (difference 3.2%, 95% CI: 0.1% to 6.3%). One patient (0.1%) from the E/C/F/TAF group had a confirmed virologic rebound and the virus showed emergent resistance (M184M/I).

Table 70. GS-US-292-0109: Virologic Outcome at Week 48 (All Subjects) Using FDA-Defined Snapshot Algorithm and HIV-1 RNA < 50 copies/mL (Week 48 [All Subjects] FAS)

			E/C/F/TAF vs. FTC/TDF+3rd Agent	
	E/C/F/TAF (N=959)	FTC/TDF+3rd Agent (N=477)	p-value[a]	Difference in Percentages (95% CI)[b]
Virologic Success at Week 48[c]; n (%)				
HIV-1 RNA < 50 copies/mL	932 (97.2)	444 (93.1)	<0.001	4.1% (1.6% to 6.7%)
Virologic Failure at Week 48[c]; n (%)	10 (1.0)	6 (1.3)		
HIV-1 RNA >= 50 copies/mL	6 (0.6)	4 (0.8)		
Discontinued Study Drug Due to Lack of Efficacy	1 (0.1)	0		
Discontinued Study Drug Due to Other Reasons and Last Available HIV-1 RNA >= 50 copies/mL[d]	1 (0.1)	0		
Added New ARV	2 (0.2)	2 (0.4)		
No Virologic Data in Week 48 Window[c]; n (%)	17 (1.8)	27 (5.7)		
Discontinued Study Drug Due to AE/Death	10 (1.0)	6 (1.3)		
Discontinued Study Drug Due to Other Reasons and Last Available HIV-1 RNA < 50 copies/mL[d]	7 (0.7)	20 (4.2)		
Missing Data During Window but on Study Drug	0	1 (0.2)		

a P-value for the superiority test comparing the percentages of virologic success was from the CMH test stratified by the prior treatment regimen (STB, ATR, ATV/boosted+TVD).

b Difference in percentages of virologic success and its 95% CI were calculated based on the MH proportion adjusted by the prior treatment regimen.

c Week 48 window is between Day 294 and 377 (inclusive).

d Discontinuation due to other reasons includes subjects who discontinued study drug due to investigator's discretion, withdrew consent, lost to follow-up, noncompliance with study drug, protocol violation, pregnancy, and study terminated by sponsor.

Virologic success rates generally favoured E/C/F/TAF in age, sex, race, geographic region, prior treatment regimen and study drug adherence subgroups. Results by prior treatment regimen are shown below.

Table 71. GS-US-292-0109: Virologic Outcome at Week 48 (All Subjects) By Prior Treatment Regimen Using FDA-Defined Snapshot Algorithm and HIV-1 RNA < 50 copies/mL (Week 48 FAS)

			E/C/F/TAF vs. FTC/TDF+3rd Agent
Virologic Success at Week 48[b]; n (%)	E/C/F/TAF (N=959)	FTC/TDF+3rd Agent (N=477)	Difference in Percentages (95% CI)[a]
ATR	241 (96.0) (n = 251)	112 (89.6) (n = 125)	6.4% (0.5% to 12.3%)
ATV/boosted+TVD	390 (97.0) (n = 402)	183 (92.0) (n = 199)	5.1% (0.9% to 9.2%)
STB	301 (98.4) (n = 306)	149 (97.4) (n = 153)	1.0% (–1.9% to 3.9%)

a Difference in response rate and its 95% CIs were from normal approximation.

b Week 48 window is between Day 294 and 377 (inclusive).

Mean (SD) increases from baseline in CD4 cell counts at Week 48 were E/C/F/TAF 35 (164.6) cells/µL and FTC/TDF+3rd Agent 24 (156.1) cells/µL.

GS-US-292-0112

Study Title: A Phase 3 Open-label Safety Study of Elvitegravir/Cobicistat/ Emtricitabine/Tenofovir Alafenamide Single-Tablet Regimen in HIV-1 Positive Patients with Mild to Moderate Renal Impairment

This open-label study was primarily designed to assess the safety profile of E/C/F/TAF in HIV-infected patients with stable renal impairment ($eGFR_{CG}$ 30-69 mL/min) at Week 24. Enrolment was in 2 cohorts:

- **Cohort 1**: switched to E/C/F/TAF from an existing ARV regimen on which they had HIV-1 RNA < 50 copies/mL for at least 6 months or they had successfully completed GS-US-236-0118.
- **Cohort 2**: ARV-naïve with plasma HIV-1 RNA ≥ 1000 copies/mL

All subjects received E/C/F/TAF once daily with food. Selection criteria were similar to those for Phase 3.

In Cohort 1 the majority was male (79.3%) with median age 58 years (63 were ≥ 65 years). Most (65.3%) were taking TDF-containing regimens prior to the switch. The baseline median CD4 count and CD4% were 632 cells/µL and 34.7%, respectively. Overall, 97.5% had baseline HIV-1 RNA < 50 copies/mL and 2.5% had ≥ 50 to ≤ 100,000 copies/mL. The majority acquired HIV via homosexual sex (52.1%), 74.4% were asymptomatic and 14.0% had AIDS. Overall, 33.1% had $eGFR_{CG}$ < 50 mL/min, 63.6% had $eGFR_{CG}$ 30-59 mL/min, 42.3% had clinically significant proteinuria (UPCR > 200 mg/g) and 48.9% had clinically significant albuminuria (UACR ≥ 30 mg/g). Dipsticks showed that 9.5% had Grade 2, 23.1% had Grade 1 and 67.4% had no proteinuria. **In Cohort 2** only 6 patients were enrolled.

Following the completion of all patients to Week 48 there were no clinically relevant differences in virologic success rates between subgroups (age, sex, race, region, or study drug adherence rate) for Cohort 1. The virologic success rate for those aged ≥ 65 years was 85.7% (54/63). The percentages with < 20 copies/mL at Week 48 using the FAS were 90.1% for Cohort 1 (baseline eGFRCG < 50 mL/min 88.8%; baseline eGFRCG ≥ 50 mL/min 90.7%) and 6/6 in Cohort 2. In Cohort 1, 2 patients (0.8%) had virus that showed resistance to multiple drug classes. One had the same resistance documented in an historic genotype and the other appeared to have had reinfection with a resistant virus.

Table 72. GS-US-292-0112: Virologic Outcome at Week 48 Using FDA Snapshot Algorithm and HIV-1 RNA < 50 copies/mL (FAS)

	Cohort 1: Switch			Cohort 2: ART-Naive
	Baseline $eGFR_{CG}$ < 50 mL/min (N = 80)	Baseline $eGFR_{CG}$ ≥ 50 mL/min (N = 162)	Total (N = 242)	Total (N = 6)
Virologic success at Week 48[a]; n (%)				
HIV-1 RNA < 50 copies/mL	72 (90.0)	150 (96.2)	222 (91.7)	6 (100.0)
95% CI[b]	81.2% to 95.6%	87.4% to 96.1%	87.5% to 94.9%	54.1% to 100.0%
Virologic failure at Week 48[a]; n (%)	0	3 (1.9)	3 (1.2)	0
HIV-1 RNA ≥ 50 copies/mL	0	1 (0.6)	1 (0.4)	0
Discontinued study drug due to lack of efficacy	0	1 (0.6)	1 (0.4)	0
Discontinued study drug due to other reasons and last available HIV-1 RNA >= 50 copies/mL[c]	0	0	0	0
Added new ARV	0	1 (0.6)	1 (0.4)	0
No virologic data in Week 48 window[a]; n (%)	8 (10.0)	9 (5.6)	17 (7.0)	0
Discontinued study drug due to AE/death	5 (6.3)	2 (1.2)	7 (2.9)	0
Discontinued study drug due to other reasons and last available HIV-1 RNA < 50 copies/mL[c]	1 (1.3)	6 (3.7)	7 (2.9)	0
Missing data during window but on study drug	2 (2.5)	1 (0.6)	23 (1.2)	0

a Week 48 window is between Day 294 and 377 (inclusive).

b The 95% CIs for virologic success rate in each cohort and baseline eGFR category was obtained using Exact method.

c Discontinuation due to other reasons includes subjects who discontinued study drug due to investigator's discretion, withdrew consent, lost to follow-up, noncompliance with study drug, protocol violation, pregnancy, and study terminated by sponsor.

CD4 cell counts remained stable during treatment with E/C/F/TAF for Cohort 1 and increased in Cohort 2 with a mean change from baseline to Week 48 of 152 [SD 152.3] cells/µL.

Analysis of viral resistance

An integrated virology analysis was performed for patients from GS-US-292-0102 (Phase 2), GS-US-292-0104 and GS-US-292-0111 (Phase 3). Genotyping of the PR/RT genes was conducted at screening and patients had to have viruses with genotypic sensitivity to FTC and TDF. Genotyping of the IN gene was conducted at screening in Phase 3 and viruses were to have genotypic sensitivity to EVG. HIV genotype distributions were comparable between treatment groups, with subtype B predominant (87%) followed by AE (6.7%).

No impact of baseline RAMs or HIV subtype was observed on treatment outcomes ($p > 0.05$).

Of 1903 treated patients only 1.9% (19/978) E/C/F/TAF and 2.4% (22/925) STB were included in the RAP and only 14 and 16 in respective groups were included in the final RAP. For those with data:

- 4/10 E/C/F/TAF patients had viruses with new RAMs, all of which had M184V associated with phenotypic resistance to FTC (FC ≥ 63 to > 89) and primary INSTI-R (2 with E92Q, one with

N155H and one with T66A). Phenotypic resistance to EVG was observed in 3 cases and the other had assay failure. One developed K65R without phenotypic resistance to TFV (FC = 1.0).

- 7/13 STB patients had viruses with new RAMs, all of which had M184V/I detected with phenotypic resistance to FTC in 5 cases. Four had emergent primary INSTI-R including 2 with E92Q, one Q148R and one E92E/Q plus Q148R; all showed phenotypic resistance to EVG (FC = 12.8 to > 156). Four had additional NRTI-R detected including 2 with K65R, one with K70K/E and one with E44E/D but 3 remained sensitive to TFV (FC = 0.5 to 1.3) and one had assay failure.

There were no additional substitutions in the RT or IN genes observed in HIV from more than one patient with virologic failure in the E/C/F/TAF or STB groups.

Viruses in virologic failures were assessed for phenotypic resistance to all commercially available NRTIs, NNRTIs, PIs and INSTIs. Cross-resistance profiles were consistent with historical data.

- Nine who had emergent M184V/I and phenotypic resistance to FTC had cross-resistance to lamivudine, with FC values of 5 to > 128. Two with K65R that were successfully phenotyped had cross-resistance to ABC (FC = 6.3 and 6.4) but not to ZDV. Viruses with K70K/E and E44E/D remained sensitive to ABC and ZDV.
- Six of the 7 with virologic failure who had emergent primary INSTI-R and phenotypic resistance to EVG showed cross-resistance to raltegravir (FC = 2.9 to 75). The virus that was not cross-resistant had emergent T66A at virologic failure, which is an EVG-specific resistance mutation. Three of the 7 were assessed for cross resistance to DTG and all remained sensitive with FC values < 4.

The results observed in the other efficacy studies (GS-US-292-0106, -0112 and -0109) were consistent with the integrated analysis.

2.5.3. Discussion on clinical efficacy

Phase 2 and 3 studies vs. STB in ART-naïve

Due to the antiviral activity of the EVG/COBI and FTC content of STB and E/C/F/TAF, the Phase 2 and 3 studies vs. STB are not sensitive enough to provide definitive evidence that the TAF dose is sufficient. Therefore the critical data to support the dose in the FDC come from the monotherapy studies (with doses ranging from 8 to 150 mg), the E_{max} modelling and the PK data that supported adjusting the dose in the FDC from 25 mg to 10 mg. These data are then supported by the Phase 3 comparative efficacy data and the analyses of emergence of mutational resistance.

The Phase 2/3 studies in ART-naïve patients were all conducted vs. STB because a major aim was to compare the safety profiles of TDF and TAF. Overall, the clinical development plan in the ART-naïve population is considered to be appropriate and acceptable. In terms of the actual results of these studies the following observations are made:

In Phase 3 the primary analyses were based on percentages with < 50 copies/mL at Week 48 using the FDA snapshot algorithm and gave very high response rates that were comparable between treatments. Results were also reported for percentages reaching < 20 copies/mL and these data, along with all the sensitivity analyses conducted, consistently support a conclusion of comparability between treatments.

In Phase 2 there was a suggestion that STB might be better in those with the highest baseline viral loads (but only 20% had >100,000 c/mL) and lowest CD4 counts (but < 5% had < 200 cells/µL). In

the Phase 3 studies slightly higher percentages had >100,000 c/mL (~23%) and < 200 cells/µL (13%). One study essentially showed no difference between treatments whereas in 0111 the point estimates were lower with E/C/F/TAF even though the CI overlapped. Since patients were documented to have susceptible virus at baseline there is no obvious explanation and the findings could have occurred by chance. Further exploration did not identify any differences in demographic details of the subgroups enrolled with the highest viral loads and lowest CD4 counts (including numbers falling into both categories) in each treatment group. The finding is mentioned in the SmPC.

Female patients accounted for ~15% of the total in Phase 3 and showed results comparable to those for men, with no appreciable difference between treatments.

In Phase 2 96% of patients had HIV-1 B. The virological responses by HIV-1 subtypes pooled across Phase 3 studies did not suggest any specific problems but data are few.

Virologically suppressed patients who switched to E/C/F/TAF

Data were obtained in the open-label extension of the Phase 2 study (switch from a DRV/COBI-containing regimen) and in GS-US-292-0109. The comparative safety data in patients who did and did not switch to E/C/F/TAF in study 0109 are considered to be more important than the efficacy data. Nevertheless, the efficacy data from both datasets do not point to unexpectedly high rates of virologic rebound at the < 50 or < 20 c/mL cut-offs after switching and the CD4 counts were well maintained. Therefore these data support a conclusion that E/C/F/TAF can suffice to maintain virologic suppression.

E/C/F/TAF in mild/moderate renal impairment

In the Phase 2 and 3 studies ART-naïve patients were to have $eGFR_{CG}$ ≥ 50 mL/min at baseline and the means/medians exceeded 100 mL/min. The same inclusion criterion was applied to the switch study in virologically suppressed patients. Therefore, study 0112 was intended to support the safety of E/C/F/TAF in HIV-infected patients with $eGFR_{CG}$ 30-69 mL/min. The FTC dose was not adjusted for those with $eGFR_{CG}$ < 50 mL/min in this study and the safety data obtained supported no dose adjustment for E/C/F/TAF for patients with $eGFR_{CG}$ ≥ 30 mL/min.

Focussing on Cohort 1, this population was expectedly older than in the ART-naïve studies. Switching to E/C/F/TAF was associated with very few virologic rebounds at the < 50 or < 20 c/mL cut-offs regardless of baseline renal function.

Use in adolescents

STB, EVG and COBI are not approved for use in adolescents and use of TDF is restricted because of safety concerns in growing adolescents, including effects on bone mineralisation. Using the PK data from 24 patients enrolled into cohort 1 of GS-US-292-0106 the POPPK analysis suggested comparable plasma exposures to TAF and TFV in adults and adolescents. There is no known reason to think that TAF uptake and intracellular conversion to TFV would be impeded in adolescents compared to adults.

Most of these patients were black, noting that ART-naïve black adults generally responded slightly better than other ethnic groups in Phase 3. Also, 58.3% were female and the median age was 15 years (range 12 to 17). The data for all 50 enrolled suggest that responses to treatment are similar to those in adults.

Virological data

The data do not point to any major concerns.

2.5.4. Conclusions on the clinical efficacy

The selected 10 mg dose of TAF in the FDC derives from monotherapy studies, the Emax modelling and PK data. These data are further supported by the Phase 3 comparative efficacy data and the analyses of emergence of mutational resistance. The Phase 3 primary analyses gave very high comparable response rates in terms of proportion of patients achieving < 50 copies/mL at Week 48. Data < 20 copies/mL along with all the sensitivity analyses conducted consistently support a conclusion of comparability between treatments. Data from the switch study d support a conclusion that E/C/F/TAF can suffice to maintain virologic suppression. Data in HIV-infected patients with renal impairment support the safety of E/C/F/TAF with eGFRCG 30-69 mL/min. PK data suggested comparable plasma exposures to TAF and TFV in adults and adolescents supporting the use in this population.

2.6. Clinical safety

The focus is on the HIV-infected patients who received E/C/F/TAF in the six studies Phase 2/3 studies.

Adverse events

ART-naive in Phase 3 (GS-US-292-0104 and 0111)

The most common AEs were diarrhoea, nausea, headache and URTI.

Table 73. GS-US-292-0104 and -0111: Drug-related AEs in ≥ 1% in either treatment group (Safety Analysis Set)

AEs by SOC and PT	E/C/F/TAF (N=866)	STB (N=867)
Number with any drug-related AE	342 (39.5%)	364 (42.0%)
Nausea	90 (10.4%)	113 (13.0%)
Diarrhoea	62 (7.2%)	74 (8.5%)
Flatulence	19 (2.2%)	25 (2.9%)
Vomiting	16 (1.8%)	27 (3.1%)
Abdominal distension	13 (1.5%)	9 (1.0%)
Abdominal pain	11 (1.3%)	11 (1.3%)
Abdominal pain upper	9 (1.0%)	11 (1.3%)
Fatigue	43 (5.0%)	35 (4.0%)
Decreased appetite	12 (1.4%)	9 (1.0%)
Osteopenia	8 (0.9%)	17 (2.0%)
Headache	52 (6.0%)	47 (5.4%)
Dizziness	26 (3.0%)	19 (2.2%)
Somnolence	9 (1.0%)	10 (1.2%)
Abnormal dreams	13 (1.5%)	26 (3.0%)
Insomnia	17 (2.0%)	14 (1.6%)
Proteinuria	7 (0.8%)	10 (1.2%)
Rash	13 (1.5%)	11 (1.3%)

Grade 3 or 4 AEs were reported for 8.2% in E/C/F/TAF and STB groups. Similar percentages in each group had any drug-related AE according to investigators, among which nausea, diarrhoea, headache and fatigue predominated. Grade 3 or 4 AEs considered drug-related were reported for 1.4% E/C/F/TAF (12) and 1% STB (9) patients.

Virologically suppressed (GS-US-292-0109)

The most common AEs with E/C/F/TAF were URTI (12.1%), diarrhoea (8.0%), nasopharyngitis (6.7%), headache (6.0%) and cough (5.1%) and for FTC/TDF+3rd Agent URTI (7.5%) and diarrhoea (7.5%). Grade 3 or Grade 4 AEs were reported in 6.4% E/C/F/TAF (61) and 6.7% FTC/TDF+3rd Agent (32) patients of whom 53 and 29, respectively, had Grade 3 AEs only. A higher percentage in the group that switched to E/C/F/TAF had a drug-related AE (19.3% vs. 12.8% in those who did not switch). Drug-related AEs were most often in the Gastro-intestinal disorders SOC.

Table 74. GS-US-292-0109: Drug-Related AEs in at least 1% per group (Safety Analysis Set)

AEs by SOC and PT	E/C/F/TAF (N=959)	FTC/TDF+3rd Agent (N=477)
Number with a drug-related AE	185 (19.3%)	61 (12.8%)
Ocular icterus	0	5 (1.0%)
Diarrhoea	24 (2.5%)	6 (1.3%)
Nausea	21 (2.2%)	2 (0.4%)
Flatulence	18 (1.9%)	1 (0.2%)
Jaundice	0	9 (1.9%)
Osteopenia	8 (0.8%)	6 (1.3%)
Dizziness	11 (1.1%)	6 (1.3%)
Headache	17 (1.8%)	0
Abnormal dreams	12 (1.3%)	6 (1.3%)
Insomnia	10 (1.0%)	6 (1.3%)

AEs of special interest (AESIs)

Fractures

In GS-US-292-0104 and GS-US-292-0111 the rates of fractures were low (E/C/F/TAF 1.3%, 11; STB 1.7%, 15). In GS-US-292-0109 the rates were E/C/F/TAF 1.5% (14) and FTC/TDF+3rd Agent 0.6% (3).

Bone Mineral Density

At Week 48 in GS-US-292-0104 and GS-US-292-0111 fewer patients in the E/C/F/TAF group had a > 3% decrease from baseline in hip BMD (E/C/F/TAF 16.8%; STB 50.1%) or in spine BMD (E/C/F/TAF 26.5%; STB 45.8%).

Virologically suppressed adults who switched to E/C/F/TAF from a TDF-based regimen in Study GS-US-292-0109 had statistically significant improvements in both hip and spine BMD. Mean (SD) percentage changes from baseline in BMD at Week 48 were 1.949% (2.9956) at the hip and 1.861% (3.0889) at the spine. There were minimal changes in the FTC/TDF+3rd Agent group ($p < 0.001$).

Table 75. Measures of bone mineral density in treatment-naïve patients (studies GS-US-292-0104 and GS-US-292-0111; Week 48 analysis)

	Genvoya	E/C/F/TDF	Treatment difference
Hip DXA analysis	(n = 780)	(n = 767)	
Mean percent change in BMD	-0.7%	-3.0%	2.29%, p < 0.001
Lumbar spine DXA analysis	(n = 784)	(n = 773)	
Mean percent change in BMD	-1.3%	-2.9%	1.56%, p < 0.001

E/C/F/TDF = Elvitegravir/cobicistat/emtricitabine/tenofovir disoproxil fumarate

BMD = Bone mineral density

DXA = Dual-energy X-ray absorptiometry

Table 76. Measures of bone mineral density in patients switching from a TDF-based regimen (study GS-US-292-0109; Week 48 analysis)

	Genvoya	Baseline regimen	Treatment difference
Hip DXA analysis	(n = 902)	(n = 452)	
Mean percent change in BMD	1.5%	-0.3%	1.81%, p < 0.001
Lumbar spine DXA analysis	(n = 912)	(n = 457)	
Mean percent change in BMD	1.6%	-0.4%	2.00%, p < 0.001

BMD = Bone mineral density

DXA = Dual-energy X-ray absorptiometry

Fracture probability (FRAX analysis)

In GS-US-292-0104 and GS-US-292-0111 the mean increase in risk from baseline to Week 48 was smaller for the E/C/F/TAF group (0.26% vs. STB 0.38%; p = 0.014). In GS-US-292-0109 the mean change from baseline to Week 48 in the 10-year probability of major osteoporotic fracture was 0.10% in the E/C/F/TAF (switch) group and 0.23% in the FTC/TDF+3rd Agent group (p = 0.002).

Bone Laboratory Parameters

In GS-US-292-0104 and GS-US-292-0111 median percentage changes from baseline to Week 48 were:

- C-telopeptide - E/C/F/TAF 9.1%; STB 21.1%
- P1NP - E/C/F/TAF 26.86%; STB 72.85%
- PTH - E/C/F/TAF 23.0%; STB 41.9%.

In GS-US-292-0109 there was no change from baseline in serum levels of C-telopeptide in the E/C/F/TAF group compared with an increase from baseline in the FTC/TDF+3rd Agent group at Week 48 (p = 0.007).

Renal safety

No cases of proximal renal tubulopathy were reported (including Fanconi syndrome) in patients who have received E/C/F/TAF for any length of time.

Ocular safety (AESI due to nonclinical findings)

ART-naive in Phase 3 (GS-US-292-0104 and GS-US-292-0111)

The incidence of AEs in the eye disorders SOC was 7.0% for E/C/F/TAF (61) and 7.3% for STB (63), of which 16 and 9 in respective groups had AEs considered related to study drugs by the investigator. There were no AEs of uveitis in the E/C/F/TAF group although 23 (2.7%) vs. 15 (1.7%) in the STB group had AEs that could represent symptoms of uveitis, of which the most common was vision blurred in E/C/F/TAF 1.3% vs. STB 1.0%.

Virologically suppressed (GS-US-292-0109)

AEs in the SOC of eye disorders were reported for 5.5% E/C/F/TAF (53) and 4.6% FTC/TDF+3rd Agent (22) patients. There were no reports of uveitis. AEs that could represent symptoms of uveitis were reported for 1.9% E/C/F/TAF and 0.8% FTC/TDF+3rd Agent patients. In the ophthalmologic sub-study most patients had normal assessments at Week 24 (26/31 vs. 12/14) and Week 48 (14/18 vs. 6/8).

Deaths

There have been very few deaths, none of which was considered related to study drug by investigators.

SAEs

ART-naïve in Phase 3 (GS-US-292-0104 and GS-US-292-0111)

SAEs were reported for 70 E/C/F/TAF (8.1%) and 59 STB (6.8%). No individual SAE occurred in ≥ 1% in either treatment group and only appendicitis (E/C/F/TAF 4; STB 3) and cellulitis (1 vs. 3) were reported for > 2 patients in either group. One E/C/F/TAF patient had a renal SAE of ureteric calculus and one had nephrotic syndrome. SAEs considered related to study drugs by investigators are shown in Table 77.

Table 77. Treatment-Emergent Study-Drug-Related Serious Adverse Events Studies GS-US-292-0104 and GS-US-292-0111 Safety Analysis Set

	E/C/F/TAF (N= 866	STB (N=867)
Number of subjects experimenting any treatment emergent study related serious adverse event	3 (0.38%)	2 (0.28%)
Number of subjects experimenting any treatment emergent study related serious adverse event by system organ class and preferred term		
Hepatobiliary disorders	0	1 (0.1%)
Cholelithiasis	0	1 (0.1%)
Immune system disorders	0	1 (0.1%)
Immune reconstitution inflammatory syndrome	0	1 (0.1%)
Infections and infestations	1 (0.1%)	0
Staphylococcal skin reaction	1 (0.1%)	0
Skin and subcutaneous tissue disorders	1 (0.1%)	0
Rash erythematous	1 (0.1%)	0
Vascular disorders	1 (0.1%)	0
Hypovolaemic shock	1 (0.1%)	0

Virologically suppressed in GS-US-292-0109

SAEs were reported in 42 E/C/F/TAF (4.4%) and 21 FTC/TDF+3rd Agent (4.4%) patients. SAEs reported for > 1 patient were aseptic meningitis (3 vs. 0), pneumonia (3 vs. 0), sepsis (2 vs. 0), sinusitis (2 vs. 0), chest pain (2 vs. 1), diarrhoea (1 vs. 2) and abdominal pain (2 vs. 0). No SAEs in the E/C/F/TAF group were considered to be related to study drugs by the investigators.

Pregnancies

Six pregnancies were reported in Phase 2/3 studies. The single pregnancy in E/C/F/TAF patients was terminated by an elective abortion.

Laboratory findings

There were no clinically relevant changes from baseline within groups or differences between treatment groups in median values for haematology parameters. Median values were within normal ranges. Approximately 20% of patients in Phase 2/3 studies had a Grade 3 or 4 laboratory abnormality, with the exception of adolescents (8.3%; 3/4 had abnormal neutrophils; data not shown in Table 78). Grade 3 or 4 CPK values occurred at a variety of time points and were not consistently present for individuals. No case of clinical rhabdomyolysis was reported.

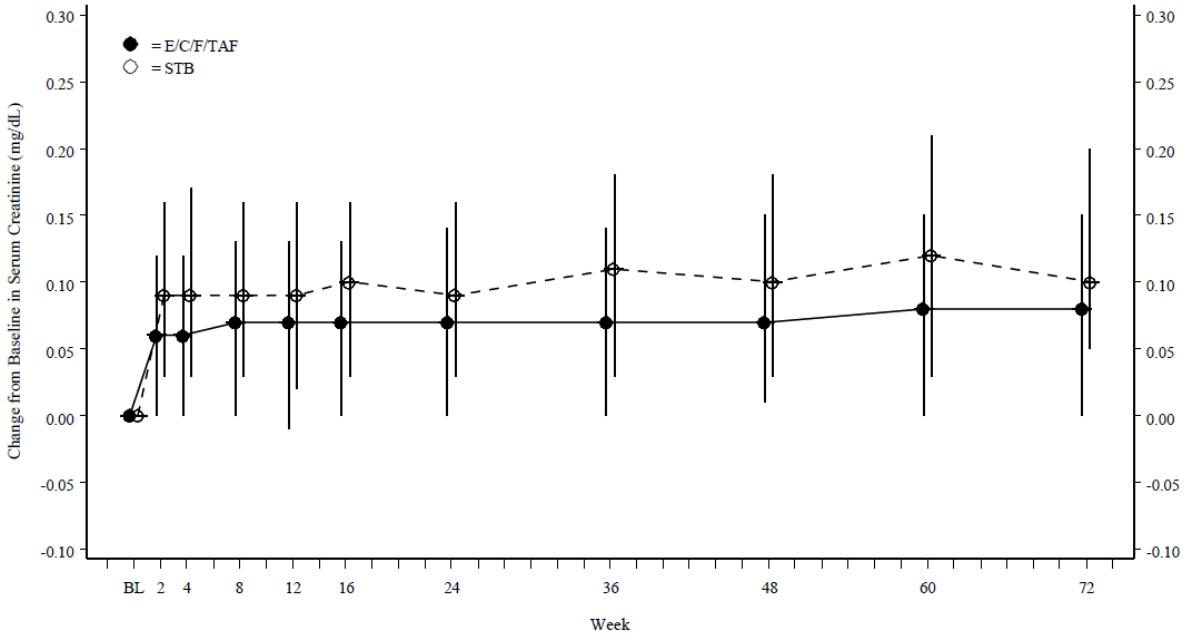
Table 78. GS-US-292-0104, -0111, -0102, -0109 and -0112: Grade 3 and 4 laboratory abnormalities in at least 1% in any group/study (Safety Analysis Set)

	GS-US-292-0104/-0111		GS-US-292-0102[a]		GS-US-292-0109		
	E/C/F/TAF (N=866)	STB (N=867)	E/C/F/TAF (N=112)	STB (N=58)	E/C/F/TAF (N=959)	FTC/TDF + 3rd Agent (N=477)	GS-US-292-0112 Cohort 1 (N=242)
Max Grade 3 or 4	172 (20.0%)	171 (19.8%)	31 (27.9%)	11 (19.0%)	190 (19.8%)	121 (25.4%)	64 (26.4%)
Neutrophils	862	865	111	58	959	477	242
Neutrophils	13 (1.5%)	21 (2.4%)	7 (6.3%)	2 (3.4%)	11 (1.1%)	3 (0.6%)	2 (0. 8%)
ALT	10 (1.2%)	12 (1.4%)	1 (0.9%)	1 (1.7%)	5 (0.5%)	3 (0.6%)	2 (0.8%)
Amylase	13 (1.5%)	26 (3.0%)	3 (2.7%)	2 (3.4%)	11 (1.1%)	9 (1.9%)	6 (2.5%)
AST	13 (1.5%)	16 (1.8%)	1 (0.9%)	0	12 (1.3%)	5 (1.0%)	1 (0.4%)
CK	59 (6.8%)	49 (5.7%)	7 (6.3%)	2 (3.4%)	50 (5.2%)	24 (5.0%)	10 (4.1%)
Creatinine	0	2 (0.2%)	0	0	0	0	5 (2.1%)
GGT	3 (0.3%)	12 (1.4%)	1 (0.9%)	1 (1.7%)	4 (0.4%)	5 (1.0%)	3 (1.2%)
Lipase	4 (4.4%)	9 (8.0%)	0	1 (10.0%)	4 (8.7%)	3 (10.0%)	4 (12.1%)
Fasting glucose	7 (0.8%)	4 (0.5%)	2 (1.8%)	1 (1.7%)	7 (0.7%)	2 (0.4%)	6 (2.5%)
Total bilirubin	0	4 (0.5%)	0	0	1 (0.1%)	68 (14.3%)	0
Fasting cholesterol	15 (1.8%)	10 (1.2%)	3 (2.7%)	0	28 (3.0%)	0	10 (4.3%)
Fasting triglycerides	4 (0.5%)	2 (0.2%)	1 (0.9%)	1 (1.7%)	8 (0.9%)	2 (0.4%)	1 (0.4%)
Fasting LDL	42 (5.0%)	18 (2.2%)	10 (9.1%)	3 (5.2%)	67 (7.2%)	4 (0.9%)	14 (6.0%)
Uric acid	0	2 (0.2%)	0	0	2 (0.2%)	0	4 (1.7%)
Glycosuria	11 (1.3%)	13 (1.5%)	2 (1.8%)	0	11 (1.1%)	5 (1.1%)	8 (3.3%)

Serum creatinine

In ART-naive patients in GS-US-292-0104 and GS-US-292-0111 increases from baseline in mean serum creatinine were smaller with E/C/F/TAF vs. STB and statistically significantly different from Weeks 2 to 48 (see figure below). Graded abnormalities for serum creatinine were reported for 3.7% E/C/F/TAF and 5.0% STB patients.

Figure 10. GS-US-292-0104 and GS-US-292-0111: Mean change from baseline in serum creatinine (Observed Data; Safety Analysis Set)



E/C/F/TAF (n=):	866	826	856	847	843	845	836	830	820	396	128
STB (n=):	867	844	858	852	841	843	834	816	806	375	118

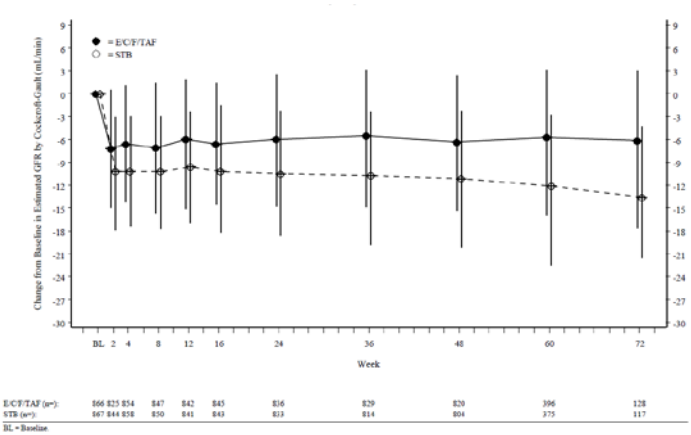
BL = Baseline.

In patients who switched from a TDF-based regimen to E/C/F/TAF in GS-US-292-0109 the mean change from baseline in serum creatinine to Week 48 was E/C/F/TAF -0.01 mg/dL vs. FTC/TDF+3rd Agent 0.04 mg/dL ($p < 0.001$).

eGFR

In ART-naive patients in GS-US-292-0104 and GS-US-292-0111 the decreases from baseline in median $eGFR_{CG}$ were smaller with E/C/F/TAF vs. STB. Consistent results were observed using $eGFR_{CKD\text{-}EPI\ serum\ creatinine}$. The difference was statistically significant from Week 2 to 48 (see Figure 11).

Figure 11. GS-US-292-0104 and GS-US-292-0111: Median change from baseline in $eGFR_{CG}$ (Safety Analysis Set)



In patients who switched from a TDF-based regimen to E/C/F/TAF (GS-US-292-0109) there were minimal changes in $eGFR_{CG}$ that varied by prior regimen (median change from -0.4 to 5.8 mg/dL).

Other renal biomarkers

In the ART-naive patients in GS-US-292-0104 and GS-US-292-0111 there were significant differences between groups for the parameters shown in Table 79. There was no change from baseline in median FEUA using adjusted serum creatinine at Week 48 in the E/C/F/TAF group vs. an increase in the STB group ($p < 0.001$ at Week 48). There were no differences in median TmP/GFR between treatment groups. The median $FEPO_4$ using adjusted serum creatinine was increased relative to baseline through Week 48 for both groups and was significantly greater in the STB group ($p = 0.005$) at Week 48.

Table 79. GS-US-292-0104 and GS-US-292-0111: Renal biomarkers at Week 48

Parameter	Median Percentage Change (%)		p-value[a]
	E/C/F/TAF	STB	
UPCR (mg/g)	-3.4%	19.8%	<0.001
UACR (mg/g)	-4.7%	7.1%	0.001
RBP to Urine Creatinine Ratio (µg/g)	9.2%	51.2%	<0.001
Beta-2-microglobulin to Urine Creatinine Ratio (µg/g)	−31.7%	24.1%	<0.001

a p-values were from the 2-sided Wilcoxon rank sum test to compare the 2 treatment groups.

In GS-US-292-0109 there were decreases from baseline in UPCR and UACR with E/C/F/TAF but increases from baseline with FTC/TDF+3rd Agent at Week 48 ($p < 0.001$). There were also decreases from baseline in the E/C/F/TAF group in urine RBP to creatinine and beta-2-microglobulin to creatinine ratios but increases at most time points in the FTC/TDF+3rd Agent group ($p < 0.001$).

Other laboratory parameters

ART-naive in Phase 3 (GS-US-292-0104 and GS-US-292-0111)

Median increases from baseline in fasting lipid parameters occurred with both treatments but were greater in the E/C/F/TAF group at Week 48 ($p < 0.001$).

More E/C/F/TAF patients had categorical changes in NCEP ATP III lipid classifications from baseline except for HDL. Grade 3 LDL abnormalities were reported for 5.0% (42) in the E/C/F/TAF group and 2.2% (18) in the STB group.

Virologically suppressed (GS-US-292-0109)

There were increases from baseline in fasting lipid parameters in the E/C/F/TAF group only, regardless of prior treatment, and categorical shifts from baseline were more frequent vs. FTC/TDF+3rd Agent. Grade 3 fasting LDL abnormalities occurred in 7.2% E/C/F/TAF (67) vs. 0.9% FTC/TDF+3rd Agent.

Safety in special populations

Patients with mild to moderate renal impairment (GS-US-292-0112)

This open-label study was primarily designed to assess the safety profile of E/C/F/TAF in HIV-infected patients with $eGFR_{CG}$ in the range 30-69 mL/min. There were no clinically significant changes from baseline in aGFR, $eGFR_{CG}$, $eGFR_{CKD\text{-}EPI}$, cysC and $eGFR_{CKD\text{-}EPI,\ creatinine}$. Significant decreases in proteinuria (UPCR), albuminuria (UACR), and tubular proteinuria from baseline occurred as early as 1 week after switch to E/C/F/TAF from a TDF-containing regimen with median levels decreasing to below the baseline levels. Improvements in BMD were also noted (see Table 81).

Table 80. Renal laboratory tests in virologically suppressed patients with renal impairment who switched to Genvoya (study GS US 292 0112; Week 48 analysis)

Renal laboratory test	All patients (n = 242)	Non-TDF containing prior regimen (n = 84)	TDF containing prior regimen (n = 158)
Median $eGFR_{CG}$ (mL/min)	Baseline: 56 Week 48: 56	Baseline: 53 Week 48: 52	Baseline: 58 Week 48: 57
Improvement in proteinuria by urine dipstick[a]	61/74 (82%)	12/18 (67%)	49/56 (88%)
Median UPCR (mg/g)	Baseline: 161 Week 48: 85	Baseline: 105 Week 48: 110	Baseline: 189 Week 48: 78
Median UACR (mg/g)	Baseline: 28.8 Week 48: 10.0	Baseline: 18.0 Week 48: 13.6	Baseline: 40.9 Week 48: 9.4

In patients with renal impairment, the prevalence of clinically significant proteinuria (UPCR > 200 mg/g) and albuminuria (UACR ≥ 30 mg/g) decreased from 42% at baseline to 16% at Week 48, and 49% at baseline to 26% at Week 48, respectively.

In a substudy, patients given Genvoya (n = 32) had no change from baseline in their actual GFR at Week 24, as measured by iohexol clearance.

Table 81. GS-US-292-0112: Changes from Baseline in Hip and Spine BMD at Weeks 24 and 48 (Observed Data; Hip and Spine DXA Analysis Sets)

		Cohort 1: Switch			Cohort 2: ART-Naive
	Statistic	With Pre-Switch TDF Use (N = 154)	Without Pre-Switch TDF Use (N = 82)	Total (N = 236)	Total (N = 6)
Hip BMD[a]					
Baseline (g/cm^2)	N	154	82	236	6
	Mean (SD)	0.918 (0.1522)	0.919 (0.1622)	0.918 (0.1554)	0.973 (0.2124)
% Change at Week 24	N	148	77	225	6
	Mean (SD)	1.151 (2.9284)	-0.071 (2.2339)	0.733 (2.7674)	-0.022 (1.6853)
% Change at Week 48	N	110	51	161	3
	Mean (SD)	1.843 (3.4939)	0.997 (6.3113)	1.575 (4.5738)	-0.222 (2.3207)
Spine BMD[b]					
Baseline (g/cm^2)	N	154	82	236	6
	Mean (SD)	1.056 (0.1908)	1.112 (0.1780)	1.076 (0.1879)	1.034 (0.2432)
% Change at Week 24	N	147	79	226	6
	Mean (SD)	2.370 (3.7131)	0.291 (3.0416)	1.643 (3.6250)	-2.686 (4.5755)
% Change at Week 48	N	107	53	160	3
	Mean (SD)	2.994 (4.0816)	1.104 (4.3644)	2.368 (4.2582)	-4.538 (6.8917)

% Change = Change from baseline at a postbaseline visit/baseline * 100%.

a Only subjects with nonmissing hip BMD for the baseline visit were included in Hip DXA Analysis Set.

b Only subjects with nonmissing spine BMD for the baseline visit were included in Spine DXA Analysis Set.

ART-naïve adolescents (GS-US-292-0106)

This open-label study was conducted in 50 ARV-naïve adolescents aged from 12 to <17 years. All have received E/C/F/TAF for at least 8 weeks while 25 have received 48 weeks.

At least 1 AE was reported for 84.0% (42/50), most of which were Grade 1 or 2 in severity. Four subjects (8.0%) had a Grade 3 or 4 AE. The most common AEs were nausea, URTI and respiratory tract infection (each reported in 24.0%; 12) and 18 (36.0%) had an AE considered related to study drug by the investigator, most of which were Grade 1 or Grade 2 in severity, most commonly nausea, abdominal pain, vomiting, upper abdominal pain and diarrhoea. Four subjects (8.0%) had a SAE but only one patient (visual impairment and intermediate uveitis) had SAEs considered related to study drug by the investigator. No subject had an AE that led to study drug discontinuation and there were no deaths. One other event of potential uveitis occurred in a subject who used illicit substances prior to sleeping and then awoke with blurred vision and photophobia. The visual changes resolved later that same day.

The median (Q1, Q3) change from baseline in serum creatinine was 0.06 (0.00, 0.12) mg/dL at Week 1 (baseline median [Q1, Q3], 0.58 [0.50, 0.79] mg/dL). Creatinine subsequently stabilized without progressive changes with a median (Q1, Q3) change from baseline at Week 24 of 0.08 (0.00, 0.15) mg/dL. No graded abnormalities of serum creatinine were reported.

The median (Q1, Q3) change from baseline in eGFR (calculated using the Schwartz formula) at Week 1 was -13.0 (-26.0, 0.0) mL/min/1.73 m^2 (baseline median [Q1, Q3], 156.0 [129.0, 185.0] mL/min/1.73 m^2). The median (Q1, Q3) change from baseline at Week 24 was -15.0 (-30.0, 0.0) mL/min/1.73 m^2. No AEs of decreased eGFR or renal failure were reported.

Post-baseline, treatment-emergent Grade 1 or Grade 2 proteinuria, generally isolated and transient, was reported for 36.0% (18/50). Proteinuria was not reported as an AE for any subject. There were no SAEs of proximal renal tubulopathy (including Fanconi Syndrome) and no subject had laboratory findings consistent with proximal renal tubulopathy.

At Week 24, 3/47 subjects (6.4%) had a ≥ 4% decrease in spine BMD but none had a ≥ 4% decrease in TBLH BMD.

Table 82. GS-US-292-0106: baseline value and percentage change from baseline in spine and TBLH BMD (spine and TBLH DXA analysis set)

Time point	Spine (N=47)			TBLH (N=45)		
	N	Mean (SD)	Median (Q1,Q3)	N	Mean (SD)	Median (Q1,Q3)
Baseline (g/cm^2)	47	0.809 (0.2031)	0.778 (0.677, 0.943)	45	0.888 (0.1229)	0.878 (.815, 0.976)
Percentage change from baseline at						
Week 24	47	1.598 (3.959)	1.252 (0.957, 4.106)	45	0.643 (2.498)	0.256 (-1.017,1.715)
Week 48	23	3.966 (4.2625)	3.249 (4.625)	23	1.578 (2.4804)	1.004 (-0.282, 3.269)

Baseline height-age adjusted spine and TBLH BMD Z-scores were higher than standard Z-scores, consistent with the below average height of the study population.

Table 83. GS-US-202-0106; Spine and TBLH height-age adjusted BMD Z-scores at baseline and change baseline (Spine and TBLH DXA Analysis set)

Time point	Spine BMD Z-score (height –age adjusted) (N=47)			TBLH BMD Z-score (height –age adjusted) (N=45)		
	N	Mean (SD)	Median (Q1,Q3)	N	Mean (SD)	Median (Q1,Q3)
Baseline	41	-0.73 (1.369)	-0.54 (-1.56,0.15)	38	-0.31 (0.34)	-0.27 (-1.12, 0.600
Change from baseline at						
Week 24	39	-0.05 (0.348)	-0.02 (-0.02, 0.13)	37	-0.10 (0.295)	-0.13 (0.31, 0.11)
Week 48	19	-0.02 (0.341)	0.04 (-0.07, 019)	18	-0.18 (0.274)	-0.14 (0.32, 0.01)

Three subjects have had > 4% decrease from baseline in spine BMD at Week 24. A corresponding > 4% decrease was not observed in TBLH BMD, and spine BMD subsequently increased in the 2 subjects with available Week 48 data. Therefore, the applicant considers that these events likely represent transient spine BMD decreases due to growth.

There were no clinically relevant changes from baseline in median values for any haematology or clinical chemistry parameter. Increases from baseline in the fasting lipid parameters were observed at Week 24 (median [Q1, Q3] change from baseline):

- fasting total cholesterol 25 [9, 37] mg/dL
- fasting LDL cholesterol 10 [0, 26] mg/dL

- fasting HDL cholesterol 7 [1, 14] mg/dL)

Most (96%, 48/50) had at least 1 treatment-emergent laboratory abnormality reported, most of which were Grade 1 or 2 in severity. Excluding Grade 3 haematuria detected by non-quantitative dipstick analysis, Grade 3 laboratory abnormalities were reported for 4 subjects. Isolated abnormalities of grade 3 decreased neutrophils were reported for 3 subjects. One subject had transient Grade 3 haematuria following quantitative analysis. None of these laboratory abnormalities was reported as an AE.

Results from GS-US-292-0106 were compared with those from a study of STB also in HIV-infected, ART-naive adolescents (GS-US-236-0112) which had similar objectives, design, procedures and assessments. The applicant acknowledges that none of STB, EVG, COBI or TDF are approved for use in ART-naïve adolescents but the studies allow for a comparison of the safety of TAF versus TDF.

The E/C/F/TAF and STB studies each enrolled 50 adolescents (median age 15 years in both studies, 56% vs. 30% female, 88% vs. 68% Black, 22% vs. 20% with baseline HIV-1 RNA > 100,000 copies/mL, median CD4 count 456 vs. 402 cells/µL, median eGFR [using the Schwartz formula] 156.0 vs. 139.5 mL/min/1.73m^2). Most AEs in both studies were mild and unrelated to treatment, with no deaths or AEs leading to treatment discontinuation.

At Week 24, the median change from baseline in serum creatinine was 0.08 mg/dL in both studies, with median eGFR (using the Schwartz formula) changes from baseline of -14.1 mL/min/1.73m^2 for E/C/F/TAF and -14.0 mL/min/1.73m2 for STB, consistent with the effect of COBI. Proteinuria (any grade) occurred in 36% E/C/F/TAF vs. 42% STB subjects, with Grade 2 proteinuria in 8% vs. 14% of participants, respectively.

Table 84. GS-US-292-0106 and GS-US-236-0112: Change from Baseline in Key Renal Laboratory Parameters at Week 24 (Safety Analysis Set)

Change from Baseline at Week 24 in:	E/C/F/TAF (N = 50)		STB (N = 50)		p-value[a]
	N	Mean (SD)	N	Mean (SD)	
Serum Creatinine (mg/dL)	47	0.08 (0.099)	49	0.08 (0.098)	0.77
eGFR (using Schwartz formula) (mL/min)	47	-4.1 (22.62)	49	-13.7 (16.84)	0.66
Proteinuria by Urinalysis (Dipstick) (%)[a]	50	36.0%	50	42.0%	NC
UPCR (mg/g)	47	2% (77.3)	25	1% (55.4)	0.16
RBP to Urine Creatinine ratio (µg/g)	47	20.3% (87.70)	15	59.2% (131.99)	0.34
Beta-2-microglobulin to Urine Creatinine ratio (µg/g)	46	3% (215.0)	15	78% (218.6)	0.13
Urine Fractional Excretion of Phosphate (using adjusted serum creatinine) (%)	46	0.50 (4.297)	25	1.02 (4.809)	0.011

eGFR = estimated glomerular filtration rate; NC = not calculated; UPCR = urine protein to creatinine ratio

a P-values, difference in least squares means (Diff in LSM), and its 95% CI for changes from baseline at postbaseline visits were from the analysis of covariance effect (ANCOVA) model including treatment as a fixed effect and baseline value as a covariate.

b Treatment-emergent graded events, any grade

Of those subjects with BMD measurements at Week 24, the mean percentage change from baseline in spine BMD was 1.252% for E/C/F/TAF subjects, with a decrease of ≥ 4% in 3 of 47 subjects (6.4%), versus a median percentage change from baseline of -0.985% for STB subjects, with a decrease of ≥ 4% in 10 of 47 subjects (21.3%).

The median change from baseline at Week 24 in spine height-age adjusted Z-scores was by -0.02 and -0.14 in the E/C/F/TAF and STB groups, respectively.

Compared with STB, E/C/F/TAF exhibited similar effects on eGFR, reduced levels of total protein and renal tubular proteins in the urine and a median increase in spine mineralization.

Table 85. GS-US-292-0106 and GS-US-236-0112: Percentage Change from Baseline in Spine BMD (g/cm2, Cross-Calibrated) by Visit (Spine DXA Analysis Set)

	E/C/F/TAF (N = 47)		STB (N = 47)		E/C/F/TAF vs. STB	
	N	Mean (SD)	N	Mean (SD)	p-value	Difference in Percentages (95% CI)
Mean Areal BMD, g/cm^2						
Baseline	47	0.804 (0.2016)	47	0.874 (0.1725)	0.074[a]	-0.070 (-0.147 , 0.007)
Week 24	47	0.813 (0.1911)	47	0.865 (0.1711)		
Week 48	23	0.802 (0.2052)	28	0.879 (0.1774)		
Mean Percentage Change from Baseline						
Week 24	47	1.606% (3.9452)	47	-0.970% (3.6642)	0.040[b]	1.575 (0.077 , 3.074)
Week 48	23	3.997% (4.3407)	28	0.229% (4.8347)	0.033[b]	2.965 (0.253 , 5.677)
Mean with ≥ 4% loss from Baseline, n/N (%)						
Week 24	47	3 (6.4%)	47	10 (21.3%)	0.070[c]	
Week 48	23	1 (4.3%)	28	5 (17.9%)	0.20[c]	

a P-values, difference in least squares means (Diff in LSM), and its 95% CI for baseline were from the analysis of variance (ANOVA) model including treatment as a fixed effect.
b P-values, difference in least squares means (Diff in LSM), and its 95% CI for % changes from baseline at postbaseline visits were from the analysis of covariance effect (ANCOVA) model including treatment as a fixed effect and baseline value as a covariate.
c P-values were from the 2-sided Fisher's exact test to compare the 2 treatment groups.

Discontinuation due to AEs

- In the ART-naive in GS-US-292-0104 and GS-US-292-0111 AEs that led to discontinuation of treatment occurred in 8 E/C/F/TAF (0.9%) and 13 STB (1.5%) patients. No AE leading to discontinuation was reported for >1 patient in the E/C/F/TAF group.
- In GS-US-292-0109 discontinuations due to AEs occurred in 9 E/C/F/TAF (0.9%) and 7 FTC/TDF+3rd Agent (1.5%) patients. In the E/C/F/TAF group none was reported for >1 patient. Most of these AEs were non-serious and considered to be related to study drug.
- In patients with mild to moderate renal impairment (GS-US-292-0112) AEs leading to study drug discontinuation were reported for 8 (3.3%) patients. Three AEs were considered related to study drug, including worsening of sleep disturbance, worsening renal insufficiency and choking. Two patients discontinued due to renal AEs of renal failure, both of which reflected declining GFR. One had labile hypertension and the other was considered unrelated to study drug.
- No adolescents in GS-US-292-0106 discontinued study drug due to an AE.
- In the TAF Phase 1 studies 5 subjects discontinued due to 7 AEs. The AEs considered drug-related were elevated CPK, headache, urticaria (2), pruritus and anxiety.

2.6.1. Discussion on clinical safety

The Phase 2/3 programme allows for direct comparisons between E/C/F/TAF and STB in previously untreated patients as well as the effects of switching from TDF to TAF within regimens, including switching from STB to E/C/F/TAF.

E/C/F/TAF vs. STB in ART-naïve patients

The safety profile of E/C/F/TAF was mostly very similar to that of STB. Rates for individual types of AEs, including Grade 3/4 and drug-related AEs, were comparable or slightly lower vs. STB. In the two Phase 3 studies the rates of fractures were low and comparable but fewer patients in the E/C/F/TAF group had a > 3% decrease from baseline in hip or spine BMD, noting nonetheless that such decreases did still occur in the 48 weeks after starting treatment with E/C/F/TAF. The FRAX analysis also suggested a benefit for E/C/F/TAF over STB as did bone biomarkers.

Switching from TDF to TAF

It could be expected that AE rates could be higher compared to those who remained on regimens that were previously tolerated. The most remarkable finding was the difference in rate of AEs considered to be drug-related by investigators. However, this finding occurred in an open-label study, which may have influenced investigator assignments to some extent. In addition, the difference seems to have been driven by gastro-intestinal AEs and there were few Grade 3 or 4 events. Fractures were reported in 1.5% E/C/F/TAF vs. 0.6% FTC/TDF+3rd Agent groups but at Week 48 there were increases from baseline in mean BMD at the hip or spine in the E/C/F/TAF group compared to no appreciable change in those who did not switch and the FRAX analysis also suggested some benefit from switching.

Ocular safety

Current data do not suggest that the nonclinical findings translate into a concern regarding the ocular safety of TAF. There was one adolescent with uveitis considered to be drug-related by the investigator. At present it seems reasonable to keep this issue under close review with appropriate reflection in the RMP.

Laboratory findings

The following issues are of note:

- Renal effects

In the two Phase 3 studies in ART-naive patients there were statistically significantly smaller increases from baseline in mean serum creatinine with E/C/F/TAF vs. STB from Weeks 2 to 48 along with lower rates of graded abnormalities. Correspondingly, the decreases from baseline in median $eGFR_{CG}$ were statistically significant smaller with E/C/F/TAF vs. STB through the same period. Other renal biomarkers suggested no difference between groups or favoured the E/C/F/TAF group.

In patients who switched from a TDF-based regimen to E/C/F/TAF there was effectively no change from baseline in mean serum creatinine to Week 48 compared to a small increase in those who continued with FTC/TDF+3rd Agent. In patients who switched to E/C/F/TAF there were minimal changes in $eGFR_{CG}$ that varied by prior regimen. Other renal biomarkers generally favoured switching to E/C/F/TAF.

The detailed assessments of renal function in the study in patients with baseline $eGFR_{CG}$ in the range 30-69 mL/min support a conclusion that E/C/F/TAF has an acceptable safety profile in terms of renal effects.

Thus far there have not been any cases of PRT or Fanconi's syndrome in patients treated with E/C/F/TAF.

- Fasting lipids

In the Phase 3 studies in previously ART-naïve patients E/C/F/TAF was associated with higher rates of abnormal fasting lipids, including Grade 3 and 4 abnormalities, than STB. Similarly, higher rates of abnormal fasting lipids were observed in those who switched to E/C/F/TAF vs. those who maintained a TDF-containing regimen. The categorical changes from baseline in NCEP ATP III lipid classifications are consistent with rates observed with ABC and other non-TDF-based regimens in ART-naive patients. The difference between TAF and TDF-containing regimens likely reflects the known lipid-lowering effect of TFV and the much lower plasma levels of TFV in those given E/C/F/TAF.

- Uric acid

Taking into account the metabolic pathway, it should be noted that there was no excess of Grade 3 or 4 hyperuricaemia with E/C/F/TAF in the ART-naïve Phase 3 studies and only a slightly higher rate of hyperuricaemia of any Grade. For mean and median uric acid there was effectively no change from baseline to Week 48 in the E/C/F/TAF group and a small decrease in the STB group.

Grade 3 or 4 hyperuricaemia occurred in 2 E/C/F/TAF and no non-switch patients in 0109. Also, any grade hyperuricaemia occurred in 13.2% vs. 5.0% although no AEs were related to abnormal uric acid. There were also four patients with renal impairment who had Grade 3 or 4 hyperuricaemia in 0112 after switching to E/C/F/TAF, two in each $eGFR_{CG}$ sub-group. There was no change from baseline in the mean and median uric acid in those with values ≤ 50 mL/min and modest increases in the group with > 50 mL/min. These four patients had raised uric acid at baseline.

Despite the laboratory data AEs that could be due to hyperuricaemia were not observed.

Safety in adolescents

The applicant proposed use of E/C/F/TAF in patients aged from 12 years and weighing at least 35 kg. The number of adolescents exposed is small but the PK analyses suggest similar exposure and the safety profile appears mostly comparable with that in adults in terms of the range of AEs reported. BMD Z-scores did not change notably from baseline through Week 24, indicating that bone mineralisation occurred at rates consistent with those of the reference population. The median change from baseline in eGFR has been ascribed to COBI.

From the safety database all the adverse reactions reported in clinical trials have been included in the Summary of Product Characteristics.

Assessment of paediatric data on clinical safety

From the safety database all the adverse reactions reported in clinical trials have been included in the Summary of Product Characteristics.

2.6.2. Conclusions on the clinical safety

The safety profile of E/C/F/TAF is similar to that of STB. The rates of fractures were low and comparable but fewer patients in the E/C/F/TAF group had a > 3% decrease from baseline in hip or spine BMD, noting that decreases did still occur in the 48 weeks after starting treatment with E/C/F/TAF. In the switch study fractures were reported in 1.5% E/C/F/TAF vs. 0.6% FTC/TDF+3rd Agent groups. At Week 48 there were increases from baseline in mean BMD at the hip or spine in the E/C/F/TAF group compared to no appreciable change in those who did not switch. The FRAX analysis also suggested benefit for E/C/F/TAF in the naïve as well as in the switch study. Overall, the renal safety profile of TAF appears to be better than that of TDF, which may be ascribed to the lower plasma TFV levels. Higher rates of abnormal fasting lipids were observed with E/C/F/TAF compared to STB. The difference between TAF and TDF-containing regimens likely reflects the known lipid lowering effect of TFV and the much lower plasma levels of TFV in those given E/C/F/TAF. Overall, the benefits in terms of renal and bone effects appear to outweigh any concerns there may be regarding the lipid profile. Hyperuricaemia has occurred at higher rates with E/C/F/TAF and this issue will be kept under monitoring as described in the RMP.

Finally, current data do not suggest that the nonclinical findings translate into a concern regarding the ocular safety of TAF. However, this issue will be monitored in the RMP.

2.7. Risk Management Plan

The CHMP received the following PRAC opinion on the submitted Risk Management Plan:

The PRAC considered that the risk management plan version 1.0 is acceptable with the following content:

Safety concerns

	Safety Concerns for E/C/F/TAF	**Attributable Component(s) of E/C/F/TAF**
Important Identified Risks	Post-treatment hepatic flares in HIV/HBV coinfected patients	FTC, TAF

Important Potential Risks	Suicidal ideation/suicide attempt in patients with a pre-existing history of depression or psychiatric illness	EVG
	Renal toxicity	TAF
	Bone events due to potential proximal renal tubulopathy/loss of BMD	TAF
	Ocular effects (posterior uveitis)	TAF
	Lipoatrophy	FTC, TAF
	Concurrent use of drugs whose coadministration with E/C/F/TAF is contraindicated	EVG, COBI
	Overdose of tenofovir occurring through accidental concurrent use of E/C/F/TAF with a TDF-containing product	TAF
Missing Information	Long-term safety information in adults and adolescents	E/C/F/TAF (as a STR)
	Safety in children aged 6 to < 12 years	EVG, COBI, TAF
	Safety in pregnancy and lactation	EVG, COBI, FTC, TAF
	Safety in patients with severe renal impairment	COBI, FTC, TAF
	Safety in patients with severe hepatic impairment (CPT score C)	EVG, COBI, TAF
	Safety in patients with cardiac conduction disorders	COBI
	Safety in patients with HCV coinfection	TAF
	Development of drug resistance in long term use	E/C/F/TAF (as a STR)
	Drug-drug interactions	COBI, TAF

Pharmacovigilance plan

Ongoing and planned studies in the PhV development plan

Study/Title	**Objectives**	**Safety Concerns Addressed**	**Status (Planned, Started)**	**Date for Submission of Interim or Final Reports (Planned or Actual)**
Interventional clinical studies (Category 3)				
Study GS-US-292-0104 A Phase 3, Randomized, Double-Blind Study to Evaluate the Safety and Efficacy of Elvitegravir/Cobicistat/ Emtricitabine/Tenofovir Alafenamide Versus Elvitegravir/Cobicistat/ Emtricitabine/Tenofovir Disoproxil Fumarate in HIV-1 Positive, Antiretroviral Treatment-Naïve Adults	To evaluate the safety of Genvoya versus Stribild® in HIV-1 infected, ARV treatment-naive adults through 96 weeks of treatment	*Important potential risk:* Suicidal ideation/suicide attempt in patients with a pre-existing history of depression or psychiatric illness *Missing information:* Long-term safety information Development of drug resistance in long term use	Started	Interim Week 96 report: Q3 2016 Week 144 report: Q3 2017

Study / Title	Objectives	Safety Concerns Addressed	Status (Planned, Started)	Date for Submission of Interim or Final Reports (Planned or Actual)
Study GS-US-292-0111 A Phase 3, Randomized, Double-Blind Study to Evaluate the Safety and Efficacy of Elvitegravir/Cobicistat/ Emtricitabine/Tenofovir Alafenamide Versus Elvitegravir/Cobicistat/ Emtricitabine/Tenofovir Disoproxil Fumarate in HIV-1 Positive, Antiretroviral Treatment-Naïve Adults	To evaluate the safety of Genvoya versus Stribild® in HIV-1 infected, ARV treatment-naive adults through 96 weeks of treatment	*Important potential risk:* Suicidal ideation/suicide attempt in patients with a pre-existing history of depression or psychiatric illness *Missing information:* Long-term safety information Development of drug resistance in long term use	Started	Interim Week 96 report: Q3 2016 Week 144 report: Q3 2017
Non-interventional studies (Category 3)				
Antiretroviral Pregnancy Registry	To collect information on the risk of birth defects in patients exposed to ARVs, including the components of Genvoya, during pregnancy	*Missing information*: Safety in pregnancy	Started	Interim reports to be included in Genvoya PSURs (DLP and periodicity as described in the List of EU reference dates and frequency of submission of PSURs)
Non-clinical studies (Category 3)				
In vitro study on the potential for significant effects on plasma TFV concentrations upon co-administration of TAF and xanthine oxidase inhibitors	The provide information on the potential for a drug-drug interaction between TAF and xanthine oxidase inhibitors	*Missing information*: Drug-drug interactions	Planned	Final report: Q4 2016

The PRAC, having considered the data submitted, was of the opinion that the proposed post-authorisation PhV development plan is sufficient to identify and characterise the risks of the product.

The PRAC also considered that routine PhV is sufficient to monitor the risks of the product.

Risk minimisation measures

Summary Table of Risk Minimization Measures

Safety Concern	Routine Risk Minimization Measures	Additional Risk Minimization Measures
Important identified risk(s)		
Post-treatment hepatic flares in HIV/HBV coinfected patients	Section 4.4 of the SmPC informs about the risk of exacerbation of hepatitis in HIV-1/HBV coinfected patients following discontinuation of E/C/F/TAF.	None
Important potential risk(s)		
Suicidal ideation/suicide attempt in patients with a pre-existing history of depression or psychiatric illness	None	None

Safety Concern	Routine Risk Minimization Measures	Additional Risk Minimization Measures
Renal toxicity	Section 4.4 of the SmPC informs that a potential risk of nephrotoxicity resulting from chronic exposure to low levels of tenofovir due to dosing with TAF cannot be excluded.	None
Bone events due to potential proximal renal tubulopathy/loss of BMD	None	None
Ocular effects (posterior uveitis)	None	None
Lipoatrophy	None	None
Concurrent use of drugs whose coadministration with E/C/F/TAF is contraindicated	Sections 4.3 and 4.5 of the SmPC provide information on drugs whose coadministration with E/C/F/TAF is contraindicated. The Package Leaflet lists medications that should never be taken with E/C/F/TAF.	None
Overdose of tenofovir occurring through accidental concurrent use of E/C/F/TAF with a TDF-containing product	Section 4.4 (and 4.5) of the SmPC warns that E/C/F/TAF should not be administered concomitantly with medicinal products containing TDF used for the treatment of HBV infection. The Package Leaflet includes TDF in a list of medicines used in treating hepatitis B infection which should not be taken with E/C/F/TAF.	None
Missing information		
Long-term safety information in adults and adolescents	None	None
Safety in children aged 6 to < 12 years	Section 4.2 of the SmPC states that the safety and efficacy of E/C/F/TAF in children younger than 12 years of age, or weighing less than 35 kg, have not yet been established, and that no data are available.	None
Safety in pregnancy and lactation	Section 4.6 of the SmPC provides information on pregnancy in humans for the FTC component and in animals for all components of E/C/F/TAF, and notes that E/C/F/TAF should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus. Section 4.6 of the SmPC also provides information on excretion of FTC in human milk, that it is unknown whether EVG, COBI and TAF are excreted in human milk, and informs that E/C/F/TAF should not be used during breastfeeding.	None
Safety in patients with severe renal impairment	Section 4.2 of the SmPC states that E/C/F/TAF should not be initiated in patients with estimated creatinine clearance < 30 mL/min as there are no data available regarding the use of E/C/F/TAF in this population, and that E/C/F/TAF should be discontinued in patients with estimated creatinine clearance that declines below 30 mL/min during treatment.	None
Safety in patients with severe hepatic impairment (CPT score C)	Section 4.2 of the SmPC informs that E/C/F/TAF is not recommended for use in patients with severe hepatic impairment (Child-Pugh Class C). Section 5.2 of the SmPC states that the effect of severe hepatic impairment on the pharmacokinetics of EVG, COBI or TAF has not been studied, and that the impact of liver impairment on the pharmacokinetics of FTC should be limited.	None
Safety in patients with cardiac	None	None

Safety Concern	Routine Risk Minimization Measures	Additional Risk Minimization Measures
conduction disorders		
Safety in patients with HCV coinfection	Section 4.4 of the SmPC states that the safety and efficacy of E/C/F/TAF have not been established in patients coinfected with HIV-1 and HCV.	None
Development of drug resistance in long term use	None	None
Drug-drug interactions	Section 4.3 of the SmPC provides a list of drugs for which coadministration with E/C/F/TAF is contraindicated. Section 4.5 of the SmPC provides information on interactions that have not been studied, potential effects on drug levels, and recommendations concerning coadministration with E/C/F/TAF.	None

The PRAC, having considered the data submitted, was of the opinion that the proposed risk minimisation measures are sufficient to minimise the risks of the product in the proposed indication.

The CHMP endorsed the Risk Management Plan version 1.0 without changes.

2.8. Pharmacovigilance

Pharmacovigilance system

The CHMP considered that the pharmacovigilance system summary submitted by the applicant fulfils the requirements of Article 8(3) of Directive 2001/83/EC.

2.9. Product information

2.9.1. User consultation

The results of the user consultation with target patient groups on the package leaflet submitted by the applicant show that the package leaflet meets the criteria for readability as set out in the *Guideline on the readability of the label and package leaflet of medicinal products for human use.*

2.9.2. Additional monitoring

Pursuant to Article 23(1) of Regulation No (EU) 726/2004, Genvoya (elvitegravir / cobicistat / emtricitabine / tenofovir alafenamide) is included in the additional monitoring list as it contains a new active substance.

Therefore the summary of product characteristics and the package leaflet includes a statement that this medicinal product is subject to additional monitoring and that this will allow quick identification of new safety information. The statement is preceded by an inverted equilateral black triangle.

3. Benefit-Risk Balance

Benefits

Beneficial effects

TAF was selected for development specifically because it had potential to be active with much lower TFV plasma levels and hence to improve safety vs. TDF. Thus selection of the TAF dose could not be based on the simple strategy of matching plasma profiles of TFV achieved with TAF vs. those observed with TDF. The critical data to support the dose in the FDC come from the monotherapy studies (with TAF doses ranging from 8 to 150 mg), the Emax modelling and the PK data that supported adjusting the dose in the FDC from 25 mg to 10 mg.

The selection of the 10 mg dose in the FDC is supported by the Phase 3 studies vs. STB in which percentages with < 50 and < 20 copies/mL at Week 48 using the FDA snapshot algorithm gave very high response rates that were comparable between treatments (difference in percentages: 2.0%; 95% CI: -0.7% to 4.7%). These data, along with all the sensitivity analyses conducted, consistently support a conclusion of comparability between treatments.

Uncertainty in the knowledge about the beneficial effects

The available data from extended follow-up in some studies further support lack of imbalance in rates of viral rebound associated with mutational resistance. Nevertheless, longer term usage is needed to better define the risk.

The Phase 2/3 studies in ART-naïve patients were all conducted vs. STB. The potential merits of using an alternative comparative regimen that is commonly used for first line treatment can be debated. Nevertheless, in this case a major aim was to compare the safety profiles of TDF and TAF and therefore selection of STB was rational.

In Phase 2 there was a suggestion that STB might be better in those with the highest baseline viral loads and lowest CD4 counts. One Phase 3 study essentially showed no difference between treatments whereas the other showed that point estimates were lower with E/C/F/TAF in these subgroups, even though the CI overlapped. Since patients were documented to have susceptible virus at baseline there is no obvious explanation and the findings could have occurred by chance. Nevertheless, the results are shown in the SmPC.

Female patients accounted for ~15% of the total in Phase 3 and showed results comparable to those for men, with no appreciable difference between treatments. Also, 96% of patients had HIV-1 B so that although there was no sign of reduced efficacy against other types no definite conclusions can be drawn.

STB, EVG and COBI are not approved for use in adolescents and use of TDF is restricted because of safety concerns in growing adolescents, including effects on bone mineralisation. POPPK analyses using data from GS-US-292-0106 suggested comparable plasma exposures to TAF and TFV in adults and adolescents (n=24). The efficacy data come from 50 adolescents and most were black, noting that ART-naïve black adults generally responded slightly better than other ethnic groups in Phase 3. Also, 58.3% were female and the median age was 15 years (range 12 to 17). The data suggest that, as predicted by the PK analyses, virological responses to treatment are similar to those in adults. However, with such limited data, specific monitoring of use in adolescents is recommended in the RMP.

Risks

Unfavourable effects

The safety profile of E/C/F/TAF was mostly very similar to that of STB. Rates for individual types of AEs, including Grade 3/4 and drug-related AEs, were comparable or slightly lower vs. STB. In the two Phase 3 studies the rates of fractures were low and comparable but fewer patients in the E/C/F/TAF group had a > 3% decrease from baseline in hip or spine BMD, noting nonetheless that such decreases did still occur in the 48 weeks after starting treatment with E/C/F/TAF. The FRAX analysis also suggested a benefit for E/C/F/TAF over STB as did various biomarkers for effects on bone.

On switching from TDF to TAF it could be expected that AE rates could be higher compared to those who remained on regimens that were previously tolerated. The difference in rate of AEs considered to be drug-related by investigators after switching may have been affected by the open-label design. The difference seems to have been driven by gastro-intestinal AEs and there were few Grade 3 or 4 events. Fractures were reported in 1.5% E/C/F/TAF vs. 0.6% FTC/TDF+3rd Agent groups but at Week 48 there were increases from baseline in mean BMD at the hip or spine in the E/C/F/TAF group compared to no appreciable change in those who did not switch. The FRAX analysis also suggested some benefit from switching.

Overall, the renal safety profile of TAF appears to be better than that of TDF, which may be ascribed to the lower plasma TFV levels.

In the Phase 3 studies in previously ART-naïve patients E/C/F/TAF was associated with higher rates of abnormal fasting lipids, including Grade 3 and 4 abnormalities, than STB. Similarly, higher rates of abnormal fasting lipids were observed in those who switched to E/C/F/TAF vs. those who maintained a TDF-containing regimen. The difference between TAF and TDF-containing regimens likely reflects the known lipid lowering effect of TFV and the much lower plasma levels of TFV in those given E/C/F/TAF. Overall, the benefits in terms of renal and bone effects appear to outweigh any concerns there may be regarding the lipid profile.

Uncertainty in the knowledge about the unfavourable effects

Thus far there have not been any cases of PRT or Fanconi's syndrome in patients treated with E/C/F/TAF. It remains to be seen whether very long term exposure to low plasma levels of TFV will be associated with tubulopathy.

Hyperuricaemia has occurred at higher rates with E/C/F/TAF although no AEs were related to abnormal uric acid. This issue will be kept under monitoring.

Current data do not suggest that the nonclinical findings translate into a concern regarding the ocular safety of TAF. However, this issue is to be monitored as an important potential risk in the RMP.

The applicant proposes use of E/C/F/TAF in patients aged from 12 years and weighing at least 35 kg. The number of adolescents exposed is small but the PK analyses suggest similar exposure and the safety profile appears mostly comparable with that in adults in terms of the range of AEs reported. MD Z-scores did not change notably from baseline through Week 24, indicating that bone mineralisation occurred at rates consistent with those of the reference population. Use in adolescents is supported but it is recommended that special attention is paid to safety in this population in the post-authorisation phase.

Benefit-risk balance

Importance of favourable and unfavourable effects

E/C/F/TAF has been shown to achieve high virologic suppression rates in the ART-naïve and to support maintenance of suppression after switching from successful regimens. The safety profile for the most part is similar or improved vs. that of STB, especially with evidence of lesser negative effects on renal function and bone mineralisation density. The fact that lipid abnormalities are more likely to occur with TAF then TDF does not impact on the overall conclusions on safety since the rates observed are in line with those that occur with other commonly used ART regimens.

Benefit-risk balance

Discussion on the benefit-risk balance

The fixed dose combination EVG/COBI/FTC/TAF offers a new single tablet treatment option with a high efficacy. Single tablet regimens are known to support patient's acceptance and compliance. The improved renal safety profile allows the administration in patients with estimated creatinine clearance $\geq$ 30 ml/min and no dose adjustments are required. However the potential risk of nephrotoxicity from chronic exposure cannot be excluded and deserves to be monitored. Based on the evaluation of quality, efficacy and safety data the benefit-risk balance is favourable.

4. Recommendations

Outcome

Based on the CHMP review of data on quality, safety and efficacy, the CHMP considers by consensus decision that the risk-benefit balance of Genvoya in the treatment of adults and adolescents (aged 12 years and older with body weight at least 35 kg) infected with human immunodeficiency virus 1 (HIV 1) without any known mutations associated with resistance to the integrase inhibitor class, emtricitabine or tenofovir is favourable and therefore recommends the granting of the marketing authorisation subject to the following conditions:

Conditions or restrictions regarding supply and use

Medicinal product subject to restricted medical prescription (see Annex I: Summary of Product Characteristics, section 4.2).

Conditions and requirements of the Marketing Authorisation

- **Periodic Safety Update Reports**

The requirements for submission of periodic safety update reports for this medicinal product are set out in the list of Union reference dates (EURD list) provided for under Article 107c(7) of Directive 2001/83/EC and any subsequent updates published on the European medicines web-portal.
The marketing authorisation holder shall submit the first periodic safety update report for this product within 6 months following authorisation.

Conditions or restrictions with regard to the safe and effective use of the medicinal product

- **Risk Management Plan (RMP)**

The MAH shall perform the required pharmacovigilance activities and interventions detailed in the agreed RMP presented in Module 1.8.2 of the Marketing Authorisation and any agreed subsequent updates of the RMP.

An updated RMP should be submitted:

- At the request of the European Medicines Agency;
- Whenever the risk management system is modified, especially as the result of new information being received that may lead to a significant change to the benefit/risk profile or as the result of an important (pharmacovigilance or risk minimisation) milestone being reached.

Conditions or restrictions with regard to the safe and effective use of the medicinal product to be implemented by the Member States

Not applicable.

New Active Substance Status

Based on the CHMP review of data on the non-clinical and clinical properties of the active substance, the CHMP considers that tenofovir alafenamide which is a derivative of tenofovir disoproxil (both prodrugs of tenofovir) is qualified as a new active substance as it differs significantly in properties with regard to safety and efficacy from the previously authorised substance.

Paediatric Data

Furthermore, the CHMP reviewed the available paediatric data of studies subject to the agreed Paediatric Investigation Plan P/0026/2014 and the results of the only available study (GS US 292 0106) is reflected in the Summary of Product Characteristics (SmPC) and, as appropriate, the Package Leaflet.