10 November 2016
EMA/793580/2016
Committee for Medicinal Products for Human Use (CHMP)

Assessment report

Vemlidy

International non-proprietary name: tenofovir alafenamide

Procedure No. EMEA/H/C/004169/0000

Note

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

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List of abbreviations

AASLD	American Association for the Study of Liver Diseases
ADME	absorption, distribution, metabolism, and elimination
ADV	adefovir dipivoxil
ALT	alanine aminotransferase
ANCOVA	analysis of covariance
ANOVA	analysis of variance
anti-HBe	antibody against hepatitis B e antigen
anti-HBs	antibody against hepatitis B surface antigen
ARV	antiretroviral
AST	aspartate aminotransferase
AUC_{inf}	area under the plasma/serum concentration versus time curve extrapolated to infinite time, calculated as $AUC_{last} + (C_{last}/\lambda_z)$
AUC_{last}	area under the plasma/serum concentration versus time curve from time zero to the last quantifiable concentration
AUC_{tau}	area under the plasma/serum concentration versus time curve over the dosing interval
BCS	Biopharmaceutics Classification System
BLQ	below the limit of quantitation
BMD	bone mineral density
bsAP	bone-specific alkaline phosphatase
CatA	cathepsin A
CES1	carboxylesterase 1
Cfu	Colony forming units
CHB	chronic hepatitis B
CL_{cr}	creatinine clearance
C_{max}	maximum observed plasma/serum concentration of drug
COBI, C	cobicistat (Tybost)
CPP	Critical Process Parameter
CPT	Child-Pugh-Turcotte
CQA	Critical Quality Attribute
C_{tau}	observed drug concentration at the end of the dosing interval

CTX	C-type collagen sequence
CV	coefficient of variation
CYP	cytochrome P450 enzyme
DNA	deoxyribonucleic acid
DSC	Differential Scanning Calorimetry
EC	European Commission
E/C/F/TAF	elvitegravir/cobicistat/emtricitabine/tenofovir alafenamide (coformulated; Genvoya)
E/C/F/TDF	elvitegravir/cobicistat/emtricitabine/tenofovir disoproxil fumarate (coformulated; Stribild)
EC_{50}	concentration of a compound inhibiting virus replication by xx%
EDQM	European Directorate for the Quality of Medicines
eGFR	estimated glomerular filtration rate
$eGFR_{CG}$	estimated glomerular filtration rate calculated using the Cockcroft-Gault equation
$eGFR_{CKD\text{-}EPI,\ creatinine}$	estimated glomerular filtration rate calculated using the Chronic Kidney Disease Epidemiology Collaboration creatinine equation
EMA	European Medicines Agency
ETV	entecavir
EVG, E	elvitegravir (Vitekta)
FDC	fixed-dose combination
FTC/RPV/TAF	rilpivirine/emtricitabine/tenofovir alafenamide (coformulated)
F/TAF	emtricitabine/tenofovir alafenamide (coformulated)
FTC, F	emtricitabine (Emtriva)
FTC/TDF	emtricitabine/tenofovir disoproxil fumarate (coformulated; Truvada)
GC	Gas Chromatography
GGT	gamma-glutamyltransferase
HBeAg	hepatitis B e antigen
HBsAg	hepatitis B surface antigen
HBV	hepatitis B virus
HCC	hepatocellular carcinoma
HDL	high-density lipoprotein
HDPE	High Density Polyethylene

HPLC	High performance liquid chromatography
HPLC-MS	High performance liquid chromatography – mass spectrometry
IC_{50}	concentration that resulted in 50% inhibition
ICH	International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
IPC	In-process control
ICP-MS	Inductively coupled plasma mass spectrometry
IR	Infrared
LAM	lamivudine
LDL	low-density lipoprotein
M = F	missing = failure
NAS	New Active Substance
NF	National Formulary
NMT	Not more than
N[t]RTI	nucleos(t)ide reverse transcriptase inhibitor
OAV	oral antiviral
OC	osteocalcin
P1NP	procollagen type 1 N-terminal propeptide
PAR	Proven Acceptable Range
PBMC	peripheral blood mononuclear cell
PD	pharmacodynamic(s)
P-gp	P-glycoprotein
Ph. Eur.	European Pharmacopoeia
PK	pharmacokinetic(s)
pol/RT	polymerase/reverse transcriptase
Q1, Q3	first quartile, third quartile
QbD	Quality by design
RBP	retinol binding protein
RH	Relative Humidity
SmPC	Summary of Product Characteristics
$t_{1/2}$	estimate of the terminal elimination half-life of the drug in plasma/serum, calculated by dividing the natural log of 2 by the terminal elimination rate constant (λ_z)

TAF	tenofovir alafenamide fumarate
TBV	telbivudine
TDF	tenofovir disoproxil fumarate (Viread)
TFV	tenofovir
TFV-DP	tenofovir diphosphate
T_{max}	time (observed time point) of C_{max}
TSE	Transmissible Spongiform Encephalopathy
UACR	urine albumin to creatinine ratio
ULN	upper limit of normal
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 27 January 2016 an application for marketing authorisation to the European Medicines Agency (EMA) for Vemlidy, through the centralised procedure falling within the Article 3(1) and point 3 of Annex of Regulation (EC) No 726/2004. The eligibility to the centralised procedure was agreed upon by the EMA/CHMP on 26 February 2015.

The applicant applied for the following indication: Tenofovir alafenamide is indicated for the treatment of chronic hepatitis B in adults.

The legal basis for this application refers to:

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

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

Information on Paediatric requirements

Pursuant to Article 7 of Regulation (EC) No 1901/2006, the application included an EMA Decision P/0209/2014 on the agreement of a paediatric investigation plan (PIP).

At the time of submission of the application, the PIP P/0209/2014 was not yet completed as some measures were deferred.

Information relating to orphan market exclusivity

Similarity

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

New active Substance status

The applicant requested the active substance tenofovir alafenamide contained in the above medicinal product to be considered as a new active substance.

Scientific Advice

The applicant received Scientific Advice from the CHMP on 18 October 2012 and 25 April 2013. The Scientific Advice pertained to the pre-clinical development of the dossier.

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: Martina Weise

- The application was received by the EMA on 27 January 2016.
- The procedure started on 25 February 2016.
- The Rapporteur's first Assessment Report was circulated to all CHMP members on 22 April 2016. The Co-Rapporteur's first Assessment Report was circulated to all CHMP members on 13 May 2016. The PRAC Rapporteur's first Assessment Report was circulated to all PRAC members on 27 May 2016.
- During the meeting on 23 June 2016, 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 23 June 2016.
- The applicant submitted the responses to the CHMP consolidated List of Questions on 15 July 2016
- The Rapporteurs circulated the Joint Assessment Report on the applicant’s responses to the List of Questions to all CHMP members on 18 August 2016.
- During the PRAC meeting on 02 September 2016, the PRAC agreed on the PRAC Assessment Overview and Advice to CHMP.
- During the CHMP meeting on 15 September 2016, 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 11 October 2016.
- The Rapporteurs circulated the Joint Assessment Report on the applicant’s responses to the List of Outstanding Issues to all CHMP members on 24 October 2016.
- During the meeting on 10 November 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 Vemlidy on 10 November 2016.

2. Scientific discussion

2.1. Problem statement

Chronic hepatitis B is a major public health care issue worldwide and one of the principal causes of chronic liver disease, cirrhosis, and hepatocellular carcinoma (HCC). The hepatitis B virus (HBV) is easily transmissible through perinatal, percutaneous, and sexual exposure. Following acute HBV infection, 5% to 10% of adults and up to 90% of children fail to produce an immune response adequate to clear the infection; these individuals become chronic carriers of the virus. Individuals who develop CHB are at substantial risk of cirrhosis, hepatic decompensation, and HCC, which will afflict 15% to 40% of patients with CHB in the absence of effective treatment. Liver cancer is the third leading cause of cancer deaths globally, with the highest burden of this disease found in regions where HBV is endemic.

Recent reports estimated that 250 to 350 million individuals are currently living with HBV (i.e. hepatitis B surface antigen [HBsAg] positive), representing a worldwide prevalence of 3.6%, with considerable geographic variability. For example, HBV prevalence rates of 0.01%, 0.76%, 4.0%, 5.5%, and 22.4% have been reported for the United Kingdom, Canada, Turkey, China, and South Sudan, respectively. In 2013, an estimated 686,000 deaths were due to HBV infection and associated complications, placing it among the top 20 causes of mortality worldwide. Despite the availability of HBV vaccine programs in many countries, new HBV infections are still common even in areas of low prevalence. The World Health Organization estimates that each year there are over 4 million acute clinical cases of HBV infection globally. In the United States (US), approximately 20,000 people become acutely infected each year according to an estimate from the Centers for Disease Control and Prevention.

2.1.1. Management

Currently, there are two approved approaches for the treatment of CHB: injectable interferons and oral antiviral (OAV) agents. Compared with OAVs, interferons are administered for a finite treatment duration (usually 48 weeks), are not associated with viral resistance, and have shown higher rates of antibody against hepatitis B surface antigen (anti-HBs) and antibody against hepatitis B e antigen (anti-HBe) seroconversion in clinical studies. However, interferons only have modest antiviral efficacy and their use is associated with treatment-limiting safety and tolerability issues, including flu-like syndrome, fatigue, cytopenias, and mood disturbances. In addition, patient acceptance of interferon therapy is low given the requirement for subcutaneous injections and the substantial adverse events (AEs) associated with therapy.

The development of the nucleos(t)ide reverse transcriptase inhibitors (N[t]RTIs) was a major breakthrough for the treatment of CHB, providing effective suppression of viral replication and reducing the risk of long-term complications. Currently available OAVs include lamivudine (LAM), adefovir dipivoxil (ADV), entecavir (ETV), telbivudine (TBV), and tenofovir disoproxil fumarate (TDF; Viread). Several N[t]RTIs possess a low barrier for virologic resistance development, including LAM, TBV, and ADV, which limit their usefulness for long term treatment of CHB. ETV and TDF are potent inhibitors of HBV replication with a higher barrier to resistance, and these OAVs are recommended as preferred initial therapies for CHB in all major treatment guidelines.

Treatment with OAV agents has been successful in achieving and maintaining a high degree of virologic suppression, which is associated with decreased morbidity and mortality related to CHB. Along with virologic suppression, the majority of patients experience normalization of serum alanine

aminotransferase (ALT) levels, and with long term OAV treatment, improvement in liver histology is observed. As few patients (< 5%) achieve an immunological cure with OAV treatment, defined as clearance of HBsAg together with seroconversion to anti-HBs, lifelong therapy is required.

ETV has a low rate of resistance development in treatment naive patients; however, with long term use, the cumulative probability of ETV resistance increases substantially in LAM refractory patients, with up to 57% of patients showing reduced susceptibility through 6 years of treatment. ETV is safe and well tolerated, but treatment is complicated by the requirement for dose adjustment for patients with creatinine clearance (CLcr) < 50 mL/min and, in patients who are refractory to LAM or have LAM or TBV resistance, a higher dose is needed.

TDF, an oral prodrug of tenofovir (TFV), is effective in both treatment naive and treatment experienced patients with LAM resistance or prior ADV therapy. After 8 years of TDF treatment for CHB, virologic suppression was well maintained and no resistance to TDF has been observed. With 5 years of treatment, a histological benefit with regard to regression of fibrosis and reversal of cirrhosis was also observed in the majority of treated subjects. However, the use of TDF is associated with a decrement in renal function, including, in rare instances, proximal tubulopathy and Fanconi syndrome. TDF requires dose adjustment and increased monitoring for patients with renal impairment (patients with CLcr < 50 mL/min and those patients with end stage renal disease maintained on haemodialysis), and reductions in bone mineral density (BMD) occur with TDF treatment in both HBV and HIV infected patients. The bone and renal toxicities associated with TDF necessitate caution when treating individuals at greatest risk, including patients with pre-existing renal impairment, patients with comorbidities that increase the risk for renal dysfunction (e.g. those with hypertension and/or diabetes mellitus), and the elderly, who are also at increased risk for comorbidities, especially those related to kidney and bone.

About the product

Tenofovir (TFV) is a nucleotide analogue with limited oral bioavailability. Tenofovir disoproxil (marketed as the fumarate TDF), an oral pro-drug of TFV, has improved bioavailability vs. TFV. While TDF is used broadly in the treatment of HIV-1 and HBV infection, an important identified risk with its use is nephrotoxicity. This may result in increased serum creatinine, increased urinary protein loss (particularly tubular) and occasional cases of proximal renal tubulopathy (PRT) including Fanconi syndrome. These risks necessitate increased renal monitoring of patients during treatment with TDF-containing products vs. other therapies, placing burden on the patient and healthcare provider. Reductions in bone mineral density (BMD) also occur, with larger decreases in BMD observed with TDF than with other treatments.

Tenofovir alafenamide (TAF) is another oral pro-drug 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. TAF is proposed to provide similar efficacy as TDF but with significantly less proteinuria, less need for renal monitoring and less impact on bone mineralisation.

2.2. Quality aspects

2.2.1. Introduction

The finished product is presented as film-coated tablets containing 25 mg of tenofovir alafenamide (as fumarate) as active substance.

Other ingredients are:

Tablet core: lactose monohydrate, microcrystalline cellulose (E460(i)), croscarmellose sodium (E468) and magnesium stearate (E470b).

Film-coating: polyvinyl alcohol (E1203), titanium dioxide (E171), macrogol (E1521), talc (E553b) and iron oxide yellow (E172).

The product is available in high density polyethylene (HDPE) bottles enclosed with a polypropylene continuous-thread, child-resistant cap and an induction-activated aluminium foil liner. 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

The active substance is identical to that used in other approved products from the same applicant (Genvoya, Odefsey and Descovy) and a declaration was received stating that the contents of module 3 are identical.

The chemical name of tenofovir alafenamide 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 it has the following structure:

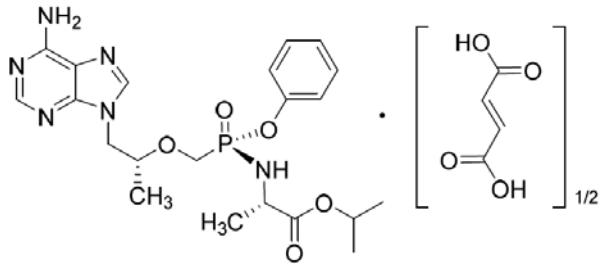


Figure 1. Structure of tenofovir alafenamide fumarate

The chemical structure of tenofovir alafenamide 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. Tenofovir alafenamide 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. Tenofovir alafenamide fumarate is freely soluble in methanol, soluble in ethanol, sparingly soluble in isopropanol and slightly soluble in acetone.

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

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

The applicant has provided justification for TAF to be considered as a new active substance (NAS) on the basis of its unique chemical structure. However, both TAF 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 is not considered a NAS on quality grounds.

Manufacture, characterisation and process controls

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

The active substance is synthesized in multiple steps. During the evaluation procedure, the active substance starting materials were 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 in-process controls 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 polyethylene bags which comply with the EC directive 2002/72/EC and EC 10/2011 as amended. The bags are held in high-density polyethylene 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 reproduced below includes tests for appearance (visual examination), identity (IR, HPLC), identity of fumaric acid (HPLC), clarity of solution (visual examination), water content (Ph. Eur.), assay (HPLC), impurities (HPLC, HPLC-MS, GC), residual solvents (GC), elemental impurities (ICP MS), and melting point (Ph. Eur.).

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

The analytical methods used have been adequately described and non-compendial methods appropriately validated in accordance with the ICH guidelines. 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 commercial scale and 3 pilot scale) of the active substance, manufactured at both proposed manufacturing sites are provided. Additional batch analysis data for development batches used in pre-clinical pharmacokinetics and toxicological studies are provided. The results are within the specifications and consistent from batch to batch.

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

Stability

Stability data on 6 commercial scale batches of active substance from the both proposed manufacturers stored in the intended commercial package for up to 36 months under long term conditions at 5 ºC and for up to 24 months under accelerated conditions at 25 ºC / 60% RH according to the ICH guidelines were provided. 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.

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

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

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

The stability results indicate that the active substance manufactured by the both proposed suppliers is sufficiently stable. The stability results justify the proposed re-test 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

Vemlidy is presented as round yellow film-coated tablets debossed with “GSI” on one face and “25” on the other.

Development work aimed to produce an immediate release tablet with suitable properties for clinical use and taking into account the properties of the active substance. Tenofovir alafenamide fumarate is a BCS class 3 compound with high solubility across the physiological pH range. It exhibits pH-dependent solubility in aqueous solution, being most stable at pH 6 and susceptible to hydrolysis at more acidic and basic pH.

During early development, the impact of filler and disintegrant type and content on product performance was evaluated by manufacturing film-coated tablets with nine different compositions with different amounts of excipients. The best of these was used in phase 1 clinical trials. This formulation was used as the basis for tablets for phase 3 studies, although the film-coat was changed and debossing added. This formulation was also selected for commercialisation.

All excipients are well known pharmaceutical ingredients and their quality is compliant with Ph. Eur. 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 2.1.1 of this report.

The dissolution method for release and stability testing was developed in line with ICH Q6A and EMA guidelines. Parameters such as pH, volume, buffer concentration, apparatus type and agitation speed were optimised in order to ensure complete dissolution whilst maintaining discriminatory power with regards to meaningful changes in tablet composition and attributes.

The commercial manufacturing process evolved in concert with the clinical development program. The impact of each step of the process on potential critical quality attributes of the finished product was evaluated by risk assessment based on prior knowledge of the physicochemical properties of the active substance and the manufacturing method. Parameters for unit operations in each step of the process (pre-blending, granulation, blending, compression and film-coating) were investigated, guided by the outcome of the risk assessment (summarised below), using a series of multivariate experiments.

Critical process parameters (CPPs) were identified and set-points for each unit operation, along with proven acceptable ranges (PARs) were defined. However, no design space is claimed and only one factor at a time will be moved from its set-point. The data presented fully supports the proposed PARs.

Based the process understanding gained during development work, finished product attributes were defined as critical and the control strategy elaborated accordingly.

The primary packaging is an HDPE bottle enclosed with a polypropylene continuous-thread, child-resistant cap and 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. The silica gel desiccant has been shown to provide adequate protection from moisture.

Manufacture of the product and process controls

The manufacturing process consists of six main steps: blending of tenofovir alafenamide fumarate with intra-granular excipients, dry granulation followed by milling, blending with extra-granular excipients, compression, film-coating and packaging. The process is considered to be a standard manufacturing process.

Holding times have been defined for final powder blend, tablet cores, and bulk film-coated tablets based on stability studies and are considered acceptable when shelf-life is defined in line with the Note for Guidance on start of shelf-life of the finished dosage form (CPMP/QWP/072/96). Major steps of the manufacturing process will be validated prior to commercialisation. A detailed validation protocol has been provided and is considered acceptable. It has been demonstrated that the manufacturing process is capable of producing the finished product of intended quality in a reproducible manner. The in-process controls are adequate for this type of manufacturing process and pharmaceutical form.

Product specification

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

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 were provided for eight 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 from four production scale batches of finished product manufactured at the commercial site and stored for up to 24 months under long term conditions (25 ºC / 60% RH), up to 24 months under intermediate conditions (30 ºC / 65% RH) and for up to 6 months under accelerated conditions (40 ºC / 75% RH) according to the ICH guidelines were provided. The batches of medicinal product are identical to those proposed for marketing and were packed in the primary packaging proposed for marketing. Samples were tested for appearance, water content, assay, impurities, dissolution, and microbiological quality. The analytical procedures used are stability indicating.

There were no significant changes to any of the measured parameters under long term conditions except for a small increase in degradation products and a decrease in water content due to the desiccant. All parameters remained within specification limits. Under intermediate conditions, more degradation was observed, along with a decrease in assay although levels remained within specification. Water content also decreased. The other parameters remained unchanged. Under accelerated conditions, significant degradation was observed after 6 months coupled with a decrease in assay but remained within specification limits. Apart from a decrease in water content, the other parameters remained unchanged.

In addition, one batch was exposed to light as defined in the ICH Guideline on Photostability Testing of New Drug Substances and Products. No significant changes to any of the measured parameters were observed indicating that the finished product is not photosensitive.

Stress studies were carried out at -20 ^{o}C (6 months), 50 ^{o}C (7 days), 60 ^{o}C (7 days) and in an open dish under long term and intermediate conditions (4 weeks). No significant changes to any of the measured parameters were observed at -20 ^{o}C or 50 ^{o}C. At 60 ^{o}C, a decrease in assay and increase in degradation was observed. In increase in water content was observed in the open dish studies, more so at higher humidity and an increase in degradation was noted at higher temperature and humidity.

Based on available stability data, the proposed shelf-life of 24 months when stored in the original package to protect from moisture as stated in the SmPC (sections 6.3 and 6.4) is acceptable.

Adventitious agents

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

Magnesium stearate is of vegetal origin. No other excipients derived from animal or human origin have been used.

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.

The applicant has applied QbD principles in the development of the finished product. However, no design spaces were claimed for the manufacturing process of the finished product. PARs have been approved for several parameters in the finished product manufacturing process.

2.2.5. Conclusions on the chemical, pharmaceutical and biological aspects

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

2.2.6. Recommendations for future quality development

Not applicable.

2.3. Non-clinical aspects

2.3.1. Introduction

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

Tenofovir alafenamide is approved as part of a fixed dose combination called Genvoya (elvitegravir / cobicistat / emtricitabine / tenofovir alafenamide EMEA/H/C/004042/0000). On 25 February 2016, the Committee for Medicinal Products for Human Use (CHMP) adopted a positive opinion, recommending the granting of a marketing authorisation for the medicinal product Descovy (emtricitabine and tenofovir alafenamide.

Tenofovir disoproxil fumarate is part of the approved FDC products Truvada (Emtricitabine (FTC)/ tenofovir disoproxil fumarate (TDF)), Atripla (efavirenz (EFV)/FTC/TDF), Eviplera (FTC/ rilpivirine (RPV)/TDF) and Stribild (EVG/ cobicistat (COBI)/FTC/TDF) for the treatment of HIV-1 infection.

TFV is currently available in marketed form of Tenofovir disoproxil fumarate (TDF), marketed by Gilead Sciences under the trade name Viread.

TAF has been identified as a next generation oral prodrug of TFV as it may improve distribution of the active substance into peripheral blood mononuclear cells (PMBCs) and to lymphatic organs following

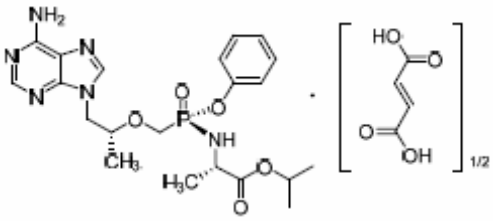
oral administration. TAF could deliver more intracellular tenofovir to PMBCs and lead to lower levels of circulating this active substance resulting in improved safety.

2.3.2. Pharmacology

After absorption, TAF is converted intracellularly to TFV, which is phosphorylated to the active metabolite, TFV-DP, that competes with natural 2'-deoxyadenosine triphosphate (dATP) for incorporation by the HBV DNA pol/RT or HIV-1 RT and, once incorporated, results in chain-termination. Tenofovir diphosphate is a very weak inhibitor of mammalian DNA polymerases α, ß, δ, ε, and mitochondrial DNA polymerase γ.

As TAF is more stable in plasma than TDF, higher intracellular levels are achieved, providing enhanced delivery of TFV and 90% lower circulating levels of TFV relative to TDF. These features translate into less risk of nephrotoxicity and less decrease (or improvements) in bone mineral density, both of which are identified risks with TDF administration.

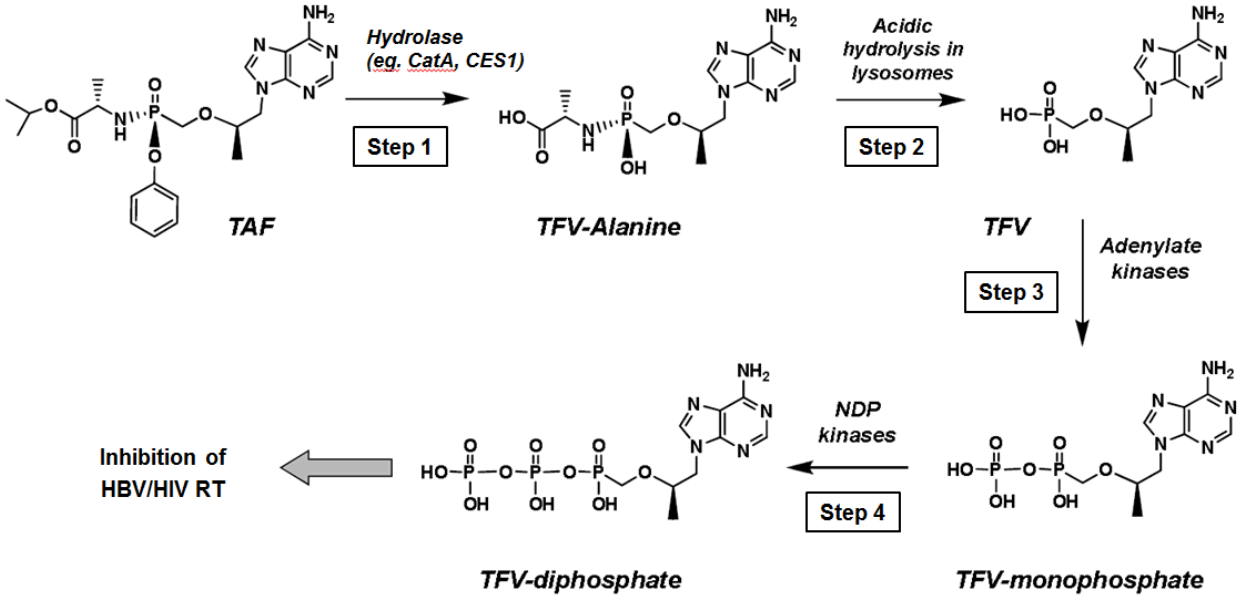
Physical chemistry

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

Primary pharmacodynamic studies

After TAF penetrates cells, the prodrug carboxylester bond is cleaved by a hydrolase (ie, CES1, CatA, etc.), releasing an intermediate metabolite TFV-alanine (TFV-Ala), which is then hydrolyzed to parent TFV and sequentially phosphorylated by adenylate kinase and nucleoside diphosphate kinase to form the active metabolite TFV-DP.

Figure 2. Intracellular activation pathway of TAF



CatA = cathepsin A; CES1 = carboxylesterase 1; NDP = nucleoside diphosphate; RT = reverse transcriptase; TAF = tenofovir alafenamide; TFV = tenofovir

The intracellular metabolism of TAF was investigated in PBMCs and macrophages. The ester bond of TAF is known to be cleaved by lysosomal CatA. TAF hydrolase activity was assessed in cell extracts in the presence or absence of known CatA inhibitors (telaprevir and boceprevir), a CES1 inhibitor (bis-p-nitrophenyl phosphate, BNPP), a CYP3A4 and Pgp inhibitor (cobicistat), or a combination of telaprevir and BNPP together. BNPP inhibited metabolism of 0.5 µM TAF in a dose dependent manner with approximately 37, 30, or 66% inhibition observed at 2, 10, or 50 µM BNPP, respectively. At concentrations of 10 µM and 50 µM of each inhibitor, inhibition of TFV-DP formation of approximately 84 and 95% inhibition, respectively, was observed. These results suggest that CES1 is the predominant enzyme activating TAF in primary human hepatocytes and that CatA also makes a minor contribution.

Due to the enzymatic role in TAF metabolism, differences in CES1 and/or CatA cellular levels may affect the antiviral activity of TAF. The expression levels of CES1 and CatA and their contribution to intracellular metabolism of TAF were assessed in two hepatic cell lines commonly used for antiviral assays (HepG2 and HepAD38). The expression level of CES1 in both hepatic cell extracts was 17- to 43-fold less than in pooled human liver S9 fractions. In comparison, the expression of CatA in bothhepatic cell extracts was 2.4- to 4.8-fold greater than in pooled human liver S9 fractions. A possible explanation for this difference is that suppression of CES1 expression levels may be common in hepatic cell lines.

Hydrolytic activation of TAF in extracts of HepG2 and HepAD38 cells was also evaluated in the presence or absence of inhibitors of CES1 (BNPP) and/or CatA (telaprevir). BNPP showed no impact on TAF hydrolysis in either HepG2 or HepAD38 cells up to the highest concentration tested (50 µM). In contrast, telaprevir was a potent inhibitor of TAF hydrolysis to TFV-Ala in both HepG2 and HepAD38 cells with similar IC50 values of 0.2 and 0.1 µM, respectively. Following incubation of HepAD38 cells with 0.5 µM of TAF, telaprevir significantly inhibited metabolism of TAF to TFV-DP by 2.2-fold, whereas the effect of BNPP was negligible (1.2-fold). When both telaprevir and BNPP were combined, a 3.7-fold greater inhibition of TFV-DP formation was observed, relative to telaprevir alone.

These results indicate that CatA is the major hydrolyzing enzyme of TAF in hepatic cell lines, while CES1 has a modest contribution.

TAF activation in the presence of protease inhibitors

Since certain viral protease inhibitors (PIs) have been shown to be potent inhibitors of CatA, the potential for drug-drug interactions between TAF and antiviral PIs were evaluated in a hydrolase activity assay using purified CatA. The HIV PIs darunavir, atazanavir, lopinavir, and ritonavir, as well as the boosting agent cobicistat did not inhibit CatA-mediated hydrolysis of TAF up to a concentration of 50 µM, well above the clinical Cmax of each drug. In contrast, the covalent anti-HCV PIs telaprevir and boceprevir were identified as potent inhibitors of CatA-mediated hydrolysis of TAF, with IC50 values of 0.3 and 0.2 µM, respectively. When adjusted for plasma binding, these IC50 values are 6- to 8-fold below the clinical maximum concentration (Cmax) levels observed in patients.

Overall, all tested HIV PIs and the majority of tested HCV PIs exhibited minimal potential to interfere with the intracellular activation of TAF. These data support the co-administration of these therapeutic PIs in coinfected patients, with the exception of telaprevir and boceprevir, in combination with TAF, without negatively affecting its intracellular conversion to TFV (see Table 7).

Table 1. Effects of COBI, HIV-1 protease inhibitors, or HCV protease inhibitors on CatA-mediated hydrolysis of 10 µM TAF

Compound	IC_{50} ± SD (µM)[a]	C_{max} (µM)[b] Total Drug	C_{max} (µM)[c] Free Fraction
COBI or HIV-1 PIs			
DRV	> 50	8.9	1.6
ATV	> 50	6.3	0.7
LPV	> 50	15.2	0.3
RTV	> 50	1.3	0.02
COBI	> 50	2.2	0.2
HCV PIs			
Telaprevir	0.3 ± 0.17	5.2	1.5
Boceprevir	0.2 ± 0.02	3.3	1.3
TMC-435	> 50	13.3	< 0.002
BI-201335	25 ± 7	20	0.08
MK-5172	50	2.5	0.06
GS-9256	> 50	10.5	0.004
GS-9451	50	1.7	0.04

ATV = atazanavir; COBI = cobicistat; DRV = darunavir; HCV = hepatitis C virus; HIV-1 = human immunodeficiency virus type 1; IC50 = 50% inhibitory concentration; LPV = lopinavir; PI = protease inhibitor; RTV = ritonavir; SD = standard deviation

a Data represent mean ± SD values from at least 2 independent experiments.

b Published data

c Concentration of free drug at Cmax based on serum protein binding as determined by Gilead Sciences.

TAF (and TFV) loading in the Rhesus monkey

The plasma pharmacokinetic profile of TAF (and TFV) and intracellular TAF metabolism in PBMCs were examined in non-human primates (NHP) given a single oral dose of tenofovir alafenamide (TAF) monofumarate at 5.0 or 50 mg/kg (study number: P2000087). At 50 mg/kg, TAF and TFV levels in the plasma increased rapidly with Tmax values of 0.5 and 1 hour, respectively. In these same animals, TAF and TFV levels in the plasma decreased with t1/2 values of 0.40 and 17.33 hours, respectively. In PBMCs, TFV levels persisted up to 96 hours with an apparently slower decline than in plasma. The TFV levels were significantly higher in the samples treated with acid phosphatase, suggesting that a significant proportion of TFV-related material in PBMCs was in phosphorylated forms. In another NHP study (study number: P2001025) in which animals were given a single subcutaneous dose of [^{14}C]TFV at 15, 30, or 60 mg/kg, TFV was efficiently taken up by PBMCs and metabolised to TFV-DP, with the intracellular concentrations of the active metabolite TFV-DP reaching 0.9 µM (30-mg/kg dose group). The t1/2 of TFV-DP in PBMCs was > 50 hours. Significant levels of TFV and its metabolites were also observed in LMNCs from axillary, inguinal, and mesenteric lymph node sites 48 hours after dosing.

Inhibition of HBV (and HIV-1) Reverse Transcriptase

HBV is a member of the Hepadnaviridae family, while HIV-1 is a member of the Retroviridae family. Both HBV and HIV-1 replicate by reverse transcription requiring RNA-dependent DNA polymerase (RDDP), DNA-dependent DNA polymerase (DDDP), and ribonuclease H activities. Both HBV and HIV-1 encode a RT enzyme with conserved homology. HBV RT has been interchangeably designated as polymerase, RT, or polymerase/reverse transctipaste (pol/RT), since it contains an additional N-terminal protein domain and spacer domain; the former of which is involved in protein-priming of viral DNA synthesis. The effect of TFV-DP on the DDDP activity of HBV pol/RT was evaluated in an enzymatic assay using recombinant HBV pol/RT expressed and purified from baculovirus. Polymerase activity of HBV pol/RT was inhibited by TFV-DP in a dose-dependent manner without a change in maximal upstroke velocity (Vmax). The kinetic inhibition constant (Ki) of DDDP inhibition by TFV-DP was determined to be 0.18 µM, which is 2.1-fold lower than the Michaelis-Menten constant (Km) of dATP.

These results showed that TFV-DP inhibits HBV pol/RT and HIV-1 RT by binding competition with dATP into DNA, which caused premature termination of DNA synthesis upon its incorporation into the nascent DNA chain.

***In Vitro* evaluation of anti-HBV activity**

The antiviral activity of TAF was assessed in HepG2 cells against a panel of 11 wild-type clinical HBV isolates representing genotypes A to H (study number PC-320-2003). Full-length genomes or pol/RT regions were amplified from treatment-naive patients infected with genotypes A to H cloned into expression vectors, and transfected into HepG2 cells. After 7 days of treatment in the presence of TAF, HBV DNA intermediates were extracted and quantified by real-time polymerase chain reaction (PCR) for determination of in vitro susceptibility. TAF showed antiviral activity against all HBV genotypes evaluated. The concentration inhibiting viral replication by 50% (EC50) values for the 11 isolates ranged from 34.7 to 134.4 nM, with an overall mean EC50 of 86.6 nM. Although genotypes D and H showed slight hypersensitivity to TAF, all other genotypes had similar TAF EC50 values compared with the control laboratory strain, pHY92 (see Table 8).

Table 2. TAF antiviral activity against genotypes A to H Clinical HBV Isolates

Type	Genotype	Isolate ID	HBeAg Status	Cloned[a]	TAF	
					EC_{50} (nM)[b]	EC_{50} FC from Control[c]
HBV	A	001	Negative	Full-length	112.0	1.1
	B	002	Negative	Full-length	109.3	1.1
	C	003	Positive	Full-length	107.5	1.1
		004	Positive	Full-length	64.6	0.6
	D	005	Negative	Full-length	70.5	0.7
		006	Negative	Full-length	62.8	0.6
	E	007	Negative	Full-length	134.4	1.3
	F	008	Negative	pol/RT	92.5	0.9
	G	009	Negative	pol/RT	120.4	1.2
		010	Negative	pol/RT	43.8	0.4
	H	011	Negative	pol/RT	34.7	0.3
	Control (A)	pHY92	NA	Full-length	102.3	1.0

FC = fold change; HBeAg = HBV e antigen; NA = not available; TAF = tenofovir alafenamide

a Full-length genomes or pol/RT regions were amplified and cloned into an expression vector pHY106 or pRTAN (containing HBV genome of pHY92 except pol/RT), respectively, followed by transfection into HepG2 cells.

b Data represent the mean from a minimum of 2 independent experiments performed in quadruplicate.

c Fold change in mean EC50 value relative to the pHY92 control (genotype A).

Anti-HBV activity and cytotoxicity of TAF

TAF was evaluated in vitro for antiviral activity against HBV (EC50) and cytotoxicity (concentration that results in 50% cytotoxicity [CC50]) in HepG2 cells (study numbers: PC-320-2003 & PC-120-2007). TAF exhibited antiviral activity against all tested HBV genotypes (A to H), with an overall mean EC50 of 86.6 nM. In addition, TAF had no observed cellular cytotoxicity up to the highest tested concentration (44400 nM). Based on these results, the selectivity (therapeutic) index (SI) for TAF was > 513 in HepG2 cells.

Drug-resistant variants

To evaluate the antiviral activity of TAF against HBV containing NRTI-R mutation(s), a panel of 11 drug resistant mutants was created in a full-length HBV clinical isolate by site-directed mutagenesis (study number: PC-320-2007). Constructs containing ADV-R (n = 5), LAM-R (n = 3), and/or ETV-R (n = 3) mutations were transfected into HepG2 cells and treated with TAF for 7 days in order to determine in vitro susceptibility. Overall, TAF showed anti-HBV activity against all LAM-R (3 of 3) and ETV-R (3 of 3) recombinants and most ADV-R (4 of 5) recombinants, with mean change in EC50 values of < 2.0-fold compared with wild type. One ADV-R recombinant (rtA181V + rtN236T), exhibited a low-level (3.7-fold) reduced susceptibility to TAF compared with wild type. In terms of fold change compared with the wild-type isolate, susceptibility to TAF for this panel of recombinants was nearly identical to TFV with minor variability. As expected, all LAM-R and ETV-R recombinants exhibited significantly reduced susceptibility to LAM (> 48.8-fold) and ETV (> 28.6-fold), respectively.

In vivo

The woodchuck hepatitis virus (WHV) in its natural host, the eastern woodchuck Marmata monax is often used as a model of HBV infection. The antiviral effect of oral administration of TDF against chronic WHV infection was evaluated in a short-term (4-week) placebo-controlled, dose-ranging study. Animals were given oral doses of TDF at 0.5, 1.5, and 5.0 mg/kg/day for 4 weeks. A significant reduced serum viral load, resulting in 0.2 ($p < 0.01$), 1.1 ($p < 0.01$), and 1.5 log10 ($p < 0.05$) decreases, respectively, from the pre-treatment levels, was seen. A dose of 15 mg/kg/day for 4 weeks reduced serum viral load by 1.2 log10 but was not considered statistically significant due to the degree of individual variation in the antiviral response.

In another long-term (48-week) study, the antiviral efficacy of oral administration of TDF, adefovir dipivoxil (ADV), LAM (lamivudine), and emtricitabine/rilpivirine/tenofovir alafenamide (coformulated) (FTC) as well as the combinations of TDF or ADV with LAM and FTC at 15 mg/kg/day were evaluated in chronic woodchuck hepatitis virus (WHV)infected woodchucks (Study number PC-174-2004). At 12 weeks of treatment, the TDF-containing groups of TDF alone, LAM+TDF, and FTC+TDF had mean serum viral load reductions of 3.6, 3.7 and 4.2 log10 copies/mL, respectively. Between Weeks 12 and 24, varying degrees of viral rebound were observed across all drug treatment groups. At Week 48, the treatment groups of TDF alone, LAM+TDF, and FTC+TDF had mean serum viral load reductions of 2.9, 5.8, and 6.1 log10 copies/mL, respectively. In the entire 48-week dosing period, there was no evidence of toxicity in woodchucks treated with any of the drugs or drug combinations.

Secondary pharmacodynamic studies

TAF is hydrolyzed to TFV in target cells resulting in high intracellular levels of TFV-DP in vivo. The in vitro specificity of TFV-DP for mammalian DNA polymerases, relative to its interaction with viral polymerases, was determined. TFV-DP was shown to be a weak inhibitor of mammalian DNA polymerases α, β, δ, ε, and mtDNA polymerase γ.

A primary screen was used to determine the effect of the prodrug TDF and the parent drug TFV (the major metabolite of TDF and TAF) on the inhibition or stimulation of binding in a series of 111 protein targets (neuroreceptors, ion channels, transporters, and nuclear receptors (Study number: V2000020)). The protein target was incubated in the presence of 10 µM TFV or TDF. The effect on the binding of the endogenous ligand was then determined. Responses of > 50% stimulation or inhibition were considered significant. There was no significant inhibition or stimulation of ligand binding to its protein target by either TFV or TDF. The results of this study demonstrate that neither TFV nor TDF significantly interact with any of the 111 protein targets tested.

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.

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

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.

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.

Safety pharmacology programme

In vivo safety pharmacology experiments were conducted using TAF as the monofumarate form (GS-7340-02) in 50 mM citric acid.

Cardiovascular system:

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.

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.

2.3.3. Pharmacokinetics

The absorption, distribution, metabolism, and excretion of 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.

Methods of analysis

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 [14C]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.

Absorption

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 tenofovir alafenamide monofumarate (GS-7340-02) or tenofovir alafenamide hemifumarate (GS-7340-03) to male CD-1 mice or GS-7340-03 (hemifumarate) to both male and female 001178-W mice via oral gavage (Study Nos. AD-120-2014 and AD-120-2016).

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

Tenofovir alafenamide monofumarate 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 Tenofovir alafenamide monofumarate 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 (monofumarate and hemifumarate) 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 Tmax of less than 1 hour. TFV exposure increased in a greater than dose proportional manner. There no significant difference in pharmacokinetic parameters between the two forms of TAF.

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

The plasma pharmacokinetic profile of tenofovir alafenamide monofumarate 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, tenofovir alafenamide monofumarate 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 (tenofovir alafenamide monofumarate [6.3 mg/kg]), or oral administration (TAF as free base [18.0 mg/kg], its diastereomer GS-7339 [18.0 mg/kg], the mixture GS-7171 [16.0 mg/kg], or GS-7340-02 [4,8, 5.0, and 20 mg/kg under fasted and 5.0 mg/kg under fed conditions]). Following oral administration, TAF and its diasteroisomer were rapidly absorbed and eliminated with a tmax of less than 0.5 h and t½ ranging from 0.2-0.9 h. The plasma exposures to the intact prodrugs were similar when TAF or GS-7339 were dosed separately, however, when the isomeric mixture, GS-7171, was dosed, the exposure to GS-7339 was approximately 3-fold higher than TAF. TFV exposure was similar for both diasteroisomers, although exposure in PBMCs was higher following dosing with TAF than with GS-7339. The effect of food led to a decrease in overall plasma exposure of TFV and TAF (2.5 fold).

When male Beagle dogs were given a single oral dose of 10 mg/kg TAF, there was rapid absorption and elimination, 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 post-dose.

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, Cmax values of 582 and 1280 ng/mL, and t½ λz values of 18 and 23 minutes, respectively. The TFV Cmax values appeared to be linear with increasing dose as well as repeat dosing. The TFV t½ was estimated to be 37 h and substantial accumulation of TFV was observed after repeat dosing. The TFV levels in PBMCs were not linear with increasing dose; however, a linear correlation was observed between TFV levels in PBMCs and corresponding trough plasma concentrations. PBMC concentrations were approximately 100-fold higher than corresponding plasma concentrations.

In a 9-month toxicology study in dog, tenofovir alafenamide monofumarate was administered once daily at doses of 0, 2, 6, and 18 mg/kg/day (Study No. TOX-120-002). The dose of 18 mg/kg/day was decreased to 12 mg/kg/day on Day 2 of Week 7 for males and Day 2 of Week 8 for females due to severe clinical signs and reduced body weight and food consumption. The concentrations of GS-7340 and tenofovir in plasma samples and total TFV in Week 39/40 PBMC samples were determined. GS-7340 was rapidly absorbed and converted to tenofovir following oral dose administration, with peak plasma concentrations of GS-7340 (free base) 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 postdose for all dose groups. The median terminal phase half-life of total tenofovir in PBMCs was estimated to be 31 hours (similar to the tenofovir plasma estimate) from the recovery animals with PBMC concentrations measured up to 72 hours. Dose-normalized PBMC mean AUC values of total tenofovir increased more than dose proportionally during Week 39/40.

Monkey:

Single dose pharmacokinetics for TAF and TFV, and TFV in PBMCs was determined using rhesus monkeys administered single oral doses of GS-7340-02 at 0.5, 5.0, and 50 mg/kg (Study No. P2000087). Tenofovir alafenamide and TFV levels increased rapidly with tmax values of approximately 0.5 and 1 hour, respectively. 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.

Distribution

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 postdose. 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 non-pigmented animals, binding to melanin was unlikely.

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

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

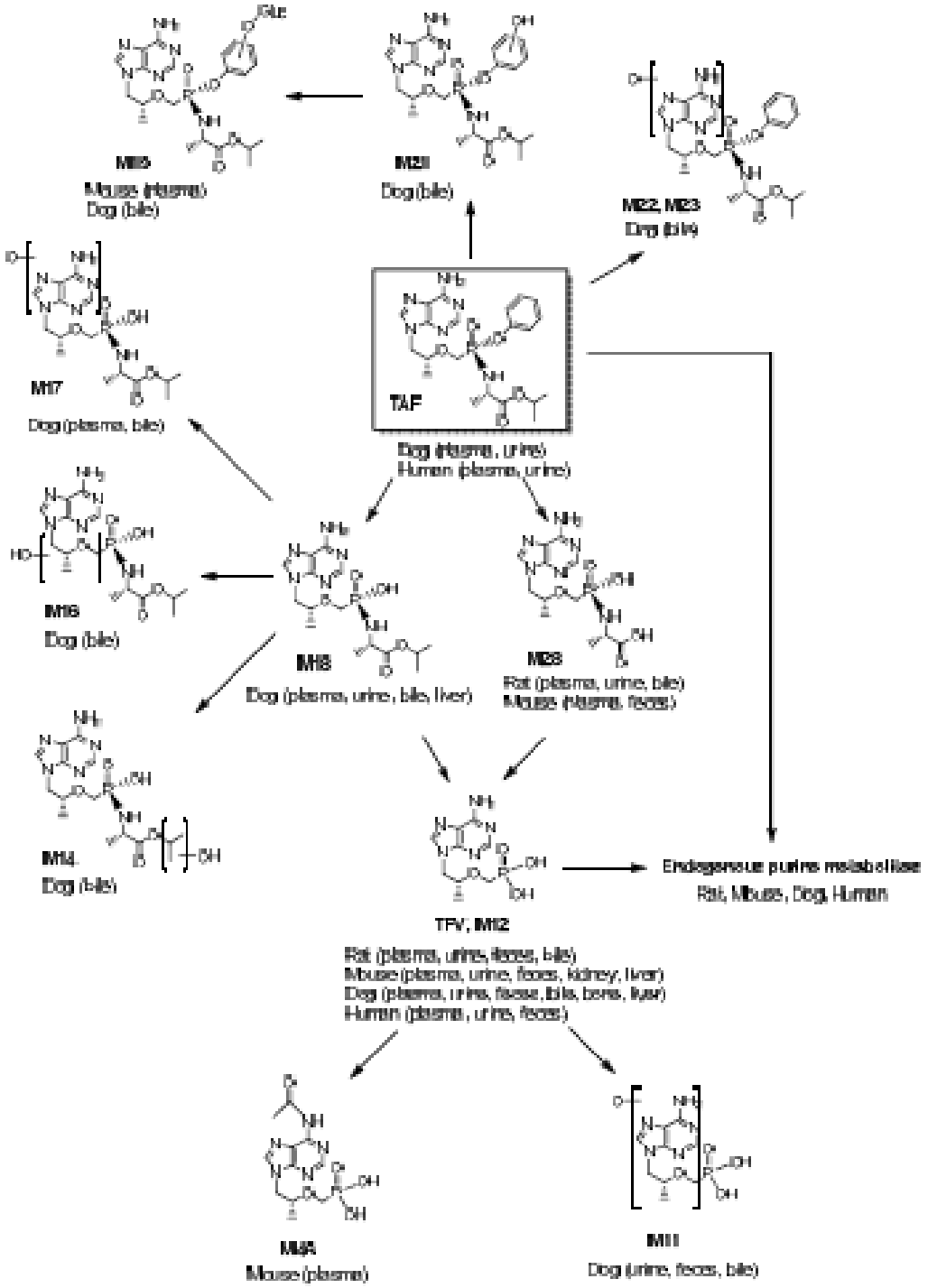
In the monkey the extent of placental transfer of TFV following subcutaneous administration was determined in pregnant rhesus monkeys (Study No. 96-DDM-1278-005). Placental transfer of TFV appeared to be significant with a foetal/maternal serum concentration ratio of 0.17 ± 0.07 (mean ± SD) at approximately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

Metabolism

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 5). 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.

Excretion

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 postdose in urine. Faecal elimination was 3.18% ± 1.85% by 24 hours, and 4.48% ± 1.89% by 7 days postdose.

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.

Pharmacokinetic drug interactions

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 (atazanavir or darunavir) or CYP inhibitors (ritonavir or COBI) at concentration of up to 100 µM. The stability of TAF was unaffected by the presence of these CYP inhibitors or PIs.

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

Induction Liability

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

There was evidence of cytotoxicity following dosing with 100 µM TAF with reduced CYP activity however 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. The relevant transporters affected by TAF (transporter substrates and inhibition) are shown in Table 9 (transport inhibition).

Table 3. Transporter Substrate and Inhibition Assessment of TAF and TFV

Transporter	Substrate Potential (y/n)		Inhibition Potential, IC_{50} (µM)	
	TAF	TFV	TAF	TFV
P-gp	y	n	>100	>1000
BCRP	y	n	>100	>100
BSEP	ND	ND	>100	>100
OATP1B1	y	ND	>100	>100
OATP1B3	y	ND	>100	>100
MATE1	ND	ND	>100	>300
OAT1	n	y	>100	33.8[a]
OAT3	n	y	>100	>1000
OCT1	n	n	>100	>100
OCT2	ND	n	>100	>300
MRP1	ND	n	ND	>500
MRP2	ND	n	ND	>100
MRP4	ND	y	ND	>1000[b]

BCRP = breast cancer resistance protein; BSEP = bile salt excretory pump; MATE = multidrug and toxin extrusion protein; MDR1 = multidrug resistance protein 1; MRP1 2, 3, or 4 = multidrug resistance associated protein 1, 2, or 4; ND = not determined; OAT1 or 3 = organic anion transporter 1 or 3; OATP1B1 or B3 = organic anion transporting polypeptide 1B1 or B3; OCT1 or 2 = organic cation transporter 1

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

b Imaoka et al 2007

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. See clinical assessment report for discussion in relation to OATP.

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. See clinical report for further discussion on transporters.

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. See clinical report for further discussion on transporters.

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).

Tenofovir alafenamide was efficiently taken up and metabolized in primary human hepatocytes. TAF was shown to be taken up by untransfected CHO cells at a rate of 9.0 pmol/min/106 cells indicating that TAF has high passive permeability. Uptake was higher with the cells expressing hepatic uptake transporter, OATP1B1 or OATP1B3 with rates of 12.0 or 24.1 pmol/min/106 cells, respectively and rifampin inhibited the transporter dependent uptake. Atorvastatin and antipyrine were used as positive and passive permeability controls, respectively. These results showed that TAF is a substrate for hepatic uptake transporters, OATP1B1 and OATP1B3 (Study number: AD-120-2022).

Effect of an OATP inhibitor, rifampicin on uptake of TAF into primary human hepatocytes was assessed in vitro. The results from four different hepatocyte donors suggested that OATP-mediated transport makes a small contribution to TAF uptake (Study number: AD-120-2042). Taken together, the

Applicant goes onto say that it is likely that the major route of TAF uptake into hepatocytes is passive permeability. The Applicant goes onto say that, while exposure to TAF may be affected slightly by inhibitors of these transporters or genetic polymorphisms that affect the transport activities, the effects of differences in OATP1B1 and OATP1B3 activity are, not expected to be clinically relevant given the high passive permeability of TAF. See clinical assessment report for discussion in relation to OATP.

2.3.4. Toxicology

In support of this application, 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.

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

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

1 µM TFV (GS-1278) = 0.287 µg/mL

1 ng/mL TFV = 3.48 nM

1 µM TAF (GS-7340) = 0.477 µg/mL

1 ng/mL TAF = 2.10 nM

Table 4. TFV Equivalents

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

Single dose toxicity

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

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

Repeat dose toxicity

Mouse

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

Rat

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

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

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

Daily oral administration of TAF (monofumarate) 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 sexes increased dose proportionally over the dose range of 5 mg/kg/day to 100 mg/kg/day at each study period.

Rabbits

Daily oral administration of TAF (monofumarate) 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 (monofumarate) 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.

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 (monofumarate) 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.

The applicant stated 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 ALP values at all doses were comparable controls except for one male at 18/12 mg/kg/day, which was outside the control ranges. At the end of the recovery period, bone marker values returned to below the control range consistent with an age effect and recovery from treatment.

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

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

A minimal to slight (Grade 1 to 2) infiltration of mononuclear cells in the ocular posterior uvea was noted in some animals at 18/12 mg/kg/day after both treatment periods. Alveolar histiocytosis was present in the lungs after 13-weeks at 18/12 mg/kg/day. Additional pulmonary findings noted following 39-weeks of treatment and consisted of macrophage accumulation with pigment, which was detected predominantly at 18/12 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 stated 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, but were however 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 were no gender differences in exposure.

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.

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 Cmax values were nonlinear with dose, with greater than expected increases in Cmax with dose. The TAF AUCtau 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 were no gender differences in exposure.

Day 28 TFV Cmax and AUC exhibited slightly greater than proportional increases with increasing dose. Comparison between Days 1 and 28 showed no statistical difference for Cmax or AUC indicating no change in clearance over time. There were no gender differences in exposure.

Key findings

Kidney

Renal tubular karyomegaly was observed in rats and dogs orally administered TAF. Focal areas of minimal renal cortical tubular basophilia and associated minimal nuclear karyomegaly were present in rats at 400 mg/kg/day (4 weeks) and 100 mg/kg/day for 26 weeks. Renal tubular karyomegaly and/or basophilia were observed in dogs at 3 and 10 mg/kg/day (4 weeks) and dogs at 6 or 18/12 mg/kg/day for at least 13 weeks. Renal cortical tubular degeneration/regeneration findings was seen at 6 or 18/12 mg/kg/day (13 weeks) in the 39-week dog toxicity study. Similar findings of renal cortical tubular degeneration/regeneration and karyomegaly were seen in dogs given 6 or 18/12 mg/kg/day for 39 weeks. These changes were minimal to slight in affected males and females at 6 mg/kg/day. At18/12 mg/kg/day the severity ranged from mild to moderate. Similar lesions (minimal karyomegaly and tubular degeneration) were seen in 2 males at 2 mg/kg/day (39 weeks). After a 13-week recovery period, test article-related histology changes were still observed in the kidney but were of reduced incidence and severity.

Bone

Atrophy of metaphyseal cancellous bone was observed in rats at 100 mg/kg/day for 26 weeks. TAF also increased biochemical markers of bone turnover and decreased serum 1,25-dihydroxy- and 25-hydroxyvitamin D3 in rats (≥ 25 mg/kg/day) and dogs (≥ 37.5 mg/kg/day for 6 days). In These findings were accompanied by statically significant decrease in serum 1,25 dihydroxy vitamin D3 in males only and a significant increase in 25-hydroxyvitamin D3 in females only.

Nasal turbinates

Adverse degenerative (olfactory) and acute inflammatory (infiltrate neutrophil) changes in the nasal mucosa were noted in mice given TAF for 13 weeks. These findings were not seen in rats, dogs, or non-human primates for longer durations of administration. It is stated that the relevance to humans is unknown and the risk of nasal inflammation in humans is very low.

Cardiovascular

Prolong PR intervals (approximately 13% to 24%), which was associated with significant decreases in triiodothyronine (T3) was noted in the 39 week dog study. After the 13-week recovery period, serum T3 values returned to levels similar to the control group animals at the end of the study.

Eye

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. In-life ophthalmologic examinations were normal. The applicant states that the histiocytic infiltration observed in multiple organs was most likely an indirect test article-effect due to general debilitation and were not observed in other repeat-dose toxicity studies. 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.

The posterior uveitis in dogs administered seen at 18/12 mg/kg/day in the 39 week dog study occurred at 3.7- and 17-fold higher exposure to TAF and TFV, respectively, than that observed in human subjects administered a 25-mg dose of TAF. No difference in distribution was observed between Sprague-Dawley and Long Evans rats, including in the skin and eyes, suggesting no binding to melanin. It is stated that because TAF has poor penetration across the blood brain and blood retinal barrier in dogs, it is unlikely that TAF directly caused the observed histiocytic infiltration in the posterior uvea. Based on the data from tissue distribution and toxicology studies (see below), it is unlikely that these findings would translate into a concern regarding the ocular safety of TAF and were probably due to the poor condition of these animals during the in-life phase of the study. However there was one adolescent with uveitis considered to be drug-related by the investigator. At present it seems appropriate to keep this issue under close review with appropriate reflection in the RMP.

Distribution of 14C-TAF to eyes has been assessed in mice, rats, and dogs (AD-120-2011, AD-120-2020, and D990173-BP). Melanin binding has specifically been assessed by comparing distribution in pigmented and non-pigmented mice (C57 black and CD-1, respectively) and rats (Long Evans and Sprague-Dawley, respectively). [14C]-TAF-related radioactivity distributed poorly to the eyes of rats and dogs (Cmax in eyes <8% that observed in plasma). Transient exposure to low levels of [14C]-TAF-related radioactivity was observed in the eyes of rats decreasing to undetectable levels at 8 hours postdose. No difference in distribution was observed between Sprague-Dawley and Long Evans rats, including in the skin and eyes, suggesting no binding to melanin. The distribution of [14C]-TAF to eyes in mice was higher than other species studied (Cmax in eyes 15%-20% that observed in plasma).

More persistent exposures in eye lens, eye uveal tract, and eyes were observed in C57 black mice compared to CD-1 mice.

Safety margins

Table 5. Estimated Safety Margins of TAF Based on AUCss When Comparing Animal No-Adverse-Effect-Level (NOAEL)

Target Organ Effect	Species	Study/Dose Duration	TAF NOAEL (mg/kg/day)	AUC_{ss} (µg·h/mL) NOAEL TFV/ TAF	Margin Relative to Human AUC_{ss} TFV[a]/TAF[b]
Nasal Turbinate Toxicity	Mouse	13 Weeks	<10	<0.213/NC	<1.5/NA
Renal Toxicity	Rat	26 weeks	25	3.8/NC	12/NA
	Dog	39 weeks	2	1.2/0.08	4/0.4
	Monkey	4-weeks	≥30	≥5.9/1.0	>18/5
Bone Mineral Loss	Rat	26 weeks	25	3.8/NC	12/NA
	Dog	39 weeks	2	1.2/0.08	4/0.4
	Monkey	4-weeks	≥30	≥5.9/1.0	>18/5
Fertility[c]	Rat	Up to 10 weeks	160	NA	NA
Embryo fetal development[c]	Rat	12 days	84	17.4/0.2	54/1
	Rabbit	14 days	100	27.3/11	85/51
Perinatal/postnatal[c]	Rat	27 days (Gestation day 7 to Lactation day 20)	150 (TDF)	7.84/NA	24/NA

NA = not applicable; NC = insufficient data to calculate

a Predicted safety margin for TFV human exposure is based on pooled PK data from TAF Phase 3 pivotal studies GS-US-320-0108 and GS-US-320-0110 where the mean TFV AUCss = 0.216 µg.h/mL.

b Predicted safety margin for TAF human exposure is based on pooled PK data from TAF Phase 3 pivotal studies GS-US-320-0108 and GS-US-320-0110 where the mean TAF AUCss = 0.322 µg.h/mL.

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

Genotoxicity

The genotoxicity studies conducted in support of this application are listed in Table 12.

Table 6. Genotoxicity studies

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

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

Carcinogenicity

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

Daily administration of TDF at dose levels of up to 300 mg/kg/day administered for 104 weeks did not reveal any evidence of carcinogenicity in mice. At 600 mg/kg/day, a low incidence of duodenal tumours was observed, possibly related to high local test article concentration in the gastrointestinal tract. In rats, daily administration of TDF at dose levels up to 300 mg/kg/day did not reveal any evidence of carcinogenicity.

Reproductive and developmental toxicity

The reproductive and developmental toxicity of TAF was evaluated in a fertility and early embryonic development to implantation study in rats, embryo-fetal development studies in rats and rabbits. Based on the scientific advice adopted by CHMP(EMA/CHMP/SAWP/214541/2013; EMEA/H/SA/2410/1/FU/1/2013/1), a peri/postnatal study in rats is not required for TAF registration due to the lack of TAF exposure in rats and lower TFV exposure compared to TDF.

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 were given 20, 80, or 160 mg free base equivalent (f.b.e.)/kg/day (22, 90, 180 mg tenofovir alafenamide (TAF) hemifumarate/kg/day. 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 (monofumarate), 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 fetal body weight associated with some delays in the rate of ossification. There was no evidence of embryolethality or teratogenicity attributed to TAF in this study. The maternal TAF NOAEL and the TAF NOAEL for embryo-fetal development were both considered to be 100 mg/kg/day, which resulted in GD17 AUC0-t values of 17.4 and 0.2 µg·hr/mL for TFV and TAF, respectively.

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

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

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

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.

2.3.5. Ecotoxicity / environmental risk assessment

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

Phase I

A PBT screening was performed. Based on an experimentally derived logKow of -4.3 at pH 7 which is below the trigger value of 4.5, it can be concluded that tenofovir is not potentially PBT. Hence, no further PBT assessment is necessary.

The initial PECsurfacewater was calculated based on the maximum daily dose of 28 mg tenofovir alafenamide fumarate under the assumption of the default market penetration factor (Fpen) of 0.01. The resulting PECsurfacewater for tenofovir alafenamide fumarat is 0.14 µg/l which clearly exceeds the trigger value of 0.01 µg/l. Consequently, a Phase II assessment was performed.

Phase II Tier A

Tenofovir alafenamide is a pro-drug which is rapidly converted into the active moiety tenofovir after absorption. Consequently, the ERA was performed with tenofovir for the aquatic compartment. The study on transformation in water/sediment systems showed a significant shifting of the active substance into the sediment. Therefore, a toxicity test on sediment dwelling organism was performed as well. Terrestrial studies are considered not necessary as the adsorption coefficient is lower than the respective trigger value of 10 000 L/kg.

Table 7. Summary of main results

Substance (INN/Invented Name): Tenofovir alafenamide/Vemlidy			
CAS-number (if available): 147127-20-6			
PBT screening		Result	**Conclusion**
Bioaccumulation potential- log K_{ow}	OECD107	pH2: -3.8 pH7: -4.3	Potential PBT No
PBT-assessment			
Parameter	**Result relevant for conclusion**		**Conclusion**
Bioaccumulation	log K_{ow}	-4.3	not B
	BCF	not required	not B
Persistence	DT50	$DT_{50\ sediment,20\ °C}$ = 142.0 d $DT_{50\ sediment,12°C}$ = 303.0 d	vP
Toxicity	NOEC or CMR	NOEC ≥ 10 mg/L	not T
PBT-statement :	The compound is not considered as PBT or vPvB		
Phase I			
Calculation	**Value**	**Unit**	**Conclusion**
$PEC_{surfacewater}$, default or refined (e.g. prevalence, literature)	0.14 (tenofovir alafenamide fumarate), default	µg/L	> 0.01 threshold (Y/N)
Other concerns (e.g. chemical class)			(Y/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 Koc des soil 968 - 2791 L/kg KF ads sludge 6.0 - 21 L/kg KF des sludge 16 - 62 L/kg	Kfoc for PECsed: 1091
Ready Biodegradability Test	OECD 301B	9% CO_2 at day 28	Not readily biodegradable
Aerobic and Anaerobic Transformation in Aquatic Sediment systems	OECD 308	$DT_{50,\ water}$ = 2.0 – 3.5 d $DT_{50,\ sediment}$ = 28.0 - 142.0 d (303.0 d at 12 °C) $DT_{50,\ whole\ system}$ = 10.4 – 32.7 d >10% parent associated with sediment from Day 7; 3 significant metabolites in water and/or sediment out of which two are considered persistent.	Tenofovir is considerd very persistent in sediments
Phase IIa Effect studies			

Study type	Test protocol	Endpoint	value	Unit	Remarks
Algae, Growth Inhibition *Pseudokirchneriella subcapitata*	OECD 201	NOEC	32 000.0	µg/L	*Pseudokirchneriella subcapitata*
Daphnia sp. Reproduction Test	OECD 211	NOEC	100 000.0	µg/L	
Fish, Early Life Stage Toxicity Test/*Pimephales promelas*	OECD 210	NOEC	10 000.0	µg/L	*Pimephales promelas*
Activated Sludge, Respiration Inhibition Test	OECD 209	NOEC (3 h)	1 000 000.0	µg/L	
Phase IIb Studies					
Bioaccumulation	OECD 305	BCF	not required	L/kg	
Aerobic and anaerobic transformation in soil	OECD 307	DT50 $\%CO_2$	not required		
Soil microorganisms: Nitrogen Transformation Test	OECD 216	%effect	not required	mg/kg	
Terrestrial Plants, Growth Test/*Species*	OECD 208	NOEC	not required	mg/kg	
Earthworm, Acute Toxicity Tests	OECD 207	NOEC	not required	mg/kg	
Collembola, Reproduction Test	ISO 11267	NOEC	not required	mg/kg	
Sediment dwelling organism	OECD 218	NOEC	NOEC ≥ 290 mg/kg_{dwt} (C_{org} 2.3%) (1261 mg/kg_{dwt} normalised for 10% OC)	mg/kg	*Chironomus riparius*

2.3.6. Discussion on non-clinical aspects

The pharmacology of tenofovir alafenamide (TAF) has been appropriately investigated in a range of in vitro and ex vivo studies.

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

Other safety pharmacology studies revealed no significant concerns for TAF. The absorption, distribution, metabolism and excretion of TFV/TAF were evaluated in vitro and in a variety of animal models.

The kidney and bone findings seen in the rat and dog toxicology studies are known toxicities of TFV.. 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 was one adolescent with uveitis considered to be drug-related by the investigator. At present it seems appropriate to keep this issue under close review as reflected in the RMP.

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).

2.3.7. Conclusion on the non-clinical aspects

No major concerns have been identified from the nonclinical data.

2.4. Clinical aspects

2.4.1. Introduction

A program of 28 clinical studies provides PK and/or PD data for TAF in support of this MAA and the overview of studies is shown in Table 14.

This includes data from studies conducted with TAF administered as a single agent or as part of the F/TAF, FTC/RPV/TAF, or E/C/F/TAF FDCs.

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 8. Tabular overview of clinical studies

Study identifier	Type of Component (Name of Leaf)
Comparative BA and Bioequivalence (BE) Study Reports	
GS-US-311-1088	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-311-1473	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-292-0103	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
Healthy Subject PK and Initial Tolerability Study Reports	
GS-US-120-0109	A Phase 1 Study to Evaluate the Pharmacokinetics, Metabolism and Excretion of GS-7340
Intrinsic Factor PK Study Reports and Related Information	
GS-US-120-0108	A Phase 1, Open-Label, Parallel-Design Study to Evaluate the Pharmacokinetics of GS-7340 in Subjects with Severe Renal Impairment
GS-US-120-0114	A Phase 1, Open-Label, Parallel-Group, Single Dose Study to Evaluate the Pharmacokinetics of Tenofovir Alafenamide in Subjects with Normal and Impaired Hepatic Function
GS-US-320-1228	A Phase 1 Single Dose Study to Investigate the Pharmacokinetics, Safety and Tolerability of Tenofovir Alafenamide in Healthy Japanese and Non-Japanese Subjects
GS-US-320-1615	A Phase 1, Open-Label, Parallel-Group, Single Dose Study to Evaluate the Pharmacokinetics of Tenofovir Alafenamide (TAF) in Subjects with Normal Hepatic Function and Subjects with Severe Hepatic Impairment
ESRD / HD Modelling Report	Prediction of Pharmacokinetic Exposures for Tenofovir following Administration of Tenofovir Alafenamide in End Stage Renal Disease Subjects with HBV Undergoing Haemodialysis
Extrinsic Factor PK Study Reports	
GS-US-120-0117	A Phase 1 Single-Dose Study Evaluating the Pharmacokinetic Drug Interaction Potential between Rilpivirine and Tenofovir Alafenamide
GS-US-120-0118	A Pharmacokinetic Study Evaluating the Drug Interaction Potential of Tenofovir Alafenamide with a Boosted Protease Inhibitor or Unboosted Integrase Inhibitor in Healthy Subjects
GS-US-120-1538	A Fixed-Sequence, Open-Label, Study Evaluating the Pharmacokinetics and Drug Interaction Potential between Tenofovir Alafenamide and Midazolam (Oral and Intravenous) in Healthy Volunteers
GS-US-120-1554	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
GS-US-320-1382	A Phase 1, Randomized, Open-Label Study to Determine the Effect of Food on the Pharmacokinetics of Tenofovir Alafenamide (TAF) in Healthy Volunteers
GS-US-311-0101	Phase 1 Study Evaluating the Drug Interaction Potential Between Once-Daily FTC/GS-7340 Fixed Dose Combination and Efavirenz or Cobicistat-Boosted Darunavir
GS-US-311-1386	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-311-1387	A Phase 1, Open-Label, Adaptive, Two-Part, Three Period, Fixed Sequence Study to Evaluate the Effect of Carbamazepine on the PK of TAF and GS-9883 in Healthy Adult Subjects
GS-US-311-1388	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
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
GS-US-366-1689	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
GS-US-292-0101	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-292-0110	A Phase 1, Randomized, Open-Label Study to Determine the Effect of Food on the Pharmacokinetics of Tenofovir Alafenamide When Administered as a Single Tablet Regimen Containing Elvitegravir/Cobicistat/Emtricitabine/Tenofovir Alafenamide in Healthy Volunteers

GS-US-292-1316	A Phase 1, Randomized, Open-Label, Three Period, Fixed Sequence Study Evaluating the Drug Interaction Potential Between Elvitegravir/Cobicistat/Emtricitabine/Tenofovir Alafenamide Single-Tablet Regimen and Sertraline in Healthy Subjects
GS-US-342-1167	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
Population PK Study Reports	
Population Pharmacokinetic Report	Population Pharmacokinetics of TAF
Population Pharmacokinetic Report	**Population Pharmacokinetics of TAF and F/TAF**
Healthy Subject PD and PK/PD Study Reports	
GS-US-120-0107	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
Patient PD and PK/PD Study Reports	
GS-US-120-0104	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
GS-US-320-0101	A Phase 1b Randomized, Open Label, Active-Controlled Study to Assess the Safety, Viral Kinetics, and Anti-HBV Activity of GS-7340 in Treatment-Naive Adults with Chronic Hepatitis B (CHB) Infection
Study Reports of Controlled Clinical Studies Pertinent to the Claimed Indication	
GS-US-320-0108	A Phase 3, Randomized, Double-Blind Study to Evaluate the Safety and Efficacy of Tenofovir Alafenamide (TAF) 25 mg QD versus Tenofovir Disoproxil Fumarate (TDF) 300 mg QD for the Treatment of HBeAg-Negative, Chronic Hepatitis B
GS-US-320-0110	A Phase 3, Randomized, Double-Blind Study to Evaluate the Safety and Efficacy of Tenofovir Alafenamide (TAF) 25 mg QD versus Tenofovir Disoproxil Fumarate (TDF) 300 mg QD for the Treatment of HBeAg-Positive, Chronic Hepatitis B

2.4.2. Pharmacokinetics

Formulation

The proposed commercial TAF 25 mg film-coated tablets are identical to those used in the pivotal bioequivalence and Phase 3 trials.

Methods

Analytical methods

The method for determination of TAF in human plasma was validated at Quest Pharmaceutical Services, L.L.C (QPS). It 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 assayed in plasma at QPS using a range of validated assays over time that involved the extraction of TFV and stable isotope internal standard (TFV-d6) from human plasma using

protein precipitation followed by LC-MS/MS with positive ionization. The linear range was from 5-300 ng/mL for Phase 3 samples and 0.3 - 300 ng/mL in the final more sensitive assay.

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.

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 or induction. The effect of P-gp inhibitors and inducers on TAF bioavailability was evaluated in several studies.

Comparative bioavailability and bioequivalence studies

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. TFV levels were not reported due to failure of the incurred sample reliability measure. The bioanalytical report states that the QCs spiked with TAF plus TFV showed conversion of TAF to TFV, particularly when the TAF:TFV ratio was high.

Table 9. GS-US-311-1088: Statistical Comparisons of Tenofovir Alafenamide PK Parameter Estimates Between Study Drugs (PK Analysis Sets)

	GLSMs by Treatment			
TAF PK Parameter	Test Treatment (F/TAF) (N = 55)	Reference Treatment (FTC+TAF) (N = 55)	GLSM Ratio (%) Test/Reference	90% CI
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-311-1473

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 a large open-label crossover study which compared F/TAF (200/25 mg) with E/C/F/TAF 10 mg. Dosing was after completion of a 600 kcal and 27% fat meal. Bioequivalence was demonstrated for TAF 25 mg without COBI and TAF 10 mg with COBI. TFV was not measured.

Table 10. 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

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

In GS-US-292-0103 healthy subjects with mean eGFRCG at baseline of 126.9 mL/min received the following treatments in randomised order:

A: EVG 150 mg/COBI 150 mg/FTC 200 mg/TAF 10 mg

C: FTC 200 mg + TAF 25 mg (i.e. co-administered)

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

The TAF and TFV exposures following administration of 10 mg TAF in Group A were comparable to those observed following administration of TAF 25 mg in Group C. Plasma TFV was slightly higher when TAF 10 mg was administered with a P-gp inhibitor (COBI) but the mean (%CV) TFV AUC and Cmax were > 90% lower than observed following administration of TDF within Stribild in GS-US-236-0110.

Table 11. 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)

Influence of food

GS-US-320-1382 – TAF food effect study

Study title: A Phase 1, Randomized, Open-Label Study to Determine the Effect of Food on the Pharmacokinetics of Tenofovir Alafenamide (TAF) in Healthy Volunteers

The study compared administration of TAF 25 mg in the fed (800 kcal and 50% fat) and fasted states.

TFV was not measured in this study. The applicant points out that the food effect on plasma TAF observed on administration of TAF 25 mg tablets (AUC 65%–68% higher vs. fasted) was consistent with the findings of the F/TAF study GS-US-311-1386 (see below), in which F/TAF (200/25 mg) was given with a similar meal type (increased TAF AUC 75% to 77%). As noted in prior dossiers, the finding contrasts with the small food effect observed when TAF 10 mg was given with COBI (see GS-US-292-0110 below), indicating that food has little effect when TAF is given with a potent P-gp inhibitor.

Table 12. GS-US-320-1382: Summary Statistics of TAF Plasma Pharmacokinetic Parameters for a Single Dose of TAF (Analysis Set: TAF PK)

TAF PK Parameter[a]	Treatment A, TAF 25 mg Fasted (N = 39)[b]	Treatment B, TAF 25 mg Fed (N = 40)
AUC_{inf} (ng•h/mL)	171.5 (33.6)	288.9 (39.2)
AUC_{last} (ng•h/mL)	170.1 (34.0)	282.7 (40.0)
C_{max} (ng/mL)	266.3 (46.9)	252.6 (46.4)
$t_{1/2}$ (h)	0.35 (0.30, 0.42)	0.45 (0.40, 0.59)
T_{max} (h)	0.50 (0.25, 0.50)	1.00 (0.50, 1.50)

a Data are mean (%CV), except t1/2 and Tmax that are reported as median (Q1, Q3).

b Subject 9191-1009 (BA sequence) withdrew consent and missed the Day 8 study drug dose, therefore did not have TAF PK concentrations with Treatment A.

Table 13. GS-US-320-1382: Statistical Comparison of TAF Pharmacokinetic Parameters for Test Versus Reference Treatments (Analysis Set: TAF PK)

	GLSM			
TAF PK Parameter	Test Treatment B, TAF 25 mg Fed (N = 40)	Reference Treatment A, TAF 25 mg Fasted (N = 39)	% GLSM Ratio (Test/Reference)	90% CI
AUC_{inf} (ng•h/mL)	268.74	160.42	167.53	(153.88, 182.38)
AUC_{last} (ng•h/mL)	262.59	158.88	165.28	(150.94, 180.97)
C_{max} (ng/mL)	225.93	239.58	94.30	(78.49, 113.30)

GLSM = geometric least-squares mean

The range of TAF exposures after dosing with the 25 mg tablet was consistent with the plasma exposures observed in the HBV monotherapy study (GS-US-320-0101; see section 2.2) in which 8 mg, 25 mg, 40 mg and 120 mg TAF doses were administered under fasted conditions. Based on the flat antiviral effect in the monotherapy study the applicant considers that the effect of food is not clinically relevant.

Study GS-US-292-110

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

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

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

Administration under fed conditions resulted in a lower TAF Cmax and delayed Tmax with a magnitude of effect that was similar between meal types. The GLSM ratios and 90% CI for TAF AUClast after each meal type exceeded 100% but the upper 90% boundaries were 124% and 126%.

Table 14. 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.

The TFV AUCinf GLSM ratios and 90% CI when E/C/F/TAF was administered after food also all exceeded 100% but fell within 80, 125%. As for TAF there was a decrease in Cmax (~15%) with a corresponding delay in median Tmax (from 0.75 to 1.50 h). These modest changes in TAF and TFV PK parameters upon E/C/F/TAF administration with food were not considered clinically relevant.

Table 15. Study GS-US-292-0110: Statistical Comparison of Selected TFV Pharmacokinetic Parameters (PK Analysis Set)

Treatment Condition (N = 42)	TFV PK Parameter		
	AUC_{inf} (ng•h/mL)	AUC_{last} (ng•h/mL)	C_{max} (ng/mL)
Test Treatment: Light/LF Meal GLSM	316.09	177.76	12.06
Test Treatment: HC/HF Meal GLSM	313.69	177.76	12.01
Reference Treatment: Fasted GLSM	278.40	175.33	14.34
Light/LF Meal vs. Fasted GLSM ratio (90% CI), %	113.54 (108.23, 119.11)	101.38 (97.77, 105.13)	84.11 (75.71, 93.45)
HC/HF Meal vs. Fasted GLSM ratio (90% CI), %	112.68 (107.40, 118.21)	101.39 (97.77, 105.13)	83.76 (75.39, 93.05)
HC/HF Meal vs. Light/LF Meal GLSM ratio (90% CI), %	99.24 (94.60, 104.11)	100.00 (96.44, 103.70)	99.58 (89.63, 110.63)

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.

Study 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 evaluated the effect of food on F/TAF 25 mg 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 Cmax and delayed Tmax (by 30 min). In contrast the GLSM ratios for TAF AUCs were ~175% (90% CI ~165, 188) for the fed vs. fasted state. TFV was not measured in this study.

Table 16. 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.

Distribution

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 levels at this time point that were close to the LLOQ.

In several human *ex vivo* studies the estimated unbound fraction of TAF was ~20%, i.e. lower than reported *in vitro*. In subjects with severe hepatic impairment (GS-US-320-1615), the mean percentage of unbound TAF ranged from 35% to 40% vs. 19-22% in the controls with normal hepatic function.

Based on the POPPK analysis provided in this dossier that takes into account PK data from patients who received F/TAF for HIV and TAF alone for HBV:

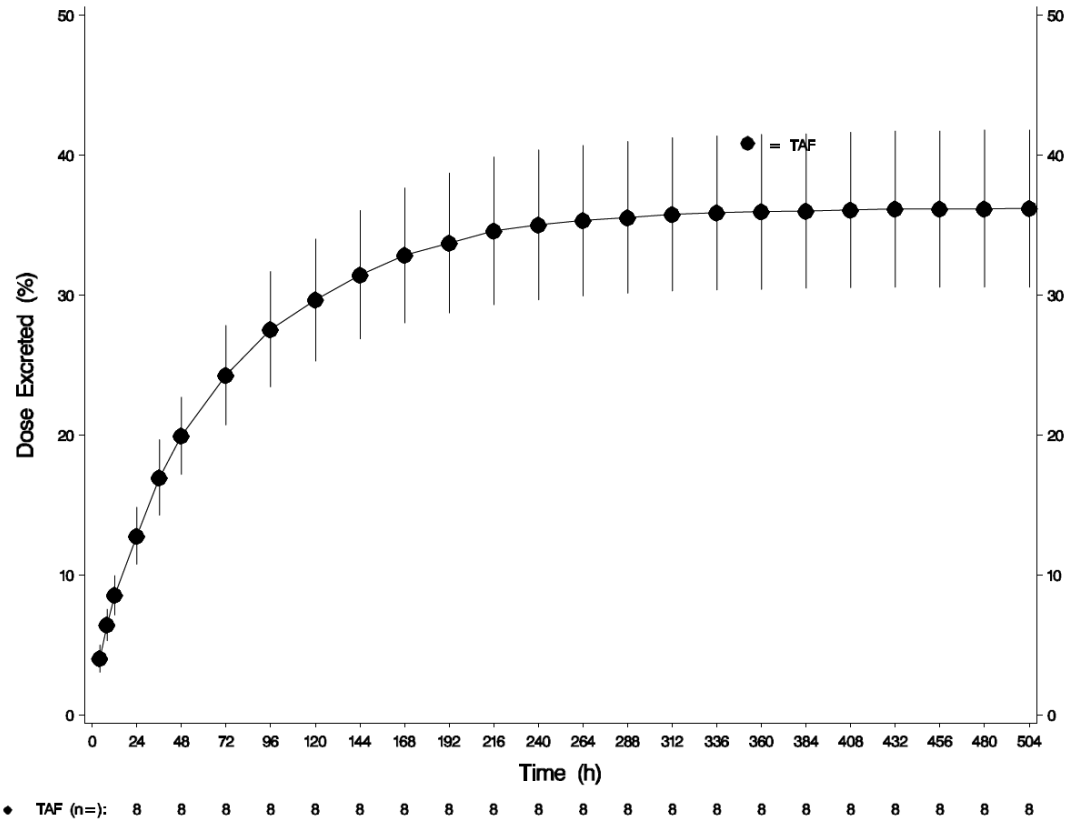
- TAF - the apparent volume of the central compartment (V_c/F) was 88.9 L, and the apparent volume of peripheral compartment (V_p/F) was 8.28 L.
- TFV - the V_c/F was 3810 L and the V_p/F was 2690 L.

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

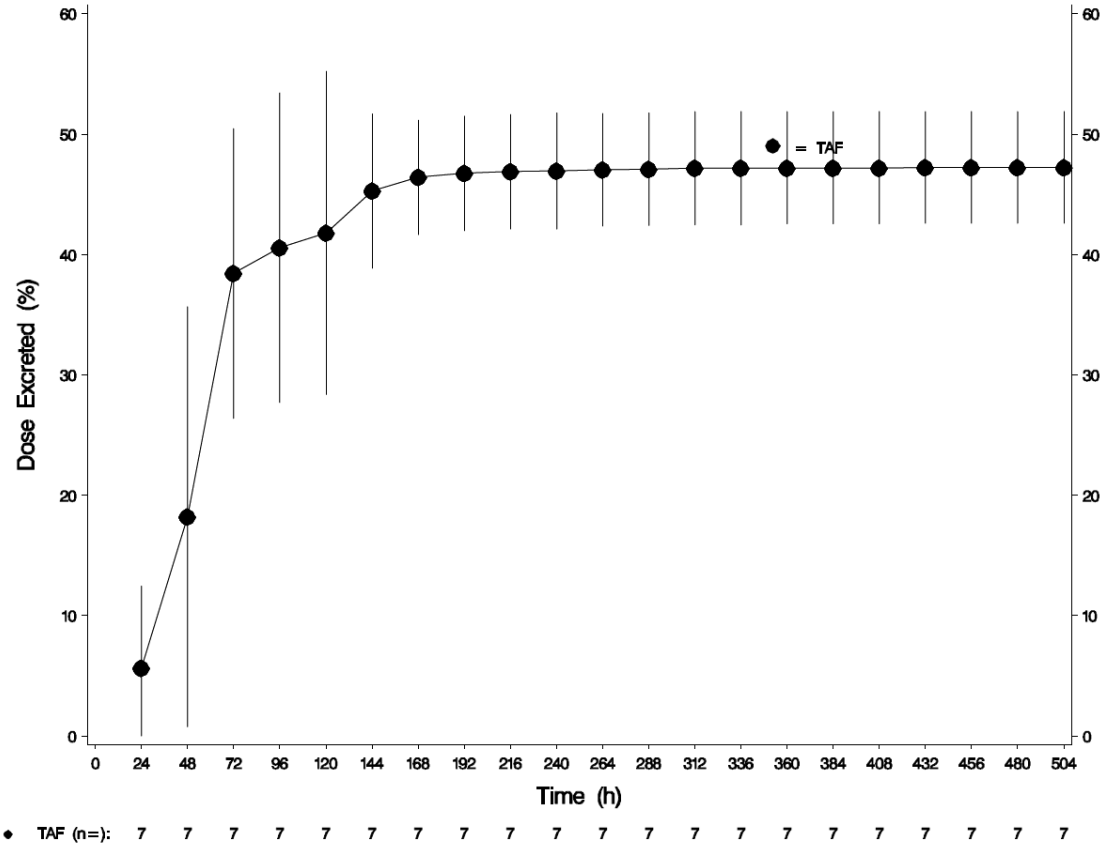


a Values below the lower limit of quantitation (BLQ) were treated as 0 for summary statistics and missing for log-normalized data.

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



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 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 17. 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)

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 18. 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)

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 19. 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

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 20. 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)

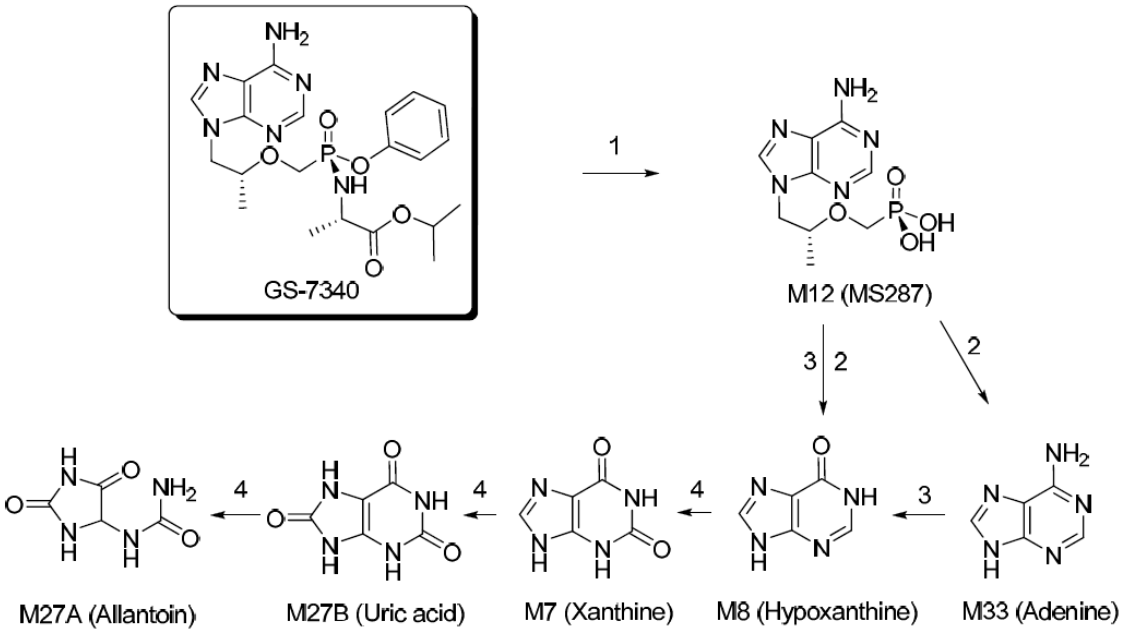
For pooled faeces a mean of 31.7% (± 10.5%) of the radioactive dose was quantified, within which the predominant species was TFV (M12), accounting for 31.4% (± 10.4%). Two unidentified metabolites appeared in trace amounts.

Table 21. 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)

The applicant's proposed biotransformation pathway of TAF is shown below (note that M12 = TFV).

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



1 Hydrolysis
2 Dealkylation
3 Deamination
4 Oxidation

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

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

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

In hepatocytes the initial step of TAF metabolism is hypothesised to be driven by a combination of CatA and CES1. This differs from the hypothesis that CatA alone effects the conversion in PBMCs.

CES1 is highly expressed in liver. Because CES1 is an enzyme with high capacity and low affinity, it is not readily inhibited by other xenobiotics. To date, there are no clinical reports of modulation of PK profiles of CES1 substrates by xenobiotics that inhibit CES1. In addition, since the in-vitro study AD-120-2031 indicated that CatA also contributes to TAF activation in hepatocytes, inhibitors of CES1 are unlikely to negatively affect the antiviral activity of TAF.

Several CES1 genetic variants that are associated with the enzyme activity have been identified at very low frequency [G143E (heterozygous: 2%-4% and homozygous: 0.05%) and D260fs (very rare)]. The G143E polymorphism modestly affected activation of oseltamivir by 18% in humans. Again, since TAF can be hydrolysed by both CES1 and CatA in liver, significant modulation of TAF PK profiles by inhibition or genetic polymorphisms of CES1 is unlikely.

Genetic polymorphisms in CatA have been described, some of which can result in depressed enzymic activity. The potential for an effect of human polymorphisms on TAF efficacy was addressed during the review of prior dossiers. The most detailed information was provided during the review of F/R/TAF.

Small interfering ribonucleic acid (siRNA)-mediated knock-down of CatA and fibroblasts isolated from patients with a genetic disorder resulting from a lack of CatA activity (galacosialidosis) had reduced intracellular accumulation of TAF metabolites in vitro. Genetic polymorphisms in CatA (CTSA gene) have been found in patients with galactosialidosis. Galactosialidosis is considered extremely rare but the exact prevalence is unknown. While greater than 100 cases have been reported, only 23 mutations have been identified as of 2013. No patients with galactosialidosis have knowingly been treated with TAF for HIV or HBV. Since conversion occurs via two routes in hepatocytes the applicant proposes that genetic polymorphisms leading to reduced CatA activity and inhibition of CatA by telaprevir or boceprevir may not effect conversion of TAF to TFV-DP in hepatocytes that express CatA and CES1.

Dose proportionality and time dependencies

Dose proportionality

In studies in which a range of TAF doses were used, including the monotherapy studies and the TQT study, 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 plasma TAF exposure (AUC) varied across studies depending on whether intensive or sparse sampling was employed (%CV: 32% to 48% across Phase 1 studies and 58% to 71% across the Phase 3 HBV studies GS-US-320-0108 and GS-US-320-0110).

Similarly, the inter-subject variability in TFV exposure (AUC) varied across studies depending on whether intensive or sparse sampling was employed (%CV: 20% to 65% across Phase 1 studies and 29% to 36% across the Phase 3 HBV studies GS-US-320-0108 and GS-US-320-0110).

Pharmacokinetics in target population

POPPK analyses were initially performed for TAF and TFV using PK data collected from E/C/F/TAF studies. A new POPPK analysis (November 2015) was included in the current application that includes PK data from the Phase 1 and Phase 3 studies in HBV-infected patients. Therefore this new analysis is the most relevant.

Population PK modeling was conducted to establish predictive model(s) to describe the plasma concentrations of TAF and TFV in healthy subjects and in patients with CHB or HIV (N = 1462). PK data used came from the following studies:

Study	Phase	Population	Sampling (Intensive/Sparse)
GS-US-120-0107	1	Healthy	Intensive
GS-US-120-0108	1	Healthy	Intensive
GS-US-120-0109	1	Healthy	Intensive
GS-US-120-0117	1	Healthy	Intensive
GS-US-120-0118	1	Healthy	Intensive
GS-US-292-0101	1	Healthy	Intensive
GS-US-320-1228	1	Healthy	Intensive
GS-US-120-0104	1	HIV-infected	Intensive, sparse
GS-US-311-1089	3	HIV-infected	Sparse
GS-US-320-0101	1b	Subjects with CHB	Intensive, sparse
GS-US-320-0108	3	Subjects with CHB	Intensive[a], sparse
GS-US-320-0110	3	Subjects with CHB	Intensive[a], sparse

a Intensive PK sampling was conducted in PK substudies.

Data from individuals who received TAF or F/TAF were combined since TAF exposures are comparable for TAF 25 mg and F/TAF 200/25 mg. The model was used to identify covariates (e.g. food effects, body weight, age, sex, race, renal and liver function, effects of co-administered ARVs and population [healthy vs. CHB or HIV]) influencing plasma concentrations. A mixed-effect modeling approach using NONMEM Version 7.3 software was applied.

TAF - The final population model that best described TAF PK was a 2-compartment model with sequential zero- and first-order absorption. The typical apparent systemic TAF CL/F was estimated to be 149 L/h. TAF was absorbed at a typical rate of 6.24 h^{-1} with no lag time. The covariates retained in the final model were dosing conditions (fed vs. fasted), effect of co-administration of ATV/r and LPV/r, disease status and sex. ATV/r and LPV/r increased TAV relative bioavailability by 82% and 39%, respectively. Relative bioavailability was 39% higher in females and apparent central volume was 34% lower in patients with HIV. Fasting increased the absorption rate by 65% and decreased bioavailability by 17%. Administration of food immediately before the dose and co-administration with ATV/r decreased the absorption rate by 45% and 31%, respectively. The final population PK model characterised TAF PK well and no covariates were considered to have a clinically meaningful effect on TAF exposure.

TFV - The final population model that best described TFV PK was a 2-compartment model with sequential zero- and first-order absorption. The typical apparent systemic TFV CL/F was estimated to be 75.6 L/h. TFV was absorbed at a typical rate of 3.58 h^{-1} with no lag time. The covariates retained in the final model were dosing conditions (fed vs. fasted), effect of co-administration of ATV/r, LPV/r and DRV/r, estimated $eGFR_{CG}$, disease status (healthy vs. treatment-experienced patients with CHB vs. others), sex and black race. The main covariate that influenced the PK of TFV was creatinine clearance.

For those with CrCL 50 mL/min, the apparent clearance (CLM/F), apparent central volume (VcM/F) and apparent peripheral volume (VpM/F) were respectively 30%, 19%, and 19% lower than for reference individuals with CrCL=90 mL/min. In those with CrCL of 150 L/min, the respective values were 36%, 20% and 20% higher vs. reference individuals. The relative bioavailability was 14% higher in females.

Apparent clearance was 11% higher in healthy volunteers. VcM/F was 22% lower in Blacks, 39% lower in healthy volunteers and 25% higher in treatment-experienced patients with HBV.

Administration of food immediately before the dose and co-administration with ATV/r or DRV/r decreased the absorption rate by 55%, 57% and 41%, respectively. Differences in TFV exposure between individuals due to statistically significant covariates were less than 32% relative to the typical reference individual. These differences were not considered clinically meaningful. It was concluded that the final population PK model characterized TFV PK well and no covariates were considered to have a clinically meaningful effect on TFV exposure.

The results of the integrated population PK modelling of TAF following administration of TAF or F/TAF were consistent with the PK of TAF following dosing as part of E/C/F/TAF. Based on POPPK analyses, disease status was retained as a covariate in the final model but was not considered clinically relevant because the differences in TAF and TFV exposures between individuals due to covariates were less than 40%. Accordingly, the range of TAF and TFV exposures observed in healthy subjects was comparable to that in patients with CHB. These data were also consistent with the established PK of TAF in HIV-infected subjects (mean [%CV] TAF and TFV AUC_{tau} of 206 [71.8] and 293 [27.4] ng•h/mL, respectively).

The range of TFV exposures was below exposures observed in historical studies after administration of TDF 300 mg in patients with CHB or HIV and in those with normal renal function (3300 ng•h/mL).

Table 22. POPPK Analysis: Steady State PK Parameter Estimates for TAF by Population

	TAF [mean (%CV)]	
TAF PK Parameter	Healthy Subjects (N = 144)[a]	Patients with CHB (N = 698)[b]
AUC_{tau} (ng•h/mL)	204.5 (35.7)	215.5 (66.6)
C_{max} (ng/mL)	133.1 (50.4)	177.6 (53.4)

Table 23. POPPK Analysis: PK Parameter Estimates for TFV Following Once Daily Dosing of TAF by Population

	TFV [mean (%CV)]	
TFV PK Parameter	Healthy Subjects (N = 144)[a]	Patients with CHB (N = 856)[b]
AUC_{tau} (ng•h/mL)	267.3 (23.4)	321.9 (31.5)
C_{max} (ng/mL)	15.9 (25.4)	17.2 (35.2)
C_{min} (ng/mL)	8.7 (24.8)	11.4 (33.0)

a Subjects from Studies GS US 120 0107, GS US 120 0108, GS US 120 0109, GS US 120 0117, GS US 120 0118, GS US 320 1228, and GS US 292 0101

b Subjects from Studies GS US 320-0108 and GS US 320 0110

Special populations

Impaired renal function

GS-US-120-0108 – Study Title: A Phase 1, Open-Label, Parallel-Design Study to Evaluate the Pharmacokinetics of GS-7340 in Subjects with Severe Renal Impairment

A single 25 mg dose of TAF was administered after a standard meal to 14 subjects with severe renal impairment (15 ≤ CrCL ≤ 29 mL/min) and 13 matched controls. There were no differences in the protein binding fractions of TAF and TFV between those with severe renal impairment and those with normal renal function.

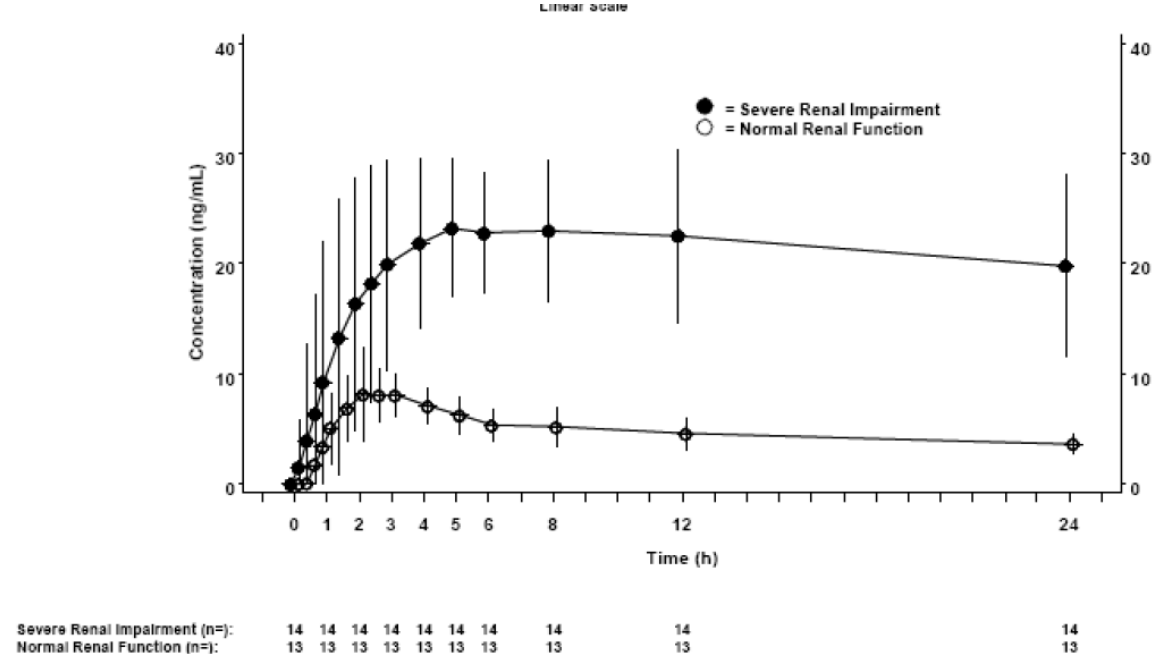
TAF - In severe renal impairment there was a 92% (< 2-fold) higher mean plasma AUCinf, 92% higher AUClast and 79% higher Cmax. Correspondingly the mean plasma TAF CL/F was significantly lower (p = 0.003) but the half-life was not statistically significantly different. At 1 and 4 h the mean percent unbound TAF was not different between those with severe renal impairment (20.0% and 14.2%) vs. controls (20.1% and 13.6%). Approximately 0.47% of the dose was excreted in urine in renally impaired subjects vs. ~2% in controls with renal clearance of 4.2 mL/min and 35.8 mL/min, respectively.

Table 24. PK TAF (25 mg SD) in Severe Renal Impairment or Normal Renal Function

Mean (%CV)	Severe Renal Impairment (n = 14)	Normal Renal Function (n = 13)
TAF		
AUC_{inf} (ng•h/mL)	513.2 (47.3)	267.3 (49.2)
AUC_{last} (ng•h/mL)	510.6 (47.4)	265.9 (49.5)
C_{max} (ng/mL)	363.7 (65.7)	198.8 (62.1)
$t_{1/2}$ (h)	0.75 (51.8)	0.53 (22.8)
CL/F (mL/h)	61,717.8 (56.8)	117,633.1 (53.9)
CL_r (mL/min)	4.2 (77.6)	35.8 (51.7)
Percent of Dose Recovered in Urine (%)	0.47 (95.6)	2.00 (34.6)
A_e (ng)	117,230.4 (95.6)	500,408.6 (34.6)

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.

Figure 7. GS-US-120-0108: Mean (SD) Plasma Concentration-Time Profiles of TFV



Note: Values below the lower limit of quantification (BLQ) were treated as 0 for summary statistics and missing for log normalized data.

Table 25. 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)

a Study GS-US-216-0114

b Br J Clin Pharmacol 2007;64 (5):655-61.

c mean (90% CI); HIV Med 2010;11 (3):193-9.

d Studies 00-909 and 01-943

e Study GS-US-104-236

f 6th International Workshop on Clinical Pharmacology of HIV Therapy; 2005 April 28-30; Québec City, Canada. Poster No. 2.11.

g Studies GS-US-236-0101 and GS-US-236-0110

h Studies GS-US-236-0120 and GS-US-334-0131

ESRD / HD Modelling Report

The 2015 POPPK model was modified to predict the TFV plasma exposures in HBV-infected patients with ESRD undergoing haemodialysis. The dataset for the established model had TAF doses that ranged from 8 mg to 125 mg. Data from 12 studies were evaluated in the original model. The following modifications were incorporated:

1) Haemodialysis clearance (CLDI) was incorporated for duration of 4 h on alternate days of the week (thrice weekly) with TAF 25 mg administration following completion of haemodialysis.

2) or All instances of inter-occasion variability were not considered as the simulations were not study phase specific.

3) The residual error (epsilons) was fixed to zero to reduce noise and to generate smoother curves.

The dataset for simulation was generated using random sampling with replacement using the individual as sampling unit from the subset of data evaluated in the original model from the Phase 3 studies in HBV patients (n = 907). All individuals were assigned a fixed CRCL value of 5 mL/min to represent ESRD. The typical value for CLDI was fixed at 9.302 L/hr and inter-individual variability for CLDI was fixed at 0.0564.

These observed values for known TFV CLDI with a representative method and haemodialysis technique were obtained from a previous study (GS-01-919) in which ESRD subjects undergoing haemodialysis received TDF 300 mg.

Simulations are shown below for duration of 2 weeks to ensure that the PK profiles are at steady state in presence of haemodialysis. The plasma PK exposure values for Cmax, Cmin and AUC are reported for the time interval of 312-336 hours where the maximum daily exposures are observed.

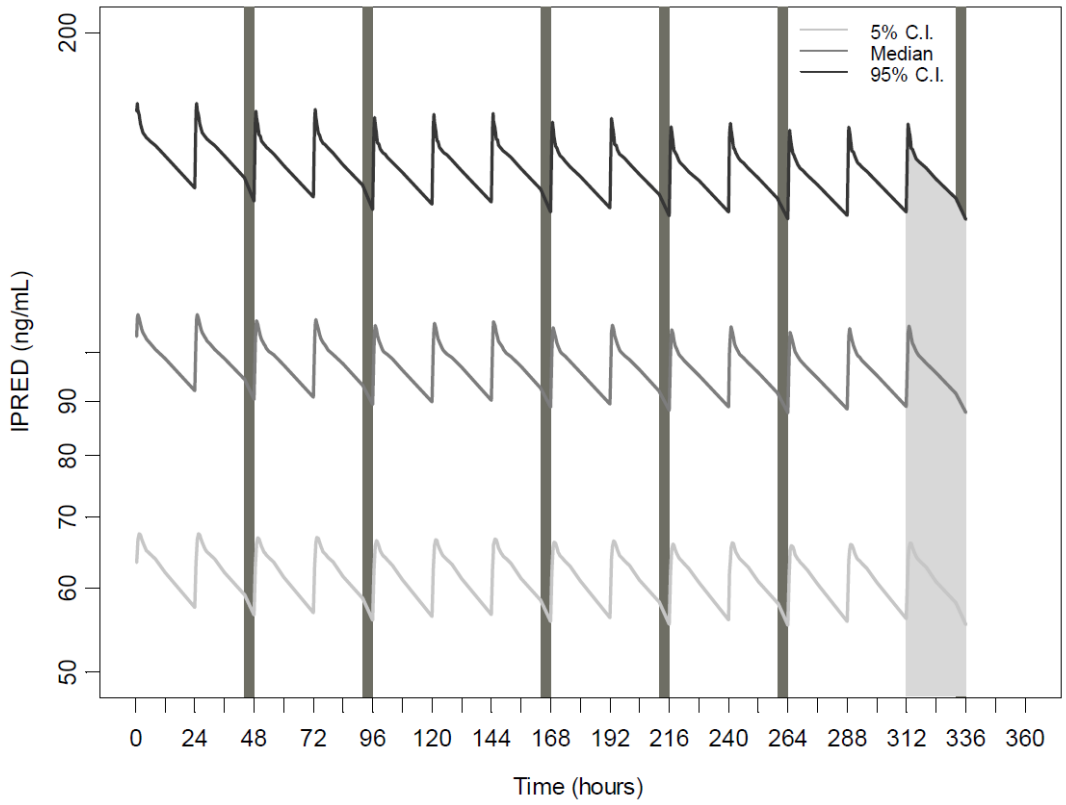
It was concluded that the modified version of the POPPK model predicted the steady state TFV exposures in HBV-infected individuals with ESRD undergoing haemodialysis. Using the modelling the applicant concluded that no dose adjustment of TAF is required in patients with renal impairment and that TAF should be administered after completion of haemodialysis on the dialysis days. This is reflected in the SmPC.

Table 26. Predicted TFV PK Following Administration of TAF in ESRD Subjects Undergoing Haemodialysis

TFV PK Parameter	Mean	Median	CV%	Range	Percentiles (5-95)
$C_{max,ss}$ (ng/mL)	110	107	27.9	46.7 – 234	66.9 – 167
$C_{min,ss}$ (ng/mL)	90.0	87.8	27.1	39.7 – 188	55.4 – 133
$AUC_{0-\tau}$ (ng•hr/mL)	2360	2300	26.7	1040 – 4810	1460 – 3510

The PK parameters are reported for 312-336 hours for maximum TFV exposures.
CV% = percent coefficient of variation

Figure 8. Model Predicted Steady State TFV PK Profile Following TAF 25 mg Daily Dosing for ESRD Subjects (CRCL: 5 mL/min) Undergoing Haemodialysis



IPRED = Individual predictions
The shaded region in "dark gray" represents hemodialysis duration and interval. Hemodialysis on the first week (0-156 hours) is represented during 44-48 hours and 92-96 hours; on the second week (156-324 hours) is represented during 164-168 hours, 212-216 hours, and 260-264 hours; and on the third week (324-336 hours) is represented during 332-336 hours.
The shaded region in "light gray" represents steady-state, maximum TFV exposure that was used in calculation of PK parameters such as Cmax, Cmin, and AUC.

The POPPK analyses of pooled data from Phase 3 studies in CHB patients showed that baseline eGFRCG was not a statistically significant or clinically relevant covariate influencing TAF PK. Note that in the Phase 3 studies all patients were to have eGFRCG > 50 mL/min at screening and only 4 patients with values < 60 mL/min provided PK data.

Table 27. POPPK Analysis: PK TAF Following Once Daily Dosing of TAF in CHB patients by Baseline Renal Function

	TAF		
TAF PK Parameter	**30 ≤ $eGFR_{CG}$ < 60 mL/min (N = 4)**	**60 ≤ $eGFR_{CG}$ < 90 mL/min (N = 162)**	**$eGFR_{CG}$ ≥ 90 mL/min (N = 532)**
AUC_{tau} (ng•h/mL)	266.4 (58.8)	234.0 (64.3)	209.5 (67.4)
C_{max} (ng/mL)	260.8 (63.9)	184.8 (52.6)	174.9 (53.4)

Data are presented as mean (%CV).

Table 28. Population PK Analysis: PK TFV Following Once Daily Dosing of TAF in CHB by Baseline Renal Function

	TAF		
TFV PK Parameter	**30 ≤ eGFRCG < 60 mL/min (N = 5)a**	**60 ≤ eGFRCG < 90 mL/min (N = 194)**	**eGFRCG ≥ 90 mL/min (N = 657)**
AUCtau (ng•h/mL)	515.8 (16.3)	387.1 (28.9)	301.1 (29.2)
Cmax (ng/mL)	27.5 (21.5)	20.2 (30.9)	16.2 (34.7)
Cmin (ng/mL)	18.6 (15.0)	13.9 (30.1)	10.6 (30.4)

Data are presented as mean (%CV).

Impaired hepatic function

GS-US-120-0114 – Study title: A Phase 1, Open-Label, Parallel-Group, Single-Dose Study to Evaluate the Pharmacokinetics of Tenofovir Alafenamide in Subjects with Normal and Impaired Hepatic Function TAF in mild or moderate hepatic impairment

In this open-label study single doses of 25 mg TAF were administered immediately after completion of a moderate-fat meal (600 calories, 27% fat). Study groups were:

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. The lower exposures vs. controls were not considered to be clinically relevant.

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

GLSMs were obtained using a mixed-effects model.

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.

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

GLSMs were obtained using a mixed-effects model.

TAF plasma protein binding at 1 and 4 h post-dose showed a mean unbound TAF range from 16% to 19% in mild hepatic impairment and from 14% to 23% in moderate hepatic impairment. TFV plasma protein binding at 2 and 24 h post-dose showed a mean unbound TFV of > 99% in mild or moderate hepatic impairment. For TAF and TFV binding was similar to controls.

GS-US-320-1615- Study title: A Phase 1, Open-Label, Parallel-Group, Single Dose Study to Evaluate the Pharmacokinetics of Tenofovir Alafenamide (TAF) in Subjects with Normal Hepatic Function and Subjects with Severe Hepatic Impairment

TAF in severe hepatic impairment

The study evaluated a single 25 mg dose of TAF subjects with normal hepatic function or severe hepatic impairment (CPT C: score 10-15). The normal controls were matched with a CP-C subject

based on age, sex and BMI. TAF 25 mg was given after a meal (~600 calories, 25% to 30% fat). The mean age was 56 years (range: 41 to 69 years), mean BMI was 27.9 (4.05) kg/m2 and mean CrCL was 122 mL/min. The plasma exposure to TAF was lower in subjects with severe hepatic impairment.

Table 29. GS-US-320-1615: Summary of TAF PK Parameters in Subjects with Severe Hepatic Impairment or Normal Hepatic Function (TAF PK Analysis Set)

TAF PK Parameter	Severe Hepatic Impairment Group (N = 10)	Normal Matched Control Group (N = 10)
AUC_{inf} (ng•h/mL)	120.6 (28.2)	228.2 (37.4)
AUC_{last} (ng•h/mL)	113.1 (27.3)	225.7 (37.7)
C_{max} (ng/mL)	79.6 (49.4)	176.0 (45.3)
T_{max} (h)	1.50 (1.00, 2.00)	1.00 (0.75, 1.50)
$t_{1/2}$ (h)	0.64 (0.38, 0.98)	0.47 (0.40, 0.52)
V_z/F (mL)	220,054.6 (62.7)	79,922.3 (22.0)
CL/F (mL/h)	220,844.9 (25.5)	121,883.7 (34.2)

Table 30. GS-US-320-1615: Statistical Comparisons of TAF PK Parameter Estimates Between Subjects with Severe Hepatic Impairment and Normal Hepatic Function (TAF PK Analysis Set)

	GLSMs		
PK Parameter	Reference Group: Matched Normal Hepatic Function Group (N = 10)	Test Group: Severe Hepatic Impairment Group (N = 10)	GLSM Ratio (%) (90% CI)
AUC_{inf} (ng•h/mL)	216.00	116.73	54.04 (41.98, 69.56)
AUC_{last} (ng•h/mL)	213.38	109.25	51.20 (40.11, 65.36)
C_{max} (ng/mL)	160.15	72.23	45.10 (31.66, 64.25)

Similarly, plasma exposures to TFV were lower in the severe hepatic impairment group.

Table 31. GS-US-320-1615: Summary of TFV PK Parameters in Subjects with Severe Hepatic Impairment or Normal Hepatic Function (TFV PK Analysis Set)

TFV PK Parameter	Severe Hepatic Impairment Group (N = 10)	Normal Matched Control Group (N = 10)
AUC_{inf} (ng•h/mL)	219.9 (54.0)	304.0 (23.8)
AUC_{last} (ng•h/mL)	184.2 (54.2)	256.7 (23.3)
C_{max} (ng/mL)	7.5 (52.4)	7.6 (24.0)
T_{max} (h)	3.00 (2.00, 4.00)	3.00 (2.00, 3.00)
$t_{1/2}$ (h)	49.59 (38.14, 63.87)	50.42 (45.31, 65.28)
V_z/F (mL)	6078637.8 (42.8)	4225647.6 (26.7)
CL/F (mL/h)	97231.1 (63.9)	52441.8 (25.3)

Table 32. GS-US-320-1615: Statistical Comparisons of TFV PK Parameter Estimates Between Subjects with Severe Hepatic Impairment and Normal Hepatic Function (TFV PK Analysis Set)

PK Parameter	GLSMs		GLSM Ratio (%) (90% CI)
	Reference Group: Matched Normal Hepatic Function Group (N = 10)	Test Group: Severe Hepatic Impairment Group (N = 10)	
AUC_{inf} (ng•h/mL)	296.01	186.67	63.06 (42.90, 92.70)
AUC_{last} (ng•h/mL)	250.59	155.46	62.04 (41.92, 91.82)
C_{max} (ng/mL)	7.42	6.66	89.88 (64.77, 124.72)

The mean percent unbound TAF was ~2-fold higher in the severe hepatic impairment subjects compared with control subjects (37.8% vs. 20.4%), such that the resulting comparison of free TAF gave similar plasma exposures between the two groups.

Table 33. GS-US-320-1615: Summary Statistics and Statistical Comparisons of Free TAF PK Parameter Estimates Between Subjects with Severe Hepatic Impairment and Normal Hepatic Function (TAF PK Analysis Set)

Free TAF PK Parameter Mean (%CV)	Severe Hepatic Impairment Group (N = 10)	Normal Matched Control Group (N = 10)	GLSM Ratio (%) (90% CI)
Free AUC_{inf} (ng•h/mL)	42.8 (27.5)	46.5 (38.3)	94.42 (72.48, 122.99)
Free AUC_{last} (ng•h/mL)	41.7 (26.8)	46.0 (38.6)	93.28 (72.62, 119.80)
Free C_{max} (ng/mL)	29.9 (58.0)	36.2 (50.8)	82.16 (56.58, 119.31)

In contrast, the percent unbound TFV was high in all subjects (> 95%) regardless of hepatic status.

Exploratory analyses indicated no clinically relevant correlations between TAF and TFV exposures (total concentrations) vs. baseline CPT score. In severe hepatic impairment the lower albumin, higher total bilirubin, prolonged PT and higher INR values observed were correlated with lower total TAF exposures. Similar but less prominent trends were observed with TFV. Figures 11-12 show the plots for total TAF and TFV AUCs.

When the free fraction of TAF was considered, no significant correlation was observed between the free TAF exposures and the individual laboratory components of the CPT score.

Figure 9. GS-US-320-1615: Scatter Plot Between TAF AUClast and Baseline CPT Score (TAF PK Analysis Set)

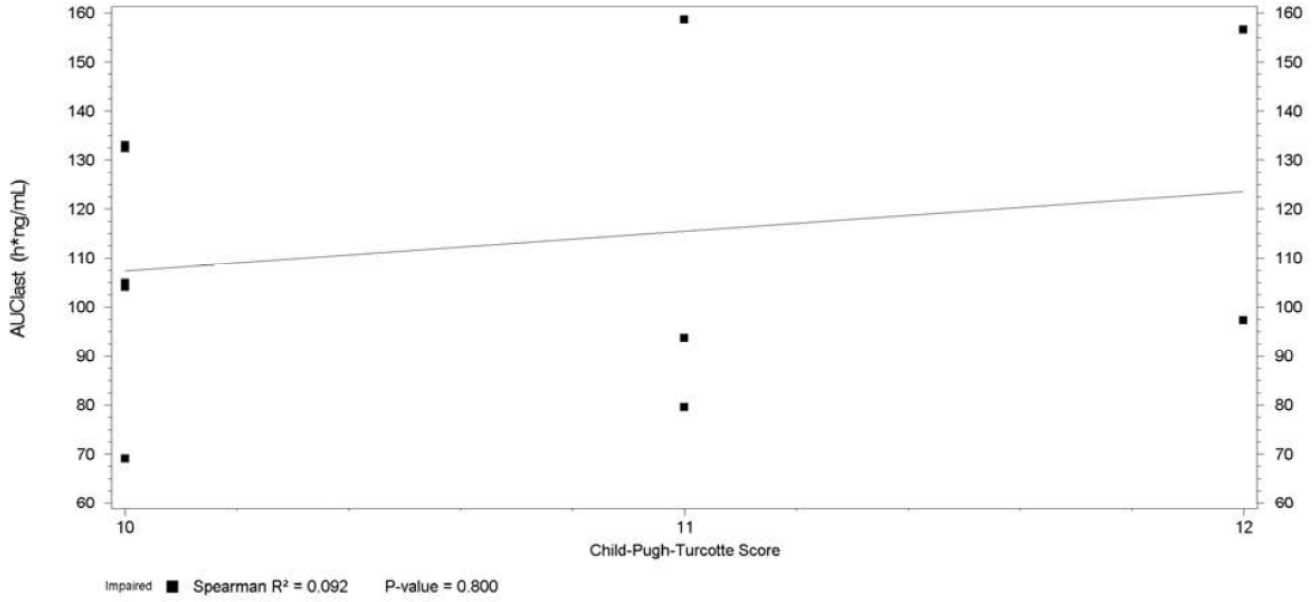
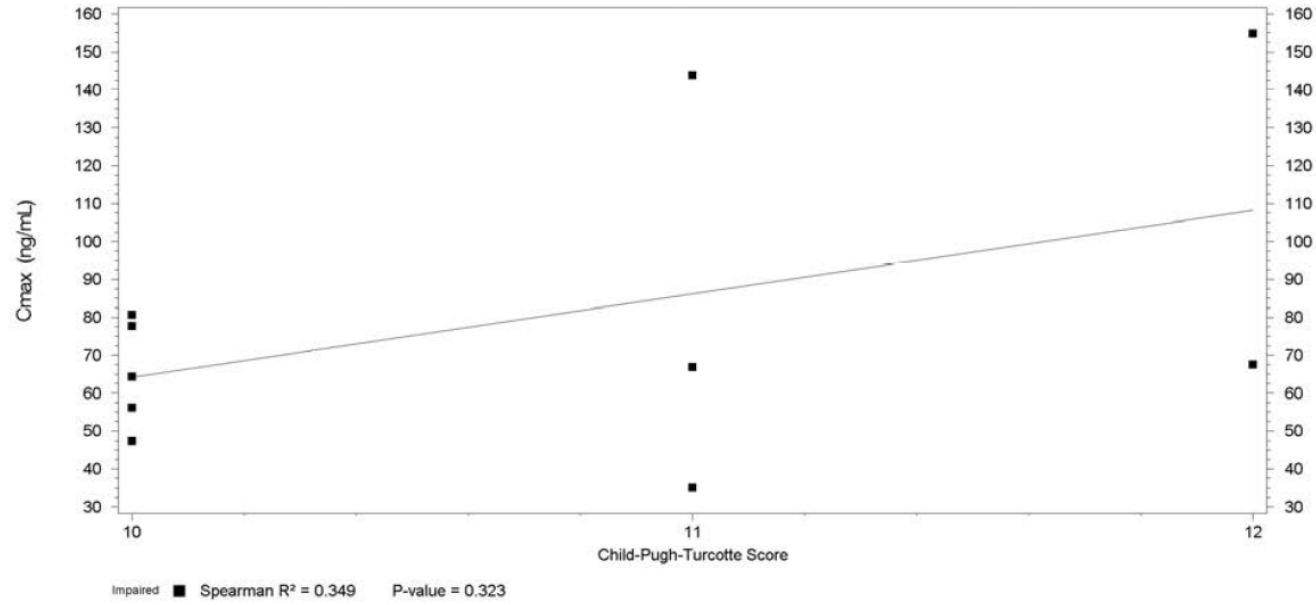


Figure 10. GS-US-320-1615: Scatter Plot Between TAF Cmax and Baseline CPT Score (TAF PK Analysis Set)



The applicant concluded that since free TAF is associated with therapeutic effect, no change in TAF efficacy is expected in patients with severe hepatic impairment.

Additional POPPK analyses assessed the relationship between TAF and TFV PK and baseline FibroTest score and concluded that the score was not a covariate for TAF or TFV PK.

Table 34. POPPK Analysis: PK TAF and TFV following once-daily dosing of TAF in CHB by baseline FibroTest score[a]

	FibroTest score[a] < 0.75	FibroTest score[a] ≥ 0.75
TAF PK Parameter	N = 624	N = 62
AUC_{tau} (ng•h/mL)	218.4 (68.5)	184.6 (36.8)
C_{max} (ng/mL)	179.6 (53.5)	152.1 (44.4)
TFV PK Parameter	N = 763	N = 73
AUC_{tau} (ng•h/mL)	321.0 (30.5)	323.4 (41.2)
C_{max} (ng/mL)	17.1 (32.9)	17.3 (54.2)
C_{min} (ng/mL)	11.4 (31.9)	11.6 (43.1)

a A FibroTest score ≥ 0.75 is considered indicative of a Metavir stage F4, which is considered cirrhosis on liver biopsy.

Elderly

The updated POPPK analyses for TAF and TFV did not show that age per se was a significant covariate in patients with CHB, noting that in the dataset the median age was 41 (18 to 78) years, and 195 subjects were ≥ 50 years of age. As age is often correlated with renal function the effects noted above are relevant.

Table 35. POPPK Analysis: PK TAF following once-daily dosing of TAF in subjects with CHB by age group

	TAF	
Age Group	**AUC_{tau} (ng•h/mL)**	**C_{max} (ng/mL)**
18 to < 50 Years of Age (N = 537)	212.6 (68.6)	177.1 (53.0)
≥ 50 Years of Age (N = 161)	225.5 (60.5)	179.4 (55.0)

Table 36. POPPK Analysis: PK TFV following once-daily dosing of TAF in subjects with CHB by age group

	TAF		
Age Group	**AUC_{tau} (ng•h/mL)**	**C_{max} (ng/mL)**	**C_{min} (ng/mL)**
18 to < 50 Years of Age (N = 661)	308.2 (29.9)	16.5 (35.0)	10.9 (31.1)
≥ 50 Years of Age (N = 195)	368.3 (31.9)	19.5 (33.0)	13.2 (33.6)

Data are presented as mean (%CV).

Paediatric population - Adolescents

This application was confined to adults. However, in the absence of specific data for single-agent TAF in regards to tolerability and antiviral activity, data from TAF fixed-dose combinations (FDCs) for the treatment of HIV infection can be used to support approval of TAF single-agent for the treatment of CHB in adolescents. The single-agent TAF 25 mg exposure is equivalent to that achieved with the 10 mg dose of TAF included in the elvitegravir/cobicistat/emtricitabine/TAF (E/C/F/TAF; Genvoya) FDC due to inhibition of TAF pharmacokinetics (PK) by cobicistat.

Study GS-US-292-0106, a Phase 2/3, multicenter, open-label study that characterized the PK, safety, tolerability, and antiviral activity of E/C/F/TAF in this population. The most recent data consist of an interim analysis performed when all adolescent subjects (Cohort 1) had either completed their Week 48 study visit or prematurely discontinued study drug.

The pharmacokinetics of tenofovir alafenamide and tenofovir were evaluated in HIV 1 infected, treatment naïve adolescents who received tenofovir alafenamide (10 mg) given with elvitegravir,

cobicistat and emtricitabine as a fixed dose combination tablet (E/C/F/TAF; Genvoya). No clinically relevant differences in tenofovir alafenamide or tenofovir pharmacokinetics were observed between adolescent and adult HIV 1 infected subjects.

These data demonstrate similar exposures between adolescents aged from 12 years and 35 kg and adults. Therefore, as TAF has been shown to be safe and effective in HIV-infected adolescents when given as E/C/F/TAF, it is possible to extrapolate that TAF 25 mg should also be safe and effective in adolescents with CHB. This extrapolation will be further confirmed via results from the planned Study GS-US-320-1092 as part of the agreed TAF PIP.

Weight and BSA

In the POPPK analyses weight and BSA did not have an effect on TAF or TFV exposure and were not statistically significant or clinically relevant covariates but there was a trend to decreasing AUC as weight increased.

Table 37. POPPK Analysis: PK TAF following once-daily dosing of TAF in subjects with CHB by weight range

	Weight bracket			
TAF PK Parameter	**Weight ≤ 57 kg (N = 183)**	**57 < Weight ≤ 67 kg (N = 175)**	**67 < Weight ≤ 75 kg (N = 168)**	**Weight > 75 kg (N = 172)**
AUC_{tau} (ng•h/mL)	262.9 (84.0)	203.0 (58.1)	199.9 (46.7)	193.2 (40.6)
C_{max} (ng/mL)	209.1 (57.7)	162.9 (48.1)	169.9 (53.0)	166.7 (44.8)

Table 38. POPPK Analysis: PK TFV following once-daily dosing of TAF in subjects with CHB by weight range

	Weight bracket			
TFV PK Parameter	**Weight ≤ 57 kg (N = 219)**	**57 < Weight ≤ 67 kg (N = 216)**	**67 < Weight ≤ 75 kg (N = 204)**	**Weight > 75 kg (N = 217)**
AUC_{tau} (ng•h/mL)	367.8 (27.9)	332.9 (30.7)	308.1 (32.6)	277.4 (27.7)
C_{max} (ng/mL)	19.6 (30.0)	17.6 (32.4)	16.6 (41.6)	15.0 (31.3)
C_{min} (ng/mL)	13.1 (29.1)	11.9 (32.1)	10.9 (34.4)	9.8 (29.0)

Gender

In the updated POPPK analysis 32% of the total dataset was female. Gender was retained as a covariate in the final model but was not considered clinically relevant as the differences in TAF and TFV exposures due to covariates were less than 40%. The overall TAF and TFV exposures observed in females were higher but in the range of those observed in males.

Table 39. POPPK Analysis: PK TAF following once-daily dosing of TAF in subjects with CHB by sex

	TAF	
TAF PK Parameter	**Male (N = 434)**	**Female (N = 264)**
AUC_{tau} (ng•h/mL)	183.9 (44.8)	267.5 (74.0)
C_{max} (ng/mL)	151.8 (47.2)	220.2 (50.8)

Table 40. POPPK Analysis: PK TFV following once-daily dosing of TAF in subjects with CHB by sex

TFV PK Parameter	TAF	
	Male (N = 535)	Female (N = 321)
AUC_{tau} (ng•h/mL)	301.1 (31.5)	356.4 (28.8)
C_{max} (ng/mL)	16.1 (37.1)	19.0 (30.2)
C_{min} (ng/mL)	10.7 (33.0)	12.7 (30.4)

Race

In the E/C/F/TAF study **GS-US-292-0108** TAF and TFV exposures were each comparable between Japanese and non-Japanese subjects after single doses. However, after multiple dosing:

- The AUCs of all analytes were lower in Japanese vs. Caucasian subjects.
- 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}.

GS-US-320-1228 – TAF in Japanese vs. non-Japanese

Study Title: A Phase 1 Single Dose Study to Investigate the Pharmacokinetics, Safety and Tolerability of Tenofovir Alafenamide in Healthy Japanese and Non-Japanese Subjects

Non-Japanese subjects were not of Japanese or Asian descent and their parents and grandparents were not born in Japan or in any Asian country. Japanese subjects were of first generation, born in Japan, had not lived outside Japan for > 10 years, and could trace their maternal and paternal Japanese ancestry of parents and grandparents. Their lifestyle, including diet, had not significantly changed since leaving Japan. A single dose of TAF 25 mg was administered after a standard Japanese breakfast.

There were similar proportions by gender in the Japanese (3 male; 7 female) and non-Japanese (4 male; 6 female) groups. Weight and height were lower for Japanese subjects but BMI was similar between groups. After a single dose the applicant concluded that PK of TAF and TFV were comparable between Japanese and non-Japanese subjects.

The difference was greater for TFV than for TAF, as shown below. The supplementary tables show that mean and median CL/F values were slightly higher in the non-Japanese for each of TAF and TFV.

Table 41. GS-US-320-1228: Geometric Least Squares Mean Pharmacokinetic Parameters, Comparison between Japanese and Non-Japanese Subjects (Analysis Set: TAF PK)

TAF Geometric Least Squares Mean PK Parameter	Japanese Subjects (N = 10)	Non-Japanese Subjects(N = 10)	GMR (90% CI)
AUC_{inf} (ng*h/mL)	191.69	167.33*	114.56 (75.66, 173.44)
$AUC_{0\text{-}last}$ (ng*h/mL)	189.77	151.02	125.66 (82.37, 191.70)
C_{max} (ng/mL)	142.79	108.42	131.70 (75.52, 229.68)

Figure 11. GS-US-320-1228: Mean (SD) Plasma Concentration-Time Profiles of TFV After a Single Dose of TFV (Linear Scale; Analysis Set: TFV PK)

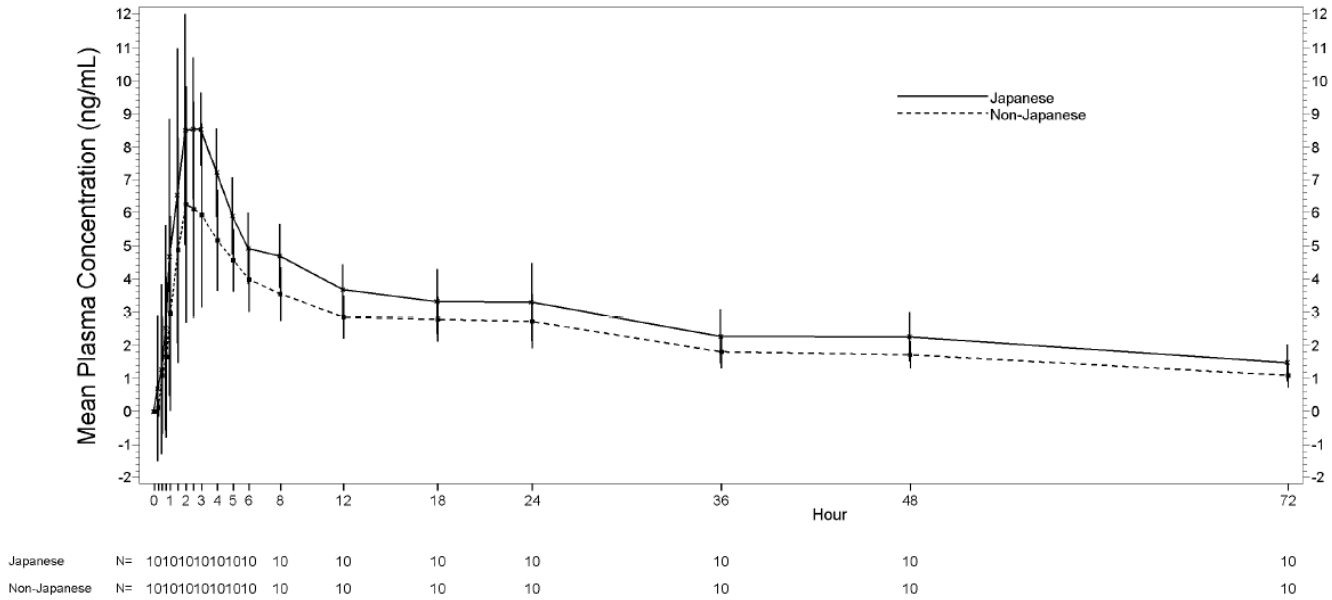


Table 42. GS-US-320-1228: Geometric Least Squares Mean Pharmacokinetic Parameters, Comparison between Japanese and Non-Japanese Subjects (Analysis Set: TFV PK)

TFV Geometric Least Squares Mean PK Parameter	Japanese Subjects (N = 10)	Non-Japanese Subjects (N = 10)	GMR (90% CI)
AUC_{inf} (ng*h/mL)	289.05	217.22	133.06 (103.71, 170.73)
$AUC_{0\text{-}last}$ (ng*h/mL)	200.32	156.29	128.17 (103.89, 158.12)
C_{max} (ng/mL)	9.76	6.73	144.99 (114.24, 184.01)

In the population PK analysis, race did not have an effect on TAF exposure in subjects with CHB and was not a statistically significant or clinically relevant covariate. Race (black vs. nonblack) was retained as a covariate for TFV in the final model but was not considered clinically relevant (differences < 40%). Although sample sizes were limited, the range of TFV exposures across races was comparable and consistently much lower than observed with TDF.

Table 43. POPPK Analysis: PK TAF Following Once-Daily Dosing of TAF in Subjects with CHB by Race

TAF PK Parameter	TAF			
	Asian (N = 561)	White (N = 127)	Black (N = 5)	Other (N = 5)
AUC_{tau} (ng•h/mL)	216.7 (70.8)	209.2 (41.5)	177.3 (26.6)	280.0 (81.5)
C_{max} (ng/mL)	176.8 (54.2)	182.5 (50.4)	158.3 (36.3)	168.7 (70.8)

Table 44. POPPK Analysis: PK TFV Following Once-Daily Dosing of TAF in Subjects with CHB by Race

TFV PK Parameter	TAF			
	Asian (N = 680)	White (N = 165)	Black (N = 6)	Other (N = 5)

AUC_{tau} (ng•h/mL)	329.9 (30.3)	296.0 (34.3)	205.1 (9.0)	228.7 (46.1)
C_{max} (ng/mL)	17.7 (34.3)	15.7 (36.8)	11.1 (15.0)	12.2 (45.4)
C_{min} (ng/mL)	11.7 (31.7)	10.5 (36.0)	7.0 (6.4)	7.9 (48.1)

Data are presented as mean (%CV).

Pharmacokinetic interaction studies

In vitro

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. All IC_{50} values were > 25 µM except for CYP3A4. 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.

CYP 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).

UGT1A1 inhibition

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

UGT1A1 and P-gp induction

In similar experiments as for CYP induction, there was no significant induction of P-gp and UGT1A1 mRNA on exposure to **TAF** (less than 2-fold increases).

Transporter studies

TAF is a substrate of P-gp, BCRP and OATP1B1/B3. TFV is a substrate of OAT1, OAT3 and MRP4.

	Substrate Assessment (yes/no)	
Transporter	**TAF**	**TFV**
P-gp	yes	no
BCRP	yes	no
OATP1B1	yes	ND

Transporter	Substrate Assessment (yes/no)	
	TAF	TFV
OATP1B3	yes	ND
OAT1	no	yes
OAT3	no	yes
OCT1	no	no
OCT2	ND	no
MRP1	ND	no
MRP2	ND	no
MRP4	ND	yes

At clinically relevant concentrations TAF and TFV do not inhibit any of the transporters tested *in vitro*.

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.

Transporter	IC_{50} (µM)	
	TAF	TFV
P-gp	> 100	> 1000
BCRP	> 100	> 100
BSEP	> 100	> 100
OATP1B1	> 100	> 100
OATP1B3	> 100	> 100
MATE1	> 100	> 300
MATE2-K	ND	ND
OAT1	> 100	33.8[a]
OAT3	> 100	> 1000
OCT1	> 100	> 100
OCT2	> 100	> 300
MRP1	ND	> 500
MRP2	ND	> 100
MRP4	ND	> 1000[b]

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.

TAF is unlikely to activate either of these human xenobiotic receptors, supporting the in-vitro induction results in human hepatocytes.

In vivo

Effect of P-gp inhibition (± other possible interaction mechanisms)

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 of E/C/F/TAF each containing 25 or 40 mg TAF as the monofumarate) with Stribild (STB) and **TAF 25 mg alone**. Dosing was for 12 days with 2-day washouts and after a meal of 550-650 kcal and 25-30% fat.

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

Following administration of E/C/F/TAF 25 mg vs. TAF 25 mg administered alone the TAF AUC_{last} and Cmax were ~2.2 and 2.3-fold higher, respectively, while TFV AUC_{tau} and Cmax were ~3.1 and 3.7-fold higher, respectively. Mean TFV AUC_{tau} and Cmax values following TAF alone were ~90% lower compared with those achieved after STB.

Table 45. TAF Pharmacokinetic Results

GS-7340 PK Parameter	Mean (%CV)		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)	552.1 (40.5)	242.4 (42.4)	221.78	199.99, 245.95
C_{max} (ng/mL)	506.9 (54.2)	215.4 (55.0)	222.62	187.11, 264.87
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)	558.7 (28.6)	244.9 (34.0)	230.93	205.52, 259.50
C_{max} (ng/mL)	472.4 (57.4)	210.8 (43.7)	223.01	188.40, 263.97

Table 46. TFV Pharmacokinetic results

TFV PK Parameter	Mean (%CV)		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)	834.9 (17.6)	3737.3 (22.3)	22.62	21.39, 23.91
C_{max} (ng/mL)	65.9 (50.9)	444.7 (28.9)	14.02	12.20, 16.11
C_{tau} (ng/mL)	28.1 (20.3)	73.2 (25.1)	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)	834.9 (17.6)	273.4 (23.5)	306.92	290.34, 324.45
C_{max} (ng/mL)	65.9 (50.9)	16.3 (24.8)	367.68	319.98, 422.50
C_{tau} (ng/mL)	28.1 (20.3)	9.4 (25.9)	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)	897.8 (12.7)	4089.6 (21.7)	22.47	21.11, 23.91
C_{max} (ng/mL)	71.9 (57.3)	505.3 (27.1)	13.54	11.62, 15.77
C_{tau} (ng/mL)	31.3 (15.0)	81.6 (22.2)	39.13	36.46, 41.99
EVG/COBI/FTC/GS-7340 Formulation 2 (25 mg) (Test) vs. GS-7340 (25 mg) (Reference)	N=17	N=18		
AUC_{tau} (ng•h/mL)	897.8 (12.7)	300.3 (13.4)	299.23	281.25, 318.37
C_{max} (ng/mL)	71.9 (57.3)	17.5 (15.1)	370.45	318.17, 431.34
C_{tau} (ng/mL)	31.3 (15.0)	10.5 (16.7)	300.33	279.91, 322.24

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

In GS-US-120-0118 FTC+TAF 10 mg was given with ATV/r, DRV/r, LPV/r and DTG once daily with a moderate fat meal.

Mean TAF AUCs after FTC+TAF alone were generally comparable with those when it was given in the presence of DRV/r (cohort 2). The effect of DRV/r was ascribed to the inductive effect of DRV combined with the inhibitory effect of RTV on P-gp. Mean plasma DRV Cmax and AUC were not affected by co-administration with FTC+TAF while Ctau was slightly higher (113% [95%–134%]).

Mean TAF AUCs and Cmax after dosing with DTG were slightly higher vs. FTC+TAF given alone. DTG Ctau and AUC were unaffected by FTC + TAF while Cmax was lower (87% [79%–96%]).

TAF AUCs and Cmax were higher following co-administration with ATV/r or LPV/r. ATV/r had the greater effect on TAF AUCs. LPV/r had the greater effect on Cmax. 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. It was concluded that F/TAF 200/10 mg should be used with these boosted PIs. Mean plasma ATV and LPV Cmax, Ctau and AUC values were not affected by co-administration

	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 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)

Mean TFV AUC and Cmax were higher on co-administration in each cohort but the greatest effect was observed with LPV/r and the least with DTG.

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

In GS-US-311-1388 F/TAF 10 mg was co-administered with ATV/co (300/150 mg using separate tablets) once daily with food for 6-7 days. TAF and TFV exposures were higher on co-administration.

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)

The magnitude of effect of COBI and RTV on TAF was similar. The effect of COBI on TFV was greater than for TAF as was observed for RTV.

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

GS-US-311-0101 evaluated multiple dose co-administration of TAF 8 mg with COBI 150 mg in the fed state. Co-administration resulted in substantially higher TAF and TFV exposures relative to TAF 8 mg dosed alone, ascribed to 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)

Effect of P-gp induction

In GS-US-311-0101 multiple dose co-administration of F/TAF 200/40 mg with EFV 600 mg in the fasted state resulted in lower exposures to TAF and TFV but the applicant concluded the effects were not clinically relevant based on the pre-defined acceptance criteria. The TAF and TFV Cmax values were also lower on co-administration.

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)

GS-US-120 1554 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

GS-US-120-1554 evaluated multiple doses of TAF 25 mg and RPV 25 mg given after standardised breakfasts for 14 days. RPV did not appear to induce P-gp since plasma PK parameters for TAF and TFV were unaffected by co-administration. The RPV AUC was unaffected whilst Cmax was slightly lower and C_{tau} was slightly higher.

GS-US-311-1387 Study Title: A Phase 1, Open-Label, Adaptive, Two-Part, Three Period, Fixed Sequence Study to Evaluate the Effect of Carbamazepine on the PK of TAF and GS-9883 in Healthy Adult Subjects

GS-US-311-1387 evaluated the effect of steady-state carbamazepine (CBZ) on the PK of TAF and TFV when administered as F/TAF 25 mg. CBZ was allowed to reach steady-state and dosing was with food. Co-administration resulted in decreases in TAF AUC_{inf}, AUC_{last} and C_{max} by approximately 54%, 55% and 57%, respectively, compared with administration of F/TAF FDC alone. A lesser decrease in TFV exposure was observed on co-administration such that AUC_{inf}, AUC_{last} and C_{max} decreased by approximately 23%, 25%, and 30%, respectively.

Table 47. GS-US-311-1387: Summary Statistics of TAF Pharmacokinetic Parameters After Administration of F/TAF FDC Alone and with Steady-State CBZ (Part A) (TAF PK Analysis Set)

PK Parameter	F/TAF FDC (200/25 mg) (N = 26)	CBZ 300 mg BID + F/TAF FDC (200/25 mg) (N = 22)
	Mean (%CV)	
AUC_{last} (h*ng/mL)	223.4 (41.7)	110.4 (61.2)
AUC_{inf} (h*ng/mL)	238.2 (36.9)	119.1 (61.3)
C_{max} (ng/mL)	219.6 (37.3)	99.4 (47.8)
T_{max} (h)[a]	0.88 (0.75, 1.00)	1.00 (0.75, 1.50)
$T_{1/2}$ (h)[a]	0.37 (0.31, 0.44)	0.42 (0.33, 0.44)
CL/F (L/h)	117.3 (31.9)	276.4 (52.8)
V_z/F (L)	64.5 (42.6)	149.3 (50.5)

TAF as a perpetrator

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

GS-US-120-1538 evaluated co-administration of TAF 25 mg with oral (2.5 mg) and IV (1 mg) midazolam when dosed after standard breakfasts. TAF had no significant effect on plasma MDZ or 1'OH-MDZ after oral or IV dosing. There was 25% to 30% decrease in TAF Cmax (25% to 30%) after co-administration with oral or IV MDZ, which the applicant proposed may reflect an effect of MDZ on gastrointestinal motility. TAF AUCs were also slightly lower but relatively less affected (~10-15% decreases).

GS-US-342-1167 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

GS-US-342-1167 evaluated co-administration of E/C/F/TAF 10 mg with Sofosbuvir and Velpatasvir (SOF/VPV 400 mg/100 mg tablets) for 8-day periods with dosing after a standard breakfast (~ 600 kcal, 25-30% fat). Co-administration resulted in increases in SOF, GS-331007 and VPV exposures, which were all attributed to COBI. VPV inhibits P-gp but there was no effect of co-administration on TAF or TFV since COBI already effected maximal P-gp inhibition.

GS-US-366-1689 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

GS-US-366-1689 evaluated co-administration of F/R/TAF 25 mg with SOF and ledipasvir (LDV) [LDV/SOF 90/400 mg] when given once daily for 11 days under fed conditions. Co-administration led to increases in TAF and TFV AUCtau of 32% and 75%, respectively. TFV Cmax was increased by 62% and Ctau 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 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 AUCtau on co-administration (467 ng*hr/mL) is approximately 5 times lower than TFV exposure following dosing with TDF.

GS-US-292-1316 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

GS-US-292-1316 evaluated co-administration of E/C/F/TAF 10 mg at steady state with a single dose of sertraline [SER; 50 mg after completing a standardised breakfast (~600 kcal and 27% fat). Co-administration had no clinically relevant effect on the PK of EVG, COBI, FTC, TAF, TFV or SER. Exposures to all analytes were consistent with historical data. All except two comparisons gave 90% CI that fell within 80, 125% and in the two exceptions (COBI Ctau and SER AUC0-∞) the lower boundary was only just below 80%.

GS-US-311-1790 Study Title: 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

GS-US-311-1790 evaluated the potential interaction between F/TAF 25 mg and Ortho Tri-Cyclen Lo, which contains norgestimate (NGM; 0.180 mg/0.215 mg/0.250 mg) and ethinyl oestradiol (EE; 0.025 mg) when each was given in the morning with food. Systemic exposures to NGMN (major metabolite of NGM), NG (minor metabolite of NGM) and EE were similar when the OC was given with and without F/TAF. The TAF and TFV exposures (given with food) were consistent with historical data.

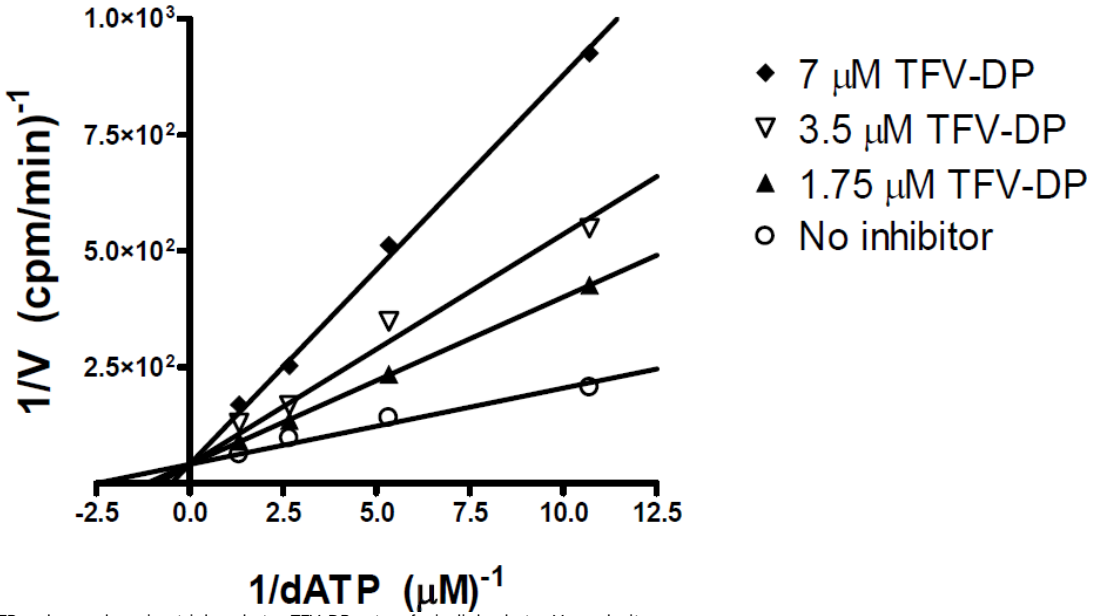
2.4.3. Pharmacodynamics

Mechanism of action

TFV-DP, the active metabolite of TAF, is an analogue of dATP that lacks a 3'-hydroxyl group. TFV-DP competes with dATP for incorporation into the nascent DNA chain during DNA polymerisation, resulting in premature termination of DNA synthesis. HBV replication involves reverse transcription and requires RNA-dependent DNA polymerase (RDDP), DNA-dependent DNA polymerase (DDDP) and ribonuclease H activities. HBV RT has been interchangeably designated as polymerase/reverse transcriptase (pol/RT), since it contains an additional N-terminal protein domain involved in protein-priming of viral DNA synthesis.

The effect of TFV-DP on the DDDP activity of HBV pol/RT was evaluated in an enzymatic assay using recombinant HBV pol/RT expressed in and purified from baculovirus.

Figure 12. Inhibition of HBV pol/RT DNA-Dependent DNA Polymerase Activity by TFV-DP



dATP = deoxyadenosine triphosphate; TFV-DP = tenofovir diphoshate; V = velocity

After incubation of HBV pol/RT with activated calf thymus DNA and dNTPs, inhibition of α-33P-labeled dATP incorporation was measured in the presence of indicated concentrations of TFV-DP. Data are presented as a Lineweaver-Burk plot.

Polymerase activity of HBV pol/RT was inhibited by TFV-DP in a dose-dependent manner without a change in maximal upstroke velocity (Vmax). The Ki of DDDP inhibition by TFV-DP was determined to be 0.18 µM, which is 2.1 fold lower than the Michaelis-Menten constant (Km) of dATP (0.38 µM).

Primary and Secondary pharmacology

Uptake of TAF into hepatocytes

A study in wild-type and transfected CHO cells evaluated whether TAF is a substrate for OATP1B1 and 1B3. TAF and control compounds were evaluated in the presence or absence of an inhibitor (40 µM rifampicin). TAF was taken up by wild-type CHO cells at a rate of 9.0 pmol/min/10^6 cells, indicating high passive permeability. The rate of TAF uptake increased by 30% and 168% in cells transfected with OATP1B1 and OATP1B3, respectively, vs. wild-type cells, but decreased by 48% and 76%, respectively, in the presence of rifampicin, demonstrating that TAF is a substrate for these transporters.

AD-120-2042 was reported during the assessment of Genvoya. The study evaluated the effect of OATP inhibition by rifampicin on the uptake of TAF into primary human hepatocytes by measuring intracellular levels of TFV-DP. The OATP substrate bosentan was used as a positive control. Following pre-incubation of primary human hepatocytes from 4 donors (deceased males aged 44-55 years) with 20 µM rifampicin for 30 min, cells were incubated with 0.5 µM TAF or bosentan for 2, 15 and 60 min and intracellular concentrations of TFV-DP or cell-associated bosentan levels were determined, respectively. A mean (of duplicate measurements) of 13% inhibition of TAF uptake by rifampicin was observed in hepatocytes from four different deceased donors (range from 4.8 to 34%) whereas the mean inhibition of accumulation of cell-associated levels of bosentan was approximately 38% (range 33-46%). Donor 1 stood out as having higher TFV-DP concentrations compared to the others but this

donor also showed very similar TFV-DP concentrations with and without pre-incubation with rifampicin. In contrast, donor 4 showed the highest bosentan levels in the absence of rifampicin with a large difference between runs but a marked reduction in both runs after pre-incubation with rifampicin.

The validity of the study was questioned due to the results with the positive control. In a published DDI study there was a ~5-fold increase in bosentan trough concentrations after the first co-administration of bosentan (125 mg BID) and rifampicin (600 mg QD). The applicant discussed that the in-vitro bosentan results likely underestimated the effect of rifampicin on bosentan uptake due to the inability to differentiate true intracellular concentrations from nonspecific association of the lipophilic pro-drug with the cell membrane. Non-specific binding would not affect the results with TAF because TFV-DP is only formed inside cells.

It was concluded by the applicant that OATP1B1/3-mediated active transport makes a small contribution to TAF uptake into human hepatocytes *in vitro*. Therefore, although TAF is a substrate for OATP1B1 and OATP1B3, these results suggested that active uptake into hepatocytes via these transporters is not a major route. It should also be noted that these transporters are affected by genetic polymorphisms, which could also affect hepatic uptake of TAF regardless of co-administered medications.

Intracellular metabolism of TAF

After hepatocyte entry, the pro-drug carboxylester bond is cleaved by CES1 and/or CatA releasing an intermediate metabolite TFV-alanine, which is followed by hydrolysis to TFV and diphosphorylation by adenylate kinase and nucleoside diphosphate kinase to form the active moiety TFV-DP.

The metabolism of TAF was compared with TDF and TFV in primary human hepatocytes by LC/MS/MS. The active moiety (TFV-DP) increased over 24 hours. Continuous incubation with 5 µM of TFV, TDF or TAF resulted in TFV-DP levels of 12.1, 302 and 1470 pmol/10^6 cells after 24 hours, respectively.

In order to assess TFV-DP persistence in primary human hepatocytes, TAF loading was evaluated using a 2-hour pulse with 5 µM TAF followed by a washout with drug-free media for 22 hours. TFV-DP levels reached approximately 700 pmol/106 cells at 3 hours and decreased slightly over 24 hour.

In a separate study that evaluated later time points in primary human hepatocytes following incubation with TFV, the t1/2 of TFV-DP of ~95 hours supported once daily dosing. These results correlated with inhibition of anti HBV activity for > 24 hours in HepG2 cells after a short incubation with TFV.

In-vitro activity against wild-type clinical HBV isolates

Full-length genomes or pol/RT regions were amplified from treatment-naive patients infected with genotypes A to H, cloned into expression vectors and transfected into HepG2 cells. After 7 days of treatment in the presence of TAF, HBV DNA intermediates were extracted and quantified by real-time PCR for determination of in-vitro susceptibility.

The TAF EC50 values ranged from 34.7 to 134.4 nM, with an overall mean EC50 of 86.6 nM. TAF EC50 values were compared with the control genotype A laboratory strain (pHY92). TAF had no observed cellular cytotoxicity up to the highest tested concentration (44400 nM). Based on these results, the selectivity (therapeutic) index (SI) for TAF was > 513 in HepG2 cells.

TDF activity against animal Hepadnavirus

The woodchuck hepatitis virus (WHV) in its natural host, the eastern woodchuck Marmata monax, is a frequently used model of HBV infection. In this model oral TDF at 0.5, 1.5 and 5.0 mg/kg/day for 4 weeks reduced serum viral load significantly, resulting in 0.2, 1.1 and 1.5 log10 decreases, respectively, from pretreatment levels. A 48-week study compared oral TDF, ADV, LAM and FTC as well as combinations of TDF or ADV with LAM and FTC at 15 mg/kg/day in the same model. After 12 weeks of TDF alone, LAM+TDF and FTC+TDF the mean serum viral load reductions were 3.6, 3.7 and 4.2 log10 copies/mL, respectively. Between Weeks 12 and 24, varying degrees of viral rebound were observed across all drug treatment groups. At Week 48, the mean serum viral load reductions in the same three groups were 2.9, 5.8 and 6.1 log10 copies/mL, respectively. In the entire 48 week dosing period, there was no evidence of toxicity in woodchucks in any treatment group.

In-vitro evaluation of HBV resistance

Despite the advent of various model systems to characterise the HBV replication cycle, primary human hepatocytes retain susceptibility for infection for only a short time and hepatoma cell lines are not susceptible to infection due to lack of receptor expression. Therefore, in the absence of a robust tissue culture system that allows for sustained virus propagation, in-vitro resistance selection against TAF has not been conducted.

In the TDF Phase 3 clinical studies (GS US 174 0102 and -0103) virologic analyses were performed to identify genotypic changes within HBV pol/RT from patients with detectable viral replication despite TDF treatment. See section 3.6 for further details of the data.

After more than 8 years of resistance surveillance, genotypic or phenotypic resistance to TDF has not been documented in HBV-infected patients.

Table 48. Antiviral susceptibility of recombinant HBV with Nucleos(t)ide-resistant mutations in RT

			EC_{50} FC from WT Control[c,d]			
Type	**RT Mutation(s)[a,b]**	**TAF**	**TFV**	**AFV**	**LAM**	**ETV**
WT	NA	1.0	1.0	1.0	1.0	1.0
ADV-R	rtA181T[e]	1.7	0.9	1.2	–	–
	rtA181T[f]	1.1	0.6	1.3	–	–
	rtA181V	1.2	1.0	1.2	–	–
	rtN236T	1.4	0.6	1.9	–	–
	rtA181V + rtN236T	3.7	2.8	2.7	–	–
LAM-R	rtM204I	1.6	1.8	–	> 48.8	–
	rtL180M + rtM204V	1.8	1.5	–	> 48.8	–
	rtV173L + rtL180M + rtM204V	0.9	1.6	–	> 48.8	–
ETV-R	rtL180M + rtM204V + rtT184G	1.7	1.2	–	–	> 28.6
	rtL180M + rtM204V + rtS202G	1.5	0.9	–	–	> 28.6
	rtL180M + rtM204V + rtM250V	1.2	1.5	–	–	> 28.6

a RT domain-specific amino acid position numbering for HBV polymerase. b Site-directed recombinants introduced into WT control (genotype D), followed by transfection into HepG2 cells c Fold change in mean EC50 value relative to WT control (genotype D). EC50 data averaged from at least 2 independent experiments performed in quadruplicate d Mean EC50 values against WT control for TAF, TFV, AFV, LAM, and ETV were 99.1 nM, 14.4 µM, 6.6 µM, 4.1 µM, and 17.5 nM, respectively e rtA181T mutation resulted in a W172* stop codon mutation in HBsAg. f rtA181T mutation resulted in a W172L mutation in HBsAg.

TAF activity against other Human and Animal viruses

The antiviral activity of TAF and TFV was assessed against a panel of 18 animal viruses, including ≥ 1 isolates of adenovirus, dengue type 2, influenza A, parainfluenza 3, respiratory syncytial virus (RSV), coxsackie B virus, rhinovirus, herpes simplex virus (HSV) type 1 (HSV-1), HSV type 2 (HSV-2), human cytomegalovirus (HCMV), varicella zoster virus (VZV), vaccinia virus, HCV, HIV-1, and simian immunodeficiency virus (SIV). Virus and cells were incubated in the presence of TAF or TFV for 2 to 7 days, and then evaluated for antiviral activity (i.e. reduction of viral cytopathic effect, EC_{50}) and cytotoxicity (CC_{50}).

TAF inhibited HIV-1 and SIV with EC_{50} value ranges of 2.04 to 5.89 nM and 0.51 to 1.21 nM, respectively. No antiviral activity was observed against other human viruses except that TAF and TFV weakly inhibited both HSV-2 strains with EC_{50} ranges of 424 to 697 nM and 146 to 278 nM, respectively. In addition, TAF showed low activity against human parainfluenza with an EC_{50} of 843 nM, which is likely attributable to cell growth inhibition observed at the highest tested concentration (1000 nM). Neither TAF nor TFV exhibited cytotoxicity up to the highest test concentrations of 1000 nM and 1000 µM, respectively.

TAF monotherapy in HBV-infected patients

GS-US-320-0101 – Study Title: A Phase 1b Randomized, Open Label, Active-Controlled Study to Assess the Safety, Viral Kinetics, and Anti-HBV Activity of GS-7340 in Treatment-Naive Adults with Chronic Hepatitis B (CHB) Infection.

This was an open-label study that compared TAF with TDF over 28 days in patients with CHB infection. Eligible patients had screening plasma HBV DNA ≥ 2 $x10^3$ IU/mL and documented CHB of at least 6 months duration (e.g. by positive serum HBsAg) with ALT ≤ 10xULN and $eGFR_{CG}$ ≥ 70 mL/min. No prior anti-HBV nucleoside/nucleotide therapy was allowed but patients who had failed interferon treatment > 6 months prior to screening were eligible. Co-infection with HDV, HIV or HCV was not allowed.

There were 51 patients randomised to TAF (8, 25, 40 or 120 mg) or TDF 300 mg orally once daily for 28 days in the fasted state and 50 completed follow-up to 30 days post-therapy. Genotypes A to E were represented but there were only 1-5 per genotype per group. About half of the overall number was HBeAg+. The majority had acquired HBV via vertical transmission.

Table 49. GS-US-320-0101: Main baseline Disease Characteristics (Full Analysis Set)

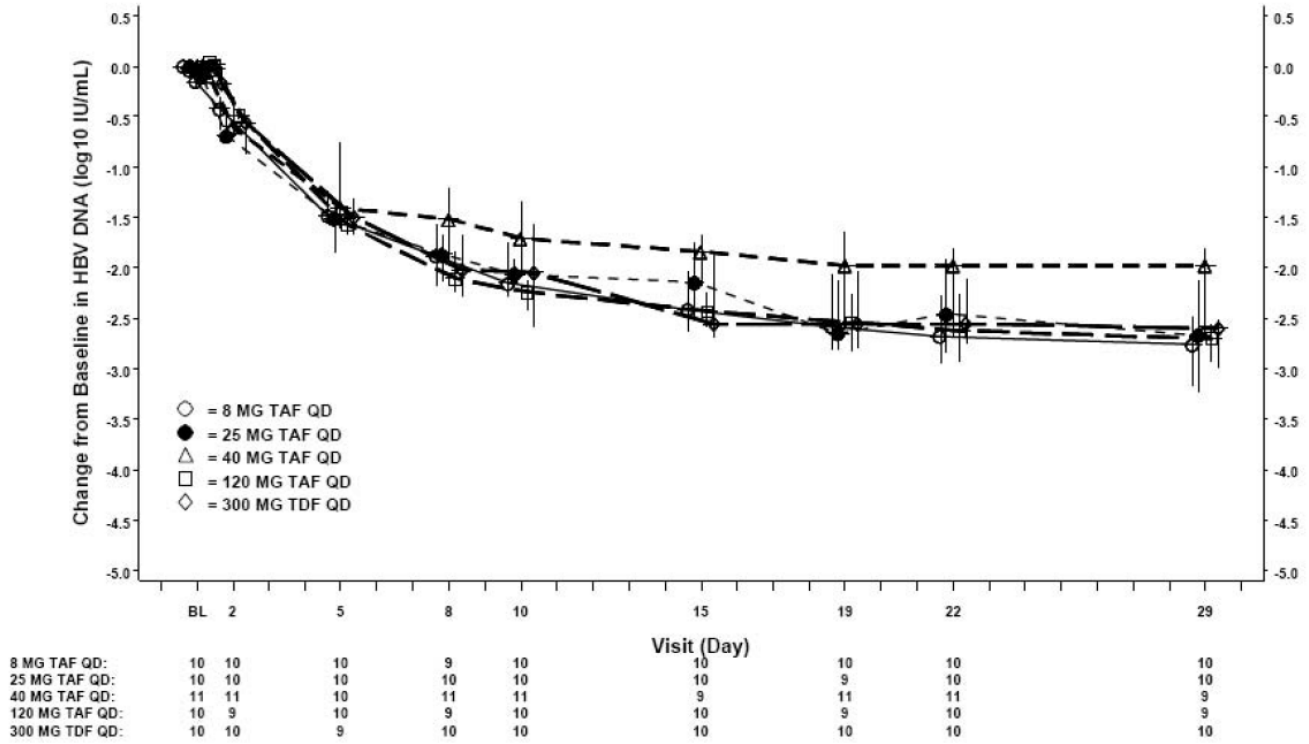
Disease Characteristic	TAF (8 mg) (n = 10)	TAF (25 mg) (n = 10)	TAF (40 mg) (n = 11)	TAF (120 mg) (n = 10)	TDF (300 mg) (n = 10)
HBV Genotype					
A	1 (10.0%)	1 (10.0%)	1 (9.1%)	1 (10.0%)	3 (30.0%)
B	1 (10.0%)	2 (20.0%)	5 (45.5%)	1 (10.0%)	1 (10.0%)
C	2 (20.0%)	4 (40.0%)	3 (27.3%)	3 (30.0%)	4 (40.0%)
D	3 (30.0%)	2 (20.0%)	1 (9.1%)	3 (30.0%)	0
E	3 (30.0%)	1 (10.0%)	1 (9.1%)	2 (20.0%)	2 (20.0%)
HBV DNA (log_{10} IU/mL)					
n	10	10	11	10	10
Mean (SD)	6.48 (1.975)	6.17 (1.893)	5.47 (2.026)	6.50 (2.488)	5.52 (1.781)
Median	6.26	6.35	5.37	5.98	4.78
Q1, Q3	5.01, 8.50	4.10, 7.68	3.42, 7.78	4.34, 8.83	4.11, 6.86
Min, Max	3.73, 8.98	3.56, 8.93	3.27, 8.57	3.71, 9.74	3.97, 8.85
Baseline HBeAg Status					
Negative	5 (50.0%)	4 (40.0%)	8 (72.7%)	5 (50.0%)	5 (50.0%)
Positive	5 (50.0%)	6 (60.0%)	3 (27.3%)	5 (50.0%)	5 (50.0%)
Quantitative HBsAg (log_{10} IU/mL)					
n	10	10	11	10	10
Mean (SD)	3.78 (1.062)	3.53 (0.851)	3.43 (1.028)	3.85 (0.852)	3.85 (0.463)
Median	4.10	3.59	3.64	3.84	3.93
Q1, Q3	2.98, 4.72	3.34, 3.97	2.44, 4.34	3.23, 4.72	3.58, 4.05
Min, Max	1.55, 4.72	1.47, 4.72	1.67, 4.72	2.64, 4.72	2.95, 4.72

The median time-weighted average change from baseline through week 4 in serum HBV DNA ranged from −1.69 log10 IU/mL to −2.18 log10 IU/mL for TAF compared with −2.31 log10 IU/mL for TDF 300 mg.

The median changes from baseline in serum HBV DNA after 4 weeks of treatment were from -1.98 to -2.76 log10 IU/mL vs. -2.60 log10 IU/mL for TDF 300 mg.

Consistently smaller declines in HBV DNA and lower median change from baseline in HBV DNA at Day 29 were observed in the TAF 40 mg group. The reason for this difference is stated to be unclear and was thought likely due to lower baseline HBV DNA values in comparison to the other TAF groups. In the TAF 40 mg group, the first quartile (Q1) at baseline (3.42 log10 IU/mL) was the smallest among all groups indicating more patients with lower baseline values were randomised into this group. The three lowest baseline values (< 3.5 log10 IU/mL) were also all in the TAF 40 mg group. The finding reflects the fact that 8/11 (72.7%) were HBeAg- in the 40 mg group. The mean HBV DNA change from baseline showed more comparable results for this group relative to the other TAF groups and TDF.

Figure 13. GS-US-320-0101: Median Change (Q1, Q3) from Baseline in Serum HBV DNA (log10IU/mL) by Treatment Group through Week 4 (Full Analysis Set)



Median first phase (treatment period) decay slopes were −0.101, −0.090, −0.071, −0.097 and −0.087 log10 IU/mL for the TAF 8, 25, 40 and 120 mg groups and TDF group, respectively.

Table 50. GS-US-320-0101: Summary of Viral Decay Slope (log10 IU/mL per Day) Baseline to Day 29 (Full Analysis Set)

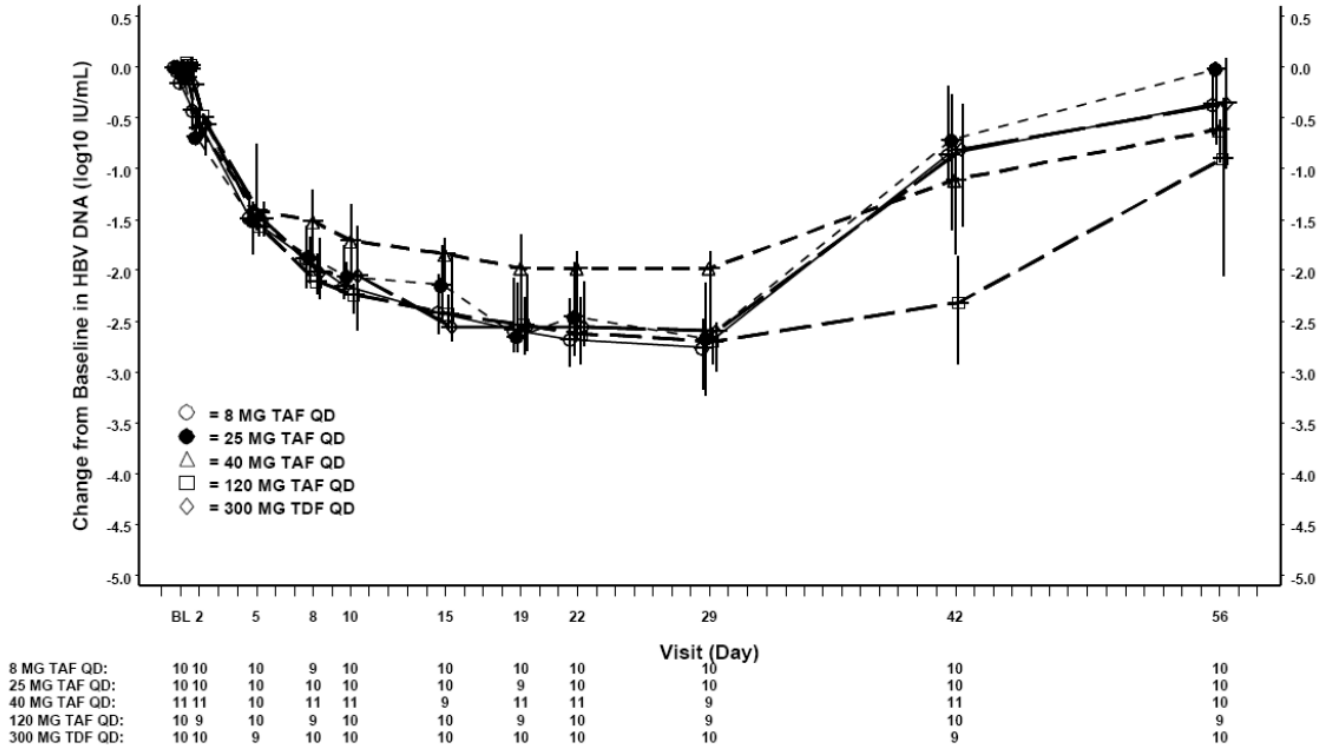
Estimate	TAF (8 mg) (n = 10)	TAF (25 mg) (n = 10)	TAF (40 mg) (n = 11)	TAF (120 mg) (n = 10)	TDF (300 mg) (n = 10)
n	10	10	11	10	10
Mean (SD)	−0.100 (0.0213)	−0.087 (0.0246)	−0.082 (0.0249)	−0.102 (0.0263)	−0.089 (0.0218)
Median	−0.101	−0.090	−0.071	−0.097	−0.087
Q1, Q3	−0.116, −0.090	−0.106, −0.069	−0.105, −0.058	−0.111, −0.091	−0.103, −0.075
Min, Max	−0.129, −0.066	−0.118, −0.038	−0.122, −0.054	−0.153, −0.064	−0.129, −0.060

At baseline, the median ALT level was lowest in the TDF group (27.50 U/L) compared with a range of 41-52.5 U/L in the TAF groups. Over 28 days of treatment, ALT levels changed minimally across treatment groups. At Day 29, median change from baseline in ALT was greatest for the TAF 25 mg group (−9.0 U/L).

At baseline, the median quantitative HBsAg levels were similar across the treatment groups and there were no significant changes from baseline after 28 days of treatment.

Although allowed by protocol, the majority (all except 4) of patients did not receive oral anti-HBV therapy during the follow-up period. In those who did not receive further anti-HBV therapy median HBV DNA levels had returned toward baseline levels at the last follow-up in all treatment groups.

Figure 14. GS-US-320-0101: Median Change (Q1, Q3) from Baseline in Serum HBV DNA (log10IU/mL) by Treatment Group through Follow-Up



The plasma TAF levels showed approximate dose proportionality. The actual levels resembled those described for similar doses in the TAF monotherapy study in HIV-infected patients.

GS-US-320-0101: Mean a (%CV) TAF Plasma PK Parameters Following Single Dose of TAF by Treatment (Analysis Set: TAF PK, Analyte: TAF)

TAF PK Parameters	TAF (8 mg) n=10	TAF (25 mg) n=10	TAF (40 mg) n=11	TAF (120 mg) n=10
AUC_{inf} (ng•h/mL)	60.6 (52.7)	154.3 (41.0)	329.9 (58.1)	855.1 (37.8)
AUC_{0-last} (ng•h/mL)	59.2 (54.1)	153.0 (41.1)	328.1 (58.4)	852.9 (38.0)
C_{max} (ng/mL)	83.2 (46.3)	249.5 (45.9)	527.4 (50.6)	1128.7 (33.7)
$t_{½}$ (h)	0.35 (0.32, 0.54)	0.48 (0.39, 0.50)	0.61 (0.48, 0.78)	0.70 (0.56, 0.85)

The plasma TFV levels showed the expected differences between the four TAF groups and the TDF group as shown in Table 57. That is, plasma TFV was markedly lower even with TAF 120 mg compared with TDF.

Table 51. GS-US-320-0101: Mean[a](%CV) TFV Plasma PK Parameters Following Single Dose of TAF by Treatment (Analysis Set: TFV PK, Analyte: TFV)

TFV PK Parameters	TAF (8 mg) (n = 10)	TAF (25 mg) (n = 10)	TAF (40 mg) (n = 11)	TAF (120 mg) (n = 10)	TDF (300 mg) (n = 10)
AUC_{inf} (ng•h/mL)	69.3 (36.3)	176.1 (32.8)	426.7 (44.1)	1518.3 (50.4)	2267.5 (26.4)
AUC_{0-last} (ng•h/mL)	33.0 (34.6)	90.7 (25.7)	213.6 (40.0)	607.8 (35.4)	1690.8 (27.6)
C_{max} (ng/mL)	3.0 (34.5)	8.3 (41.6)	20.3 (43.2)	61.0 (33.5)	306.8 (24.5)
$t_{½}$ (h)	24.06 (21.13, 26.69)	21.30 (13.56, 31.82)	25.11 (18.55, 27.74)	26.32 (24.63, 39.94)	10.26 (8.78, 11.80)

%CV = percent of coefficient of variation

a All parameters are reported as mean (%CV) except t½ which is reported as median (Q1, Q3)

PK-PD for efficacy

In GS US 320 0101, taking into account the likely explanation for the results in the TAF 40 mg group, the applicant concluded that there was a lack of dose effect and no trends in exposure-response. Based on the efficacy and safety profile of TAF 25 mg in HIV-infected patients (or 10 mg with P-gp inhibitors) the 25 mg dose of TAF was selected for Phase 3 studies in HBV.

The PK/PD relationship between TAF plasma exposure and efficacy (proportion with plasma HBV DNA < 29 IU/mL at Week 48 using a missing = failure approach) was evaluated using data from the Phase 3 studies (GS US 320 0108 and GS US 320 0110) and estimated TAF exposures (AUCtau and Cmax) derived from the POPPK analysis. In HBeAg-negative and HBeAg-positive patients there were no trends in exposure response relationship observed, consistent with the findings from the POPPK analyses that identified no clinically relevant covariates on TAF PK. In addition, no relationship was observed between quartiles of PK exposure and the maximum decrease from baseline in ALT.

PK-PD for safety

Analyses were performed between AUCtau and Cmax of TAF or TFV estimated from POPPK and selected safety endpoints using data from the Phase 3 studies.

For diarrhoea, nausea and GI/abdominal pain, no exposure AE trends were observed. For vomiting, there was a trend towards a higher rate with higher TAF (but not TFV) exposure, which was discordant with the PK/PD analyses of the TAF exposure-safety relationship following administration of F/TAF in HIV-infected patients, which showed no trend in the presence of vomiting across a wide range of TAF exposures. No exposure effect relationships were noted for the percentage change in hip and spine BMD from baseline at Week 48. No exposure effect relationship was found for serum creatinine trends. The change in total cholesterol from baseline at Week 48 was comparable across TAF and TFV AUCtau quartiles. A lack of relationship was also observed between TAF and TFV exposure quartiles and change in fasted LDL, HDL and triglycerides from baseline at Week 48.

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 was a TQT study. Single doses of 25 mg and 125 mg TAF were administered in a cross-over design so that healthy subjects received each of the following with 11-day washouts:

- Treatment A: 25 mg TAF tablet plus 4 x placebo
- Treatment B: 125 mg TAF (5 x 25 mg TAF tablets)
- Treatment C: 5 x placebo
- Treatment D: 400 mg moxifloxacin

Treatments A, B and C were administered double blind and moxifloxacin (D) was open label. All dosing was in the morning at ~08:00 with water and within 5 minutes of consuming a standard breakfast. There were 48 subjects (32 male) who completed all doses and were evaluable for the PK and PK-PD analyses.

Pharmacokinetic parameters for TAF and TFV following single oral doses of 25 or 125 mg TAF were approximately proportional to dose.

The lower bound of the 2-sided 90% CI for the mean difference between moxifloxacin and placebo was greater than 5 ms at 2 post-dose time points (3 and 4 h) establishing assay sensitivity. 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.

2.4.4. Discussion on clinical pharmacology

P-gp inhibition or induction

TAF is a substrate of P-gp and BCRP. TAF uptake from the gut after oral administration is mainly determined by the presence or absence of co-administered inhibitors and inducers of P-gp.

During review of the Genvoya (E/C/F/TAF) and Descovy (F/TAF) application dossiers it was agreed that 10 mg TAF should be used when it is given with a strong inhibitor of P-gp. For Genvoya this is inevitable since cobicistat is in the FDC. For F/TAF the 10 mg TAF formulation was recommended when it is given with HIV PIs boosted with COBI or RTV. It was agreed that the use of F/TAF with any other strong inhibitor of P-gp should advise a reduction in dose so that the F/TAF 10 mg strength is used (e.g. this is advised when co-administering Descovy with ketoconazole, itraconazole and ciclosporin).

However, when treating HBV a dose reduction to TAF 10 mg daily is not possible since there is only a 25 mg tablet to be marketed. This same problem applied to F/R/TAF, for which only one strength tablet (containing 25 mg TAF) is marketed. Therefore the SmPC for TAF reflects the position reached on co-administration of F/R/TAF with inhibitors of P-gp such that co-administration with strong P-gp inhibitors is not recommended. In the F/R/TAF SmPC the agents not recommended on these grounds are triazole antifungals and ciclosporin.

In addition, it was agreed during the review of Descovy that use of F/TAF (10 or 25 mg) with inducers of P-gp should be not recommended. Co-administration with CYP3A inducers that are also P-gp inducers is contraindicated in the F/R/TAF SmPC due to RPV. However, for TAF alone a statement that co-administration with P-gp inducers is not recommended was considered to suffice.

Co-infection with HIV

There are no clinical data on the use of TAF 25 mg to treat HBV and simultaneously, in conjunction with other antiretroviral agents, to simultaneously treat HIV. Correspondingly, there are no data on the use of the various TAF-containing HIV FDCs to treat concurrent HBV infection. In reality, both potential uses of TAF-containing products should be successful and the safety profile should not be any different with respect to TAF per se. Therefore, the SmPC for the TAF 25 mg tablet in section 4.4 includes advice that in patients who are co-infected with HBV and HIV TAF should be co-administered with other antiretroviral agents to ensure that the patient receives an appropriate regimen for treatment of HIV.

Effect of food

It was previously established that food increases TAF absorption but the magnitude of effect is notable only when TAF is given without a strong P-gp inhibitor. The applicant has reported an additional food effect study (GS-US-320-1382) in which the TAF 25 mg tablet was given after a meal of 800 kcal and 50% fat. The food effect in this study (AUC ~65% higher vs. fasted; AUClast 262 vs. 158 ng.h/mL) was consistent with the effect noted in the F/TAF study (AUC ~75% higher vs. fasted; AUClast 235 vs. 134 ng.h/mL). During the review of Descovy a concern was raised regarding the adequacy of TAF absorption if the F/TAF 25 mg tablet was taken without food and without a P-gp inhibitor. However, after further review of plasma and intracellular levels in the HIV monotherapy and switch studies by third agent, it was finally agreed that for treatment of HIV Descovy may be taken with or without food.

Based on the exposure-response observed in GS-US-320-0101 the applicant considered that the effect of food was not clinically relevant. In this study dosing was in the fasting state and the AUClast value for the 25 mg dose was 153 ng.h/mL. This mean value is similar to fasted state values in the two food effect studies mentioned above. Short-term viral suppression in the 25 mg group was at least as good as that achieved with 8, 40 mg and 120 mg doses and the 120 mg dose showed an advantage over lower doses only in a slower recovery of serum HBV DNA.

However, in the Phase 3 HBV studies patients were instructed to take study drugs with food (unspecified meal content). The applicant reported that 5024/6051 plasma samples with measureable TAF concentrations were obtained after administration of TAF in the fed state. The POPPK estimate of the effect of fasting on TAF plasma levels (17.5% reduction in AUC for fasted vs. fed, which is a 21.2% increase in fed vs. fasted, with no change in Cmax) was less than that observed in GS US 320 1382, which showed that TAF AUCinf and AUClast were increased by 68% and 65%, respectively and Cmax was unchanged when administered with a high fat meal. Nevertheless, the applicant was unable to substantiate the reliability of information on whether TAF was taken with or without food on the sampling days in Phase 3 studies. Therefore the SmPC recommends that TAF 25 mg tablets should be taken with food.

Uptake from plasma and onward metabolism

TAF is a pro-drug with a short half-life in plasma. The mechanisms of uptake of TAF from plasma into hepatocytes and onward conversion to the active moiety TFV-DP are discussed further in the next section Although TAF is a substrate of OATP1B1/1B3, the in-vitro data suggest that active transport by OATP1B1/1B3 plays a minor role in its uptake into hepatocytes. A clinical interaction study with TAF and an inhibitor of OATP1B without effects on P-gp or BCRP has not been conducted to assess whether

plasma levels could be higher only as a result of blocking hepatocyte uptake. In the Phase 3 studies co-administration with OATP1B inhibitors was not precluded but it seems that most recognised inhibitors would likely have potential to affect TAF levels due to effects on P-gp. Therefore a POPPK analysis that attempts to identify any possible effects of OATP1B inhibition on plasma TAF is unlikely to be fruitful.

Carboxylesterase 1 (CES1) is proposed to be the predominant enzyme involved in the conversion of TAF to TFV in primary human hepatocytes, although CatA also makes a contribution. In support of this hypothesis, the anti-HBV EC50 of TAF in HepAD38 cells was not significantly affected by inhibition of CatA or CES1 alone. A marked effect was observed only when both CatA and CES1 were inhibited by telaprevir and BNPP, respectively; resulting in a significant reduction in TAF derived metabolites.

The applicant describes CatA conversion as a high capacity and low affinity pathway not readily inhibited by other xenobiotics. In-vitro studies did not suggest significant inhibition of the conversion step by HIV protease inhibitors (known to inhibit CatA) but during review of the E/C/F/TAF dossier the applicant acknowledged that the HCV PIs telaprevir and boceprevir could have an effect intracellularly and the SmPC was amended such that co-administration with these agents is not recommended. There are also rare genetic polymorphisms resulting in low Cat A activity. However, since CES1 appears to be more important than CatA in hepatocytes, inhibition of CatA by telaprevir or boceprevir may not affect the conversion of TAF to TFV-DP in HepAD38 cells, which express both CatA and CES1. Therefore omission of the advice not to co-administer TAF with telaprevir or boceprevir is acceptable. In any case it is not at all likely that this will now occur due to the advent of DAA-only anti-HCV regimens.

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 in the metabolite profiling study <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, induction of this pathway should not affect the TAF levels and, therefore, should 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 are xanthine oxidase inhibitors. This enzyme 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. There is a need to understand the possible effects of allopurinol and febuxostat on TAF metabolism and whether TFV levels in plasma could increase, approaching those observed on dosing with TDF, with associated changes in the anticipated safety profile of TAF compared to TDF.

Renal impairment

Use of the HIV FDCs containing TAF is limited to persons with CrCL at least 30 mL/min due to the FTC content and inability to adjust the dose for those with lower renal function. In the additional study

reported in the TAF dossier in subjects with CrCL between 15-29 mL/min the actual plasma TAF exposures were approximately double those observed in the matched controls, with mean plasma AUClast ~500 ng.h/mL and a CV% value of 47%. The proportion of TAF unbound was similar in the two groups. As expected, severe renal impairment had a much greater effect on plasma TFV such that after a 25 mg dose the mean plasma AUC (2070 ng.h/mL) was at the lower end of the range documented for TDF 300 mg doses in other studies.

Since the actual plasma levels of TFV after a 25 mg TAF dose did not exceed the exposures that occur with the licensed dose (TDF 300 mg) in patients with normal renal function there is no reason to preclude use of TAF in patients with CrCL between 15-29 mL/min. Nevertheless, it should be expected that the safety profile of TAF 25 mg in this patient sub-group will resemble that of TDF. Therefore section 4.4 of the SmPC includes warnings about the risks of the expected TFV exposures.

Use of TAF 25 mg in patients with CrCL < 15 mL/min who are on haemodialysis is based only on modelling and only on the predicted TFV exposures.

In those on thrice weekly haemodialysis plasma TFV exposures are predicted to be in the range documented for dosing TDF 300 mg in patients with normal renal function. TAF 25 mg is not recommended for those with CrCL < 15 mL/min who are not on haemodialysis due to lack of data.

Hepatic impairment

In the Phase 3 HBV studies patients <15% had compensated cirrhosis and the remainder (large majority) was non-cirrhotic (based on Fibrotest scores). Due to the exclusion criteria (taking into account the clinical criteria excluding decompensation and the screening laboratory values), it is not at all likely that patients with HBV and CP-C scores have been treated.

In a study in subjects with mild or moderate hepatic impairment TAF and TFV plasma exposures were not markedly different vs. matched controls. In a separate study the TAF AUClast in subjects with severe hepatic impairment group was half that in the controls, with higher apparent volume of distribution in the terminal phase (Vz/F) and apparent total plasma clearance (CL/F). Correspondingly the plasma TFV in those with severe hepatic impairment was about 63% of that in matched controls, also with higher Vz/F and CL/F. The effect of severe hepatic impairment on plasma TAF cannot be explained by existing data on liver fibrosis since the POPPK model did not identify Fibrotest (FT) score as a predictor of PK (although few had scores $\geq$ 0.75). The calculated free plasma TAF was comparable between those with severe hepatic impairment and their matched controls. However, similar plasma free TAF levels do not necessarily mean that uptake into hepatocytes is similar. Since there are no data on the efficacy of TAF in patients with CP-C (based on the patient selection criteria in Phase 3 studies) it is not possible to assume that there would be no difference in virological response between patients with normal hepatic function and those with severe impairment.

Pharmacodynamics

The intracellular conversion of TFV to the active moiety TFV-DP is the same regardless of whether dosing is with TDF or TAF. Hence the mechanism of action against HBV is also the same. The critical issue is to identify an appropriate dose of TAF for treatment of HBV since it cannot be assumed that a dose that is adequate for HIV is necessarily appropriate for HBV. For this application dossier, taking into account that:

- TDF is already shown to be efficacious for treatment of HBV when dosed at 300 mg once daily
- The in-vitro studies that can be conducted to assess activity of agents against HBV are more limited compared to what can be done to assess activity against HIV

- Subject to an adequate dose of TAF the risk of selecting for resistance and the potential for cross-resistance should be similar to that of TDF
- Intracellular TFV-DP cannot be assessed in hepatocytes from HBV-infected patients undergoing treatment without liver biopsy but in-vitro data suggested higher TFV-DP levels in hepatocytes after exposure to TAF compared to TFV or TDF
- The applicant's claim that contribution of OATP1B to hepatocyte uptake of TAF is minimal is based only on in-vitro data from a study in which the control substance did not behave as expected

The data reported by the applicant from the TAF dose effect study vs. TDF are very important for an expectation that an adequate dose of TAF should be able to achieve at least similar efficacy to TDF.

Uptake of TAF into, and onward metabolism within, human hepatocytes was discussed in the previous section. 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.

The initial study in CHO cells clearly demonstrated that TAF is a substrate of OATP1B1 and, especially, 1B3. Due to the issues with the control (bosentan) in the in-vitro study (AD-120-2042), a definitive conclusion regarding the relative importance of OATP1B for TAF uptake into hepatocytes cannot be reached. In this study the effect of rifampicin on TAF uptake was at most 34% based on intracellular TFV-DP but ranged from 4.8 to 34% across the four donors. Since the SmPC states that use of TAF 25 mg with strong inhibitors and inducers of P-gp is not recommended this will anyway cover use with several known agents that affect the activity of OATP1B.

The applicant's final choice of TAF 25 mg once daily for the HBV Phase 3 studies is supported by the short-term efficacy data (GS-US-320-0101). This was a small study (10 subjects per treatment group) with an uneven distribution of subjects according to HBeAg status, HBV genotype and baseline viral load (~5.5 to 6.5 log10 IU/mL). Supplementary figures provided, show the change from baseline for each subject in each dose group and demonstrate that all subjects in the study did have some decline from baseline, although of variable degree. The plot for the TAF 120 mg dose demonstrates that all subjects had more than 1 log10 drop during treatment. This was also observed for the TDF group but it was not a uniform finding for TAF doses lower than 120 mg. In addition, the follow-up data indicated that recovery of HBV DNA was delayed only in the TAF 120 mg dose group.

This study actually suggested that 120 mg could be the most reliable dose of TAF across genotypes, viral loads and HBeAg status. The TAF 120 mg dose might even be somewhat more efficacious than TDF and was associated with TFV AUC substantially below that observed with TDF 300 mg; however, the TFV AUCs with 120 mg and 125 mg (QTc study) doses were 5-6-fold higher than observed with TAF 25 mg in each study. Therefore, selection of a TAF dose higher than 25 mg once daily, for which there is already substantial safety experience in HIV-infected patients, runs the risk of a safety profile (including renal and bone effects) that more resembles that of TDF.

In such small groups and mixed disease characteristics it is not appropriate to analyse viral responses in even smaller sub-groups from this preliminary dose-finding study. More importantly, the PK-PD analyses derived from the Phase 3 studies did not demonstrate an exposure-response relationship based on POPPK-derived TAF AUCs in HBeAg-positive or -negative populations. These analyses do support a conclusion that doses higher than 25 mg would be unlikely to result in substantially higher virological response rates.

Other observations

The observation that plasma exposures to all analytes measured after dosing with Genvoya for several days was lower in Japanese vs. Caucasian subjects (for TAF the lower bound of the 90% CI was well below 80% for AUClast and for TFV the lower bounds of the 90% CI were well below 80% for AUCtau and Ctau) was never satisfactorily explained but for the purposes of treating HIV it was agreed that the differences should not impact on efficacy. These racial differences were not observed after a single dose.

In a further study with a single dose of TAF 25 mg the TAF and TFV AUCs were slightly higher in the Japanese subjects, as were the mean and median CL/F values. It is unsatisfactory that this study was conducted with a single dose in light of the previous observations and lack of explanation for them. Nevertheless, the actual TAF and TFV AUCs that were observed after dosing Japanese subjects with Genvoya would be expected to apply to dosing HBV-infected Japanese subjects with TAF 25 mg. On this basis, taking into account the PK-PD analyses that have been conducted, if lower exposures were to occur in HBV-infected Japanese vs. non-Japanese subjects during daily dosing they would not be predicted to impact on efficacy.

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. TAF is a substrate of P-gp and BCRP. TAF uptake from the gut after oral administration is mainly determined by the presence or absence of co-administered inhibitors and inducers of P-gp. Co-administration of TAF with P-gp inducers is not recommended.

TAF should be administered with food. No dose adjustment of TAF is required in patients with hepatic impairment. No dose adjustment is required in adults or adolescents with estimated creatinine clearance (CrCl) ≥ 15 mL/min or in patients with CrCl < 15 mL/min receiving haemodialysis.

2.5. Clinical efficacy

The two Phase 3 efficacy studies were as follows:

GS-US-320-0108	A Phase 3, Randomized, Double-Blind Study to Evaluate the Safety and Efficacy of Tenofovir Alafenamide (TAF) 25 mg QD versus Tenofovir Disoproxil Fumarate (TDF) 300 mg QD for the Treatment of HBeAg-Negative, Chronic Hepatitis B
GS-US-320-0110	A Phase 3, Randomized, Double-Blind Study to Evaluate the Safety and Efficacy of Tenofovir Alafenamide (TAF) 25 mg QD versus Tenofovir Disoproxil Fumarate (TDF) 300 mg QD for the Treatment of HBeAg-Positive, Chronic Hepatitis B

These studies differed in study population (HBeAg status) but were otherwise of very similar design. The results are shown separately since, as expected, virological response rates were different.

Methods

Eligible patients were to meet the following criteria:

- HBeAg-negative CHB (0108) or HBeAg positive (0110) meeting both the following criteria at screening:
- Plasma HBV DNA ≥ 2 × 104 IU/mL
- Serum ALT > 60 U/L (males) or > 38 U/L (females) and ≤ 10 × ULN (central laboratory range)

- Treatment-naive (< 12 weeks of oral antiviral [OAV] treatment with any nucleoside or nucleotide analogue or treatment-experienced (> 12 weeks of previous OAV). Any OAV was discontinued before starting assigned study drug. Any previous treatment with interferon (pegylated or non-pegylated) must have ended at least 6 months prior to the baseline visit
- eGFRCG ≥ 50 mL/min based on serum creatinine and actual body weight. Patients with confirmed eGFR < 30 mL/min while on study were required to permanently discontinue treatment. Patients with confirmed eGFR < 50 mL/min and > 20% decline in eGFR (eGFRCKD-EPI, cysC) at any time during the study underwent dose modification to every other day dosing of study drugs

Excluded were patients with any of:

- Co-infection with hepatitis C virus (HCV), HIV or hepatitis D virus (HDV)
- Evidence of HCC (e.g. by recent imaging) or hepatic decompensation (e.g. ascites, encephalopathy or variceal haemorrhage)
- Abnormal specified haematological and biochemical parameters
- Received solid organ or bone marrow transplant
- Significant organ or bone disease or multiple bone fractures
- Also excluded were patients on any of the following prohibited concomitant medications (if stopped at screening there was to be at least 30 days washout before study baseline):
- Nephrotoxic agents (e.g. aminoglycosides, amphotericin B, vancomycin, cidofovir, foscarnet, cisplatin, pentamidine, ciclosporin, tacrolimus)
- Probenecid
- Agents that reduced renal function or competed for active tubular secretion with TFV (e.g. cidofovir, aciclovir, valaciclovir, ganciclovir, valganciclovir)
- Systemic chemotherapeutic agents, systemic corticosteroids (except short-term prednisone), immunosuppressants or immunomodulating agents
- Bisphosphonates

Both studies were double-blind and double-dummy in design. Patients were randomised in a 2:1 ratio to:

- TAF group: TAF 25 mg once daily and matched placebo of TDF 300 mg once daily for 96 weeks
- TDF group: TDF 300 mg once daily and matched placebo of TAF 25 mg once daily for 96 weeks

It was recommended but not mandated that study drugs were taken in the morning at approximately the same time each day and dosing was to be with food.

Patients who lost HBsAg and seroconverted to anti-HBs were to discontinue study drugs within 3 to 6 months or after Week 48 if seroconversion occurred prior to this visit.

Plasma HBV DNA was measured using the Roche COBAS Taqman HBV test for use with the high pure System (LLOQ 29 IU/mL). HBsAg was quantified at centralised laboratories in serum using the Abbott Architect assay (LLOQ ≤0.05 IU/mL). A single laboratory analysed sera for genotype using the

INNO-LiPA HBV Genotyping Assay, for the presence of drug resistance mutations in HBV pol/RT using the INNO-LiPA Multi-DR vs2/v3 hybridization assay and sequenced the HBV pol/RT.

In 0108: Sample sizes of 130 TDF and 260 TAF patients were planned to give 90% power to rule out the non-inferiority margin of 10% at a 1-sided significance level of 0.025. This sample size was based on the assumption that the difference (TAF-TDF) in proportions with HBV DNA < 29 IU/mL was 0 and the proportion with HBV DNA < 29 IU/mL in the TDF group was 91% (derived from the TDF study GS-US-174-0102). Randomisation was stratified by plasma HBV DNA level (< 7 $\log_{10}$ IU/mL, ≥ 7 to < 8 $\log_{10}$ IU/mL, ≥ 8 $\log_{10}$ IU/mL) and OAV treatment status.

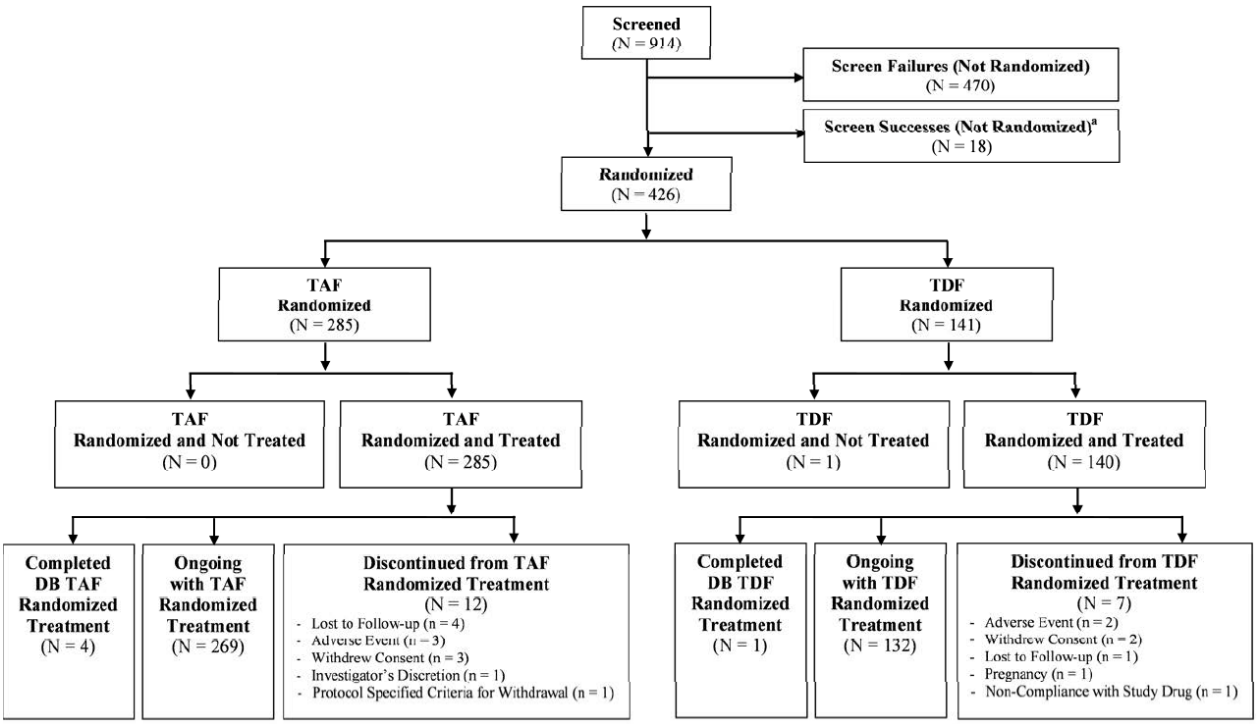
In 0110: Sample sizes of 288 TDF and 576 TAF patients were planned to give 84% power to rule out the non-inferiority margin of 10% at a 1-sided significance level of 0.025. This sample size was based on the assumption that the difference (TAF-TDF) in the proportions with HBV DNA < 29 IU/mL was 0 and the proportion with HBV DNA < 29 IU/mL in the TDF group was 69% (derived from the TDF study GS-US-174-0103). Randomisation was stratified by plasma HBV DNA level (≥ 8 or < 8 $\log_{10}$ IU/mL) and OAV treatment status.

The primary efficacy endpoint was the proportion of subjects with HBV DNA < 29 IU/mL at Week 48 and the primary efficacy analysis was conducted after the last randomised patient reached Week 48 or discontinued study drugs prematurely. An M = F approach was employed. The baseline stratum-weighted difference in the proportion (P1 – P2) and its 95% CI was calculated based on stratum-adjusted Mantel-Haenszel (MH) proportion, where stratification factors included baseline HBV DNA and OAV status.

Results – study 0108

There were 426 patients randomised and 425 received at least one dose of study drugs.

Figure 15. GS-US-320-0108: Disposition of Study Subjects



DB = double blind

a For screen successes but not randomized, 14 were due to withdrawal of consent and 4 were due to outside of visit window.
Data cut-off date was 01 October 2015.

Demographic and baseline characteristics were similar between the treatment groups except that patients in the TAF group were slightly younger with median age 46 years (range: 19 to 80) vs. 50 years (range: 25 to 72) in the TDF group. The majority was male (60.9%) and Asian (72.0%), 25% were white and 8 subjects were Black.

Regarding baseline disease characteristics (see below) only 11.1% TAF and 14.4% TDF patients had a baseline FibroTest score > 0.75. The baseline HBV DNA ranges show that not all patients met the inclusion criterion applied at screening for minimum ≥ 2×10^4 IU/mL. Similarly, the baseline ALT ranges show that not all patients met the inclusion criterion applied at screening for minimum ALT >60 U/L (although 82.8% TAF and 86.4% TDF had values > ULN) and some exceeded 10 x ULN.

Table 52. GS-US-320-0108: Baseline Disease Characteristics (Safety Analysis Set)

	TAF 25 mg (N = 285)	TDF 300 mg (N = 140)	Total (N = 425)	TAF 25 mg vs TDF 300 mg P-Value[a]
HBV DNA (log_{10} IU/mL)				
N	285	140	425	0.63
Mean (SD)	5.7 (1.34)	5.8 (1.32)	5.8 (1.33)	
Median	5.6	5.7	5.7	
Q1, Q3	4.8, 6.7	5.0, 6.6	4.9, 6.7	
Min, Max	1.8, 9.9	1.4, 8.2	1.4, 9.9	
HBV DNA Categories				
< 7 log_{10} IU/mL	230 (80.7%)	116 (82.9%)	346 (81.4%)	0.69
≥ 7 log_{10} IU/mL - < 8 log_{10} IU/mL	42 (14.7%)	20 (14.3%)	62 (14.6%)	
≥ 8 log_{10} IU/mL	13 (4.6%)	4 (2.9%)	17 (4.0%)	
ALT (U/L)				
N	285	140	425	0.74
Mean (SD)	94 (88.3)	94 (80.8)	94 (85.8)	
Median	67	67	67	
Q1, Q3	44, 102	47, 102	45, 102	
Min, Max	17, 720	9, 491	9, 720	
ALT Level[b]				
≤ ULN	49 (17.2%)	19 (13.6%)	68 (16.0%)	0.77
> ULN - 5 × ULN	209 (73.3%)	109 (77.9%)	318 (74.8%)	
> 5 × ULN – 10 × ULN	22 (7.7%)	10 (7.1%)	32 (7.5%)	
> 10 × ULN	5 (1.8%)	2 (1.4%)	7 (1.6%)	
HBeAg Status[c]				
Positive	2 (0.7%)	2 (1.4%)	4 (0.9%)	0.47
Negative	283 (99.3%)	138 (98.6%)	421 (99.1%)	
HBV Genotype Group				
A	15 (5.3%)	6 (4.3%)	21 (4.9%)	0.13
B	60 (21.1%)	40 (28.6%)	100 (23.5%)	
C	115 (40.4%)	47 (33.6%)	162 (38.1%)	
D	90 (31.6%)	42 (30.0%)	132 (31.1%)	
E	5 (1.8%)	2 (1.4%)	7 (1.6%)	
H	0	2 (1.4%)	2 (0.5%)	
Unknown	0	1 (0.7%)	1 (0.2%)	

Years Positive for HBV				
N	285	140	425	0.31
Mean (SD)	8.5 (7.85)	9.3 (8.72)	8.8 (8.14)	
Median	6.0	6.5	6.0	
Q1, Q3	2.0, 12.0	3.5, 11.0	3.0, 12.0	
Min, Max	1.0, 39.0	1.0, 49.0	1.0, 49.0	
Previous Oral Nucleoside/Nucleotide Treatment[d]				
Yes	60 (21.1%)	31 (22.1%)	91 (21.4%)	0.80
No	225 (78.9%)	109 (77.9%)	334 (78.6%)	
Cirrhosis History				
Yes	24 (11.0%)	14 (12.4%)	38 (11.4%)	0.70
No	195 (89.0%)	99 (87.6%)	294 (88.6%)	
Indeterminate/Unknown	66	27	93	
FibroTest Score				
N	280	139	419	0.60
Mean (SD)	0.43 (0.223)	0.45 (0.229)	0.44 (0.225)	
Median	0.41	0.42	0.42	
Q1, Q3	0.26, 0.58	0.27, 0.62	0.27, 0.59	
Min, Max	0.05, 0.97	0.04, 0.97	0.04, 0.97	
eGFR by CG (mL/min)				
N	285	140	425	0.13
Mean (SD)	104.7 (27.83)	100.3 (24.23)	103.2 (26.74)	
Median	99.6	98.4	98.5	
Q1, Q3	86.4, 120.6	83.2, 112.2	85.2, 117.6	
Min, Max	39.0, 214.2	59.4, 187.8	39.0, 214.2	
eGFR by CKD-EPI Creatinine (mL/min/1.73 m^2)				
N	285	140	425	0.040
Mean (SD)	99.8 (14.97)	96.7 (13.48)	98.8 (14.55)	
Median	100.9	97.1	99.4	
Q1, Q3	90.0, 109.6	87.5, 106.8	88.9, 108.7	
Min, Max	46.4, 132.9	53.5, 122.3	46.4, 132.9	

The primary analysis met the pre-defined non-inferiority margin as shown below.

Table 53. GS-US-320-0108: HBV DNA Outcome at Week 48 Using HBV DNA of < 29 IU/mL, Missing = Failure (Full Analysis Set)

	TAF 25 mg (N = 285)	TDF 300 mg (N = 140)	TAF 25 mg vs TDF 300 mg	
			P-Value[a]	Prop Diff (95% CI)[b]
Success				
HBV DNA < 29 IU/mL	268 (94.0%)	130 (92.9%)	0.47	1.8% (−3.6% to 7.2%)
Failure				
HBV DNA ≥ 29 IU/mL	7 (2.5%)	4 (2.9%)		
Discontinued Study Drugs Due to Lack of Efficacy	0	0		
Discontinued Study Drugs Due to AE/Death	3 (1.1%)	1 (0.7%)		
Discontinued Study Drugs Due to Other Reasons[c]	6 (2.1%)	4 (2.9%)		
Missing Data During Window but on Study Drugs	1 (0.4%)	1 (0.7%)		

Prop Diff = difference in proportions

The Week 48 window was between Days 322 and 363 (inclusive).

a P-value for the superiority test comparing the percentages of HBV DNA < 29 IU/mL was from the Cochran-Mantel-Haenszel test stratified by baseline HBV DNA categories and oral antiviral treatment status strata.

b Difference in the proportion between treatment groups and its 95% CI were calculated based on the Mantel-Haenszel proportions adjusted by baseline HBV DNA categories and oral antiviral treatment status strata.

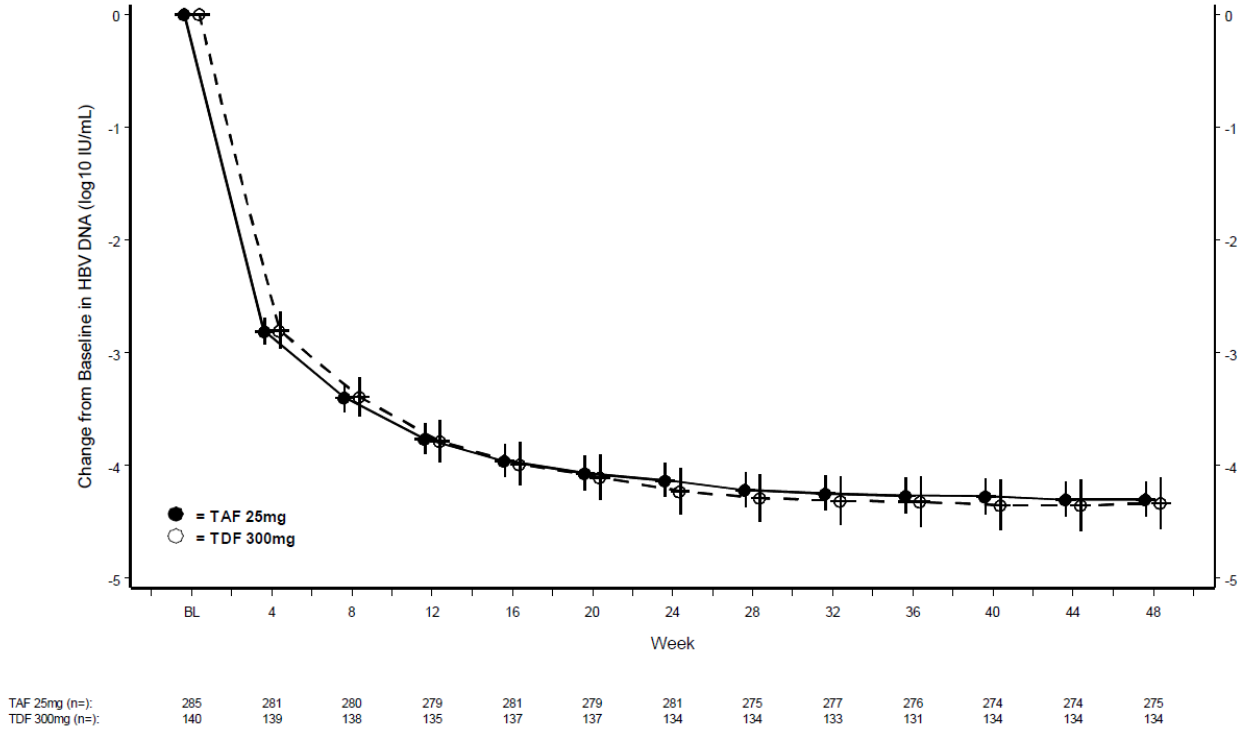
c Discontinuation due to other reasons included subjects who prematurely discontinued study drugs due to investigator's discretion, withdrew consent, lost to follow-up, noncompliance with study drugs, protocol violation, pregnancy, and study termination by sponsor.

The pre-defined non-inferiority margin was also met in the PP population, in which the percentages with < 29 IU/mL at Week 48 were TAF 97.4% vs. TDF 97.7% (difference in proportions [stratum-adjusted]: 0.5%, 95% CI: −3.3% to 4.4%).

In the FAS the proportions with HBV DNA < 29 IU/mL (target not detected) were numerically higher in the TAF group by both M = F (21.1% vs. 17.1%) and M = E analyses (TAF 21.8% vs. TDF 17.9%).

The kinetics of HBV DNA decline and magnitude of decline was similar between the groups.

Figure 16. GS-US-320-0108: Mean and 95% CIs of Change from Baseline in HBV DNA (log10 IU/mL) by Visit (Observed Data) (Full Analysis Set)



At Week 48, the rates of HBV DNA ≤ 29 IU/mL for subgroups did not differ statistically between TAF and TDF groups (see below). Those with baseline HBV DNA ≥ 7 $\log_{10}$ IU/mL had a higher rate of HBV DNA < 29 IU/mL at Week 48 in the TDF group compared with the TAF group. Additional analyses performed by baseline HBV DNA level (< 7, ≥ 7 or ≥ 8 $\log_{10}$ IU/mL; M = F analysis) did not demonstrate significant differences at the highest HBV DNA level. In the subgroup with HBV DNA ≥ 8 $\log_{10}$ IU/mL at baseline, 76.9% (10/13) in the TAF group and 75% (3/4) in the TDF group achieved HBV DNA <29 IU/mL at Week 48 (p = 0.67).

Table 54. GS-US-320-0108: Proportion of Subjects with HBV DNA < 29 IU/mL at Week 48 by Subgroup, Missing = Failure (Full Analysis Set)

	TAF 25 mg (N = 285)	TDF 300 mg (N = 140)	TAF 25 mg vs TDF 300 mg Prop Diff (95% CI)[a]
Age (years)			
< 50	171/176 (97.2%)	64/69 (92.8%)	4.9% (−2.7% to 12.4%)
≥ 50	97/109 (89.0%)	66/71 (93.0%)	−4.4% (−13.9% to 5.0%)
Sex			
Male	162/173 (93.6%)	80/86 (93.0%)	0.6% (−6.7% to 7.9%)
Female	106/112 (94.6%)	50/54 (92.6%)	2.8% (−6.5% to 12.0%)
Race			
Asian	192/205 (93.7%)	92/101 (91.1%)	2.7% (−4.2% to 9.7%)
Non-Asian	76/80 (95.0%)	38/39 (97.4%)	−3.0% (−13.1% to 7.2%)
Baseline HBV DNA[b]			
< 7 $\log_{10}$ IU/mL	221/230 (96.1%)	107/116 (92.2%)	3.8% (−1.9% to 9.6%)
≥ 7 $\log_{10}$ IU/mL	47/55 (85.5%)	23/24 (95.8%)	−10.4% (−25.2% to 4.5%)
Oral Antiviral Treatment Status			
Treatment Experienced	56/60 (93.3%)	28/30 (93.3%)	0.2% (−12.4% to 12.7%)
Treatment Naive	212/225 (94.2%)	102/110 (92.7%)	1.6% (−4.3% to 7.6%)
Region			
East Asia	110/114 (96.5%)	58/64 (90.6%)	6.1% (−2.8% to 15.0%)
Europe	69/73 (94.5%)	35/36 (97.2%)	−2.7% (−13.8% to 8.4%)
North America	46/53 (86.8%)	28/30 (93.3%)	−6.9% (−22.8% to 8.9%)
Other	43/45 (95.6%)	9/10 (90.0%)	9.0% (NC)
Study Drug Adherence (%)[c]			
< 95	3/4 (75.0%)	5/6 (83.3%)	−5.0% (NC)
≥ 95	265/281 (94.3%)	125/134 (93.3%)	1.2% (−4.2% to 6.6%)
Genotype			
A/D	98/105 (93.3%)	46/48 (95.8%)	−2.9% (−12.3% to 6.6%)
B/C	165/175 (94.3%)	79/87 (90.8%)	3.5% (−4.1% to 11.2%)
Other	5/5 (100.0%)	5/5 (100.0%)	NC (NC)
Baseline ALT by Central Lab Normal Range			
≤ ULN	46/49 (93.9%)	17/19 (89.5%)	5.5% (NC)
> ULN	222/236 (94.1%)	113/121 (93.4%)	0.8% (−5.0% to 6.6%)
Baseline FibroTest Score			
< 0.75	237/249 (95.2%)	110/119 (92.4%)	3.2% (−2.6% to 9.1%)
≥ 0.75	27/31 (87.1%)	19/20 (95.0%)	−6.2% (−29.3% to 17.0%)

NC = not calculable; Prop Diff = difference in proportions

The Week 48 window was between Days 322 and 363 (inclusive).

a Difference in response rates and its 95% CI were calculated based on the Mantel-Haenszel proportions adjusted by baseline HBV DNA categories and oral antiviral treatment status strata (if not the subgroup factor).

b For this subgroup analysis, 2-level baseline HBV DNA categories (< 7 log10 IU/mL and ≥ 7 log10 IU/mL) were used.

c Study drug adherence subgroups analysis was based on the adherence up to Week 48 visit for active study drug.

Possible imbalances between the TAF and TDF groups for subjects enrolled in the European and North American regions were explored using four identified risk factors for treatment failure. The imbalances in demographics and disease characteristics do not seem to explain the differences in virologic outcomes at Week 48 between TAF and TDF in these regions.

Table 55. Baseline Characteristics among regions by treatment Group – Safety Analysis Set

	North America		Europe	
	TAF 25 mg (N=53)	TDF 300 mg (N=30)	TAF 25 mg (N=73)	TDF 300 mg (N=36)
Proportion of subjects with baseline HBV DNA ≥ 8 log10 (IU/mL)	3 (5.7)	2 (6.7%)	5 (6.8%)	2 (5.6%)
Proportion of subjects with baseline BMI ≥ 25 (kg/m2)	23 (43.4%)	10 (33.3%)	45 (61.6%)	22 (61.1%)
Proportion of treatment experienced subjects	13 (24.5%)	9 (30.0%)	19 (26.0%)	8 (22.2%)
Proportion of subjects with baseline ALT ≤ 5XULN (AASLD)	43 (81.1%)	27 (90.0%0	57 (78.1%)	27 (75.0%)

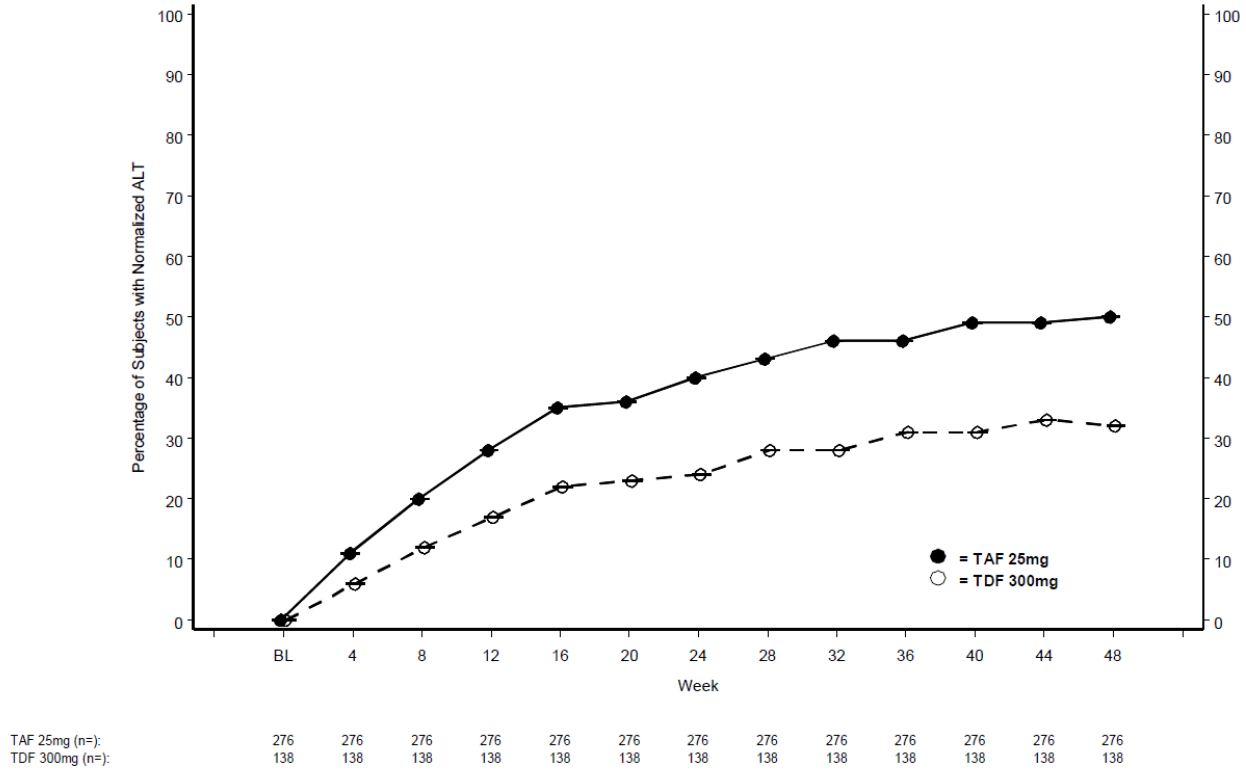
The Week 72 rates for HBV DNA < 29 IU/mL in North America were more similar than results seen at Week 48 but rates for Europe showed a similar small magnitude of difference as observed at Week 48.

- **Europe:** TAF 91.8%; TDF 94.4%
- **North America:** TAF 83.0%; TDF 86.7%

No patient experienced HBsAg loss by Week 48. There were very small and similar decreases in mean HBsAg level in the two treatment groups at Weeks 12, 24 and 48.

At Week 48, the mean (SD) change in ALT from the baseline value was −66.8 [90.58] U/L in the TAF group and −62.4 [84.85] U/L in the TDF group (p = 0.68). Using the central laboratory criteria, the percentage with normalised ALT was numerically higher for the TAF group from Weeks 4 through 48. At Week 48, rates of ALT normalisation were similar between treatment groups by the M = F method for the FAS. Using the AASLD criteria (≤ 30 U/L for men and ≤ 19 U/L for women) the percentage with normalised ALT was significantly higher in the TAF group from Week 8 onward using the M = F method.

Figure 17. GS-US-320-0108: Proportion of Subjects with ALT Normalization by Visit, Missing = Failure (AASLD Criteria) (Full Analysis Set)

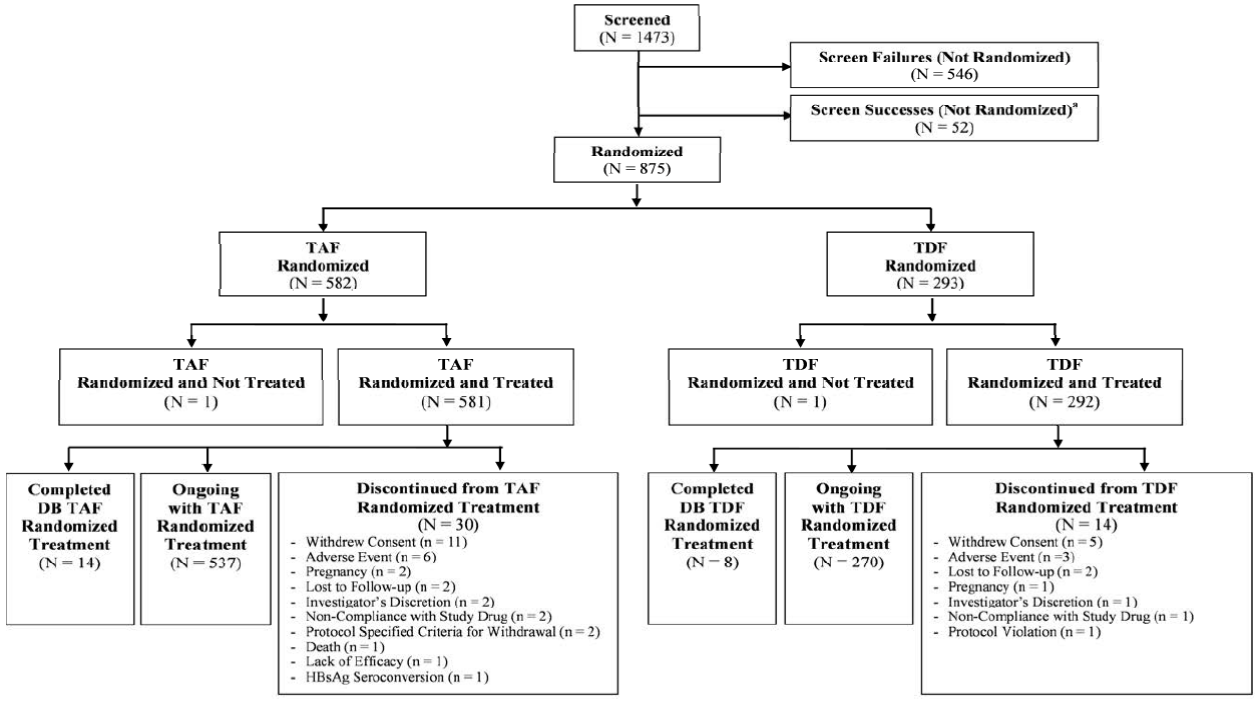


At Week 48, the mean (SD) change from baseline in FibroTest scores was −0.05 (0.108) in the TAF group and −0.03 (0.131) in the TDF group (LSM difference: −0.03, 95% CI: −0.05 to 0.00; p = 0.028).

Results – study 0110

There were 875 patients randomised and 873 received at least one dose of study drugs.

Figure 18. GS-US-320-0110: Disposition of Study Subjects



Demographic and baseline characteristics were similar between the treatment groups. Five participants were Black and 17% were White (TAF: 16.5%; TDF 18.2%). There were 18 patients (14 TAF) who were HBeAg positive at screening but were negative at baseline and 14 of the 18 were HBeAg negative at all subsequent visits. These 18 were excluded from the analysis of HBeAg loss/seroconversion but were included in the FAS. Other baseline data indicate that not all patients still met the eligibility criteria applied at screening for ALT but the minimum for HBV DNA appears to have been met. At baseline 8% TAF and 7.8% TDF patients had FibroTest scores > 0.75.

Table 56. GS-US-320-0110: Baseline Disease Characteristics (Safety Analysis Set)

	TAF 25 mg (N = 581)	TDF 300 mg (N = 292)	Total (N = 873)	TAF 25 mg vs. TDF 300 mg P-Value[a]
HBV DNA (log_{10} IU/mL)				
N	581	292	873	0.51
Mean (SD)	7.6 (1.34)	7.6 (1.41)	7.6 (1.36)	
Median	7.9	8.0	7.9	
Q1, Q3	6.9, 8.5	6.8, 8.6	6.9, 8.6	
Min, Max	2.5, 9.9	2.6, 9.9	2.5, 9.9	
HBV DNA Categories				
< 8 log_{10} IU/mL	309 (53.2%)	150 (51.4%)	459 (52.6%)	0.61
≥ 8 log_{10} IU/mL	272 (46.8%)	142 (48.6%)	414 (47.4%)	
ALT (U/L)				
N	581	292	873	0.64
Mean (SD)	117 (105.1)	125 (128.2)	120 (113.4)	
Median	85	86	85	
Q1, Q3	61, 139	57, 137	60, 138	
Min, Max	13, 1160	21, 872	13, 1160	
ALT Level[b]				
≤ ULN	44 (7.6%)	24 (8.2%)	68 (7.8%)	0.16
> ULN – 5 × ULN	470 (80.9%)	225 (77.1%)	695 (79.6%)	
> 5 × ULN - 10 × ULN	56 (9.6%)	30 (10.3%)	86 (9.9%)	
> 10 × ULN	11 (1.9%)	13 (4.5%)	24 (2.7%)	
HBeAg Status[c]				
Positive	567 (97.6%)	288 (98.6%)	855 (97.9%)	0.31
Negative	14 (2.4%)	4 (1.4%)	18 (2.1%)	

HBV Genotype Group				
A	39 (6.7%)	25 (8.6%)	64 (7.3%)	0.78
B	100 (17.2%)	48 (16.4%)	148 (17.0%)	
C	303 (52.2%)	152 (52.1%)	455 (52.1%)	
D	134 (23.1%)	63 (21.6%)	197 (22.6%)	
E	2 (0.3%)	1 (0.3%)	3 (0.3%)	
F	3 (0.5%)	2 (0.7%)	5 (0.6%)	
Unknown	0	1 (0.3%)	1 (0.1%)	
Years Positive for HBV				
N	579	290	869	0.80
Mean (SD)	6.3 (6.24)	6.3 (6.33)	6.3 (6.27)	
Median	4.0	4.0	4.0	
Q1, Q3	2.0, 8.0	2.0, 8.0	2.0, 8.0	
Min, Max	1.0, 43.0	0.0, 36.0	0.0, 43.0	
Previous Oral Nucleoside/Nucleotide Treatment[d]				
Yes	151 (26.0%)	77 (26.4%)	228 (26.1%)	0.90
No	430 (74.0%)	215 (73.6%)	645 (73.9%)	
Cirrhosis History				
Yes	41 (9.8%)	24 (11.3%)	65 (10.3%)	0.58
No	376 (90.2%)	189 (88.7%)	565 (89.7%)	
Indeterminate/Unknown	164	79	243	
FibroTest Score				
N	566	282	848	0.20
Mean (SD)	0.34 (0.227)	0.32 (0.225)	0.34 (0.227)	
Median	0.29	0.25	0.27	
Q1, Q3	0.16, 0.48	0.14, 0.47	0.15, 0.48	
Min, Max	0.04, 0.98	0.03, 0.99	0.03, 0.99	
eGFR by CG (mL/min)				
N	581	292	873	0.53
Mean (SD)	113.7 (27.78)	112.5 (29.33)	113.3 (28.29)	
Median	108.6	109.2	109.2	
Q1, Q3	94.9, 128.4	93.0, 128.7	94.4, 128.4	
Min, Max	54.6, 235.8	39.6, 227.4	39.6, 235.8	

The primary analysis just met the pre-defined non-inferiority margin. The pre-defined non-inferiority margin was also met in the PP population, in which the percentages with < 29 IU/mL at Week 48 were TAF 66.9% vs. TDF 69.0% (difference -2.6%, 95% CI: −8.9% to 3.6%).

GS-US-320-0110: HBV DNA Outcome at Week 48 Using HBV DNA Cut-off at < 29 IU/mL, Missing = Failure (Full Analysis Set)

	TAF 25 mg (N = 581)	TDF 300 mg (N = 292)	TAF 25 mg vs TDF 300 mg	
			P-Value[a]	Prop Diff (95% CI)[b]
Success				
HBV DNA < 29 IU/mL	371 (63.9%)	195 (66.8%)	0.25	−3.6% (−9.8% to 2.6%)
Failure				
HBV DNA ≥ 29 IU/mL	183 (31.5%)	88 (30.1%)		
Discontinued Study Drug Due to Lack of Efficacy	1 (0.2%)	0		
Discontinued Study Drug Due to AE/Death[c]	6 (1.0%)	3 (1.0%)		
Discontinued Study Drug Due to Other Reasons[d]	19 (3.3%)	6 (2.1%)		
Missing Data During Window but on Study Drug	1 (0.2%)	0		

Prop Diff = difference in proportions

The Week 48 window was between Day 322 and 363 (inclusive).

a P-value for the superiority test comparing the percentages of HBV DNA < 29 IU/mL was from the Cochran-Mantel-Haenszel test stratified by baseline HBV DNA categories and oral antiviral treatment status strata.

b Difference in the proportion between treatment groups and its 95% CI were calculated based on the Mantel-Haenszel proportions adjusted by baseline HBV DNA categories and oral antiviral treatment status strata.

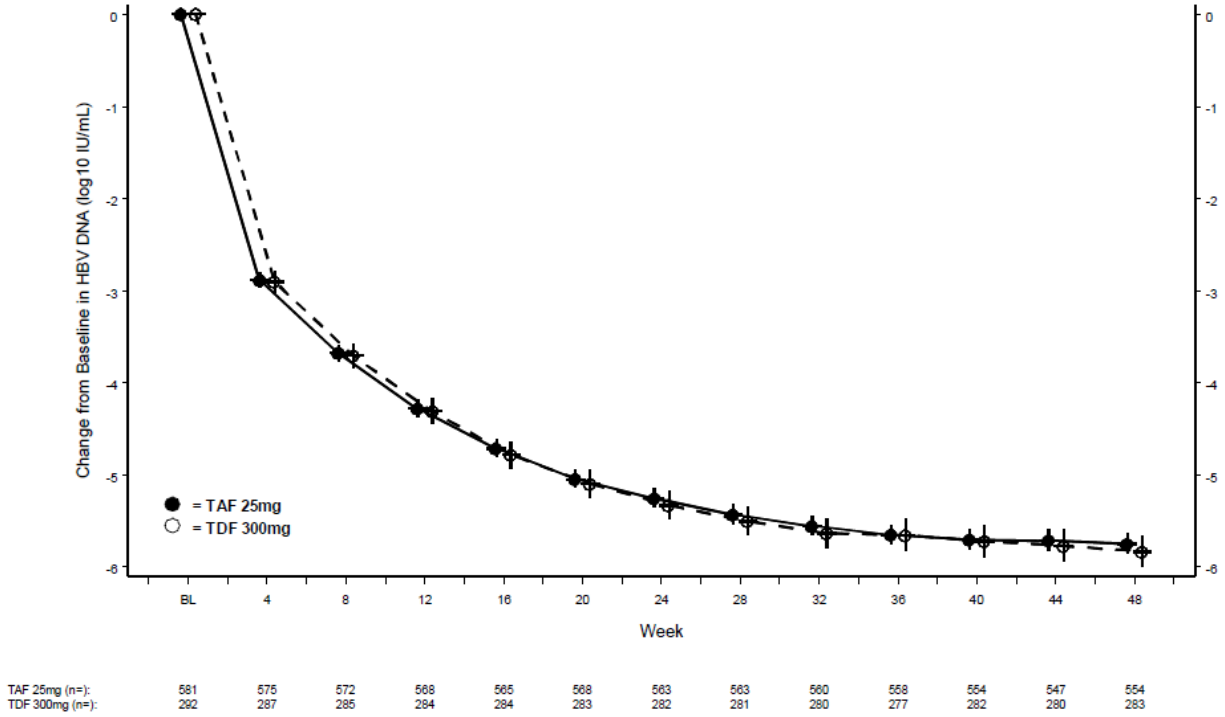
c No deaths occurred in any subject on treatment. Subject 09695-5212 discontinued study drugs and died 3 days after the last dose (Appendix 16.2, Listing 22). After the database was closed, the investigator clarified that the subject discontinued due to coma.

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, and pregnancy.

The proportions with HBV DNA < 29 IU/mL (target not detected) were the same by both M = F (3.1%) and M = E analyses (3.2%).

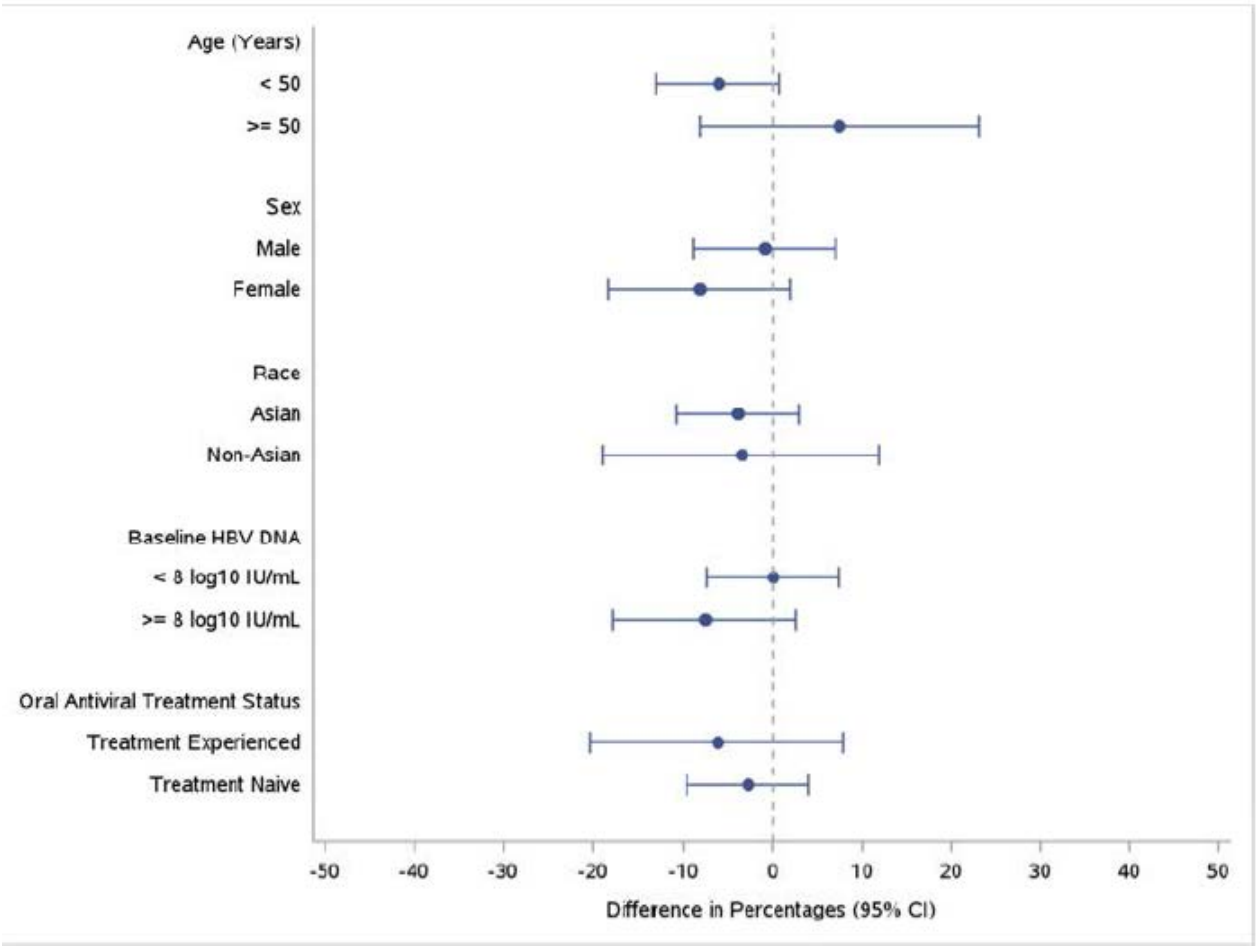
The kinetics and magnitude of HBV DNA decline was similar between treatment groups. Between treatment groups, generally no significant differences in the mean change from baseline at Weeks 4, 24, and 48 were observed with the exception of the subgroup with baseline HBV DNA ≥ 7 to < 8 $\log_{10}$ IU/mL (−5.96 and −6.11 $\log_{10}$ IU/mL for the TAF and TDF groups, respectively; p = 0.031).

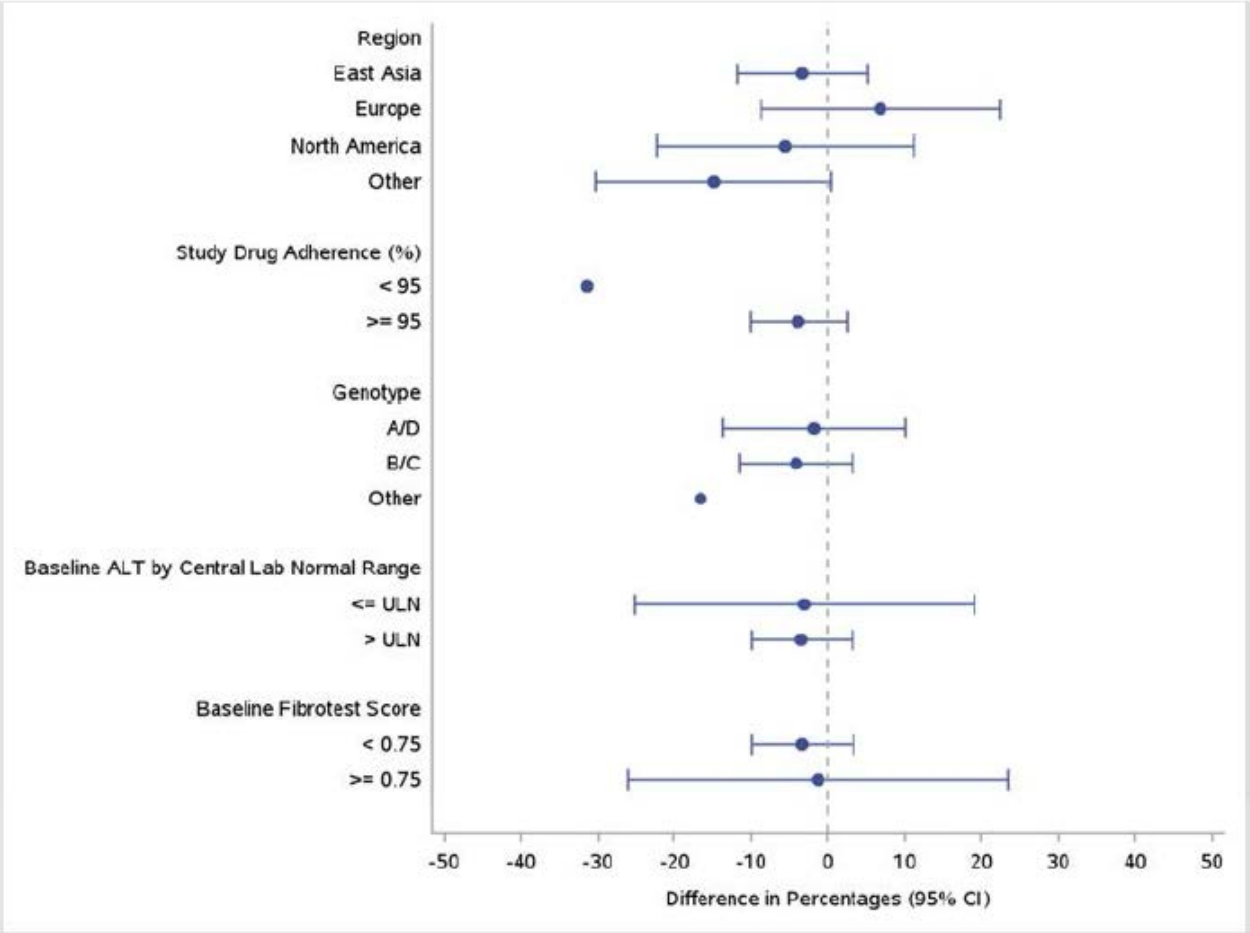
Figure 19. GS-US-320-0110: Mean and 95% CIs of Change from Baseline by Visit in HBV DNA (log_{10} IU/mL) (Full Analysis Set)



At Week 48, the rates of HBV DNA ≤ 29 IU/mL for subgroups did not differ statistically between TAF and TDF groups but most numerically favoured TDF, reflecting the overall primary analysis.

Figure 20. GS-US-320-0110: Forest Plot of Treatment Difference in Proportion of Subjects with HBV DNA < 29 IU/mL at Week 48 (Full Analysis Set)





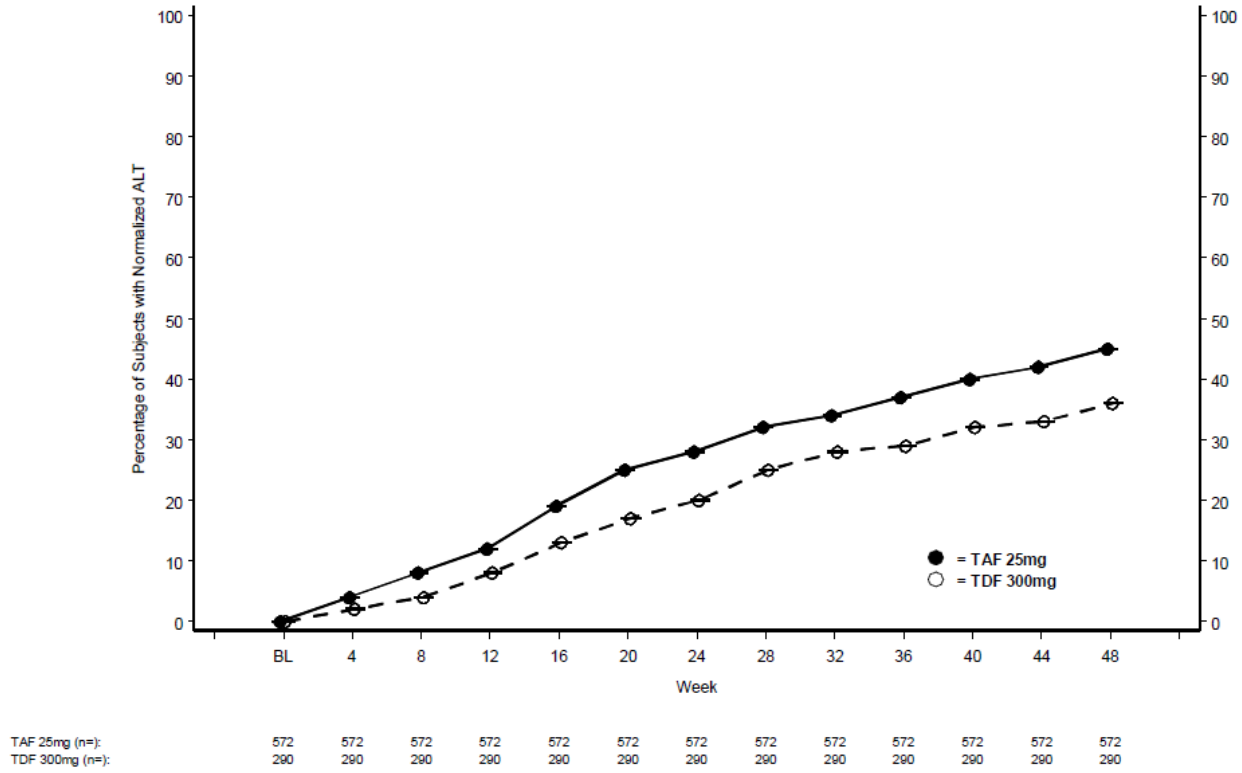
Difference in response rates and its 95% CI were calculated based on the Mantel-Haenszel proportions adjusted by baseline HBV DNA categories and oral antiviral treatment status strata (if not the subgroup factor).

The 95% CIs of the difference in response rates were not provided for some subgroups since they were not calculable.

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

Using the central laboratory criteria, the percentage with normalised ALT was numerically higher for the TAF group from Weeks 4 through 48. At Week 48, rates of ALT normalisation were similar between treatment groups by the M = F method for the FAS. Using the AASLD criteria the percentage with normalised ALT was significantly higher in the TAF group from Week 8 onward using the M = F method.

Figure 21. GS-US-320-0110: Proportion of Subjects with ALT Normalization by Visit, Missing = Failure (AASLD Criteria) (Full Analysis Set)



At Week 48, the mean (SD) change in ALT from the baseline value was −84.4 [110.16] U/L in the TAF group and −84.2 [127.19] U/L in the TDF group (p = 0.95).

Four TAF patients and one TDF patient had HBsAg loss at Week 48 and 3 of the 4 TAF patients also had HBsAg seroconversion. Also, 78 (13.8%) in the TAF group and 34 (11.9%) in the TDF group had HBeAg loss at Week 48. HBeAg loss with seroconversion occurred in 58 (10.3%) and 23 (8.1%), respectively.

At Week 48, the mean (SD) change in FibroTest scores from the baseline value was −0.07 (0.127) in the TAF group and −0.04 (0.121) in the TDF group (LSM difference: −0.03, 95% CI: −0.04 to -0.01; p = 0.007).

Details of failures across studies 0108 and 0110

Of the 1298 patients in FAS across Phase 3 studies, 282 (TAF 190; TDF 92) had HBV DNA ≥ 29 IU/mL at Week 48. Of the 282 patients, 30 (TAF 17; TDF 13) had virologic breakthrough (29 at Week 48 and one at early discontinuation at Week 24). Univariate analysis identified higher HBV DNA level (≥ 8 $\log_{10}$ IU/mL), hepatitis B e antigen (HBeAg) positive status, prior OAV, decreased FibroTest (FT) score and HBV genotype D as being predictive of virologic failure. Multivariate analysis demonstrated that the 6 factors most significantly associated with virologic failure (p < 0.001) were baseline HBV DNA ≥ 8 $\log_{10}$ IU/mL, baseline HBeAg seropositivity, baseline BMI ≥ 25 kg/m^2, prior treatment experience, HBV genotype D and baseline ALT ≤ 5 × ULN by AASLD criteria. Treatment with TAF 25 mg was not identified as an independent predictor of virologic failure.

Table 57. Multivariate predictors of Virologic Failure – Full Analysis Set

Predictor	Contrast	Odd ratio	%95 Lower CI	%95 Upper CI	p-value
Baseline HBV DNA category	≥ 8 log vs < 8 log	7.16	4.98	10.29	<.001
Baseline HBeAg	Positive vs negative	10.13	5.27	19.46	<.001
Genotype D	Y vs N	4.05	2.54	6.46	<.001
Baseline BMI category	≥25 vs 25 (normal/under wt)	2.52	1.77	3.59	<.001
Oral antiviral trt status	Treatment experienced vs treatment naive	2.25	1.52	3.33	<.001
Baseline ALT category (AASLD)	≤ 5x ULN vs > 5 x ULN	2.05	1.36	3.07	<.001
Asian	Y vs N	2.20	1.29	3.73	0.004

Using the 6 factors identified in multivariate analysis, subjects in the TAF and TDF treatment groups were classified by the number of risk factors present at baseline (0−1 risk factors, 2−3 risk factors and 4−6 risk factors).

Table 58. HBV DNA outcome at Week 48 (HBV DNA at 29 IU/mL) Full analysis set by baseline composite risk factors

	TAF 25 mg (N=839)			TDF 300 mg (N=417)		
	0-1 risk factor (N=154	2-3 risk factor (N=512)	4-6 risk factor (N=173)	0-1 risk factor (N=71)	2-3 risk factor (N=260)	4-6 risk factor (N=86)
Virologic success at Week 48	151 (98.1%)	426 (83.24%)	62 (35.8%)	70 (98.6%)	219 (84.2%)	36 (41.9%)
Virologic failure at Week 48	3 (1.9%)	86 (16.8%)	111 (64.2%)	1 (1.4%)	41 (15.8%)	50 (58.1%)

In both the TAF and TDF groups, rates of virologic failure increased in the presence of more risk factors, most markedly in the subgroup with 4 or more risk factors. Although the presence of multiple predictors of virologic failure reduces the likelihood of achieving virologic suppression consistently to a larger extent for TAF, the difference between the treatments is small and does not suggest a relevant difference in efficacy in the subgroups.

Update of efficacy to Week 72

Across both studies 70.2% patients remained on double-blind study drug treatment and 22.5% had completed double-blind study drug treatment through the Week 72 data-cut date. A total of 32 subjects had discontinued double-blinded study drug between the Week 48 and 72 data cut-off dates. The 32 comprised 2 TAF and 4 TDF subjects in 0108 and 17 TAF and 9 TDF subjects in 0110.

There were no differences between treatments in reasons to discontinue. Similar rates of HBV DNA suppression were achieved in the TAF and TDF groups when assessed using the M = F method at Week 72.

Table 59. GS-US-320-0108 and GS-US-320-0110: HBV DNA Outcome at Weeks 48 and 72 Using HBV DNA of < 29 IU/mL, Missing = Failure (Full Analysis Set)

	GS-US-320-0108		GS-US-320-0110	
	TAF 25 mg (N = 285)	TDF 300 mg (N = 140)	TAF 25 mg (N = 581)	TDF 300 mg (N = 292)
Week 48				
HBV DNA < 29 IU/mL	269 (94.4%)	131 (93.6%)	371 (63.9%)	195 (66.8%)
P-Value[a]	0.55		0.25	
Difference in Proportions (95% CI)[b]	1.4% (−3.8% to 6.6%)		−3.6% (−9.8% to 2.6%)	
HBV DNA ≥ 29 IU/mL	7 (2.5%)	4 (2.9%)	183 (31.5%)	88 (30.1%)
No Virologic Data at Week 48	9 (3.2%)	5 (3.6%)	27 (4.6%)	9 (3.1%)
Discontinued Study Drug Due to Lack of Efficacy	0	0	1 (0.2%)	0
Discontinued Study Drug Due to AE/Death	3 (1.1%)	1 (0.7%)	6 (1.0%)	3 (1.0%)
Discontinued Study Drug Due to Other Reasons[c]	6 (2.1%)	4 (2.9%)	19 (3.3%)	6 (2.1%)
Missing Data During Window but on Study Drug	0	0	1 (0.2%)	0
Week 72				
HBV DNA < 29 IU/mL	264 (92.6%)	129 (92.1%)	416 (71.6%)	210 (71.9%)
P-Value[a]	0.84		0.78	
Difference in Proportions (95% CI)[b]	0.6% (−5.3% to 6.4%)		−0.9% (−7.0% to 5.2%)	
HBV DNA ≥ 29 IU/mL	8 (2.8%)	2 (1.4%)	131 (22.5%)	59 (20.2%)
No Virologic Data at Week 72	13 (4.6%)	9 (6.4%)	34 (5.9%)	23 (7.9%)
Discontinued Study Drug Due to Lack of Efficacy	0	0	1 (0.2%)	0
Discontinued Study Drug Due to AE/Death	4 (1.4%)	2 (1.4%)	8 (1.4%)	4 (1.4%)
Discontinued Study Drug Due to Other Reasons[c]	9 (3.2%)	7 (5.0%)	25 (4.3%)	16 (5.5%)
Missing Data During Window but on Study Drug	0	0	0	3 (1.0%)

a P-value for the superiority test comparing the percentages of HBV DNA < 29 IU/mL was from the CMH test stratified by baseline HBV DNA categories and OAV treatment status strata.

b Difference in the proportion between treatment groups and its 95% CI were calculated based on the MH proportions adjusted by baseline HBV DNA categories and OAV treatment status strata.

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

The Week 48 window was between Days 322 and 363 (inclusive), and the Week 72 window was between Days 476 and 531 (inclusive).

Percentages with HBV DNA < 29 IU/mL at Week 72 were:

- Study GS-US-320-0108: TAF 92.6%, TDF 92.1%; difference (baseline stratum-adjusted): 0.6%, 95% CI: −5.3% to 6.4%
- Study GS-US-320-0110: TAF 71.6%, TDF 71.9%; difference (baseline stratum-adjusted): −0.9%, 95% CI: −7.0% to 5.2%.

Table 60. GS-US-320-0110: Proportion of Subjects with HBV DNA < 29 IU/mL at Weeks 48 and 72 by Baseline HBV DNA Subgroup, Missing = Failure (Full Analysis Set)

	TAF 25 mg (N = 581)	TDF 300 mg (N = 292)
Week 48		
All Subjects	371/581 (63.9%)	195/292 (66.8%)
Baseline HBV DNA		
< 7 $\log_{10}$ IU/mL	132/150 (88.0%)	60/77 (77.9%)
≥ 7 to < 8 $\log_{10}$ IU/mL	122/159 (76.7%)	63/73 (86.3%)
≥ 8 $\log_{10}$ IU/mL	117/272 (43.0%)	72/142 (50.7%)
Week 72		
All Subjects	416/581 (71.6%)	210/292 (71.9%)
Baseline HBV DNA		
< 7 $\log_{10}$ IU/mL	131/150 (87.3%)	61/77 (79.2%)
≥ 7 to < 8 $\log_{10}$ IU/mL	134/159 (84.3%)	63/73 (86.3%)
≥ 8 $\log_{10}$ IU/mL	151/272 (55.5%)	86/142 (60.6%)

In both studies, the kinetics of HBV DNA decline as assessed by the proportion of subjects with HBV DNA < 29 IU/mL over 72 weeks were similar between treatment groups.

In GS-US-320-0108, a slightly lower percentage had HBV DNA levels < 29 IU/mL in both treatment groups at Week 72 compared with Week 48. This decrease reflected a higher number (difference = 8) with no virologic data at Week 72 due to premature discontinuation of study drug compared with Week 48. However, in GS-US-320-0110, a higher percentage had HBV DNA levels < 29 IU/mL in both treatment groups at Week 72 compared with Week 48, indicating that some HBeAg positive subjects required longer treatment to achieve virologic suppression. At Week 72 using the M=E method the percentages with ≤ 29 IU/mL were 76.1% in the TAF and 78.1% in the TDF group (difference [baseline stratum-adjusted] −2.8% [95% CI: −8.6% to 3.1%], p = 0.35). The mean change from baseline in HBV DNA at Week 72 was not significantly different between the TAF and TDF groups in either study.

Proportions with ALT normalisation at Weeks 48 and 72 using the central laboratory criteria and the AASLD criteria continued to show advantages for TAF over TDF as shown below.

Table 61. GS-US-320-0108 and GS-US-320-0110: Proportion of Subjects with ALT Normalization at Weeks 48 and 72, Missing = Failure (Full Analysis Set)

	GS-US-320-0108		GS-US-320-0110	
Normalized ALT	**TAF 25 mg**	**TDF 300 mg**	**TAF 25 mg**	**TDF 300 mg**
Week 48				
Central Laboratory Criteria[a]	196/236 (83.1%)	91/121 (75.2%)	384/537 (71.5%)	179/268 (66.8%)
P-Value[b]	0.076		0.18	
Difference in Proportions (95% CI)[c]	8.0% (−1.3% to 17.2%)		4.6% (−2.3% to 11.4%)	
AASLD Criteria[d]	137/276 (49.6%)	44/138 (31.9%)	257/572 (44.9%)	105/290 (36.2%)
P-Value[b]	< 0.001		0.014	
Difference in Proportions (95% CI)[c]	17.9% (8.0% to 27.7%)		8.7% (1.8% to 15.6%)	
Week 72				
Central Laboratory Criteria[a]	196/236 (83.1%)	93/121 (76.9%)	392/537 (73.0%)	173/268 (64.6%)
P-Value[b]	0.19		0.015	
Difference in Proportions (95% CI)[c]	5.9% (−3.3% to 15.0%)		8.3% (1.4% to 15.2%)	
AASLD Criteria[d]	137/276 (49.6%)	55/138 (39.9%)	279/572 (48.8%)	112/290 (38.6%)
P-Value[b]	0.057		0.005	
Difference in Proportions (95% CI)[c]	9.9% (−0.2% to 20.0%)		10.1% (3.1% to 17.1%)	

AASLD = American Association for the Study of Liver Diseases

a For the analysis of ALT normalization using the central laboratory criteria, only subjects with ALT > ULN of the central laboratory range at baseline were included. Central laboratory ULN for ALT are as follows: ≤ 43 U/L for males 18 to < 69 years and ≤ 35 U/L for males ≥ 69 years; ≤ 34 U/L for females 18 to < 69 years and ≤ 32 U/L for females ≥ 69 years.

b P-value was from the CMH tests stratified by baseline HBV DNA categories and OAV treatment status strata.

c Difference in the proportion between treatment groups and its 95% CI were calculated based on the MH proportions adjusted by baseline HBV DNA categories and OAV treatment status strata.

d For the analysis of ALT normalization using the AASLD criteria, only subjects with ALT > ULN of the AASLD criteria (> 30 U/L males and > 19 U/L females) at baseline were included [Lok et al 2009], [Terrault et al 2015].

Serology was not analysed at Week 72 but the serology results from Week 64 are reported.

In GS-US-320-0108, one TAF subject (0.4%) experienced HBsAg loss at Week 64 without seroconversion. This 44 year-old Asian female with genotype A CHB had HBsAg seroconversion at Week 80.

In GS-US-320-0110, two TAF subjects had HBsAg loss between Weeks 48 and 64 so that 6/576 (1.0%) TAF and 3/288 (1.0%) TDF subjects had HBsAg loss at Week 64 (M = F analysis).

HBsAg seroconversion was achieved by 2 TAF subjects between Weeks 48 and 64 to give overall rates from baseline of 5 (0.9%) in the TAF group and 0 in the TDF group (M = F analysis).

At Week 64, 90/565 (15.9%) in the TAF group and 42/285 (14.7%) in the TDF group experienced HBeAg loss (M = F analysis; p = 0.68).

HBeAg seroconversion was achieved in 71/565 (12.6%) in the TAF group and 28/285 (9.8%) in the TDF group (M = F analysis; p = 0.25).

Virology data pooled across studies 0108 and 0110

For the baseline analyses conducted i, subjects were assigned to a resistance category using the classifications defined in Table 68.

Table 62. Resistance Mutation Categories Defined by Phenotypic Analyses

Resistance Category		Mutations
Primary Resistance Mutations	Lamivudine Resistance[a]	rtM204V/I/S
	Adefovir Resistance[b]	rtA181T/V, rtN236T
	Entecavir Resistance[c]	rtM204V/I ± rtT184X[e] ± rtS202X ± rtM250X
Other Mutations[d]		rtL80V/I, rtV173L, rtL180M, rtT184X, rtA194T, rtS202X, rtM250X, unknown variants

Baseline virology analyses

The HBV genotype distribution was comparable between treatment groups, with genotype C predominant in both groups (47.5% overall, 617/1298; see below). Proportions with < 29 IU/mL at Week 48 was analysed for genotypes A-D, there being too few or no patients with other genotypes. In the TAF group the proportion with < 29 IU/mL at Week 48 was significantly lower for genotype D vs. genotypes A-C. The rate in genotype D patients treated with TAF was also significantly lower than the rates for patients with genotypes B and C in the TDF group.

Table 63. TAF Integrated Analysis: Proportion of Subjects with HBV DNA < 29 IU/mL at Week 48 by Baseline HBV Genotype

	TAF		TDF		
Genotype[a]	Mean Baseline HBV DNA (log_{10} IU/mL ± SD)	Number of Subjects with HBV DNA < 29 IU/mL, n (%)[b,c]	Mean Baseline HBV DNA (log_{10} IU/mL ± SD)	Number of Subjects with HBV DNA < 29 IU/mL, n (%)[b,c]	p-value[c]
A	7.15 ± 1.54	45/54 (83.3)	7.52 ± 1.55	23/31 (74.2)	0.3998
B	7.08 ± 1.52	118/160 (73.8)	6.68 ± 1.66	70/88 (79.5)	0.3541
C	6.85 ± 1.54	328/418 (78.5)	7.03 ± 1.47	156/199 (78.4)	1.0
D	7.12 ± 1.74	140/224 (62.5)	7.16 ± 1.80	69/105 (65.7)	0.6239

a Only the predominant viral genotypes are presented due to small numbers of genotypes E, F, G, and H

b Number of subjects as a proportion of total subjects in that category

c Comparison of treatment outcome between treatment groups performed using Fisher's Exact Test in a pair-wise fashion

Most (89.2%) HBV at baseline were classified as wild type (TAF: 89.5% wild type, 10.5% mutant; TDF: 88.7% wild type, 11.3% mutant). A higher percentage of HBV in the OAV-naive (92.4%) were classified as wild type vs. OAV-experienced (78.4%). The remaining 10.8% (140/1298) were found to have mutations in pol/RT, with a higher rate for the OAV-experienced (21.6% vs. 7.6%).

- Primary resistance mutations were observed in 5.4% (70/1298), with a higher percentage in the OAV-experienced (17.2% vs. 1.9%). LAM-R mutations were predominant (in 32/1298; 2.5%).
- The other 70 (5.4%) had other mutations, with similar percentages for OAV-naive and OAV-experienced (5.7% vs. 4.4%).
- The distributions of primary and other resistance mutations were similar between treatment groups (TAF: 4.7% primary and 5.8% other mutations; TDF: 6.7% primary and 4.6% other mutations).

The impact of baseline resistance mutations as determined by INNO-LiPA on proportions achieving HBV DNA < 29 IU/mL at Week 48 was assessed for all patients. No significant differences were observed in the proportions with HBV DNA < 29 IU/mL in the TAF vs. TDF group at Week 48 ($p > 0.05$).).

Table 64. TAF Integrated Analysis: Proportion of Subjects with Treatment Success at Week 48 by Baseline Resistance Mutations

	TAF				TDF				
Resistance Category[a]	Mean Baseline HBV DNA[b]	HBV DNA < 29 IU/mL, n (%)[c,d]	HBV DNA > 29 IU/mL, n (%)[c,d]	ED or Missing, n (%)[c,d]	Mean Baseline HBV DNA[b]	HBV DNA < 29 IU/mL, n (%)[c,d]	HBV DNA > 29 IU/mL, n (%)[c,d]	ED or Missing, n (%)[c,d]	p-value[c]
Wild Type	6.94 ± 1.60	587/775 (75.7)	161/775 (20.8)	27/775 (3.5)	7.02 ± 1.63	293/383 (76.5)	77/383 (20.1)	13/383 (3.4)	0.8264
Primary Resistance Mutation(s)[a]	7.54 ± 1.26	19/41 (46.3)	17/41 (41.5)	5/41 (12.2)	7.29 ± 1.33	18/29 (62.1)	10/29 (34.5)	1/29 (3.4)	0.2301
Other Mutations[a]	7.14 ± 1.67	33/50 (66.0)	12/50 (24.0)	5/50 (10.0)	6.52 ± 1.85	14/20 (70.0)	5/20 (25.0)	1/20 (5.0)	1.0

ED = Early Discontinuation

a Primary resistance mutations and other mutations are defined in Table 68.

b Mean baseline HBV DNA as log10 IU/mL ± SD

c Comparison between treatment groups performed using Fisher's Exact Test in a pair-wise fashion

d Number of subjects as a proportion of total subjects in that category

In both treatment groups, those with primary resistance mutations were less likely to achieve HBV DNA < 29 IU/mL (TAF 46.3% vs. TDF 62.1%). The lower rate of HBV DNA suppression at Week 48 in those with baseline primary resistance mutations was not associated with increased rates of virologic breakthrough. The applicant proposed that the lower proportion with primary resistance mutations achieving HBV DNA < 29 IU/mL in the TAF treatment group may reflect in some part the higher proportion with early discontinuation or missing data vs. the TDF group.

Table 65. Percentage of Subjects Qualifying for Resistance Testing at Week 48 by Baseline Resistance Mutation

Resistance Category[a]	TAF Virologic Breakthrough, n (%)[b]	TDF Virologic Breakthrough, n (%)[b]
Wild type	15/775 (1.9)	11/383 (2.9)
Primary Resistance Mutation(s)[a]	1/41 (2.4)	1/29 (3.4)
Other Mutations[a]	0/50	1/20 (5.0)

a Primary resistance mutations and other mutations are defined in Table 68.

b Number of subjects as a proportion of total subjects in that category

Week 48 Virology Analyses

In the TAF group 24/866 866 (2.8%) qualified for sequence analysis, including 16 with virologic breakthrough and 8 discontinuing at or after Week 24 with HBV DNA ≥ 69 IU/mL. Of the 24, 15 had no changes detected in the pol/RT sequence from baseline, 4 were unable to be sequenced and 5 had polymorphic site substitutions.

Table 66. TAF Integrated analyses: sequence analysis in the TAF group through Week 48

Subject	Baseline HBV DNA[a]	HBV DNA at Qualifying Time point[a]	Changes in HBV pol/RT at Qualifying Time point
2826-4527	8.24	2.31 (Week 36)	rtD134E, rtM309K
4296-5147	4.62	4.68 (Week 48)	rtS256S/C
8017-4565	8.21	2.61 (Week 48)	rtI80L/I, rtI91I/L, rtI204M/I, rtE271A/E
8758-5188	4.74	1.96 (Week 48)	rtR153Q
9035-5187	4.94	2.21 (Week 48)	rtS13N/S, rtS117S/P, rtL267Q/L, rtL269I/L

a HBV DNA expressed as log10 IU/mL

In the TDF group,_14/432 (3.2%) qualified for sequence analysis, including 13 with virologic breakthrough and one who discontinued at or after Week 24 with HBV DNA ≥ 69 IU/mL. Six of the 14 had no changes detected in the pol/RT sequence from baseline, 4 were unable to be sequenced, 2 had polymorphic site substitutions and 2 had conserved site substitutions.

Table 67. TAF Integrated analyses: sequence analysis in the TDF group through Week 48

Subject	Baseline HBV DNA[a]	HBV DNA at Qualifying Time point[a]	Changes in HBV pol/RT at Qualifying Time point[b]
1507-4546	8.07	3.00 (Week 48)	rtD134D/E, rtV214V/A, rtA317A/S
9035-4845	6.62	4.38 (Week 48)	rtR110R/G, rtL269I/L
5620-5225	7.68	2.29 (Week 48)	**rtQ67Q/H**, rtN118N/T, rtN123N/D, rtM207V/M
8569-5131	5.87	1.96 (Week 48)	**rtQ288Q/stop**

a HBV DNA expressed as log10 IU/mL b Conserved site substitutions noted in bold

Overall, no HBV pol/RT amino acid substitutions associated with resistance to TFV were detected through 48 weeks of the study in either treatment group.

Of the 38 reported above who qualified for population sequencing analysis through Week 48, 8 (0.9%) from the TAF group and 8 (1.9%) from the TDF group qualified for deep sequencing analyses. One ADV resistance-associated substitution was detected in one TDF patient (rtN236T) and two polymorphic substitutions in pol/RT were detected in two patients each (rtN123D in one TAF and one TDF patient and rtH124D in two TAF patients). All of the patients with these substitutions who remained on study through Week 48 achieved HBV DNA < 69 IU/mL with continued treatment. Thus, no substitutions associated with sustained virologic breakthrough were identified through 48 weeks of this study. Nine of the 38 patients (TAF and 4 TDF) qualified for phenotypic analysis. No patient in the TAF or TDF group had HBV with reduced susceptibility to TAF or tenofovir, respectively, through Week 48.

Summary of efficacy analysis

Table 68. HBV DNA efficacy parameters at Week 48[a]

	Study 108 (HBeAg-Negative)		Study 110 (HBeAg-Positive)	
	Vemlidy (N=285)	TDF (N=140)	Vemlidy (N=581)	TDF (N=292)
HBV DNA < 29 IU/mL	94%	93%	64%	67%
Treatment difference[b]	1.8% (95% CI = -3.6% to 7.2%)		-3.6% (95% CI = -9.8% to 2.6%)	
HBV DNA ≥ 29 IU/mL	2%	3%	31%	30%
No Virologic data at Week 48	4%	4%	5%	3%
Discontinued study drug due to lack of efficacy	0	0	< 1%	0
Discontinued study drug due to AE or death	1%	1%	1%	1%
Discontinued study drug due to other reasons[c]	2%	3%	3%	2%
Missing data during window but on study drug	< 1%	1%	< 1%	0

TDF = tenofovir disoproxil fumarate

a. Missing = failure analysis

b. Adjusted by baseline plasma HBV DNA categories and oral antiviral treatment status strata

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

Table 69. Additional efficacy parameters at Week 48[a]

	Study 108 (HBeAg-Negative)		Study 110 (HBeAg-Positive)	
	Vemlidy (N=285)	TDF (N=140)	Vemlidy (N=581)	TDF (N=292)
ALT Normalized ALT (Central lab)[b]	83%	75%	72%	67%
Normalized ALT (AASLD)[c]	50%	32%	45%	36%
Serology HBeAg loss / seroconversion[d]	N/A	N/A	14% / 10%	12% / 8%
HBsAg loss / seroconversion	0 / 0	0 / 0	1% / 1%	< 1% / 0

N/A = not applicable

TDF = tenofovir disoproxil fumarate

a. Missing = failure analysis

b. The population used for analysis of ALT normalization included only patients with ALT above upper limit of normal (ULN) of the central laboratory range at baseline

c. The population used for analysis of ALT normalization included only patients with ALT above ULN of the American Association of the Study of Liver Diseases (AASLD) criteria (> 30 U/L males and > 19 U/L females) at baseline

d. The population used for serology analysis included only patients with antigen positive and antibody negative or missing at baseline

2.5.1. Discussion on clinical efficacy

Assay of HBV DNA

The applicant selected the Roche COBAS Taqman HBV test for use with the high pure System (LLOQ 29 IU/mL and LOD 10 IU/mL. The use of this assay is acceptable and it allows for expression of results in International Units.

Comparison of TAF with TDF

The comparison with TDF in both Phase 3 studies is acceptable taking into account the aim to provide a direct comparison between TAF and TDF for safety and efficacy. The applicant also justified the use of TDF as the comparator based on the fact that in the prior TDF Phase 3 studies the primary endpoint was a composite of HBV DNA suppression and histological improvement demonstrated in a repeat liver biopsy. Since then it has become unfeasible to demand that patients are enrolled only if they meet specific histological criteria applied to liver biopsies and it is no longer routine to monitor progress on treatment with repeat biopsies. Therefore a primary endpoint based on suppression of HBV DNA below the LLOQ using the same assay as applied in the prior TDF Phase 3 studies was considered appropriate and supportive of assumptions that virological responses would be accompanied by some beneficial changes in histology if this had been assessed.

Primary endpoint and non-inferiority margin

The assumptions made regarding the anticipated HBV DNA suppression rates for TDF in HBeAg-positive and -negative populations were based on the prior TDF Phase 3 studies in which TDF was compared with adefovir. A brief consideration of prior development programmes seems appropriate.

Adefovir was compared to placebo in HBeAg positive and HBeAg negative populations. Significantly more patients treated with adefovir vs. placebo in each sub-population (53% vs. 25% and 64% vs. 33%) had histological improvement from baseline at week 48 (≥ 2 points in the Knodell necro-inflammatory score with no concurrent worsening in the Knodell fibrosis score). Baseline ALT ≥ 2 x ULN, Knodell HAI ≥ 10 and HBV DNA < 7.6 log10 copies/mL were associated with greater histological improvement. Adefovir was associated with significant reductions in serum HBV DNA (3.52 and 3.91 log10 copies/mL, respectively, vs. 0.55 and 1.35 log10 copies/mL), increased rate of normalisation of ALT (48 and 72% vs. 16 and 29%) and higher rate of HBV DNA < 400 copies/mL (Roche Amplicor

Monitor PCR assay; 21 and 51% vs. 0%). HBeAg seroconversion (12 %) and HBeAg loss (24%) was observed significantly more frequently with adefovir (vs. 6 % and 11 %, respectively, for placebo) after 48 weeks of treatment.

In the subsequent TDF Phase 3 studies TDF was significantly superior to adefovir for the primary efficacy endpoint of complete response (HBV DNA < 400 copies/mL and Knodell necroinflammatory score improvement ≥ 2 points without worsening in Knodell fibrosis). The rates were 71% vs. 49% in the HBeAg negative and 67% vs. 12% in the HBeAg positive populations. Rates for < 169 c/mL [<29 IU/mL] were 91% and 69% for TDF vs. 56% and 9% for adefovir in respective populations. Also, in the HBeAg positive study a significantly greater proportion of TDF patients had normalised ALT and achieved HBsAg loss at week 48.

According to Marcellin et al. (2008) more than half of the patients in the two Phase 3 TDF studies were Caucasian while one quarter to one third were Asian. The HBeAg positive population was slightly younger than the HBeAg negative population, with mean ages 34 vs. 44 years. Mean baseline HBV DNA was > 8 log10 c/mL in the HBeAg positive and > 6 log10 c/mL in the HBeAg negative study, mean baseline ALT was in the range 120-160 IU/mL, genotypes D and C predominated and 20% per study had cirrhosis. There were some differences in factors potentially impacting on outcomes compared with the populations enrolled into the TAF Phase 3 studies. Nevertheless, as discussed below, the virologic response rates in the TDF groups in the TAF Phase 3 studies closely resembled those in the prior TDF Phase 3 studies.

Although TDF was not directly compared with entecavir, the effects of TDF on HBV DNA are in keeping with those for entecavir when compared with lamivudine (LAM) in LAM-naïve patients. Percentages with < 300 c/mL at Week 48 were 90% and 67% in HBeAg-negative and -positive populations compared with 72% and 36%, respectively, for LAM.

Overall, the primary efficacy endpoint as determined at Week 48 in the TAF Phase 3 studies is acceptable. The non-inferiority margin causes no concern regarding differentiating the effect of TAF from placebo. The 10% margin could be questioned when comparing active treatments, especially since the same margin was applied for the HBeAg-positive and -negative populations even though higher rates of virologic response were anticipated and achieved in the latter. The fact that the margin was barely met in the former group but easily met in the latter population is discussed below.

Virologic failures

In GS-US-320-0108 in the primary analysis 27 patients (17 TAF) did not have < 29 IU/mL HBV DNA at Week 48. Of these, 7 TAF and 4 TDF patients were known to have ≥ 29 IU/mL HBV DNA at week 48 while 10 TAF and 6 TDF patients did not have a value that could be used in the analysis. Three patients had virologic breakthrough through Week 48 (2 TAF) while 4 patients (all TAF) were persistently viraemic.

In GS US 320 0110 in the primary analysis 210 TAF and 97 TDF patients (total 307) had < 29 IU/mL at Week 48. Of the rest, 183 TAF and 88 TDF patients were known to have ≥ 29 IU/mL HBV DNA at Week 48 while 27 and 9 in respective groups had no value for the analysis. Virologic breakthrough through Week 48 occurred in 14 TAF patients (5/14 had baseline HBV DNA < 8 log10 IU/mL) and in 12 TDF patients (6 with < and 6 with ≥ 8 log10 IU/mL). Persistent viraemia was confirmed at Week 48 for 114 TAF (19.6%) and 53 TDF (18.2%) patients.

In summary, the data from the Virology Report demonstrate that the majority of patients who did not have < 29 IU/mL HBV DNA at Week 48 could be classified as primary virologic failures. The relatively low proportion with virologic breakthrough is in keeping with the results of the Phase 3 TDF studies in which only 10/426 TDF patients (10/39 with > 400 c/mL) met the definition of breakthrough.

Univariate and multivariate analyses did not identify treatment (i.e. TAF vs. TDF) as a predictor of failure. The analysis of outcome according to number of risk factors for failure (multivariate analysis) showed that ≥4 factors in an individual patient had an important effect on outcome regardless of the treatment.

In patients without virologic breakthrough who remained viraemic (HBV DNA ≥ 29 IU/mL) after 48 weeks of treatment, a longer duration of treatment may be required to achieve complete suppression, particularly those with high HBV DNA levels at baseline.

At Week 72, both the TAF and TDF groups in GS US 320 0110 demonstrated increased rates of HBV DNA suppression vs. Week 48 (at Week 72 rates were GS US 320 0108: TAF 92.6%, TDF 92.1%; GS US 320 0110: TAF 71.6%, TDF 71.9%). Given the clinically significant viral declines in those with primary virologic failure, the increased rates of HBV DNA suppression at Week 72 and the lack of resistance among patients with viral breakthrough, switching to another agent just because viral suppression has not been achieved at Week 48 does not appear to be warranted.

General considerations on study populations

Although both studies required that eligible patients at screening should have HBV DNA ≥2 x 104 IU/mL and serum ALT > 60 U/L (males) or > 38 U/L (females) and ≤ 10 x ULN (central laboratory range) the baseline data show that some patients did not meet these same criteria by the time they were enrolled. Further analyses showed similar outcomes for those who fell outside of these criteria at baseline compared to the total study population.

Prior (> 12 weeks) previous OAV with any nucleoside or nucleotide analogue was reported for ~21% in 0108 and ~26% in 0110. Only in the latter study was there a negative impact of prior treatment on virologic response. It is not known whether pre-treated patients had pre-study primary failure or rebound since the data were not collected. To explore the lower response rates in treatment experienced patients, multivariate logistic regression analysis was performed. No baseline factor appeared clearly responsible for the difference. When treatment experienced was defined as ☐12 weeks prior treatment with any individual OAV (grouping those who took ADV, CLE, TBV and other OAV into “Other Agents” due to small numbers) similar response rates were observed between the TAF and TDF groups for those pre-treated with ETV and LAM. Responses rates were lower for those who had taken TDF. At present there is no clear explanation for the lower response rates in pre-treated HBeAg positive patients.

As discussed under Pharmacokinetics, there are no data on use of TAF to treat HBV in patients co-infected with HIV or HCV. Patients with hepatitis D virus (HDV) were excluded from the Phase 3 studies. The lack of data is stated in the SmPC.

Patients were not classified or excluded at baseline according to their Child-Pugh scores. However, patients with a history or current evidence of hepatic decompensation (e.g. ascites, encephalopathy or variceal haemorrhage) were excluded. In addition, there were exclusions based on haematology and chemistry laboratory results. As discussed under Pharmacokinetics, it is not at all likely that patients with severe hepatic impairment were enrolled. The SmPC reflects the lack of data in this subset.

Based on FibroTest scores a low percentage of patients in the TAF Phase 3 studies had cirrhosis. In study 0108 viral suppression rates in those with FibroTest scores ≥0.75 were 27/31 (87%) for TAF and 19/20 (95%) for TDF. The corresponding rates in study 0110 were 31/45 (69%) vs. 17/22 (77%). Therefore, although numbers were small, there was consistent numerical inferiority for TAF. Further analyses showed that treatment responses were high in both treatment groups and consistent across FT categories < 0.49 and ≥ 0.49 to < 0.75 in study 01018. A lower response was observed in the TAF group compared with the TDF group by M = F analysis for those with baseline FT scores ≥ 0.75 but this may reflect the higher percentage in the TAF group that discontinued treatment early for “other

reasons" (i.e. withdrew consent, lost to follow up). In 0110, treatment responses were higher in the 0.75 category compared with < 0.49, indicating no impact of cirrhosis on viral suppression by either TAF or TDF.

The baseline demographic and disease characteristics of those with FT scores did not fail treatment (HBV DNA ≥ 29 IU/mL or < 29 IU/mL at Week 48) showed that those who failed treatment were older and more likely to have co-morbidities (diabetes, hypertension and hyperlipidemia). Although the sample size was small, those assumed to have cirrhosis who failed treatment were more likely to have HBV DNA ≥ 8 log10 IU/mL, ALT ≤ 5 x ULN, to be HBeAg positive, to have HBV genotype D and to be treatment experienced compared with those who did not fail treatment. Overall, the findings were consistent with factors predictive of treatment failure in the overall study population.

Efficacy in the HBeAg-negative population in study 0108

TAF was non-inferior to TDF based on the proportions with HBV DNA < 29 IU/mL at Week 48, with a lower bound of the 95% CI around the difference that was -3.6%. There were 10 TAF and 5 TDF FAS patients without a viral load value at Week 48, indicating that there was no obvious bias in favour of either treatment. Results in the PP population supported the FAS results. Additionally, numerically higher percentages in the TAF group with < 29 IU/mL had undetectable HBV DNA. On this basis, regardless of any concern there could be regarding a 10% margin (which was not pre-agreed with CHMP) TAF was similarly efficacious to TDF based on proportions with < 29 IU/mL at Week 48.

Week 48 rates of HBV DNA < 29 IU/mL by major sub-group showed that in those with baseline HBV DNA ≥ 7 log10 IU/mL there was a numerically higher rate of viral suppression in the TDF group (47/55 [85.5%) vs. 23/24 [95.8%]). However, 5/55 TAF and 0/24 TDF patients had undetectable HBV DNA at Week 48.

Additional analyses performed by baseline HBV DNA level (< 7 log10 IU/mL; ≥ 7 log10 IU/mL; and ≥ 8 log10 IU/mL; M = F) did not demonstrate significant differences in HBV suppression at the highest HBV DNA level. In fact there were only 13 TAF and 4 TDF patients with ≥ 8 log10 IU/mL of which 10/13 (77%) and 3/4 (75%) had < 29 IU/mL at Week 48.

A numerically higher proportion in the TAF group achieved ALT normalisation by central laboratory criteria over 48 weeks with a significant difference obtained based on the AASLD criteria. Both curves showed an early divergence between treatments in effects on ALT. It should be noted that mean baseline ALT tended to be lower in the TAF studies compared to the prior TDF studies.

Efficacy in the HBeAg-positive population in study 0110

TAF was concluded to be non-inferior to TDF based on the pre-defined non-inferiority margin applied to the 95% CI around the difference in proportions with plasma HBV DNA < 29 IU/mL at Week 48. The actual lower bound was very near to the pre-defined limit (-9.8%) with a numerical difference of TAF 63.9% vs. TDF 66.8%. Relatively few patients in this study had < 29 IU/mL and undetectable HBV DNA at Week 48 but there was no difference between treatments in this respect.

The applicant points to the difference in percentages with no Week 48 value, affecting 27 TAF (one of whom discontinued due to lack of efficacy) and 9 TDF patients, i.e. a 3:1 ratio compared to the 2:1 randomisation ratio. However, in the PP analysis non-inferiority was also only just about shown (TAF 66.9% vs. TDF 69.0%; diff -2.6%, 95% CI: −8.9% to 3.6%). An additional analysis excluding 14 patients who lost HBeAg between screening and randomisation and stayed negative throughout the study gave response rates of TAF 63.3% vs. TDF 66.4% (difference -3.6%, 95% CI: −9.9%, 2.7%).

There were no statistical differences in proportions with HBV DNA < 29 IU/mL across the major subgroups but the comparisons almost uniformly favoured TDF, the exceptions being patients aged

over 50 years and those enrolled in Europe. Despite the apparent difference between treatments for effects on HBV DNA, a higher proportion in the TAF group achieved ALT normalisation by central and AASLD laboratory criteria with a significant difference vs. TDF for the latter. As in 0108, the curves show an early divergence in ALT by treatment. There was also no disadvantage for TAF in terms of numbers with HBsAg loss, HBeAg loss or HBeAg loss with seroconversion (10.3% vs. 8.1%).

Responses by HBV genotype

There were no statistically significant differences between treatment groups in viral suppression rates for the commonest genotypes (A-D). TAF was numerically better for genotype A and numerically worse for genotype B, with very similar viral suppression rates for C and D. TAF and TDF achieved the lowest rates for genotype D, which accounted for about 30% in 0108 and 20% in 0110. As reported above, the multivariate analyses identified genotype D as a significant factor associated with virologic failure.

Responses by baseline mutations

All patients were assessed for the presence of pre-existing resistance mutations in pol/RT at baseline using the HBV INNO-LiPA Multi-DR v2/v3 hybridization assay. Not surprisingly a higher percentage of HBV in the OAV-naive (92.4%) were classified as wild type vs. OAV-experienced (78.4%). Primary resistance mutations occurred in 17.2% OAV experienced vs. 1.9% of the OAV naïve, the commonest being associated with resistance to LAM. For each resistance category, no significant differences were observed in the proportions with HBV DNA < 29 IU/mL in the TAF vs. TDF group at Week 48. However, in the subset with primary resistance mutations 19/41 (46%) TAF and 18/29 (62%) TDF patients had <29 IU/mL at Week 48. It appeared that the difference was driven by primary virologic failure and not breakthrough.

Development of new mutations

The number of patients qualifying for population sequencing of Week 48 samples was 24 in the TAF group (2.8%) and 14 in the TDF group (3.2%). In the TAF group 15/24 had no changes detected in the pol/RT sequence from baseline, 4 were unable to be sequenced and 5 had polymorphic site substitutions.

In the TDF group 6/14 had no changes detected in the pol/RT sequence from baseline, 4 were unable to be sequenced, 2 had polymorphic site substitutions and 2 had conserved site substitutions. No HBV pol/RT amino acid substitutions associated with resistance to TFV were detected through 48 weeks of the study in either treatment group. Additional limited data were reported from deep sequencing and phenotypic analyses that raise no new concerns. The studies will now continue to Week 384, providing useful information on long-term exposure and emergence of resistance.

Further data from 0108 and 0110

Data to Week 72 were provided and did not raise new concerns. The data from study 0110 demonstrated that some HBeAg positive patients needed very long exposures to achieve virologic suppression. The applicant has extended both studies and committed to provide the Clinical Study Reports (CSRs) at timed intervals (Week 96, 144, and 384 data-cuts).

2.5.2. Conclusions on the clinical efficacy

The applicant conducted two studies in a population characterised by HBeAg status and having HBV DNA ≥ 2 x 104 IU/mL (i.e. ≥ 20,000 IU/mL) and serum ALT > 60 U/L (males) or > 38 U/L (females) and ≤ 10 x ULN. According to EASL, HBeAg-positive and HBeAg-negative patients with ALT > 2 x ULN (AASLD defines normal ALT as ≤ 30 U/L for men and ≤ 19 U/L for women) and serum HBV DNA above 20,000 IU/ml may start treatment without a liver biopsy. On this basis, the trials enrolled patients

eligible for treatment. According to EASL, tenofovir or entecavir would be the two preferred first-line monotherapies for treatment for patients who do not wish to try, or are not eligible for, PEG-IFN alfa 2.

TAF is similarly efficacious to TDF in the HBeAg-negative population. Based on effect on HBV DNA, TAF is clearly effective but it may be not quite as good as TDF in the HBeAg-positive population, even though the lower bound of the 95% CI was within the applicant's pre-defined criterion. Nevertheless, there is no reason to preclude the use of TAF as an alternative to TDF in HBeAg positive patients.

2.6. Clinical safety

Patient exposure

In the Phase 3 HBV studies 866 patients took TAF. The median ([Q1], [Q3]) exposures were nearly identical between TAF (56.1 [48.1, 64.4] weeks) and TDF (56.1 [48.1, 64.7] weeks). More than half in each treatment group had received assigned treatment for ≥ 56 weeks at the time of the Week 48 data cut-off date (TAF 60.5 %; TDF 62.0 %). There was no statistically significant difference between groups in the overall Kaplan-Meier estimate of time to premature discontinuation of study drug.

Adverse events

The overall summary of safety in Phase 3 studies is shown below.

Table 70. GS-US-320-0108 and GS-US-320-0110: Overall Summary of AEs (Safety Analysis Set)

Adverse Events	TAF 25 mg (N = 866)	TDF 300 mg (N = 432)
Subjects Experiencing Any AE	608 (70.2%)	291 (67.4%)
Subjects Experiencing Any Grade 2, 3, or 4 AE	221 (25.5%)	120 (27.8%)
Subjects Experiencing Any Grade 3 or 4 AE	39 (4.5%)	17 (3.9%)
Subjects Experiencing Any Study Drug-Related AE	123 (14.2%)	68 (15.7%)
Subjects Experiencing Any Grade 2, 3, or 4 Study Drug-Related AE	33 (3.8%)	21 (4.9%)
Subjects Experiencing Any Grade 3 or 4 Study Drug-Related AE	6 (0.7%)	2 (0.5%)
Subjects Experiencing Any SAE	36 (4.2%)	21 (4.9%)
Subjects Experiencing Any Study Drug-Related SAE	0	0
Subjects Experiencing Any AE Leading to Premature Study Drug Discontinuation	9 (1.0%)	5 (1.2%)
Subjects Experiencing Any AE Leading to Dose Modification or Study Drug Interruption	17 (2.0%)	7 (1.6%)
Death[a]	0	0

a. Treatment-emergent death refers to the death occurred between the first dose date and the last dose date (inclusive).

The most frequently reported AEs by treatment group were as follows:

- TAF - URTI (9.9%), nasopharyngitis (9.9%) and headache (9.5%)
- TDF - headache (8.3%), URTI (7.4%) and nasopharyngitis (7.2%)

Table 71. GS-US-320-0108 and GS-US-320-0110: AEs in ≥ 5% (Safety Analysis Set)

Adverse Events by System Organ Class and Preferred Term[a,b,c]	TAF 25 mg (N = 866)	TDF 300 mg (N = 432)
Number of Subjects Experiencing Any Adverse Event	608 (70.2%)	291 (67.4%)
Gastrointestinal disorders	227 (26.2%)	108 (25.0%)
Nausea	43 (5.0%)	22 (5.1%)
General disorders and administration site conditions	125 (14.4%)	62 (14.4%)
Fatigue	49 (5.7%)	23 (5.3%)
Infections and infestations	259 (29.9%)	121 (28.0%)
Upper respiratory tract infection	86 (9.9%)	32 (7.4%)
Nasopharyngitis	86 (9.9%)	31 (7.2%)
Nervous system disorders	149 (17.2%)	60 (13.9%)
Headache	82 (9.5%)	36 (8.3%)
Respiratory, thoracic and mediastinal disorders	106 (12.2%)	44 (10.2%)
Cough	55 (6.4%)	27 (6.3%)

a Adverse events were mapped according to MedDRA Version 18.

b SOC were presented alphabetically, and PT was presented by decreasing order of the total frequencies.

c Multiple AEs were counted only once per subject for each SOC and PT, respectively.

There were no Grade 4 clinical AEs. The only Grade 3 AE that occurred in >2 patients in either group were increased ALT (TAF 0.6%, 5; TDF 0.7%, 3) and HCC (TAF 0; TDF 0.7%, 3).

Similar percentages had any AE considered related to study drug by the investigator (TAF 14.2% vs. TDF 15.7%) and any Grade 3 AE considered related (TAF 0.7% vs. TDF 0.5%; 6 vs. 2). The most commonly reported treatment-related AEs were as follows:

- TAF - nausea (2.0%) and fatigue and headache (each 1.4%)
- TDF - nausea (3.7%), fatigue (2.1%) and dyspepsia and headache (each 1.9%)

Table 72. GS-US-320-0108 and GS-US-320-0110: Drug-related AEs in ≥ 1% (Safety Analysis Set)

Preferred Term[a,b,c]	TAF 25 mg (N = 866)	TDF 300 mg (N = 432)
Number (%) Experiencing Treatment-Related AE	123 (14.2%)	68 (15.7%)
Gastrointestinal disorders	61 (7.0%)	34 (7.9%)
Nausea	17 (2.0%)	16 (3.7%)
Dyspepsia	8 (0.9%)	8 (1.9%)
Abdominal distension	9 (1.0%)	2 (0.5%)
Diarrhoea	4 (0.5%)	5 (1.2%)
General disorders and administration site conditions	15 (1.7%)	13 (3.0%)
Fatigue	12 (1.4%)	9 (2.1%)
Nervous system disorders	24 (2.8%)	17 (3.9%)
Headache	12 (1.4%)	8 (1.9%)

a Adverse events were mapped according to MedDRA Version 18.

b SOC was presented alphabetically, and PT was presented by decreasing order of the total frequencies.

c Multiple AEs were counted only once per subject for each SOC and PT, respectively.

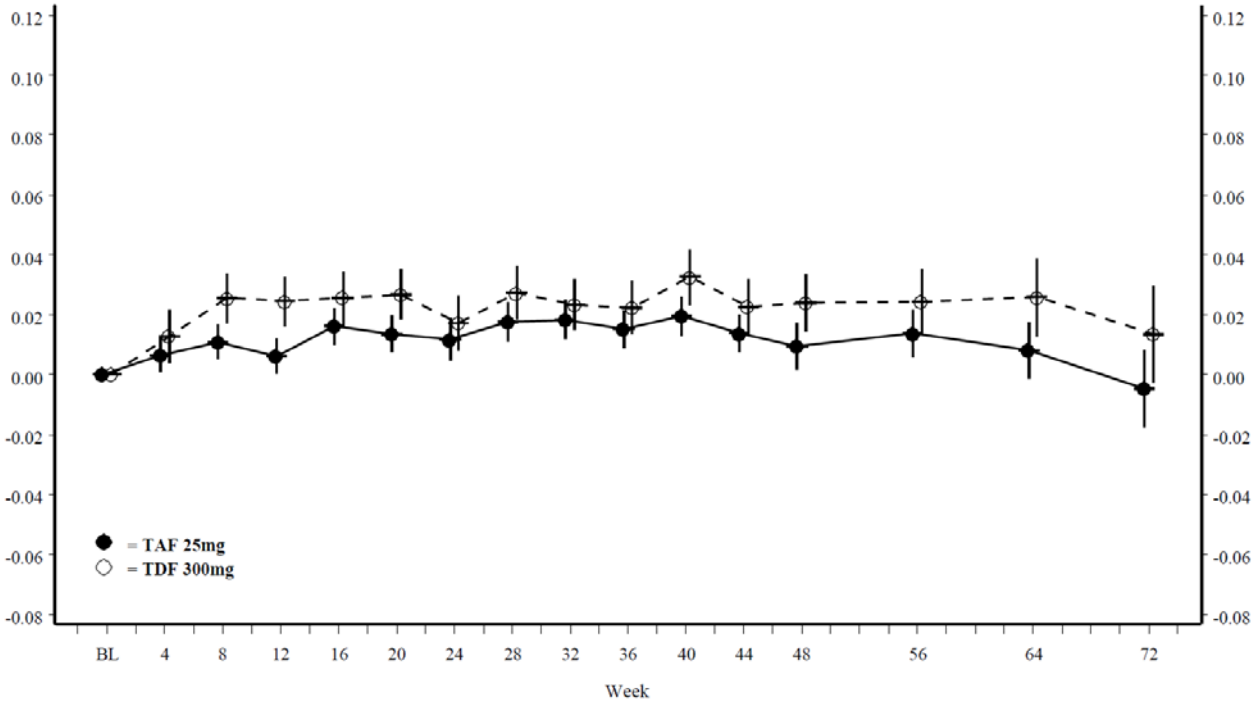
Fractures were uncommon (6 TAF, 1 TDF), the result of trauma, considered by the investigators as unrelated to the study drugs and did not result in permanent discontinuation of study drugs. Patients who received TAF experienced significantly less BMD reduction than those receiving TDF. At Week 48, the mean (SD) percentage decreases from baseline were as follows:

- Hip: TAF −0.163% (2.2437 %); TDF −1.860 % (2.4525 %)
- Spine: TAF −0.570 % (2.9147 %); TDF −2.366 % (3.2051 %)

The distribution of the clinical BMD status adjusted for baseline status was significantly different between treatment groups at Week 48 at the hip ($p < 0.001$) and at Weeks 24 and 48 at the spine ($p < 0.001$), with fewer in the TAF group having worsening BMD status vs. TDF group. The TAF group also demonstrated a smaller effect on bone turnover markers.

There were no cases of proximal renal tubulopathy (including Fanconi syndrome) or renal failure in either treatment group. Increases from baseline in mean serum creatinine were smaller in the TAF group at Week 48 (0.010 vs. 0.024 mg/dL; $p = 0.012$). Graded serum creatinine abnormalities were reported for 6 TAF patients (0.7%), all of which were Grade 1 or 2, and no TDF patients. Five of the 6 had isolated serum creatinine elevations that were not associated with decreased eGFR. The other had a history of hypertension and diabetes, multiple instances of graded creatinine elevations and had eGFR ≤ 50 mL/min.

Figure 22. GS-US-320-0108 and GS-US-320-0110: Mean (95% CI) Change from Baseline in Serum Creatinine (mg/dL) by Visit in the TAF Phase 3 Safety Population (Observed Data; Safety Analysis Set)



Decreases from baseline in median $eGFR_{CG}$ values were significantly smaller in the TAF group (median changes at Week 48 were −1.2 mL/min vs. −5.4 mL/min; $p < 0.001$).

The median changes in $eGFR_{CKD\text{-}EPI,\ creatinine}$ from baseline at Week 48 were −1.2 mL/min/1.73m^2 in the TAF group and −2.2 mL/min/1.73m^2 in the TDF group ($p = 0.007$). Confirmed renal laboratory abnormalities (increases from baseline in creatinine ≥ 0.5 mg/dL or $eGFR_{cg}$ < 50 mL/min or confirmed phosphorus < 2 mg/dL) occurred in 5 TAF patients (0.6%) and 7 TDF patients (1.6%). Most instances were isolated, transient and resolved without treatment.

Serious adverse event/deaths/other significant events

There were no deaths that occurred during treatment. One TAF patient died 3 days after the last dose of study drug (H1N1 influenza and cardiorespiratory arrest subsequent to organ failure).

No SAEs were considered related to study drugs by the investigators. Most occurred in only one patient and therefore SAEs were very varied in nature. One TAF patient had a SAE of acute kidney injury 2 days after discontinuation of study drug. This was the patient who died and had H1N1 influenza.

Table 73. GS-US-320-0108 and GS-US-320-0110: SAEs in >1 patient (Safety Analysis Set)

Preferred Term[a,b]	TAF 25 mg (N = 866)	TDF 300 mg (N = 432)
Number of Subjects (%) Experiencing Any SAE	36 (4.2%)	21 (4.9%)
Hepatocellular carcinoma	1 (0.1%)	5 (1.2%)
Cellulitis	0	3 (0.7%)
Hand fracture	2 (0.2%)	0
Dizziness	2 (0.2%)	0
Calculus ureteric	2 (0.2%)	0

a Adverse events were mapped according to MedDRA Version 18.

b Multiple AEs were counted only once per subject for each SOC and PT, respectively.

Four pregnancies have been reported so far in the HBV Phase 3 studies (2 TAF and 2 TDF) and all discontinued study drugs. One TAF patient underwent elective abortion and the other had a FTND. One TDF patient underwent elective abortion and the other continued with pregnancy (due 26 February 2016).

Table 74. AEs leading to discontinuation of TAF or TDF (Safety Analysis Set)

System Organ Class and Preferred Term[a,b,c]	TAF 25 mg (N = 866)	TDF 300 mg (N = 432)
Number of Subjects (%) Experiencing Any AE Leading to Permanent Discontinuation of TAF or TDF	9 (1.0%)	5 (1.2%)
Blood and lymphatic system disorders	0	1 (0.2%)
Anaemia	0	1 (0.2%)
Leukocytosis	0	1 (0.2%)
Gastrointestinal disorders	3 (0.3%)	2 (0.5%)
Dyspepsia	1 (0.1%)	1 (0.2%)
Nausea	2 (0.2%)	0
Abdominal discomfort	0	1 (0.2%)
Abdominal pain upper	0	1 (0.2%)
Diarrhoea	1 (0.1%)	0
Vomiting	1 (0.1%)	0
General disorders and administration site conditions	0	1 (0.2%)
Fatigue	0	1 (0.2%)
Investigations	3 (0.3%)	1 (0.2%)
Alanine aminotransferase increased	1 (0.1%)	1 (0.2%)
Amylase increased	1 (0.1%)	0
Lipase increased	1 (0.1%)	0
Musculoskeletal and connective tissue disorders	1 (0.1%)	0
Musculoskeletal chest pain	1 (0.1%)	0
Neoplasms benign, malignant and unspecified (including cysts and polyps)	1 (0.1%)	2 (0.5%)
Hepatocellular carcinoma	1 (0.1%)	2 (0.5%)
Nervous system disorders	3 (0.3%)	0
Basilar artery occlusion	1 (0.1%)	0
Dizziness	1 (0.1%)	0
Dizziness postural	1 (0.1%)	0
Psychiatric disorders	1 (0.1%)	1 (0.2%)
Anxiety	0	1 (0.2%)
Depressed mood	1 (0.1%)	0
Respiratory, thoracic and mediastinal disorders	0	1 (0.2%)
Pulmonary embolism	0	1 (0.2%)
Skin and subcutaneous tissue disorders	1 (0.1%)	0
Pruritus	1 (0.1%)	0
Rash maculo-papular	1 (0.1%)	0

Similar percentages (TAF 1.8%; TDF 1.6%) had AEs that led to interruption of study drug. Increased ALT was the only AE resulting in interruption in > 1 patient (TAF 2).

Laboratory findings

Most patients (TAF 94.8%; TDF 91.1%) had at least one laboratory abnormality, of which 28.4% vs. 29.4% in respective groups were Grade 1 and 35.0% vs. 32.2% were Grade 2 in severity. Grade 3/4 AEs were reported in 31.3% TAF and 29.4% TDF patients, of which most were Grade 3 (26.2% vs. 22.4%).

Median values of all haematology parameters remained within normal ranges for all time points in both studies. Abnormalities of individual haematology parameters were generally balanced between treatment groups but a lower percentage in the TAF group (6.2%) had low haemoglobin compared with the TDF group (13.4%). There were no clinically relevant changes from baseline within either treatment group and no differences between the treatment groups in median values for assessed serum chemistry parameters. Median values of all serum chemistry parameters remained within normal ranges. Hepatic laboratory abnormalities occurred with lower overall incidence in the TAF group compared with the TDF group.

- Graded ALT increased occurred in 22.8% TAF and 30.4% patients with AST increased in 22.2% vs. 25.2% and total bilirubin increased in 12.7% vs. 10.0%.
- Grade 3 or 4 ALT elevation occurred in 8.1% TAF and 9.3% TDF patients.
- Grade 3 or 4 elevations of AST occurred in 3.3% TAF vs. 5.4% TDF patients.
- Grade 3 or 4 bilirubin elevations occurred in TAF 0.3% and TDF 0.2% and no patient had Grade 3 or 4 ALT or AST elevations coincident with Grade 3 or 4 total bilirubin elevations.

Five (0.6%) in the TAF group and 3 (0.7%) in the TDF group had elevations in ALT or AST > 3×ULN concurrent with elevations in total bilirubin > 2×ULN. All elevations > 3×ULN in ALT or AST plus > 2×ULN in total bilirubin were isolated and transient. Two had AEs associated with the elevation:

- One TAF patient who had Grade 1 elevated ALT and AST and Grade 3 elevated total bilirubin at baseline experienced a Grade 3 SAE of pancreatitis at Week 48 coincident with Grade 2 elevated ALT, Grade 4 elevated total bilirubin, Grade 3 elevated amylase and Grade 2 elevated lipase. The event resolved 5 days after onset, was considered by the investigator to be unrelated to study drugs and did not result in discontinuation of study drugs. No additional laboratory data were available after the Week 48 visit.
- One TAF patient had Grade 4 hepatic encephalopathy with sepsis associated with H1N1 influenza. This patient had a Grade 3 increase in AST at Week 12 shortly before the onset of the events.

Table 75. TE Grade 3 or 4 laboratory abnormalities (≥ 1% in either group) (Safety Analysis Set)

	TAF 25mg (N=866)	TDF 300mg (N=432)
Maximum Post-baseline Toxicity Grade (N)	859	428
Grade 3	225 (26.2%)	96 (22.4%)
Grade 4	44 (5.1%)	30 (7.0%)
Chemistry		
Alanine Aminotransferase (N)	859	428
Grade 3	52 (6.1%)	27 (6.3%)
Grade 4	18 (2.1%)	13 (3.0%)
Amylase (N)	859	427
Grade 3	22 (2.6%)	9 (2.1%)
Aspartate Aminotransferase (N)	859	428
Grade 3	25 (2.9%)	18 (4.2%)
Grade 4	3 (0.3%)	5 (1.2%)
Creatine Kinase (N)	859	428
Grade 3	16 (1.9%)	7 (1.6%)
Grade 4	9 (1.0%)	6 (1.4%)
Fasting Glucose (Hyperglycemia) (N)	857	425
Grade 3	9 (1.1%)	0
Fasting LDL Cholesterol (N)	837	417
Grade 3	37 (4.4%)	1 (0.2%)
Non-fasting Glucose (Hyperglycemia) (N)	856	426
Grade 3	25 (2.9%)	7 (1.6%)
Urinalysis		
Occult Blood (N)	859	426
Grade 3	66 (7.7%)	30 (7.0%)
Urine Erythrocytes (N)	768	386
Grade 3	59 (7.7%)	35 (9.1%)
Urine Glucose (N)	859	426
Grade 3	41 (4.8%)	5 (1.2%)

Subjects were counted once for the maximum post-baseline severity for each laboratory test.

For urinalysis (i.e. urine glucose, urine protein, and urine RBC), the highest grade is Grade 3.

For non-fasting glucose, the maximum post-baseline toxicity grades, instead of treatment-emergent abnormalities, were summarized, because non-fasting glucose test was not done at baseline.

ALT elevations defined as ALT > 2×baseline and > 10×ULN, with or without associated symptoms, were observed for 16 (1.8%) in the TAF group and 9 (2.1%) in the TDF group. Most of the events were at isolated time points within the first 8 weeks of dosing and resolved without recurrence while the patient remained on study drug. One additional TAF patient had an ALT elevation during the treatment-free follow-up period, 66 days following discontinuation of study drugs.

An ALT elevation that was confirmed at 2 consecutive post-baseline visits was considered an ALT flare. Five (0.6%) TAF and 4 (0.9%) TDF patients had ALT flares. All except two flares occurred early in the dosing period (baseline to Week 8). In 7/9 the ALT flares resolved without recurrence despite continuing study drug. One TAF patient discontinued early and the ALT level remained elevated 4 weeks after the discontinuation (the last time point available). These flares typically were not associated with coincident elevations in bilirubin (one TDF patient had a concurrent Grade 1 bilirubin elevation).

Median decreases from baseline in total cholesterol, LDL, HDL and triglycerides were greater in the TDF group with reductions in all parameters at Week 48. The difference between groups in median change from baseline was statistically significant at Week 48 for total cholesterol, direct LDL, HDL and triglycerides ($p < 0.001$). Eight (0.9%) TAF patients had Grade 3 elevated fasting cholesterol, of which 7 had a history of hyperlipidaemia and/or elevated fasting cholesterol at baseline. No TAF patients had Grade 4 elevated fasting cholesterol and no TDF patients had Grade 3 or 4 elevated fasting cholesterol but 37 (4.4%) TAF and one (0.2%) TDF patient had Grade 3 elevated fasting LDL.

- Male patients had a lower overall incidence of AEs (TAF: male 66.0% vs. female 77.3%; TDF: male 65.8% vs. female 70.1%). The overall difference was primarily driven by higher rates of a small number of specific AEs, such as URTI, dyspepsia, nausea or vomiting.
- Percentages with any AE were slightly higher in those aged ≥ 50 years in both treatment groups (TAF: < 50 years 68.9% and ≥ 50 years 74.6%; TDF: < 50 years 64.7% and ≥ 50 years 73.6%). Incidences for individual AE PTs generally were consistent between treatment groups by age.
- Percentages experiencing any AE were comparable in Asian or non-Asian racial subgroups.
- Percentages with any AE were comparable in patients with normal or elevated baseline ALT (TAF: ≤ ULN 71.0% and > ULN 70.1%; TDF: ≤ ULN 62.8% and > ULN 67.9%).
- Baseline HBV DNA levels (< 8 log10 IU/mL or ≥ 8 log10 IU/mL) did not impact on AE rates.

The AEs that led to discontinuation in Phase 3 studies occurred at similar rates between treatment groups. Two AEs leading to discontinuation were reported for > 1 patient in either treatment group. These were nausea [TAF 2; TDF 0] and HCC [TAF 1; TDF 2 subjects].

Additional safety data to Week 72 or more

In the safety update provided during the procedure the median (Q1, Q3) exposures were similar between treatment groups (88.1 [80.1, 96.0] weeks in the TAF group and 88.1 [80.1, 96.0] weeks in the TDF group across the two studies. Up to the cut-off date no clinically relevant differences in the type, frequency and severity of AEs, including SAEs, were observed between treatments. Study drug-related AEs and AEs leading to premature study drug discontinuation were infrequent. None of the SAEs was considered by the investigator to be related to study drugs.

Table 76. GS-US-320-0108 and GS-US-320-0110: Overall Summary of Adverse Events Through Week 72 (Safety Analysis Set)

	GS-US-320-0108 and GS-US-320-0110	
Subjects Experiencing Any, n (%)	**TAF 25 mg (N = 866)**	**TDF 300 mg (N = 432)**
AE	654 (75.5%)	316 (73.1%)
Grade 3 or 4 AE	51 (5.9%)	21 (4.9%)
Study Drug-Related AE	128 (14.8%)	70 (16.2%)
Grade 3 or 4 Study Drug-Related AE	6 (0.7%)	2 (0.5%)
SAE	51 (5.9%)	25 (5.8%)
Study Drug-Related SAE	0	0
AE Leading to Premature Study Drug Discontinuation	12 (1.4%)	4 (0.9%)
AE Leading to Dose Modification or Study Drug Interruption	20 (2.3%)	8 (1.9%)
Treatment-Emergent Death	0	0

The rate and types of AEs were similar in the two treatment groups. As shown below, the most common were nasopharyngitis, headache and URTI.

Table 77. GS-US-320-0108 and GS-US-320-0110: Adverse Events in ≥ 5% of Subjects by System Organ Class and Preferred Term Through Week 72 in Either Treatment Group (Safety Analysis Set)

	GS-US-320-0108 and GS-US-320-0110	
Subjects Experiencing, n (%)	**TAF 25 mg (N = 866)**	**TDF 300 mg (N = 432)**
AE	654 (75.5%)	316 (73.1%)
Gastrointestinal disorders	258 (29.8%)	120 (27.8%)
Nausea	50 (5.8%)	22 (5.1%)
General disorders and administration site conditions	135 (15.6%)	65 (15.0%)
Fatigue	52 (6.0%)	23 (5.3%)
Infections and infestations	305 (35.2%)	142 (32.9%)
Nasopharyngitis	101 (11.7%)	40 (9.3%)
Upper respiratory tract infection	95 (11.0%)	41 (9.5%)
Musculoskeletal and connective tissue disorders	182 (21.0%)	94 (21.8%)
Arthralgia	38 (4.4%)	28 (6.5%)
Back pain	52 (6.0%)	25 (5.8%)
Nervous system disorders	171 (19.7%)	66 (15.3%)
Headache	97 (11.2%)	40 (9.3%)
Respiratory, thoracic, and mediastinal disorders	127 (14.7%)	48 (11.1%)
Cough	67 (7.7%)	32 (7.4%)

Three (one TAF) new potential uveitis events were observed between Week 48 and 72. No additional subject experienced an eye disorder SAE after Week 48.

Up to Week 48 two deaths had occurred after study drugs were discontinued and were considered not treatment emergent. In the update another three deaths (one TAF) were reported to have occurred after study drugs were discontinued and were considered not treatment emergent.

The TAF patient died as a consequence of HCC (diagnosed Day 401) on Day 459, 17 days after receiving the last dose of study drug. HCC was the only SAE reported at a frequency > 1% in either treatment group: 0.3% (3/866) in the TAF group and 1.2% (5/432) in the TDF group.

The rate and types of AEs leading to premature discontinuation of study drugs were similar in the two treatment groups. Nausea was the only AE leading to premature discontinuation of study drugs that occurred in > 1 subject in either treatment group (TAF 2 subjects).

Grade 3 or 4 chemistry laboratory abnormalities that occurred in > 5% included increased ALT (TAF 8.4% [72/859]; TDF 9.3% [40/428]), increased fasting LDL cholesterol (TAF 5.7% [48]; TDF 0.5% [2]) and increased AST (TAF 3.5% [30]; TDF 5.4% [23]). Nine (1.1%) in the TAF group and no TDF subjects had Grade 3 or 4 fasting increased glucose but there were no additional cases between weeks 48 and 72. No Grade 3 or 4 haematology laboratory abnormalities occurred in > 1% of subjects.

Six (5 TAF) of 1298 patients met Hy's Law criteria up to the Week 72 data cut-off. Three of the 5 TAF patients had concurrent AEs (biliary pancreatitis, H1N1 influenza and hepatitis E) associated with transaminase and bilirubin elevations. The other 3 had ALT elevations early on during treatment that resolved without sequelae and were consistent with on-treatment ALT elevation or flare. All 6 had elevated total bilirubin levels at baseline (mostly indirect bilirubin) and in 3/6 this did not worsen or actually declined on treatment. Thus, the events are most likely due to treatment of underlying viral

hepatitis and not drug-induced liver toxicity. Resolution of the events with continued TAF or TDF therapy in 5 cases is not consistent with DILI.

The pattern of median changes in fasting lipid parameters was similar at Weeks 48 and 72.

Table 78. GS-US-320-0108 and GS-US-320-0110: Measures of Fasting Lipid Parameters at Weeks 48 and 72 (Safety Analysis Set)

	Median Change From Baseline (Q1, Q3)				
Fasting Metabolic Assessment[a]	N	TAF 25 mg (N = 866)	N	TDF 300 mg (N = 432)	P-Value[b]
Week 48					
Total Cholesterol (mg/dL)	773	−2 (−17, 16)	394	−24 (−42, −6)	< 0.001
Direct LDL Cholesterol (mg/dL)	773	4 (−8, 20)	394	−9 (−25, 5)	< 0.001
HDL Cholesterol (mg/dL)	772	−3 (−10, 2)	394	−9 (−17, −3)	< 0.001
Total Cholesterol to HDL Ratio	772	0.2 (−0.1, 0.5)	394	0.2 (−0.2, 0.5)	0.16
Triglycerides (mg/dL)	774	6 (−13, 26)	394	−7 (−27, 10)	< 0.001
Week 72					
Total Cholesterol (mg/dL)	761	1 (−17, 19)	373	−22 (−42, −3)	< 0.001
Direct LDL Cholesterol (mg/dL)	761	7 (−8, 22)	373	−9 (−25, 6)	< 0.001
HDL Cholesterol (mg/dL)	761	−4 (−11, 2)	373	−10 (−17, −3)	< 0.001
Total Cholesterol to HDL Ratio	761	0.2 (−0.1, 0.6)	373	0.2 (−0.1, 0.5)	0.096
Triglycerides (mg/dL)	762	5 (−14, 29)	373	−8 (−29, 12)	< 0.001

a Only laboratory measurements under fasting status were summarized.

b P-values were calculated from the 2-sided Wilcoxon rank sum test to compare the 2 treatment groups.

No further reductions in fasting lipid parameters were observed in the TDF group from Week 48 to 72. Fasting lipid parameters remained relatively stable through Week 72 in the TAF group.

Differences in mean percentage decreases from baseline in hip or spine BMD were statistically significant for the TAF group compared with the TDF group at both Weeks 48 and 72 ($p < 0.001$). For both hip and spine BMD, the difference in least-squares mean (LSM) between the TAF and TDF groups was greater at Week 72 compared with Week 48. There was little change in the TAF group but further decreases in hip and spine (latter changed less than hip) BMD from Week 48 to 72 in the TDF group.

Table 79. GS-US-320-0108 and GS-US-320-0110: Measures of Bone Mineral Density at Weeks 48 and 72 (Hip DXA Analysis Set and Spine DXA Analysis Set)

	Week 48				Week 72			
	N	TAF 25 mg	N	TDF 300 mg	N	TAF 25 mg	N	TDF 300 mg
Hip DXA Analysis Set								
Mean (SD) Percent Change in Hip BMD	811	−0.160 (2.2405)	406	−1.862 (2.4466)	753	−0.287 (2.3630)	373	−2.432 (2.9285)
P-Value[a]	< 0.001				< 0.001			
Difference in LSM (95% CI)	1.702 (1.426, 1.977)				2.145 (1.827, 2.464)			
Subjects with > 3% Decrease in Hip BMD, n (%)	811	68 (8.4%)	406	108 (26.6%)	753	81 (10.8%)	373	149 (39.9%)
P-Value[b]	< 0.001				< 0.001			
Subjects with > 3% Increase in Hip BMD, n (%)	811	55 (6.8%)	406	8 (2.0%)	753	48 (6.4%)	373	8 (2.1%)
P-Value[b]	< 0.001				0.002			
Subjects with no Decrease (≥ Zero %Change) in Hip BMD, n (%)	811	386 (47.6%)	406	83 (20.4%)	753	356 (47.3%)	373	75 (20.1%)
P-Value[b]	< 0.001				< 0.001			
Spine DXA Analysis Set								
Mean (SD) Percent Change in Spine BMD	819	−0.581 (2.9263)	409	−2.375 (3.2089)	757	−0.603 (3.2204)	374	−2.515 (3.5440)
P-Value[a]	< 0.001				< 0.001			
Difference in LSM (95% CI)	1.794 (1.435, 2.153)				1.912 (1.499, 2.325)			
Subjects with > 3% Decrease in Spine BMD, n (%)	819	160 (19.5%)	409	156 (38.1%)	757	159 (21.0%)	374	169 (45.2%)
P-Value[b]	< 0.001				< 0.001			
Subjects with > 3% Increase in Spine BMD, n (%)	819	90 (11.0%)	409	11 (2.7%)	757	81 (10.7%)	374	20 (5.3%)
P-Value[b]	< 0.001				0.003			
Subjects with no Decrease (≥ Zero %Change) in Spine BMD, n (%)	819	332 (40.5%)	409	89 (21.8%)	757	319 (42.1%)	374	91 (24.3%)
P-Value[b]	< 0.001				< 0.001			

a P-values, difference in least squares means, and its 95% CI were calculated from the ANOVA model including treatment as a fixed effect.

b P-values were calculated from the CMH test.

Increases from baseline in mean serum creatinine were observed in both treatment groups at Weeks 48 and 72 with significantly smaller increases in the TAF group at Week 48 but no significant difference between treatments at Week 72 ($p = 0.11$). While the mean change from baseline was unchanged in the TAF group, the mean change from baseline in the TDF group was lower at Week 72 compared with Week 48. Decreases from baseline in median $eGFR_{CG}$ were less in the TAF group compared with the TDF group at Weeks 48 and 72 ($p < 0.001$).

Table 80. GS-US-320-0108 and GS-US-320-0110: Measures of Renal Function at Week 72 (Safety Analysis Set)

	Week 48					Week 72				
Parameter	**N**	**TAF 25 mg**	**N**	**TDF 300 mg**	**P-Value**	**N**	**TAF 25 mg**	**N**	**TDF 300 mg**	**P-Value**
Mean (SD) Change from Baseline in Serum Creatinine (mg/dL)	828	0.010 (0.1140)	418	0.024 (0.0974)	0.012[a]	818	0.009 (0.0933)	399	0.016 (0.0911)	0.11[a]
Median (Q1, Q3) Change from Baseline in $eGFR_{CG}$ (mL/min)	828	−1.2 (−8.4, 7.3)	418	−5.1 (−12.0, 3.0)	< 0.001[b]	818	−0.6 (−9.0, 7.8)	399	−4.2 (−12.0, 3.6)	< 0.001[b]
Median (Q1, Q3) Change from Baseline in $eGFR_{CKD\text{-}EPI,\ Creatinine}$ (mL/min/1.73m^2)	828	−1.2 (−5.5, 3.4)	418	−2.2 (−7.0, 2.0)	0.007[b]	818	−1.7 (−5.9, 2.8)	399	−2.1 (−7.0, 2.1)	0.086[b]
Median Percentage Change (%) (Q1, Q3)										
UPCR (mg/g)	828	6.1 (−31.0, 59.0)	414	16.5 (−21.6, 72.4)	0.010[b]	814	11.2 (−28.4, 82.6)	399	23.8 (−14.7, 75.0)	0.033[b]
UACR (mg/g)	829	7.1 (−25.6, 46.7)	416	12.2 (−21.0, 63.5)	0.076[b]	815	17.2 (−21.8, 70.8)	400	22.8 (−11.3, 82.4)	0.057[b]
Urine RBP to Creatinine Ratio (µg/g)	828	−0.4 (−23.1, 33.3)	414	25.1 (−7.9, 73.2)	< 0.001[b]	811	13.0 (−16.0, 51.4)	394	42.9 (7.9, 108.5)	< 0.001[b]
Urine Beta-2-microglobulin to Creatinine Ratio (µg/g)	825	−3.8 (−34.2, 31.8)	413	38.3 (−4.4, 153.1)	< 0.001[b]	805	5.9 (−25.2, 53.8)	399	54.0 (0.9, 221.3)	< 0.001[b]

a P-values for changes from baseline at postbaseline visits were from the ANCOVA model including treatment as a fixed effect and baseline serum creatinine as a covariate.

b P-values were calculated from the 2-sided Wilcoxon rank sum test to compare the 2 treatment groups.

No subjects experienced an AE of proximal tubulopathy (including Fanconi syndrome).

Four TAF (0.5%) subjects experienced 5 renal SAEs (nephrolithiasis [2]; ureterolithiasis and haematuria [1]; ureterolithiasis [1]) and one (0.2%) TDF subject had 2 renal SAEs of calculus urinary. These renal SAEs were considered not related to study drug by the investigator and resolved. One TAF patient with H1n1 influenza (who died) had a SAE of acute kidney injury 2 days after discontinuation of study drug and prior to Week 48 that was considered not to be related to study drug by the investigator.

2.6.1. Discussion on clinical safety

The most relevant safety database for this application concerns the 866 patients exposed to TAF in the Phase 3 HBV studies. Data were reported initially when ~60% per group had taken TAF for ≥ 56 weeks. The applicant provided an update of all available safety data. With the exception of renal and bone effects and the plasma lipid profiles the safety profile of TAF was very similar to that of TDF even though plasma levels of TFV are much lower when dosing with TAF.

Regarding effects on bone, the various measurements presented consistently indicated a lesser effect of TAF, including an apparently minimal effect of TAF on hip and spine BMD. Although relatively more fractures occurred with TAF when taking 2:1 randomisation into account these do not seem to be treatment-related. Therefore the data were consistent with the results in the Phase 3 studies that compared Genvoya with Stribild in the HIV-infected population.

So far there have been no cases of proximal renal tubulopathy (including Fanconi syndrome) or renal failure in either treatment group. Although increases from baseline in mean serum creatinine were significantly smaller in the TAF group at Week 48 there was no significant difference at Week 72 but there was a lesser effect of TAF on eGFR at Weeks 48 and 72.

Graded hepatic laboratory abnormalities (all and ≥ Grade 3) occurred slightly less often in the TAF group. Hy's Law was potentially met in 5 TAF and one TDF patient but details of these cases indicate that they had alternative explanations or that they had resolution despite continuing treatment, which does not suggest DILI. ALT flares (as defined in the protocols) have occurred on and post-treatment and both types of event should be carefully evaluated in the post-approval period (see further below).

As in the Phase 3 studies that compared Genvoya with Stribild, the HBV studies showed an apparent beneficial effect of TDF on fasting cholesterol, LDL cholesterol, HDL cholesterol and triglycerides. In the TAF group there were either small decreases from baseline (fasting cholesterol and HDL cholesterol) that were less than the decreases in the TDF group or modest increases (LDL cholesterol and triglycerides) from baseline. The fasting total cholesterol to HDL ratios were not different between treatments. Overall, in the absence of concomitant HIV and medications that can themselves increase plasma lipids, the effect of TAF per se was either negative or gave small increases. The data do not raise a major concern.

More patients in the TAF group developed hyperglycaemia and/or elevated urine glucose during treatment. In some cases there is no plausible explanation for development of hyperglycaemia. As for the TAF-containing HIV FDCs, special attention will be paid to cases of hyperglycaemia reported post-approval but at present there is a lack of clear association with TAF.

In the Phase 3 studies 7 (0.8%) in the TAF group vs. 1 (0.2%) in the TDF group developed an AE of vision blurred which could potentially be a symptom of uveitis. The applicant provided details of the cases, which do not point to a clear association with TAF at present.

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

TAF was well tolerated as demonstrated by the low percentages of subjects who had Grade 3 or 4 adverse events, SAEs, or who discontinued study drugs due to AEs in the Phase 3 program. The rate and types of AEs were similar in the 2 treatment groups, and a similar percentage of subjects had Grade 3 or 4 and laboratory abnormalities. Various measurements performed consistently indicated a lesser bone effect of TAF. No proximal tubulopathy was observed and lesser effect of TAF on eGFR was observed. Treatment with TAF was associated with slight increases from baseline of lipid laboratory parameters. The safety data do not raise a major concern.

2.7. Risk Management Plan

Safety concerns

Important Identified Risks	Post-treatment hepatic flares
Important Potential Risks	Overdose of tenofovir occurring through accidental concurrent use of TAF with a TDF- or TAF-containing product
	Renal toxicity
	Bone events due to potential proximal renal tubulopathy/loss of BMD
	Ocular effects
Missing Information	Long-term safety information in adults and adolescents
	Safety in children aged 2 years to < 12 years
	Safety in elderly patients
	Safety in pregnancy
	Safety in lactation
	Safety in HBV infected patients with renal impairment (CrCl < 50 mL/min)
	Safety in patients with HCV co-infection
	Safety in patients with HIV co-infection
	Development of resistance in long-term use

Pharmacovigilance plan

Study / Title	**Objectives**	**Safety Concerns Addressed**	**Status (Planned, Started)**	**Date for Submission of Interim or Final Reports (Planned or Actual)**
Interventional clinical studies (Category 3)				
Study GS-US-320-0108 Phase 3, randomized,	To evaluate the safety and efficacy of TAF vs TDF in	*Missing information:* Long-term	Started	Interim Week 96, 144 report: Q4 2017, Q1

Study / Title	Objectives	Safety Concerns Addressed	Status (Planned, Started)	Date for Submission of Interim or Final Reports (Planned or Actual)
double-blind study to evaluate the safety and efficacy of TAF vs TDF in HBeAg-negative subjects with CHB	HBeAg-negative subjects with CHB.	safety information in adults. Development of drug resistance in long-term use.		2019 Final Week 384 report: Q2 2023
Study GS-US-320-0110 Phase 3, randomized, double-blind study to evaluate the safety and efficacy of TAF vs TDF in HBeAg-positive subjects with CHB	To evaluate the safety and efficacy of TAF vs TDF in HBeAg-positive subjects with CHB	*Missing information:* Long-term safety information in adults. Development of drug resistance in long-term use	Started	Interim Week 96, 144 report: Q4 2017, Q1 2019 Final Week 384 report: Q2 2023
Study GS-US-320-1092 A Randomized, Double-Blind Evaluation of the Pharmacokinetics, Safety, and Antiviral Efficacy of TAF in Adolescents with Chronic Hepatitis B Virus Infection	To evaluate the safety and efficacy of TAF in adolescents with CHB	*Missing information:* Long-term safety information in adolescents	Started	December 2019

Study / Title	Objectives	Safety Concerns Addressed	Status (Planned, Started)	Date for Submission of Interim or Final Reports (Planned or Actual)
Study GS-US-320-3912 Phase 2, randomized, open-label study to evaluate the efficacy and safety of TAF vs TDF-containing regimens in subjects with chronic HBV infection and stage 2 or greater chronic kidney disease who have received a liver transplant	To evaluate the safety and efficacy of TAF in subjects with chronic HBV infection and stage 2 or greater chronic kidney disease who have received a liver transplant	*Missing information:* Safety in HBV infected patients with renal impairment (CrCl < 50 mL/min)	Started	Q3 2018
Study GS-US-320-4018 Phase 3, randomized, double blind study to evaluate the efficacy and safety of switching from TDF 300 mg QD to TAF 25 mg QD in subjects with CHB who are virologically suppressed.	To compare the safety and tolerability and evaluate efficacy in virologically suppressed subjects with chronic HBV.	*Missing information:* Long-term safety information in adults. Development of drug resistance in long-term use	Planned	Interim Week 48 Report: Q4 2019 Final Week 96 Report: Q4 2021
Study **GS-US-367-1657** Phase 1, multiple dose study to evaluate the pharmacokinetic drug-drug interaction potential between sofosbuvir/velpatasvir/GS-9857 (SOF/VEL/VOX) fixed-dose combination (FDC) and HIV antiretrovirals in healthy subjects	To evaluate the PK of sofosbuvir (SOF), its metabolites GS-566500 and GS-331007, velpatasvir (VEL) and voxilaprevir (VOX) upon administration of SOF/VEL/VOX FDC with FTC/RPV/TAF FDC, EVG/COBI/FTC/TAF FDC + FTC/TDF FDC. To evaluate the safety and tolerability of SOF/VEL/VOX and FTC/RPV/TAF, EVG/COBI/FTC/TAF, DRV+RTV+FTC/TDF administered alone or in combination	*Missing information:* Safety in patients with HCV coinfection	Started	Q4 2017
Planned studies in TAF PIP	To address missing information.	*Missing information:* Safety information in children (aged 2 to <12 years)	Planned	Milestone to be determined
Non-interventional studies (Category 3)				

Study / Title	Objectives	Safety Concerns Addressed	Status (Planned, Started)	Date for Submission of Interim or Final Reports (Planned or Actual)
Antiretroviral Pregnancy Registry	To collect information on the risk of birth defects in patients exposed to TAF, during pregnancy	*Missing information*: Safety in pregnancy	Started	Interim reports to be included in TAF PSURs/PBRERs (DLP and periodicity as described in the List of EU reference dates and frequency of submission of PSURs/PBRERs)

Risk minimisation measures

Safety Concern	Routine Risk Minimization Measures	Additional Risk Minimization Measures
Important identified risk(s)		
Post-treatment hepatic flares patients	Sections 4.4 of the SmPC inform about the risk of exacerbation of hepatitis in HBV patients following discontinuation of TAF.	None
Important potential risk(s)		
Overdose of tenofovir occurring through accidental concurrent use of TAF with a TDF- or TAF-containing product	Section 4.4 of the SmPC warns that TAF should not be administered concomitantly with medicinal products containing TDF or TAF. The Package Leaflet includes TDF and TAF in a list of medicines which should not be taken with 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. Section 5.1 of the SmPC states that in both studies (0108 and 0110) TAF was associated with smaller changes in renal safety parameters (smaller reductions in estimated CrCl by Cockcroft-Gault and smaller percentage increases in urine protein to creatinine ratio and urine albumin to creatinine ratio) compared to TDF after 72 weeks of treatment.	None
Bone events due to potential proximal renal tubulopathy/loss of BMD	Section 5.1 of the SmPC states that in both studies (0108 and 0110) TAF was associated with smaller percentage decreases in BMD (as measured by hip and lumbar spine DXA analysis)	None

Safety Concern	Routine Risk Minimization Measures	Additional Risk Minimization Measures
	compared to TDF after 72 weeks of treatment.	
Ocular effects	None	None
Missing information		
Long-term safety information in adults and adolescents	None	None
Safety in children aged 2 to < 12 years	Section 4.2 of the SmPC states that the safety and efficacy of 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	Section 4.6 of the SmPC provides TAF information on pregnancy in humans. Studies in animals have shown no evidence of teratogenicity (rats and rabbits) or an effect on reproductive function (rats) due to TAF. The use of TAF may be considered during pregnancy, if necessary.	None
Safety in lactation	Section 4.6 of the SmPC provides information that it is unknown whether TAF is excreted in human milk, however, in animal studies it has been shown that tenofovir is secreted into milk. There is insufficient information on the effects of tenofovir in newborns/infants. A risk to the breastfed child cannot be excluded; therefore, TAF should not be used during breastfeeding.	None
Safety in HBV infected patients with renal impairment (CrCl < 50 mL/min)	Section 4.2 of the SmPC states that in renal impairment no dose adjustment of TAF is required for adults or adolescents (aged at least 12 years and of at least 35 kg body weight) with estimated CrCl ≥ 15 mL/min or in patients with CrCl < 15 mL/min who are receiving hemodialysis. On days of hemodialysis, TAF should be administered after completion of hemodialysis treatment. Section 4.4 of the SmPC states that there are no safety data on the use of TAF to treat HBV-infected patients with CrCl <30 mL/min, and the use of TAF is not recommended in patients with CrCl < 15 mL/min who are not receiving hemodialysis. Section 5.2 of the SmPC states that no clinically relevant differences in TAF or TFV PK were observed in between healthy subjects and subjects with renal impairment (estimated CrCl from 15 to <30 mL/min).	None

Safety Concern	Routine Risk Minimization Measures	Additional Risk Minimization Measures
Development of drug resistance in long term use	None	None
Safety in patients with HIV co-infection	Section 4.4 of the SmPC states that HIV antibody testing should be offered to all HBV-infected patients whose HIV-1 infection status is unknown before initiating therapy with TAF. In patients who are co-infected with HBV and HIV, TAF should be co-administered with other antiretroviral agents to ensure that the patients receive an appropriate regimen for treatment of HIV.	None
Safety in patients with HCV co-infection	Section 4.4 of the SmPC states that there are no data on the safety and efficacy of TAF in patients co-infected with HCV.	None

Conclusion

The CHMP and PRAC considered that the risk management plan version 0.6 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. New Active Substance

The CHMP, based on the available data, considers that tenofovir alafenamide is not a new active substance, as it is a constituent of a medicinal product previously authorised within the European Union. Tenofovir alafenamide is contained in the marketing authorisation Genvoya which was authorised in the European Union on 19/11/2015.

2.10. Product information

2.10.1. User consultation

The results of the user consultation with target patient groups on the package leaflet submitted by the applicant show that the package leaflet meets the criteria for readability as set out in the *Guideline on the readability of the label and package leaflet of medicinal products for human use.*

2.10.2. Additional monitoring

Pursuant to Article 23(1) of Regulation No (EU) 726/2004, Vemlidy (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

3.1. Favourable effects

TAF was selected for development for treatment of HIV and HBV because it had potential to be similarly active with much lower TFV plasma levels and hence improved safety vs. TDF. Study GS-US-320-0101 provided preliminary evidence that TAF 25 mg could exert a similar effect on HBV as 300 mg TDF in the short term despite achieving plasma TFV levels ~6-fold lower. The selection of the 25 mg TAF dose for treatment of HBV is further supported by two Phase 3 studies in which TAF was compared with TDF. In HBeAg-negative and HBeAg-positive populations the lower bound of the 95% CI around the difference in percentages of patients achieving HBV DNA < 29 IU/mL at Week 48 fell within the pre-defined non-inferiority margin (10%). In the HBeAg-negative population the lower bound of the 95% CI fell within -5%. In the HBeAg-positive population the lower bound of the 95% CI fell near to -10% in the FAS and PP analyses. In both populations TAF had a greater beneficial effect (see effects table) than TDF on ALT.

Same as in the Phase 3 studies that compared Genvoya with Stribild in the HIV-infected population, the Phase 3 HBV studies showed that TAF had a lesser effect on BMD and other markers of bone turnover compared with TDF (see effects Table). Another beneficial effect is that the renal safety profile of TAF appears to be better than that of TDF, with lesser effects on several markers of renal and proximal renal tubular function in the HIV- and HBV-infected populations.

3.2. Uncertainties and limitations about favourable effects

There is a risk of reduced efficacy if TAF 25 mg is given with inducers of P-gp and CHMP agreed that co-administration is not recommended.

There is no information on the use of TAF 25 mg in HIV/HBV co-infected patients but there is no reason why TAF 25 mg tablets, if used in conjunction with other appropriate antiretroviral agents, could not be used to treat both infections. Although there are no data on use of TAF 25 mg in HBV-HCV co-infected patients, there is no reason why patients could not be treated for HCV while taking TAF 25 mg for HBV. The lack of clinical safety and efficacy data in HCV as well as hepatitis D-infected patients is stated in the SmPC.

Regarding the efficacy of TAF in the HBeAg-positive population, further analyses of covariates associated with virologic failure do not explain the differences vs. TDF although the Week 72 analyses suggest that the difference gets smaller over time.

There were very few black patients (13; 7 TAF) enrolled in the Phase 3 studies. In this very small subset virologic responses were not lower in blacks compared with other racial groups. Within each study, the HBV DNA responses for whites were similar across the TAF and TDF treatment groups. In study GS US 320 0108, similar response rates were observed in white and non-white patients but in GS US 320 0110 lower response rates were observed in whites in both treatment groups. Further exploration of the latter study showed that whites had higher rates of several factors identified to be associated with lack of virological response to TAF and TDF.

3.3. Unfavourable effects

The most relevant safety database for this application concerns 866 patients exposed to TAF in the Phase 3 HBV studies. With the exception of beneficial effects of TAF vs. TDF on renal and bone markers and the lack of beneficial effect of TAF vs. TDF on plasma lipid profiles, the safety profile of TAF was very similar to that of TDF even though plasma levels of TFV are much lower when dosing with TAF.

3.4. Uncertainties and limitations about unfavourable effects

Data from Phase 3 studies were initially provided when ~60% of patients per treatment group had taken TAF for ≥ 56 weeks. Markers of renal and bone function/turnover support beneficial effects of TAF vs. TDF but there were no significant differences between treatments in clinical renal and bone AEs. The applicant provided an update of available safety data in which median (Q1, Q3) exposures were 88.1 [80.1, 96.0] weeks for TAF and 88.1 [80.1, 96.0] weeks for TDF. The additional data generally suggest a continued benefit for TAF on the markers previously reported. The two Phase 3 studies have since been extended to Week 384 and therefore very long term safety data will eventually emerge, including data on the effects of switching from TDF to TAF at Week 144. These long-term and switch data should help to confirm whether any apparent safety benefits of TAF are sustained.

Grade 3 or 4 hepatic laboratory abnormalities occurred in 8.1% TAF and 9.3% TDF patients. Hy's Law was potentially met in 5 TAF and 1 TDF patient but detailed review of the cases did not indicate that DILI was likely to have occurred.

As in the Phase 3 studies that compared Genvoya with Stribild, the HBV studies showed an apparent beneficial effect of TDF on fasting cholesterol, LDL cholesterol, HDL cholesterol and triglycerides. In the TAF group there were either small decreases from baseline (fasting cholesterol and HDL cholesterol) that were less than the decreases in the TDF group or modest increases (LDL cholesterol and triglycerides) from baseline. The fasting total cholesterol to HDL ratios did not differ between treatments. Overall, in the absence of concomitant HIV and medications that can themselves increase plasma lipids, the effect of TAF per se was either negative or gave small increases. The clinical implications, if any, are unknown.

Regarding the rates of AEs of hyperglycaemia reported for TAF in the Phase 3 studies, a causal relationship between TAF and hyperglycaemia is not established but cannot be ruled out and will be re-assessed in the post-approval period.

In the Phase 3 safety population numerically more patients in the TAF group developed an AE of vision blurred which could potentially be a symptom of uveitis (TAF 0.8%, 7 subjects; TDF 0.2 %, 1 subject). Cases of vision blurred were further explained but at present it does not seem that these AEs reflected uveitis.

During review of Genvoya and Descovy it was agreed that 10 mg TAF should be used when it is given with a strong inhibitor of P-gp. When treating HBV a dose reduction to TAF 10 mg daily is not possible since there is only a 25 mg tablet to be marketed. Chronic exposure to plasma TFV that is higher than would occur with TAF 25 mg without a P-gp inhibitor could result in some loss of beneficial effects on safety vs. TDF. Therefore co-administration of TAF 25 mg with strong P-gp inhibitors is not recommended.

In patients with CrCL between 15-29 mL/min the plasma levels of TFV after a 25 mg TAF dose did not exceed the exposures that occur with TDF 300 mg in patients with normal renal function. Also, in ESRD patients on thrice weekly haemodialysis plasma TFV exposures are predicted to be in the range documented for dosing TDF 300 mg in patients with normal renal function. The safety profile of TAF 25 mg in these patient sub-groups can be expected to resemble that of TDF in patients with normal renal

function. For patients with CrCL <15 mL/min and not on haemodialysis the SmPC now clarifies that use of 25 mg TAF is not recommended due to lack of any data to support this.

Taking into account the metabolic pathway, thus far there does not appear to be a link between TAF-associated hyperuricaemia and gout or other AEs that could be due to hyperuricaemia (including renal stones). Up to Week 72 four TAF (0.5%) patients experienced 5 renal SAEs (nephrolithiasis [2]; ureterolithiasis and haematuria [1]; ureterolithiasis [1]) and 1 (0.2%) TDF patient had 2 renal SAEs of calculus urinary. These renal SAEs were considered not related to study drug by the investigator and resolved. With 2:1 randomisation and lack of details on stones it is not known whether TAF is associated with uric acid stones and the total rates of renal calculi were 1% for TAF and 0.9% for TDF. Two AEs of gout (1 in each treatment group) and 2 AEs of hyperuricaemia (1 in each treatment group) were reported through the Week 72 data cut. These events were Grade 1 (mild) in severity with the exception of 1 AE of hyperuricaemia (TDF group; Grade 2). All AEs resolved with continued study drug treatment, and none was considered to be related to study drug by the investigator. In each case patients had evidence of elevated serum uric acid levels at baseline and the patient with the AE of gout in the TAF group had a history of gouty arthritis. Nevertheless, this matter needs to be kept under review.

3.5. Effects Table

Table 81. Effects Table for Vemlidy for the treatment of chronic hepatic B

Effect	Short Description	TAF	TDF	Uncertainties / Strength of evidence
HBV DNA < 29 IU/mL at Week 48 HBeAg-negative	FAS PP	94% 97.4%	92.9% 97.7%	Lower bound of 95% CI around the difference in suppression rates -3.6% FAS and -3.3% PP
ALT normalisation at Week 48 HBeAg-negative	Central lab AASLD criteria	83.1% 49.6%	75.2% 31.9%	Significant difference for normalisation by AASLD criteria
HBV DNA < 29 IU/mL at Week 48 HBeAg positive	FAS PP	63.9% 66.9%	66.8% 69.0%	Lower bound of 95% CI around the difference in suppression rates -9.8% FAS and -8.9% PP
ALT normalisation at Week 48 HBeAg-positive	Central lab AASLD criteria	71.5% 44.9%	66.8% 36.2%	Significant difference for normalisation by AASLD criteria
HBV DNA < 29 IU/mL at Week 48 by HBV genotype	Types A-D only could be analysed	Lowest for D	Lowest for D	No significant differences between TAF and TDF by HBV genotype
ALT or ALT >3xULN and total bilirubin >2xULN	Potentially meeting Hy's Law	5 (0.6%)	1 (0.2%)	Lack of information on alternative explanations
ALT elevations >2x baseline and >10x ULN		16 (1.8%)	9 (2.1%)	
ALT flares		5 (0.6%	4 (0.9%)	
Bone effects over 48 weeks	Change in BMD at hip Change in BMD at spine	-0.163 -0.570	-1.860 -2.366	Statistically significant differences that favour TAF
Renal effects over 48 weeks	Change in serum creatinine Markers of proximal tubular nephropathy	0.010 mg/dL -1.2 mL/min	0.024 mg/dL -5.4 mL/min	Statistically significant differences that favour TAF
Plasma lipid effects over 48 weeks	Median change at Week 48 Cholesterol LDL HDL	mg/dL -2 4 -3	mg/dL -24 -9 -9	Statistically significant differences that favour TDF

Effect	Short Description	TAF	TDF	Uncertainties / Strength of evidence
	Triglycerides	6	-7	

Additional Week 72 safety and efficacy data provided do not change the conclusions stated above.

3.6. Benefit-risk balance

3.6.1. Importance of favourable and unfavourable effects

TAF has been shown to achieve high virologic suppression rates in the HBeAg-negative population that are similar to those of TDF. TAF is associated with numerically lower virologic suppression rates vs. TDF in the HBeAg-positive population. The safety profile of TAF 25 mg in HBV-infected patients is similar or improved vs. that of TDF.

3.6.2. Conclusions

The overall the risk/benefit balance of Vemlidy is positive.

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 Vemlidy is favourable in the following indication:

Vemlidy is indicated for the treatment of chronic hepatitis B in adults and adolescents (aged 12 years and older with body weight at least 35 kg) (see section 5.1).

The CHMP 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).

Other 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 review of the available data, the CHMP considers that tenofovir alafenamide is a derivative of tenofovir disoproxil (both prodrugs of tenofovir). The CHMP has also considered all the arguments presented by the applicant in the marketing authorisation dossier and concluded that 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.