Janssen Research & Development

Nonclinical Overview

MODULE 2.4

Darunavir/Cobicistat/Emtricitabine/Tenofovir Alafenamide Fixed Dose Combination Tablet for the Treatment of HIV-1 Infection in Adults and Adolescents

D/C/F/TAF (Darunavir/Cobicistat/Emtricitabine/Tenofovir Alafenamide)

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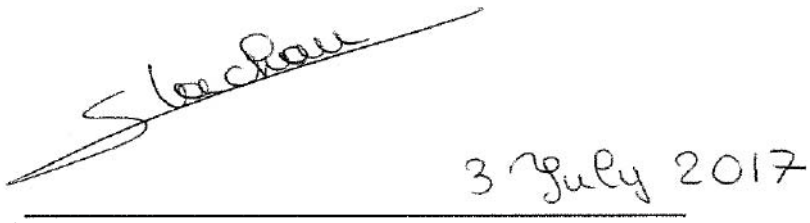
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EXPERT SIGNATURE:



3 July 2017

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LIST OF ABBREVIATIONS

[I]1	inhibitor concentration corresponding to steady state C_{max}
[I]2	inhibitor concentration corresponding to theoretical maximum concentration in the intestinal lumen
AhR	aryl hydrocarbon receptor (AHR gene product)
ALT	alanine aminotransferase
ALP	alkaline phosphatase
APD	action potential duration
APTT	activated partial thromboplastin time
AST	asparate aminotransferase
ATV	atazanavir (Reyataz®, Bristol-Myers Squibb)
AUC	area under the plasma concentration-time curve
AUCss	area under the plasma concentration-time curve at steady state
AUC_{tau}	the area under the plasma concentration-time curve from time zero to time tau over a dosing interval at steady state (AUC0-tau), where tau is the length of the dosing interval.
BCRP	breast cancer resistance protein (ABCG2)
BDC	bile-duct cannulated
BMD	bone mineral density
BSEP	bile salt excretory pump
BUN	blood urea nitrogen
Caco-2	human colon carcinoma cell line
CatA	cathepsin A
CC_{50}	50% cytotoxic concentration
CD4	cluster determinant 4
C_{max}	maximum plasma concentration
$C_{max,u}$	unbound concentration of drug at C_{max}
CNS	central nervous system
COBI	cobicistat (GS-9350, [Tybost®, Gilead])
CsA	cyclosporine A
CYP	cytochrome P450
D/C/F/TAF FDC	DRV 800 mg/COBI 150 mg/FTC 200 mg/TAF 10 mg once daily fixed-dose combination
DMSO	dimethyl sulfoxide
DRV	darunavir (Prezista®, Janssen)
EAD	early after depolarization
ECG	electrocardiograph, electrocardiogram
EVG	elvitegravir (Vitekta®, Gilead)
F1	first generation
FDC	fixed-dose combination
FTC	emtricitabine (Emtriva®, Gilead)
GD	gestation day
GFR	glomerular filtration rate
GI	gastrointestinal
Gilead	Gilead Sciences International Limited
GLP	Good Laboratory Practice
GMP	Good Manufacturing Practice
GS-7340	tenofovir alafenamide (TAF) free base
GS-7340-02	tenofovir alafenamide (TAF) monofumarate
GS-7340-03	tenofovir alafenamide (TAF) hemifumarate

HEK	human embryonic kidney (cell line)
HEPES	4-(2-Hydroxyethyl)piperazine-1-ethanesulfonic acid, N-(2-Hydroxyethyl)piperazine-N′-(2-ethanesulfonic acid)
hERG	human ether-à-go-go related gene
HIV	human immunodeficiency virus
HIV-1	human immunodeficiency virus type 1
IC_{50}	concentration resulting in 50% of maximum inhibition
Ig	immunoglobulin
IV	intravenous
Ki	kinetic inhibition constant
KI	affinity constant for enzyme inactivation
k_{inact}	inactivation constant
KLH	keyhole limpet hemocyanin
LD	lactation day
LD_{50}	the estimated dose that results in lethality in 50 percent of a group
LPV	lopinavir
LV	left ventricular
MAPD	monophasic action potential duration
MATE	multidrug and toxin extrusion protein
MBI	mechanism based inhibitor
MDR1	multidrug resistance protein 1 (P-gp)
MRP	multidrug resistance associated protein (ABCC)
MTD	maximal tolerated dose
NRTI	nucleoside reverse transcriptase inhibitor
NtRTI	nucleotide reverse transcriptase inhibitor
NNRTI	nonnucleoside reverse transcriptase inhibitor
NOAEL	no-observed-adverse-effect level
NOEL	no-observed-effect level
NRTI	nucleoside reverse transcriptase inhibitor
OAT	organic anion transporter
OATP	organic anion transporter protein
OCT	organic cation transporter
OCTN	organic cation transporter novel
PBMC	peripheral blood mononuclear cell
PEG	polyethylene glycol
P-gp	p-glycoprotein
PI	protease inhibitor
PK	pharmacokinetic(s)
PPB	plasma protein binding
PR	interval representing the time between the onset of atrial depolarization and the onset of ventricular depolarization
PT	prothrombin time
PXR	pregnane X receptor
QT	interval representing the time for both ventricular depolarization and repolarization to occur (start of the Q wave and the end of the T wave)
QTc	QT interval duration corrected for heart rate
QTcF	QT interval duration corrected for heart rate according to Fridericia
RBC	red blood cell(s)
RTV	ritonavir (Norvir®, Abbvie)

S9	tissue post-mitochondrial (9,000 x g) supernatant
SIV	simian immunodeficiency virus
T4	thyroxine
TAF	tenofovir alafenamide (GS-7340)
TDAR	T-cell dependent antibody response
TDF	tenofovir disoproxil fumarate, tenofovir DF (Viread®, Gilead)
TFV	tenofovir, PMPA
TFV-MP	tenofovir monophosphate
TFV-DP	tenofovir diphosphate
TSH	thyroid-stimulating hormone
T_{max}	time to reach C_{max}
UGT	uridine diphosphate glucuronosyltransferase

1. OVERVIEW OF THE NONCLINICAL TESTING STRATEGY

This dossier is being submitted in support of a new drug application for a fixed-dose combination (FDC) that contains darunavir (DRV, PREZISTA®, formerly TMC114), cobicistat (COBI, TYBOST®), emtricitabine (FTC, Emtriva®) and tenofovir alafenamide (TAF, formerly GS-7340). The FDC tablet of DRV 800 mg/COBI 150 mg/FTC 200 mg/TAF 10 mg once daily (further referred to as the D/C/F/TAF FDC) is developed by the Applicant in collaboration with Gilead Sciences International Limited (Gilead). Darunavir is a registered human immunodeficiency virus type 1 (HIV-1) protease inhibitor (PI) (Janssen Products L.P.; NDA21976), COBI is a registered pharmacokinetic (PK) enhancer for DRV (Gilead; NDA203094), FTC is a registered nucleoside reverse transcriptase inhibitor (NRTI) (Gilead, NDA21500), and the next-generation tenofovir (TFV, a nucleotide reverse transcriptase inhibitor [NtRTI]) prodrug TAF is a registered NtRTI (Gilead; Genvoya® NDA207561). The F/TAF 200/25 mg FDC is currently approved for use in combination with DRV/COBI for the treatment of adults and adolescents (aged 12 years and older) infected with HIV-1 (Discovy® NDA208215, January 2015). The D/C/F/TAF FDC tablet is therefore intended as a complete regimen for the treatment of HIV-1 infection in adults and adolescents (aged 12 years and older).

No nonclinical studies have been conducted with DRV in combination with COBI, FTC and TAF as no additive or synergistic toxicology/safety effects are expected for the D/C/F/TAF FDC beyond the expected pharmacokinetic boosting of DRV by COBI. This is in accordance with the FDA Guidance for Industry on the Nonclinical Safety Evaluation of Drug or Biologic Combinations (US Department of Health and Human Services, FDA, CDER, [redacted]).

Therefore, the nonclinical development program of the D/C/F/TAF FDC is entirely based on the individual development programs of the 4 components, and supports the use of the FDC tablet D/C/F/TAF as proposed in the Prescribing Information in Mod1. In this document, a summary of the nonclinical data for the separate components is described and a nonclinical assessment on the combined use of DRV, COBI, FTC and TAF is given in the integrated overview and conclusion. The original individual applications have been submitted previously under NDA21976 (DRV), NDA203094 (COBI), and NDA21500 (FTC), while TAF has been initially submitted as part of a combination therapy with elvitegravir, COBI and TAF (Genvoya® NDA207561), and as agreed via pre-NDA discussions with FDA ([redacted]), these data are not re-submitted.

A comprehensive package of nonclinical data is available for DRV, including studies of DRV in combination with ritonavir (RTV). Similarly, a comprehensive nonclinical program has been carried out by Gilead, documenting the safety pharmacology, nonclinical PK and toxicology of COBI, FTC and TAF. In the development of TAF, 3 forms of the active drug substance have been used: GS-7340, synonym for GS-7340 as the free base; GS-7340-02, synonym for GS-7340 as the monofumarate (1:1 ratio of free base to fumarate), and GS-7340-03, synonym for the hemifumarate (2:1 ratio of free base to fumarate). The hemifumarate, GS-7340-03 (TAF fumarate) was selected as the form for final development. GS-7340-03 is considered comparable to GS-7340-02 based on physical/chemical properties and PK data. In addition, due to the lack of TAF exposure in rats and mice and lower TFV exposure in rats and mice compared to tenofovir

disoproxil fumarate (TDF ; tenofovir DF; Viread®) other nonclinical studies conducted with TDF are included in this document

The program and design of the studies of each compound were consistent with the best scientific principles and international guidelines. Pivotal safety studies have been conducted in compliance with Good Laboratory Practices (GLP) standards issued by the Organization for Economic Cooperation and Development (OECD Principles of GLP). The animal species used in the various studies were from recognized sources and are standard models for nonclinical safety testing.

Throughout this summary, clinical exposure data for DRV, COBI, FTC and TAF were derived from Mod5.3.5.1/GS-US-299-0102 PK substudy in which HIV-1 infected subjects were treated with fixed dose D/C/F/TAF at 800/150/200/10 mg once daily.

Table 1: Mean Clinical Exposure Values At Steady State From Mod5.3.5.1/GS-US-299-0102 PK substudy

Compound	C_{max} (µg/mL)	AUC_{0-24h} (µg.h/mL)
DRV	8.83	99.3
COBI	1.13	8.74
FTC	2.1	11.9
TAF	0.163	0.130
TFV	0.019	0.339

2. PHARMACOLOGY

2.1. Primary and Secondary Pharmacodynamics

Darunavir is an inhibitor of the dimerization and of the catalytic activity of the HIV-1 protease. It selectively inhibits the cleavage of HIV encoded Gag-Pol polyproteins in virus infected cells, thereby preventing the formation of mature infectious virus particles. Darunavir has potent in vitro activity against both wild type and multi-drug resistant HIV-1 strains.

Cobicistat is a structural analog of RTV, which retains its potent mechanism-based inhibition of human CYP3A, but lacks anti-HIV activity. No remarkable cytotoxicity was observed with COBI in vitro in human MT-2 and HepG2 cells, with 50% cytotoxic concentration (CC_{50}) values of 89 and 44 µM, respectively (69.1 and 34.1 µg/mL). In vitro data indicate that COBI shows low potential for inhibition of host proteases (50% inhibitory concentration [IC_{50}] >30 µM) and a low potential for effects on adipocyte functions (lipid accumulation and glucose uptake).

Emtricitabine and TAF/TFV are potent and selective inhibitors of HIV-1. Both drugs show potent antiretroviral activity against diverse subtypes of HIV-1 in vitro. Emtricitabine and TAF/TFV are phosphorylated intracellularly through nonoverlapping pathways, and in combination show no antagonism for the formation of their active metabolites. Additive to synergistic effects were observed in in vitro interaction studies of TFV, the active metabolite of TAF, with NRTIs, non-NRTIs (NNRTIs), PIs and the integrase inhibitor elvitegravir (EVG) [34]. The potential of FTC and TFV to interfere with mitochondrial functions is low, whether administered alone or in combination with other NRTIs.

2.2. Safety Pharmacology

Darunavir, COBI, FTC and TAF were studied in a series of in vitro and in vivo safety pharmacology studies.

2.2.1. Darunavir

Darunavir has been tested in 2 in vitro safety pharmacology studies at concentrations significantly exceeding the DRV free plasma concentration determined in patients treated with a dose of 800/150/200/10 mg D/C/F/TAF (human free C_{max} for DRV is 0.52 µg/mL calculated using a human total C_{max} of 8.83 µg/mL and an estimated plasma protein binding [PPB] = 94%). In vitro, DRV at a concentration of 10 µM (5.9 µg/mL) showed no significant effect on membrane potassium current in human-ether-à-go-go related gene (hERG).T.human embryonic kidney (HEK) 293 cells and there were no effects on the electrophysiological cardiac action potential parameters in sheep isolated cardiac Purkinje fibers at the same concentration corresponding to 11-fold the clinical concentration.

In vivo, DRV had no effect on cardio-hemodynamic and electrocardiogram (ECG) parameters in telemetered dogs following single oral (gavage) doses of up to 120 mg/kg. Although DRV systemic exposure was not determined in the telemetry study, mean C_{max} and $AUC_{0-\infty}$ values for male and female dogs were 16.6 and 15.0 µg/mL and 69.4 and 53.4 µg.h/mL, respectively, after a single administration at a similar dose level (120 mg/kg) at the start of the 12-month dog study. Although DRV peak plasma levels in dogs are higher (1.7- to 1.9-fold) than that attained in humans (mean C_{max} = 8.83 µg/mL) at the therapeutic dose (D/C/F/TAF 800/150/200/10 mg once daily), AUC values are below the clinical exposure (mean AUC_{0-24h} = 99.3 µg.h/mL). In addition, no treatment-related effects on heart rate or ECG morphology were noted in vivo after repeated administration in dogs.

In rats, after single oral administration of up to 2000 mg/kg DRV, there was no effect on gastrointestinal transit time of a charcoal solution, no relevant effects on neurobehaviour and motor activity and no acute effects on respiration.

2.2.2. Cobicistat

Safety pharmacology studies were conducted to determine the potential effects of COBI on the central nervous, cardiovascular and respiratory systems. In the rat central nervous system (CNS) study, there were no significant neurotoxic effects; changes were limited to salivation, decreases in arousal, locomotor and motor activities, and decreases in body temperature at doses of 150 mg/kg and above. The no observed adverse effect level (NOAEL) was 50 mg/kg. Decreases in body temperature are commonly observed in rodents after xenobiotic exposure, and most likely represent an adaptive thermoregulatory response unique to rodents, rather than a direct effect on the CNS [62, 61, 43]. Similarly, decreases in arousal and motor activity may represent a general toxicity response rather than a direct CNS response. No adverse effects were observed in the rat respiratory study (NOAEL 500 mg/kg).

Patch clamp studies indicated that COBI inhibited the hERG potassium current (IC_{50} = 1.8 µM [1.4 µg/mL] and the hCav1.2 L type calcium channel (IC_{50} = 6 µM [4.7 µg/mL]), but was a weak

inhibitor of the hNav1.5 sodium channel (IC_{50} = 86.5 µM [67.5 µg/mL]). In rabbit Purkinje fibers (protein-free environment), which are considered more sensitive to drug-induced action potential duration (APD) prolongation and early after depolarizations (EADs) than fibers isolated from dog and several other species [31], COBI caused a shortening of the APD at ≥1 µM (0.78 µg/mL); there was no evidence of triangulation, instability, or alternans predictive of prolongation of the QT interval (i.e. interval representing the time for both ventricular depolarization and repolarization to occur).

In a Langendorff study in rabbit hearts (protein-free environment) conducted with COBI alone, negative inotropic effects and shortening of the APD was noted at ≥1 µM. In a second Langendorff study in rabbit hearts, COBI produced similar effects (PR interval prolongation [i.e. interval representing the time between the onset of atrial depolarization and the onset of ventricular depolarization] and decreases in left ventricular [LV] function) at concentrations ≥1.5 µM (1.17 µg/mL). When hearts were exposed to COBI in combination with ATV, effects on PR interval and LV function were similar to the decreases noted with COBI alone. Cobicistat had no notable effects alone, or in combination with atazanavir (ATV), on QRS and QT intervals, monophasic action potential duration (MAPD), or triangulation; and there were no EADs.

In conscious telemetered dogs, there were no adverse effects on hemodynamic and ECG parameters up to 45 mg/kg, the highest dose administered. Cobicistat plasma levels 1 hour after dose administration at 45 mg/kg were between 2530 and 8950 ng/mL (3.3 to 11.5 µM; mean of 7.7 µM). Compared to vehicle control values, mild prolongation in PR intervals were noted primarily from 1 to 6 hours postdose, although mean PR intervals never exceeded the upper limits of normal for canines at any time point [45, 48]. Further, based on the results of the Japanese QT PRODACT studies and others, the mild increases in QT interval duration corrected for heart rate (QTc) (<4%) noted from 13 to 24 hours postdose at 45 mg/kg are unlikely to be biologically significant [42, 52].

Although COBI inhibits the L type calcium ion channel and K^+ hERG current at low micromolar concentrations, data from the Purkinje fiber assay, the cardiovascular dog study, and ECG evaluations in the repeat-dose toxicity studies in dogs up to 39 weeks duration suggest that COBI has a low potential for QT prolongation, but may have a tendency to slightly prolong the PR interval. Of note, in the 39-week dog toxicity study, there were no notable effects on the QT and PR intervals at dose levels up to 20 mg/kg/day. Mean COBI C_{max} values during Week 39 at 20 mg/kg were between 7090 to 8405 ng/mL (9.1 to 10.8 µM). The shortening of the APD in rabbit Purkinje fibers and the mild delay in the PR interval in dogs may be a consequence of interaction with cardiac calcium channels [11, 23].

Cobicistat has shown the potential to decrease LV function and prolong the PR interval in the isolated rabbit heart at ≥1 µM (0.78 µg/mL), which is approximately 10-fold above the anticipated clinical unbound exposure at the 150 mg COBI dose. However, as the fraction of unbound COBI is lower in plasma samples obtained in clinical studies (2.49% to 3.23%) compared to the in vitro studies, including clinical studies in subjects with moderate hepatic

impairment or severe renal impairment, the potential of COBI to decrease LV function and prolong PR is expected to be low in patients. In a thorough QT clinical study, COBI demonstrated a lack of prolongation effects on the QTcF interval in healthy adult subjects at therapeutic and supratherapeutic exposures. A small but statistically significant negative association between COBI plasma concentration and QTc interval, and a modest, dose related increase in PR interval, were observed in the QT/QTc study, which are not considered to be clinically significant. Further, echocardiograms performed in healthy subjects (NDA203094/Mod5.3.4.1/GS-US-216-0116) at baseline and after receiving 150 mg COBI for at least 15 days indicated no clinically significant change in LV function.

2.2.3. Emtricitabine

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 (10- to more than 50-fold). No effects on the cardiovascular system were reported in anesthetized dogs administered a cumulative dose of 38.5 mg/kg of FTC intravenously (IV) over a 1-hour period. In addition, there were no abnormalities reported on the ECG data obtained from the repeated-dose toxicity studies in monkeys, where AUC exposures were up to 23-fold higher than in humans administered the 200-mg dose.

2.2.4. Tenofovir Alafenamide

Tenofovir alafenamide was evaluated in safety pharmacology studies of the rat central nervous, renal, gastrointestinal (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. In the in vitro hERG assay, TAF as GS-7340-03 was dissolved in dimethyl sulfoxide (DMSO) and diluted with HEPES-buffered physiological saline to a final concentration of 0.3% DMSO.

The IC_{50} for the inhibitory effect of TAF on hERG potassium current was estimated to be greater than 10 μM, far above human exposure.There were no adverse effects detected in the CNS in rats dosed at 1000 mg/kg, in the renal system in rats administered 1000 mg/kg or in the cardiovascular system of dogs dosed at 100 mg/kg (80 mg free base equivalents/kg). There was reduced gastric emptying in rats dosed at 1000 mg/kg, but not at 100 mg/kg.

Although TAF showed some potential to prolong the PR interval in the 39-week dog study at 18/12 mg/kg/day, the slight change was associated with decreased weight gain, bone and renal toxicity, and significant decreases in triiodothyronine (T_3) [27, 53]. 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 (Genvoya® NDA207561/GS-US-120-0107).

2.2.5. Assessment of Safety Pharmacology

Darunavir safety pharmacology studies did not detect any significant nonclinical safety signals.

Safety pharmacology studies with COBI did not reveal any significant safety findings, with the exception of the Langendorff studies, in which effects on PR interval and LV function were shown. However, no clinically significant cardiovascular changes have been observed at clinical exposures up to 4-fold higher than those achieved at the clinical dose of 150 mg COBI.

Emtricitabine and TAF had 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 at 18/12 mg/kg/day, 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. There was no evidence of toxicity to mitochondrial toxicity in vitro or in vivo for FTC and TAF.

The favorable pharmacodynamic and safety pharmacology assessment of DRV, COBI, FTC and TAF supports the effective and safe use of the D/C/F/TAF FDC at the proposed doses for the treatment of HIV-1 disease.

3. PHARMACOKINETICS

The nonclinical PK of DRV, COBI, FTC and TAF were investigated in a series of in vitro and in vivo.

3.1. Absorption

3.1.1. Darunavir

The transepithelial permeability of DRV was intermediate to high in Caco-2 cells and passive transcellular diffusion is proposed as the predominant mechanism for DRV intestinal absorption. One or more efflux transporters modulated DRV permeation, but the impact of these systems was limited at higher DRV concentrations (>100 µM or 55 µg/mL).

In animals, DRV absorption was rapid following oral administration in all species with observed values for T_{max} in the range of 0.5 to 6 hours. The absolute oral bioavailability was 37% to 58% in adult rats and was very likely influenced by the high first pass effect as demonstrated by the presence of a large amount of metabolites in the bile. Bioavailability was higher in dogs (60 to 122%). In rats and dogs, the plasma clearance and the volume of distribution were moderate to high. The elimination was rapid in all species.

After single oral administration and across the dose range studied, the plasma kinetics of DRV in mice, rats, rabbits, minipigs and dogs was less than dose proportional, especially at high dose levels, in line with the low solubility of the compound.

In adult rodents including pregnant rats, repeated oral dosing resulted in a decrease in systemic exposure, mainly due to induction of CYP3A iso-enzymes, which are extensively involved in the metabolism of DRV. In dogs, no decrease in exposure or enzymatic induction was observed after repeated administration. No conclusion could be drawn from the minipig study.

3.1.2. Cobicistat

In Caco-2 cells, COBI showed high forward permeability and no evidence for efflux.

Single-dose PK was studied in rats, dogs, and monkeys. Clearance values were high relative to hepatic blood flow (likely due to the lack of self-limiting enzyme inactivation in nonclinical species) and volumes of distribution were similar to those for total body water. After oral dosing, bioavailability was low or low/moderate, likely due to high first-pass elimination.

After repeated oral dosing, there were species differences in auto-induction during these studies, with hepatic microsomal fractions from treated mice and rats showing higher levels of CYP3A, but with no increases in treated dogs. This is consistent with the species differences observed in induction studies in vitro where COBI was found to activate rat pregnane X receptor (PXR) but did not activate human PXR or induce human drug metabolizing enzymes or multidrug resistance protein 1 (MDR1) in hepatocytes.

3.1.3. Emtricitabine

Single-dose PK of FTC has been studied in mice, rats [17], and cynomolgus monkeys. 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.

The multiple-dose PK parameters for FTC were derived as part of the repeat-dose toxicity studies in mice (80 to 3000 mg/kg/day), rats (60 to 3000 mg/kg/day), and monkeys (40 to 2000 mg/kg/day) 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.

3.1.4. Tenofovir Alafenamide

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 (CsA) diminished the efflux ratio and increased the forward permeability. In the presence of 90 µM COBI in the Caco-2 bidirectional permeability assay, TAF forward permeability increased 4.6-fold and the efflux ratio significantly decreased suggesting P-glycoprotein (P-gp) mediated drug interaction.

Single-dose plasma PK of TFV and/or TAF was 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, to rats via oral gavage, to dogs via IV 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. Tenofovir alafenamide was not detected in any of the rat studies. Additionally, the plasma PK profiles for TAF and TFV and TFV concentrations in peripheral blood mononuclear cells (PBMCs) were determined in rhesus monkeys following a single oral dose of GS-7340-02. Tenofovir alafenamide is generated at sufficient exposures in nonclinical species chosen for assessment of toxicology. Consistent with dose-dependent permeability observed in vitro, the oral bioavailability of TAF increased with increasing dose in dogs and the observed oral bioavailability was 14.3% at the 10 mg/kg dose [1]. Following an oral dose of ^{14}C-TAF to a bile-duct cannulated (BDC) dog, the fraction

absorbed was at least 41% based on excretion in urine and bile. Since 41% of the total dose was absorbed approximately 65% of the absorbed drug was hepatically extracted. This was consistent with hepatic extraction estimated from the in vitro stability study in dog hepatic tissue post-mitochondrial (9,000 x g) supernatant (S9) fractions (60.5%).

The multiple-dose PK of TFV was characterized in a PK study in dogs orally administered TAF and in toxicokinetic studies following oral administration of TAF in mice, rats, dogs, and monkeys. 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.

3.2. Plasma Protein Binding and Tissue Distribution

3.2.1. Darunavir

The PPB of DRV was moderate to high in all tested species (i.e., mouse, rat, rabbit, dog and human). In human plasma, DRV was mostly bound to α_1-acid glycoprotein and to a lesser extent to albumin. The free fraction ranged from 5% (rat) to 38% (rabbit), and was 5% in humans. Plasma protein binding in most species was concentration-dependent. The blood to plasma concentration ratios ranged from 0.64 to 1.11 across all species at 0.5 µg/mL, indicating some distribution of DRV to blood cells, especially in the rabbit and dog in which the PPB was lower than in the other species.

After oral administration of ^{14}C-DRV in rats, the tissue distribution of ^{14}C-DRV was extensive and rapid. The highest concentrations of radioactivity were measured in the liver and adrenal gland. No undue retention or accumulation was observed, except in melanin-rich tissues such as the pigmented parts of the eye. However, from these tissues a gradual decrease of the radioactivity levels could be demonstrated, showing the reversibility of this binding.

3.2.2. Cobicistat

Binding of COBI in plasma was moderately high, yielding a fraction unbound of 6.3% in human plasma at 1 µM COBI (0.78 µg/mL). Binding to mouse, rat, and monkey plasma was similar and showed modest concentration-dependence, but binding in dog and human plasma was largely unchanged over the range 1–30 µM. Fraction unbound values were similar in nonclinical species (mean values 4.75%–6.54%) to humans. In addition, mean fraction unbound values were between 2.49% and 2.71% in normal subjects, 3.23% in subjects with moderate hepatic impairment, and 2.47% in subjects with severe renal impairment. Cobicistat does not distribute well into the cellular fraction of blood from mouse, rat, dog, or human.

After oral administration of ^{14}C-COBI to albino and pigmented rats, radioactivity was rapidly and widely distributed to most tissues. Generally, the radioactivity was preferentially distributed into glandular tissues and organs of elimination. The tissues showing the highest concentrations of radioactivity, excluding the gastro intestinal tract, included liver, adrenal, kidney, and pituitary. The tissues with the lowest C_{max} values were eye, spinal cord, and brain, bone, and secondary sex organs. Low levels of radioactivity in the brain, spinal cord, and testes suggest

minimal transport across the blood:brain and blood:testes barriers. Compared to albino rats, the pigmented rats showed very similar patterns of distribution of radioactivity, but with higher concentrations in the uveal tract of the eye. There were also higher concentrations of radioactivity in pigmented skin compared to nonpigmented skin, suggesting that COBI was associated with melanin. Tissue concentrations of radioactivity declined largely in parallel with those in plasma. In pigmented animals there was more prolonged retention of radioactivity in melanin-containing tissues, but dosimetry analysis confirmed that concentrations were declining and association with the tissues was reversible.

3.2.3. Emtricitabine

The protein binding of FTC was very low (<5%) in mouse, rabbit, monkey, and human plasma.

The tissue distribution of ^{14}C-FTC was characterized in rats and cynomolgus monkeys after a single oral dose of 200 mg/kg. 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. Pharmacokinetic parameters for FTC in pregnant animals were similar to those reported for nonpregnant animals.

3.2.4. Tenofovir Alafenamide and Tenofovir

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. In vitro protein binding of TAF was moderate in dog and human plasma with the percent unbound values of 48.0% and 46.8%, respectively. These in vitro values were higher than those observed in multiple human ex vivo studies with the mean percent unbound TAF ranging from 14% to 23% in all subjects (Genvoya® NDA207561/GS-US-120-0108 and GS-US-120-0114). Since the ex vivo results should be more clinically relevant than the in vitro values, percent unbound TAF of 20% 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.

Following oral administration of ^{14}C-TAF to mouse, rat, and dog, ^{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 (iliac, axillary, inguinal and mesenteric lymph nodes, and spleen) 24 hours following administration of an equivalent dose of ^{14}C-TAF relative to ^{14}C-TDF [30].

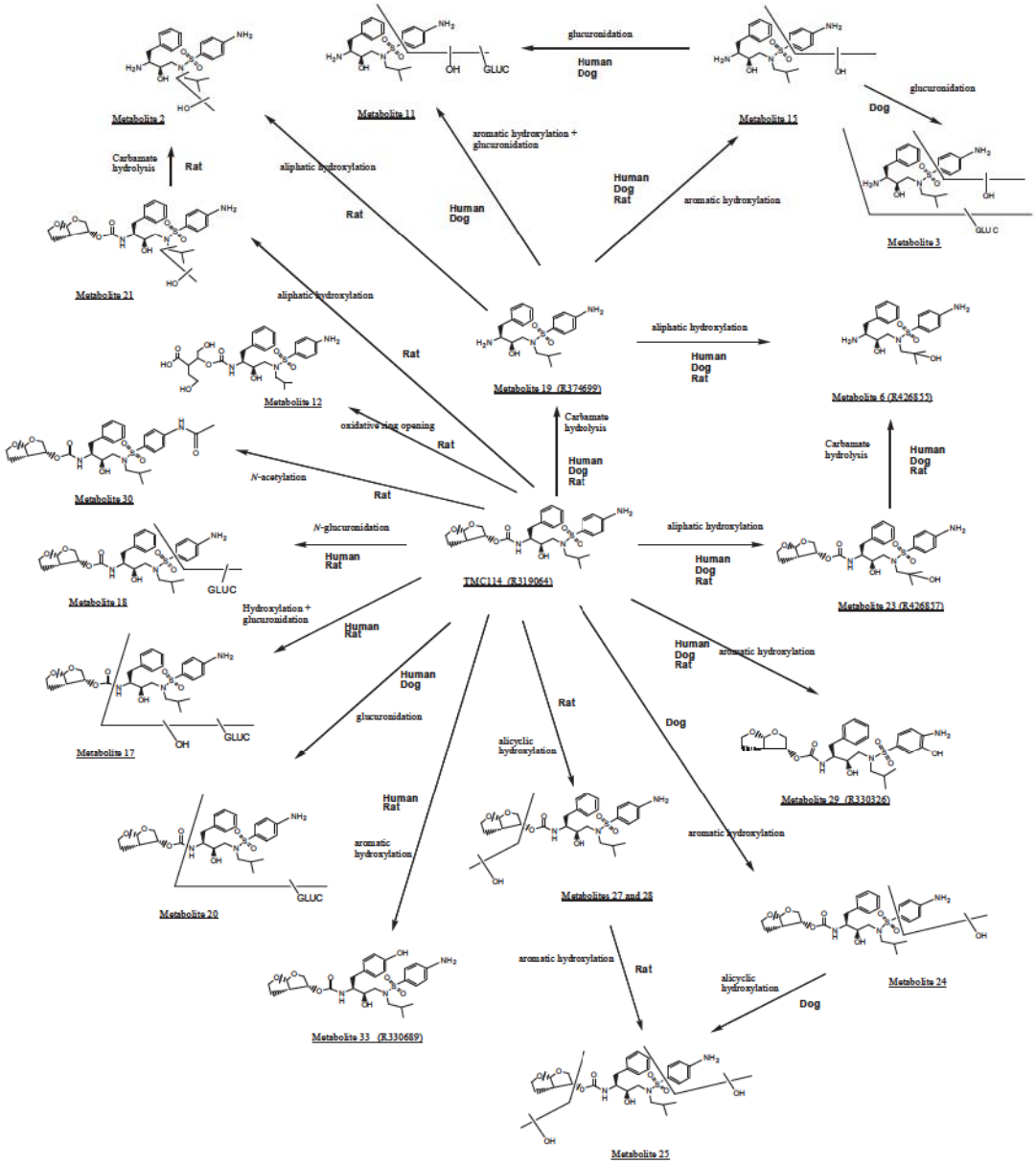
3.3. Metabolism and Interaction Potential

3.3.1. Darunavir

The metabolism of DRV following single oral administration was extensive and qualitatively similar in all species, including humans. In vitro and in vivo studies in rats, dogs and humans identified three major Phase I metabolic reactions: carbamate hydrolysis, aliphatic hydroxylation at the isobutyl moiety and aromatic hydroxylation at the aniline moiety (see Figure 1). In dogs and humans, the major Phase I metabolic pathway was the carbamate hydrolysis whereas in rats, hydroxylation in a different part of the molecule was more important. Phase II glucuronidation was a minor pathway in rats, dogs and humans. No unique human metabolites were identified. Metabolic pathways proposed on the basis of in vitro studies were consistent with those observed following in vivo studies in rats, dogs and humans.

In human liver, CYP3A was almost exclusively involved in the metabolism of DRV. Darunavir inhibited CYP3A in human liver microsomes with an inhibitory constant (K_i) value of 0.40 µM (0.22 µg/mL). Given this low value, this inhibition is considered to be clinically relevant. The K_i values for the other CYP450 enzymes (CYP2B6, CYP2C9, CYP2C1, CYP2D6, CYP2C8 and uridine disphosphate glucuronosyltransferase [UGT] 1A1) were at least 60-fold higher, indicating a much lower affinity and less potential for clinically relevant interactions. Darunavir also showed a concentration dependent effect on CYP3A4 induction in vitro. This effect may have limited clinical relevance, as induction was significant in vitro at concentrations several fold higher than those attained in the clinic. In Caco-2 cells, DRV has P-gp inhibitory properties with an apparent IC_{50} of 32.9 µM (18.0 µg/mL). Darunavir has low potential to inhibit organic cation transporter (OCT) 2 and multidrug and toxin extrusion protein (MATE) 1. A comprehensive drug-drug interaction clinical program with DRV/RTV was conducted, including a cocktail study to assess the effects of DRV/RTV on the metabolism of probe substrates. Interactions are most likely a result of the RTV component because of its inhibitory effects on CYP2D6, CYP3A and certain transporters, and/or its induction effects on CYP2C9, CYP2C19 and UGT [15].

Figure 1: In Vivo Metabolic Pathways of DRV in Animals and Humans

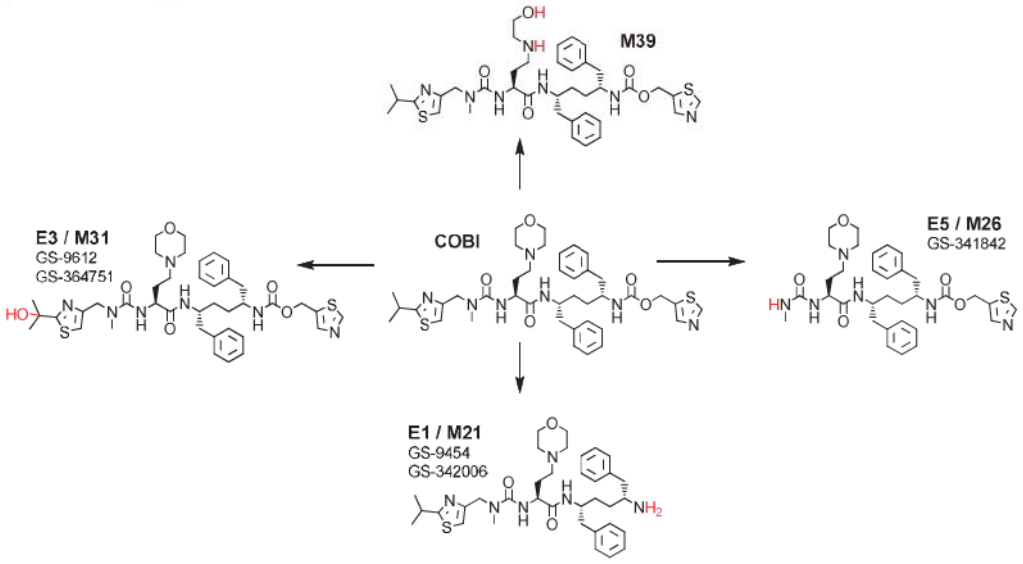


3.3.2. Cobicistat

The primary metabolic pathways for COBI are illustrated in Figure 2 and are methine oxidation of the isopropyl moiety (M31, GS-9612), cleavage adjacent to the methylurea (M26, GS-341842), cleavage of the carbamate (M21, GS-9454), and cleavage and deethylation of the morpholine (M39). Combinations of these routes and other routes of oxidative metabolism were also detected. Oxidation is primarily catalyzed by CYP3A, which can generate all metabolites, with a minor role for CYP2D6 (which contributes to the generation of M31). In vitro metabolism in nonclinical species was relatively rapid but rates of metabolism by human hepatic microsomal fractions, human hepatocytes, and recombinant CYP3A4 were relatively slow due to concurrent CYP3A inactivation. After oral administration of ^{14}C-COBI to mice, COBI was the most abundant analyte in plasma. Parent COBI, M21, and M31 were the most abundant analytes in

feces, with M39 also being significant in dog feces. Profiles in bile from rats and dogs were complex, with many small peaks being detected (each accounting for ≤5.3% of the dose).

Figure 2: Proposed Primary Metabolic Pathway of COBI



Cobicistat is a potent inhibitor of human CYP3A with inactivation kinetics (inactivation constant [k_{inact}] = 0.47 min-1, affinity constant for enzyme inactivation [K_I] = 1.1 µM), similar to those of RTV. Inhibition of CYP3A is relatively specific as COBI did not inhibit human CYP1A2, CYP2C9, or CYP2C19, is a very weak inhibitor of CYP2C8 (IC_{50} = 30.1 µM), a weak inhibitor of CYP2D6 (IC_{50} = 9.2 µM), and a modest inhibitor of CYP2B6 (IC_{50} = 2.8 µM). This is in contrast to RTV, which is a more potent inhibitor of CYP2D6 (IC_{50} = 3.4 µM), CYP2C9 (IC_{50} = 3.9 µM), and CYP2C8 (IC_{50} = 5.5 µM) [45, 48]. This higher specificity for COBI has been confirmed in clinical drug interaction studies in which COBI had no effect on the PK of efavirenz (CYP2B6) and little effect on the PK of desipramine (CYP2D6). Cobicistat is a weak inhibitor of human hepatic microsomal UGT1A1 activity (IC_{50} = 16.3 µM), being less potent than RTV (IC_{50} = 4.7 µM). Cobicistat treatment should thus not raise serum bilirubin concentrations due to inhibition of UGT1A1.

To aid in the interpretation of the data and allow a quantitative estimate of the potential drug interaction liability from the IC_{50} values, the key human PK data are summarized in Table 2.

Table 2: Clinical Concentrations for Drug Interaction Liability Assessment

Parameter	Value	Rationale
Total C_{max} ($[I]_1$)	1.45 µM	C_{max} 1.13 µg/mL
$C_{max,u}$	0.092 µM	f_u 6.33% at 1 µM
$[I]_2$	770 µM	150 mg/250 mL

f_u = fraction unbound; the f_u value is from Mod4.2.2.3/AD-216-2026 study $[I]_1$ = inhibitor concentration corresponding to steady state C_{max}; $[I]_2$ = inhibitor concentration corresponding to theoretical maximum concentration in the intestinal lumen

In xenobiotic receptor transactivation studies, COBI showed no ability to activate human aryl hydrocarbon receptor and was a very weak activator of human PXR (2.2-fold activation at

10 µM, compared to 10.1-fold activation by 10 µM RTV). This was confirmed in human hepatocyte studies where COBI, at concentrations up to 30 µM, increased CYP1A2 activity and mRNA and protein by <2% of the positive control and increased CYP3A4 mRNA expression by an average of 27.4%. CYP3A activity was below that of the vehicle control, due to inhibition by COBI, but a slight increase in immunodetectable CYP3A was detected. Other targets for induction (UGT1A1 mRNA, MDR1 mRNA, and CYP2B6 mRNA and protein) were all unaffected or weakly affected by COBI treatment.

In contrast to its lack of effect on human PXR, COBI activates rodent PXR and increases the expression of proteins regulated by this receptor, such as rat CYP3A, UGT1A1, and presumably organic anion transporter protein (OATP) 2.

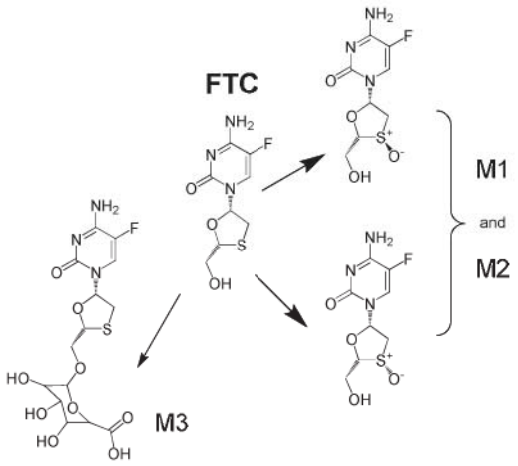
At systemic concentrations achieved in plasma at the 150 mg COBI dose, COBI would not inhibit the drug transporters MDR1, multidrug resistance associated protein (MRP) 1, MRP2, breast cancer resistance protein (BCRP), organic anion transporter (OAT) 1, or OAT3 ($[I]_1/IC_{50}$ <0.1). However, at concentrations achievable briefly in the intestinal lumen during absorption ($[I]_2$ = 770 µM) COBI can inhibit intestinal efflux transporters such as MDR1 and BCRP ($[I]_2/IC_{50}$ >10). This was consistent with a clinical drug interaction study in which COBI was found to increase the C_{max} of the MDR1 substrate, digoxin, but to have less effect on the area under the curve. With respect to hepatic uptake transporters, COBI is a moderate inhibitor of OATP1B1 and OATP1B3 ($[I]_1/IC_{50}$ = 0.4 and 0.7, respectively). Inhibition of OATP transporters was consistent with a clinical drug interaction study in which, after dosing with 150 mg EVG and 150 mg COBI, there was a modest increase (38%) in exposure of co dosed rosuvastatin that was not considered clinically relevant.

With respect to renal transporters, COBI is a weak inhibitor of MRP4, MATE 2K and OCT2, and a more potent inhibitor of MATE1 and organic cation transporter novel, type 1 (OCTN1), with similar potencies to RTV. Since OCT2 and MATE1 transporters appear to play a role in the active tubular secretion of creatinine by the kidney [56, 46, 50] , inhibition of these transporters by COBI provides a plausible explanation for the clinical finding of a reduction in renal creatinine clearance without a change in glomerular filtration rate (GFR), i.e., COBI effects the active secretion of creatinine, but not passive filtration. This phenomenon has been reported for a variety of other compounds including cimetidine [57], trimethoprim [36], pyrimethamine [37], amiodarone [38], ranolazine [40], dronedarone [54], rilpivirine [13], dolutegravir [35], the antitubercular agent PA-824 [19], the fluoroquinolone DX-619 [26], and the thrombin inhibitor AZD0837 [47].

3.3.3. Emtricitabine

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. A minor direct glucuronide metabolite, M3, was also detected (Figure 3) [17].

Figure 3: Oxidative Metabolism of FTC



Emtricitabine was not extensively metabolized and is eliminated primarily as unchanged drug by renal excretion in mice, rats, and cynomolgus monkeys. Over 90% of the radioactivity in mouse and rat urine and 64% of the radioactivity in monkey urine was unchanged drug. Only trace levels of metabolites were found in feces [17, 16]. In all 3 species, metabolism accounted for only a minor percentage of FTC elimination. Emtricitabine is subject to Phase I metabolism (oxidation to a diastereomeric sulfoxide) and to some direct conjugation (glucuronidation of hydroxymethyl group) as minor metabolic routes.

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.

Emtricitabine did not activate human AhR (aryl hydrocarbon receptor) or PXR at concentrations up to 50 μM.

In vitro studies indicated that FTC is not a substrate (P-gp, BCRP, OAT1 and OCT2) or an inhibitor (P-gp, BCRP, bile salt excretory pump [BSEP], OATP1B1, OATP1B3, MATE1, OAT1, OAT3, OCT1, OCT2 MRP2 and MRP4) of any of the transporters tested except for being a substrate of OAT3. There is no clinical evidence for FTC to be involved in transporter-mediated drug interactions.

3.3.4. Tenofovir Alafenamide

3.3.4.1. Intracellular Metabolism

Tenofovir alafenamide is subject to intracellular metabolism to TFV, which is further phosphorylated to the anabolites, tenofovir monophosphate (TFV-MP) and tenofovir diphosphate (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 cathepsin A (CatA) [2, 3]. In contrast to PBMCs, TAF is primarily hydrolyzed by carboxylesterase 1 in primary hepatocytes. Of the HIV PIs (DRV, ATV, lopinavir [LPV], and RTV), the boosting agent COBI, and HCV PIs (telaprevir, boceprevir, TMC-435, BI-201355,

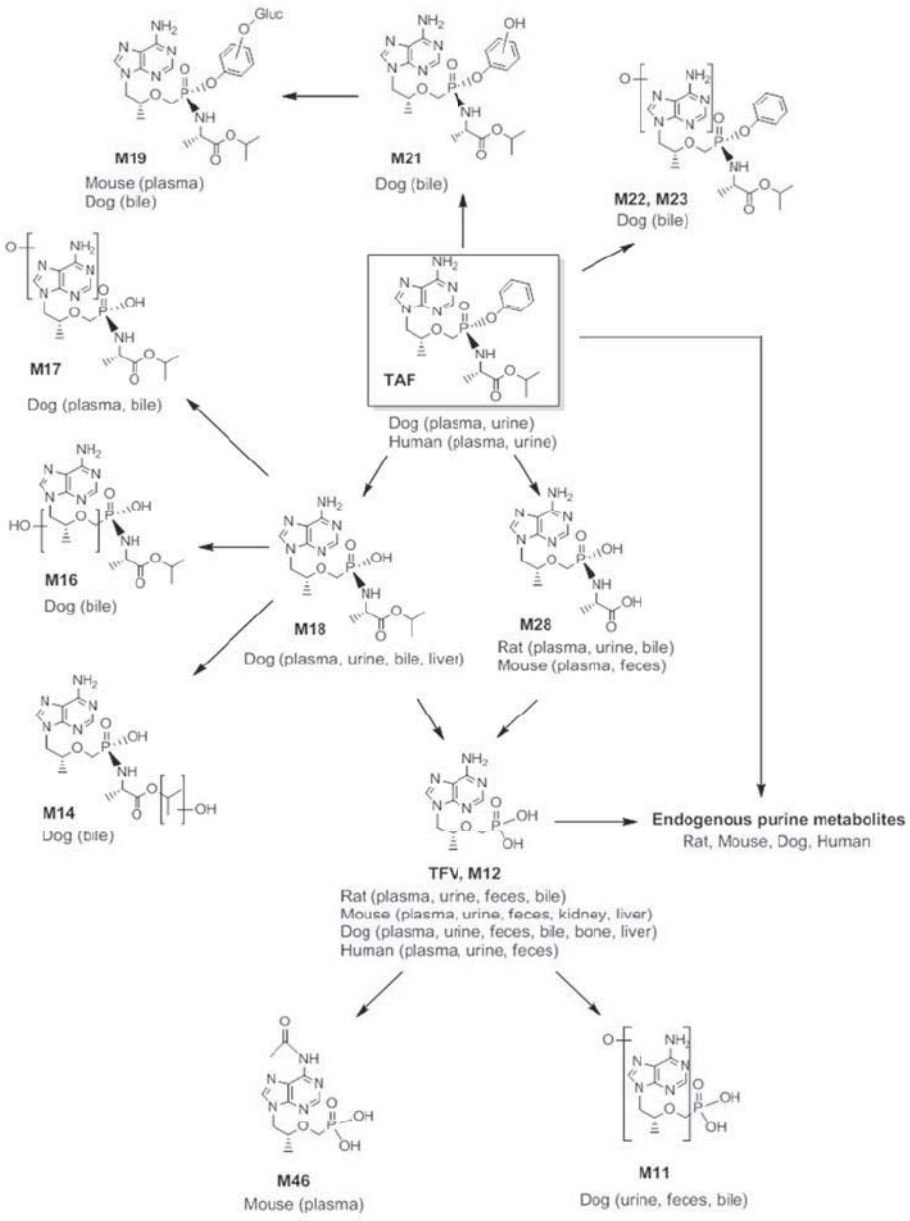
MK-5172, GS-9256, and GS-9451), the HCV PIs telaprevir and boceprevir, which are known to inhibit CatA, were the only ones that changed the antiretroviral effect of TAF in primary CD4+ T lymphocytes (reduced 23-fold and 3-fold, respectively). These data support the coadministration of the tested therapeutic 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.

3.3.4.2. Metabolism

The metabolic profiles of TAF were determined in plasma, urine, feces, kidney and liver from mice, in plasma, urine, bile, and feces from rats, and in plasma, urine, bile, feces, bone, and liver from dogs. The metabolite profiles were also determined in human plasma, urine, and feces following administration of a single oral dose of ^{14}C-TAF (Genvoya® NDA207561/GS-US-120-0109). Based on the results from mouse, rat, dog, and human, a proposed biotransformation pathway is summarized (Figure 4).

Figure 4: Metabolites of TAF



The potential for CYP enzymes to metabolize TAF was assessed. 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.

Based on the studies from mouse, rat, dog and human, 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. 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.

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 post dose [1]. 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.

The potential for TAF and TFV to inhibit human CYP-mediated drug metabolism was examined in vitro using hepatic microsomal fractions and enzyme-selective activities. The inhibitory activity of TAF with human liver microsomal CYP isozymes, CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, and CYP3A were assessed at concentrations up to 25 μM. The inhibition constant (IC_{50}) values calculated for CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, and CYP2D6 were greater than 25 μM. Tenofovir alafenamide weakly inhibited CYP3A-mediated oxidation of midazolam or testosterone with IC_{50} values of 7.6 or 7.4 μM, respectively. The weak inhibition of CYP3A, however, is unlikely to be clinically relevant as TAF did not affect the exposure to CYP3A substrates, midazolam or rilpivirine (Genvoya® NDA207561/GS-US-120-1538 and GS-US-120-1554). Tenofovir at 100 μM did not inhibit CYP1A2, CYP2C9, CYP2D6, CYP2E1, and CYP3A.

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. There was no evidence for time- or cofactor-dependent inhibition of any enzyme by TAF, with the maximum change in activity of 17.4% with CYP2C8 relative to control.

Incubation of TAF with the HIV-1 PIs ATV or DRV, or the CYP inhibitors, RTV or COBI, did not markedly affect the stability of TAF in intestinal subcellular fractions. Similarly, because of the high specificity of the enzymes catalyzing the phosphorylation of the nucleoside analogs, FTC and TFV and COBI are unlikely to interact with this process, and no antagonistic effects on antiviral potency have been seen in vitro.

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).

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, TAF is unlikely to be a clinically relevant inducer as it did not affect the exposure to midazolam or rilpivirine (Genvoya® NDA207561/GS-US-120-1538 and GS-US-120-1554).

In vitro studies demonstrated that TAF did not inhibit any of the transporters tested (P-gp, BCRP, BSEP, OATP1B1, OATP1B3, MATE1, OAT1, OAT3, OCT1 and OCT2) at clinically relevant concentrations. In addition, TFV did not inhibit any of the transporters tested (P-gp, BCRP, BSEP, OATP1B1, OATP1B3, MATE1, OAT1, OAT3, OCT1, OCT2, MRP1, MRP2 and MRP4) at clinically relevant concentrations. Therefore, TAF and TFV are unlikely to be perpetrators of transporter-mediated drug interactions.

Tenofovir alafenamide is 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. The effect of CsA on TAF oral bioavailability was also assessed in vivo in dogs. 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 cells 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 found to be a substrate for hepatic uptake transporters, OATP1B1 and OATP1B3. However, OATP1B1 and OATP1B3 only make a small contribution to TAF uptake into primary human hepatocytes due to the high passive permeability of the prodrug. Unlike TFV, TAF is 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. In order to understand the role of transporters in the renal secretion of TFV and to explore potential drug interactions based on these transport systems, the interactions of TFV with a variety of both uptake and efflux transporters were studied in vitro. Results of in vitro transport studies indicate that the active tubular secretion of TFV is mediated by the human OAT1 and MRP4 acting in series as the major uptake and efflux transporters in proximal tubules, respectively, [9, 8, 41]. Human organic anion transporter type 3 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 [9]. Furthermore, the PIs ATV, LPV, and RTV did not exhibit any

effect on the active cellular elimination of TFV mediated by the MRP4 efflux pump [41]. 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 indicated that MRP1 is not involved in the reabsorption of TFV at the basolateral membrane of proximal tubule cells.

Neither FTC nor TFV interact with drug metabolizing enzymes as substrates, inhibitors, or inducers (oxidative metabolism of FTC plays only a minor role in the elimination of the compound) and so will not take part in metabolic drug interactions with COBI or RTV. Cobicistat and RTV are shown to be weak inhibitors of efflux transporters, P-gp and BCRP. Since TAF, but not FTC, is a substrate for both P-gp and BCRP, high concentrations of COBI or RTV achieved briefly in the intestinal lumen can inhibit the intestinal efflux of TAF, thereby increasing its absorption. Therefore, a lower TAF dose for the FDC (F/TAF, 200/10 mg) is recommended when used with boosted antiretrovirals. Cobicistat and RTV showed either weak or undetectable inhibition of OAT1, OAT3, and MRP4 in vitro. Consistently, transport of TFV by OAT1, OAT, and MRP4 was not meaningfully inhibited by COBI or RTV under physiological conditions and clinically relevant concentrations. In addition, COBI and RTV had no effect on the accumulation of TFV in human renal tissue slices at clinically relevant concentrations. Since both COBI and RTV are inhibitors and TAF is a substrate of OATP transporters in vitro, the exposure to TAF may be affected by COBI or RTV via inhibition of hepatic uptake. The effects of differences in OATP1B1 and OATP1B3 activity are, however, not expected to be clinically relevant given the high passive permeability of TAF. Because of the highly restricted substrate specificity of the enzymes catalyzing the phosphorylation of FTC and TFV, inhibition of pharmacological activation by COBI or RTV is unlikely.

3.4. Excretion

3.4.1. Darunavir

In all examined species, the predominant route of excretion for ^{14}C-DRV was via the feces (94% in rats, 86% in dogs and 82% in humans). Urinary excretion was about 4% of the administered dose in rats and dogs but was higher (12.2%) in humans. Unchanged DRV was mainly excreted in feces and amounted to up to 12.3% in rats, 26% in dogs and 6.8% in humans. In plasma, unchanged compound accounted for the largest fraction of the radioactivity in the 3 species.

In rats, DRV was excreted in milk with milk to plasma AUC ratios up to 2.3 in dams. The systemic exposure in pups exposed to DRV via milk was very limited when compared to levels observed in dams or in pups after direct dosing.

3.4.2. Cobicistat

After oral administration of ^{14}C-COBI to mice, rats, and dogs, recovery of radioactivity was high (≥86.1% in all groups) with the majority being found in feces (≤2.06% in urine). Recovery was largely complete by 48 hours postdose. After oral administration of ^{14}C-COBI to bile duct

cannulated animals, an average of 69.3% and 63.9% of dosed radioactivity was recovered in bile in rats and dogs, respectively.

Cobicistat was present in rats in milk samples 2 hours post dose on lactation day (LD) 10 with milk to plasma ratios ranging from 1.3 to 1.9.

3.4.3. Emtricitabine

The primary route of elimination of ^{3}H-FTC and ^{14}C-FTC was via renal excretion (66.8 % in mice, 74% in rats and 41% in monkeys) after oral administration in mice, rats, and cynomolgus monkeys [17]. 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.

Excretion into milk has not been evaluated for FTC.

3.4.4. Tenofovir Alafenamide

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. In mice, at of 100 mg/kg ^{14}C-TAF, an average of 41.3 and 27.7% of the administered radioactivity were excreted in feces and urine, respectively, by 168 hours postdose. Overall, recovery of radioactivity after oral dosing to CD-1 mice was 83.2%. In rats, at 5 mg/kg of ^{14}C-TAF the mean values of 71.9 and 22.2% of the administered radioactivity were excreted in feces and urine, respectively, by 168 hours postdose. Overall recovery of radioactivity was 96.7%. In BDC rats, the mean values 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%. In dogs, at 15 mg/kg, the mean values of 37.4% and 35.9% of the administered radioactivity were excreted in feces and urine, respectively, by 168 hours postdose. Overall mean recovery of radioactivity was 80.4%. 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. Based on excretion in urine and bile, the fraction absorbed was at least 41% in dogs. 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 by a combination of glomerular filtration and tubular secretion. Following IV 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.

Tenofovir was excreted into the breast milk of lactating rats and rhesus monkeys. The TFV milk to plasma ratios ranged from 0.11 to 0.24 in rats and 0.19 to 0.22 in rhesus monkeys.

3.5. Other pharmacokinetics

The impact of RTV, a structural analogue of COBI and a potent mechanism based inhibitor (MBI) of CYP3A, on the PK of DRV was evaluated in a series of experiments in mice, rats, rabbits, minipigs and dogs. Ritonavir had a modest effect on DRV exposure in mice (2-fold increase) and (pregnant) rats (4-fold increase) but had no clear effect in minipigs and dogs. In juvenile rats (23 to 50 days of age) DRV exposure (AUC) increased by approximately 2-fold when given in combination with RTV. The highest impact, however, was in rabbits where a 15-fold increase in exposure was seen. In pigmented and pregnant rats, the blood/tissue ratios were similar after oral administration of DRV alone or in combination with RTV. In line with its CYP3A effect, RTV had inhibitory effects on different metabolic pathways in rats such as aromatic hydroxylation, oxidative ring opening and carbamate hydrolysis. No difference in excretion of radioactivity was seen after single oral administration of ^{14}C-DRV alone or in combination with RTV. Overall, RTV had limited or no impact on exposure, distribution, metabolism and elimination of DRV in mice, rats, dogs and minipigs. The highest impact, however, was in rabbits where a 15-fold increase in exposure was seen. In humans, however, the impact was noticeable. Ritonavir markedly reduced the metabolism of DRV and consequently increased its oral bioavailability from 37% to 82%. The exposure to DRV in humans was equivalent when co-administered with COBI as a booster compared to RTV as a booster.

4. TOXICOLOGY

4.1. Single Dose Toxicity Studies

4.1.1. Darunavir

DRV was generally well tolerated after single oral doses up to 100 mg/kg in mice, 2000 mg/kg in rats and 80 mg/kg in dogs.

4.1.2. Cobicistat

The maximum tolerated dose (MTD) was 100 mg/kg in mice (moribund euthanasia occurred at 300 mg/kg), and the NOAEL was 500 mg/kg in rats.

4.1.3. Emtricitabine

Emtricitabine has demonstrated minimal acute toxicity in rodents (oral 50% lethal dose [LD_{50}] >4000 mg/kg and IV LD_{50} >200 mg/kg).

4.1.4. Tenofovir Alafenamide

The NOAEL for a single oral dose TAF as GS-7340-02 in the rat was determined to be >1000 mg/kg. The no observed effect level (NOEL) in dogs administered a single dose of TAF was 30 mg/kg (treatment-related clinical signs, renal lesions in the kidneys at 90 and 270 mg/kg.