25 February 2016
EMA/192941/2016
Committee for Medicinal Products for Human Use (CHMP)

Assessment report

Descovy

International non-proprietary name: emtricitabine / tenofovir alafenamide

Procedure No. EMEA/H/C/004094/0000

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
CHMP	Committee for Medicinal Products for Human use
COBI, C	cobicistat (Tybost)
CPP	Critical process parameter
CQA	Critical quality attribute
C telopeptide	type I collagen C telopeptide
ddI	didanosine
dNTP	2' deoxynucleoside triphosphate
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)
EC	European Commission
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
HPLC-MS	High performance liquid chromatography-mass spectrometry
ICH	International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
INSTI	integrase strand transfer inhibitor
IPC	In-process control
ICP-MS	Inductively coupled plasma-mass spectrometry
IR	Infrared
KF	Karl Fischer titration
LDL	low-density lipoprotein
LOCF	last observation carried forward
LSM	least-squares mean
M = F	missing = failure
MAH	Marketing authorisation holder
MCC	Micorcrystalline cellulose
mtDNA	mitochondrial DNA
N or n	number of subjects in a population (N) or subset (n)
NAS	New active substance
NCEP	National cholesterol education programme
NMR	Nuclear magnetic resonance
NMT	Not more than
NNRTI	nonnucleoside reverse transcriptase inhibitor
NOR	Normal operating range
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)
PE	Polyethylene
P-gp	P-glycoprotein
Ph. Eur.	European Pharmacopoeia
PI	protease inhibitor
PP	Polypropylene
PRT	proximal renal tubulopathy
PSP	Paediatric Study Plan
PTH	parathyroid hormone
Q1, Q3	first quartile, third quartile
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 analog mutation
TDF	tenofovir disoproxil fumarate (Viread)
TFV	tenofovir
TFV DP	tenofovir diphosphate
TVD	emtricitabine/tenofovir disoproxil fumarate (coformulated; Truvada)

UACR	urine albumin to creatinine ratio
UGT	uridine diphosphate glucuronosyltransferase
UPCR	urine protein to creatinine ratio
USP	United States Pharmacopoeia
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 21 April 2015 an application for Marketing Authorisation to the European Medicines Agency (EMA) for Descovy, 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 25 September 2014.

The applicant applied for the following indication: Descovy is indicated in combination with other antiretroviral agents for the treatment of human immunodeficiency virus type 1 (HIV-1) infection in adults without any known mutations associated with resistance to the individual components of Descovy.

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 submission 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 of Regulation (EC) No 1901/2006, the application included an EMA Decision P/0032/2015 on the agreement of a paediatric investigation plan (PIP).

At the time of submission of the application, the PIP P/0032/2015 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.

Applicant's request for consideration

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 itself, as the applicant claims that it is not a constituent of a

product previously authorised within the Union.

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 and 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: Joseph Emmerich

- The application was received by the EMA on 21 April 2015.
- The procedure started on 28 May 2015.
- The Rapporteur's first Assessment Report was circulated to all CHMP members on 03 August 2016. The Co-Rapporteur's first Assessment Report was circulated to all CHMP members on 19 August 2015.
- The PRAC Rapporteur's RMP Assessment Report was circulated to all CHMP and PRAC members on 31 August 2016.
- During the meeting on 24 September 2015, the CHMP agreed on the consolidated List of Questions to be sent to the applicant. The final consolidated List of Questions was sent to the applicant on 25 September 2015.
- The applicant submitted the responses to the CHMP consolidated List of Questions on 16 October 2015.
- The Rapporteurs circulated the Joint Assessment Report on the applicant's responses to the List of Questions to all CHMP members on 16 November 2015.
- The Updated PRAC RMP Assessment Report was circulated to all CHMP and PRAC members on 03 December 2015.
- The Updated Joint Assessment Report was circulated to all CHMP members on 10 December 2015.
- During the CHMP meeting on 17 December 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 26 January 2016.
- The Rapporteurs circulated the Joint Assessment Report on the applicant's responses to the List of outstanding issues to all CHMP members on 08 February 2016.
- The Rapporteurs circulated the updated Joint Assessment Report on the applicant's responses to the List of outstanding issues to all CHMP members on 18 February 2016.
- During a meeting of a SAG Working Party on 15 February 2016, experts were convened to address

questions raised by the CHMP.

- During the meeting on 25 February 2016, 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 Descovy.

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 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.

The Applicant has co-formulated TAF (as 11 mg or 28 mg of the fumarate) in FDC tablets of two strengths, each with 200 mg emtricitabine (FTC; Emtriva), which is a NRTI approved for treatment of HIV-1 infection in adults and children (generally 3 months of age or older).

2.2. Quality aspects

2.2.1. Introduction

The finished product is presented as film-coated tablets containing 200 mg of emtricitabine (FTC) and either 10 or 25 mg of tenofovir alafenamide (as fumarate - TAF) as active substances.

Other ingredients are:

Tablet core: microcrystalline cellulose, croscarmellose sodium and magnesium stearate

Film-coating: polyvinyl alcohol, titanium dioxide, macrogol 3350, talc and iron oxide black (E172)

The product is available in high density polyethylene (HDPE) bottles with polypropylene (PP) continuous-threads, child-resistant caps and lined with an induction activated aluminium foil liners. Each bottle contains silica gel desiccant and a polyester coil as described in section 6.5 of the SmPC.

2.2.2. Active Substance

General information

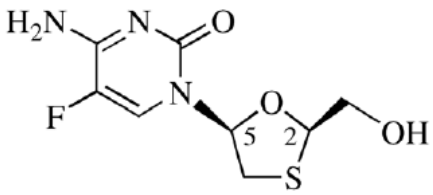
Emtricitabine (FTC)

The information on chemistry, manufacturing and control of emtricitabine (FTC) active substance has been previously assessed through centralised procedure and approved in the EU as part of the marketing authorisation applications for Emtriva, Truvada, Atripla, Eviplera and Stribild.

The Module 3.2.S sections of the dossier for FTC 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 FTC is 4-amino-5-fluoro-1-[(2*R*,5*S*)-2-(hydroxymethyl)-1,3-oxathiolan-5-yl]-1,2-dihydropyrimidin-2-one and has the following structure:



The structure of the active substance was elucidated by a combination of ^{1}H and ^{13}C NMR spectroscopy, IR spectroscopy, UV spectroscopy, mass spectrometry and single crystal x-ray determination.

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

Manufacture, characterisation and process controls

FTC 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 FTC enantiomer. Five manufacturing sites are involved. Adequate in-process controls (IPCs) 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 FTC that meets the required quality standards.

The active substance is packaged in double polyethylene (PE) bags inside HDPE drums which comply with the EC directives 2002/72/EC and EC 10/2011 as amended.

Specification

FTC 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 FTC. 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, microbial testing of the active substance is not required. Satisfactory information regarding the reference standards used for assay testing has been presented.

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 FTC 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 (25 ºC/60% RH) for up to 36 months. Of the above batches, eight commercial scale and five pilot batches were stored under accelerated conditions (40 ºC/75% RH) for up to 6 months. In addition another three batches were stored under intermediate conditions (30 ºC/65% RH) for up to 12 months. Samples were tested for appearance, impurities, assay, water content and enantiomeric purity by validated stability indicating methods. Stability data for FTC manufactured by both synthetic routes was comparable. The majority of tested parameters remained within the specification limits throughout the study 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 FTC stored under accelerated conditions. Four batches stored under accelerated conditions exceeded the specification limit at 6 months. These data indicate that FTC should not be exposed to elevated temperatures for extended periods of time.

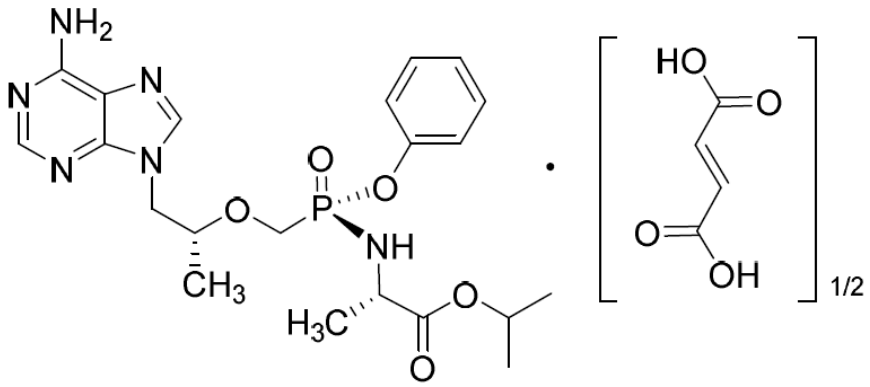
A photostability study was conducted on one batch of FTC. The results showed no significant changes in appearance, purity, and impurity content and indicate that FTC is not sensitive to light.

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

Tenofovir alafenamide fumarate (TAF fumarate)

General information

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



The chemical structure of TAF fumarate has been adequately demonstrated by infrared spectroscopy, nuclear magnetic resonance spectroscopy (^{1}H, ^{13}C, and ^{31}P), 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. TAF fumarate is a BCS Class III compound, with pH-dependent aqueous. solubility decreasing with increasing basicity. 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. TAF 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 has the *S*-configuration at the alpha-carbon. The remaining stereocentre is located at the phosphorus atom and is in the *S*-onfiguration. Enantiomeric purity is monitored routinely by chiral HPLC.

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

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

Manufacture, characterisation and process controls

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

During the evaluation procedure, the active substance starting materials were re-defined to ensure enough of the process is documented in the dossier in line with ICH Q11. Commercially available well-defined starting materials with acceptable specifications are used.

Adequate IPCs are applied during the synthesis. The specifications and control methods for intermediate products, starting materials and reagents have been presented. Potential and actual impurities were well discussed with regards to their origin and characterised. Critical process parameters were identified using a risk assessment approach.

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 is packaged in double-lined PE bags which comply with the EC directive 2002/72/EC and EC 10/2011 as amended. The bags are held in HDPE drums (or other suitable secondary container) with lids of appropriate size and fitted with a security seal.

The characterisation of the active substance and its impurities is 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.). The active substance specifications are based on the active substance critical quality attributes (CQAs).

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. Satisfactory information regarding the reference standard used for assay testing has been presented.

Batch analysis data (n=16 using the proposed commercial process; 13 of which were production 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.

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 (5 ºC) and for up to 24 months under accelerated conditions (25 ºC / 60% RH) according to the ICH guidelines were provided. Results under stressed 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.

Samples were tested for appearance, impurities, assay, water content, and solid state characteristics (XRD and melting point). 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 ICH guideline Q1B was performed on one batch, indicating 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 condition in the proposed container.

2.2.3. Finished Medicinal Product

Description of the product and Pharmaceutical development

Descovy tablets are available in 2 strengths. The 200 mg FTC / 10 mg TAF tablet is grey, rectangular and debossed with 210 on one side and GSI on the other. The 200 mg FTC / 25 mg TAF tablet is blue, rectangular and debossed with 225 on one side and GSI on the other. The tablets are the same size and weight.

The aim of development was to formulate a single tablet containing the required amounts of the two active substances. FTC is a BCS Class I compound with high aqueous solubility across the physiological pH range and high permeability. It hydrolyses in aqueous solution and to a small extent under warm moist conditions. TAF fumarate is a BCS Class III compound with high aqueous solubility and low permeability. The active substances were shown to be mutually compatible and stable in combination with excipients in stability studies. The choice of manufacturing process and excipients was based on the properties of the active substances.

All excipients are well known pharmaceutical ingredients and their quality is compliant with Ph. Eur. or other relevant standards. 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 2.2.1 of this report.

The formulation evolved throughout development. The commercial formulation is the same as that used in phase III clinical trials which was shown to be equivalent to earlier clinical formulations by suitable bridging studies.

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.

Both active substances dissolve rapidly from the formulated tablet across the physiological pH range. Various manufacturing parameters with potential to impact the dissolution rate were examined during development and have been set at levels guaranteed to produce Descovy tablets of the required quality. The dissolution method was developed in order to identify tablets manufactured outside of the defined process and its discriminatory power is considered adequate.

The primary packaging is an HDPE bottle with PP continuous-thread, child-resistant cap and lined with an induction activated aluminium foil liner. Each bottle contains silica gel desiccant and a polyester coil. The materials comply 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 six main steps: blending of excipients followed by lubrication; roller compaction; milling and lubrication of granules; compression; film-coating; packaging. The process is considered to be a standard manufacturing process.

Major steps of the manufacturing process have been validated by a number of studies. It has been demonstrated that the manufacturing process is capable of producing the finished product of intended quality in a reproducible manner. Full validation will be carried out on commercial scale for 3 batches of each tablet strength prior to release to the market and a suitable process validation scheme has been provided. The control strategy consists of a series of input material attributes, PARs, NORs and set-points for unit operations, and IPCs. The IPCs are adequate for this type of manufacturing process. Adequate justification for holding times of bulk intermediates has been provided and is discussed in the stability section.

Product specification

The finished product release specifications are appropriate for this kind of dosage form and include tests for appearance, identification (UV, HPLC), water content (KF), assay (HPLC), degradation products (HPLC), uniformity of dosage units (Ph. Eur.), dissolution (Ph. Eur.) and microbiological quality (Ph. Eur.). Limits for specific impurities are set in line with manufacturing batch data and levels qualified clinically, in line with ICH Q3B(R2).The CHMP considers that the dissolution specification is wide compared to the batch results available to date and the applicant is recommended to tighten these if appropriate once additional commercial scale manufacturing experience has been gained.

The analytical methods used have been adequately described and appropriately validated in accordance with the ICH guidelines. Satisfactory information regarding the reference standards used for assay and impurities testing has been presented.

Batch analysis results are provided for five production scale batches confirming the consistency of the manufacturing process and its ability to manufacture to the intended product specification.

Stability of the product

Stability data on three production scale batches of each strength stored for up to 18 months under long term conditions (25 °C / 60% RH), for up to 18 months under intermediate conditions (30 °C / 75% RH), and for up to 6 months under accelerated conditions (40 °C / 75% RH) in line with the ICH guidelines was provided. The batches of finished product are identical to and packaged in the same primary packaging as those proposed for marketing. Samples were tested for appearance, strength, degradation products, dissolution, water content and microbiological quality. All attributes were within their specification limits throughout the study period. No trends were observed for any of the measured parameters except for TAF strength, assay and degradation products. An increase in TAF degradation products was observed, larger in the 200/10 mg tablets than the 200/25, and more significant as storage temperature and humidity increase. This is accompanied by a related drop in TAF assay and TAF strength. No degradation of the FTC component was noted under any condition.

In addition, one batch of each strength was exposed to light as defined in the ICH Guideline on Photostability Testing of New Drug Substances and Products. No changes to any of the measured parameters were observed, save for a slight increase in water as the tablets were exposed unprotected from atmospheric moisture.

Stressed studies were carried out to evaluate conditions which may be experienced during shipping and handling. Studies were conducted on one batch of each strength in both primary and bulk packaging at -20 ^{o}C for 4 days and at 50 or 60 ^{o}C for 7 days. No changes to any of the measured parameters were observed other than a slight increase in TAF degradation products at the higher temperature. All parameters were within their specification limits.

Studies on bulk storage of intermediate products, powder blend, tablet cores, and film-coated tablets under warehouse conditions (20-25 ^{o}C / 30-60% RH) were also carried out. Results support the proposed bulk holding times for powder blend, tablet cores and film-coated tablets.

Based on available stability data, the proposed shelf-life of 24 months without specific storage conditions as stated in the SmPC (section 6.3) is acceptable.

Adventitious agents

No excipients derived from animal or human origin have been used. The magnesium stearate is of vegetable origin.

2.2.4. Discussion on chemical, pharmaceutical and biological aspects

Information on development, manufacture and control of the active substance 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.

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.

2.2.6. Recommendation for future quality development

In the context of the obligation of the MAHs to take due account of technical and scientific progress, the CHMP recommends the following points for investigation:

The dissolution specification should be revised and tightened in line with batch data, if appropriate, once additional commercial scale manufacturing experience has been gained.

2.3. Non-clinical aspects

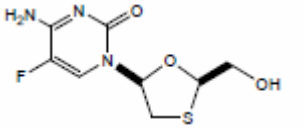
2.3.1. Introduction

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.

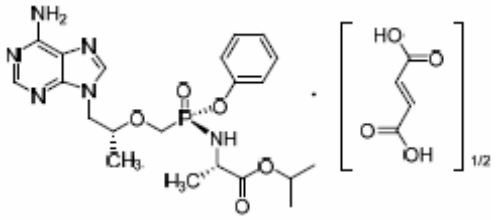
Physical chemistry

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. Stereo isomer - GS-7339

2.3.2. Pharmacology

Primary pharmacodynamic studies

The primary pharmacology to examine the antiviral activity of FTC and TAF have been described in the virology summary section in the dossier and so the following is only discussed briefly in respect to the relevant non-clinical aspects. Considering there have been extensive previous reviews for each component and a similar product known as Stilbild (STB) where TAF is substituted for TDF only a limited review for primary pharmacology for the TAF component has been undertaken.

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:

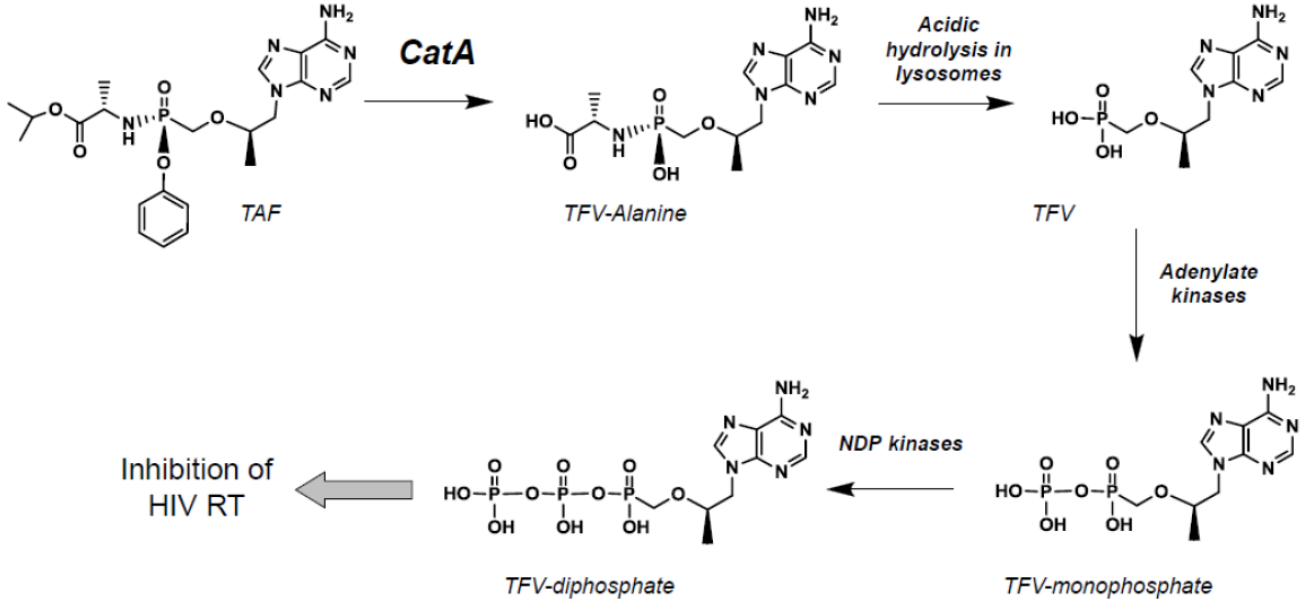
Tenofovir alafenamide is hydrolysed to tenofovir (TFV) by the lysosomal carboxypeptidase, Cathepsin A (CatA). To investigate the intracellular activity of TAF in lymphoid cells and tissues, $CD4^+$ T lymphocytes and monocyte-derived macrophage (MDMs) were isolated from PBMCs from viable donors (Study No. PC-120-2017) and extent of CatA activity determined by measuring the rate of conversion of TAF to TFV-alanine (Figure 1). 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,

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 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).

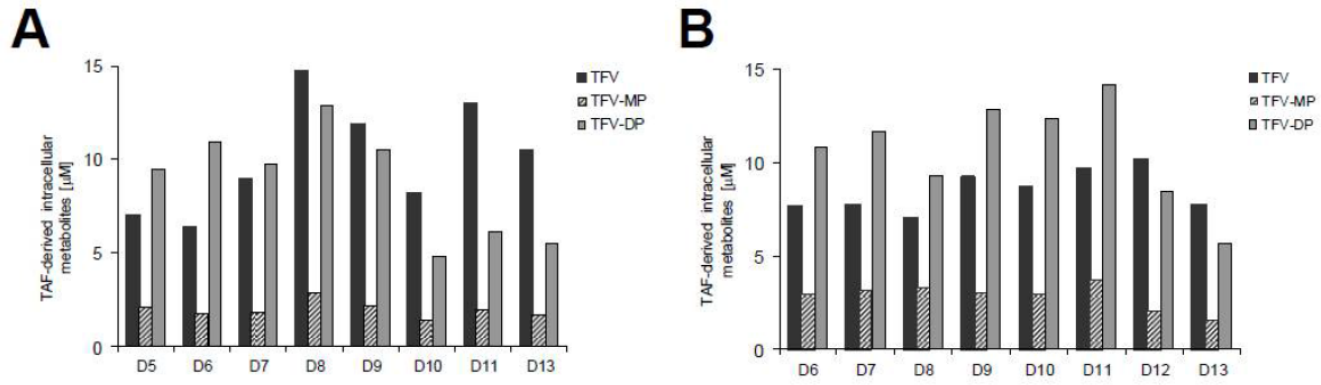
In MT-2 cells, TAF shows low cytotoxicity with a selectivity index (SI) of > 10,000). Based on data generated with the parent nucleotide TFV, TAF is expected to be active against a wide range of HIV-1 subtypes and also against HIV-2. In addition, TAF is a potent inhibitor of HBV replication, exhibiting *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



D= Donor; TAF = tenofovir alafenamide; TFV = tenofovir; TFV-MP = tenofovir monophosphate; TFV-DP = tenofovir diphosphate

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 has also been completed to evaluate 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 Cmax 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 (Cmax) levels observed in patients.

No *in vivo* work with TAF was conducted however this has been 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.

FTC / TAF

No additional studies for the F/TAF FDC were conducted in animal models in view of the extensive clinical experience with the use of FTC, and TDF, FTC/TDF containing regimens, and the E/C/F/TAF FDC for the treatment of HIV-1 infection.

Secondary pharmacodynamic studies

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

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 (CC50 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 (CC50 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 CC50 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 CC50 in renal HEK293 cells expressing OAT1 or OAT3 relative to EC50 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 or with other combinations, no additional secondary pharmacodynamic studies have been conducted for the F/TAF combination.

Safety pharmacology programme

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

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 at 200 mg.

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 post-dose. 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 post-dose. 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 IC50 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 doses 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.

Combination

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.

Pharmacodynamic drug interactions

2.3.3. Pharmacokinetics

Pharmacokinetic studies

The absorption, distribution, metabolism, and excretion of 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 FTC/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 FTC/TAF combination.

Methods of analysis

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.

FTC/TAF: *In vitro* transporter methods were developed to measure the impact of 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

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 (Table 3.2.1). Gender differences in plasma TFV levels were less than 2-fold in Cmax and AUC0-t values (Table 3.2.2). The pharmacokinetic profiles for the 2 different fumarate forms of TAF were observed to be generally similar.

Table 1. 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 2. 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 Cmax 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 AUC0-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 Cmax and AUC0-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 (Table 3). 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 3. 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 C_{max} 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 t_{max} 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, t_{max} 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 posdose.

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 (C_{max}) of 1.47 and 2.12 µM on Day 1 and 7, respectively. The pharmacologically active diphosphate metabolite, TFV-DP, was efficiently formed in dog livers achieving concentrations of 242 and 153 µM at 4.0 and 24 hours post-dose 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, C_{max} 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). he 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 t_{max} values of approximately 0.5 and 1 hour, respectively (Table 4). 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 5).

Table 4. 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 5. 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.

FTC / TAF:

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

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

Distribution

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 [14C]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 [14C]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 [14C]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 nonpigmented 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.

FTC / TAF:

Drug interactions, within the combination, that affect distribution would not be expected from the data available. Interactions through binding displacement would not be anticipated.

Metabolism

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.

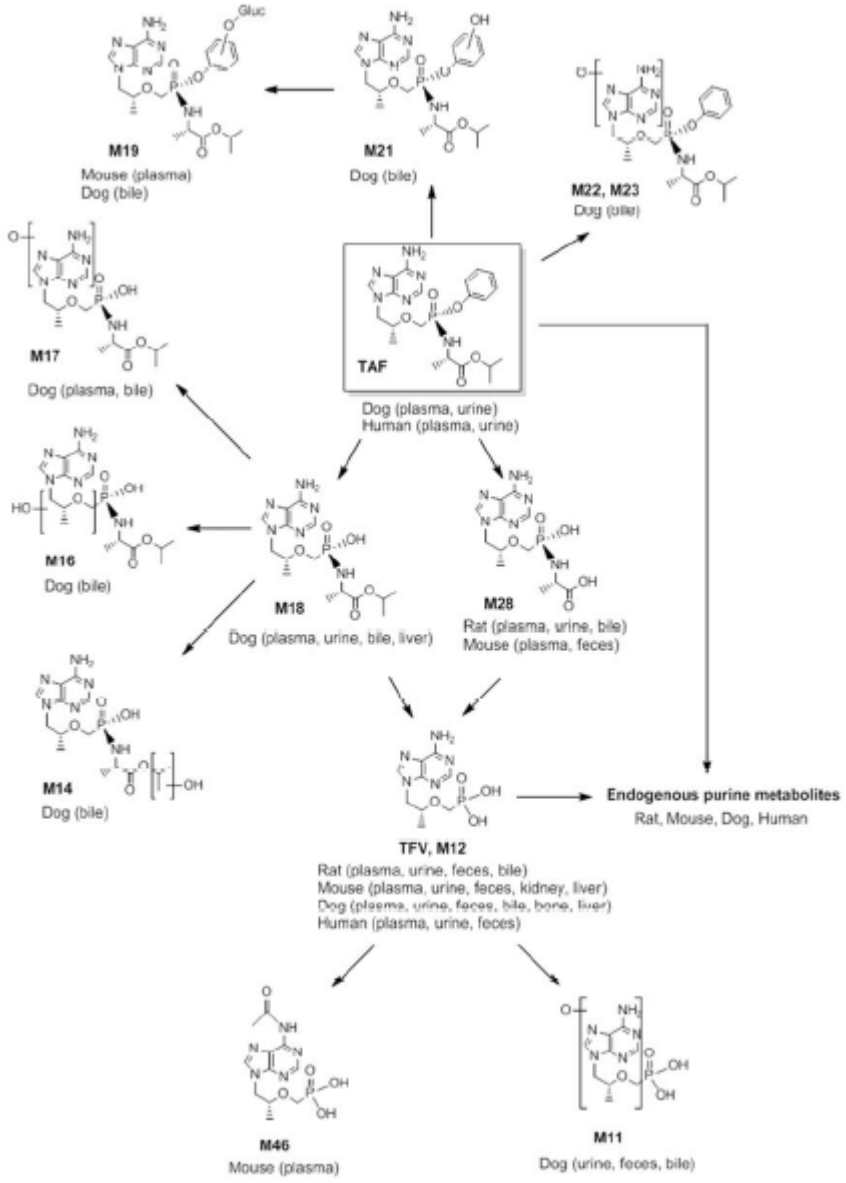
FTC was not extensively metabolised and is eliminated primarily as unchanged drug by renal excretion in mice, rats, and cynomolgus monkeys. Over 90% of the radioactivity in mouse and rat urine and 64% of the radioactivity in monkey urine was unchanged drug. Only trace levels of metabolites were found 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). 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. 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 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 [14C]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 [14C]TAF (Study No. GS-US-120-0109).

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 [14C]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.

FTC / TAF:

TAF is a weak substrate and inhibitor of CYP3A.

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.

Excretion

FTC:

The primary route of elimination of [3H]FTC and [14C]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 [14C]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 14C]TAF (Study No. AD-120-2020). [14C]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 [14C]TAF was determined after administration of a single 15-mg/kg oral dose of 14C-TAF to bile duct-intact and BDC male dogs (Study No. AD-120-2007). [14C]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 [14C]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 [14C]TAF was determined following oral administration of a single 15-mg/kg dose of [14C]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 [14C]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.

FTC / TAF:

FTC and TFV are almost exclusively eliminated by renal excretion.

Pharmacokinetic drug interactions

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 (Tables 3.6.2 and 3.6.3).

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 IC50 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 enzymes 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 (IC50 > 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 helpatocytes 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 6.

Table 6. Effect of TAF Treatment on CYP mRNA Levels and Activity in Cultured Human Hepatocytes (mean, n = 3 donors)

	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.

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 (IC50 >300 µM) so TFV is unlikely to cause drug interactions through inhibition of these transporters (Study No. AD-104-2012).

FTC/TAF:

In addition to the studies described for the individual component of F/TAF FDC, a number of transporter studies were conducted with the STB components, elvitegravir (EVG), cobicistat (COBI), FTC, and TFV. Since TFV is the major circulating metabolite for both TDF and TAF, these results are relevant to evaluate the potential for transporter-mediated drug-drug interactions among these components.

Emtricitabine, TAF, and TFV do not inhibit any of the transporters tested at clinically relevant concentrations in vitro. Therefore, FTC, TAF, and TFV are unlikely to be a perpetrator 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 as it shows undetectable inhibition of OAT1, OAT3, and MRP4 in vitro.

As F/TAF FDC may be used in combination with boosted ARVs, the potential for drug-drug interactions with a pharmacokinetic enhancer, COBI or ritonavir (RTV), was examined. Neither FTC nor TFV interact with drug metabolising enzymes as substrates, inhibitors, or inducers (oxidative metabolism of FTC plays a minor role in the elimination of the compound) and so is unlikely to influence metabolic drug interactions with COBI or RTV. Cobicistat and RTV were shown to be weak inhibitors of efflux transporters, P-gp and BCRP. Since TAF, but not FTC, is a substrate for both P-gp and BCRP, high concentrations of COBI or RTV achieved briefly in the intestinal lumen can inhibit the intestinal efflux of TAF, increasing absorption. Therefore, a lower TAF dose for the FDC (F/TAF, 200/10 mg) is recommended when used with boosted ARVs. This is adequately addressed in the SmPC.

Cobisistat and RTV showed either weak or undetectable inhibition of OAT1, OAT3, and MRP4 in vitro. Transport of TFV by OAT1, OAT, and MRP4 was not meaningfully inhibited by COBI or RTV under physiological conditions and clinically relevant concentrations. In addition, COBI and RTV had no effect on the accumulation of TFV in human renal tissue slices at clinically relevant concentrations. Since both COBI and RTV are inhibitors and TAF is a substrate of OATP transporters in vitro, the exposure to TAF may be affected by COBI or RTV via inhibition of hepatic uptake. The effects of differences in OATP1B1 and OATP1B3 activity are, however, not expected to be clinically relevant given the high passive permeability of TAF. Due to the highly restricted substrate specificity of the enzymes catalyzing the phosphorylation of FTC and TFV, inhibition of pharmacological activation by COBI or RTV is unlikely.

2.3.4. Toxicology

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).

In agreement with the 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.

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. These data will not be discussed in this report.

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 7. Mean repeat-dose toxicokinetics of TFV following oral administration of GS-7340-02 (monofumarate)

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).

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 8. Mean repeat-dose pharmacokinetics of TFV following oral administration of GS-7340-02

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

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 (Study number TX-120-2003). 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) (Study number D990175) 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 9. 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 at18/12 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 (2x males at 18 mg/kg). One of these was considered to be due to a gavage accident. A different male at 18 mg/kg was killed on Day 45 due to deteriorating clinical condition, which was considered to be treatment related. Prior to necropsy this 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 consisted of 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.

Table 10. Electrocardiograph findings

Daily Dose (mg/kg/day)	0 (Control)		2		6		18/12 [a]	
Gender: Number of Animals	M: 6	F: 6	M: 6	F: 6	M: 6	F: 6	M: 8	F: 8
Electrocardiography [c]								
Heart Rate (beats/min)								
Week 13	117.0	106.0	-	-	-	-	-32.5% [B]	-29.2% [B]
Week 39	111.0	-	-	-	-	-	-49.5% [C]	-
PR (msec)								
Week 13	-	86.7	-	-	-	-	-	+26.2% [B]
Week 39	83.8	87.5	-	-	+28.3% [B]	+25.7%	+31.3% [B]	+41.9% [D]
QT								
Week 13	189.2	200.8	-	-	-	-	+17.2% [B]	+10.2% [B]
Week 39	200.0	217.5	-	-	-	-	+26.3% [C]	+10.7% [A]

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 (no further details are provided in the non-clinical toxicology summary). 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) was 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 Applicant goes onto sat that 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.

Toxicokinetic analysis showed that TAF was rapidly absorbed and converted to TFV following oral dose administration, with peak plasma concentrations of TAF and TFV occurring 0.5 and 1 hour after dosing, respectively. The systemic exposure of TAF was dose dependent. Plasma Cmax and AUC values for GS-7340 increased more than proportionally over the dose range. Plasma Cmax and AUC, increased roughly in

proportion to the administered dose. There was some accumulation of TFV following repeat dosing (approximately 3-fold). There was no sex difference.

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

Table 11. 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 12. 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 C_{max} or AUC indicating no change in clearance over time. There was no sex difference.

Table 13. 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

TAF was shown to be negative in 2 in vitro and one in vivo genotoxicity study.

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

Based on the CHMP scientific advice (EMA/CHMP/SAWP/629722/2012; EMEA/H/SA/2410/1/2012/1), carcinogenicity studies are not required for TAF registration based on the lack of TAF exposure in rats and TgRasH2 mice and lower TFV exposure in rats and mice compared to TDF.

Reproduction Toxicity and Toxicokinetic data

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 foetal 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-Foetal 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. Foetal weights (males, females and sexes combined) were decreased dose dependently and remained within the range of historical control data, however foetal weights at 250 mg/kg/day were at the lower extreme of this range. The incidences of foetal 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 foetal 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-foetal 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.

Oral Embryo-Foetal 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 foetuses, dead foetuses, resorptions, the sex ratio and the pre and post implantation losses were not affected. There was no effect of TAF on foetal 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-foetal development was 100 mg/kg/day (AUC0-t = 11.0 and 27.3 µg·h/mL for TAF and TFV, respectively.

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

FTC

FTC in combination with 1% (by weight) TP-0296 (FTU, degradant), was tested in the Bacterial Reverse Mutation Assay to qualify degradant TP-0296 using *Salmonella typhimurium* tester strains TA98, TA 100, TA 1535, and TA 1537 and *Escherichia coli* tester strain WP2*uvr*A in the presence and absence of S9 metabolic activation. In the initial toxicity-mutation assay, dose levels of FTC tested were 1.5, 5.0, 15, 50, 150, 500, 1500, and 5000 µg/plate, and the highest dose of TP-0296 tested was 50 µg/plate. No toxicity and no

positive mutagenic response were observed at any dose. Based on these findings, the maximum doses plated in the confirmatory mutagenicity assay were 5000 µg/plate for FTC and 50 µg/plate for TP-0296.

In the confirmatory mutagenicity assay, no positive mutagenic response was observed **(**FTC tested were 50, 150, 500, 1500, and 5000 µg/plate.

The chromosome aberration assay was used to evaluate the clastogenic potential of the combination of FTC and 1% TP-0296 (FTU, degradant). This GLP-compliant study of FTC was conducted to qualify TP-0296 using Chinese Hamster ovary (CHO) cells in the absence and presence of S9 metabolic activation. The percentage of cells with structural or numerical aberrations in the FTC/TP-0296 groups was not significantly increased above that of the solvent control at any dose level ($p>0.056$, Fisher's exact test). Based on the results of this study, it was concluded that FTC with 1% TP-0296 was negative for the induction of structural and numerical chromosome aberrations in CHO cells.

There was no toxicity in a 28-day mouse study at doses (FTC/FTU) of 50/1 mg/kg/day, 150/3 mg/kg/day, and450/9 mg/kg/day. A 28-day mouse bridging study was performed to qualify impurities in FTC (specifically FTC-carboxylic acid - menthol Process). There was no toxicity of FTC at doses of 50, 150, and 450 mg/kg/day.

Based on the impurity profiles, the multiple GLP batches of FTC tested in the toxicology program are considered, in composite, to be representative of the GMP material and support the specified limits of impurities and degradation products proposed for commercial production. This is considered acceptable.

TAF

A 2 week oral rat (males) study was conducted to evaluate the toxicity and to qualify potential impurities of TAF. Animals were given GS-7340-02 at 5 or 50 mg/kg/day (10 mL/kg) from 2 different purity lots (Lot No. 1 - 97.7% and Lot No. 2 - 83.1%)]. The impurities, including 13% GS-7339, were added to the more pure lot. No test article-related findings were noted, and no differences were found between the 2 lots tested.

A 4 week oral rat (males and females) study was conducted. Three lots of GS-7340-03 were each administered at 25 and 50 mg/kg/day (free base equivalents). Test article 1 was 99.3% pure GS-7340-03. Test article 2 was 98% pure GS-7340-03 containing 11 spiked impurities. Test article 3 was 97.8% pure GS-7340-03 containing 4 spiked impurities. There were no significant in-life or histopathological differences between the 3 lots tested. The NOAEL for all 3 lots is 50 mg f.b.e./kg/day.

Based on their impurity profiles, the multiple GLP batches of TAF tested in the toxicology program are considered, in composite, to be representative of the GMP material and support the specified limits of impurities and degradation products proposed for commercial production. This is considered acceptable.

FTC / TDF

Four degradation products not present in the individual drug substances have been observed in FTC/TDF tablets placed on accelerated stability at high temperature. The name for these degradation products are mono-POC PMPA/FTC Adduct (Adduct 1), bis-POC PMPA/FTC Adduct (Adduct 2), cyclic FTU1 (cFTU1) and cyclic FTU2 (cFTU2). The adducts of FTC/TDF form when formaldehyde, formed by the hydrolysis of TDF (bis-POC PMPA), reacts with one molecule of each TDF and FTC to form an adduct. FTC may undergo hydrolytic deamination and may additionally cyclize via an intramolecular Michael addition of the hydroxyl group of the sugar moiety onto the double bond of the uracil ring, thereby creating cyclic-FTU (cFTU; cyclic 5-fluro-1-(2-

hydroxymethyl-[1,3]oxathiolan-5-yl)-1H-pyrimidine-2,4-dione). While cyclic-FTU has the potential to exist as 4 diastereomers, only 2 of these diastereomers have been observed in FTC/TDF containing products. The 2 observed diastereomers of cyclic-FTU are GS-9237 (cyclic-FTU1, cFTU1) and GS-492127 (cyclic-FTU2, cFTU2).

Two 14-day GLP oral toxicity studies were conducted in rats to qualify impurities and degradants in the FTC/TDF tablets (TX-164-2001 & TX-164-2005). In the first study, animals were given 20/30, 67/100, or 200/300 mg/kg/day of non-degraded or degraded FTC/TDF in suspension vehicle. Organ weight evaluation showed marginal increases in the weights of adrenal glands (noted in all treated groups except those receiving degraded FTC/TDF at 20/30). No gross or histological changes were seen which might account for this increased adrenal weight. No treatment-related gross changes were observed. Microscopic evaluation revealed hyperplasia of the anterior duodenal mucosa overlying Brunner's glands. It was seen in 7/10 animals treated at 200/300 with non-degraded FTC/TDF and in 2/10 animals given degraded FTC/TDF and was considered to be treatment-related. The NOAEL was considered to be 67/100 mg/kg/day FTC/TDF.

The second qualification study (TX-164-2005) was conducted to verify the qualification of cFTU1 and cFTU2 as these degradants were identified later in development due to a new analytical assay. In these studies, rats were given formulations prepared from crushed tablets that were experimentally degraded by humidity and high temperatures or formulations prepared from crushed tablets that were not degraded. The doses were 0/0, 20/30, 67/100, and 200/300 mg/kg/day FTC/TDF in both studies.

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

The NOAEL was considered to be 200/300 mg/kg/day for both non-degraded and degraded TDF/FTC. No differences in toxicity were observed between non-degraded and degraded material**.**

There were slight differences in the findings from both studies, however there were no new toxicities or exacerbation of previously defined toxicities, and there was no difference in toxicity between non-degraded and degraded material. The NOAEL in the initial study (TX-164-2001) was considered to be 67/100 mg/kg/day, and 200/300 mg/kg/day FTC/TDF in the second study (TX-164-2005).

The impurities and degradation products present in FTC and TAF and in 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). This is considered acceptable.

Other studies

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

Male rats were given 400 mg/kg TAF (GS-7430-02) by oral gavage daily for 6 days. One animal was due to non-drug related injury (study number: R990177). There were no effects on serum parathyroid hormone (PTH) concentrations. Serum 1,25-dihydroxycholcalciferol was decreased by 80% compared to controls. Urinary deoxypyridinoline (Dpd) was decreased by 38% compared controls. The urinary calcium/creatinine and phosphorus/creatinine ratios were increased 444% and 202%, respectively, compared to controls by Day 6. The results of this study demonstrated that TAF had effects on selected urinary and serum parameters. The applicant states that due to the lack of strong statistical comparison to controls and lack of correlation between data, interpretation on overall toxicity was not possible.

In the second study, an additional dose was evaluated. This study examined changes in markers of calcium homeostasis in male Sprague-Dawley rats after once daily oral administration of TAF. Animals were given 100 or 400 mg/kg TAF (GS-7340-02) by oral gavage on Days 0, 1, 2, 3, 4, and 5. At 400 mg/kg/day statistically significant decreases in body weight gain was noted. No changes in plasma calcium, phosphorus, or ionized calcium were observed during the first day of the study at either dose. No changes in serum 25-hydroxyvitamin D3, parathyroid hormone, or total carbon dioxide (CO2) were observed at either dose. Significant dose-dependent reductions of 35% and 46% were observed in 1,25-dihydroxyvitamin D3 on Day 6 at 100 or 400 mg/kg/day TAF, respectively. Significant non-dose-dependent reductions of 15% and 14% were observed in serum calcium on Day 6 at 100 or 400 mg/kg/day TAF, respectively. Significant dose-dependent decreases of 20% and 31% in serum phosphate were observed at 100 or 400 mg/kg/day TAF, respectively. Urinary calcium:creatinine ratio values were significantly decreased by approximately 71% and 53% on Days 1 and 2, respectively, in at 100 mg/kg/day TAF. Urinarycalcium:creatinine ratio values were significantly decreased by 42% (Day 1) then significantly increased by 213% (Day 6) at 400 mg/kg/day TAF. No changes in urinary phosphorus:creatinine ratio, N-acetyl-ß-glucosaminidase (NAG):creatinine, cyclic adenosine monophosphate (cAMP):creatinine, pH, protein, urobilinogen, or specific gravity were observed.

These data indicated that daily administration of TAF to rats for 6 days altered plasma vitamin D, calcium and phosphate levels as well as urinary excretion of calcium. It is stated that the mechanistic basis of these changes and their biological relationships in terms of cause and effect are unknown.

Dogs were given oral doses of 37.5 or 75 mg/kg/day of TAF (GS-7340-02) for 5 days (1/dose). Emesis, excessive salivation and diarrhoea were observed along with weight loss and reduced food intake. On day 6, dogs had decreased WBC, neutrophil, lymphocyte, monocyte, and eosinophil counts with a left shift in the myeloid series with under representation of mature segmented neutrophils as the predominant bone marrow finding. Animals also had increased total protein, globulin, triglyceride, cholesterol, AST, ALP, and creatinine kinase (CK) values. A trend toward proteinuria was observed in the urinalysis data.

The Applicant states that there were no biologically important changes observed in mean values for ionized calcium or N telopeptide. The applicant states that the lack of the expected age-related reduction in mean serum bone ALP (B-ALP) suggested increased bone remodelling. The mean serum calcitriol (1, 25 dihydroxycholecalciferol) concentration was reduced by approximate 68% at both doses. The mean serum 25-hydroxyvitamin D (25 hydroxycholecalciferol) concentration was reduced approximately 35% and 33% at 37.5 and 75 mg/kg/day. Gastrointestinal lesions characterised by epithelial cell necrosis, regenerative hyperplasia, and cyst formation were most prevalent in animals at 75 mg/kg/day. Lymphoid cell depletion was noted in the lymph nodes, thymus and tonsilat 75 mg/kg/day animals. Bile duct hyperplasia and peri-portal inflammation was observed at both doses.

Renal Function

An investigative study was conducted in SD rats to evaluate renal parameters. Animals were given a single oral dose of 100 or 1000 mg/kg/day TAF (GS-7340-02). Urinary output of calcium was increased at 1000 mg/kg/day which correlated with an increase in serum calcium concentration and indicated that the kidneys were functioning in order to reduce the serum calcium load.

2.3.5. Ecotoxicity / environmental risk assessment

The environmental risk assessment (ERA) of Descovy consists of a full assessment for the active substance tenofovir alafenamide (TAF) in the overall assessment for the FDC. The ERA data from the Genvoya MAA (EMEA/H/C/004042) has been used for the assessment of the active substance emtricitabine (FTC).

Emtricitabine

The n-octanol/water partition coefficient (Logkow) of FTC was determined to be below 4.5, the Phase I action limit for a PBT assessment.

The predicted environmental concentration in surface water (PECsw) was calculated using a refined Fpen based on the prevalence of HIV in the member states and the value obtained was above the Phase I action limit of 0.01 µg/L, thus triggering a Phase II A assessment.

The risk quotient RQ (PEC:PNEC) for the sewage treatment plant, surface water and groundwater compartments were all below 1, thus indicating that the active substance poses a low environmental risk for these compartments.

In the aerobic and anaerobic transformation in aquatic sediment systems study (OECD 308), FTC demonstrated significant shifting to the sediment (> 10%), thus triggering the sediment dweller organisms study (OECD 218) in Phase II B. The PEC:PNEC sediment ratio was determined to be below 1 and, therefore, FTC it is not expected to pose a risk to the sediment compartment.

The results of the environment risk assessment of FTC are summarised in the Table 14

Table 14. Summary of main study results

Substance (INN/Invented Name): Emtricitabine/ Descovy					
CAS-number (if available): 143491-57-0					
PBT screening		Result			**Conclusion**
Bioaccumulation potential- log *K*ow	OECD107	-0.693 – -0.670			Potential PBT (N)
PBT-assessment					
Parameter	**Result relevant for conclusion**				**Conclusion**
Bioaccumulation	log *K*ow	-0.693 – -0.670			not B
	BCF	Not required			not B
Persistence	DT50 or ready biodegradability	DT50 (dissipation) 36-151 days; DT50 (degradation) > 100 days. No significant metabolites formed			
Toxicity	NOEC or CMR				not T
PBT-statement :	The compound is not considered as PBT nor vPvB				
Phase I					
Calculation	**Value**	**Unit**			**Conclusion**
PEC surfacewater, refined	1.2 (F*pen* 0.012)	□g/L			> 0.01 threshold (Y)
Other concerns (e.g. chemical class)					(N)
Phase II Physical-chemical properties and fate					
Study type	**Test protocol**	**Results**			**Remarks**
Adsorption-Desorption	OECD 106	*Kd* sludge =12.9 L.kg-1			
Ready Biodegradability Test	OECD 301	Not readily biodegradable			
Aerobic and Anaerobic Transformation in Aquatic Sediment systems	OECD 308	DT_{50} (dissipation) 36-151 days; DT_{50} (degradation) > 100 days; No significant metabolites formed. % shifting to sediment =>10% AR associated with sediment from Day 7			Sediment toxicity assessment is triggered
Phase IIa Effect studies					
Study type	**Test protocol**	**Endpoint**	**value**	**Unit**	**Remarks**
Algae, Growth Inhibition Test/*Species*	OECD 201	NOEC	110	mg.L-1	*Pseudokirchneriella subcapitata*
Daphnia sp. Reproduction Test	OECD 211	NOEC	110	mg.L-1	
Fish, Early Life Stage Toxicity Test/*Species*	OECD 210	NOEC	6.10	mg.L-1	*Pimephales promelas*
Activated Sludge, Respiration Inhibition Test	OECD 209	EC	≥ 1000	mg.L-1	Sewage microorgnisms
Phase IIb studies					
Sediment dwelling organism	OECD 218	NOEC	38	mg/kgd wt	*Chironomus riparius* normalised for 10% OC 200 mg.kgdwt -1)

Tenofovir alafenamide

TAF is extensively transformed to TFV and the latter can, therefore, be considered the environmentally relevant residue of TAF.

The n-octanol/water partition coefficient for TFV was determined to be below 4.5 and, therefore, a PBT assessment is not required.

The predicted environmental concentration in surface water (PECsw) was calculated using a refined Fpen based on the prevalence of HIV in the member states and the value obtained was above the Phase I action limit of 0.01 µg/L, thus triggering a Phase II A assessment.

The PEC:PNEC ratios for the sewage treatment plant, surface water and groundwater compartments were all below 1, thus indicating that the active substance poses a low environmental risk for these compartments.

The adsorption /desorption study (OECD 106) indicated TFV to be persistent in the environment however it is not bioaccumulative (B) or toxic (T) and, therefore, not classified as a PBT substance.

In the aerobic and anaerobic transformation in aquatic sediment systems study (OECD 308), more than 10% of the applied radioactivity was found associated with the sediment at or after day 7 of the study, thus triggering the assessment of toxicity to sediment dwelling organisms in phase II B.

The PEC:PNEC sediment ratio was determined to be is below 1, thus indicating that TFV poses a low risk to the sediment compartment.

The results of the environment risk assessment of TAF are summarised in the Table 15.

Table 15. Summary of main study results

Substance (INN / Invented Name): Tenofovir alafenamide (as environmentally relevant TFV) / Descovy					
CAS-number (if available): 1392275-56-7					
PBT screening		Result			**Conclusion**
Bioaccumulation potential- log *K*ow	OECD107	-3.8 – -4.3			Potential PBT (N)
PBT-assessment					
Parameter	**Result relevant for conclusion**				**Conclusion**
Bioaccumulation	log *K*ow	-3.8 – -4.3			not B
	BCF	Not required			not B
Persistence	DT50 or ready biodegradability	DT50 (degradation) 10.4-32.7 days; Water DT50 (dissipation) 2.0-3.5 days Three significant metabolites formed			P
Toxicity	NOEC or CMR				not T
PBT-statement :	The compound is not considered as PBT nor vPvB				
Phase I					
Calculation	**Value**	**Unit**			**Conclusion**
PEC surfacewater, refined	0.07 µg/L (F*pen* 0.012)	□g/L			> 0.01 threshold (Y)
Other concerns (e.g. chemical class)					(N)
Phase II Physical-chemical properties and fate					
Study type	**Test protocol**	**Results**			**Remarks**
Adsorption-Desorption	OECD 106	Koc ads soil 351 - 1091 L.kg-1 Koc des soil 968 - 2791 L.kg-1 KF ads sludge 6.0 - 21 L.kg-1 KF des sludge 16 - 62 L.kg-1			
Ready Biodegradability Test	OECD 301	Not readily biodegradable			
Aerobic and Anaerobic Transformation in Aquatic Sediment systems	OECD 308	DT50 (degradation) 10.4-32.7 days; Water DT50 (dissipation) 2.0-3.5 days; Three significant metabolites formed. >10% AR associated with sediment from Day 7			Sediment toxicity assessment is triggered
Phase IIa Effect studies					
Study type	**Test protocol**	**Endpoint**	**value**	**Unit**	**Remarks**
Algae, Growth Inhibition Test/*Species*	OECD 201	NOEC	32	mg.L-1	*Pseudokirchneriella subcapitata*
Daphnia sp. Reproduction Test	OECD 211	NOEC	100	mg.L-1	
Fish, Early Life Stage Toxicity Test/*Species*	OECD 210	NOEC	≥10	mg.L-1	*Pimephales promelas*
Activated Sludge, Respiration Inhibition Test	OECD 209	NOEC	≥1000	mg.L-1	Sewage microorganisms
Phase II b studies					
Sediment dwelling organism	OECD 219	NOEC	290	mg/kg dwt	*Chironomous riparius* normalised for 10% OC 17.06 mg.kgdwt-1

2.3.6. Discussion on non-clinical aspects

Detailed nonclinical data was provided in support of this application for FTC/TAF, including study reports for FTC and TAF and for FTC/TDF.

In-vitro and ex-vivo studies demonstrated TAF uptake into PBMCs, conversion to TFV and phosphorylation to form the active form 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 metabolism toxicity. Safety pharmacology studies revealed no significant concerns for TAF. The absorption, distribution, metabolism and excretion of 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 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 F/TAF combination.

The kidney and bone findings seen in the rat and dog toxicology studies are known toxicities of TFV. 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 18/12 mg/kg/day occurred at 3.7- and 17-fold higher exposures to TAF and TFV, respectively, than that observed in human subjects administered a 25 mg dose of TAF. 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. Adverse degenerative (olfactory) and acute inflammatory (infiltrate neutrophil) changes in the nasal mucosa in mice given TAF for 13 weeks were not seen in other species and 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-foetal 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. Given that no peri-/postnatal study was conducted with TAF, the product literature should contain the reproductive findings seen with TDF (i.e. reduced viability index and weight of pups in peri-/postnatal toxicity studies at maternally toxic doses) or an omission of this information should be justified using relative exposures of TFV and TFV-DP after TAF exposure.

2.3.7. Conclusion on the non-clinical aspects

No major concerns have been identified from the nonclinical data.

The environmental risk assessment of Descovy has shown that 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 identified as persistent in the environment, but it is considered to be of low risk to aquatic organisms, it is not bioaccumulative and does not pose significant risk to the environment.

2.4. Clinical aspects

2.4.1. Introduction

F/TAF resembles Truvada (TVD) except that TDF is substituted with TAF [GS-7340], which allows for ingestion of a very much lower oral dose (25 mg; 10 mg when boosted by COBI) compared to 245 mg TD. TAF is presented for clinical use as the fumarate (11 mg or 28 mg GS-7340-03 vs. 300 mg TDF) but all doses reported refer to the TAF content of the various formulations. The studies listed in Table 1 were conducted with TAF alone or with F/TAF in uninfected or HIV-infected individuals. The efficacy of F/TAF is heavily based on the studies conducted with E/C/F/TAF and bridging to these data via two bioequivalence studies (GS US 311 1472 and GS US 311 1473). Details are not presented for studies that supported the approval of FTC or TVD or those submitted to support E/C/F/TAF except for those in which a group received TAF alone or F/TAF, which are repeated for convenience. Furthermore, some additional pharmacokinetic data were provided during the evaluation.

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.

Table 16. Overview of clinical studies

Study Number Phase	Test Treatments Dose and Formulation	n	Reference Treatment(s) Dose and Formulation
Comparative Bioavailability/Bioequivalence			
GS-US-311-1088 Phase 1	**F/TAF** 200/25-mg tablet	56	**FTC** 200-mg capsule + **TAF** 25-mg tablet
GS-US-311-1472 Phase 1	**F/TAF** 200/10-mg + **EVG** 150-mg + **COBI** 150-mg	99	**E/C/F/TAF** 150/150/ 200/10-mg tablet
GS-US-311-1473 Phase 1	**F/TAF** 200/25-mg tablet	116	**E/C/F/TAF** 150/150/ 200/10-mg tablet
GS-US-311-0103 Phase 1	**E/C/F/TAF** 150/150/200/10-mg tablet	33	**EVG** 150-mg + **COBI** 150-mg **FTC** 200-mg capsule + **TAF** 25-mg tablet
Mass Balance (PK, Metabolism, and Excretion)			
GS-US-120-0109 Phase 1	**TAF** [^{14}C]-labeled, 25-mg capsule	8	Not applicable
Effect on QT/QTc Interval			
GS-US-120-0107 Phase 1	**TAF** 25-mg tablet and 5 × **TAF** 25-mg tablet	58	**Moxifloxacin** 400-mg tablet
Study Supporting PK and Initial Tolerability in Healthy Subjects			
GS-US-292-0101 Phase 1	**E/C/F/TAF** 150/150/200/25-mg tablet (F1 or F2) **E/C/F/TAF** 150/150/200/40-mg tablet (F1 or F2))	38	**STB** 150/150/200/300-mg tablet **TAF** 25-mg tablet
Studies Supporting PK and Initial Tolerability in HIV-Infected Subjects (Including PD and PK/PD Relationships)			
GS-US-311-1089 PK report for Phase 3 study	**F/TAF** 200/25 mg-tablet + **unboosted 3rd Agent F/TAF** 200/10 mg-tablet + **boosted 3rd Agent**	333	**FTC/TDF** 200/300 mg-tablet + **3rd Agent**
GS-US-120-0104 Phase 1	**TAF** 8-mg, 25-mg or 40-mg tablet	25	**TDF** 300-mg tablet
Studies Evaluating the Effect of Intrinsic Factors			

Study Number Phase	Test Treatments Dose and Formulation	n	Reference Treatment(s) Dose and Formulation
Studies of the Effect of Renal Impairment			
GS-US-120-0108 Phase 1; non-HIV infected subjects	**TAF** 25-mg tablet	27	Not applicable
Studies of the Effect of Hepatic Impairment			
GS-US-120-0114 Phase 1; non-HIV infected subjects	**TAF** 25-mg tablet	40	Not applicable
Studies Evaluating the Effect of Extrinsic Factors			
Studies of the Effect of Food in Healthy Subjects			
GS-US-311-1386 Phase 1	**F/TAF** 200/25-mg tablet	40	Not applicable
Studies of Potential Drug-Drug Interaction in Healthy Subjects			
GS-US-311-0101 Phase 1	**F/TAF** 200/40-mg tablet + **EFV** 600-mg tablet **F/TAF** 200/25-mg + 2 × **DRV** 400-mg + **COBI** 150-mg **TAF** 8-mg tablet + **COBI** 150-mg tablet	50	**F/TAF** 200/40-mg tablet **F/TAF** 200/25-mg tablet 2 × **DRV** 400-mg + **COBI** 150-mg **TAF** 8-mg tablet
GS-US-342-1167 Phase 1	**D/C/F/TAF** F1, 800/150/200/25-mg, monolayer tablet **D/C/F/TAF** F2, 800/150/200/25-mg, bilayer tablet **D/C/F/TAF** F3, 800/150/200/10-mg, monolayer tablet 2 × **DRV** 400-mg + **COBI** 150-mg + **FTC/TDF** 200/300-mg tablet	101	2 × **DRV** 400-mg tablet + **COBI** 150-mg tablet **FTC/TDF** 200/300-mg tablet **FTC** 200-mg capsule + **TAF** 25-mg tablet
GS-US-120-0118 Phase 1	**TAF** 10-mg + **FTC** 200-mg capsule **ATV** 300-mg capsule + **RTV** 100-mg tablet 2 × **DRV** 400-mg tablet + **RTV** 100-mg tablet **LPV/r** 4 × 200/50-mg tablet **DTG** 50-mg tablet	40	**TAF** 10-mg tablet + **FTC** 200-mg capsule **ATV** 300-mg + **RTV** 100-mg 2 × **DRV** 400-mg + **RTV** 100-mg tablets **LPV/r** 4 × 200/50-mg tablet **DTG** 50-mg tablet
GS-US-120-1538 Phase 1	**TAF** 25-mg tablet + **MDZ** 2.5-mg oral syrup **TAF** 25-mg tablet + **MDZ** 1-mg solution for injection	18	**TAF** 25-mg tablet
GS-US-120-0117 Phase 1	**TAF** 25-mg tablet (CM1208B1) + **RPV** 25-mg tablet	36	**TAF** 25-mg tablet **RPV** 25-mg tablet
GS-US-120-1554 Phase 1	**TAF** 25-mg tablet **RPV** 25-mg tablet **TAF** 25-mg tablet + **RPV** 25-mg tablet	34	Not applicable
Controlled Multiple-Dose Studies with Sparse PK Sampling in HIV-Infected Subjects			
GS-US-299-0102	**D/C/F/TAF** 800/150/200/10-mg tablet	103	2 × **DRV** 400-mg + **COBI** 150-mg + **FTC/TDF** 200/300-mg tablet

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.

Formulations

Two forms of TAF were used during clinical development:

TAF monofumarate (GS-7340-02; 1:1 GS-7340 to fumarate) (GS-US-292-0101 and 120-1101)

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

TAF fumarate (GS-7340-03) was selected for further clinical development in various FDCs due to its enhanced purging capability of the process impurity GS-7339, increased thermodynamic stability in organic solvents and improved thermal stability compared to TAF monofumarate.

The proposed commercial F/TAF FDC film-coated tablets are identical to those used in the pivotal bioequivalence studies (GS-US-311-1472 and 1473) and in Phase 3 (GS-US-311-1089).

Methods

Analytical methods

- The bioanalytical method for the determination of TAF in human plasma was validated at Quest Pharmaceutical Services, L.L.C (QPS). The method involved protein precipitation extraction of TAF and its internal standard (TAF-d7) from human plasma followed by LC MS/MS with positive ionization. The linear range was from 1 to 1000 ng/mL.
- Tenofovir (TFV) was initially assayed in plasma at QPS using an assay with a linear range from 0.3 - 300 ng/mL. An improved version was used for assay of samples obtained from GS-US-120-0114 and 0118 and another improvement led to the assay used for GS-US-311-1088.
- The bioanalytical method for the simultaneous determination of TAF and TFV in human urine was developed and validated at QPS. The method involved protein precipitation extraction of TAF, TFV and internal standards (TAF-d7 and TFV-d6, respectively) from human urine followed by LC MS/MS with positive ionization. The linear range was 2-1000 ng/mL for TAF and 10-5000 ng/mL for TFV.
- The bioanalytical method for the simultaneous determination of FTC and TFV in human plasma was initially developed at Gilead but further improved by QPS. The linear range was 5-3000 ng/mL for both analytes and this assay was used for GS-US-342-1167 and GS-US-311-0101.

2.4.2. Pharmacokinetics

Absorption

Absolute bioavailability

Absolute bioavailability has not been determined for TAF but the applicant estimates that this is ~ 40% in the absence of P-gp inhibition and ~90% when it is ingested with an inhibitor of P-gp (e.g. COBI or RTV).

FTC-110 demonstrated that the absolute bioavailability of FTC is 93%.

- TAF dose selection when given ± a P-gp inhibitor and resulting TFV concentrations

GS-US-292-0101

Study Title: A Phase 1, Multiple-Dose Study Evaluating the Relative Bioavailability of Two Elvitegravir/Cobicistat/ Emtricitabine/GS-7340 Single Tablet Regimen Formulations vs Elvitegravir/Cobicistat/Emtricitabine/Tenofovir Disoproxil Fumarate Single Tablet Regimen and GS-7340

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 (STB) and TAF 25 mg alone. Healthy subjects were assigned to 1 of 2 cohorts and randomised to 1 of 4 treatment sequences:

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 (STB)
- 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 after a meal of 550-650 kcal and 25-30% fat.

Dosing was for 12 days with 2-day washout periods between treatments.

Following administration of E/C/F/TAF 25 mg in either formulation 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. Mean TFV exposures (AUC_{tau} and C_{max}) following TAF alone were ~90% lower compared with those achieved after STB. TAF and TFV exposures after STRs containing 25 or 40 mg TAF were generally dose-proportional. For FTC there was bioequivalence between the E/C/F/TAF formulations and STB.

Table 17. 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 18. 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

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. FTC exposures were unaffected by formulation into E/C/F/TAF 10 mg.

Table 19. 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)
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-311-1386

Study Title: A Phase 1, Randomized, Open-Label Study to Determine the Effect of Food on the Pharmacokinetics of Tenofovir Alafenamide When Administered as Emtricitabine/Tenofovir Alafenamide Fixed-Dose Combination Tablet in Healthy Volunteers

GS-US-311-1386 was an open-label crossover study that evaluated the effect of food on F/TAF tablets. Dosing was on days 1 and 8 with a single F/TAF 200/25 mg tablet in the fasted state (A; reference) or after a high-calorie/high-fat meal of approximately 800 kcal and 50% fat (B; test).

Administration of F/TAF under fed conditions resulted in a slightly lower TAF C_{max} and delayed T_{max} (by 30 min). In contrast the GLSM ratios for TAF AUCs were ~175% (90% CI ~165, 188) for the fed vs. fasted state. Of note, TFV was not measured in this study.

Table 20. GS-US-311-1386: Statistical Comparisons of TAF PK Parameter Estimates Between Study Treatments (TAF PK Analysis Set)

TAF PK Parameter (Test/Reference)	GLSMs by Treatment		GLSM Ratio Test/Reference (%)	90% CI
	Test Treatment B: (F/TAF Fed) (N = 38)	Reference Treatment A: (F/TAF Fasted) (N = 40)		
AUC_{inf} (h•ng/mL)	234.86[a]	133.91	175.38	(163.93, 187.63)
AUC_{last} (h•ng/mL)	234.02	132.53	176.57	(166.19, 187.60)
C_{max} (ng/mL)	180.00	212.94	84.53	(74.92, 95.37)

a N = 33; AUCinf could not be calculated in 5 subjects for analyte TAF with Treatment B.

As expected, FTC AUCs were unaffected by food, although slightly lower. C_{max} was clearly reduced.

Table 21. GS-US-311-1386: Statistical Comparisons of FTC PK Parameter Estimates Between Study Treatments (FTC PK Analysis Set)

FTC PK Parameter (Test/Reference)	GLSMs by Treatment		GLSM Ratio Test/Reference (%)	90% CI
	Test Treatment B: (F/TAF Fed) (N = 38)	Reference Treatment A: (F/TAF Fasted) (N = 40)		
AUC_{inf} (h•ng/mL)	9114.01	10,002.77	91.11	(88.84, 93.44)
AUC_{last} (h•ng/mL)	8901.52	9758.42	91.22	(88.90, 93.60)
C_{max} (ng/mL)	1513.12	2058.61	73.50	(69.26, 78.00)

Compared to GS-US-292-0110, which assessed the PK of TAF after dosing E/C/F/TAF 10 mg STR under fasted and fed conditions (including the same meal type as above), the effect of food on TAF was greater after dosing with 200/25 mg F/TAF.

Table 22. 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.

Bioequivalence studies to support the F/TAF formulation

GS-US-311-1088

Study Title: A Phase 1, Randomized, Open Label, Single Dose, Two-way Cross-Over Study to Evaluate the Bioequivalence of Emtricitabine/Tenofovir Alafenamide Fixed Dose Combination Tablet

GS-US-311-1088 was an open-label crossover study in which subjects received F/TAF (200/25 mg) and FTC 200 mg + TAF 25 mg. Dosing was after completion of a 600 kcal and 27% fat meal. Bioequivalence was demonstrated for each of TAF and FTC. The CSR states that TFV levels are not reported due to failure of the incurred sample reliability measure. The bioanalytical report states that the QCs spiked with GS-7340 plus TFV showed conversion of GS-7340 to TFV so the method did not control conversion effectively, particularly when the GS-7340: TFV ratio was high.

Table 23. GS-US-311-1088: Statistical Comparisons of Tenofovir Alafenamide PK Parameter Estimates Between Study Drugs (PK Analysis Sets)

TAF PK Parameter	GLSMs by Treatment		GLSM Ratio (%) Test/Reference	90% CI
	Test Treatment (F/TAF) (N = 55)	Reference Treatment (FTC+TAF) (N = 55)		
AUC_{last} (ng•h/mL)	245.91	239.48	102.68	(95.78, 110.09)
AUC_{inf} (ng•h/mL)	254.18[a]	240.33[b]	105.77	(97.26, 115.01)
C_{max} (ng/mL)	209.36	226.11	92.59	(82.31, 104.16)

GLSM = geometric least-squares mean

a N = 43

b N = 48

GS-US-311-1472

Study Title: A Phase 1, Randomized, Open-Label, Single-Dose, Two-Way Cross-Over Study to Evaluate the Bioequivalence of Emtricitabine and Tenofovir Alafenamide between Emtricitabine/Tenofovir Alafenamide (200/10 mg) and Elvitegravir/Cobicistat/Emtricitabine/Tenofovir Alafenamide (150/150/200/10 mg) Fixed-Dose Combination Tablets

GS-US-311-1472 was a large (>100 subject) open-label crossover study which compared F/TAF (200/10 mg) plus single tablets of EVG 150 mg and COBI 150 mg with E/C/F/TAF 10 mg. Dosing was after completion of a 600 kcal and 27% fat meal. Bioequivalence was demonstrated for each of TAF and FTC. COBI and EVG concentrations were the same for the two groups. TFV was not measured.

Table 24. GS-US-311-1472: Statistical Comparisons of TAF PK Parameter Estimates Between Study Drugs (TAF PK Analysis Set)

TAF PK Parameter	N	Test Mean (%CV)	N	Reference Mean (%CV)	GLSM Ratio (Test/Reference) (%)	90% CI (%)
F/TAF (200/10 mg) +EVG+COBI (Test) vs E/C/F/TAF (150/150/200/10 mg) (Reference)						
AUC_{last} (h•ng/mL)	97	336.6 (33.9)	99	340.2 (33.8)	97.96	94.69, 101.34
AUC_{inf} (h•ng/mL)	97	351.8 (31.0)	99	354.1 (32.9)	98.34	94.81, 101.99
C_{max} (ng/mL)	97	301.6 (48.8)	99	310.3 (48.7)	96.86	89.36, 104.99

Table 25. GS-US-311-1472: Statistical Comparisons of FTC PK Parameter Estimates Between Study Drugs (FTC PK Analysis Set)

FTC PK Parameter	N	Test Mean (%CV)	N	Reference Mean (%CV)	GLSM Ratio (Test/Reference) (%)	90% CI (%)
F/TAF (200/10 mg) +EVG+COBI (Test) vs E/C/F/TAF (150/150/200/10 mg) (Reference)						
AUC_{last} (h•ng/mL)	97	10159.2 (17.2)	99	10086.8 (15.9)	99.84	98.41, 101.29
AUC_{inf} (h•ng/mL)	97	10535.1 (27.0)	99	10294.4 (15.8)	100.67	98.24, 103.16
C_{max} (ng/mL)	97	1660.8 (20.6)	99	1662.6 (19.1)	99.57	96.78, 102.44

GS-US-311-1473

Study Title: A Phase 1, Randomized, Open-Label, Single-Dose, Two-Way Cross-Over Study to Evaluate the Bioequivalence of Emtricitabine and Tenofovir Alafenamide between Emtricitabine/Tenofovir Alafenamide (200/25 mg) and Elvitegravir/Cobicistat/Emtricitabine/Tenofovir Alafenamide (150/150/200/10 mg) Fixed-Dose Combination Tablets

GS-US-311-1473 was of the same design as GS-US-311-1472 except that the F/TAF tablet was 200/25 mg and it was given alone, i.e. without EVG/COBI. Bioequivalence was demonstrated for each of TAF (i.e. between 25 mg in F/TAF and 10 mg in E/C/F/TAF) and FTC. TFV was not measured.

Table 26. GS-US-311-1473: Statistical Comparisons of TAF Pharmacokinetic Parameter Estimates Between Study Drugs (TAF PK Analysis Set)

TAF PK Parameter	N	Test Mean (%CV)	N	Reference Mean (%CV)	GLSM Ratio (Test/Reference) (%)	90% CI (%)
F/TAF (200/25 mg) (Test) vs E/C/F/TAF (150/150/200/10 mg) (Reference)						
AUC_{last} (h*ng/mL)	116	374.0 (43.4)	116	369.3 (40.6)	100.32	96.48, 104.31
AUC_{inf} (h*ng/mL)	95	396.4 (42.6)	97	389.5 (39.3)	98.54	94.61, 102.62
C_{max} (ng/mL)	116	280.5 (62.9)	116	267.8 (59.8)	103.63	95.46, 112.49

Table 27. GS-US-311-1473: Statistical Comparisons of FTC Pharmacokinetic Parameter Estimates Between Study Drugs (FTC PK Analysis Set)

FTC PK Parameter	N	Test Mean (%CV)	N	Reference Mean (%CV)	GLSM Ratio (Test/Reference) (%)	90% CI (%)
F/TAF (200/25 mg) (Test) vs E/C/F/TAF (150/150/200/10 mg) (Reference)						
AUC_{last} (h*ng/mL)	116	9423.9 (19.3)	116	10475.3 (19.7)	90.01	88.88, 91.16
AUC_{inf} (h*ng/mL)	116	9654.6 (19.3)	116	10706.6 (19.6)	90.20	89.06, 91.35
C_{max} (ng/mL)	116	1577.4 (26.8)	116	1601.7 (19.6)	97.26	94.57, 100.03

Distribution

GS-US-120-109

Study title: A Phase 1 Study to Evaluate the Pharmacokinetics, Metabolism and Excretion of GS-7340

In the TAF metabolite profiling study GS-US-120-0109 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-7430 (%)
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:

- 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.

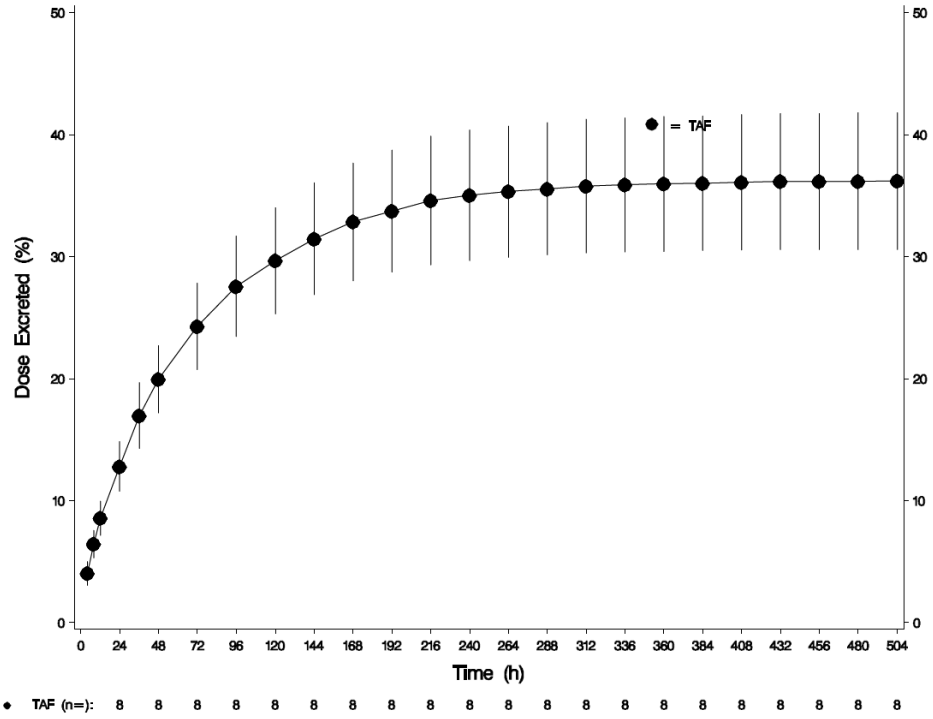
- TFV Vc/F and Vp/F were lower in moderate renal impairment and higher in those with supra-normal eGFR compared to normals. Both were also lower in in healthy subjects vs. HIV-infected patients.

Elimination

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]

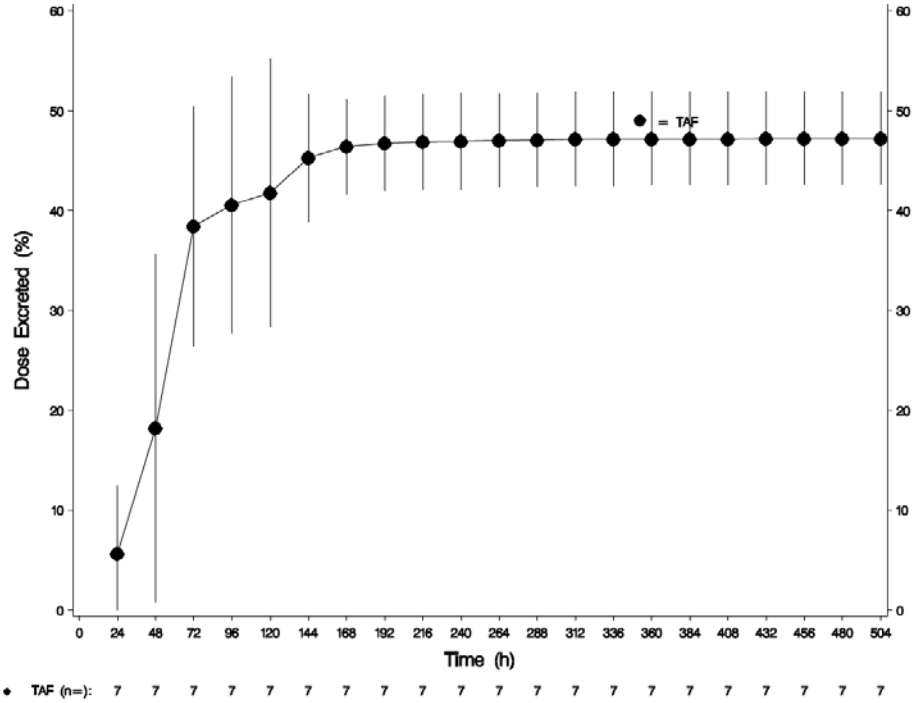


a Values below the lower limit of quantitation (BLQ) were treated as 0 for summary statistics and missing for log-normalized data.

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 1007 (PK Analysis Set)a,b,c,d



TAF (n=): 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7

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 Subject 1007 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 28. 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 29. 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 30. 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 31. 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 32. GS-US-120-0109: Percent of Total [14C]-Radioactivity Present as [14C] Metabolites in Pooled Faeces From All Sampling Intervals by HPLC (PK Analysis Set)

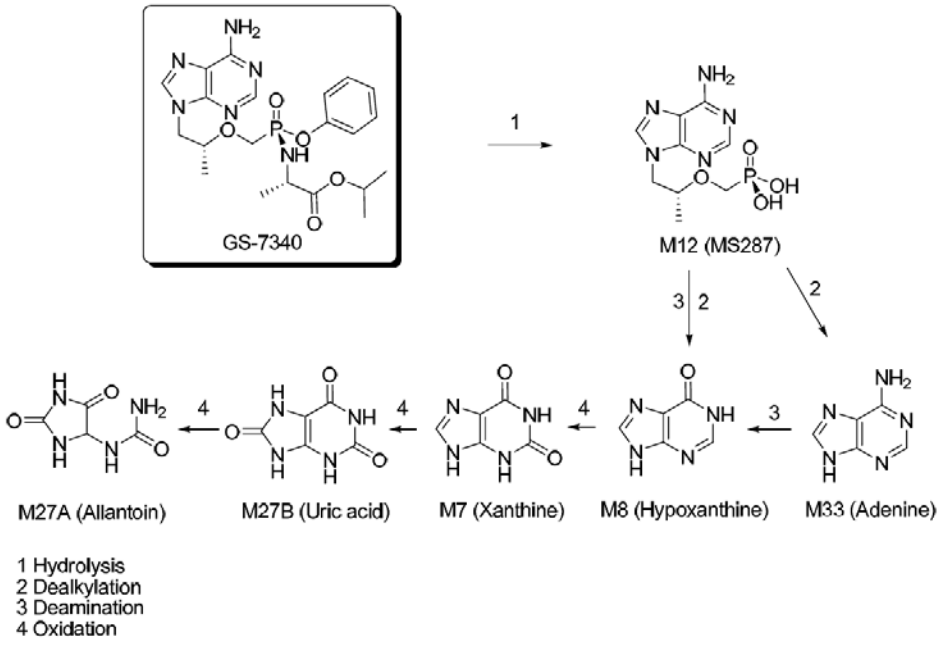
[^{14}C]-TAF Metabolite	Mean (SD) Percent of Total [^{14}C]-Radioactivity
M29	0.224 (0.328)
M12	31.4 (10.4)
M43	0.0628 (0.178)

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

Pharmacokinetics of metabolites

The biotransformation pathway of TAF is shown in Figure 3 (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.

Inter-conversion

TAF has 3 chiral centres. The potential for in-vivo isomerisation to occur was addressed as part of the E/C/F/TAF review and is considered to be negligible.

Consequences of possible genetic polymorphism

Genetic polymorphisms in CatA have been described, some of which can result in depressed enzymatic activity. The potential for human polymorphisms in CatA to affect conversion of TAF to TFV was addressed and significant modulation of TAF PK profiles by inhibition or genetic polymorphisms of CES1 seems to be unlikely. However, the HCV PIs telaprevir and boceprevir can form covalent bonds with the serine carboxypeptidase and so inhibit CatA, which could reduce intracellular TFV-DP formation. The SmPC reflects this since co-administration with these agents is not recommended.

Dose proportionality and time dependency

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 and 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). The intra-subject variability in E/C/F/TAF Phase 1 studies was modest (<20%). The inter-subject variability in intracellular TFV-DP is greater than for TAF in plasma (see pharmacodynamics, mechanism of action).

Pharmacokinetics in target population

GS-US-299-0102

Study Title: A Phase 2, Randomized, Double-Blinded Study of the Safety and Efficacy of Darunavir/Cobicistat/Emtricitabine/GS-7340 Single Tablet Regimen Versus Cobicistat-boosted Darunavir plus Emtricitabine/Tenofovir Disoproxil Fumarate Fixed Dose Combination in HIV-1 Infected, Antiretroviral Treatment-Naïve Adults

This Phase 2 study GS-US-299-0102 compared D/C/F/TAF 10 mg with DRV/co + TVD.

All patients had a single sample for PK at any time pre-or post-dose at Weeks 2, 4, 12, 16, 32 and 40 as well as trough levels at Weeks 8, 24 and 48. On or between the Week 4 and 8 visits, intensive profiling in plasma (21 TAF and 11 TDF) and/or PBMCs (14 vs. 8 per treatment group) was conducted in subsets of patients at selected study sites.

The TAF PK parameters were consistent with historical data following D/C/F/TAF 10 mg and TAF 25 mg (GS-US-120-0104, GS-US-299-0101).

Table 33. GS-US-299-0102: Summary of TAF Pharmacokinetic Parameters (All Substudy PK Analysis Set)

	AUC_{last} (ng·h/mL) Mean (%CV) (N = 21)	C_{max} (ng/mL) Mean (%CV) (N = 21)	T_{max} (h) Median (Q1, Q3) (N = 21)	$t_{1/2}$ (h) Median (Q1, Q3) (N = 21)
TAF	130.5 (34.1)	163.0 (51.9)	0.53 (0.50, 1.00)	0.45 (0.38, 0.66)

The TFV plasma concentrations following D/C/F/TAF were markedly lower vs. DRV+COBI+TVD. Concentrations in the latter group were consistent with historical data for EVG/COBI/FTC/TDF. These results were consistent with those observed in other studies that compared TAF 25 mg with TDF 300 mg (GS-120-1101 and GS-US-120-0104).

Figure 7. GS-US-299-0102: Mean (SD) Plasma Tenofovir Concentrations (Semi-Logarithmic Scale; PK Substudy Analysis Set)

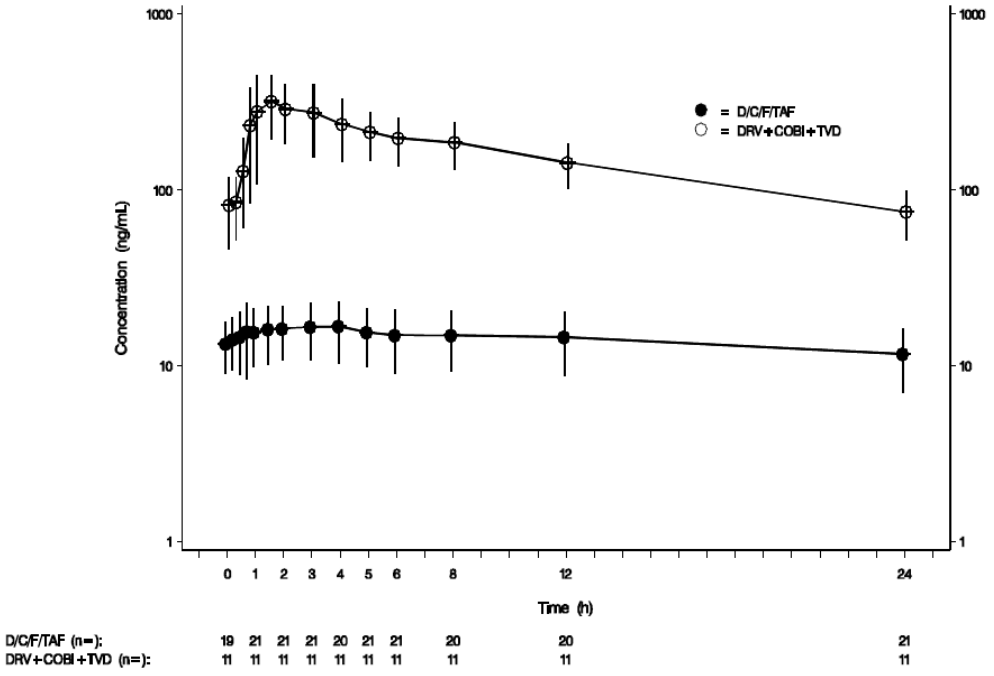


Table 34. GS-US-299-0102: Summary of TFV Pharmacokinetic Parameters (All Substudy PK Analysis Set)

Parameter	Units	D/C/F/TAF (N = 21)	DRV+COBI+TVD (N = 11)
AUC_{tau}	ng·h/mL	339.0 (37.1)	3737.0 (26.8)
C_{max}	ng/mL	18.8 (37.6)	413.2 (28.3)
T_{max} [a]	h	2.00 (1.50, 3.13)	1.00 (1.00, 3.00)
C_{tau}	ng/mL	11.7 (39.3)	75.4 (30.9)
$t_{½}$ [a]	h	43.82 (32.03, 59.23)	11.85 (11.35, 16.15)

Table 35. GS-US-299-0102: Statistical Comparisons of Pharmacokinetic Parameter Estimates Between Test and Reference Treatments (All Substudy PK Analysis Set)

	GLSM by Treatment			
TFV PK Parameter	D/C/F/TAF (N = 21)	DRV+COBI+TVD (N = 11)	GLSM Ratio (%)	90% CI (%)
AUC_{tau} (ng·h/mL)	312.95	3620.60	8.64	(6.99, 10.68)
C_{tau} (ng/mL)	10.68	72.20	14.79	(11.67, 18.75)
C_{max} (ng/mL)	17.44	397.70	4.39	(3.53, 5.45)

The intracellular TFV-DP AUC_{tau} was markedly higher in patients who received D/C/F/TAF vs. DRV+COBI+TVD. The geometrical least square means (GLSM) ratio of 652.09% was consistent with the 5- to 7-fold increase in TFV-DP observed with TAF 25 mg vs. TDF 300 mg in GS-US-120-1101 and GS-US-120-0104.

Table 36. GS-US-299-0102: Statistical Comparisons of AUCtau Between Test and Reference Treatments (All Substudy PK Analysis Set)

TFV-DP PK Parameter	GLSM by Treatment		GLSM Ratio (%)	90% CI (%)
	D/C/F/TAF (N = 14)	DRV+COBI+TVD (N = 8)		
AUC_{tau} (µM·h)	17.12	2.62	652.09	(268.28, 1585.00)

The plasma PK DRV, COBI and FTC at steady-state following administration of D/C/F/TAF for patients who participated in the intensive PK sub-study were consistent with those observed for the same formulation in GS-US-299-0101.

Table 37. GS-US-299-0102: Summary of DRV, COBI and FTC pharmacokinetic parameters (PK substudy analysis set =21)

	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)
DRV	99301.8 (45.3)	8826.2 (33.3)	1651.0 (108.0)	3.00 (2.00, 4.00)	9.42 (6.31, 13.87)
COBI	8744.5 (43.9)	1128.7 (35.3)	30.5 (135.1).	3.03 (3.00, 4.00)	3.16 (2.77, 3.70)
FTC	11918.0 (35.9)	2056.4 (25.3)	93.1 (58.3)	1.52 (1.50, 2.00)	7.51 (6.40, 8.79)

POPPK analysis

POPPK analyses were performed for TAF and TFV using PK data collected from E/C/F/TAF studies.

POPPK analyses were performed for TAF and TFV are shown in Table 23.

Table 38. 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 39.

Table 39. 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 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. AUCs by covariates are shown in Table 40.

Table 40. Summary of Individual Steady-State Predictions of TAF AUC by Covariates: All Subjects

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.

It was concluded that HIV disease status did not have ab effect on TAF exposure and was not a statistically significant relevant covariate.

Table 41. Population PK Analysis: summary of Steady State PK Parameters estimates for TAF Following Once-Daily Dosing of E/C/F/TAF by Subject Population

TAF PK Parameter	E/C/F/TAF	
	Healthy Subjects (N = 79)[a]	HIV-Infected Subjects (N = 539)[b]
AUC_{last} (ng•h/mL)	250 (31.6)	206 (71.8)
C_{max} (ng/mL)	178 (46.4)	162 (51.1)

a Subjects from Studies GS-US-292-0103, GS-US-292-0108, and GS-US-292-0110

b Subjects from Studies GS-US-292-0104 and GS-US-292-0111

Data are presented as mean (%CV).

TFV pharmacokinetics

The predicted steady-state AUC, C_{max}, and C_{min} (95% CI; % CV) parameters in the E/C/F/TAF 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%), respectively. The main covariate was CrCL. Although there was a statistically significant effect of HIV disease stratus on TFV PK, the range of TVF exposures across healthy and HIV infected was comparable and it was considered that the differences were not clinically significant.

Table 42. Summary of Individual Steady-State Predictions of TFV AUC by Covariates: All Subjects

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.

Additional integrated ad hoc PK analysis

The analysis used data from studies conducted with TAF 25mg or TAF 10 mg + COBI in healthy subjects or HIV-infected patients who received any of TAF 25 mg, F/TAF 200/25mg, E/C/F/TAF or F/TAF 200/10 mg + COBI and who had at least one Cmax or AUC for the respective analyte. Separate analyses were performed for data collected in the presence and absence of a boosting agent.

TAF - Exposure following multiple administrations of TAF 25mg was similar to that with TAF 10 mg + COBI. There was no evidence of accumulation or time-dependent kinetics.

	TAF 25 mg		TAF 10 mg + COBI	
	Single dose (N=336)	Multiple dose (N=112)	Single dose (N=278)	Multiple dose (n=123)
TAF AUClast (h*ng/mL)				
N	336	112	278	123
Mean (%CV)	270.2 (53, 57)	254.2 (37, 25)	322.0 (40, 37)	251.8 (40, 67)
Median	239,9	249.5	293.5	235.3
Q1, Q3	168,4, 330.9	177.9, 313,3	232.9, 388.5	184.9, 311.4
Min, Max	39.6, 1171.4	49.6, 512.6	94.5, 1028.3	95.6, 723.6
TAF Cmax (ng/mL)				
N	336	112	278	123
Mean (%CV)	224.6 (62, 76)	209.8 (44, 27)	289.0 (51, 68)	230.4, (56, 55)
Median	197.5	197.7	254.0	199.6
Q1, Q3	134.0, 267.0	142.1, 255.1	181.0, 368.0	135, 290.0
Min, Max	45.7, 1380.0	59.6, 521.0	57.2, 1160.0	58.2, 708.0

The analysis was considered by the applicant to show no clinically relevant differences in TAF exposure between healthy subjects and HIV-infected patients based on descriptive summaries of TAF PK.

Table 43. Integrated PK Analysis: Summary of Steady-State PK Parameter Estimates for TAF Following Once-Daily Dosing of TAF 25 mg or TAF 10 mg + COBI by Subject Population (TAF PK Analysis Set)

	TAF 25 mg		TAF 10 mg + COBI	
TAF PK Parameter	Healthy Subjects (N = 432)	HIV-Infected Subjects (N = 8)	Healthy Subjects (N = 330)	HIV-Infected Subjects (N = 54)
AUC_{last} (ng•h/mL)	270.2 (48.95)	127.4 (48.96)	311.1 (40.06)	239.8 (56.04)
C_{max} (ng/mL)	220.3 (58.57)	227.7 (66.42)	279.0 (52.69)	228.6 (60.80)

Data are presented as mean (%CV)

Weight (as a continuous covariate), age, BSA and race did not have a significant effect on TAF exposure in healthy subjects or HIV-infected patients. The integrated *ad hoc* PK analysis detected a statistically significant effect of sex, with higher exposure in females. The applicant considered that the finding was of no consequence due to the wide range of safe and efficacious TAF exposures.

Table 44. Integrated Ad Hoc PK Analysis: Summary of Steady-State PK Parameter Estimates for TAF Following Once-Daily Dosing of TAF 25 mg or TAF 10 mg + COBI in Healthy or HIV-Infected Subjects by Sex (TAF PK Analysis Set)

	TAF 25 mg		TAF 10 mg + COBI	
TAF PK Parameter	Male (N = 271)	Female (N = 169)	Male (N = 257)	Female (N = 127)
AUC_{last} (ng•h/mL)	241.8 (49.02)	305.7 (47.69)	276.1 (42.40)	355.9 (36.30)
C_{max} (ng/mL)	206.6 (61.06)	243.7 (54.28)	262.9 (54.40)	294.8 (51.54)

Data are presented as mean (%CV)

TFV - The integrated *ad hoc* PK analysis indicated comparable TFV exposures after TAF 25 mg and TAF 10 mg + COBI.

GS-US-311-1089 - F/TAF Phase 3 Clinical Study

Additional patient plasma TAF data became available during the evaluation from GS-US-311-1089, in which virologically suppressed patients switched to F/TAF from FTC/TDF or continued with their TDF-based regimen. A range of third agents was allowed in the regimens. Using the POPPK model the estimated mean TAF AUCtau and Cmax values and the estimated mean TFV AUC_{tau}, C_{max} and C_{tau} values in this switch study were consistent with those observed in E/C/F/TAF Phase 3 studies. The comparable TAF and TFV exposure data (at a dose of 25 mg in an unboosted regimen and 10 mg in a boosted regimen) supported bridging F/TAF to the E/C/F/TAF efficacy and safety database.

Table 45. GS-US-311-1089: Summary of Estimated TAF Exposures

TAF Exposures Mean (%CV)	n	AUC_{tau} (ng*h/mL)	C_{max} (ng/mL)
All	292	137.2 (48.1)	140.5 (54.0)
By third agent			
ATV/r	44	149.6 (50.8)	127.1 (60.3)
DRV/r	72	73.5 (41.0)	74.0 (44.2)
DTG	25	155.6 (18.5)	166.3 (36.9)
EFV	6	141.6 (18.7)	126.9 (52.8)
LPV/r	15	89.8 (25.0)	97.1 (42.4)
MVC	1	191.3 (N/A)	249.8 (N/A)
NVP	67	167.4 (32.1)	178.1 (30.2)
RAL	61	170.8 (37.2)	185.4 (44.4)
RPV	1	273.1 (N/A)	238.5 (N/A)

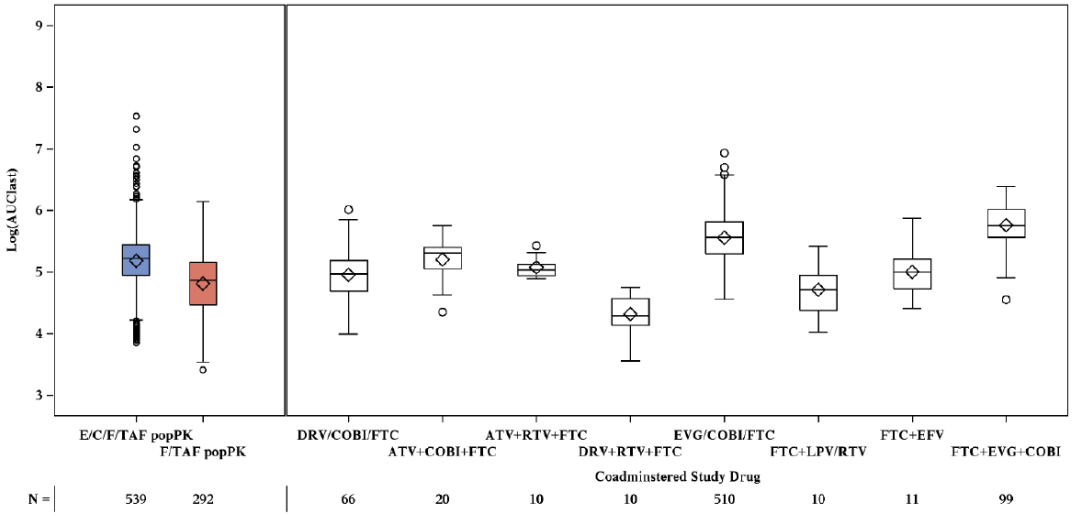
Table 46. GS-US-311-1089: Summary of Estimated TFV Exposures from F/TAF Administration

TFV Exposures Mean (%CV)	n	AUC_{tau} (ng*h/mL)	C_{max} (ng/mL)	C_{tau} (ng/mL)
All	328	346.3 (40.0)	18.2 (40.9)	12.4 (42.5)
By third agent				
ATV/r	53	343.7 (36.4)	17.7 (35.8)	12.3 (39.0)
DRV/r	82	313.8 (45.5)	16.1 (44.0)	11.2 (49.5)
DTG	26	305.8 (44.0)	16.1 (45.5)	10.9 (46.8)
EFV	8	311.6 (92.9)	16.5 (91.5)	11.2 (100.1)
LPV/r	17	417.1 (44.2)	22.4 (49.6)	14.8 (46.6)
MVC	1	351.3	18.3	12.6
NVP	73	410.4 (29.8)	21.9 (31.5)	14.7 (31.0)
RAL	65	321.8 (29.1)	17.2 (29.5)	11.4 (30.7)
RPV	3	297.6 (15.7)	15.4 (21.5)	10.6 (13.6)

TAF exposures by third agent

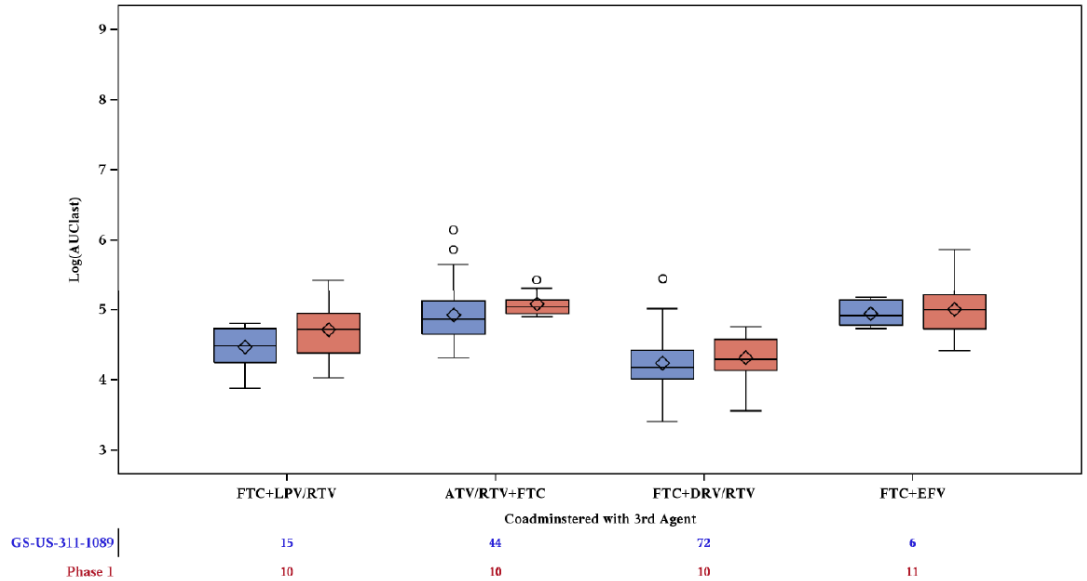
Figure 8 presents TAF exposures across several studies by third agents along with those from the E/C/F/TAF, F/TAF and D/C/F/TAF studies. These data support achievement of therapeutic TAF exposures when TAF at recommended doses is administered with those third agents. Importantly, these results are concordant with those from Phase 1 studies as illustrated in Figure 9, where GS-US-311-1089 exposure data are shown to be similar to those from Phase 1 DDI studies. The analysis includes data from a DDI study of F/TAF (10 mg) with ATV+COBI provided during the procedure that shows a mean (%CV) TAF AUC_{last} of 192 ng*h/mL (30.9 % CV) when co-administered with ATV+COBI (GS-US-311-1388).

Figure 8. Comparison of TAF Exposures When Administered with Third Agents of Interest



Study drugs presented on the right panel were coadministered with TAF 10 mg except for FTC+EFV, which was coadministered with TAF 40 mg and dose normalized to TAF 25 mg (unboosted) based on dose proportionality results from Study GS-US-120-0104. Studies presented: GS-US-120-0118, GS-US-292-0102, GS-US-292-0103, GS-US-292-0108, GS-US-292-0110, GS-US-292-1316, GS-US-299-0101, GS-US-299-0102, and dose normalized to TAF 25 mg (unboosted) based on dose proportionality results from Study GS-US-120-0104. Boxplot whiskers include data within 1.5 times the interquartile range above and below the box. Circle = outlier; line inside box = median.

Figure 9. Comparison of TAF Exposures from Phase 3 (GS-US-311-1089) and Phase 1 (GS-US-120-0118 and GS-US-311-0101) Studies When Administered with LPV/RTV, ATV/RTV, DRV/RTV, and EFV



Study drugs were coadministered with TAF 10 mg except for FTC+EFV, which was coadministered with TAF 40 mg and dose normalized to TAF 25 mg (unboosted) based on dose proportionality results from Study GS-US-120-0104. Studies presented: GS-US-120-0118 and GS-US-311-0101. Boxplot whiskers include data within 1.5 times the interquartile range above and below the box. Circle = outlier; line inside box = median.

Impaired renal function

TAF study GS-US-120-0108

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.

TAF

In severe renal impairment there was a 92% (< 2-fold) higher mean plasma AUCinf, 92% higher AUClast and 79% higher Cmax. 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.

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 AUCinf 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

GS-US-120-0108: Comparison of Plasma TFV Exposures after a Single Dose of TAF 25 mg Versus TDF 300 mg

Severe Renal Impairment (eGFR 15-29 mL/min)	
	TFV AUC (mean [%CV])
TAF 25 mg (n=14)	2070 (47)
TDF-containing Regimens in Normal Renal Function	
Atazanavir/r (n=26)[a]	3940 (30)
Darunavir/r (n=12)[b]	4630 (16)
Fosamprenavir/r (n=15)[c]	2930 (1780, 4280)
Lopinavir/r (n=45)[d]	3500 (27)
Saquinavir/r (n=35)[e]	3110 (24)
Rilpivirine (n=15)[f]	3590 (22)
Emtricitabine+TDF (n=80)[g]	2870 (25)
Efavirenz/Emtricitabine/TDF (n=59)[h]	2270 (19)

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

This open-label study in HIV-infected patients with $eGFR_{CG}$ in the range 30-69 mL/min included an intensive PK/PD sub-study in which plasma was collected ≤ 30 min pre-iohexol and then at 5 min and 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 5, 8 and 24 h post-dose. Single PK samples were also obtained from all patients for use in POPPK analyses. In summary

TAF - TAF PK parameters were consistent with data obtained from HIV-infected patients with normal renal function in E/C/F/TAF studies (mean AUC_{last} 254.2 ng*h/mL compared to 227.5 ng*h/mL, 229.8 ng*h/mL and 259.0 ng*h/mL) as well as from healthy subjects (AUC_{last} 244.8 ng*h/mL). Exposure to TAF was

numerically higher in those with baseline $eGFR_{CG}$ < 50 vs. ≥ 50 mL/min but it was less than the mean AUC_{last} (510.6 ng*h/mL) in subjects with $eGFR_{CG}$ 15-29 mL/min in GS-US-120-0108.

TFV – TFV plasma levels were higher in those with screening $eGFR_{CG}$ 30 to 69 mL/min compared to the HIV-infected patients in E/C/F/TAF studies (mean TFV AUCtau 552.7 ng*h/mL vs. 326.2, 311.8 and 286.2 ng*h/mL; see section 2.1.8 above) but well below the TFV exposure in those with $eGFR_{CG}$ 15-29 mL/min in GS-US-120-0108 (mean AUCinf 2073.8 ng*h/mL) and after TDF-containing regimens (see 2.1.8). As expected, exposure to TFV was higher in those with baseline $eGFR_{CG}$ < 50 vs. ≥ 50 mL/min.

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

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)

Impaired hepatic function

TAF study GS-US-120-0114

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

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 as follows:

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)

The plasma exposure parameters of TAF and TFV were considered to be comparable between subjects with mild hepatic impairment and matched controls. In those with moderate hepatic impairment the plasma exposure parameters for TAF were slightly higher and exposures to TFV were slightly lower vs. matched controls. The differences observed were not considered to be clinically relevant.

TAF plasma protein binding was measured in all subjects 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. TFV plasma protein binding was measured in all subjects 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.

Table 47. GS-US-120-0108: Comparison of Plasma TFV Exposures after a Single Dose of TAF 25 mg Versus TDF 300 mg

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)

Race

In the E/C/F/TAF study **GS-US-292-0108** after multiple dosing:

The AUCs of all analytes were lower in Japanese vs. Caucasian subjects. For FTC the lower bounds of the 90% CI were well below 80% for AUC_{tau} and C_{tau}. For TAF the lower bound of the 90% CI was well below 80% for AUC_{last}. For TFV the lower bounds of the 90% CI were well below 80% for AUC_{tau} and C_{tau}.

Age

Plasma exposures to TAF in adolescents treated with E/C/F/TAF in **GS-US-292-0106** were very similar to those in adults. This open-label study was conducted in 2013-2014 at 9 sites across 4 countries 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. All patients received E/C/F/TAF QD with food, at approximately the same time each day.

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 and confirm use of the adult dose of E/C/F/TAF. Based on cross-study comparisons with HIV-infected adults treated with E/C/F/TAF the data suggested no difference between ARV-naïve adolescents and adults (data from GS-US-292-0102) for TAF or TFV plasma exposures as shown below.

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)

The POPPK predicted values were also comparable between adolescents and adults for TAF and TFV.

Multiple-Dose TAF exposure in ARV-naive adolescents and adults

Age Group	AUC_{last} (ng•h/mL)	C_{max} (ng/mL)
Adolescent Subjects (N = 23)[a]	242.8 (57.8)	121.7 (46.2)
Adult Subjects (N = 539)[b]	206.4 (71.8)	162.2 (51.1)

Multiple-Dose TFV exposure in ARV-naive adolescents and adults

Age Group	AUC_{tau} (ng•h/mL)	C_{max} (ng/mL)	C_{min} (ng/mL)
Adolescent Subjects (N = 23)[a]	275.8 (18.4)	14.6 (20.0)	10.0 (19.6)
Adult Subjects (N = 841) [b]	292.6 (27.4)	15.2 (26.1)	10.6 (28.5)

a Adolescents from Study GS-US-292-0106 b Adults from Studies GS-US-292-0104 and GS-US-292-0111

FTC exposures were also comparable between age groups (noting that it is already approved for use from 4 months of age and use of the adult dose is recommended from 33 kg upwards).

Table 48. GS-US-292-0106: Statistical Comparisons of FTC Plasma PK Parameter Estimates Between Adolescents and Adults (FTC Substudy Analysis Set)

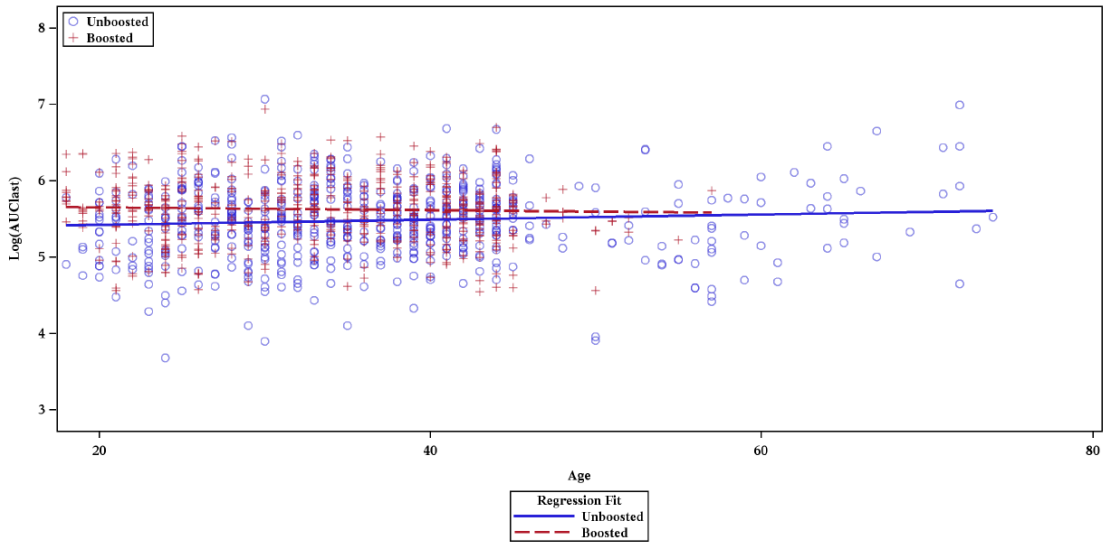
FTC PK Parameter	GLSM		%GLSM Ratio (90% CI) Test/Reference
	GS-US-292-0106 (Test) (N = 24)	GS-US-292-0102, GS-US-292-0103 (Reference) (N = 52)	
AUC_{tau} (ng•h/mL)	14007.48	11964.30	117.08 (106.68, 128.49)
C_{max} (ng/mL)	2209.14	1947.42	113.44 (103.49, 124.35)
C_{trough} (ng/mL)[a]	94.98	97.42	97.49 (83.42, 113.94)

GLSM = geometric least-squares mean

a N = 23 for the test group

In the POPPK analysis of TAF (E/C/F/TAF in HIV-infected patients), which consisted of 915 aged 18 to < 55 years and 197 aged ≥ 55 years of age, age was not a covariate and did not have an effect on TAF exposures. In addition, the individual estimates of AUClast showed no notable trend of TAF exposures with increasing age, as shown in the scatter plot from the integrated ad hoc PK dataset.

Figure 10. Scatter Plot of AUClast Versus Age from Ad Hoc PK Analysis Dataset



Pharmacokinetic interaction studies

Cytochrome P450 inhibition

The inhibitory activity of **TAF** with human liver microsomal CYP isoenzymes (CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6 and CYP3A) was assessed at concentrations up to 25 µM. TAF showed weak inhibition of CYP3A-mediated oxidation of midazolam or testosterone with IC_{50} values of 7.6 or 7.4 µM, respectively.

The potential for **TAF** to be a mechanism-based inhibitor of human CYP enzymes (CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19 and CYP2D6) was assessed at 50 µM. There was no evidence for time- or cofactor-dependent inhibition of any enzyme by TAF. The maximum change in activity observed was 17.4% with CYP2C8 relative to control.

TFV at 100 µM did not inhibit CYP1A2, CYP2C9, CYP2D6, CYP2E1 or CYP3A.

Cytochrome P450 induction

CYP induction was assessed from mean fold increases in mRNA in cultured human hepatocytes from 3 separate donors treated with 1, 10, and 100 µM **TAF** once daily for 3 consecutive days. 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 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, which correspond to 3% and 6% of the induction levels observed with respective positive controls. TAF showed little or no potential for CYP induction at clinically relevant concentration (1 µM).

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 did not inhibit UGT1A1 up to 50 µM. There was no significant induction of P-gp and UGT1A1 mRNA on exposure to TAF.

TAF is a substrate for P-gp and BCRP. An increase in TAF absorption was observed in the presence of efflux transport inhibitors CsA or COBI. 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 affecting activities.

TAF was not a substrate for renal transporters OAT1 and OAT3.

TFV

Active tubular secretion of TFV is mediated by human OAT1 and MRP4 acting in series in proximal tubules. Human OAT3 may play a secondary role in the tubular uptake of TFV.

Under physiologically relevant conditions, none of the tested drugs affected OAT1-mediated transport of TFV.

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

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

Tenofovir did not inhibit human OCT2 or MATE1 (IC50 > 300 µM).

Other relevant information

There is no evidence for inhibition of TFV renal excretion by FTC. 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.

In vivo

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

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

This open-label crossover study evaluated co-administration of FTC/TAF with EFV or DRV/co and the effects of co-administration of TAF and COBI. There were no washout periods within each cohort and treatments were administered for 10 or 12 days. Cohorts were treated as follows.

Treatments by Cohort		
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 or Cmax compared with FTC/TAF (40 mg) dosed alone based on the pre-defined acceptance criteria but they were all lower in the presence of EFV. The applicant considered that the differences were not clinically meaningful.

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)

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)

FTC 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)	10,339.5 (16.8)	11,251.2 (14.7)	91.63	(87.38, 96.09)
C_{max} (ng·h/mL)	2344.7 (22.5)	2643.7 (26.7)	89.66	(81.30, 98.86)
C_{tau} (ng/mL)	59.5 (20.6)	64.7 (20.1)	91.94	(86.05, 98.22)

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. There was an increase in TAF AUClast and Cmax following single doses, suggesting an inhibitory drug interaction giving increased availability of TAF that abated following multiple dosing. The increase in TFV but not TAF exposures after multiple-dose co-administration was thought to be due to a mixed inhibitory/inductive effect of COBI on P-gp, influencing TAF absorption.

GS-7340 PK Parameter	Test Mean (%CV)	Reference Mean (%CV)	GLSM Ratio (Test/Reference) (%)	90% Confidence Interval
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)

TFV PK Parameter	Test Mean (%CV)	Reference Mean (%CV)	GLSM Ratio (Test/Reference) (%)	90% Confidence Interval
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)

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** COBI exposures were in the range of historical data (GS-US-216-0112 and 0124). Co-administration of TAF 8 mg plus COBI 150 mg resulted in substantially higher TAF and TFV exposures relative to TAF 8 mg dosed alone. The effects of COBI on TAF and TFV were consistent with the data from GS-US-292-0101, in which E/C/F/TAF was compared with TAF alone. The applicant ascribed the effect to COBI-mediated inhibition of P-gp-mediated intestinal secretion of TAF.

GS-7340 PK Parameter	Test Mean (%CV)	Reference Mean (%CV)	GLSM Ratio (Test/Reference) (%)	90% Confidence Interval
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)

TFV PK Parameter	Test Mean (%CV)	Reference Mean (%CV)	GLSM Ratio (Test/Reference) (%)	90% Confidence Interval
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-299-0101

Study Title: A Phase 1, Adaptive-Design, Multiple-Dose Study Evaluating the Bioavailability of Three Formulations of Darunavir/Cobicistat/Emtricitabine/GS-7340 Single Tablet Regimen (STR) Relative to the Administration of Individual Components Cobicistat-Boosted Darunavir, Emtricitabine, and GS-7340

GS-US-299-0101 was in three parts of which Parts 1 and 3 provide the data of some relevance for use of F/TAF 200/10 and 200/25 mg tablets.

Part 1 was primarily designed to identify a possible formulation for a STR containing DRV/co plus F/TAF using either 10 or 25 mg. Subjects were randomised (1:1:1 ratio) to one of:

A: F1 monolayer tablet containing DRV 800 mg/COBI 150 mg/FTC 200 mg/TAF **25 mg**

B: **F2 bilayer** tablet containing DRV 800 mg/COBI 150 mg/FTC 200 mg/TAF **25 mg**

C: F3 monolayer tablet containing DRV 800 mg/COBI 150 mg/FTC 200 mg/TAF **10 mg**

Plasma exposures to TAF and TFV were proportionately lower with F3 (C), were highest on day 1 and did not change between days 6 and 21 (TAF) or days 12 and 21 (TFV). DRV, COBI and FTC plasma concentrations were similar between the three formulations. Using a non-validated LC-MS/MS assay the Day 12 TFV-DP AUC_{0-12} values as measured in PBMCs were higher with F1 and F2 vs. F3 and were within a range associated with antiviral activity in the TAF monotherapy study GS-US-120-0104.

GS-US-299-0101: Summary of Tenofovir DP in PBMCs, Multiple- Dose PK Parameters (Part 1, Day 12; Tenofovir-DP PK Analysis Set)

	DRV/COBI/FTC/GS-7340 STR		
Tenofovir-DP PK Parameter	**Formulation 1 (N = 10)**	**Formulation 2 (N = 10)**	**Formulation 3 (N = 10)**
AUC_{0-12} (µM•h)	22.8 (46.1)	31.3 (55.0)	9.3 (61.9)

Note: Data are presented as mean (%CV).

Note: Formulation 1 was a monolayer tablet with 25 mg GS-7340, Formulation 2 was a bilayer tablet with 25 mg GS-7340,and Formulation 3 was a monolayer tablet with 10 mg GS-7340.

Part 3 had 2 cohorts, each of crossover design:

Cohort 1 received C as above and separately received DRV 800 mg + COBI 150 mg

Cohort 2 received C as above and separately received FTC 200 mg + **TAF 25 mg**

All dosing was after completion of a 400 kcal meal containing 13 g fat.

In Part 3 Cohort 1 bioequivalence was demonstrated for DRV and COBI.

In Part 3 Cohort 2

TAF plasma concentrations were lower following treatment C vs. FTC + TAF, with a difference that was similar on days 1 and 24 after single or multiple dosing. In contrast, TFV plasma concentrations were higher following multiple (but not single) once-daily administrations of treatment C vs. FTC + TAF. Nevertheless, TFV exposures were ~88% lower than observed following DRV/co + TVD in Part 2 (GLSM AUC_{tau} 3352 ng.h/mL and Cmax 342 ng/mL).

Table 49. GS-US-299-0101: Statistical Analysis of GS-7340 Pharmacokinetics Parameters (Part 3, Cohort2: GS 7340 PK Analysis Set)

GS-7340 PK Parameter	Geometric Least-Squares Mean		Geometric Least-Squares Means Ratio (%) (90% CI)
	Test DRV/COBI/FTC/GS-7340 STR Formulation 3 (N = 16)	Reference FTC + GS-7340 (N = 16)	
AUC_{last} (ng•h/mL)	160.27	307.63	52.10 (46.53, 58.33)
C_{max} (ng/mL)	171.96	291.70	58.95 (48.30, 71.95)

Table 50. GS-US-299-0101: Statistical Analysis of Tenofovir Pharmacokinetics Parameters (Part 3, Cohort2: Tenofovir PK Analysis Set)

Tenofovir PK Parameter	Geometric Least-Squares Mean		Geometric Least-Squares Means Ratio (%) (90% CI)
	Test DRV/COBI/FTC/GS-7340 STR Formulation 3 (N = 16)	Reference FTC + GS-7340 (N = 16)	
AUC_{tau} (ng•h/mL)	413.85	276.24	149.81 (143.48, 156.43)
C_{max} (ng/mL)	23.89	16.92	141.21 (134.20, 148.59)
C_{tau} (ng/mL)	14.35	9.49	151.16 (143.55, 159.18)

FTC exposures were higher following treatment C vs. FTC + TAF. Although the GLSM values and 90% CI were all > 100% the upper limit of 125% was not exceeded.

Table 51. GS-US-299-0101: Statistical Analysis of Emtricitabine Pharmacokinetics Parameters (Part 3, Cohort2: Emtricitabine PK Analysis Set)

Emtricitabine PK Parameter	Geometric Least-Squares Mean		Geometric Least-Squares Means Ratio (%) (90% CI)
	Test DRV/COBI/FTC/GS-7340 STR Formulation 3 (N = 16)	Reference FTC + GS-7340 (N = 16)	
AUC_{tau} (ng•h/mL)	13,198.61	11,381.87	115.96 (112.94, 119.07)
C_{max} (ng/mL)	2329.91	2152.89	108.22 (103.06, 113.64)
C_{tau} (ng/mL)	91.95	76.67	119.93 (112.86, 127.43)

As in GS-US-311-0101, the TAF AUC_{last} and Cmax were higher (~30% to 50%) following a single dose of each STR formulation vs. multiple doses, which the applicant considers is consistent with a mixed inhibitory/inductive effect on TAF absorption.

The TFV exposures with treatment C (10 mg TAF) were in the range of those observed after TAF 25 mg given as a single agent. The applicant considered that the substantial increase in TFV exposures without a change in TAF exposures after multiple-doses reflected a mixed inhibitory/inductive effect on P-gp influencing TAF absorption. The decrease in TAF levels following multiple doses was thought likely to be due to an inductive effect of DRV on P-gp.

Having selected treatment C for further development, the comparison with FTC + TAF 25 mg was considered to support the FDC since, although TAF exposures were lower after the FDC, they were in a range associated with antiviral activity in the monotherapy study (GS-US-120-0104).

GS-US-120-0118

Study Title: A Pharmacokinetic Study Evaluating the Drug Interaction Potential of Tenofovir Alafenamide with a Boosted Protease Inhibitor or Unboosted Integrase Inhibitor in Healthy Subjects

This was an open-label study in which 40 healthy subjects (10 per cohort) received the following treatments once daily with a moderate fat meal:

Treatment A:	FTC 200 mg + TAF 10 mg
Treatment B:	ATV 300 mg + RTV 100 mg
Treatment C:	DRV 800 mg + RTV 100 mg
Treatment D:	4 × LPV/r 200/50 mg
Treatment E:	DTG 50 mg
Treatment F (same as A):	FTC 200 mg + TAF 10 mg

FTC plasma levels were not measured in this study.

TAF AUCs and Cmax were higher following FTC+TAF plus ATV/r (cohort 1) or LPV/r (cohort 3) vs. FTC+TAF alone. ATV/r had the greater effect on TAF AUCs. LPV/r had the greater effect on Cmax.

Mean TAF AUCs after FTC+TAF alone were generally comparable with those when it was given in the presence of DRV/r (cohort 2) but Cmax was higher on co-administration and all 90% CI suggested slightly greater exposures on co-administration with DRV/r.

Mean TAF AUCs and Cmax after FTC+TAF plus DTG were higher vs. dosing alone (cohort 4).

The applicant ascribed the increases in TAF exposure seen with ATV/r and LPV/r to the known effect of RTV as P-gp inhibitor. The effect of DRV/r was ascribed to the inductive effect of DRV combined with the inhibitory effect of RTV on P-gp. It was concluded that F/TAF 200/10 mg should be used with these boosted PIs. The applicant considered that there was no effect of DTG on TAF and therefore F/TAF 200/25 mg should be used in conjunction with INSTIs and with other classes not known to have an effect on P-gp, such as NNRTIs.

Mean TFV AUC and C_{max} were higher on co-administration in each cohort but the greatest effect was observed with LPV/r (cohort 3) and the least with DTG (cohort 4).

Table 52. GS-US-120-0118: Statistical Comparisons of TAF Plasma Pharmacokinetic Parameter Estimates between Test and Reference Analysis Set (Analysis Set: All PKs)

	GLSMs by Treatment			
	Cohort 1			
TAF PK Parameter	FTC+TAF+ATV/r (Test) (N = 10)	FTC+TAF (Reference) (N = 10)	GLSM Ratio (%)	90% CI (%)
AUC_{inf} (ng•h/mL)	162.62	86.08	188.92	(155.37, 229.71)
AUC_{last} (ng•h/mL)	160.28	83.89	191.06	(155.08, 235.40)
C_{max} (ng/mL)	130.85	74.04	176.72	(128.19, 243.63)
	Cohort 2			
	FTC+TAF+DRV/r (Test) (N = 10)	FTC+TAF (Reference) (N = 10)		
AUC_{inf} (ng•h/mL)	76.73	73.54	104.34	(84.14, 129.39)
AUC_{last} (ng•h/mL)	74.76	70.35	106.27	(83.59, 135.10)
C_{max} (ng/mL)	91.16	64.29	141.80	(96.11, 209.22)
	Cohort 3			
	FTC+TAF+LPV/r (Test) (N = 10)	FTC+TAF (Reference) (N = 10)		
AUC_{inf} (ng•h/mL)	113.27	78.25	144.75	(114.15, 183.55)
AUC_{last} (ng•h/mL)	111.07	75.70	146.73	(116.60, 184.65)
C_{max} (ng/mL)	145.42	66.41	218.97	(171.88, 278.97)
	Cohort 4			
	FTC+TAF+DTG (Test) (N = 9)	FTC+TAF (Reference) (N = 10)		
AUC_{inf} (ng•h/mL)	106.61	91.42	116.62	(93.49, 145.48)
AUC_{last} (ng•h/mL)	105.29	88.47	119.02	(95.83, 147.82)
C_{max} (ng/mL)	81.74	66.11	123.64	(87.79, 174.13)

Mean plasma ATV and LPV C_{max}, C_{tau} and AUC values were not affected by co-administration with FTC+TAF with all 90% CI within 80, 125% and spanning 100%.

Similarly, mean plasma DRV C_{max} and AUC were not affected by co-administration with FTC+TAF while C_{tau} was slightly higher (113% [95%–134%]).

Also, DTG C_{tau} and AUC were unaffected by FTC + TAF while C_{max} was slightly lower (87% [79%–96%]).

Table 53. GS-US-120-0118: Statistical Comparisons of TFV Plasma Pharmacokinetic Parameter Estimates Between Test and Reference Analysis Set (Analysis Set: All PKs)

TFV PK Parameter	GLSMs by Treatment		GLSM Ratio (%)	90% CI (%)
	Cohort 1			
	FTC+TAF+ATV/r (Test) (N = 10)	FTC+TAF (Reference) (N = 10)		
AUC_{inf} (ng•h/mL)	278.69	106.54	261.59	(213.95, 319.84)
AUC_{last} (ng•h/mL)	100.74	40.66	247.77	(216.82, 283.14)
C_{max} (ng/mL)	8.62	4.06	212.35	(185.83, 242.65)
	Cohort 2			
	FTC+TAF+DRV/r (Test) (N = 10)	FTC+TAF (Reference) (N = 10)		
AUC_{inf} (ng•h/mL)	254.44	124.35	204.61	(153.78, 272.25)
AUC_{last} (ng•h/mL)	102.99	42.43	242.74	(207.17, 284.41)
C_{max} (ng/mL)	9.03	3.74	241.54	(198.10, 294.51)
	Cohort 3			
	FTC+TAF+LPV/r (Test) (N = 10)	FTC+TAF (Reference) (N = 10)		
AUC_{inf} (ng•h/mL)	400.79	96.26	416.36	(349.56, 495.93)
AUC_{last} (ng•h/mL)	128.10	39.78	322.01	(298.02, 347.93)
C_{max} (ng/mL)	12.33	3.29	374.52	(319.28, 439.30)
	Cohort 4			
	FTC+TAF+DTG (Test) (N = 9)	FTC+TAF (Reference) (N = 10)		
AUC_{inf} (ng•h/mL)	113.98	91.23	124.94	(106.46, 146.62)
AUC_{last} (ng•h/mL)	42.73	40.99	104.25	(98.74, 110.08)
C_{max} (ng/mL)	3.77	3.43	109.91	(96.39, 125.32)

GS-US-311-1388

Study Title: A Fixed-Sequence, Open-Label, 3-Period Cross-Over Pharmacokinetic Study Evaluating the Drug Interaction Potential between Emtricitabine/Tenofovir Alafenamide Fixed Dose Combination Tablet and Atazanavir Boosted by Cobicistat in Healthy Subjects

Study data became available during the evaluation. In this multiple dose study F/TAF 10 mg and ATV/co (300/150 mg; co-administered as separate tablets) were each given alone and together once daily with food for periods of 6-7 days. The PK data were as follows:

Table 54. Statistical Comparisons PK parameters for TAF, TFV, and FTC following administration F/TAF alone or ATV+COBI in combination with F/TAF

PK Parameter	GLSMs by Treatment		%GLSM Ratio (90% CI)
	Test: ATV+COBI+F/TAF (N = 20)	Reference: F/TAF (N = 20)	
TAF			
AUC_{last} (h•ng/mL)	182.21	104.08	175.06 (154.81, 197.96)
C_{max} (ng/mL)	133.52	74.28	179.76 (148.45, 217.67)
TFV			
AUC_{tau} (h•ng/mL)	340.26	97.92[a]	347.49 (329.34, 366.65)
C_{tau} (ng/mL)	12.37	3.31	373.16 (353.91, 393.45)
C_{max} (ng/mL)	18.43	5.83	315.98 (299.75, 333.10)
FTC			
AUC_{last} (h•ng/mL)	11,576.49	10,023.15	115.50 (110.68, 120.53)
C_{tau} (ng/mL)	90.87	66.52	136.62 (131.55, 141.89)
C_{max} (ng/mL)	1844.78	1696.16	108.76 (100.15, 118.11)

GLSM = geometric least-squares mean

a n = 19

Co-administration with ATV/co increased the plasma exposures to both TAF (~1.75-fold) and TFV (~3.5-fold) as expected from strong intestinal P-gp inhibition by COBI.

It is pertinent to note that in the previously reported DDI study GS-US-120-0118 FTC 200 mg + TAF 10 mg were given alone on Day 1 and also co-administered with ATV/r (300/100 mg) on day 15, after 13 days dosing with ATV/r alone. All dosing was in the fed state. FTC was not measured in this study. As reported above, the TAF and TFV AUCs and Cmax were higher following FTC+TAF plus ATV/r vs. FTC+TAF alone. The magnitude of effect of RTV on TAF AUC and Cmax was very similar to that exerted by COBI above and the effect of RTV on TFV was greater than for TAF as was observed for COBI.

GS-US-120-1538

Study Title: A Fixed-Sequence, Open-Label, Study Evaluating the Pharmacokinetics and Drug Interaction Potential between Tenofovir Alafenamide and Midazolam (Oral and Intravenous) in Healthy Volunteers

This study evaluated co-administration of TAF with oral and IV midazolam dosed after standard breakfasts:

Treatment A (Day 1): MDZ 2.5 mg oral syrup

Treatment B (Day 3): MDZIV 1 mg IV over 1 min

Treatment C (Days 4-15 and 17): TAF 25-mg tablet

Treatment D (Day 16): TAF 25-mg tablet + MDZ 2.5 mg oral syrup

Treatment E (Day 18): TAF 25 mg tablet + MDZ 1 mg IV over 1 min

TAF had no significant effect on plasma MDZ or 1'OH-MDZ after oral dosing based on 90% CI that fell within 80, 125%. The 90% CI for MDZ AUCs were slightly higher on co-administration (did not span 100%) but the metabolite was not similarly affected.

TAF had no significant effect on plasma MDZ or 1'OH-MDZ after IV dosing based on 90% CI that fell within 80, 125% but the 90% CI for MDZ and metabolite AUCs did not span 100%.

There was 25% to 30% decrease in TAF Cmax (25% to 30%) after co-administration with oral or IV MDZ, which the Company proposed may reflect an effect of MDZ on gastrointestinal motility. The AUCs were also slightly lower on co-administration but relatively less affected (~10-15% decreases).

GS-US-120-1554 – Multiple doses of TAF + RPV

Study title: A Fixed-Sequence, Randomized, Open-Label, 2-Cohort, 2-Period, Multiple-Dose Study Evaluating the Pharmacokinetics and Drug Interaction Potential between Tenofovir Alafenamide and Rilpivirine in Healthy Subjects

RPV is primarily metabolised by CYP3A but is expected to induce CYP3A4 at 25 mg QD. It also inhibits P-gp *in vitro* but did not affect PK of digoxin *in vivo*. This study was in two cohorts who received either TAF 25 mg or RPV 25 mg followed by co-administration after standardised breakfasts.

Plasma PK parameters for TAF were unaffected by co-administration for 14 days in that 90% CI around Cmax and AUC ratios all fell within 80, 125% and spanned 100%. For TFV the 90% CI fell within 80, 125% but all exceeded 100%, indicating slightly higher exposures on co-administration. The RPV AUC was unaffected by co-administration whilst Cmax was slightly lower and Ctau was slightly higher.

Table 55. GS-US-120-1554: Statistical Comparisons of RPV Plasma PK Parameter Estimates by ANOVA Model (RPV PK Analysis Set)

RPV PK Parameter	GLSM by Treatment				Statistical Comparison	
	TAF+RPV (Test)		RPV (Reference)		GLSM Ratio (%)	90% CI (%)
	N	GLSM	N	GLSM		
TAF+RPV (Treatment C) vs RPV (Treatment B)						
AUC_{tau} (h*ng/mL)	32	2926.33	16	2889.75	101.27	96.42, 106.36
C_{max} (ng/mL)	32	193.24	16	207.98	92.91	87.44, 98.72
C_{tau} (ng/mL)	32	116.44	16	103.25	112.77	103.58, 122.77

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

Study Title: A Phase 1, Open-Label, Fixed Sequence Study Evaluating the Pharmacokinetics and Drug Interaction Potential Between Elvitegravir/Cobicistat/Emtricitabine/Tenofovir Alafenamide Single-Table Regimen and Sertraline in Healthy Subjects

SER is eliminated by several CYP450 enzymes including CYP2D6, CYP2C9, CYP2B6, CYP2C19 and CYP3A4. The percentage contribution of each isoform has been estimated to be ~35%, 29%, 14%, 13% and 9%, respectively. COBI is an inhibitor of CYP3A (major) and CYP2D6 (minor). Dosing was after standardised breakfasts as follows:

A: SER 50 mg tablet, single dose on day 1

B: E/C/F/TAF (10 mg) once daily for 12 days on days 2-14

C: SER 50 mg single dose on day 14 (i.e. with the last dose of E/C/F/TAF 10 mg)

Co-administration had no clinically relevant effect on the PK of EVG, COBI, FTC, TAF, TFV or SER. All except two comparisons gave 90% CI that fell within 80, 125% and in the two exceptions (COBI C_{tau} and SER $AUC_{0-\infty}$) the lower boundary was only just below 80%.

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

Study Title: A Phase 1 Study to Evaluate the Pharmacokinetic Drug-Drug Interactions between Sofosbuvir/GS-5816 Fixed-Dose Combination (FDC) Tablet and Antiretrovirals Efavirenz/Emtricitabine/Tenofovir Disoproxil Fumarate (EFV/FTC/TDF; Atripla), Emtricitabine/Rilpivirine/Tenofovir Disoproxil Fumarate (FTC/RPV/TDF; Complera), Dolutegravir (DTG; Tivicay), or Elvitegravir/Cobicistat/Emtricitabine/Tenofovir Alafemamide Fumarate (EVG/COBI/FTC/TAF) in Healthy Subjects

This was an open-label, multiple-dose, 4-cohort study to evaluate co-administration of SOF/VEL (400 mg/100 mg tablets) with each of EFV/FTC/TDF (Atripla), FTC/RPV/TDF (Eviplera), dolutegravir (DTG) and E/C/F/TAF (10 mg).

Only Cohort 4 has some potential relevance to F/TAF. This cohort was dosed within 5 minutes of completing breakfast (~ 600 kcal, 25-30% fat) and with randomised order of treatments as follows:

J: SOF/VEL once daily for 8 days

K: E/C/F/TAF once daily for 8 days

L: SOF/VEL plus E/C/F/TAF once daily for 8 days

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

Exposures to EVG and FTC were not altered following co-administration with SOF/VEL.

There was ~100% increase in COBI C_{tau} when E/C/F/TAF was given with SOF/VEL. Due to the relatively short COBI $t_{1/2}$ (~3.5 h) there was no impact on AUC COBI.

VEL 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.

The applicant concluded that SOF/VEL may be administered with E/C/F/TAF 10 mg.

GS-US-366-1689 F/R/TAF plus sofosbuvir (SOF) and ledipasvir (LDV)

Study title: A Phase 1 Study to Evaluate Pharmacokinetic Drug-Drug Interaction Potential between Emtricitabine/Rilpivirine/Tenofovir Alafenamide Fumarate (FTC/RPV/TAF) and Ledipasvir/Sofosbuvir (LDV/SOF)Fixed-Dose Combination (FDC) Tablets

This was an open label, multiple-dose, 3-way cross-over study to evaluate the potential for interactions between F/R/TAF and SOF, its metabolites (GS-566500 and GS-311007) or LDV. Healthy subjects (42) were randomised to one of six treatment sequences and received the following 3 treatments:

A: LDV/SOF 1 × 90/400 mg tablet once daily under fed conditions in the morning

B: FTC/RPV/TAF 1 × 200/25/25 mg tablet once daily under fed conditions in the morning

C: LDV/SOF + FTC/RPV/TAF using the two FDCs as above under fed conditions in the morning

Each treatment in the sequence was taken for 11 days with no washout periods.

On the days of intensive PK sampling (Days 11, 22 and 33) study drug was administered in the morning following an overnight fast and within 5 minutes of completing a standardised breakfast (approximately 600 kcal and 27% fat). On non-intensive PK days study drug was administered following at least a 6 hour fast and within 5 minutes of completion of a standardised moderate fat breakfast.

Co-administration with LDV/SOF did not notably affect the PK of FTC or RPV. Compared to FTC/RPV/TAF alone co-administration led to increases in TAF and TFV AUC_{tau} of 32% and 75%, respectively. TFV Cmax was increased by 62% and C_{tau} by 85% but there was no effect on TAF Cmax. Co-administration with FTC/RPV/TAF did not notably affect the PK of LDV or SOF (including its metabolites GS-566500 or GS-331007). The applicant discussed that the increase in TFV exposures of approximately 75% was consistent with the mechanistic understanding of the interaction between the P-gp inhibitor LDV and the P-gp substrate TAF. It was pointed out that the mean TFV AUC_{tau} on co-administration (467 ng*hr/mL) is approximately 5 times lower than TFV exposure following dosing with TDF (AUC_{tau} 2290 ± 690 ng*hr/mL). As such, the differences in overall TFV exposure were not considered to be clinically relevant and no dose adjustment of FTC/RPV/TAF is proposed.

Table 56. Statistical analysis of mean and percentage coefficient of variation (%CV) PK parameters for FTC, RPV, TAF, TFV, SOF, GS-566500, GS-331007, and LDV following administration FTC/RPV/TAF and LDV/SOF alone or in combination

PK Parameter	Mean (%CV)		%GLSM Ratio (90% CI)
	FTC/RPV/TAF (Treatment B) (N = 42)	LDV/SOF+FTC/RPV/TAF (Treatment C) (N = 42)	
FTC			
AUC_{tau} (h*ng/mL)	10,764.1 (14.3)	10,805.1 (15.3)	100.29 (98.43,102.19)
C_{max} (ng/mL)	1707.6 (20.2)	1650.3 (17.6)	97.02 (92.72,101.53)
C_{tau} (ng/mL)	87.9 (28.2)	88.7 (25.1)	101.59 (98.47,104.81)
RPV			
AUC_{tau} (h*ng/mL)	3040.1 (27.3)	2857.6 (25.6)	94.59 (91.20,98.10)
C_{max} (ng/mL)	203.3 (25.4)	197.1 (28.3)	96.65 (91.73,101.84)
C_{tau} (ng/mL)	109.1 (31.6)	100.0 (26.0)	93.33 (89.38,97.45)
TAF			
AUC_{last} (h*ng/mL)	277.2 (37.5)	362.3 (34.4)	132.39 (124.99,140.22)
C_{max} (ng/mL)	200.0 (43.5)	204.5 (45.7)	103.12 (93.58,113.63)
TFV			
AUC_{tau} (h*ng/mL)	268.4 (22.6)	467.2 (21.0)	174.72 (168.78,180.86)
C_{max} (ng/mL)	15.8 (21.7)	25.4 (20.0)	161.50 (155.60,167.62)
C_{tau} (ng/mL)	9.0 (24.8)	16.7 (22.0)	184.86 (177.57,192.46)

PK Parameter	Mean (%CV)		%GLSM Ratio (90% CI)
	LDV/SOF (Treatment A) (N = 41)	LDV/SOF+FTC/RPV/TAF (Treatment C) (N = 42)	
SOF			
AUC_{tau} (h*ng/mL)	2909.4 (32.8)	3068.9 (30.5)	104.69 (100.51,109.04)
C_{max} (ng/mL)	1469.5 (35.4)	1390.6 (32.3)	95.99 (88.80,103.76)
GS-566500			
AUC_{tau} (h*ng/mL)	2504.0 (16.4)	2575.4 (16.2)	102.05 (99.34,104.83)
C_{max} (ng/mL)	510.2 (20.1)	502.2 (18.2)	99.03 (95.24,102.97)
GS-331007			
AUC_{tau} (h*ng/mL)	11,766.4 (12.8)	12,883.3 (16.1)	107.98 (106.20,109.79)
C_{max} (ng/mL)	884.4 (13.7)	960.4 (14.8)	108.09 (105.05,111.20)
C_{tau} (ng/mL)	339.8 (16.4)	378.1 (18.9)	109.92 (107.46,112.44)
LDV			
AUC_{tau} (h*ng/mL)	11,590.4 (40.3)	11,944.8 (42.7)	101.53 (97.36, 105.88)
C_{max} (ng/mL)	647.4 (35.8)	658.4 (37.7)	100.62 (96.76, 104.63)
C_{tau} (ng/mL)	419.7 (45.9)	434.3 (47.4)	102.40 (98.02, 106.99)

GS-US-337-1624 - E/C/F/TAF plus sofosbuvir (SOF) and ledipasvir (LDV)

Study title: A Phase 1 Study to Evaluate Pharmacokinetic Drug-Drug Interaction Potential between Ledipasvir/Sofosbuvir (LDV/SOF) Fixed-Dose Combination (FDC) Tablet and HIV Antiretroviral Regimen Elvitegravir/Cobicistat/Emtricitabine/Tenofovir Alafenamide (E/C/F/TAF) FDC Tablet

This was a randomised, open label, multiple-dose study to assess co-administration of the LDV/SOF and E/C/F/TAF FDCs. Healthy subjects were randomized to 1 of 6 treatment sequences and received the following 3 treatments for 10 days each, all administered with a moderate far meal in the morning:

- **LDV/SOF:** LDV/SOF (90 mg/400 mg tablet once daily) (Treatment A)
- **E/C/F/TAF:** E/C/F/TAF (150 mg/150 mg/200 mg/10 mg tablet once daily) (Treatment B)
- **LDV/SOF+E/C/F/TAF:** A+B (Treatment C)

Following co-administration there were increases in SOF (28% to 47%) and GS-331007 (48% to 66%) primary PK parameters were observed relative to administration of LDV/SOF alone. The 90% CIs for the GLSM ratios for GS-566500 PK parameters were contained within the protocol predefined lack of PK alteration boundaries. Co-administration resulted in higher LDV AUC_{tau}, Cmax and C_{tau} of approximately 79%, 65%, and 93%, respectively, consistent with intestinal P-gp and/or BCRP inhibition by COBI.

These effects on LDV/SOF are consistent with those observed following co-administration of LDV/SOF+EVG+COBI. The exposures observed were within the exposure-safety range, as defined by the PK/PD analyses from the LDV/SOF clinical development programme. Following co-administration no effect of LDV/SOF was observed on FTC, TAF and TFV. There was no effect on EVG AUCtau and Cmax but Ctau was approximately 46% higher. There was also no effect on COBI Cmax but AUCtau and Ctau were approximately 53% and 225% higher.

The higher COBI exposure was not considered to be clinically relevant since prior data indicated no association between COBI exposure and the incidence of common AEs or renal function parameters. Additionally, the Ctau and AUCtau are not expected to introduce further drug interaction potential based on prior study of the dose/exposure-response relationship between COBI and CYP3A inhibition. Thus, no dose adjustment of E/C/F/TAF or LDV/SOF was thought necessary when they are co-administered.

Table 57. Statistical analysis of mean and percentage coefficient of variation PK parameters for LDV, SOF, GS-566500, GS-331007, EVG, COBI, FTC, TAF, and TFV following the administration E/C/F/TAF and LDV/SOF alone or in combination

PK Parameter	Mean (%CV)		%GLSM Ratio (90% CI)
	LDV/SOF FDC (N = 30)	LDV/SOF + E/C/F/TAF (N = 30)	
LDV			
AUC_{tau} (h*ng/mL)	12,657.2 (36.26)	22,939.3 (35.71)	178.99 (163.62, 195.81)
C_{max} (ng/mL)	684.2 (32.25)	1136.0 (32.14)	164.74 (152.60, 177.86)
C_{tau} (ng/mL)	459.2 (40.34)	896.1 (37.74)	193.16 (173.91, 214.55)
SOF			
AUC_{tau} (h*ng/mL)	2336.2 (37.40)	3308.4 (24.84)	146.53 (135.44, 158.53)
C_{max} (ng/mL)	1220.3 (37.87)	1573.2 (34.88)	128.48 (112.51, 146.72)
GS-566500			
AUC_{tau} (h*ng/mL)	2541.0 (18.04)	2826.7 (16.16)	111.64 (107.85, 115.57)
C_{max} (ng/mL)	516.8 (23.42)	556.3 (21.87)	107.78 (99.46, 116.79)
GS-331007			
AUC_{tau} (h*ng/mL)	12,611.4 (20.91)	18,739.0 (22.60)	148.15 (143.54, 152.91)
C_{max} (ng/mL)	950.9 (18.74)	1228.7 (18.57)	129.36 (123.92, 135.04)
C_{tau} (ng/mL)	358.7 (24.66)	599.5 (27.40)	165.96 (159.61, 172.57)

PK Parameter	Mean (%CV)		%GLSM Ratio (90% CI)
	E/C/F/TAF (N = 30)	LDV/SOF + E/C/F/TAF (N = 30)	
EVG			
AUC_{tau} (h*ng/mL)	26,888.6 (22.74)	29,750.7 (23.26)	110.62 (102.40, 119.50)
C_{max} (ng/mL)	2184.3 (23.29)	2155.3 (27.49)	97.85 (89.57, 106.91)
C_{tau} (ng/mL)	513.3 (40.06)	731.3 (35.57)	145.70 (127.99, 165.86)
COBI			
AUC_{tau} (h*ng/mL)	11,354.2 (36.34)	17,046.0 (30.36)	153.17 (144.87, 161.95)
C_{max} (ng/mL)	1381.4 (25.98)	1668.0 (17.14)	123.35 (115.35, 131.91)
C_{tau} (ng/mL)	56.1 (99.59)	175.2 (90.42)	325.08 (287.62, 367.43)
FTC			
AUC_{tau} (h*ng/mL)	12,035.7 (15.39)	11,622.9 (15.53)	96.59 (93.22, 100.08)
C_{max} (ng/mL)	1777.0 (18.40)	1840.3 (22.12)	103.04 (95.93, 110.67)
C_{tau} (ng/mL)	103.5 (26.84)	98.5 (27.24)	95.02 (90.82, 99.42)
TAF			
AUC_{last} (h*ng/mL)	238.6 (45.87)	194.8 (29.21)	85.67 (77.63, 94.54)
C_{max} (ng/mL)	165.9 (50.79)	148.2 (48.24)	90.45 (73.41, 111.45)
TFV			
AUC_{tau} (h*ng/mL)	314.8 (18.68)	396.8 (15.90)	126.61 (122.55, 130.81)
C_{max} (ng/mL)	17.8 (20.43)	20.7 (16.14)	116.85 (111.71, 122.23)
C_{tau} (ng/mL)	11.7 (20.05)	15.5 (16.62)	132.89 (127.74, 138.25)

2.4.3. Pharmacodynamics

Mechanism of action

TAF is predominantly hydrolysed to TFV by Cathepsin A (CatA) cleavage in target lymphoid cells. TFV (which is itself a monophosphate) is then metabolised to the active moiety TFV diphosphate (TFV-DP). TFV-DP is a competitive inhibitor of HIV-1 RT. In T-cells, macrophages and PBMCs TAF EC50 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. For TAF intracellular activation pathways in lymphoid cells and tissues, refer to Figure 1.

Cathepsin A levels and the intracellular activation of TAF were evaluated in primary CD4+ T lymphocytes and MDMs isolated from PBMCs from 13 donors of variable gender, age and ethnicity. Cathepsin A activity was determined by measuring the rate of conversion of TAF to TFV-alanine in extracts prepared from quiescent and PHA/interleukin-2 (IL-2) activated CD4+T cells and MDMs. For both primary cell types 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.

Results from in-vitro experiments are consistent with passive diffusion of TAF into HIV-target cells. While TAF was found in vitro to be a substrate for the hepatic uptake transporters OATP1B1 and OATP1B3, PBMCs express only low levels of transporters and do not express OATP1B1 and OATP1B3. Based on available data, there is no evidence for transporter-mediated uptake of TAF into PBMCs.

Following incubation of PBMCs with increasing concentrations of TAF from 13.7 nM to 10 µM, the intracellular concentration of TFV-DP increased proportionally with TAF loaded dose (Table 58). These results are consistent with an in-vivo uptake mechanism into PBMCs via passive diffusion because saturation was not observed at TAF levels significantly higher than those achieved in the clinic.

Table 58. Intracellular TFV-DP Levels Detected in PBMC Loading Studies with TAF

TAF (nM)	TFV-DP Concentration (µM)					
	Continuous Incubation			2 Hour Pulse		
	6 hours	24 hours	48 hours	6 hours	24 hours	48 hours
13.7	0.548	1.18	2.12	BLQ	BLQ	BLQ
41.2	0.818	3.35	6.00	BLQ	BLQ	BLQ
124	2.89	10.3	19.4	BLQ	BLQ	BLQ
370	5.93	25.4	49.9	1.89	1.35	0.95
1111	20.2	69.0	128	7.05	4.01	3.17
3333	59.5	204	357	19.9	13.5	7.03
10000	176	453	840	73.5	46.7	24.5

BLQ = below limit of quantitation (limit of quantitation: 0.5 µM)

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 EC50 values are affected to a similar extent by various mutations and combinations of mutations. However, the

applicant postulated that the in-vivo resistance profile may differ when dosing with TAF or TDF since the level of TFV DP achieved after TAF is significantly higher than that after TDF.

Viral breakthrough experiments were conducted using known TDF-resistant HIV-1 isolates in MT-2 cells to model the impact of the higher TFV-DP concentrations. These experiments were conducted at a higher multiplicity of infection (MOI) compared to typical EC50 assays; the EC50 values for TAF and TFV were 0.02 and 5 µM, respectively. The cells were incubated in the presence of TAF or TFV (concentrations were equivalent to the EC95 [estimated at 10 × EC50] with a 5-fold increase for TAF) 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. 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.

Primary and Secondary pharmacology

TAF monotherapy studies

GS-120-1101

Study Title: A Phase 1/2 Randomized, Double-Blind, Active-Controlled, Dose Escalation Study of the Safety, Tolerance, Pharmacokinetics, and Antiviral Activity of GS-7340-02 in Antiretroviral-Naïve Patients Who Are Chronically Infected with HIV-1

This study explored the antiviral activity of TAF (50 mg and 150 mg QD as the monofumarate) vs. TDF during 14 days monotherapy in 30 ARV-naïve patients (27 male) with plasma HIV-1 RNA ≥ 15,000 copies/mL and CD4 cell count ≥ 200 cells/mm^3 at screening. TFV was detectable within PBMCs earlier, more consistently, and at greater concentrations after TAF vs. TDF. DAVG1, 2 and 3 were all significantly greater in the TAF groups compared to TDF but there was no significant difference between the TAF doses.

GS-US-120-0104

Study Title: A Phase I Randomized, Partially-Blinded, Active and Placebo-Controlled Study of the Safety, Pharmacokinetics, and Antiviral Activity of GS-7340 Monotherapy in Subjects with HIV-1

TAF monotherapy was compared to TDF and placebo over 10 days in patients with HIV-1 RNA > 2000 copies/mL, CD4 counts ≥ 200 cells/mm^3 and without use of ARVs within 90 days. Randomisation (2:2:2:1:2) was to TAF at 8, 25 or 40 mg or to TDF 300 mg or placebo. At steady state, the mean TFV AUC_{tau} after TAF doses were 97%, 86% and 79% lower, respectively, vs. the mean after TDF dosing and Cmax values were 98%, 94% and 89% lower, respectively.

The peripheral blood mononuclear cell TFV-DP concentrations were highly variable but 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. The antiviral effect of GS-7340 8 mg was similar to that of TDF 300 mg. There were statistically significantly greater decreases in viral load with 25 mg and 40 mg TAF doses compared to TDF 300 mg. The first phase decay slopes for plasma HIV-1 RNA in the TAF 25 mg and 40 mg groups were also significantly steeper than for TDF 300 mg.

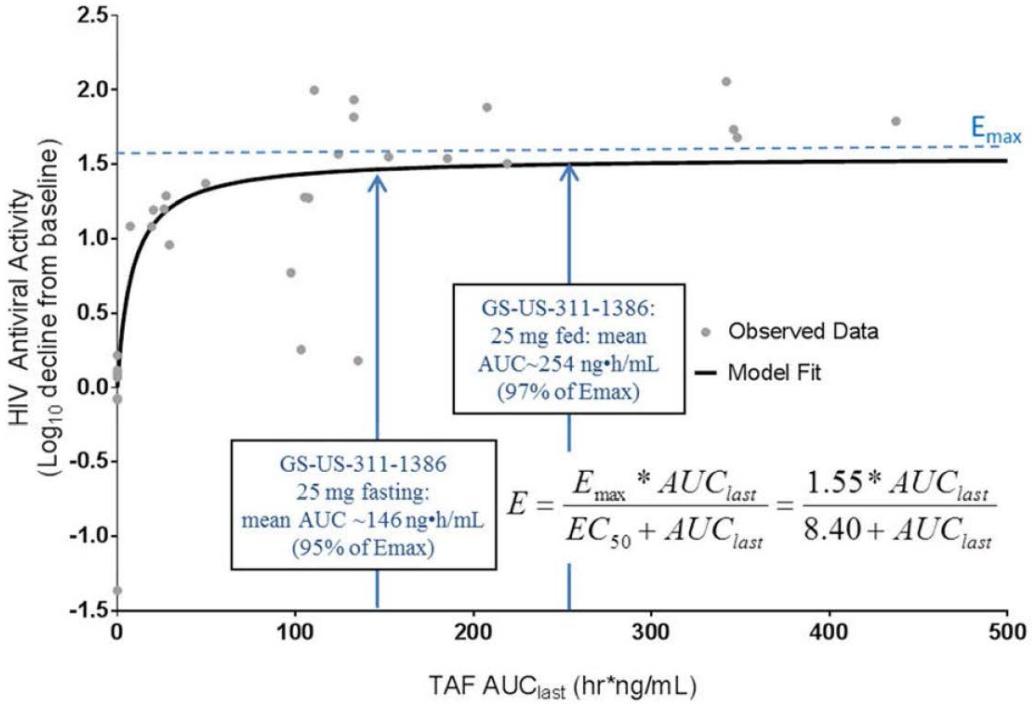
Table 59. Time-Weighted average change from baseline up to Day 11 (DAVG11) in plasma HIV-1 RNA (Full analysis set)

	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				

PK-PD analyses

Using data from TAF monotherapy GS-US-120-0104 study the PK/PD relationships between TAF and TFV plasma exposures and antiviral activity were explored using a maximum (PD) effect (E_{max}) model. 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}, which was somewhat expected given its brief plasma half-life and the resulting contribution of the C_{max} to the overall AUC. Upon comparison of antiviral activity with 40 mg and previous data with 150 mg (GS-120-1101), TAF 25 mg was expected to provide near-maximal activity (~1.7 to 1.8 log_{10} copies/mL). Plasma TFV exposure, which was substantially lower with TAF versus TDF, did not correlate with antiviral activity. The Figure below also shows the AUCs on dosing healthy subjects with 25 mg TAF without a P-gp inhibitor in fed and fasted states in GS-US-311-1386.

Figure 11. GS-US-120-0104: TAF Exposure-Response Model for AUClast and HIV Antiviral Activity



GS-US-292-0104 and 0111- Phase 3 E/C/F/TAF studies

The PK/PD analysis sets from these Phase 3 E/C/F/TAF studies included all ARV-naïve patients who received a dose of E/C/F/TAF and had at least one TAF or TFV PK parameter (AUC_{tau} or C_{max}) estimated from the POPPK analysis. Virologic success was uniformly high across TAF AUC_{tau} quartiles with no trends in exposure-response relationship observed.

Table 60. Virologic success rates at Week 48 across quartiles of TAF exposure (TAF PK/PD analysis set)

TAF AUC_{tau} Quartile (ng•h/mL)	**Virologic Success at Week 48 (HIV-1 RNA < 50 copies/mL, Snapshot Analysis) (%) (N = 135)**
47.2 to 140.2	96.3[a]
140.5 to 184.8	92.6
184.9 to 229.7	94.8
230.8 to 1869.3	91.9

These results support the selection of TAF 10 mg for use with COBI (or other agent with similar PK effects on TAF) based on equivalent exposure to unboosted TAF 25 mg, which was concluded to provide near maximal antiviral efficacy in the monotherapy study. The results are also in accordance with the lack of statistically significant covariates on PK in the POPPK analyses and the comparable efficacy observed across patient subgroups according to demographics.

The data were also used to explore the exposure-safety relationships in these two studies, focusing on diarrhoea and nausea (very common), vomiting and GI/abdominal pain (common).

- For diarrhoea and GI/abdominal pain, both the TAF and TFV exposure was comparable regardless of the presence or absence of either of those symptoms and no exposure-AE trends were observed.

- For nausea and vomiting, the TFV exposure was comparable regardless of the presence or absence of either of those symptoms with no exposure-AE trends noted. Logistic regression analysis showed a trend between the highest TAF exposure and the presence of these AEs (nausea correlated with the highest 4% of TAF exposure; vomiting correlated with the highest 19% of TAF exposure). No trend was observed between TAF exposure and severity.
- Across all TAF and TFV AUC_{tau} quartiles, the percentage change from baseline at Week 48 in BMD was comparable; no trends in exposure-changes in BMD were noted.
- Across all TAF and TFV AUC_{tau} quartiles, the maximum increase from baseline in serum creatinine was comparable; no trends in exposure-changes in serum creatinine were noted.

These Phase 3 E/C/F/TAF studies also provided intracellular TFV-DP concentrations following administration of E/C/F/TAF vs. STB. There was a >4-fold higher intracellular concentration of TFV-DP (based on the AUC_{tau} GLSM ratio) when TAF was given vs. TDF.

GS-US-311-1089

Study Title: Phase 3, randomized, double-blind, switch study to evaluate F/TAF in HIV-1 positive subjects who are virologically suppressed on regimens containing FTC/TDF

The results from the PK/PD analysis of this Phase 3 F/TAF study, in which virologically suppressed patients switched to F/TAF from FTC/TDF (Truvada), were similar to those from the E/C/F/TAF Phase 3 studies. F/TAF and FTC/TDF were administered without regard to food.

TAF AUCs from GS-US-311-1089 were estimated from a TAF population PK model. Based on the population PK model as well as the observed plasma concentration data, predicted individual PK parameters were estimated and used to simulate TAF concentration-time profiles that were subsequently used for non-compartmental analysis to obtain individual AUC and Cmax values. TAF AUCs were estimated for 292 subjects.

The estimated TAF exposures and clinical efficacy of F/TAF by third agent in this study are shown below. Maintenance of viral suppression was similar across the different third agents when used with recommended F/TAF doses, regardless of the variable TAF exposures achieved, and comparable to the FTC/TDF group.

Table 61. GS-US-311-1089: TAF PK Exposure and Virologic Outcome at Week 48 Using Snapshot Algorithm and HIV-1 RNA < 50 copies/mL by Third Agent (Full Analysis Set)

	F/TAF+3rd Agent with Available PK Data		F/TAF+3rd Agent (N = 333)			FTC/TDF+3rd Agent (N = 330)		
Third Agent	n	Mean (%CV) TAF AUC_{tau} (ng*h/mL)	n	Percent of Virologic Success at Week 48 (HIV-1 RNA < 50 copies/mL)	Percent of Virologic Failure at Week 48 (HIV-1 RNA ≥ 50 copies/mL)	n	Percent of Virologic Success at Week 48 (HIV-1 RNA < 50 copies/mL)	Percent of Virologic Failure at Week 48 (HIV-1 RNA ≥ 50 copies/mL)
ATV/r	44	149.6 (50.8)	53	90.6	0	50	94.0	2.0
DRV/r	72	73.5 (41.0)	84	90.5	1.2[a]	82	91.5	2.4
DTG	25	155.6 (18.5)	26	96.2	0	23	91.3	0
EFV	6	141.6 (18.7)	8	100	0	6	83.3	16.7
LPV/r	15	89.8 (25.0)	18	100	0	18	100	0
MVC	1	191.3 (NA)	1	100	0	6	100	0
NVP	67	167.4 (32.1)	74	97.3	0	66	98.5	0
RAL	61	170.8 (37.2)	66	95.5	0	73	89.0	0
RPV	1	273.1 (NA)	3	100	0	6	83.3	16.7

a Subject had TAF AUC of 197.3 ng*h/mL

NA = not applicable

Across a wide range of TAF exposures (range: 30 to 467 ng*h/mL), which correspond to 78% to 98% of Emax of the TAF exposure-response model, the rates of virologic success were 93.2 to 97.3%. There were no apparent trends with virologic failure and TAF exposure (only 1 patient had virologic failure at Week 48, and the TAF exposure was in Quartile 4; this subject developed M184V with reduced susceptibility to emtricitabine at virologic failure [Week 36]).

Table 62. GS-US-311-1089: Percentage of Virologic Success and Virologic Failure at Week 48 Across Quartiles of TAF Exposure (TAF PK/PD Analysis Set)

Quartile	TAF AUC_{tau} Quartile Range (ng*h/mL)	N	Percentage of Virologic Success at Week 48 (HIV-1 RNA < 50 copies/mL, Snapshot Analysis)	Percentage of Virologic Failure at Week 48 (HIV-1 RNA ≥ 50 copies/mL, Snapshot Analysis)
1	30.3 to 87.6	73	93.2	0
2	87.6 to 129.5	73	95.9	0
3	129.8 to 173.1	73	97.3	0
4	173.8 to 466.7	73	95.9	1.4[a]

a N = 1 virologic failure at Week 48

Data on intracellular TFV-DP concentrations were provided in the Week 48 report from this study. Based on trough blood samples collected at Week 4 (20 to 24 h post-dose) F/TAF resulted in intracellular TFV-DP concentrations >4-fold those observed with FTC/TDF.

Table 63. GS-US-311-1089: Statistical Comparisons of Intracellular PBMC TFV-DP Concentrations Between F/TAF and FTC/TDF (PBMC PK Analysis Set)

	Intracellular PBMC TFV-DP Concentration (pg/10^6 cell)	
	F/TAF (N = 308)	FTC/TDF (N = 271)
Geometric Mean (95% CI)	14.2 (12.6, 16.0)	3.4 (3.0, 3.8)
GLSM	14.1	3.3
TAF/TDF GLSM Ratio (90% CI) x 100%	424.62 (370.26, 486.97)	

Further breakdowns according to co-administered agents, noting the variable denominators, showed:

- A very clear difference between use with EFV (n=8) vs. other agents.
- Lower values with DRV/r and LPV/r compared to ATV/r.

Table 64. GS-US-311-1089: Summary of PBMC TFV-DP Concentrations by Treatment and 3rd Agent Stratum (PBMC PK Analysis Set)

	Mean (%CV)				Median (Q1, Q3)	
PBMC TFV-DP Concentration[a,b,c]	n	F/TAF+3rd Agent (Test)	n	FTC/TDF+3rd Agent (Reference)	F/TAF+3rd Agent (Test)	FTC/TDF+3rd Agent (Reference)
pg/million						
Overall Boosted 3rd Agents[d]	148	144.954 (105.5)	117	40.261 (156.8)	92.450 (47.350, 186.500)	23.000 (12.800, 41.600)
ATV/r	50	187.214 (93.5)	34	33.054 (87.2)	133.000 (67.600, 227.000)	23.050 (12.100, 40.200)
DRV/r	82	123.681 (116.2)	69	35.778 (127.1)	71.850 (39.000, 157.000)	21.000 (12.800, 37.800)
LPV/r	16	121.913 (75.0)	14	79.861 (180.1)	86.650 (58.750, 182.500)	37.650 (13.200, 93.800)
Overall Unboosted 3rd Agents[e]	156	237.354 (109.7)	148	47.423 (127.0)	154.500 (65.550, 349.000)	32.650 (18.300, 56.500)
DTG	24	154.921 (80.7)	19	53.583 (152.5)	104.000 (64.650, 215.000)	34.900 (15.400, 58.600)
EFV	8	99.900 (122.3)	6	44.050 (98.8)	50.900 (23.000, 143.700)	31.200 (19.600, 41.800)
MVC	1	268.000 (—)	5	34.220 (68.9)	268.000 (268.000, 268.000)	28.200 (21.800, 37.900)
NVP	65	227.936 (91.2)	56	43.924 (98.9)	157.000 (87.100, 335.000)	32.650 (18.650, 55.750)
RAL	55	295.804 (115.7)	57	50.355 (142.7)	159.000 (75.900, 462.000)	31.700 (15.500, 59.900)
RPV	3	385.633 (113.2)	5	47.020 (81.0)	271.000 (17.900, 868.000)	21.800 (21.300, 65.300)

GS-US-299-0102

Study Title: A Phase 2, Randomized, Double-Blinded Study of the Safety and Efficacy of Darunavir/Cobicistat/Emtricitabine/GS-7340 Single Tablet Regimen Versus Cobicistat-boosted Darunavir plus Emtricitabine/Tenofovir Disoproxil Fumarate Fixed Dose Combination in HIV-1 Infected, Antiretroviral Treatment-Naïve Adults

Use with a boosted-DRV regimen provides support for efficacy at the lower end of TAF exposure. In GS-US-299-0102 the percentages with < 50 c/mL in the D/C/F/TAF vs. DRV+COBI+FTC/TDF groups were 74.8% vs. 74.0% at Week 24 and 76.7% vs. 84.0% at Week 48.

TAF AUCs from 52 patients in this study were estimated from a TAF population model. The virologic success rates were similar across the quartiles of TAF AUCs, with no trends in exposure-response relationship.

Similarly, no apparent trend was observed with virologic failure at Week 48 and TAF exposures. The exposure range observed in this D/C/F/TAF study was similar to that in the E/C/F/TAF pivotal studies and F/TAF Phase 3 study. In addition, the intracellular TFV-DP exposure ratio of 6.5 (compared to TDF) was consistent with the E/C/F/TAF studies.

Pharmacodynamic interactions with other medicinal products or substances

TFV is presented for clinical use as a nucleotide analogue, i.e. it is already monophosphorylated. The effects of combination of TFV with other agents that require intracellular phosphorylation has been investigated in in-vitro studies. Combination with ABC did not change the rates of phosphorylation of either compound to the triphosphate (active) forms.

Secondary pharmacology

GS-US-120-0107

Study Title: A Phase 1, Partially-Blinded, Randomized, Placebo- and Positive-Controlled Study to Evaluate the Effect of GS-7340 on the QT/QTc Interval in Healthy Subjects

GS-US-120-0107 evaluated single doses of 25 mg and 125 mg TAF. 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 > 5 ms at 2 post-dose time points (3 and 4 h) establishing assay sensitivity. At these respective time points the actual changes were -1.4 and +4.1 ms for moxifloxacin compared to -12.9 and -7.1 ms for placebo.

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. Analyses of secondary endpoints (QTcB, QTcN and QTcI) were consistent with the primary analysis.

In the categorical analyses no subject had a treatment-emergent absolute QTc interval > 500 ms or a change from pre-dose baseline QTc > 60 ms with any correction factor following any treatment. 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.

2.4.4. Discussion on clinical pharmacology

Doses of TAF in F/TAF

Based on the combined observations from TAF monotherapy studies GS-US-120-1101 and -0104, there did not appear to be an advantage in terms of antiviral effect for TAF doses higher than 25 mg QD administered alone in the fasting state. For the 25 mg dose the day 10 mean and median TAF AUC_{last} values were 115 and 109 ng.h/mL, respectively while the mean and median AUC_{tau} values for intracellular TFV-DP levels were 21.4 and 15.8 µM.h.

It was calculated that when TAF is co-administered with strong inhibitors of P-gp (such as COBI and RTV) the dose should be ~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 E/C/F/TAF containing 10 mg TAF with FTC 200 mg + TAF 25 mg. After multiple daily dosing with

food, the plasma TAF AUC_{last} values were 250.2 and 278.2 ng.h/mL for respective formulations and both C_{max} and AUC met the bioequivalence criteria although the TAF AUC was numerically lower for 10 mg TAF in the presence of COBI vs. F/TAF 25 mg.

In contrast, the plasma TFV was slightly higher after dosing 10 mg TAF with COBI vs. F/TAF 25 mg, although the upper bound of the 90% CI was 131% (see GS-US-292-0103 pharmacokinetic results).

In order to support presentation of F/TAF containing 10 mg or 25 mg and to support bridging these formulations to the efficacy observed in the Phase 3 E/C/F/TAF studies in the ART-naive three bioequivalence studies were conducted in healthy subjects dosed after standard meals. However, it should be noted that TFV was not measured in these three studies so that the apparent discrepant effects on TFV noted in GS-US-292-0103 could not be further verified.

Study GS-US-311-1088 established that F/TAF (200/25 mg) was bioequivalent to co-administration of FTC 200 mg + TAF 25 mg. The TAF AUC_{last} values were 245.9 and 238.5 ng.h/mL, respectively.

Study GS-US-311-1472 established that F/TAF (200/10 mg) co-administered with EVG 150 mg and COBI 150 mg was bioequivalent to E/C/F/TAF 10 mg in terms of TAF and FTC. COBI and EVG concentrations were the same for the two groups. The TAF AUC_{last} values were 336.6 and 340.2 ng.h/mL, respectively.

Study GS-US-311-1473 established that F/TAF 200/25 mg given without EVG/COBI was bioequivalent to E/C/F/TAF 10 mg in terms of TAF and FTC. The TAF AUC_{last} values were 374 and 369 ng.h/mL, respectively.

Bridging to efficacy data

The prediction of efficacy when using F/TAF 10 mg or 25 mg with third antiretroviral (ART) agents rests mainly on PK bridging of F/TAF doses to the efficacy documented for E/C/F/TAF 10 mg in Phase 3 studies in ART-naïve patients. Bridging is supported by comparisons with exposures documented with D/R/F/TAF 10 mg in a Phase 2 study in ART-naïve patients (GS-US-299-0102) and those reported from a switch study in virologically-suppressed patients who received either strength of F/TAF with a range of third agents (GS-US-311-1089). For each dataset the contribution of F/TAF to the overall efficacy observed cannot be determined and has to be inferred from comparisons of plasma levels.

In study GS-US-292-103, although plasma TAF was very slightly lower on dosing with Genvoya, the plasma TFV AUC was lower after dosing with F/TAF 25 mg. This finding, which remains mechanistically unexplained, suggested an effect of COBI on the compartmental disposition of TFV.

The finding raised a question regarding the basis for extrapolation of efficacy from Genvoya to F/TAF regardless of the third agent co-administered and whether or not the regimen included P-gp inhibitors COBI or RTV.

It also raised a question regarding the CNS levels of TFV-DP that may be achieved with F/TAF, and, hence, the efficacy of various TAF-containing regimens against HIV within the CNS.

The applicant responded to these concerns during the procedure and the questions were referred to the SAG HIV/Viral Diseases. When considering these issues the following observations have some relevance.

To ascribe the difference in TFV plasma levels that was observed when TAF was administered with or without COBI to a differential effect on the whole body distribution of TFV would require that systemic concentrations of COBI are sufficient to exert effects on relevant transporters outside of the gut.

In vitro, COBI inhibits the transporters P-gp, BCRP, MATE1, MRP-2, OATP1B1 and OATP1B3. Its effect on plasma exposures to substrates of P-gp and/or BCRP via inhibition at the gut level is clear. There are modest

changes in TFV exposure when TDF (a known substrate of P-gp) is given with inhibitors of intestinal P-gp, including COBI and RTV. In addition, COBI is predicted to inhibit intestinal BCRP and MRP2 at concentrations theoretically achievable in the intestinal lumen following a 150 mg oral dose. The results of a study to evaluate whether MRP2 or BCRP could contribute to the intestinal efflux transport of TDF were submitted during the evaluation of the Tybost dossier and indicated that this could occur. However, in the context of plasma TFV levels after oral administration of TAF, it should be noted that TFV is not a substrate for P-gp, MRP2 or BCRP and its renal elimination should not be affected by COBI based on the calculated $C_{max,u}/IC_{50}$ ratios.

Regarding the potential for systemic effects on other transporters it is clear that COBI reaches sufficient concentrations to inhibit MATE1 in the kidney, with consequent effects on serum creatinine. It is also predicted to have weak to modest effects resulting in increased exposures to OATP1B substrates. In the interaction study with digoxin, in which Cmax and AUC_{0-last} increased but AUC_{inf} remained unchanged on co-administration, there was a numerical decrease in the digoxin t1/2 from 38 to 30 h, which was unexpected in light of the calculations described above suggesting that COBI would not reach sufficient systemic concentrations to inhibit renal P-gp and so affect elimination of digoxin in urine. This effect on t1/2 remains unexplained and in a small DDI study it may not represent a true difference.

Overall, existing knowledge regarding COBI and its effects on transporters, as well as the substrate profile of TFV, do not explain the modest difference in TFV plasma levels observed when TAF was given with and without COBI to healthy subjects. There is no clear basis for concluding that the difference truly reflects different whole body distribution of TFV when TAF is administered with or without COBI.

TFV is not the active moiety but is the moiety associated with adverse renal and bone effects. Since TFV plasma levels were slightly lower for F/TAF 25 mg vs. TAF 10 mg given with COBI there is no reason to expect a worse safety profile for F/TAF when used without COBI compared to Genvoya. In addition, the safety data from GS-US-311-1089, in which subjects either switched to TAF or remained on TDF, each with FTC and a wide range of third agents (including PI/r combinations), indicated that the safety profile for TAF-treated patients vs. TDF-treated patients was consistent with observations made in the Phase 3 Genvoya studies that compared Genvoya with Stribild.

The remaining issue was whether the supposed difference in whole body distribution of TFV in the presence and absence of COBI could bring into question the validity of bridging F/TAF (either strength, with or without COBI or RTV) to the Genvoya Phase 3 efficacy data by means of TAF bioequivalence. Thus, to consider whether the durable anti-viral activity observed so far in plasma when dosing TAF as part of Genvoya, accompanied by lack of any signal for neurocognitive disorders caused by HIV-1 in the CNS, would apply when dosing TAF within other ART regimens.

As part of this concern regarding bridging of efficacy, it was questioned whether CNS penetration of TAF and formation of the active moiety TFV-DP in brain parenchyma could be different in the presence vs. absence of COBI. In addition, it was questioned whether control of HIV-1 within the CNS could vary depending on the third agent used with F/TAF. On this latter theme, in light of the concurrent application for F/R/TAF and in relation to the uncertainties of the CHMP regarding the efficacy of RPV 25 mg QD, it was questioned whether patients taking this FDC could be at particular risk of HIV-related neurocognitive disorders. Thus, several issues culminated in a series of questions that covered both the efficacy of F/TAF when used with third agents other than EVG/co, including but not limited to RPV, and the associated potential for HIV-1 neurocognitive disorders to occur.

With regard to the brain levels of the active moiety (TFV-DP) when TAF is given orally, the theoretical concern is that systemic levels of inhibitors of transporters known to be located at the BBB (including P-gp, BCRP and MRP-4) could suffice to have a clinically important impact. Hence, if COBI or RTV were to exert some inhibition of these transporters at the BBB, the efflux of their substrates from the brain might be reduced. TAF is a substrate of P-gp and BRCP but TFV is not a substrate of either of these transporters. Theoretically brain parenchyma concentrations of TAF and TFV-DP could be relatively higher when TAF is dosed with vs. without COBI or RTV.

Regarding possible effects of COBI and RTV on P-gp or BCRP activity in the CNS the most important transporters that restrict the permeability of toxins and drugs across the BBB are the ABC transporters P-gp, BCRP and MRP-4. Hence, whilst they are neuroprotective, these transporters can restrict the entry of drugs that are substrates. Potentially, induction or inhibition of important transporters at the BBB could reduce or increase brain parenchyma concentrations of substrates, respectively. Substrates of P-gp include most HIV PIs, abacavir, maraviroc and raltegravir.

In-vitro data have shown that P-gp induction could occur at the BBB during chronic treatment with ARTs identified as ligands of hPXR and/or hCAR. *In vitro*, AhR was not induced by COBI or the three human metabolites tested (COBI showed no induction up to 3 µM and 1.6-fold induction at 10 µM, which is similar to RTV (fold induction ~0.80). COBI showed weak activation of PXR *in vitro* (1.6-fold at 3 µM and 2.2-fold at 10 µM) but had a lesser effect than RTV (3.6 to 10.1-fold across the same concentrations). For comparison, COBI plasma Cmax is about 1-2 µg/mL while 10 µM is about 7.76 µg/mL.

COBI and RTV are (net effect) weak inhibitors of P-gp. The total effects of COBI and RTV on transporters that can result in increased systemic bioavailability of many drugs are exerted at the intestinal level. There is a low probability that they could inhibit transporters at the BBB when given at the recommended clinical doses.

TFV and TAF poorly penetrate into the CSF, likely resulting in concentrations well below EC_{50} for HIV reverse transcriptase. However, as reported by Kalvass *et al*. (2013) in a review conducted by the International Transporter Consortium (ITC), transport processes at the blood-CSF barrier can be functionally different than those at the BBB. For example, P-gp and BCRP secrete substrates into the CSF at the blood–CSF barrier, but at the BBB they act as efflux transporters pumping substrates into blood. In contrast, MRP4 acts as an efflux transporter at both the BBB and the blood–CSF barrier. The authors point out that effects on the activity of BCRP or MRP4 at the BBB are not likely to result in clinically relevant effects. As elaborated by the authors, drug detection and drug concentrations in CSF may not provide an accurate reflection of concentrations in brain parenchyma. CSF concentrations are often not representative of unbound brain concentrations for substrates of P-gp, BCRP and/or MRP4. Furthermore, TFV-DP is the active moiety but it is formed only within cells. Hence it cannot be assumed that TAF and TFV levels measured in CSF necessarily correlate with inhibition of HIV-1 replication by TFV-DP within the brain.

Kalvass *et al*. (2013) also discuss uptake transporters at the BBB. In light of the possibility that COBI could have effects on other (as yet untested or unrecognised transporters) this potential effect of COBI cannot be ruled out but it would only be important whenever TAF was a sensitive substrate.

In the switch study GS-US-311-1089 there were no differences in efficacy or safety of F/TAF when used with a boosted PI or unboosted third agents, including no signal for neurocognitive disorders. However, the demonstration of efficacy in a population in which HIV-1 replication is already profoundly suppressed may not be relevant to initiation of treatment in the ART-naïve. In addition, only 3 patients received F/TAF+RPV in this switch study. Therefore the switch study efficacy data cannot *per se* overcome the theoretical concerns that have been raised.

HIV-1 replication in the CNS is thought to occur in perivascular macrophages and/or microglia within the brain parenchyma. In patients with cognitive impairment, HIV-1 RNA in the CSF is derived primarily from enriched perivascular macrophages and migrating monocytes. Although plasma TFV exposures with TAF are ~90% lower vs. TDF, intracellular TFV-DP levels are typically 4- to 7-fold higher. Theoretically it may be that TAF is able to load migrating monocytes more effectively than TDF prior to entry into the CNS.

Neuro-symptomatic replication of HIV-1 in the CNS (in which CSF HIV-1 RNA is higher than in plasma) does occur but appears to be rare and is mostly documented anecdotally via case reports or series. In reported cases of concurrent virologic failure in the CSF and plasma it is not possible to determine whether plasma virologic failure followed CSF virologic failure or *vice versa*. Generally the evidence supports a conclusion that clinically significant escape replication of HIV-1 in the CNS is extremely unlikely to occur when there is successful suppression of plasma HIV-1 RNA regardless of the regimen sued and the predicted CNS penetration of the active moieties. In addition, there is only a very remote possibility that escape replication in the CNS would lead to failure to control plasma HIV-1 RNA levels.

In summary, although there remains a theoretical possibility that the presence of a P-gp inhibitor as part of an overall TAF-containing ART regimen could affect distribution of TAF into the brain and, thus, TFV-DP levels, the overall picture at present suggests that this is a remote possibility. CSF levels of TFV or TAF cannot be regarded as highly predictive of TFV-DP in the brain. There is at least a theoretical possibility that use of TAF rather than TDF could improve on levels of TFV-DP achieved in the CNS replicating sites.

The general experience with the more highly effective ART regimens that have become available especially in the last decade support a conclusion that effective and sustained virologic suppression in plasma is associated with CSF virologic suppression or, at least, only asymptomatic and usually temporary detection of HIV-1 RNA in CSF. There is no reason to think that rates of plasma virologic failure or CSF virologic failure are more likely to occur with regimens containing TAF vs. otherwise identical regimens containing TDF. Thus, F/R/TAF should exert similar activity to Eviplera when used in accordance with identical restrictions and warnings and there is no good reason to expect that the risk of escape HIV-1 replication in the brain is any greater with the former vs. the latter. The Applicant committed to provide any publicly available data from planned studies that will evaluate CSF penetration of TAF and/or TDF.

Bridging for efficacy

When making comparisons across studies presented in the dossier it is pertinent to remember that in the POPPK analysis based on the E/C/F/TAF studies the applicant concluded that HIV disease status did not have an effect on TAF exposure and was not a statistically significant or clinically relevant covariate. The estimated TAF AUC_{last} values were 250 ng.h/mL for healthy subjects vs. 206 ng.h/mL for HIV-infected patients, noting that patients were to take E/C/F/TAF with food. In the additional integrated *ad hoc* PK analysis in the F/TAF dossier, from which the applicant again concluded that there was no clinically relevant difference in TAF exposures between healthy subjects and HIV-infected patients, the estimated TAF AUC_{last} for TAF 10 mg + COBI in HIV-infected patients, derived from studies in which patients received E/C/F/TAF with food, was ~240 ng.h/mL. It could be argued then that the target TAF AUC to support bridging of efficacy to the most robust dataset (the Phase 3 E/C/F/TAF studies) would be somewhere between ~200-250 ng.h/mL.

When F/TAF 25 mg is administered without a P-gp inhibitor the estimated AUC_{last} in patients is 127 ng.h/mL, i.e. similar to that observed with 25 mg TAF in the monotherapy study and also similar to the value of 133 ng.h/mL observed when F/TAF 25 mg was administered in the absence of food and without a P-gp inhibitor in the food effect study GS-US-311-1386 (the TAF AUC in the fed state in this same study was 234 ng.h/mL). In contrast, when TAF 10 mg was administered as a component of E/C/F/TAF the TAF AUC last was 206

ng.h/mL in the fasted state vs. 240 ng.h/mL after a meal. Therefore when F/TAF 10 mg was administered with a strong P-gp inhibitor there was no important effect of food. For F/TAF 25 mg (i.e. the dose to be used without a strong inhibitor of P-gp as the third agent in the regimen), the TAF AUC approaches that derived from E/C/F/TAF efficacy studies only when F/TAF is given with food. This observation raised a question regarding the applicant's proposal in the SmPC that F/TAF could be given without food regardless of co-administration with a P-gp inhibitor.

In this regard it was appropriate to also consider the PK data from the Phase 2 study GS-US-299-0102 that compared DRV/COBI/FTC/TAF 10 mg with DRV/co plus TVD in ART-naïve patients. The mean TAF AUC_{last} was 130 ng.h/mL but the intracellular TFV-DP value was 17.1 vs. 2.6 µM.h in the TDF group. The TAF exposures were therefore similar to 25 mg given alone in the fasting state in the monotherapy study. However, in this small Phase 2 study the regimen containing TAF was numerically inferior to the regimen containing TDF.

Additional data came from the Week 48 results of the switch study in the virologically suppressed. Due to the design the efficacy data can only be viewed as supportive but, as in the Phase 2 and 3 studies described above, there was no detectable relationship between TAF plasma levels and efficacy. The plasma TAF AUCs when F/TAF 25 mg was taken without a strong intestinal P-gp inhibitor and *without regard to food* overlapped with those for co-administration of F/TAF 10 mg with ATV/r.

Furthermore, although TFV-DP levels must be viewed with some caution (data is expressed in pg/million cells and not in µM), they supported consistently higher levels with TAF vs. TDF regardless of the regimen.

Table 65. GS US 311-1089: Statistical Comparison of Intracellular PBMC TFV DP Concentrations (pg/million cells) Between F/TAF and FTC/TDF Group

	F/TAF		FTC/TDF		GLSM Ratio (%) (90% CI)
	N	TFV-DP GLSM	N	TFV-DP GLSM	
Overall	304	114.00	265	27.78	410.35 (356.90, 471.81)
Boosted PI (F/TAF 200/10 mg)	148	94.46	117	24.10	391.99 (322.62, 476.26)
Unboosted 3rd Agent (F/TAF 200/25 mg)	156	136.26	148	31.09	438.31 (359.57, 534.29)

CI=confidence interval; GLSM=geometric least-squares mean; In the F/TAF and FTC/TDF group, 4 and 6 subjects, respectively, were excluded from PBMC analysis because the PBMC samples were out of the 61 days window of stability

In conclusion, the data generally supported expectations that the efficacy of F/TAF 10 mg administered regardless of food in the presence of a P-gp inhibitor and F/TAF 25 mg administered regardless of food in the absence of a P-gp inhibitor, should provide broadly comparable efficacy.

Dose of F/TAF when given with other strong inhibitors of P-gp

The applicant proposed that F/TAF 10 mg should be used when it is co-administered with one of the specified boosted PIs. Thus, F/TAF 25 mg was to be recommended when the third agent in the ART regimen is not a strong inhibitor of P-gp regardless of whether or not the patient is taking another concomitant medication that is a strong P-gp inhibitor. This proposal did not raise efficacy concerns, however it cannot be ruled out that chronic exposure to plasma TFV concentrations higher than observed with Genvoya (even though much lower than observed with TDF) could eventually result in the types of safety issues associated with TDF on bones and renal function. As a consequence, section 4.5 advises that F/TAF 10 mg is given when non-ART potent P-gp inhibitors (including ciclosporin, ketoconazole and itraconazole) are concomitantly administered.

Dose of F/TAF when given with various ARTs

The applicant proposed that F/TAF 10 mg should be the dose used when it is given with a boosted PI (specifically ATV/co, ATV/r, DRV/co, DRV/r and LPV/r) whereas F/TAF 25 mg should be used with other ARTs (specifically mentioning DTG, RPV or EFV). To support these recommendations the applicant conducted several DDI studies. The data supported co-administration of F/TAF 25 mg with DTG or RPV.

There remained some concern regarding the adequacy of F/TAF 25 mg when given with EFV, especially if TAF were to be taken with EFV on an empty stomach. In the DDI study conducted in the fasted state the C_{max} and AUC_{last} for each of TAF, TFV and FTC were lower on co-administration and failed to meet the usual bioequivalence criteria but the applicant dismissed the finding. Although GS-US-311-1089 indicated that for patients who took F/TAF 25 mg with EFV the plasma TAF AUCs were relatively high vs. other third agents, the intracellular TFV-DP levels were the lowest observed by third agent. With < 10 patients treated with F/TAF + EFV in a switch study the fact that 8/8 maintained suppression was not necessarily reassuring, especially when it is not known if TAF was taken at a different time and possibly with food. However, TFV-DP levels were comparable with those associated with efficacy in the monotherapy study. It was finally concluded that F/TAF 25 mg could be given with EFV, and this is reflected in section 4.5 of the SmPC where actual changes are shown.

In addition, there remained some concern regarding the adequacy of F/TAF 10 mg when given with DRV/co or DRV/r. This was mainly due to the plasma TAF and intracellular TFV-DP levels observed with DRV/COBI/FTC/TAF in GS-US-299-0101 in healthy subjects, which fell considerably short of those in patients in Phase 3 E/C/F/TAF studies. Also, in the Phase 2 study GS-US-299-0102 in which DRV/COBI/FTC/TAF 10 mg was compared with DRV/co plus TVD, the mean TAF AUC_{last} was 130 ng.h/mL, i.e. very similar to the value documented in GS-US-299-0101 in healthy subjects but admittedly also similar to the TAF AUC with 25 mg monotherapy. In GS-US-299-0102 the percentages with < 50 c/mL in the D/C/F/TAF vs. DRV+COBI+FTC/TDF groups were unusually low for an ART-naïve patient study at 74.8% vs. 74.0% at Week 24. At Week 48 rates were 76.7% vs. 84.0%. Additional data indicated that there was also a disadvantage for D/C/F/TAF at week 24 based on the <20 c/mL cut-off. However, this was a relatively small Phase 2 study that was not powered for inferential testing. Rather than preclude use of F/TAF 10 mg with DRV/r or DRV/co, the CHMP considered that it was important to add the efficacy results based on the <20 c/mL cut-off to section 5.1 and to cross refer to this section from the posology for DRV combinations in 4.2. In addition, section 4.2 should specify that DRV 800 mg QD was the dose used.

When F/TAF 10 mg was given with ATV/r or LPV/r TAF exposures were in the range observed with TAF 25 mg monotherapy. Taking into account the applicant's additional TAF AUCs from patients treated with F/TAF 10 mg and these PI/r combinations in GS-US-311-1089 and a recently completed DDI study of F/TAF (10 mg) with ATV+COBI, the plasma levels provided some degree of reassurance regarding the adequacy of the TAF 10 mg dose with ATV/r or ATV/c.

Additional data on the differential effects of PI/P-gp inhibitors on TAF vs. TFV

Concomitant use of FTC/TAF 25 mg with DRV/co (800/150 mg) in the fed state for 10-12 days resulted in comparable exposures to TAF and FTC but ~3-fold higher TFV exposures vs. FTC/TAF (25 mg) dosed alone. The increase in TFV but not TAF exposures after multiple-dose co-administration was ascribed to a mixed inhibitory/inductive effect of COBI and DRV on P-gp, influencing TAF absorption. Furthermore, co-administration with ATV/co increased the plasma exposures to both TAF (~1.75-fold) and TFV (~3.5-fold) but, as was also observed in the DDI study with ATV/r (~1.9-fold TAF and ~2.5-fold TFV increases), the magnitude of effect on TFV was considerably more than that on TAF.

The applicant acknowledged that the extent of increases in TFV was higher than increases in TAF when co-administered with a P-gp inhibitor but the exact mechanism for the relative change in TAF and TFV exposures remains unknown. The interaction mediated by P-gp inhibition (and mixed inhibition/ induction effects in the case of DRV/r) likely involves interplay of P-gp and metabolizing enzymes such as esterases both in the intestine and liver.

Taking into account data with TDF, it was considered that the effects observed were unlikely to reflect changes in elimination of TFV. GS-US-216-0134 investigated the PK of COBI and TFV following multiple dose administration of COBI and TDF alone or in combination. Results showed a ~50% increase in TFV Cmax but moderate increase in AUC (~20% increase) when TDF was co-administered with COBI, with no change in TFV $t_{1/2}$. This suggested a local, gut-related interaction between a P-gp inhibitor and TDF at the intestinal level and that elimination of TFV was not affected.

Irrespective of the potential mechanism for changes in metabolite exposure, any increase in TFV exposure resulting from co-administration of TAF with other concomitant medications is expected to be well below the TFV exposures observed in patients receiving TDF-containing regimens. Therefore, although lack of a clear explanation remains somewhat unsatisfactory, it seems unlikely that there are unidentified DDI mechanisms that will result in TFV exposures after TAF dosing that approach those observed with TDF.

Co-administration of TAF with inducers of P-gp

The applicant reported the results of GS-US-311-1387, which assessed the magnitude of the interaction between TAF and a strong P-gp inducer (carbamazepine). The ~50 % reduction in plasma TAF observed in the presence of a potent inducer of P-gp may not matter too much in terms of intracellular TFV-DP if F/TAF is also given with a potent P-gp inhibitor. However, if F/TAF is not given with a potent P-gp inhibitor the consequences for efficacy could be very variable and unpredictable. The situation could be exacerbated if F/TAF is also taken without food and/or with EFV, which would not be precluded in the SmPC.

Initially the applicant proposed to recommend dosing with food to address the effect of P-gp induction but TAF AUC increased 175% in the food effect study only when it was given with a high-calorie/high-fat meal of approximately 800 kcal and 50% fat. It certainly cannot be assumed that patients would take it with such a meal routinely. Overall, there is a risk of inadequate TAF and hence TFV-DP levels if F/TAF is taken with a potent inducer of P-gp. There does not seem to be a good reason to accept such a risk. Therefore, section 4.5 was modified to advise that co-administration of F/TAF with potent inducers of P-gp is not recommended.

Uptake of TAF into PBMCs

The applicant has not been able to identify any specific mechanism(s) of uptake of TAF into PBMCs and proposes that this occurs via passive diffusion. If there were to be any reason why TAF uptake into PBMCs was reduced to a level below that achieved with TDF 300 mg there would have to be a concern for efficacy. Results from in-vitro experiments are consistent with passive diffusion of TAF into HIV-target cells. While TAF was found *in vitro* to be a substrate for the hepatic uptake transporters OATP1B1 and OATP1B3, PBMCs express only low levels of transporters and do not express OATP1B1 and OATP1B3. Furthermore, anti-HIV activity of TAF in combination with various third agents, including many known to act as transport inhibitors, has been tested *in vitro* in an HIV-1 IIIB infected T-lymphoblastoid cell line. There was no antagonistic anti-HIV effect observed between TAF and any of the other agents tested. Based on available data, there is currently no evidence for transporter-mediated uptake of TAF into PBMCs.

The major route of TAF uptake into primary hepatocytes is passive diffusion so that OATP1B1 and OATP1B3 make a small contribution. This hypothesis is supported by combination intracellular metabolism experiments

in primary hepatocytes where the effects of COBI, telaprevir and boceprevir on intracellular levels of TFV-DP were assessed. These compounds are known to inhibit OATP1B1 with IC_{50} values of 2.5 µM, 2.2 µM and 18 µM, respectively, and to inhibit OATP1B3 with corresponding IC_{50} values of 1.9 µM, 6.8 µM and 4.9 µM. None of these compounds affected intracellular TFV-DP levels at concentrations up to 50 µM. Despite the results with the positive control bosentan in this study, the results support a conclusion that OATP1B1/1B3 inhibition in primary human hepatocytes does not have a very marked effect on TAF uptake.

Conversion of TAF to TFV-DP and onward metabolism

The applicant selected TAF for potential clinical use based on its rapid disappearance from plasma, efficient loading into PBMCs and efficient intracellular conversion to TFV. The latter is proposed to occur via CES1 and CatA to form TFV-alanine which is then hydrolysed in lysosomes to form TFV. The applicant has concluded that TAF is primarily hydrolysed by CatA in PBMCs. Cathepsin A is a ubiquitously expressed multifunctional enzyme with deamidase, esterase and carboxypeptidase activities. Genetic polymorphisms in CatA have been described, some of which can result in depressed enzymic activity. The potential for human polymorphisms in CatA to affect conversion of TAF to TFV was addressed and significant modulation of TAF PK profiles by inhibition or genetic polymorphisms of CES1 seems to be unlikely. However, the HCV PIs telaprevir and boceprevir can form covalent bonds with the serine carboxypeptidase and so inhibit CatA, which could reduce intracellular TFV-DP formation. The SmPC reflects this since co-administration with these agents is not recommended.

After conversion of TAF to TFV, TFV-DP is formed via intracellular phosphorylation 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 with none in faeces whereas TFV accounted for the majority of the radioactive dose recovered from urine and faeces. Although TAF accounted for most of the plasma radioactivity in the first few hours, most of the radioactivity in plasma (74%) was associated with uric acid over 96 h post-dose. After conversion to TFV it is proposed that metabolism proceeds via the purine catabolic pathway. This includes formation of uric acid. Since uric acid levels in pooled plasma increased over time and reached a maximum at 72 h, TAF was undetectable by the 6-h time point, indicating that the depurination reaction proceeded even after TAF was depleted from plasma. The applicant considered that in theory complete inhibition of the depurination pathway could result in increase of plasma TFV levels up to 4-fold. Even under these conditions, the plasma TFV levels would be lower than those after administration of TDF (300 mg). Since the depurination reaction is likely to occur after TAF is converted to TFV, potential induction of this pathway should not affect the TAF levels and, therefore, not affect efficacy which is mainly delivered by intact TAF. On this basis the applicant concluded that clinically important DDIs associated with the depurination pathway are unlikely.

However, allopurinol and febuxostat inhibit xanthine oxidase, the enzyme that is responsible for the successive oxidation of hypoxanthine and xanthine, resulting in the production of uric acid. Therefore they decrease uric acid formation and may also inhibit purine synthesis. The clinical data do not point to clear increases in plasma uric acid or cases of gout but the potential for this to occur cannot be dismissed and is reflected in the RMP.

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. Treatment should not be initiated if CrCL is < 30 mL/min and is not recommended in patients with Class C severe hepatic impairment due to lack of data. The advice is compatible with that accepted for Genvoya.

The available pharmacokinetic data from adolescents aged 12 years or more and at least 35 kg body weight treated with E/C/F/TAF support use of F/TAF at the same doses as recommended for adults. Further data will be available from study GS-US-311-1269.

Although the POPPK analysis did not detect an effect of racial group on TAF or TFV PK that was considered significant the small study GS-US-292-0108 showed lower exposure to all analytes (i.e. not just TAF and TFV) in the Japanese group. The applicant has no mechanistic explanation for the findings but, based on the actual exposures observed in Japanese subjects and the lack of trends in exposure-response relationships observed in clinical studies with E/C/F/TAF, it does not appear likely that the differences would translate into lower efficacy.

Pharmacodynamics

The evidence to support selection of 25 mg TAF once daily in the absence of an inhibitor or inducer of P-gp comes from the monotherapy studies. Doses of 25 mg and 40 mg TAF achieved effects on viral load that were similar to those of 50 mg and 150 mg in the preliminary 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 but 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 in the monotherapy and other studies in which data were generated. Any attempt to interpret the differences in TFV-DP levels within and between studies must take into account the technical challenges associated with the assessment of intracellular nucleotide levels, including TFV-DP in PBMCs. However, it seems that the consistent pattern of differences in TFV-DP concentrations between TDF and TAF treatment groups is most likely real. It is notable then that in the monotherapy study 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 data on plasma HIV RNA from the two 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. 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. Thus, 25 mg TAF should provide near maximal activity.

After 10 days dosing with TAF 25 mg alone the mean and median TAF AUClast values were 115 and 109 ng.h/mL, respectively. The POPPK TAF analysis gave a mean (95% CI; % CV) predicted steady-state AUC from Phase 3 studies with E/C/F/TAF 10 mg 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 F/TAF 10 mg presentation when given with a P-gp inhibitor in the fed state is predicted to give AUC values that comfortably exceed the EC_{50} in the majority.

The observation of higher levels of TFV-DP in PBMCs after dosing with TAF 25 mg compared to TDF 300 mg is not mechanistically explained. However, TAF is a more stable prodrug than TDF and it is more efficiently loaded into PBMCs (including lymphocytes and other HIV-target cells), although the mechanism of cell uptake is not known. Due to its poor cellular permeability, plasma TFV does not meaningfully contribute to intracellular levels of TFV-DP in patients receiving F/TAF. This is supported by the kinetic profile of TFV-DP in PBMCs since TFV-DP accumulates rapidly after dosing, when plasma TAF levels are still high, and are

maintained based on its long intracellular half-life. Additionally, the TAF AUC fitted well with an Emax model but TFV exposure did not correlate with antiviral activity.

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.

There is no evidence to suggest that substitution of TDF with TAF in the FDC will increase the risk of selection of resistance. Also, in-vitro studies with FTC and TAF indicated no intracellular interference in formation of their respective phosphorylated active moieties.

2.4.5. Conclusions on clinical pharmacology

TAF is a prodrug 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 when TAF 25 mg is compared with TDF 300 mg.

The TAF and FTC components were bioequivalent between both doses of F/TAF (200/10 mg with COBI and 200/25 mg without COBI) and E/C/F/TAF. The demonstration of bioequivalence enables the pharmacokinetic bridging between the F/TAF and E/C/F/TAF FDCs.

TAF is transported by P-gp. Therefore, drugs that induce P-gp activity may lead to a decreased plasma concentration of TAF, whereas drugs that inhibit P-gp increase the absorption and plasma concentration of TAF. Generally, F/TAF 200/25 mg is recommended with third agents that do not have clinically relevant effects on TAF exposure (EFV, RPV, DTG), and F/TAF 200/10 mg is recommended with third agents that substantially increase TAF exposure (ATV with RTV or COBI, DRV with RTV or COBI and LPV/r).

The PK of TAF in adolescents was consistent with the range of exposures associated with antiviral activity of E/C/F/TAF in adults, which supports the extrapolation of efficacy data from adults to paediatric subjects aged at least 12 years and of at least 35 kg body weight.

No dose adjustment of F/TAF is necessary in patients with estimated creatinine clearance greater or equal to 30 mL/min. No dose adjustment of F/TAF is necessary in patients with mild or moderate hepatic impairment as no clinically relevant changes in the PK were observed in these subjects.

2.5. Clinical efficacy

2.5.1. Dose response studies

There were no dose-finding studies with F/TAF since the dose of TAF was identified from the monotherapy study GS-120-1101, study GS-120-0104 and modelling plus the PK data indicating the need to manufacture two F/TAF tablets so that the TAF dose could be adjusted in accordance with the content of the total ART regimen, i.e. whether or not it included a P-gp inhibitor.

2.5.2. Main studies

There were no efficacy and safety studies conducted in treatment naïve patients with F/TAF. The anticipated efficacy of F/TAF is primarily based on PK bridging to the E/C/F/TAF Phase 3 studies in the ART-naïve patients

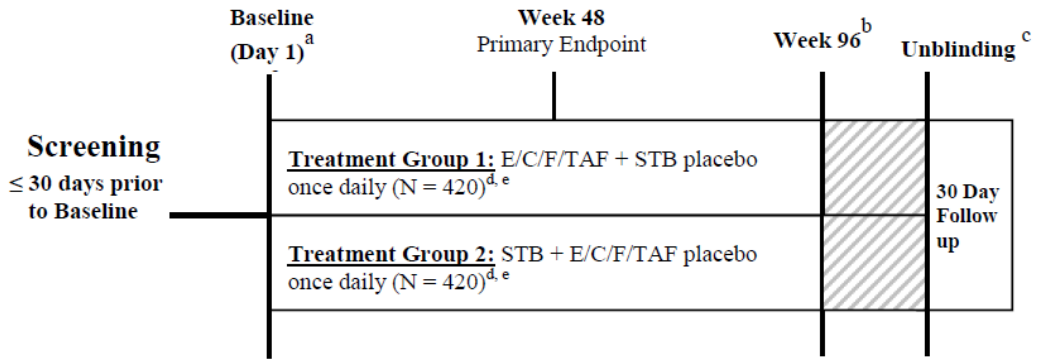
(GS-US-292-0104 and 0111). To some extent it is also based on bridging to the Phase 2 study GS-US-299-0102 and, to a lesser extent, the switch study in virologically suppressed patients GS-US-311-1089. In addition data is available from E/C/F/TAF in adolescents.

GS-US-292-0104 and 0111

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 two double-blind studies in ART-naïve patients were of the same design as summarised in Figure 7.

Figure 12. Study design



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

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 66. 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 67. 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 68. 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.

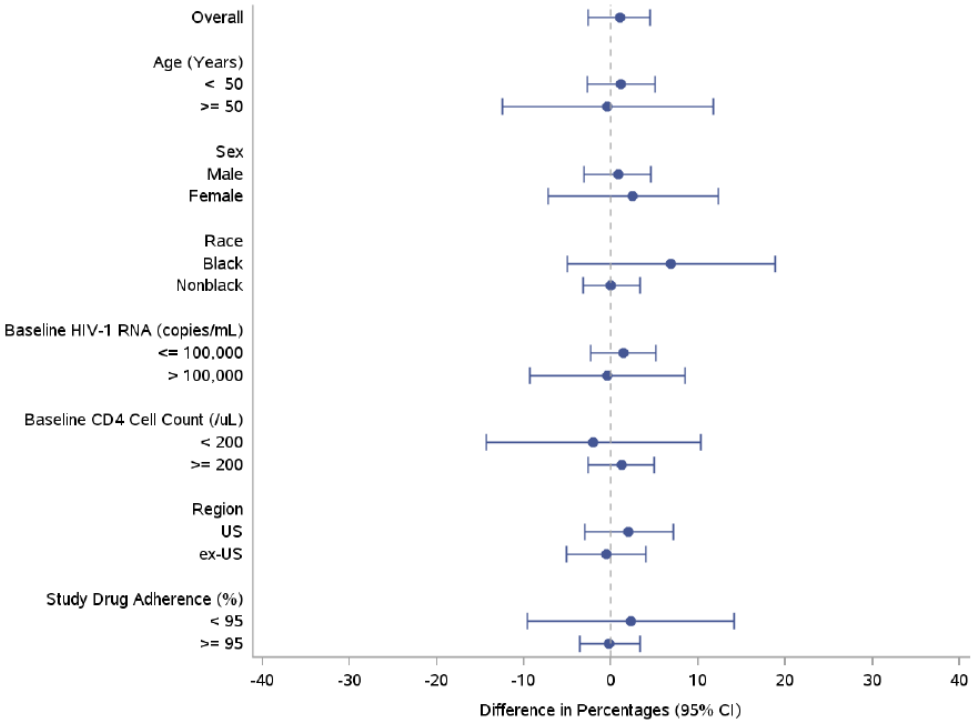
c Week 48 window is between Day 294 and 377 (inclusive).

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 13. 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 69. 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 70. 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 71. 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.

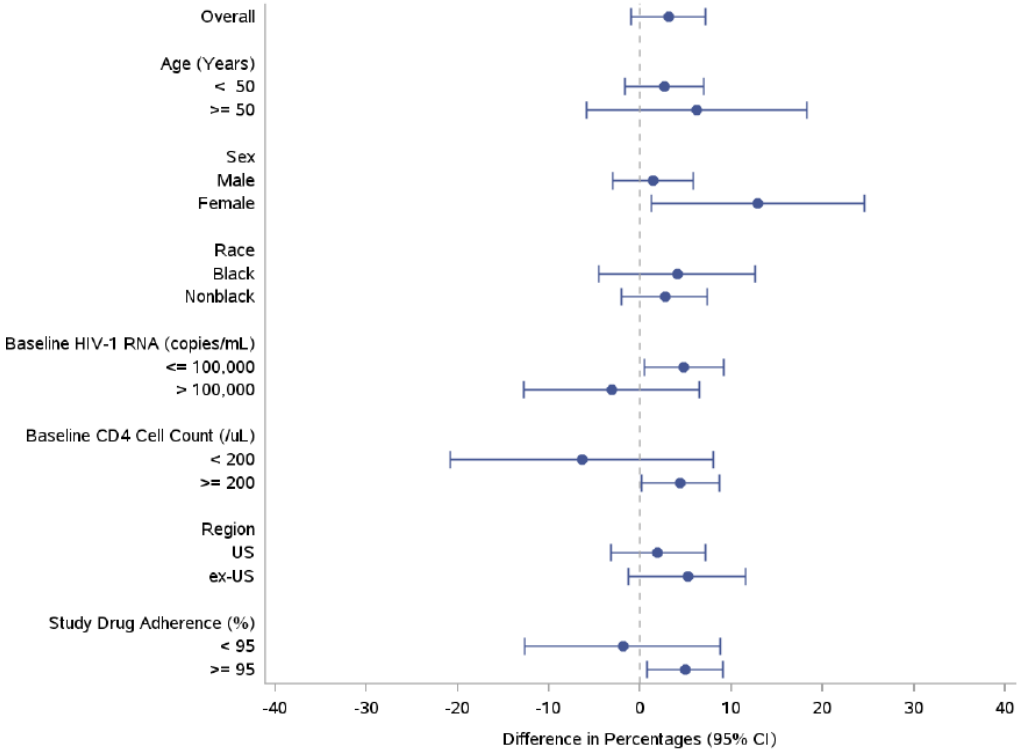
Virologic outcomes at Week 48 were also similar between treatments when assessed using the FDA-defined snapshot algorithm applied to HIV-1 RNA < 20 copies/mL. Rates of virologic failure in this analysis were the same for the two treatments (13.9% vs. 13.8%) and similar findings applied in the PP Analysis 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 14. 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%.

Table 72. Pooled virologic outcomes of studies GS US 292 0104 and GS US 292 0111 at Week 48[a,b]

	E/C/F/TAF (n=866)	E/C/F/TDF[e] (n=867)
HIV-1RNA <50copies/mL	92%	90%
Treatment difference	2.0% (95% CI: -0.7% to 4.7%)	
HIV-1RNA ≥50copies/mL[c]	4%	4%
No virologic data at Week48 window	4%	6%
Discontinued study drug due to AE or death[d]	1%	2%
Discontinued study drug due to other reasons and last available HIV-1RNA <50copies/mL[e]	2%	4%
Missing data during window but on study drug	1%	< 1%
Proportion (%) of patients with HIV-1 RNA <50copies/mL by subgroup		
Age < 50 years ≥ 50 years	 716/777 (92%) 84/89 (94%)	 680/753 (90%) 104/114 (91%)
Sex Male Female	 674/733 (92%) 126/133 (95%)	 673/740 (91%) 111/127 (87%)
Race Black Non-black	 197/223 (88%) 603/643 (94%)	 177/213 (83%) 607/654 (93%)
Baseline viral load ≤100,000copies/mL >100,000copies/mL	 629/670 (94%) 171/196 (87%)	 610/672 (91%) 174/195 (89%)
Baseline CD4+cell count <200cells/mm^3 ≥200cells/mm^3	 96/112 (86%) 703/753 (93%)	 104/117 (89%) 680/750 (91%)
HIV-1RNA <20copies/mL	84.4%	84.0%
Treatment difference	(0.4% (95% CI: -3.0% to 3.8%)	

E/C/F/TAF = elvitegravir/cobicistat/emtricitabine/tenofovir alafenamide

E/C/F/TDF = elvitegravir/cobicistat/emtricitabine/tenofovir disoproxil fumarate

a Week 48 window was between Day 294 and 377 (inclusive).

b In both studies, patients were stratified by baseline HIV 1 RNA (≤ 100,000 copies/mL, > 100,000 copies/mL to ≤ 400,000 copies/mL, or > 400,000 copies/mL), by CD4+ cell count (< 50 cells/μL, 50 199 cells/μL, or ≥ 200 cells/μL), and by region (US or ex US).

c Included patients who had ≥ 50 copies/mL in the Week 48 window; patients who discontinued early due to lack or loss of efficacy; patients who discontinued for reasons other than an adverse event (AE), death or lack or loss of efficacy and at the time of discontinuation had a viral value of ≥ 50 copies/mL.

d Includes patients who discontinued due to AE or death at any time point from Day 1 through the time window if this resulted in no virologic data on treatment during the specified window.

e Includes patients who discontinued for reasons other than an AE, death or lack or loss of efficacy; e.g., withdrew consent, loss to follow-up, etc.

GS-US-299-0102

Study Title: A Phase 2, Randomized, Double-Blinded Study of the Safety and Efficacy of Darunavir/Cobicistat/Emtricitabine/GS-7340 Single Tablet Regimen Versus Cobicistat-boosted Darunavir plus Emtricitabine/Tenofovir Disoproxil Fumarate Fixed Dose Combination in HIV-1 Infected, Antiretroviral Treatment-Naïve Adults

Eligible patients were adults with: HIV-1 RNA ≥ 5,000 copies/mL at screening; no prior use of any approved or investigational ARV; documented viral susceptibility to DRV, FTC and TDF. Patients were randomised (1:1) to D/C/F/TAF (800/150/200/10 mg) or DRV 800 mg (2 x 400 mg) + COBI 150 mg + TVD (FTC 200 mg/TDF 300 mg). Randomisation was stratified by HIV-1 RNA ≤ 100,000 vs. > 100,000 copies/mL) and race (Black vs. non-Black). All study drugs were to be taken once daily at about the same time and with food. After Week 48, patients continued on blinded medications until treatment assignments were unblinded, when they were offered open label E/C/F/TAF.

HIV 1 RNA plasma concentrations were assessed using the COBAS AmpliPrep/COBAS TaqMan HIV 1 Test (Version 2.0). The primary efficacy endpoint was the percentage of patients that achieved HIV-1 RNA < 50 copies/mL at Week 24 as defined by the FDA snapshot analysis algorithm. The primary analysis assessed the non-inferiority of D/C/F/TAF relative DRV+COBI+TVD using a conventional 95% CI approach, with a non-inferiority margin of 12%. The primary analysis used the FAS and a secondary analysis used the PP analysis set.

The study was conducted between in 2012 and 2014 at 40 sites in the USA and Puerto Rico. Demographic and general baseline characteristics were similar between the treatment groups. The majority was male (92.8%) with a mean age of 35 years (range 18 to 68 years). All patients had eGFR (as measured by CG) >70 mL/min with means between 115-120 mL/min. The mean baseline HIV-1 RNA value was 4.68 $\log_{10}$ copies/mL, mean CD4 count was 417 cells/µL and mean CD4% was 23.7. Overall, 80.4% had ≤ 100,000 copies/mL and 5.2% had > 400,000 copies/mL. The majority (89.5%) was asymptomatic, 7.2% had symptomatic HIV-1 infection and 3.3% had AIDS.

Most (97.4 %) had HIV-1 subtype B. Six patients had HIV-1 that contained one DRV-specific RAM at screening, which did not adversely influence treatment response. NNRTI-associated RAMs were found in nearly 20% with the K103N/S mutation being the most prevalent (10.5%) and 9.8% had HIV-1 with nucleoside-associated RAMs, with the V118I being the most prevalent (6.5%).

In the primary analysis the virologic outcomes at Week 24 were similar between treatments groups.

Table 73. GS-US-299-0102: Virologic Outcome at Week 24 using Snapshot Analysis Algorithm and HIV-1 RNA < 50 copies/mL (FAS)

	D/C/F/TAF (N=103)	DRV+COBI +TVD (N=50)	D/C/F/TAF vs. DRV+COBI+TVD	
			p-value[a]	Difference in Percentages (95% CI)[b]
Virologic Success at Week 24[c]				
HIV-1 RNA < 50 copies/mL	77 (74.8%)	37 (74.0%)	0.64	3.3% (-11.4% to 18.1%)
Virologic Failure at Week 24[c]	21 (20.4%)	12 (24.0%)		
HIV-1 RNA ≥ 50 copies/mL	14 (13.6%)	11 (22.0%)		
Discontinued Study Drug Due to Other Reasons and Last Available HIV-1 RNA ≥ 50 copies/mL[d]	7 (6.8%)	1 (2.0%)		
Added New ARV	0	0		
No Virologic Data in Week 24 Window[c]	5 (4.9%)	1 (2.0%)		
Discontinued Study Drug Due to AE/Death	1 (1.0%)	0		
Discontinued Study Drug Due to Other Reasons and Last Available HIV-1 RNA < 50 copies/mL	4 (3.9%)	1 (2.0%)		

a P-value for the superiority test comparing the percentages of virologic success was from the CMH test stratified by baseline HIV-1 RNA and race strata.

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.

Virologic success rates in the Week 24 PP analysis set were D/C/F/TAF 84.6% (77/91) vs. DRV+COBI+TVD 78.7% (37/47) with a difference of 8.3% and 95% CI: -5.3% to 22.0%.

In contrast, the virologic success rates through Week 48 were lower for D/C/F/TAF vs. DRV+COBI+TVD, which reflected numbers that discontinued study drug and had a last available HIV-1 RNA ≥ 50 copies/mL (D/C/F/TAF: 8.7%, 9 subjects; DRV+COBI+TVD: 4.0%, 2 subjects). Percentages with documented virologic failure at Week 48 were more comparable (6.8% vs. 8%).

Table 74. GS-US-299-0102: Virologic Outcome at Week 48 using Snapshot Analysis Algorithm and HIV-1 RNA < 50 copies/mL (FAS)

	D/C/F/TAF (N=103)	DRV+COBI +TVD (N=50)	D/C/F/TAF vs. DRV+COBI+TVD	
			p-value[a]	Difference in Percentages (95% CI)[b]
Virologic Success at Week 48[c]				
HIV-1 RNA < 50 copies/mL	79 (76.7%)	42 (84.0%)	0.35	-6.2% (-19.9% to 7.4%)
Virologic Failure at Week 48[c]	16 (15.5%)	6 (12.0%)		
HIV-1 RNA ≥ 50 copies/mL	7 (6.8%)	4 (8.0%)		
Discontinued Study Drug Due to Lack of Efficacy	0	0		
Discontinued Study Drug Due to Other Reasons and Last Available HIV-1 RNA ≥ 50 copies/mL[d]	9 (8.7%)	2 (4.0%)		
Added New ARV	0	0		
No Virologic Data in Week 48 Window[c]	8 (7.8%)	2 (4.0%)		
Discontinued Study Drug Due to AE/Death	1 (1.0%)	1 (2.0%)		
Discontinued Study Drug Due to Other Reasons and Last Available HIV-1 RNA < 50 copies/mL	7 (6.8%)	1 (2.0%)		

a P-value for the superiority test comparing the percentages of virologic success was from the CMH test stratified by baseline HIV-1 RNA and race strata.

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.

The data at the <20 c/mL cut-off were provided on request and showed that there was a disadvantage for D/C/F/TAF at both Weeks 24 and 48.

Table 75. GS-US-299-0102: Virologic Outcome at Week 24 and Week 48 Using Snapshot Algorithm and HIV-1 RNA < 20 copies/mL (Full Analysis Set)

Week	Virologic Outcome	D/C/F/TAF n/N (%)	DRV+COBI+TVD n/N (%)
Week 24	Success	57/103 (55.3)	31/50 (62.0)
	Failure	44/103 (42.7)	18/50 (36.0)
	No Data	2/103 (1.9)	1/50 (2.0)
Week 48	Success	65/103 (63.1)	38/50 (76.0)
	Failure	33/103 (32.0)	10/50 (20.0)
	No Data	5/103 (4.9)	2/50 (4.0)

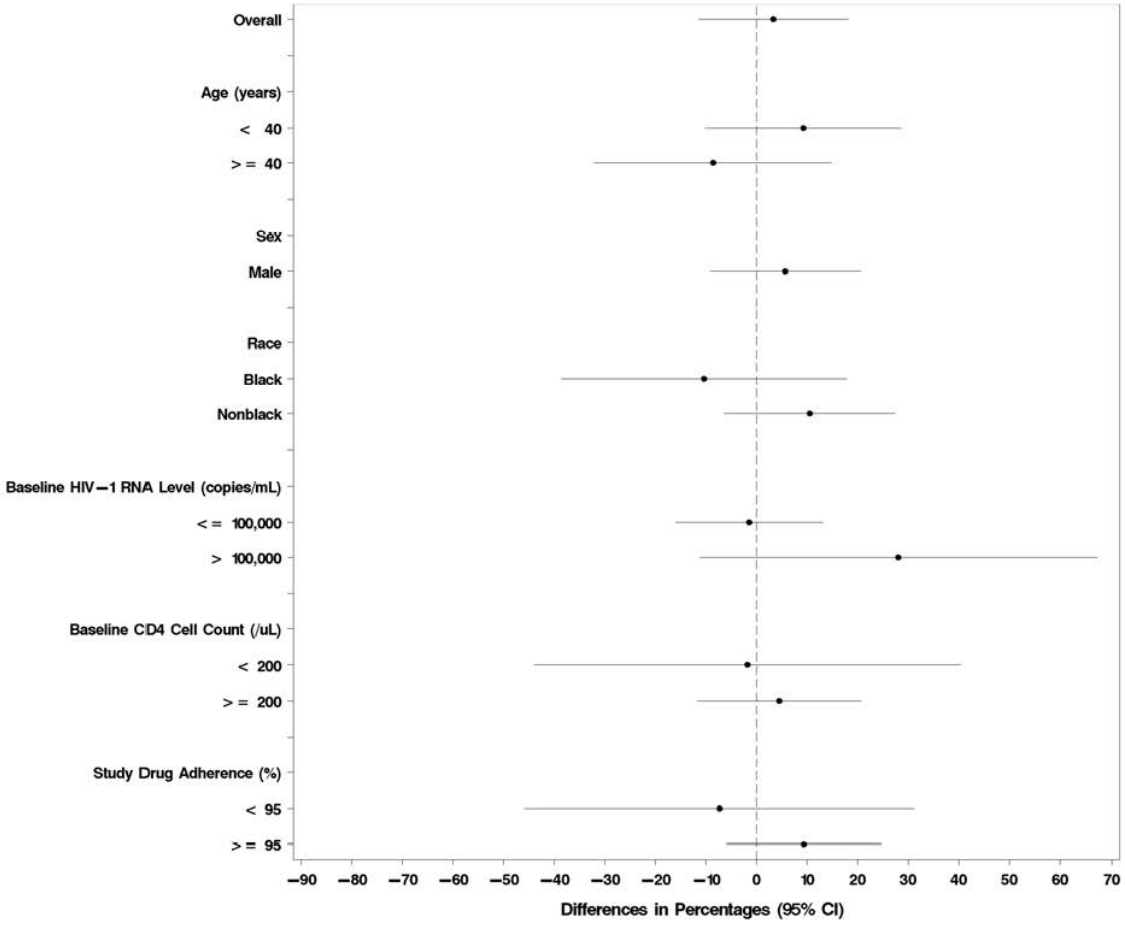
In the Week 48 PP analysis set the virologic success rates were D/C/F/TAF 92.9% (79/85) vs. DRV+COBI+TVD 91.3% (42/46); difference 2.4%, 95% CI: -8.8% to 13.7%. Other approaches to analysis of the FAS gave generally similar results to the primary analysis and also applied to the PP sets.

A pure virologic response (HIV-1 RNA < 50 copies/mL on 2 consecutive visits) through Week 48 was observed for D/C/F/TAF 81.6% (84/103) vs. DRV+COBI+TVD 86.0% (43/50). Similar percentages in each treatment group did not achieve confirmed suppression (15% vs. 12%). At Week 48, the KM estimates for the percentages with PVF were 18% in the D/C/F/TAF group and 14% in the DRV+COBI+TVD group (overall p-value = 0.89).

CD4 cell counts increased for each treatment group with mean increases through Week 48 (observed data) using the FAS of D/C/F/TAF 231 (141.9) cells/µL vs. DRV+COBI+TVD 212 (151.5) cells/µL. The CD4% also increased in each treatment group with mean increases from baseline at Week 48 of D/C/F/TAF 8.2% vs. DRV+COBI+TVD 9.3%.

At Week 24, the rate of virologic success by FDA-defined snapshot algorithm for subgroups according to age, sex, race, baseline HIV-1 RNA level, baseline CD4 cell count or study drug adherence rate was similar for the D/C/F/TAF and DRV+COBI+TVD. Data for Week 48 were consistent with Week 24.

Figure 15. GS-US-299-0102: Forest Plot of Treatment Difference in Virologic Success by Subgroup at Week 24 using Snapshot Analysis Algorithm and HIV-1 RNA < 50 copies/mL (FAS)



Difference in response rate and its 95% CI were from baseline HIV-1 RNA stratum-adjusted MH proportion and normal approximation (except for baseline HIV-1 RNA subgroup).

For baseline HIV-1 RNA subgroup, difference in response rate and its 95% CI were from normal approximation.

Relative to the vertical line at 0, differences on the right favour the D/C/F/TAF group and differences on the left favour the DRV+COBI+TVD group.

Point estimate of the treatment difference and 95% CI were not calculable for female subgroup.

One patient in the D/C/F/TAF arm had HIV containing a new NRTI-resistance mutation at the unblinding visit post Week 48 with a mutant/wild-type mixture at position K65 (K65K/R) and a mutant/wild-type mixture at position M184 (M184M/I), which are associated with resistance to TDF/TAF and FTC, respectively. However, phenotypic susceptibilities to both FTC and TDF were in the sensitive range. This patient had a prior episode of virologic failure at Week 40 followed by re-suppression of HIV-1 RNA <50 copies/mL, suggesting a history of poor adherence. The patient has had undetectable HIV-1 RNA for >6 months while receiving E/C/F/TAF in GS-US-292-0102.

GS-US-311-1089 – Study Title: A Phase 3, Randomized, Double-Blind, Switch Study to Evaluate F/TAF in HIV-1 Positive Subjects who are Virologically Suppressed on Regimens Containing FTC/TDF

This is an ongoing randomised, double-blind, multicentre, active-controlled study to evaluate the efficacy and safety of switching to F/TAF vs. continuing FTC/TDF while maintaining the same third agent in virologically suppressed (HIV-1 RNA < 50 copies/mL) patients on a stable regimen containing FTC/TDF. The primary efficacy endpoint was the percentage with HIV-1 RNA < 50 copies/mL at Week 48 (FDA snapshot algorithm). The distribution of third agent use by treatment groups was well balanced (Table 41).

Table 76. GS-US-311-1089: Baseline Third Agent (interim Week 48)

	F/TAF+ 3rd Agent (N = 333)	FTC/TDF+ 3rd Agent (N = 330)	Total (N = 663)	F/TAF vs. FTC/TDF
Baseline Third Agent (N)				
ATV/r	53 (15.9%)	50 (15.2%)	103 (15.5%)	
DRV/r	84 (25.2%)	82 (24.8%)	166 (25.0%)	
LPV/r	18 (5.4%)	18 (5.5%)	36 (5.4%)	
DTG	26 (7.8%)	23 (7.0%)	49 (7.4%)	
EFV	8 (2.4%)	6 (1.8%)	14 (2.1%)	0.65
MVC	1 (0.3%)	6 (1.8%)	7 (1.1%)	
NVP	74 (22.2%)	66 (20.0%)	140 (21.1%)	
RAL	66 (19.8%)	73 (22.1%)	139 (21.0%)	
RPV	3 (0.9%)	6 (1.8%)	9 (1.4%)	

Virologic outcomes at Week 48 were similar between treatments in the FAS and the analysis met the applicant's pre-defined non-inferiority margin. Virologic failure at Week 48 occurred in 0.3% in the switch group vs. 1.5% in the FTC/TDF group while similar percentages had no virologic data.

Table 77. GS-US-311-1089: Virologic Outcome at Week 48 Using Snapshot Algorithm and HIV-1 RNA < 50 copies/mL (Full Analysis Set)

	F/TAF+ 3rd Agent (N = 333)	FTC/TDF+ 3rd Agent (N = 330)	F/TAF vs. FTC/TDF	
			P-value[a]	Difference in Proportions (95.002% CI)[b]
Virologic Success at Week 48				
HIV-1 RNA < 50 copies/mL	314 (94.3%)	307 (93.0%)	0.50	1.3% (−2.5% to 5.1%)
Virologic Failure at Week 48	1 (0.3%)	5 (1.5%)		
HIV-1 RNA ≥ 50 copies/mL	0	5 (1.5%)	—	—
Discontinued Study Drug Due to Lack of Efficacy	0	0	—	—
Discontinued Study Drug Due to Other Reasons and Last Available HIV-1 RNA ≥ 50 copies/mL[c]	1 (0.3%)	0	—	—
Added New ARV	0	0	—	—
No Virologic Data in Week 48 Window	18 (5.4%)	18 (5.5%)	—	—
Discontinued Study Drug Due to AE/Death	7 (2.1%)	3 (0.9%)	—	—
Discontinued Study Drug Due to Other Reasons and Last Available HIV-1 RNA < 50 copies/mL[c]	10 (3.0%)	15 (4.5%)	—	—
Missing Data During Window but on Study Drug	1 (0.3%)	0	—	—

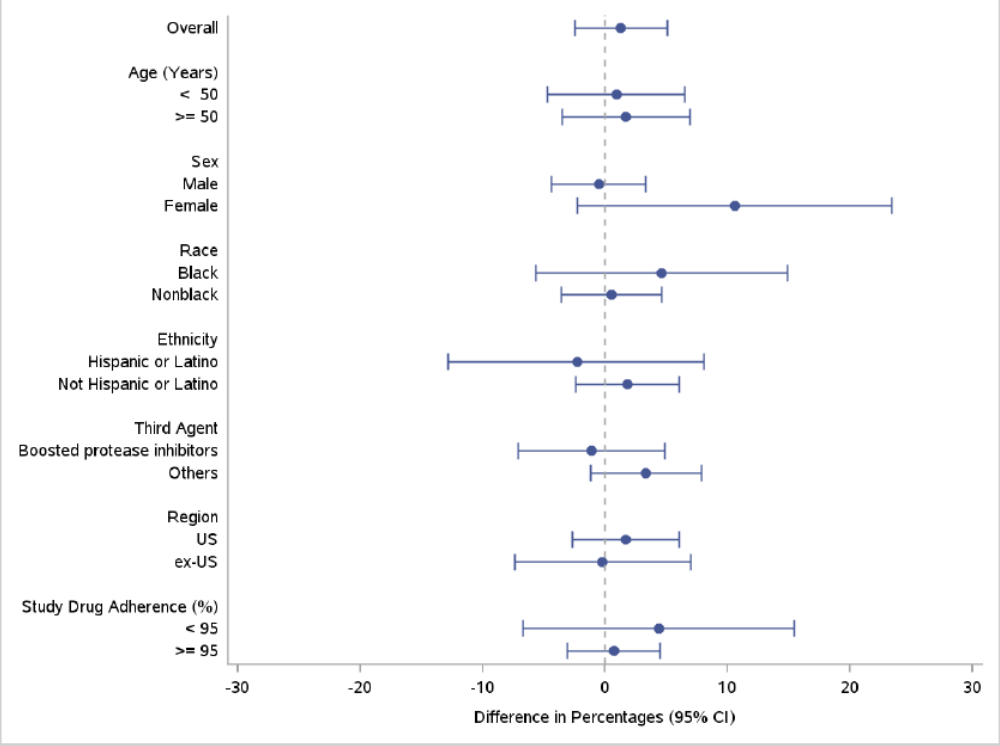
a P-value for the superiority test comparing the percentages of virologic success was from the CMH test stratified by third agent (ritonavir-boosted protease inhibitors vs. others).

b Difference in percentages of virologic success between treatment groups and its 95.002% CI were calculated based on the MH proportions adjusted by the third agent stratum.

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

The proportion of patients with HIV-RNA < 50 copies/mL by prior treatment regimen with boosted PIs was 91.6% in the F/TAF containing arm versus 92.7% in the baseline arm. The proportion of patients with HIV-RNA < 50 copies/mL by prior treatment regimen with other third agents was 96.6% in the F/TAF containing arm versus 93.3% in the baseline arm.

Figure 16. GS-US-311-1089: Forest Plot of Treatment Difference in Virologic Success at Week 48 (HIV-1 RNA < 50 copies/mL, Snapshot Algorithm) by Subgroup (Full Analysis Set)



Difference in response rates and its 95% CI were calculated based on the MH proportions adjusted by the third agent (ritonavir-boosted protease inhibitors vs. others) stratum (if not the subgroup factor).

Relative to the vertical line at 0, differences on the right favor the F/TAF group and differences on the left favor the FTC/TDF group.

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.

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.

Overall, 56% were female, the median age was 15 years (range 12 to 17) and patients were either black (88%) or Asian (12%). The median BMI at baseline was 20.0 kg/m2 and the median eGFR values calculated using the Schwartz and modified Schwartz formulas were 156 mL/min/1.73 m2 and 110.8 mL/min/1.73 m2, respectively. Pubertal stage at baseline varied widely.

The median baseline HIV-1 RNA value was 4.65 $\log_{10}$ copies/mL and 20.8% had > 100,000 copies/mL. The median baseline CD4 cell count was 456 cells/µL and median CD4% was 23%. Sixty-four percent had acquired HIV through vertical transmission. The majority (83.3%) was asymptomatic.

Mean (SD) adherence to study drug was 97.2%. Most subjects (83.7%) had an adherence rate ≥ 95%, and 93.9% had an adherence rate ≥ 90%.

Table 78. GS-US-292-0106: Virologic Outcome at Week 48 (HIV-1 RNA Cut-off at 50 copies/mL, Snapshot Algorithm, FAS)

	E/C/F/TAF (N=50)
Virologic Success at Week 48[a]	
HIV-1 RNA < 50 copies/mL	46 (92.0%)
Virologic Failure at Week 48[a]	3 (6.0%)
HIV-1 RNA >= 50 copies/mL	2 (4.0%)
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 48 Window[a]	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 48 window is between Day 308 and 377 (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.

Resistance analysis of Phase 2 and Phase 3 E/C/F/TAF studies

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) based on data to week 48. No patient excluded from the final RAP had viruses with emergent resistance mutations. The impact of baseline RAMs as well as subtype on treatment outcomes was assessed for all patients.

Across the 3 studies that compared E/C/F/TAF with STB in ART-naive patients the baseline RAMs and HIV-1 subtypes were comparable between treatment groups.

- Baseline RAMs and HIV-1 subtype had no impact on treatment outcomes.
- After 48 weeks of treatment the emergence of resistance mutations was rare. There were 11 patients with viruses that developed any treatment-emergent genotypic resistance – 4/978 (0.4%) in the E/C/F/TAF group and 7/925 (0.8%) in the STB group.
- In the E/C/F/TAF group, M184V was observed in conjunction with primary INSTI-R mutations in 4 patients, all of whom had > 350,000 copies/mL at baseline and 3 had baseline CD4 counts <50 cells/µL.
- The patterns of emergent resistance were similar in the E/C/F/TAF and STB groups but numerically lower for the E/C/F/TAF group.

- Overall, the cross-resistance profiles for the subjects with virologic failure who had emergent resistance to EVG, FTC, and TFV were consistent with historical data.

During the assessment resistance data became available from the F/TAF Study GS-US-311-1089 and from studies of E/C/F/TAF GS-US-292-0106, 0109 and 1249.

In the Week 48 analysis of **GS-US-311-1089** 3/663 (0.5%) patients met the protocol-defined virologic failure criteria with confirmed virologic rebound and were included in the resistance analysis population. All three had received FTC plus TAF or TDF with DRV/r.

Two were from the 333 in the F/TAF+3rd Agent group and one was from the 330 in the FTC/TDF+3rd Agent group. One was classified as a Week 48 FDA-snapshot virologic failure on F/TAF+3rd Agent (see the efficacy data in the previous question) and two were classified as virologic success because they had re-suppression of HIV-1 RNA to < 50 copies/mL at Week 48. One of the two in the F/TAF+3rd Agent group (0.3%) developed M184V with reduced susceptibility to FTC at virologic failure (Week 36) and then discontinued study drug 36 days later. The single subject in the FTC/TDF+3rd agent group did not develop resistance to any regimen components.

The Week 48 analysis of the E/C/F/TAF switch study **GS-US-292-0109** described 5/1436 (0.3%) patients who had virologic failure with confirmed virologic rebound. Four of 959 had switched to E/C/F/TAF and one of 477 remained on FTC/TDF+3rd Agent. One of the switched patients developed M184M/I with reduced susceptibility to emtricitabine at virologic failure (Week 8) and then re-suppressed with HIV-1 RNA < 50 copies/mL by ESDD when the patient switched to a new drug regimen. The single patient in the FTC/TDF+3rd agent group did not develop resistance to any regimen components.

No emergent resistance to E/C/F/TAF was detected through Week 48 in **GS-US-292-0106** in adolescents.

After 48 weeks of E/C/F/TAF in **GS-US-292-1249** none of the 75 patients co-infected with HBV in the FAS met criteria for inclusion in the resistance analysis population.

2.5.3. Discussion on clinical efficacy

The Phase 2 and 3 E/C/F/TAF studies vs. STB and the D/C/F/TAF study vs. DRV/co + TVD evaluated the use of TAF with other highly active agents to which ART-naïve patients' viruses were known to be susceptible. The design of these studies, while appropriate for patient care, cannot provide definitive evidence that the TAF dose within these highly active regimens was necessarily sufficient.

In addition, while GS-US-311-1089 has so far suggested high rates of maintenance of viral suppression, the efficacy data from switch studies can only be viewed as supportive.

Therefore, as indicated in the sections on pharmacokinetics and pharmacodynamics, the critical data to support the TAF doses in the F/TAF FDCs come from the monotherapy studies (with doses ranging from 8 to 150 mg), the Emax modelling and the PK data that supported adjusting the TAF dose from 25 mg to 10 mg depending on the content of the regimen.

Regarding use of F/TAF 10 mg with DRV/r and DRV/c, the only PK and efficacy data concern use of F/TAF with 800 mg DRV once daily when dosing DRV/co within the candidate FTC presentation. Nevertheless, in terms of PK interaction, it seems likely that multiple dosing with DRV 400 mg BID to steady state would likely exert the same impact on TAF as DRV 800 mg QD. The results cannot be dismissed on grounds that the differences between treatments reflect discontinuation rates and missing data rather than documented failure

rates. In addition, this study was not intended to provide definitive evidence of the efficacy of the test STR. In this small study with 2:1 randomisation it is difficult to interpret the subgroup analyses.

In conclusion, this study, in which the virologic suppression rates were unusually low for an ART-naïve population, cannot *per se* support the sufficiency of F/TAF 10 mg when co-administered with DRV/r or DRV/co and it is not possible to dismiss potential concerns regarding the Week 48 data. The additional data from the subgroup in GS-US-311-1089 that received F/TAF 10 mg with DRV/r cannot address this concern. However, it is difficult to justify precluding the use of F/TAF with boosted DRV and, as discussed under PK, this use is considered acceptable with the reporting of the data < 20 c/mL in section 5.1 and cross-referring from section 4.2.

The Applicant committed to submit the 96-week results of studies GS-US-311-1089 and GS-US-366-1216, which should include a detailed analysis of all patients who fail to maintain suppression <20 cp/mL.

The concern regarding the adequacy of F/TAF-containing regimens to prevent HIV-1-related CNS disorders is discussed in full under PK. Thus far the virological resistance data do not point to any major concerns.

2.5.4. Conclusions on the clinical efficacy

The efficacy of F/TAF 10 mg given with a P-gp inhibitor such as COBI was demonstrated in the Phase 3 studies of E/C/F/TAF vs. STB in which very high percentages achieved < 50 and < 20 copies/mL at Week 48 using the FDA snapshot algorithm. Supportive evidence of efficacy comes from the Phase 2 study that compared D/C/F/TAF 10 mg with D/C/F/TDF and from the E/C/F/TAF and F/TAF switch studies in which TAF-containing regimens have maintained viral suppression with no excess of rebound compared to patients who stayed on their TDF-containing regimens. The critical data to support the dose recommendations for F/TAF come from the monotherapy studies (with TAF doses ranging from 8 to 150 mg), the Emax modelling and the PK data on use of F/TAF 10 mg or 25 mg with various potentially interacting antiretroviral agents. These data supported a standard TAF dose of 25 mg and generally supported a reduction from 25 mg to 10 mg when TAF is given with a strong P-gp inhibitor, such as COBI or RTV.

The data in adolescents are currently limited and confined to use of E/C/F/TAF but the PK data support use of the adult dose. Since E/C/F/TAF has been accepted for use in adolescents there is no reason to object to use of F/TAF. Nevertheless, the RMP reflects the paucity of data and the need to obtain more experience in this population.

2.6. Clinical safety

The comparisons between TAF and TDF facilitated by the E/C/F/TAF studies are important and highly relevant to assessing the safety profile that can be expected from F/TAF. These data were assessed during the review of Genvoya. A Safety Update from ongoing and recently reported studies was provided during the review of F/TAF. Essentially this did not indicate any new concerns.

This section discusses the new safety data from the Phase 2 study with E/C/F/TAF GS-US-299-010, the safety data from the switch study GS-US-311-1089 (which not only compared TAF with TDF but also TAF 10 mg and 25 mg in conjunction with a range of third agents) and data from the E/C/F/TAF study in adolescents (GS-US-292-0106).

In **GS-US-299-0102** the median (Q1, Q3) duration of exposure was similar between treatments (D/C/F/TAF 68.0 [65.4, 72.7] weeks; DRV+COBI+TVD 69.1 [66.0, 73.6] weeks). The majority (>75%) in each treatment group received study drug for ≥ 60 weeks.

Almost patients reported at least one AE in both treatment groups. The most commonly reported were:

- D/C/F/TAF - diarrhoea (21.4%, 22); URTI (15.5%, 16); fatigue (13.6%, 14); nausea (12.6%, 13); rash (11.7%, 12). Arthralgia was reported for 8.7% in the D/C/F/TAF group compared with none in the DRV+COBI+TVD group.
- DRV+COBI+TVD - diarrhoea (26.0%, 13); fatigue (18.0%, 9); URTI (14.0%, 7); flatulence (12.0%, 6); and nausea, vomiting, pain in extremity, vitamin D deficiency each in 10.0% (5)

Table 79. GS-US-299-0102: Overall Summary of Adverse Events (Safety Analysis Set)

Subjects Experiencing Any	D/C/F/TAF (N = 103)	DRV+COBI+TVD (N = 50)
Treatment-Emergent AE	95 (92.2%)	47 (94.0%)
Any Grade 2, 3, or 4 Treatment-Emergent AE	57 (55.3%)	24 (48.0%)
Any Grade 3 or 4 Treatment-Emergent AE	7 (6.8%)	4 (8.0%)
Any Treatment-Emergent Study Drug–Related AE	43 (41.7%)	19 (38.0%)
Any Grade 2, 3, or 4 Treatment-Emergent Study Drug–Related AE	10 (9.7%)	3 (6.0%)
Any Grade 3 or 4 Treatment-Emergent Study Drug–Related AE	1 (1.0%)	1 (2.0%)
Any Treatment-Emergent SAE	5 (4.9%)	2 (4.0%)
Any Treatment-Emergent Study Drug–Related SAE	1 (1.0%)	0
Any Treatment-Emergent AE Leading to Premature Study Drug Discontinuation	2 (1.9%)	2 (4.0%)
Treatment-Emergent Death	0	0

Table 80. GS-US-299-0102: Adverse Events Reported for ⩾5% of Subjects in Either Treatment Group (Safety Analysis Set

Adverse Events by System Organ Class and Preferred Term[a,b]	D/C/F/TAF (N = 103) n (%)	DRV+COBI+TVD (N = 50) n (%)
Any Treatment-Emergent AE	95 (92.2)	47 (94.0)
Gastrointestinal Disorders	54 (52.4)	26 (52.0)
Diarrhoea	22 (21.4)	13 (26.0)
Nausea	13 (12.6)	5 (10.0)
Flatulence	5 (4.9)	6 (12.0)
Abdominal pain	6 (5.8)	3 (6.0)
Vomiting	4 (3.9)	5 (10.0)
Haemorrhoids	3 (2.9)	4 (8.0)
General Disorders and Administration Site Conditions	26 (25.2)	11 (22.0)
Fatigue	14 (13.6)	9 (18.0)
Pyrexia	7 (6.8)	2 (4.0)
Infections and Infestations	62 (60.2)	32 (64.0)
Upper respiratory tract infection	16 (15.5)	7 (14.0)
Bronchitis	9 (8.7)	2 (4.0)
Sinusitis	7 (6.8)	4 (8.0)
Nasopharyngitis	5 (4.9)	3 (6.0)
Folliculitis	3 (2.9)	4 (8.0)
Influenza	2 (1.9)	3 (6.0)
Pharyngitis	1 (1.0)	3 (6.0)
Tooth abscess	0	3 (6.0)
Metabolism and Nutrition Disorders	16 (15.5)	13 (26.0)
Decreased appetite	4 (3.9)	3 (6.0)
Vitamin D deficiency	2 (1.9)	5 (10.0)
Musculoskeletal and connective tissue disorders	28 (27.2)	18 (36.0)
Pain in extremity	8 (7.8)	5 (10.0)
Arthralgia	9 (8.7)	0
Back pain	1 (1.0)	3 (6.0)
Neoplasms benign, malignant and unspecified (incl cysts and polyps)	8 (7.8)	5 (10.0)
Anogenital warts	3 (2.9)	3 (6.0)
Nervous system disorders	16 (15.5)	11 (22.0)
Headache	7 (6.8)	4 (8.0)
Psychiatric disorders	19 (18.4)	6 (12.0)
Insomnia	6 (5.8)	2 (4.0)
Respiratory, thoracic and mediastinal disorders	20 (19.4)	9 (18.0)
Cough	7 (6.8)	3 (6.0)
Oropharyngeal pain	5 (4.9)	4 (8.0)
Sinus congestion	6 (5.8)	3 (6.0)
Skin and subcutaneous tissue disorders	29 (28.2)	13 (26.0)
Rash	12 (11.7)	4 (8.0)
Vascular disorders	4 (3.9)	3 (6.0)
Hypertension	2 (1.9)	3 (6.0)

Similar percentages in each group had any Grade 3 AEs and no Grade 4 AEs were reported. Grade 3 AEs included a E/C/F/TAF patient with a SAE of Grade 3 hypersensitivity and non-serious AE of Grade 3 rash, which resulted in study drug discontinuation. AEs considered by the investigator to be related to study drug were reported for E/C/F/TAF 41.7% (43) and DRV+COBI+TVD 38.0% (19). The most common were diarrhoea (13.6% vs. 14.0%), flatulence (3.9% vs. 10.0%), nausea (9.7% vs. 6.0%) and fatigue (8.7% vs. 8.0%).

A higher percentage of patients experienced AEs of arthralgia in the D/C/F/TAF group (8.7%, 9/103 patients with 10 AEs) compared with none of 50 patients in the DRV+COBI+TVD group.

Of the 10 arthralgia AEs, 8 were Grade 1 and 2 were Grade 2 while none was serious or led to discontinuation. Two both Grade 1 AEs were related to study drug as assessed by the investigator. Seven resolved with continued treatment with study drug. No consistent manifestation of arthralgia was identified in terms of unilateral vs. bilateral or joint distributions and there were no trends with respect to time to onset (range Day 3 to 480; median Day 106.5). The distribution by gender and age reflected the overall study population. One had a medical history of osteoarthritis in the left ankle reported as ongoing. Concurrent self-limited systemic or gastrointestinal symptoms were reported by two patients (one had concurrent vomiting, nausea and fatigue) and one had bloody diarrhoea, loose stool and cough.

No selected fracture AEs were reported during this study. Decreases from baseline in BMD at the hip or spine were smaller in the D/C/F/TAF group compared with the DRV+COBI+TVD group. The differences in the categorical distribution of percentage change from baseline in hip or spine BMD were statistically significant ($p \leq 0.002$ for both at Weeks 24 and 48).

At Week 48 there was a > 3% decrease from baseline in hip BMD in E/C/F/TAF 18.3% vs. DRV+COBI+TVD 61.7% and in spine BMD in 32.5% vs. 55.3%. The distribution of the clinical BMD status adjusted for baseline status was significantly different between treatment groups at Week 48 at the hip and at Weeks 24 and 48 at the spine. Few patients in either group had worsening hip or spine BMD clinical status at Week 48 (hip 2 vs. 7 and spine 6 vs. 10).

No case of PRT occurred in the E/C/F/TAF group. Two patients in the E/C/F/TAF group had increased serum creatinine that was reported as an AE.

There were no AEs of uveitis during the study. AEs in the eye disorders SOC were reported for 5.8% (n = 6) in the D/C/F/TAF group and none in DRV+COBI+TVD group. One AE of photophobia was considered to be drug-related but did not result in discontinuation and resolved on the same day without treatment.

Patient exposure

Adverse events

Serious adverse event / deaths / other significant events

There have been no deaths in the additional studies in the F/TAF development programme.

In GS-US-299-0102 SAEs were reported in 5 D/C/F/TAF (4.9%) and 2 DRV+COBI+TVD (4.0%) patients. One SAE was considered related to study drug in the D/C/F/TAF group (the case of Grade 3 hypersensitivity on day 1 and Grade 3 rash starting on day 8 above). Both events resolved on day 16.

Discontinuations due to adverse events

In GS-US-299-0102 two patients in each treatment group (D/C/F/TAF 1.9%; DRV+COBI+TVD 4.0%) discontinued from the study due to AEs:

One in the D/C/F/TAF group had a SAE of hypersensitivity and a non-serious AE of rash (see above)

One in the D/C/F/TAF group had a SAE of substance abuse

One in the DRV+COBI+TVD group had a SAE of Grade 2 renal tubular disorder (reported as PRT)

One in the DRV+COBI+TVD group had an AE of Grade 3 worsening of diarrhoea

In GS-US-311-1386 one subject discontinued due to neutropenia.

In GS-US-311-1342 one subject discontinued due to Grade 2 macular rash.

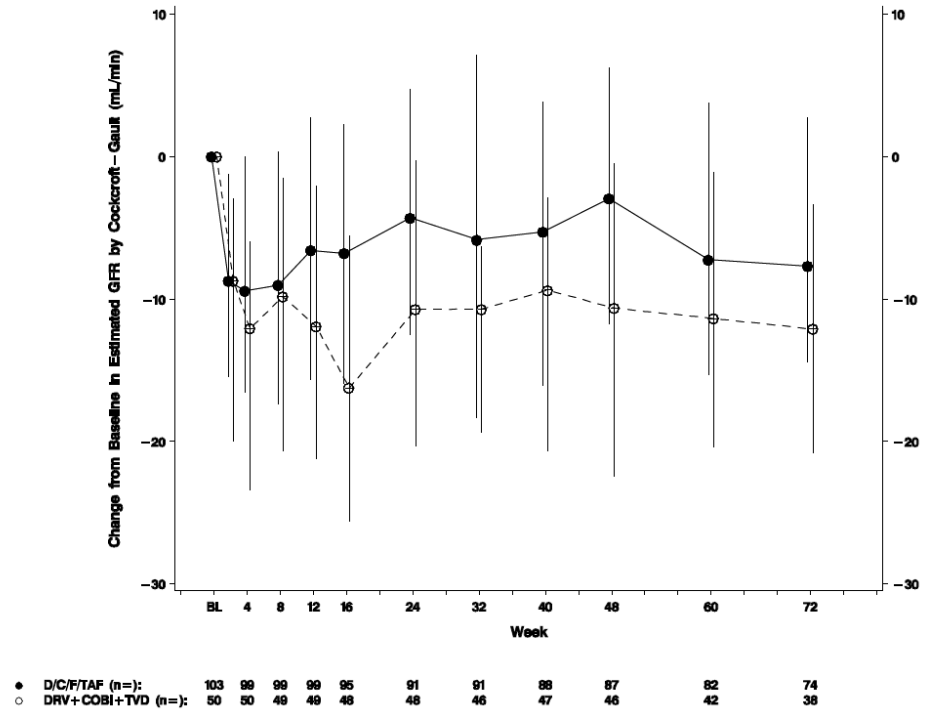
Laboratory findings

Renal laboratory parameters

In GS-US-299-0102 increases from baseline in mean values for serum creatinine occurred in both groups but were smaller at weeks 24 and 48 in the D/C/F/TAF group. Note that the actual changes were small in both groups (e.g. increases from baseline at Week 48 were D/C/F/TAF 0.06 mg/dL vs. DRV+COBI+TVD 0.09 mg/dL [p = 0.053]).

Decreases from baseline in median eGFRCG occurred in both groups but were smaller in the D/C/F/TAF group. For example, at Week 48 the median changes from baseline were D/C/F/TAF -2.9 mL/min vs. DRV+COBI+TVD -10.6 mL/min (p = 0.017). The changes from baseline in eGFRCKD-EPI, creatinine and eGFRCKD-EPI, cysC all followed the observations for eGFRCG.

Figure 17. GS-US-299-0102: Median (Q1, Q3) of Change from Baseline in Estimated GFR by Cockcroft-Gault (mL/min) by Visit (Safety Analysis Set)



Proteinuria was reported for 32.4% in the D/C/F/TAF group and 34.0% in the DRV+COBI+TVD group. Most proteinuria by dipstick was Grade 1 or 2; one in the DRV+COBI+TVD group had Grade 3. There were

numerical but not statistically significant differences between treatment groups in median percentage change from baseline in UPCR or UACR (UPCR: D/C/F/TAF -8.22% vs. DRV+COBI+TVD -27.52%; UACR -13.1% vs. -22.6%).

The median percent increase from baseline at Week 48 in RBP to creatinine ratio was D/C/F/TAF 9% vs. DRV+COBI+TVD 54% (p = 0.003) whereas the median percent change from baseline at Week 48 in beta-2-microglobulin to creatinine ratio was -42.0% vs. 2.3% (p = 0.002), respectively.

There were no clinically relevant changes from baseline in median values for the other renal biomarkers in either group (FEPO4 and FEUA using serum creatinine adjusted or unadjusted values) and no statistically significant differences between groups.

Other laboratory parameters

There were no clinically relevant changes from baseline within groups or differences between the treatment groups in median values for haematology or chemistry parameters and median values were within normal ranges except for lipase, which was measured only for subjects with elevated amylase.

Nevertheless, the majority had at least laboratory abnormality (D/C/F/TAF 96.1% vs. DRV+COBI+TVD 94.0%) including Grade 3 or 4 laboratory abnormalities in 27.5% vs. 28.0%. The most commonly reported Grade 3 or 4 abnormality was creatine kinase (D/C/F/TAF 8.8%; DRV+COBI+TVD 12.0%). No patients had elevations > 3 × ULN in AST or ALT, in addition to total bilirubin > 2 × ULN and ALP < 1.5 × ULN.

There were increases from baseline in fasting total cholesterol, fasting direct LDL cholesterol, fasting HDL cholesterol and fasting triglycerides at Weeks 24 and 48 for both treatments except that there was a decrease in fasting triglycerides at Week 48 in the DRV+COBI+TVD group. The median increases from baseline were for D/C/F/TAF at Week 24 and Week 48, with significant differences in fasting total cholesterol and fasting direct LDL cholesterol at Week 24 and for all four parameters at Week 48.

Graded fasting hypercholesterolemia was more common in the D/C/F/TAF group (57.6% vs. 36.7%). Graded abnormalities in fasting LDL occurred in 53.5% vs. 34.7%, with Grade 2 in 19.2% vs. 10.2% but Grade 3 in 5.1% vs. 8.2%. Similar percentages received lipid-modifying agents (14.6% vs. 14.0%).

Safety in special populations

GS-US-292-0106 Adolescents treated with E/C/F/TAF

At the time of the Week 24 interim analysis 39/48 subjects (81.3%) had at least 1 AE. Very common AEs included nausea (22.9%, 11 subjects), upper respiratory tract infection (20.8%, 10 subjects), diarrhoea (16.7%, 8 subjects), abdominal pain, headache, respiratory tract infection (each 14.6%, 7 subjects), vomiting (12.5%, 6 subjects) and dizziness, vitamin D deficiency (both 10.4%, 5 subjects). Four subjects (8.3%) had a Grade 3 or 4 AE including Grade 3 neuralgia, Grade 3 bipolar disorder, conduct disorder, mania and substance abuse, Grade 3 chorioretinitis and Grade 4 suicide attempt. Eighteen subjects (37.5%) had an AE considered related to study drug by the investigator, most of which were gastrointestinal or nervous system disorders.

Grade 2 visual impairment, Grade 3 chorioretinitis and Grade 2 intermediate uveitis that were considered drug-related by the investigator were reported for one subject. This case of potential uveitis was one of the four SAEs reported and the only one considered drug-related. The patient responded to treatment and did

not discontinue study drug. One renal SAE was reported, which was Grade 2 urinary retention in a 17-year-old black male with a prior history of urinary retention.

An updated report was provided. At that time enrolment was complete and 50 subjects had received E/C/F/TAF for at least 8 weeks while 25 had received 48 weeks. The median (Q1, Q3) duration of exposure to E/C/F/TAF was 48.0 (40.1, 72.1) weeks. Data reflected 2650 subject-weeks of exposure (vs. 922 subject-weeks in the original submission). At the time of the data cut 48 subjects were continuing study drug (25 main phase and 23 in the extension phase). Two had prematurely discontinued study drug and from the study (withdrew consent and lost to follow up).

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). Eighteen (36.0%) had an AE considered related to study drug by the investigator, most of which were Grade 1 or Grade 2 in severity and most commonly nausea, abdominal pain, vomiting, upper abdominal pain and diarrhoea. Four subjects (8.0%) had a SAE but except for the previously reported case of visual impairment and intermediate uveitis these were not considered related to study drug by the investigator. No subject had an AE that led to study drug discontinuation and there were no deaths.

An additional event of potential uveitis occurred in a subject who used illicit substances prior to sleeping and then awoke with blurred vision and photophobia. He presented to the ED the same day, where the vision changes were attributed to migraine headaches. The subject denied any previous history of migraine headaches. The visual changes resolved later that same night and have not been reported since. The investigator assessed this event as moderate, non-serious, and not related to E/C/F/TAF, with a possible alternative aetiology of substance abuse. The subject remains virologically suppressed on E/C/F/TAF.

One pregnancy occurred. The subject underwent an abortion and then resumed treatment with E/C/F/TAF.

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 stabilised 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 concerning decreases in eGFR or of 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.

Although limited data are available through Week 48, renal safety results at Week 48 were generally similar to Week 24. 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 81. GS-US-292-0106: Baseline Value and Percentage Change from Baseline in Spine and Total-Body-Less-Head BMD at Weeks 24 and 48 (Spine and TBLH DXA Analysis Sets)

	Spine BMD (N = 47)		TBLH BMD (N = 45)	
Time Point	Mean (SD)	Median (Q1, Q3)	Mean (SD)	Median (Q1, Q3)
Baseline (g/cm^2)	0.809 (0.2031)	0.778 (0.677, 0.943)	0.887 (0.1222)	0.878 (0.815, 0.962)
% Change at Week 24	1.598 (3.9359)	1.252 (−0.957, 4.106)	0.567 (2.5071)	0.248 (-1.141, 1.715)
% Change at Week 48	4.162 (4.9777)	3.261 (0.806, 7.050)	1.259 (2.6793)[a]	0.931 (-0.521, 2.598)[a]

a N = 44

Because the study population was shorter than the general population at the same age (median Z-scores for height at baseline and Week 24 were -0.75 and -0.72, respectively), adjustment of BMD Z-scores is considered critical for interpretation of the adolescent data. 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). Changes from baseline in spine and TBLH height-age adjusted BMD Z-scores at Weeks 24 and 48 are shown in Table 82.

Table 82. GS-US-292-0106: TBLH Standard and Height-Age BMD Z-Scores at Baseline, and Change from Baseline at Weeks 24 and 48 (TBLH DXA Analysis Set)

	TBLH BMD Z-Score (Standard) (N = 45)		TBLH BMD Z-Score (Height-Age) (N = 45)[a]	
	Mean (SD)	Median (Q1, Q3)	Mean (SD)	Median (Q1, Q3)
Baseline	−1.19 (1.154)	−1.07 (−1.79, −0.30)	−0.33 (1.034)[b]	−0.42 (−1.12, 0.60)[b]
Change from Baseline at Week 24	−0.11 (0.269)	−0.16 (−0.25, 0.02)	−0.11 (0.292)[b]	−0.13 (−0.31, 0.11)[b]
Change from Baseline at Week 48	−0.20 (0.276)[c]	−0.24 (−0.38, −0.01)[c]	−0.15 (0.310)[d]	−0.09 (−0.33, 0.07)[d]

a Some subjects had missing height-age Z-scores because their heights were outside the median height in the CDC growth chart, or the height-ages were outside the BMD reference data for Z-scores.

b N = 38

c N = 44

d N = 35

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.

Comparison of F/TAF vs. F/TDF and F/TAF 10 mg vs. 25 mg

During the procedure the Week 48 CSR for the switch study **GS-US-311-1089** (F/TAF 333 vs. FTC/TDF 330) was provided. The F/TAF group included 155 dosed with TAF 10 mg and 178 dosed with TAF 25 mg. This study is currently the only one with comparative safety data F/TAF vs. F/TDF when used with a range of third agents and also the only study that provides comparative safety data for the TAF 10 mg and 25 mg doses.

TAF vs. TDF

Table 83. GS-US-311-1089: Treatment-Emergent Adverse Events – Overall Summary (Safety Analysis Set)

	F/TAF+3rd Agent (N = 333)	FTC/TDF+3rd Agent (N = 330)
Subjects Experiencing Any Treatment-Emergent Adverse Event	281 (84.4%)	262 (79.4%)
Subjects Experiencing Any Grade 2, 3, or 4 Treatment-Emergent Adverse Event	130 (39.0%)	120 (36.4%)
Subjects Experiencing Any Grade 3 or 4 Treatment-Emergent Adverse Event	17 (5.1%)	12 (3.6%)
Subjects Experiencing Any Treatment-Emergent Study-Drug-Related Adverse Event	31 (9.3%)	40 (12.1%)
Subjects Experiencing Any Grade 2, 3, or 4 Treatment-Emergent Study-Drug-Related Adverse Event	5 (1.5%)	7 (2.1%)
Subjects Experiencing Any Grade 3 or 4 Treatment-Emergent Study Drug–Related Adverse Event	2 (0.6%)	1 (0.3%)
Subjects Experiencing Any Treatment-Emergent Serious Adverse Event	18 (5.4%)	14 (4.2%)
Subjects Experiencing Any Treatment-Emergent Study-Drug-Related Serious Adverse Event	0	1 (0.3%)
Subjects Experiencing Any Treatment-Emergent Adverse Event Leading to Premature Study Drug Discontinuation	7 (2.1%)	3 (0.9%)
Subjects who had Treatment-Emergent Death[a]	1 (0.3%)	0

a Treatment-emergent death refers to a death occurring between the first dose date and the last dose date plus 30 days (inclusive).

Safety conclusions for the comparisons between patients treated with TAF vs. those treated with TDF through 48 weeks (median 48.2 and 48.3 weeks) in this study were consistent with those in the studies of E/C/F/TAF vs. Stribild, as follows:

- There were low rates of SAEs and AEs leading to study drug discontinuation

- Renal laboratory parameters improved upon switching to F/TAF from FTC/TDF
- Spine and hip bone mineral density (BMD) improved upon switching to F/TAF from FTC/TDF
- Greater increases from baseline in fasting total cholesterol, fasting LDL cholesterol and fasting triglycerides were observed in the F/TAF group.

The most common AEs by treatment group were:

- F/TAF+3rd Agent: diarrhoea and URTI (9.0%, 30 patients each) and headache (8.1%, 27 patients)
- FTC/TDF+3rd Agent: URTI (13.6%, 45), diarrhoea (10.0%, 33) and sinusitis (6.7%, 22).

Grade 3 or 4 AEs were reported for 5.1% TAF and 3.6% TDF patients but none was considered drug-related by investigators.

AEs considered drug-related by investigators were reported for 9.3% TAF and 12.1% TDF patients of which nausea and diarrhoea were the most frequent in both treatment groups.

One and two patients per treatment group had fractures but all were the result of trauma and considered by the investigator as unrelated to the study drugs. There were increases from baseline in mean (SD) BMD at the hip and at the spine in the F/TAF+3rd Agent group compared with minimal changes from baseline in both parameters in the FTC/TDF+3rd Agent group ($p < 0.001$ at Weeks 24 and 48).

Differences between groups in the categorical distribution of percentage change from baseline in hip or spine BMD were also statistically significant ($p < 0.001$ at Weeks 24 and 48). At Week 48, more patients in the F/TAF+3rd Agent group had a $\geq$ 3% increase from baseline in hip (16.7% vs. FTC/TDF+3rd Agent 8.6%) or spine BMD (30.3% vs. 13.7%) and there was a decrease in bone turnover after switching to F/TAF from FTC/TDF.

Table 84. GS-US-311-1089: Bone Mineral Density (Observed Data, Hip or Spine DXA Analysis Set)

				F/TAF vs FTC/TDF	
		F/TAF+ 3rd Agent	FTC/TDF+ 3rd Agent	P-Value[a]	Diff in LSM (95% CI)[a]
Hip BMD[b]					
Baseline	N	321	317	0.64	0.005 (−0.015, 0.025)
	Mean (SD)	0.982 (0.1290)	0.977 (0.1288)		
% Change at Week 24	N	309	305	< 0.001	0.625 (0.274, 0.977)
	Mean (SD)	0.573 (2.1720)	−0.053 (2.2656)		
% Change at Week 48	N	300	303	< 0.001	1.287 (0.864, 1.710)
	Mean (SD)	1.135 (2.7526)	−0.152 (2.5317)		
Spine BMD[c]					
Baseline	N	321	320	0.76	0.004 (−0.022, 0.030)
	Mean (SD)	1.081 (0.1669)	1.077 (0.1663)		
% Change at Week 24	N	310	310	< 0.001	1.473 (1.022, 1.924)
	Mean (SD)	0.852 (2.8881)	−0.612 (2.8473)		
% Change at Week 48	N	300	306	< 0.001	1.735 (1.223, 2.246)
	Mean (SD)	1.527 (3.1816)	−0.206 (3.2233)		

% Change = Change from baseline at a postbaseline visit/baseline × 100%; Diff = difference

a For baseline, p-value and difference in least squared means (Diff in LSM), and its 95% CI were from an ANOVA model including treatment as a fixed effect. For postbaseline visits, p-values, difference in least squared means (Diff in LSM), and its 95% CI were from an ANOVA model including treatment and third agent randomization stratum as fixed effects.

b Only subjects with nonmissing baseline hip BMD were included in the Hip DXA Analysis Set.

c Only subjects with nonmissing baseline spine BMD were included in the Spine DXA Analysis Set.

In the F/TAF+3rd Agent group, no patient had renal AEs that were serious, resulted in discontinuation of study drug or were considered by the investigator as related to study drug. One patient in the FTC/TDF+3rd Agent group had a SAE of nephrolithiasis considered related to study drug and one had a non-serious renal AE of creatinine increased considered related and leading to study drug discontinuation. There were no AEs of proximal renal tubulopathy (including Fanconi Syndrome) reported.

In the switched group there were decreases from baseline in serum creatinine at most time points as compared with minimal changes from baseline with FTC/TDF+3rd Agent. At Week 48, the mean (SD) changes from baseline in serum creatinine were F/TAF+3rd Agent –0.08 [0.238] mg/dL vs. FTC/TDF+3rd Agent –0.04 [0.126] mg/dL (p = 0.005). There were increases from baseline in eGFRCG in the F/TAF+3rd Agent group vs. minimal changes in the FTC/TDF+3rd Agent group at Weeks 4 through 48.

Table 85. GS-US-311-1089: Changes from Baseline in Estimated GFR at Week 48 (Safety Analysis Set)

	F/TAF+3rd Agent (N = 333)			FTC/TDF+3rd Agent (N = 330)			
	N	Median	Q1, Q3	N	Median	Q1, Q3	P-Value[a]
$eGFR_{CG}$ (mL/min)							
Baseline	333	99.4	83.8, 120.3	329	100.2	83.8, 121.2	0.74
Change at Week 48	317	8.4	0.2, 15.6	310	2.8	−5.1, 10.9	< 0.001
$eGFR_{CKD\text{-}EPI,\ creatinine}$ (mL/min/1.73 m^2)							
Baseline	333	85.9	74.4, 97.4	329	86.9	76.1, 98.7	0.74
Change at Week 48	317	5.5	−1.3, 11.4	311	2.4	−2.6, 8.8	< 0.001

a P-values comparing the 2 treatment groups were from the 2-sided Wilcoxon rank sum test.
For postbaseline visits, p-values were from rank analysis of covariance adjusting for baseline value and the third agent randomization stratum for treatment comparison.

At Week 24, there was improvement in proteinuria in the F/TAF+3rd Agent group. There were decreases from baseline in urine protein to creatinine ratio, urine albumin to creatinine ratio, and in urine retinol binding protein to creatinine and beta-2-microglobulin to creatinine ratios in the F/TAF+3rd Agent group compared with increases from baseline in the FTC/TDF+3rd Agent group in all of these parameters at Week 48 ($p < 0.001$ for the differences between groups).

There were no clinically relevant changes from baseline within groups, or differences between the treatment groups in median values for haematology or clinical chemistry parameters. With the exception of lipase, which was measured only for subjects with elevated amylase, all median values were within normal ranges. Patients taking ATV/r showed the expected effects on bilirubin.

There were increases from baseline in fasting values of total cholesterol, LDL cholesterol and triglycerides in the F/TAF+3rd Agent group vs. little change in the FTC/TDF+3rd Agent group at both Week 24 and Week 48 ($p < 0.001$ for the differences between groups for total cholesterol and LDL cholesterol; $p = 0.016$ at Week 24 and $p = 0.002$ at Week 48 for triglycerides). There were no clinically relevant changes from baseline in the total cholesterol to HDL ratio in either treatment group.

One patient in the F/TAF+3rd Agent group died during the study as a result of lymphoma and increased lipase; these events were considered by the investigator as not related to study drug. One patient in the F/TAF+3rd Agent group had a confirmed pregnancy.

No AE that led to study drug discontinuation was reported for more than 1 patient in either group. Four patients in F/TAF+3rd Agent group (including the patient who died) and 3 subjects in the FTC/TDF+3rd Agent group had study-drug related AEs that led to treatment discontinuation.

F/TAF 10 mg vs. F/TAF 25 mg

The comparison of safety between subgroups that received F/TAF 10 mg and F/TAF 25 mg should be treated with caution since patients were not randomised in this regard and the data are confounded by the use of a PI vs. no PI. Nevertheless, the data by TAF dose suggest similar safety profiles. The only AE that had ≥ 5% difference between TAF dose groups was diarrhoea (TAF 10 mg 12.3%; TAF 25 mg 6.2%). This could have been driven by the association of PI/r with diarrhoea.

When compared with TDF, the incidence of diarrhoea was similar for those on a PI/r (F/TAF 12.3% vs. FTC/TDF 10.6%) and for those not on a PI/r (F/TAF 6.2% vs. FTC/TDF 9.5%). No other notable differences were observed in the percentages with specific AEs when comparing TAF dose groups.

At Week 48 there were improvements from baseline in mean (SD) BMD at the hip and at the spine that were similar between TAF dose groups and there were decreases from baseline in mean (SD) serum creatinine and increases in median eGFRCG values that were similar between TAF dose groups.

2.6.1. Discussion on clinical safety

In the E/C/F/TAF Phase 2/3 studies there were direct comparisons of safety with STB in previously untreated patients and assessments of safety after switching from TDF to TAF within regimens. The AE profile of E/C/F/TAF was mostly very similar to that of STB. Overall the data suggested benefits in terms of renal and bone effects for TAF vs. TDF, which was apparent in prospective comparisons as well as post-switching. Detailed assessment of renal function in patients with eGFRCG 30-69 mL/min supported no adjustment of the FTC dose when CrCL is ≥30 mL/min. Thus far there have not been any cases of PRT or Fanconi's syndrome with TAF.

Current data, including updates provided during the review of E/C/F/TAF, 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. This potential risk is reflected in the RMP.

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. The differences between TAF and TDF-containing regimens are thought to mainly reflect the known lipid lowering effect of TFV and the loss of this effect due to the much lower plasma levels of TFV in those given E/C/F/TAF vs. STB. Also, based on the finding that median changes from baseline decreased in renally impaired patients in 0112 who switched to E/C/F/TAF from a non TDF containing regimen the applicant suggests that the effect on lipids was not due to TAF per se. Furthermore, the lipid changes after initiation of E/C/F/TAF are in line with those observed with several other ART regimens that do not contain TDF. There is no indication that there are excessive changes when initiating E/C/F/TAF.

Taking into account the metabolic pathway, 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 (13.8% vs. 10.9% in 0104 and 0.5% vs. 0.2% in 0111). For mean and median uric acid these studies both showed that 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. AEs that could be due to hyperuricaemia were not observed.

The new safety data from the Phase 2 study that compared D/C/F/TAF with DRV/co + TVD are very much in keeping with those observed in E/C/F/TAF vs. STB studies, the only difference in the regimen being use of TAF instead of TDF. This was a small study with 2:1 randomisation so that it is difficult to place too much stress on differences in rates for individual AEs between treatments. However, the rate of arthralgia (9 TAF vs. no TDF patients) stands out. In this relatively small Phase 2 study with 2:1 randomisation in which the only difference between regimens was TAF vs. TDF, the imbalance in reports of arthralgia was notable but the details of these cases do not reveal any pattern suggesting a very clear relationship to TAF. Nevertheless, in the E/C/F/TAF studies arthralgia was reported by 7% on E/C/F/TAF vs. 4.5% on Stribild and in the uncontrolled study GS-US-292-0112 in patients with renal impairment 20/242 (8.3%) reported arthralgia. Arthralgia was not included in the ADRs in the Genvoya SmPC (which did not include the D/C/F/TAF study),

however it is included in the F/TAF SmPC as an uncommon ADR (based on overall rates across studies).There were no new concerns raised after review of the Safety Update.

In light of the unexplained differential effects on the plasma exposures to TAF and TFV that have been observed in several studies the safety data from GS-US-311-1089 are important to support a conclusion that F/TAF when administered with a range of agents provides a similar profile and apparent benefits on renal and bone safety as observed in the E/C/F/TAF vs. Stribild studies.

Overall the safety data do support this conclusion. In addition, the limited comparison that can be made between TAF dose groups indicates that the differential effects vs. TDF apply regardless of use of F/TAF 10 mg or 25 mg depending on the third agent (even though the DDI data indicate that TFV exposures are likely higher with the former than the latter due to co-administered agents) but it should be noted that the study was not designed to support definitive conclusions in this respect.

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 Phase 3 studies with E/C/F/TAF 10 mg vs. STB in ART-naïve patients, the Phase 2 study with D/C/F/TAF vs. D/C F/TDF and the switch studies, including GS-US-311-1089 (in which F/TAF or F/TADF were each given with a range of third agents in the ART regimen) all pointed to benefits for TAF over TDF in terms of effects on hip or spine BMD. The renal safety profile of TAF also appears to be better than that of TDF in patients previously naïve to ART and in those who switched from TDF to TAF. Additionally, detailed assessments of renal function in the E/C/F/TAF study in patients with baseline eGFRCG in the range 30-69 mL/min support a conclusion that F/TAF has an acceptable renal safety profile in patients in the 30-50 mL/min range. These data also support use of FTC without dose adjustment in patients with CrCL ≥ 30 mL/min.

In comparative studies in ART-naïve patients the AE profile of E/C/F/TAF was mostly very similar to that of STB while that of D/C/F/TAF was similar to that of DRV/co + TVD. In ART-naïve patients both E/C/F/TAF and D/C/F/TAF were associated with higher rates of abnormal fasting lipids, including Grade 3 and 4 abnormalities, than TDF-containing comparative regimens. Similarly, higher rates of abnormal fasting lipids were observed in those who switched to 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, which is reduced in line with the much lower plasma levels of TFV in those given E/C/F/TAF. In conclusion, the effect of TAF-containing regimens on lipids resembles that of other commonly used ART regimens without TDF and the benefits of TAF vs. TDF in terms of renal and bone effects appear to outweigh any concerns there may be regarding the lipid profile.

An imbalance in cases of arthralgia was observed suggesting to be an ADR.

The available data in adolescents suggest a benign safety profile. Since E/C/F/TAF has been accepted for use in adolescents there is no reason to object to use of F/TAF. Nevertheless, the RMP reflects the paucity of data and the need to obtain more experience in this population.

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

Safety concerns

The applicant proposed the following summary of safety concerns in the RMP:

	Safety Concerns for F/TAF	Attributable Component(s) of F/TAF
Important Identified Risks	Post-treatment hepatic flares in HIV/HBV coinfected patients	FTC, TAF
Important Potential Risks	Overdose of tenofovir occurring through accidental concurrent use of F/TAF with a TDF-containing product	TAF
	Renal toxicity	TAF
	Bone events due to potential proximal renal tubulopathy/loss of BMD	TAF
	Ocular effects (posterior uveitis)	TAF
Missing Information	Long-term safety information in adults and adolescents	F/TAF
	Safety in children aged 4 weeks to < 12 years	TAF
	Safety in elderly patients	FTC, TAF
	Safety in pregnancy and lactation	FTC, TAF
	Safety in patients with moderate to severe renal impairment	FTC, TAF
	Safety in patients with severe hepatic impairment (CPT score C)	TAF
	Safety in patients with HCV coinfection	TAF
	Development of drug resistance in long term use	F/TAF
	Drug-drug interactions	TAF

Having considered the data in the safety specification, the CHMP agrees that the updated safety concerns listed by the applicant are appropriate.

Pharmacovigilance plan

Study/Title	Objectives	Safety Concerns Addressed	Status (Planned, Started)	Date for Submission of Interim or Final Reports (Planned or Actual)
Interventional studies (Category 3)				
Study GS-US-311-1089 A Phase 3, Randomized, Double-Blind, Switch Study to Evaluate F/TAF in HIV 1 Positive Subjects who are Virologically Suppressed on Regimens containing FTC/TDF	A switch study to evaluate F/TAF in HIV-1 positive subjects who are virologically suppressed on regimens containing FTC/TDF	Long-term safety information in adults and adolescents	Ongoing	Week 96 report: Q3 2017
Study GS-US-311-1269 A Phase 2/3, Open Label, Multi-Cohort Switch Study to Evaluate Emtricitabine/Tenofovir Alafenamide (F/TAF) in HIV 1 Infected Children and Adolescents Virologically Suppressed on a Tenofovir Disoproxil Fumarate (TDF)-Containing Regimen	A switch study to evaluate F/TAF in HIV-1 infected children and adolescents virologically suppressed on a tenofovir disoproxil fumarate (TDF)-containing regimen	Safety in children aged 4 weeks to < 12years	Ongoing	Final report: November 2018

Study/Title	Objectives	Safety Concerns Addressed	Status (Planned, Started)	Date for Submission of Interim or Final Reports (Planned or Actual)
Study GS-US-311-1717 A Phase 3b, Randomized, Double-Blind, Switch Study to Evaluate F/TAF in HIV-1 Positive Subjects who are Virologically Suppressed on Regimens containing ABC/3TC	To collect information on the efficacy of switching ABC/3TC to F/TAF versus maintaining ABC/3TC in HIV-1 infected subjects who are virologically suppressed on regimens containing ABC/3TC as determined by the proportion of subjects with HIV-1 RNA < 50 copies/mL at Week 48	Long-term safety information in adults and adolescents	Ongoing	Week 96 report: Q1 2019
Study GS-US-311-1790 A Phase 1, Randomized, Open Label, Drug Interaction Study Evaluating the Effect of Emtricitabine/Tenofovir Alafenamide Fixed-Dose Combination Tablet or GS-9883 on the Pharmacokinetics of a Representative Hormonal Contraceptive Medication, Norgestimate/Ethinyl Estradiol	To collect information on the effect of FTC and TAF administered as the F/TAF 200/25 mg fixed-dose combination tablet or GS-9883 on the PK of a representative hormonal contraceptive medication, norgestimate /ethinyl estradiol.	Long-term safety information in adults and adolescents	Ongoing	Final report: Q4 2016
Non-interventional studies (Category 3)				
Antiretroviral Pregnancy Registry	To collect information on the risk of birth defects in patients exposed to ARVs, including F/TAF, during pregnancy	Missing information: Safety in pregnancy	Started	Interim reports to be included in F/TAF PSURs (DLP and periodicity as described in the List of EU reference dates and frequency of submission of PSURs)
Nonclinical studies (Category 3)				
In vitro study on the potential for significant effects on plasma TFV concentrations upon coadministration of TAF and xanthine oxidase inhibitors	To provide information on the potential for a drug-drug interaction between F/TAF and xanthine oxidase inhibitors	Missing information: Drug-drug interactions	Planned	Final report: Q4 2016

The Applicant's proposal to address the safety concerns listed above within the pharmacovigilance plan is considered acceptable.

Risk minimisation measures

Safety Concern	Routine Risk Minimization Measures	Additional Risk Minimization Measures
Important identified risk(s)		
Post-treatment hepatic flares in HIV/HBV coinfected patients	Sections 4.4 of the SmPC informs about the risk of exacerbation of hepatitis in HIV-1/HBV coinfected patients following discontinuation of F/TAF.	None

Safety Concern	Routine Risk Minimization Measures	Additional Risk Minimization Measures
Important potential risk(s)		
Overdose of tenofovir occurring through accidental concurrent use of F/TAF with a TDF-containing product	Section 4.4 (and 4.5) of the SmPC warns that 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 F/TAF.	None
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
Missing information		
Long-term safety information in adults and adolescents	None	None
Safety in children aged 4 weeks to < 12 years	Section 4.2 of the SmPC states that the safety and efficacy of F/TAF in children younger than 12 years of age have not yet been established and that no data are available.	None
Safety in elderly patients	None	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 F/TAF, and notes that 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 TAF is excreted in human milk, and informs that F/TAF should not be used during breastfeeding.	None
Safety in patients with moderate to severe renal impairment	Section 4.2 of the SmPC states that 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 F/TAF in this population, and that 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 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 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 HCV coinfection	Section 4.4 of the SmPC states that the safety and efficacy of 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 4.5 of the SmPC provides information on interactions that have not been studied, potential effects on drug levels, and recommendations concerning coadministration with F/TAF.	None

The applicant's proposal for routine risk minimisation measures is considered sufficient to address these safety concerns.

Conclusion

The CHMP and PRAC considered that the risk management plan version 1.0 is acceptable.

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

No full user consultation with target patient groups on the package leaflet has been performed on the basis of a bridging report making reference to Stribild and Genvoya. The bridging report submitted by the applicant has been found acceptable.

2.9.2. Additional monitoring

Pursuant to Article 23(1) of Regulation No (EU) 726/2004, Descovy (Emtricitabine/Tenofovir alafenamide) is included in the additional monitoring list as it contains a new active substance which, on 1 January 2011, was not contained in any medicinal product authorised in the EU.

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 improved 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 efficacy of F/TAF 10 mg given with a P-gp inhibitor such as COBI was demonstrated in the Phase 3 studies of E/C/F/TAF vs. STB in which very high percentages achieved < 50 and < 20 copies/mL at Week 48 using the FDA snapshot algorithm. Supportive evidence of efficacy comes from the Phase 2 study that compared D/C/F/TAF 10 mg with D/C/F/TDF and from the E/C/F/TAF and F/TAF switch studies in which TAF-

containing regimens have maintained viral suppression with no excess of rebound compared to patients who stayed on their TDF-containing regimens.

The Phase 3 studies with E/C/F/TAF 10 mg vs. STB in ART-naïve patients, the Phase 2 study with D/C/F/TAF vs. D/C F/TDF and the switch studies, including GS-US-311-1089 (in which F/TAF or F/TADF were each given with a range of third agents in the ART regimen) all pointed to benefits for TAF over TDF in terms of effects on hip or spine BMD. The renal safety profile of TAF also appears to be better than that of TDF in patients previously naïve to ART and in those who switched from TDF to TAF. Additionally, detailed assessments of renal function in the E/C/F/TAF study in patients with baseline eGFRCG in the range 30-69 mL/min support a conclusion that F/TAF has an acceptable renal safety profile in patients in the 30-50 mL/min range. These data also support use of FTC without dose adjustment in patients with CrCL ≥ 30 mL/min.

The data in adolescents are currently limited and confined to use of E/C/F/TAF but the PK data support use of the adult dose and available data suggest a benign safety profile. Since E/C/F/TAF has been accepted for use in adolescents there is no reason to object to use of F/TAF. Nevertheless, the RMP reflects the paucity of data and the need to obtain more experience in this population.

Uncertainty in the knowledge about the beneficial effects

Although the Phase 3 E/C/F/TAF studies gave very high response rates they are not sensitive to confirm the adequacy of the 10 mg TAF dose when used with COBI due to the major antiviral effects of the other agents in the regimen. Additional efficacy data from the Phase 2 D/C/F/TAF study and the switch studies can only be viewed as supportive. Therefore, the critical data to support the dose recommendations for F/TAF come from the monotherapy studies (with TAF doses ranging from 8 to 150 mg), the Emax modelling and the PK data on use of F/TAF 10 mg or 25 mg with various potentially interacting antiretroviral agents. These data supported a standard TAF dose of 25 mg and generally supported a reduction from 25 mg to 10 mg when TAF is given with a strong P-gp inhibitor, such as COBI or RTV.

However, GS-US-292-103 showed that although plasma TAF was very slightly lower on dosing with Genvoya compared with F/TAF 25 mg, the plasma TFV AUC was lower after dosing with F/TAF 25 mg vs. Genvoya. This finding, which remains mechanistically unexplained, suggested an effect of COBI on the compartmental disposition of TFV.

The finding raised a question regarding the basis for extrapolation of efficacy from Genvoya to F/TAF regardless of the third agent co-administered and whether or not the regimen included P-gp inhibitors COBI or RTV. It also raised a question regarding the CNS levels of TFV-DP that may be achieved with F/TAF, and, hence, the efficacy of various TAF-containing regimens against HIV within the CNS.

The applicant responded to these concerns during the procedure and the questions were referred to the Virology SAG. When considering these issues the following observations have some relevance.

To ascribe the difference in TFV plasma levels that was observed when TAF was administered with or without COBI to a differential effect on the whole body distribution of TFV would require that systemic concentrations of COBI are sufficient to exert effects on relevant transporters outside of the gut.

In vitro, COBI inhibits the transporters P-gp, BCRP, MATE1, MRP-2, OATP1B1 and OATP1B3. Its effect on plasma exposures to substrates of P-gp and/or BCRP via inhibition at the gut level is clear. However, in the context of explaining effects on plasma TFV levels after oral administration of TAF, it should be noted that TFV is not a substrate for P-gp, MRP2 or BCRP and its renal elimination should not be affected by COBI based on the calculated Cmax,u/IC50 ratios. Regarding the potential for systemic effects on other transporters it is

clear that COBI reaches sufficient concentrations to inhibit MATE1 in the kidney, with consequent effects on serum creatinine. However, it is not expected to reach sufficient concentrations to inhibit P-gp at the BBB and inhibition of BCRP and MRP4 at the BBB does not seem to have marked effects on their substrates. Overall, existing knowledge regarding COBI and its effects on transporters, as well as the substrate profile of TFV, do not explain the modest difference in TFV plasma levels observed when TAF was given with and without COBI to healthy subjects. There is no clear basis for concluding that the difference truly reflects different whole body distribution of TFV when TAF is administered with or without COBI or that dosing with and without COBI will affect brain parenchyma levels of the active moiety TFV-DP.

TFV is not the active moiety but is the moiety associated with adverse renal and bone effects. Since TFV plasma levels were slightly lower for F/TAF 25 mg vs. TAF 10 mg given with COBI there is no reason to expect a worse safety profile for F/TAF when used without COBI compared to Genvoya. In addition, the safety data from GS-US-311-1089, in which subjects either switched to TAF or remained on TDF, each with FTC and a wide range of third agents (including PI/r combinations), indicated that the safety profile for TAF-treated patients vs. TDF-treated patients was consistent with observations made in the Phase 3 Genvoya studies that compared Genvoya with Stribild.

In summary, although there remains a theoretical possibility that the presence of a P-gp inhibitor as part of an overall TAF-containing ART regimen could affect entry of TAF into the brain and, thus, TFV-DP levels, the overall picture at present suggests that this is a remote possibility. CSF levels of TFV or TAF cannot be regarded as highly predictive of TFV-DP in the brain. There is at least a theoretical possibility that use of TAF rather than TDF could improve on levels of TFV-DP achieved in the CNS replicating sites.

The general experience with the more highly effective ART regimens that have become available especially in the last decade support a conclusion that effective and sustained virologic suppression in plasma is associated with CSF virologic suppression or, at least, only asymptomatic and usually temporary detection of HIV-1 RNA in CSF. There no reason to think that rates of plasma virologic failure or CSF virologic failure are more likely to occur with regimens containing TAF vs. otherwise identical regimens containing TDF. Thus, F/R/TAF should exert similar activity to Eviplera when used in accordance with identical restrictions and warnings and there is no good reason to expect that the risk of escape HIV-1 replication in the brain is any greater with the former vs. the latter.

The SAG concurred with a conclusion that F/TAF should pose no difference vs. Truvada in control over HIV replication in plasma and in the CNS when each is given with the same third agent.

Regarding the nature of the third agent, the Phase 2 study that compared D/C/F/TAF 10 mg with D/C/F/TDF suggested numerical inferiority for the TAF vs. TDF regimen based on percentages with < 50 c/mL at week 48 and percentages with < 20 c/mL at weeks 24 and 48. This relatively small study was not powered for inferential testing. The plasma TAF level was comparable with that observed with 25 mg TAF given alone and the intracellular TFV-DP levels were higher in the TAF vs. TDF group as observed in other studies. The findings of this study are currently unexplained. There is a Phase 3 study ongoing, which is not sponsored by the current applicant but the applicant is kept informed. It is expected that results may be reported within ~1 year and should provide more definitive evidence on the performance of the D/C/F/TAF 10 mg regimen in ART-naïve patients. Meanwhile, the data from this study have been reflected in section 5.1.

Other uncertainties regarding efficacy included concern over the adequacy of F/TAF 25 mg if it was given without food and without a P-gp inhibitor, use of F/TAF 25 mg with the P-gp inducer EFV and use with stronger inducers of P-gp, such as carbamazepine. Additionally, a concern arose regarding maintenance of the beneficial effects of TAF vs. TDF on safety if F/TAF 25 mg was given with a strong inhibitor of P-gp other

than RTV or COBI. These various issues were further addressed during the procedure. The applicant addressed these various concerns based on comparisons of plasma TAF (and to some extent intracellular TFV-DP) across various regimens and by amendments to the SmPC.

Ultimately it was concluded that the data support expectations that the efficacy of F/TAF 10 mg administered regardless of food in the presence of a P-gp inhibitor and F/TAF 25 mg administered regardless of food in the absence of a P-gp inhibitor should provide broadly comparable efficacy. The applicant added advice in section 4.5 that F/TAF 10 mg should be used when it is given with non-ART potent P-gp inhibitors, including ciclosporin, ketoconazole and itraconazole. It was finally concluded that plasma TAF and intracellular TFV-DP data from the switch study GS-US-311-1089 and the Phase 2 study with D/C/F/TAF 10 mg supported use of F/TAF 25 mg with EFV and use of F/TAF 10 mg with DRV/co or DRV/r (with which the interaction with TAF seems complex). The DDI study with carbamazepine indicated a risk of inadequate TAF and hence TFV-DP levels if F/TAF is taken with a potent inducer of P-gp. There does not seem to be a good reason to accept such a risk. Therefore, it is considered that co-administration of F/TAF with potent inducers of P-gp should be not recommended.

Risks

Unfavourable effects

In comparative studies in ART-naïve patients the AE profile of E/C/F/TAF was mostly very similar to that of STB while that of D/C/F/TAF was similar to that of DRV/co + TVD. In ART-naïve patients both E/C/F/TAF and D/C/F/TAF were associated with higher rates of abnormal fasting lipids, including Grade 3 and 4 abnormalities, than TDF-containing comparative regimens. Similarly, higher rates of abnormal fasting lipids were observed in those who switched to 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, which is reduced in line with the much lower plasma levels of TFV in those given E/C/F/TAF.

Nevertheless, the effect of TAF-containing regimens on lipids resembles that of other commonly used ART regimens without TDF and the benefits of TAF vs. TDF in terms of renal and bone effects appear to outweigh any concerns there may be regarding the lipid profile.

Arthralgia was noted to show an imbalance in cases suggesting that this could be an ADR. Arthralgia was added to the SmPC as an uncommon ADR.

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 TAF. More extensive and longer-term data are needed to confirm the observation. In addition, although data thus far support a conclusion that TAF is much less likely than TDF to exert negative effects on BMD or on renal function it remains to be seen whether very long term exposure to low TFV plasma levels could have an effect. This matter can really only be addressed in routine use. It is particularly important that this matter is addressed in adolescents, in whom the use of TDF is restricted at present due to the concerns regarding effects on BMD.

Taking into account the metabolic pathway, it was noted that Grade 3 or 4 hyperuricaemia occurred in 2 E/C/F/TAF and no non-switch patients in GS-US-292-0109. Also, any grade hyperuricaemia occurred in 13.2% vs. 5.0% although no AEs were related to abnormal uric acid. Thus far there does not appear to be a link between the hyperuricaemia and gout or other AEs that could be due to hyperuricaemia (including renal

stones). However, this matter needs to be kept under review as a potential risk. The possible effect of co-administering xanthine oxidase inhibitors (allopurinol or febuxostat) on the final metabolic fate of TAF also needs to be addressed.

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.

In conclusion, only wider and longer-term exposures to TAF and relatively low levels of TFV in adults and in adolescents can inform whether chronic exposure to TFV plasma concentrations much lower than observed with TDF can eventually lead to the types of ADRs associated with TDF.

Benefit-risk balance

Importance of favourable and unfavourable effects

F/TAF when given with EVG and COBI achieved high virologic suppression rates in the ART-naïve and maintained suppression after switching from successful regimens. The bridging of F/TAF when used with a wide range of third agents in the ART regimen to that observed with Genvoya in the Phase 3 studies is based on demonstrations of comparable plasma TAF levels, supported by the TFV-DP concentrations for TAF regimens vs. TDF regimens. Taking into account these PK data, as well as the TAF monotherapy data and the Emax modelling, there are no major concerns regarding the anticipated efficacy of F/TAF provided that it is used in accordance with the SmPC.

Nevertheless, it remains possible that the efficacy that is observed with F/TAF-containing regimens may vary according to the third agent. It is also not possible to rule out entirely that different combination regimens could result in variable control of HIV-1 replication in the CNS. Nevertheless, the substantial experience gained with effective regimens indicates that so long as profound viral suppression is maintained based on measuring plasma levels the risk of escape replication of HIV-1 in the CNS leading to symptoms seems remote. The risk that escape replication of HIV-1 in the CNS could ultimately lead to a resurgence of HIV-1 RNA in plasma also seems to be remote.

The safety profile of TAF-containing regimens is for the most part similar or improved vs. that of TDF-containing regimens, especially notable for the reduced renal and bone effects. The fact that lipid abnormalities are more likely to occur with TAF then TDF does not impact on the overall conclusions on safety and the rates observed are in line with those that occur with other commonly used regimens.

Benefit-risk balance

Discussion on the benefit-risk balance

The fixed dose combination F/TAF offers an alternative option to F/TDF with which similar efficacy can be expected. The improved renal safety profile allows the administration in patients with estimated creatinine clearance > 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 that the risk-benefit balance of Descovy in combination with other antiretroviral agents for the treatment of adults and adolescents (aged 12 and older with body weight at least 35 Kg) infected with human immunodeficiency virus type 1 (HIV-1) (see sections 4.2 and 5.1), 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.

New Active Substance Status

Based on the CHMP review of data, the CHMP considers that tenofovir alafenamide is a derivative of tenofovir disoproxil (both prodrugs of tenofovir). The active substance tenofovir alafenamide is contained in the

marketing authorisation Genvoya which was authorised in the Union on 19/11/2015. Tenofovir alafenamide is therefore not a new active substance in itself, as it is a constituent of a medicinal product previously authorised within the Union.