SECTION 2.6 NONCLINICAL SUMMARY

Section 2.6.1 Introduction

EMTRICITABINE/RILPIVIRINE/ TENOFOVIR DISOPROXIL FUMARATE FIXED-DOSE COMBINATION

Gilead Sciences International Limited

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GLOSSARY OF ABBREVIATIONS AND DEFINITION OF TERMS

ARV	antiretroviral
bisPOC	bis-isopropyloxycarbonylmethyl; disoproxil
DAPY	diarylpyrimidine
dATP	deoxyadenosine triphosphate
DLV	delaviridine, Rescriptor®
EC_{50}	median effective concentration
EFV	efavirenz, Sustiva®, Stocrin®
EFV/FTC/TDF	efavirenz/emtricitabine/tenofovir DF, Atripla®
EMA	European Medicines Evaluation Agency
ETR	etravirine, Intelence®
FDC	fixed-dose combination
FTC	emtricitabine
FTC/TDF	emtricitabine, Truvada®
HIV-1	human immunodeficiency virus type 1
MAA	marketing authorization application
monoPOC PMPA	tenofovir soproxil
NNRTI	nonnucleoside reverse transcriptase inhibitor
NRTI	nucleoside reverse transcriptase inhibitor
NtRTI	nucleotide reverse transcriptase inhibitor
NVP	nevirapine, Viramune®
PBMC	peripheral blood mononuclear cell
PMPApp	tenofovir diphosphate
RPV, TMC278	rilpivirine (27.5 mg rilpivirine hydrochloride is equivalent to 25 mg RPV)
TDF	tenofovir DF, Viread®
TFV	tenofovir, PMPA
TMC	Tibotec medicinal compound
US	United States
WT	wild type

2.6. NONCLINICAL SUMMARY

2.6.1. INTRODUCTION

This dossier is being submitted in support of a marketing authorization application (MAA) for a fixed-dose combination (FDC) film-coated tablet that contains the active substances emtricitabine (FTC), rilpivirine (RPV, which is also referred to as TMC278 throughout this document), and tenofovir disoproxil fumarate (tenofovir DF, TDF). Emtricitabine and TDF are antiretroviral agents developed by Gilead Sciences that have been approved for the treatment of human immunodeficiency virus type 1 (HIV-1) infection as stand-alone agents Emtriva® (Commission Decision granted on [redacted]) and Viread® (Commission Decision granted on [redacted]), and in a FDC product Truvada® (emtricitabine/tenofovir DF [FTC/TDF]; Commission Decision granted on [redacted]). Emtricitabine, a nucleoside reverse transcriptase inhibitor (NRTI), and TDF, a nucleotide reverse transcriptase inhibitor (NtRTI), are listed as preferred agents in United States (US) and international treatment guidelines {15207}, {12716}, {14065}. Emtricitabine and TDF are also approved for the treatment of HIV-1 infection in combination with efavirenz (EFV), a nonnucleoside reverse transcriptase inhibitor (NNRTI). This FDC product is Atripla® (efavirenz/emtricitabine/tenofovir DF [EFV/FTC/TDF]; Commission Decision granted on [redacted]). These products are currently approved in the US, the European Community, and other countries worldwide for use in adults. In some regions, Emtriva and Viread are approved for use in adolescents; Emtriva, which is also available as an oral solution formulation, may be administered to children as young as 4 months of age. Rilpivirine, an NNRTI, is an investigational agent that is being submitted for approval by Tibotec BVBA.

Gilead Sciences has coformulated FTC and TDF, the standard of care NRTI backbone, with RPV into a FDC tablet. This FDC tablet is referred to as emtricitabine/rilpivirine/tenofovir disoproxil fumarate (FTC/RPV/TDF; dose strength 200/25/300 mg, respectively) throughout this document. Each FTC/RPV/TDF FDC tablet contains FTC, RPV, and TDF at the same dosages as recommended for the individual components, i.e., 200 mg of FTC, 25 mg RPV (27.5 mg rilpivirine hydrochloride is equivalent to 25 mg RPV), and 300 mg of tenofovir disoproxil fumarate (equivalent to 245 mg tenofovir disoproxil or 136 mg of tenofovir [TFV]). The FTC/RPV/TDF FDC tablet has demonstrated bioequivalence to each of the individual dosage forms (FTC, TDF, and RPV). It is proposed that the FTC/RPV/TDF FDC tablet be indicated for the treatment of HIV-1 infection in adults and taken orally once daily with a meal.

In accordance with the advice received from the European Medicines Evaluation Agency (EMA) at the EMA Strategy meeting held on [redacted] (see meeting minutes provided in Module 1.2, Annex 5.14), the MAAs for RPV as a single agent and for the FTC/RPV/TDF FDC tablet are being submitted in parallel by Tibotec BVBA and Gilead Sciences, respectively.

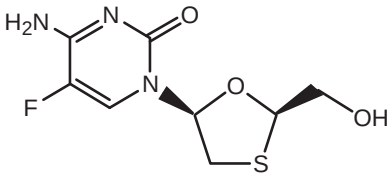
Emtricitabine, RPV, and TDF have been assessed individually in comprehensive nonclinical studies. As the RPV component is a new chemical entity, this FDC MAA dossier contains full data on this new component while providing the key data on the Truvada (i.e. FTC, TDF, and FTC/TDF) components. All Truvada studies considered to support the FDC are included to ensure that this is a 'stand-alone dossier.' This is in agreement with the feedback received at the presubmission meeting with the EMA on [redacted] 20[redacted] and with the meeting with the Rapporteur/Co-Rapporteur on [redacted] 20[redacted] (see Module 1.2.5.14, final minutes). To assist the reviewer, a listing of all the FTC, TDF, FTC/TDF, and EFV/FTC/TDF nonclinical reports is provided in Module 2.4, Section 2.4.7.

Information from all nonclinical studies with FTC, RPV, and TDF should be considered in the context of the substantial clinical experience with FTC and TDF within antiretroviral combination therapy for the treatment of HIV-1 infection, the Phase 2 and Phase 3 clinical experience with RPV, and with RPV administered in combination with Truvada (FTC/TDF).

2.6.1.1. Emtricitabine

Emtricitabine is a NRTI. It is the active ingredient in Emtriva® 200 mg capsules and 10mg/mL oral solution that have been approved in the US, the European Community, and other countries worldwide in combination with other antiretroviral agents for the treatment of HIV-1 infection. The international birthdate for FTC is [redacted] 20[redacted].

Emtricitabine is a synthetic analogue of the naturally occurring 2′-deoxycytidine, a pyrimidine nucleoside. The chemical name of FTC is 5-fluoro-1-[(2R,5S-2-hydroxymethyl)-1,3-oxathiolan-5-yl]cytosine. Emtricitabine is the (-) enantiomer of the compound and has also been referred to as (-)-2',3'-dideoxy-5-fluoro-3'-thiacytidine. It has a molecular formula of $C_8H_{10}FN_3O_3S$ and a molecular weight of 247.24. The chemical structure of FTC is as follows:



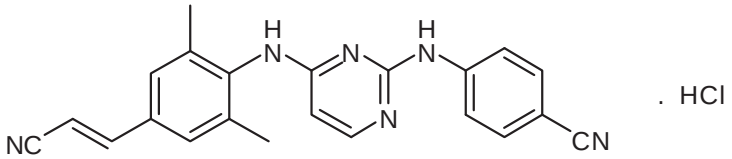
Following absorption, FTC is phosphorylated by cellular enzymes to emtricitabine 5'-triphosphate (FTC-TP), the active metabolite. Emtricitabine 5'-triphosphate inhibits the activity of HIV-1 reverse transcriptase through high affinity binding, competing with the natural substrate deoxycytidine 5'-triphosphate. Emtricitabine 5'-triphosphate is efficiently incorporated into the nascent (viral) DNA chain by HIV-1 RT resulting in termination of DNA synthesis due to the lack of a hydroxyl group in the 3'- position of the sugar moiety of FTC, which in turn inhibits viral replication. In a clinical study, the intracellular half-life of FTC-TP in peripheral blood mononuclear cells was 39 hours. Intracellular triphosphate levels increased with dose, but reached a plateau at doses of 200 mg or greater. Emtricitabine has activity against retroviruses and hepadnaviruses.

2.6.1.2. Rilpivirine

Tibotec Medicinal Compound 278 (TMC278, rilpivirine, RPV), a substituted diarylpyrimidine (DAPY) derivative, is a potent NNRTI with in vitro activity against wild type (WT) HIV-1 and HIV-1 NNRTI-resistant mutants, which has been developed for long term treatment of HIV-infected treatment-naive adults in combination with commercialized antiretroviral (ARV) agents. TMC278 has not yet been approved for use in any country.

TMC278 has a median effective concentration (EC_{50}) in vitro for HIV-1/IIIb subtypes ranging from 0.13 to 0.73 nM (0.05 to 0.27 ng/mL) and for HIV-1 Group M ranging from 0.07 to 1.01 nM (0.03 to 0.37 ng/mL). Based on in vitro experiments, TMC278 has a higher genetic barrier to the development of HIV-1 resistance than the currently approved NNRTIs in the US and/or Europe for use in HIV-infected, treatment-naive patients, including nevirapine (NVP, Viramune®), delaviridine (DLV, Rescriptor®), and efavirenz (EFV, Sustiva®, Stocrin®). Currently, one novel NNRTI etravirine (ETR, Intelence®), developed by Tibotec, is approved for use in HIV-1 infected, treatment-experienced patients with evidence of NNRTI resistance (Commission Decision granted [redacted] 20[redacted]).

The chemical name for TMC278 is 4-[[4-[[4-[(E)-2-cyanovinyl]-2,6-dimethylphenyl]amino]-2-pyrimidinyl]amino]benzonitrile hydrochloride. The molecular formula is $C_{22}H_{18}N_6$.HCl and its molecular weight is 402.88 Dalton. TMC278 has the following structural formula:

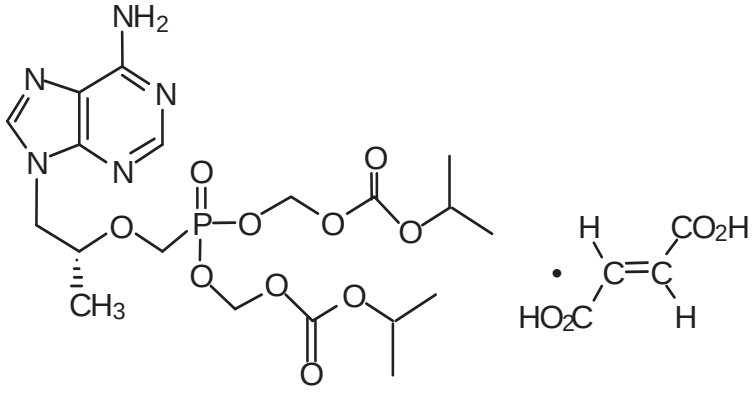


2.6.1.3. Tenofovir Disoproxil Fumarate

Tenofovir disoproxil fumarate (tenofovir DF; TDF), the oral prodrug of tenofovir (TFV; PMPA), is a NtRTI. It is the active ingredient in Viread® that has been approved in the US, the European Community, and other countries worldwide as a once a day tablet (300 mg, equivalent to 245 mg tenofovir disoproxil), in combination with other antiretroviral agents, for the treatment of HIV-1 infection. Viread® is also approved for the treatment of chronic hepatitis B in adults in the US, the European Community, and other markets worldwide, including Australia, New Zealand, and Canada. The international birthdate for tenofovir DF is 31 October 2001.

Tenofovir DF is an alkoxycarbonyloxymethyl ester prodrug of TFV with high water solubility (13.4 mg/mL) and a molecular weight of 635.52. Tenofovir is not well absorbed from the intestine because of the presence of the negative charges associated with the phosphonate groups. Therefore the prodrug, TDF was developed to mask the charge and improve oral bioavailability. As the drug is absorbed through the intestinal wall, the disoproxil (bisPOC) prodrug moiety is rapidly cleaved by esterases so that free TFV along

with a minor component of tenofovir soproxil (monoPOC PMPA), is detected in the circulation. The chemical structure of TDF is shown below.



Following absorption, TDF is rapidly converted to TFV, which is metabolized intracellularly to the active metabolite, tenofovir diphosphate (PMPApp). Tenofovir is converted to PMPApp by constitutively expressed cellular enzymes through 2 phosphorylation reactions. This conversion occurs in both resting and activated T cells. The active intracellular anabolite, PMPApp, exerts its antiviral effect by terminating the extension of the developing viral DNA chain. The viral DNA chain termination occurs because PMPApp can be incorporated in place of the natural substrate, deoxyadenosine triphosphate (dATP), and the DNA chain cannot be extended significantly beyond the point of its incorporation. In nonproliferating human peripheral blood mononuclear cells (PBMCs), the half-life of PMPApp was found to be approximately 50 hours, whereas the half-life in phytohaemagglutinin-stimulated PBMCs was found to be approximately 10 hours. Tenofovir has activity against retroviruses and hepadnaviruses.

2.6.1.4. Fixed Combination of Emtricitabine and Tenofovir Disoproxil Fumarate

Truvada, the fixed dose, FTC/TDF combination film-coated tablet contains FTC and TDF at the same dosages as recommended for the individual components, i.e., 200 mg FTC and 300 mg of TDF (equivalent to 245 mg tenofovir disoproxil). Truvada® is approved in the US, the European Community, and other countries worldwide and is indicated in combination with other antiretroviral medicinal products for the treatment of HIV-1 infected adults over 18 years of age, providing a dual NRTI backbone in a once-a-day single tablet for use in standard of care antiretroviral therapy regimens. The international birthdate for FTC/TDF is 02 August 2004.

2.6.1.5. Fixed Combination of Emtricitabine, Rilpivirine, and Tenofovir Disoproxil Fumarate

The proposed FDC is based on the complimentary pharmacological mechanisms of action of FTC, RPV, and TDF and the body of clinical experience with NRTI and NNRTI inhibitors in HIV-infected patients. As expected, combinations of these agents are not antagonistic and are synergistic in cell-based in vitro studies. The mechanisms of elimination of these compounds

differ from one another and no pharmacokinetic interactions have been observed. The FTC/RPV/TDF FDC tablet has demonstrated bioequivalence to each of the individual dosage forms (FTC, TDF, and RPV).

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SECTION 2.6
NONCLINICAL SUMMARY

Section 2.6.2 — Pharmacology Written Summary

EMTRICITABINE/RILPIVIRINE/
TENOFOVIR DISOPROXIL FUMARATE
FIXED-DOSE COMBINATION

Gilead Sciences International Limited

17AUG2010

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GLOSSARY OF ABBREVIATIONS AND DEFINITION OF TERMS

3TC	lamivudine
AAG	alpha-1 acidic glycoprotein
ABC	abacavir
APD90	action potential duration at 90% repolarization
AK	adenylate kinase
APV	amprenavir
ATPase	adenosine triphosphatase
ATV	atazanavir
BCO	biological cut-off
BID	twice daily
CBV-TP	carbovir triphosphate
CC_{50}	drug concentration that results in a 50% reduction in cell viability
(c)DNA	(complementary) deoxyribonucleic acid
CHO	Chinese hamster ovary
CNS	central nervous system
d4T	stavudine
dATP	deoxyadenosine triphosphate
ddC	zalcitabine
ddI	didanosine
DLV	delavirdine
DMSO	dimethylsulfoxide
DNA	deoxyribonucleic acid
dNTP	2'-deoxynucleoside triphosphates
DRV	darunavir
EAD	early afterdepolarization
EC_{50}	half maximal effective concentration
ECG	electrocardiogram
ENF	enfuvirtide
EFV	efavirenz
EGFP	enhanced green fluorescent protein
ERP	effective refractory period (frequency of external stimulations that does not elicit continuous contractions)
FBS	fetal bovine serum
FC	force of contraction
FDC	fixed-dose combination
FIV	feline immunodeficiency virus
FTC, 524W91	emtricitabine
FTC-TP	emtricitabine 5'-triphosphate
GI	gastrointestinal
HBV	hepatitis B virus

GLOSSARY OF ABBREVIATIONS AND DEFINITION OF TERMS (CONTINUED)

HCl	hydrochloride
HEK	human embryonic kidney
hERG	human ether-à-go-go related gene
HIV-1	human immunodeficiency virus type 1
HPMC	hydroxypropylmethylcellulose
IC_{50}	median inhibitory concentration
$I_{Ca,L}$	high threshold L-type calcium current
I_{K1}	inward rectifying potassium current
I_{Kr}	rapidly activating rectifying potassium current
I_{Ks}	slowly activating rectifying potassium current
I_{Na}	fast sodium current
I_{to}	transient outward potassium current
LV dp/dt max/pd	maximum positive rate of change of isovolumic left ventricular pressure divided by the developed pressure at maximum positive rate of change (contractility index)
LV dp/dt max	maximum positive rate of change of isovolumic left ventricular pressure
LV dp/dt min	maximum negative rate of change of isovolumic left ventricular pressure
LVEDP	left ventricular pressure measured at the end of diastole
MAA	marketing authorization application
MOI	multiplicity of infection
MVC	maraviroc
NDP	Nucleoside diphosphate kinase
NFV	nelfinavir
NNRTI	nonnucleoside reverse transcriptase inhibitor
NRTI	nucleoside/nucleotide reverse transcriptase inhibitor
NVP	nevirapine
PBMC	peripheral blood mononuclear cell
PCR	polymerase chain reaction
PEG400	polyethylene glycol with molar mass between 380 and 420
PMPAp	tenofovir monophosphate
PMPApp	tenofovir diphosphate
PNP	purine nucleoside phosphorylase
PQ	interval between the start of the P wave and the start of the Q wave on ECG
QRS	part of the ECG complex comprising the Q, R, and S-waves
QT	interval between the start of the Q wave and the end of the T wave on ECG
QTc	QT interval corrected for heart rate
RAL	raltegravir
RBV	ribavirin
RC	rate of contraction
RR	interval between the peak of R waves of 2 consecutive ECG complexes
rNTP	ribonucleoside triphosphates

GLOSSARY OF ABBREVIATIONS AND DEFINITION OF TERMS (CONTINUED)

RPV, TMC278	rilpivirine (27.5 mg rilpivirine hydrochloride is equivalent to 25 mg rilpivirine)
RT	reverse transcriptase
RT-PCR	reverse transcriptase-polymerase chain reaction
rTp-Te interval	Tp-Te/QT ratio; reflects the potential for phase 2 afterdepolarizations.
RTV	ritonavir
SCID	severe combined immunodeficiency
SHIV	HIV-1 RT-containing SIV chimera
SIV	simian immunodeficiency virus
SQV	saquinavir
TC_{50}	drug concentration that results in a 50% reduction in cell growth
TDF	tenofovir DF
TdP	torsade de pointes
TDR	transmural dispersion of repolarization
TFV	tenofovir
Tp-Te interval	time between peak and end of the T wave on ECG
TPV	tipranavir
TQT study	thorough clinical QTc-prolongation study according to ICH E14
Tween 80	polyoxyethylenesorbitan monooleate 80
US	United States
VF	ventricular fibrillation
VT	ventricular tachycardia
WHV	woodchuck hepatitis virus
ZDV	zidovudine

2.6. NONCLINICAL SUMMARY

2.6.2. PHARMACOLOGY WRITTEN SUMMARY

2.6.2.1. Brief Summary

This dossier is being submitted in support of a marketing authorization application (MAA) for a fixed-dose combination (FDC) film-coated tablet that contains the active substances emtricitabine (FTC), rilpivirine (RPV, which is also referred to as TMC278 throughout this document), and tenofovir disoproxil fumarate (tenofovir DF, TDF). Emtricitabine and TDF are antiretroviral agents developed by Gilead Sciences that have been approved for the treatment of human immunodeficiency virus type 1 (HIV-1) infection as stand-alone agents Emtriva® (Commission Decision granted on 24 October 2003 [EU/1/03/261/001-003]) and Viread® (Commission Decision granted on 05 February 2002 [EU/1/01/200/001-2]), and in a FDC product Truvada® (emtricitabine/tenofovir DF [FTC/TDF]; Commission Decision granted on 21 February 2005 [EU/1/04/305/001]). Emtricitabine, a nucleoside reverse transcriptase inhibitor (NRTI), and TDF, a nucleotide reverse transcriptase inhibitor (NtRTI), are listed as preferred agents in United States (US) and international treatment guidelines {15207}, {12716}, {14056}. Emtricitabine and TDF are also approved for the treatment of HIV-1 infection in combination with efavirenz (EFV), a nonnucleoside reverse transcriptase inhibitor (NNRTI). This FDC product is Atripla® (efavirenz/emtricitabine/tenofovir DF [EFV/FTC/TDF]; Commission Decision granted on 13 December 2007 [EU/1/07/430/001-2]). These products are currently approved in the US, the European Community, and other countries worldwide for use in adults. In some regions, Emtriva and Viread are approved for use in adolescents; Emtriva is also available as an oral solution and may be administered to children as young as 4 months of age. Rilpivirine, an NNRTI, is an investigational agent that is being submitted for approval by Tibotec BVBA.

Gilead Sciences has coformulated FTC and TDF, the standard of care NRTI backbone, with RPV into a FDC tablet. This FDC tablet is referred to as emtricitabine/rilpivirine/tenofovir disoproxil fumarate (FTC/RPV/TDF; dose strength 200/25/300 mg, respectively) throughout this document. Each FTC/RPV/TDF FDC tablet contains FTC, RPV, and TDF at the same dosages as recommended for the individual components, i.e., 200 mg of FTC, 25 mg RPV (27.5 mg rilpivirine hydrochloride is equivalent to 25 mg RPV), and 300 mg of tenofovir disoproxil fumarate (equivalent to 245 mg tenofovir disoproxil or 136 mg of tenofovir [TFV]). The FTC/RPV/TDF FDC tablet has demonstrated bioequivalence to each of the individual dosage forms (FTC, TDF, and RPV). It is proposed that the FTC/RPV/TDF FDC tablet be indicated for the treatment of HIV-1 infection in adults and taken orally once daily with a meal.

In accordance with the advice received from the European Medicines Evaluation Agency (EMA) at the EMA Strategy meeting held on [redacted] 20[redacted] (see meeting minutes provided in Module 1.2, Annex 5.14), the MAAs for RPV as a single agent and for the FTC/RPV/TDF FDC tablet are being submitted in parallel by Tibotec BVBA and Gilead Sciences, respectively.

Emtricitabine, RPV, and TDF have been assessed individually in comprehensive nonclinical studies. As the RPV component is a new chemical entity, this FDC MAA dossier contains full data on this new component while providing the key data on the Truvada (i.e. FTC, TDF, and FTC/TDF) components. All Truvada studies considered to support the FDC are included to ensure that this is a 'stand-alone dossier.' This is in agreement with the feedback received at the presubmission meeting with the EMA on [redacted] 20[redacted] and with the meeting with the Rapporteur/Co-Rapporteur on [redacted] 20[redacted] (see Module 1.2.5.14, final minutes). To assist the reviewer, a listing of all the FTC, TDF, FTC/TDF, and EFV/FTC/TDF nonclinical reports is provided in Module 2.4, Section 2.4.7.

Comprehensive programs of nonclinical studies with FTC, RPV, and TDF have been conducted. Within this Module 2.6.2 and in the pharmacology Tabulated Summary 2.6.3, rilpivirine (RPV) is referred to as TMC278 in the text and tables describing the nonclinical studies of rilpivirine alone.

Information from all nonclinical studies with FTC, RPV, and TDF should be considered in the context of the substantial clinical experience with FTC and TDF within antiretroviral combination therapy for the treatment of HIV-1 infection, the Phase 2 and Phase 3 clinical experience with RPV, and with RPV administered in combination with Truvada (FTC/TDF).

Of note, the following conversions are provided to aid the reviewer; for
FTC 1 μM = 0.247 μg/mL, RPV 1μM = 0.366 μg/mL, TDF 1 μM = 0.636 μg/mL, and
TFV 1 μM = 0.287 μg/mL.

Emtricitabine

Intracellularly, FTC is sequentially phosphorylated to FTC 5'-monophosphate and 5'-diphosphate, and finally to FTC 5'-triphosphate (FTC-TP), the intracellular form active as an antiviral substance. Emtricitabine 5'-triphosphate inhibits viral polymerases by direct binding competition with the natural deoxyribonucleotide substrate (deoxycytidine triphosphate) and by deoxyribonucleic acid (DNA) chain termination after incorporation into DNA. Emtricitabine triphosphate is a very weak inhibitor of mammalian DNA polymerases α, β, ε, and mitochondrial DNA polymerase γ.

Studies in established human T-cell lines and peripheral blood mononuclear cells (PMBCs) have demonstrated that FTC was active against laboratory and clinical isolates of HIV-1, including virus with reduced sensitivity to other NRTIs. The concentration of FTC required to inhibit 50% of the viral replication (half maximal effective concentration [EC_{50}] values) of laboratory adapted strains of HIV-1 and HIV-2 ranged from 0.0013 to 0.5 μM and 0.08 to 1.5 μM, respectively, depending on cell type and virus strain used in the assay. With clinical isolates of HIV-1, EC_{50} values ranged from 0.002 to 0.028 μM. EC_{50} values were comparable for all subtypes of HIV-1 tested (subtypes A, B, C, D, E, F, and O).

The emergence of viruses resistant to FTC has been examined in vitro by serial passage of the virus in human PBMCs in the presence of increasing FTC concentrations. Resistance to FTC developed more slowly than resistance to 3TC under similar conditions. Resistance

developed as the result of base changes at codon 184 causing the methionine to be changed to a valine (M184V). An isoleucine intermediate has also been observed. The mutant virus was highly resistant to FTC and 3TC (100-fold increase in EC_{50} values) and slightly less sensitive to inhibition by didanosine (ddI) and zalcitabine (ddC; 3-fold increase in EC_{50} values). Conversely, viruses resistant to zidovudine (ZDV), ddC, ddI, and NNRTIs, including to RPV, retained their sensitivity to FTC (EC_{50} values = 0.002 μM to 0.38 μM).

The in vivo antiviral activity of FTC has been described in the published literature. Severe combined immunodeficient (SCID) mice were reconstituted with human PBMCs and subsequently infected with HIV-$1_{A018.}$ Emtricitabine (30 mg/kg twice daily [BID] given intraperitoneally) completely inhibited viral infection. In a separate study, using the same mouse model infected with HIV-$1_{A018,}$ both FTC and lamivudine (3TC) at oral dose levels of about 60 mg/kg/day for 7 days reduced viral loads to a similar extent.

The in vitro anti-hepatitis B virus (HBV) activity of FTC was determined in HepG2 2.2.15 cells with EC_{50} values ranging from 0.01 to 0.04 μM. Hepatitis B virus resistance to FTC has been observed associated with mutations in the YMDD motif of the HBV polymerase (M204V). The in vivo efficacy of FTC was demonstrated in the woodchuck hepatitis virus (WHV) chronically infected woodchuck model and in a chimeric mouse model of HBV infection.

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

Emtricitabine showed a low potential for mitochondrial toxicity. Emtricitabine did not reduce mitochondrial DNA in Molt-4 cells or affect cell growth, lactic acid concentration, or mitochondrial DNA content in human HepG2 cells and MT-2 cells. Emtricitabine had no effect on mitochondrial ultrastructure.

Emtricitabine showed additive to synergistic anti-HIV activity in vitro in combination with NRTIs (abacavir [ABC], 3TC, stavudine [d4T], tenofovir [TFV or PMPA], ddC, and ZDV), NNRTIs (delavirdine [DLV], EFV, nevirapine [NVP], and RPV), protease inhibitors (amprenavir [APV], nelfinavir [NFV], ritonavir [RTV], and saquinavir [SQV]), and the integrase inhibitor elvitegravir.

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 have been reported in anaesthetized dogs given a cumulative dose of 38.5 mg/kg of FTC intravenously over a 1-hour period. In addition, there were no abnormalities reported on the electrocardiogram (ECG) data obtained from the repeated-dose toxicity studies in monkeys, where AUC exposures were up to 26-fold higher than in humans given the 200 mg dose.

Rilpivirine

The primary pharmacodynamics of rilpivirine (RPV, TMC278) are presented in Modules 2.6.2, 2.6.3 and 2.7.2.

The following convention is applied throughout this module: reference is made to "TMC278" when the hydrochloride (HCl) salt was administered and to "TMC278 base" when the base was administered. The dose or concentration is always given as base equivalent. The analyte in bioanalytical determinations is referred to as "TMC278." TMC278 base has been applied in the early phases of development and TMC278 in the later phases, after selection of the final chemical form.

TMC278 is an NNRTI that shows subnanomolar EC_{50} values against wild-type HIV-1 group M isolates A, B, C, D, E, F, and G (0.07 to 1.01 nM), HIV-1_{IIIB} (0.73 nM), and nanomolar EC_{50} values against HIV-1 group O isolates (2.88 to 8.45 nM). The TMC278 EC_{50} values observed in human monocyte-derived-macrophages infected with HIV-1_{Ba-L} or HIV-1_{ADA} were comparable to those observed for HIV-1 group M isolates. TMC278 had antiviral activity in the micromolar range against HIV-2 and simian immunodeficiency virus (SIV). TMC278 is bound more than 99% to plasma proteins of the species involved in nonclinical safety studies and 99.7% to human plasma proteins. TMC278 did not show inhibition of human DNA polymerase α, β, or γ at concentrations up to 1000 μM.

In vitro selection experiments performed at high multiplicity of infection (MOI) showed that TMC278 was capable of inhibiting viral replication at concentrations where first-generation NNRTIs fail to do so. The rate of in vitro selection of TMC278-resistant strains at low MOI was comparable among HIV-1 group M subtypes. The NNRTI mutations emerging in HIV-1 under selective pressure of TMC278 included combinations of V90I, L100I, K101E, V106A/I, V108I, E138G/K/Q/R, V179F/I, Y181C/I, V189I, G190E, H221Y, F227C, and M230I/L, where E138R represented a newly identified NNRTI mutation.

Analysis of sensitivity to TMC278 in a panel of 4,786 HIV-1 recombinant clinical isolates resistant to at least one of the first-generation NNRTIs (EFV or NVP) showed that the majority of the isolates (62%) retained sensitivity to TMC278. Considerable in vitro cross-resistance was observed between TMC278 and etravirine (ETR). In a study of HIV-1_{HXB2} site-directed mutants carrying single, double, triple, and quadruple RT mutations, TMC278 retained antiviral activity against 63% (136 of 216) of the viruses. Only 3 mutants with K101P, Y181I, or Y181V were resistant to TMC278. For other NNRTI mutations, more than 1, and usually more than 2 NNRTI resistance-associated mutations were necessary to confer resistance to TMC278 in vitro. Resistance to TMC278 was mostly driven by the combination of specific NNRTI mutations rather than the total number of NNRTI mutations. K103N in isolation was not associated with resistance to TMC278. TMC278 retained activity against HIV-1 harboring exclusively single or multiple NRTI mutations, including M184V/I (FTC-selected) and K65R (TDF-selected). The addition of M184V to any of the combinations of RT mutations did not modify their sensitivity to TMC278, EFV, ETR, or NVP. The current in vitro data demonstrated that TMC278 is highly potent against a wide

range of wild type HIV-1 group M and NNRTI-resistant HIV-1 variants that typically emerged after failing EFV- or NVP-containing regimens.

The crystal structure of TMC278 bound to the HIV-1 RT complex was determined and revealed that TMC278 was bound to the NNRTI-binding pocket. Study of RT with NNRTI mutations showed that TMC278 adapted to changes in the NNRTI-binding pocket which could explain the increased genetic barrier to the development of resistance in vitro to this compound.

No activity of TMC278 was observed against several non-HIV related viruses such as human hepatitis B virus (HBV), herpes simplex virus 2, human corona virus, influenza A virus, and vaccinia virus at concentrations up to 10 μM.

The in vitro cytotoxicity experiments performed in cell lines of various origins confirmed that the TMC278 CC_{50} was > 5 μM. A selectivity index of approximately 8,000 indicated that TMC278 was a potent and specific inhibitor of HIV-1.

TMC278 did not show antagonism when studied in combination with other antiretroviral agents. TMC278 showed additive to synergistic antiviral activity in combination with the N(t)RTIs ABC, ddI, FTC, 3TC, d4T, TFV, and ZDV; the PIs APV, atazanavir (ATV), darunavir (DRV), indinavir (IDV), lopinavir (LPV), NFV, RTV, SQV, and tipranavir (TPV); the NNRTIs EFV, ETR, and NVP; the fusion inhibitor enfuvirtide (ENF); the entry inhibitor maraviroc (MVC); and the integrase inhibitor raltegravir (RAL).

TMC278 did not cause any inhibition in vitro of α- or β-adrenergic, dopaminergic, muscarinergic, serotonergic, opioid, interleukin, or chemokine receptors (at up to 10 μM). TMC278 showed no agonistic or antagonistic activity on histamine H_2 receptors in the isolated guinea pig right atrium and no inhibition of adenosine triphosphatase (ATPase) in isolated pig stomach. In vivo, TMC278 did not cause any significant inhibition of pentagastrin-induced gastric acidity in rats. Therefore, TMC278 is essentially devoid of any clinically relevant secondary pharmacodynamic effect at unbound concentrations that exceed the median maximum systemic exposure to total TMC278 in man (0.55 μM or 0.2 μg/mL) following an oral dose of 25 mg once daily.

The standard battery of cardiovascular safety studies showed a concentration-dependent inhibition of TMC278 on the rapidly activating rectifying potassium current (I_{Kr}) from 33% at 0.3 μM (0.11 μg/mL) to 80% at 3 μM (1.1 μg/mL). However, no relevant effects by TMC278 were noted on other cardiovascular or electrocardiographic parameters in vitro in the right atrium of the guinea pig; in vivo in anesthetized guinea pigs and dogs given a single intravenous dose; or in conscious instrumented or telemetered dogs given a single oral dose.

Delayed-onset (after 11 days of treatment) prolongation of the QT-interval corrected for heart rate according to Fridericia (QTcF) was reported in the clinical thorough QTc (TQT) study, TMC278-TiDP6-C131 (C131). To investigate the mechanism of action of the QTc prolongation and of the delayed onset, additional nonclinical studies were done. Moreover,

the potential of TMC278 to induce proarrhythmic effects, in particular torsade des pointes (TdP), was evaluated.

Additional studies showed inhibition of the slowly activating rectifying potassium current (I_{Ks}) from 17% at 1 μM (0.37 μg/mL) up to 73% at 10 μM (3.7 μg/mL), with an IC_{50} of 3.1 μM (1.15 μg/mL). In addition, the transient outward potassium current (I_{to}) was reduced by 14% at 0.3 μM (0.11 μg/mL) and up to 36% at 1 μM (0.37 μg/mL). However, no effects were observed on the inward rectifying potassium current (I_{K1}), the fast sodium current (I_{Na}), or the high threshold L-calcium current ($I_{Ca,L}$).

In vitro, a concentration-dependent inhibition of trafficking of the human ether à go-go (hERG) channel by TMC278 was observed from 1 μM (0.37 μg/mL), and above. However, no signs of trafficking, determined as delayed onset of QT prolongation, were noted in an in vivo model. In this model, telemetered guinea pigs orally dosed for 16 days at 10 mg/kg/day had maximum measured plasma concentrations of TMC278 ranging from 0.6 to 0.9 μg/mL. This concentration was similar to the steady state median maximum measured plasma concentration at 75 mg of TMC278 once daily, a dose that caused delayed-onset QTc-prolongation in TQT study C131.

TMC278 showed only a marginal potential to induce proarrhythmic effects in the rabbit arterially perfused left ventricular wedge model. Up to 10 μM (3.7 μg/mL), TMC278 caused maximally 9% QT-prolongation, but showed no effects on dispersion of the repolarization across the ventricular wall (TDR) or any early afterdepolarizations, resulting in a TdP score of 0.5. True arrhythmogenic drugs have a TdP score of around 5 in this model.

TMC278 had no effect on respiratory parameters in anesthetized dogs (single 1-hour intravenous infusion of 5 mg/kg) or conscious telemetered dogs (single oral dose of 20 mg/kg).

No compound-related neurological, behavioral changes, or delayed neurotoxicity were observed in a modified Irwin test in rats (single oral dose up to 400 mg/kg) or in conscious telemetered (single oral dose up to 160 mg/kg) or instrumented dogs (single oral dose of 20 mg/kg).

Overall, TMC278 had no effects on secondary pharmacodynamic parameters or on the core battery of safety pharmacology tests, apart from inhibitory effects on some cardiac potassium currents and channels and moderate QT-prolongation in the rabbit ventricular wedge. The observed inhibition of trafficking of the hERG channel may be involved in the delayed onset of the QTcF-prolongation observed in the clinical TQT study C131. However, pharmacokinetic mechanisms may also be involved. Importantly, the effects in nonclinical models allow the conclusion that TMC278 has only a marginal potential to induce proarrhythmic effects.

Tenofovir disoproxil fumarate

Tenofovir disoproxil fumarate is converted to TFV by serum esterases. Intracellularly, TFV is then converted through 2 phosphorylation reactions to its active phosphorylated anabolite,

tenofovir diphosphate (PMPApp). Tenofovir diphosphate inhibits viral polymerases by direct binding competition with the natural deoxyribonucleotide substrate (deoxyadenosine triphosphate [dATP]) and by DNA chain termination after incorporation into DNA. Tenofovir diphosphate is a very weak inhibitor of mammalian DNA polymerases α, β, δ, ε, and mitochondrial DNA polymerase γ.

Tenofovir has been shown to have broad spectrum in vitro antiviral activity against retroviruses. The concentration of TFV required for 50% inhibition of wild-type HIV-1_{IIIB} is 1 to 6 μM in MT-2 or MT-4 cells (based on inhibition of viral cytopathic effect) and 0.2 to 0.6 μM in PBMCs (based on inhibition of virus production). The mean EC_{50} values of TFV against HIV-1 subtypes A, C, D, E, F, G, and O in PBMCs were within 2-fold of subtype B (0.55 to 2.2 μM). Tenofovir was demonstrated to have an EC_{50} of 0.04 μM against HIV-1_{BaL} in primary monocyte/macrophage cells and to be active against HIV-2 in vitro.

Pharmacodynamic studies have indicated that TFV remains active (EC_{50} within 2-fold of wild type) against recombinant HIV-1 with reverse transcriptase (RT) mutations conferring resistance to ddI (L74V), ddC resistance (T69D), and multiple nucleosides (Q151M complex). HIV-1 expressing the FTC-associated M184V mutation shows a mild hypersusceptibility to TFV. HIV-1 with NNRTI resistance-associated mutations, including those that show resistance to RPV, remained susceptible to TFV. The TFV susceptibility of HIV-1 with mutations associated with ZDV and d4T appears to depend on the type and number of these mutations. However, most samples remain within 4-fold of wild-type. In vitro selection yielded strains of HIV-1 with the mutation K65R that show reduced susceptibility to TFV. The K65R mutation also results in reduced replication capacity compared to wild-type HIV-1. The molecular mechanism of resistance for the K65R mutant HIV-1 is a decreased incorporation of PMPApp. The K65R mutation has also been selected in vitro by ddC, ABC, and d4T; and in vivo by ddI, ddC, and ABC. In vivo, however, the K65R mutation is rarely observed to develop with these antiretroviral drugs and is observed in the plasma HIV of 2% to 4% of antiretroviral experienced patients.

In a study of SIV-infected juvenile macaques, TFV (30 or 75 mg/kg/day) administered subcutaneously once daily for 4 weeks was more efficacious than ZDV (100 mg/kg/day), as assessed by surrogate markers of SIV infection and clinical status. In the macaque model of SIV infection, TFV treatment selected for mutant SIV that contained the RT mutation K70E that was present transiently and was replaced by the K65R mutation. In a subset of macaques that were chronically infected with SIV and for which prolonged TFV treatment failed to significantly suppress viral RNA levels in plasma, the survival time of the animals was significantly improved.

Controlled studies of prevention of infection in macaques have shown that oral or subcutaneous treatment with TDF or TFV, respectively, prevented or delayed the onset of viremia when administered either before or shortly after rectal, vaginal, or intravenous inoculation with SIV or HIV. Macaques that became infected while under treatment had longer median time to infection or lower levels of viral DNA than untreated controls. Studies regarding use of TFV as intermittent preexposure prophylaxis demonstrated that intermittent preexposure prophylaxis combined with a postexposure dose was highly protective. By

topical vaginal administration, TFV was also completely protective against vaginal transmission of SIV in another simian model. Topically administered TDF gel at a very low dose was unable to protect infant macaques from SIV infection by the oral route. In a follow-up study, orally administered TDF lowered the infection rate at birth, but had lower efficacy against virus infection at 4 weeks of age (against oral challenge with multiple low doses of SIV in infant macaques).

In studies of acute and chronic feline immunodeficiency virus (FIV) infection in cats, TFV treatment (30 mg/kg/day) reduced circulating viral FIV RNA, but not PBMC-associated, coculture-detected virus burden. The antiviral activity of TFV administered by subcutaneous injection and TDF administered orally was demonstrated in murine sarcoma virus (MSV)-infected SCID mice.

Tenofovir has shown in vitro activity against human HBV with EC_{50} values of 1.1 to 2.5 μM in cells lines replicating human HBV. Tenofovir has also shown in vitro activity against duck HBV in primary duck hepatocytes.

In quiescent human PBMCs, no cytotoxic effect of TFV was detected at concentrations as high as 100 μM. Similar findings were observed in HepG2 cells, skeletal muscle cells of human origin, and human renal proximal epithelial tubule cells (RPTECS). Similarly, TFV has shown no effect on hematopoietic progenitor cells in vitro.

A variety of clinical symptoms observed in HIV patients treated with prolonged NRTI therapy may be linked to mitochondrial toxicity. These include myopathy, cardiomyopathy, polyneuropathy, lactic acidosis, pancreatitis, lipodystrophy, and possibly others. Tenofovir was studied and compared with other NRTIs for its in vitro effects on mitochondrial deoxyribonucleic acid (mtDNA) synthesis and lactic acid production. Because the in vivo effects of NRTIs appear to be most pronounced in liver and muscle tissues, the experiments were carried out in HepG2 human liver cells and in normal human skeletal muscle cells (SkMCs). No effect of TFV was seen on the synthesis of mtDNA or lactic acid production in these studies. The results of these studies indicate a low potential for TFV to interfere with mitochondrial functions.

In vitro interaction studies have demonstrated TFV to have additive to synergistic activity with the following antiretroviral drugs: ABC, ddI, FTC, 3TC, d4T, ddC, ZDV, EFV, NVP, RPV, EVG, APV, DLV, IDV, NFV, RTV, and SQV.

Tenofovir DF was evaluated in safety pharmacology studies of the central nervous system (CNS), cardiovascular system, gastrointestinal (GI) system, and renal system. There were no adverse effects detected in the CNS in rats dosed at 500 mg/kg, or of the cardiovascular system of dogs dosed at 30 mg/kg. There was reduced gastric emptying in rats dosed at 500 mg/kg, but not at 50 mg/kg. There was increased urinary electrolyte excretion and urine volume in rats dosed at 500 mg/kg but not at 50 mg/kg.

Emtricitabine/Tenofovir DF

Emtricitabine and TDF are potent and selective inhibitors of HIV-1, weak inhibitors of human DNA polymerases, and show low potential to induce mitochondrial damage. Both drugs show potent antiretroviral activity against HIV-1 in vitro and are active in vivo in several animal retrovirus models. The drugs are phosphorylated intracellularly through nonoverlapping pathways and in combination show no antagonism for the formation of their active metabolites. The combination of FTC and TFV consistently shows synergistic anti-HIV-1 activity in vitro. The combination also showed no evidence of cytotoxicity or mitochondrial toxicity. One potential mechanism behind the synergy of FTC and TFV in cell culture correlates with approximately 1.4-fold increased levels of the fully phosphorylated active anabolites of both compounds when dosed together compared to single drug dosing, as measured in CEM cells. The increased phosphorylation of FTC and TFV in combination was not reproduced in cultured PBMCs. A second potential mechanism involved in this synergy was shown in vitro at the enzymatic level where the presence of FTC-TP enhanced the formation of stable, dead-end complexes by HIV-1 reverse transcriptase and TFV-terminated DNA that potentiates the chain-termination activity of TFV.

In a study in the SIV macaque model, the combination of FTC and TFV administered subcutaneously once daily resulted in full suppression of plasma SIV in treated animals. The response was durable throughout the study period of 32 weeks, with no detectable development of resistance. Additional studies in macaques provided evidence that FTC and TFV protected animals from infection against rectal or vaginal exposure to a SIV-HIV-1 chimeric virus (SHIV).

In a 48-week study of WHV-infected woodchucks, treatment with the combination of FTC plus TDF showed a greater decline in serum viral load as compared to treatment with either drug alone.

HIV-1 resistance to FTC develops readily in vitro with a mutation in the M184 codon of HIV RT either to valine or isoleucine. The M184V/I mutation results in high-level resistance to FTC and 3TC, but increased susceptibility to TFV. HIV-1 resistance to TFV is associated with the K65R mutation in HIV RT that is slow to develop both in vitro and in vivo, and the drug maintains partial in vivo activity against K65R mutant SIV strains. The K65R mutation showed reduced susceptibility to FTC and 3TC; however, much higher fold resistance is associated with M184V/I for FTC, suggesting that this mutation is more clinically relevant for FTC.

In vitro resistance selection with the combination of TFV and FTC results first in the selection of M184V/I. After prolonged culture with increasing concentrations of TFV, resistance to TFV was observed in the form of the K65R mutation. Both the K65R and M184V mutations were observed together upon subsequent increases in the FTC concentration. These results predict that in the case of treatment failure in vivo, there would be step-wise development of resistance as is the case for most antiretroviral regimens, with the M184V/I mutation developing initially and then potentially the K65R mutation. Clinical study data using the combination of TDF with FTC support this conclusion as the M184V

mutation was observed to develop in the absence of K65R in patients with virologic failure (see Module 2.5). Overall, the pharmacodynamic assessment of TFV and FTC supports the effective use of these 2 agents together in combination therapy for HIV-1 disease.

Emtricitabine/Rilpivirine/Tenofovir DF

Emtricitabine, RPV, and TFV are potent and selective inhibitors of HIV-1. All 3 drugs show potent antiretroviral activity against diverse subtypes of HIV-1 in vitro. Emtricitabine and TFV are phosphorylated intracellularly through nonoverlapping pathways and in combination show no antagonism for the formation of their active metabolites. Rilpivirine does not require modification for activity. The dual-drug combinations of FTC, RPV, and TFV show additive to synergistic anti-HIV-1 activity in vitro. The anti-HIV activity of the triple combination of FTC, RPV, and TFV in vitro demonstrates synergy for anti-HIV activity and no evidence of cytotoxicity.

In vitro dose-escalation resistance selection with the combination of FTC, RPV, and TFV resulted in the selection of M184I. Resistance selection using fixed-dose breakthrough experiments with the combination of FTC, RPV, and TFV resulted in the selection of M184I at drug concentrations fixed at 1.7-fold the EC_{50} for each drug, and K65R at drug concentrations that were fixed at 3.3-fold the EC_{50} for each drug. At higher drug concentrations, no virus was able to replicate. No resistance to RPV was detected in either of the selection experiments with the triple combination. Clinical study data of subjects with virologic failure using the combination of FTC/TDF with RPV was associated with the emergence of NNRTI and N(t)RTI mutations, with the combination of E138K and M184I being the most common, and a loss of phenotypic susceptibility to TMC278 in approximately 50% of subjects (see Module 2.7.2).

Emtricitabine and TDF had little effect on vital organ systems in safety pharmacology studies. Rilpivirine has shown the potential for QT prolongation, an effect confirmed in the TQT study, but only a marginal potential to induce proarrhythmic effects. At the 25 mg dose of RPV, the observed change in QTcF was not considered clinically relevant, and the combination product is not anticipated to exacerbate the small cardiovascular effect seen with the RPV 25 mg dose alone.

Overall, the pharmacodynamic assessment of FTC, RPV, and TFV supports the effective use of these 3 agents together in combination therapy for HIV-1 disease.

2.6.2.2. Primary Pharmacodynamics

The primary pharmacodynamic effects of FTC are presented in Sections 2.6.2.2.1 to 2.6.2.2.5. The primary pharmacodynamic effects of RPV are presented in Sections 2.6.2.2.6 to 2.6.2.2.9 and in Module 2.7.2 (Summary of Clinical Pharmacology). Discussion of the primary pharmacodynamics of TDF is provided in Sections 2.6.2.2.10 to 2.6.2.2.14.

The primary pharmacodynamics of FTC/TDF and FTC/RPV/TDF combinations are presented in Sections 2.6.2.2.15 and 2.6.2.2.16, respectively.

2.6.2.2.1. Emtricitabine: Mechanism of Action

2.6.2.2.1.1. Emtricitabine: Cellular Uptake

Transport studies were conducted in confluent cultures of HepG2 cells to determine the route(s) of cellular uptake of FTC {4527}. Assays were performed at 20°C using a modified rapid, cold buffer stop method. The influx of FTC into the cells did not depend on the concentration of FTC or the presence of a Na^+ gradient, and was only partially inhibited by competing nucleosides and nucleoside transport inhibitors. This observation along with the negligible impact of the protein modification agents N-ethylmaleimide, 4,4'-diisothiocyanato-2, 2'-stilbenedisulfonic acid, and 4-aceamido-4'-isothiocynatostilbene-2, 2'-disulfonic acid on FTC uptake suggests that a component of FTC entry into HepG2 cells was not transporter-mediated.

2.6.2.2.1.2. Emtricitabine: Intracellular Metabolism

In vitro

Emtricitabine is efficiently phosphorylated in HepG2 cells to the corresponding 5'-monophosphate, 5'-diphosphate, and 5'-triphosphate. A time course showed that the nucleotides of FTC were formed rapidly and reached a steady-state intracellular concentration by 3 to 6 hours {4527}. The intracellular concentrations of the 5'-phosphorylated forms produced as a function of the extracellular concentration are presented in Figure 1.. The concentrations of these derivatives increase in a linear manner indicating that the anabolic pathway is not saturated over the concentration range tested. The concentration of FTC 5'-diphosphate was somewhat higher than those of the 5'-monophosphate and 5'-triphosphate derivatives (Tabulated Summary 2.6.3.1.6, {4535}) {4527}.

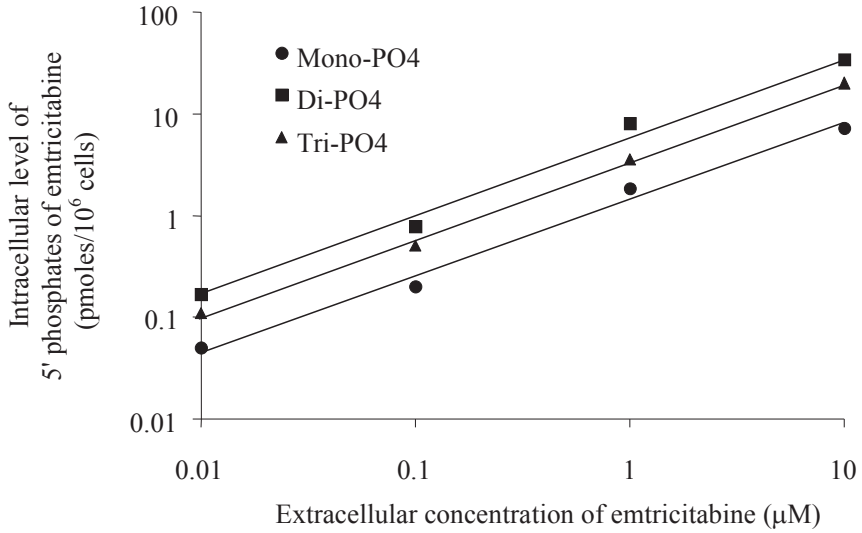
In studies to determine which enzymes were responsible for phosphorylating FTC to the 5'-triphosphate, 2'-deoxycytidine kinase was identified as the enzyme that catalyzes the phosphorylation of FTC to the corresponding 5'-monophosphate. An apparent K_m value of 11.8 μM and an apparent relative V_{max} of 9.3 $nmol \times mL^{-1} \times h^{-1}$ was determined when UTP was the phosphate donor. When ATP was the phosphate donor, an apparent K_m value of 7.4 μM and an apparent relative V_{max} of 3.8 $nmol \times mL^{-1} \times h^{-1}$ was obtained {4544}. Using calf thymus 2'-deoxycytidine kinase, the relative rate of phosphorylation was 3.4% of that measured for 2'-deoxyguanosine and 40% of that observed for 2'-deoxycytidine (Tabulated Summary 2.6.3.1.1**,** TESF/91/0014 and TESF/92/0002). Phosphorylation of the 5'-monophosphate was catalyzed by 2'-deoxycytidine monophosphate kinase (Tabulated Summary 2.6.3.1.6, {4535}) {4250}. The efficiency with which the enzyme purified from calf thymus phosphorylated the 5'-monophosphate of FTC was approximately 32% of that observed when using the natural substrate, 2'-deoxycytidine 5'-monophosphate (Tabulated Summary 2.6.3.1.1, TEIT/92/0005). Emtricitabine 5'-monophosphate can also be phosphorylated by nucleoside monophosphate kinase purified from beef liver. However, the phosphorylation catalyzed by this enzyme is relatively inefficient. The formation of the 5'-triphosphate of FTC from the 5'-diphosphate has been proposed to be catalyzed by nucleoside diphosphate kinase (NDP) (Tabulated Summary 2.6.3.1.6, {4535}), a cytosolic enzyme with a broad specificity for nucleoside 5'-diphosphates

(Tabulated Summary 2.6.3.1.1, TGZZ/93/0025). However, Cheng *et al.* have suggested that L-nucleoside 5'-diphosphates cannot be utilized as substrates by NDP kinases and are selectively phosphorylated by 3-phosphoglyceride kinase {6289}.

The metabolism of FTC was studied in HepG2.2.2.15 human hepatocellular carcinoma cells (Tabulated Summary 2.6.3.1.1, TEZA/92/0062) and CEM human T-lymphoblasts (Tabulated Summary 2.6.3.1.1, TEZA/92/0103). Both cell lines anabolize FTC to FTC-TP, the intracellular form of the compound having antiviral activity. The intracellular half-life of the triphosphate in these cells ranged from 2 to 5 hours.

The anabolism of FTC was evaluated as a function of time and drug concentration in HepG2.2.2.15 and CEM cells. The concentrations of the monophosphate, diphosphate, and triphosphate of FTC increased in a concentration-dependent manner and reached their maximum intracellular concentration by 4-hours postdose (Tabulated Summary 2.6.3.1.1, TEZA/92/0062). Two other metabolites were observed, although at much lower concentrations than the monophosphate, diphosphate, and triphosphate. A study was performed to determine the intracellular metabolism of FTC in normal HepG2 cells and to compare it to that in HBV-infected HepG2.2.2.15 and P5A cells (Tabulated Summary 2.6.3.1.1, TEZA/92/0111). The metabolites were identified as FTC-diphosphoethanolamine and FTC-diphosphocholine.

Figure 1. Intracellular Levels of Emtricitabine 5'-Phosphates as a Function of Extracellular Emtricitabine Concentration



Data from reference: {4527}

In vivo

The half-life ($t_{1/2}$) for FTC-TP has been determined directly in PBMCs, taken from healthy volunteers dosed orally with 200 mg of FTC QD. This in vivo value was found to be approximately 39 hours (Module 2.7.2, Summary of Clinical Pharmacology,

Section 2.7.2.2.1.2.1 [Trial FTC-106]). The long intracellular half-life supports once daily dosing.

2.6.2.2.2. Emtricitabine: Inhibition of Viral and Human DNA Polymerases

2.6.2.2.2.1. Emtricitabine: HIV-1 Reverse Transcriptase

Emtricitabine 5'-triphosphate serves as an alternative substrate inhibitor of HIV-1 RT and is incorporated into nascent chain viral DNA. Incorporation results in the termination of DNA synthesis due to the lack of a hydroxyl group in the 3'-position of the sugar moiety of FTC, which in turn inhibits viral replication. The K_i values for FTC-TP inhibition of HIV-RT catalyzed RNA-dependent DNA synthesis and DNA-dependent DNA synthesis were calculated to be 0.6 μM and 0.43 μM, respectively {4537}. In comparison, the K_i values for 3TC 5'-triphosphate inhibition of HIV-RT catalyzed RNA-dependent and DNA-dependent DNA synthesis were comparable of 0.97 μM and 0.7 μM, respectively. In separate experiments in which calf thymus DNA was used as the template primer, a K_i value of 0.17 μM was obtained for FTC-TP against HIV-1 RT {4541}.

Using rapid quench techniques, Feng *et al.* compared the pre-steady state kinetics of single nucleotide incorporation of dCTP, 3TC 5'-triphosphate, and FTC-TP opposite a template guanosine in RNA-dependent DNA synthesis catalyzed by HIV-1 RT (Tabulated Summary 2.6.3.1.1, {4545}). The results are presented in Table 1. The overall incorporation rate of the oxathiolane nucleoside analogues is significantly slower than that observed for the natural substrate dCTP, as evidenced by the values of k_{pol}. However the K_d values reveal that the oxathiolane substrates bind much tighter to the active site of the enzyme-DNA complex than does the natural substrate, with the K_d values of the analogues being approximately 6- to 30-fold lower than those of the natural substrate. The K_d and k_{pol} values both indicate that FTC-TP is a better overall substrate for the enzyme than 3TC 5'-triphosphate. This efficiency advantage can account in part for the higher activity seen for FTC compared to 3TC in cell culture.

Table 1. Kinetic Constants for the Incorporation of Deoxycytidine 5'-Triphosphate, Emtricitabine 5'-Triphosphate, and Lamivudine 5'-Triphosphate into an RNA/DNA Template/Primer

Compound	Template/Primer	k_{pol} (s^{-1})	K_d (μM)	k_{pol}/K_d ($\mu M^{-1}s^{-1}$)
Deoxycytidine 5'-triphosphate	r44/d23	9 ± 2	16 ± 5	—
	R45/D23	22.9 ± 0.7	30 ± 4	0.76
FTC 5'-triphosphate	r44/d23	0.240 ± 0.02	1.7 ± 0.3	—
	R45/D23	0.082 ± 0.005	1.4 ± 0.4	0.06
3TC 5'-triphosphate	R45/D23	0.033 ± 0.002	5.0 ± 0.8	0.0067

Data from reference: {4545}

2.6.2.2.2. Emtricitabine: Cellular DNA Polymerases

The inhibition of human HeLa cell DNA polymerases α, β, γ, and ε by FTC-TP was examined under steady-state conditions (Tabulated Summary 2.6.3.1.1, TEZZ/93/0007) {4541}. Activated calf thymus DNA was used as the template for analysis of each enzyme. Under these conditions, FTC-TP was a weak inhibitor of each of the human DNA polymerases when compared to HIV-1 RT. Apparent K_i values were 6.0 μM for polymerase α; 17 μM for polymerase β; 6.0 μM for polymerase γ; and 150 μM for polymerase ε.

Inhibition of human DNA polymerase γ (Pol γ) is one of the proposed mechanisms for nucleoside analogue-derived toxicity. Therefore the potential for FTC-TP and 3TC 5'-triphosphate to serve as substrates for Pol γ was investigated using pre-steady state kinetics (Tabulated Summary 2.6.3.1.1, TPI 9501). For dCTP (the natural substrate), FTC-TP, and 3TC 5'-triphosphate, the order of incorporation efficiency is dCTP (k_{pol}/K_d = 40 $\mu M^{-1}s^{-1}$) > 3TC 5'-triphosphate (k_{pol}/K_d = 0.014 $\mu M^{-1}s^{-1}$) > FTC-TP (k_{pol}/K_d = 0.0006 $\mu M^{-1}s^{-1}$). The low rate of incorporation and poor binding affinity of FTC-TP makes it the least favorable substrate for Pol γ in this analysis.

2.6.2.2.3. Emtricitabine: In Vitro Evaluation of Antiretroviral Activity

2.6.2.2.3.1. Emtricitabine: Anti-HIV Activity

The ability of FTC to inhibit replication of HIV-1 and HIV-2 in cell culture has been studied extensively using various human T-lymphoid cell lines (MT-2, MT-4, CEM, and HT4-6C), and PBMCs infected with laboratory-adapted strains of HIV-1 (IIIB, LAI or LAV) and HIV-2 (ZY, ROD2). The results are summarized in Table 2. Several investigators have compared directly the anti-HIV activity of FTC with that of 3TC in the same assay. The results are summarized in Table 3. Although different laboratory strains of virus, different cell types, and different assay methods were used, FTC consistently showed greater activity than 3TC with the activity advantage ranging from 3- to 11-fold.

Table 2. Inhibitory Effect of Emtricitabine on the Replication of Laboratory Strains of HIV-1 and HIV-2

Virus	Cell Type	Assay	EC_{50} (µM)
$HIV\text{-}1_{IIIB}$	CEM	RT	0.1 [a,b]
	MT-4	cytoprotection	0.5 [a,b]
	PBMC	RT	0.01 [b]
$HIV\text{-}1_{LAV}$	CEM	RT	0.009 [a]
	HT4-6C	RT	0.02 [a]
	PBMC	p24	0.009 [b]
	PBMC	RT	0.001 [d]
$HIV\text{-}1_{LAI}$	CEM	cytoprotection	0.04 [c]
	MT-2	cytoprotection	0.62 [c]
	PBMC	p24	0.03 [c]
	PBMC	p24	0.0014 [e]
$HIV\text{-}2_{ZY}$	MT-4	RT	1.5 [a]
	CEM	RT	0.1 [a]
$HIV\text{-}2_{ROD2}$	PBMC	p24	0.007 [b]

a Data from reference: {4534}
b Data from reference: {4541}
c Data from reference: TPI 462-v2
d Data from reference: {4526}
e Data from reference: TPI 10498-v2

Table 3. Comparison of the Antiviral Activities of Emtricitabine and Lamivudine against Various Laboratory Strains of HIV-1

HIV-1 Strain	Cell Line	EC_{50} (µM)		Sensitivity Ratio[d]
		FTC	3TC	
LAI[a]	PBMC	0.018	0.19	11
IIIB[b]	PBMC	0.01	0.07	7
IIIB[b]	MT-4	0.5	3.2	6
LAI[a]	MT-2	0.3	1.6	5
HXB2[b]	MT-4	0.09	0.24	3
LAI[c]	CD4+ HeLa	0.06	0.18	4

a Data from reference: TPI 10498-v2
b Data from reference: {4534}
c Data from reference: {1794}
d Ratio of 3TC (lamivudine) to FTC (emtricitabine) EC_{50} values.

Schinazi *et al.* tested 2 low passage HIV-1 clinical isolates, J6 and 2:DR2, in PHA-stimulated PBMCs isolated from uninfected donors for FTC activity (Tabulated Summary 2.6.3.1.1,

{4534}). The EC_{50} values were similar to those calculated using laboratory strains of virus in PBMCs (Table 4). The sensitivities to FTC reported for 2 additional wild-type clinical isolates, WT-pre-AZT and WT-MKC09-day 29, were similar to those determined against J6 and 2:DR2 (Tabulated Summary 2.6.3.1.1, TPI 462-v2). In the single experiment in which a direct comparison was made between the activity of FTC and 3TC, FTC demonstrated the same 5-fold potency advantage observed using laboratory strains of virus.

Table 4. Inhibition of HIV-1 Clinical Isolates by Emtricitabine

Virus	EC_{50} (μM)	
	FTC	3TC
J6[a]	0.002	0.01
2:DR2[a]	0.002	ND[c]
WT – pre-AZT[b]	0.008	ND[c]
WT – MKC09-day 29[b]	0.02	ND[c]

a Data from reference: {4534}
b Data from reference: TPI 462-v2
c ND = Not Determined

The potency of FTC has also been determined using a coculture assay {4248} and compared directly to the potencies of 3TC, ddC, ddI, ZDV, and the nonnucleoside RT inhibitor TIBO R82913. At the end of the coculture period, viral replication was estimated by HIV-1 p24 ELISA. Results from this study expressed as mean EC_{50}, EC_{90}, and EC_{99} values are given in Table 5. A potency ranking (based on EC_{90} values) showed FTC to be the most potent compound. The low potency ranking for ZDV compared to that observed in laboratory strains may be the result of inclusion of PBMCs from ZDV-experienced patients in the coculture.

Table 5. Comparative Potency of RT Inhibitors in HIV-1 Infected PBMCs Using a Coculture Method

Inhibitor	EC_{50} (μM)	EC_{90} (μM)	EC_{99} (μM)
FTC	0.0085	0.055	0.43
3TC	0.11	0.3	0.85
ddC	0.011	0.074	0.6
ddI	0.76	6.4	65.8
AZT	0.055	0.53	6.4
TIBO R82913	0.17	0.67	2.95

Data from reference: {4248}

2.6.2.2.3.2. Emtricitabine: Activity Against HIV-1 Subtypes

The activity of FTC on non-B subtypes of HIV-1 clinical isolates (group M subtypes A, C, D, E, F, and G, and O group) was evaluated. EC_{50} values were determined in MAGI-CCR5 and PBMCs. Within each model, EC_{50} values were comparable for all subtypes of HIV-1. Results are presented in Table 6. Emtricitabine was more active than 3TC and ddI, and had activity comparable to that of ZDV for all subtypes of HIV-1 tested. Overall, the EC_{50} values of HIV-1 subtypes were 2- to 5-fold higher in MAGI-CCR5 than in PBMCs (Tabulated Summary 2.6.3.1.1, TPI 10498-v2 and TPI 11419-v2).

Table 6. EC_{50} Values of NRTIs Against HIV-1 Group M and Group O Isolates in PBMCs and MAGI-CCR5 Cells

Isolate	Subtype	Host Cell	AZT (μM)	3TC (μM)	ddI (μM)	FTC (μM)
Group M						
RW/92/008	A	PBMCs[a]	0.008	0.054	0.26	0.012
		MAGI-CCR5[b]	0.085	0.20	3.0	0.055
BR/92/025	C	PBMCs[a]	0.035	0.027	0.49	0.017
		MAGI-CCR5[b]	0.033	0.17	0.95	0.032
UG/92/024	D	PBMCs[a]	0.003	0.026	0.21	0.007
		MAGI-CCR5[b]	0.035	0.11	1.70	0.030
Tha/92/019	E	PBMCs[a]	0.039	0.069	0.5	0.028
		MAGI-CCR5[b]	0.080	0.15	1.50	0.065
Br/930/20	F	PBMCs[a]	0.003	0.022	0.34	0.009
		MAGI-CCR5[b]	0.045	0.15	1.50	0.050
RU570	G	PBMCs[a]	0.008	0.090	0.34	0.030
		MAGI-CCR5[b]	0.150	0.18	2.50	0.075
Group O						
BCF03 O		MAGI-CCR5[b]	0.09	0.20	2.20	0.065
		PBMCs[a]	0.028	2.5	4.75	0.14

a Data from reference: TPI 10498-v2

b Data from reference: TPI 11419-v2

2.6.2.2.3.3. Emtricitabine: Effect of Multiplicity of Infection (MOI)

The effect of MOI on the antiviral activity of FTC was assessed in 2 cell lines using 2 different assay methods and was found to be minimal. In MAGI-CCR5 cells (Figure 2), an increase of 5.7-fold in infectious titer resulted in no change in EC_{50} (Tabulated Summary 2.6.3.1.1, 10518v2). In PBMCs (Figure 3), using p24 ELISA, increasing the MOI from 0.01 to 0.1 caused approximately a 10-fold shift in apparent EC_{50} (Tabulated Summary 2.6.3.1.1, 11773). Increasing the MOI above 0.1 produced no further change in apparent EC_{50}.

Figure 2. Effect of MOI on the EC_{50} of Emtricitabine in MAGI-CCR5 Cells

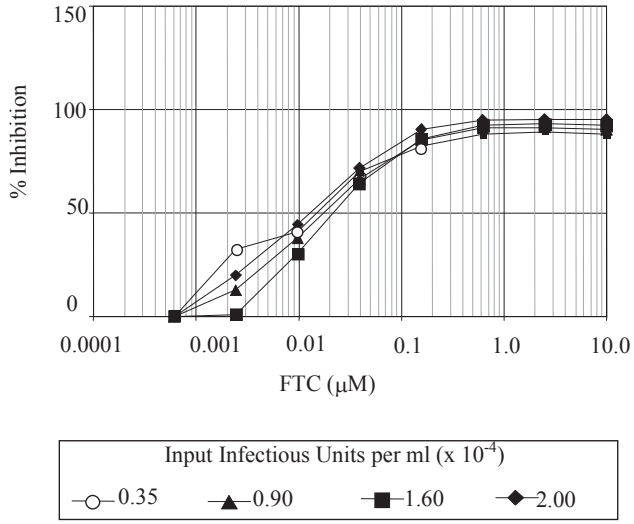
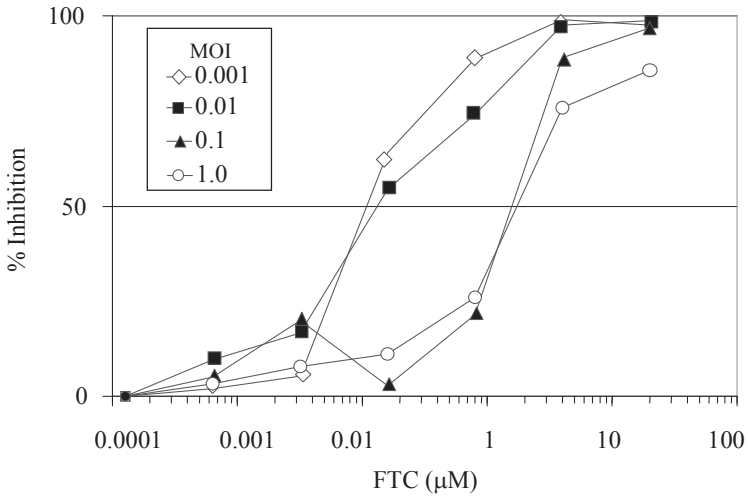


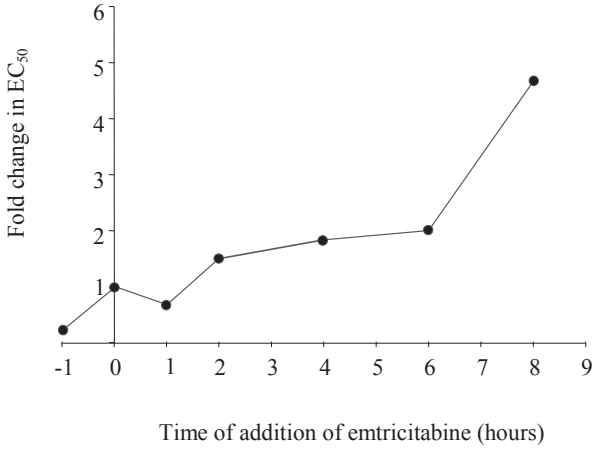
Figure 3. Effect of MOI on the EC_{50} of Emtricitabine in PBMCs



2.6.2.2.3.4. Emtricitabine: Effect of Time of Addition

Various concentrations of FTC were added to MAGI cells infected with HIV-1_{LAI} (Tabulated Summary 2.6.3.1.1, 10247) and EC_{50} values determined at −1, 0, +1, +2, +4, +6, and +8 hours postinfection. Results of the study are presented in Figure 4 as fold change in EC_{50} over time of addition. Emtricitabine maintained maximal antiviral activity when added as late as 6-hours postinfection. However, adding FTC 8-hours postinfection resulted in a decrease in antiviral activity consistent with the mechanism of action of an NRTI inhibitor.

Figure 4. Fold Change in Emtricitabine EC_{50} as a Function of Time of Addition



2.6.2.2.3.5. Emtricitabine: Effect of Serum Proteins and α-1 Acidic Glycoprotein

The potential effect of protein binding on the antiviral activity of FTC was evaluated in CEM cells. Cells were infected with HIV-1_{LAI} and maintained in culture in the presence of various concentrations of FTC in standard 10% fetal bovine serum (FBS) containing media, or in modified media where FBS was replaced with 25% human serum albumin or 25% human serum albumin plus 1 mg/mL alpha-1 acidic glycoprotein (AAG). Neither the medium containing 25% human serum albumin alone or in combination with 1 mg/mL AAG affected the antiviral activity of FTC (Tabulated Summary 2.6.3.1.1, 463), indicating that FTC activity is not affected by binding to serum proteins.

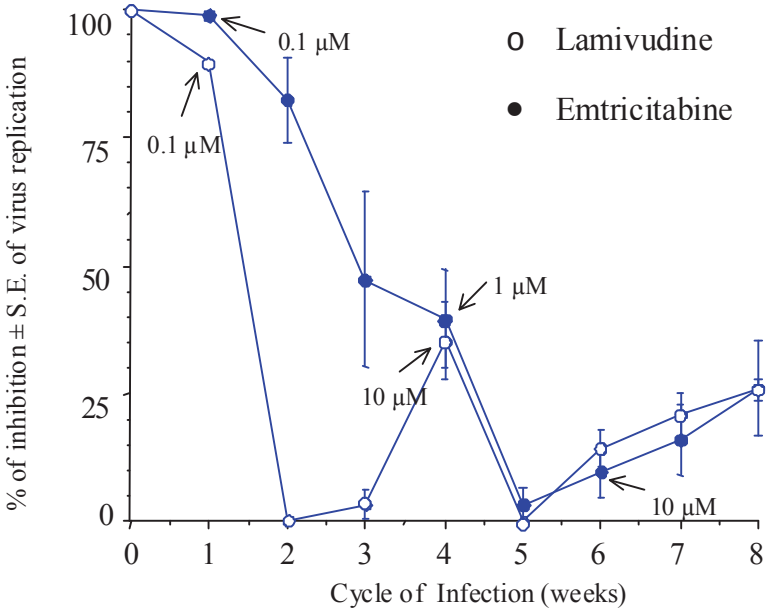
2.6.2.2.4. Emtricitabine: In Vitro HIV-1 Resistance

The development of resistance to FTC was examined by passaging virus in vitro in the presence of drug. In a study reported by Tisdale *et al*., the wild-type virus HIV-1_{HXB2} or the ZDV-resistant mutant virus HIV-1_{RTMC} (contains D67N, K70R, T215Y, and K219Q RT mutations), was passaged in MT-4 cells in the presence of increasing concentrations of FTC or 3TC (Tabulated Summary 2.6.3.1.1, {1794}). Rapid emergence of resistance occurred with both compounds. By the fourth passage of HIV-1_{HXB2} and the second passage of HIV-

1_{RTMC}, EC_{50} values exceeded 50 µM and by passage 6, EC_{50} values were in excess of 250 µM. These FTC-induced variants were highly cross-resistant to 3TC but showed no cross-resistance to ZDV, ddI, or NVP. DNA sequence analysis showed a change in RT of M184V. Passaging virus in the presence of increasing concentrations of FTC and 50 µM ZDV was able to delay appreciably, but not prevent the emergence of FTC-resistant virus.

In experiments performed by Schinazi *et al*., the relative potential for HIV-1 to develop resistance to 3TC and FTC was evaluated by serial passage of the virus in human PBMCs in the presence of increasing drug concentrations (Tabulated Summary 2.6.3.1.1, {1777}). Results presented in Figure 5 show that after 2 weeks of infection, 0.1 µM 3TC was no longer able to inhibit virus replication and drug resistant variants dominated the replicating virus population. In contrast, FTC remained highly active under identical conditions, reducing virus replication by 80%. Emtricitabine was still able to inhibit virus replication by 50% at Week 3. At Week 5, the concentration of 3TC was increased 100-fold to 10 µM and the concentration of FTC was increased 10-fold to 1 µM to produce the same level of inhibition. Emtricitabine retained up to a 10-fold potency advantage over 3TC after 5 weeks of passaging. These data suggest that, due to greater potency, FTC may delay the breakthrough of M184V resistant viruses longer than 3TC.

Figure 5. **HIV-1 Breakthrough in the Presence of Emtricitabine and Lamivudine**



Data from reference: {1777}

DNA sequence analysis of the RT gene amplified from resistant viruses generated in these passaging experiments consistently identified mutations at M184V/I. Resistant variants were cross-resistant to both FTC and 3TC, but remained sensitive to ddC, ddI, ZDV, PFA, 3'-fluoro-3'-deoxythymidine (FLT), and 2 NNRTIs, the TIBO (compound R82150) and the bis(heteroaryl) piperazine derivative U-87201E (Tabulated Summary 2.6.3.1.1, {1777}).

2.6.2.2.4.1. Emtricitabine: Mechanisms of Resistance

Biochemical studies were performed to quantify the change in susceptibility of HIV-1 RT derived from virus resistant to FTC-TP, 3TC 5'-triphosphate, and ddC 5'-triphosphate (Tabulated Summary 2.6.3.1.1, {1777}). Virus particle-derived RT was obtained from the supernatant of human PBMCs that were infected with M184V mutant virus. The mutated enzyme was 15-fold less sensitive to inhibition by FTC-TP or 3TC 5'-triphosphate than was wild-type (LAI) HIV-RT. However, only a 3-fold decrease in susceptibility was noted for ddC 5'-triphosphate. Similar results were reported using a highly purified cloned RT containing the M184V mutation for which the K_i values of FTC-TP and 3TC 5'-triphosphate were increased 320- and 80-fold, respectively, compared to wild-type HIV-1 RT (Tabulated Summary 2.6.3.1.1, {4545}). Using steady state and pre-steady state kinetic analysis, Wilson *et al*. examined the effect of the M184V mutation on HIV-1 RT catalytic function (Tabulated Summary 2.6.3.1.1, {4249}). These kinetic studies showed that the M184V mutation did not alter either the K_m or the k_{cat} values for the natural substrates, but did cause a 300-fold increase in the K_i for FTC-TP.

The M184V mutant HIV-1 is also associated with a decrease in replication capacity of HIV-1 expressing this mutation. This has been observed previously for a site-directed mutant expressing the M184V mutation {3852}, and is shown in Table 8 for a panel of clinical isolates expressing the M184V mutation that were obtained from a random set of plasma samples submitted to Monogram Biosciences for phenotypic analyses {5049}, (Tabulated Summary 2.6.3.1.3, PC-104-2004). The combination of K65R with M184V showed the greatest reduction in replication capacity (Tabulated Summary 2.6.3.1.3, {5476}). Decreases in natural substrate binding (M184V), incorporation (K65R), and reduced initiation of minus-strand single-stranded DNA synthesis (K65R+M184V) (Tabulated Summary 2.6.3.1.3, {10671}) are likely responsible for these observed additive decreases in viral replication capacity.

2.6.2.2.4.2. Emtricitabine: Activity Against Drug-Resistant Variants of HIV-1

Emtricitabine activity has been evaluated extensively against a panel of clinical isolates (Table 7 and Table 8). The panel consists of a series of recombinant wild-type clinical isolates and recombinant isolates containing anywhere from 1 to 12 mutations. Consistent with earlier passaging experiments, a high level of resistance is imparted by introduction of the M184V mutation onto any mutation background. Moderate resistance to FTC is seen for the highly mutated isolate (M41L, E44D, D67N, T69D, L74I, K101E, V108I, V118I, Y181C, G190A, L210W, T215Y) which contains 2 mutations associated with moderate resistance to 3TC (E44D, V118I) {2359}. In addition, moderate resistance was observed for both FTC and 3TC, with isolates harboring the K65R mutation or a multi-drug resistance (MDR) genotype containing the T69S(SS) insertion as previously described for 3TC {2359}, {1003}, {4536}. The antiviral activity of FTC against HIV-1 containing K65R, Q151M, and K65R+Q151M showed reduced susceptibility (8.4-, 2.3-, > 20-fold, respectively) in cell culture. Evaluation of the mutant RT enzymes suggested that resistance was mainly due to a significant decrease in the rate of incorporation of FTC using pre-steady state kinetics studies in vitro. Similar

levels of virus resistance and incorporation defects were measured for FTC and 3TC in this study (Tabulated Summary 2.6.3.1.1, {8887}).

Table 7. Phenotypic Analysis of a Panel of Recombinant Viruses

Genotype	EC_{50} (μM)		
	FTC	3TC	ZDV
WT ($HIV\text{-}1_{HXB2}$)[a]	0.058	0.3235	0.0305
K65R, F116Y, Q151M, V106I[a]	2.607	3.327	0.696
M41L, D67N, K70R, A98S, Y181C, M184V[a], G190A, L219W, T215Y[a]	> 5	> 31.25	0.47
V75I, M184V[a]	> 125	> 31.25	0.028
A62V, T69S(SS), K70R, T215Y[a]	0.893	2.566	0.965
K103N, V108I/V, P225H[a]	0.123	0.618	0.0358
G190A, K238T[a]	< 0.122	0.541	< 0.05
V106A, G190A[a]	0.292	2.16	> 125
$HIV\text{-}1_{IIIB}$[b]	0.01	0.02	0.01
V106A[b]	0.03	0.03	0.014
V106A, F227L[b]	0.003	0.007	0.003
V106A, F227L, K101I, Y181C[b]	0.01	0.03	0.01

a Data from reference: TPI 11148

b Data from reference: {4538}

Table 8. Phenotypic Analysis of Recombinant Viruses Generated from Clinical Isolates

Genotype	EC_{50} (μM)[a]		
	FTC	3TC	ZDV
WT (LAI)	0.619[b]	2.567[b]	0.487[b]
WT (n=16)	0.64[c]	2.998[c]	0.917[c]
L100I (A)	0.176	2.125	0.037
L100I (B)	0.595	2.350	0.160
G190A	0.220	0.900	0.205
G333E	1.350	1.265	0.260
M184V	>20	>50	0.140
K103T	0.330	1.100	0.140
V108I	0.205	0.650	0.135
K103N (A)	0.825	3.150	0.595
K103N (B)	0.680	4.000	0.250
K103N, M184V (A)	>20	>50	0.185
K103N, M184V (B)	>20	>20	3.02
E138K, M184V (A)	>20	>50	0.145
E138K, M184V (B)	>20	>50	0.130
E13Q, M184V	>20	>50	0.890
A98S, M184V	>20	>50	0.120
L74V, K103N	2.34	>2.26	1.87
K101Q, E138K	1.38	1.94	0.54
K103R, Y188C	1.13	1.16	0.72
K103N, Y181C	0.55	1.17	0.29
K70R, L74V, M184V	>20	>20	0.90
K103T, V106I, M184V	>20	>20	0.36
K101Q, E138K, K103N	0.55	0.48	0.67
K103N, V108I, M184V	>20	>20	0.31

(Continued on following page)

Table 8. Phenotypic Analysis of Recombinant Viruses Generated from Clinical Isolates (Continued)

Genotype	EC_{50} (μM)[a]		
	FTC	3TC	ZDV
T215Y, K103N, L210W	0.73	1.05	>2
M41L, K101R, M184V, T215Y	>20	>20	>2
A98S, F116Y, Q151M, T215Y	1.45	0.55	>2
T69N, K70R, M184V, K219Q	>20	>20	1.23
D67N, K70R, M184V, G190A	>20	>50	0.35
D67N, T69D, K103R, T219Q	2.58	2.92	5.75
A62V, A98S, K101D, K102Q, M184V	>20	>50	0.35
M41L, D67N, M184V, L210W, T215Y	>20	>20	>2
D67N, K70R, E138A, M184V, T215Y, K219E	>20	>20	>2
M41L, D67N, Y181C, M184V, L210W, T215Y	>25	>20	>2
M41L, D67N, T69D, V108I, M184V, T215Y	>20	>20	>2
A62V, V75M, K103N, F116Y, Q151M, M184V	>20	>20	>2
D67N, T69D, K70R, K103N, M184V, T215Y, K219Q	>20	>20	>2
M41L, D67N, A98G, K101E, K103N, M184V, G190A, L210W, T215Y, G333E	>20	>20	>2
M41L, E44D, D67N, T69D, L74I, K101E, V108I, V118I, Y181C, G190A, L210W, T215Y	7.27	6.63	1.85

a EC_{50} values are expressed as the median value of at least 3 replicates unless otherwise noted.

b EC_{50} is the average value of at least 10 replicates.

c EC_{50} values are the average of replicates from 16 different recombinants displaying a wild-type genotype.

2.6.2.2.5. Emtricitabine: In Vivo Efficacy Against Animal Retroviruses

The anti-HIV activity of FTC has been tested in SCID mice. Mice were reconstituted with human PBMCs and after 2 weeks, infected with HIV-1_{A018} (Tabulated Summary 2.6.3.1.1, {4525}, TPI 11985). Drug therapy was initiated 1 day before infection. Test compounds were administered intraperitoneally at 30 mg/kg BID. Viral inhibition was measured by quantitative cocultures for infectious HIV-1, and quantitative RNA viral load measurement

on peritoneal wash cells, lymph nodes, spleen cells, and plasma. At the concentration used in this study, FTC completely inhibited viral infection.

Black and Furman evaluated the anti-HIV-1 activity of orally administered FTC and 3TC side-by-side in the HuPBMC-SCID mouse model (Tabulated Summary 2.6.3.1.1, TPI 11985). Groups of 12 or 15 female C.N-17 SCID mice were reconstituted by the intraperitoneal injection of 1.3×10^8 human PBMCs. Two weeks later, the mice were infected intraperitoneally with 2000 tissue culture infectious doses (TCID) of HIV-1_{A018}. Drugs were then administered in drinking water, which contained 0.3 mg/mL of FTC or 3TC. Seven days after infection, viral load in plasma was measured using a real-time reverse transcriptase-polymerase chain reaction (RT-PCR). Both FTC and 3TC were well tolerated during the 7 days of the study with no evident toxicity. The average daily doses of the 2 drugs were similar, about 60 mg/kg. In the control group the geometric mean viral load was 2.5×10^4 copies/mL. Emtricitabine reduced plasma viral loads to below 50 copies/mL in all 12 treated mice and 3TC reduced plasma viral loads to below the limit of detection in 11 of the 12 treated mice. The reductions in viral loads in both treatment groups were statistically significant ($p < 10^{-5}$) compared to control, but did not differ significantly from each other.

2.6.2.2.6. Rilpivirine: Mechanism of Action and Inhibition of Viral and Human DNA Polymerases

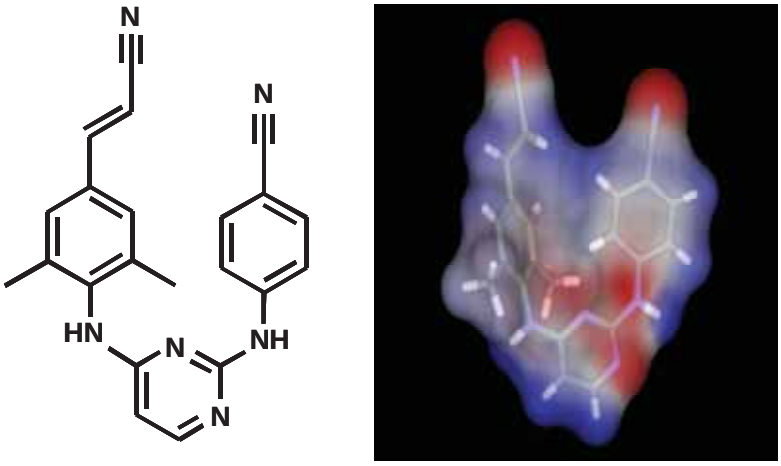
TMC278 is an NNRTI. For additional primary pharmacodynamics of RPV (TMC278), please refer to Module 2.7.2 (Summary of Clinical Pharmacology).

All in vitro studies described in this section were conducted with serum free medium unless noted otherwise.

2.6.2.2.6.1. Rilpivirine: HIV-1 Reverse Transcriptase

The inhibitory effect of TMC278 (chemical structure shown in Figure 6) on HIV-1 reverse transcriptase (RT) activity was determined by an enzymatic assay (Quan-T-RT; Amersham) described previously {15847}. Briefly, the inhibition of incorporation of [^{3}H]TTP and TTP by RT in a p(rA)p(dT)primer/template was measured via biotin/streptavidin linkage. The median inhibitory activity of HIV-1 RT for TMC278 IC_{50} was 42 nM, with an interquartile range (IQR) ranging from 31 to 44 nM (Tabulated Summary 2.6.3.1.2, TMC278-IV1-AVMR).

Figure 6. Chemical Structure and 3-dimensional Model of TMC278



The chemical structure of TMC278, $C_{22}H_{18}N_6$, or 4-[[4-[[4-[(1*E*)-2-cyanoethenyl]-2,6-dimethylphenyl]amino]-2-pyrimidinyl]amino]benzonitrile, is shown. Red represents CN groups or the nitrogen of the pyrimidine and the nitrogen of the aniline (highest density of charge in the structure), and blue represents the aromatic group (no charge).

Crystal structures of the binding site between TMC278 and the HIV-1 RT complex revealed that TMC278, similarly to other members of the diarylpyrimidine family of inhibitors, binds to HIV-1 RT and adapts to the conformational changes in the NNRTI-binding pocket {15849}, {15850}, {15866}, {15854}.

In a first study, the structure of wild-type HIV-1 RT in complex with TMC278 was determined at 1.8 Å resolution, using an RT crystal form engineered by systematic RT mutagenesis {15850}. This high resolution structure revealed that TMC278 makes important contacts with a number of key amino acids in the NNRTI-binding pocket, for example the second linker nitrogen in a water-mediated hydrogen bond network with the main-chain carbonyl group of E138 of the p51 subunit and the positioning of the cyanovinyl group of TMC278 in a hydrophobic tunnel connecting the NNRTI-binding pocket to the nucleic acid-binding cleft.

2.6.2.2.6.2. Rilpivirine: Human DNA Polymerases

TMC278 had no effects on DNA synthesis by human polymerase α, β, or γ at concentrations up to 1000 μM as determined by PCR (Tabulated Summary 2.6.3.1.2, Report TMC278-1646_0005343).

2.6.2.2.7. Rilpivirine: In Vitro Evaluation of Antiretroviral Activity

2.6.2.2.7.1. Rilpivirine: Anti-HIV Activity

The antiviral activities of TMC278 against HIV-1_{IIIB} and HIV-2_{ROD} were compared with those of other NNRTIs: EFV, ETR, and NVP (Table 9). The median EC_{50} value for TMC278 against HIV-1_{IIIB} observed in the MT-4 T-cell line is in the subnanomolar range (0.73 nM). This median EC_{50} value was lower than that obtained for EFV, ETR, and NVP by a factor of

2.4, 3.7, and 46.7, respectively (Tabulated Summary 2.6.3.1.2, TMC278-IV1-AVMR). The antiviral activity of TMC278 against HIV-2_{ROD} was compared with that of EFV, ETR, and NVP in MT-4-LTR-EGFP cells (Tabulated Summary 2.6.3.1.2, TMC278-IV2-AVMR). The median EC_{50} values for TMC278 against HIV-2_{ROD} observed in the MT-4-LTR-EGFP cell line was 5.22 µM. The median EC_{50} value for HIV-2_{ROD} was lower than that obtained for EFV and NVP, and similar to that obtained for ETR.

Table 9. In Vitro Antiviral Activity of TMC278, EFV, ETR and NVP Against HIV-1 and HIV-2

Viral Type	Median EC_{50}			
	TMC278	**EFV**	**ETR**	**NVP**
HIV-1_{IIIb}	0.73 nM	1.73 nM	2.73 nM	34.09 nM
HIV-2_{ROD}	5.22 µM	24.83 µM	5.67 µM	> 31.25 µM

The antiviral activity was determined in a cell-based assay which directly measured the ongoing replication of viruses in MT-4-LTR-EGFP cells, using the specific interaction of HIV tat with the HIV-1 LTR coupled to the GFP reporter gene, as a marker of the ongoing HIV-1 infection {2381}, {15876}. Briefly, 384-well plates were filled with various concentrations of the test compound obtained by serial dilutions in the cell culture medium (RPMI-1640 containing 10% FCS). MT-4 cells and HIV were added into the 384-well plate at a concentration of 1.5 x 10^5 cells/mL and at a MOI of 0.01 in 40 µL. Subsequently, the cell cultures were incubated at 37°C in a 5% CO_2 atmosphere for 3 days.

The in vitro antiviral activity of TMC278, EFV, and ETR, and the control NRTI ZDV was determined against HIV-1_{Ba-L} and HIV-1_{ADA} in monocyte-derived macrophages (MDMs) (Tabulated Summary 2.6.3.1.2, TMC278-IV2-AVMR). The data presented in Table 10 show EC_{50} values of 0.18 and 0.22 nM (0.07 to 0.08 ng/mL) for TMC278 for HIV-1_{Ba-L} and HIV-1_{ADA}, respectively.

Table 10. In Vitro Antiviral Activity of TMC278, EFV and ETR Against HIV1$_{Ba-L}$ and HIV-1$_{ADA}$

HIV-1 Primary Isolate	Antiviral Activity (nM)							
	TMC278		EFV		ETR		Control (ZDV)	
	EC_{50}	EC_{90}	EC_{50}	EC_{90}	EC_{50}	EC_{90}	EC_{50}	EC_{90}
Ba-L	0.18	1.28	1.15	7.85	0.65	4.44	2.44	29.4
ADA	0.22	1.07	0.64	5.67	0.40	2.52	5.74	35.5

Reported values are the result of one experiment run in triplicate (see Tabulated Summary 2.6.3.1.2, TMC278-IV2-AVMR).

Briefly human peripheral blood mononuclear cells (PBMCs) were isolated from screened donors, seronegative for HIV and hepatitis B virus (HBV) and used as source for isolation of MDMs. PBMCs were incubated for 2 to 18 hours at 37ºC, 5% CO_2 to allow MDMs to adhere to the wells. Following adherence, the cultures were washed to remove nonadherent cells. After 6 to 14 days in culture, MDMs and serially diluted test compounds were added to the wells, and subsequently a pretitered amount of HIV was added. Cultures were washed a final time by media removal 24 hours post infection, fresh compound was added, and the cultures continued for an additional 6 days. At assay termination, virus replication was measured by collecting cell-free supernatant samples, which were analyzed for HIV p24 antigen content using a commercially available p24 ELISA assay (PerkinElmer).

2.6.2.2.7.2. Rilpivirine: Activity Against HIV-1 Subtypes

TMC278 was determined to be active against HIV-1 Group M primary isolates from diverse origins, with EC_{50} values ranging from 0.07 to 1.01 nM for Group M and EC_{50} values ranging from 2.88 to 8.45 nM for Group O (Table 11). These values were similar to those observed in MT-4 cells with HIV-1$_{IIIB}$ (Tabulated Summary 2.6.3.1.2, TMC278-IV1-AVMR and TMC278-IV2-AVMR). The median EC_{50} of TMC278 against all the HIV-1 primary clinical isolates tested in PBMC cultures was 0.26 nM (IQR: 0.15 to 0.52 nM). There was no apparent difference in the antiviral activity of TMC278 based on virus tropism.

Compared to the HIV-1 Group M primary isolates, similar results were obtained testing the antiviral activity of TMC278 against a panel of nine Group M recombinant HIV-1 clinical isolates of subtypes A1, AE, AG, BG, C, D, F1, G, and H (Tabulated Summary 2.6.3.1.2, TMC278-IV1-AVMR).

Table 11. In Vitro Antiviral Activities of TMC278, EFV, and ETR Against Primary HIV-1 Isolates

HIV-1 strain	Subtype	Tropism	EC_{50} (nM)		
			TMC278	EFV	ETR
92UG029	A	CXCR4	0.44	0.62	1.45
92UG037	A	CCR5	0.24	1.11	1.42
92RW020	A	CCR5	0.07	0.30	0.48
JR-CSF	B	CCR5	0.51	0.76	1.02
93BR021	B	CCR5	0.23	1.17	1.35
WEJO	B	CXCR4	0.08	0.16	0.43
92BR025	C	CCR5	0.33	2.08	1.70
93IN101	C	CCR5	0.53	1.47	2.72
93MW959	C	CCR5	0.11	0.19	1.01
92UG001	D	DUAL	0.26	0.53	0.90
92UG024	D	CXCR4	0.38	0.72	3.18
92UG035	D	CCR5	0.07	0.34	0.44
92TH006	E	CCR5	0.08	0.48	0.50
93TH073	E	CCR5	0.07	0.39	0.21
CMU08	E	CXCR4	1.01	0.78	3.47
93BR019	F	CXCR4	0.95	0.15	0.46
93BR020	F	DUAL	0.16	0.74	1.08
93BR029	F	CCR5	0.19	0.42	1.06
G3	G	CCR5	0.25	0.12	0.66
JV1083	G	CCR5	0.26	0.70	1.45
RU132	G	CCR5	0.51	0.52	1.70
BCF01	Group O	CCR5	3.13	72.8	16.4
BCF02	Group O	CCR5	2.88	57.2	52.8
BCF03	Group O	CCR5	8.45	194	149

The median EC_{50} values for TMC278, EFV, and ETR were 0.26, 0.66, and 1.22 nM, respectively, and the corresponding first- to third-quartile values were 0.15 to 0.52, 0.38 to 1.13, and 0.62 to 1.96 nM, respectively.

CCR5, chemokine receptor 5; CXCR4, CXC chemokine receptor 4 (see Report TMC278-IV1-AVMR-RD) {15541}.

2.6.2.2.7.3. Rilpivirine: Effect of Multiplicity of Infection

The antiviral activity of TMC278 against HIV-1_{IIIB} was studied at 3 MOIs (0.25, 0.0025, and 0.00025; Tabulated Summary 2.6.3.1.2, TMC278-IV2-AVMR). The data presented in Table 12 summarizes the median EC_{50} values at each MOI for TMC278, EFV, ETR, NVP, and the control (ZDV). Comparable EC_{50} values were obtained at the 3 MOIs for TMC278, EFV, and ETR. EC_{50} values clearly increased with increasing MOI for NVP and the NRTI control ZDV.

Table 12. Influence of the MOI on the EC_{50} Values of TMC278, EFV, ETR, NVP and Control (ZDV) Against HIV-1_{IIIB}

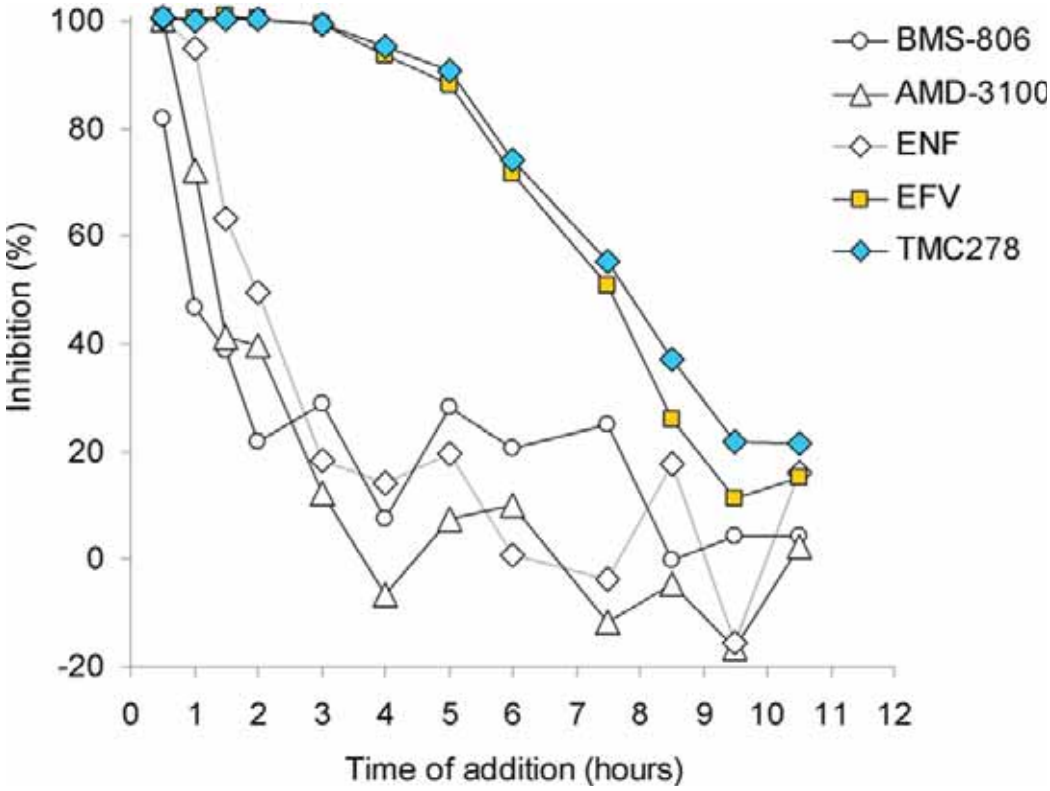
MOI	Median EC_{50} (IQR) (nM)				
	TMC278	EFV	ETR	NVP	Control (ZDV)
0.00025	0.18 (0.17-0.19)	0.22 (0.20-0.24)	0.78 (0.77-0.80)	5.00 (4.85-73.50)	13.00 (11.50-14.50)
0.0025	0.42 (0.40-0.44)	0.79 (0.75-0.83)	0.94 (0.92-1.07)	37.00 (31.55-39.0)	39.30 (36.65-46.15)
0.25	1.40 (1.40-1.78)	4.25 (4.20-4.30)	5.30 (0.51-0.65)	5835.00 (5477.50-6012.50)	890.00 (805.0-922.0)

Antiviral assays were carried out as described previously {15876}, {2381}. MT-4 cells (3.0×10^4 cells/well in a final volume of 200 µL) were added to serial dilutions of drugs in a 96-well microtiter plate. HIV-1_{IIIB} was added to the wells and cell cultures were incubated at 37°C in a 5% CO_2 atmosphere for 5 days. Cell viability was determined by the MTT method and EC_{50} values were calculated from dose response curves. The results are expressed as the median and interquartile range of 2 independent experiments.

2.6.2.2.7.4. Rilpivirine: Effect of Time of Addition

A time of addition assay was conducted in order to evaluate the stage at which TMC278 acts in the HIV-1 replication cycle (Tabulated Summary 2.6.3.1.2, TMC278-IV1-AVMR). The antiviral activity of TMC278, ENF (fusion inhibitor), AMD-3100 (CXCR4 entry inhibitor), BMS-806 (CD4/HIV-1 gp120 attachment inhibitor), and EFV were studied at different times of addition of the drugs to the infected HIV-1_{IIIB} cell culture. The data presented in Figure 7 show that the activity profile of TMC278 was similar to that of EFV. Both TMC278 and EFV inhibited virus replication by at least 50% up to 8 hours after initiation of infection. This confirmed a similar mode of action for EFV and TMC278, i.e., inhibition of the HIV-1 RT.

Figure 7. Effect of the Time of Addition of Compound on the Antiviral Activity of TMC278



Note: MT-4-LTR-Luc cells at a concentration of 3.5 x 10^5 cells/mL were infected with HIV-1_{IIIB} at high MOI. TMC278, EFV, AMD-3100 (CXCR4 inhibitor), BMS-806 (CD4/gp120 inhibitor) and ENF (fusion inhibitor) were added to each culture at a final concentration of 0.05, 1.00, 1.00, 10.00, and 1.00 μM, respectively at 30 minutes and then every 60 to 90 minutes for the next 11 hours post infection. At 24 hour post infection, the antiviral activity of the compounds was calculated by determination of the induction of the Luclite® luminescence in cell cultures.

2.6.2.2.7.5. Rilpivirine: Effect of Serum Proteins and α-1 Acid Glycoprotein

The antiviral activity of TMC278 was compared to those of other NNRTIs in the presence or absence of human serum proteins (Tabulated Summary 2.6.3.1.2, TMC278-IV1-AVMR). The data presented in Table 13 show a reduction in the antiviral activity of TMC278 in the presence of 1 mg/mL alpha-1 acid glycoprotein (AAG), 45 mg/ml human serum albumin (HSA), and 50% human serum as demonstrated by median EC_{50} ratios of 1.8, 39.2, and 18.5, respectively. Comparable results were obtained for EFV.

Table 13. Influence of Human Serum Proteins on the In vitro Anti-HIV Activity of TMC278, EFV, ETR, and NVP

	Median EC_{50} ratio[a,b (Q1–Q3)]					
NNRTI	**1 mg/mL AAG**	**n**	**45 mg/ml HSA**	**n**	**50% human serum**	**n**
TMC278	1.8 (1.5–2.1)	13	39.2 (25.7–54.1)	12	18.5 (11.0–40.4)	13
EFV	3.8 (3.2–4.1)	5	17.3 (13.2–19.4)	5	12.3 (10.2–16.2)	5
ETR	3.4 (2.0–4.4)	17	4.9 (4.0–5.9.)	17	5.7 (4.9–12.1)	16
NVP	2.0 (1.5–2.9)	7	2.9 (1.5–3.3)	7	3.8(1.8–5.1)	7

Ratios of the 50% effective concentration (EC_{50}) values in the presence and in the absence of serum proteins; Q1–Q3, first and third quartile; n, number of experiments

The antiviral activity was determined in a cell-based assay, which directly measured the ongoing replication of HIV in MT-4-LTR-EGFP cells, using the specific interaction of HIV-tat with the HIV-1 LTR coupled to the GFP reporter gene as a marker of the ongoing HIV-1 infection {15876}, {2381}. Briefly, the test compound was serially diluted, using a calibrated automatic pipetting station, in 96-well plates prefilled with 100 µL of cell media containing the human serum proteins studied, at twice the desired final concentration. Simultaneously, MT-4-LTR-EGFP cells were infected with HIV-1 for 1 hour at an MOI of 0.001 to 0.01. Subsequently, 100 µL of infected cells (1.5×10^5 cells/mL) were added to the 96-well plates described above. Finally, the cell cultures were transferred to a 384-well plate (40 µL/well, 4 replicates per well of a 96-well plate) and incubated at 37°C in a 5% CO2 atmosphere for 3 days. GFP production was measured at 488 nm.

2.6.2.2.8. Rilpivirine: In Vitro HIV-1 Resistance

2.6.2.2.8.1. Rilpivirine: Mechanisms of Resistance

In vitro selection experiments starting from wild type HIV-1 or from HIV-1 harboring mutations in the RT were performed to determine the rate of emergence of resistant viruses, as well as the mutations associated with decreased susceptibility to TMC278 upon drug selective pressure (Tabulated Summary 2.6.3.1.2, TMC278-IV1-AVMR). Two methods were used to isolate viruses under the selective pressure of TMC278. The first series of selections were high MOI selection experiments, where a high initial virus inoculum was propagated for several passages at a fixed drug concentration. This methodology was used to determine the rate of emergence of resistant viruses under selective pressure of TMC278, allowing for a standardized comparison with other NNRTIs. The second series of selections were low MOI selection experiments, where a low virus inoculum was propagated in the presence of increasing drug concentrations. This methodology was used to determine the mutations that were associated with decreased susceptibility to TMC278 in vitro.

2.6.2.2.8.2. In Vitro Selection from Wild-type and Mutant HIV-1 at High MOI and Fixed Drug Concentrations

The phenotype and genotype of the emerging HIV-1 strains were determined after sequential passage of wild type and mutant HIV-1 at a high MOI in the presence of fixed drug concentrations (2, 10, 40, 200, and 1000 nM). After 32 days in culture, no viral replication was detected in cells infected with HIV-1 subtype B (HIV_{IIIB}) at concentrations of TMC278 ≥ 40 nM (Figure 8). The same results were observed for recombinant clinical

HIV-1 isolates from group M subtypes A1, AE, AG, BG, C, D, F1, G, and H (Table 14) and from 2 HIV-1_{HXB2} site-directed mutants containing either the NNRTI mutation K103N or Y181C. The concentration to prevent viral replication of various wild-type HIV-1 group M subtypes inoculated at high MOI was lower than that needed for any of the other studied NNRTIs, indicating a high genetic barrier to the development of resistance. Another set of fixed-drug concentration selection experiments with TMC278 resulted in the selection of HIV-1 with V179D at Day 12 in culture with 40 µM TMC278, and HIV-1 with K101E at Day 22 in culture with 100 µM TMC278; however, these experiments were not internally controlled for timing of mutation development in comparison to other NNRTIs (Tabulated Summary 2.6.3.1.2, PC-264-2003).

Figure 8. Selection of Viruses Resistant to TMC278, ETR, EFV, or NVP Starting from Wild-type HIV-1_{IIIB}

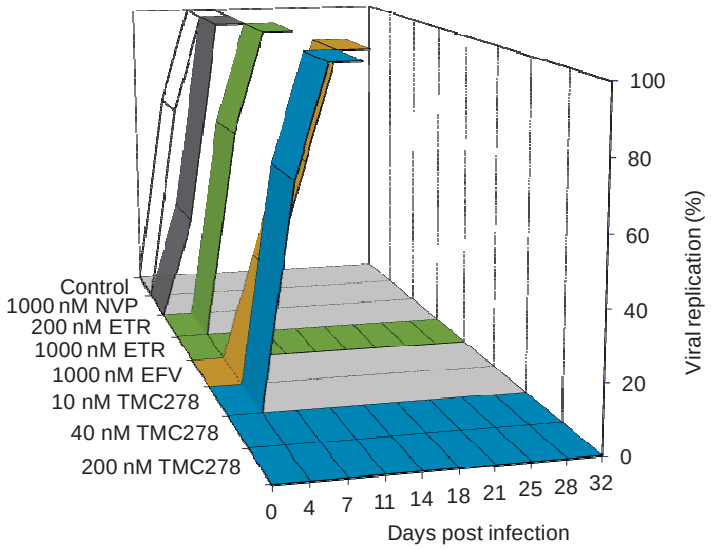


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Table 14. Time to Viral Replication of HIV 1 Group M Subtypes in the Presence of 3 Concentrations of TMC278

HIV-1 subtypes	Days to Viral Replication Breakthrough		
	TMC278		
	10 nM	40 nM	200 nM
A1	10	>31	>31
AE	>31	>31	>31
AG	>31	>31	>31
B	7	>31	>31
BG	10	13	>31
C	>31	>31	>31
D	20	>31	>31
F1	>31	>31	>31
G	>31	>31	>31
H	>31	>31	>31

Note: The time to breakthrough of resistant viruses was determined in cell culture, over a 32-day period, under selective pressure from various concentrations of TMC278, ETR, EFV, and NVP, or in the absence of NNRTIs (control).

For high MOIs and fixed drug concentration experiments, MT-4 cells were infected with wild-type HIV-1 at an MOI of 0.1 to 1.0 in the absence (control) or presence of different concentrations of the inhibitor. Cell cultures were maintained by repeat passages up to a maximum of 32 days in the presence of the initial concentration of each inhibitor and were examined for signs of viral replication. Assessment of cytopathic effect was used to determine viral replication.

2.6.2.2.8.3. In Vitro Selection from Wild-type and Mutant HIV-1 at Low MOI and Escalating Drug Concentrations

For the low-MOI and escalating drug concentration experiments, mutant strains resistant to TMC278 were isolated from cell cultures containing initial TMC278 concentrations ranging from 5 to 16 µM starting from wild-type and NNRTI resistant strains from various HIV-1 group M subtypes. The resistance profiles of the 12 HIV-1 strains are presented in Table 15.

2.6.2.2.8.4. Description of HIV-1 Mutant Strains Selected in the Presence of TMC278

The results from the high MOI selection experiments showed that 10 nM TMC278 was a suboptimal permissive concentration at which new HIV-1 strains emerged from subtypes A1, B, BG, and D and from the 2 HIV-1 site-directed mutants resistant to TMC278. These emerging HIV-1 strains were sequenced and harbored combinations of the NNRTI mutations L100I, K101E, V106I, Y181C, Y181I, and/or M230I. Diversity in the number of emergent NNRTI mutations was observed under the selective pressure of TMC278.

For the low MOI and escalating-drug concentration experiments, resistance profiles of the emerging HIV-1 strains are presented in Table 15. The data presented are limited to the emerging strains that were resistant to TMC278 (TMC278 fold change ranging from 3.8 to

13920.7). These isolates exhibited up to 5 NNRTI mutations. A greater number of emerging mutations was associated with a higher TMC278 fold change. The HIV-1 strain emerging from r13817 (subtype AE) harbored E138G in combination with M230I and H221Y. In this experiment, E138G evolved under further selective pressure to E138R; this final substitution from glutamic acid to arginine resulted in a significant increase in fold change for TMC278 (from 66.8 to > 1932.0), for EFV (from 268.3 to 4849.9), and for ETR (from 180.2 to > 3787.9). The increase in fold change for TMC278, EFV, and ETR in these isolates cannot only be attributed to the presence of E138R, because the HIV-1 site-directed mutants containing E138R had a fold change value of 3.2, 2.4, and 3.7 for TMC278, EFV, and ETR, respectively. The E138R mutation was also observed to emerge in combination with H221Y from the mutant strain SM041, which contained E138K at Day 0.

Genotypic analyses of the viral RT of the strains emerging under selective pressure of TMC278, which were performed with a range of viruses of different origins and genotypic profiles, suggest that the in vitro resistance profile of TMC278 may include the mutations V90I, L100I, K101E, V106A/I, V108I, E138G/K/Q/R, V179F/I, Y181C/I, V189I, G190E, H221Y, F227C, and M230I/L.

Table 15. Evolution of Viral Variants Selected With TMC278, Starting from Wild-type Strains from Subtypes AE, AG, B, C, and D, or from Mutant HIV-1 Strains

Subtype	Strain	Exp[a]	Day[b]	Conc (nM)[c]	Fold Change[d]			Mutations in HIV-1 RT[e]
					TMC278	EFV	ETR	
Wild-types								
AE	r13816[f]		0	0	0.4	1.5	0.9	
		1	69	10	3.8	7.4	5.0	**E138K**
		2	109	40	5.9	13.1	24.3	**E138K**, R358G
	r13817[f]		0	0	0.7	1.6	0.7	
			107	40	66.8	268.3	180.2	**E138G, V189I, H221Y, M230I**
			293	5000	>1932.0	4849.9	>3787.9	I50T, **E138R**, **V189I**, K219E, **H221Y**, **M230I**, E297K
AG	r13813[f]		0	0	0.7	0.8	0.9	
			108	40	5.4	13.4	26.2	E6A, **E138Q**, I178M, T200V, V245E
B	IIIB[g]		0	0	1.0	1.1	0.9	
		1	69	80	12.0	NA	17.9	**E138K**, **G190E**
			90	600	114.1	>12345.8	787.4	E6K, **E138K**, **G190E**
			121	5000	>1830.2	>15923.6	>3906.3	E6K, **L100I**, **E138K**, **G190E**, K219N

Subtype	Strain	Exp[a]	Day[b]	Conc (nM)[c]	Fold Change[d]			Mutations in HIV-1 RT[e]
					TMC278	EFV	ETR	
		2	66	62	34.6	19.7	45.3	**K101E**, **Y181C**, T386A
			108	4000	5490.6	3895.1	NA	**L100I**, **K101E**, **Y181C**, **F227C**, T386A
			122	16000	13920.7	>21240.8	4878.0	**L100I**, **K101E**, A158T, **V179F**, **Y181C**, **F227C**, T386A
		3	34	62	100.1	173.5	286.0	**V179F**, **Y181C**, **F227C**, **M230I**
			59	4000	7716.3	546.1	7381.9	**V179F**, **Y181C**, **F227C**, **M230I**
		4	34	5	5.0	5.4	5.7	E40K, **Y181C**
			62	80	315.4	427.8	1722.2	E40K, **V179F**, **Y181C**, **F227C**, **M230I**
			90	1500	626.9	783.6	244.8	E40K , V60A, **V90I**, **Y181C**, **F227C**, **M230I**
		5	32	40	23.7	30.9	27.1	E40K, **K101E**, **V108I**, **Y181C**, **H221Y**
			52	200	144.4	402.8	117.1	E40K, **K101E**, **V108I**, **Y181C**, K219E, **H221Y**, R358K
			70	5000	2154.6	1688.0	2566.5	E40K, **K101E**, **V108I**, **Y181C**, **F227C**, **M230I**
		6	116	2000	2022.6	1025.2	112.0	**L100I**, **K101E**, **Y181C**, P225L, **F227C**
C	v071130[f]		0	0	1.9	0.9	1.6	
			128	80	15.9	174.8	19.9	**E138K**, V241M, E399G
			276	5000	>1467.1	6191.1	>759.9	**V108I**, **E138K**, **M230L**, Y232F, V241M, G335Y, E399G
D	v071038[f]		0	0	0.4	0.3	0.3	
			93	80	26.0	287.8	224.0	L74V, **L100I**, T139K, **Y181C**, T240I, R284K
			139	3000	978.9	2455.1	2796.7	R72K, L74V, **L100I**, T139K, **Y181C**, T240I, E396K

Subtype	Strain	Exp[a]	Day[b]	Conc (nM)[c]	Fold Change[d]			Mutations in HIV-1 RT[e]
					TMC278	EFV	ETR	
			163	5000	>1984.1	15027.6	>2883.5	L74V, **L100I**, **V106A**, **V108I**, T139K, **Y181C**, T240I, V241M
Mutants								
B	SM041[h]		0	0	3.1	1.5	2.0	**E138K**
			90	40	13.2	6.4	3.1	**E138R**, K173Q, **H221Y**, G273E
			160	150	185.6	27.5	60.7	**L100I**, **E138R**, K173Q, **V179I**, **H221Y**, V241I, G273E
			212	5000	>3004.8	159.9	>41.3	**L100I**, **V108I**, I135T, **E138R**, K173Q, **V179I**, L214F, **H221Y**, V241I, G273E
	SM051[h]		0	0	0.7	17097.0	6.1	**L100I, K103N**
			93	5000	>1830.2	>15923	333.1	**L100I, K103N, E138G, V179I**, G196R, P225Y
	SM052[h]		0	0	3.2	348	1.7	**K101E, K103N**
			118	5000	>1947.0	4238.0	269.4	E28K, **K101E, K103N, V108I, E138G, V179I, Y181C**, K219R, L228R, T376S
	SDM020001[h]		0	0	3.1	1.8	9.1	**V179I, Y181C**
			161	5000	970.2	130.6	1674.2	K30I, **V106I**, **V108I**, **E138K**, T165A, **V179I, Y181C**, **V189I**, L214F, **H221Y**, N348I, E370K
	SDM020059[h]		0	0	8.0	4.5	184.0	**V179F, Y181C**
			171	5000	>2784.0	>24630.3	>3041.4	**L100I**, **V108I**, **V179F, Y181C**, L214F, N348I, E370G, V381I
	r9602[f]		0	0	3.2	ND	3.2	K102Q, **K103N**, E122K, **Y181C**, T200I, L214F, S322T, D324E, K366R, T400A

Subtype	Strain	Exp[a]	Day[b]	Conc (nM)[c]	Fold Change[d]			Mutations in HIV-1 RT[e]
					TMC278	EFV	ETR	
		1	28	40	7.4	102.2	ND	T69I, K102Q, **K103N**, E122K, **E138G**, S163N, **Y181C**, **V189I**, T200I, L214F, S322T, D324E, K366R, T386A, T400A
			52	200	237.7	91.7	409.4	K102Q, **K103N**, E122K, **E138G**, **V179I, Y181C**, T200I, L214F, S322T, D324E, N348I, V365I, K366R, T386A, T400A
			63	1000	156.9	26.1	159.6	K102Q, **K103N**, E122K, **E138G**, **V179I, Y181C**, T200I, L214F, S322T, D324E, N348I, K366R, T386A, T400A
		2	74	1000	142.4	312.8	485.1	L74I, K102Q, **K103N**, E122K, **E138G**, **V179I, Y181C**, T200I, L214F, S322T, D324E, N348I, K366R, T386A, T400A
			109	10000	460.3	332.8	635.2	M41I, L74I, K102Q, **K103N**, **V108I**, E122K, **E138G**, **V179I, Y181C**, T200I, E203K, L214F, S322T, D324E, N348I, K366R, T386A, T400A

a The experiments of which results are presented are those in which viruses resistant to TMC278 (fold change > 3.7) were obtained.

b Number of days in cell culture.

c TMC278 concentration at which the resistant isolate was selected.

d Calculated as the ratio between the EC50 of the compound for the emerging HIV-1 strain and that obtained in the same experiment with HIV-1IIIB. Each result is the mean for a single experiment run in duplicate. NA, not applicable; ND, not done

e The first 400 amino acids of the RT were sequenced. Emerging mutations are underscored and NNRTI resistance-associated mutations are in boldface.

f Recombinant HIV-1 clinical isolate

g Laboratory derived HIV-1 strain

h Site-directed mutant derived from HIV-1HXB2

Note: For low MOIs and escalating drug concentration experiments, MT-4 cells were infected at an MOI of 0.001 to 0.01 with wild-type HIV-1 ($HIV\text{-}1_{IIIB}$), NNRTI-resistant recombinant clinical HIV-1 isolates of various subtypes, or various NNRTI resistant $HIV\text{-}1_{HXB2}$ site-directed mutants in the presence of TMC278 at initial concentrations ranging from 1 to 10 nM. The cultures were maintained by repeat cell passages, and cells were examined for signs of viral replication. At 100% cytopathic effect, virus supernatants were collected and used to infect fresh cells at 0- to 5-fold-incremented concentrations of TMC278.

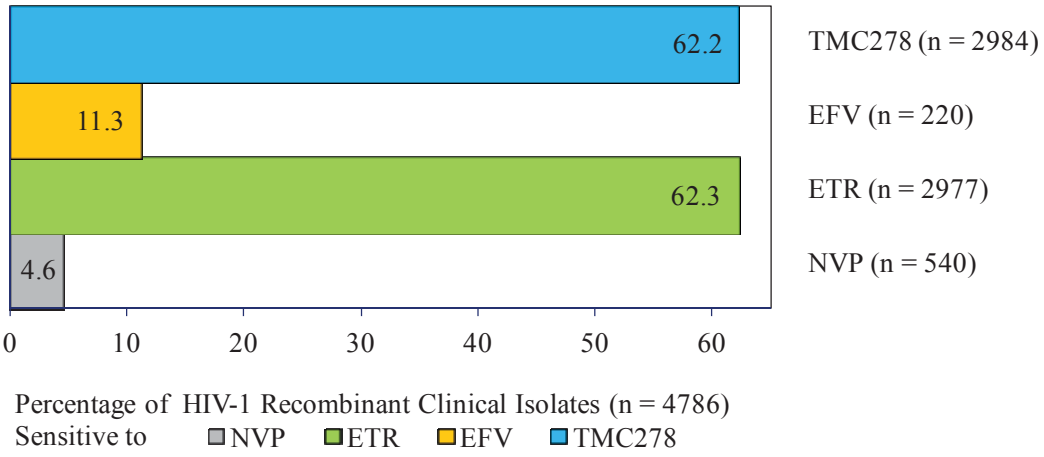
The crystal structures of TMC278 in complexes with the double mutant RT enzymes with K103N/Y181C (at 2.1 Å resolution) and L100I/K103N (at 2.9 Å resolution) demonstrated that TMC278 can adapt to bind mutant RTs. In the K103N/Y181C RT/TMC278 structure, loss of the aromatic ring interaction caused by the Y181C mutation was counterbalanced by interactions between the cyanovinyl group of TMC278 and the aromatic side chain of Y183, which was facilitated by a 1.5 Å shift of the conserved Y183MDD motif. In the L100I/K103N RT/TMC278 structure, the binding mode of TMC278 was significantly altered so that the drug conformed to changes in the binding pocket primarily caused by the L100I mutation. The flexible binding pocket acted as a molecular ‘‘shrink wrap’’ that made a shape complementary to the optimized TMC278 in wild-type and drug-resistant forms of HIV-1 RT. These crystal structures provided a better understanding of how the flexibility of an inhibitor could compensate for drug-resistance mutations.

Recently, another study reported data on the crystal structures of wild-type and K103N HIV-1 RT in complexes with etravirine (ETR) and TMC278 {15866}. TMC278 was positioned in a similar fashion as ETR in the NNRTI binding pocket, adopting the expected horseshoe type shape. The pyrimidine ring of TMC278 was situated between V179 and L100 with the secondary amine making the conserved hydrogen bond with K101 (2.7 Å). The K103 side chain was well ordered and was located below the benzonitrile and pyrimidine rings. The dimethylphenylacrylonitrile group was situated across the Y181 and Y188 side chains. The electron density for the acrylonitrile dropped off toward the end of the group, consistent with an increase in temperature factors for these atoms compared to the better positioned aromatic rings of TMC278. As previously observed, the acrylonitrile protruded through a hydrophobic opening formed by residues W229, Y188, F227, and L234 {15850}. TMC278 was also cocrystallized with K103N RT to gain insight into the nature of retained activity against this important resistance mutation. The position of TMC278 in the pocket revealed no evidence of shifting in response to the K103N mutation. In the crystal structure, the K103N was situated below TMC278, and the amide formed a long-range hydrogen bond to the secondary amine linker (3.1 Å) and the carbonyl of K101 (3.0 Å). These data confirmed that the diarylpyrimidine NNRTIs have inherent flexibility, helping to maintain activity against a wide range of resistance mutations. The crystal structures revealed a similar binding mode for etravirine and TMC278, whether bound to wild-type or K103N RT {15850}, {15866}.

2.6.2.2.8.5. Rilpivirine: Activity Against Drug-Resistant Variants of HIV-1

A large-scale evaluation of the antiviral activity of TMC278 was done on a panel of 4786 HIV-1 recombinant clinical isolates chosen from a library of 10,990 on the basis of resistance to a first-generation NNRTI (i.e., fold change > biological cut-off [BCO] for either or both EFV and NVP) (Tabulated Summary 2.6.3.1.2, TMC278-IV1-AVMR). The percentages of HIV-1 recombinant clinical isolates with sensitivity (i.e., FC ≤ BCO) to TMC278, EFV, ETR, and NVP are presented in Figure 9. The data show that, in vitro, 62% of these HIV-1 recombinant clinical isolates, resistant to at least 1 first-generation NNRTI, retained sensitivity to TMC278 or ETR as compared with 11.3% to EFV and 4.6% to NVP.

Figure 9. **Analysis of the Prevalence of HIV-1 Recombinant Clinical Isolates Sensitive to TMC278, ETR, EFV, or NVP Among Those Resistant to at Least One First-Generation NNRTI (n = 4,786)**



The analysis of a panel of HIV-1_{HXB2} site-directed mutants presented in Tabulated Summary 2.6.3.1.2 (TMC278-IV1-AVMR and TMC278-IV2-AVMR) showed that TMC278 retains antiviral activity against 63.0% (136 of 216) of the HIV-1_{HXB2} mutants carrying single, double, triple and quadruple RT mutations; as compared to 57.7% for ETR, 45.8% for EFV and 36.2% for NVP.

The 216 HIV-1_{HXB2} site-directed mutants were classified according to the number of RT mutations (panels of 1, 2, 3, or 4 mutations) in the viral genome and the sensitivity to TMC278, EFV, ETR, and NVP (Table 16). The data show that the proportion of HIV-1 mutants sensitive to TMC278 and ETR decreased as the number of mutations in the HIV-1_{HXB2} increased. The panel of HIV-1 mutants presented in Table 16 was designed on the basis of resistance to ETR and the information on emerging RT mutations in patients failing TMC278 enrolled in the Phase 2b and Phase 3 trials (Module 5.3.5.1, TMC278-C204-W96; Module 5.3.5.1, TMC278-TiDP6-C209; and Module 5.3.5.1, TMC278-TiDP6-C215).

Table 16. Proportion of HIV-1_{HXB2} Site-directed Mutants Sensitive to TMC278, EFV, ETR, and NVP on the Basis of the Number of Reverse Transcriptase Mutations in the Viral Clone

[n/N (%)]	Sensitivity to the NNRTI (fold change ≤ biological cut-off)			
HIV-1_{HXB2} with:	TMC278	EFV	ETR	NVP
1 RT Mutation	64/67 (95.5)	50/67 (74.6)	60/67 (89.6)	41/63 (65.1)
2 RT Mutations	49/79 (62.0)	30/77 (39.0)	40/79 (50.6)	15/71 (21.1)
3 RT Mutations	21/62 (33.9)	14/60 (23.3)	21/62 (33.9)	12/57 (21.1)
4 RT Mutations	2/8 (25.0)	3/8 (37.5)	3/7 (42.9)	4/8 (50.0)
Total	136/216 (63.0)	97/212 (45.8)	124/215 (57.7)	72/199 (36.2)

In Table 17, the median fold change for TMC278, EFV, ETR, and NVP for all 216 HIV-1_{HXB2} site-directed mutants are presented. The results of the analysis of this panel of HIV-1_{HXB2} site-directed mutants are discussed below.

TMC278 retained activity against 64 of 67 single HIV-1_{HXB2} site-directed mutants analyzed and only the 3 HIV-1 mutants with K101P, Y181I, or with Y181V were resistant to TMC278. More than 1, and usually more than 2 NNRTI mutations, were necessary to confer resistance to TMC278 in vitro. Resistance to TMC278 was mostly driven by the combination of specific NNRTI mutations rather than the total number of NNRTI mutations. K103N in isolation was not associated with resistance to TMC278 or ETR.

From all the double HIV-1_{HXB2} site-directed mutants with a combination containing V90I, only those with E138Q or Y181I were resistant to TMC278 in vitro. From the various combinations with K101E, only those with L100I, E138K, or M184V were sensitive to TMC278 in vitro.

The combination of E138K+M184I was resistant to TMC278 and ETR, but not to EFV and NVP. In contrast, the combinations of E138A+M184I, E138G+M184I, E138K+M184V, and K101E+E138K+M184V were sensitive to TMC278.

The addition of M184V to any of the combinations of RT mutations did not modify their sensitivity to TMC278, EFV, ETR, or NVP. M184I and M184V were not selected in vitro in the presence of TMC278.

TMC278 and ETR retained activity against most of the single and a small number of the double and triple HIV-1_{HXB2} site-directed mutants resistant to NVP and EFV. However, considerable cross-resistance between TMC278 and ETR was observed among double, triple, and quadruple site-directed mutants.

TMC278, as well as EFV, ETR, and NVP, retained activity against HIV-1_{HXB2} site-directed mutants harboring exclusively single or multiple NRTI mutations (Table 18).

These results confirmed that, in vitro, TMC278 had a greater activity against wild type HIV-1 than EFV, ETR, and NVP, and shows an improved resistance profile compared to EFV or NVP.

Table 17. Antiviral Activity of TMC278, EFV, ETR, and NVP Against HIV-1_{HXB2} Site-directed Mutants Containing Reverse Transcriptase Mutations

RT Mutation(s)	Median Fold Change			
	TMC278	EFV	ETR	NVP
V90I	1.7	1.6	1.5	4.4
L100I	0.9	20.3	1.3	7.3
K101A	2.2	4.1	1.8	12.8
K101D	1.4	5.7	1.0	17.0
K101E	2.4	3.8	2.7	ND
K101N	1.3	0.9	1.3	ND
K101P	51.7	72.3	5.3	>166.1
K101Q	1.6	2.4	1.4	2.5
K103H	0.5	14.4	0.6	7.6
K103N	0.9	32.5	0.9	>42.1
K103R	1.1	1.0	1.0	1.8
K103S	1.6	4.8	0.8	59.4
K103T	0.4	1.5	0.6	33.7
V106A	0.6	2.0	0.5	49.0
V106I	1.2	1.3	1.1	1.4
V106L	0.2	0.8	0.3	0.5
V106M	0.9	2.6	0.9	6.3
V108I	0.7	1.5	0.7	3.1
V108L	0.9	0.4	0.5	0.3
Y115F	1.0	1.7	1.1	4.3
E138A	2.5	1.6	2.9	2.3
E138G	1.6	0.9	2.4	2.8
E138K	2.8	2.0	2.6	1.1
E138Q	2.7	3.4	3.0	5.1
E138R	3.3	2.2	3.6	4.4
E138S	2.7	1.4	2.8	3.8
T139K	2.2	1.9	2.8	4.8

RT Mutation(s)	Median Fold Change			
	TMC278	EFV	ETR	NVP
T139R	1.4	2.2	1.2	7.3
I178L	1.7	2.0	2.1	4.4
V179D	1.7	2.7	1.9	4.6
V179E	1.2	5.7	1.3	2.1
V179F	<0.1	<0.4	<0.2	0.6
V179G	0.2	0.3	0.4	0.1
V179I	0.9	1.0	0.9	1.3
V179L	0.8	0.7	1.0	1.7
V179M	0.5	0.3	0.7	1.2
V179N	0.9	1.1	0.8	1.6
V179T	1.5	1.0	0.7	0.8
V179Y	0.1	0.2	0.2	0.2
Y181C	2.7	2.1	4.0	>43.0
Y181F	0.3	0.4	0.4	1.5
Y181I	15.3	1.6	12.5	>65.7
Y181V	12.2	3.0	15.1	2155.9
M184V	0.8	0.9	0.8	1.2
Y188C	0.1	1.5	0.2	25.5
Y188F	0.5	0.4	0.4	1.7
Y188L	2.8	42.6	1.1	>42.1
V189I	1.0	1.0	0.8	1.4
G190A	1.1	8.1	1.1	>86.6
G190S	0.2	94.8	0.2	96.9
G190T	0.6	11.3	0.7	ND
K219D	1.6	1.3	1.1	3.7
K219H	1.3	1.1	1.7	2.1
H221L	1.3	1.6	1.1	4.3
H221Y	1.8	1.6	1.6	4.3
P225H	0.4	1.5	0.6	2.1
F227C	3.1	4.5	2.5	ND
F227L	0.4	0.6	0.5	5.1
F227Y	0.8	1.0	1.1	1.1

RT Mutation(s)	Median Fold Change			
	TMC278	EFV	ETR	NVP
M230I	2.7	5.0	3.5	13.6
M230L	3.4	5.9	3.6	20.4
M230V	2.1	1.1	0.8	1.4
M236L	1.1	1.0	1.2	3.4
K238N	0.9	1.3	1.4	3.3
K238T	1.4	2.1	1.3	9.2
Y318F	1.3	0.8	1.1	2.8
Y318W	0.5	2.0	0.5	17.4
V90I E138K	2.9	2.0	2.8	2.6
V90I E138Q	5.5	4.1	6.1	ND
V90I H221Y	1.8	1.8	1.6	3.9
V90I Y181C	2.3	2.6	5.1	269.3
V90I Y181I	30.2	3.7	36.8	>501.3
A98G K101E	6.1	12.8	3.1	53.8
L100I T139K	1.2	37.1	1.8	9.5
L100I Y181C	2.0	20.3	12.2	222.7
L100I Y188L	293.7	12785.0	42.8	ND
L100I K101E	2.1	49.1	0.9	18.1
L100I K103N	7.0	575.8	4.0	>42.5
K101E E138K	1.9	1.8	2.6	3.6
K101E F227C	10.6	28.5	5.0	106.4
K101E H221Y	8.1	9.3	5.7	ND
K101E M184I	5.8	6.1	5.8	25.8
K101E M184V	2.2	4.7	2.8	10.6
K101E M230L	7.0	22.7	7.5	69.0
K101E V189I	5.0	6.1	3.3	24.2
K101H Y181C	11.3	8.2	13.1	>542.4
K101P G190A	20.2	3414.0	3.2	ND
K101P I167V	31.7	27.8	2.7	237.0
K101P K103S	429.3	1788.5	20.0	>747.9
K101P V108I	41.5	61.4	2.2	>718.1
K101E, K103N	2.0	56.4	1.8	>41.6

RT Mutation(s)	Median Fold Change			
	TMC278	EFV	ETR	NVP
K102L Y188L	10.0	230.5	2.6	>22.7
K103N E138R	1.3	11.0	1.6	116.0
K103N H221L	1.7	ND	0.9	>79.6
K103N K238T	0.9	42.9	0.8	133.0
K103N M184V	1.1	40.2	0.9	121.9
K103N V179I	1.1	14.7	0.9	34.8
K103N Y181F	0.3	5.8	0.2	23.7
K103N Y188F	0.9	ND	0.7	>61.8
K103N Y318F	1.0	57.5	1.0	124.2
K103R V179D	2.2	8.7	2.7	15.2
K103R Y181C	3.4	1.3	3.9	>130.7
K103N F227L	0.5	8.9	0.5	141.1
K103N V108I	0.5	96.0	0.7	>205.2
K103N Y181C	3.5	36.5	4.1	>41.8
K103N Y181I	94.9	6.4	16.1	>71.5
V106A F227L	2.4	11.0	1.2	>478.2
V106A Y181C	1.8	2.8	2.2	>456.4
V106I E138K	2.8	1.9	3.9	2.2
V106I V179I	1.5	1.4	1.3	1.7
V108I E138K	2.6	2.3	2.6	4.4
V108I E138R	3.0	3.1	3.4	7.6
V108I M184V	0.7	1.4	0.9	2.6
Y115F M184V	0.9	1.0	0.9	1.8
V118I V179L	0.7	0.8	0.9	1.9
E138A M184I	2.8	1.4	3.3	1.3
E138A Y181I	92.1	2.7	56.4	>449.2
E138G M184I	1.7	1.1	2.3	ND
E138G V179G	0.5	0.4	0.7	0.2
E138K M184I	6.7	2.4	5.1	3.8
E138K M184V	3.1	1.9	3.2	1.8
E138K T139K	3.8	3.0	4.6	ND
E138K V189I	4.9	3.6	4.8	ND

RT Mutation(s)	Median Fold Change			
	TMC278	EFV	ETR	NVP
E138R M184V	3.0	2.2	3.5	3.5
E138R V189I	3.2	3.8	5.2	7.7
E138K M230L	22.6	18.7	19.3	>63.7
I167V Y181C	2.8	1.6	4.1	90.0
V179I G190A	1.1	4.7	1.0	203.4
V179T Y181C	6.0	2.6	13.0	170.8
V179Y G190A	<0.1	1.0	<0.1	16.5
V179D Y181C	6.0	9.3	10.8	>71.5
V179E Y181C	6.8	13.1	30.6	>71.5
V179F Y181C	8.7	4.6	158.9	>358.3
V179F Y181I	11.4	1.0	122.8	>71.5
V179I Y181C	3.7	1.3	4.6	139.9
Y181C Y188C	0.4	1.4	0.7	>373.4
Y181C G190A	2.1	13.3	2.7	511.8
Y181C L234I	1.6	3.6	4.9	>43.4
Y181C M184I	2.5	2.0	3.0	156.4
Y181C F227C	23.6	12.1	25.3	>77.3
Y181C G190S	3.4	486.0	20.4	>71.5
Y181C Y188L	30.2	237.5	6	>157.7
M184V F227C	1.4	2.8	2.5	ND
M184V T215Y	0.3	0.4	0.3	0.7
F223C L234I	2.3	30.0	4.2	>59.6
F227C M230L	20.2	43.0	16.5	234.3
C38R V108I Y181C	1.7	3.6	3.1	>22.7
I50T L100I K103N	4.4	1511.4	3.8	>85.9
L74V L100I K103N	6.2	2354.3	2.8	>105.0
V90I E138A M184I	2.8	1.2	2.0	1.3
V90I E138A Y181I	150.9	2.1	87.6	>501.3
V90I E138K H221Y	5.6	3.6	7.2	3.9
V90I E138K M184I	4.6	3.7	3.9	2.3

RT Mutation(s)	Median Fold Change			
	TMC278	EFV	ETR	NVP
V90I E138K M184V	2.9	2.0	3.7	1.6
V90I E138Q V189I	6.1	6.3	9.8	20.5
V90I H221Y M230L	6.9	12.6	7.3	ND
V90I Y181C M184I	1.8	2.1	3.0	166.4
L100I E138K K219E	12.9	8.0	3.9	3.7
L100I E138K M184V	1.8	4.5	1.0	1.5
L100I E138K T139K	2.9	6.7	3.3	ND
L100I K101E F227C	132.3	520.3	5.1	>433.7
L100I K103N E138Q	52.9	3334.9	12.9	299.3
L100I K103N M184V	4.1	1531.1	1.7	>68.6
L100I K103N V179I	20.3	2396.6	4.0	>118.4
L100I, K103N, E138G	33.2	1695.3	20.8	>58.1
L100I K103N T386A	22.8	10866.5	12.2	>51.6
L100I K103N V179L	46.1	5660.6	13.4	>71.5
L100I K103N Y181C	80.8	1812.0	58.1	468.1
L100I V179I Y181C	15.2	16.9	34.1	>205.2
K101E E138K H221Y	4.2	3.7	3.7	9.6
K101E E138K M184I	2.0	1.3	2.6	2.5
K101E E138K M184V	1.8	1.6	1.6	ND
K101E Y181C H221Y	12.8	13.3	14.2	>628.7

RT Mutation(s)	Median Fold Change			
	TMC278	EFV	ETR	NVP
K101E Y181C M184V	4.5	4.7	2.7	247.3
K101H K103R Y181C	7.6	11.1	11.2	>503.2
K101Q K103N V108I	0.9	96.2	0.7	33.4
K101P K103N V108I	>162.1	12931.1	18.4	>51.6
K103N E138Q Y181C	10.5	25.3	8.2	>85.9
K103N K219D Y181C	13.1	72.9	20.2	>79.6
K103N T139R Y181C	7.4	61.2	7.0	>22.7
K103N V108I Y181C	4.1	110.2	2.1	>456.4
K103N V179I G190A	0.4	208.0	0.6	>415.9
K103N V179T Y181C	5.2	21.8	8.4	>591.5
K103N Y181C E194G	4.3	ND	3.9	ND
K103N Y181C G190A	1.0	555.5	1.9	>456.4
K103N Y181C K219H	11.3	49.0	14.5	>79.6
K103N Y181C Y318F	7.8	89.2	6.9	>376.7
K103N Y188S Y318F	0.5	32.4	0.6	241.5
K103R V179D Y181C	14.1	60.4	46.3	>542.4
K103R V179F Y181C	3.5	3.4	7.3	ND
K103N V179I Y181C	10.5	16.7	14.5	>429.9
V106A E138K F227L	39.9	12.0	5.5	>501.3
V106A E138K V179I	4.9	1.0	1.6	111.6

RT Mutation(s)	Median Fold Change			
	TMC278	EFV	ETR	NVP
V106I E138K V179I	2.7	1.1	2.1	1.2
V106M V179D Y181C	2.4	2704.1	28.8	>484.9
V108I E138K M184V	2.1	1.9	2.2	3.3
V108I E138K V179I	5.4	2.0	3.4	7.6
V108I Y181C G190A	1.2	55.7	1.9	>456.4
E138A Y181I M184I	33.0	1.2	20.0	>501.3
E138K M184V M230L	1.8	1.9	2.7	6.0
E138K V179I M184V	2.2	1.1	2.8	1.4
E138K V189I H221Y	7.5	5.7	10.9	9.1
T139R Y181C Y188L	114.3	ND	26.6	>22.7
V179I Y181C G190A	1.2	9.8	2.1	661.8
V179F Y181C F227C	553.8	25.7	638.6	>71.5
Y181C M184V H221Y	2.1	3.5	3.5	>494.3
M184V K219N M230V	4.1	2.4	3.4	2.1
F227C M230L M184V	19.3	56.2	14.3	352.1
L74V L100I K103N M184V	5.5	1642.4	3.2	>79.0
V90I E138A Y181I M184I	20.5	0.8	17.3	>478.3
V90I E138K M184V H221Y	5.2	4.2	5.1	4.3
L100I E138K M184V K219E	6.9	7.7	2.9	2.6
K101E Y181C M184V H221Y	8.0	5.8	7.3	>470.0

RT Mutation(s)	Median Fold Change			
	TMC278	EFV	ETR	NVP
V106A E138K V179I F227L	47.5	13.2	9.2	>501.3
V106I E138K V179I M184V	2.2	1.3	3.0	1.4
E138K V179I M184V K219E	1.7	1.3	ND	1.2

Note: Median fold change values above the respective biological cut-off (BCO) of the tested drug were shaded in grey.
Source: TMC278-IV1-AVMR and TMC278-IV2-AVMR

Table 18. Antiviral Activity of TMC278, EFV, ETR and NVP Against HIV-1_{HXB2} Site-directed Mutants Harboring NRTI Mutations

NRTI Mutation(s)	In Vitro Phenotypic Susceptibility Median Fold Change			
	TMC278	EFV	ETR	NVP
None[a]	1.0	1.0	1.0	1.0
M41L[a]	0.7	0.5	0.9	1.3
A62V	0.7	0.9	0.8	2.0
K65R[a]	1.0	1.4	ND	ND
L74V	1.0	1.0	1.1	2.0
Y115F[b]	1.0	1.7	1.1	4.3
Q151M	1.8	1.7	1.8	4.5
M184V[b]	0.8	0.9	0.8	1.2
M41L T215Y	0.4	0.7	0.4	1.1
K65R M184V	0.6	0.7	0.6	1.2
Y115F M184V[b]	0.9	1.0	0.9	1.8
M184V T215Y[b]	0.3	0.4	0.3	0.7
M41L L210W T215Y	0.5	0.4	0.3	0.9
M41L T69S-SG T215Y[a]	0.5	<0.4	0.3	0.9
T69S-SS L210W T215Y[a]	0.7	0.6	0.5	0.8
M41L D67N K70R T215Y	0.3	0.6	0.2	1.5
M41L V75M L210W T215Y	0.6	0.7	0.5	1.1
M41L D67N L210W T215Y[a]	0.7	1.0	0.7	1.8
D67N K70R K219Q T215F	0.9	0.7	0.7	0.8
V75I F77L F116Y Q151M	2.2	2.0	2.0	1.9

NRTI Mutation(s)	In Vitro Phenotypic Susceptibility			
	Median Fold Change			
	TMC278	EFV	ETR	NVP
M41L D67N T69D L210W T215Y[a]	<0.5	0.5	1.0	1.4
M41L D67N V75M L210W T215Y[a]	0.6	0.8	0.2	0.4
M41L D67N M184V L210W T215Y	0.3	0.4	0.3	0.7
M41L D67N T69D V118I L210W T215Y	0.4	0.4	0.3	0.7
M41L D67N K70Q M184V L210W T215Y	0.3	0.3	0.3	0.5
M41L M184V L210W R211K L214F T215Y[a]	<0.5	0.8	0.9	0.6
M41L T69S-SG L210W R211K L214F T215Y[a]	<0.5	<0.4	0.4	0.4
M41L T69S-SS L210W R211K L214F T215Y[a]	<0.5	<0.4	<0.2	0.3
M41L D67N T69D L210W R211K L214F T215Y	0.8	0.5	0.6	1.4
A62V T69S M184V L210W R211K L214F T215Y[a]	0.6	<0.4	0.5	0.7
M41L D67N M184V L210W R211K L214F T215Y[a]	0.7	0.5	0.6	0.7
M41L E44D D67N L210W R211K L214F T215Y[a]	0.7	0.9	0.8	1.4
M41L E44D D67N T69D L210W R211K L214F T215Y[a]	0.7	0.7	0.9	1.6
M41L E44D D67N V118I L210W R211K L214F T215Y[a]	<0.5	0.9	0.4	1.0
D67N T69D V75M M184V L210W R211K L214F T215Y[a]	1.0	1.0	1.0	1.3
M41L E44D D67N T69D V118I L210W R211K L214F T215Y[a]	<0.5	<0.4	0.9	0.9
M41L E44D D67N V118I M184V L210W R211K L214F T215Y[a]	<0.5	<0.4	0.4	0.8
M41L E44D D67N T69D V118I M184V L210W R211K L214F T215Y[a]	0.8	0.6	0.5	0.6

a Except for those marked (single experiment), data are presented as the median of 3 independent experiments. All experiments were run in duplicate.

b Some of the site-directed mutants are repeated from Table 17 for completeness.

ND = not done.

2.6.2.2.9. Rilpivirine: In Vitro Efficacy Against Animal Retroviruses

The antiviral activity of TMC278 against SIV_{mac251} was compared with that of EFV, ETR, and NVP in MT-4-LTR-EGFP cells (Table 19; Tabulated Summary 2.6.3.1.2, TMC278-IV2-AVMR). The median EC_{50} value for TMC278 against SIV_{mac251} observed in the MT-4-LTR-EGFP cell line was 8.55 µM.

Table 19. In Vitro Antiviral Activity of TMC278, EFV, ETR, and NVP Against SIV_{mac251}

Virus	Median EC_{50}			
	TMC278	EFV	ETR	NVP
$HIV\text{-}1_{IIIb}$	0.73 nM	1.73 nM	2.73 nM	34.09 nM
SIV_{mac251}	8.55 µM	24.40 µM	>19.67 µM	> 31.48 µM

The antiviral activity was determined in a cell-based assay which measured the ongoing replication of viruses in MT-4-LTR-EGFP cells, and using the specific interaction of HIV tat with the HIV-1 LTR coupled to the GFP reporter gene as a marker of the ongoing HIV-1 infection {15876}, {2381}. Briefly, 384-well plates were filled with various concentrations of the test compound obtained by serial dilutions in the cell culture medium (RPMI-1640 containing 10% FCS). MT-4 cells and HIV or SIV were added into the 384-well plate at a concentration of 1.5 x 10^5 cells/mL and at a MOI of 0.01 in 40 µL. Subsequently, the cell cultures were incubated at 37°C in a 5% CO_2 atmosphere for 3 days.

2.6.2.2.10. Tenofovir DF: Mechanism of Action

2.6.2.2.10.1. Tenofovir DF: Intracellular Metabolism

In vitro

Tenofovir is a nucleotide analog (i.e., a nucleoside monophosphate analog) and therefore not dependent on an intracellular nucleoside kinase activity for the first step in the conversion to the active metabolite, PMPApp. In some cells, intracellular concentrations of nucleoside kinases are cell-cycle dependent. In particular, thymidine kinase, which is responsible for the phosphorylation of ZDV and d4T, is expressed at low levels in nonproliferating lymphocytes and macrophages. This difference in intracellular metabolism suggests that TFV may be a more effective inhibitor of HIV in macrophages and other nondividing cells as compared to some other nucleoside analogs. The cellular enzymes responsible for TFV anabolism to the phosphorylated forms are adenylate kinase (AK) (Tabulated Summary 2.6.3.1.3, {13}) and nucleotide diphosphate kinase, which are highly active and ubiquitous. Adenylate kinase exists as 2 isozymes, AK1 and AK2, with the phosphorylation of TFV mediated more efficiently by AK2.

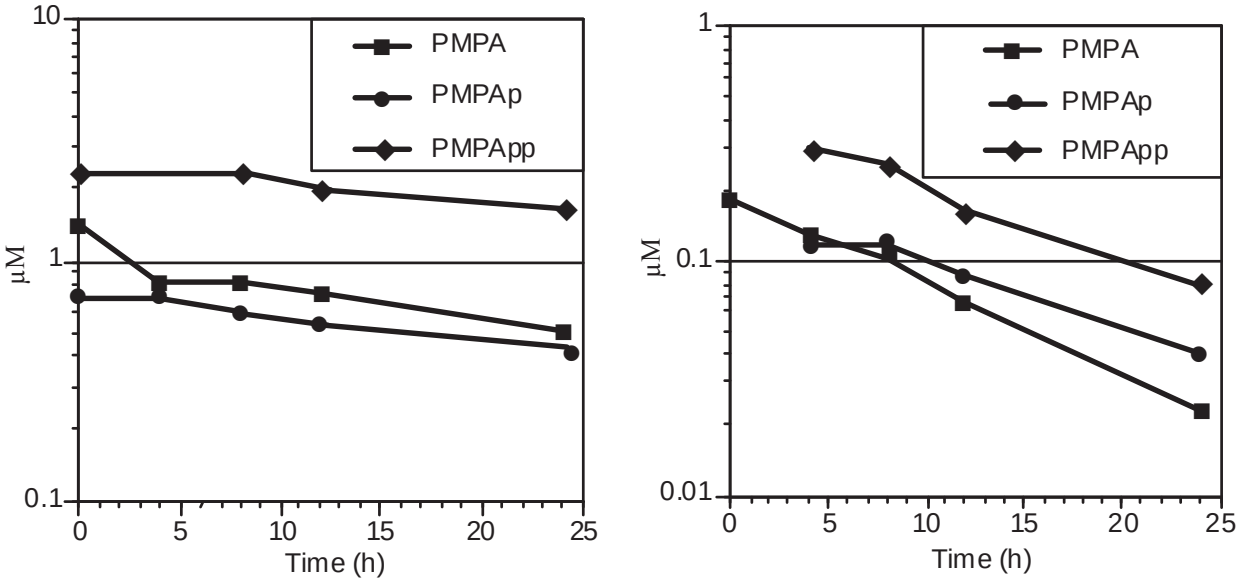
The intracellular metabolism of TFV was studied in PBMCs (Tabulated Summary 2.6.3.1.3, {1574}). The intracellular half-life of TFV and the phosphorylated metabolites tenofovir monophosphate (PMPAp) and PMPApp differs in resting cells versus activated PBMCs, as presented in Figure 10. In resting human PBMCs the half-life of PMPApp was found to be approximately 50 hours, whereas the half-life in PHA-stimulated PBMCs was found to be approximately 10 hours (Tabulated Summary 2.6.3.1.3, {1574}). This long intracellular half-life supports once daily dosing.

The intracellular phosphorylation of TFV and ABC to their active anabolites, PMPApp and carbovir triphosphate (CBV-TP), respectively, was investigated in vitro in CEM-SS cells for potential antagonism (Tabulated Summary 2.6.3.1.3, PC-104-2008). The results demonstrate no difference in the intracellular phosphorylation of either compound when present in combination as compared to when given alone (Table 20 and Table 21). These in vitro results

indicate that there is no overlap between the anabolic routes of these NRTIs and thus limited potential for a drug interaction. These results are in agreement with combination results that show additive anti-HIV activity of these compounds and a lack of antiviral antagonism (see Section 2.6.2.5.1).

Figure 10. Tenofovir Intracellular Metabolism in Human PBMCs

Resting PBMCs: **PHA-Stimulated PBMCs:**



Data from reference: {1574}

Table 20. Metabolism of 10 µM Tenofovir (PMPA) either Alone or in Combination with 10 µM Abacavir (ABC)

Time	PMPA Alone (pmols/million cells)[a]			PMPA with ABC (pmols/million cells)[a]		
(h)	PMPA	PMPAp	PMPApp	PMPA	PMPAp	PMPApp
2	1.27 ± 0.18	0.05 ± 0.01	0.17 ± 0.01	1.13 ± 0.03	0.05 ± 0.01	0.14 ± 0.02
24	1.71 ± 0.33	0.45 ± 0.02	1.54 ± 0.12	1.59 ± 0.14	0.41 ± 0.01	1.57 ± 0.04

a Values determined by LC/MS/MS.

Data from reference: PC-104-2008

Table 21. Metabolism of 10 μM Abacavir (ABC) either Alone or in Combination with 10 μM Tenofovir (PMPA)

Time	ABC Alone (pmols/million cells)[a]	ABC with PMPA (pmols/million cells)[a]
(h)	CBV-TP[b]	CBV-TP
2	0.03 ± 0.01	0.05 ± 0.02
24	0.17 ± 0.01	0.24 ± 0.03

a Values determined by LC/MS/MS.

b Carbovir triphosphate (CBV-TP) is the active anabolite of abacavir (ABC).

Data from reference: PC-104-2008

Co-administration of TDF and ddI has been clinically associated with increased plasma concentrations of ddI. In vitro studies have shown that tenofovir monophosphate and tenofovir diphosphate can inhibit purine nucleoside phosphorylase (PNP) which is involved in the metabolic degradation of ddI (Tabulated Summary 2.6.3.1.3, {6054}). Thus, the clinical observation of increased ddI plasma concentrations appears to be due to inhibition of its degradation via inhibition of PNP by TFV.

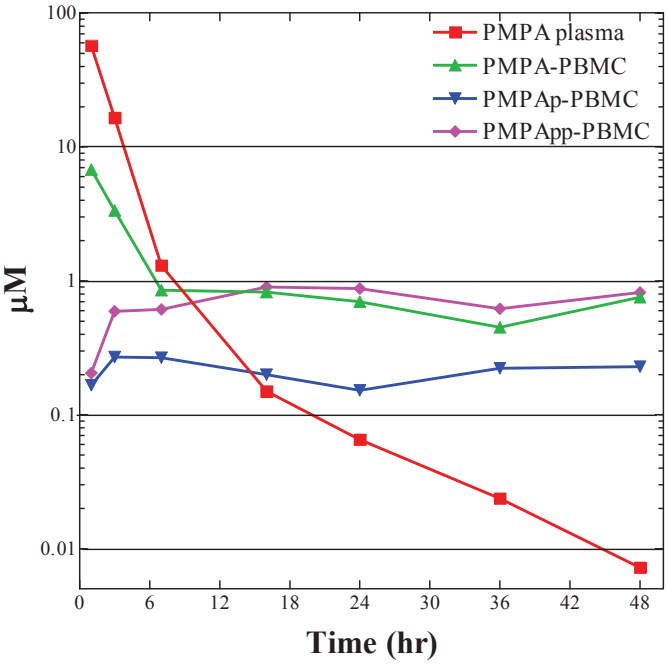
In order to assess the potential effect of TFV on the natural pools of 2'-deoxynucleoside triphosphates (dNTP) and ribonucleoside triphosphates (rNTP), the T-cell line CEM-CCRF was treated with TFV, either alone or in combination with other NRTIs (Tabulated Summary 2.6.3.1.3, {8573}). There was no effect of TFV alone, or TFV plus ABC, ddI, or 3TC on either the dNTP or rNTP pool concentrations intracellularly. Zidovudine was the only tested NRTI that significantly altered dNTP pools. Incubation of 10 μM ZDV, either alone or in combination with other NRTIs, increased dATP, dGTP, and TTP levels by up to 44%. In contrast to a control potent PNP inhibitor, TFV was unable to alter intracellular dNTP pools upon addition of exogenous 2'-deoxyguanosine. While inhibition of PNP-dependent ddI degradation is the most plausible mechanism for the drug interaction between ddI and tenofovir, TFV does not appear to be a potent enough inhibitor of PNP to cause changes in nucleotide pools.

In vivo

The kinetics of intracellular TFV metabolism were studied in monkeys that received a single dose of 30 mg/kg [^{14}C]TFV subcutaneously. After dosing, serial blood samples were obtained and were analyzed. Plasma TFV levels and PBMC intracellular TFV and TFV metabolite concentrations were determined (Tabulated Summary 2.6.3.1.3, P2001025). The TFV concentration in plasma reached a maximum of approximately 50 μM and declined with a half-life of 5 to 7 hours. Consistent with the in vitro studies, TFV is efficiently taken up by PBMCs and is metabolized to PMPApp, with the intracellular concentrations of the active metabolite PMPApp reaching 0.9 μM. The half-life of PMPApp in this experiment was > 50 hours (Figure 11).

To assess the metabolism of TFV in lymph nodes, axillary, inguinal, and mesenteric lymph nodes were obtained at 48 hours after dosing of [^{14}C]TFV in the monkey. As observed in PBMCs, significant intracellular concentrations of TFV and its metabolites were observed in lymph node mononuclear cells from all 3 sites (Table 22).

Figure 11. Plasma Tenofovir Concentrations and Concentrations of Tenofovir, Tenofovir Monophosphate (PMPAp), and Tenofovir Diphosphate (PMPApp) in PBMCs After Administration of a Single Dose of [^{14}C]Tenofovir to Monkeys



Data from reference: P2001025

Table 22. Tenofovir and Metabolites in Selected Lymph Nodes of a Monkey 48 Hours After Administration of a Single Dose of [^{14}C]Tenofovir

Node	Intracellular Concentration (µM)		
	Tenofovir	PMPAp	PMPApp
Axillary	0.37	0.26	0.27
Inguinal	1.3	0.65	ND
Mesenteric	5.2	0.17	0.13

ND = not determined.

Data from reference: P2001025

2.6.2.2.11. Tenofovir DF: Inhibition of Viral and Human DNA Polymerases

2.6.2.2.11.1. Tenofovir DF: HIV-1 Reverse Transcriptase

Inside the cells, TFV is converted to its active metabolite PMPApp. Tenofovir diphosphate efficiently inhibits both RNA- and DNA-directed HIV-1 RT DNA polymerization. It competes with dATP for incorporation into DNA and since it lacks a 3' hydroxyl group, causes premature termination of DNA synthesis upon its incorporation into the nascent DNA chain. The kinetic inhibition constants (K_i) for PMPApp against HIV-1 RT have been determined using both RNA and DNA templates (Table 23). K_i values for reverse transcription (RNA-dependent DNA synthesis) and the second strand DNA synthesis (DNA-dependent DNA synthesis) are 0.02 and 1.6 μM, respectively (Tabulated Summary 2.6.3.1.3, {1131}). The removal of incorporated TFV can occur by wild-type RT and RT with mutations that enhance excision (notably T215F/Y or T69S-SS) by both pyrophosphorolysis and ATP-mediated excision mechanisms; however, TFV-terminated primers are efficiently translocated which can result in partial protection from the excision process (Tabulated Summary 2.6.3.1.3, {7583}, {11304}).

Table 23. Kinetic Inhibition Constants of Tenofovir Diphosphate Against HIV-1 Reverse Transcriptase

Template	K_i (μM)	K_m dATP (μM)	K_i/K_m
RNA template	0.02	0.05	0.40
DNA template	1.6	4.6	0.35

Data from reference: {1131}

2.6.2.2.11.2. TDF: Cellular DNA Polymerases

The in vitro specificity of PMPApp for viral polymerases relative to its interaction with mammalian DNA polymerases was determined.

Table 24 summarizes the inhibitory effects of PMPApp on DNA synthesis catalyzed by the mammalian DNA polymerases α, β, and γ, and by the rat DNA polymerases δ and ε (Tabulated Summary 2.6.3.1.3, {1131}), {2516}. The K_m for the natural substrate dATP is also shown. Tenofovir diphosphate showed specificity for HIV-1 RT with K_i/K_m ratios 5- to 200-fold higher for mammalian DNA polymerases compared to HIV-1 RT. The K_i/Km ratio was particularly high (85.3) for mitochondrial DNA polymerase γ, suggesting a low potential of TFV to interfere with the synthesis of mitochondrial DNA (Tabulated Summary 2.6.3.1.3, {1131}). Additional studies have shown that 1 mM PMPApp exhibited little effect on the in vitro replication of SV40 DNA indicating a significant specificity of PMPApp towards the viral RT in comparison to the host DNA replication complex {2517}. Similar conclusions of strong specificity of PMPApp towards HIV-1 RT have been made using pre-steady state enzyme kinetic experiments {2518}.

Table 24. Kinetic Inhibition Constants of Tenofovir Diphosphate Against DNA Polymerases α, β, γ, δ, and ε

Enzyme	K_i (μM)	K_m dATP (μM)	K_i/K_m
Human DNA pol α	5.2	2.7	1.92
Human DNA pol β	81.7	5.6	14.6
Human DNA pol γ	59.5	0.7	85.3
Rat DNA pol δ/PCNA	7.1	0.7	10.2
Rat DNA pol ε	95.2	6.1	15.6

Data from references: {1131}, {2516}

In order to evaluate PMPApp as a potential substrate for host polymerases, its incorporation efficiency into a DNA primer/template by human DNA polymerases α, β, and γ relative to the natural dNTPs has been determined and compared with that of the triphosphates of other NRTIs (Tabulated Summary 2.6.3.1.3, {2005}). Tenofovir diphosphate showed similar or lower incorporation by DNA polymerases α and β compared to ddATP (the active metabolite of ddI), ddCTP, 3TC-TP, and d4T-TP (Table 25). Importantly, DNA pol γ incorporates PMPApp into a DNA primer/template with a very low efficiency (0.06%) relative to the natural substrate. This observation confirms the conclusions from the inhibition studies above.

Table 25. Relative Efficiencies of Incorporation into DNA of Tenofovir Diphosphate and NRTI-Triphosphates by Human DNA Polymerases α, β, and γ

dNTP Analog	Relative Efficiency of Incorporation (%)[a]		
	Pol α	Pol β	Pol γ
PMPApp	1.4	1.3	0.06
ddATP	0.25	80	20
ddCTP	0.1	125	25
3TC-TP	0.05	9.0	0.13
d4T-TP	6.3	142	8.0

a f inc (%) = 100 × $[V_{max}(\text{dNTP analog})/K_m(\text{dNTP analog})]/[V_{max}(\text{dNTP})/K_m(\text{dNTP})]$.

Data from reference: {2005}

2.6.2.2.12. Tenofovir DF: In Vitro Evaluation of Antiretroviral Activity

2.6.2.2.12.1. Tenofovir DF: Anti-HIV Activity

Tenofovir and TDF were evaluated in vitro for antiviral activity (EC_{50}) and cytotoxicity (CC_{50}) using the HIV-1_{IIIb} strain in MT-2 cells and PBMCs (Table 26). The selectivity index ($SI = CC_{50}/EC_{50}$) for TFV was > 2,000. Due to its increased cellular permeability, the anti-HIV activity of TDF was increased by 17- to 90-fold over TFV. The selectivity index also was higher for TDF (Tabulated Summary 2.6.3.1.3, {1574}). In primary macrophage/monocyte cultures, TFV has an EC_{50} of 0.04 µM against the macrophage-tropic HIV-1_{Bal} strain (Table 26) (Tabulated Summary 2.6.3.1.3, {625}). In other assay systems and cell types, the antiviral activity of TFV against wild-type laboratory strains of HIV-1 ranged from 0.2 to 6.0 µM (Tabulated Summary 2.6.3.1.3, {39}, {2078}, {1649}, {1574}). Data from Virco NV (Mechelen, Belgium) have shown a mean TFV EC_{50} value of 0.9 µM against a panel of 10 wild-type clinical isolates in the Antivirogram™ susceptibility assay (Tabulated Summary 2.6.3.1.3, P4331-00035). Data from a large panel of recombinant wild-type patient viruses (n = 2536) from Monogram Biosciences showed a median TFV EC_{50} value of 0.7 µM with a biological cut-off determined at 1.2-fold from their wild-type control (Tabulated Summary 2.6.3.1.3, {7289}. Tenofovir has also been shown to be active against HIV-2, with similar potency as observed against HIV-1 (range: 1.6 to 5.5 µM) (Tabulated Summary 2.6.3.1.3, {39}, PC-104-2003, PC-104-2013).

Table 26. Anti-HIV Activity (EC_{50}) and Cytotoxicity (CC_{50}) of Tenofovir and Tenofovir DF

	Cells / Virus						
	MT-2 / HIV-1 IIIb			PBMC / HIV-1 IIIb			MØ-monocytes / HIV-1 Bal
Drug	EC_{50} (µM)	CC_{50} (µM)	SI[a]	EC_{50} (µM)	CC_{50} (µM)	SI[a]	EC_{50} (µM)
Tenofovir	0.63	1250	1984	0.18	1200	6666	0.04
TDF	0.007	22	3142	0.005	29	5800	ND

a Selectivity index; ratio of CC_{50} to EC_{50}.

ND = not determined.

Data from references: {1574}, {625}

Tenofovir was investigated in a novel antiviral persistence assay to assess the ability of the intracellular drug to maintain antiviral suppression over extended periods of time (Tabulated Summary 2.6.3.1.3, {9497}). Tenofovir demonstrated long-term antiviral persistence through 24 hours in primary $CD4^+$ T cells (58.7% persistence at 24 hours), whereas, ABC was much less persistent (< 0.5% persistence at 4 hours) in comparison. Consistent with the sustained antiviral persistence of TFV, the calculated in vitro half-life of tenofovir diphosphate in primary $CD4^+$ T cells was ~21 hours compared to ~5 hours for the active moiety of ABC, carbovir triphosphate.

2.6.2.2.12.2. Tenofovir DF: Activity Against HIV-1 Subtypes

The activity of TFV against clinical HIV-1 isolates from non-B subtypes has also been studied (Tabulated Summary 2.6.3.1.3, {5044}). The mean EC_{50} values for TFV against HIV-1 subtypes A, C, D, E, F, G, and O in primary PBMC cultures were all within 2-fold of the subtype B EC_{50} value (range: 0.54 to 2.2 μM). For comparison, similar results were obtained for ZDV, with all non-B subtypes having EC_{50} values with 3-fold of the subtype B EC_{50} value.

Table 27. Mean EC_{50} Values for HIV-1 Subtypes A, B, C, D, E, F, G, and Group O Strains

Subtype	Mean Drug EC_{50} (μM)[a]	
	Zidovudine	Tenofovir
A (n = 4)	0.01 ± 0.01	1.4 ± 0.3
B (n = 3)	0.01 ± 0.005	1.1± 0.2
C (n = 3)	0.03 ± 0.04	2.1 ± 2.1
D (n = 3)	0.01 ± 0.01	0.5 ± 0.4
E (n = 3)	0.02 ± 0.01	2.2 ± 0.8
F (n = 3)	0.02 ± 0.02	0.7 ± 0.4
G (n = 3)	0.004 ± 0.003	0.6 ± 0.3
O (n = 3)	0.01 ± 0.01	0.9 ± 0.5

a Results are expressed as the mean ± the standard deviation.
Data from reference: {5044}

2.6.2.2.12.3. Tenofovir DF: Effect of Multiplicity of Infection

The effect of MOI on the antiviral activity of TFV was assessed in MT-2 using wild-type HIV-1_{LAI} (Tabulated Summary 2.6.3.1.3, PC-180-2018). The historical EC_{50} for TFV of 3.4 μM was measured at HIV-1 MOI ≤ 0.004. The EC_{50} for TFV was increased with increasing MOI, with the final EC_{50} value approximately 10-fold increased at the highest MOI tested of 0.3. This trend was similar to that observed for FTC and other NRTIs.

2.6.2.2.12.4. Tenofovir DF: Effect of Serum Proteins

The protein binding of TFV was studied in the presence or absence of human plasma and human serum (Tabulated Summary 2.6.3.1.3, Report P0504-00039.1). The percentage of TFV that remain unbound in human plasma and human serum were 99.3% and 92.8%, respectively.

2.6.2.2.13. Tenofovir DF: In Vitro HIV-1 Resistance

Successive passage of HIV-1 in increasing concentrations of TFV resulted in virus with a K65R mutation in RT (Tabulated Summary 2.6.3.1.3, {2078}). A site-directed recombinant virus expressing this mutation showed 3-fold reduced susceptibility to TFV, 3- to 12-fold reduced susceptibility to ABC, ddI, FTC, 3TC, ddC, and d4T, but full susceptibility to ZDV (Table 28). The K65R mutation has also been selected in vitro by ddC, d4T, and ABC; and in vivo by ddI, ddC and ABC {1003}, {1004}, {1793}, {2141}, {4573}. However, in vivo the K65R mutation is infrequently observed to develop with these antiretroviral drugs and is observed in the plasma HIV of 2% to 4% of antiretroviral-experienced patients {2187}, {3799}, {12143}.

A study demonstrated a greater propensity for HIV-1 of subtype C to develop a K65R mutation under in vitro selection with TFV as compared to subtype B or other non-B subtypes (Tabulated Summary 2.6.3.1.3, {9276}). The mechanistic basis for this in vitro observation was not clear, as the single nucleotide change is identical for the K65R substitution in either subtype B or subtype C. Gilead Sciences evaluated patients with subtype C, subtype B, and other non-B HIV-1 subtypes for virologic failure and development of resistance in two phase 3 clinical studies of TDF {11322}. The virologic failure rates were similar between subjects with subtype B or non-B HIV-1 subtypes (15.5% versus 19%, respectively, p = 0.75 for Study GS-99-903 at Week 144; 19% versus 13%, respectively, p = 1.0 for Study GS-01-934 at Week 48). Among the 10 subjects with subtype C treated with TDF in both studies, only 1 subject was classified as virologic failure and this subject did not develop a K65R mutation or any other resistance mutations. For Studies TMC278-TiDP6-C209 and TMC278-TiDP6-C215, there were a total of 5 subjects in the FTC+TMC278+TDF group and 2 subjects in the EFV+FTC+TDF group that had emergent K65R during the treatment period. Of those, 2/5 in the TMC278 group and 2/2 in the EFV group with K65R were subtype C HIV-1, compared to the overall prevalence of 69.2% and 12.1%, respectively, for HIV-1 subtype B versus subtype C in the pooled Phase 3 trials (see Module 2.7.3 and Report PC-264-2005).

Table 28. Drug Susceptibilities for HIV-1 Containing the K65R or K65R + M184V RT Mutations

Virus	Mean EC_{50} in µM (Fold change from wild-type[a])													
	Tenofovir		ZDV		FTC		3TC		ddI		d4T		ABC	
Wild-type	3.4	(1.0)	0.11	(1.0)	0.77	(1.0)	3.9	(1.0)	3.5	(1.0)	5.9	(1.0)	0.34	(1.0)
K65R SDM[a]	10.2	(3.0)	0.10	(0.9)	6.4	(8.4)	46.0	(11.7)	9.8	(2.8)	14.3	(2.4)	1.3	(3.9)
K65R (n=5)	7.4	(2.1)	0.07	(0.6)	9.1	(11.8)	71.6	(18.3)	8.0	(2.3)	10.0	(1.7)	0.89	(2.6)
K65R + M184V (n=3)	6.0	(1.7)	0.06	(0.6)	>167	ND	>167	ND	11.5	(3.3)	7.0	(1.2)	2.0	(6.0)

a Site-directed mutant containing only the K65R mutation.

Data from reference: TPI 15883

The FTC/3TC-associated M184V mutation in combination with the K65R mutation results in an increase in TFV susceptibility. This has been shown in numerous experimental settings, including site-directed mutagenesis studies with a laboratory-derived RT sequence and of patient-derived HIV-1 expressing the K65R mutation. The range of susceptibilities to TFV for the K65R + M184V double mutants is from 1.1- to 2.0-fold of wild-type in the various assay systems (Tabulated Summary 2.6.3.1.3, PC-104-2004, {2078}; Tabulated Summary 2.6.3.1.1, TPI 15883), {3852}. Shown in Table 28 are the results from TDF-treated subjects who had developed the K65R mutation in the clinical Study GS-99-903. The M184V mutation appears to also result in improved susceptibility to d4T, but further reductions in susceptibility to ABC, ddI and, naturally, FTC and 3TC.

Due to the instability of TDF in tissue culture media, most drug susceptibility assays use TFV rather than TDF to measure susceptibility. However, as shown in Table 26, TDF shows notably greater activity against HIV-1 when used in a phenotypic assay. A panel of patient-derived recombinant viruses (n = 19) expressing a variety of drug resistance mutations was assessed by Monogram Biosciences for susceptibility using both TFV and TDF (Tabulated Summary 2.6.3.1.3, PC-104-2017). Consistent with previous data, the EC_{50} value for TDF against wild-type HIV was 26.2 nM, whereas the EC_{50} value for TFV was 0.766 µM. However, among the resistant viruses, there were no significant differences in the fold change from wild-type when using either TFV or TDF (ranges 0.46- to 23.0-fold for TFV and 0.34- to 17.0-fold for TDF). These results confirm that use of TFV in vitro is sufficient for the detection of changes in phenotypic susceptibility to TDF.

2.6.2.2.13.1. Tenofovir DF: Mechanisms of Resistance

Reduced susceptibility to TFV associated with the K65R mutation is mediated by a decrease in the incorporation of the active metabolite of TFV, tenofovir diphosphate, relative to the natural substrate dATP. This can be observed enyzmologically as an increase in the inhibitory constant (K_i) in steady state kinetics experiments and as a decrease in the k_{pol} in pre-steady state enzyme kinetics experiments with the K65R mutant RT (Tabulated Summary 2.6.3.1.3, {5476} and {7583}) {3852}. Similarly, pre-steady state kinetics showed an approximate 5-fold decrease in incorporation (decreased k_{pol}) for both FTC-TP and 3TC-TP for the K65R mutant RT in accordance with the observed decreased susceptibility of the K65R mutant HIV for these drugs (Tabulated Summary 2.6.3.1.1, TPI 15883). Significant decreases in the incorporation of natural substrates, most notably the purines dATP and dGTP, were also observed in this enzymatic analysis. The K65R mutant RT also shows decreased ATP-mediated excision of incorporated NRTIs that results in increased stability of the chain-termination activity of NRTIs. The combined effects of altered NRTI incorporation and excision explain the susceptibility profile of K65R, which shows low-level decreased susceptibility to all NRTIs with the exception of ZDV, which remains fully active due to the strongly decreased excision caused by the mutation (Tabulated Summary 2.6.3.1.3, {7583}, {11304}).

As noted above, addition of the M184V mutation to a virus expressing K65R results in an increase in TFV susceptibility to near wild-type values. The mechanism by which this occurs appears to be a reduction in the affinity of the natural substrate dATP that is associated with

the M184V mutation and results in an increase in the incorporation of tenofovir diphosphate relative to dATP and, thus, improved TFV susceptibility for the double mutant virus (Tabulated Summary 2.6.3.1.1, TPI 15883; Tabulated Summary 2.6.3.1.3, {5476}).

The K65R mutant HIV-1 is also associated with a decrease in replication capacity for HIV-1 expressing this mutation. This has been observed previously for a site-directed mutant expressing the K65R mutation {3852} and is shown in Table 29 for a panel of clinical isolates expressing the K65R mutation that were obtained from a random set of plasma samples submitted to Monogram Biosciences for phenotypic analyses {5049}, (Tabulated Summary 2.6.3.1.3, PC-104-2004). Reduced replication capacity of K65R mutant HIV-1 was also found in macrophages (Tabulated Summary 2.6.3.1.3, {11307}). Interestingly, A62V and S68G are 2 mutations in RT that can occur with K65R and can act as partial compensatory mutations to increase the fitness of K65R-containing viruses without significantly altering the resistance to TFV (Tabulated Summary 2.6.3.1.3, {12688}). The M184V mutation is also associated with reduced replication capacity and the combination of mutations (K65R+M184V) showed the greatest reduction in replication capacity (Tabulated Summary 2.6.3.1.3, {5476}). Decreases in natural substrate binding (M184V) and incorporation (K65R) and reduced initiation of minus strand single stranded DNA synthesis (K65R+M184V) (Tabulated Summary 2.6.3.1.3, {10671}) for these HIV mutants are likely responsible for these observed additive decreases in viral replication capacity.

Table 29. Replication Capacity of Primary HIV-1 Isolates Without PI Resistance

	N	Replication Capacity			P value[a]
		Mean	Median	SD	
No NRTI-associated mutations	1307	94.9	92.7	45.7	-
M184V/I alone	291	65.4	60.8	41.5	<0.0001
K65R alone	17	57.6	58.7	38.6	0.0008
K65R + M184V/I alone	12	37.7	27.5	29.3	<0.0001

a P value from Student's unpaired t-test.
Data from reference: PC-104-2004

2.6.2.2.13.2. TDF: In Vitro Activity Against Drug-Resistant Variants of HIV-1

The activity of TFV against drug-resistant HIV-1 has been extensively studied in vitro. As shown in Table 30, TFV remains active (within 2-fold of wild-type) against recombinant mutant molecular clones of HIV-1 expressing ddI resistance (L74V), ddC resistance (T69D), ZDV resistance (D67N + K70R, D67N + K70R + K219Q, or T215Y), or multinucleoside drug resistance (Q151M complex) mutations in HIV-1 RT (Tabulated Summary 2.6.3.1.3, {2078}, {2191}, {3200}, {2064}) {1793}. Tenofovir was fully active against recombinant

HIV-1 expressing the K70E mutation that was observed to develop in 2 patients treated with adefovir dipivoxil (ADV) (Tabulated Summary 2.6.3.1.3, {2078}, {1648}). However, biochemical studies of the K70E mutant RT have suggested low-level reduced susceptibility of this mutant to TFV (Tabulated Summary 2.6.3.1.3, {10898}). The M184V substitution is commonly observed in HIV-1 isolates from 3TC- and FTC-treated patients and is associated with high-level resistance to FTC and 3TC and low-level resistance to ABC. Tenofovir shows slightly increased activity against HIV-1 expressing the ABC/3TC/FTC resistance mutation M184V or the combination of the high-level ZDV resistance mutation T215Y and M184V (Tabulated Summary 2.6.3.1.3, {2078}, {1649}).

Table 30. Antiviral Susceptibilities of Molecular Clones of HIV-1 Expressing Nucleoside-Associated Resistance Mutations in RT

RT Mutation	EC_{50} Fold Increase Above Wild-Type (HXB2D or IIIb)						
	TFV	ZDV	d4T	ddI	3TC	ddC	ABC
D67N + K70R	0.7	5.7	ND	ND	ND	ND	1.7
D67N + K70R + K219Q	1.8	23	ND	ND	3.1	ND	1.2
T69D	2	1.7	4	ND	19	4.3	ND
K70E	1.3	0.6	1.1	1	3.8	0.7	ND
L74V	1	2	1.1	2.8	2.4	3.9	3.7
Q151M[a]	0.8	39	69	42	1.9	7.3	ND
M184V	0.2 – 0.9	0.7	1.5	2.8	> 50	1.8	4.9
T215Y	1.8	6.9	1.6	2.2	1.1	1.3	2.2
T215Y + M184V	0.5	0.7	1.1	2	< 50	1.7	7

a Site-directed recombinant also includes A62V, V75I, F77L, and F116Y RT mutations.

ND = not determined.

Data from references: {2078}, {3200}, {2064}, {1793}, {2191}

2.6.2.2.13.3. Tenofovir DF: In Vitro Activity Against Nucleoside-Resistant Clinical HIV-1 Isolates

The susceptibility results with molecular clones of HIV-1 have been confirmed and extended with phenotypic analyses of a panel of recombinant HIV-1 clinical isolates from antiretroviral experienced patients. Seventy patient HIV-1 isolates expressing multiple patterns of resistance mutations have been analyzed by Virco (Mechelen, Belgium) using the Antivirogram™ phenotypic resistance assay (Table 31) (Tabulated Summary 2.6.3.1.3, P4331-00035, {2152}). In this assay, TFV showed a mean EC_{50} value of 0.9 µM against a panel of 10 wild-type clinical isolates. Phenotypic classification is based upon EC_{50} changes relative to the wild-type reference where “sensitive” is < 4-fold, “intermediate” is 4- to 10-fold, and “resistant” is > 10-fold.

Table 31. Tenofovir Susceptibility of a Panel of HIV-1 Clinical Isolates Expressing Nucleoside or Multinucleoside Resistance Mutations (n = 70)

Resistance Group	N	Mean Fold Change in Susceptibility from Wild-Type[a] (range)					
		TFV	ZDV	3TC	ddI	d4T	ABC
M184V	10	0.7 (0.3-1.3)	0.9 (0.2-1.5)	**> 50** (> 50)	1.0 (0.3-2.4)	1.4 (0.4-2.8)	1.3 (0.9-2.4)
ZDV-HI	10	3.7 (0.8-8.4)	**47** (9.3-82)	**4.3** (0.4-12)	1.6 (0.3-3.4)	2.5 (0.5-6.7)	2.6 (0.5-5.6)
ZDV-HI + M184V	10	2.4 (0.9-3.8)	**15** (2.1-34)	**> 50** (> 50)	1.8 (0.7-4.5)	1.7 (0.7-4.3)	**4.6** (1.8-9.5)
Q151M	5	1.8 (1.1-3.0)	**43** (9.6-85)	2.1 (1.3-2.6)	**13** (6.4-31)	**20** (6.1-57)	**11** (3.0-24)
Q151M + M184V	5	1.6 (0.8-3.3)	**46** (19-70)	**> 50** (> 50)	**19** (4.8-38)	**11** (3.7-20)	**16** (3.9-24)
T69S Ins	5	**23** (14-35)	**101** (60-149)	**28** (8.3-53)	**4.1** (1.4-6.4)	**9.3** (2.4-20)	**20** (10-29)
T69S Ins + M184V	10	**6.0** (2.1-15)	**31** (2.1-54)	**> 50** (> 50)	1.8 (0.4-3.3)	**4.2** (1.3-15)	**8.1** (2.1-28)
L74V	5	0.7 (0.4-1.0)	1.3 (0.6-2.5)	1.4 (1.1-1.9)	0.8 (0.4-1.0)	0.9 (0.4-1.4)	1.4 (0.8-2.2)
L74V + M184V	5	0.5 (0.2-0.8)	0.6 (0.4-1.0)	**> 39** (> 39)	3.0 (0.4-8.8)	1.0 (0.3-1.6)	2.9 (1.3-4.7)
L74V + Y115F + M184V	5	0.6 (0.5-1.0)	0.6 (0.4-0.9)	**> 39** (> 39)	1.5 (0.7-2.3)	0.5 (0.3-1.1)	**7.3** (3.1-13.3)

a Antivirogram assay results by Virco Central Virological Laboratories (Mechelen, Belgium). Fold changes of between 4- and 10-fold indicate reduced susceptibility, above 10-fold indicate possible resistance (both shown shaded). The wild-type EC_{50} values for tenofovir ranged from 0.8-1.7 μM in these analyses.

Data from reference: P4331-00035

Clinical HIV-1 isolates expressing M184V alone showed mild hypersusceptibility to TFV (0.7-fold). High-level ZDV-resistant HIV-1 (ZDV-HI; mean 47-fold ZDV resistant; mean 3.4 ZDV mutations including T215Y/F in all cases) remained sensitive to TFV, demonstrating a mean 3.7-fold reduced susceptibility, with only 3 samples having an intermediate phenotype. Furthermore, the combination of M184V and high-level ZDV mutations exhibited a mean 2.4-fold change from wild-type (mean 3.2 ZDV mutations). HIV-1 expressing the multinucleoside-resistant T69S double amino acid insertion mutations (T69S Ins) was resistant to TFV (23-fold). Intermediate susceptibility to TFV (6-fold) was observed when these insertions were combined with M184V. The frequency of the T69S Ins multinucleoside-resistant virus was 1% in a survey of over 12,000 samples from patients {2187}. The multinucleoside-resistant HIV-1 with Q151M showed full sensitivity to TFV (1.6- to 1.8-fold) regardless of the presence of M184V. HIV-1 expressing the ddI-associated L74V mutation, with or without M184V, was slightly hypersusceptible to TFV (0.5- to 0.7-fold). HIV-1 expressing a common pattern of resistance mutations associated with ABC (L74V + Y115F + M184V) were also hypersusceptible to TFV (0.6-fold) while showing reduced susceptibility to ABC (7.3-fold).

The activity of TFV was also evaluated against primary PBMC-derived HIV-1 isolates directly in donor PBMCs (Tabulated Summary 2.6.3.1.3, {5044}). The 10 primary HIV-1 isolates in this study were derived from nucleoside-experienced patients and all isolates expressed one or more nucleoside-associated RT mutations. The drug susceptibility results, as well as the resistance genotype for each isolate, are presented Table 32 in order of increasing ZDV resistance. Nine of these primary isolates demonstrated high-level 3TC resistance as a result of the M184V RT mutation. The mean fold change in TFV susceptibility of all 10 primary HIV-1 isolates was unchanged from the wild-type reference (range 0.4 to 2.2). In contrast, these primary HIV-1 isolates showed a mean resistance to ZDV, ABC, and 3TC of 20.4-fold, 11.9-fold, and > 45-fold, respectively.

Table 32. Drug Susceptibilities of Nucleoside-Resistant Primary HIV-1 Isolates

Virus ID	Nucleoside-Associated RT Mutations[b]	Drug Susceptibility (Fold Change from Wild-Type [a])			
		TFV	ZDV	ABC	3TC
1-MLS	M184V	0.5	0.3	1.7	> 50
2-JMG	M184V	0.4	0.6	1.9	> 50
3-OT	K70R M184V	0.5	0.7	1.8	> 50
4-RJN	T69N K70R	0.7	1.4	0.8	0.4
5-AB	M41L M184V T215Y	0.4	2.8	8.3	> 50
6-EDM	M41L M184V L210W T215Y	1.0	3.1	6.8	> 50
7-WK	M41L D67N M184V T215F K219Q	1.0	15	4.6	> 50
8-RON	M41L M184V T215Y	1.3	16	26	> 50
9-EMS	D67N K70R M184V T215F K219E	1.5	50	43	> 50
10-GGR	M41L D67N T69D M184V L210W T215Y	2.2	> 100	21	> 50
	Mean (n = 10):	1.0	20.4	11.9	> 45
	Range	0.4 – 2.2	0.3 – > 100	0.8 – 43	0.4 – > 50

a Wild-type HIV_{IIIb} EC_{50} values for zidovudine (ZDV), tenofovir (TFV), abacavir (ABC), and lamivudine (3TC) were 0.012, 1.7, 0.09, and 0.27 μM, respectively, for these experiments. The fold change from these wild-type EC_{50} values was calculated as the mean of 2-3 independent experiments for each nucleoside-resistant primary isolate analyzed.

b Nucleoside-associated RT mutations are any of the following amino acid changes in RT: M41L, A62V, K65R, D67N, T69N/D, K70R, L74V/I, V75T, F77L, Y115F, F116Y, Q151M, M184V/I, L210W, T215Y/F or K219Q/E.

Data from reference: {5044}

The distribution of TFV susceptibility in over 1,000 antiretroviral-naive, HIV-1 infected individuals worldwide was determined using the Virco Antivirogram assay. In addition, phenotypic susceptibility to TFV and other RT inhibitors was determined in a panel of nearly 5,000 recombinant HIV-1 clinical isolates from predominantly treatment-experienced patients analyzed as a part of routine drug resistance testing.

Greater than 97.5% of isolates from treatment-naive patients had TFV susceptibility < 3-fold above the wild-type controls using the Antivirogram. The clinically derived panel of 5,000 samples exhibited a broad range of antiretroviral drug susceptibilities, including 69%, 43%, and 16% having > 10-fold decreased susceptibility to at least 1, 2, and 3 antiretroviral drug classes, respectively. Greater than 88% of these 5,000 clinical isolates were within the 3-fold susceptibility range for TFV, and > 99% exhibited < 10-fold reduced susceptibility to TFV. The results suggest that the majority of treatment-naive and treatment-experienced individuals harbor HIV-1 that remains within the normal range of TFV susceptibility and may be susceptible to TDF therapy (Tabulated Summary 2.6.3.1.3, {3961}).

A retrospective analysis was undertaken to look for patterns of resistance among TFV-naive, treatment-experienced patients that can result in a reduction in TFV susceptibility {5482}. In this study, 321 samples from 294 patients that had been sent to the German National Reference Centre for Retroviruses for drug resistance testing between January 1998 and July 2002 were analyzed. When the susceptibility of viruses to TFV was examined, viruses with insertion mutations (n = 6) were highly resistant. Viruses with Q151M could be divided into 2 subsets of susceptible (n = 12) and highly resistant (n = 8), where 7/8 viruses in the resistant set also contained K65R. A clonal analysis of viruses containing thymidine-analog associated mutations (M41L, D67N, L210W, T215F/Y, and K219Q/E/N [TAMs]) showed that when present as single mutations, resistance to TFV could be ranked as increasing for mutations at position 41 > 215 > 70. Additionally, viral clones with M184V or M184I exhibited slightly increased susceptibilities to TFV (0.7-fold), and almost all clones with TAM-induced resistance were resensitized when M184V was present. However, accumulation of at least 2 TAMs resulted in more than 2.0-fold reduced susceptibility to TFV, irrespective of the presence of M184V. Overall, a total of 40.4%, 34.3%, and 25.3% of the viruses had less than 1.5-fold, 1.5- to 4.0-fold, and more than 4.0-fold reduced susceptibilities to TFV, respectively.

Two studies have confirmed the resensitizing effect of the M184V/I mutation on TFV susceptibility with recombinant clinical isolates using the Monogram Biosciences PhenoSense assay {7279}, {12143}. The effect appears across all genotypic backgrounds, including K65R, L74V, and TAMs. For viruses with TAMs (n = 3805), the resensitization effect for viruses containing M184V/I resulted in 19% of isolates showing reduced susceptibility to TFV above the clinical cut-off of 1.4-fold versus 52% of isolates in the absence of M184V/I. The resistance mechanisms of HIV-1 RT with K65R+M184V mutations suggest that the increased susceptibility conferred by M184V to TFV is mediated by decreased incorporation of the competing natural substrate dATP relative to TFV compared to the K65R mutant (Tabulated Summary 2.6.3.1.3, {12142}, {5476}).

Although they do not often occur together, recent studies have found that K65R can be found on the same genome as L74V or T215Y using clonal sequencing analyses of viral populations (Tabulated Summary 2.6.3.1.3, {10318}). A set of studies describe that K65R and TAMs were antagonistic both for their prevalence in database searches of patient-isolates, as well as for their resistance profiles and mechanisms of resistance (Tabulated Summary 2.6.3.1.3, {8925}, {9037}, {9494}, {11306}). Genotypic and molecular antagonism was also found between the RT mutations K65R and K70E using clonal sequencing of virus from patients, which was primarily explained by the dramatically reduced replication capacity of the constructed double mutant (Tabulated Summary 2.6.3.1.3, {11303}).

2.6.2.2.13.4. Tenofovir DF: In Vitro Activity Against Nucleoside-Resistant Clinical HIV-2 Isolates

A panel of HIV-2 clinical isolates with pre- and post-Q151M mutation development and laboratory strain HIV-2_{ROD} were evaluated for drug susceptibilities in a PBMC-based assay {9389}. The specific components of the antiretroviral therapy used that led to the development of Q151M was not described. Prior to the development of the Q151M mutation,

the EC_{50} value for TFV for HIV-2_{ROD} was 0.3 μM, and the clinical isolates had EC_{50} values that ranged from 0.14 to 0.52 μM (n = 4). Of the HIV-2 that showed development of the Q151M mutation in their RT, 2 of 4 patients showed no change in susceptibility to TFV, while the remaining 2 showed 8.6- and 29-fold reductions in TFV susceptibility as compared to pre-Q151M HIV-2. The patients with phenotypic changes also developed either a V111I or a K65R mutation in addition to Q151M. For comparison, all 4 patients had developed reductions in susceptibility to d4T and ABC associated solely with Q151M.

2.6.2.2.13.5. Tenofovir DF: Removal of Tenofovir by HIV-1 RT

The removal of NRTIs by HIV-1 RT using a pyrophosphate acceptor molecule or a similar mechanism using ATP as an acceptor have been proposed as mechanisms of NRTI resistance (Tabulated Summary 2.6.3.1.3, {2043}). Removal of the inhibitors by these mechanisms frees the HIV-1 RT to incorporate the natural nucleotide substrate and resume/rescue HIV-1 replication. The excision of TFV from TFV-chain-terminated primers was originally not detected (Tabulated Summary 2.6.3.1.3, {2252}). However, newer methodology has demonstrated that TFV can be efficiently excised by pyrophosphorolysis and ATP-mediated excision mechanisms (Tabulated Summary 2.6.3.1.3, {11304}, {7583}, {8925}). The excision of NRTIs occurs only when the nucleotide exists in its pretranslocational state. The binding of the next complementary nucleotide causes the formation of a stable dead-end complex in the posttranslocational state, which blocks the excision reaction. The excision of TFV was highly sensitive to dead-end complex formation, allowing greater chain termination stability. Thus, the rate of excision of TFV is partially neutralized by the facile switch to the posttranslocational state and by dead-end complex formation (Tabulated Summary 2.6.3.1.3, {11304}).

Drug resistant HIV-1 RT with mutations that are known to cause high-level NRTI excision such as TAMs either with or without a T69S Ins mutation also excise TFV at high rates to contribute to resistance (Tabulated Summary 2.6.3.1.3, {11304}), {5479}. These mutants also show greater phenotypic changes to TFV in vitro. The greater levels of resistance associated with the M41L-L210W-T215Y pattern of TAM resistance have also been shown to be clinically significant in terms of reduced responses to TDF therapy {5010}.

2.6.2.2.13.6. Tenofovir DF: In Vitro Activity Against NNRTI-Resistant HIV-1

Nonnucleoside RT inhibitors bind to a structurally distinct portion of RT and cross-resistance to nucleoside and/or nucleotide RT inhibitors is not expected. Nevertheless, RT amino acid changes associated with NNRTIs may induce structural changes in RT potentially altering the antiviral susceptibility of any RT inhibitor. In published studies, HIV-1 molecular clones expressing the primary NNRTI-associated resistance mutations Y181C and K103N demonstrated increased susceptibility to TFV of 4- to 8-fold (Tabulated Summary 2.6.3.1.3, {2191}). The TFV and NNRTI susceptibilities of 10 HIV-1 clinical isolates expressing these mutations in the context of additional NNRTI-associated mutations is shown in Table 33. For these clinical isolates expressing Y181C or K103N, the mean TFV EC_{50} values were 0.82 μM and 1.01 μM, respectively, in comparison to 0.96 μM for wild-type clinical isolates. Thus, mutations associated with high-level NNRTI resistance, remain fully susceptible, or

show increased susceptibility to TFV in vitro. A panel of 139 HIV-1 with NNRTI mutations showed full susceptibility to TFV, and showed full susceptibility to FTC for the subset of those lacking M184V/I in vitro (Report PC-264-2004).

Table 33. Tenofovir Susceptibility of HIV-1 Clinical Isolates and Molecular Clones Expressing High-Level NNRTI Resistance Mutations

Virus Source	Primary NNRTI Resistance Mutation	Mean EC_{50} (μM)			
		Tenofovir	Nevirapine	Efavirenz	Delavirdine
Clinical Isolates (n = 8)	Wild-Type	0.96	0.019	0.0007	0.008
Clinical Isolates (n = 5)	Y181C	0.82	> 1.21	0.0027	> 0.725
Clinical Isolates (n = 5)	K103N	1.01	> 0.89	0.095	0.484

Data from reference: P4331-00035

2.6.2.2.14. Tenofovir DF: In Vivo Efficacy Against Animal Retroviruses

2.6.2.2.14.1. Tenofovir DF: Murine Retroviral Infection

The efficacy of orally administered TDF was studied in Moloney murine sarcoma virus (MSV)-infected SCID mice (Tabulated Summary 2.6.3.1.3, {1133}, {2477}). SCID mice were inoculated intramuscularly with MSV on Day 0 and treated with TFV subcutaneously or TDF orally (50, 100, or 200 mg/kg) once daily for 5 days starting on Day 0. Both subcutaneous TFV and oral TDF had a significant effect on time-to-tumor appearance and tumor size compared to the untreated control group. The efficacy of oral TDF was comparable to subcutaneous TFV in both dose groups, whereas orally administered TFV had no effect, consistent with its poor oral bioavailability.

2.6.2.2.14.2. Tenofovir DF: Feline Immunodeficiency Virus Infection

The efficacy of TFV against FIV was studied in both acute and chronic FIV infection models in cats (Tabulated Summary 2.6.3.1.3, {1576}). Tenofovir (30 mg/kg/day) was administered to cats subcutaneously either at the time of FIV challenge or beginning 8 weeks after infection. All animals that received TFV at the time of infection remained free of FIV infection as determined by PBMC coculture, PCR, and FIV antibody assay, in the absence of hematological or other toxicity. By contrast, 100% of the placebo-treated cats became persistently infected and developed symptoms of immunodeficiency. Tenofovir treatment of cats with established infection reduced circulating viral FIV RNA, but not PBMC-associated, coculture-detected virus burden.

2.6.2.2.14.3. Tenofovir DF: Simian Immunodeficiency Virus Infection

Tenofovir shows potent anti-SIV activity in monkeys in several models of infection. SIV infection of rhesus macaques is similar to HIV infection in humans and has been used to study the pathogenesis of HIV disease and to test potential antiretroviral therapies.

In a study of juvenile macaques chronically infected with SIV, TFV (30 or 75 mg/kg) or ZDV (100 mg/kg) was administered subcutaneously once daily for 4 weeks and virologic results compared to those in mock-treated controls (Tabulated Summary 2.6.3.1.3, {1367}). SIV was undetectable by PBMC coculture from animals treated with either dose of TFV (5 animals/group). In contrast, all untreated controls continued to have isolatable SIV from PBMC. At Week 4, plasma SIV RNA levels, as determined by the Chiron branched DNA (bDNA) assay, were reduced 100- to 1,000-fold from baseline in the 2 TFV treatment groups and CD4 cell counts had risen to a mean of 158 to 314 cells/mm^3 in each group. However, both control and TFV-treated groups continued to have SIV DNA in PBMC as determined by a more sensitive PCR assay. Tenofovir at either dose was more efficacious than ZDV as assessed by surrogate markers of SIV infection and clinical status.

In a study designed to evaluate the efficacy of TFV in the prevention of acute infection (i.e., needle stick injuries), TFV was administered subcutaneously at a dose of 30 mg/kg/day for 28 to 30 days beginning either 48 hours before, 4 hours after, or 24 hours after inoculating macaques intravenously with SIV. Treatment was continued once daily for 4 weeks; clinical and virologic parameters were monitored for up to 56 weeks after inoculation. As shown in Table 34, none of the animals treated with TFV showed evidence of SIV infection, whereas all mock-treated animals showed both virologic evidence and clinical signs of SIV infection (Tabulated Summary 2.6.3.1.3, {17}). Importantly, SIV was undetectable by PCR in PBMC and lymph node biopsies from TFV-treated animals. In addition, TFV was well-tolerated in the treatment groups, without signs of significant drug-related toxicity. In contrast, ZDV prevented infection in only 1 of 18 monkeys in this model when administered at 100 mg/kg for 4 weeks beginning 24 hours preinoculation {45}.

Table 34. Viral, Antibody, and Clinical Status of Macaques Inoculated With SIVmne and Treated With Tenofovir or Mock-Treated

Group	Tenofovir Treatment[a]		Viral Load		SIV DNA in PBMCs[d]	Lymph Node Biopsy[e]	Clinical Status[f]
	Time Started	Dose (mg/kg)	Plasma[b]	PBMCs[c]			
1 (n = 5)	48 Hours Preinoculation	20	—	—	—	—	Four healthy, 56 weeks; one euthanized at 40 weeks, healthy
2 (n = 10)	48 Hours Preinoculation	30	—	—	—	—	Nine healthy, 36 to 56 weeks; one euthanized at 40 weeks, healthy
3 (n = 5)	4 Hours Postinoculation	30	—	—	—	—	Healthy, 36 weeks
4 (n = 5)	24 Hours Postinoculation	30	—	—	—	—	Healthy, 36 weeks
5 (n = 10)	Control, Mock-Treated	None	+	+	+	+	Transient rash, diarrhea, enlarged lymph nodes 20 to 36 weeks postinoculation

a A single daily dose of tenofovir was administered subcutaneously for 28 to 30 days.

b Cell-free viral load was determined in polyethylene glycol-treated plasma samples; (—) not detected; (+) detected (for the control macaques, cell-free SIV was persistently detected at dilutions of 10^{-2} to 10^{-3}).

c Cell-associated (PBMC) viral load was determined by a limiting dilution assay; for the tenofovir-treated macaques, 5×10^6.

d PBMCs were cocultured; for the control macaques a minimum of 10 to 10^3 PBMCs were needed to detect SIV. SIV DNA in PBMCs was detected by PCR.

e Inguinal lymph nodes were biopsied from each macaque at 16 or 26 weeks postinoculation and examined for the presence of SIV by co-culture, PCR, and immunohistochemistry.

f The clinical status of the treated macaques was monitored for 36 to 56 weeks; two healthy macaques were euthanized at 40 weeks postinoculation for complete necropsy; mock-treated macaques were monitored for 20 to 36 weeks postinoculation.

Data from reference: {17}

In a study designed to evaluate the efficacy of TFV in the prevention of acute infection after rectal exposure to an HIV-SIV chimeric virus (SHIV) (Tabulated Summary 2.6.3.1.3, {9496}), animals were exposed rectally to SHIV weekly for 14 weeks, with groups receiving oral TDF (22 mg/kg) daily, weekly, or not receiving therapy. Most macaques were infected, although infection was delayed in treated macaques, suggesting that treatment with oral TDF provided partial protection against SHIV infection but ultimately did not protect all treated animals against multiple virus challenges.

The use of TFV as postexposure prophylaxis has been further explored in the SIV macaque model to investigate the effects of delayed treatment (Tabulated Summary 2.6.3.1.3, {1796}). Twenty-four cynomolgus macaques were inoculated intravenously with SIV_{mne} and then treated subcutaneously with TFV at 30 mg/kg starting at 24, 48, or 72 hours postexposure. The duration of treatment was either 28, 10, or 3 days. With 4 animals per test group, all 4 animals receiving TFV 24 hours postexposure for 28 days were fully protected from SIV infection. One of 4 animals treated 24 hours postexposure, but for only 10 days, became

SIV-infected, and 2 of 4 animals treated for only 3 days became infected. Moreover, delaying treatment until 48 or 72 hours also resulted in reduced effectiveness of postexposure prophylaxis. Thus, complete protection from SIV infection can be obtained, but requires early intervention (within 24 hours) and 10 to 28 days of treatment. However, in the animals that were SIV-infected, there is evidence of an attenuated infection in many of the animals, with 50% of these animals only showing transiently detectable SIV RNA in their plasma. Evidence of an attenuated SIV infection resulting from starting TFV treatment 5 days after oral SIV infection of neonatal macaques has also been reported (Tabulated Summary 2.6.3.1.3, {2144}). These studies suggest that even delayed early intervention may have clinical benefits.

Two studies in neonatal macaques have also shown that TFV treatment is effective as a prophylaxis against oral SIV infection of newborn macaques. In one study (Tabulated Summary 2.6.3.1.3, {1800}), one TFV dose at 4 hours prior to inoculation and a second dose at 24 hours postinoculation resulted in complete protection in 4 of 4 animals. In the second study (Tabulated Summary 2.6.3.1.3, {1802}), animals received TFV starting 24 hours postinoculation, and treatment was continued for 14 days. In this group, 3 of 4 animals were protected from SIV infection. However, a single dose of TFV just prior to cesarean section was insufficient to protect from neonatal transmission. These results demonstrate that TFV treatment of animals directly after birth can be highly effective in preventing SIV infection and suggest possible prophylaxis options for HIV-infected pregnant women.

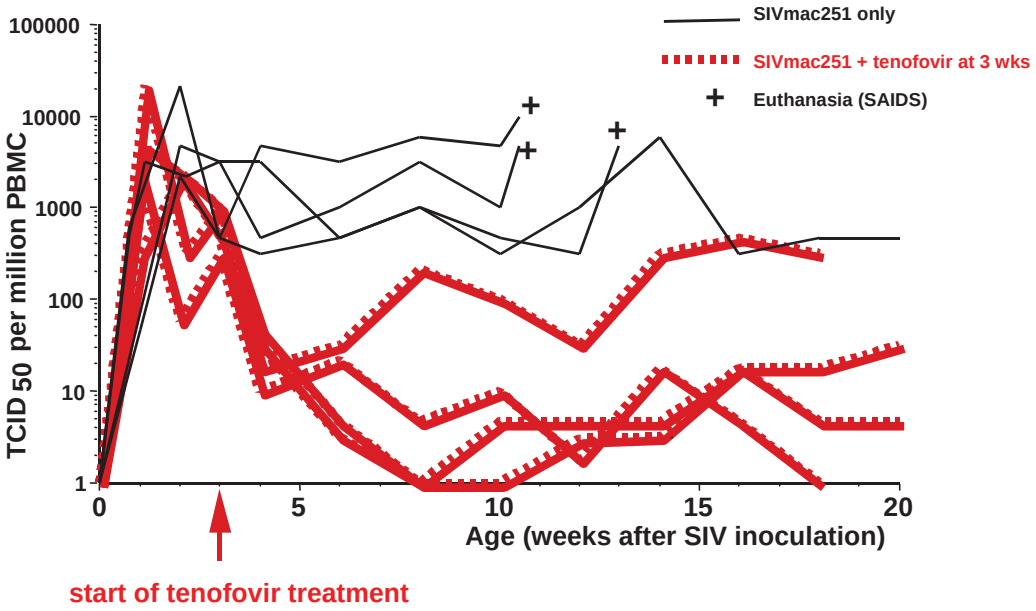
SIV infection of infant macaques was used to determine whether a topical (oral) formulation of TDF to the nursing infant could reduce transmission of SIV through breast feeding (Tabulated Summary 2.6.3.1.3, {3873}). This study showed that in both the control group that received placebo and the TDF group that received 0.037 mg of TDF/day (an approximately 200 to 2700 times lower dose than the oral doses used in prior studies that had been shown to protect against oral SIV infection [Tabulated Summary 2.6.3.1.3, {1800}]), about half of the animals became infected after multiple low-dose exposures to SIV. In a subsequent study of juvenile macaques treated with a mixture of TDF and SIV_{mac251} (Tabulated Summary 2.6.3.1.3, {3873}), protection against oral SIV infection did not occur. In a follow-up study of an oral administration of TDF (10 mg/kg) to prevent infection of infant macaques against low-dose oral exposures to SIV_{mac251} (Tabulated Summary 2.6.3.1.3, {9457}), TFV lowered the infection rate at birth, but had lower efficacy against virus infection at 4 weeks of age, potentially due to suboptimal drug levels compared to the TDF regimen in humans.

Tenofovir also has shown profound anti-SIV activity when administered to neonatal rhesus macaques with established SIV infection. Eight macaques were inoculated with uncloned SIV_{mac251} within 72 hours after birth. Beginning 3 weeks postinfection, 4 animals began once daily dosing with TFV (30 mg/kg subcutaneous) and 4 animals served as untreated controls. As shown in Figure 12, TFV treatment resulted in a rapid and durable suppression of SIV replication as determined by PBMC coculture (Tabulated Summary 2.6.3.1.3, {35}). The TFV-treated animals showed no clinical evidence of resistance to TFV. In addition to decreased viremia, all TFV-treated animals were alive at 2 years, whereas 3 of 4 untreated animals were euthanized due to progressive signs of simian AIDS less than 4 months

postinfection. Also, newborn macaques tolerated TFV well, as determined by weight gain, complete blood counts, serum chemistries, and clinical observations.

A subsequent study was conducted that followed a subset of infant macaques that were chronically infected with highly virulent SIV_{mac251} for which prolonged TFV treatment failed to significantly suppress viral RNA levels in plasma (Tabulated Summary 2.6.3.1.3, {7288}). Untreated animals with similarly high viremia developed fatal immunodeficiency within 3 to 6 months, whereas the TFV-treated animals had significantly improved survival (up to 3.5 years). TFV had little or no effect on CD4+ and CD8+ lymphocyte counts and antibody responses to SIV and test antigens.

Figure 12. Tenofovir Effect on PBMC-Associated Viremia in SIV-Infected Rhesus Infants



Data from reference: {35}

Tenofovir was also studied for safety and efficacy in SIV models of vertical transmission {1787}. Eighteen pregnant rhesus macaques were included in these studies. The fetuses of 12 macaques were directly infected with SIV in utero. Three weeks later, 6 of these 12 fetuses were treated with TFV subcutaneously (30 mg/kg) for the duration of gestation. Additionally, 6 uninfected fetuses were similarly treated with TFV. Tenofovir treatment was continued in the infants after cesarean-section births. Pharmacokinetic results showed that TFV can readily cross the placenta. Moreover, there was a significant reduction in viral load in the SIV-infected fetuses and infants treated with TFV as compared to control animals. However, with this dose of TFV, significant effects on body weight gain, serum phosphorus, and serum ALP occurred in most infants and significant bone-related toxicity occurred in 2 animals, one SIV-infected and one TFV-treated uninfected macaque. Thus, once daily TFV

treatment in this model of vertical transmission resulted in a marked reduction of viral load and significantly enhanced survival, but was accompanied by clinical signs of toxicity and changes in laboratory markers at the high doses used. Toxicological findings of TFV in the SIV efficacy studies are presented in Module 2.6.6, Toxicology Summary, Section 2.6.6.8.5.

2.6.2.2.14.4. TDF: In Vivo Simian Immunodeficiency Virus Resistance

The development of SIV resistance to TFV during long-term treatment was evaluated in SIV-infected newborn rhesus macaques (Tabulated Summary 2.6.3.1.3, {35}). Four untreated SIV-infected newborn macaques developed persistently high viremia, whereas long-term TFV treatment of 4 newborn macaques, starting 3 weeks after virus inoculation, resulted in a rapid, pronounced, and persistent reduction of viremia in 3 of the 4 animals. In the animals demonstrating reduced viremia, there was a 100- to 1,000-fold reduction in PBMC-associated SIV titer within 3 weeks of treatment and undetectable SIV titers from plasma. Emergence of virus with a 5-fold decreased susceptibility to TFV in vitro occurred in all 4 TFV-treated animals, and was associated with the development of a K65R mutation and additional mutations in RT after 5 to 15 weeks of TFV treatment. However, the clinical implications of this low-level resistance are not clear since viremia levels have remained low for 3 of the 4 monkeys during > 2 years of TFV therapy.

Virus from 3 of these animals had K65R, N69S, and I118V mutations in the RT. The fourth animal had persistently high viremia and died at 20 months; virus from this animal had K65R, N69T, R82K, A158S, and S211N mutations in RT (Tabulated Summary 2.6.3.1.3, {35}). To directly assess whether TFV resistance alters viral virulence and/or the therapeutic efficacy of TFV treatment, 2 virus stocks resistant to TFV were made (SIV_{mac385}: K65R, N69S, I118V; SIV_{mac055}: K65R, N69T, R82K, A158S, S211N) (Tabulated Summary 2.6.3.1.3, {2145}). Two groups of 6 newborn macaques were inoculated with either SIV_{mac385} or SIV_{mac055}. Three animals of each group were started on TFV treatment at 3 weeks of age. The 6 untreated animals developed persistently high viremia and had rapid immunosuppression; all died within 3 months. In contrast, the 6 infants inoculated with virus resistant to TFV but treated with TFV also had high viremia, but had a delayed disease progression. The 3 SIV_{mac055}-infected animals treated with TFV developed fatal disease between 5 to 9 months of age, while the 3 SIV_{mac385}-infected animals treated with TFV survived greater than 20 months. The genotype and phenotype of resistance to TFV in most animals, even those that did not get TFV treatment, were stable. Specifically, K65R was not lost and phenotypic expression of altered susceptibility was maintained. Thus, although SIV resistant to TFV is fully virulent, TFV treatment has strong therapeutic benefits even in the presence of virus resistant to TFV.

The persisting therapeutic benefits of TFV against SIV with reduced susceptibility to TFV have been confirmed in a prophylaxis and short-term treatment study in neonatal macaques (Tabulated Summary 2.6.3.1.3, {2146}). Five animals were TFV treated 24 hours prior to inoculation with the K65R-expressing SIV_{mac055} isolate, and then for a period of 4 weeks after inoculation. Two animals had no evidence of infection, while the remaining 3 animals demonstrated delayed viremia, enhanced antibody responses, and a slower disease course than control animals. Thus, despite a 5-fold reduction in TFV susceptibility with the highly

virulent SIV_{mac055} isolate, short-term TFV treatment resulted in complete or partial protection from SIV disease.

A study was designed to model HIV-1 RT responses and resistance development to TFV in macaques infected with RT-SHIV, a chimeric SIV containing HIV-1 RT, and started on prolonged TFV therapy 5 months after infection (daily subcutaneous doses of TFV at 10 mg/kg; Tabulated Summary 2.6.3.1.3, {12759}). For all 12 animals, there was transient emergence of K70E RT mutants within 4 weeks of initiating therapy, which were then replaced by K65R mutants within 12 weeks of therapy. For most animals, the occurrence of these mutations preceded a partial rebound of plasma viremia to levels that remained on average 10-fold below baseline values. One animal eventually suppressed K65R viremia to undetectable levels for more than 4 years.

2.6.2.2.15. Emtricitabine/Tenofovir DF

2.6.2.2.15.1. In Vitro Phosphorylation of Tenofovir and Emtricitabine

The intracellular phosphorylation of TFV and FTC to their active anabolites, TFV diphosphate and FTC triphosphate, respectively, was investigated for potential antagonism (Tabulated Summary 2.6.3.1.4, PC-164-2001). All forms of TFV, the free drug, the monophosphate, and diphosphorylated forms (PMPA, PMPAp, and PMPApp, respectfully) were quantified in this analysis. The activated form of FTC (FTC-TP) was also measured. When 10 μM TFV was incubated alone in CEM cells, conversion to the active form, PMPApp, was readily observed over the 24-hour time course (Table 35). Similarly, FTC-TP was also readily observed to form over the same time period when incubated alone (Table 36).

When TFV and FTC were incubated together (10 μM each), slightly higher levels of each respective active anabolite (PMPApp and FTC-TP) were observed at 2 and 24 hours as compared to the NRTIs incubated alone in CEM cells (Figure 13, Table 35 and Table 36). The slightly higher concentrations of active anabolites observed in the combination experiment may suggest slight enhancement of anabolism of both TFV and FTC; however, no difference in anabolite production was observed in cultured PBMCs {8998}.

Table 35. Metabolism of 10 μM Tenofovir (PMPA) either Alone or in Combination with 10 μM Emtricitabine (FTC)

Time	PMPA Alone (pmols/million cells)[a]			PMPA with FTC (pmols/million cells)[a]		
(hrs)	PMPA	PMPAp	PMPApp	PMPA	PMPAp	PMPApp
2	0.74 ± 0.31	0.02 ± 0.01	0.06 ± 0.01	0.50 ± 0.10	0.04 ± 0.01	0.09 ± 0.01
24	1.32 ± 0.48	0.54 ± 0.06	1.64 ± 0.08	0.97 ± 0.78	0.34 ± 0.03	2.24 ± 0.07

a Values determined by LC/MS/MS.

Data from reference: PC-164-2001

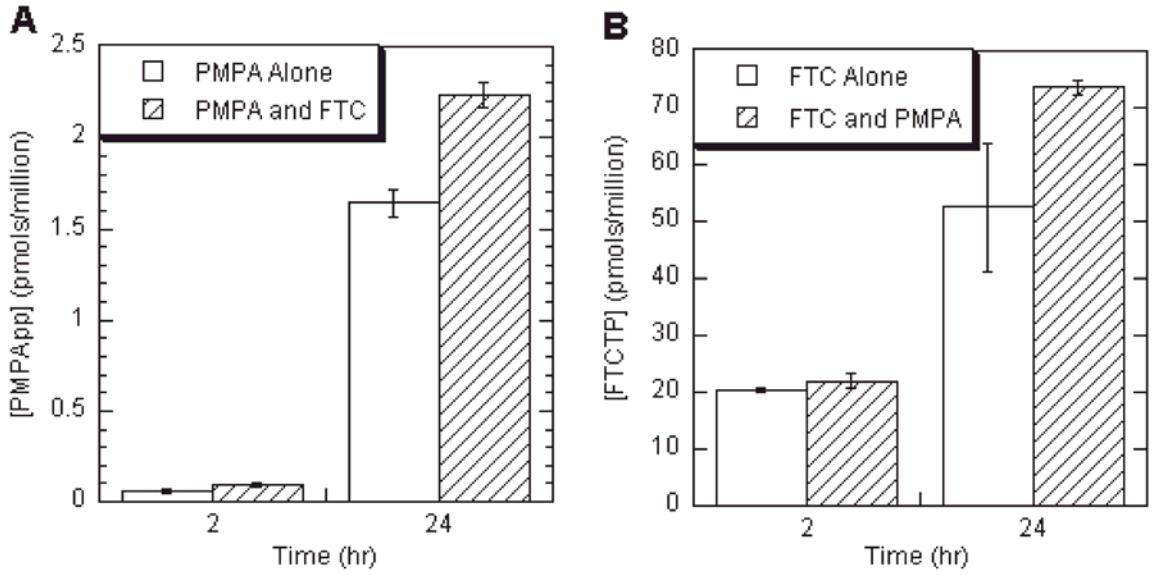
Table 36. Metabolism of 10 μM Emtricitabine (FTC) either Alone or in Combination with 10 μM Tenofovir (PMPA)

Time	FTC Alone (pmols/million cells)[a]	FTC with PMPA (pmols/million cells)[a]
(h)	FTC-TP	FTC-TP
2	20.4 ± 0.5	22.0 ± 1.3
24	52.5 ± 11.3	71.6 ± 1.4

a Values determined by LC/MS/MS.

Data from reference: PC-164-2001

Figure 13. Formation of the Active Metabolites of Tenofovir and Emtricitabine



Concentrations of the active anabolites of tenofovir (A) and emtricitabine (B), (PMPApp and FTCTP, respectively) formed after incubation of CEM-CCRF cells with 10 μM concentrations of NRTIs either alone or in combination.
Data from reference: PC-164-2001

Experiments were also carried out using a prodrug of TFV (GS-7340), which more efficiently delivers PMPA, PMPAp, and PMPApp to cells, to determine if higher levels of TFV metabolites could affect the metabolism of FTC to FTC-TP (Tabulated Summary 2.6.3.1.4, PC-164-2001). At these higher concentrations of TFV anabolites, no significant effect on the anabolism of FTC was observed with the combination as compared to the NRTI given alone. Additionally, no effects were seen on GS-7340 anabolism with and without FTC.

These results demonstrate that the intracellular phosphorylation of both TFV and FTC to their respective activated metabolites (PMPApp and FTC-TP) is not negatively affected by the simultaneous presence of the other NRTI. These results were observed using concentrations of drugs that are in excess of the plasma concentrations obtained in patients treated with these drugs (C_{max} values of 1 and 7 μM for TFV and FTC, respectively). A similar lack of an intracellular phosphorylation interaction has been described for TFV and ddI, 2 adenosine analogs that are known to have a higher degree of similarity in their phosphorylation pathways {5468}. Taken together, these results show that even at super physiological concentrations there is little potential for a negative metabolic drug interaction at the level of phosphorylation between TFV and FTC and evidence for increased production of anabolites was detected in some cell lines.

2.6.2.2.15.2. In Vitro Anti-HIV Activity of Tenofovir and Emtricitabine

Both TFV and FTC have been tested in parallel for their anti-HIV activity (Tabulated Summary 2.6.3.1.9, PC-164-2002). Two HIV-1 isolates were used in these analyses, the laboratory-adapted LAI strain and a recombinant HIV-1 strain containing a wild-type patient derived RT and protease sequence (MM-317). The infections were carried out at 2 different MOIs (0.03 and 0.1) and the results calculated as EC_{50} and EC_{90} values for each drug (Table 37). Overall, there was some effect of MOI observed, with increased EC_{50} and EC_{90} values noted for FTC with both viruses and increased values for TFV with the MM-317 virus. The drug susceptibility values derived from the laboratory strain and the patient recombinant virus were similar for both drugs. The EC_{90} values were approximately 3-fold greater than the EC_{50} values. The combined effects of TFV and FTC together on HIV-1 replication are described in the pharmacodynamic drug interactions section (Section 2.6.2.5).

Table 37. Mean EC_{50} and EC_{90} Values for Tenofovir and Emtricitabine Against HIV-1_{LAI} and HIV-1_{MM-317} Strains

Viral Isolate	MOI	TFV EC_{50} (μM)	TFV EC_{90} (μM)	FTC EC_{50} (nM)	FTC EC_{90} (nM)
LAI	0.03	5.1 ± 1.8	19.1 ± 5.8	132.3 ±± 38.5	439.7 ± 127.9
LAI	0.1	5.1 ± 0.9	17.0 ± 6.0	220.6 ± 65.4	720.8 ± 127.5
LAI	Mean	5.1	18	176	580
MM-317	0.03	6.2 ± 1.4	20.5 ± 4.8	126.6 ± 25.2	420.7 ± 83.7
MM-317	0.1	11.2 ± 0.9	37.0 ± 3.3	216.9 ± 38.4	720.8 ± 127.5
MM-317	Mean	8.7	29	172	571

Date from reference: PC-164-2002

2.6.2.2.15.3. In Vitro Selection of Resistant HIV-1 with Tenofovir and Emtricitabine

In vitro resistance selection experiments were performed with the wild-type HIV-1_{IIIB} in the immortalized MT-2 cell line with the combination of TFV and FTC (Tabulated Summary 2.6.3.1.4, PC-164-2005). In parallel, resistance selections were performed with each drug separately. The concentrations of drugs used to initiate the selection experiments were at the EC_{50} values for each individual drug for the single drug selections and one-half the EC_{50} values for the 2 drug selections to allow for adequate virus replication. As summarized in Table 38, FTC-resistant virus was selected in both cultures containing FTC when the concentration of FTC had reached 1.6 μM, or 2-fold the EC_{50} value. The genotype of this virus showed an M184I mutation in RT as the dominant mutation, with some evidence of M184V but no other genotypic changes in the first 250 amino acids of RT. Resistant virus continued to grow in the FTC-alone culture at an FTC concentration of 230 μM (> 200-fold resistance to FTC), with further evolution to dominance of the M184V mutation. Previous resistance selection experiments have also resulted in the M184I mutation which later converted to an M184V mutation upon further in vitro passage (Tabulated Summary 2.6.3.1.1, {1777}).

Table 38. In Vitro Selection of Tenofovir and Emtricitabine Resistant HIV-1

Selecting Drugs	Starting Drug Concentration	Ending Conditions		Days in Culture	HIV RT Genotype
		Drug Concentration	Fold above EC_{50}		
TFV	3.5 μM	112 μM	32	99	K65R (at 56 μM TFV; day 56)
FTC	0.8 μM	230 μM	287	48	M184I/V (at 1.6 μM FTC; day 17) M184V/I (at 230 μM FTC; day 44)
FTC + TFV	0.4 μM FTC	19.2 μM	24	155	M184I L214F (at 1.6 μM FTC, 7 μM TFV; day 24)
	1.8 μM TFV	84 μM	24		K65R (at 12.8 μM FTC, 56 μM TFV; day 114)

TFV = tenofovir; FTC = emtricitabine

Data from reference: PC-164-2005

In the TFV alone culture, resistance to TFV was observed with the K65R mutation in RT at Day 56 at a TFV concentration of 56 μM, 16-fold above the TFV EC_{50} value. In the FTC alone culture, resistance to FTC was observed with the M184V/I mutations in RT at Day 17 at an FTC concentration of 1.6 μM. In the combination culture, an M184I mutation was detected at Day 24 in culture and at an FTC concentration of 1.6 μM and a K65R mutation was observed later at Day 114 at a TFV concentration of 56 μM. The M184I mutation was not observed at this time point; however, the M184V mutation was observed upon subsequent increases in the FTC concentration to 25.6 μM. Attempts to increase the TFV concentration have resulted in a loss of HIV-1 growth in both cultures. The results obtained show that resistance development to FTC occurs much more readily and quickly than resistance development to TFV in vitro. These in vitro results suggest that under conditions of continued viral replication in vivo, the combination of TFV and FTC would initially select for the M184V/I mutation, and then potentially for TFV resistance in the form of the K65R mutation. Clinical study data using the combination of TDF with FTC support this conclusion as the M184V mutation was observed to develop in the absence of K65R in patients with virologic failure (see Module 2.5).

2.6.2.2.15.4. In Vivo Antiviral Activity of the Combination of Tenofovir and Emtricitabine in SIV-infected Macaque Monkeys

In a published study, the SIV latent cell reservoir in SIV-infected pig-tailed macaques on a regimen of TFV and FTC was analyzed (Tabulated Summary 2.6.3.1.4, {5477}). Macaques were infected with SIV and showed primary viremia of 10^5 to 10^7 copies/mL at 2 weeks postinoculation (n = 4). At 50 days postinoculation, viral loads dropped to 10^3 to 10^5 copies/mL and animals were either treated subcutaneously with TFV (20 mg/kg) and FTC (50

mg/kg) (n = 2) or were not given any drugs (n = 2). The treated macaques achieved SIV levels that were below the limit of detection of 100 copies of viral RNA per mL, whereas only 1 of the non-treated macaques showed a decrease in SIV RNA. Except for an isolated "blip" of detectable viremia, all treated animals remained close to or below the limit of detection for up to 6 months, suggesting that this regimen was effective against SIV in this macaque model. Given that viral load remained controlled in the treated animals, there was no evidence of resistance development through 6 months of dual therapy with TFV and FTC in this study.

A study was presented that explored preexposure chemoprophylaxis with antiretrovirals as a strategy to prevent the transmission of HIV (Tabulated Summary 2.6.3.1.4, {11074}). All animals were subjected to 14 weekly rectal exposures with a low dose of SHIV (SIV/HIV chimeric virus). Three groups of 6 rhesus macaques were injected subcutaneously with TFV/FTC daily, at 2 hours before and 24 hours after the first virus exposure, or at 2 hours before first virus exposure only. Twenty-one control animals did not receive any antiretroviral treatment. Twenty of the 21 control animals became infected after a median of 2.5 challenges. In contrast, all 6 animals treated with TFV/FTC daily or before and 24 hours after the first challenge were fully protected after 14 challenges. In the single-dose group, 1 of 6 animals was infected (data from 10 challenges was analyzed). In this animal model, the multiple dose TFV/FTC combination provided a high level of protection against repeated virus challenges, whereas the single dose was highly effective, but not sufficient to fully protect against rectal transmission.

2.6.2.2.16. Emtricitabine/Rilpivirine/Tenofovir DF

2.6.2.2.16.1. In Vitro HIV-1 Resistance Selection with Emtricitabine, Rilpivirine, and Tenofovir

In vitro resistance selection experiments were performed with wild-type HIV-1 (IIIB and LAI strains) in the immortalized MT-2 cell line with the combination of FTC + RPV + TFV (Tabulated Summary 2.6.3.1.5, PC-264-2003). In parallel, resistance selections were performed with the combination of FTC + TFV + EFV, and with each drug separately. Two series of selection experiments were performed. The first were traditional dose-escalation experiments where the doses of the drugs were increased over time (Table 39). The second were fixed-dose breakthrough experiments, where the doses of the drugs were kept constant (Table 40). In dose-escalation experiments, the combination of FTC + RPV + TFV resulted in the selection of HIV-1 with the M184I RT mutation by 47 days that was maintained with no additional mutations present by Day 74. The FTC + TFV + EFV combination selected for HIV-1 with the M184V RT mutation by 29 days followed by the addition of the G190A mutation for EFV by Day 74, and resulted in HIV-1 with K65R + M184V + G190A by Day 110. In fixed-dose breakthrough experiments, the combination of FTC + RPV + TFV resulted in the selection of HIV-1 with the M184I RT mutation in the culture with drug concentrations at 1.7 × their EC_{50} values for each drug, and K65R with drugs at 3.3 × their EC_{50} values. At higher drug concentrations, no virus was able to replicate at up to 64 days in culture. The triple combination of FTC + TFV + EFV was not tested in breakthrough experiments. Single-drug selection experiments yielded viruses with characteristic resistance

mutations for each drug, including RPV. No resistance mutations to RPV were observed in any of the FTC + RPV + TFV triple combination selections.

Table 39. Genotypic Changes in HIV-1 Isolates Selected by Dose-Escalation

Drug Combination[a]	Time in Culture	Concentrations Reached (Fold Change EC_{50}) [b]	Mutation in RT Gene
FTC+RPV+TFV	21 days	0.64 μM + 0.12 nM + 4.6 μM (4)	No mutations
	47 days, 57 days, 74 days	1.28 μM + 0.24 nM + 9.2 μM (8)	M184I
FTC+TFV+EFV	12 days	0.32 μM + 2.32 μM + 1.2 nM (2)	No mutations
	29 days	0.64 μM + 4.6 μM + 2.4 nM (4)	M184V
	53 days	1.28 μM + 9.2 μM + 4.8 nM (8)	M184V
	74 days, 91 days	1.28 μM + 9.2 μM + 4.8 nM (8)	M184V + G190A
	110 days	2.56 μM + 18.4 μM + 8.6 nM (16)	K65R + M184V + G190A
FTC	18 days, 44 days	1 μM (2), 16 μM (32)	M184I
	78 days	128 μM (256), 512 μM (1024)	M184V
RPV	18 days	0.4 nM (4)	No mutations
	40 days	1.6 nM (16)	V531I
	61 days, 88days	12.8 nM (128), 102.4 nM (1024)	E138G, T240I, V531I
TFV	18 days, 46 days	14 μM (4), 28 μM (8)	No mutations
EFV	18 days	8 nM (4)	No mutations
	44 days, 65 days	64 nM (32), 512 nM (256)	L100I, V179D
	88 days	2048 nM (1024)	L100I, V106I, V179D

a FTC, emtricitabine; RPV, rilpivirine; TFV, tenofovir; EFV, efavirenz

b Triple combination experiments were initiated with 0.3x the EC_{50} values for each drug: FTC + RPV + TFV used 0.16 μM + 0.03 nM + 1.2 μM, respectively; FTC + TFV + EFV used 0.16 μM + 1.2 μM + 0.6 nM, respectively. Single drug experiments were initiated with 1x the EC_{50} values with the following drug concentrations: FTC (0.5 μM), TFV (3.5 μM), RPV (0.1 nM), and EFV (2 nM). The dose-escalation selections used HIV-1 strain xxLAI.

Data from reference: PC-264-2003

Table 40. Genotypic Changes in HIV-1 Isolates Selected by Fixed-Dose Breakthrough Selections

Drug Combination	Drug Concentration (Fold Change EC_{50})[a]	Days to Breakthrough	Mutation in RT Gene
FTC + RPV + TFV	1.7 + 1.7 + 1.7[b]	22	M184I
	3.3 + 3.3 + 3.3 [b]	49	K65R
	6.7 + 6.7 + 6.7 [b]	—	—
	33.3 + 33.3 + 33.3 [b]	—	—
FTC	10	15	M184I
	20	15	M184V
	40	19	M184I
	100	15	M184V
RPV	20	8	L214F
	40	12	V179D, L214F
	100	22	K101E, L214F, S251N
TFV	5	22	No mutations
	10	46	K65R
	20	64	K65R
	100	—	—

a Triple combination experiments were initiated with multiples of the EC_{50} values for each drug. The EC_{50} values of the drugs used were FTC (0.5 μM), TFV (3.5 μM), and RPV (0.1 nM). The fixed-dose breakthrough selections used HIV-1 strain IIIb.

b Fold change EC_{50} for FTC, RPV, and TFV, respectively.

— denotes that there was no breakthrough of viral replication in these cultures by 64 days.

Data from reference: PC-264-2003

2.6.2.3. Secondary Pharmacodynamics

The secondary pharmacodynamic effects of FTC are presented in Sections 2.6.2.3.1 to 2.6.2.3.4. The secondary pharmacodynamic effects of RPV are presented in Sections 2.6.2.3.5 to 2.6.2.3.7. The secondary pharmacodynamic effects of TDF are described in Sections 2.6.2.3.9 to 2.6.2.3.12, and discussions of the secondary pharmacodynamics of FTC/TDF and FTC/RPV/TDF are presented in Sections 2.6.2.3.13 and 2.6.2.3.14, respectively.

2.6.2.3.1. Emtricitabine: Anti-Hepatitis B Virus Activity

2.6.2.3.1.1. In Vitro

HepG2 2.2.15

The in vitro anti-HBV activity of FTC has been studied extensively using the stably HBV-transfected cell line HepG2 2.2.15. In this system, FTC decreased levels of extracellular and intracellular HBV DNA in a dose-dependent manner. The EC_{50} values determined by various investigators using extracellular HBV DNA levels ranged from 0.01 to 0.04 μM. The FTC EC_{50} value based on intracellular DNA was somewhat higher, 0.16 μM, than the EC_{50} values based on extracellular DNA (Table 41) {4535}. In contrast to the situation with HIV, the EC_{50} values for FTC and 3TC are comparable against HBV.

Primary Hepatocytes

Condreay *et al.* examined the effect of FTC on HBV replication in primary human hepatocytes {4532}. Although EC_{50} values were not calculated, FTC at 2 μM completely inhibited the production of intracellular HBV DNA, even when added 24 hours after infection. The EC_{50} value calculated from inhibition of extracellular virus production is < 0.02 μM, a value that is comparable to that determined in HepG2 2.2.15 cells (Table 41).

Table 41. Anti-HBV Activity in HepG2 2.2.15 Cells of Compounds Approved and Under Development for HBV Infection

Compound	EC_{50} Values
Emtricitabine (FTC)	0.01 ± 0.005 μM[a], 0.04 ± 0.006 μM[b]
β-L-Fd4C	< 0.1 μM[d]
Adefovir	0.03 ± 0.01 μg/mL[c]
Amdoxovir	13 ± 2.1 μg/mL[c]
Clevudine	0.1 ± 0.06 μg/mL[c]
Entecavir	0.004 μM[e]
L-dT	0.19 ± 0.09 μM[d]
L-dC	0.24 ± 0.08 μM[d]
Lamivudine (3TC)	0.008 ± 0.003 μM[a], 0.0016 ± 0.0005 μg/mL[c]
Lobucavir	0.1 ± 0.1 μg/mL[c]
Penciclovir	3.5 ± 0.2 μg/mL[c], 2.5 μM[d]
Tenofovir	0.04 ± 0.02 μg/mL[c]

a Data from reference: {4535}
b Data from reference: {4533}
c Data from reference: {6287}
d Data from reference: {6288}
e Data from reference: {6295}

Inhibition of HBV DNA Polymerase

The replication cycle of hepadnavirus includes the reverse transcription of an RNA template. This process is carried out by a polymerase that shares significant sequence homology with the RT of retroviruses, including HIV. Since all attempts to date to purify the human HBV DNA polymerase have been unsuccessful, examination of the effect of FTC-TP on HBV DNA polymerase was carried out using an endogenous polymerase assay. In this assay, intact virus particles are treated with 1% Nonidet-P40, a nonionic detergent that partially disrupts the virus particles and allows nucleotide 5'-triphospate substrates to enter the virus particle so that DNA synthesis can occur. Davis *et al.*, using the endogenous polymerase assay, demonstrated that the HBV DNA polymerase could incorporate [α-^{32}P] FTC-TP into minus strand DNA {4539}. Endogenous polymerase assays using HBV particles isolated from cell cultures treated with FTC-TP showed either reduced or no polymerase activity depending on the concentration of FTC-TP used. Furthermore, the particles produced in FTC-TP treated cells did not contain any detectable HBV plus strand DNA, which is consistent with the chain terminating activity of incorporated FTC 5'-monophosphate. Taken together, the results demonstrate that FTC-TP serves as an alternative substrate inhibitor of the HBV DNA polymerase.

HBV Resistance

Treatment of HBV-infected patients with 3TC has been shown to be effective in suppressing virus replication and in reducing inflammatory activity. However, resistance to this agent has been documented to be associated with mutations in the YMDD motif (amino acids 203 to 206) in domain C of the viral DNA polymerase, analogous to changes seen in the YMDD motif of HIV-RT. Inhibition assays performed using the hepatoma derived cell lines AD38 and AD79 {6293}, which replicate wild-type and the M204V mutant HBV, respectively, confirmed that the HBV M204V mutation conferred resistance to FTC as well as to 3TC.

2.6.2.3.1.2. In Vivo

The in vivo anti-hepatitis activity of FTC was first tested in a chimeric mouse model {4530}. NIH *bg-nu-xid* mice were subcutaneously injected with suspensions of 10^7 HepG2 2.2.15 cells. Subcutaneous injection of these cells in mice resulted in the development of HBV-producing tumors in all animals. HBV could be detected in serum samples from the tumor-bearing mice using an immunoaffinity system linked to quantitative PCR. Beginning 1 week after injection, mice were orally dosed with 0.9, 3.5, 18.4, and 88.8 mg/kg/day FTC for 21 days. Comparison of tumor progression and human α-fetoprotein levels in control versus drug-dosed mice indicated that FTC did not have anti-tumor activity. However, FTC at the 18.4 and 88.8 mg/kg/day doses did significantly reduce circulating levels of HBV DNA. Examination of tumor extracts in these 2 dose groups revealed a marked reduction in intracellular levels of replicative HBV DNA intermediates, including double-stranded linear DNA.

Transgenic SCID Mice

Anti-HBV activity has also been reported for FTC in HBV transgenic SCID mice {6290}. A group of 5 mice were treated with FTC at 100 mg/kg/day intraperitoneal for 6 days, and observed for an additional 6 days posttreatment. By Day 3 of treatment, 2 mice had undetectable HBsAg, and an additional 2 mice cleared HBsAg on Day 5. The remaining mouse cleared HBsAg on Day 8. Hepatitis B virus DNA levels in blood were determined by semiquantitative PCR at Days 3, 5, 8, and 12. By Day 3 of treatment, PCR signals had dropped 10-fold in all of the mice. By Day 8, none of the mice had detectable HBV DNA levels by PCR. In contrast to 3TC, there were no signs of rebound in the levels of HBV DNA during the course of treatment.

Woodchuck Hepatitis Virus (WHV)

The WHV in its natural host, the eastern woodchuck *Marmata monax*, is the most frequently used model of HBV infection. In an oral dosing study, 5 groups of chronically infected woodchucks were given FTC once daily at 1 of 5 doses (0.3, 1.0, 3.0, 10, or 30 mg/kg) for 4 weeks {6292}. At doses of 3.0 mg/kg and greater, FTC induced a statistically significant reduction in both serum viremia and replicative intermediates. The largest reduction in viremia, approximately 4.9 $\log_{10}$, and in replicative intermediates, approximately 80-fold, was seen at the 30 mg/kg dose. No significant effect on the levels of intrahepatic RNA, serum levels of WHsAg, or the appearance of antibodies to WHsAg or WHcAg in the serum were observed. Viremia returned to pretreatment levels within 1 to 2 weeks following the cessation of treatment at all doses. This rapid rebound is consistent with a lack of significant impact on levels of WHV cccDNA.

Cullen *et al.* have studied the effect of FTC on WHV in naturally infected, wild caught woodchucks {6293}. Animals were dosed intraperitoneally at either 20 or 30 mg/kg BID for 4 weeks. Administration of the 20 mg/kg dose suppressed WHV DNA levels from 6- to 49-fold (average of 27-fold in the 6 animal groups). Serum DNA polymerase activity measured by the incorporation of [^{32}P]dCTP into WHV DNA was reduced in a similar fashion. A more profound effect was seen at the 30 mg/kg dose. Serum WHV DNA levels were reduced from 20- to 150-fold (average of 56-fold) in the 6 animal groups. Serum DNA polymerase activity was similarly reduced. WHV DNA levels in the liver (determined in biopsy specimens) were also reduced in all 6 of the animals in the 30-mg/kg treatment group. Reductions ranged from 68% to 98% of the pretreatment levels. The authors stated that while the level of replicative intermediates remained close to those seen pretreatment, the WHV genome was being shifted toward shorter fragments.

2.6.2.3.2. Emtricitabine: In Vitro Cytotoxicity

2.6.2.3.2.1. Emtricitabine: Cytotoxicity in Human Cells

The cytotoxicity of FTC has been evaluated extensively in vitro (Table 42). In all the cell lines examined, cell growth was not affected at concentrations of FTC ≥ 100 μM. Results are shown in Table 42.

Table 42. Cytotoxicity of Emtricitabine in Comparison to Lamivudine and Zidovudine

Cells	CC_{50} (µM)		
	FTC	3TC	ZDV
MT-4	> 100[a], > 200[b]	> 100[a], > 33[b]	20[a], > 100[b]
PBMC	> 100[a]	> 100[a]	> 100[a]
CEM	> 100[a], > 100[b]	> 100[a], > 100[b]	14.3[a], > 6[b]
Vero	> 100[a]	> 100[a]	28.0[a]
IM9	> 100[b]	> 100[b]	70[b]
Molt 4	> 100[b]	> 100[b]	10[b]
HepG2 2.2.15	> 200[b], > 200[c]	> 200[b]	> 200[b]

a Data from reference: {4533}
b Data from reference: {4531}
c Data from reference: {4535}

Because of the apparent correlation between toxicity to bone marrow progenitor cells in vitro and bone marrow suppression in vivo, human bone marrow progenitor colony-forming assays were performed. The concentration of FTC required to inhibit the formation of granulocyte-macrophage (CFU-GM) colonies by 50% (CC_{50}) was 300 ± 40 µM (n = 6). The CC_{50} for erythroid colonies (BFU-E) was 220 ± 8 µM (n = 6). CC_{50} values for 3TC were comparable, with a value for CFU-GM of 260 ± 8 µM and a value for BFU-E of 180 ± 2 µM (Tabulated Summary 2.6.3.1.6, TPI 11963 and {4535}) {4531}. The CC_{50} values of the (+) isomer of 3TC in the bone marrow progenitor cell assay were 10 ± 2 µM for CFU-GM and 4 ± 1 µM for BFU-E. It is interesting that the (+) isomer of 3TC showed bone marrow toxicity, while the (+) isomer of FTC did not.

2.6.2.3.2.2. Emtricitabine: Mitochondrial Toxicity

To evaluate the potential for mitochondrial toxicity of FTC, HepG2 cells were incubated with FTC at concentrations ranging between 0.1 and 10 µM for 2 weeks {4550} (Tabulated Summary 2.6.3.1.6, TPI 11963), and MT-2 cells were incubated with FTC at concentrations up to 100 µM for up to 8 weeks (Tabulated Summary 2.6.3.1.6, TPI 11963). Under these conditions, FTC had no adverse effects on cell growth, mitochondrial DNA synthesis, or lactic acid production. In a separate study conducted in HepG2 cells exposed to concentrations of FTC ranging from 0.1 to 10 µM for 7 days, no effects on mitochondrial morphology were observed by transmission electron microscopy (Tabulated Summary 2.6.3.1.6, 233).

The inhibition of mitochondrial DNA synthesis was assessed in an in vitro cell culture assay using Molt-4 cells (a T-lymphoblast cell line). The ratio of mitochondrial to cellular DNA was determined after prolonged exposure of cells to clinically relevant concentrations of FTC

(0.1, 1, 10, and 100 μM) (Tabulated Summary 2.6.3.1.6, TGZZ/93/0016 and TGZZ/93/0023). The ratio of mitochondrial DNA to genomic DNA for Molt-4 cells treated with several nucleoside analogues is shown in (Table 43).

Treatment of Molt-4 cells with various concentrations of ddC resulted in a reduction of the mitochondrial DNA content of the cells. After 5 days of treatment with 0.05 μM ddC, there was an 80% reduction in the ratio of mitochondrial to cellular DNA; however, there was no apparent increase in doubling time of the treated cells (data not shown). At 0.5 μM ddC, there was a 92% reduction in the ratio of mitochondrial DNA to cellular DNA.

Emtricitabine did not reduce the ratio of mitochondrial to cellular DNA when tested at concentrations of up to 100 μM after 7 days of continuous cell exposure.

Table 43. Ratio of Mitochondrial DNA to Cellular DNA

Compound	N	Concentration (μM)	Days	Mitochondrial DNA/Cellular DNA	
				Control	Percent of Control
Zalcitabine (ddC)	2	0.05	5	102 ± 3[b]	20 ± 10
		0.1	5		11 ± 8
		0.5	5		8 ± 1
		5.0	5[a]		ND
Emtricitabine (FTC)	2	0.1	7	104 ± 12	104 ± 10
		1.0	7		121 ± 11
		10.0	7		98 ± 15
		100.0	7		123 ± 19
Zidovudine (ZDV)	1	0.5	9	100 ± 39	130[c]
		5.0	9		174[c]
Fialuridine (FIAU)	2	0.1	7	105 ± 4	82 ± 27
		0.5	7		89 ± 37
		5.0	2[a]		173 ± 78
Alovudine (FLT)	1	0.05	7	103 ± 14	41
		0.1	7		17
		0.5	7		14
		5.0	5[a]		7
Stavudine (d4T)	1	0.5	9	100 ± 39	113
		5.0	9		60

a Cell death

b Standard deviation, n > 1

c n = 1

Data from references: TGZZ/93/0016, TGZZ/93/0023

Thymidine analogues were also examined. After 7 days of treatment with 0.05 μM FLT (alovudine, 3'-dideoxy-3'-fluorothymidine), there was a 59% reduction in the ratio of mitochondrial to cellular DNA. After 9 days exposure to 5 μM d4T (stavudine, (2R,5S)-1-(2,5-dihydro)-5-(hydroxymethyl)-2-furyl thymidine), there was a 40% reduction in the ratio of mitochondrial to cellular DNA. In contrast, fialuridine (FIAU) caused no reduction in the ratio of mitochondrial to cellular DNA after 7 days exposure to 0.1 or 0.5 μM of drug; however, significant cell death was noted after exposure to 5 μM FIAU for 2 days. Zidovudine (3'-azido-3'-deoxythymidine) at 0.5 or 5.0 μM caused no significant decrease in the ratio of mitochondrial to cellular DNA after 9 days exposure. It should be noted that cell death occurred at 5 μM ddC, FLT, and FIAU.

Cell death was not noted in ZDV- or FTC-treated cells at concentrations up to 100 μM.

The lack of impact of FTC on mitochondrial DNA content and function is consistent with its very low affinity for γ polymerase.

2.6.2.3.3. Emtricitabine: In Vitro Receptor Binding Potencies

The effects of FTC (524W91) on the specific binding of various radioactively labeled ligands were studied in 19 different receptor binding assays (Tabulated Summary 2.6.3.1.6, TPZZ/93/0002). Tissues were obtained in all but 2 assays from Sprague Dawley rats. A heart preparation was obtained from beagle dogs for use as a calcium release channel-binding assay, and platelets which were isolated from New Zealand White rabbits were used for a platelet-activating-factor assay. Emtricitabine had no pharmacologically significant binding affinity at the adenosine (A1 and A2), adrenergic (alpha1, alpha2, and beta), angiotensin II, benzodiazepine, calcium channel (dihydropyridine and phenylalkylamine), calcium release channel (ryanodine), cholinergic (M1 and M2), dopamine$_2$, GABA$_a$ gated chloride channel (TBPS), glutamate, neurotensin, platelet activating factor, and serotonergic (5HT1A and 5HT2) receptors.

2.6.2.3.4. Emtricitabine: In Vitro Autonomic Pharmacology Effects on Peripheral Autonomic Receptors

A variety of isolated-muscle preparations were used in vitro to assess effects of FTC on autonomic function and peripheral receptors (Tabulated Summary 2.6.3.1.6, TPZZ/92/0055). Receptor activity functions that were investigated were cholinergic (guinea pig ileum), adrenergic (rabbit aorta, guinea pig atria, and trachea), histaminergic (guinea pig atria), and serotonergic (rat fundus), as well as tissue responsiveness to arachidonic acid (rat fundus), bradykinin (guinea pig ileum), and angiotensin II (rabbit aorta). Emtricitabine (0.1 μM or 1.0 μM) had little or no direct effect on various isolated muscle preparations and had no major inhibitory effects on the contractile responses to acetylcholine, norepinephrine, serotonin, isoproterenol, arachidonic acid, histamine, bradykinin, and angiotensin II.

2.6.2.3.5. Rilpivirine: Activity Against Other Viruses

No antiviral activity of TMC278 (EC_{50} values > 10 µM) was observed against human HBV, herpes simplex virus 2, human corona virus, influenza A virus, and vaccinia virus (Tabulated Summary 2.6.3.1.2, TMC278-IV2-AVMR).

2.6.2.3.6. Rilpivirine: In Vitro Cytotoxicity

2.6.2.3.6.1. Rilpivirine: Cytotoxicity in Human Cells

The in vitro effects of TMC278 on the cell proliferation and the viability of HeLa (epithelial cervix, adenocarcinoma), HepG2 (epithelial liver, hepatoblastoma), HEp-2 (epithelial cervix adenocarcinoma), MRC-5 (normal fetal lung fibroblast), and A549 (epithelial lung carcinoma) cells were investigated (Tabulated Summary 2.6.3.1.2, TMC278-IV2-AVMR). EFV, ETR, and NVP were tested in the same assays.

The CC_{50} value (as a measure of inhibition of cell proliferation) was determined as the concentration of a compound that resulted in a drop of 50% cell growth compared to the control. The median CC_{50} values of TMC278, EFV, ETR, and NVP on the different cells were measured at Day 3 and Day 5. The data presented in Table 44 show that the median CC_{50} values for TMC278 range between 17.34 to 34.51 µM (6.35 to 12.65 µg/mL) and between 16.90 to 35.59 µM (6.19 to 13.04 µg/mL) at Day 3 and Day 5, respectively. Median CC_{50} values for EFV, ETR, and NVP are all > 40 µM, except for NVP on MRC-5 cells.

Table 44. In Vitro TMC278 and NNRTI CC_{50} Values

Cells	Median CC_{50} (µM) (IQR)			
	TMC278	EFV	ETR	NVP
Day 3				
A549	25.48 (25.05 - 25.69)	>40	>40	>40
HeLa	17.34 (17.22 - 17.37)	>40	>40	>40
HEp-2	34.38 (31.88 - 35.43)	>40	>40	>40
HepG2	33.18 (32.52 - 33.21)	>40	>40	>40
MRC-5	34.51 (32.84 - 34.86)	>40	>40	33.03 (32.02 - 33.74)
Day 5				
A549	26.70 (26.60 - 27.52)	>40	>40	>40
HeLa	16.90 (16.41 - 18.51)	>40	>40	>40
HEp-2	26.27 (25.46 - 28.88)	>40	>40	>40
HepG2	27.13 (26.99 - 30.83)	>40	>40	>40
MRC-5	35.59 (35.34 - 37.79)	>40	>40	35.96 (35.09 - 36.03)

Cell lines (MRC-5, HepG2, HeLa, HEp-2, and A549) were seeded one day before compound addition (Day −1), in flat-bottom 96-well plates. Cells were incubated at 37°C in a 5% CO_2 atmosphere in the presence of 2-fold serial dilutions of the compounds. Rezasurin (50 µl) was added for 6 hours incubation at 37°C. The CC_{50} of TMC278 was estimated by the rezasurin assay on Day 0 (control), Day 3, and Day 5. The experiments were performed in triplicate.

The TC_{50} value (as a measure of inhibition of cell viability) was defined as the concentration of a compound that resulted in a 50% reduction in cell growth on Day 3 or Day 5 compared to Day 0. Median TC_{50} values of TMC278, EFV, ETR, and NVP on the different cells were determined at Day 3 and Day 5. The data presented in Table 45 show that the median TC_{50} values for TMC278 range between 33.94 to > 40.00 µM (12.44 to >14.66 µg/mL) and between 31.93 to > 40.00 µM (11.70 to >14.66 µg/mL) at Day 3 and Day 5, respectively. Median TC_{50} values for EFV, ETR, and NVP at Day 3 and Day 5 are all > 40 µM.

Table 45. In Vitro TMC278 and NNRTI TC_{50} Values

Cells	Median TC_{50} (µM) (IQR)			
	TMC278	**EFV**	**ETR**	**NVP**
Day 3				
A549	>40	>40	>40	>40
HeLa	33.94 (33.89 - 34.17)	>40	>40	>40
HEp-2	>40	>40	>40	>40
HepG2	>40	>40	>40	>40
MRC-5	>40	>40	>40	>40
Day 5				
A549	>40	>40	>40	>40
HeLa	31.93 (31.77 - 32.65)	>40	>40	>40
HEp-2	>40	>40	>40	>40
HepG2	>40	>40	>40	>40
MRC-5	>40	>40	>40	>40

The results are expressed as the median and interquartile ranges of 3 independent experiments.

Cell lines (MRC-5, HepG2, HeLa, HEp-2, and A549) were seeded one day before compound addition (Day −1), in flat-bottom 96-well plates. Cells were incubated at 37°C in a 5% CO_2 atmosphere in the presence of 2-fold serial dilutions of the compounds. Rezasurin (50 µl) was added for 6 hours incubation at 37°C. The TC_{50} effect of TMC278 was estimated by the rezasurin assay on Day 0 (control), Day 3, and Day 5. The experiments were performed in triplicate.

Median CC_{50} values for TMC278, EFV, ETR, and NVP on MT-4-LTR-EGFP cells were measured at Day 3. The median CC_{50} for TMC278 was 5.91 µM, whereas it was > 32 µM for EFV, ETR, and NVP (Table 46).

Table 46. In Vitro TMC278 and NNRTI CC_{50} Values on MT-4

Cells	Median CC_{50} (µM) (IQR) (n)			
	TMC278	**EFV**	**ETR**	**NVP**
MT-4	5.91 (5.02–8.31) (381)	40.02 (36.46–43.06) (805)	> 64.00 (820)	> 32.00 (457)

A selectivity index (ratio of the CC_{50} to the EC_{50} value);(EC_{50} = 0.73 nM and CC_{50} = 5.91 µM) of 8096 was calculated in MT-4 cells, indicating that TMC278 is a potent and selective inhibitor of HIV-1 in vitro (Tabulated Summary 2.6.3.1.2, TMC278-IV1-AVMR).

2.6.2.3.7. Rilpivirine: In Vitro Receptor Binding and Neurotransmitter Uptake Inhibition

The interaction of TMC278 base with 19 receptors in tissue or cellular preparations was studied at a concentration of 10 µM (3.7 µg/mL) in buffer containing 1% DMSO (Tabulated Summary 2.6.3.1.7, TMC278-870219). TMC278 did not cause any significant inhibition of binding to α- or β-adrenergic, dopaminergic, muscarinergic, serotonergic, opioid, interleukin, or chemokine receptors.

No agonistic or antagonistic activity of TMC278 was noted on histamine H2 receptors in the isolated guinea pig right atrium at 30 µM (11 µg/mL) (Tabulated Summary 2.6.3.1.7, TMC278-NC204 [2]). No inhibition of adenosine triphosphatase (ATPase) by TMC278 was evident in isolated pig stomach at 10 µM (3.7 µg/mL) (Tabulated Summary 2.6.3.1.7, TMC278-NC204 [3]). The incubation in these 2 studies was done in buffer containing 0.1% DMSO.

2.6.2.3.8. Rilpivirine: In Vivo Effect on Gastic Antisecretory Activity

TMC278 was evaluated for possible gastric antisecretory activity in the pentagastrin-stimulated gastric acidity assay (Tabulated Summary 2.6.3.1.7, TMC278-NC204 [1]). Three groups of 5 male Wistar rats fasted overnight were treated intraperitoneally with TMC278 at 9.1 mg/kg at a volume of 5 mL/kg in vehicle (2% Tween 80 in 0.9% NaCl in water), or with the positive control cimetidine (10 mg/kg). Thirty minutes later, gastric acid production was stimulated by intraperitoneal injection of 5 µg/kg pentagastrin dissolved in distilled water. TMC278 caused a 20% increase in gastric acidity compared to vehicle. The positive control reduced acidity by 74%. The significance of the slightly increased gastric acidity effect by TMC278 is considered limited.

2.6.2.3.9. Tenofovir DF: Anti-Hepatitis B Virus Activity

2.6.2.3.9.1. In Vitro

Tenofovir is a potent and selective inhibitor of HBV. Tenofovir inhibits HBV production in HepG2 2.2.15 and HB611 cells with EC_{50} values of 1.1 and 2.5 µM, respectively, and corresponding CC_{50} values of > 100 and 260 µM, respectively (Tabulated Summary 2.6.3.1.8, {21}, P4331-00038). As observed with anti-HIV activity, TDF showed increased in vitro potency against HBV in comparison with TFV (EC_{50} = 0.018 µM in HEPG2 2.2.15 cells). Tenofovir activity against 4 laboratory strains of HBV with up to 3 3TC-associated HBV polymerase mutations was shown to be within 2.2-fold of the wild-type EC_{50} (Tabulated Summary 2.6.3.1.8, {8381}). A range of 0.7- to 3.3-fold reductions in TFV susceptibility was observed for 3TC-resistant HBV clinical isolates with multiple mutations

in HBV pol {10426}, {10916}, {7060}. In contrast, 3TC demonstrated >200-fold reduced activity against these HBV mutants. The rtA194T HBV mutant, either alone or in the presence of two 3TC-resistance mutations, showed < 2.4-fold change in TFV susceptibility (Tabulated Summary 2.6.3.1.8, PC-104-2012). Laboratory strains of HBV expressing ADV-associated HBV mutations (rtN236T, rtA181V/T) showed reductions in TFV susceptibility ranging from 1.5- to 10-fold as compared to wild-type {10926}. A panel of entecavir-associated HBV mutations showed reductions in TFV susceptibility ranging from 0.6- to 6.9-fold as compared to wild-type (Tabulated Summary 2.6.3.1.8, PC-174-2003). Tenofovir has also been shown to inhibit the replication of duck HBV (DHBV) in primary duck hepatocytes with an EC_{50} of 0.11 μM (Tabulated Summary 2.6.3.1.8, {10}).

2.6.2.3.9.2. In Vivo

The WHV in its natural host, the eastern woodchuck *Marmata monax*, is a frequently used model of HBV infection. The antiviral activity of TDF was evaluated in woodchucks chronically infected with WHV. In a short dose-ranging study, oral administration of TDF at 0.5 , 1.5, and 5.0 mg/kg of body weight/day for 4 weeks reduced serum viral load significantly, at 0.2 $\log_{10}$ ($p < 0.01$), 1.1 $\log_{10}$ ($p < 0.01$), and 1.5 $\log_{10}$ ($p < 0.05$) from the pretreatment levels. The 15 mg/kg of body weight/day dose for 4 weeks reduced serum viral load by 1.2 $\log_{10}$, but was not considered statistically significant due to the degree of individual variation in the antiviral response {8176}.

2.6.2.3.10. Tenofovir DF: In Vitro Anti-HTLV-1 Virus Activity

The antiviral effects of TFV and several other NRTIs on the human retrovirus human T cell leukemia virus type I (HTLV-I) has been studied {5483}. There are currently no accepted therapies for HTLV-I disease; however, ZDV or interferon-α in combination with antiviral nucleoside analogs have been tested in the clinic with some success. Using a single-cycle, quantitative cell culture replication system for HTLV-I infection, the NRTIs 3TC, ABC, TFV, d4T, ZDV, and ddC were assayed for HTLV-I antiviral activity. Tenofovir was found to be the most potent inhibitor of HTLV-I replication, with an EC_{50} of 5.4 nM, followed by ZDV, ddC, ABC, d4T, and 3TC, with EC_{50} values ranging from 0.11 to 22 μM. This study suggests that TFV is more potent than ZDV and other NRTIs for the inhibition of HTLV-I in vitro.

2.6.2.3.11. Tenofovir DF: In Vitro Cytotoxicity

2.6.2.3.11.1. Tenofovir DF: Cytotoxicity in Human Cells

PBMCs and T-lymphocytic Cells

The cytotoxicity of TFV was determined both in quiescent and activated PBMCs, and in an established T-lymphocytic cell line. In PBMCs and MT-2 cells, TFV exhibited low cytotoxicity with CC_{50} values > 1 mM. In quiescent PBMCs, no cytotoxic effect of TFV was detected at concentrations as high as 100 μM {1574}.

Liver Cells

In vitro cytotoxicity of TFV and other NRTIs was determined in HepG2 cells, an established cell line derived from human hepatocytes. After an 8-day incubation with proliferating HepG2 cells, TFV showed only a weak inhibition of cell growth, as indicated by a CC_{50} value of 588 µM (Table 47). Under the same conditions, only 3TC was less cytotoxic than TFV. Abacavir, d4T, ddI, ZDV, and ddC showed more pronounced inhibition of HepG2 cell growth than TFV (Tabulated Summary 2.6.3.1.8, P4331-00037).

Skeletal Muscle Cells

Similar to the observations in lymphocytes and liver cells, low in vitro cytotoxicity of TFV was also observed in normal human skeletal muscle cells (SkMCs). In a 6-day assay with proliferating SkMCs, TFV showed a CC_{50} of 870 µM, which was comparable to that of 3TC and ddI (Table 47). In contrast, ZDV was approximately 2-fold more cytotoxic than TFV; and ABC, ddC, and d4T were significantly more cytotoxic towards SkMCs than TFV with CC_{50} values < 100 µM (Tabulated Summary 2.6.3.1.8, P4331-00037).

Table 47. In Vitro Cytotoxicity of Tenofovir and Other NRTIs in Human Liver and Skeletal Muscle Cells

Drug	In vitro Cytotoxicity – CC_{50} [µM] [a]	
	Human Liver Cells (HepG2)	Human Skeletal Muscle Cells (SkMCs)
TFV	588 ± 223	870 ± 275
ABC	320 ± 35	97 ± 13
ZDV	87 ± 11	497 ± 19
ddC	7.7 ± 0.2	90 ± 38
ddI	279 ± 190	846 ± 253
d4T	290 ± 22	66 ± 38
3TC	1021 ± 66	1230 ± 331

a Mean ± standard deviation from two independent experiments.

Data from Reference: P4331-00037

Hematopoietic Toxicity

The hematopoietic toxicity of TFV and 4 other NRTIs (ZDV, d4T, ddC, and 3TC) was evaluated in human CD34+ bone marrow progenitor stem cells exposed to specific cytokines, which programmed their differentiation and expansion into the erythroid and myeloid lineages {4077}. The expansion of the 2 lineages in the presence of the tested drugs was determined with progenitor cells from 2 independent donors by immunofluorescence detection of lineage-specific cell surface markers. Irrespective of the donor, TFV, at concentrations as high as 200 µM, showed no significant effect on the expansion of the erythroid lineage from the progenitor stem cells as determined by the level of expression of

glycophorin A (Table 48). Likewise, TFV showed only limited effects on the expansion of the myeloid lineage based on the expression of CD11b, with the inhibition being more pronounced in progenitor cells from donor 2 (CC_{50} of 85 μM). Likewise, 3TC exhibited only a weak cytotoxicity against both the erythroid and myeloid lineages, with a moderate degree of inhibition observed at a concentration of 200 μM. In contrast, ZDV and d4T produced notable suppression of the erythroid and myeloid lineage expansion. Zalcitabine (ddC) caused by far the most severe suppression of both the erythroid and myeloid lineages with CC_{50} values ranging from < 0.06 to 0.38 μM.

Table 48. In Vitro Hematopoietic Toxicity of Tenofovir in Comparison with Other NRTIs

Drug	Hematopoietic Toxicity – CC_{50} [μM] [a]			
	Myeloid Lineage		Erythroid Lineage	
	Donor 1	Donor 2	Donor 1	Donor 2
TFV	> 200	85	> 200	> 200
ZDV	49	3.6	0.85	0.62
d4T	200	10.5	5.0	3.3
ddC	0.38	0.24	0.14	< 0.06
3TC	> 200	140	> 200	170

a Concentration of each drug inhibiting production of the myeloid or erythroid lineage from progenitor stem cells by 50%. The results are from a single experiment performed in triplicate.

Data from reference: {4077}

Renal Proximal Tubule Epithelial Cells

Effects of TFV have been studied in several in vitro models for renal proximal tubular toxicity and compared with those of cidofovir and adefovir in order to better understand the in vivo differences in nephrotoxicity observed between the 3 structurally related nucleotide analogs. In normal human renal proximal tubule epithelial cells (RPTECs), TFV showed a negligible effect on the cell growth with a CC_{50} of > 2,000 μM (Table 49). Moreover, TFV did not exhibit any marked effect on the long-term viability of quiescent RPTECs during a 25-day incubation (Table 49). In contrast, the half-life of quiescent RPTECs in the presence of cidofovir and adefovir was approximately 10 and 21 days, respectively (Tabulated Summary 2.6.3.1.8, P4331-00037). In a separate study, TFV did not cause significant changes in cell viability in RPTECs after 22 days at concentrations up to 300 μM {9864}.

Integrity of the proximal tubule epithelium is essential for maintaining the selective barrier between blood and urine. As shown in Table 49, TFV at concentrations as high as 3 mM did not significantly affect the in vitro integrity of the differentiated proximal tubule epithelium when assessed by measuring the transepithelial resistance after a 10-day incubation with the drug {2520}. By comparison, cidofovir and adefovir reduced the tubular epithelium integrity by 50% at 105 μM and 1.2 mM, respectively.

Human renal organic anion transporter 1 (hOAT1), a protein localized in the basolateral membrane of the renal proximal tubule epithelium, has been implicated in the etiology of cidofovir- and adefovir-associated nephrotoxicity {2087}. Transport kinetics experiments revealed similar transport efficiency (calculated as V_{max}/K_m ratio) for cidofovir, adefovir, and TFV (Table 49) {2520} suggesting that a lack of interference with essential intracellular function(s) rather than a difference in renal transport is responsible for the improved nephrotoxicity profile of TFV.

Table 49. Profile of Tenofovir, Cidofovir, and Adefovir in In Vitro Models of Renal Proximal Tubular Toxicity

In vitro Assay	Tenofovir	Cidofovir	Adefovir
Inhibition of RPTECs growth; CC_{50} [μM]	> 2,000	260	495
Viability of RPTECs; $t_{1/2}$ [days] [a]	> 25	9.7	21
Integrity of RPTEC epithelium; $CTER_{50}$ [b] [μM]	> 3,000	110	1,100
Efficiency of hOAT1-mediated transport [V_{max}/K_m]	3.26	1.77	1.93

a In the presence of 500 μM drug.

b $CTER_{50}$, concentration reducing the transepithelial resistance of RPTEC monolayer cultured on microporous membrane by 50%.

Data from references: P4331-00037, {2520}

2.6.2.3.11.2. Tenofovir DF: Mitochondrial Toxicity

A variety of clinical symptoms observed in HIV patients treated with prolonged NRTI therapy may be linked to mitochondrial toxicity. These include myopathy and cardiomyopathy, polyneuropathy, lactic acidosis, pancreatitis, lipodystrophy, and possibly others {2522}. Tenofovir was studied and compared with other NRTIs for its in vitro effects on mitochondrial DNA (mtDNA) synthesis and lactic acid production. Results of these studies indicate a low potential of TFV to interfere with mitochondrial functions.

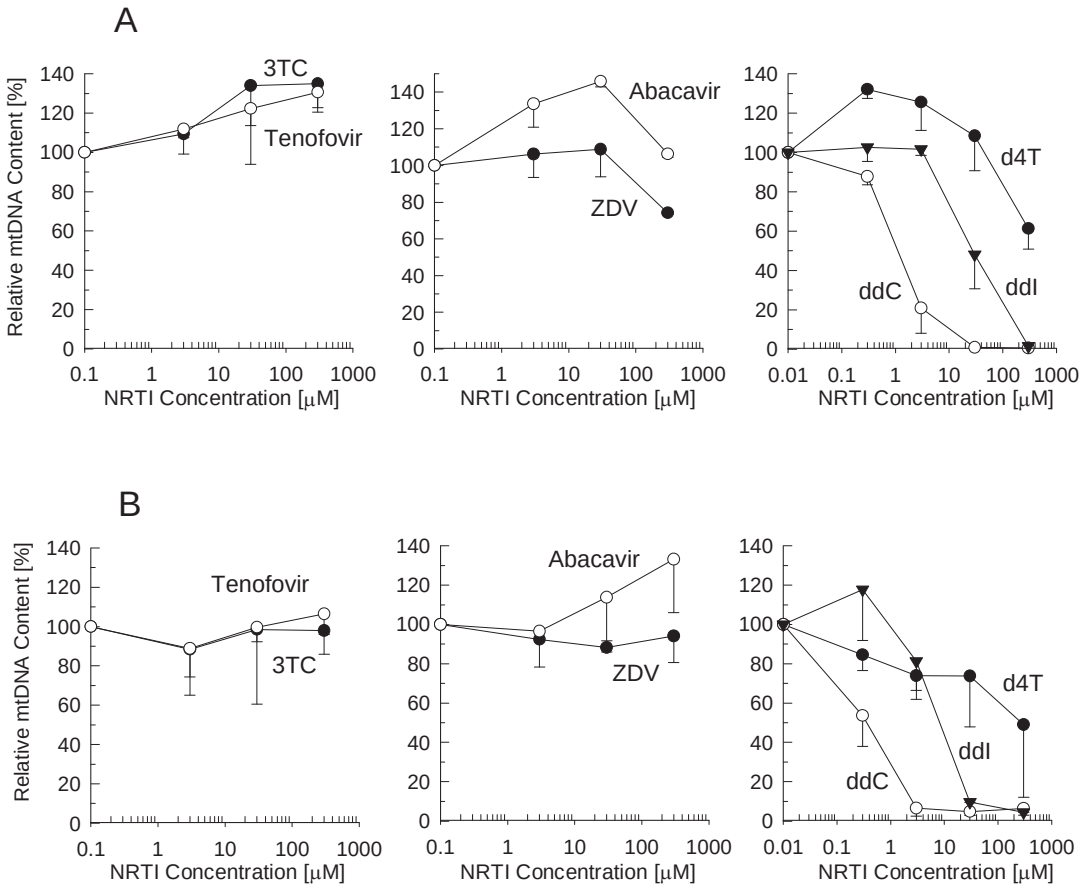
Synthesis of Mitochondrial DNA

Hybridization analyses to quantify mitochondrial DNA and chromosomal DNA levels were performed to assess any relative impairment in mtDNA synthesis. In HepG2 cells following a 9-day treatment with TFV at concentrations ranging from 3 to 300 mM; no effect of TFV on the synthesis of mtDNA was observed (Figure 14 A). In contrast, ddC and ddI showed marked depletion of mtDNA in HepG2 cells. Stavudine and ZDV showed less pronounced effects, with a reduction of relative mtDNA content of 30% to 40% at 300 μM. Similar to TFV, 3TC, and ABC did not significantly change the relative levels of mtDNA (Table 50; Tabulated Summary 2.6.3.1.8, P1278-00042).

Treatment of SkMCs with TFV and other NRTIs yielded very similar conclusions. Proliferating SkMCs did not show any decrease in mtDNA levels following a 9-day incubation with up to 300 μM of TFV (Figure 14 B). Lamivudine, ABC, and ZDV also did not deplete mtDNA in these cells. Stavudine moderately reduced mtDNA, while ddC and ddI

showed marked depletion of mtDNA in SkMCs. Similar effects of the tested drugs, including no depletion of mtDNA by TFV, were observed upon a 3-week treatment of quiescent nonproliferating SkMCs (Table 50; Tabulated Summary 2.6.3.1.8, P1278-00042).

Figure 14. Effect of Tenofovir and Other NRTIs on Mitochondrial DNA Content in Human Liver and Skeletal Muscle Cells



HepG2 human liver cells (A) and human skeletal muscle cells (B) were incubated with various drug concentrations for 9 days and DNA content was determined by hybridization analysis. Data are the means ± standard deviation from two independent experiments.

Data from reference: P1278-00042

Table 50. Effect of Tenofovir and Other NRTIs on Mitochondrial DNA Content in Differentiated Human RPTECs

Drug	Drug Concentration [μM]	Content of mtDNA [% of control][a]	
		12 days	21 days
TFV	300	118 ± 5.9	109 ± 9.2
ZDV	200	109 ± 3.0	104 ± 14.7
d4T	200	92.3 ± 13.9	77.0 ± 10.9
ddC	2	12.1 ± 1.1	5.1 ± 0.6
ddI	40	47.5 ± 5.3	25.7 ± 0.1

a Relative content of mtDNA in RPTECs after 12- and 21-day drug treatment given as mean ± standard deviation from a representative experiment performed in duplicates.

Data from reference: P1278-00042

Because of the potential renal accumulation of TFV, effects of the drug on mtDNA in human RPTECs were also characterized. As shown in Table 50, no changes in relative mtDNA levels were observed following up to a 21-day treatment of differentiated RPTECs with TFV or ZDV. While d4T produced minor decrease in mtDNA content, ddC and ddI showed the most pronounced effects on mtDNA in RPTECs (Table 50; Tabulated Summary 2.6.3.1.8, P1278-00042). In a separate study, levels of mtDNA or COXII mRNA were not affected by TFV treatment of RPTECs for 22 days at concentrations up to 300 μM {9864}.

Overall, the relative effects of the drugs on mtDNA content were similar in all human cell types tested, and correlated well with the efficiencies of incorporation into DNA of the respective dNTP analogs by DNA polymerase γ determined in vitro (Table 25).

Production of Lactic Acid

Lactic acid production is one of the widely used mitochondrial markers. Drug-related deficiencies in the mitochondrial oxidative phosphorylation system may induce a shift in the pyruvate/lactate ratio leading to increased production of lactic acid {2522}. As shown in Table 51, TFV does not increase the lactic acid production in HepG2 cells and SkMCs after 3- and 6-day incubations, relative to the untreated controls. Similarly, no effect was observed with 3TC. However, ZDV produced a concentration-dependent increase in the lactate production in both cell types tested (Tabulated Summary 2.6.3.1.8, P1278-00042). Lactic acid production appears to be one of the few methods by which mitochondrial toxicity can be detected for ZDV in vitro. There were no measurable increases with TFV at concentrations up to 300 μM.

Table 51. Effects of Tenofovir and Other NRTIs on the In Vitro Production of Lactic Acid

Drug	Concentration (μM)	Lactic Acid Production (mg /10^6 cells)[a]	
		HepG2 cells[b]	SkMCs[b]
None	--	1.61 ± 0.25 (100)	7.53 ± 0.83 (100)
TFV	30	1.34 ± 0.18 (83)	7.23 ± 1.21 (96)
	300	1.62 ± 0.06 (101)	8.79 ± 1.97 (116)
ZDV	30	2.26 ± 0.04 (141)	10.39 ± 0.56 (138)
	300	3.32 ± 0.05 (207)	21.94 ± 4.04 (291)
3TC	30	1.92 ± 0.67 (119)	7.29 ± 1.47 (97)
	300	1.94 ± 0.14 (121)	8.13 ± 0.95 (108)

a Extracellular lactate production given as a mean ± standard deviation from a representative experiment performed in duplicate. Data in parentheses represent percentage change from the no drug control.

b HepG2 cells and SkMCs were incubated with drugs for 3 and 6 days, respectively.

Data from reference: P1278-00042

2.6.2.3.12. Tenofovir DF: Spectrum Screen of Tenofovir DF and Tenofovir

A primary screen was used to determine the effect of TFV and TDF on the inhibition or stimulation of binding in a series of 111 protein targets (neuroreceptors, ion channels, transporters, and nuclear receptors) (Tabulated Summary 2.6.3.1.8, V2000020). The protein target was incubated in the presence of 10 μM TFV or TDF. The effect on the binding of the endogenous ligand was then determined. Responses of > 50% stimulation or inhibition were considered significant. There was no significant inhibition or stimulation of ligand binding to its protein target by either TFV or TDF. The results of this study demonstrate that neither TFV nor TDF significantly interacts with any of the 111 protein targets tested.

2.6.2.3.13. Emtricitabine/Tenofovir DF

The anti-HBV activities of TFV and FTC were determined alone and in combination using a cell-based HBV replication assay (Tabulated Summary 2.6.3.1.9, PC-164-2004). The in vitro EC_{50} values of TFV and FTC were 0.63 and 0.088 μM, respectively, when tested alone in this study. The combination of TFV and FTC demonstrated additive anti-HBV activity in vitro.

The combination of TFV and FTC has also been studied in vitro for potential synergistic cellular toxicity in the MT-2 cell line (Tabulated Summary 2.6.3.1.9, PC-164-2002). In combination studies of up to 5 μM FTC and 50 μM TFV, no effect on cell viability was observed using an XTT-based enzymatic analysis. In all drug combinations, cell viability values were > 95% of controls that did not contain any drugs. Thus, there was no evidence of synergistic cellular toxicity for the combination of TFV with FTC in vitro. These results are in agreement with the high CC_{50} values for each individual drug (> 100 μM) and the overall

high selectivity ratios for the individual drugs with reference to their anti-HIV-1 activities (> 100-fold).

In vitro combination studies have been conducted in HepG2 cells to evaluate the potential mitochondrial toxicity of FTC and TFV (as well as other nucleosides) (Tabulated Summary 2.6.3.1.9, TX-104-2001). HepG2 cells were exposed to FTC and TFV (as well as other nucleosides), either alone or in combination. HepG2 cells were treated for up to 25 days with concentrations of NRTIs equal to 1 and 10 times the maximal therapeutic plasma levels. Assay endpoints included cell growth, extracellular production of lactic acid, relative cellular content of mitochondrial DNA (mtDNA) and mtDNA-encoded cytochrome c oxidase II (COX II), and intracellular lipid accumulation.

Tenofovir and FTC alone or in combination with each other or other nucleosides generally had no time- or concentration-dependent effects on cytotoxicity (cell counts) or mitochondrial parameters in HepG2 liver cells. The dual combination of high-dose FTC + ZDV with or without TFV appeared to have greater cytotoxicity than the agents alone, but showed no increase in mitochondrial effects.

These studies confirmed that the potential of FTC and TFV to interfere with mitochondrial functions is low, whether administered alone or in combination with other licensed NRTIs.

2.6.2.3.13.1. In Vivo Studies

The efficacy of TDF, ADV, 3TC, and FTC, as well as the combinations of TDF or ADV each with 3TC or FTC, were evaluated by treating chronic WHV-infected woodchucks for 48 weeks (Tabulated Summary 2.6.3.1.9, PC-174-2004). At 12 weeks of treatment, the TDF-containing groups, TDF alone, 3TC + TDF, and FTC + TDF, each had a mean serum viral load reduction of 3.6, 3.7, and 4.2 $\log_{10,}$ respectively. Varying degrees of viral recrudescence were then observed between Weeks 12 and 24 across all drug treatment groups, with further continued reduction in viral load throughout the remainder of drug treatment. At Week 48, the treatment groups of TDF alone, 3TC + TDF, and FTC + TDF had a mean serum viral load reductions of 2.9, 5.8, and 6.1 $\log_{10}$ respectively. In the 48-week dosing period, there was no evidence of toxicity in woodchucks treated with any of the drugs or drug combinations.

2.6.2.3.14. Emtricitabine/Rilpivirine/Tenofovir DF

No additional secondary pharmacodynamic studies have been conducted for the triple combination of FTC + RPV + TDF.

2.6.2.4. Safety Pharmacology

In vitro and in vivo safety pharmacology data for FTC, RPV, TDF, and the FTC/RPV/TDF combination are presented in Sections 2.6.2.4.1 to 2.6.2.4.15.

Neither TDF nor FTC had significant unwanted pharmacologic activity as determined in a variety of in vitro and in vivo safety pharmacology studies. Rilpivirine had no effects on the

core battery of safety pharmacology tests, apart from inhibitory effects on some potassium currents and channels, and moderate QT prolongation in the rabbit ventricular wedge. While the study designs of these studies varied between the individual products, the major organ systems have been comprehensively evaluated. Given the lack of effects for FTC and TDF on the cardiovascular system, no additional cardiovascular system studies with the combination were considered warranted.

2.6.2.4.1. Emtricitabine: Overt Pharmacodynamic Effects

2.6.2.4.1.1. Mice

In a single-dose modified Irwin screen with toxicity observations, male ICR mice (10/dose) were given FTC orally at 0, 10, 30, or 100 mg/kg, then observed for 7 days to collect data on behavioral effects (Tabulated Summary 2.6.3.1.13, 477). Emtricitabine did not affect behavior at any dose. In a subsequent single-dose general pharmacology study, male CD-1 mice (4/dose) were given FTC orally at 0, 100, 250, 500, 750, or 1000 mg/kg, then observed for 7 days to collect data on body weight, rectal temperature, and behavior (Tabulated Summary 2.6.3.1.13, TPZZ/93/0001). Emtricitabine did not affect body weight, rectal temperature, or behavior at any dose.

2.6.2.4.1.2. Rats

In a single-dose general pharmacology study, male CD (SD) rats (4/dose) were given FTC orally at 0, 250, 500, or 1000 mg/kg, then observed for 7 days to collect data on body weight, rectal temperature, and behavior (Tabulated Summary 2.6.3.1.13, TPZZ/93/0001). Emtricitabine did not affect body weight, rectal temperature, or behavior at any dose. In a separate single-dose study, male Wistar rats (5/dose) were given FTC orally at 0, 10, 30, or 100 mg/kg (Tabulated Summary 2.6.3.1.13, 477). Rectal temperature was measured before dosing at intervals up to 2 hours postdose. Again, FTC did not affect rectal temperature at any dose.

2.6.2.4.2. Emtricitabine: Nervous System Effects

2.6.2.4.2.1. Mice

In a single-dose modified Irwin screen with toxicity observations, male ICR mice (10/dose) were given FTC orally at 0, 10, 30, or 100 mg/kg, then observed for 7 days to collect data on neurological and autonomic effects (Tabulated Summary 2.6.3.1.13, 477). Emtricitabine did not produce neurological or autonomic effects at any dose. In a subsequent single-dose general pharmacology study, male CD-1 mice (4/dose) were given FTC orally at 0, 100, 250, 500, 750, or 1000 mg/kg, then observed for 7 days to collect data on reflexes (Tabulated Summary 2.6.3.1.13, TPZZ/93/0001). Emtricitabine did not affect reflexes at any dose.

In a single-dose spontaneous locomotor activity study, male ICR mice (8/dose) were given FTC orally at 0, 10, 30, or 100 mg/kg (Tabulated Summary 2.6.3.1.13, 477). Spontaneous

activity was recorded at 15-minute intervals for 2 hours postdose. Emtricitabine did not affect spontaneous locomotion at any dose.

In a single-dose motor coordination (rotorod) study, male ICR mice (10/dose) were given FTC orally at 0, 10, 30, or 100 mg/kg (Tabulated Summary 2.6.3.1.13, 477). Ability to remain on a rotating rod was evaluated at 0.5, 1, and 2 hours postdose. Emtricitabine did not affect motor coordination at any dose.

In a single-dose hexobarbital potentiation (sleeping time) study, male ICR mice (10/dose) were given FTC orally at 0, 10, 30, or 100 mg/kg, and then anesthetized with hexobarbital 1 hour later (Tabulated Summary 2.6.3.1.13, 477). Time to recovery from anesthesia (return of righting reflex) was measured. Emtricitabine did not affect duration of anesthesia at any dose.

In a single-dose anticonvulsant activity study, male ICR mice (10/dose) were given FTC orally at 0, 10, 30, or 100 mg/kg, then given a maximal electrical shock at 1 hour postdose (Tabulated Summary 2.6.3.1.13, 477). The occurrence of death or tonic/clonic convulsions was recorded. Emtricitabine did not affect mortality and had no anticonvulsant activity at any dose. In a second single-dose anticonvulsant activity study, male ICR mice (10/dose) were given FTC orally at 0, 10, 30, or 100 mg/kg, then given metrazole at 100 mg/kg at 1 hour postdose (Tabulated Summary 2.6.3.1.13, 477). The occurrence of death or tonic/clonic convulsions was recorded. Again, FTC did not affect mortality and had no anticonvulsant activity at any dose.

In a single-dose proconvulsant activity study, male ICR mice (10/dose) were given FTC orally at 0, 10, 30, or 100 mg/kg, then given a subthreshold electrical shock at 1 hour postdose (Tabulated Summary 2.6.3.1.13, 477). The occurrence of death or tonic/clonic convulsions was recorded. Emtricitabine did not affect mortality and had no proconvulsant activity at any dose. In a second single-dose proconvulsant activity study, male ICR mice (10/dose) were given FTC orally at 0, 10, 30, or 100 mg/kg, then given metrazole at 70 mg/kg at 1 hour postdose (Tabulated Summary 2.6.3.1.13, 477). The occurrence of death or tonic/clonic convulsions was recorded. Again, FTC did not affect mortality and had no proconvulsant activity at any dose.

In a single-dose analgesic activity study, male ICR mice (10/dose) were given FTC orally at 0, 10, 30, or 100 mg/kg (Tabulated Summary 2.6.3.1.13, 477). The time to tail-flick response to an uncomfortable heat stimulus was recorded before and 1 hour after dosing. Emtricitabine had no analgesic activity at any dose. In a second single-dose analgesic activity study, male ICR mice (10/dose) were given FTC orally at 0, 10, 30, or 100 mg/kg, then given phenylquinone at 2 mg/kg at 1 hour postdose (Tabulated Summary 2.6.3.1.13, 477). The number of writhes occurring during the next 10 minutes was recorded. Again, FTC had no analgesic activity at any dose. In a subsequent single-dose general pharmacology study, male CD-1 mice (4/dose) given FTC orally at 0, 100, 250, 500, 750, or 1000 mg/kg were evaluated for analgesia by the tail-flick test (Tabulated Summary 2.6.3.1.13, TPZZ/93/0001). Once again, FTC had no analgesic activity at any dose.

2.6.2.4.2.2. Rats

In a single-dose general pharmacology study, male CD (SD) rats (4/dose) were given FTC orally at 0, 250, 500, or 1000 mg/kg, and then observed for 7 days to collect data on reflexes and analgesia (Tabulated Summary 2.6.3.1.13, TPZZ/93/0001). Emtricitabine did not affect reflexes and had no analgesic activity at any dose.

In a single-dose conditioned avoidance response study, trained ovariectomized female Long-Evans rats (6/dose) were given FTC by intraperitoneal injection at 0, 30, or 100 mg/kg, then tested for their ability to respond to an audio-visual cue to avoid foot-shock (Tabulated Summary 2.6.3.1.13, TPZZ/93/0119). Emtricitabine did not affect conditioned avoidance response at any dose.

2.6.2.4.3. Emtricitabine: Cardiovascular Effects

2.6.2.4.3.1. Effects on Isolated Cardiac Muscle of Rat, Guinea Pig, and Cat

Emtricitabine was tested on different cardiac preparations to determine its effect on cardiac function in vitro (Tabulated Summary 2.6.3.1.10, TPZZ/92/0056). Parameters measured following treatment with 1 μM FTC included: cardiac chronotropy (rat and guinea pig), cardiac inotrophy (cat and guinea pig), and the incidence of ventricular arrhythmias (rat). Results from these in vitro studies suggested that FTC was free of negative cardiac effects at 1 μM.

2.6.2.4.3.2. Rats

In a single-dose cardiovascular effects study, conscious male Wistar rats (5/dose) were given FTC orally at 0, 5, 10, or 50 mg/kg (Tabulated Summary 2.6.3.1.13, 477). Heart rate and arterial blood pressure were measured before dosing and at 5, 30, and 60 minutes postdose. Emtricitabine did not affect heart rate or blood pressure at any dose. In a second single-dose cardiovascular effects study, conscious male CD (SD) rats (8/dose) were given FTC orally at 0 or 250 mg/kg (Tabulated Summary 2.6.3.1.13, TPZZ/92/0057). Heart rate and blood pressure were measured at intervals for 4 hours postdose. Again, FTC did not affect heart rate or blood pressure.

2.6.2.4.3.3. Dogs

In a single-dose cardiovascular and respiratory effects study, 4 anesthetized male Beagle dogs were given FTC intravenously as consecutive bolus injections of 1.0, 2.5, 5, 10, and 20 mg/kg (cumulative dose = 38.5 mg/kg) over an hour, then monitored for 30 more minutes (Tabulated Summary 2.6.3.1.13, TPZZ/92/0076). The average plasma concentration of FTC at 30 minutes postinfusion was 34.6 mg/mL. Heart rate, arterial blood pressure, and lead II ECG were measured at intervals, and blood pressure responses to norepinephrine, acetylcholine, carotid artery occlusion, and vagal nerve stimulation were evaluated 30 minutes after the last dose. Emtricitabine did not affect the ECG, cardiovascular function parameters, or blood pressure response to stimuli at any dose or time point.

2.6.2.4.4. Emtricitabine: Respiratory Effects

2.6.2.4.4.1. Mice

In a single-dose general pharmacology study, male CD-1 mice (4/dose) were given FTC orally at 0, 100, 250, 500, 750, or 1000 mg/kg, then observed for 7 days to collect data on respiratory rate (Tabulated Summary 2.6.3.1.13, TPZZ/93/0001). Emtricitabine did not affect respiratory rate at any dose.

2.6.2.4.4.2. Rats

In a single-dose general pharmacology study, male CD (SD) rats (4/dose) were given FTC orally at 0, 250, 500, or 1000 mg/kg, and then observed for 7 days to collect data on respiratory rate. Emtricitabine did not affect respiratory rate at any dose (Tabulated Summary 2.6.3.1.13, TPZZ/93/0001).

2.6.2.4.4.3. Dogs

In a single-dose cardiovascular and respiratory effects study, 4 anesthetized male Beagle dogs were given FTC intravenously as consecutive bolus injections of 1, 2.5, 5, 10, and 20 mg/kg (cumulative dose = 38.5 mg/kg) over an hour, then monitored for 30 more minutes (Tabulated Summary 2.6.3.1.13, TPZZ/92/0076). The average plasma concentration of FTC at 30 minutes postinfusion was 34.6 mg/mL. Respiratory rate and respiratory minute volume were measured at intervals. Emtricitabine did not affect respiratory function parameters at any dose or time point.

2.6.2.4.5. Emtricitabine: Renal Effects

In a single-dose renal function study, male Long Evans-derived rats (6/dose) were given FTC orally at 0, 10, 30, or 100 mg/kg and urine was collected for 6 hours postdose to measure pH, volume, and electrolyte concentrations (Tabulated Summary 2.6.3.1.13, 477). Emtricitabine did not affect urine output, pH, or electrolyte excretion at any dose.

2.6.2.4.6. Emtricitabine: Gastrointestinal Effects

In a single-dose GI motility study, male ICR mice (10/dose) were given FTC orally at 0, 10, 30, or 100 mg/kg (Tabulated Summary 2.6.3.1.13, 477), then given a charcoal suspension orally at 1 hour postdose and killed 15 minutes later to record intestinal transit of charcoal. Emtricitabine did not affect GI motility at any dose.

2.6.2.4.7. Rilpivirine: Central Nervous System Effects

2.6.2.4.7.1. Neurobehaviour and Motor Activity in Rat

TMC278 was tested in a modified test according to Irwin {9454} in Sprague Dawley rats (5 males/group) in order to evaluate the neurofunctional integrity (Tabulated Summary 2.6.3.1.14, TMC278-Exp5560). TMC278 base, dissolved in PEG400 with citric

acid, was administered orally by gavage in a volume of 10 mL/kg at 40, 120, and 400 mg/kg. Mortality, observations of motor-affective and sensori-motor behavior and neurologic and autonomic functioning upon manipulation and made at cage side, general clinical signs, and body weight were evaluated during a 7-day observation period following the single oral administration.

No mortality was seen in any group and no adverse clinical signs were noted up to 400 mg/kg. Neurofunctional integrity of rats was not affected at 40 or 120 mg/kg, and there were no signs of neurotoxic effects recorded up to Day 7 after dosing. At a dose of 400 mg/kg, occasionally animals showed a single slightly abnormal behavioral parameter 8 hours after dosing, and all animals showed a slightly reduced pupil size 2 hours after dosing. These parameters returned to normal 24 hours after dosing. No compound-related neurological changes or delayed neurotoxicity were noted.

Data from a separate toxicokinetic study in male rats with the same formulation and oral doses (Tabulated Summary 2.6.7.6.A, FK 4243 [Addendum to TMC278-Exp5535]) indicated that T_{max} values were reached within 3 hours (40 mg/kg) or within 8 hours (120 and 400 mg/kg) after dosing. Mean C_{max} values were 1.6, 2.8, and 9.9 µg/ml at 40, 120, and 400 mg/kg, respectively (Module 2.6.4, Pharmacokinetics Written Summary, Section 2.6.4.4.1.2).

2.6.2.4.7.2. Neurobehavior Assessment in Instrumented, Awake Dogs

Behavioral effects of a single oral dose of 20 mg/kg of TMC278 base dissolved in PEG400 were assessed upon cage-side observations in instrumented awake dogs (Tabulated Summary 2.6.3.1.14, TMC278-CPF654).

TMC278 had no overt effects on behavior. The median maximum plasma concentration of TMC278 was 1.5 µg/mL.

2.6.2.4.7.3. Locomotor Activity in Telemetered Conscious Dogs

Four telemetered male beagle dogs were dosed orally via gavage at escalating doses of 0 (vehicle), 20, 80, and 160 mg/kg of TMC278 base dissolved in PEG400 plus citric acid (Tabulated Summary 2.6.3.1.14, TMC278-Exp5555), with 1 week dosing intervals.

TMC278 did not influence locomotor activity at cage side observations over 12 hours following dosing. For exposure data, see Section 2.6.2.4.8.1.6.

2.6.2.4.8. Rilpivirine: Cardiovascular Effects

Additional cardiovascular safety studies were conducted to investigate the mechanism of action of the delayed onset of the prolongation of the QT-interval corrected for heart rate according to Fridericia {15639} (QTcF) noted in the thorough clinical QTc-prolongation (TQT) study, TMC278-TiDP6-C131 (C131). In this study, TMC278 was given to healthy subjects for 11 days. Another objective of the additional nonclinical studies was to evaluate the proarrhythmic potential of TMC278 at doses that gave QT-prolongation.

As a result of duration-related reduced responses to TMC278 in some of the in vitro studies, recovery of freshly prepared solutions of TMC278 base in the buffers used in these studies was evaluated using [^{14}C]TMC278 base. The radioactive concentration of a TMC278 base stock solution of 10 mM (10 MBq/mL) in DMSO was 99.1% of the theoretical radioactive concentration. However, the radioactive concentration of a solution of 10 µM and 3 µM of TMC278 base in Tyrode buffer containing 0.1% and 0.03% DMSO was 84.7% and 58.0% of the theoretical radioactive concentration, respectively (Report TMC278-NC338). Further studies (TMC278-TiDP6-NC381) showed that recovery could be significantly improved by adding 5% bovine serum albumin to the solutions, indicating that the low recovery in the serum-free conditions was likely due to adsorption of TMC278 base to the equipment. This conclusion is in line with the high (more than 99%) degree of plasma protein binding of TMC278 in all species including man (see Module 2.6.4, Pharmacokinetics Written Summary, Section 2.6.4.4.1.2). As the in vitro models were validated with protein-free incubation fluids, it was decided not to add serum albumin. The results of the studies are presented with the nominal bath concentrations.

2.6.2.4.8.1. Standard Battery Studies

2.6.2.4.8.1.1. Inhibition of Rapidly Activating Rectifying Potassium Membrane Current (I_{Kr})

Chinese hamster ovary (CHO) cells were stably transfected with the hERG encoding for the I_{Kr} channel (Tabulated Summary 2.6.3.1.11, TMC278-CPF730). These cells were incubated with TMC278 base at nominal bath concentrations of 0.1, 0.3, and 3 µM (0.037, 0.111, and 1.11 µg/mL) in buffer containing 0.1 % DMSO. Astemizole and terfenadine were used as positive controls.

TMC278 caused a concentration-dependent inhibition of I_{Kr} ranging from 10% at 0.1 µM (0.037 µg/mL) to 80% at 3 µM (1.11 µg/mL).

2.6.2.4.8.1.2. Cardiac Contractility in Isolated Guinea Pig Right Atrium

Rate and force of (spontaneous) contraction (RC and FC, respectively) and effective refractory period (ERP), defined as the frequency of external stimulations that does not elicit continuously a contraction, were evaluated in 2 series of experiments with isolated spontaneously beating right atrium of guinea pigs. In the first series, incubations occurred with TMC278 base at increasing concentrations of 0.01, 0.03, and 0.1 µM (0.004, 0.011, and 0.037 µg/mL) in buffer containing 0.01%, 0.03%, 0.1% DMSO, respectively. In the second series, increasing concentrations of 1, 3, and 10 µM (0.369, 1.11, and 3.69 µg/mL) TMC278 base in buffer containing 1%, 3%, and 10% DMSO were tested (Tabulated Summary 2.6.3.1.11, TMC278-N168576). The ERP was determined after the highest concentration in each of the series was applied.

TMC278 caused a concentration-dependent decrease of the rate of spontaneous contractions to 86%, 72%, and 44% of baseline at 1, 3, and 10 µM (0.369, 1.11, and 3.69 µg/mL), respectively. The compound at 0.1 and 10 µM (0.037 and 3.69 µg/mL) had no effects on ERP

compared to vehicle. TMC278 did not cause any effects on the force of the spontaneous contraction compared to vehicle over the full range of concentrations.

2.6.2.4.8.1.3. Cardiovascular Parameters in Anesthetized Guinea Pigs

In this assay, anesthetized guinea pigs were used to evaluate the effects of TMC278 base relative to vehicle on heart rate and mean arterial blood pressure and electrophysiological parameters (Tabulated Summary 2.6.3.1.14, TMC278-CPF643). These parameters comprised the duration of the QRS-complex and of the PQ, RR, and QT interval. The duration of QT interval corrected for heart rate using Bazett's formula {14029} ($QT = QT/\sqrt{RR}$) was calculated. In addition, the incidence of cardiac conduction disturbances, such as type II and III atrioventricular blocks and intraventricular bundle branch block, was recorded. Moreover, the occurrence of ventricular arrhythmias was evaluated on the basis of occurring ventricular premature beats, VT and VF. A group of 7 animals received a series of 6 intravenous injections of increasing concentrations of TMC278 dissolved in PEG400 at a volume of 0.5 mL/kg with a 15 minute interval. Individual animals received doses ranging from 0.16 up to 5 mg/kg (cumulative dose of 9.87 mg/kg over 75 minutes). Plasma, heart, and lung tissue of the first 3 animals were sampled immediately after the last injection for determination of TMC278.

TMC278 had no effect on heart rate and mean arterial blood pressure, and had no statistically significant effect on the duration of the PQ and the QRS intervals of the ECG. The QT interval tended to be increased (+18% versus +10% with solvent) after administration of the highest dose of 5 mg/kg. After correction for heart rate using Bazett's formula, no drug-induced effect was observed on QTc. No differences in the incidence of cardiac conduction disturbances were observed. Plasma levels of TMC278 reached a median value of 9.2 µg/mL, 5 minutes after the last injection. The concentration of TMC278 in lung tissue was 4 times higher than that in heart tissue (47.7 µg/g and 11.7 µg/g, respectively).

2.6.2.4.8.1.4. Cardiovascular Parameters in Anesthetized Dog

Groups of 4 anesthetized beagle dogs (Tabulated Summary 2.6.3.1.14, TMC278-CPF648) received an intravenous infusion for 1 hour at a flow of 2 mL/kg of a formulation of 2.5 mg TMC278 base/mL in PEG400 (dose: 5 mg TMC278 base/kg) or the vehicle, PEG400. The effects of TMC278 base were compared with those of the vehicle, and evaluated during infusion and for 3 hours following administration on general and regional cardio-hemodynamic, ECG, cardio-electrophysiological, pulmonary, and arterial blood parameters. The general hemodynamic parameters comprised heart rate, systolic and diastolic aortic and pulmonary artery pressure, systolic pressure rate product, cardiac output, and stroke volume. Moreover, left ventricular (LV) pressure parameters were determined, including end diastolic pressure (LVEDP), and at maximum positive and negative isovolumic rate of change (LV dp/dt max and LV dp/dt min, respectively); contractility index (LV dp/dt max/pd); and the time constant of relaxation. The regional hemodynamic parameters were common carotid artery blood flow, systemic and pulmonary vascular resistance, common carotid arterial vascular resistance, systolic and diastolic intracranial pressure, and cerebral perfusion pressure. Electrocardiogram morphology was evaluated and the duration of the QRS complex

and of the PQ and QT interval was determined; the latter also corrected for heart rate according to Bazett, Fridericia, and Van de Water {15700}. Cardio-electrophysiological effects were evaluated on the morphology and the duration of the right endocardial monophasic action potential (MAP, at 90% of repolarization [APD90]). The pulmonary parameters and arterial blood parameters are presented in Section 2.6.2.4.9.1.

TMC278 had no effects on the ECG or cardio-electrophysiological parameters. From cardio-hemodynamic parameters, systemic vascular resistance (–25% versus baseline; vehicle: +29% versus baseline), pulmonary vascular resistance (–20% versus baseline; vehicle: +31% versus baseline), and cardiac output (+31% versus baseline; vehicle: –12% versus baseline) were affected at the end of the infusion. The vehicle, PEG400, had its own cardio-hemodynamic effects. The mean plasma concentration of TMC278 at the end of the infusion was 2.62 µg/mL and decreased to 0.484 µg/mL, 3 hours after cessation of the infusion. The results on the pulmonary and arterial blood parameters are presented in Section 2.6.2.4.9.1.

2.6.2.4.8.1.5. Cardiovascular Parameters in Instrumented, Awake Dogs

Instrumented dogs were allocated to 2 groups of 7 dogs each. One group was administered a single oral dose of 20 mg/kg of TMC278 base dissolved in PEG400 and the second group was dosed with vehicle (PEG400) (Tabulated Summary 2.6.3.1.14, TMC278-CPF654).

TMC278 had no effects on heart rate, blood pressure, systolic pressure rate product, LV dp/dt max, LV dp/dt min, LV dp/dt max/pd, cardiac output, stroke volume, systemic vascular resistance, and ECG intervals. No differences in the morphology of the ECG were observed between the 2 groups. The median maximum plasma concentration of TMC278 was 1.5 µg/mL.

2.6.2.4.8.1.6. Cardiovascular Parameters in Telemetered Conscious Dogs

Four telemetered male beagle dogs were administered orally via gavage escalating doses of 0 (vehicle), 20, 80, and 160 mg/kg of TMC278 base dissolved in PEG400 plus citric acid (Tabulated Summary 2.6.3.1.14, TMC278-Exp5555). The interval between administrations was 1 week. Cardio-hemodynamic (heart rate, systolic and diastolic blood pressure, and pressure rate product) and ECG parameters (RR, PQ, and QT intervals) recorded for 12 hours following dosing were evaluated.

TMC278 did not show any effects on cardio-hemodynamic or ECG parameters. Plasma samples taken during the days of dosing were rejected following erroneous processing. Exposure to TMC278 was evaluated on the basis of oral single-dose toxicokinetic data from a separate dog study (Tabulated Summary 2.6.7.6.A, FK4244 [Addendum to TMC278-Exp5534]), with the same formulation at oral doses of 20, 40, and 80 mg TMC278/kg. The mean maximal plasma concentrations of TMC278 were fairly comparable between the different dose groups: 1.5, 1.7, and 1.5 µg/mL, respectively. The T_{max} values were variable, occurring up to 24 hours after dosing. The $AUC_{0\text{-}24h}$ values also did not differ between the 3 dose levels (18.1, 26.8, and 18.8 µg.h/mL, respectively).

2.6.2.4.8.2. Additional Mechanistic Studies

2.6.2.4.8.2.1. Membrane Currents Involved in Cardiac Action Potential Depolarization and Repolarization

The effects of TMC278 base were studied on ion channels involved in the depolarization and repolarization phase of the cardiac action potential. The parameters evaluated included the slowly activating rectifying potassium current (I_{Ks}), the transient outward potassium current (I_{to}), the inward rectifying potassium current (I_{K1}), the fast sodium current (I_{Na}), and the high threshold L-calcium current ($I_{Ca,L}$). The effects on the currents were studied at single cell level using the single-electrode-whole-cell configuration of the patch clamp technique. Positive controls were used in each study.

The effect of TMC278 base at nominal bath concentrations of 0.3 to 10 µM (0.11 to 3.7 µg/mL) in a buffer containing 0.3% DMSO was studied in a CHO cell line transfected with human KvLQT1 and minK, 2 subunits encoding for the I_{Ks} channel (Tabulated Summary 2.6.3.1.11, TMC278-NC342). The positive control was HMR 1556. TMC278 caused a concentration-dependent block of I_{Ks} from 1 µM (0.37 µg/mL) and above, with an IC_{50} of 3.1 µM (1.15 µg/mL).

In addition, TMC278 base was tested in the human embryonic kidney (HEK) 293 cells (Tabulated Summary 2.6.3.1.11, TMC278-NC331) transfected with human cDNA encoding for channels for I_{Na}, I_{Ks}, I_{to}, $I_{Ca,L}$, and I_{K1}. The positive controls used were lidocaine for I_{Na}, chromanol 293B for I_{Ks}, flecinide for I_{to}, nifedipine for $I_{Ca,L}$, and $BaCl_2$ for I_{K1}. TMC278 base at nominal concentrations of 0.1, 0.3, and 1 µM (0.037, 0.11, and 0.37 µg/mL) caused 19.1% inhibition of I_{Ks} at 1 µM (0.37 µg/mL), and 13.6% and 35.5% inhibition of I_{to} at 0.3 and 1 µM (0.1 and 0.37 µg/mL), respectively. TMC278 had no biologically relevant effects on I_{K1}, I_{Na}, or $I_{Ca,L}$ at 1 µM.

2.6.2.4.8.2.2. Trafficking of hERG Channel

One of the mechanisms possibly responsible for the delayed-onset QTcF-prolongation observed in clinical TQT study C131 is inhibition by TMC278 of the synthesis, assembly in the endoplasmic reticulum, and/or transport of the hERG channel to the cell membrane. This effect - also known as trafficking - on the most prominent ion channel involved in the repolarization of the cardiac action potential was assessed in the HERG-Lite® test ({15701}, Tabulated Summary 2.6.3.1.11, TMC278-NC330). In this test, the effects of TMC278 on the expression of the wild type hERG channel (hERG-WT) or a single mutant channel (hERG-SM) at the surface of HEK293 cells transfected with these channels were determined, following overnight incubation. The mildly misfolded mutant channels show reduced trafficking. However, hERG channel blockers have shown to act as pharmacological chaperones for the mutant channels in stabilizing their correct conformation and rescuing their expression by allowing their export from the endoplasmic reticulum and movement to the cell surface. So, in this test the potential of TMC278 to inhibit trafficking of the hERG-WT channel was evaluated in parallel with its potential to behave as a hERG blocker by rescuing the surface expression of the hERG-SM channel. TMC278 base was incubated at

nominal bath concentrations of 1, 10, and 30 µM (0.37, 3.7, and 11.1 µg/mL) in 0.1% DMSO in aqueous buffer. Geldanamycin (1 µM) served as positive control for trafficking of hERG-WT. Astemizole (1 µM) was used as positive control hERG blocker.

On the basis of the reduced expression of the hERG-WT on the cell membrane (29% and 36% of control expression at 10 and 30 µM (3.7 and 11.0 µg/mL), respectively), it is concluded that TMC278 has the potential to reduce trafficking of the hERG channel. The overexpression of hERG-SM on the cell membrane of 146%, 155%, and 213% of control values at TMC278 base concentrations of 1, 10, and 30 µM (0.37, 3.7, and 11.0 µg/mL), respectively, is an indication of the potential of TMC278 to inhibit the hERG channel. This result is in line with that of the patch clamp study with CHO cells expressing the hERG channel (Section 2.6.2.4.8.1.1, TMC279-CPF730).

2.6.2.4.8.2.3. Electrophysiological Parameters of the Isolated Arterially Perfused Rabbit Left Ventricular Wedge

Electrophysiological and other cardiophysiological parameters were studied in isolated, arterially-perfused rabbit ventricular wedge preparations (Tabulated Summary 2.6.3.1.11, TMC278-NC341). The effects of TMC278 base at concentrations increasing from 10 nM to 10 µM (3.7 ng/mL to 3.7 µg/mL) were evaluated relative to vehicle control (buffer containing 0.1% DMSO). Parameters determined were the duration of the QT and QRS intervals; the time between the peak and the end of the T wave (Tp-Te) as a measure of transmural dispersion of repolarization (TDR); and the Tp-Te/QT ratio (rTp-Te) as an indicator of the potential to induce phase 2 early afterdepolarizations (EADs). Moreover, the force of contraction (FC), in-excitability (inability of the preparation to follow the stimulation), and the occurrence of ventricular tachycardia (VT) and ventricular fibrillation (VF) were recorded. The potential to induce torsade de pointes (TdP) was assessed semiquantitatively on the basis of QT duration, TDR measured as (Tp-Te)/QT, and the occurrence of phase 2 EADs.

TMC278 caused 6% and 9% prolongation of the QT-interval from baseline at nominal bath concentrations of 1 and 10 µM (0.37 and 3.7 µg/mL), respectively. At 10 µM (3.7 µg/mL) only, the assessment of the potential to induce TdP resulted in a marginal score of 0.5. No other significant or physiologically relevant changes were noted in any of the other parameters.

2.6.2.4.8.2.4. Cardiovascular Parameters in Telemetered Guinea Pig

The potential of orally administered TMC278 to inhibit in vivo trafficking of the hERG channel was evaluated through the occurrence of delayed-onset QT-prolongation in a repeat-dose telemetered guinea pig model ({15702}, Tabulated Summary 2.6.3.1.14, TMC278-NC327). The dose of TMC278 was selected in a preliminary pharmacokinetics study as that giving a C_{max} value of approximately 2 times the human C_{max} at 75 mg TMC278 once daily (0.466 µg/mL) in a clinical Phase 2b study (C204). This dose gave approximately 10 milliseconds QT prolongation in the TQT study C131, at a steady state median maximum plasma concentration of 0.636 µg/mL. TMC278 was suspended in an aqueous mixture of

hydroxypropylmethylcellulose (HPMC; 0.5%) and Tween 80, and dosed by gavage at 10 mg/kg for 16 consecutive days. Recordings of heart rate, body temperature, and ECG made for 4 hours after dosing were evaluated. From ECGs, the durations of the QRS complex, and the PQ and QT interval (with or without correction for heart rate according to Bazett {14029} or Fridericia {15639}) were reported every 30 minutes.

TMC278 at a dose of 10 mg/kg/day for 16 consecutive days, which gave mean C_{max} values of 0.689 to 0.911 µg/mL throughout the dosing period, had no notable effects on the measured ECG parameters, heart rate, or body temperature.

2.6.2.4.9. Rilpivirine: Pulmonary Safety

2.6.2.4.9.1. Respiratory Parameters in Anesthetized Dog

Respiration pressure and flow, tidal volume, expiratory airway resistance, dynamic lung compliance, and arterial blood parameters (pH, oxygen tension and saturation, CO_2 tension, and concentration of electrolytes, hemoglobin, glucose, lactate, chloride, and bicarbonate) were studied in groups of 4 anesthetized beagle dogs (Tabulated Summary 2.6.3.1.14, TMC278-CPF648). The animals received an intravenous infusion for 1 hour at a flow of 2 mL/kg of 2.5 mg TMC278 base/mL in PEG400, resulting in a dose of 5 mg/kg, or the vehicle PEG400. Parameters were recorded during the infusion and for 3 hours after the end of the administration.

None of the respiratory or arterial blood parameters were affected during or for 3 hours after the 1-hour intravenous infusion. The mean plasma concentration of TMC278 at the end of the infusion was 2.62 µg/mL, and 3 hours after cessation of the infusion decreased to 0.484 µg/mL.

2.6.2.4.9.2. Respiratory Parameters in Telemetered Conscious Dog

Respiratory rate and tidal volume were not affected in 4 telemetered conscious male beagle dogs at 1, 2, and 4 hours after single oral (gavage) escalating doses of 0 (vehicle), 20, 80, and 160 mg TMC278 base/kg dissolved in PEG400 plus citric acid (Tabulated Summary 2.6.3.1.14, TMC278-Exp5555), with a dosing interval of 1 week. For exposure data, see Section 2.6.2.4.8.1.6.

2.6.2.4.10. Tenofovir DF: Central Nervous System Effects

The pharmacological effects of a single oral (gavage) dose of TDF at dosages of 0, 50, or 500 mg/kg on the CNS of the male albino rat (10/group) were examined (Tabulated Summary 2.6.3.1.15, R990152). A functional observation battery of assessments was performed by technicians blinded to the treatment groups, on Day –1 (predose) and again 2 hours following treatment. Motor activity evaluations, using automated figure 8 mazes, were performed on Day –3 and again approximately 2 hours following treatment. On Day 2, animals were euthanized and subjected to a gross pathological examination and samples of brain, spinal cord, and sciatic nerve were retained in fixative.

There were no mortalities or clinical signs of reaction to treatment. There were no statistically or biologically significant differences from controls for any of the qualitative or quantitative functional assessments. Group mean total activity counts for the high-dose group were statistically ($P < 0.05$) reduced as compared to the controls 2 hours following treatment. However, since the absolute difference was of a relatively low magnitude, this difference was considered to be of unlikely biological significance. There were no gross lesions observed in control or high dose animals. Therefore, a single oral (gavage) dose of TDF at 0, 50, or 500 mg/kg to albino male rats produced no biologically significant signs of a pharmacological effect on the CNS (Tabulated Summary 2.6.3.1.15, R990152).

2.6.2.4.11. Tenofovir DF: Cardiovascular Effects

The objective of this study was to evaluate the hemodynamic effects of a single 30 mg/kg dose of TDF following oral administration to 3 conscious male beagle dogs (Tabulated Summary 2.6.3.1.15, D990155). Under isoflurane/oxygen anesthesia, a catheter was inserted into the abdominal aorta for measurement of systemic blood pressure and heart rate. Limb leads were placed for recording of ECGs. A jacket and tether system allowed the animals to move freely within the cage. All animals were observed for mortality and clinical signs of ill health, and a detailed exam was performed at least once prior to treatment. Body weights were recorded for calculation of anesthesia/test article dose volume. Each animal was returned to its cage and allowed to stabilize after surgical preparation for a period of at least 16 hours. For at least 1.5 hours prior to the control article dose, at least 60 minutes after the control dose, and continuing until up to 24 hours after the test article dose, the cardiovascular profile was continuously monitored. Blood pressure/heart rate values were averaged every 60 seconds. Electrocardiogram recordings were obtained 3 times during a 1.5-hour predose period (at least 15 minutes apart) approximately 5, 15, 30, and 60 minutes following the control dose, and approximately 5, 15, and 30 minutes and 1, 2, 4, 12, and 24 hours following the test article dose. Monitoring took place from outside the animal room. On completion of the observation period, each animal was euthanized and discarded without further examination.

There were no deaths. There were no test article-related effects on clinical signs, heart rate, systemic blood pressure, or ECGs. In conclusion, the oral administration of TDF to the conscious male beagle dog at a dose level of 30 mg/kg did not result in any test article-related effects on clinical signs, heart rate, systemic blood pressure, or ECGs. In this study, the no-hemodynamic-effect level was considered to be 30 mg/kg (Tabulated Summary 2.6.3.1.15, D990155).

2.6.2.4.12. Tenofovir DF: Effects on the Renal System

The pharmacological effects of a single dose of TDF at dosages of 0, 50, or 500 mg/kg administered by oral gavage on the renal system of the male albino rat (10/group) (Tabulated Summary 2.6.3.1.15, R990154) were examined. The following were evaluated: clinical observations, creatinine clearance, clinical biochemistry (serum and urine), urinalysis, kidney weights, and macroscopic observations at necropsy.

There were no deaths during the course of the study. There were no treatment-related clinical signs. There were no remarkable differences between creatinine clearance values for the control and treated groups. In the high-dose group, serum concentrations of potassium and chloride were elevated. The serum concentrations of the other parameters were unaffected in this group and there were no remarkable differences between any of the control and low dose values. In the high-dose group, the volume of urine excreted was significantly reduced; the quantities of excreted calcium, sodium, potassium, chloride, and bicarbonate were decreased. There were no remarkable differences between any of the control and low dose values. Kidney weights, both absolute and relative to body weight, were reduced in the high-dose group. Values for the low-dose group were not remarkably different from the controls. There were no treatment-related gross pathology findings.

In conclusion, the administration of TDF (GS-4331-05) by oral gavage at dosages of 50 or 500 mg/kg indicated that although urinary output was reduced, kidney function was unimpaired at 500 mg/kg. The reduced quantities of excreted electrolytes and urine suggested that the kidneys were adequately maintaining vascular fluid volume and electrolyte concentrations. The immediacy of the reduction in the weight of the kidneys (i.e., 1 day postdose) indicated that it was unlikely to have been due to a pathological effect, but may have been related to a transfer of interstitial fluid into the vascular compartment. The NOEL was considered to be 50 mg/kg (Tabulated Summary 2.6.3.1.15, R990154).

2.6.2.4.13. Tenofovir DF: Gastrointestinal Motility

The pharmacological effects of a single dose of TDF at dosages of 0, 50, or 500 mg/kg administered by oral gavage on GI motility in the male albino rat (9/group) were evaluated (Tabulated Summary 2.6.3.1.15, R990153). All animals were examined BID for mortality and signs of ill health or reaction to treatment, and body weights were recorded. The animals were food and water deprived overnight prior to treatment. Thirty minutes after dosing, approximately 0.5 mL of activated charcoal was given by oral gavage to act as a GI marker. At this point, animals had access to water, but no food. Three animals from each group were euthanized by carbon dioxide asphyxiation either at 2, 4, or 6 hours after the administration of the control or the test article. The abdominal cavity was opened and the GI tract was removed and extended to its full length. The distance from the pyloric sphincter to the most proximal and distal traces of indicator were measured and recorded. In addition, the stomach (including contents) was weighed. The GI tract and the carcass were discarded without further examination.

There were no deaths or treatment-related clinical signs. At the 2-hour timepoint, the distal distance was above 90% for most animals. Among the remaining animals at the 2-hour timepoint, the proximal distance ranged from 0% to 37.7%, with no overt differences between groups. In all animals in each group, charcoal was detected in the stomach. However the weight of the stomach and contents was elevated in the high-dose group. At the 4-hour timepoint, the distal distance was above 90% for all animals in each group. Proximal distance was 0% in all 3 control animals and 1 low-dose animal, resulting in an apparent dose-related increase in proximal distance at this timepoint. At the 6-hour timepoint, there were no remarkable differences between group mean distal or proximal travel of the low-dose group

and that of the controls (proximal values ranged from 0% to 54%; distal distances were 100% in all instances). However, only control animals and 1 low-dose animal showed evidence of small particles in the stomach. Two low-dose and all high-dose animals clearly exhibited the presence of the indicator in the stomach. In the high-dose group, no indicator was present along the length of the intestinal tract; however, a yellow mucoid material was present along the length of the tract in 2 of the animals. As at the earlier time points, group mean stomach (and contents) weights were comparable between the control and low-dose groups, but elevated in the high-dose group.

In conclusion, the increased weight of the stomach and contents and observations of indicator in the stomachs of all high-dose animals at all time points indicated that at 500 mg/kg, TDF reduced the rate of gastric emptying. At 50 mg/kg, there was no clear effect on gastric emptying; the NOAEL was 50 mg/kg (Tabulated Summary 2.6.3.1.15, R990153).

2.6.2.4.14. Tenofovir DF: Guinea Pig Ileum Contractile Response

The objective of the study was to determine if TFV or TDF directly affected the contraction of guinea pig ileum after stimulation by agonists (Tabulated Summary 2.6.3.1.12, V2000009). Guinea pig ileum was harvested and preincubated for 5 minutes with either 0, 10, 30, or 100 μM TFV or TDF. The ileal tissues were subsequently exposed to agonists that stimulate contractions (acetylcholine, histamine, or $BaCl_2$). The percent inhibition of the contractile response compared to that at 0 μM was then calculated. Tenofovir had no inhibitory effect on the contractile response. Tenofovir DF significantly inhibited the contractile response up to 14% for each agonist tested. The findings suggest that TDF, the prodrug of TFV, but not TFV itself, can alter cellular pathways and impair the normal functional tissue response.

2.6.2.4.15. Emtricitabine/Rilpivirine/Tenofovir DF

Emtricitabine and TDF had little effect on vital organ systems in safety pharmacology studies as determined in a variety of in vitro and in vivo safety pharmacology studies. Given the minimal effects observed with high-dose administration of the individual agents (FTC or TDF) alone (decreased urinary output and reduced gastric emptying with high-dose TDF alone), additional safety pharmacology studies on the combination product are considered unwarranted as they would be unlikely to reveal new pharmacologic activities. Rilpivirine has shown the potential for QT prolongation, an effect confirmed in a thorough QT study in healthy subjects. At the 25-mg dose of RPV, the observed change in QTcF was not considered clinically relevant, and the combination product is not anticipated to exacerbate the small cardiovascular effect seen with the 25-mg RPV dose alone.

2.6.2.5. Pharmacodynamic Drug Interactions

The potential for pharmacodynamic drug interactions for FTC, RPV, TDF, FTC/TDF, and FTC/RPV/TDF are presented in Sections 2.6.2.5.1 to 2.6.2.5.5 below.

2.6.2.5.1. Emtricitabine: In Vitro Antiviral Combination Studies

The effect of combining FTC with other anti-HIV agents in the NRTI, NNRTI, and PI classes has been studied extensively using isobologram analysis. Results of 2-drug combination assays are presented in Table 52.

In all cases, in vitro combination studies with FTC and other anti-HIV agents resulted in additive to synergistic anti-HIV activity. No antagonism was observed under any of the reported conditions.

Emtricitabine has also been tested with the integrase inhibitor EVG (Tabulated Summary 2.6.3.1.18, PC-183-2004). The results showed strong antiviral synergy of these 2 drugs paired together with a synergy volume of 167.45 nM^2%.

Table 52. Synergistic Activity of Emtricitabine with Other Antiretroviral Agents

Compounds	Class	Cell line	HIV Strain	Results	References
Delavirdine	NNRTI	MT-2	LAI	Additive to Synergistic	(Report No. 470), (Report No. 10804)
Efavirenz	NNRTI	MT-2	LAI	Additive to Synergistic	(Report No. 470), (Report No. 10804)
Emivirine	NNRTI	MT-2	LAI	Additive to Synergistic	(Report No. 470), (Report No. 10804)
Nevirapine	NNRTI	MT-2	LAI	Additive to Synergistic	(Report No. 470), (Report No. 10804)
Abacavir	NRTI	MT-2	LAI	Additive to Synergistic	(Report No. 470), (Report No. 10804)
Adefovir	NRTI	MT-2	LAI	Additive to Synergistic	(Report No. 470), (Report No. 10804)
Amdoxovir	NRTI	MT-2	LAI	Additive to Synergistic	(Report No. 470), (Report No. 10804)
Didanosine	NRTI	MT-4	IIIB	Strongly Synergistic	{4541}
Didanosine	NRTI	MT-2	IIIB	Additive	{4543}
Didanosine	NRTI	MT-2	LAI	Additive to Synergistic	(Report No. 470), (Report No. 10804)
Elvitegravir	INI	MT-2	IIIB	Synergistic	(Report No. 183-2004)
Lamivudine	NRTI	MT-2	LAI	Additive to Synergistic	(Report No. 470), (Report No. 10804)
Rilpivirine	NNRTI	MT-2	IIIB	Strongly Synergistic	(Report No. PC-264-2001)
Stavudine	NRTI	MT-2	IIIB	Additive	{4543}

Compounds	Class	Cell line	HIV Strain	Results	References
Stavudine	NRTI	MT-2	LAI	Additive to Synergistic	(Report No. 470), (Report No. 10804)
Zalcitabine	NRTI	MT-4	IIIB	Strongly Synergistic	{4541}
Zalcitabine	NRTI	MT-2	IIIB	Additive	{4543}
Zidovudine	NRTI	MT-4	IIIB	Strongly Synergistic	{4541}
Zidovudine	NRTI	MT-2	IIIB	Additive	{4543}
Zidovudine	NRTI	MT-2	LAI	Additive to Synergistic	(Report No. 470), (Report No. 10804)
Amprenavir	PI	MT-2	LAI	Additive to Synergistic	(Report No. 470), (Report No. 10804)
Indinavir	PI	MT-2	LAI	Additive to Synergistic	(Report No. 470), (Report No. 10804)
Nelfinavir	PI	MT-2	LAI	Additive to Synergistic	(Report No. 470), (Report No. 10804)
Ritonavir	PI	MT-2	LAI	Additive to Synergistic	(Report No. 470), (Report No. 10804)

2.6.2.5.2. Rilpivirine: In Vitro Antiviral Combination Studies

For the pharmacodynamic drug interactions, please also refer to Module 2.7.2 (Summary of Clinical Pharmacology).

The in vitro antiviral activity of TMC278 in combination with current HIV-1 inhibitors was examined and was scored as additive, synergistic, or antagonistic. The following combinations with TMC278 were studied: the NRTIs 3TC, ABC, ddI, d4T, FTC, TFV, and ZDV, the NNRTIs EFV, ETR, and NVP, the PIs APV, ATV, DRV, IDV, LPV, NFV, RTV, SQV, and TPV, the integrase inhibitor RAL, the fusion inhibitor ENF, and the binding inhibitor MVC. TMC278 did not show antagonism when studied in combination with other antiretroviral agents. All combinations were scored as additive to synergistic (Tabulated Summary 2.6.3.1.17, TMC278-IV1-AVMR).

2.6.2.5.3. Tenofovir DF: In Vitro Antiviral Combination Studies

2.6.2.5.3.1. Anti-HIV-1 Combination Studies

Tenofovir, paired individually with 17 other antiretroviral compounds, was tested for additive, synergistic, or antagonistic antiviral activity against HIV-1 in MT-2 cells (Tabulated Summary 2.6.3.1.18, C1278-00005; Tabulated Summary 2.6.3.1.9, PC-164-2002; Tabulated Summary 2.6.3.1.20, PC-264-2001) {1469}. The data, shown in Table 53, were analyzed and defined using the MacSynergy™ II program {42}. Based on this analysis, TFV showed minor to moderate synergy with ddI, FTC, and NFV, and strong synergy with ZDV, APV, and all nonnucleoside RT inhibitors tested, including RPV (synergy volume of 156.0 $\mu M^2\%$). The

other combinations were additive, and no significant antiviral antagonism was observed. Tenofovir has also been tested with the integrase inhibitor EVG (Tabulated Summary 2.6.3.1.18, PC-183-2004). The results showed strong antiviral synergy of these 2 drugs paired together with a synergy volume of 133.2 $\mu M^2\%$. It should be noted that in these synergy studies, the numerical value is the measure of the combination effect, and the units (nM or uM) do not impact the data interpretation.

Table 53. Tenofovir Combinations Against HIV-1 in MT-2 Cells

Drug Combination	Volume ($\mu M^2\%$)[a]		Combined Effect
	Synergy	Antagonism	
Tenofovir + Nucleoside Reverse Transcriptase Inhibitors (NRTIs)			
TFV + d4T	4.1 ± 2.8	–2.8 ± 1.3	Additive
TFV + ABC	12.0 ± 12.1	–20.3 ± 10.8	Additive
TFV + 3TC	18.2 ± 9.6	–3.9 ± 3.4	Additive
TFV + ddC	22.0 ± 7.9	–8.1 ± 8.1	Additive
TFV + ddI	36.6 ± 2.0	–22.0 ± 3.5	Minor Synergy
TFV + FTC	81.1 ± 8.5	0 ± 0	Moderate Synergy
TFV + ZDV	105.5 ± 12.9	–0.5 ± 0.5	Strong Synergy
Tenofovir + Nonnucleoside Reverse Transcriptase Inhibitors (NNRTIs)			
TFV + DLV	138.3 ± 97	0 ± 0	Strong Synergy
TFV + NVP	167.8 ± 29.9	0 ± 0	Strong Synergy
TFV + EFV	210.5 ± 76.6	–10.6 ± 0.8	Strong Synergy
TFV + RPV	156.0 ± 40.4	-5 ± 5.5	Strong Synergy
Tenofovir + Protease Inhibitors			
TFV + IDV	12.0 ± 2.9	–8.7 ± 5.8	Additive
TFV + SQV	17.5 ± 1.9	–3.6 ± 3.6	Additive
TFV + RTV	20.3 ± 5.3	–12.6 ± 8.7	Additive
TFV + NFV	49.9 ± 11.5	–7.9 ± 7.9	Minor/Moderate Synergy
TFV + APV	108.9 ± 95	–12.5 ± 10.9	Strong Synergy
Tenofovir + Integrase Inhibitor			
TFV + Elvitegravir	133.2	–7.84	Strong Synergy

a Volume of synergy and antagonism were computed by the MacSynergy™ II program using a 95% confidence interval and are defined by the program as follows: values < 25 $\mu M^2\%$ indicate insignificant synergy (additive); values ≥ 25 and < 50 $\mu M^2\%$ indicate minor synergy/antagonism; values ≥ 50 and < 100 $\mu M^2\%$ indicate moderate synergy (+)/antagonism (-); and values ≥ 100 $\mu M^2\%$ indicate strong synergy (+)/antagonism (-).

Data from references: {1469}, C1278-00005, PC-164-2002, 183-2004, and PC-264-2001)

Triple combinations of TFV with 3TC and either ddI or ABC have also been evaluated in vitro for potential synergistic or antagonistic interactions (Tabulated Summary 2.6.3.1.18, PC-104-2005 and PC-104-2006). In these studies, activated PMBCs were utilized in order to more closely approximate the in vivo conditions required for the anti-HIV activity. The results show that all 2-drug combinations as well as the 3-drug combinations were additive to synergistic in their anti-HIV-1 activity (Table 54).

Table 54. Tenofovir Combinations Against HIV-1 in PBMCs

Drug 1	Drug 2	3TC Overlay	Synergy (95% confidence)	Antagonism (95% confidence)	Net Effect (Synergy + Antagonism)[a]
ABC	3TC	None	45.1 $\mu M^2\%$	-14.3 $\mu M^2\%$	Minor synergy
TFV	3TC	None	33.8 $\mu M^2\%$	0 $\mu M^2\%$	Minor synergy
ABC	TFV	None	6.1$\mu M^2\%$	-20.6 $\mu M^2\%$	Additive
ABC	TFV	25 nM	1.7 $\mu M^2\%$	-0.5 $\mu M^2\%$	Additive
ABC	TFV	50 nM	2.8 $\mu M^2\%$	-9.8 $\mu M^2\%$	Additive
ddI	TFV	None	0 $\mu M^2\%$	-1.1 $\mu M^2\%$	Additive
ddI	TFV	25 nM	4.05 $\mu M^2\%$	-2 $\mu M^2\%$	Additive
ABC	TFV	150 nM	10.3 $\mu M^2\%$	0 $\mu M^2\%$	Additive
3TC	3TC	None	19.1 $\mu M^2\%$	-1.8 $\mu M^2\%$	Additive

a Volume of synergy and antagonism were computed by the MacSynergy™ II program using a 95% confidence interval and are defined by the program as follows: values < 25 $\mu M^2\%$ indicate insignificant synergy (additive); values ≥ 25 and < 50 $\mu M^2\%$ indicate minor synergy (+)/antagonism (-); values ≥ 50 and < 100 $\mu M^2\%$ indicate moderate synergy (+)/antagonism (-); and values ≥ 100 $\mu M^2\%$ indicate strong synergy (+)/antagonism (-).

Data from references: PC-104-2005; PC-104-2006

In addition to the MacSynergy analyses presented above, isobologram analyses were also performed. In these analyses, additive to synergistic anti-HIV-1 activity was also determined. Statistically significant anti-HIV activity was observed for the combination of TFV with 3TC. In all studies, there was no evidence for antiviral antagonism with these drug combinations.

Hydroxyurea has been previously shown to reduce the concentrations of dNTP intracellularly by inhibiting the cellular enzyme ribonucleotide reductase. The effect on dATP pools is most notable. As a potential consequence of this, ddI, which is converted to the active metabolite ddATP, competes with dATP, shows greater anti-HIV activity both in vitro and in vivo, {1582} in combination with hydroxyurea. Tenofovir, another dATP analog, also shows increased anti-HIV activity in vitro when combined with hydroxyurea (Table 55) {3952}. Combinations of TFV and 50 μM hydroxyurea show greater than a 26-fold decrease in the TFV EC_{50} value for the wild-type HIV molecular clone NL4-3. Notably, HIV isolates with RT mutations associated with slightly decreased susceptibility to TFV show hypersusceptibility to TFV in the presence of hydroxyurea. However, this synergy was not demonstrated in vivo in Study 901 (see GS-97-901 Clinical Study Report for additional information).

Table 55. Combination of Tenofovir and Hydroxyurea Show Potent Anti-HIV Activity In Vitro Against HIV with Multiple Nucleoside Resistance Mutations

HIV Isolate	TFV EC_{50} (µM)[a]	TFV EC_{50} (µM) with 50 µM Hydroxyurea
HIV-1 (NL4-3 wild-type)	1.32	< 0.05
HIV-1 (41,67,210,215,219)[b]	2.58	0.43

a EC_{50} values are determined by infecting PBMCs and quantifying the inhibition of p24 production.

b RT resistance mutations at these amino acids.

Data from reference: {3952}

The combination of ribavirin (RBV) and TFV was also studied in vitro in order to provide insights into the safety of coadministration of TDF and RBV in HIV/hepatitis C virus (HCV) coinfected patients (Tabulated Summary 2.6.3.1.18, PC-104-2007). In this study, cells were infected with HIV-1 in the presence of RBV with either TFV, ddI, ZDV, 3TC, d4T, ABC, or NFV, and the antiviral effect of the combinations were measured. In cell culture, RBV on its own did not exhibit any anti-HIV-1 activity, but its presence had an effect on the anti-HIV activity of all of the NRTIs tested (Table 56). Ribavirin with ddI showed strong enhancement of the anti-HIV activity of ddI, confirming a previously defined synergistic interaction. In contrast to ddI, RBV with either TFV or ABC resulted in moderate levels of antagonism. Strong antagonism was shown for RBV with either ZDV, d4T, or 3TC. The observed levels of RBV interaction in the drug combination studies correlated closely with the antiretroviral effect observed for the nucleoside analogs, such that the antiviral activity of ddI increased 2.5-fold; decreased 1.4- and 1.6-fold for ABC and TFV, respectively; and decreased 3.2-, 3.5-, and 3.4-fold for 3TC, AZT, and d4T, respectively (Tabulated Summary 2.6.3.1.18, PC-104-2007). These results suggest a low potential for additive toxicity upon coadministration of TDF with RBV in patients. Since no clinical significance for the high-level anti-HIV antagonism between ZDV and RBV has been reported, the low-level antagonism observed for both TFV and ABC with RBV is unlikely to be clinically significant.

Table 56. Ribavirin-Antiretroviral Interaction Studies Against HIV-1

Drug Combination	Volume ($\mu M^2\%$)[a]		
	Synergy	Antagonism	Combined Effect[a]
RBV + ddI	359 ± 111	–4.8 ± 7.8	Strong Synergy
RBV + NFV	6.9 ± 2.9	–9.0 ± 8.2	No Effect
RBV + ABC	3.6 ± 5.3	–64.1 ± 23.0	Moderate Antagonism
RBV + TFV	3.8 ± 8.4	–84.3 ± 39.6	Moderate Antagonism
RBV + 3TC	0 ± 0	–165 ± 83.7	Strong Antagonism
RBV + ZDV	0 ± 0	–339 ± 119	Strong Antagonism
RBV + d4T	0.5 ± 1.0	–455 ± 217	Strong Antagonism

a Volume of synergy and antagonism were computed by the MacSynergy™ II program using a 95% confidence interval and are defined by the program as follows: values < 25 $\mu M^2\%$ indicate insignificant synergy (additive); values ≥ 25 and < 50 $\mu M^2\%$ indicate minor synergy (+)/antagonism (-); values ≥ 50 and < 100 $\mu M^2\%$ indicate moderate synergy (+)/antagonism (-); and values ≥ 100 $\mu M^2\%$ indicate strong synergy (+)/antagonism (-).

Data from reference: PC-104-2007

2.6.2.5.3.2. Anti-HBV Combination Studies

The anti-HBV activity of TFV in combination with other nucleoside anti-HBV polymerase inhibitors, FTC, 3TC, telbivudine, entecavir, and adefovir, was evaluated using AD38 cells (Tabulated Summary 2.6.3.1.19, PC-174-2006). By MacSynergy analysis, the results demonstrated that TFV in combination with FTC, 3TC, telbivudine, entecavir, and adefovir each produced an additive anti-HBV effect. Similarly, using a different HBV-expressing stable cell line, an additive effect of TFV in combination with FTC or adefovir was demonstrated (Tabulated Summary 2.6.3.1.9, PC-164-2004).

2.6.2.5.4. Emtricitabine/Tenofovir DF

The combination of TFV and FTC has been extensively studied in vitro for its anti-HIV activity (Tabulated Summary 2.6.3.1.9, PC-164-2002; Tabulated Summary 2.6.3.1.19, 14379 and PC-177-2001). The first studies were performed using the LAI strain of HIV-1 at a MOI of 0.03 in MT-2 cells. The results showed synergistic activity of TFV and FTC in an isobologram analysis (Tabulated Summary 2.6.3.1.19, 14379). These experiments were repeated using both the LAI laboratory-adapted strain of HIV-1, as well as a clinical HIV-1 recombinant containing a wild-type RT and protease gene (MM-317). For each virus infection, 2 MOIs were assessed (0.03 and 0.1). The results were analyzed by both MacSynergy (Table 57) and isobolograms (Table 58). Both analyses for all infections showed that the combination of TFV and FTC resulted in synergistic anti-HIV-1 activity. As a control, the combination of FTC with FTC showed additive anti-HIV activity. The isobologram analysis method permits an assessment of the level of statistical significance of the observed synergy versus the null hypothesis of additivity. P-values of ≤ 0.0017 were

obtained for all infections (Table 58). There was no evidence of antiviral antagonism in these experiments.

Table 57. Tenofovir and Emtricitabine Combinations – Synergy Volumes Analysis

Virus	MOI	Synergy[a]	Antagonism[a]	Combined Effect[a]
LAI	0.03	52.4 $\mu M^2\%$	-1.4 $\mu M^2\%$	Moderate Synergy
LAI	0.1	81.1 $\mu M^2\%$	0 $\mu M^2\%$	Moderate Synergy
MM-317	0.03	81.7 $\mu M^2\%$	0 $\mu M^2\%$	Moderate Synergy
MM-317	0.1	74.8 $\mu M^2\%$	0 $\mu M^2\%$	Moderate Synergy
LAI control (FTC-FTC)	0.03	6.0 $\mu M^2\%$	-10.1 $\mu M^2\%$	Additive

a Volume of synergy and antagonism were computed by the MacSynergy™ II program using a 95% confidence interval and are defined by the program as follows: values < 25 $\mu M^2\%$ indicate insignificant synergy (additive); values ≥ 25 and < 50 $\mu M^2\%$ indicate minor synergy (+)/antagonism (-); values ≥ 50 and < 100 $\mu M^2\%$ indicate moderate synergy (+)/antagonism (-); and values ≥ 100 $\mu M^2\%$ indicate strong synergy (+)/antagonism (-).

Data from reference: PC-164-2002

Table 58. Tenofovir and Emtricitabine Combinations – Isobologram Analysis

			D, Average Deviation from Additivity			
Combination	Virus	MOI	Mean	S.E.M.	p (t test)	Net effect
TFV + FTC	LAI	0.03	-4.6743	0.0662	0.0008	Synergy
TFV + FTC	LAI	0.1	-3.9498	0.0756	0.0017	Synergy
TFV + FTC	MM317	0.03	-8.5804	0.0383	< 0.0001	Synergy
TFV + FTC	MM317	0.1	-4.2563	0.0420	0.001	Synergy

S.E.M. = standard error of the mean

Data from reference: PC-164-2002

One potential mechanism behind the synergy of TFV and FTC in cell culture correlates with approximately 1.4-fold increased levels of the fully phosphorylated active anabolites of both compounds when dosed together compared to single drug dosing, as measured in CEM cells. However, the increased phosphorylation of TFV and FTC in combination was not reproduced in cultured PBMCs {8998}. A second potential mechanism involved in this synergy was shown in vitro at the enzymatic level, where the presence of FTC-TP enhanced the formation of stable, dead-end complexes by HIV-1 reverse transcriptase and TFV-terminated DNA that potentiates the chain-termination activity of TFV (Tabulated Summary 2.6.3.1.19, PC-177-2001).

2.6.2.5.5. Emtricitabine/Rilpivirine/Tenofovir DF

The antiviral activity of RPV in combination with nucleoside/nucleotide RT inhibitors FTC or TFV was evaluated in 5-day cytopathic assays with acutely HIV-1 infected MT-2 cells (Tabulated Summary 2.6.3.1.20, PC-264-2001; Table 59). The combination effects of 2-drug combinations were analyzed by the Prichard and Shipman method using MacSynergy II software {42}. The combinations of TFV/FTC, RPV/FTC, and RPV/TFV all showed strong antiviral synergy with no evidence of antiviral antagonism in cells.

Table 59. Mean Synergy/Antagonism Volumes for the Two-Drug Combinations of Emtricitabine, Rilpivirine, and Tenofovir

Drug Combination[a]	Synergy ($\mu M^2\%$) ± SD[b]	Antagonism ($\mu M^2\%$) ± SD[b]	Combined Effect[b]
TFV + FTC	152.7 ± 28.7	0 ± 0	Strong Synergy
RPV + TFV	156.0 ± 40.4	-5.0 ± 5.5	Strong Synergy
RPV + FTC	135.0 ± 68.1	-14.4 ± 10.3	Strong Synergy
RBV + ddI	250.1 ± 186.6	-13.4 ± 18.9	Strong Synergy
RBV + d4T	0 ± 0	-493.1 ± 286.9	Strong Antagonism

a TFV, tenofovir; FTC, emtricitabine; RPV, rilpivirine; ddI, didanosine

b Synergy/antagonism volumes represent the mean and standard deviation of at least two independent experiments. Volume of synergy and antagonism were computed by the MacSynergy™ II program using a 95% confidence interval and are defined by the program as follows: values < 25 $\mu M^2\%$ indicate insignificant synergy (additive); values ≥ 25 and < 50 $\mu M^2\%$ indicate minor synergy (+)/antagonism (-); values ≥ 50 and < 100 $\mu M^2\%$ indicate moderate synergy (+)/antagonism (-); and values ≥ 100 $\mu M^2\%$ indicate strong synergy (+)/antagonism (-).

Data from reference: PC-264-2001

The antiviral activity of RPV in combination with FTC and TFV was evaluated in 5-day cytopathic assays in MT-2 cells acutely infected with HIV-1 (Tabulated Summary 2.6.3.1.20, Report PC-264-2002; Table 60). The antiviral effect of the 3-drug combination was analyzed using the combination index method of the CalcuSyn software. The triple drug combination, FTC/RPV/TFV displayed moderate synergy (CI score of 0.73 ± 0.13). These results suggest that the components of the FDC tablet of FTC/RPV/TDF show in vitro synergy that was similar to that observed for the components of Atripla (EFV/FTC/TDF).

Table 60. Triple Drug Combination Index Values for Emtricitabine, Rilpivirine, and Tenofovir

Drug Combination[a]	CI ± SD[b]	Net Effect
FTC + RPV + TFV	0.73 ± 0.13	Moderate synergy
FTC + TFV + EFV	0.57 ± 0.08	Synergy
d4T + ZDV + RPV	0.92 ± 0.04	Additive

a FTC, emtricitabine; RPV, rilpivirine; TFV, tenofovir ; EFV, efavirenz ; d4T, stavudine ; ZDV, zidovudine.

b Mean Combination Index (CI) and standard deviation calculated from n=3-5 experiments for all combinations. CalcuSyn interprets the net effect as antagonism (CI > 1.1), additivity (0.9 ≤ CI ≤ 1.1), and synergy (CI < 0.9).

Data from reference: PC-264-2002

2.6.2.6. Discussion and Conclusions

Emtricitabine

Emtricitabine has antiviral activity against HIV-1, HIV-2, and HBV. Emtricitabine has demonstrated additive to synergistic activity with a variety of other antiretroviral drugs. Antiretroviral resistance is associated with an M184V or I mutation that shows cross-resistance with 3TC.

Emtricitabine has shown no in vitro cytotoxicity in a variety of human cell types. It also shows no toxicity against hematopoietic progenitor cells. Emtricitabine has a high selectivity for HIV RT versus cellular DNA polymerases α, β, γ, and ε. Emtricitabine has also shown no effect on mitochondrial function as measured by mitochondrial DNA synthesis, cellular content of COX II, intracellular lipid accumulation, lactic acid production, and mitochondrial ultrastructure.

The results of the safety pharmacology program suggest that FTC is without undesirable effects on any organ system at systemic (AUC) exposures approximately 10- to > 50-fold higher than human exposure at the recommended clinical dose (rodent exposures for 100 and 1000 mg/kg doses, respectively, as compared to human AUC).

Rilpivirine

Rilpivirine is a nonnucleoside RT inhibitor that has antiviral activity against wild-type and many drug resistant HIV-1 at the subnanomolar level. Rilpivirine also has antiviral activity against HIV-2 and SIV, but at the micromolar level. Rilpivirine has demonstrated additive to synergistic activity with a variety of other antiretroviral drugs. Antiretroviral resistance to RPV in vitro is associated with combinations of mutations in RT at V90I, L100I, K101E, V106A/I, V108I, E138G/K/Q/R, V179F/I, Y181C/I, V189I, G190E, H221Y, F227C, and M230I/L.

Rilpivirine has shown a low potential for in vitro cytotoxicity in a variety of human cell types. The selectivity index of approximately 8000 indicates that RPV is a potent and selective inhibitor of HIV-1.

Rilpivirine did not show antagonism when studied in combination with other antiretroviral agents. Rilpivirine showed additive to synergistic antiviral activity in combination with the N(t)RTIs ABC, ddI, FTC, 3TC, d4T, TFV, and ZDV; the PIs APV, ATV, DRV, IDV, LPV, NFV, RTV, SQV, and TPV; the NNRTIs EFV, ETR, and NVP; the fusion inhibitor ENF; the entry inhibitor MVC; and the integrase inhibitor RAL.

The testing on a variety of receptors, human DNA polymerases, stomach ATPase, and gastric acidity did not reveal any significant effects secondary to the primary anti-HIV effect of TMC278. Therefore, and in line with ICH Guideline S7A, safety pharmacology evaluations focused on the battery of core vital systems (nervous, cardiovascular, and pulmonary).

At the level of the nervous system, TMC278 had no effects in dogs up to a maximum plasma concentration of 1.5 µg/mL. In rats, incidental motor-affective and sensori-motor behavior parameters were affected and pupil size (autonomic parameter) was slightly reduced on the day of dosing, at an estimated maximum plasma concentration of 10 µg/mL. The repeat-dose general toxicity studies have not indicated any effect of TMC278 the on the nervous system (see Module 2.6.6, Toxicology Written Summary). It is concluded that the margin of exposure for effects of TMC278 on the nervous system at a clinical dose of 25 mg once daily is approximately 11.

The standard battery of cardiovascular safety studies indicates that TMC278 has the potential to inhibit partially the hERG channel at the nominal unbound concentration of 37 ng/mL and higher. In the isolated guinea pig right atrium, no effects possibly associated with potassium current reduction were noted up to 3.69 µg/mL. Even the lowest concentration of TMC278 that caused significant potassium current reduction in protein-free medium (37 ng/mL) is much higher than the unbound concentration that can be achieved in the in vivo cardiovascular safety studies in guinea pigs and dogs. These species have only 0.14% and 0.65% unbound TMC278, respectively. No other cardiovascular or cardio-electrophysiological parameters were affected by TMC278. The relevance of the decreased vascular resistance and increased cardiac output noted in the anesthetized dog study (Section 2.6.2.4.9.1) is questionable as the vehicle PEG400 appeared to have a clear effect on these parameters, opposite to that of TMC278.

In view of the results of the standard battery of studies, the QTc-prolongation in the TQT study C131 was unexpected (see Table 61 and also Module 2.7.4, Summary of Clinical Safety, Section 2.7.4.5.4). Study C131 with 75 and 300 mg of RPV once daily for 11 days showed dose-related clinically relevant QTcF-prolongation which became manifest after 11 days of treatment.

Table 61. Exposure and QTcF-data in QTc-prolongation studies with TMC278

	25 mg Once Daily				75 mg Once Daily				300 mg Once Daily			
	Day 1		Day 11		Day 1		Day 11		Day 1		Day 11	
	ΔQTcF	C_{max}	ΔQTcF	C_{max}	ΔQTcF	C_{max}	ΔQTcF	C_{max}	ΔQTcF	C_{max}	ΔQTcF	C_{max}
TQT C131					1.0	0.867 (289)	10.4	1.908 (636)	3.9	2.514 (838)	23.8	4.995 (1665)
Pilot C151			2.2	0.687 (229)								
TQT C152			2.0	0.741 (247)								

ΔQTcF: median increase compared to baseline of QT-interval corrected for heart rate according to Fridericia in ms; C_{max}: maximum unbound plasma concentration of TMC278 in ng/mL (0.3% of measured C_{max}; measured C_{max} within parentheses); TQT: thorough QTc-prolongation study according to ICH E14.

In order to investigate the mechanism of action of the clinical QTc-prolongation, additional nonclinical cardiovascular safety studies were done with the following objectives:

- To investigate the mechanism of action of the QT-prolongation seen in the TQT study with a dose of 75 mg once daily, and above
- To investigate the mechanism of action of the delayed onset of the QTc-prolongation
- To assess the potential of TMC278 to induce proarrhythmic effects

In order to find a dose that would not cause QTc-prolongation, additional clinical studies were done. Pilot study C151 with 25 mg of TMC278 once daily for 11 days showed no QTcF-prolongation. The TQT study C152 with 25 mg of TMC278 once daily for 11 days confirmed the absence of a clinically relevant QTcF-prolongation of 25 mg of TMC278 once daily, the dose demonstrating efficacy in the clinical Phase 3 studies, C209 and C215 (see Module 2.7.4).

Mechanism of QT-prolongation:

The potential of TMC278 to inhibit potassium currents I_{Kr}, I_{Ks}, and I_{to} involved in the repolarization phase of the cardiac action potential is likely a contributing factor, but cannot explain some aspects of the QTcF-prolongation seen in TQT study C131. The inhibition of these potassium currents in the cells expressing their channels is a direct concentration-related effect. For that reason, this effect cannot explain the delayed onset of the QTc-prolongation in the TQT study. Moreover, in TQT study C131, the median maximum plasma concentration on Day 1 in subjects treated with 300 mg was higher than that on Day 11 with 75 mg. On Day 1, 300 mg did not elicit a clinically relevant QTc-prolongation whereas 75 mg did on Day 11. Unfortunately, due to the adsorption of TMC278 to the in

vitro equipment, the margin between the unbound concentrations of TMC278 at which the inhibition of the potassium currents occurred in the serum-free in vitro conditions (nominally 0.11 µg/mL and higher) and the free C_{max} values associated with QTcF-prolongation in TQT study C131 (see Table 61) cannot be accurately determined but is likely significant.

Mechanism of delayed onset:

Several drugs (e.g., pentamidine, As_2O_3) are now known to prolong the QT interval in man by interfering with hERG trafficking {15693}, and this can be demonstrated in repeat-dose animal models. The concentration-related inhibition of trafficking of the hERG channel seen in the hERG Lite® test could contribute to the delayed onset of the QTcF-prolongation seen in TQT study C131. However, it is to be noted that the in vitro inhibition of trafficking becomes manifest after hours, whereas the QTc-prolongation by TMC278 needs days to develop in the TQT study. In addition, the repeat-dose study with telemetered guinea pigs failed to induce QT-prolongation even after treatment for 16 days (Tabulated Summary 2.6.3.1.14, TMC278-NC327). The hERG trafficking inhibition seen in vitro did not occur in vivo in the guinea pigs, whereas delayed-onset QTcF-prolongation occurred in subjects treated with 75 mg in the TQT study at the median maximum plasma concentration (636 ng/mL) of TMC278 that was similar to that in the repeat-dose guinea pig study. Moreover, ECG recordings in a repeat-dose study with cynomolgus monkeys (Tabulated Summary 2.6.6.3.5.4, TMC278-TiDP38-NC326 [FK6541]) and in the repeat-dose general toxicity studies with TMC278 base in dogs (see Module 2.6.6, Toxicology Written Summary, Section 2.6.6.3.4) did not indicate any QT prolongation. The highest C_{max} value in the 12-month study in male dogs was 4.1 µg/mL, with a plasma protein binding of 99.3% (see Module 2.6.4, Pharmacokinetics Written Summary, Section 2.6.4.3.4.7). This value was approximately 6 times higher than that in man at 75 mg of TMC278 once daily in Trial C131 after 11 days of administration.

Noncardio-electrophysiological mechanisms to explain the delayed onset of the QTc-prolongation comprise an increase over time of the exposure to TMC278 or to one or more of its metabolites or an increase in the concentration of TMC278 or of one or more of its metabolites in the myocardium.

In man, exposure to TMC278 increased over time until steady state was reached before Day 11. However, in study C131, this increase in exposure from Day 1 to Day 11 at the dose of 75 mg once daily was less than the difference in exposure between 75 and 300 mg on Day 1 (see Table 61). Therefore, it cannot explain the delayed onset of the QTcF-prolongation noted at 300 mg in TQT study C131, and likely also not that at 75 mg. TMC278 is metabolized in man predominantly by oxidation to metabolites that are likely to have a shorter residence time than the parent compound. After a single radiolabelled dose, 76% of the radioactivity in plasma on the basis of C_{max} is unchanged drug, whereas it is 51% on the basis of AUC. There are no major human metabolites (> 10% of C_{max} of the parent compound) and man does not produce a unique metabolite compared to the nonclinical species (see Module 2.7.2, Summary of Clinical Pharmacology for human exposure data and Module 2.6.4, Pharmacokinetics Written Summary, Section 2.6.4.5 for the metabolism data). Exposure data of metabolites after repeat dosing to man are sparse, but indicate that while exposure to the

3 most prominent metabolites increased approximately 10-fold after 14 days of treatment (R278474-CDE-102), the levels of the individual metabolites still remained less than 10% of the exposure to the parent compound, TMC278. TMC278 accumulated 2- to 3-fold from Day 1 to Day 11 in TQT study C131. These increases in exposure over time probably contributed to the longer QT intervals on Day 11 than Day 1, but do not entirely explain them.

Single-dose quantitative whole body autoradiography studies in rats showed heart to plasma ratios of radioactivity to be 2 or less during maximally 8 hours following dosing. The maximum levels of radioactivity in all tissues were reached 4 to 8 hours after dosing (see Module 2.6.4, Pharmacokinetics Written Summary, Section 2.6.4.4.2). The data do not indicate any particular affinity of TMC278 or its metabolites for heart tissue. Single-dose mass balance data show complete excretion in rats and dogs after 4 and 7 days, respectively; whereas it takes 14 days to recover 91% of the radioactivity from a dose of 150 mg in man (see Module 2.6.4, Pharmacokinetics Written Summary, Section 2.6.4.6). It cannot be excluded that this slow excretion plays a role in the delayed-onset development of the QTcF-prolongation. However, no indications are available about these contributing elements. It is to be noted, that whatever the correlation between the slow excretion and the delayed-onset QTcF-prolongation may be, no QTcF-prolongation occurred after 11 days of administration, at steady state of 25 mg TMC278 once daily, in the pilot study C151 or in the TQT study C152.

Potential to induce TdP:

The perfused rabbit ventricular wedge study indicated that TMC278 has at most a marginal potential to induce arrhythmogenic effects, like TdP. QTc-prolongation as such does not lead necessarily to TdP. The dispersion of the repolarization of the cardiac action potential over the ventricular wall plays an equally important role as QT-prolongation, whereas the occurrence of EADs adds even more weight in the assessment of the potential to induce TdP according to Liu et al. {15697}. TMC278 did not induce EADs or any other proarrhythmic effects and had no effect on TDR. Only the QT-prolongation contributed to the TdP score of 0.5. Compounds with a history of TdP such as clarithromycin, erythromycin, and cisapride typically have a score of around 5 in this model {15697}. The margin between the concentration of TMC278 that caused a marginal TdP score of TMC278 and the unbound C_{max} at 75 mg TMC278 once daily, 0.3% of 636 ng/mL (see Table 61), after 11 days of treatment in TQT study C131 cannot be determined accurately due to the loss in the test system. However, it is likely that the margin is very high, almost 1000, based on nominal bath concentrations. The median C_{max} values in HIV-infected treatment-naive subjects treated for 48 weeks with 25 mg once daily in clinical Phase 3 studies C209 and C215 are 139 and 133 ng/mL, respectively; again 5 times lower.

TMC278 tested up to a maximum plasma concentration of 2.6 µg/mL in anesthetized or conscious instrumented dogs showed no potential to affect respiratory parameters. The margin of exposure for respiratory effects by TMC278 at a clinical dose of 25 mg once daily is 20.

In summary, TMC278 had no effects on secondary pharmacodynamic parameters or on the core battery of safety pharmacology tests, apart from inhibitory effects on some cardiac potassium currents and channels and moderate QT-prolongation in the rabbit ventricular wedge. The observed inhibition of trafficking of the hERG channel in vitro, but not confirmed in vivo in guinea pigs may be involved in the delayed onset of the QTcF-prolongation observed in clinical TQT study C131. However, kinetic mechanisms may also be involved. Importantly, the effects in nonclinical models allow a conclusion that TMC278 has only a marginal potential to induce proarrhythmic effects.

Tenofovir DF

Tenofovir has potent antiretroviral activity against wild-type or drug-resistant strains of HIV-1 in vitro and in vivo it is active in several animal retrovirus models. Tenofovir was demonstrated to have additive to synergistic activity with a variety of other antiretroviral drugs. Antiretroviral resistance is slow to develop both in vitro and in vivo, and the drug maintains partial in vivo activity against known SIV strains with reduced susceptibility to TFV. Tenofovir also shows activity against human HBV and HIV-2.

Tenofovir has low in vitro cytotoxic potential in a variety of human cell types. It also shows no toxicity against hematopoietic progenitor cells. Tenofovir diphosphate is a very weak inhibitor of mammalian DNA polymerases α, β, δ, ε, and mitochondrial DNA polymerase γ. Tenofovir has also shown no effect on mitochondrial function as measured by mitochondrial DNA synthesis, cellular content of COX II, intracellular lipid accumulation, and lactic acid production.

Safety pharmacology studies demonstrated that TDF has no effect on the CNS or cardiovascular system. A dose of 500 mg/kg of TDF reduced urinary and electrolyte output; however, kidney function was unimpaired and a dose of 50 mg/kg was considered to be the no-effect level. In addition, there was reduced gastric emptying in rats dosed at 500 mg/kg, but not at 50 mg/kg.

Emtricitabine/Tenofovir DF

Tenofovir and FTC are analogues of 2 different nucleosides, adenosine and cytosine, respectively, and do not share a common intracellular metabolism pathway. When both drugs are incubated together in vitro at concentrations higher than achieved in the plasma (10 μM each), complete conversion to the fully activated forms of each drug, TFV diphosphate and FTC triphosphate, was observed. As TFV diphosphate and FTC triphosphate are alternative substrates for different natural substrates, dATP and dCTP, respectively, there should be no competition for incorporation by HIV RT and subsequent chain termination. In support of this, the antiviral activity of the combination of TFV and FTC was found to be synergistic in multiple in vitro assay systems.

In vitro resistance selection experiments with the combination of TFV and FTC have demonstrated the initial development of the M184V/I mutation with high-level resistance to FTC followed by development of the K65R mutation with low-level resistance to TFV.

These results are in agreement with the results obtained from resistance selection with the individual components, that is quick selection of M184V/I by FTC and a delayed selection of resistance by TFV. These results suggest that the combination of TFV and FTC in vivo would select for the M184V/I mutation first and then potentially TFV resistance in a step-wise fashion. Clinical trial data using the combination of TDF with FTC support this conclusion as the M184V mutation was observed to develop in the absence of K65R in patients with virologic failure (see Module 2.5).

The antiviral activity of the combination of TFV and FTC was demonstrated in SIV-infected macaques. Daily subcutaneous administration of TFV (20 mg/kg) and FTC (50 mg/kg) resulted in complete suppression (< 100 copies/mL) of SIV for the duration of the 32-week study. There was no development of detectable resistance to either drug over the period of study. A prophylaxis study also demonstrated the efficacy of the combination of TFV and FTC for protection against rectal exposure of macaques to an HIV-1/SIV chimeric virus. These results in a model system of HIV-1 infection suggest the potency of the combination of TFV and FTC for the control of a retroviral infection.

The combination of TFV and FTC were studied for cytotoxicity in MT-2 cells. No cytotoxicity was observed at concentrations up to 50 μM TFV and 5 μM FTC. Additive mitochondrial toxicity was not observed in HepG2 cells.

Neither TDF nor FTC had significant unwanted pharmacologic activity as determined in a variety of in vitro and in vivo safety pharmacology studies. While the study designs for these studies varied between the 2 individual products, the major organ systems were comprehensively evaluated. Given the minimal effects observed with high-dose administration of individual products (decreased urinary output and reduced gastric emptying with high-dose TDF alone), additional safety pharmacology studies on the FTC/TDF combination are considered unwarranted.

Emtricitabine/Rilpivirine/Tenofovir DF

The HIV-1 NNRTI, RPV, and the NRTIs, FTC and TFV, have potent antiretroviral activity against wild-type and many drug-resistant strains of HIV-1 in vitro and in vivo. The combination of FTC, RPV, and TFV in 2-drug combination experiments showed additive to synergistic anti-HIV-1 activity, and synergistic anti-HIV-1 activity in 3-drug combination experiments. Additive antiretroviral activity would be expected given that the active metabolites of FTC and TFV compete with different natural substrates for incorporation into viral DNA during the reverse transcription step, and RPV would also be expected to be additive as it binds at a distinct binding pocket on RT to inhibit reverse transcription. The observation of synergistic anti-HIV-1 activity for the FTC/RPV/TFV combination suggests potentiation of the individual anti-HIV activities of these compounds within cells.

NRTIs carry a class labeling for mitochondrial toxicity; however, both FTC and TDF have shown a low potential for mitochondrial toxicity in long-term toxicity studies. The potential for mitochondrial toxicity of RPV was low by in vitro assessment of the inhibitory activity on human polymerase γ. As mitochondrial toxicity is generally less relevant for NNRTIs than

NRTIs, and as RPV is not anticipated to significantly increase the exposure of FTC or TFV, the potential for exacerbating mitochondrial toxicity is low.

From in vitro data, pharmacokinetics studies in dogs (Module 2.6.4) and clinical experience (Module 2.7.2), there are no anticipated pharmacokinetic interactions between FTC, RPV, and TDF. Emtricitabine and TDF had little effect on vital organ systems in safety pharmacology studies. Rilpivirine has shown the potential for QT prolongation, an effect confirmed in a thorough QT study in healthy subjects. At the 25-mg dose of RPV, the observed change in QTcF was not considered clinically relevant, and the combination product is not anticipated to exacerbate the cardiovascular effect seen with RPV alone. No additional safety pharmacology studies are considered necessary with the FTC/RPV/TDF combination.

The absence of nonclinical safety studies with the combination is in accordance with the CHMP Guideline on the Non-Clinical Development of Fixed Combinations of Medicinal Products (EMEA/CHMP/SWP/258498/2005, January 2008). There are no anticipated clinically relevant pharmacokinetic or toxicological interactions expected in the FTC/RPV/TDF combination. The Scientific Advice Working Party agreed with the assessment that no further nonclinical studies are needed to support the FTC/RPV/TDF FDC based on the lack of relationship between the primary pharmacodynamics of RPV and the individual NRTIs (FTC and TDF); the toxicities of these compounds; the comprehensive nonclinical data set for FTC, RPV and TDF; and the long-term safety from the Phase 2b Study TMC278-C204 (EMEA/CHMP/SAWP/670243/2009 corrigendum, Module 1.2.5.14). Further, extensive clinical safety data are available for the approved drugs FTC, TDF, and the FTC/TDF FDC product Truvada. Additionally, data from the Phase 2b and Phase 3 studies with RPV and Truvada supports the use of these products (see Section 2.7). The clinical data, along with the lack of overlapping toxicity in animals, support the safety of the new combination product.

Overall, the pharmacodynamic and pharmacological assessment of FTC, RPV, and TDF supports the effective and safe use of these 3 agents together in combination therapy for HIV-1 disease.

2.6.2.7. Tables and Figures

Tables and figures have been integrated within the textual summaries.

2.6.2.8. References

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SECTION 2.6
NONCLINICAL SUMMARY

Section 2.6.3 — Pharmacology Tabulated Summary

EMTRICITABINE/RILPIVIRINE/
TENOFOVIR DISOPROXIL FUMARATE
FIXED-DOSE COMBINATAION

Gilead Sciences International Limited

17 August 2010

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2.6. NONCLINICAL SUMMARY

2.6.3. PHARMACOLOGY TABULATED SUMMARY

Type of Study	Test System	Method of Administration	Testing Facility	Study Number & [Reference]
2.6.3.1. Pharmacology Overview				
2.6.3.1.1. Primary Pharmacodynamics of Emtricitabine				
Phosphorylation of FTC	Calf Thymus Deoxycytidine Kinase	In vitro	Burroughs Wellcome Co., Research Triangle Park, NC 27709 USA	TESF/91/0014
Phosphorylation of FTC	Calf Thymus Deoxycytidine Kinase	In vitro	Burroughs Wellcome Co., Research Triangle Park, NC 27709 USA	TESF/92/0002
Phosphorylation of FTC	dCMP Kinase and Nucleoside Monophosphate Kinase.	In vitro	Burroughs Wellcome Co., Research Triangle Park, NC 27709 USA	TEIT/92/0005
Phosphorylation of FTC	Nucleoside Monophosphate Kinase and Nuclear Diphosphate Kinase	In vitro	Burroughs Wellcome Co., Research Triangle Park, NC 27709 USA	TGZZ/93/0025
Inhibition of human immunodeficiency virus-1 (HIV-1) reverse transcriptase	Endogenous RT assay Chain-termination substrate assays Purified HIV-1 reverse transcriptase enzyme assay	In vitro	Division of Virology, Burroughs Wellcome Co., Research Triangle Park, North Carolina	*Antimicrob Agents Chemother* 1993;37:1270-2 {4249}

FTC = emtricitabine

2.6.3.1 Pharmacology — Overview — Test Article: Emtricitabine

Type of Study	Test System	Method of Administration	Testing Facility	Study Number & [Reference]
Inhibition of HIV-1 reverse transcriptase	Pre–steady state kinetic analysis of HIV-1 reverse transcriptase	In vitro	Gilead Sciences, Inc., USA	*FASEB J.* 1999; **13**:1-7 {4545}
Antiviral activity vs. HIV-1 and HIV-2	Human peripheral blood mononuclear cells (PBMC) and various T cell lines	In vitro	Veterans Affairs Medical Center, Decatur, Georgia	*Antimicrob Agents Chemother* 1992;36:2423-31 {4534}
Antiviral activity vs. HIV-1	Human PBMC, Human T-cell lines MT2 and CEM	In vitro	Gilead Sciences, Inc., USA	TPI 462-v2
Antiviral activity against various subtypes of HIV-1	Human PBMC	In vitro	Gilead Sciences, Inc., USA	TPI 10498-v2
Antiviral activity against various subtypes of HIV-1	HeLa cells	In vitro	Gilead Sciences, Inc., USA	TPI 11419-v2
MAGI-LU assay validation 1: inhibitory effect of FTC on HIV-1 xxLAI viral infection is independent of multiplicity of infection (MOI) of the infecting virus	MAGI cells	In vitro	Gilead Sciences, Inc., USA	10518v2
Effect of MOI on inhibition of HIV-1 replication by FTC	Human PBMC	In vitro	Gilead Sciences, Inc., USA	11773
DXG, FTC, and zidovudine (AZT): Time of addition.	MAGI cells	In vitro	Gilead Sciences, Inc., USA	10247

FTC = emtricitabine

2.6.3.1 Pharmacology

Overview

Test Article: Emtricitabine

Type of Study	Test System	Method of Administration	Testing Facility	Study Number & [Reference]
Effect of human serum on the antiviral activity of FTC assessed in CEM cells infected with the LAI strain of HIV-1	CEM cells	In vitro	Gilead Sciences, Inc., USA	463
Selection of FTC resistant virus	Human T-cells line, MT-4 cells	In vitro	Burroughs Wellcome Co., Research Triangle Park, NC 27709 USA	*Proc. Natl. Acad. Sci. U. S. A.* 1993; **90**:5653-5656 {1794}
Selection of FTC resistant virus	Human PBMC	In vitro	Emory University, Atlanta, GA, USA	*Antimicrob. Agents Chemother.* 1993; **37**:875-881 {1777}
Antiviral activity vs. nucleoside reverse transcriptase inhibitor-resistant recombinant HIV-1 clinical isolates	MT-4 cells	In vitro	Virco NV, Mechelen, Belgium	TPI 11148
Activity against drug-resistant HIV-1 with K65R mutation	Recombinant mutant enzymes and viruses in MT-2 cells	In vitro	Gilead Sciences, Inc., USA	TPI 15883
Virologic and enzymatic studies revealing the mechanism of K65R- and Q151M-associated HIV-1 drug resistance towards FTC and lamivudine (3TC)	MT-2 cells and in vitro	In vitro	Gilead Sciences, Inc., USA	*Nucleosides, Nucleotides, and Nucleic Acids,* 2006; 25:89-107 {8887}
Metabolism of FTC	$HepG_2$ 2.2.15 (P5A) cells	In vitro	Burroughs Wellcome Co., Research Triangle Park, NC 27709 USA	TEZA/92/0062

FTC = emtricitabine

2.6.3.1 Pharmacology **Overview** **Test Article:** Emtricitabine

Type of Study	Test System	Method of Administration	Testing Facility	Study Number & [Reference]
Metabolism of FTC	CEM T-lymphoblast cells	In vitro	Burroughs Wellcome Co., Research Triangle Park, NC 27709 USA	TEZA/92/0103
Metabolism of FTC in hepatitis B infected cells	HepG2 (Human Hepatocellular Carcinoma) cells	In vitro	Burroughs Wellcome Co., Research Triangle Park, NC 27709 USA	TEZA/92/0111
Effect of FTC on human DNA polymerase	HeLa S3 cells and purified Human polymerases α, β, γ, and ε	In vitro	Burroughs Wellcome Co., Research Triangle Park, NC 27709 USA	TEZZ/93/0007
Effect of FTC on human polymerase γ	Human polymerase γ	In vitro	Gilead Sciences, Inc., USA	TPI 9501
Effect of orally administered FTC and lamivudine in the HuPBMC-SCID mouse model of HIV-1 infection	HuPBMC-SCID mouse model of HIV-1 infection	Oral	Gilead Sciences, Inc., USA	TPI 11985
Antiviral activity of FTC *in vivo*	HuPBMC-SCID mouse model of HIV-1 infection	Intraperitoneal (IP)	Emory University, Atlanta, GA, USA	2nd International Workshop on HIV Drug Resistance and Treatment Strategies, 1998, Lake Maggiore, Italy, Abstract 9 {4525}

FTC = emtricitabine

Type of Study	Test System	Method of Administration	Testing Facility	Study Number & [Reference]
2.6.3.1.2. Primary Pharmacodynamics of Rilpivirine				
Mechanism of drug action: HIV-1 RT inhibition	RT primer-extension based scintillation proximity assay	In vitro	Tibotec, Belgium	TMC278-IV1-AVMR
Mechanism of drug action: RPV inhibition of human DNA polymerases	Enzymatic assay	In vitro	[redacted], USA	TMC278-1646_0005343
Mechanism of drug action: cocrystallization and structural modeling of TMC278 and HIV-1 RT	X-ray crystallography	In vitro	Gilead Sciences, Inc, USA	*J Med Chem 2010;53 (10):4295-9.* {15866} *Proc Natl Acad Sci U S A 2008;105 (5):1466-71 .*{15850}
Mechanism of drug action: time of addition (TOA)	Phenotypic assay in MT-4 cells Quantification of supernatant HIV-1 p24 Ag	In vitro	Tibotec, Belgium	TMC278-IV1-AVMR
I*n vitro* antiviral activity against WT HIV-1, HIV-2, and SIV in human cell lines	Phenotypic assays in MT-4 cells and in derived engineered cell lines (MT-4-LTR-EGFP cells) Direct measurement of viral replication in MT-4-LTR-EGFP cells and determination of cell viability by resazurin or MTT	In vitro	Tibotec, Belgium	TMC278-IV1-AVMR and TMC278-IV2-AVMR
I*n vitro* antiviral activity against HIV-1 BaL and ADA in human primary cells	Phenotypic assay in monocyte-derived-macrophages infected with HIV-1 Quantification of HIV-1 p24 Ag in the cell supernatant	In vitro	[redacted], USA	TMC278-IV2-AVMR

2.6.3.1 Pharmacology

Overview

Test Article: Rilpivirine

Type of Study	Test System	Method of Administration	Testing Facility	Study Number & [Reference]
I*n Vitro* Antiviral Activity Against Primary HIV-1 Isolates Group M and Group O in PBMCs	HIV-1 primary isolates in human PBMCs based assays Quantification of RT activity in cell supernatant	In vitro	SRI, University of Alabama, USA	TMC278-IV1-AVMR and TMC278-IV2-AVMR
Multiplicity of infection (MOI)	Phenotypic assay in MT-4 cells Direct measurement of viral replication in MT-4-LTR-EGFP cells	In vitro	Tibotec, Belgium	TMC278-IV2-AVMR
Effect of human serum proteins	Phenotypic assay in MT-4 cells in the presence of AAG, HS or HSA Direct measurement of viral replication in MT-4-LTR-EGFP cells	In vitro	Tibotec, Belgium	TMC278-IV1-AVMR
In Vitro Cytotoxicity and Susceptibility Index	*In vitro* TMC278 inhibitory effect on cell proliferation and viability assessed using resazurin. Inhibition of proliferation and anti-viral activity of TMC278 assessed on MT-4-EGFP cells	In vitro	Tibotec, Belgium	TMC278-IV1-AVMR and TMC278-IV2-AVMR
In vitro drug resistance: determination of the biological cut-off for TMC278	Phenotypic susceptibility to a given drug was determined by interpreting fold-change values in EC_{50} with current biological cut-offs (BCO), reported in the Antivirogram (version 2.5.00).	In vitro	Tibotec-Virco, Belgium	TMC278-IV1-AVMR

2.6.3.1 Pharmacology

Overview

Test Article: Rilpivirine

Type of Study	Test System	Method of Administration	Testing Facility	Study Number & [Reference]
In vitro drug resistance: antiviral activity of TMC278 against a large panel of NNRTI-resistant recombinant HIV-1 clinical isolates	Phenotypic assays (Antivirogram®)	In vitro	Tibotec-Virco, Belgium	TMC278-IV1-AVMR
In vitro drug resistance: *in vitro* selection from WT and mutant HIV-1 at high MOI	Sequential passage of WT and mutant HIV-1 at a high MOI in the presence of fixed drug concentrations (2, 10, 40, 200, and 1000 nM) Phenotypic (Antivirogram®) and genotypic (virco®TYPE HIV-1) assays	In vitro	Tibotec, Belgium	TMC278-IV1-AVMR PC-264-2003
In vitro drug resistance: *in vitro* selection from WT and mutant HIV-1 at high MOI	Sequential passage of WT and mutant HIV-1 at a high MOI in the presence of fixed drug concentrations (2, 10, 40, 200, and 1000 nM) Phenotypic (Antivirogram®) and genotypic (virco®TYPE HIV-1) assays	In vitro	Tibotec, Belgium	TMC278-IV1-AVMR PC-264-2003
In vitro drug resistance: antiviral activity against HIV-1 SDMs carrying mutations in the HIV-1 RT	Phenotypic (Antivirogram®) and genotypic (virco®TYPE HIV-1) assays	In vitro	Tibotec- Virco, Belgium	TMC278-IV1-AVMR and TMC278-IV2-AVMR

Type of Study	Test System	Method of Administration	Testing Facility	Study Number & [Reference]
2.6.3.1.3. Primary Pharmacodynamics of Tenofovir DF				
Mechanism of action (activation of tenofovir via phosphorylation)	Human T lymphoid cells (CEMss, CEMss $^{r-1}$)	In vitro	St Jude Children's Hospital and Univ. of Tennessee, Memphis, TN, USA	*Antimicrob Agents Chemother.*, 1995 Oct;39(10):2304-8 {13}
Antiviral activity of vs wild-type HIV $_{IIIB.}$ & evaluation of cytotoxicity	Human resting/activated peripheral blood mononuclear cells (PBMC) & T lymphocyte (MT-2) cells	In vitro	St Jude Children's Hospital and Univ. of Tennessee, Memphis, TN, USA	*Antimicrob Agents Chemother.*, 1998, Mar, 42 (3): 612-7. {1574}
Mechanism of action (intracellular metabolism): in vitro phosphorylation of tenofovir and abacavir	CEM-CCRF cells	In vitro	Gilead Sciences, Inc., USA	PC-104-2008
Mechanism of action (intracellular metabolism): role of purine nucleoside phosphorylase in drug interactions between 2',3'-dideoxyinosine and allopurinol, ganciclovir, or tenofovir	CEM-CCRF cells and activated PBMC	In vitro	Gilead Sciences, Inc., USA	*Antimicrob. Agents Chemother.*, 2004; 48 (4):1089-95. {6054}
Mechanism of action (intracellular metabolism): effect of tenofovir in combination with other anti-HIV nucleoside reverse transcriptase inhibitors (NRTIs) on intracellular nucleotide pools	CEM-CRFF cells	In vitro	Gilead Sciences, Inc., USA	45th Interscience Conference on Antimicrobial Agents and Chemotherapy Poster H-1901 {8573}

2.6.3.1 Pharmacology

Overview

Test Article: Tenofovir DF, Tenofovir

Type of Study	Test System	Method of Administration	Testing Facility	Study Number & [Reference]
Mechanism of action (intracellular metabolism in vitro following in vivo dosing)	PBMC	In vitro	[redacted], USA	P2001025
	Macaque monkeys	In vivo, subcutaneous (SC)		
Inhibitory effects of tenofovir & TDF on human HIV $_{IIIB}$ RT & human DNA polymerases	HIV $_{IIIB}$ RT, human DNA polymerase (α, β, γ)	In vitro	Gilead Sciences, Inc., USA	*Antiviral Chemistry and Chemotherapy* 1995; 6 (4):217-21. {1131}
Structural features of acyclic nucleotide analogs conferring inhibitory effects on cellular replicative DNA polymerases	In vitro	In vitro	Gilead Sciences Inc., USA	*Institute of Organic Chemistry and Biochemistry, Academy of Sciences of Czech Republic* 1996; 1:188-91 {2516}
Incorporation into DNA by DNA polymerase	Primer template DNA, DNA polymerases (α, β, γ)	In vitro	Gilead Sciences, Inc., USA	*Antiviral Chemistry and Chemother.,* 1997 May;8 (3):187-95. {2005}
Antiviral activity vs. HIV-1	Human primary macrophage/ monocyte cells	In vitro	Catholic University, Leuven, Belgium	*Biochem. Biophys. Res. Commun.,* 1996;219:337-341 {625}
Antiviral activity vs. HIV-1	293T cells	In vitro	Monogram Biosciences, Inc., South San Francisco, CA	*Antimicrob. Agents Chemother.* 2004; 48 (2):437-43 {7289}

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Test Article: Tenofovir DF, Tenofovir

Type of Study	Test System	Method of Administration	Testing Facility	Study Number & [Reference]
Antiviral activity vs. HIV-1 & HIV-2	JC53BL cells	In vitro	National Cancer Institute, Frederick, MD and Gilead Sciences, Inc., USA	PC-104-2003
Antiviral activity vs. HIV-1 & HIV-2	MT-2 cells	In vitro	Gilead Sciences, Inc., USA	PC-104-2013
Antiviral activity vs. HIV-1 & HIV-2 *in vitro* & *in vivo* activity vs. retroviruses	Murine C3H/3TC cells, human MT-4/CEM cells	In vitro	Catholic University, Leuven, Belgium, & Czech Academy of Sciences, Prague.	*Antimicrob. Agents Chemother.*, 1993 Feb;37 (2):332-8. {39}
	MSV tumour formation in NMRI mice	In vivo; SC		
Antiviral activity of tenofovir vs. HIV-1 non-B subtypes (A, C, D, E, F, G, O) & HIV-1 isolates with nucleoside-associated resistance	PBMC	In vitro	Gilead Sciences, Inc., USA & Southern Research Institute, Frederick, MD, USA	*AIDS Res. Hum. Retroviruses;* 2001; 17: 1167-73 {5044}
Effect of increasing multiplicity of infection on the EC_{50} of GS-9131, GS-9148, and tenofovir	MT-2	In vitro	Gilead Sciences, Inc., USA	PC-180-2018
Anti-HIV activity: the long intracellular half-life of tenofovir diphosphate correlates with persistent inhibition of HIV-1 replication in vitro	PBMC	In vitro	Gilead Sciences, Inc., USA	19th International Conference on Antiviral Research, 2005, San Juan, Puerto Rico. Poster 60 {9497}

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Test Article: Tenofovir DF, Tenofovir

Type of Study	Test System	Method of Administration	Testing Facility	Study Number & [Reference]
Antiviral activity vs. animal retroviruses	Moloney Murine Sarcoma Virus (MSV)-Infected Severe Combined Immunodeficient (SCID) mice	SC or oral	St Jude Children's Hospital and Univ. of Tennessee, Memphis, TN, USA, Catholic University, Leuven, Belgium	4th Conference on Retroviruses and Opportunistic Infections; 1997, Washington DC, Abstract 214 {1133}
Antiviral activity & resistance properties vs wild type HIV_{IIIB}	MT-2 cells	In vitro	McGill University AIDS Center, Montreal, Canada	*Antiviral Therapy*, 1999;4 (2):87-94 {2078}
Selection of resistant virus with subtype C HIV-1	Cord blood mononuclear cells	In vitro	McGill University AIDS Center, Montreal, Canada	*AIDS*, 2006; 20 (9):F9-F13 {9276}
Comparison of activity of TDF vs. tenofovir in drug susceptibility assays	293T cells	In vitro	[redacted]	PC-104-2017
Antiviral activity vs. HIV-1 expressing the K70E mutation	Clinical isolates from AIDS patients treated with adefovir dipivoxil long-term therapy	In vitro	Gilead Sciences, Inc., USA	*Antimicrob. Agents Chemother.*, 1998 Jul;42 (7):1620-8. {1648}
Molecular mechanism by which the K70E mutation in HIV-1 reverse transcriptase confers resistance to NRTIs	In vitro	In vitro	University of Pittsburgh, School of Medicine, Pittsburgh, PA, USA	*Antimicrob. Agents Chemother.*, 2007; 51:48-53 {10898}

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Test Article: Tenofovir DF, Tenofovir

Type of Study	Test System	Method of Administration	Testing Facility	Study Number & [Reference]
HIV-1: resistance to nucleoside analogues and replication capacity in primary human macrophages	Primary human macrophages	In vitro	Hôpital Bichat-Claude Bermard, Paris, France	*J. Virol.,* 2007; 81:4540-4550 {11307}
A combination of decreased NRTI incorporation and decreased excision determines the resistance profile of HIV-1 K65R RT	MT-2 cells, RT enzyme assay	In vitro	Gilead Sciences Inc., USA	*AIDS,* 2005; 19:1751-1760 {7583}
Effects of the translocation status of HIV-1 reverse transcriptase on the efficiency of excision of tenofovir	RT enzyme assay	In vitro	McGill University, Montreal, Quebec, Canada and Gilead Sciences Inc., USA	*Antimicrob. Agents Chemother.,* 2007; 51:2911-2919 {11304}
The K65R reverse transcriptase mutation in HIV-1 reverses the excision phenotype of AZT resistance mutations	MT-2 cells, RT enzyme assay	In vitro	Gilead Sciences Inc., USA	*Antiviral Ther.,* 2006; 11:155-163 {8925}
Antagonism between the HIV-1 reverse transcriptase mutation K65R and thymidine analogue mutations at the genomic level	In vitro	In vitro	University of Pittsburgh, School of Medicine, Pittsburgh, PA, USA	*J. Infec. Dis.*, 2006, 194:651-660 {9494}
Molecular mechanisms of bidirectional antagonism between K65R and thymidine analogue mutations in HIV-1 reverse transcriptase	In vitro	In vitro	University of Pittsburgh, School of Medicine, Pittsburgh, PA, USA	*AIDS,* 2007; 21:1405-1414 {11306}

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Test Article: Tenofovir DF, Tenofovir

Type of Study	Test System	Method of Administration	Testing Facility	Study Number & [Reference]
The K65R mutation in HIV-1 reverse transcriptase exhibits bidirectional phenotypic antagonism with thymidine analogue mutations	In vitro	In vitro	University of Pittsburgh, School of Medicine, Pittsburgh, PA, USA	*J. Virol.,* 2006, 80:4971-4977 {9037}
Coexistence of the K65R/L74V and/or K65R/T215Y mutations on the same HIV-1 genome	In vitro	In vitro	Timone Hospital and CNRS UMR 6020, France	*J. Clin. Virol.,* 2006; 37:227-230 {10318}
The A62V and S68G mutations in HIV-1 reverse transcriptase partially restore the replication defect associated with the K65R mutation	MT-2 cells, RT enzyme assay	In vitro	Gilead Sciences Inc., USA	*J. Acquir. Immune. Defic. Syndr.,* 2008; 48:428-436 {12688}
Diminished efficiency of HIV-1 reverse transcriptase containing the K65R and M184V drug resistance mutations	In vitro	In vitro	McGill AIDS Centre, Montreal, Quebec, Canada	*AIDS,* 2007; 21:665-675 {10671}
The balance between NRTI discrimination and excision drives the susceptibility of HIV-1 RT mutant K65R, M184V, and K65R+M184V	MT-2 cells, RT enzyme assay	In vitro	Gilead Sciences Inc., USA	*Antivir. Chem. Chemother.,* 2008; 18:307-316 {12142}

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Test Article: Tenofovir DF, Tenofovir

Type of Study	Test System	Method of Administration	Testing Facility	Study Number & [Reference]
Molecular basis of antagonism between K70E and K65R tenofovir-associated mutations in HIV-1 reverse transcriptase	In vitro	In vitro	Quest Diagnostics Nichols Institute, USA, Glaxo SmithKline USA, University of Minnesota, MN, USA, and Research Think Tank Inc., USA	*Antivir. Res.,* 2007; 75:210-218 {11303}
Mechanistic basis for reduced viral and enzymatic fitness of HIV-1 reverse transcriptase containing both K65R and M184V mutations	In vitro	In vitro	Université Aix-Marseille, France and Gilead Sciences Inc., USA	*J. Biol, Chem.,* 2004; 279:509-516 {5476}
Antiviral activity vs. nucleoside reverse transcriptase inhibitor-resistant recombinant HIV-1 clinical isolates	MT-4 cells	In vitro	Virco NV, Mechelen, Belgium	P4331-00035
Antiviral activity vs. drug resistant HIV variants	Clinical isolates of drug resistant HIV-1 variants, MT-2 cells	In vitro	St Jude Children's Hospital and Univ. of Tennessee, Memphis, TN, USA	*Antimicrob. Agents. Chemother.,* 1998;42:1484-7. {2191}
Patterns of HIV resistance of clinical isolates from study GS-96-408	MT-2 cells	In vitro	Gilead Sciences, Inc., USA	*JAIDS,* 2001; 27:450-8 {3200}

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Type of Study	Test System	Method of Administration	Testing Facility	Study Number & [Reference]
Antiviral activity vs. HIV-1 variants from clinical isolates	MOLT-4 cells, PBMCS & H9 cells	In vitro	National Cancer Institute, Bethesda, MD, USA	*Proc. Natl. Acad. Sci USA.*1995;92: 2398-2402 {2064}
Activity against nucleoside resistant HIV-2	PBMC	In vitro	Hopital Bichat-Claude Bernard, Paris, France	*Antiviral Therapy*,2005;10:861-5 {8389}
Unblocking of chain-terminated primer by HIV-1 reverse transcriptase	In vitro	In vitro	University of Miami, Miami, FL	*Proc. Natl. Acad. Sci.*,1998 Nov; 95 (23): 13471-6. {2043}
Unblocking of chain-terminated primer by HIV-1 reverse transcriptase	In vitro	In vitro	Gilead Sciences, Inc., USA	XIV International Roundtable on Nucleosides, Nucleotides and their Biological Applications; 2000; San Francisco, CA. {2252}
Antiviral activity & replication capability vs. HIV-1 expressing the 3TC-associated M184V mutation	MT-2 cells & PBMC	In vitro	Gilead Sciences, Inc., USA	*J. Infect. Dis.,* 1999 Jan;179 (1):92-100. {1649}
Effects of K65R mutation on HIV-1 replication capacity	293T cells	In vitro	[redacted]	PC-104-2004
Antiviral activity vs. clinical HIV samples expressing M184V, T215Y, Q151M, T69S, K65R insertions/resistant mutations	Clinical HIV isolates from HIV patients expressing mutations	In vitro	Gilead Sciences, Inc., USA	4th International Workshop on HIV Drug Resistance and Treatment Strategies; 2000; Sitges, Spain {2152}

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Test Article: Tenofovir DF, Tenofovir

Type of Study	Test System	Method of Administration	Testing Facility	Study Number & [Reference]
Antiviral activity vs. wild-type and drug-resistant HIV-1	MT-4 cells	In vitro	Virco NV, Mechelen, Belgium and Gilead Sciences, Inc., USA	*Antimicrob. Agents Chemother.*, 2002 Apr;46 (4):1067-72. {3961}
Antiviral activity vs. animal retroviruses	Moloney Murine Sarcoma Virus (MSV)-Infected Severe Combined Immunodeficient (SCID) mice	SC or oral	Rega Institute for Medical Research and Catholic University, Leuven, Belgium	*Antimicrob Agents Chemother.*, 1998, 42: 1568-73. {2477}
Use of topical tenofovir to prevent vaginal transmission of simian immune deficiency virus (SIV)	SIV-infected monkeys	Intravaginal	California Regional Primate Research Center, Davis, CA, USA	9th International Conference on Antiviral Research, 1996; Urabandai, Fukushima, Japan {670}
Antiviral activity vs. animal retroviruses	Feline Immunodeficiency Virus (FIV)-Infected cats	SC or oral	Colorado State University, Denver, CO, USA, & Gilead Sciences, Inc., USA	11th International Conference on Antiviral Research; 1998, San Diego, CA {1576}
Effects of TFV monotherapy on chronic SIV infection in macaques	SIV-infected macaques monkeys	SC	Washington Regional Primate Research Center, Seattle, WA, USA	*AIDS Research and Human Retroviruses*, 1997; 13:707-712. {1367}
Antiviral activity vs. animal retroviruses	SIV-infected macaques monkeys	SC	Washington Regional Primate Research Center, Seattle, WA, USA	*Science*, 1995, Nov 17; 270 (5329): 1197-9. {17}

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Test Article: Tenofovir DF, Tenofovir

Type of Study	Test System	Method of Administration	Testing Facility	Study Number & [Reference]
Antiviral activity vs. animal retroviruses	SIV-infected macaques monkeys	SC	Washington Regional Primate Research Center, Seattle, WA, USA	*AIDS Res. Hum. Retroviruses,* 1997, May 20; 13 (8): 707-12. {1367}
Antiviral activity vs. animal retroviruses	SIV-infected macaques monkeys	SC	Washington Regional Primate Research Center, Seattle, WA, USA	*J. Virol.,* 1998 May;72 (5):4265-73. {1746}
Antiviral activity vs. animal retroviruses	Macaque monkeys post exposure to SIV, orally	SC	California Regional Primate Research Center, Davis, CA, USA	*J. Virol.,* 1999 Apr;73 (4):2947-55. {2144}
Antiviral activity vs. animal retroviruses	Neonatal macaque monkeys post exposure to oral administration of SIV	SC	California Regional Primate Research Center, Davis, CA, USA	*AIDS*, 1998 Jun 18;12 (9):F79-F83. {1800}
Antiviral activity vs. animal retroviruses	Neonatal macaque monkeys post exposure to oral or intravenous administration of SIV	SC	California Regional Primate Research Center, Davis, CA, USA	*AIDS Res. Hum. Retroviruses*, 1998 Jun 10;14 (9):761-73. {1802}
Antiviral activity vs. animal retroviruses	SIV-infected neonatal macaques monkeys	SC	California Regional Primate Research Center, Davis, CA, USA	*Antimicrob Agents Chemother.,* 1996, Nov 40 (11) : 2586-91 {35}

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Test Article: Tenofovir DF, Tenofovir

Type of Study	Test System	Method of Administration	Testing Facility	Study Number & [Reference]
Antiviral activity vs. animal retroviruses	SIV-infected infant macaques monkeys, infected with strains of SIV resistant to tenofovir (SIV_{mac} 385 & SIV_{mac} 055)	SC	California Regional Primate Research Center, Davis, CA, USA	*Antimicrob. Agents Chemother.*, 1999 Apr;43 (4):802-12. {2145}
Antiviral activity vs. animal retroviruses	SIV-infected infant macaques monkeys, infected with strains of SIV resistant to tenofovir (SIV_{mac} 055)	SC	California Regional Primate Research Center, Davis, CA, USA	*J. Virol.*, 2000 Feb;74 (4):1767-74. {2146}
Antiviral activity vs. animal retroviruses: topical (oral) administration of low-dose TDF to protect infant macaques against multiple oral exposures of low doses of SIV	SIV-challenged infant macaque monkeys	Oral	California National Primate Research Center, Davis, CA, USA	*J. Infect. Dis.*, 2002; 186:1508-1513 {3873}
Antiviral activity vs. animal retroviruses: evaluation of oral TDF and topical (oral) tenofovir prodrug (GS-7340) to protect infant macaques against repeated oral challenges with virulent SIV	SIV-challenged infant macaque monkeys	Oral	California National Primate Research Center, Davis, CA, USA	*J. Acquir. Immune Defic. Syndr.*, 2006; 43:6-14 {9457}
Antiviral activity vs. animal retroviruses: the clinical benefits of tenofovir for SIV-infected macaques are larger than predicted by its effects on standard viral and immunologic parameters	SIV-infected infant macaque monkeys	SC	California National Primate Research Center, Davis, CA, USA	*J. Acquir. Immune Defic. Syndr.*, 2004; 36:900-914 {7288}

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Test Article: Tenofovir DF, Tenofovir

Type of Study	Test System	Method of Administration	Testing Facility	Study Number & [Reference]
Administration of TFV to gravid and infant rhesus macaques: safety and efficacy studies	SIV-infected gravid and infant macaque monkeys	SC	California National Primate Research Center, Davis, CA, USA	*J. Acquir. Immune Defic. Syndr.*, 1999; 20:323-333 {1878}
Antiviral activity vs. animal retroviruses: chemoprophylaxis with TDF provided partial protection against infection with SIV in macaques given multiple virus challenges	SIV-challenged macaque monkeys	Oral	National Center for HIV, STD, & TB Prevention, Atlanta, GA, USA	*J. Infect. Dis.*, 2006; 194:904-911 {9496}
Effectiveness of postinoculation TFV treatment for prevention of persistent simian immunodeficiency virus SIV_{mne} infection depends critically on timing of initiation and duration of treatment	SIV-challenged macaque monkeys	SC	Regional Primate Research Center, Seattle, WA, USA	*J. of Virology*, 1998; 72:4265-4273 {1796}
Antiviral activity vs. animal retroviruses: sequential emergence and clinical implications of viral mutants with K70E and K65R mutation in reverse transcriptase during prolonged tenofovir monotherapy in rhesus macaques with chronic RT-SHIV infection	Simian Immune Deficiency Virus/HIV chimera (SHIV)-infected macaque monkeys	SC	California National Primate Research Center, Davis, CA, USA	*Retrovirology*, 2007; 4:25 {12759}
Protein binding of cidofovir, cyclic HPMPC, PMEA and PMPA in human plasma and serum	In vitro	In vitro	Gilead Sciences, Inc., USA	P0504-00039.1

Type of Study	Test System	Method of Administration	Testing Facility	Study Number & [Reference]
2.6.3.1.4. Primary Pharmacodynamics of Emtricitabine/Tenofovir DF				
Phosphorylation of tenofovir and FTC	CEM CRFF cells	In vitro	Gilead Sciences, Inc., USA	PC-164-2001
Selection of resistant HIV-1 with combinations of tenofovir and FTC	MT-2 cells	In vitro	Gilead Sciences, Inc., USA	PC-164-2005
Antiviral activity of the combination of tenofovir and FTC	SIV-infected rhesus monkeys	SC	John Hopkins University, Baltimore, MA, USA	*Journal of Virology* 2003; 77:4938-4949 {5477}
Intermittent antiretroviral prophylaxis with tenofovir and FTC protects macaques against repeated rectal SHIV exposures	SHIV-challenged rhesus monkeys	SC	Centers for Disease Control and Prevention, Atlanta, GA, USA; Emory University, Decatur, GA, USA	16th International HIV Resistance Workshop, 2007, Barbados, Abstract 85 {11074}

FTC = emtricitabine; TDF = tenofovir disoproxil fumarate, tenofovir DF

Type of Study	Test System	Method of Administration	Testing Facility	Study Number & [Reference]
2.6.3.1.5. Primary Pharmacodynamics of Emtricitabine/Rilpivirine/Tenofovir DF				
HIV-1 triple drug resistance selection by FTC, RPV, and TFV	MT-2 cells	In vitro	Gilead Sciences, Inc., USA	PC-264-2003

FTC = emtricitabine

Type of Study	Test System	Method of Administration	Testing Facility	Study Number & [Reference]
2.6.3.1.6. Secondary Pharmacodynamics of Emtricitabine				
Antiviral activity vs. human hepatitis B virus (HBV), Cytotoxicity	Human Hepatoma cell line HepG2.2.15	In vitro	Emory University, Atlanta, GA, USA	*Antimicrob Agents Chemother*. 1994; 38:2172-2174 {4533}
Antiviral activity vs. human hepatitis B virus (HBV)	Human Hepatoma cell line HepG2.2.15	In vitro	Yale University, New Haven, CT, USA	{6287}
Antiviral activity vs. human hepatitis B virus (HBV)	Human Hepatoma cell line HepG2.2.15	In vitro	Victorian Infectious Diseases Reference Laboratory, Fairfield Hospital, Victoria, Australia	{6288}
Antiviral activity vs. human hepatitis B virus (HBV)	Primary Human heptocytes	In vitro	Burroughs Wellcome Co., Research Triangle Park, NC 27709 USA	{4532}
Antiviral activity vs. human HBV, Cytotoxicity	Human Hepatoma cell line HepG2.2.15	In vitro	Burroughs Wellcome Co., Research Triangle Park, NC 27709 USA	*Antimicrob Agents Chemother*. 1992; 36:2686-2692 {4535}
Effect on HBV replication *in vivo*	Mouse	Oral	Burroughs Wellcome Co., Research Triangle Park, NC 27709 USA	*Antimicrob. Agents Chemother*. 1994; 38:616-619 {4530}

FTC = emtricitabine

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Test Article: Emtricitabine

Type of Study	Test System	Method of Administration	Testing Facility	Study Number & [Reference]
Effect on HBV replication *in vivo*	In vitro	In vitro	Glaxo Wellcome Research Triangle Park, NC, USA	{4539}
Effect on HBV replication *in vivo*	SCID mouse	IP	Thomas Jefferson University, PA, USA	*Frontiers in Viral Hepatitis* 2002. Elsevier science, Printed in the Netherlands. {6290}
Effect on HBV replication *in vivo*	Woodchuck	Oral	Georgetown University, MA, USA	*Antimicrob. Agents Chemother.* 2000; 44:1757-1760 {6290}
Effect on HBV replication *in vivo*	Woodchuck	IP	Glaxo Wellcome Research Triangle Park, NC, USA	*Antimicrob. Agents Chemother.* 1997; 41:2076-2082 {6293}
Cytotoxicity assay	Molt-4 cells	In vitro	Department of Pharmacology, School of Medicine, University of North Carolina, Chapel Hill	*J Biol Chem* 264:11934-7 {4249}
Effect of FTC on mitochondrial DNA	Molt-4 cell culture assay	In vitro	Burroughs Wellcome Co., Research Triangle Park, NC 27709 USA	TGZZ/93/0016
Effect of FTC on mitochondrial DNA	Molt-4 cell culture assay	In vitro	Burroughs Wellcome Co., Research Triangle Park, NC 27709 USA	TGZZ/93/0023

FTC = emtricitabine

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Test Article: Emtricitabine

Type of Study	Test System	Method of Administration	Testing Facility	Study Number & [Reference]
Mitochondrial toxicity	Human hepatoma cell line HepG2	In vitro	University of Alabama, USA	*Biochem. Pharmacol.* 1996; 52:1577-1584 {4550}
An in vitro evaluation of the effects on cell growth and mitochondrial functions in the MT2 cell line after long term exposure to antiviral xenobiotics	Human T cell line, MT-2 cells	In vitro	Gilead Sciences, Inc., USA	TPI 11963
Data from clonogenic assays CFU-GM and BFU-E and mitochondrial assays for TP0001 and TP0004 as compared to AZT	Human bone marrow progenitor cells	In vitro	Gilead Sciences, Inc. USA	2336081
In vitro receptor binding potencies of FTC (524W91)	In vitro binding assay	In vitro	Burroughs Wellcome Co., Research Triangle Park, NC 27709 USA	TPZZ/93/0002
In vitro autonomic pharmacology		In vitro	Burroughs Wellcome Co., Research Triangle Park, NC 27709 USA	TPZZ/92/0055
Cholinergic (muscarinic) activity	Guinea pig ileum			
Alpha-adrenoceptor activity	Rabbit aortic strips			
Beta-adrenoceptor activity	Guinea pig trachea and atria			
Serotonin receptor activity	Rat fundus strips			

FTC = emtricitabine

Type of Study	Test System	Method of Administration	Testing Facility	Study Number & [Reference]
2.6.3.1.7. Secondary Pharmacodynamics of Rilpivirine				
Antiviral activity vs. human HBV, HSV 2, human corona virus, influenza virus A and vaccinia virus	Cell culture virus replication assays	In vitro	Tibotec, Belgium	TMC278-IV2-AVMR
Cytotoxicity	HeLa, HepG2, MRC-5 and A549 cells	In vitro	Tibotec, Belgium	TMC278-IV2-AVMR
Receptor binding	Cells & enzymes	In vitro	[redacted], France	TMC278-870219
Receptor binding	Guinea pig right atrium	In vitro	[redacted], Taiwan	TMC278-NC204(2)
Receptor binding	Pig stomach	In vitro	[redacted], Taiwan	TMC278-NC204(3)
Gastric acidity	Rat/Wistar	Intraperitoneal injection.	[redacted], Taiwan	TMC278-NC204(1)

Type of Study	Test System	Method of Administration	Testing Facility	Study Number & [Reference]
2.6.3.1.8. Secondary Pharmacodynamics of Tenofovir DF				
Antiviral activity vs. human HBV	Human hepatoblastoma cell line (HB611)	In vitro	Rational Drug Design Laboratories, Fukushima, Japan	*Antiviral Chemistry and Chemother.*, 1994;5 (2):57-63. {21}
Antiviral activity vs. human HBV & duck HBV	Human hepatoma cell line HepG2, primary duck hepatocytes	In vitro	Erasmus University Rotterdam, Holland, Catholic University, Leuven Belgium	*Antimicrob. Agents Chemother.*, 1994 Sep;38 (9):2180-2. {10}
Antiviral activity vs. 3TC-resistant human HBV	HepG2 cells	In vitro	Gilead Sciences, Inc., USA	*Antiviral Therapy.*, 2005; 10:625-33. {8381}
Antiviral activity vs. wild type & 3TC-resistant human HBV	HepG2, HepAD38 & HepAD79 cells	In vitro	Gilead Sciences, Inc., USA	P4331-00038
Antiviral activity against HBV with the rtA194T mutation	HepG2 cells	In vitro	Gilead Sciences, Inc., USA	PC-104-2012
Antiviral activity against HBV with entecavir-associated resistance mutations	HepG2 cells	In vitro	[redacted], Australia	PC-174-2003
Antiviral activity against Woodchuck Hepatitis Virus	Woodchucks	Oral	College of Veterinary Medicine, Cornell Univ., Ithaca, NY, USA	*Antimicrob. Agents Chemother.* 2005; 49(7) :2720-8 {8176}

TDF = tenofovir disproxil fumarate, tenofovir DF; HBV = hepatitis B virus

2.6.3.1 Pharmacology

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Test Article: Tenofovir DF, Tenofovir

Type of Study	Test System	Method of Administration	Testing Facility	Study Number & [Reference]
Cytotoxicity	HepG2, human skeletal muscle cells (SKMC), human renal proximal tubule epithelial cells (RPTECS)	In vitro	Gilead Sciences, Inc., USA	P4331-00037
Cytotoxicity	RPTECS	In vitro	Hospital Universitari de Tarragona, Spain	*Antimicrob. Agents Chemother.*, 2006;50 (11):3824-32. {9864}
Mitochondrial toxicity	HepG2, SKMC, RPTECS	In vitro	Gilead Sciences, Inc., USA	P1278-00042
Binding screen to neuroreceptors, ion channels, transporters, nuclear receptors	Protein targets	In vitro	[redacted], USA	V2000020

TDF = tenofovir disproxil fumarate, tenofovir DF; HBV = hepatitis B virus

Type of Study	Test System	Method of Administration	Testing Facility	Study Number & [Reference]
2.6.3.1.9. Secondary Pharmacodynamics of Emtricitabine/Tenofovir DF				
Anti-HBV activity	HepG2 cells	In vitro	Gilead Sciences, Inc., USA	PC-164-2004
Mitochondrial toxicity	HepG2 cells	In vitro	[redacted], Germany	TX-104-2001
Antiviral activity against Woodchuck Hepatitis Virus	Woodchucks	Oral	[redacted], USA	PC-174-2004

FTC = emtricitabine; TDF = tenofovir disproxil fumarate, tenofovir DF

Type of Study	Test System	Method of Administration	Testing Facility	Study Number & [Reference]
2.6.3.1.10. Safety Pharmacology: Emtricitabine In Vitro				
Isolated guinea pig ileum	Guinea Pig, Duncan Hartley	In vitro	[redacted]	477
Isolated Cardiac Muscle		In vitro	Burroughs Wellcome Co., Research Triangle Park, NC 27709 USA	TPZZ/92/0056
Isolated perfused rat heart	Rat heart			
Electrically driven papillary and atrial muscle of the cat	Cat papillary and atrial muscle strips			
Spontaneously-beating guinea pig paired atria	Guinea pig paired atria			

FTC = emtricitabine

Type of Study	Test System	Method of Administration	Testing Facility	Study Number & [Reference]
2.6.3.1.11. Safety Pharmacology: Rilpivirine In Vitro				
Cardiovascular safety (cardiac membrane potassium current)	I_{Kr} in transfected CHO cells expressing hERG	In vitro	[redacted], Germany	TMC278-CPF730
Cardiovascular safety (cardiac membrane potassium current)	I_{Ks} in transfected CHO cells expressing KvLQT1/minK	In vitro	J&J PRD, Belgium	TMC278-NC342
Cardiovascular safety (cardiac membrane potassium current)	Surface expression of hERG channels in HEK293 cells	In vitro	[redacted], USA	TMC278-NC330
Cardiovascular safety (cardiac membrane ion currents)	I_{Ks} in transfected HEK293 cells expressing KvLQT1/minK I_{to} in transfected HEK293 cells expressing $hK_v4.3$ I_{K1} in transfected HEK293 cells expressing $hK_{ir}2.1$ I_{Na} in transfected HEK293 cells expressing $hNa_v1.5$ $I_{Ca,L}$ in transfected HEK293 cells expressing $hCa_v1.2$	In vitro	[redacted], USA	TMC278-NC331
Cardiovascular safety (cardiac action potential)	Guinea pig isolated atrium	In vitro	Janssen Research Foundation, Belgium	TMC278-N168576
Cardiovascular safety (cardiac action potential)	Arterially perfused rabbit ventricular wedge	In vitro	J&J PRD, Belgium	TMC278-NC341

RPV = rilpivirine, TMC278; hERG: human ether-à-go-go related gene, CHO: Chinese hamster ovary, HEK293 = human embryonic kidney cell line, $I_{Ca,L}$ = high threshold calcium current, I_{Ki} = inward rectifying potassium current, I_{Kr} = rapidly activating rectifying potassium current; I_{Ks} = Slowly activating rectifying potassium current, I_{Na} = fast sodium current, I_{to} = transient outward potassium current

Type of Study	Test System	Method of Administration	Testing Facility	Study Number & [Reference]
2.6.3.1.12. Safety Pharmacology: Tenofovir DF In Vitro				
Inhibition of agonist-induced contractile responses	Guinea pig ileum	In vitro	[redacted], USA	V2000009

2.6.3.1.13. Safety Pharmacology: Emtricitabine In Vivo

Type of Study	Test System	Method of Administration	Testing Facility	Study Number & [Reference]
General High Dose Pharmacology Testing Results for FTC			[redacted] Taiwan	477
Modified Irwin Screen	Mouse, ICR	Oral		
CNS – Spontaneous locomotor activity	Mouse, ICR	Oral		
CNS – Motor incoordination (roto-rod test)	Mouse, ICR	Oral		
CNS – Hexobarbital potentiation (sleeping time)	Mouse, ICR	Oral		
CNS – Anticonvulsant activity (maximal electroshock)	Mouse, ICR	Oral		
CNS – Anticonvulsant activity (metrazole)	Mouse, ICR	Oral		
CNS – Preconvulsant activity (electroshock)	Mouse, ICR	Oral		
CNS – Preconvulsant activity (metrazole)	Mouse, ICR	Oral		
CNS –Analgesic activity (tail flick)	Mouse, ICR	Oral		
CNS – Analgesic activity (phenylquinone writhing)	Mouse, ICR	Oral		
CNS – Body temperature	Rat, Wistar	Oral		
Cardiovascular Function	Rat Wistar	Oral		
Renal Function	Rat, Long Evans	Oral		
Gastrointestinal Motility	Mouse, ICR	Oral		

2.6.3.1 Pharmacology

Overview

Test Article: Emtricitabine

Type of Study	Test System	Method of Administration	Testing Facility	Study Number & [Reference]
Overt Pharmacological Effects	Mouse, CD-1 Rat, CD(SD)	Oral gavage	Burroughs Wellcome Co., Research Triangle Park, NC 27709 USA	TPZZ/93/0001
Conditioned Avoidance Response	Rat, Long Evans	IP	Burroughs Wellcome Co., Research Triangle Park, NC 27709 USA	TPZZ/93/0119
Systolic Blood Pressure and Heart Rate	Conscious Normotensive Rat, CD (SD)	Oral	Burroughs Wellcome Co., Research Triangle Park, NC 27709 USA	TPZZ/92/0057
Cardiovascular, Respiratory and Autonomic Function			Burroughs Wellcome Co., Research Triangle Park, NC 27709 USA	TPZZ/92/0076
Cardiovascular and respiratory effects in spontaneously breathing dogs anesthetized with allobarbital-urethane	Anesthetized Dogs, Beagle	IV		
Effects on the changes in mean arterial blood pressure induced by norepinephrine, carotid occlusion, acetylcholine and vagal nerve stimulation in the anesthetized dog	Anesthetized Dogs, Beagle	IV		

Type of Study	Test System	Method of Administration	Testing Facility	Study Number & [Reference]
2.6.3.1.14. Safety Pharmacology: Rilpivirine In Vivo				
Cardiovascular safety (ECG and cardio-hemodynamic parameters)	Anesthetized Guinea pig/Dunkin Hartley	Intravenous/ bolus	J&J PRD, Belgium	TMC278-CPF643
Cardiovascular safety (ECG, cardio-hemodynamic and respiratory parameters)	Telemetered Guinea pig/Dunkin Hartley	Oral/gavage	J&J PRD, Belgium	TMC278-NC327
Cardiovascular safety (ECG, cardio-hemodynamic and respiratory parameters)	Anesthetized dog/beagle	Intravenous/ infusion	J&J PRD, Belgium	TMC278-CPF648
Cardiovascular safety (ECG and cardio-hemodynamic parameters)	Conscious instrumented dog/beagle	Oral/gavage	J&J PRD, Belgium	TMC278-CPF654
Cardiovascular and respiratory safety (ECG, cardio-hemodynamic and respiratory parameters) [a]	Conscious telemetered dog/beagle	Oral/gavage	[redacted], Switzerland	TMC278-Exp5555
Neurobehavior and motor activity (modified Irwin test) [a]	Rat/Sprague Dawley	Oral/gavage	J&J PRD, Belgium	TMC278-Exp5560

a GLP compliant studies; ECG: electrocardiogram

Type of Study	Test System	Method of Administration	Testing Facility	Study Number & [Reference]
2.6.3.1.15. Safety Pharmacology: Tenofovir DF In Vivo				
Pharmacological effects on the central nervous system	Rat, Sprague Dawley	Oral gavage	[redacted], Canada	R990152
Pharmacological effects on gastrointestinal motility	Rat, Sprague Dawley	Oral gavage	[redacted], Canada	R990153
Pharmacological effects on renal system	Rat, Sprague Dawley	Oral gavage	[redacted], Canada	R990154
Pharmacological effects on cardiovascular system	Dog, Beagle	Oral gavage	[redacted], Canada	D990155

2.6.3.1.16. Pharmacodynamic Drug Interactions of Emtricitabine

Type of Study	Test System	Method of Administration	Testing Facility	Study Number & [Reference]
Antiviral activity in combination with other antiretroviral drugs	MT 2 cells	In vitro	Gilead Sciences, Inc., USA	470
In vitro synergy studies with FTC and other anti-HIV compounds.	MT 2 cells	In vitro	Gilead Sciences, Inc., USA	10804
Antiviral activity in combination with other antiretroviral drugs	PBMC	In vitro	Gilead Sciences, Inc., USA	12207

2.6.3.1.17. Pharmacodynamic Drug Interactions of Rilpivirine

Type of Study	Test System	Method of Administration	Testing Facility	Study Number & [Reference]
Combination studies: antiviral activity in combination with current HIV-1 inhibitors	HIV-1_{IIIB} -infected M-T4 cells or CEM-SS and MAGI-CCR5 cells infected with the HIV-1_{Ba-L}.	In vitro	Tibotec, Belgium	TMC278-IV1-AVMR

Type of Study	Test System	Method of Administration	Testing Facility	Study Number & [Reference]
2.6.3.1.18. Pharmacodynamic Drug Interactions of Tenofovir DF				
Antiviral activity in combination with other antiretroviral nucleoside analogues	MT-2 cells	In vitro	Gilead Sciences, Inc., USA	*Antiviral Res.,* 1997 Nov; 36(2):91-7 {1469}
Antiviral activity combination with other antiretroviral drugs	MT-2 cells	In vitro	Gilead Sciences, Inc., USA	C1278-00005
Antiviral activity in combination with other antiretroviral drugs	PBMC	In vitro	Gilead Sciences, Inc., USA	PC-104-2005
Antiviral activity in combination with other antiretroviral drugs	PBMC	In vitro	Gilead Sciences, Inc., USA	PC-104-2006
Antiviral activity in combination with other antiretroviral drugs	MT-2 cells	In vitro	Gilead Sciences, Inc., USA	PC-104-2007
Antiviral activity in combination with other antiretroviral drugs	MT-2 cells	In vitro	Gilead Sciences, Inc., USA	PC-183-2004

2.6.3.1.19. Pharmacodynamic Drug Interactions of Emtricitabine/Tenofovir DF

Type of Study	Test System	Method of Administration	Testing Facility	Study Number & [Reference]
In vitro synergy between tenofovir & FTC	MT-2 cells	In vitro	Gilead Sciences, Inc., USA	14379 {4559}
Anti-HBV activity in combination with other drugs	AD38 cells	In vitro	Gilead Sciences, Inc., USA	PC-174-2006
Enzymatic combination study of tenofovir diphosphate, emtricitabine triphosphate, and efavirenz as inhibitors of HIV-1 reverse transcriptase	MT-2 cells and HIV-1 RT enzymes	In vitro	Gilead Sciences, Inc., USA	PC-177-2001
Antiviral activity in combination with other antiretroviral drugs	MT-2 cells	In vitro	Gilead Sciences, Inc., USA	PC-164-2002

Type of Study	Test System	Method of Administration	Testing Facility	Study Number & [Reference]
2.6.3.1.20. Pharmacodynamic Drug Interactions of Emtricitabine/Rilpivirine/Tenofovir DF				
Antiviral HIV-1 dual drug combination study of FTC, RPV, and TFV	MT-2 cells	In vitro	Gilead Sciences, Inc., USA	PC-264-2001
Antiviral HIV-1 triple drug combination study of FTC, RPV, and TFV	MT-2 cells	In vitro	Gilead Sciences, Inc., USA	PC-264-2002

2.6.3.2. Primary Pharmacodynamics

Studies of the primary pharmacodynamics of FTC, RPV, TDF, and the FDC of FTC/TDF and FTC/RPV/TDF are presented in Sections 2.6.3.1.1 to 2.6.3.1.5.

2.6.3.3. Secondary Pharmacodynamics

Studies of the secondary pharmacodynamics of FTC, RPV, TDF, and the FDC of FTC/TDF are presented in Sections 2.6.3.1.6 to 2.6.3.1.9.

Organ Systems Evaluated	Species Strain	Method of Admin.	Doses (mg/kg)	Gender and No. per Group	Noteworthy Findings	GLP Compliance	Study Number
2.6.3.4. Safety Pharmacology							
2.6.3.4.1. In Vitro Studies with Emtricitabine							
Contractile Responses	Isolated Guinea Pig Ileum	In vitro	10, 30, 100 µM	-	FTC did not elicit any significant agonist effect upon guinea pig ilea or alter contractile responses induced by acetylcholine, histamine or $BaCl_2$	No	477
Isolated perfused rat heart	Rat heart	In vitro	10^{-4} M	-	Perfusion of isolated rat hearts at 10^{4} M produced decreases in heart rate of 5 ± 4% that were similar to that of control hearts (6 ± 5% decrease). The incidence of ventricular premature extrasystoles in FTC-treated hearts was no greater than that observed in control hearts. No ventricular tachycardia or fibrillation occurred in hearts treated with FTC or vehicle control.	No	TPZZ/92/0056

FTC = emtricitabine; M = molar

2.6.3.4 Safety Pharmacology

Test Article: Emtricitabine

Organ Systems Evaluated	Species Strain	Method of Admin.	Doses (mg/kg)	Gender and No. per Group	Noteworthy Findings	GLP Compliance	Study Number
Electrically driven papillary and atrial muscle of the cat	Cat papillary and atrial muscle strips	In vitro	10^{-4} M	-	Minor positive inotropism (12 ± 6% increase in developed tension) was observed on papillary muscle preparations (n = 7) after incubation for 30 minutes with FTC. Negligible positive inotropism (2 ± 3% increase in developed tension) occurred on cat atrial preparations (n = 5) following a 30 minute incubation. The maximum following rate at which atrial muscle would follow electrical stimulation was not affected by FTC.	No	TPZZ/92/0056
Spontaneously-beating guinea pig paired atria	Guinea pig paired atria	In vitro	10^{-4} M	-	Fifteen-minute incubation with FTC produced no direct chronotropic or inotropic effects on spontaneously-beating guinea-pig atria.	No	TPZZ/92/0056

FTC = emtricitabine; M = molar

2.6.3.4 Safety Pharmacology

Test Article: Emtricitabine

Organ Systems Evaluated	Species Strain	Method of Admin.	Doses (mg/kg)	Gender and No. per Group	Noteworthy Findings	GLP Compliance	Study Number
2.6.3.4.2. In Vivo Studies with Emtricitabine							
Modified Irwin Screen	Mouse, ICR	Oral	10, 30, 100	10 M	FTC did not cause any noteworthy findings on behavioural, autonomic, or neurological signs in animals at all doses.	No	477
CNS – Spontaneous locomotor activity	Mouse, ICR	Oral	10, 30, 100	8 M	FTC had no significant effect on spontaneous locomoter activity.	No	477
CNS – Motor incoordination (roto-rod test)	Mouse, ICR	Oral	10, 30, 100	10 M	FTC had no significant effect on motor incoordination.	No	477
CNS – Hexobarbital potentiation (sleeping time)	Mouse, ICR	Oral	10, 30, 100	10 M	FTC had no significant prolongation of hexobarbital sleeping time.	No	477
CNS – Anticonvulsant activity (maximal electroshock)	Mouse, ICR	Oral	10, 30, 100	10 M	FTC had no significant effect on the number of animals experiencing clonic/tonic convulsions and mortality.	No	477
CNS – Anticonvulsant activity (metrazole)	Mouse, ICR	Oral	10, 30, 100	10 M	FTC had no significant effect on the number of animals experiencing clonic/tonic convulsions and mortality.	No	477

FTC = emtricitabine; M = male

2.6.3.4 Safety Pharmacology

Test Article: Emtricitabine

Organ Systems Evaluated	Species Strain	Method of Admin.	Doses (mg/kg)	Gender and No. per Group	Noteworthy Findings	GLP Compliance	Study Number
CNS – Preconvulsant activity (electroshock)	Mouse, ICR	Oral	10, 30, 100	10 M	FTC had no significant effect on the incidence of clonic/tonic convulsions and mortality.	No	477
CNS – Preconvulsant activity (metrazole)	Mouse, ICR	Oral	10, 30, 100	10 M	FTC had no significant effect on the incidence of clonic/tonic convulsions and mortality.	No	477
CNS –Analgesic activity (tail flick)	Mouse, ICR	Oral	10, 30, 100	10 M	FTC caused no significant increase in latency of the tail flick response.	No	477
CNS – Analgesic activity (phenylquinone writhing)	Mouse, ICR	Oral	10, 30, 100	10 M	No significant attenuation of PQ-inducing writhing was observed after all tested doses.	No	477
CNS – Body temperature	Rat, Wistar	Oral	10, 30, 100	10 M	FTC did not cause any significant change in body temperature.	No	477
General Pharmacology	Mouse, CD-1	Oral	100, 250, 500, 750, 1000	4 M	Mice exhibited no visible effects. Minimal respiratory rate effects were only noted at 750 mg/kg (21% decrease) and in the control animals (29% increase). Behavioral reflexes were unaffected at doses up to 1000 mg/kg.	No	TPZZ/93/0001

FTC = emtricitabine; M = male

2.6.3.4 Safety Pharmacology

Test Article: Emtricitabine

Organ Systems Evaluated	Species Strain	Method of Admin.	Doses (mg/kg)	Gender and No. per Group	Noteworthy Findings	GLP Compliance	Study Number
General Pharmacology	Rat, CD (SD)	Oral	250, 500, 1000	4 M	Rats exhibited no visible effects. Minimal respiratory rate effects were only noted at 750 mg/kg (21% decrease) and in the control animals (29% increase). Behavioral reflexes were unaffected at doses up to 1000 mg/kg.	No	TPZZ/93/0001
Conditioned Avoidance Response	Rat, Long Evans	IP	30,100	6 F	Control days – avoided shock on 95 ± 2 trials (mean ± SEM). Vehicle – avoided shock on 96 ± 1 trials. Emtricitabine 30 or 100 mg/kg – avoided shock 97 ± 1 and 94 ± 2 trials, respectively. In neither control, vehicle, nor the drug conditions did the rats fail to escape shock on the trials in which they failed to avoid. After 100 mg/kg, inter-trial crosses declined significantly (to 42 ± 12) but inter-trial crosses also declined significantly after vehicle injection (to 34 ± 8), an injection effect that has been seen before.	No	TPZZ/93/0119
Cardiovascular Function	Rat, Wistar	Oral	5, 10, 50	5 M	No significant effect on mean arterial blood pressure and heart rate was found.	No	477
Systolic Blood Pressure and Heart Rate	Conscious Normotensive Rat, CD (SD)	Oral	250	8 M	No statistically significant differences in SBP or HR were found.	No	TPZZ/92/0057

FTC = emtricitabine; M = male

2.6.3.4 Safety Pharmacology

Test Article: Emtricitabine

Organ Systems Evaluated	Species Strain	Method of Admin.	Doses (mg/kg)	Gender and No. per Group	Noteworthy Findings	GLP Compliance	Study Number
Cardiovascular, Respiratory and Autonomic Function	Anesthetized Dogs, Beagle	IV	Total cumulative dose 38.5	4 M	No statistically significant changes in mean arterial blood pressure or heart rate. No drug-related arrhythmias were observed in the Lead II electrocardiogram. There were no decreases in respiratory parameters. Increases in respiratory rate of up to 3.8 breaths/minute and minute volume of up to 0.5 l/minute were seen 10 minutes after dosing FTC; however, they were not statistically significant. The average plasma level of 2 FTC-treated dogs 30 minutes following the last dose was 140 mM. This represents 93-fold the anticipated anti-HIV/HBV IC_{50} values *in vitro* at a minimum.	No	TPZZ/92/0076
Renal Function	Rat, Long Evans	Oral	10, 30, 100	6 M	FTC failed to cause any significant change in urine volume output, electrolyte excretion or pH values.	No	477
Gastrointestinal Motility	Mouse, ICR	Oral	10, 30, 100	10 M	No significant change in G.I. motility was observed.	No	477

FTC = emtricitabine; M = male

2.6.3.4 Safety Pharmacology

Test Article: Rilpivirine

Organ Systems Evaluated	Species Strain	Method of Admin.	Doses (mg/kg)	Gender and No. per Group	Noteworthy Findings	GLP Compliance	Study Number
2.6.3.4.3. In Vitro Studies with Rilpivirine							
Cardiovascular safety (cardiac membrane potassium current)	I_{Kr} in transfected CHO cells expressing hERG	In vitro	0.1, 0.3, 3 µM (0.037, 0.111, 1.11 µg/mL) (Positive controls: Astemizole, Terfenadine) (Vehicle: 0.1% DMSO in aqueous buffer)	NA	0.1 µM: 10% inhibition 0.3 µM: 33% inhibition 3 µM: 80% inhibition	No	TMC278-CPF730
Cardiovascular safety (cardiac membrane potassium current)	I_{Ks} in transfected CHO cells expressing KvLQT1/minK	In vitro	0.3, 1, 3, 10 µM (0.111, 0.370, 1.11, 3.7 µg/mL) (Positive control: HMR1556) (Vehicle: 0.3% DMSO in aqueous buffer	NA	1 µM: 17% inhibition 3 µM: 47% inhibition 10 µM: 73% inhibition IC_{50}: 3.1 µM (1.15 µg/mL)	No	TMC278-NC342

I_{Kr} = rapidly activating rectifying potassium current; I_{Ks} = Slowly activating rectifying potassium current, CHO: Chinese hamster ovary, DMSO = dimethylsulfoxide, hERG = human-ether-à-go-go related gene, NA = not applicable, IC_{50}: median inhibitory concentration

2.6.3.4 Safety Pharmacology

Test Article: Rilpivirine

Organ Systems Evaluated	Species Strain	Method of Admin.	Doses (mg/kg)	Gender and No. per Group	Noteworthy Findings	GLP Compliance	Study Number
Cardiovascular safety (cardiac membrane ion currents)	I_{Ks} in transfected HEK293 cells expressing KvLQT1/minK	In vitro	0.1, 0.3 , 1 µM (0.037, 0.11, 0.37 µg/mL) (Positive control: Chromanol 293B) (Vehicle: 0.3% DMSO in aqueous buffer)	NA	1 µM: 19.1% inhibition	No	TMC278-NC331
	I_{to} in transfected HEK293 cells expressing $hK_v4.3$	In vitro	0.1, 0.3 , 1 µM (0.037, 0.11, 0.37 µg/mL) (Positive control: flecinide) (Vehicle: 0.3% DMSO in aqueous buffer)	NA	0.3 µM: 13.6% inhibition 1 µM: 35.5% inhibition	No	
	I_{Ki} in transfected HEK293 cells expressing $hK_{ir}2.1$	In vitro	0.1, 0.3 , 1 µM (0.037, 0.11, 0.37 µg/mL) (Positive control: $BaCl_2$) (Vehicle: 0.3% DMSO in aqueous buffer)	NA	No effects	No	

I_{Ks} = Slowly activating rectifying potassium current, $I_{Ca,L}$ = high threshold calcium current, I_{Ki} = inward rectifying potassium current, I_{Na} = fast sodium current, I_{to} = transient outward potassium current, HEK293 = human embryonic kidney cell line, NA = not applicable, DMSO = dimethylsulfoxide

2.6.3.4 Safety Pharmacology

Test Article: Rilpivirine

Organ Systems Evaluated	Species Strain	Method of Admin.	Doses (mg/kg)	Gender and No. per Group	Noteworthy Findings	GLP Compliance	Study Number
Cardiovascular safety (cardiac membrane ion currents) (continued)	I_{Na} in transfected HEK293 cells expressing $hNa_v1.5$	In vitro	0.1, 0.3 , 1 µM (0.037, 0.11, 0.37 µg/mL) (Positive control: lidocaine) (Vehicle: 0.3% DMSO in aqueous buffer)	NA	No effects	No	TMC278-NC331 (continued)
	$I_{Ca,L}$ in transfected HEK293 cells expressing $hCa_v1.2$	In vitro	0.1, 0.3 , 1 µM (0.037, 0.11, 0.37 µg/mL) (Positive control: nifedipine) (Vehicle: 0.3% DMSO in aqueous buffer)	NA	No effects	No	

I_{Ks} = Slowly activating rectifying potassium current, $I_{Ca,L}$ = high threshold calcium current, I_{Ki} = inward rectifying potassium current, I_{Na} = fast sodium current, I_{to} = transient outward potassium current, HEK293 = human embryonic kidney cell line, NA = not applicable, DMSO = dimethylsulfoxide

2.6.3.4 Safety Pharmacology

Test Article: Rilpivirine

Organ Systems Evaluated	Species Strain	Method of Admin.	Doses (mg/kg)	Gender and No. per Group	Noteworthy Findings	GLP Compliance	Study Number
Cardiovascular safety (cardiac membrane potassium current)	hERG blocking and/or trafficking in transfected HEK293 cells	In vitro	1, 10, 30 µM (0.37, 3.7, 11.1 µg/mL) (Positive controls: Astemizole, Geldanamycin) (Vehicle: 0.1% DMSO in aqueous buffer)	NA	1 µM: 146% expression hERG-SM 10 µM: 155% expression hERG-SM 29% block hERG-WT 30 µM: 213% expression hERG-SM 36% block hERG-WT TMC278 has the potential to reduce trafficking of the hERG channel and the potential to inhibit the hERG channel.	No	TMC278-NC330
Cardiovascular safety (cardiac action potential)	Isolated Guinea pig right atrium	In vitro	0.01, 0.03, 0.1, 1, 3, 10 µM (0.004, 0.011, 0.037, 0.37, 1.11, 3.7 µg/mL (Vehicle: 0.01 – 10% DMSO in aqueous buffer)	NA	1 µM: RC 86% of baseline 3 µM: RC 72% of baseline 10 µM: RC 44% of baseline No effects on force of contraction compared to vehicle over the whole conc. range. No effects on ERP compared to vehicle at 0.1 and 10 µM	No	TMC278-N168576

hERG = human-ether-à-go-go related gene, hERG-WT = wild type hERG channel, hERG-SM = chaperone resistant single mutant hERG channel, DMSO = dimethylsulfoxide, HEK293 = human embryonic kidney cell line, NA = not applicable; RC = rate of spontaneous contraction, ERP = effective refractory period = maximal frequency of stimulations not followed by contraction, QT-interval = time between peak Q wave and end T wave, EAD = early afterdepolarization, TdP = torsade des pointes, VT = ventricular tachycardia, VF = ventricular fibrillation

2.6.3.4 Safety Pharmacology

Test Article: Rilpivirine

Organ Systems Evaluated	Species Strain	Method of Admin.	Doses (mg/kg)	Gender and No. per Group	Noteworthy Findings	GLP Compliance	Study Number
Cardiovascular safety (cardiac action potential)	Isolated arterially perfused rabbit left ventricular wedge	In vitro	0.01, 0.1, 1, 10 µM (0.004, 0.037, 0.37, 3.7 µg/mL) (Vehicle: 0.1% DMSO in aqueous buffer)	NA	1 µM: QT-interval 106% of baseline 10 µM: QT-interval 109% of baseline. TdP score 0.5. No EADs, TdP, VT, VF, or in-excitability over the whole conc. range	No	TMC278-NC341

hERG = human-ether-à-go-go related gene, hERG-WT = wild type hERG channel, hERG-SM = chaperone resistant single mutant hERG channel, DMSO = dimethylsulfoxide, HEK293 = human embryonic kidney cell line, NA = not applicable; RC = rate of spontaneous contraction, ERP = effective refractory period = maximal frequency of stimulations not followed by contraction, QT-interval = time between peak Q wave and end T wave, EAD = early afterdepolarization, TdP = torsade des pointes, VT = ventricular tachycardia, VF = ventricular fibrillation

2.6.3.4 Safety Pharmacology

Test Article: Rilpivirine

Organ Systems Evaluated	Species Strain	Method of Admin.	Doses (mg/kg)	Gender and No. per Group	Noteworthy Findings	GLP Compliance	Study Number
2.6.3.4.4. In Vivo Studies with Rilpivirine							
Behavior and neurologic and autonomic functioning	Rat/ Sprague Dawley	Oral/gavage Single dose	40, 120, 400 mg/kg (vehicle: PEG400)	5M	400 mg/kg: Slightly abnormal single behavioral parameter, 8 hrs after dosing Slightly reduced pupil size, 2 hrs after dosing.	Yes	TMC278-Exp5560
Cardiovascular safety	Anesthetized Guinea pig/Dunkin Hartley	Intravenous escalating doses 15 min interval	0.16, 0.32, 0.64, 1.25, 2.5, 5 mg/kg (vehicle: PEG400)	7F	No effects on HR, MABP, or ECG Plasma conc.:9.2 µg/mL Heart conc.: 47.7 µg/g Lung conc.: 11.7 µg/g All conc. 5 min after dosing at 5 mg/kg	No	TMC278-CPF643
Cardiovascular safety	Telemetered Guinea pig/Dunkin Hartley	Oral/gavage for 16 days	10 mg/kg (vehicle: HPMC 0.5% + 0.11 – 0.2% tween 80 in water)	6F	No effects on HR; ECG, BT Plasma C_{max} = 0.689 – 0.911 µg/mL throughout the dosing period	No	TMC278-NC327

GLP = good laboratory practices, F = female, M = male, PEG = polyethylene glycol, HPMC = hydroxypropylmethylcellulose, HR = heart rate, MABP = mean arterial blood pressure, ECG = electrocardiogram, BT = body temperature, SVR = systemic vascular resistance, PVR = peripheral vascular resistance, BL = baseline, C_{max} = maximal concentration

2.6.3.4 Safety Pharmacology

Test Article: Rilpivirine

Organ Systems Evaluated	Species Strain	Method of Admin.	Doses (mg/kg)	Gender and No. per Group	Noteworthy Findings	GLP Compliance	Study Number
Cardiovascular and pulmonary safety	Anesthetized dog/beagle	Intravenous infusion 1 hour	5 mg/kg (vehicle: PEG400)	4 (gender not reported)	5 mg/kg: SVR: -25% to BL; vehicle: +29% to BL PVR: -20% to BL; vehicle: +31% to BL Cardiac output: +31% to BL; vehicle: -12% to BL No effect on other parameters Plasma $C_{end\ of\ inf}$ = 2.62 µg/mL Plasma $C_{3h\ after\ inf}$ = 0.721 µg/mL	No	TMC278-CPF648
Cardiovascular safety	Conscious instrumented dogs/beagle	Oral/gavage Single dose	20 mg/kg (vehicle: PEG400)	3M and 11F	No effects Median C_{max} = 1.5 µg/mL (at 240 min)	No	TMC278-CPF654
Cardiovascular and pulmonary safety and locomotor activity	Conscious telemetered dog/beagle	Oral/gavage Single dose	20, 80, 160 mg/kg (vehicle: PEG400)	4M	No effects on cardiac hemodynamic, electrophysiological, respiratory, or locomotor activity parameters.	Yes	TMC278-Exp5555

GLP = good laboratory practices, F = female, M = male, PEG = polyethylene glycol, HPMC = hydroxypropylmethylcellulose, HR = heart rate, MABP = mean arterial blood pressure, ECG = electrocardiogram, BT = body temperature, SVR = systemic vascular resistance, PVR = peripheral vascular resistance, BL = baseline, C_{max} = maximal concentration

2.6.3.4 Safety Pharmacology

Test Article: Tenofovir DF, Tenofovir

Organ Systems Evaluated	Species Strain	Method of Admin.	Doses (mg/kg)[a]	Gender and No. per Group	Noteworthy Findings	GLP Compliance	Study Number
2.6.3.4.5. In Vitro Studies with Tenofovir DF							
Contractile responses	Guinea pig ileum	in vitro	Tissues pre-incubated with 0, 10, 30, 100μM tenofovir or TDF	-	Tenofovir has no effect on contractile responses to agonists (Acetylcholine histamine, $BaCl_2$) TDF (100 μM) inhibited contractile responses to agonists by 14%	No	V2000009

TDF = tenofovir DF
[s] unless otherwise noted

2.6.3.4 Safety Pharmacology

Test Article: Tenofovir DF, Tenofovir

Organ Systems Evaluated	Species Strain	Method of Admin.	Doses (mg/kg)	Gender and No. per Group	Noteworthy Findings	GLP Compliance	Study Number
2.6.3.4.6. In Vivo Studies with Tenofovir DF							
CNS	Rat, Sprague Dawley	oral gavage	0, 50, 500	10M	No biologically significant signs or pharmacological effects on the CNS were observed.	Yes	R990152
Cardiovascular	Dog, Beagle	oral gavage	30	3M	No test article-related effects on heart rate, clinical signs, blood pressure or ECG observed. No haemodynamic effect level was 30mg/kg.	Yes	D990155
Renal system	Rat, Sprague Dawley	oral gavage	0, 50, 500	10M	50mg/kg was considered to be the NOEL.	Yes	R990154
GI motility	Rat, Sprague Dawley	oral gavage	0, 50, 500	9M	TDF at high dose reduced gastric emptying. There was no clear effect on GI motility at either dose level.	Yes	R990153

CNS = central nervous system, ECG = electrocardiogram, M = males, NOEL = no observed effect level, GI = gastrointestinal, TDF = tenofovir DF

2.6.3.5. Pharmacodynamic Drug Interactions

Studies of the pharmacodynamic drug interactions of FTC, RPV, TDF, and the FDCs of FTC/TDF and FTD/RPV/TDF are presented in Sections 2.6.3.1.16 to 2.6.3.1.20.

2.6.3.6. References

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