SECTION 2.4—NONCLINICAL OVERVIEW

BICTEGRAVIR/EMTRICITABINE/TENOFOVIR ALAFENAMIDE FIXED-DOSE COMBINATION (B/F/TAF FDC)

Gilead Sciences

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TABLE OF CONTENTS

LIST OF IN-TEXT TABLES

LIST OF IN-TEXT FIGURES

GLOSSARY OF ABBREVIATIONS AND DEFINITION OF TERMS

AhR	aryl hydrocarbon receptor
ALT	alanine aminotransferase
ARV	antiretroviral
AUC	area under the concentration versus time curve
AUC_{ss}	area under the concentration versus time curve at steady state
AUC_{tau}	area under the concentration versus time curve over the dosing interval
AUCR	AUC ratio
B/F/TAF	bictegravir/emtricitabine/tenofovir alafenamide (coformulated)
BCRP	breast cancer resistance protein
BDC	bile duct cannulated
BIC	bictegravir (GS-9883)
BSEP	bile salt export pump
CatA	cathepsin A
CC_{50}	concentration that resulted in 50% cytotoxicity
cDNA	complementary DNA
cFTU1	cyclic FTU1
cFTU2	cyclic FTU2
CHB	chronic hepatitis B
CHMP	Committee for Medicinal Products for Human Use
C_{max}	maximum observed concentration of drug
C_{min}	minimum observed concentration of drug
CNS	central nervous system
COBI	cobicistat (Tybost®)
CsA	cyclosporine (cyclosporin A)
CYP	cytochrome P450 enzyme
DDI	drug-drug interaction
DMSO	dimethylsulfoxide
DNA	deoxyribonucleic acid
DTG	dolutegravir
E/C/F/TAF	elvitegravir/cobicistat/emtricitabine/tenofovir alafenamide (coformulated; Genvoya®)
EC_{50}	half-maximal effective concentration
EC_{95}	95% effective concentration
ECG	electrocardiogram
EMA	European Medicines Agency
EVG	elvitegravir (Vitekta®)
F/TAF	emtricitabine/tenofovir alafenamide (coformulated; Descovy®)
FDA	Food and Drug Administration
FDC	fixed-dose combination
FMO	flavin monooxygenase

FTC	emtricitabine (Emtriva®)
FTC/RPV/TAF	emtricitabine/rilpivirine/tenofovir alafenamide (coformulated; Odefsey®)
FTC/RPV/TDF	emtricitabine/rilpivirine/tenofovir disoproxil fumarate (coformulated; Complera®/Eviplera®)
FTC/TDF	emtricitabine/tenofovir disoproxil fumarate (coformulated; Truvada®)
FTC-TP	emtricitabine 5′-triphosphate
GD	gestation day
GGT	gamma-glutamyltransferase
GI	gastrointestinal
Gilead	Gilead Sciences
GLP	Good Laboratory Practice
GMP	Good Manufacturing Practice
HBV	hepatitis B virus
HCV	hepatitis C virus
hERG	human ether-a-go-go-related gene
HIV	human immunodeficiency virus
HIV-1	human immunodeficiency virus type 1
HIV-2	human immunodeficiency virus type 2
IC_{50}	50% inhibitory concentration
ICH	International Council for Harmonisation (of Technical Requirements for Pharmaceuticals for Human Use)
IgM	immunoglobulin M
IND	investigational new drug
INSTI	integrase strand-transfer inhibitor
LAM	lamivudine
LD_{50}	median lethal dose
MATE1	multidrug and toxin extrusion 1
mRNA	messenger RNA
MRP	multidrug resistance-associated protein
mtDNA	mitochondrial DNA
NA	not applicable
NADPH	nicotinamide adenine dinucleotide phosphate, reduced
ND	not determined
NDA	new drug application
NNRTI	nonnucleoside reverse transcriptase inhibitor
NOAEL	no observed adverse effect level
NOEL	no observed effect level
NRTI	nucleoside reverse transcriptase inhibitor
NtRTI	nucleotide reverse transcriptase inhibitor
OAT	organic anion transporter
OATP	organic anion transporting polypeptide

OCT	organic cation transporter
$PAEC_{95}$	protein-adjusted EC_{95}
PBMC	peripheral blood mononuclear cell
PD	pharmacodynamics(s)
PDCO	Pediatric Development Committee
P-gp	P-glycoprotein
PI	protease inhibitor
PIP	pediatric investigation plan
PK	pharmacokinetic(s)
PR interval	electrocardiographic interval occurring between the onset of the P wave and the QRS complex representing time for atrial and ventricular depolarization, respectively
PSP	pediatric study plan
PXR	pregnane X receptor
QT	electrocardiographic interval between the beginning of the Q wave and termination of the T wave, representing the time for both ventricular depolarization and repolarization to occur
QWBA	quantitative whole body autoradiography
RAL	raltegravir
RBC	red blood cell
RPV	rilpivirine
RT	reverse transcriptase
SIV	simian immunodeficiency virus
$t_{1/2}$	estimate of the terminal elimination half-life of the drug, calculated by dividing the natural log of 2 by the terminal elimination rate constant (λ_z)
T3	triiodothyronine
TAF	tenofovir alafenamide (Vemlidy®)
TDF	tenofovir disoproxil fumarate (Viread®)
TFV	tenofovir
TFV-DP	tenofovir diphosphate
TFV-MP	tenofovir monophosphate
UGT	uridine diphosphate glucuronosyltransferase

NOTE TO THE REVIEWER

This overview summarizes nonclinical data for the fixed-dose combination (FDC) tablet containing bictegravir (BIC, B, previously referred to as GS-9883), emtricitabine (FTC, F) and tenofovir alafenamide (TAF, previously referred to as GS-7340), the B/F/TAF (50/200/25 mg) FDC. Bictegravir is a low molecular weight HIV-1 integrase strand-transfer inhibitor (INSTI) active against a broad panel of HIV-1 viral lab strains and clinical isolates and is fully active against a panel of mutant viruses with resistance to nucleoside reverse transcriptase inhibitors (NRTIs), nonnucleoside reverse transcriptase inhibitors (NNRTIs) and protease inhibitors (PIs). Emtricitabine is an NRTI and is approved for the treatment of HIV-1 infection as a single agent (Emtriva®) for use in combination with other antiretrovirals (ARVs) for the treatment of HIV-1 infection, and in the FDC products Truvada® (FTC/tenofovir disoproxil fumarate [TDF]), Atripla® (efavirenz/FTC/TDF), Complera®/Eviplera® (FTC/rilpivirine [RPV]/TDF), Stribild® (elvitegravir [EVG; E]/cobicistat [COBI; C]/FTC/TDF), Genvoya® (E/C/F/TAF), Descovy® (F/TAF) and Odefsey® (FTC/RPV/TAF). Tenofovir alafenamide is a prodrug of tenofovir (TFV), a nucleotide reverse transcriptase inhibitor (NtRTI). Tenofovir alafenamide is approved for the treatment of HIV-1 infection in the FDC products Genvoya®, Descovy® and Odefsey®. Tenofovir alafenamide is also approved for the treatment of hepatitis B virus (HBV) infection as a single agent (Vemlidy®). Information from all nonclinical studies with FTC and TAF/TFV should be considered in the context of their clinical experience within ARV combination therapy for the treatment of HIV-1 infection.

A brief summary of the pharmacology/virology, pharmacokinetics, and toxicology of BIC, FTC, and TAF, as well as a nonclinical assessment of the combination product, is provided.

1. OVERVIEW OF THE NONCLINICAL TESTING STRATEGY

This document provides an overview of the nonclinical information that is relevant to the assessment of the fixed-dose combination (FDC; F) of bictegravir (BIC; B), emtricitabine (FTC), and tenofovir alafenamide (TAF). The overview is structured as a logical summary of the studies in the various disciplines, including primary pharmacodynamics (PD), secondary PD, safety pharmacology, pharmacokinetics (PK), and toxicology. A critical assessment of the completeness and relevance of the nonclinical testing program and the key findings are included. An integrated safety assessment of B/F/TAF for the treatment of HIV-1 infected adults is included in the section, "Integrated Overview and Conclusions" of this document. Specific cross-disciplinary topics and proposals for the inclusion of nonclinical items in the product labeling are discussed throughout the text, as appropriate, and summarized at the end of the document.

All of the definitive safety pharmacology, toxicology, and toxicokinetic studies reported in this summary for BIC, FTC, and TAF were conducted in accordance with guidelines issued by the International Council for Harmonisation (ICH) and with Good Laboratory Practice (GLP) or other applicable regulations promulgated by international health authorities. Pilot, exploratory, and mechanistic studies were either conducted in full compliance with GLP procedures or were conducted using appropriate protocols and documentation to assure data integrity.

1.1. BIC

Bictegravir (2R,5S,13aR)-8-hydroxy-7,9-dioxo-N-(2,4,6-trifluorobenzyl)-2,3,4,5,7,9,13,13a-octahydro-2,5-methanopyrido[1',2':4,5]pyrazino[2,1-b][1,3]oxazepine-10-carboxamide) is a potent investigational integrase strand-transfer inhibitor (INSTI) with in vitro activity against wild-type HIV-1 and HIV-1 with INSTI-resistance associated mutations.

The nonclinical testing strategy for BIC is aimed at evaluating the primary and secondary pharmacodynamic effects of BIC. Moreover, a variety of models and tests were applied to detect adverse effects of BIC. The profile of BIC, in terms of absorption, kinetics, distribution, metabolism, and excretion, in the major models was studied.

Bictegravir is well absorbed, generating sufficient systemic exposure in animal species chosen for toxicity assessment. The rat and monkey were demonstrated to have similar in vitro and in vivo metabolic profiles to humans and were used for repeat dose toxicology studies. The rat was also used for fertility, developmental and reproductive toxicity studies, and the rabbit was used for developmental and reproductive toxicity studies. All in vivo studies utilized oral administration, the clinical route of administration. With the exception of the initial investigational new drug (IND)-enabling studies conducted with BIC as a free acid form, a sodium salt was utilized for the toxicology program; all dose levels stated are represented as free base equivalents (f. b. e.) of BIC. Bictegravir was orally administered in vivo as an aqueous vehicle consisting of 0.5% hydroxypropyl methylcellulose [HPMC; Methocel K100 LV] and 0.1% Tween®20 in reverse osmosis water (%w/w), with the exception of one dose range-finding embryofetal development study in rabbits (100% organic vehicle). For in vitro studies, BIC was generally dissolved in dimethyl sulfoxide (DMSO) and subsequently diluted with incubation

medium. The high dose in transgenic mice was selected based on single dose oral pharmacokinetic studies with the aqueous vehicle formulation showing saturation of absorption at 1000 mg/kg/day (m2.6.4, Section 3.2.1.2.1). The high dose in rats was selected based on single dose oral pharmacokinetic studies with multiple organic and aqueous formulations showing saturation of absorption at 300 mg/kg/day (m2.6.4, Section 3.2.1.2.2). The high dose in monkeys was selected based on the ICH recommended limit dose of 1000 mg/kg/day (m2.6.4, Section 3.2.1.2.4). Blood was collected and BIC plasma concentration was determined in all pivotal toxicology studies. For BIC exposure margin calculations, the BIC AUC plasma values in the toxicology studies were divided by the BIC AUC plasma values in HIV-1 infected subjects who received 50 mg BIC once daily in the B/F/TAF FDC (BIC AUC_{tau} of 102 µg·h/mL; clinical studies GS-US-380-1489, GS-US-380-1490, GS-US-380-1844, GS-US-380-1878).

The oral toxicity of BIC was studied in transgenic mice, rats, and monkeys for treatment periods up to 39 weeks. The only target organ toxicity observed was hepatobiliary toxicity in a 39 week repeat dose chronic toxicology study in monkeys at an exposure margin of 16-fold; the no observed effect level (NOEL) correlated to a margin of 7.0-fold. No adverse findings were noted in transgenic mouse or rat repeat dose toxicology studies up to 26 weeks dosing duration with an exposure margin of ≥18-fold at the no observed adverse effect levels (NOAELs). Bictegravir tested negative in the bacterial mutation, chromosomal aberration, and rat micronucleus assays, and is considered nongenotoxic. A 6 month carcinogenicity study in transgenic mice showed no evidence of carcinogenicity at an exposure margin of ≥15-fold. The 2 year rat carcinogenicity study will be completed by [redacted] 20[redacted] and the final carcinogenicity report will be submitted to specific health authorities according to agreed upon timelines.

Developmental and reproductive toxicity studies in rats indicated no effects at a dose up to 300 mg/kg/day (highest dose tested), corresponding to exposure margins ranging from 29- to 36-fold. In pregnant rabbits, the high dose of 1000 mg/kg/day showed significant maternal toxicity that was associated with abortion and decreased fetal weights. The NOEL for maternal and embryofetal toxicity in pregnant rabbits was 300 mg/kg/day, which provided plasma exposure that was subtherapeutic (0.59-fold margin).

Bictegravir is nonphototoxic, noncorrosive and nonirritating to skin, and moderately irritating to eyes, and did not show the potential to cause skin sensitization.

1.2. FTC

Emtricitabine (FTC) is a NRTI and a (-) enantiomer of a thio analogue of cytidine, which differs from other cytidine analogues in that it has a fluorine in the 5-position. Intracellularly, FTC is phosphorylated by enzymes to form emtricitabine triphosphate (FTC-TP), the active metabolite. Emtricitabine is an NRTI that has activity against HIV and hepatitis B virus (HBV).

The general systemic (single and repeat dose) toxicity, genotoxicity, carcinogenicity, reproductive toxicity, and immunotoxicity of FTC have been characterized in a variety of in vitro and in vivo studies. All nonclinical studies required to support chronic use have been performed as part of the safety assessment. For FTC exposure margin calculations, the FTC AUC plasma values in the toxicology studies were divided by the FTC AUC plasma values in HIV-1 infected subjects who received 200 mg FTC once daily in the B/F/TAF FDC (FTC AUC_{tau} of

12.3 µg·h/mL; clinical studies GS-US-380-1489, GS-US-380-1490, GS-US-380-1844, and GS-US-380-1878). The nonclinical toxicity studies demonstrate that there was no adverse effect of FTC for up to 26 weeks in the mouse and up to 52 weeks in the monkey at exposure margins ranging from 8.0- to 22-fold.

1.3. TAF

Tenofovir alafenamide is a prodrug of tenofovir (TFV), a nucleotide reverse transcriptase inhibitor (NtRTI). After absorption, TAF is converted to TFV intracellularly, which is phosphorylated to the active metabolite, tenofovir diphosphate (TFV-DP) {Robbins 1998}. Tenofovir diphosphate is a very weak inhibitor of mammalian DNA polymerases α, β, δ, and ε and mitochondrial DNA (mtDNA) polymerase γ.

Tenofovir alafenamide is metabolized by cellular enzymes including carboxylesterase 1 and cathepsin A (CatA) and has minimal interaction with typical xenobiotic metabolizing enzymes. TAF is stable in plasma, and delivers high levels of TFV to HIV-target cells including lymphocytes and macrophages.

Tenofovir alafenamide is well absorbed, generating sufficient exposure in animal species chosen for toxicity assessment. Tenofovir alafenamide was evaluated in mouse, rat, dog, and monkey repeat-dose toxicity studies up to 39 weeks in duration. In vitro and in vivo genotoxicity studies were conducted. The mouse was used for the in vivo genetic toxicity study and local lymph node assay. The rat was used for fertility and developmental toxicity studies and the rabbit was used for developmental and reproductive toxicity studies and local irritation. All in vivo studies utilized oral administration, the clinical route of administration, with the exception of the sensitization and dermal irritation studies. The rat and dog were demonstrated to have similar in vitro and in vivo metabolic profiles to humans. The vehicle used for toxicity studies was 1) 25 mM 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) HPMC.

Per separate agreements with FDA and the Committee for Medicinal Products for Human Use (CHMP), carcinogenicity studies, and a perinatal and postnatal study were not required for TAF registration due to the lack of TAF exposure in rats and TgRasH2 mice and lower TFV exposure in rats and mice compared to the same studies in which TDF, another prodrug of tenofovir, was administered.

For TAF or TFV exposure margin calculations, the TFV or TAF (when available) AUC plasma values in the toxicology studies were divided by the TFV or TAF AUC plasma values in HIV-1 infected subjects, respectively, who received 25 mg TAF once daily. Specifically, for TFV, exposure margins were based upon TFV AUC plasma values in HIV-infected subjects who received BIC (75 mg) + F/TAF (200/25 mg) once daily (TFV AUC_{tau} = 0.316 µg·h/mL; Phase 2 clinical study GS-US-141-1475). For TAF, exposure margins were based upon TAF AUC plasma values in HIV-1 infected subjects who received 25 mg TAF once daily in the B/F/TAF FDC (TAF AUC_{tau} of 0.142 µg·h/mL; clinical studies GS-US-380-1489 and GS-US-380-1490). Results from the nonclinical toxicity studies demonstrate that there were no adverse effects of TAF for up to 26 weeks in the rat, up to 39 weeks in the dog, and 1 month in the monkey at doses producing TFV systemic exposure margins of 12-, 3.7- and >19-fold.

1.4. B/F/TAF

Comprehensive nonclinical pharmacology/virology, pharmacokinetic, and toxicology programs were undertaken in support of the development and/or registration of the individual agents of BIC, FTC and TAF. The clinical data, along with the lack of overlapping toxicity data shown in animals, support the safety of the new combination product. The overall program, including the data from the combination and individual agent studies, is considered adequate to support the safety of the B/F/TAF combination tablets.

The proposed FDC is based on the complimentary pharmacology of BIC, FTC and TAF and the body of clinical experience with INSTI or N[t]RTIs in HIV-infected patients. Combinations of these agents in cell-based in vitro assays show favorable anti-HIV activity and no evidence for antagonism. The toxicity profiles of the 3 agents differ substantially with no clinically significant overlapping toxicity. Because the target organ profiles are different, and there is no evidence of genotoxicity, carcinogenicity, or reproductive toxicity in studies of the single agents, administration of the B/F/TAF combination product is unlikely to introduce new toxicities or to exacerbate known toxicities of the individual components. The ample nonclinical safety databases on these drugs strongly indicate further toxicological investigations are unlikely to yield new data relevant to humans. Additionally, the extensive clinical safety data available from other FTC and/or TDF- (TAF-) containing regimens (Emtriva, Truvada, Atripla, Complera/Eviplera, Stribild, Genvoya, Odefsey, Descovy, or Vemlidy) demonstrate an acceptable benefit/risk profile for the proposed use of the B/F/TAF FDC for the treatment of HIV-1 infection.

The absence of nonclinical safety studies with the B/F/TAF combination is in accordance with the FDA Guidance for Industry, Nonclinical Safety Evaluation of Drug or Biologic Combinations, March 2006; the CHMP Guideline on the Non-Clinical Development of Fixed Combinations of Medicinal Products (EMEA/CHMP/SWP/258498/2005, January 2008); and Human Immunodeficiency Virus-1 Infection: Developing Antiretroviral Drugs for Treatment, Guidance for Industry (November 2015).

2. PHARMACOLOGY

2.1. Primary Pharmacodynamics

2.1.1. BIC

Bictegravir is a novel strand transfer inhibitor of HIV-1 integrase with high potency and selectivity in antiviral assays and does not require metabolic modification to exert ARV activity (m2.6.3, Section 1.1, PC-141-2032, PC-141-2034, and PC-141-2036). Using lymphoblastoid T-cell lines and primary human T-lymphocytes in HIV-1 antiviral assays, the estimated concentration of drug for a half-maximal response (EC_{50}) of BIC ranged from 1.5 to 2.4 nM and the selectivity indices ranged from 1500 to 8800 (m2.6.3, Section 1.1, PC-141-2032 and PC-141-2034). When tested in primary human PBMCs against clinical isolates of all HIV-1 groups (M, N, O), including subtypes A, B, C, D, E, F, and G, BIC displayed similar antiviral activity across all clinical isolates with mean and median EC_{50} values of 0.60 and 0.55 nM, respectively, based on a range of EC_{50} values between < 0.05 and 1.71 nM (m2.6.3, Section 1.1, PC-141-2035 and PC-141-2057). HIV-2 was similarly susceptible to BIC with an EC_{50} value of 1.1 nM (m2.6.3, Section 1.1, PC-141-2035). Bictegravir is a specific inhibitor of HIV with no measurable antiviral activity against non-HIV viruses, including HBV, hepatitis C virus (HCV), influenzas A and B, human rhinovirus, and respiratory syncytial virus (RSV) (m2.6.3, Section 1.4, PC-141-2043).

Bictegravir maintained potent antiviral activity against HIV-1 variants resistant to currently approved ARVs from the NRTI, NNRTI, and protease inhibitor (PI) classes (m2.6.3, Section 1.1, PC-141-2039). Bictegravir displays a resistance profile similar to that of dolutegravir (DTG) and markedly improved compared with that of raltegravir (RAL) and elvitegravir (EVG; E). Bictegravir maintained full activity against clonal isolates from virologic failures treated with Stribild (m2.6.3, Section 1.1, PC-141-2040 and PC-141-2050). Bictegravir had an improved resistance profile compared to EVG, RAL, and DTG in patient isolates, particularly for isolates with high-level INSTI resistance containing combinations of mutations such as E92Q + N155H or G140C/S + Q148R/H/K ± additional INSTI mutations, and may have unmet clinical utility in these patients (m2.6.3, Section 1.1, PC-141-2051). Bictegravir had a longer dissociation half-life from HIV-1 integrase-DNA complexes compared with DTG, RAL, and EVG (m2.6.3, Section 1.1, PC-141-2058).

HIV-1 isolates with reduced susceptibility to BIC have been selected in cell culture (m2.6.3, Section 1.1, PC-141-2041, PC-141-2052, and PC-141-2056). These selections showed that BIC displayed a comparable barrier to resistance emergence as DTG, and a higher barrier than EVG. Bictegravir selected the M50I + R163K combination and S153F with a transient T66I substitution in HIV-1 integrase. The R263K single mutant and M50I + R263K double mutant viruses had low-level reduced susceptibility to BIC, but the single M50I mutant was fully sensitive to BIC. The M50I + R263K selected variants exhibited low-level cross-resistance to RAL and DTG and intermediate cross-resistance to EVG but remained susceptible to other classes of ARVs. The effect of the T66I and S153F/Y single mutants and the T66I + S153F double mutant in integrase on BIC susceptibility was minimal.

Similar to a number of other ARV agents, the in vitro activity of BIC was reduced in the presence of human serum due to significant protein binding. Bictegravir exhibited approximately 70-fold increase in the EC_{50} value in the presence of 100% serum relative to its activity in cell culture medium. The 95% effective concentration (EC_{95}) calculated from the high density antiviral dose response was used in conjunction with the human serum shift determined by equilibrium dialysis to calculate the protein-adjusted EC_{95} ($PAEC_{95}$) of 361 nM (m2.6.3, Section 1.1, PC-141-2033 and m2.6.5, Section 6.1, AD-141-2287).

2.1.2. FTC

Emtricitabine, an NRTI, is a synthetic analogue of the naturally occurring pyrimidine nucleoside, 2′-deoxycytidine. Intracellularly, FTC is converted through 3 phosphorylation reactions to its active tri-phosphorylated anabolite emtricitabine 5′-triphosphate (FTC-TP) {Furman 1992, Paff 1994}. Emtricitabine 5'-triphosphate inhibits the activity of viral polymerases, including HIV-1 RT by direct binding competition with the natural deoxyribonucleotide substrate (deoxycytidine triphosphate) and by being incorporated into nascent viral DNA, which results in chain termination {Wilson 1993}. FTC has activity that is specific to HIV (HIV-1 and HIV-2) and HBV. The EC_{50} of FTC against laboratory adapted strains of HIV-1 ranged from 0.001 to 0.62 μM depending on cell type and virus strain used in the assay {Jeong 1993, Painter 1995, Schinazi 1992}. With clinical isolates of HIV-1, EC_{50} values ranged from 0.002 to 0.028 μM {Schinazi 1992}. Emtricitabine 5’-triphosphate is a weak inhibitor of mammalian DNA polymerases α, β, and ε and mtDNA polymerase γ {Painter 1995}. There was no evidence of toxicity to mitochondria in vitro and in vivo.

The antiviral activity of FTC against laboratory and clinical isolates of HIV-1 was assessed in lymphoblastoid cell lines, the MAGI-CCR5 cell line, and PBMCs. The EC_{50} values for FTC were in the range of 0.001 to 0.62 μM. FTC displayed antiviral activity in cell culture against HIV-1 clades A, B, C, D, E, F, G, and O (EC_{50} values ranged from 0.007 to 0.140 μM) and showed activity against HIV-2 (EC_{50} values ranged from 0.007 to 1.5 μM).

HIV-1 isolates with reduced susceptibility to FTC have been selected in cell culture. Reduced susceptibility to FTC was associated with M184V/I mutations in HIV-1 RT.

2.1.3. TAF

TAF is a phosphonamidate prodrug of TFV (2’-deoxyadenosine monophosphate analogue). Cells are permeable to TAF, and due to increased plasma stability and intracellular activation through hydrolysis by cathepsin A, TAF is more efficient than TDF in loading TFV into PBMCs, including T cells and macrophages {Birkus 2008, Birkus 2007}. Intracellular TFV is subsequently phosphorylated to the pharmacologically active metabolite tenofovir diphosphate (TFV-DP) {Robbins 1998}. Tenofovir diphosphate inhibits HIV replication through incorporation into viral DNA by the HIV RT, which results in DNA chain-termination {Cherrington 1995, Yokota 1994}. Tenofovir has activity that is specific to human immunodeficiency virus (HIV-1 and HIV-2) and HBV {Delaney 2006, Kalayjian 2003, Lee 2005}. In vitro studies have shown that both FTC and TFV can be fully phosphorylated when combined in cells (m2.6.3, Section 1.3, PC-164-2001). Tenofovir diphosphate is a weak

inhibitor of mammalian DNA polymerases that include mtDNA polymerase γ {Cherrington 1994, Kramata 1998}, and there is no evidence of mitochondrial toxicity *in vitro* based on several assays including mtDNA analyses {Birkus 2002, Stray 2017}.

The antiviral activity of TAF against laboratory and clinical isolates of HIV-1 subtype B was assessed in lymphoblastoid cell lines, PBMCs, primary monocyte/macrophage cells, and CD4-T lymphocytes. The EC_{50} values for TAF were in the range of 2.0 to 14.7 nM. TAF displayed antiviral activity in cell culture against all HIV-1 groups (M, N, O), including subtypes A, B, C, D, E, F, and G (EC_{50} values ranged from 0.10 to 12.0 nM) and activity against HIV-2 (EC_{50} values ranged from 0.91 to 2.63 nM) (m2.6.3, Section 1.3, PC-120-2004). The antiviral activity of two TAF metabolites, M18 (GS-645552; isopropylalaninyl TFV) and M28 (GS-652829; alaninyl TFV), were evaluated in two T-lymphoblastoid cell lines (MT-2 and MT-4) following 5 days of compound exposure (m2.6.3, Section 1.3, PC-120-2021). GS-645552 is also a drug product degradant. Both metabolites showed weak inhibition of HIV-1 replication with 1723 to 2630-fold lower inhibitory potency relative to TAF (EC_{50} values of 7.41 to 21.0 µM) for metabolite M28 and 121 to 130-fold lower inhibitor potency relative to TAF (EC_{50} values of 0.56 to 0.97 µM) for metabolite M18.

HIV-1 isolates with reduced susceptibility to TAF have been selected in cell culture. HIV-1 isolates selected by TAF expressed a K65R mutation in HIV-1 RT; in addition, a K70E mutation in HIV-1 RT has been transiently observed {Margot 2006}. HIV-1 isolates with the K65R mutation have low-level reduced susceptibility to abacavir, FTC, TFV, and lamivudine (LAM) {Kagan 2007, Margot 2006} (m2.6.3, Section 1.3, PC-120-2011). In vitro drug resistance selection studies with TAF have shown no development of high-level resistance after extended time in culture.

Tenofovir has activity that is specific to HBV in addition to HIV-1 and HIV-2. The antiviral activity of TAF against a panel of HBV clinical isolates representing genotypes A-H was assessed in HepG2 cells. The EC_{50} values for TAF ranged from 34.7 to 134.4 nM, with an overall mean EC_{50} of 86.6 nM (m2.6.3, Section 1.3, PC-320-2003). The concentration that resulted in 50% cytotoxicity (CC_{50}) in HepG2 cells was >44,400 nM (m2.6.3, Section 1.3, PC-320-2003 and PC-120-2007). The combination of TFV and FTC was studied for cytotoxicity in MT-2 cells. No cytotoxicity was observed at the highest concentrations tested, up to 50 µM TFV and 5 µM FTC (m2.6.3, Section 1.12, PC-164-2002). Cytotoxicity studies were also conducted on the combination of TFV and FTC in HepG2 cells and no cytotoxicity was observed (m2.6.3, Section 1.6, TX-104-2001).

The antiviral activity of TAF was evaluated against a panel of HBV isolates containing nucleos(t)ide RT inhibitor mutations in HepG2 cells. HBV isolates expressing the rtV173L, rtL180M, and rtM204V/I substitutions associated with resistance to LAM remained susceptible to TAF (< 2-fold change in EC_{50}) (m2.6.3, Section 1.3, PC-320-2007). HBV isolates expressing the rtL180M, rtM204V plus rtT184G, rtS202G, or rtM250V substitutions associated with resistance to entecavir remained susceptible to TAF. HBV isolates expressing the rtA181T, rtA181V, or rtN236T single substitutions associated with resistance to adefovir remained susceptible to TAF; however, the HBV isolate expressing rtA181V plus rtN236T exhibited reduced susceptibility to TAF (3.7-fold change in EC_{50}). The clinical relevance of these substitutions is not known.

2.1.4. B/F/TAF

Bictegravir, FTC, and TAF are potent and selective inhibitors of HIV-1. All 3 drugs show potent ARV activity against diverse subtypes of HIV-1 in vitro. Emtricitabine and TFV are phosphorylated intracellularly through non-overlapping pathways, and in combination show no antagonism for the formation of their active metabolites (m2.6.3, Section 1.3, PC-164-2001). Bictegravir does not require metabolic modification for activity. The anti-HIV-1 activity of the 3-drug combination of BIC, FTC, and TAF was found to be highly synergistic with no evidence of antagonism in vitro, supporting the use of these agents in combination in HIV-1 infected patients (m2.6.3, Section 1.10, PC-141-2038). In addition, in vitro combination studies have shown that in 2-drug combination studies BIC, FTC, and TFV have additive to synergistic anti-HIV-1 activity with other approved NRTIs, NNRTIs, and PIs {Hill 1997, Miller 1999, Rimsky 2001}, (m2.6.3, Section 1.10, PC-141-2038). The resistance profiles of the individual agents BIC, TFV, and FTC are distinct and non-overlapping (m2.6.3, Section 1.1, PC-141-2039).

2.2. Secondary Pharmacodynamics

2.2.1. Cytotoxicity

For BIC, the CC_{50} in primary CD4+ T-lymphocytes, MT-4, MT-2, resting and activated PBMCs, and monocyte-derived macrophages cells ranged from of 3700 to 29,800 nM (m2.6.3, Section 1.1, PC-141-2032 and PC-141-2034).

The cytotoxicity of FTC has been evaluated extensively in vitro. In all the cell lines examined, cell growth was not affected at concentrations of FTC ≥ 100 µM {Furman 1992, Schinazi 1994, Van Draanen 1994}.

Both TAF and its metabolites M18 and M28 had no cytotoxicity up to the highest tested concentration (57 µM) (m2.6.3, Section 1.3, PC-120-2021).

2.2.2. Off-Target Activity

Bictegravir had no pharmacologically significant binding affinity to a diverse panel of 68 protein targets, including neuroreceptors, ion channels, and nuclear receptors (m2.6.3, Section 3.1, PC-141-2029).

Emtricitabine had no pharmacologically significant binding affinity to 19 different receptors (m2.6.3, Section 1.5, TPZZ/93/0002), showed little or no direct effect on various isolated muscle preparations (cholinergic, adrenergic, histaminergic, and serotonergic), and had no major inhibitory effects on the contractile responses to acetylcholine, norepinephrine, serotonin, isoproterenol, arachidonic acid, histamine, bradykinin, and angiotensin II (m2.6.3, Section 1.5, TPZZ/92/0055).

Tenofovir showed no significant inhibition of, or increased binding to a series of 111 protein targets (neuroreceptors, ion channels, transporters, and nuclear receptors) (m2.6.3, Section 1.6, V2000020).

2.3. Safety Pharmacology

2.3.1. BIC

Bictegravir was evaluated in safety pharmacology studies of the central nervous system (CNS), respiratory, and cardiovascular systems (m2.6.3, Section 4.2.1, PC-141-2047, PC-141-2048, and PC-141-2046, respectively). At the highest doses tested, BIC had no effects on the central nervous and respiratory systems of rats (300 mg/kg), no effects on the cardiovascular system of monkeys (1000 mg/kg), and no notable inhibition of the human ether-a-go-go-related gene (hERG) potassium channel current at a concentration up to 7.1 µM (m2.6.3, Section 1.7, PC-141-2049). Plasma exposures (free [unbound] C_{max}) in the in vivo studies were at least 0.92-fold (rats) and 22-fold (monkeys) of the free BIC C_{max} concentration following clinical administration of the B/F/TAF FDC. In the hERG study, exposures were at least 200-fold above free BIC C_{max} concentration following clinical administration of the B/F/TAF FDC.

2.3.2. FTC

A comprehensive range of safety pharmacology studies revealed no treatment-related adverse effects on any organ system at systemic exposure levels much higher than those anticipated in patients at the recommended clinical dose (m2.6.3, Section 4.2.2, 477, TPZZ/93/0001, TPZZ/93/0119, and TPZZ/92/0057; m2.6.3, Section 4.1.2, TPZZ/92/0056). No effects on the cardiovascular system were reported in anesthetized dogs administered a cumulative dose of 38.5 mg/kg of FTC intravenously over a 1-hour period (m2.6.3, Section 4.2.2, TPZZ/92/0076). In addition, there were no abnormalities reported on the electrocardiogram (ECG) data obtained from the repeated-dose toxicity studies in monkeys, where plasma AUC exposure margins were at least 28-fold (m2.6.7, Section 7.2.6, TOX600; Section 7.2.7, TOX627; and Section 7.2.8, TOX032).

2.3.3. TAF

Tenofovir alafenamide was evaluated in safety pharmacology studies of the rat central nervous, renal, GI, and cardiovascular systems. In vivo safety pharmacology experiments were conducted using TAF as the monofumarate form (GS-7340-02) in 50 mM citric acid. The 50% inhibitory concentration (IC_{50}) for the inhibitory effect of TAF on hERG potassium current was > 10 µM, far above human exposure (m2.6.3, Section 4.1.3, PC-120-2005).There were no adverse effects detected in the CNS in rats dosed at 1000 mg/kg (m2.6.3, Section 4.2.3, R990188) or in the renal system in rats administered 1000 mg/kg (m2.6.3, Section 4.2.3, R990186). In the chronic repeat dose dog study (m2.6.7, Section 7.3.5, TOX-120-002), a dose-related prolongation of PR interval was noted at Week 39; however, in the single dose cardiovascular safety pharmacology study in dogs (m2.6.3, Section 4.2.3, D2000006) dosed at 100 mg/kg (80 mg free base equivalents/kg), there were no findings. There was reduced gastric emptying in rats dosed at 1000 mg/kg but not at 100 mg/kg (m2.6.3, Section 4.2.3, R990187).

2.3.4. B/F/TAF

A comprehensive safety pharmacology program has been conducted for the 3 individual components of the B/F/TAF regimen. While the designs for these safety studies varied between the agents, the major organ systems were evaluated. Bictegravir had no effect on vital organ systems in safety pharmacology studies. Neither FTC nor TAF had clinically relevant effects on vital organ systems in safety pharmacology studies. Although TAF showed some potential to prolong the PR interval in the 39-week dog study (m2.6.7, Section 7.3.5, TOX-120-002), no PR prolongation or any change in ECG results occurred in the cardiovascular safety pharmacology study (m2.6.3, Section 4.2.3, D2000006) or in the thorough QT study (GS-US-120-0107). Neither BIC nor FTC had an effect on PR interval in safety pharmacology studies; therefore, there is no potential for overlapping toxicity. Overall, the pharmacological assessment of BIC, FTC, and TAF supports the effective use of these 3 agents together in combination therapy for HIV-1 infection. Additional safety pharmacology studies on the B/F/TAF combination are considered unwarranted.

2.4. Pharmacodynamic Drug Interactions

The anti-HIV-1 activity of 2-drug and 3-drug combinations of BIC, FTC, and TAF were found to be additive to highly synergistic with no evidence of antagonism in multiple in vitro assay systems, supporting the use of these agents in combination in HIV-infected patients (m2.6.3, Section 1.10, PC-380-2001).

In vitro two-drug combination studies have shown that BIC has additive to synergistic anti-HIV-1 activity with other approved NRTIs, NNRTIs, and PIs, including synergistic activity with TAF, FTC, and darunavir. No antagonistic antiviral interaction was found between BIC and the tested clinically relevant classes of ARVs (m2.6.3, Section 1.10, PC-141-2038).

In two-drug combination studies of FTC with NRTIs, NNRTIs, PIs, and INSTIs, additive to synergistic effects were observed. No antagonism was observed for these combinations {Hill 1997, Rimsky 2001}.

In a study of TAF with a broad panel of representatives from the major classes of approved anti-HIV agents (NRTIs, NNRTIs, INSTIs, and PIs), additive to synergistic effects were observed. No antagonism was observed for these combinations (m2.6.3, Section 1.12, PC-104-2005, PC-104-2006, PC-264-2001, and PC-120-2002).

In cell culture combination antiviral activity studies of TFV with the HBV NRTIs FTC, entecavir, LAM, and telbivudine, no antagonistic activity was observed (m2.6.3, Section 1.12, PC-120-2001 and PC-120-2032). The anti-HCV PIs telaprevir and boceprevir were identified as the only potent inhibitors of CatA-mediated hydrolysis of TAF in a biochemical assay. The tested HIV PIs, host serine PIs, and the majority of other HCV PIs exhibit minimal potential to interfere with the intracellular activation of TAF (m2.6.3, Section 1.12, PC-120-2001). These data support the co-administration of the tested therapeutic PIs, with the exception of telaprevir and boceprevir, in combination with TAF, without negatively affecting its clinical pharmacology and intracellular conversion to TFV.

2.5. Summary of Pharmacology

The INSTI BIC and the NRTIs FTC and TAF are potent and selective inhibitors of HIV-1 and HIV-2. All 3 drugs show potent ARV activity against diverse subtypes of HIV-1 in vitro. FTC and TAF are also potent inhibitors of HBV. Emtricitabine and TAF are phosphorylated intracellularly through nonoverlapping pathways, and in combination show no antagonism for the formation of their active metabolites. Bictegravir does not require metabolic modification for activity. Two and 3-drug combinations of BIC, FTC, and TAF consistently show synergistic anti-HIV-1 activity in vitro and no evidence of toxicity.

The resistance profiles for the individual agents of BIC, FTC, and TAF have been well characterized. There is no cross-resistance between the NRTI and INSTI classes.

Both FTC and TAF have shown a low potential for mitochondrial toxicity in long term toxicity studies and there was no evidence of toxicity to mitochondria in vitro and in vivo. Emtricitabine triphosphate and TFV-DP have high selectivities for HIV RT and are very weak inhibitors of mammalian DNA polymerases α, β, δ, and ε and mtDNA polymerase γ. However, as mitochondrial toxicity is not associated with INSTIs as a class and BIC is not anticipated to significantly increase the exposure of FTC or TFV, the potential for exacerbating mitochondrial toxicity is low.

Bictegravir, FTC, and TAF have no pharmacologically significant off-target binding affinity to the receptors tested. Bictegravir, FTC, and TAF have low in vitro cytotoxicity in a variety of human cell types. Bictegravir, FTC, and TAF had no or little effect on vital organ systems in safety pharmacology studies. Although TAF showed some potential to prolong the PR interval in the 39-week dog study, no findings were noted in the cardiovascular safety pharmacology study in dogs or in a clinical QT study conducted with TAF 125 mg, 5-fold higher than the approved clinical dose of 25 mg once daily.

There are no anticipated pharmacological interactions expected for the B/F/TAF FDC. Additional safety pharmacology studies on the B/F/TAF FDC are not warranted. The absence of nonclinical safety pharmacology studies with the combination is in accordance with the FDA Guidance for Industry, Nonclinical Safety Evaluation of Drug or Biologic Combinations, March 2006 and the CHMP Guideline on the Non-Clinical Development of Fixed Combinations of Medicinal Products (EMEA/CHMP/SWP/258498/2005, January 2008).

Overall, the pharmacodynamic and pharmacological assessment of BIC, FTC, and TAF supports the effective and safe use of these 3 agents as combination therapy for HIV-1 disease.

3. PHARMACOKINETICS

The absorption, distribution, metabolism, and excretion of BIC, FTC, and TFV/TAF were evaluated in vitro and in a variety of animal models in vivo. In addition, the drug-drug interaction (DDI) profile was also evaluated. The pharmacokinetics (PK) of the B/F/TAF FDC is discussed based on the results of clinical DDI studies completed with the 3 agents.

3.1. Analytical Methods

Bioanalytical methods for BIC, FTC, TDF/TAF and where appropriate, for their metabolites, were validated to support toxicokinetic analysis from GLP safety studies. Some of these methods for early nonclinical studies did not strictly conform to GLP guidelines but were evaluated for appropriate selectivity, sensitivity, and linearity as well as intra-assay accuracy and precision. All bioanalytical methods were conducted using appropriate protocols and documentation to assure data integrity.

3.2. In Vitro Absorption and Single Dose Pharmacokinetics

3.2.1. BIC

Bictegravir was highly permeable and showed efflux transport in Caco-2 cell monolayers (m2.6.5, Section 3.1.1, AD-141-2295). Bictegravir was a substrate of P-glycoprotein (P-gp) (m2.6.5, Section 14.1.1, AD-141-2278). The PK of BIC was determined in male rats, dogs, and monkeys following administration of oral solutions (m2.6.5, Section 3.1, AD-141-2279, AD-141-2280, and AD-141-2281). Bictegravir systemic plasma clearance was low in nonclinical species (0.1% to 1.3% of hepatic blood flow). Bictegravir volume of distribution (V_{ss}; 0.09 to 0.22 L/kg) in animals was lower than total body water. Bictegravir showed moderate to high oral bioavailability (42% to 74%) in nonclinical species. Overall, these data support high intestinal absorption for BIC in humans wherein the oral bioavailability is expected to be high (71% to 88%; GS-US-141-1481).

3.2.2. FTC

Single-dose PK of FTC was studied in mice (m2.6.5, Section 3.2, TEIN/93/0003, TEIN/93/0004 and IUW00101), rats {Frick 1993}, and cynomolgus monkeys (m2.6.5, Section 3.2, IUW00301 and TEZZ/93/0019). In these species, FTC was rapidly and well absorbed with oral bioavailability ranging from 58% to 97% over the dose range of 10 to 600 mg/kg.

3.2.3. TAF

In Caco-2 cell monolayers, 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 cyclosporin A diminished the efflux ratio and increased the forward permeability (m2.6.5, Section 3.3.1, AD-120-2037).

Single-dose plasma PK of TFV and/or TAF were evaluated following administration of TAF by dosing either GS-7340-02 or GS-7340-03 to male CD-1 mice or GS-7340-03 to both male and female 001178-W mice via oral gavage (m2.6.5, Section 3.3, AD-120-2014 and AD-120-2016),

to rats via oral gavage (m2.6.5, Section 3.3, AD-120-2015, R990130 and R2000065), to dogs via intravenous bolus of GS-7340-02 or oral administration of TAF as free base, its diastereomer GS-7339, the mixture GS-7171, or GS-7340-02 under fasted and under fed conditions (m2.6.5, Section 3.3, 99-DDM-1278-001-PK and AD-120-2034). Tenofovir alafenamide was not detected in plasma in any of the rat studies. Additionally, the plasma PK profiles for TAF and TFV and TFV concentrations in PBMCs were determined in rhesus monkeys following a single oral dose of GS-7340-02 (m2.6.5, Section 3.3.9, P2000087). The liver pharmacokinetic profiles were determined following oral administration of 10 mg/kg TAF to dogs and the pharmacologically active metabolite, TFV-DP was the major metabolite in liver achieving a maximal concentration (C_{max}) of 126 µM at 4.0 hours postdose and persisting for over 24 hours (m2.6.5, Section 3.3.8, AD-120-2034). Tenofovir alafenamide generated sufficient exposure of TFV in nonclinical species chosen for assessment of toxicology.

3.2.4. B/F/TAF

No nonclinical studies of the absorption kinetics of the B/F/TAF FDC have been conducted. However, comprehensive clinical studies on the combination product have been performed (m2.7.2, Section 1.2).

3.3. Repeat Dose Pharmacokinetics

3.3.1. BIC

The multiple-dose pharmacokinetic parameters for BIC were determined as part of the repeat-dose pivotal GLP toxicity studies in rats dosed 5 to 300 mg/kg/day for up to 26 weeks and in monkeys dosed 30 to 1000 mg/kg/day for up to 39 weeks (m2.6.7, Section 7.1, TX-141-2031 and TX-141-2032). Bictegravir plasma exposure increased following repeat oral administration of BIC; the increases in C_{max} and AUC were less than dose proportional. In rats, females had higher BIC C_{max} and AUC_{0-24} values than males, with gender-based differences of 2-to 3-fold on study days 90 and 181 for animals administered the high dose (300 mg/kg/day). No accumulation (< 2-fold) of BIC was observed in rats after repeat dosing with the exception of females administered the low dose (5 mg/kg/day), wherein a slight accumulation (~ 3-fold) was observed (m2.6.7, Section 7.1.3, TX-141-2031). In monkeys, gender-based differences in BIC C_{max} and AUC values were less than 2-fold and no accumulation (< 2-fold) of BIC was observed after repeat dosing (m2.6.7, Section 7.1.5, TX-141-2032).

3.3.2. FTC

The multiple-dose pharmacokinetic parameters for FTC were derived as part of the repeat-dose toxicity studies in mice (80 to 3000 mg/kg/day; m2.6.5, Section 4.2.1, TOX-109; m2.6.7, Section 7.2, TOX001, TOX599, and TOX628), rats (60 to 3000 mg/kg/day; m2.6.5, Section 4.2.2, TOX108; m2.6.7, Section 7.2.5, TOX097), and monkeys (40 to 2000 mg/kg/day; m2.6.7, Section 7.2, TOX600, TOX627, and TOX032) dosed for periods of 3 days to 104 weeks. In general, there were no significant differences in PK following single and multiple dosing. Systemic exposure to FTC (C_{max} and AUC) increased approximately proportionally with dose and was similar between males and females.

3.3.3. TAF

The multiple-dose PK of TFV were characterized in a pharmacokinetic study in dogs orally administered TAF (m2.6.5, Section 4.3.1, AD-120-2033) and in toxicokinetic studies following oral administration of TAF in mice (m2.6.7, Section 7.3.1, TX-120-2007), rats (m2.6.7, Section 7.3, R990182 and TOX-120-001), dogs (m2.6.7, Section 7.3, D990175 and TOX-120-002), and monkeys (m2.6.7, Section 7.3.6, P2000114). After repeat dosing in mice or monkeys for up to 13 weeks or 4 weeks, respectively, no accumulation of TFV occurred; slight accumulation (up to ~3-fold) of TFV occurred in rats and dogs dosed for up to 26 and 39 weeks, respectively.

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 Days 1 and 7 (m2.6.5, Section 4.3.1, AD-120-2033). TAF was rapidly absorbed and exhibited a short terminal half-life of 0.3 hours in plasma on both Days 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 C_{max} of 1.47 and 2.12 µM on Days 1 and 7, respectively. The pharmacologically active diphosphate metabolite, TFV-DP, was efficiently formed in dog livers achieving concentrations of 242 and 153 µM at 4.0 and 24 hours postdose on Day 7, respectively.

3.3.4. B/F/TAF

No additional repeat-dose pharmacokinetic studies were considered warranted with the combination of BIC, FTC, and TAF due to the lack of pharmacokinetic interactions between the 3 agents in humans (GS-US-141-1218) and the clinical experience with the components in ARV combination therapy for the treatment of HIV-1 infection.

3.4. Distribution

3.4.1. Protein Binding

3.4.1.1. BIC

Bictegravir was highly bound to plasma proteins in vitro in all species (> 98% bound) and was 99.75% bound in humans (m2.6.5, Section 6.1.1, AD-141-2287). Bictegravir has minimal partitioning into erythrocytes; the blood to plasma BIC concentration ratio was close to 0.6 in all species (m2.6.5, Section 5.1.1, AD-141-2312).

3.4.1.2. FTC

The protein binding of FTC was very low (< 5%) in mouse, rabbit, monkey, and human plasma (m2.6.5, Section 6.2.1, TBZZ/93/0025).

3.4.1.3. TAF and TFV

Since TAF is highly unstable in rodent plasma due to hydrolytic cleavage by plasma esterases, the extent of TAF binding to plasma was determined in dog and human plasma in vitro (m2.6.5, Section 6.3.1, AD-120-2026). In vitro protein binding of TAF was moderate in dog and human plasma with the percentage bound values of 52.0% and 53.2%, respectively. These in vitro values were lower than those observed in multiple human ex vivo studies with the mean percentage bound TAF ranging from 77% to 86% in all subjects (GS-US-120-0108 and GS-US-120-0114). Since the ex vivo results should be more clinically relevant than the in vitro values, percentage bound TAF of 80% was used for the assessments for potential drug interactions.

The protein binding of TFV was very low (< 10%) in the plasma and serum of humans and all other species examined (m2.6.5, Section 6.3.2, P0504-00039.1).

3.4.1.4. B/F/TAF

The plasma protein binding of BIC, FTC and TAF has been well characterized; no further studies have been conducted with the combination.

3.4.2. Tissue Distribution

3.4.2.1. BIC

The tissue distribution of BIC in rats has been studied using quantitative whole body autoradiography (QWBA) following single oral administration of [^{14}C]BIC at 2 mg/kg (m2.6.5, Section 5.1.2, AD-141-2276). Studies were performed in male Wistar Han (non-pigmented) and Long Evans (pigmented) rats. The [^{14}C]BIC-derived radioactivity was rapidly (0.25 hours postdose) and widely distributed to most tissues and was similar in both Wistar Han and Long Evans rat. Concentrations in tissues were lower than in blood and decreased throughout the course of the study (168 hours). Low levels of radioactivity were detected in brain (< 4% relative to blood), suggesting that [^{14}C]BIC-derived radioactivity poorly crossed the blood brain barrier. By 168 hours, quantifiable radioactivity was observed in tissues, but concentrations were declining, suggesting reversible binding. Distribution trends in the pigmented uveal tract of the eye and pigmented skin suggested that [^{14}C]BIC-related radioactivity was not selectively associated with melanin-containing tissues.

3.4.2.2. FTC

The tissue distribution of [^{14}C]FTC was characterized in rats and cynomolgus monkeys after a single oral dose of 200 mg/kg (m2.6.5, Section 5.2.1, TOX092; m2.6.5, Section 8.2.2, TOX063). Emtricitabine was widely distributed in the body, with measurable concentrations found in all tissues within 1 hour following oral administration. Tissue concentrations generally declined in parallel with plasma concentrations, with no indication of accumulation in any tissue examined. Virtually no radioactivity remained in the body at 72 hours after dosing. The highest concentrations of FTC were found in the kidneys and liver. Concentrations in CNS tissues were 2% to 10% of the concentration in plasma.

3.4.2.3. TAF and TFV

Following oral administration of [^{14}C]TAF to mouse (m2.6.5, Section 5.3.1, AD-120-2011), rat (m2.6.5, Section 5.3.2, AD-120-2020), and dog (m2.6.5, Section 5.3, AD-120-2009 and D990173-BP), [^{14}C]TAF-derived radioactivity was widely distributed to most of the tissues in all species. Consistent with high hepatic extraction, high levels of radioactivity were observed in the liver; high radioactivity was also measured in the kidney. Low levels of radioactivity were observed in brain and testis in mouse. No melanin binding was observed in rats. Distribution trends in the pigmented uveal tract of the eye and pigmented skin suggested that [^{14}C]TAF-related radioactivity was not selectively associated with melanin-containing tissues in the pigmented mouse. Distribution studies in dogs showed 5.7 to 15-fold higher ^{14}C-radioactivity in lymphoid tissues 24 hours following administration of an equivalent dose of [^{14}C]TAF relative to [^{14}C]TDF {Lee 2005}. The concentration of TAF in dogs was relatively high also in lungs, thyroid, spleen, skeletal muscle, bone marrow, and some other tissues relative to TDF. Since the clinical TAF dose is > 10-fold less than TDF, accumulation of TAF and/or its metabolites in these tissues should be similar (or less) to that with TDF.

3.4.2.4. B/F/TAF

Tissue distribution studies have not been conducted with the B/F/TAF combination, as each of the components in the combination tablet has been evaluated thoroughly and a distribution interaction is unlikely.

3.4.3. Distribution in Pregnant Animals

Pharmacokinetic parameters for BIC and FTC in pregnant animals were generally similar to those reported for nonpregnant animals. In pregnant rats, no accumulation of BIC was observed at the 30 and 300 mg/kg/day dose levels, while accumulation was observed at the 5 mg/kg/day dose level between the first and last dose (AUC_{0-24}; approximately 3.5-fold higher) (m2.6.7, Section 11.1, TX-141-2034). In general, no accumulation of BIC was observed after multiple dosing in pregnant rabbits (m2.6.7, Section 11.1, TX-141-2038). While accumulation of TFV was observed after multiple dosing of TAF as GS-7340-02 up to 200 mg/kg/day in pregnant rats in a range-finding study (m2.6.7, Section 11.3, TX-120-2001), no accumulation of TAF and TFV was observed up to 250 mg/kg/day in the definitive embryo-fetal development study (m2.6.7, Section 13.5, TX-120-2002). No accumulation of TAF and TFV occurred in pregnant rabbits (m2.6.7, Section 11.3, TX-120-2004; m2.6.7, Section 13.6, TX-120-2005).

The plasma exposure of BIC in nursing pups was determined in a prenatal and postnatal development study in rats (m2.6.7, Section 14.1, TX-141-2045). Bictegravir was detected in the plasma of neonates on lactation day 10. Bictegravir exposure in maternal rats was roughly similar to pups at the 2 mg/kg/day dose level, slightly higher (approximately 1.5-fold) in maternal rats than in pups at the 10 mg/kg/day dose level, and greater than 2-fold higher (approximately 2.8-fold) in maternal rats than in pups at the 300 mg/kg/day dose level. These data suggested that BIC present in maternal rat systemic circulation was distributed to milk and transferred to nursing pups.

Placental transfer studies were conducted for TFV (rhesus monkeys) and FTC (mice and rabbits). Both drugs are transferred across the placenta but did not concentrate in fetal tissues. Fetal/maternal exposure ratios, determined on appropriate gestation days (GDs) by the concentrations of TFV in serum and FTC in plasma and umbilical cord blood, were ≤ 0.5 (m2.6.5, Section 7.3.2, 96-DDM-1278-005; m2.6.5, Section 7.2, TOX103 and TOX038).

3.5. Metabolism

The metabolism of BIC, FTC, and TAF has been well characterized. In addition, the potential for drug interactions based on in vitro intracellular metabolism and the effect on drug metabolizing enzymes has been well studied.

3.5.1. Intracellular Metabolism

Bictegravir is not subject to intracellular activation.

Tenofovir alafenamide is subject to intracellular metabolism to TFV, which is further phosphorylated to the anabolites tenofovir monophosphate (TFV-MP) and TFV-DP with TFV-DP being the pharmacologically active form. Intracellular metabolic activation of TAF in PBMCs or HIV-target cells including lymphocytes involves conversion to TFV by CatA {Birkus 2008, Birkus 2007}. In contrast to PBMCs, TAF is primarily hydrolyzed by carboxylesterase 1 in primary hepatocytes. Tenofovir is then further phosphorylated to TFV-DP by cellular nucleotide kinases. These steps are high capacity and low affinity and are not readily inhibited by other xenobiotics. Of the HIV PIs (darunavir, atazanavir, lopinavir, and ritonavir), the boosting agent COBI, and HCV PIs (telaprevir, boceprevir, TMC-435, BI-201355, MK-5172, GS-9256, and GS-9451), the HCV PIs telaprevir and boceprevir, which are known to inhibit CatA, were the only ones that changed the ARV effect of TAF in primary CD4+ T lymphocytes (reduced 23-fold and 3-fold, respectively). These data support the co-administration of the tested therapeutic PIs, with the exception of telaprevir or boceprevir, in combination with TAF, without negatively affecting its clinical pharmacology and intracellular conversion to TFV.

Emtricitabine and TFV are analogues of 2 different nucleosides, cytosine and adenosine, respectively, and do not share a common intracellular metabolism pathway. In experiments where both FTC and TFV were incubated together at concentrations higher than achieved in the plasma (10 µM), the intracellular phosphorylation of FTC and TFV to their active intracellular anabolites was not affected (m2.6.3, Section 1.3, PC-164-2001).

The in vitro activation of TAF in human primary hepatocytes was evaluated and compared with that of TDF and TFV (m2.6.5, Section 9.3.5, AD-120-2017). Following a 24-hour continuous incubation of primary hepatocytes with 5 µM TAF, TDF, or TFV, the levels of TFV-DP increased to 1470, 302, and 12.1 pmol/million cells illustrating that incubation with TAF resulted in 5- and 120-fold higher intracellular levels of TFV-DP compared to TDF and TFV, respectively. In primary human hepatocytes, the half-life of intracellular TFV-DP was estimated to be greater than 24 hours {Murakami 2015}.

3.5.2. In Vitro Metabolism

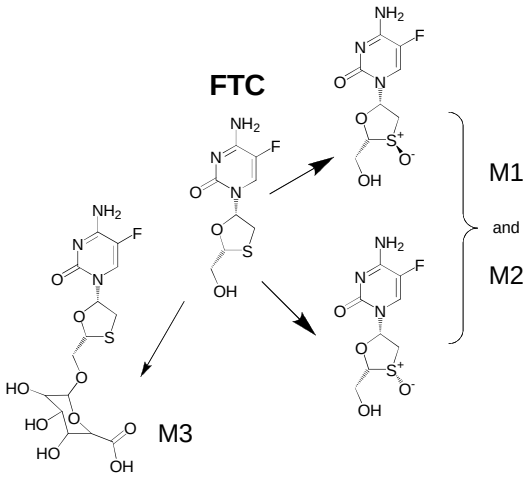
3.5.2.1. BIC

The rate of hepatic metabolism of BIC was assessed in vitro (m2.6.5, Section 9.1.1, AD-141-2289). Bictegravir was highly stable in human microsomal fraction and moderate to highly stable in dog, rat and monkey microsomal fractions. Bictegravir metabolism was determined in cryopreserved hepatocytes from rat, dog, monkey and human (m2.6.5, Section 9.1.4, AD-141-2288). Metabolic pathways included hydroxylation, N-dealkylation, and direct glucuronidation. All metabolites observed in human hepatocyte incubations were also found in rat and monkey; these were the species used in chronic toxicology studies. No unique human metabolites were observed in vitro. Bictegravir metabolism was predominantly mediated by cytochrome P450 enzyme (CYP) 3A and uridine diphosphate glucuronosyltransferase (UGT) 1A1 (m2.6.5, Section 9.1, AD-141-2290 and AD-141-2291).

3.5.2.2. FTC

Emtricitabine is not subject to significant metabolism by CYP enzymes. Generation of a minor (~1%) sulfoxide metabolite (M1 and/or M2) was catalyzed by CYP3A4, and inhibitor studies suggested that at least one other enzyme, possibly flavin-containing monooxygenase, may play a role (m2.6.5, Section 9.2.1, 15396v1). A minor direct glucuronide metabolite, M3, was also detected (Figure 1) {Frick 1993}.

Figure 1. Oxidative Metabolism of FTC



An in vitro metabolism study was performed to identify the potential human CYP enzyme(s) responsible for the metabolism of FTC using human liver microsomes and Bactosomes containing complementary DNA (cDNA)-expressed human CYP enzymes (m2.6.5, Section 9.2.1, 15396v1). The results showed that FTC was relatively stable in the incubation medium. One minor metabolite (~1%) was detected only in incubations with cDNA-expressed CYP3A4 incubations. It was not formed by CYP1A2, 2A6, 2B6, 2D6, 2E1, 2C8, 2C9, or 2C19. Human hepatic

microsomal incubations in the presence and absence of selective inhibitors of various CYPs confirmed the low rate of FTC metabolism, and due to incomplete inhibition by the CYP3A-selective inhibitor ketoconazole, also suggested the possible involvement of flavin monooxygenases (FMOs) in the metabolism of FTC. In vitro glucuronidation of FTC was not detected.

3.5.2.3. TAF

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

Stability of TAF was assessed in plasma, intestinal S9, and hepatic S9 fractions from dogs and humans (m2.6.5, Section 9.3, AD-120-2023, AD-120-2024, AD-120-2025, and AD-120-2027). Tenofovir alafenamide was moderately stable in plasma and intestinal S9 with half-lives of 74.7 and 58.3 minutes for human and 69.5 and 47.1 minutes for dog, respectively (m2.6.5, Section 9.3, AD-120-2025 and AD-120-2024). The stability of TAF in human intestinal S9 fractions was also determined in a separate study assessing the effect of HIV-PIs on TAF stability in intestinal S9 and a somewhat lower but similar half-life for TAF was observed with 24.5 minutes (m2.6.5, Section 9.3.7, AD-120-2027). Relative to plasma or intestinal S9, TAF was somewhat less stable in human and dog hepatic S9 fractions with half-lives of 20.6 and 31.1 minutes, respectively. Based on these data, predicted hepatic extraction ratios for human and dog were calculated to be 76.2% and 60.5%, respectively (m2.6.5, Section 9.3.2, AD-120-2023).

The potential for CYP enzymes to metabolize TAF was assessed by incubating TAF with 6 individual bacterially expressed human CYP enzyme preparations (Bactosomes) coexpressed with human nicotinamide adenine dinucleotide phosphate, reduced (NADPH) CYP reductase (m2.6.5, Section 9.3.4, AD-120-2004). Metabolism of TAF was not detected by CYP1A2, CYP2C8, CYP2C9, CYP2C19 or CYP 2D6. Tenofovir alafenamide was slowly metabolized by CYP3A4 at a rate of 1.9 min^{-1}, which was 26.6% of the positive control, testosterone. While TAF is a weak inhibitor of CYP3A in vitro, it is not a clinically meaningful inhibitor or inducer of CYP3A.

The in vitro metabolism of [^{14}C]TFV was studied in dog plasma, in control and induced (Aroclor™ 1254) rat liver microsomes, and also in dog liver and intestinal S9 fractions (m2.6.5, Section 9.3.6, 96-DDM-1278-003). No metabolites were detected in either rat microsomal preparation, with or without the addition of NADPH cofactor. There was no evidence of chiral inversion. Similarly, there was no apparent loss of TFV following incubation with dog plasma, liver, or intestinal S9 fractions, and no metabolites were detected.

3.5.2.4. B/F/TAF

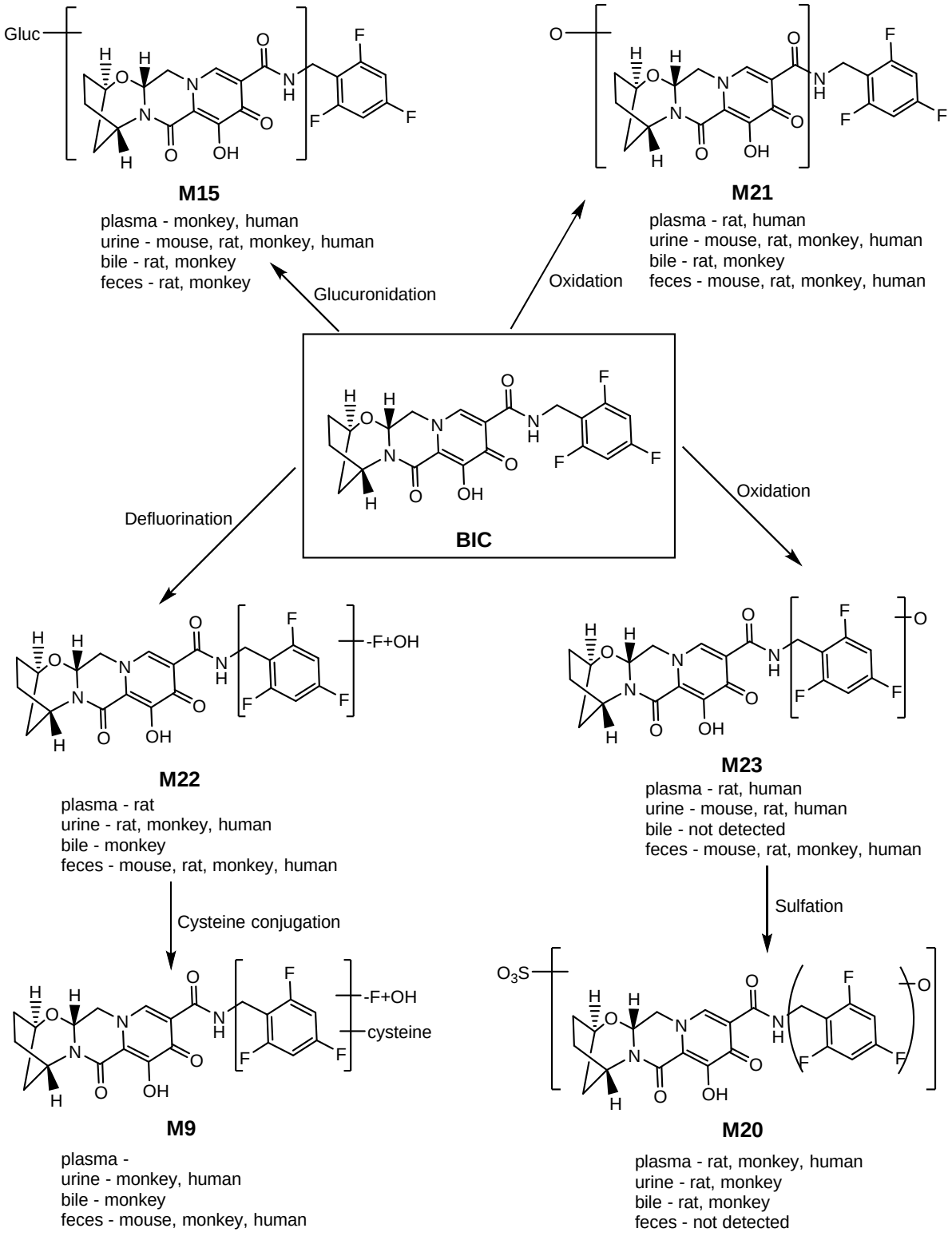
In vitro metabolism studies have not been conducted with B/F/TAF together, as each agent in the combination tablet has been evaluated thoroughly. Based on in vitro metabolism of the individual agents, any interactions are unlikely.

3.5.3. In Vivo Metabolism

3.5.3.1. BIC

Bictegravir metabolism was determined following a single oral administration of [^{14}C]BIC to mouse, rat, monkey and human (m2.6.5, Section 8.1, AD-141-2304, AD-141-2277, and AD-141-2299; and GS-US-141-1481). The proposed metabolic pathways are shown in Figure 2. The combined results demonstrate that BIC is predominantly eliminated by hepatic metabolism followed by excretion of the biotransformed products into feces and urine. Metabolic pathways included hydroxylation, oxidative defluorination, direct glucuronidation and oxidation followed by phase II conjugation. In the monkey, BIC was metabolized via the oxidative pathways to a greater extent compared to rat and human.

Figure 2. Metabolites Identified in Mouse, Rat, Monkey and Human Following a Single Dose of [^{14}C]BIC



3.5.3.2. FTC

Emtricitabine is primarily eliminated as unchanged drug by renal excretion in mice, rats, and cynomolgus monkeys. Over 90% of the radioactivity in mouse and rat urine and 64% of the radioactivity in monkey urine was unchanged drug. Only trace levels of metabolites were found in feces {Frick 1994, Frick 1993} (m2.6.5, Section 8.2, TEIN/93/0015, TEIN/93/0016, and TOX063). In all 3 species, metabolism accounted for only a minor percentage of FTC elimination. Of the fraction metabolized, FTC was oxidized to a diastereomeric sulfoxide and to a lesser extent as a direct hydroxymethyl glucuronide conjugate.

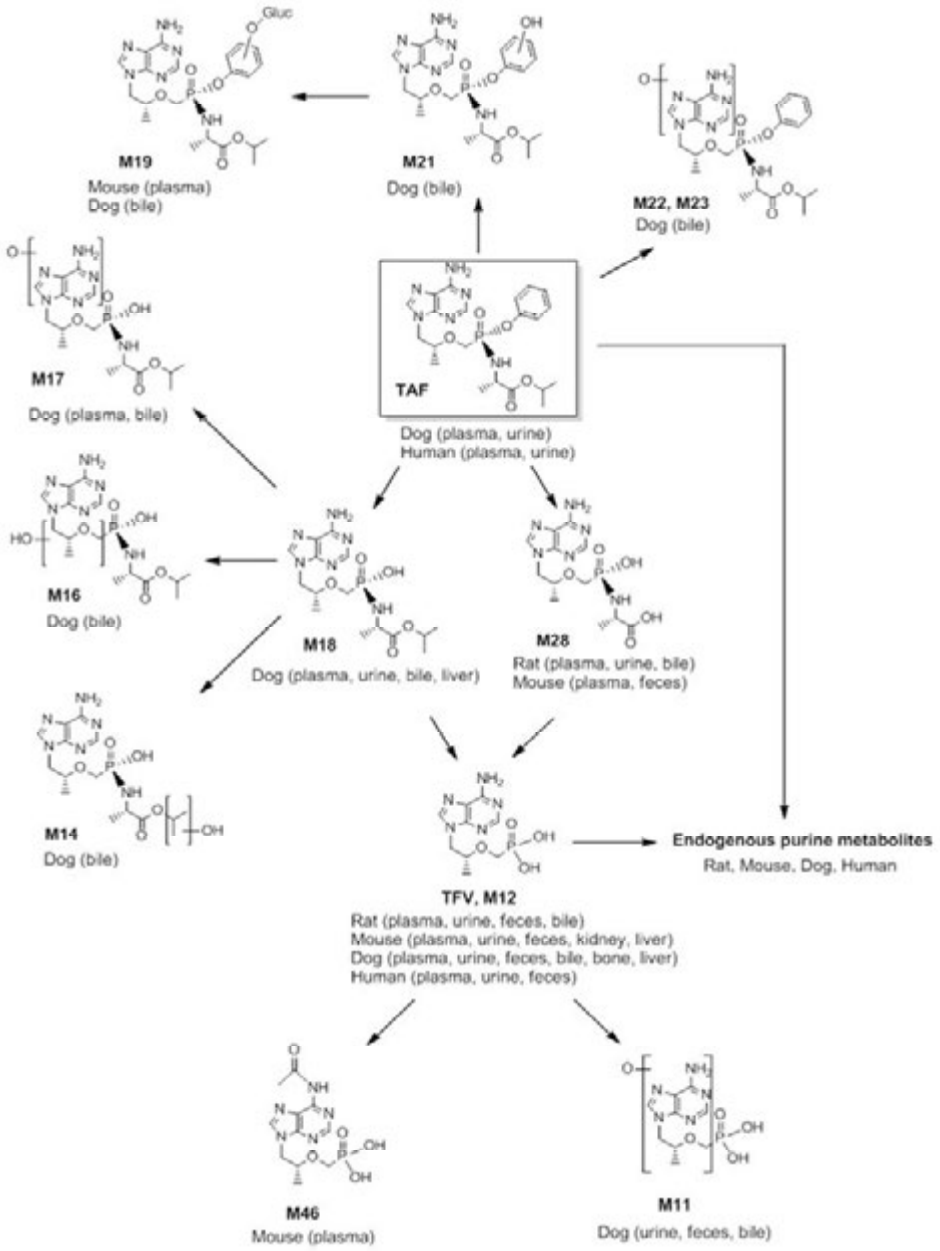
3.5.3.3. TAF

The metabolic profiles of TAF were determined in plasma, urine, feces, kidney, liver, and nasal turbinate from mice (m2.6.5, Section 8.3.1, AD-120-2012); in plasma, urine, bile, and feces from rats (m2.6.5, Section 8.3.2, AD-120-2021); and in plasma, urine, bile, feces, bone, and liver from dogs (m2.6.5, Section 8.3.3, AD-120-2008). The metabolite profiles were also determined in human plasma, urine, and feces following administration of a single oral dose of [^{14}C]TAF (GS-US-120-0109). Based on the results from mouse, rat, dog, and human, a proposed biotransformation pathway is summarized (Figure 3). Endogenous purine metabolites including hypoxanthine, xanthine, allantoin, and uric acid were observed in all species. Tenofovir accounted for a majority of drug related material in plasma, urine, and feces from all species except for human plasma, in which uric acid was the predominant metabolite accounting for 73.9% of the total AUC over 96 hours. M18 was the major metabolite in rat bile accounted for 63% of total radioactivity recovered in bile. M18 and its oxidized metabolite M16 were the major metabolites in dog bile accounted for 29% and 38% of total radioactivity recovered in bile. Various oxidative metabolites were found in dog bile. No metabolites unique to human were observed.

Tenofovir alafenamide-related metabolites were also monitored in kidney, liver, and nasal turbinate from mice (m2.6.5, Section 8.3.1, AD-120-2012). Most of the radioactivity was associated with TFV in kidney and liver and xanthine (M7) was the major identified metabolite in nasal turbinates. In dog, TAF-related metabolites were monitored in bone and liver and most of the radioactivity in these tissues was associated with TFV (m2.6.5, Section 8.3.3, AD-120-2008).

M18 (isopropylalaninyl TFV) and M28 (alaninyl TFV) are considered to be intermediate metabolites during intracellular conversion of TAF to TFV. In the metabolite profiling study in dog, M28 was not detected in this study although it has been qualitatively detected previously in dog plasma at 15 minutes postdose {Babusis 2013}. M18 was detected as a minor metabolite in plasma, urine, and liver. Relatively high level of M18 was observed in bile. Low levels of M28 were observed in rat and mouse plasma and relatively high levels were in rat bile.

Figure 3. Metabolites of TAF



3.5.3.4. B/F/TAF

No nonclinical studies have been completed assessing the metabolism of the 3-drug combination of BIC, FTC, and TAF because each agent has distinct metabolic and excretion pathways. Bictegravir is metabolized by CYP3A mediated oxidation and via conjugation by UGT enzymes, FTC is cleared by renal excretion and TAF is metabolized to TFV by hydrolysis.

3.6. Excretion

3.6.1. Recovery in Excreta

3.6.1.1. BIC

The excretion of radioactivity was determined following a single oral administration of [^{14}C]BIC to male mouse, rat, and monkey (m2.6.5, Section 12.1, AD-141-2303, AD-141-2276 and AD-141-2298). The average cumulative overall recovery of dosed radioactivity was > 80% in all species studied. The excretion routes in intact animals were consistent across species, with the majority of the excreted dose in feces (> 40% of dose) and with minor amounts in urine (< 21% of dose). The excretion into bile in bile duct cannulated (BDC) rat and monkey was approximately 34% to 40% of dose, respectively. In both species, the amount of unchanged BIC in urine or bile was negligible. In combination with metabolite profiling, these results demonstrate that BIC is mainly eliminated through metabolism by the liver followed by excretion into feces and urine.

3.6.1.2. FTC

The primary route of elimination of [^{3}H]FTC and [^{14}C]FTC was via renal excretion of parent drug after oral and intravenous administration in mice, rats, and cynomolgus monkeys {Frick 1993} (m2.6.5, Section 8.2, TEIN/93/0015, TOX063, and TEIN/93/0016; m2.6.5, Section 5.2.1, TOX092). The majority of the FTC recovered in the feces after oral administration most likely represents unabsorbed drug, rather than biliary excretion. Although FTC is metabolized to only a minor extent, its metabolites are also excreted via the kidneys.

3.6.1.3. TAF and TFV

Following oral dosing of mice, rats, and dogs with [^{14}C]TAF, the majority of radiolabel is recovered in the feces or urine in all species (m2.6.5, Section 5.3, AD-120-2011, AD-120-2020; m2.6.5, Section 13.2.1, AD-120-2007). The excretion of [^{14}C]TAF was determined after administration of a single 5-mg/kg oral dose of [^{14}C]TAF to bile duct-intact and BDC male Sprague-Dawley rats (m2.6.5, Section 5.3.2, AD-120-2020). In BDC rats, means of 72.6%, 23.2%, and 2.11% of the administered radioactivity were excreted in feces, urine, and bile, respectively, by 168 hours postdose. Recoveries of radioactivity in bile and urine from BDC rats indicated that at least 25% of the dose was absorbed. The mean overall recovery of radioactivity after oral dosing to BDC rats was 99.9%. The excretion of [^{14}C]TAF was determined after administration of a single 15-mg/kg oral dose of [^{14}C]TAF to bile duct-intact and BDC male dogs (m2.6.5, Section 13.2.1, AD-120-2007). In BDC dogs, means of 42.7%, 26.5%, and 14.0% of the administered radioactivity were excreted in feces, urine, and bile, respectively, through 168 hours postdose. The elimination of a large amount of radioactivity in bile of BDC dogs indicates that biliary excretion is a major route of elimination of [^{14}C]TAF-derived radioactivity. The overall recovery of radioactivity in BDC dogs was 86.2%.

Renal excretion is the primary systemic route of elimination of TFV in all preclinical species tested. Following intravenous administration of [^{14}C]TFV, the majority of radioactivity was recovered in the urine in rats and dogs with 85.2% by 24 hours and 70.03% by 48 hours, respectively (m2.6.5, Section 12.3.1, 96-DDM-1278-001; m2.6.5, Section 13.2.2, 96-DDM-1278-002).

3.6.1.4. B/F/TAF

No nonclinical excretion studies have been done with the combination of BIC, FTC, and TAF. Bictegravir is metabolized by CYP3A mediated oxidation and conjugation by UGT enzymes and then eliminated into bile, feces and urine. FTC is eliminated primarily intact by renal excretion. TAF is metabolized by hydrolysis to TFV and is then eliminated by renal excretion. Since BIC, FTC, and TAF have distinct metabolic and excretion pathways for elimination, the coadministration of BIC, FTC, and TAF is not anticipated to change the excretion of the individual compounds.

3.6.2. Excretion into Breast Milk

Excretion of BIC into milk was assessed in a rat prenatal and postnatal development study (m2.6.7, Section 14.1, TX-141-2045). Bictegravir was detected in the plasma of neonates on lactation day 10; BIC exposure in maternal rats was up to 2.8-fold higher than in pups. These data indicated that BIC present in maternal rat systemic circulation was distributed to milk and transferred to nursing pups.

Excretion into milk has not been evaluated for FTC.

Tenofovir was excreted into the breast milk of lactating rats and rhesus monkeys (m2.6.7, Section 14.3, R990202; m2.6.5, Section 7.3.2.2, P2000116). The TFV milk to plasma ratios ranged from 0.11 to 0.24 in rats and 0.19 to 0.22 in rhesus monkeys.

Because of the potential for HIV transmission and the potential for serious adverse reactions in nursing infants, mothers will be instructed not to breastfeed if they are receiving the B/F/TAF FDC.

3.7. Pharmacokinetic Drug Interactions

3.7.1. Cytochrome P450 and UGT1A1 Inhibition

3.7.1.1. BIC

In vitro, UGT1A1 and CYP3A played a major role in the biotransformation of BIC (m2.6.5, Section 9.1, AD-141-2290 and AD-141-2291). These in vitro findings were consistent with the results from a clinical DDI study in healthy subjects. Administration of rifampin, a strong CYP3A4 and UGT1A1 inducer, reduced BIC plasma AUC by 75% and administration of atazanavir (a UGT1A1/CYP3A4 inhibitor) increased BIC plasma AUC by 315%; and administration of voriconazole, a strong CYP3A4 inhibitor, increased BIC plasma AUC by 61% (GS-US-141-1485).

In vitro, BIC had little or no inhibitory effect (IC_{50} > 100 µM) on the activities of CYPs 1A2, 2B6, 2C8, 2C9, 2C19, 2D6, 3A or UGT1A1 (m2.6.5, Section 11.1, AD-141-2293 and AD-141-2294). BIC is unlikely to cause significant drug interactions in vivo through inhibition of human CYP enzymes or UGT1A1 based on the calculated AUC ratio (AUCR) values (< 1.2; m2.6.5, Section 14.1.7, AD-141-2313) using the FDA net effect model as well as the calculated $[I]/K_{i,u}$ (< 0.02; m2.6.5, Section 14.1.7, AD-141-2313) based on the European Medicines Agency (EMA) guidance.

Bictegravir showed no time-dependent inhibition against CYPs 1A2, 2B6, 2C8, 2C9, 2C19, or 2D6 (m2.6.5, Section 11.1.3, AD-141-2308). Bictegravir was detectable as a very weak mechanism based inhibitor of CYP3A by IC_{50} shift (K_I > 100 µM). Further determination of kinetic parameters was not possible due to solubility limitations and weak activity. BIC is unlikely to be a clinically relevant mechanism-based inhibitor of CYP3A.

3.7.1.2. FTC

Emtricitabine was not an inhibitor of activities catalyzed by CYP1A2, 2A6, 2B6, 2C9, 2C19, 2D6, 2E1, or 3A in human hepatic microsomal fractions. Emtricitabine also did not show inhibition of the glucuronidation of 7-hydroxycoumarin, a general UGT substrate (m2.6.5, Section 11.2.1, 15247).

3.7.1.3. TAF and TFV

The potential for TAF and TFV to reversibly inhibit human CYP-mediated drug metabolism was examined in vitro using hepatic microsomal fractions and enzyme-selective activities (m2.6.5, Section 11.3, AD-120-2003 and V990172-104). In vitro, TAF had little or no inhibitory effect (IC_{50} > 25 µM) on the activities of CYPs 1A2, 2B6, 2C8, 2C9, 2C19, or 2D6. Tenofovir alafenamide was a weak inhibitor of CYP3A activity (IC_{50} of 7.6 µM). The weak inhibition of CYP3A was not clinically relevant as TAF did not affect the exposure to CYP3A substrates, midazolam or rilpivirine (GS-US-120-1538 and GS-US-120-1554). Tenofovir at 100 µM did not inhibit CYPs 1A2, 2C9, 2D6, 2E1, and 3A.

The potential for TAF to be a mechanism-based inhibitor of the human CYP enzymes, CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, and CYP2D6 was assessed at TAF concentration at 50 µM (m2.6.5, Section 11.3.3, AD-120-2040). Tenofovir alafenamide showed no time-dependent inhibition against the assessed CYPs.

3.7.2. Induction Liability

3.7.2.1. BIC

The potential for BIC to induce CYP enzymes, UGT1A1, and P-gp was assessed in vitro in cultured human hepatocytes from 3 different donors (m2.6.5, Section 11.1.5, AD-141-2305). Bictegravir was a weak inducer of CYP3A4 with other enzymes and transporters unaffected or modulated to a lesser extent. Concentration dependent CYP3A4 messenger RNA (mRNA) increases were observed resulting up to 16.7-fold with 60 µM BIC. Although in vitro data suggested weak induction of CYP3A4, clinical studies confirmed that BIC is not an inducer of CYP3A in vivo (GS-US-380-4270, GS-US-380-1999, GS-US-141-1218, and GS-US-311-1790).

3.7.2.2. FTC

Emtricitabine did not activate human aryl hydrocarbon receptor (AhR) or pregnane X receptor (PXR) at concentrations up to 50 µM (m2.6.5, Section 11.2.2, AD-162-2005).

3.7.2.3. TAF

The induction of CYP, P-gp and UGT1A1 mRNA and CYP activity by TAF was assessed in cultured human hepatocytes from 3 separate donors treated with 1, 10, and 100 µM TAF once daily for 3 consecutive days (m2.6.5, Section 11.3.5, AD-120-2032). Due to cytotoxicity, the 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 noncytotoxic concentrations of TAF (1 and 10 µM), no significant increases in the mRNA levels and the CYP activities were observed. 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 the induction levels observed with the respective positive controls. Therefore, TAF showed little or no potential for CYP induction at clinically relevant concentration (1 µM). No significant induction of P-gp and UGT1A1 mRNA was observed (less than 2-fold).

The potential for TAF to induce human drug metabolizing enzymes and drug transporters through the activation of human AhR or human PXR was further evaluated in cell-based systems (m2.6.5, Section 11.3.4, AD-120-2005). For PXR activation 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. Tenofovir alafenamide did not activate AhR up to 50 µM, the highest concentration tested. Therefore, TAF is unlikely to activate either of these human xenobiotic receptors supporting the in vitro induction results in human hepatocytes. Furthermore, clinical studies confirmed that TAF is not an inducer in vivo (GS-US-120-1538 and GS-US-120-1554).

3.7.3. Transporter Drug Interactions

3.7.3.1. BIC

Bictegravir was a substrate for human P-gp and breast cancer resistance protein (BCRP), and was not a substrate of organic anion transporting polypeptide (OATP) 1B1 or OATP1B3 (m2.6.5, Section 14.1, AD-141-2275 and AD-141-2278). Bictegravir did not inhibit OATP1B1, OATP1B3 or organic anion transporter (OAT) 1 mediated transport. Bictegravir, at the highest concentration tested (80 µM or 100 µM), weakly inhibited P-gp (20%), BCRP (6%), bile salt export pump (BSEP; 46%), OCT1 (13%) and OAT3 (64%) (m2.6.5, Section 14.1, AD-141-2273, AD-141-2274, and AD-141-2310). Bictegravir showed dose-dependent inhibition of MATE1 with an IC_{50} value of 8.0 µM (m2.6.5, Section 14.1.5, AD-141-2285). Bictegravir was an inhibitor of renal uptake transporter OCT2 in vitro, with an IC_{50} value of 0.42 µM (m2.6.5, Section 14.1.5, AD-141-2285). A clinical study was conducted with B/F/TAF and metformin (an OCT2 and MATE1 substrate), which showed no clinically relevant changes in the PK and PD of metformin (GS-US-380-3908).

3.7.3.2. FTC

In vitro studies indicated that FTC was not a substrate or an inhibitor of any of the transporters tested except for being a substrate of OAT3. There is no clinical evidence that FTC is involved in transporter-mediated drug interactions.

3.7.3.3. TAF and TFV

In vitro studies demonstrated that TAF and TFV do not inhibit any of the transporters tested at clinically relevant concentrations. Therefore, TAF and TFV are unlikely to be perpetrators of transporter-mediated drug interactions.

Tenofovir alafenamide was a substrate for intestinal efflux transporters, P-gp and BCRP. An increase in TAF absorption was observed in the presence of efflux transport inhibitors, CsA or COBI in vitro (m2.6.5, Section 14.3.3, AD-120-2013; m2.6.5, Section 3.3.1, AD-120-2037). Cobicistat is a weak inhibitor of intestinal efflux transporters, but high concentrations of COBI in the intestinal lumen, achievable briefly during absorption, may inhibit P-gp and result in increase in TAF exposure. The effect of CsA on TAF oral bioavailability was also assessed in vivo in dogs (m2.6.5, Section 14.3.14, AD-120-2035). Following oral administration of TAF at 2 mg/kg to untreated or pretreated dogs with 75 mg CsA, the CsA pretreatment increased the plasma exposure to TAF and oral bioavailability by approximately 10-fold, while the PK profile of TFV was not significantly affected by CsA. Consistent with the increased TAF plasma exposure, the exposure to TFV-DP in PBMCs isolated from the CsA pretreated dogs was approximately 2-fold higher than that in PBMCs from untreated animals. These results suggest that coadministration of efflux inhibitors increases TAF absorption and may potentiate the antiviral effect by increasing the TFV-DP levels in PBMCs.

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

The route of elimination of TFV is renal excretion by a combination of glomerular filtration and tubular secretion.

Results of in vitro transport studies indicate that the active tubular secretion of TFV was mediated by the human OAT1 and multidrug resistance-associated protein (MRP) 4 acting in series as the major uptake and efflux transporters in proximal tubules, respectively (m2.6.5, Section 14.3, PC-103-2001, AD-104-2001 and AD-104-2002) {Cihlar 2004, Cihlar 2001, Ray 2005}. Human OAT3 may play a secondary role in the tubular uptake of TFV. Neither P-gp nor MRP2 appear to be involved in the tubular efflux of TFV. As the primary transporter handling the tubular uptake of TFV, human 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 human OAT1-mediated transport of TFV, indicating a low potential for renal interactions with TFV due to inhibition of this pathway (m2.6.5, Section 14.3, PC-104-2010 and PC-104-2011) {Cihlar 2001}. Furthermore, the PIs

atazanavir, lopinavir, and ritonavir did not exhibit any effect on the active cellular elimination of TFV mediated by the MRP4 efflux pump {Ray 2005}. The results of in vitro drug interaction studies indicate that PIs are unlikely to exert any substantial effect on the accumulation of TFV in renal proximal tubules.

The results from in vitro studies investigating the contribution from MRP1 in tubular reabsorption of TFV (m2.6.5, Section 14.3.11, PC-104-2014) indicated that MRP1 is not involved in the reabsorption of TFV at the basolateral membrane of proximal tubule cells.

3.7.4. B/F/TAF Pharmacokinetic Drug Interactions

No in vitro DDI studies were conducted with the B+F+TAF. In vivo PK drug interaction studies with the combination are described in m2.7.2.

3.8. Summary of Pharmacokinetics

A comprehensive nonclinical program defining the absorption, distribution, metabolism, excretion and drug interaction potential of BIC, FTC and TAF/TFV has been completed. The nonclinical pharmacokinetic and disposition studies discussed in this section provide an adequate basis for comparing and interpreting results from toxicology and clinical studies.

Based on the data supporting the individual components and the B/F/TAF combination, adverse pharmacokinetic interactions that would negatively affect pharmacological efficacy are not anticipated. This conclusion is based on the differences in physicochemical properties between the compounds that influence drug distribution and their discrete routes of disposition; each agent has a distinct metabolic and excretion pathway for elimination. Bictegravir is metabolized by CYP3A oxidation and UGT conjugation, FTC is cleared intact by renal excretion and TAF by hydrolysis. This was confirmed in a clinical drug interaction study (GS-US-141-1218) dosing BIC (100 mg), FTC (200 mg), and TAF (25 mg).

Bictegravir is metabolized by CYP3A oxidation and UGT conjugation and, hence, BIC plasma exposure may be altered by inducers or inhibitors of these enzymes. Bictegravir was not an inhibitor of UGT1A1 or CYP enzymes known to metabolize xenobiotics in vitro except for a very weak mechanism based inhibition of CYP3A. Bictegravir was a weak inducer of CYP3A4. Bictegravir is highly bound to plasma proteins (human > 99%) and is, therefore, unlikely to be a perpetrator of drug interactions through induction or inhibition of UGT1A1 or CYP enzymes. The low potential for clinically meaningful DDIs was confirmed in dedicated clinical studies.

Bictegravir was a substrate for intestinal efflux transporters P-gp and BCRP and its intestinal absorption may be decreased by inducers or increased by coadministered inhibitors of P-gp and BCRP. Bictegravir was not an inhibitor of the hepatic transporters OATP1B1, OATP1B3, OCT1, or BSEP, or the renal transporters OAT1 and OAT3 at clinically relevant concentrations. Although BIC was an inhibitor of renal transporters OCT2 and MATE1 in vitro, a clinical study with B/F/TAF FDC showed a lack of clinically relevant changes in the PK and PD of metformin.

Tenofovir alafenamide was not an inhibitor or an inducer of UGT1A1 or CYP enzymes known to metabolize xenobiotics except for weak inhibition observed for CYP3A in vitro. However, the weak inhibition of CYP3A is not clinically relevant as TAF did not affect the exposure to CYP3A substrates, midazolam or rilpivirine, in clinical DDI studies. Tenofovir alafenamide is unlikely to be a perpetrator of transporter-mediated drug interactions. TAF is a substrate for intestinal efflux transporters (P-gp and BCRP) and hepatic uptake transporters (OATP1B1 and OATP1B3); hence, TAF exposure may be affected by inhibitors and by inducers of the intestinal efflux transporters and inhibitors or genetic polymorphisms of OATPs. Tenofovir alafenamide was not a substrate for renal transporters OAT1 and OAT3. While TFV is a substrate for OAT1, OAT3, and MRP4, none of these transporters was inhibited by BIC or FTC. Therefore, interactions between the compounds during excretion are unlikely.

No additional pharmacokinetic studies are considered warranted with the combination of BIC, FTC, and TAF in view of the results of extensive nonclinical and clinical pharmacokinetic studies of the 3 components.

4. TOXICOLOGY

4.1. Brief Summary

Comprehensive nonclinical toxicology programs with BIC, FTC and TAF have been conducted. These studies have characterized the repeat dose toxicity, mutagenicity, carcinogenicity (TDF studies in place of TAF; 2 year rat study with BIC is ongoing), and reproductive toxicity of each of the individual agents (pre- and postnatal study with TDF in place of TAF), and the toxicity of the FTC/TDF combination. The nonclinical toxicology studies discussed in this section provide an adequate basis to evaluate potential toxicities of the individual components and the 3-drug combination, and for comparing and interpreting results from clinical studies. For exposure margin calculations, AUC plasma values in the toxicology studies were divided by the AUC plasma values in HIV-1 infected subjects for BIC, FTC, or TAF (TFV) at the respective dosages in the B/F/TAF FDC. References to the clinical studies which provide the basis for margin calculations are shown for BIC, FTC, or TAF (TFV) in Table 2, Table 3, and Table 4, respectively.

4.2. Single Dose Toxicity

4.2.1. BIC

No formal single dose toxicity studies were conducted with BIC. However, single dose PK studies in transgenic mice, rats and monkeys were performed. Single oral doses of BIC up to 1500 mg/kg were well-tolerated in transgenic mice, with saturation of absorption at 1000 mg/kg (m2.6.4, Section 3.2.1.2.1, AD-141-2307). Single oral doses of BIC up to 1000 mg/kg were well-tolerated in Wistar-Han rats (m2.6.4, Section 3.2.1.2.2, AD-141-2286). The increase in exposure was limited between 100 and 300 mg/kg and similar exposure was observed between 300 and 1000 mg/kg, suggesting saturation of absorption at 300 mg/kg. In monkeys, single oral doses of BIC up to 1000 mg/kg were well-tolerated (m2.6.4, Section 3.2.1.2.4, AD-141-2284). The increase in exposure was limited between 300 to 1000 mg/kg.

4.2.2. FTC

Emtricitabine has demonstrated minimal acute toxicity in rodents with oral LD_{50} values at > 4000 mg/kg and intravenous LD_{50} values at > 200 mg/kg (m2.6.7, Section 5.2, TTEP/93/0020, TTEP/93/0023, TTEP/93/0021, and TTEP/93/0024).

4.2.3. TAF

The NOAEL for a single oral dose TAF (as GS-7340-02) in the rat was determined to be >1000 mg/kg (m2.6.7, Section 5.3, R990185). The NOEL in dogs administered a single dose of TAF was 30 mg/kg with treatment-related clinical signs and renal lesions in the kidneys at 90 and 270 mg/kg (m2.6.7, Section 5.3, D990181).

4.2.4. B/F/TAF

No single-dose studies have been performed with the combination of BIC, FTC, and TAF. Coadministration in single-dose studies is unlikely to provide significant information based on results of the repeat dose studies of the individual agents.

4.3. Repeat Dose Toxicity

4.3.1. BIC

The oral toxicity of BIC has been studied in transgenic mice, rats, and monkeys for treatment periods up to 39 weeks. The only target organ toxicity observed was hepatobiliary toxicity in a 39 week repeat dose chronic toxicology study in monkeys at an exposure margin of 16-fold; the no observed effect level (NOEL) correlated to a margin of 7.0-fold (m2.6.7, Section 7.1.5, TX-141-2032). No adverse findings were noted in transgenic mouse (m2.6.7, Section 7.1.1, TX-141-2042) or rat (m2.6.7, Section 7.1.3, TX-141-2031) toxicology studies up to 26 weeks dosing duration with an exposure margin of ≥18-fold at the NOAELs.

4.3.1.1. Hepatobiliary system

The only notable adverse effect of BIC in chronic animal studies was hepatobiliary toxicity in monkeys following 39 weeks of administration at 1000 mg/kg/day at a 16-fold margin (m2.6.7, Section 7.1.5, TX-141-2032). No target organs were identified following repeat oral administration of high dose of 1000 mg/kg/day BIC in cynomolgus monkeys for up to 13 weeks. Administration of 1000 mg/kg/day for 39 weeks showed minimal to marked bile duct hyperplasia and minimal or moderate hepatocyte hypertrophy in both sexes, and minimal regenerative hyperplasia and minimal or slight neutrophil infiltrate. After a 4-week recovery period, hepatobiliary toxicity was partially reversible; findings were noted in one of two males and one of two females, while the other two animals in the 1000 mg/kg/day recovery group had no hepatobiliary findings. Minimally to mildly increased mean alanine aminotransferase (ALT) activity (≤ 3.5-fold versus mean baseline values) at 1000 mg/kg/day was not clearly correlated with BIC-related microscopic hepatobiliary changes at 1000 mg/kg/day. Additionally, hepatocyte degeneration/necrosis was not a feature of those hepatobiliary changes noted. Slight increases in ALT activity were apparent for males by Week 4 of the dosing phase but not for females until Week 26 of the dosing phase. There were no other adverse findings in the study. The NOEL after 39-weeks administration was 200 mg/kg/day, corresponding to an exposure margin of at least 7.0-fold.

4.3.2. FTC

A series of GLP oral repeat-dose toxicity studies were conducted with FTC in mice (4 weeks [m2.6.7, Section 7.2.2, TOX599; m2.6.7, Section 17.2, TOX118] and 26 weeks [m2.6.7, Section 7.2.3, TOX022; m2.6.7, Section 7.2.4, TOX628]), rats (13 weeks [m2.6.7, Section 7.2.5, TOX097]), and cynomolgus monkeys (4 weeks [m2.6.7, Section 7.2.6, TOX600], 13 weeks [m2.6.7, Section 7.2.7, TOX627], and 52 weeks [m2.6.7, Section 7.2.8, TOX032]).

Effects associated with the administration of FTC in the toxicology studies were confined to high-dose groups. Changes in red blood cell (RBC) parameters, interpreted as a mild, reversible anemia, occurred at the highest dose levels. Exposure margins at the NOELs for the longest treatment period in each species ranged from 8.0-fold (monkeys) to 22/28-fold (mice/rats).

4.3.3. TAF

The repeat-dose oral toxicity of TAF has been studied in mice, (2 weeks [m2.6.7, Section 6.2, TX-120-2006] and 13 weeks [m2.6.7, Section 7.3.1, TX-120-2007]), rats (6-7 days [m2.6.7, Section 6.2, R000139; m2.6.7, Section 17.3, R2000044; m2.6.7, Section 17.3, R990177], 4 weeks [m2.6.7, Section 7.3.2, R990182] and 26 weeks [m2.6.7, Section 7.3.3, TOX-120-001]), dogs (4-weeks [m2.6.7, Section 7.3.4, D990175] and 39 weeks [m2.6.7, Section 7.3.5, TOX-120-002]) and monkeys (4 weeks [m2.6.7, Section 7.3.6, P2000114]). In chronic studies, kidneys (karyomegaly, tubular degeneration), and bone (atrophy of metaphyseal cancellous bone) were the primary target organs. TAF also appeared to increase biochemical markers of bone turnover and decrease serum 1, 25-dihydroxy- and 25-hydroxyvitamin D_3 in rats and dogs. The NOAEL TFV exposure margins for chronic toxicology studies were 12- (rat) and 3.7-fold (dog), respectively.

4.3.3.1. Kidney

Renal tubular karyomegaly was observed in rats and dogs orally administered TAF. Focal areas of minimal renal cortical tubular basophilia and associated minimal nuclear karyomegaly were present in rats administered 400 mg/kg/day for 4 weeks and 100 mg/kg/day for 26 weeks. Renal tubular karyomegaly and/or basophilia were observed in dogs administered 3 and 10 mg/kg/day for 4 weeks and dogs administered 6 or 18/12 mg/kg/day for at least 13 weeks. Renal cortical tubular degeneration/regeneration findings were limited to animals administered ≥6 mg/kg/day for at least 13 weeks. Similar findings of renal cortical tubular degeneration/regeneration and karyomegaly were present in dogs administered ≥6 mg/kg/day for 39 weeks; similar lesions of lesser severity (karyomegaly and tubular degeneration) were also present in 2 males at 2 mg/kg/day of TAF for 39 weeks. After a 13-week recovery period, treatment-related histology changes were still observed in the kidney but were of reduced incidence and severity. The NOAEL TFV/TAF exposure margins for bone findings were 12- (rat; TFV only) and 3.7-/0.6-fold (dog), respectively.

4.3.3.2. Bone

Atrophy of metaphyseal cancellous bone was observed in rats administered TAF at 100 mg/kg/day for 26 weeks. TAF also increased biochemical markers of bone turnover and decrease serum 1,25-dihydroxy- and 25-hydroxyvitamin D_3 in rats (≥ 25 mg/kg/day) and dogs (≥ 37.5 mg/kg/day for 6 days). In the 39-week dog study, bone mineral density changes at 18/12 mg/kg/day were most likely due to body weight loss, but these changes were accompanied by a slight but significant decrease in serum 1,25-dihydroxyvitamin D_3 in males only and a significant increase in 25-hydroxyvitamin D_3 in females only. The NOAEL TFV/TAF exposure margins for bone findings were 12- (rat; TFV only) and 3.7-/0.6-fold (dog), respectively.

4.3.3.3. Other

TAF administered by oral gavage for up to 13 weeks to mice at ≥ 10 mg/kg/day resulted in adverse degenerative (olfactory) and acute inflammatory (infiltrate neutrophil) changes in the nasal mucosa. The relevance to humans is unknown and the risk of nasal inflammation in humans is very low because these changes were not observed in rats, dogs, or monkeys following long durations of administration.

TAF had no discernible electrocardiographic effect at the low dose of 2 mg/kg/day. There was some evidence for an effect to slightly prolong PR intervals associated with significant decreases in triiodothyronine (T3) {Kienle 1994, Tribulova 2010}. After the 13-week recovery period, serum T3 values returned to levels similar to the vehicle control group animals. No PR prolongation or any change in ECG results occurred in the safety pharmacology study that evaluated a TAF dose up to 100 mg/kg (m2.6.3, Section 4.2.3, D2000006) or in the thorough QT study (GS-US-120-0107).

At the highest dose of 18/12 mg/kg/day (highest dose) in the 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. Inlife ophthalmologic examinations were normal. Histiocytic infiltration observed in multiple organs was likely an indirect drug effect due to general debilitation and was not observed in other repeat dose toxicity studies. There were no drug-related effects on ophthalmic exams or microscopic exams of ocular tissue observed in repeat-dose toxicity studies in mice (up to 13 weeks), rats (up to 26 weeks), dogs (up to 4 weeks), and nonhuman primates (4 weeks).

Distribution of [^{14}C]TAF to eyes has been assessed in mice, rats, and dogs (m2.6.5, Section 5.3.1, AD-120-2011; m2.6.5, Section 5.3.2, AD-120-2020; m2.6.5, Section 5.3.4, D990173-BP). [^{14}C]TAF-related radioactivity distributed poorly to the eyes of rats and dogs (C_{max} in eyes < 8% that observed in plasma). In rats and mice, no difference in distribution between pigmented and nonpigmented skin was observed, illustrating that [^{14}C]TAF-related radioactivity was not selectively associated with melanin-containing tissues. In dogs, TAF was found to poorly penetrate across the blood brain and blood retinal barrier. In summary, it is unlikely that TAF directly caused the observed histiocytic infiltration in the posterior uvea. Based on the evidence from tissue distribution and toxicology studies, Gilead Sciences (Gilead) concludes that the risk of posterior uveitis in humans is very low.

The nonclinical toxicity studies demonstrate that there was no adverse effect of TAF for up to 26 weeks in the rat, 39 weeks in the dog, and 4 weeks in the monkey at doses producing TFV systemic exposure margins of 12-, 3.7- and >19- fold greater, respectively.

4.3.4. FTC/TDF

Two week rat oral gavage studies were conducted to investigate the potential toxicity of FTC/TDF, and to qualify potential impurities in nondegraded and degraded FTC/TDF tablets following daily oral administration (m2.6.7, Section 17.4, TX-164-2001 and TX-164-2005). There were no toxicologically significant differences between groups treated with nondegraded and degraded FTC/TDF, and no exacerbation of toxicity with the FTC/TDF combination compared to data with the individual agents.

A 4-week toxicity study was conducted with FTC and TDF in dogs to examine the possible exacerbation of renal toxicity with combination treatment and to assess possible effects on the immune system (m2.6.7, Section 7.4.1, TX-164-2004). There were no overall differences in the incidences or mean severities of renal findings between the single agent TDF or FTC/TDF dosing groups, renal findings were reversible (examined for combination only), and exposure was not altered with combination dosing when compared to the agents dosed individually.

4.3.5. B/F/TAF

Administration of BIC in combination with TAF and FTC is unlikely to exacerbate known toxicities of the individual agents. Bictegravir, FTC and TAF exhibit different patterns of target organ toxicity. Hepatobiliary toxicity in monkeys at 16-fold clinical exposures was the only BIC-related toxicity observed in chronic studies with no evidence of hepatobiliary toxicity at 7.0-fold clinical exposures. The only toxicity observed in chronic animal studies with FTC was mild, reversible anemia at large multiples of clinical exposure, which is not considered relevant to clinical use. Extensive nonclinical investigations of the toxicity of TAF have shown that unlike BIC or FTC, the bone marrow or liver are not targets for this agent, and that the target organs for TAF are distinctly different (bone and kidney). Hepatobiliary (BIC), or renal or bone toxicity (TAF) is not anticipated to be exaggerated with the B/F/TAF combination product.

Comprehensive nonclinical safety databases on these individual agents, including combination toxicity studies with FTC and TDF, indicate that further toxicological investigations are unlikely to yield new data relevant to humans. Further studies of longer duration with the combinations are not warranted because of the lack of significant overlapping toxicities, and the use of additional animals that would be required to obtain such information. Based on the well-characterized toxicity profiles of BIC, FTC and TDF/TAF, the low potential for toxicologic interaction noted in combination toxicology studies with FTC and TDF, along with the clinical safety data available for B/F/TAF, and other FTC and/or TDF- (TAF-) containing regimens (Emtriva, Truvada, Atripla, Complera/Eviplera, Stribild, Genvoya, Odefsey, Descovy, or Vemlidy), support the lack of clinically relevant overlapping toxicity for B/F/TAF for the treatment of HIV-1 infection.

4.4. Genotoxicity

4.4.1. BIC

Bictegravir was not genotoxic in a reverse mutation bacterial test (Ames test) (m2.6.7, Section 8.1, TX-141-2026), chromosomal aberration assay (m2.6.7, Section 8.2, TX-141-2027), or in an in vivo evaluation that consisted of a 2-week repeat dose rat bone marrow micronucleus study (m2.6.7, Section 9.1, TX-141-2029).

4.4.2. FTC

Emtricitabine was not genotoxic in a reverse mutation bacterial test (Ames test) (m2.6.7, Section 8.3, 18637-0-409R; m2.6.7, Section 8.4, MUT203; m2.6.7, Section 8.5, K01-3154), mouse lymphoma (m2.6.7, Section 8.6, TOX012), or mouse micronucleus assay (m2.6.7, Section 9.2, TOX011).

4.4.3. TAF

Tenofovir alafenamide was not genotoxic in a reverse mutation bacterial test (m2.6.7, Section 8.7, V990212), a L5178Y gene mutation assay in mouse lymphoma cells (m2.6.7, Section 8.8, V990213) or mouse micronucleus assay (m2.6.7, Section 9.3, M2000113).

4.4.4. FTC/TDF

No exacerbation of mutagenicity was apparent in either the bacterial reverse mutation assay (Ames assay) or the in vitro mammalian cell gene mutation assay (L5178Y/$TK^{+/-}$ mouse lymphoma assay) when FTC and TDF were administered together compared with each agent alone (m2.6.7, Section 8.9, TX-164-2002; m2.6.7, Section 8.10, TX-164-2003, respectively).

4.4.5. B/F/TAF

Bictegravir, FTC, and TAF were negative in genotoxicity studies. The combination of the 3 components is, therefore, not expected to have an altered genotoxicity profile as compared with that of the individual agents.

4.5. Carcinogenicity

4.5.1. BIC

Bictegravir demonstrated no carcinogenic potential in a 6-month transgenic mouse study (m2.6.7, Section 10.1, TX-141-2047), at exposures that exceeded 15- to 23-fold. It is considered unlikely that combination dosing would change this profile as no exposure difference would be expected and no exacerbation of toxicity is expected.

A 2-year carcinogenicity study with BIC in Wistar-Han rats is ongoing. The 2 year rat carcinogenicity study will be completed by [redacted] 20[redacted] and the final carcinogenicity report will be submitted to specific health authorities during the marketing application review or after approval, in accordance with agreed upon timelines.

4.5.2. FTC

In long-term carcinogenicity studies of FTC, no drug-related increases in tumor incidence were found in mice (m2.6.7, Section 10.3, TOX109) or rats (m2.6.7, Section 10.4, TOX108) at exposures that exceeded 25-fold.

4.5.3. TAF

Per separate agreements with the FDA and with the CHMP (EMEA/CHMP/SAWP/629722/2012; EMEA/H/SA/2410/1/2012/I), carcinogenicity studies were not required for TAF registration due to the lack of TAF exposure in rats and TgRasH2 mice and lower TFV exposure in rats and mice compared to TDF.

Tenofovir disoproxil fumarate/TFV has demonstrated low carcinogenic potential in conventional 2-year bioassays. Long-term oral carcinogenicity studies of TDF in mice and rats were carried out at TFV exposures up to approximately 151- or 51-fold (m2.6.7, Section 10.5, M990205; m2.6.7, Section 10.6, R990204, respectively). Female mice showed a low incidence of liver adenomas at the highest dose of 600 mg/kg/day at exposures 151-fold those observed in humans.

4.5.4. B/F/TAF

Bictegravir demonstrated no carcinogenic potential in a 6-month transgenic mouse study. The 2-year rat bioassay will complete in [redacted] 20[redacted]. FTC and TDF have demonstrated low carcinogenic potential in conventional 2-year bioassays. Given the data available, it is considered unlikely that combination dosing would change these profiles, and no exacerbation of toxicity/genotoxicity is expected. Based on the overall lack of genotoxicity and the available carcinogenicity study data with individual agents, the conduct of carcinogenicity studies with the B/F/TAF combination is not considered necessary nor would they add to the safety evaluation.

4.6. Reproductive Toxicity

4.6.1. BIC

Bictegravir had no remarkable effects on reproductive performance or embryonic viability in the rat fertility study (m2.6.7, Section 12.1, TX-141-2039) and the NOEL was the high dose of 300 mg/kg/day corresponding to exposure levels of approximately 29-fold higher than the BIC AUC in the B/F/TAF FDC. In the rat embryo-fetal development studies (m2.6.7, Section 11.1, TX-141-2034; m2.6.7, Section 13.1, TX-141-2036), there were no maternal or embryofetal development findings and the high dose of 300 mg/kg/day was the NOEL with exposures corresponding to a 36-fold exposure margin. In rabbits, abortion and decreased fetal body weights are considered secondary to overt maternal toxicity (adverse clinical observations, body weight loss and low food consumption) and are regarded as species-specific (m2.6.7, Section 11.1, TX-141-2035 and TX-141-2038; m2.6.7, Section 13.2, TX-141-2037). At the NOEL in the rabbit embryofetal development study (300 mg/kg/day), the margin of exposure is 0.59-fold. In the pre- and postnatal development study in rats (m2.6.7, Section 14.1, TX-141-2045), there were no BIC-related effects at any dosage level, and the high dose of 300 mg/kg/day was the NOEL for maternal systemic toxicity, F_1 neonatal/developmental toxicity, F_1 parental systemic toxicity, F_1 reproductive toxicity, and F_2 neonatal/early postnatal toxicity of BIC. Slight decreases in F_1 generation male and female fertility at 300 mg/kg/day were not statistically significant, remained within historical control ranges, and were not considered BIC-related. At the NOEL, maternal and pup BIC exposures were approximately 30-fold and 11-fold, respectively.

4.6.2. FTC

Emtricitabine did not affect fertility in male rats or in male and female mice (m2.6.7, Section 12.2, TOX036; m2.6.7, Section 12.3, TTEP/95/0028) at exposures at least 60-fold higher than human exposures. There were no adverse effects in embryo-fetal development studies in mice at exposures approximately 60-fold higher and in rabbits at exposures approximately 108-fold higher than human exposures (m2.6.7, Section 13.3, TOX037; m2.6.7, Section 13.4, TOX038). In the pre/postnatal study in mice, F_1 dams at 1000 mg/kg/day had slightly longer estrous cycles than controls, but fertility was normal in the offspring exposed daily from before birth (in utero) through sexual maturity at margins of approximately 60-fold (m2.6.7, Section 14.2, TOX039).

4.6.3. TAF

The TAF fumarate (GS-7340-03) oral rat fertility study (m2.6.7, Section 12.4, TX-120-2012) data indicate dose-related decreases in body weight gain in males and females, but no drug related changes occurred in male or female fertility endpoints including mating index, fertility index, sperm motility, sperm concentration, number of corpora lutea, implantation sites, early and late resorptions and live fetuses at doses up to 160 mg free base equivalents/kg/day. At the TAF NOAEL for fertility and early embryonic toxicity (160 mg/kg/day), the margin of TAF was at a dose equivalent of 155 times the human dose based on body surface area comparisons (25 mg TAF).

There was no effect on fetal viability or fetal development in pregnant rats administered doses of TAF (as GS-7340-02) up to 250 mg/kg/day (m2.6.7, Section 13.5, TX-120-2002), or in pregnant rabbits up to 100 mg/kg/day (m2.6.7, Section 13.6, TX-120-2005). The highest doses were maternally toxic. In the rat, decreased fetal body weight associated with some minor transitory delays in the rate of ossification was observed at 250 mg/kg/day, a maternally toxic dose. At the TAF NOAEL for embryo-fetal development in rats (100 mg/kg/day), the margins of exposure for TAF and TFV (Day 17) were 2 and 55, respectively. At the TAF NOAEL for embryo-fetal development in rabbits (100 mg/kg/day), the margins of exposure for TAF and TFV (Day 20) were 78 and 86, respectively.

Tenofovir disoproxil fumarate, but not TAF, has been tested in a perinatal/postnatal study. Per separate agreements with the FDA (FDA Reference ID 3231054) and with the CHMP (EMA/CHMP/SAWP/214541/2013, EMEA/H/SA/2410/1/FU/1/2013/I) a perinatal/postnatal study in rats was not required for TAF registration due to the lack of TAF exposure in rats and lower TFV exposure after TAF administration compared to TDF administration. There was an alteration of the estrous cycle in female rats in the perinatal study in rats (m2.6.7, Section 14.3, R990202). The NOEL for behavioral, reproductive, and development toxicity was 150 mg/kg/day corresponding to TFV exposure margin of 37-fold. Maternally toxic doses (≥ 450 mg/kg/day) had effects on pup survival, pup body weights, and sexual maturation.

4.6.4. B/F/TAF

Bictegravir, FTC, and TAF have not shown significant adverse effects in reproductive and developmental toxicity studies in the absence of maternal toxicity, and the combination of BIC, FTC and TAF is not expected to have an altered reproductive toxicity profile compared with that of the individual agents. In rabbits, BIC was associated with abortion and decreased fetal body weights at low exposure margins. These findings are considered secondary to overt maternal toxicity and are regarded as species-specific; there were no effects on reproductive or developmental effects in rats at exposure margins of at least 30-fold. Slightly longer estrous cycles were observed in F_1 generation rats after exposure to high doses of FTC and a delay in sexual maturation was observed in F_1 generation rats after exposure to high (maternally toxic) doses of TDF with a clearly defined NOEL and NOAEL. Neither TDF nor TAF showed an effect on rabbit fetuses. FTC has not shown any adverse effects in reproductive and developmental toxicity studies. No additive effects are anticipated with the 3-drug combination.

4.7. Juvenile Toxicity

4.7.1. BIC

Per agreement with FDA and the EMA's Pediatric Committee (PDCO), pediatric study plan (PSP) (Ref ID 3952985) and pediatric investigation plan (PIP) (EMEA-001766-PIP01-15-M01), respectively, juvenile toxicity studies with BIC are not required to support future pediatric development of B/F/TAF FDC based upon the lack of findings in the rat repeat dose toxicology and reproductive and developmental toxicology studies conducted with the components of the FDC.

4.7.2. FTC

Emtricitabine is approved for use in infants (aged 4 months of age or older), children, adolescents, and adults. No specific juvenile toxicity studies are considered warranted with FTC.

4.7.3. TAF/TFV

TAF as FTC/TAF (Descovy®; 200 mg/25 mg) is approved for use in patients 12 years of age and older. Although no specific juvenile toxicity studies have been conducted with TAF or TDF, data are available from efficacy studies of TFV in simian immunodeficiency virus (SIV)-infected and non-infected rhesus macaques {Tarantal 1999, Van Rompay 2004, Van Rompay 2008}. These studies included 12 gravid rhesus macaques, and more than 85 infant and juvenile rhesus macaques treated from ages ranging from 1 day to 7.5 years at initiation of dosing. This age range covers the human equivalent of prenatal, infant, juvenile and adolescent phases of growth. The duration of treatment ranged from 12 weeks to 13 years. Clinically relevant renal and bone pathology (including reduced bone mineral density, joint swellings, and bone fractures) occurred only in animals in which TFV was chronically administered at 30 mg/kg/day by daily subcutaneous injection. Exposure levels (TFV AUC 150 µg·h/mL) at this dose were more than 475-fold higher than those of adults after a 25 mg dose of TAF. Effects in rhesus monkeys were reversible by decreasing or stopping exposure. Administration of lower doses of TFV (10 mg/kg/day, ~15 µg·h/mL) did not cause renal dysfunction or abnormal bone density or growth.

Tenofovir administered to newborn or infant rhesus monkeys at doses of 4 to 30 mg/kg/day did not cause adverse effects in short term studies (up to 12 weeks). However, prolonged TFV treatment (generally more than 4 months of daily treatment at 30 mg/kg/day administered subcutaneously) resulted in a Fanconi-like syndrome with glucosuria, aminoaciduria, hypophosphatemia, growth restriction, and bone pathology (osteomalacia) {Van Rompay 2004}. Clinical, biochemical, and radiographic resolution/improvement occurred with dose reduction (from 30 to ≤ 10 mg/kg/day) or discontinuation of treatment.

Three animals (1 SIV-infected) were dosed chronically, beginning as neonates, with 10 mg/kg/day TFV administered subcutaneously. After more than 5 years of treatment, there were no clinical, radiographic, or dual-emission X-ray absorptiometry scan {Van Rompay 2004} findings of an adverse effect on bone. The TFV plasma exposure margin associated with this dosage (18 µg·h/mL) was 57-fold.

4.7.4. B/F/TAF

No studies of juvenile toxicity were conducted with BIC, FTC, TAF, or any combination of the agents. This application supports the proposed registration of B/F/TAF for treatment of HIV-infected adults. No pediatric indication is proposed at this time.

4.8. Local Tolerance

BIC was predicted to be nonphototoxic (m2.6.7, Section 16.1, TX-141-2046), noncorrosive and nonirritating (m2.6.7, Section 16.1, TX-141-2058 and TX-141-2059) to skin, and a moderate eye irritant (m2.6.7, Section 16.1, TX-141-2060). No local tolerance studies have been conducted with FTC. TAF was predicted to be a noncorrosive/nonsevere eye irritant (m2.6.7, Section 16.2, TX-120-2013) and nonirritating/noncorrosive to skin (m2.6.7, Section 16.2, TX-120-2011).

The B/F/TAF tablet is intended for oral use. No local tolerance studies were conducted for the B/F/TAF combination. Evaluation of local tolerance to the gastrointestinal (GI) tract has been conducted during the repeat-dose oral toxicity studies with each of the individual agents.

4.9. Other Toxicity Studies

4.9.1. Antigenicity

Bictegravir (m2.6.7, Section 17.1.2, TX-141-2061) or TAF (m2.6.7, Section 17.3, TX-120-2014) showed no potential for sensitization.

4.9.2. Immunotoxicity

The immunotoxicity of FTC was evaluated in a 28-day study in rats at doses up to 1000 mg/kg/day (m2.6.7, Section 17.2, TOX146). There were no adverse effects of FTC during the dosing period and FTC did not affect the immunoglobulin M (IgM) antibody titers to sheep RBCs at any of the doses administered. The NOEL for immunotoxicity was 1000 mg/kg/day.

Data from repeat-dose toxicity studies with BIC, FTC or TAF (hematology, lymphoid organ weights, microscopy of lymphoid tissues, bone marrow cellularity) did not suggest immunotoxic potential. There were no notable effects of the combination of FTC/TDF on immune cells or NK cell assay values in a 4-week dog study (m2.6.7, Section 7.4, TX-164-2004). No further studies were deemed necessary with the B/F/TAF FDC.

4.9.3. Metabolites, Impurities, Degradation Products

4.9.3.1. BIC

No toxicology studies with metabolites were conducted as there were no unique human metabolites in humans with BIC. The impurity profiles for batches of BIC active pharmaceutical ingredient or drug product used in nonclinical toxicology studies are provided (m2.6.7, Section 4.1). A 4-week repeat dose oral toxicity study was conducted in rats to qualify impurities and potential impurities in the Good Manufacturing Practice (GMP) material (m2.6.7, Section 17.1.2, TX-141-2043). The impurities spiked into the drug substance did not alter the NOAEL in rats (300 mg/kg/day).

Based on their impurity profiles, the multiple GLP batches of BIC 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 (m3.2.P.5.5, Characterization of Impurities [B/F/TAF Tablets] and m3.2.P.5.6, Justification of Specifications [B/F/TAF Tablets]).

4.9.3.2. FTC

No toxicology studies with metabolites were conducted as there were no unique human metabolites in humans with FTC. Process impurities and degradation products of FTC have been qualified in animal studies. The major degradation product, FTU, was qualified in 2 in vitro genotoxicity studies (m2.6.7, Section 17.2, TOX151; TOX152), which were negative for genotoxicity. In addition, there was no toxicity in a 28-day mouse studies to qualify FTU (m2.6.7, Section 17.2, TOX153, TOX118) and no toxicity in a 28-day mouse bridging study (m2.6.7, Section 17.2, TX-162-2001) to qualify impurities (specifically FTC-carboxylic acid).

Based on their impurity profiles, the multiple GLP batches of FTC tested in the toxicology program are considered, in composite, to be representative of the GMP material and support the specified limits of impurities and degradation products proposed for commercial production (m3.2.P.5.5, Characterization of Impurities [B/F/TAF Tablets] and m3.2.P.5.6, Justification of Specifications [B/F/TAF Tablets]).

4.9.3.3. TAF

No metabolite toxicology studies were conducted as there were no unique human metabolites in humans with TAF. Two repeat dose impurity qualification studies were conducted in rats to evaluate potential drug substance impurities. In the first study, no differences were found between the two lots of GS-7340-02, each administered by oral gavage for 14 days to male rats (m2.6.7, Section 17.3, TX-120-2008). In the second study, no differences were found between three lots of GS-7340-03, each administered by oral gavage to male and female rats for 28 days (m2.6.7, Section 17.3, TX-120-2021).

Based on their impurity profiles, the multiple GLP batches of BIC 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 (m3.2.P.5.5, Characterization of Impurities [B/F/TAF Tablets] and m3.2.P.5.6, Justification of Specifications [B/F/TAF Tablets]).

4.9.3.4. FTC/TDF

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

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

Two 14-day oral repeat dose studies were conducted in rats to qualify impurities and degradants in the FTC/TDF tablets (m2.6.7, Section 17.4, TX-164-2001 and TX-164-2005). The second qualification study (m2.6.7, Section 17.4, TX-164-2005) was conducted to verify the qualification of cFTU1 and cFTU2 as these degradants were identified later in development by virtue of a new analytical assay. No new toxicities or exacerbation of previously defined toxicities or differences in toxicity between non-degraded and degraded material were noted.

The qualification of degradation products is summarized in m3.2.P.5.6, Justification of Specifications [B/F/TAF Tablets]. The impurities and degradation products in the 3 active ingredients, BIC, FTC and TDF, as well as the tableted drug product have been identified and qualified in toxicology studies. The safety margins support the specified limits proposed for these impurities and degradation products.

4.9.3.5. B/F/TAF

The B/F/TAF tablets are bilayer tablets. The degradation products of BIC, FTC or TAF observed in the B/F/TAF tablets are consistent with those in the BIC, FTC or TAF drug substance, respectively. There are no unique impurities or degradants in the B/F/TAF tablets.

The impurities and degradation products present in BIC, FTC or TAF drug substance and in B/F/TAF tablets have been qualified through toxicology studies, which employed drug substance from normal productions batches, laboratory scale batches with enhanced levels of impurities, or samples subjected to forced degradation conditions (high heat and humidity) (m3.2.P.5.5, Characterization of Impurities [B/F/TAF Tablets] and m3.2.P.5.6, Justification of Specifications [B/F/TAF Tablets]).

4.10. Relationship of BIC Key Findings to Exposure

The plasma concentrations of BIC associated with key responses and the associated exposure margins are presented in Table 1. Margins of exposures presented are based on a comparison of plasma AUC values from the toxicology studies to plasma AUC values from HIV-1 infected subjects who received 50 mg BIC once daily in the B/F/TAF FDC (BIC AUC of 102 µg·h/mL; clinical studies GS-US-380-1489, GS-US-380-1490, GS-US-380-1844, GS-US-380-1878).

Table 1. Concentrations of BIC Associated with Key Responses

Key Response(s)	Dose (mg/kg)	AUC_{0-24}[a] (µg•h/mL)	C_{max}[a] (µg/mL)	Exposure Margin[b]
Safety Pharmacology Studies				
In vitro hERG – PC-141-2049				
1% inhibition	0.8 µM	NA	NA	23
10% inhibition	7.1 µM	NA	NA	203
Cardiovascular study in male telemetered cynomolgus monkeys (4/dose) – PC-141-2046				
No noteworthy findings.	30	306	26.9	5.4
	100	342	29.8	6.0
	1000 (NOEL)	1250	109	22
Respiratory study in male rats (8/dose) – PC-141-2048				
No noteworthy findings.	10	712	42.2	0.27
	30	1670	97.0	0.63
	100	2470	138	0.90
	300 (NOEL)	2860	141	0.92
CNS (Irwin) study in male rats (8/dose) – PC-141-2047				
No noteworthy findings.	10	712	42.2	0.27
	30	1670	97.0	0.63
	100	2470	138	0.90
	300 (NOEL)	2860	141	0.92
Repeat-Dose Toxicity Studies				
4-week dose range-finding study in 001178-W (Wild Type) RasH2 Mice (10/sex/dose) – TX-141-2042				
No noteworthy findings.	30	629	57.7	6.2
	100	1180	96.8	12
Clin signs: ♂ ↓BW **Microscopic:** ♂ Liver; ↓centrilobular hepatocellular glycogen	1000 (NOAEL)	2330	158	23
2-week toxicity study in rats with bone marrow micronucleus assay (10/sex/dose) – TX-141-2029				
No noteworthy findings.	10	1160	68.4	11
	30	1790	103	18
	100	2550	150	25
Clin path: ↓RBC and HCT ♀; ↑PHOS♂	300 (NOAEL)	2970	150	29

Key Response(s)	Dose (mg/kg)	AUC_{0-24}[a] (µg•h/mL)	C_{max}[a] (µg/mL)	Exposure Margin[b]
26-week toxicity study in rats with a 4-week recovery period (15/sex/dose [terminal phase]; 5/sex/group in vehicle and high dose [recovery phase]) – TX-141-2031[c]				
No noteworthy findings.	5	457/1290	28.8/70.0	4.5/13
Clin path: ↑PHOS♀; reversible	30	1010/2910	76.9/169	9.9/29
Clin path: ↑PHOS♂♀; reversible	300 (NOAEL)	1830/4680	100/213	18/46
2-week toxicity study in cynomolgus monkeys (3/sex/dose) – TX-141-2030				
No noteworthy findings.	30	212	17.6	2.1
	100	324	24	3.2
Enzymes: ↓CYP1A activity♂	1000 (NOAEL)	1090	79.1	11
39-week toxicity study in monkeys with a 13–week interim sacrifice and a 4-week recovery (3/sex/dose [interim phase], 4/sex/dose[terminal phase], 2/sex/group in vehicle and high dose [recovery phase]) – TX-141-2032				
No noteworthy findings.	30	251	26.4	2.5
	200 (NOEL)	709	69.1	7.0
13 week: **Clin path:** ↑ ALT ♂ 39 weeks: **Clin signs:** ♂ vomitus/emesis **Clin path:** ↑ ALT reversible (6/12); ↑GGT (2/12; uncertain relationship to BIC; no clear correlation to the observed bile duct hyperplasia) **Macroscopic:** Liver; rough surface and discolored (n = 1;♂) **Microscopic:** Bile duct hyperplasia (8/8), hepatocyte hypertrophy (2/8), regenerative hyperplasia (1/4 ♂), neutrophil infiltrate (2/4 ♂); partially reversible	1000	1600	106	16
Repeat Dose Toxicity Studies – Other				
2-week investigative toxicity study in male rats (13-15/dose) – TX-141-2033				
No noteworthy findings; histopathological evaluation limited to CNS tissues.	300 (NOAEL)	NA[d]	NA[d]	NA[d]
Carcinogenicity				
6-month carcinogenicity study in transgenic mice (25/sex/dose) – TX-141-2047				
Not carcinogenic.	5 (Males)	190	18.2	1.9
	10 (Females)	341	25.8	3.3
	15 (Males)	526	42.3	5.2
	30 (Females)	861	69.2	8.4
	100 (Males)	1560	104	15
	300 (Females)	2340	143	23
2-year carcinogenicity study in rat (55/sex/dose) – TX-141-2040				
In Progress				

Key Response(s)	Dose (mg/kg)	AUC_{0-24}[a] (µg•h/mL)	C_{max}[a] (µg/mL)	Exposure Margin[b]
Developmental and Reproductive Toxicity Studies				
Embryo-fetal development dose range finding study in female rats (6/dose) – TX-141-2034				
No noteworthy findings.	5	1630	80.1	16
	30	3080	164	30
	300 M-(NOEL) D-(NOEL)	3650 (GD17)	180	36
Embryo-fetal development study in female rats (22/dose) – TX-141-2036[e]				
No noteworthy findings.	5	ND	ND	ND
	30	ND	ND	ND
	300 M-(NOEL) D-(NOEL)	ND	ND	ND
Embryo-fetal development dose range finding study in female rabbits (8/dose) – TX-141-2035				
No NOAELs for maternal and developmental toxicity determined. Maternal toxicity in BIC-treated (dose-dependent) and in vehicle control groups. High dose group sacrificed on GD 14 or 16 due to overt toxicity. Abortions at 300 mg/kg/day (n = 5) and 100 mg/kg/day (n = 1). Organic vehicle used not suitable for developmental toxicity studies.	100	145	4.67	1.4
	300	412	14.1	4.0
	1000	ND	ND	ND
Embryo-fetal development dose range finding study in female rabbits (8/dose) - TX-141-2038				
No noteworthy findings.	100	38.5	3.96	0.38
Maternal: Ungroomed coat, pale or light brown feces.	300 M-(NOAEL) D-(NOEL)	60.3	4.05	0.59
Maternal: Adverse clinical signs, abnormal fecal observations, ↓ BW and FC, abortion (n = 1) **Fetal:** ↓BW (within Test Facility historical controls)	1000	138	11.0	1.4
Embryo-fetal development study in female rabbits (22/dose) - TX-141-2037[f]				
No noteworthy findings.	100	ND	ND	ND
	300 M-(NOAEL) D-(NOEL)	ND	ND	ND
Maternal: Adverse clinical signs, abnormal fecal observations, ↓ BW and FC, abortion (n = 2) **Fetal:** ↓BW	1000	ND	ND	ND
Male and female combined fertility study in rats (25/sex/dose) – TX-141-2039				
No noteworthy findings.	5	ND	ND	ND
	30	ND	ND	ND
	300 (NOEL)	ND	ND	ND

Key Response(s)	Dose (mg/kg)	AUC_{0-24}[a] (µg•h/mL)	C_{max}[a] (µg/mL)	Exposure Margin[b]
Peri- and post-natal development study in female rats (25/dose) – TX-141-2045[g]				
No findings on F_0 maternal systemic toxicity, F_1 neonatal/developmental toxicity, F_1 parental systemic toxicity, F_1 reproductive toxicity, or F_2 neonatal/early postnatal toxicity.	2	335/334	20.0/16.6	3.3/3.3
	10	1170/762	74.7/33.5	11/7.5
	300 $F_0/F_1/F_2$ (NOEL)	3110/1120	156/48.0	30/11

↑ = increase; ↓ = decrease; ♂ = male; ♀ = female; ALT = alanine aminotransferase; BW = body weight; CNS = central nervous system; D = developmental; GD = gestation day; GGT = gamma-glutamyltransferase; FC = food consumption; M = maternal; MTD = maximum tolerated dose; ND = not determined; NOAEL = no observed adverse effect level; NOEL = no observed effect level

a In repeat-dose studies, AUC and C_{max} values indicate BIC mean plasma concentrations. Reported values were obtained near termination, or as specified. Values reported as combined male/female, unless otherwise noted.

b Margin obtained by dividing the steady state mean AUC_{0-24} of BIC with the projected BIC steady-state AUC_{tau} of 102 µg•h/mL for BIC/F/TAF (50/200/25 mg) FDC once daily under fed conditions. For safety pharmacology studies, C_{max} values were obtained from Day 1 values (males) from 2-week repeat dose monkey or rat studies. Exposure margins for safety pharmacology studies are calculated based on rat or monkey free C_{max} divided by human free C_{max} value for BIC/F/TAF (50/200/25 mg) under fed conditions. Note: human free C_{max} = 0.035 µM or 0.016 µg/mL based upon human C_{max} = 13.7 µM or 6.15 µg/mL; 0.25% free), rat protein binding is 0.01% free, and monkey protein binding is 0.31% free.

c Due to gender difference in exposure, mean rat AUC_{0-24} values and margins are reported separately as males/females.

d Bioanalysis evaluated only at 4 hours postdose (estimated T_{max}) on Day 14, measured as 119 µg/mL.

e No toxicokinetics evaluated; exposure values for identical dose levels as noted in Study TX-141-2034.

f No toxicokinetics evaluated; exposure values for identical dose levels as noted in Study TX-141-2038.

g Exposures and margins listed as maternal Lactation Day 10/F_1 pup Postnatal Day 10.

4.11. Summary of Toxicology and Target Organ Effects

4.11.1. Target Organ Effects

4.11.1.1. BIC

The oral toxicity of BIC has been studied in transgenic mice, rats, and monkeys for treatment periods up to 39 weeks. In a 39 week repeat dose chronic toxicology study in monkeys, hepatobiliary toxicity at the high dose of 1000 mg/kg/day was the only notable adverse finding. No target organs were identified following repeat oral administration of 1000 mg/kg/day BIC in cynomolgus monkeys for up to 13 weeks. No adverse findings were noted in transgenic mouse or rat repeat dose toxicology studies up to 26 weeks dosing duration with BIC plasma exposures at the NOAELs of ≥18-fold the BIC plasma exposure at the clinical dose of 50 mg in the B/F/TAF FDC. Bictegravir demonstrated no carcinogenic potential in a 6-month transgenic mouse study at an exposure margin of ≥15-fold.

4.11.1.1.1. Hepatobiliary system

The only significant effect identified in chronic animal studies with BIC was hepatobiliary toxicity in monkeys following 39 weeks of administration at a dose (1000 mg/kg/day) which produced BIC plasma exposures which were 16-fold higher than those in patients at a 50 mg BIC dose in the B/F/TAF FDC. The effects were partially reversible after a four week period of non-exposure (recovery). At 39 weeks, livers from all high dose animals showed evidence of bile duct hyperplasia. Clinical pathology changes at this dose level were confined to elevations in

ALT and GGT but a clear association with individual histologic findings was not obvious in most animals (m2.6.6, Section 3.1.5, Table 6). Mean bilirubin concentrations were not affected. Following a 4 week recovery period, biliary hyperplasia was only observed in 2 of 4 animals (1/sex). Neither ALT nor GGT activity were increased following the 4 week recovery period. No toxicity was observed at the mid-dose of 200 mg/kg/day which provided a margin of exposure of at least 7.0-fold.

4.11.1.2. FTC

No specific concerns were identified in the safety pharmacology, genotoxicity, carcinogenicity and reproductive toxicity studies with FTC. The only significant effect of FTC identified at dose levels constituting large clinical multiples was minor anemia.

4.11.1.3. TAF

No specific concerns were identified in the safety pharmacology, genotoxicity, carcinogenicity and reproductive toxicity studies with TAF. Target organ toxicity from repeat dose toxicology studies is summarized below.

4.11.1.3.1. Kidney

Renal tubular karyomegaly was observed in rats and dogs orally administered TAF. Focal areas of minimal renal cortical tubular basophilia and associated minimal nuclear karyomegaly were present in rats administered 400 mg/kg/day for 4 weeks and 100 mg/kg/day for 26 weeks. Renal tubular karyomegaly and/or basophilia were observed in dogs administered 3 and 10 mg/kg/day for 4 weeks and dogs administered 6 or 18/12 mg/kg/day for at least 13 weeks.

Renal cortical tubular degeneration/regeneration findings were limited to animals administered 6 or 18/12 mg/kg/day for at least 13 weeks in the 39-week dog toxicity study. Similar findings of renal cortical tubular degeneration/regeneration and karyomegaly were present in dogs administered either 6 or 18/12 mg/kg/day for 39 weeks. These changes were minimal to slight in affected males and females at 6 mg/kg/day. In high-dose males (18/12 mg/kg/day) the severity ranged from mild to moderate. Similar lesions (karyomegaly and tubular degeneration) but of only minimal severity were also present in 2 males administered 2 mg/kg/day of TAF for 39 weeks. After a 13-week recovery period, treatment-related histology changes were still observed in the kidney but were of reduced incidence and severity. The NOAEL TFV/TAF exposure margins for bone findings were 12- (rat; TFV only) and 3.7-/0.6-fold (dog), respectively.

4.11.1.3.2. Bone

Atrophy of metaphyseal cancellous bone was observed in rats administered TAF at 100 mg/kg/day for 26 weeks. TAF also increased biochemical markers of bone turnover and decrease serum 1, 25-dihydroxyvitamin D_3 and 25-hydroxyvitamin D_3 in rats ($\geq$ 25 mg/kg/day) and dogs ($\geq$ 37.5 mg/kg/day for 6 days). In the 39-week dog study, bone mineral density changes at 18/12 mg/kg/day may have been secondary to body weight loss but these changes were accompanied by a slight but significant decrease in serum 1, 25-dihydroxyvitamin D_3 in males

only and a significant increase in 25-hydroxyvitamin D_3 in females only. The NOAEL TFV/TAF exposure margins for bone findings were 12- (rat; TFV only) and 3.7-/0.6-fold (dog), respectively.

4.11.1.3.3. Other

TAF administered by oral gavage for up to 13 weeks to mice at ≥ 10 mg/kg/day resulted in adverse degenerative (olfactory) and acute inflammatory (infiltrate neutrophil) changes in the nasal mucosa. The relevance to humans is unknown and the risk of nasal inflammation in humans is very low because these changes were not observed in rats, dogs, or monkeys following long durations of administration.

Slightly prolong PR intervals (~13-24%) secondary to poor clinical condition in the 39 week chronic dog study were associated with significant and reversible decreases in serum T3 levels {Kienle 1994, Tribulova 2010}. 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 thorough QT study (GS-US-120-0107).

Minimal infiltration of histiocytes in the eye (choroid plexus, ciliary body) in the 39-week repeat dose chronic dog study was noted; in-life ophthalmologic examinations were normal. There were no 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), and nonhuman primates (4 weeks) or in the 4-week dog toxicology study. The histopathological findings, at 43- and 14-fold higher exposure to TFV and TAF, respectively, did not correlate with the tissue distribution where TAF was found to poorly penetrate across the blood brain and blood retinal barrier in dogs. Findings were likely an indirect drug effect due to general debilitation and based on the evidence from tissue distribution and toxicology studies, Gilead Sciences (Gilead) concludes that the risk of posterior uveitis in humans is very low.

4.11.1.4. B/F/TAF

Administration of the combination of BIC, FTC, and TAF is unlikely to exacerbate known toxicities as individual agents exhibit different patterns of target organ toxicity. The only adverse effects observed in chronic animal studies with BIC were hepatobiliary findings at plasma exposures approximately 16-fold greater than the efficacious BIC exposure. Findings with FTC were limited to mild, reversible anemia in mice and minor decreases in erythrocyte counts/increases in mean corpuscular hemoglobin in monkeys at large multiples of clinical exposure and not considered relevant to clinical use. Extensive nonclinical investigations of the toxicity of TAF have shown that the target organs are bone and kidney. As pathological changes are not overlapping, exaggerated toxicity is not anticipated with the B/F/TAF FDC.

4.11.2. Exposure Margins

Exposure margins for BIC (50 mg), FTC (200 mg) and TAF (25 mg) are calculated based upon PK data from clinical studies cited below in Table 2, Table 3, and Table 4, respectively.

4.11.2.1. BIC

Bictegravir plasma exposure for hepatobiliary toxicity and the margin relative to human plasma exposure are shown in Table 2. The NOEL in the 39 week cynomolgous toxicity study represents systemic exposures in animals compared to those in humans administered the daily recommended dose of 50 mg BIC in the B/F/TAF FDC.

Table 2. Exposure Margins of BIC Based on AUC When Comparing Animal NOEL

Target Organ Effect	Species	Study Duration	NOEL (mg/kg/day)	AUC_{0-24} (µg·h/mL) NOEL	Margin Relative to Human AUC[a]
Hepatobiliary toxicity	Monkey	9 months	200	709	7.0

a Predicted exposure margin for BIC human exposure is based on clinical population PK data in HIV-1 infected subjects in the following clinical studies where mean BIC AUC_{tau} = 102 µg·h/mL for 50 mg BIC as B/F/TAF FDC; m2.7.2, Section 3.2, GS-US-380-1489, GS-US-380-1490, GS-US-380-1844, and GS-US-380-1878.

4.11.2.2. FTC

Cross-species comparisons of FTC exposure for the major target organs are shown in Table 3. The NOELs obtained in the toxicity studies represent systemic exposures in animals compared to those in humans administered the daily recommended dose of 200 mg FTC in the B/F/TAF FDC.

Table 3. Exposure Margins of Emtricitabine Based on AUC When Comparing Animal NOELs

Target Organ Effect	Species	Study Duration	NOEL (mg/kg/day)	AUC_{0-24} (µg·h/mL) NOEL	Margin Relative to Human AUC[a]
Anemia	Mouse	6 months	500	266	22
	Rat	3 months	600	346	28
	Monkey	1 year	200	98	8.0

a Predicted exposure margin for FTC human exposure is based on pooled Phase 3 PK data in in HIV-1 infected subjects in the following clinical studies where mean FTC AUC_{tau} = 12.3 µg·h/mL for 200 mg FTC as B/F/TAF FDC; m2.7.2, Section 3.2, GS-US-380-1489, GS-US-380-1490, GS-US-380-1844, and GS-US-380-1878.

4.11.2.3. TAF

Cross-species comparisons of exposure for the major target organs are shown in Table 4. Because TFV is a more relevant analyte for nonclinical comparisons, both the TAF and TFV margins are presented below (when available). Dog was the most sensitive species to renal and bone effects of TAF. The rat and dog showed some loss of bone mineral density at relatively high doses; however, clinically evident osteomalacic lesions occurred only in juvenile monkeys in which TFV was chronically administered at exposure levels at an exposure margin of >475-fold. The NOELs obtained in the toxicity studies represent systemic exposures in animals compared to those in humans administered the daily recommended dose of 25 mg TAF in the B/F/TAF FDC.

Table 4. Safety Margins of TAF Based on AUC When Comparing Animal NOAELs

Target Organ Effect	Species	Study/Dose Duration	TAF NOAEL (mg/kg/day)	AUC_{0-24} (µg·h/mL) NOAEL	Margin Relative to Human AUC
				TFV/ TAF	TFV[a]/TAF[b]
Nasal Turbinate Toxicity	Mouse	13 Weeks	<10	<0.213/NC	<0.7/NA
Renal Toxicity	Rat	26 weeks	25	3.8/NC	12/NA
	Dog	39 weeks	2	1.2/0.08	3.7/0.6
	Monkey	4-weeks	≥30	≥5.9/1.0	>19/7
Bone Mineral Loss	Rat	26 weeks	25	3.8/NC	12/NA
	Dog	39 weeks	2	1.2/0.08	3.7/0.6
	Monkey	4-weeks	≥30	≥5.9/1.0	>19/7

NA = not applicable; NC = insufficient data to calculate

a Predicted exposure margin for TFV human exposure is based on steady state PK data from Phase 2 clinical study (GS-US-141-1475) in HIV-infected subjects in clinical study with BIC (75 mg) + F/TAF (200/25 mg) where the mean TFV AUC_{tau} = 0.316 µg·h/mL.

b Predicted exposure margin for TAF human exposure is based on clinical population PK data in HIV-1 infected subjects in the following clinical studies where mean TAF AUC_{tau} = 0.142 µg·h/mL for 25 mg TAF as B/F/TAF FDC; m2.7.2, Section 3.2, GS-US-380-1489 and GS-US-380-1490.

5. INTEGRATED OVERVIEW AND CONCLUSIONS

The pharmacologic basis to recommend the B/F/TAF FDC tablet for the treatment of HIV-1 infection is scientifically sound based on the nonclinical in vitro and in vivo efficacy data for the individual components and the combination of the agents presented in this dossier.

The overall program including the data from the combination and individual agent studies is considered supportive for the development of B/F/TAF FDC tablet based on the following considerations. All 3 drugs show potent ARV activity against diverse subtypes of HIV-1 in vitro. FTC and TAF are phosphorylated intracellularly through nonoverlapping pathways, and in combination show no antagonism for the formation of their active metabolites. Bictegravir does not require metabolic modification for activity and exhibits synergistic antiviral effect in vitro in combination with TAF and FTC in 2- and 3-drug combinations.

The pharmacokinetic and toxicologic profiles of BIC, FTC, TAF, and TFV are well characterized in multiple animal species and the findings are pertinent in consideration of the use of these agents in combination. Adverse pharmacokinetic interactions that would negatively affect safety or pharmacological efficacy are not anticipated. This is based on the well-characterized routes of elimination demonstrated for each compound and the differences in physicochemical properties between the compounds, which influence drug distribution. This was confirmed in a clinical drug interaction study with BIC (100 mg), FTC (200 mg) and TAF (25 mg). As such, cumulative data from nonclinical and clinical studies demonstrated acceptable tolerability and safety profiles to support use of B/F/TAF in patients.

The toxicity profiles of BIC, FTC and TAF differ substantially with no clinically significant overlapping toxicity. Target organ toxicities in chronic animals studies with BIC, FTC, and TAF were hepatobiliary, anemia, and renal/bone toxicities, respectively. Due to the lack of overlapping toxicities, exacerbation of respective single agent toxicities in the B/F/TAF FDC is not anticipated. Bictegravir, FTC, and TAF are nongenotoxic; the B/F/TAF FDC is not anticipated to alter the genotoxicity profiles of the individual agents. FTC and TDF/TFV have demonstrated low carcinogenic potential in conventional 2-year bioassays; BIC demonstrated no carcinogenic potential in a 6-month transgenic mouse study and has not been evaluated to date in the 2-year rat study. Combination dosing would not be expected to change these individual profiles. Bictegravir, FTC, and TAF have not shown significant adverse effects in reproductive and developmental toxicity studies in the absence of maternal toxicity, and the combination of BIC, FTC and TAF is not expected to have an altered reproductive toxicity profile compared with that of the individual agents.

The absence of nonclinical safety studies with the B/F/TAF combination is in accordance with the ICH Guidance on Nonclinical Safety Studies for the Conduct of Human Clinical Trials and Marketing Authorization for Pharmaceuticals M3(R2) (June 2009). There are no anticipated clinically relevant pharmacokinetic or toxicological interactions expected in the B/F/TAF FDC, and administration of the B/F/TAF combination product is unlikely to introduce new toxicities or to exacerbate known toxicities of the individual agents. The ample nonclinical safety databases for FTC and TAF and nonclinical studies conducted to date for BIC strongly indicate further toxicological investigations are unlikely to yield new data relevant to humans. Additionally, the

clinical safety data available from the approved FTC/TAF(TDF) regimens and with the E/C/F/TAF FDC supports the safety of the F/TAF FDC in combination with BIC for the treatment of HIV-1 infection.

5.1. Justification for Text in Labeling

The proposed prescribing information for B/F/TAF FDC includes all relevant nonclinical safety findings. Based on findings in the nonclinical studies, the key safety points for consideration related to BIC, FTC, and TAF include the following: potential DDIs with potent inhibitors of CYP3A and UGT1A1, potent inducers of CYP3A and UGT1A1, potent inhibitors of P-gp and BCRP, and potent inducers of P-gp and BCRP, use by women of childbearing potential and during pregnancy, and use during lactation. In regard to these possible concerns, the following should be considered:

1) Bictegravir is primarily eliminated through hepatic metabolism by CYP3A and UGT1A1. Drugs that are potent inducers of both CYP3A and UGT1A1, such as rifampin, may significantly decrease plasma exposures of BIC leading to reduced therapeutic effect of BIC, and coadministration is not recommended. Potent inhibitors of both CYP3A and UGT1A1, such as atazanavir, may significantly increase BIC exposure, and coadministration is not recommended.

2) Tenofovir alafenamide is a substrate of P-gp and BCRP. Drugs that strongly affect P-gp and BCRP activity may lead to changes in TAF absorption. Drugs that induce P-gp activity are expected to decrease the absorption of TAF, resulting in decreased plasma concentration of TAF, which may lead to loss of therapeutic effect of the product and development of resistance. Coadministration of B/F/TAF FDC with other drugs that inhibit P-gp and BCRP may increase the absorption and plasma concentration of TAF.

3) Bictegravir is an inhibitor of renal transporters OCT2 and MATE1 in vitro with an IC_{50} of 0.42 and 8.0 μM, respectively. A clinical DDI study was conducted with an OCT2/MATE1 substrate to assess the DDI potential, and labeling is supported by these clinical data as summarized in the m2.5.

4) Animal data indicate that BIC, FTC, and TAF do not cause reproductive or fetal toxicity.

5) Animal data indicate that BIC, FTC and TAF are nongenotoxic. Bictegravir was not carcinogenic in a transgenic mouse study; a rat 2-year carcinogenicity study for BIC is ongoing. Long-term carcinogenicity studies of FTC in rats and mice did not show any carcinogenicity potential. Because there is a lower tenofovir exposure in rats and mice after TAF administration compared to TDF, carcinogenicity studies were conducted only with TDF. TDF did not show any carcinogenic potential in a long-term oral carcinogenicity study in rats. A long-term oral carcinogenicity study in mice showed a low incidence of duodenal tumors, considered likely related to high local concentrations in the gastrointestinal tract at the high dose of 600 mg/kg/day. The mechanism of tumor formation in mice and potential relevance for humans are uncertain.

6) In animal studies, BIC was detected in the plasma of nursing rat pups likely due to the presence of BIC in milk, without effects on nursing pups. In animal studies it has been shown that TFV is secreted into milk. It is not known whether BIC or TAF is secreted in human milk.

5.2. Overall Conclusion

The overall program including the data from the combination and individual agent studies is considered adequate to support the efficacy and safety of B/F/TAF FDC tablet based on the following considerations. Bictegravir has potent antiviral activity against HIV-1 and HIV-2 and has demonstrated additive to synergistic activity with a variety of other ARV drugs. Emtricitabine and TAF have antiviral activity against HIV-1, HIV-2, and HBV and have demonstrated additive to synergistic activity with a variety of other ARV drugs.

Based on the data supporting the individual components and the B/F/TAF combination, adverse pharmacokinetic interactions that would negatively affect pharmacological efficacy are not anticipated. This conclusion is based on the differences in physicochemical properties between the compounds that influence drug distribution and their discrete routes of disposition; each agent has a distinct metabolic and excretion pathway for elimination. Bictegravir is metabolized by CYP3A oxidation and UGT conjugation, FTC is cleared intact by renal excretion and TAF by hydrolysis. This was confirmed in a clinical drug interaction study (GS-US-141-1218) of BIC (100 mg), FTC (200 mg), and TAF (25 mg). As such, cumulative data from nonclinical and clinical studies demonstrated acceptable tolerability and safety profiles to support use in patients.

The toxicity profiles of BIC, FTC and TAF differ substantially with no clinically significant overlapping toxicity. Bictegravir, FTC, and TAF did not have positive findings in genotoxicity studies. Emtricitabine and TDF/TFV have demonstrated low carcinogenic potential in conventional 2-year bioassays, and BIC was negative for carcinogenicity in the transgenic mouse study. Bictegravir, FTC, and TAF/TDF have not shown significant adverse effects in reproductive and developmental toxicity studies in the absence of maternal toxicity. The combination of BIC, FTC and TAF is not expected to have an altered toxicity profile compared with that of the individual agents.

Identified impurities and degradants have been assessed as part of the routine toxicology or qualification studies with the individual agents and with the FTC/TDF combinations.

The absence of nonclinical safety studies with the B/F/TAF combination is in accordance with the FDA Guidance for Industry, Nonclinical Safety Evaluation of Drug or Biologic Combinations, March 2006; the CHMP Guideline on the Non-Clinical Development of Fixed Combinations of Medicinal Products (EMEA/CHMP/SWP/258498/2005, January 2008); and Human Immunodeficiency Virus-1 Infection: Developing Antiretroviral Drugs for Treatment, Guidance for Industry (November 2015).

There are no anticipated clinically relevant pharmacokinetic or toxicological interactions expected in the B/F/TAF FDC. Because the target organ profiles are different, and there is no evidence of genotoxicity, carcinogenicity, or reproductive toxicity, administration of the B/F/TAF combination product is unlikely to introduce new toxicities or to exacerbate known toxicities of the individual agents. The ample nonclinical safety databases on these drugs strongly indicate further toxicological investigations are unlikely to yield new data relevant to humans.

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Justification for Absent Nonclinical Data in Module 4

Section		Comment
All Module 4		*Bictegravir/Emtricitabine/Tenofovir alafenamide* All nonclinical studies required to support the proposed use of BIC, FTC and TAF have been completed. Based on the well-defined toxicity profiles of the single agents, the combination of BIC, FTC and TAF is not anticipated to exacerbate known toxicities or lead to new toxicities. In accordance with the EMA Guideline on clinical development of fixed combination medicinal products, combination toxicity studies with BIC, FTC and TAF were not conducted. Consequently the "Bictegravir/Emtricitabine/Tenofovir alafenamide" node extension is not used throughout Module 4, with the exception of a drug interaction study included in m4.2.1.4.
4.2.1.4	Pharmacodynamic Drug Interactions	*Bictegravir* Studies were conducted to evaluate the interactions of the fixed-dose combination (FDC) Bictegravir/Emtricitabine/Tenofovir alafenamide (B/F/TAF), so it was not considered relevant to conduct drug interaction studies on the individual component bictegravir. *Emtricitabine* Studies were conducted to evaluate the interactions of the FDCs B/F/TAF, Emtricitabine/Tenofovir disoproxil fumarate and Emtricitabine/Rilpivirine/Tenofovir disoproxil fumarate, so it was not considered relevant to conduct drug interaction studies on the individual component emtricitabine Drug interactions are discussed in m2.7.2 Section 2.5.
4.2.2.5	Excretion	*Emtricitabine* Report TOX063 in m4.2.2.4 covers both the metabolism and excretion of FTC.
4.2.2.7	Other Pharmacokinetic (PK) Studies	*Bictegravir, Emtricitabine & Tenofovir alafenamide* "Other" PK studies were not conducted. All PK study information is included elsewhere in the dossier (see m2.6.5).
4.2.3.1	Single-Dose Toxicity	*Bictegravir* No formal single dose toxicity studies were conducted with BIC. However, single dose PK studies in transgenic mice, rats and monkeys were performed (m2.6.4).

Section		Comment
4.2.3.4.1	Carcinogenicity (including supportive toxicokinetics evaluations): Long term studies	*Bictegravir* The 2 year rat carcinogenicity study will be completed by [redacted] 20[redacted], and the final study report will be submitted during the MAA procedure with the responses to the Day 120 List of Questions; the EMA accepted this proposal at the EMA [redacted] Meeting held on [redacted] 20[redacted]. *Tenofovir alafenamide* As agreed with the EMA and CHMP (EMA/CHMP/SAWP/629722/2012, FAL 2410-1-2012), carcinogenicity studies are not required for TAF due to the lack of TAF exposure in rats and TgRasH2 mice, and lower TFV exposure in rats and mice administered TAF compared to after administration of TDF. Reports of the carcinogenicity studies conducted with TDF and FTC are provided in this section (see also m2.6.6).
4.2.3.4.2	Carcinogenicity (including supportive toxicokinetics evaluations): Short- or medium-term studies	*Emtricitabine & Tenofovir alafenamide* Short- or medium-term carcinogenicity studies were not performed since long term carcinogenicity studies relevant to each component were conducted and are reported in m4.2.3.4.1 (see also m2.6.6). All supportive or dose-range finding studies are included elsewhere in the dossier.
4.2.3.4.3	Carcinogenicity (including supportive toxicokinetics evaluations): Other studies	*Bictegravir, Emtricitabine & Tenofovir alafenamide* "Other" carcinogenicity studies were not performed since short-, medium- and long-term carcinogenicity studies relevant to the components were conducted and are reported in m4.2.3.4.2 and m4.2.3.4.1 respectively (see also m2.6.6). All supportive or dose-range finding studies are included elsewhere in the dossier.
4.2.3.5.3	Prenatal and postnatal development, including maternal function	*Tenofovir alafenamide* As agreed with the EMA and CHMP (EMA/CHMP/SAWP/214541/2013, FAL 2410-1-FU-1-2013) a perinatal/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 after TDF administration. The perinatal/postnatal study conducted with TDF is provided in this section (see also m2.6.6).
4.2.3.5.4	Studies in which the offspring (juvenile animals) are dosed and/or further evaluated	*Bictegravir, Emtricitabine & Tenofovir alafenamide* No specific studies on offspring were conducted with any of the components (see m2.6.6 and m2.4 Section 4.7). Data are available from efficacy studies of TFV in nonhuman primates.
4.2.3.6	Local Tolerance	*Emtricitabine* Based on the extensive clinical data for FTC, local tolerance studies were not considered necessary.

Section		Comment
4.2.3.7.1	Antigenicity	*Emtricitabine* In view of the absence of indications from repeat-dose toxicity studies that FTC might elicit allergic reactions, no specific antigenicity studies were conducted.
4.2.3.7.2	Immunotoxicity	*Bictegravir* No specific immunotoxicity studies were conducted with BIC. There were no findings in the repeat dose toxicity studies with BIC to indicate an immunological concern. *Tenofovir alafenamide* No specific immunotoxicity studies were conducted with TAF. There were no findings in the repeat dose toxicity studies with TAF to indicate an immunological concern.
4.2.3.7.3	Mechanistic studies	*Bictegravir* Based on the extensive pharmacology, toxicology and clinical data, mechanistic studies with BIC were not considered necessary. *Emtricitabine* Based on the extensive pharmacology, toxicology and clinical data, mechanistic studies with FTC were not considered necessary. Specific endpoints were included in repeat dose toxicity studies in mice and cynomolgus monkeys to evaluate the potential for mitochondrial toxicity.
4.2.3.7.4	Dependence	*Bictegravir, Emtricitabine & Tenofovir alafenamide* No specific studies have been conducted to evaluate dependency of BIC, FTC, or TAF. Integrase inhibitors or nucleoside analogs, as classes, have no known properties that would suggest development of dependence and there was no evidence of development of dependence in nonclinical studies. Low levels of [^{14}C]BIC radioactivity were detected in brain (< 2% relative to blood) suggesting low BIC permeability across the blood brain barrier (see m2.6.5). In the tissue distribution studies using [^{14}C]TAF, little or no radioactivity was observed in brain tissues from dog and rat (see m2.6.5), while low levels of radioactivity was detected in mouse brain tissues, suggesting low permeability across the blood brain barrier in mouse (see m2.6.5). Consequently, dependency studies were considered not warranted.

Section		Comment
4.2.3.7.5	Metabolites	No specific studies with the metabolites of any of the components were conducted (see m2.6.6). *Bictegravir* There were no unique human BIC metabolites; all human metabolites were also found in nonclinical species. *Emtricitabine* Emtricitabine does not undergo extensive first-pass or systemic metabolism, and is eliminated primarily by renal excretion of unchanged drug. *Tenofovir alafenamide* There were no unique human TAF metabolites.
4.2.3.7.7	Other	*Emtricitabine & Tenofovir alafenamide* "Other" toxicity studies were not conducted. All toxicity study information is included elsewhere in the dossier (see m2.6.6).