MODULE 2.4. NONCLINICAL OVERVIEW

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List of Abbreviations

3TC	Lamivudine
AAALAC	Association for Assessment and Accreditation of Laboratory Animal Care
ACTH	Adrenocorticotropic hormone
A/G	Albumin/globulin
AIDS	Acquired immunodeficiency syndrome
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
API	Active pharmaceutical ingredient
APTT	Activated partial thromboplastin time
ARV	Antiretroviral
AST	Aspartate aminotransferase
ATPase	Adenosine triphosphatase
AUC	Mean area under the concentration versus time curve
AZT	Zidovudine
BCO	Biological cut-off
BCOP	Bovine corneal opacity and permeability
b.i.d.	Bis in diem = twice daily
BID	Twice daily
CA	Citric acid
CAC	Carcinogenicity Assessment Committee
CC50	Median cellular toxic concentration
CFR	Code of Federal Regulations (US)
Clb	Blood clearance
C_{max}	Maximum plasma concentration
CRF	Corticotrophin releasing factor
CYP	Cytochrome P450
DHEA	Dehydroepiandrosterone
DLV	Delavirdine
DMSO	Dimethylsulfoxide
DNA	Deoxyribonucleic acid
DTG	Dolutegravir
EADs	Early afterdepolarizations
EC_{50}	Median effective concentration
ECGs	Electrocardiographs
EFV	Efavirenz
ETR	Etravirine
EVG	Elvitegravir
F	Female
F_0 generation	Parents
F_1 generation	Offspring
FC	Fold change
FDA	Food and Drug Administration
FDC	Fixed dose combination
GD	Gestation day
GI	Gastrointestinal

GLP	Good Laboratory Practices
HAART	Highly active antiretroviral therapy
HCl	Hydrochloride
HCV	Hepatitis C virus
hERG	Human ether-à-go-go
HET-CAM	Hen's Egg Test Chorioallantoic Membrane
HIV-1	Human immunodeficiency virus type 1
HPMC	Hydroxypropylmethylcellulose
IC_{50}	Median concentration resulting in 50% of maximum inhibition
ICH	International Conference on Harmonisation
IKr	Rapidly activating rectifying potassium current
INI	Integrase inhibitor
KLH	Keyhole limpet hemocyanin
LA	Long-acting
LC-MS/MS	Liquid chromatography with tandem mass spectrometry
LD	Lactation day
LH	Luteinizing hormone
LLNA	Local lymph node assay
LOAEL	Lowest Observed Adverse Effect Level
M	Male or molar
MPS	Mononuclear phagocytic system
m/v	Mass per volume
N(t)RTIs	Nucleoside or nucleotide reverse transcriptase inhibitor
NNRTI	Non-nucleoside reverse transcriptase inhibitor
NOAEL	No observed adverse effect level
NRTI	Nucleoside reverse transcriptase inhibitor
NVP	Nevirapine
OECD	Organization for Economic Cooperation and Development
PEG 400	Polyethylene glycol 400
PFC	Plaque forming cells
P-gp	P-glycoprotein
PIs	Protease inhibitors
PK	Pharmacokinetics
PPB	Plasma protein binding
PT	Prothrombin time
q.d.	Once daily
QD	Once daily
QT	Interval between the start of the Q wave and the end of the T wave on ECG
QTc	QT interval duration corrected for heart rate
QTcF	QT interval duration corrected for heart rate according to Fridericia
QWBA	Quantitative whole body autoradiography
RAL	Raltegravir
RAM	Resistance associated mutation
RPV	Rilpivirine
RT	Reverse transcriptase
RTV	Ritonavir
S9	Metabolic activation system

STR	Single tablet regimen
T3	Triiodothyronine
T4	Thyroxine
TBG	Thyroid binding globulin
TdP	Torsade de Pointes
TDAR	T-cell-dependent antibody response
TDR	Transmural dispersion of repolarization
TK	Toxicokinetics
TMC	Tibotec Medicinal Compound
TQT	Thorough QT prolongation study according to ICH E14
TR	Total radioactivity
TSH	Thyroid stimulating hormone
TTC	Threshold of toxicological concern
UDPGT	Uridine diphosphate-glucuronosyltransferase
Vd_{ss}	Volume of distribution at steady state
γGT	γ-glutamyltransferase

1. OVERVIEW OF THE NONCLINICAL TESTING STRATEGY

1.1. Introduction

Dolutegravir (DTG) is a potent, selective and novel integrase inhibitor (INI) and rilpivirine (RPV) is a potent non-nucleoside reverse transcriptase inhibitor (NNRTI) against human immunodeficiency virus type 1 (HIV-1). DTG/RPV FDC is a two-drug, NRTI-sparing regimen, differing from the current standard of care, which typically includes 2 NRTIs plus a third agent from the PI, NNRTI or INI class. The proposed new combination treatment regimen of RPV and DTG targets HIV replication at the early and late stage of the virus life cycle, respectively, resulting in suppression of HIV-1 replication in a substantial proportion of infected cells. Based on the established tolerability and combined resistance profiles of DTG and RPV, this simplified two-drug regimen offers favorable tolerability and suppression of resistance similar to other DTG based HIV-1 treatment regimens.

Dolutegravir (DTG; GSK1349572) is an HIV integrase inhibitor (INI) approved for use in antiretroviral treatment-naïve and treatment-experienced HIV-infected adults and adolescents at a dose of 50 mg once daily (TIVICAY®) [NDA 204790]. Rilpivirine (RPV; TMC278, GSK1329758) is a non-nucleoside reverse transcriptase inhibitor (NNRTI) approved for use in antiretroviral treatment-naïve HIV-1 infected adults with a viral load ≤100,000 HIV-1 RNA copies/mL, at a dose of 25 mg once daily taken with meal (EDURANT®) [NDA 202022].

This application is in support of DTG/RPV fixed dose combination (FDC) as indicated for the treatment of HIV-1 infection in virologically suppressed (HIV-1 RNA <50 copies/mL) adults without known or suspected resistance to either antiretroviral component. The recommended oral dose for adults is one tablet administered once a day with a meal.

The dose will be orally administered as an FDC tablet. Each film coated tablet contains 50 mg of dolutegravir as dolutegravir sodium and 25 mg rilpivirine. The structure of DTG and RPV is shown in Figure 1 and Figure 2 below.

Figure 1 Structure of DTG

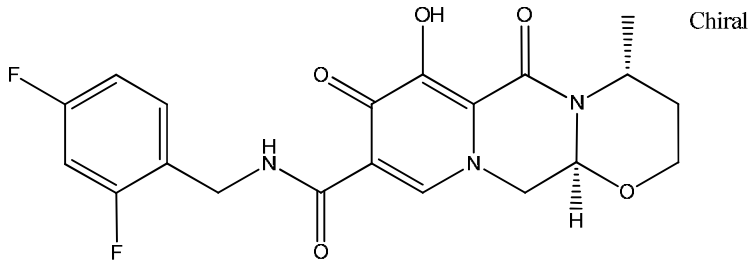
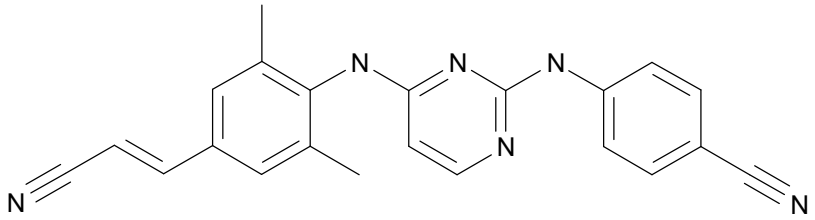


Figure 2 Structure of RPV



1.2. Nonclinical Development Programme

1.2.1. DTG/RPV in combination

In agreement with ICH Guidance M3 (R2), nonclinical combination studies with DTG/RPV were not considered warranted to support the current application. However, single dose combination PK studies were conducted in dogs.

Refer to Section 5 for a complete discussion of the nonclinical considerations regarding the combined use of DTG/RPV FDC.

The key nonclinical information relating to the virology of DTG and RPV, effects in repeat dose toxicity, genotoxicity, carcinogenicity and reproductive toxicity studies, and use during pregnancy and lactation is addressed in the proposed product label [m1.14.1 (US) or m1.3 (EU)].

1.2.2. DTG and RPV as monotherapies

Full packages of nonclinical safety studies have been completed with DTG and RPV individually to support their development and marketing as monotherapies.

Preliminary nonclinical toxicity studies, experiments undertaken to determine the virology of DTG and RPV to establish suitable dose levels for use in repeat dose toxicity and pharmacokinetics studies, were conducted in line with Company Divisional Standard Operating Procedures and Policies, and in general accordance with the principles of Good Laboratory Practice (GLP). All definitive toxicity studies were carried out in full compliance with GLP regulations in an OECD member country in accordance with the OECD Test Guidelines.

Except where indicated, all studies described in this section were performed using either the sodium salt of DTG or the HCl salt of RPV (RPV base is referred to when the base form was administered). All doses and concentrations quoted in this summary are expressed in terms of the parent compounds (referred to simply as DTG and RPV).

An overview of the nonclinical studies for each of the individual actives, DTG and RPV, is presented below, and the nonclinical study reports are provided in Module 4. An integrated assessment of nonclinical considerations for the DTG/RPV FDC is also presented in Section 5 and supports the conclusion that DTG/RPV FDC can be used safely in patients for the treatment of HIV.

Throughout this section, exposure margins for DTG are presented based upon comparison of the animal systemic exposure (end of study gender averaged) with that reported for patients receiving 50 mg DTG once daily (QD) (C_{max} = 3.7 μg/mL; AUC = 53.6 μg.h/mL) [pooled data from Clinical Studies ING112276 and ING113086]. For RPV, exposure margins are presented based upon comparison of the animal systemic exposure (end of study gender averaged) with that reported for patients receiving 25 mg RPV QD (C_{max} = 0.13 μg/mL; AUC = 2.4 μg.h/mL) [pooled data from Clinical Studies C209 and C215] [see Section 3.1, m2.7.2].

1.2.2.1. DTG

Nonclinical studies carried out to support the development of DTG include primary pharmacology studies demonstrating inhibition of integrase activity and HIV-1 replication in vitro as well as studies to determine the potential for HIV resistance to develop via mutations. Secondary pharmacologic activity was assessed and safety pharmacology studies were conducted to investigate any untoward pharmacologic actions of DTG on the respiratory, cardiovascular, and central and peripheral nervous systems. The Primary Pharmacodynamics/Virology studies are listed in m2.7.2.4, Special Studies.

The absorption, distribution, metabolism and excretion of DTG were investigated to characterize the disposition of DTG in the toxicology test species, and appropriate toxicokinetic evaluations were included during toxicological evaluation of DTG.

To assess the nonclinical safety of DTG, repeat dose toxicity was studied in rats and monkeys following oral administration for up to 26 weeks and 38 weeks, respectively. A series of genotoxicity studies were performed to determine the mutagenic and clastogenic potential of DTG. Toxicology studies were also conducted to assess potential carcinogenicity in mice and rats. Fertility studies were conducted in male and female rats, and embryofetal development studies in pregnant rats and rabbits. Juvenile toxicity studies and a pre- and post-natal development toxicity study were conducted in rats. Other studies were performed to assess the local tolerance and potential immunotoxicity of DTG, and the genotoxicity of potential impurities.

1.2.2.2. RPV

The nonclinical testing strategy for the RPV oral tablet indication aimed at evaluating the primary and secondary pharmacodynamic effects of RPV. Moreover, a variety of models and tests was applied to detect adverse effects of RPV, its metabolites as well as its impurities in drug substance and drug product, if relevant. The behavior of RPV, in terms of absorption, kinetics, distribution, metabolism and excretion in the major models was studied. Considerable effort was made to elucidate the mechanism of action of the observed effects for a better understanding of their relevance and to allow an adequate

safety assessment for man. The Primary Pharmacodynamics/Virology studies are listed in m2.7.2.4, Special Studies.

Safety pharmacology studies included cardiovascular safety evaluations in in vitro hERG and guinea pig right atrium studies and in vivo in anesthetized guinea pig and dog and in single dose conscious telemetered dog studies. Additional cardiovascular studies to investigate a delayed onset QT prolongation in a clinical setting included investigations into trafficking with an in vivo telemetered guinea pig study and into potential Torsade de Pointes (TdP) in the rabbit perfused left ventricle wedge preparation. Respiratory safety evaluations were included in the anesthetized dog and conscious telemetered dog studies. Compound-related neurological, behavioral changes or delayed neurotoxicity were studied in a modified Irwin test in rats or included in the conscious telemetered dog study. The objectives of the drug metabolism and pharmacokinetic program were to characterize the pharmacokinetics of RPV in various animal species, measure exposure after single and repeated administration to animals, characterize the distribution in the body, identify metabolic pathways and metabolites formed in man and animal species, determine the rates and routes of elimination in animals, predict the potential for drug-drug interactions, and where appropriate to examine the inter-relationships between dose, exposure and effects observed. Several in vitro and in vivo studies were performed. In vivo studies were conducted in CD-1 and CB6F1-nonTgrasH2-transgenic mice, pigmented Long Evans and Sprague Dawley rats, New Zealand white rabbits, beagle dogs and cynomolgus monkeys. With the exception of pigmented rats, all species and strains were the same as those used in the nonclinical pharmacology and toxicology studies. Radiolabeled RPV was used in plasma protein binding, tissue distribution and metabolism and elimination studies.

No formal single dose studies were done as single dose evaluations were part of the initial dose range finding studies or, in the case of mice, part of the bone marrow micronucleus test. These studies were all done with the maximum feasible dose. Repeat dose toxicity studies were done in mice as (preparation for) a 3 month dose range finding carcinogenicity study, in rats for up to 6 months, in non-pregnant rabbits for 5 days as preparation for the dose range finding early embryonic development studies, in dogs up to 12 months, and in immature female cynomolgus monkeys up to 8 weeks as part of the assessment of the effects of RPV on juvenile animals. Juvenile rats were involved in a 2 week oral dosing study starting on lactation Day 12. The dogs in studies up to 1 month duration are also considered immature. Since the animals were approximately 6 months old at the start of these studies they were not yet sexually mature at the end of the dosing period. Reversibility (after a 1 month recovery period) of the effects of RPV on the high dose group was investigated in the 6 month rat study and the 1 month dog study. The genotoxic testing of RPV comprised in vitro bacterial reverse mutation (Ames) tests and mouse lymphoma assays and the in vivo mouse bone marrow micronucleus test. An Ames test with human liver microsomal fraction was done to evaluate further the genotoxic potential of the unsaturated nitrile moiety of RPV. The carcinogenicity of RPV was evaluated in 2 year studies in mice and rats. The reproductive and developmental effects of RPV were studied on male and female rat fertility, rat and rabbit early development, and rat peri- and post-natal development. Moreover, phototoxicity in 3T3 cells, skin irritation in rabbits, eye irritation by means of the bovine corneal opacity and permeability (BCOP) test, delayed type sensitization in the mouse local lymph node

assay (LLNA) and immunotoxicity in rats were evaluated. The mechanism of action of adrenal and gonadal effects observed in repeat dose studies in mice, rats and dogs was further evaluated in vitro with dog adrenal cortex homogenate, in vivo in guinea pigs, dogs and immature female cynomolgus monkeys. In the absence of unique or major human metabolites of RPV, no evaluation of the safety of individual metabolites was performed. Several drug substance impurities of RPV were investigated and qualified, either on the basis of structural alerts or on the basis of their specification. In the majority of the studies and in all pivotal studies, the exposure to RPV was determined.

2. PHARMACOLOGY

2.1. Presentation Order of Pharmacology Summaries

Pharmacology Summary	Section
Pharmacology for DTG and RPV in combination	Not applicable.
Pharmacology of DTG alone	Section 2.2
Pharmacology of RPV alone	Section 2.3
Overall summary of all pharmacological studies performed with DTG or RPV	Section 5, Integrated Assessment of the Fixed Dose Combination Product

2.2. DTG

A summary of each secondary and safety pharmacology study and its findings is presented in m2.6.2, DTG NDA. A listing of each secondary and safety pharmacology study conducted, and a tabular summary of each safety pharmacology study and its findings, is presented in m2.6.3, DTG NDA. All secondary and safety pharmacology study reports are available in m4.2.1, Nonclinical Study Reports.

2.2.1. Virology (primary pharmacodynamics)

A range of in vitro virology studies have been conducted to determine the mechanism of action, antiviral activity and the potential for development of drug resistance via mutations. In accordance with ICH M4S Q&A (R4), an overview of these studies is provided within the Clinical Overview [see m2.5, Section 4.7]. However, for the reviewer's convenience, a brief summary of the key findings from these studies is also provided below.

2.2.1.1. Mechanism of action

Integration of viral DNA into the host chromosome of infected cells is an important step in the HIV replication cycle and is facilitated by viral integrase protein [Pommier, 2005]. Integration requires two metal-dependent consecutive steps in the viral replication cycle: 3'-processing and strand transfer. Viral cDNA is primed for integration in the cytoplasm by integrase-mediated trimming of the 3'-ends of the viral cDNA. Integrase remains bound to the viral cDNA ends in the pre-integration complexes (PICs). Following nuclear translocation of the PICs, integrase catalyzes the insertion of the viral cDNA ends into the host chromosomes. DTG inhibits HIV integrase by binding to the integrase active site and blocking the strand transfer step of retroviral DNA integration which is essential for the HIV replication cycle.

2.2.1.2. In vitro antiviral activity and potential resistance

DTG has low nM activity against wildtype HIV-1 and HIV-2 in a variety of cell lines, regardless of subtype. DTG has little activity against non-HIV viruses, displaying the highest antiviral activity against HCV. Human serum causes an approximately 75-fold increase in the DTG IC_{50}. DTG is additive or synergistic when assayed in combination with other antiretroviral agents.

When HIV-1 Strain IIIB was passaged in the presence of DTG for 112 days, viruses with a 4.1-fold maximum increase in IC_{50} and S153Y or S153F substitutions in integrase polymorphic sites were observed. Passage of the wildtype HIV-1 NL432 in the presence of 6.4 nM DTG selected for E92Q (FC=3.1) and G193E (FC=3.2) substitutions in the IN region on Day 56. Passage of HIV-1 NL432 with Q148H, Q148K or Q148R RAL-resistant mutations resulted in selection of additional mutations and an increase in DTG FC. Passage of HIV-1 subtypes B and A/G in TZM-bl cells selected for integrase mutation R263K.

Comparative susceptibilities to DTG and RAL were obtained from 60 RAL-resistant site directed HIV-1 mutants and 6 site directed HIV-2 mutants. DTG retained activity against a vast majority of these mutants. Additionally, susceptibilities to DTG and RAL were determined for over 700 RAL-resistant clinical isolates, with DTG retaining activity (<10 FC) against >90% of them.

The dissociation of DTG, RAL and EVG from wildtype and mutant IN proteins complexed with DNA was investigated to obtain a better understanding of INI dissociation kinetics. DTG demonstrated slower dissociation from all IN-DNA complexes tested, including those with single and double residue IN substitutions.

2.2.2. Secondary pharmacology

DTG (up to 10 μM) was tested in vitro against a variety of proteins which included 16 enzyme assays and 65 physiological receptors and ion channels binding sites [Report RH2007/00072, m4.2.1.2]. DTG at 10 μM did not significantly affect (defined as ≥50%) 80 of the 81 in vitro assays. The only effect greater than 50% was a 64% inhibition in the melanocortin (MC4) receptor binding assay. Inhibition at 10 μM was >100-fold above the free clinical C_{max} for DTG when administered 50 mg QD [C_{max} = 3.7 μg/mL (~8.8 μM) and because DTG is ~99% protein bound the free unbound concentration was 0.037 μg/mL (0.088 μM). In an additional study conducted to assess the potential for dolutegravir to bind the melanocortin receptors MC1, MC3, MC4 and MC5, similar results were observed with IC_{50} values ranging from 5.31 to 56.1 μM with all exposure margins >60X [Report 2014N204265, DTG NDA].

No findings associated with MC4R agonism or antagonism have been observed in toxicity or clinical studies with DTG. No significant effects on body weight in healthy or HIV-infected subjects administered DTG have been observed to date. Taken together, these data indicate a lack of apparent biological activity at the melanocortin receptors.

2.2.3. Safety pharmacology

2.2.3.1. Neurobehavioral and pulmonary safety

No treatment-related behavioral or overt pharmacological effects were noted in conscious male rats at ≤500 mg/kg (the highest dose tested) [m2.6.3, DTG NDA, Report RD2007/01038]. Systemic exposure at 500 mg/kg is estimated to be ~24X above the expected human C_{max} of dolutegravir administered 50 mg QD, respectively, based on extrapolation from Day 1 exposure in the rat 14 day toxicity study (87.1 μg/mL).

Single oral doses of DTG at ≤500 mg/kg did not produce any effect on respiratory functional parameters in male rats when monitored for up to 6 hours following dosing [m2.6.3, DTG NDA, Report RD2007/01037]. Systemic exposure at 500 mg/kg is estimated to be ~25X above the expected human AUC_{0-24} of DTG administered 50 mg QD (53.6 μg.h/mL), based on extrapolation from Day 1 exposure in the rat 14 day toxicity study (1360 μg.h/mL).

2.2.3.2. Cardiovascular safety

In male monkeys, single oral doses of DTG at doses up to 1000 mg/kg (C_{max} = 20.1 μg/mL; AUC_{0-24} = 259 μg.h/mL) had no effect on arterial blood pressures, heart rate or electrocardiographic (ECG) parameters when monitored for 24 hours after dosing at a C_{max} ~5X above the expected human C_{max} of DTG when administered 50 mg QD [m2.6.3, DTG NDA, Report RD2007/01141]. Additionally, there were no treatment-related effects in ECG parameters measured during the repeat dose monkey toxicity studies up to 38 weeks at doses ≤1000 mg/kg/day.

The effect of a series of DTG concentrations (≤8.38 μg/mL) on hERG tail current was studied [m2.6.3, DTG NDA, Report RD2007/01039]. An IC_{50} could not be determined as only 16.1% inhibition of hERG channel tail current occurred at the highest concentration, 20 μM. The high dose (20 μM or 8.4 μg/mL) is approximately 227X above the free C_{max} obtained with a 50 mg QD oral dose of dolutegravir (0.037 μg/mL for 50 mg QD based on 99% protein binding).

There were no findings from safety pharmacology studies that indicate an unacceptable risk for oral administration of DTG to patients in accordance with the proposed indication. Additionally, a supratherapeutic clinical dose of DTG (250 mg as a suspension, which achieved exposures ~3X higher than a 50 mg QD dose) was well tolerated and had no effect on cardiac repolarization [see m2.7.2 Section 2.3.4].

2.2.4. Pharmacodynamic drug interactions

A number of in vitro studies have been conducted with DTG in combination with approved agents from all anti-HIV therapy classes (e.g., nucleoside/nucleotide reverse transcriptase [RT] inhibitors, non-nucleoside RT inhibitors and protease inhibitors) and was shown to be additive or synergistic in all cases. These studies are discussed as part of the virology discussion [see m2.7.2.4, Special Studies].

2.3. RPV

A summary of each secondary and safety pharmacology study and its findings is presented in m2.6.2, RPV NDA. A listing of each secondary and safety pharmacology study conducted, and a tabular summary of each safety pharmacology study and its findings, is presented in m2.6.3, RPV NDA. All secondary and safety pharmacology study reports are available in m4.2.1, Nonclinical Study Reports.

2.3.1. Virology (primary pharmacodynamics)

A range of in vitro virology studies have been conducted to determine the mechanism of action, antiviral activity and the potential for development of drug resistance via mutations. In accordance with ICH M4S Q&A (R4), an overview of these studies is provided within the Clinical Overview [m2.5, Section 4.7]. However, for the reviewer's convenience, a brief summary of the key findings from these studies is also provided below.

2.3.1.1. Mechanism of action

RPV is a non-nucleoside reverse transcriptase inhibitor (NNRTI) that showed sub-nanomolar EC_{50} values against wildtype HIV-1 group M isolates A, B, C, D, E, F and G (0.07 to 1.01 nM or 0.03 to 0.37 ng/mL), HIV-1/IIIB (0.73 nM or 0.27 ng/mL) and nanomolar EC_{50} values against HIV-1 group O isolates (2.88 to 8.45 nM or 1.06 to 3.10 ng/mL). The RPV 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 showing the broad and robust spectrum of the antiviral activity exhibited by this NNRTI. RPV had antiviral activity in the micromolar range against HIV-2 and SIV, but no activity was observed against several non-HIV-related viruses like the human hepatitis B virus, herpes simplex virus 2, human corona virus, influenza A virus and vaccinia virus. A selectivity index of ±8,000 indicated that RPV was a potent and specific inhibitor of HIV-1. The in vitro cytotoxicity experiments performed in cell lines of various origins confirmed that the RPV selectivity index was robust.

The crystal structure of RPV bound to the HIV-1 RT complex revealed that RPV 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.

The antiviral activity of RPV in the presence of human serum proteins was comparable to that of EFV. RPV did not show antagonism when studied in combination with other antiretroviral agents. Low level synergy was observed in the presence of lamivudine (3TC), zidovudine (AZT) and raltegravir (RAL).

2.3.1.2. Resistance

RPV retained antiviral activity against 63.0% (136 of 216) of the HIV-1/HXB2 SDMs carrying single, double, triple and quadruple RT mutations; as compared to 57.7% for etravirine (ETR), 45.8% for efavirenz (EFV) and 36.2% for nevirapine (NVP). This panel of HIV-1/HXB2 SDMs was created on the basis of resistance to ETR and the information on emerging RT mutations in patients failing RPV enrolled in the Phase IIb and Phase III trials. The analysis of this panel showed that RPV retained activity against 64 of 67 single HIV-1/HXB2 SDMs analyzed in vitro and only the 3 HIV-1/HXB2 SDMs with K101P, Y181I or with Y181V were resistant to RPV. More than one, and usually more than two NNRTI resistance associated mutations (RAMs), were necessary to confer resistance to RPV in vitro. Resistance to RPV was mostly driven by the combination of specific NNRTI RAMs rather than the total number of NNRTI RAMs. K103N in

isolation was not associated with resistance to RPV or ETR. Furthermore, some of the NNRTI RAMs present in the SDMs that showed resistance to RPV were uncommon in NNRTI treatment-experienced patients.

In vitro selection of RPV-resistant strains was performed at high and low virus inoculum using wildtype HIV-1 of different origins and subtypes as well as NNRTI-resistant HIV-1. At high virus inoculum, emergence of resistant strains from wildtype HIV-1 was prevented at concentrations equal or greater than 0.0040 μM (0.00147 μg/mL). The same was observed with resistant strains harboring the single NNRTI resistance associated mutations (RAMs) K103N or Y181C or the NRTI RAM M184V. NNRTI RAMs emerging in vitro under selective pressure of RPV included: V90I, L100I, K101E, V106A, V106I, V108I, E138G, E138K, E138Q, E138R, V179F, V179I, Y181C, Y181I, G190E, H221Y, F227C, M230I and M230L.

2.3.1.3. Cross-resistance

Analysis of sensitivity to RPV of 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 in vitro cross-resistance that existed between EFV and NVP. This cross-resistance was not shared to the same extent by RPV and ETR, with the majority of the isolates (62%) retaining sensitivity to these novel NNRTIs. Considerable in vitro cross-resistance was observed between RPV and ETR.

2.3.2. Secondary pharmacology

RPV was investigated in a series of in vitro secondary pharmacodynamic studies [m2.6.2, RPV NDA]. The studies conducted are listed in m2.6.3 RPV NDA.

RPV did not cause any inhibition in vitro of α- or β-adrenergic, dopaminergic, muscarinergic, serotonergic, opioid, interleukin or chemokine receptors (up to 10 μM, 3.7 μg/mL), or human DNA polymerase α, β or γ (up to 1000 μM, 366 μg/mL).

RPV 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, RPV did not cause any significant inhibition of pentagastrin-induced gastric acidity in rats.

It is concluded that RPV is essentially devoid of any clinically relevant secondary pharmacodynamic effect at unbound concentrations that greatly exceed the median pooled total C_{max} of RPV of 0.13 μg/mL in Clinical Phase III Studies TMC278-C209 and TMC278-C215, following an oral dose of 25 mg q.d. The total C_{max} value corresponds with 0.3% (unbound fraction RPV in human plasma protein binding (PPB) study) of 0.13 μg/mL = 0.00039 μg/mL unbound RPV.

2.3.3. Safety pharmacology

RPV was studied in a series of in vitro and in vivo safety pharmacology studies [m2.6.2, RPV NDA]. The studies conducted are listed in m2.6.3, RPV NDA and individual study details are given in Tabulated Summary Tables, see m2.6.3 RPV NDA.

2.3.3.1. Cardiovascular safety

The standard battery of cardiovascular safety studies showed a concentration-dependent inhibition of RPV 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) [m2.6.3, RPV NDA, Report 2017N315903]. However, no relevant effects by RPV were noted on other cardiovascular or electrocardiographic parameters in vitro in the right atrium of the guinea pig [m2.6.3, RPV NDA, Report 2017N315532], in vivo in anesthetized guinea pigs [m2.6.3, RPV NDA, Report 2017N315533] and dogs [m2.6.3, RPV NDA, Report 2017N315527] given a single intravenous dose, or in conscious instrumented [m2.6.3, RPV NDA, Report 2017N315534] or telemetered dogs [m2.6.3, RPV NDA, Report 2017N317705] given a single oral dose. The mean C_{max} in the conscious dog studies at the highest dose was 1.5 to 1.7 μg/mL (extrapolated from a single dose PK study in dogs).

Delayed onset (after 11 days of treatment) prolongation of the QTcF interval was reported in the TQT study, TMC278-C131 [see Section 2.4.4, m2.7.2]. To investigate the mechanism of the QTc prolongation and of the delayed onset, additional nonclinical studies were done. Moreover, the potential of RPV to induce proarrhythmic effects, in particular TdP, was evaluated.

The 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 by 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 ($I_{Ca,L}$) [m2.6.3, RPV NDA, Reports 2017N316078 and 2017N315959].

In vitro, a concentration-dependent inhibition of trafficking of the hERG channel by RPV was observed from 1 μM (0.37 μg/mL) upwards [m2.6.3, RPV NDA, Report 2017N315957]. 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 were orally dosed for 16 days at 10 mg/kg/day associated with maximum plasma concentrations of RPV ranging from 0.6 to 0.9 μg/mL [m2.6.3, RPV NDA, Report 2017N315955]. This concentration was similar to the steady state median C_{max} at 75 mg q.d. RPV, a dose that caused delayed onset QTc prolongation in TQT Study [see Section 2.4.4, m2.7.2].

RPV showed only a marginal potential to induce proarrhythmic effects in the rabbit arterially perfused left ventricular wedge model [m2.6.3, RPV NDA, Report 2017N315973]. Up to 10 μM (3.7 μg/mL), RPV caused maximally 9% QT prolongation but showed no effects on the transmural dispersion of the repolarization (TDR) across the ventricular wall or any early after depolarizations (EADs), resulting in a TdP score of 0.5. Arrhythmogenic drugs have a TdP score of around 5 in this model.

The standard battery of cardiovascular safety studies indicated that RPV has the potential to inhibit partially the hERG channel at unbound concentrations of 0.111 μg/mL and higher. However, no correlates of this effect were found in subsequent studies with models that are considered more physiologically relevant. In the isolated guinea pig right atrium, no effects possibly associated with potassium current reduction were noted although the maximum unbound concentration was 3.69 μg/mL. In the anesthetized guinea pigs and dogs, no associated effects were noted at maximum plasma concentrations of, respectively, 9.2 and 2.6 μg/mL. It is to be noted that the unbound plasma concentrations must have been lower than the concentration in the hERG study due to the high plasma protein binding of RPV, in these species.

No other cardiovascular or cardio-electrophysiological parameters were affected by RPV. The relevance of the decreased vascular resistance and increased cardiac output noted in the anesthetized dog study is questionable as the vehicle PEG 400 appeared to have a clear effect on these parameters, opposite to that of RPV.

Given the results of the standard battery studies, the observed QTc prolongation in the TQT Study TMC278-C131 [see Section 2.4.4, m2.7.2] was unexpected (see Table 2.1). TMC278-C131 with 75 and 300 mg RPV q.d. for 11 days showed dose-related clinically relevant QTcF prolongation which became manifest after 11 days of treatment.

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

- Investigating the mechanism of action of the QT prolongation seen in the TQT study with a dose of 75 mg q.d. and above.
- Investigating the mechanism of action of the delayed onset of the QTc prolongation.
- Assessing the potential of RPV to induce proarrhythmic effects.

In order to find a dose that would not cause QTc prolongation, additional clinical studies were done. Pilot Study TMC278-C151 with 25 mg RPV q.d. for 11 days showed no QTcF prolongation. TQT Study TMC278 C152 with 25 mg RPV q.d. for 11 days confirmed the absence of a clinically relevant QTcF prolongation of 25 mg RPV q.d., the recommended dose [see Section 2.4.4, m2.7.2].

Table 2.1 QTcF and C_{max} Data in Clinical QTc Prolongation Studies with RPV

Study	RPV 25 mg q.d.						RPV 75 mg q.d.						RPV 300 mg q.d.					
	Day 1			Day 11			Day 1			Day 11			Day 1			Day 11		
	Δ	TC	UC	Δ	TC	UC	Δ	TC	UC	Δ	TC	UC	Δ	TC	UC	Δ	TC	UC
TQT C131							1.0	0.289	0.0009	10.4	0.636	0.0019	3.9	0.838	0.0025	23.8	1.665	0.005
Pilot C151				2.2	0.229	0.0007												
TQT C152				2.0	0.247	0.0007												

Δ: median increase from baseline of QT-interval corrected for heart rate according to Fridericia in ms; TC: total C_{max} of RPV in µg/mL; UC: unbound C_{max} of RPV = 0.3% of TC in µg/mL; TQT: thorough QTc prolongation study according to ICH E14 [see Section 2.4.4, m2.7.2].

Mechanism of QT prolongation

The potential of RPV to inhibit potassium currents I_{Kr}, I_{Ks} and I_{to} involved in the repolarization phase of the cardiac action potential is probably a contributing factor but cannot explain some aspects of the QTcF prolongation seen in TQT Study RPV-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 for the delayed onset of the QTc prolongation in the TQT study. On Day 1, 300 mg RPV q.d. did not elicit a clinically relevant QTc prolongation whereas 75 mg q.d. did on Day 11 [see Section 2.4.4, m2.7.2].

The actual unbound concentrations of RPV at which the inhibition of the potassium currents occurred in the serum-free in vitro conditions, nominally 0.111 µg/mL and higher [see m2.6.3, RPV NDA], was not determined but was probably less than the nominal concentrations, due to the adsorption of RPV to the in vitro equipment. For this reason, the margin between the unbound concentrations inhibiting potassium currents and the unbound C_{max} values associated with QTcF prolongation in TQT Study RPV-C131 (see Table 1) cannot be accurately determined but is probably significant.

Mechanism delayed onset

Several drugs (e.g., pentamidine, As_2O_3) are known to prolong the QT interval in man by interfering with hERG trafficking [Dennis, 2007] and this can be demonstrated in repeat dose animal models. The concentration-related inhibition of trafficking of the hERG channel seen in the in vitro test using RPV could contribute to the delayed onset of the QTcF prolongation seen in TQT study [see Section 2.4.4, m2.7.2]. However, it is to be noted that the in vitro inhibition of trafficking becomes manifest after hours whereas

the QTc prolongation by RPV needed days to become maximal 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. So, the trafficking inhibition seen in vitro did not occur in vivo whereas delayed onset QTcF prolongation occurred in subjects treated with 75 mg RPV q.d. in the TQT study at a median RPV C_{max} of 0.636 µg/mL. This value was similar to that in the repeat dose guinea pig study [see m2.6.3, RPV NDA]. Moreover, in a repeat dose study with RPV in cynomolgus monkeys [see m2.6.6, RPV NDA] and in the repeat dose general toxicity studies with RPV base in dogs [see m2.6.6, RPV NDA]. ECG recordings 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% close to that in man (99.7%). This value was approximately 6 times higher than that in man (0.636 µg/mL) at 75 mg RPV q.d. in TMC278-C131, after 11 days of administration (Table 1).

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

In healthy volunteers exposure to RPV increased over time until steady state was reached before Day 7 [see Section 2.4.4, m2.7.2 Clinical Study TMC278-C131]. However, this increase in exposure from Day 1 to Day 11 at the dose of 75 mg q.d. was less than the difference in exposure between 75 and 300 mg q.d. on Day 1 (see Table 1). Therefore, it cannot explain the delayed onset of the QTcF prolongation noted at 300 mg q.d.in TQT Study TMC278-C131 and likely also not that at 75 mg q.d. RPV 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 Section 3.3, m2.7.2].

Single dose quantitative whole body autoradiography (QWBA) 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 m2.6.4, RPV NDA]. The data do not indicate any particular affinity of RPV or its metabolites for heart tissue. It is to be noted that no QTcF prolongation occurred after 11 days of 25 mg RPV q.d., in the Pilot Study C151 or in the TQT Study C152.

Potential to induce TdP

The perfused rabbit ventricular wedge study indicated that RPV 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 a role as important as QT prolongation whereas the occurrence of EADs adds even more weight in the assessment of the potential to induce TdP [Liu, 2006]. RPV did not induce EADs or any other proarrhythmic effects and had no effect on TDR. So, 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 [Liu, 2006]. The margin between the nominal concentration of RPV that caused a marginal TdP score of 0.5 in the rabbit ventricular wedge model, 3.7 μg/mL, and the unbound C_{max} of 0.0019 μg/mL at 75 mg RPV q.d., after 11 days of treatment in TQT Study TMC278-C131 (Table 1), cannot be determined accurately due to the adsorption in the test system. However, it is likely that the margin is very high. The unbound fraction of the pooled median C_{max} in HIV-1-infected treatment-naïve subjects treated for 48 weeks with 25 mg RPV q.d. in Clinical Phase III studies, 0.3% of 0.13 μg/mL, 0.00039 μg/mL is again 5 times lower[see Clinical Study TMC278-C209-C215-C904-W48-AVMR, RPV NDA].

2.3.3.2. Pulmonary safety

RPV tested up to a maximum plasma concentration of 2.6 μg/mL in anesthetized dogs dosed with a single 1 hour intravenous infusion of 5 mg/kg or conscious instrumented dogs dosed with a single oral dose of 20 mg/kg showed no potential to affect respiratory parameters [see m2.6.2, RPV NDA].

It is concluded that the margin of exposure for effects on the respiratory system by RPV at a clinical dose of 25 mg q.d. is 13.

2.3.3.3. Nervous system safety

At the level of the nervous system, RPV had no effects in conscious telemetered or instrumented dogs that received a single oral dose up to 160 mg/kg rendering a C_{max} of up to 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 RPV on the nervous system [see m2.6.6, RPV NDA].

It is concluded that the margin of exposure for effects of RPV on the nervous system at a clinical dose of 25 mg q.d. is approximately 7.

2.3.4. Pharmacodynamic drug interactions

No specific nonclinical pharmacodynamic drug interaction studies have been performed with RPV. Studies conducted with RPV in combination with other antivirals are summarized in m2.7.2.4, Special Studies.

3. PHARMACOKINETICS

Extensive programs of studies investigating the absorption, distribution, metabolism and excretion have been carried out individually with DTG and RPV in animals used in toxicity studies. In general, the systemic exposure and metabolism defined in the animal species used for toxicological assessment indicates that the species used were appropriate for predicting the safety of DTG and RPV and their metabolites in humans.

3.1. Presentation Order of Pharmacokinetics Summaries

Pharmacokinetics Summary	Section
Pharmacokinetics for DTG and RPV in combination	Section 3.2.
Pharmacokinetics of DTG alone	Section 3.3
Pharmacokinetics of RPV alone	Section 3.4
Overall summary of all pharmacokinetics studies performed with DTG and RPV	Section 5, Integrated Assessment of the Fixed Dose Combination Product

3.2. DTG and RPV in Combination

The pharmacokinetics of DTG and RPV were assessed in the beagle dog, to investigate the effect of on the bioavailability of either DTG or RPV, when co-administered in a number of FDC tablet formulations, in the fed and fasted states [Table A3.1, m2.6.5, Reports 2015N239161, 2015N239160, 2015N228863]. Generally, there was no notable (i.e., >2-fold) difference in exposure to either DTG or RPV with the various FDC formulations. However, there was a notable increase in DTG and RPV systemic exposure in the fed state (compared to fasted state dosing).

Additionally, clinical bioequivalence was confirmed in terms of both DTG and RPV for DTG/RPV FDC compared with separate tablet formulations of DTG and RPV when administered after a moderate fat meal [see Section 2, m2.5].

3.3. DTG

A summary of each pharmacokinetics study and its findings is presented in m2.6.4, DTG NDA. A listing of each pharmacokinetics study conducted, tabular summaries and the findings are presented in m2.6.5, DTG NDA. All pharmacokinetics study reports are available in m4.2.1, Nonclinical Study Reports.

3.3.1. Analytical methods and validation

In pharmacokinetic and toxicity studies, plasma DTG concentrations were measured following protein precipitation with chiral or achiral liquid chromatographic tandem mass spectrometric (LC/MS/MS) methods. For toxicity and human studies, the chiral and achiral methods used for analysis were fully validated across each calibration range. All methods and limits of quantification were adequate with regard to specificity and sensitivity to support the pharmacokinetic analyses of DTG.

In investigations where [^{14}C]-DTG was used, determination of the radioactivity in in vitro or in vivo biological samples was carried out by either direct liquid scintillation counting (LSC) or by LSC following combustion of the sample. For analysis of radioactivity concentrations in tissues, quantitative whole body autoradiography was used. The profiling and identification of metabolites of DTG was performed using LC-MS^n. Nuclear magnetic resonance (NMR) methods were used to confirm structures not confirmed by mass spectrometric methods.

3.3.2. Absorption and pharmacokinetics

The nonclinical pharmacokinetics of DTG are characterized by low plasma clearance and low volume of distribution. Absorption was rapid with high oral bioavailability from a solution formulation. In repeat oral administration studies, systemic exposure to DTG was dissolution or solubility limited leading to an increase that was less than proportional with dose.

3.3.2.1. Single dose

Intravenous: Following a single intravenous administration, DTG exhibited low plasma clearance (<15% liver plasma flow) in the rat, dog and monkey [m2.6.5, DTG NDA, Reports RH2007/00101, RH2007/00102 and RH2007/00103]. The low steady state volume of distribution reflects the restrictive high protein binding of the compound (Section 3.2.3.1). The terminal half-life in rats, dogs, and monkeys was 5.2 to 6.2 hours.

Oral: DTG absorption from an oral solution was rapid, reaching peak plasma concentrations within 2 hours with high oral bioavailability (76 to 87%) in fasted rats and monkeys [m2.6.5, DTG NDA, Reports RH2007/00101 and RH2007/00103]. When DTG was administered as a suspension, the increase in systemic exposure (C_{max} and AUC_{0-t}) was less than proportional to the increase in dose. The oral bioavailability of DTG from a suspension formulation was lower (bioavailability range of 25% to 52%) and suggested that the absorption is limited by dissolution rate or solubility.

Administration of DTG with food to rats reduced exposure whereas in humans exposure was increased [see Section 2, m2.7.2].

3.3.2.2. Repeat dose toxicokinetics

Oral: The repeat dose toxicokinetics of DTG were assessed as part of general toxicity, reproductive toxicity and juvenile toxicity studies. A comparison of systemic exposure values (C_{max} and AUC_{0-24}) for DTG is presented in Table 4.2.

The increase in systemic exposure (C_{max} and AUC_{0-24}) to DTG was less than proportional with the increase in dose during repeated oral administration toxicity studies in mice, non-pregnant rats, rabbits and monkeys. Differences (>2-fold) in systemic exposure between single and repeated administration, regardless of pregnancy status, or between the sexes were generally not observed.

Higher systemic exposure to DTG was observed in pre-weaning rat pups (Day 13 post partum) compared to juvenile rats on Day 32 post partum. Because DTG is primarily metabolized by uridine glucuronosyl transferase (UGT) in the rat (Section 4.2.7), this difference reflects the early differential expression of UGT in the rat [Kishi, 2008; Saghir, 2012; de Zwart, 2008]. No apparent sex-related differences (>2-fold) in systemic exposure were observed in juvenile rats.

3.3.3. Distribution

DTG has high passive membrane permeability, is highly protein bound and is widely distributed. DTG crosses the placental barrier and is secreted into the milk of lactating rats.

3.3.3.1. Protein binding and blood cell association

The in vitro protein binding of DTG was high (≥99%) across species (rat, monkey and human) and similar to an ex-vivo assessment (>99%) in plasma from healthy human subjects [m2.6.5, DTG NDA, Reports RH2007/00106 and 2011N119355]. The association of DTG-related material with blood cellular components was minimal [m2.6.5, DTG NDA, Reports RD2009/00562, RD2008/00108, CD2008/00195 and RD2008/01300] [see m2.7.2, DTG NDA].

3.3.3.2. Efflux-mediated transport and cell membrane permeability

In vitro, DTG was a substrate for the human efflux transporters P-glycoprotein (P-gp) and human breast cancer resistance protein (BCRP). DTG was determined to have high passive membrane permeability (333 nm/s at pH 7.4). The absorptive membrane permeabilities also were high in the presence of FaSSIF at pH 7.4 and pH 5.5 ($P_{7.4[abs]}$ value of 253 nm/s and a $P_{5.5[abs]}$ value of 265 nm/s, respectively) [m2.6.5, DTG NDA, Report RD2008/00360]. Based on solubility and permeability determinations, DTG sodium is classified as a Biopharmaceutics Classification System (BCS) Class 2 drug.

3.3.3.3. Tissue distribution

After a single oral dose of [^{14}C]-DTG, radioactivity was widely, but not highly distributed in a similar pattern between male Lister Hooded partially pigmented rats and pregnant Sprague Dawley rats. Radioactivity in tissues generally peaked 4 to 6 hours post dose with concentrations typically less than those in blood [m2.6.5, DTG NDA, Reports CD2008/00195 and 2012N137348]. The concentration of radioactivity in the brain was low (~2% of the blood radiocarbon concentration) due in part to restrictive protein binding. By 28 days post dose, only bone and pigmented skin contained quantifiable concentrations of radioactivity. Radioactivity was not associated with melanin in the uveal tract, lowering the concern for a phototoxicity liability (Section 4.2.10). DTG crossed the placental barrier but appeared to exert no adverse effects on fetal development [see m1.14.1 (US) or m1.3 (EU)]. Radioactivity rapidly equilibrated to fetal tissues with fetal tissue to fetal blood ratios generally higher than corresponding maternal tissue to blood ratios. DTG concentrations in fetal bone marrow exceeded those in fetal blood.

In lactating rats at 10 days post partum, radioactivity was detected in milk at concentrations typically higher than blood, with unchanged DTG constituting most (97% to 83%) of the drug-related material [m2.6.5, DTG NDA, Report 2012N132387]. These results suggested that pre-weaned pups were exposed to DTG (Section 4.2.7) by nursing in the pre- and post-natal development study.

3.3.4. Metabolism

The comparative biotransformation pathways between animals and humans are presented in Figure 3. The predominant circulating component in the nonclinical species and humans is unchanged DTG and there were no human plasma metabolites representing >10% total drug-related material - the threshold for further consideration in nonclinical species [ICH M3 (R2)]. The main metabolic route in each species and humans was by conjugation to form the ether glucuronide (M3). The drug metabolism in animal species used for toxicology testing was relevant to the safety assessment of DTG for human use.

3.3.4.1. In vitro biotransformation

The in vitro metabolic turnover of DTG was low (<10%), indicating low intrinsic clearance consistent with the low plasma clearance. The primary biotransformation common to all species was glucuronidation to form the ether glucuronide (M3) that also was observed in vivo. Other common metabolic products included a glucose conjugate (M2) and an N-dealkylated product (M1) [m2.6.5, DTG NDA, Reports RH2007/00076, RD2007/01557, RD2007/01496 and RH2007/00058]. The generation of a glutathione or cysteine conjugate through oxidative defluorination and microsomal binding with rat, monkey and human liver microsomes suggested evidence for the formation of an electrophilic metabolic intermediate by bioactivation in vitro [m2.6.5, DTG NDA, Reports RH2007/00058 and RD2007/01557]. However, in vivo in mice, rats, monkeys and humans, these metabolic products have represented only a small fraction of the metabolic clearance.

No notable metabolic conversion of DTG to any of its possible stereoisomers occurred in vitro following incubations of DTG with cryopreserved rat, dog, monkey and human hepatocytes [m2.6.5, DTG NDA, Report RH2007/00105].

3.3.4.2. In vivo studies

In vivo, absorbed [^{14}C]-DTG was extensively metabolized in male and female mice, rats and monkeys. A comparative metabolic summary of the products identified in the nonclinical species with products identified in humans is presented in Figure 3 [m2.6.5, DTG NDA, Reports RD2008/00220, RD2008/00899 and RD2009/00723; see Section 3.3, m2.7.2]. Metabolic profiles in nonclinical species were qualitatively similar to humans, with adequate coverage for the circulating human metabolites in at least one nonclinical species.

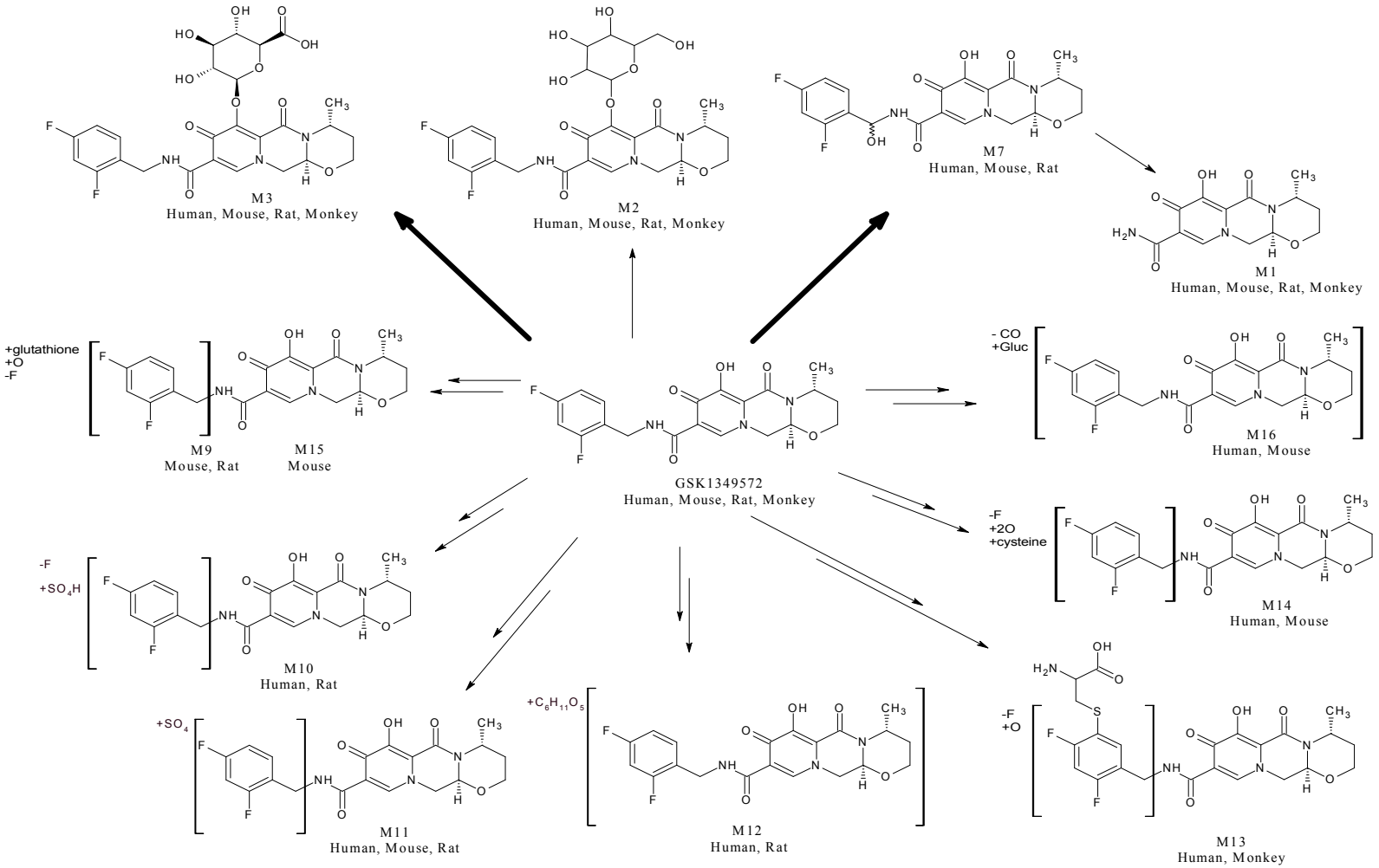
Plasma metabolic profile

DTG was the predominant component in plasma of mice, rats, monkeys and humans with the glucuronide as the principal metabolite. No metabolite was present in the plasma at concentrations greater than 10% of parent or drug-related material. In humans, the steady state plasma metabolic profile of DTG was similar to the single dose metabolic profile, indicating data obtained after single dose administration was an adequate predictor of the profile at steady state [see Section 3.3, m2.7.2]. Based on the kinetics of DTG in animals, the systemic exposures to DTG and circulating metabolites found in the nonclinical metabolism studies adequately reflected exposures in the toxicity studies. No disproportionate human metabolites were noted.

Biotransformation

The predominant biotransformation product in mice, rats and humans was an ether glucuronide (M3). The glucuronide metabolite was formed in approximately equal proportions with a glucose conjugate (M2) in monkeys. These conjugated metabolites, M2 and M3, are not pharmacologically active because they disrupt the two-metal binding capability of the carbamoyl pyridone motif of DTG thereby completely abrogating any antiviral activity resulting from the active site binding to the integrase enzyme. Although these conjugates were the primary constituents of the drug-related material in bile of the nonclinical species, they were not observed in the feces of animals or humans. Thus, these DTG conjugates are likely deconjugated in the intestine by host or bacterial enzymes after secretion in the bile to reform DTG. In animals, fecal metabolites were not quantifiable, but in human fecal samples, an N-dealkylation product (M1) and a product of oxidative defluorination with cysteine addition (M13) was quantifiable at less than 2% of the dose.

Figure 3 Comparative Metabolic Profile of DTG Between Nonclinical Species and Humans



Key: Bolded arrows indicate the primary metabolic products in humans (M3 the predominant product, M7 a notable metabolite).

DTG constituted a very small percentage of drug-related material in the urine (≤0.7%) and bile (≤0.3%) in mice, rats and monkeys or in urine of humans. The primary components of rat urine, but represented to a smaller extent in mouse and monkey urine, were products of oxidation at the benzylic carbon (M7) and its hydrolysis to an N-dealkylation product (M1). These components also represented notable products in human urine. Following co-administration of DTG and efavirenz (an approved NNRTI) to healthy human volunteers, an increase of DTG glucuronide (M3) was noted as compared to the metabolic profile of DTG given alone.

In mice, rats, monkeys and humans, the oxidative defluorination with glutathione or cysteine addition was present, indicating the formation of an electrophilic arene oxide intermediate. Except in mice, these products were a small fractional part of the overall clearance.

Following repeat oral administration of DTG for 10 days to male and female juvenile rats or to healthy human volunteers, no evidence for the in vivo metabolic conversion of DTG to any of its stereoisomers was observed [m2.6.5, DTG NDA, Report RD2010/00173; and Clinical Study Report RD2008/00860].

No notable qualitative differences in the metabolic profile between male and female animals were observed.

3.3.5. Excretion

Fecal excretion of radioactivity consisted primarily of unchanged DTG and was the predominant route of elimination of administered radioactivity in all species [m2.6.5, DTG NDA, Reports RD2008/00108, RD2008/01299, RD2008/01300 and RD2009/00562]. Following oral administration of [^{14}C]-DTG, urinary excretion of radioactivity was greater in humans (approximately 32%) than in animals (≤6%) [see Section 3.4, m2.7.2], which is consistent with the hypothesis of a higher molecular weight threshold for biliary secretion in humans [Yang, 2009].

Excretion of administered radioactivity was essentially complete in all species and was eliminated quicker in animals than in humans, consistent with the longer half-life and gastrointestinal transit time in humans. The radiolabel location was metabolically stable with no notable sequestration or covalent binding of DTG to plasma or excreta. Biliary excretion in animals accounted for the major portion of the absorbed dose and represented the predominant excretion route for DTG glucuronide. Thus, DTG conjugates are deconjugated in the intestine, after secretion in the bile, to reform DTG allowing it to be available for enterohepatic circulation.

3.3.6. Pharmacokinetic drug interactions

No nonclinical studies have been performed specifically to evaluate potential interactions with drugs that may be co-administered with DTG. However, a series of in vitro studies have been conducted to help evaluate the mechanisms and drug interaction potential of DTG, and, except for sensitive OCT2 substrates (e.g., dofetilide, pilsicainide), dolutegravir has a low propensity to cause drug interactions based on in vitro results.

DTG is a substrate of P-gp and BCRP, but inhibition of these transporters is unlikely to affect absorption of dolutegravir to a clinically relevant extent [see m2.7.2, Section 1.2].

3.3.6.1. Potential effect of co-administered agents on DTG

In vitro and in vivo, DTG is primarily metabolized by UGT1A1 with a notable contribution from CYP3A4. UGT1A3 and 1A9 were minor glucuronidation pathways [m2.6.5, DTG NDA, Reports RD2008/00373 and RD2008/01339]. Therefore, drugs that are strong inducers of UGT1A1 or CYP3A4 may decrease DTG plasma concentrations. Although drugs that inhibit UGT1A1 and CYP3A4 may increase DTG plasma concentrations, based on the clinical interaction study with atazanavir, a potent UGT1A1 and CYP3A4 inhibitor, any increases in DTG concentrations are not expected to be clinically meaningful. Although DTG is a substrate for efflux transporters, no notable effect on DTG pharmacokinetics was observed in humans following co-administration with lopinavir/ritonavir, inhibitors of the efflux transporters P-gp and BCRP [see m2.7.2, DTG NDA]. These data, together with the rapid absorption in humans, low to moderate pharmacokinetic variability and high intrinsic permeability suggests a low potential for drug interactions with BCRP and P-gp inhibitors that would result in clinically significant changes to DTG exposure.

3.3.6.2. Effect of DTG on co-administered agents

In vitro, DTG was noted to have little or no inductive effects on the human Pregnane X receptor (PXR) on CYP1A2, 2B6 or 3A4 mRNA (as determined by the increase in mRNA relative to vehicle control). DTG demonstrated little or no inhibition (IC_{50} values >30 μM) in vitro on the transporters BCRP, bile salt export pump (BSEP), multidrug resistance protein (MRP) 2, MRP4, organic anion transporting polypeptide (OATP) 1B1, OATP1B3, organic cation transporter (OCT) 1, OCT3, Equilibrative Nucleoside Transporter (ENT) 4 and P-gp, or the enzymes CYP1A2, 2A6, 2B6, 2C8, 2C9, 2C19, 2D6, 3A4, UGT1A1 or 2B7, demonstrating a low propensity to cause drug interactions through modulation of these systems. DTG glucuronide (M3) did not inhibit MRP2, thus, inhibition of biliary clearance of bilirubin glucuronides or glucuronide conjugates of co-administered drugs is not expected. In vitro DTG did not alter the paracellular permeability of the probe substrate Lucifer yellow, nor did it alter transport of metformin across the Caco2 cells up to concentrations of 100 μM, suggesting that DTG is unlikely to alter the permeability of these compounds across the intestinal lumen.

As a weak inhibitor of UGT1A1, DTG has the potential to interfere with the conjugation of bilirubin which could result in a mild increase in total or unconjugated bilirubin on prolonged treatment with DTG [see m2.6.5, DTG NDA, Reports RH2007/00104]. Because bilirubin has low solubility and low permeability, it is transported to the UGT1A1 enzymatic site by hepatic uptake transporter OATP1B1. Since DTG has high permeability and does not rely on transport to the enzyme site, this favors rapid access by DTG to UGT1A1, although the affinity of bilirubin for UGT1A1 is higher than that of DTG.

In vitro, DTG inhibited OAT1 and OAT3 with IC_{50} values of 2.12 μM and 1.97 μM, respectively; however, in vivo, no notable changes in plasma concentrations of the OAT substrates tenofovir [see Section 3.6.1.2, m2.7.2] or p-aminohippurate [see Section 2.3.4, m2.7.2, ING114819] were observed in healthy subject Phase 1 studies. Multidrug resistance associated protein (MRP) 2 and MRP4 are anion transporters responsible for the transport of anions (e.g., tenofovir) from the renal tubule to the urine with MRP4 as the predominate transporter for tenofovir excretion. DTG did not inhibit MRP2 and weakly inhibited MRP4 (IC_{50} value of 84 μM) with a 50-fold unbound C_{max} value less than the K_i estimate. Furthermore, polymorphic MRP4 that decreased tenofovir renal clearance by 15% also increased plasma tenofovir concentrations by 32% [Kiser, 2008], which was not observed in the drug interaction study assessing the impact of DTG on tenofovir PK. In addition, a physiological based pharmacokinetic (PBPK) mechanistic kidney model (Simcyp v,12 R1) developed for steady state concentrations of tenofovir (300 mg once daily) predicts that co-administration of DTG at 50 mg once daily would result in a minimal decrease in tenofovir renal clearance with no notable change in tenofovir exposure within the proximal tubule cells of the kidney. Based on these collective data, no clinically significant interaction with tenofovir by DTG at the renal tubule is expected.

In vitro, DTG inhibited the basolateral renal organic cation transporter 2 (OCT2; IC_{50} = 1.9 μM) and the renal apical transporters, multidrug and toxin extrusion transporter (MATE) 1 (IC_{50} = 6.34 μM) and MATE2-K (IC_{50} = 24.8 μM) [see m2.6.5, DTG NDA, Reports 2010N104937 and 2013N161621] which provides a mechanistic basis for the non-pathological mild serum creatinine increases observed in clinical studies. Because DTG inhibits OCT2, but only weakly OCT1, and both OCT1 and OCT2 are equally expressed in rat proximal tubules [Tahara, 2005], this effect on creatinine was not observed in rats. These in vitro results indicate caution should be used due to the potential for a drug interaction in vivo when DTG is co-administered with cationic compounds that have a narrow therapeutic index drugs and in which a significant part of their clearance is by renal proximal tubule secretion by OCT2. DTG is contraindicated for co-administration with the OCT2 substrates dofetilide and pilsicainide because they possess narrow therapeutic indices that present the potential for toxicity due to higher exposure [m2.7.2, Section 3.6.1.1]. DTG has a low potential to affect the transport of MATE2-K substrates with a 50-fold unbound Cmax value less than the K_i estimate.

3.4. RPV

A summary of each pharmacokinetics study and its findings is presented in m2.6.4, RPV NDA. A listing of each pharmacokinetics study conducted, tabular summaries and the findings are presented in m2.6.5, RPV NDA. All pharmacokinetics study reports are available in m4.2, Nonclinical Study Reports. The studies conducted are listed in m2.6.5, RPV NDA.

3.4.1. Analytical methods and validation

RPV and/or its metabolites were determined in various in vitro and biological samples (plasma, urine, bile and feces).

An LC-MS/MS method was validated for the determination of RPV in mouse, rat, rabbit, dog and monkey ethylenediamine tetra-acetic acid plasma and in dog heparin plasma. The stability of RPV was assessed in the biological matrices at different conditions. RPV was sufficiently stable under the conditions tested which allowed the use of the developed assays under normal laboratory testing conditions.

The tissue distribution of ^{14}C-RPV base was studied by QWBA. The total radioactivity in biological samples was measured by liquid scintillation counting. The metabolites were identified by co-chromatography with authentic substances using different high performance liquid chromatography methods and detection systems such as radiodetection and tandem mass spectrometry (LC-MS/MS).

3.4.2. Absorption and pharmacokinetics

After repeated administration of RPV for 11 days in healthy subjects at 75 and 300 mg q.d., there was no disproportionate increase in exposure of any of the relevant metabolites compared to the parent compound exposure [see Section 2.4.4, m2.7.2, Clinical Study C131].

In nonclinical species after oral administration of RPV base, the absolute oral bioavailability of RPV was 32%, 54%, 31% and 24% to rats, rabbits, dogs and monkeys, respectively. Adding citric acid (CA) in the formulation administered to rats and dogs usually increased the exposure showing that the absorption of RPV is pH-dependent in these species as in humans [see Section 3.6.2.1, m2.7.2].

After oral administration of RPV (base and HCl forms), peak plasma concentrations were generally reached rapidly followed by a decline at lower dose levels, whereas at higher dose levels the plasma profiles showed a plateau until at least 8 hours in all species.

Across the dose range studied and species including humans, plasma concentrations of RPV increased dose-proportionally or more often less than dose-proportionally, due to low solubility. At very high dose levels in animals, no further increase in exposure was seen.

There were no major gender differences in pharmacokinetics in mice at low dose levels and in dogs whereas in mice at high dose levels and in rats, exposures in females were higher than in males (up to 2-fold in mice and 2- to 4-fold in rats). In rats, the gender effect was less pronounced after administration of the HCl salt particularly at Day 1 in the carcinogenicity study. In humans, no major difference in pharmacokinetics was noticed between males and females [see Section 3.5.2, m2.7.2].

In mice, exposure after repeated administration was comparable to or slightly lower than at Day 1 in males and females (only at 20 mg/kg/day). In females, at higher dose levels (60 to 320 mg/kg/day) exposure after repeated administration was higher (up to 2.2-fold) than at Day 1. In rats, after repeated administration of the base, systemic exposure increased slightly (up to 1.6-fold) in comparison to Day 1 in females while in males particularly at high dose levels, exposure decreased slightly (up to 40%). After repeated administration of the HCl salt, the decrease in exposure seen in males was more pronounced (up to 76%) compared to base, particularly in the carcinogenicity study, while no further decrease was observed between Week 27 and Week 39. In female rats no clear tendency for time-dependent pharmacokinetics was seen. In rabbits, the exposures obtained after repeated administration were similar to those obtained at Day 1. In dogs, after repeated administration (base and HCl forms), exposure increased after repeated administration as compared to Day 1 mainly due to the long elimination half-life ($t_{½}$ = 31 hours) of RPV. In monkeys, after repeated administration of the HCl salt, exposure tend to increase but due to the high inter-individual variability it was difficult to reach firm conclusions. In humans, an increase in exposure was seen after repeated administration mainly due to the long-half life ($t_{½}$ = 45 to 50 hours) [see Section 3.4.2, m2.7.2]. In the species (rats and dogs) where the base and the HCl salt were administered, the exposure was comparable at low dose in rats.

At high dose in rats, exposure after administration of the base was higher (1.7- to 2.7-fold) than that after administration of the HCl salt. In dogs no clear difference was noted at the 2 dose levels tested. However, inter-individual variability was high in dogs especially after administration of the HCl salt.

3.4.3. Distribution

3.4.3.1. Plasma protein binding

The plasma protein binding of RPV was studied in vitro by equilibrium dialysis in mice, rats, rabbits, guinea pigs, dogs, monkeys and healthy adult male subjects [see m2.6.5, RPV NDA].

RPV was extensively bound to plasma proteins in all species and the plasma protein binding was found to be concentration-independent. Plasma protein binding values ranged between 99.08% and 99.97%. RPV was extensively bound to human albumin (99.5% at the physiological protein concentration of 4.3% and irrespective of the RPV concentration) and to lesser extent to α_1-acid glycoprotein (48.8% at the physiological protein concentration of 0.07% and a RPV concentration of 1 μg/mL). The rank order of blood to plasma concentration ratio in all species was monkey > dog > rat > man > guinea pig > rabbit > mouse and ranged from 0.96 to 0.58. The distribution of RPV to red blood cells is limited in all species.

3.4.3.2. Tissue distribution

The tissue distribution of RPV in rats has been studied using QWBA following single oral administration of ^{14}C-labelled RPV (40 mg/kg). Studies were performed in

pigmented Long Evans rats and pregnant female Sprague Dawley rats. Distribution to placenta and fetuses was examined in the latter study [see m2.6.5, RPV NDA].

In rats, tissue distribution of ^{14}C-RPV base and its metabolites after single dose was rapid and extensive. The highest concentrations of radioactivity were measured in the liver, adrenal gland, brown fat and kidney. There was no evidence of undue retention and there were no indications of irreversible binding of RPV and its metabolites to melanin. In pregnant rats, there was distribution of ^{14}C-RPV base to the placenta and the fetus. Total radioactivity (TR) exposure values in the placenta and in whole fetus were 0.94- and 0.64-fold those of maternal blood, respectively, suggesting that the placenta presents a partial barrier for RPV and/or its metabolites.

The transepithelial permeability of RPV was intermediate in human colon carcinoma-derived Caco-2 cells. Passive transcellular diffusion is proposed as a mechanism for RPV intestinal absorption. RPV was not a substrate of P-glycoprotein (P-gp). RPV showed P-gp inhibitory properties with an apparent 50% inhibitory concentration (IC_{50}) of 9.2 μM (3.4 μg/mL). Inhibition of transepithelial permeation of P-gp substrates by RPV, therefore, cannot be excluded. However, for most drugs it is unlikely that any effect on intestinal absorption will be clinically relevant [see m2.6.4, RPV NDA].

3.4.4. Metabolism

3.4.4.1. In vitro biotransformation

In vitro, RPV metabolism was studied in hepatocytes and liver subcellular fractions (microsomes and 12,000 g supernatant fractions) of mice, rats, guinea pigs, rabbits, dogs, monkeys and humans. In each species, a large number of metabolites were detected. The main metabolic pathways proposed on the basis of in vitro studies were consistent with those observed in vivo. For the minor pathways such as the release of the nitrile group followed by reduction/oxidation, resulting in the formation of an alcohol metabolite (M31) and a carboxylic acid metabolite (M30), they were not observed in vitro in rodents but were observed in vivo.

3.4.4.2. In vivo biotransformation

RPV is metabolized by Phase I and Phase II pathways including aromatic and aliphatic hydroxylation, glutathione conjugation, N-glucuronidation and nitrile split-off followed by reduction/oxidation, whether or not in combination with secondary pathways like glucuronidation, dehydration and catabolism of the glutathione conjugate. In mice, oxidation of RPV and to a lesser extent glutathione conjugation were the predominant pathways. In rats the glutathione conjugation pathway was predominated whereas in dog and man, oxidation of RPV was the predominant one. No unique human metabolites were observed (Figure 4 and Table 3.1). In plasma of animals and human, unchanged RPV was more abundant than all metabolites combined.

Figure 4 In Vivo Metabolic Pathways of RPV in Animals and Humans

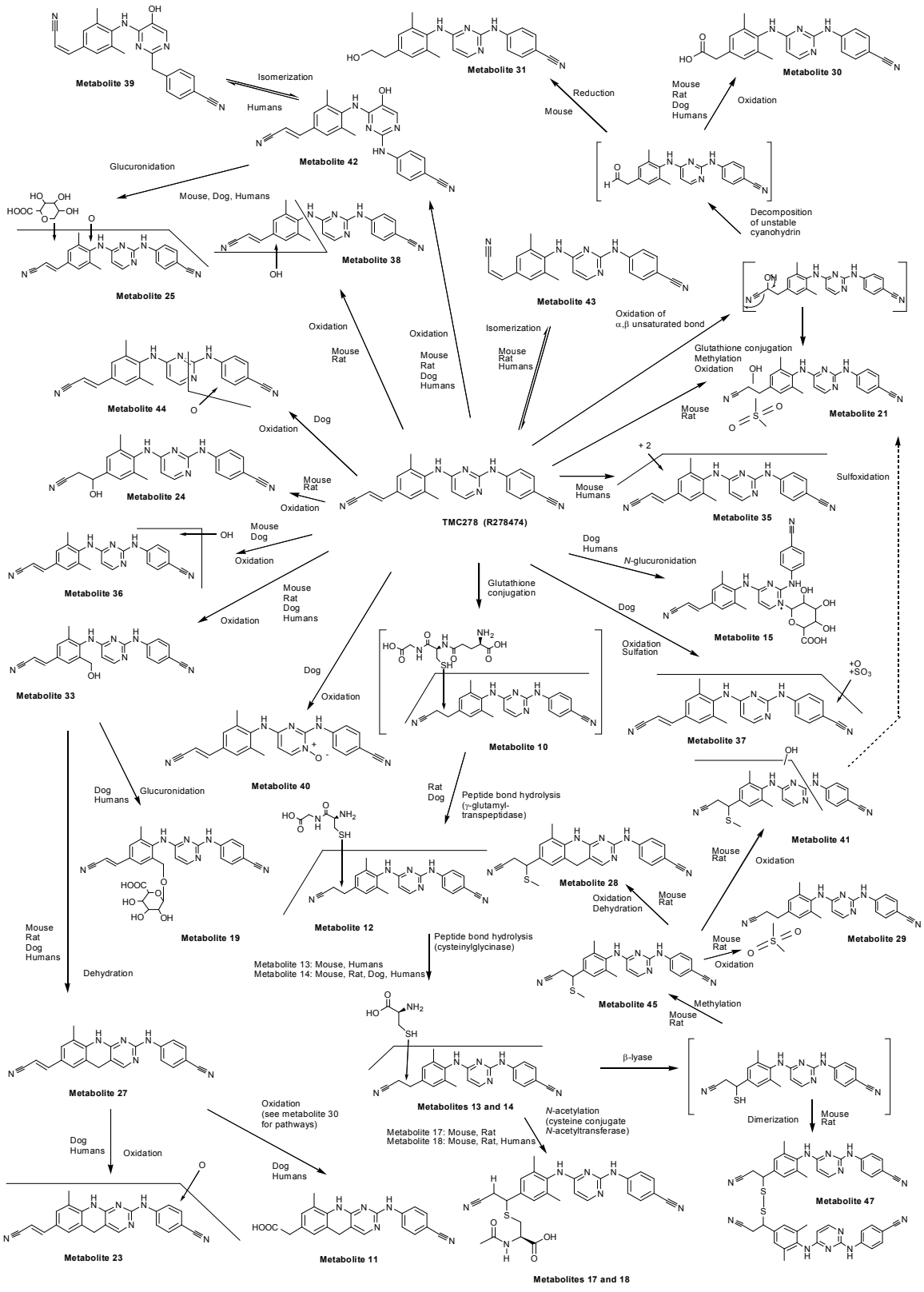


Table 3.1 Total Percentage of the Administered Dose Metabolized per Major Pathways in Human and its Corresponding Percentages in Mice, Rats and Dogs After Oral Administration of ^{14}C-RPV Base

Metabolites	Mice		Rats	Dogs	Human
	20 mg/kg	320 mg/kg	40 mg/kg	5 mg/kg	150 mg
5-Hydroxyl RPV at the pyrimidinyl moiety (M42)	18 – 26 [a]	9.2 – 13[a]	2.8-3.6[b]	5.3	16
Hydroxymethyl of RPV (M33)	0.5 – 0.7	1.3 – 1.0	0.54-0.54	8.7 (traces in plasma)	3.0 (seen in plasma)
Carboxylic acid metabolite of the cyanoethenyl moiety (M30)	1.6 – 3.1	1.5 – 1.2	0.47 - 0.05	3.1[c]	2.7
Unknown (M35)	< 0.2	< 0.2	-	-	2.2
Tricyclic metabolite (M27) and carboxylic metabolite of M27 (M11)	0.3 - <0.2 [d]	<0.2 – 0.1 [d]	0.99–1.60 [f]	3.1[g] (traces of M27 in plasma)	2.2 (M27 seen in plasma)
Glutathione derived conjugates (M13, M14, and M18)	9.6 – 7.9[e]	8.7 – 7.3 [e]	0.03 - 0.46 [i]	< 0.08 [h]	1.2
N-glucuronide of RPV (M15)	-	-	-	traces in plasma	0.6 (seen in plasma)
Unchanged compound	8.8 – 7.9	33 – 34	47 - 43	45	26

[a] co-eluted with M41, M42 was estimated at 13.9-16.6% (20 mg/kg) and at 5.9-8.0% (320 mg/kg); [b] co-eluted with M41; [c] co-eluted with M48; [d] co-eluted with M28 and M29; [e] includes M17; [f] co-eluted with M24, M28 and M29; [g] including M23; [h] each of them; [i] M14 co-eluted with M12; In mice and rats, the first number in each box is male data and the second one female data

3.4.5. Excretion

Some differences were seen in blood clearance (Cl_b) across species. In rats, the Cl_b of RPV was moderate whereas in rabbits, dogs and monkeys the Cl_b was very low compared to the hepatic blood flow. The volume of distribution at steady-state (Vd_{ss}) was larger in rats, dogs and monkeys and very low in rabbits. Large differences in plasma half lives were also observed between the rat (4.4 hours), rabbit (12 hours), dog (31 hours) and monkey (7.1 hours) after intravenous administration. In humans, the terminal elimnation half-life was longer (45-50 hours) after oral administration.

The routes and extent of RPV excretion were studied after a single oral administration of ^{14}C-RPV base in CD 1 mice (20 and 320 mg/kg), Sprague-Dawley rats (40 mg/kg), dogs (5 mg/kg) and humans (150 mg). In all animal species, the predominant route of ^{14}C-RPV excretion was via feces and amounted to 87-96%, 93%, 95% and 85% of the administered radioactive dose in mice, rats, dogs and humans, respectively. ^{14}C-RPV was mainly excreted in feces as unchanged RPV in mice (33-34% at 320 mg/kg), in rats (43-47%) and in dogs (43%) by 48 hours after dosing. Only, in mice at 20 mg/kg, one

metabolite M42 was the most abundant in feces. Urinary excretion of radioactivity was less than 4.2% in mice, rats and dogs but was slightly higher, 6.1%. in humans. In all species including humans the amount of unchanged RPV in urine was negligible. Therefore, the renal clearance of RPV is negligible. RPV was also excreted in the bile in rats (18% and 25% of the administered radioactivity, in restrained and nonrestrained animals, respectively). The amount of unchanged RPV excreted in bile was negligible (about 0.2 % within 24 hours).

3.4.6. Pharmacokinetic drug interactions

No nonclinical studies have been performed specifically to evaluate potential interactions with drugs that may be co-administered with RPV. However, a series of in vitro studies have been conducted to help evaluate the mechanisms and drug interaction potential of RPV. The clinical drug-drug interaction studies are described in detail in the Clinical Pharmacology summary see Section 2.4.3, m2.7.2].

3.4.6.1. Potential Effects of co-administered agents on RPV

In vitro, the CYP3A4 isoenzyme played a major role in the biotransformation of RPV. Therefore, some effects of drugs modulating CYP3A4 enzyme activity on plasma concentrations of RPV were expected in humans. Such effects were seen in drug-drug interaction trials with CYP3A4 inducers such as rifampicin and rifabutin, which both decreased the exposure of RPV and CYP3A4 inhibitors such as ketoconazole and ritonavir-boosted protease inhibitors, which increased the exposure of RPV [see Section 2.4, m2.7.2].

3.4.6.2. Effect of RPV on co-administered agents

Ex vivo induction studies in rodents showed that RPV is an inducer of the cytochrome P450 (CYP)3A-family (up to 1.7-fold in mice and up to 6-fold in rats) and CYP4A-family (up to 25-fold in mice and up to 4.7-fold in rats). Additionally, RPV induced uridine diphosphate-glucuronosyltransferase (UDP-GT) activity in mice (up to 2.3-fold) and to a lesser extent in rats (up to 1.3-fold only at high dose in males). In dogs, treatment with RPV did not result in any enzyme induction. The main objective to generate these data was to explain the toxicity findings in animals. The findings in carcinogenicity studies could be associated with the observed enzyme induction.

In vitro, RPV was an inhibitor of CYP3A4 (IC_{50}> 4.2 µM (1.5 µg/mL) and CYP2C19 (IC_{50} < 0.06 µM (0.021 µg/mL). Further in vitro data indicated a possible inhibitory effect of RPV on the metabolism of substrates of CYP3A4 e.g. clarithromycin (IC_{50} = 2 µM (0.72 µg/mL), norethindrone (IC_{50} = 3.9 µM (1.4 µg/mL) and sildenafil (IC_{50} = 1.4 µM (0.5 µg/mL), and on the metabolism of omeprazole (IC_{50} = 12 µM (4.3 µg/mL), substrate of CYP3A4 and CYP2C19. However, no impact was expected in the clinic taking into account the mean C_{max} of 0.13 µg/mL (Week 4-8) obtained in HIV-infected treatment-naïve patients at 25 mg q.d, the recommended dose. In vivo, some drug-drug interaction studies were performed using substrates of CYP3A4 (atorvastatin, omeprazole (omeprazole sulfone pathway) and norethindrone) and substrate of CYP2C19

(omeprazole (hydroxyomeprazole pathway) [see m2.6.5, RPV NDA]. No increase in exposure of these 3 compounds with RPV was observed [see Section 2.4.3, m2.7.2].

In addition, in vitro, RPV was a moderate inducer of CYP3A4 and CYP2C19 activities. In the clinic a modest decrease in exposure of atorvastatin, norethindrone and CYP2C19-mediated metabolism of omeprazole was observed at high dose of RPV [150 mg q.d; see Section 2.4.3, m2.7.2]. RPV at the recommended dose of 25 mg q.d. does not inhibit or induce CYP3A4 and is unlikely to result in clinically relevant interaction for CYP2C19.

In vitro results on CYP2E1 inhibition by RPV are considered not relevant due to contradictory results. In one study, RPV showed to be a strong inhibitor of CYP2E1 with chlorzoxazone (IC_{50} = 0.03 µM (0.010 µg/mL) as a substrate but not with lauric acid as a substrate. However, in another in vitro drug-drug interaction study no inhibition of chlorzoxazone by RPV was observed. In addition, no conclusion could be drawn on the induction effect of RPV on CYP2E1. In vivo, no impact of RPV at a dose of 150 mg q.d was seen on the chlorzoxazone exposure.

In vitro, RPV is an inhibitor of CYP2C8 and CYP2C9 with a K_i of 10 µM (3.7 µg/mL) and 1.7 µM (0.62 µg/mL), respectively. Taking into account the mean C_{max} of 0.13 µg/mL obtained in HIV-infected subjects, inhibition of CYP2C8 and CYP2C9 by RPV is not expected.

For other CYPs (CYP1A1, CYP2A6 and CYP2D6), taking into account the mean C_{max} of 0.13 µg/mL, inhibition by RPV is unlikely. RPV might be a very weak inducer of CYP1A2 and CYP2B6.

Inhibition of OCT2 transporter by RPV was evaluated in vitro, in transfected Chinese hamster ovary (CHO) cells. The in vitro IC_{50} for inhibition of OCT2 by RPV was 5.46 µM (2.0 µg/mL) [see m2.6.5 RPV NDA]. Given the fact that RPV plasma concentrations are low as compared with the IC_{50} (C_{max}/IC_{50} = 0.07), the clinical relevance of this finding is not clear. The inhibition of MATE-mediated transport by RPV was investigated in vitro in CHO cells overexpressing MATE-1 and MATE-2K (FK10420). The uptake of ^{14}C-Tetra Ethyl Ammonium (TEA) was inhibited by RPV with an IC_{50} value of 7.51 µM (2.75 µg/mL) for MATE-1 and of < 0.05 µM (< 18 ng/mL) for MATE-2K [see see m2.6.5 RPV NDA]. In conclusion, the effect of RPV on MATE-1 is unlikely to be clinically relevant, but it cannot be excluded that RPV would inhibit MATE-2K at clinically relevant concentrations [see m2.7.2, RPV NDA].

The clinical drug-drug interaction studies are described in detail in the Clinical Pharmacology summary see m2.7.2, RPV NDA].

4. TOXICOLOGY

4.1. Presentation Order of Toxicology Summaries

Toxicology Summary	Section
Toxicology for DTG and RPV in combination	Not applicable.
Toxicology of DTG alone	Section 4.2
Toxicology of RPV alone	Section 4.3
Overall summary of all toxicology studies performed with DTG and RPV	Section 5, Integrated Assessment of the Fixed Dose Combination Product

4.2. DTG

The species studied in the definitive toxicology evaluations (rats and monkeys) were selected on the basis of similarities in their pharmacokinetic and metabolic profiles to humans and extensive historical background data available for these species. The dog was not an appropriate species for toxicity testing with DTG due to intolerance vomiting) observed after a single dose at ≥150 mg/kg. Exposure in this single dose TK study at 100 mg/kg/day was ~44 μg.h/mL, which is approximately one-eighth that achieved in the monkey 14 day study at the high dose of 1000 mg/kg/day.

4.2.1. Single dose toxicity

Single dose oral acute toxicity studies have not been conducted in rats or monkeys with DTG; however, the potential for acute toxicity was assessed in repeat dose studies at the highest possible systemic exposure based on saturation of absorption (rat) or highest tolerable dose (monkey). No adverse clinical observations were noted following administration of DTG to rats at ≤1000 mg/kg/day in the 4 week toxicity study [m2.6.7, DTG NDA, Report RD2008/01628]. DTG was not tolerated at doses ≥300 mg/kg/day in the 14 day monkey toxicity study and resulted in severe gastrointestinal intolerance leading to morbidity and mortality (Section 4.2.3.1).

A single dose TK study in dogs was conducted at doses up to 500 mg/kg. DTG was not tolerated and resulted in vomiting at doses ≥150 mg/kg [m2.6.7, DTG NDA, Report RD2009/00963].

4.2.2. Repeat dose toxicity

The toxicity of repeated oral gavage doses of DTG has been assessed in rats and monkeys in studies of up to 26 and 38 weeks, respectively [m2.6.7, DTG NDA, Reports RD2009/00410 and RD2009/00036].

Principal treatment-related effects of DTG in rats and monkeys were related to gastrointestinal toxicity. The NOAEL in the 26 week rat toxicity study was 50 mg/kg/day (Day 180 gender mean C_{max} = 47 μg/mL, AUC_{0-24} = 765 μg.h/mL). Systemic exposure at the NOAEL is ~14X above the expected human exposure for a 50 mg QD. The NOAEL in the 38 week monkey toxicity study was 15 mg/kg/day (Day 270 gender mean C_{max} = 5.1 μg/mL, AUC_{0-24} = 39 μg.h/mL). Systemic exposure (AUC) at the NOAEL is ~0.7X the expected human exposure for a 50 mg QD. However, it should be noted that the exposure margins at the NOAEL in the monkey are greater when compared on a mg/m^2 basis (see Section 4.2.3.2).

Drug-related morbidity and mortality occurred in monkeys when DTG was administered at doses ≥50 mg/kg/day. Signs of GI effects (emesis, diarrhea) were observed at these doses. Body weight loss and the morbidity/mortality were considered secondary to profound dehydration due to GI intolerance as a result of local drug administration and not systemic toxicity.

The main findings from these studies are discussed in more detail below and summarized together with their effect and no effect doses in Table 4.1.

4.2.2.1. Treatment-related mortality/morbidity

In a 14 day study, one female monkey given 1000 mg/kg/day died on Day 13 after experiencing daily emesis and diarrhea [m2.6.7, DTG NDA, Report RD2007/01142]. This animal's condition deteriorated over the dosing phase and the moribund condition was considered secondary to treatment-related effects on the digestive tract (emesis, diarrhea, ulcer in colon) and resultant significant changes in blood electrolytes. This animal's systemic exposure (AUC_{0-24}) on Day 1 was 277 μg.h/mL. Gender mean Day 14 exposure (AUC_{0-24}) at 1000 mg/kg/day was 360 μg.h/mL, which corresponds to 7X above the expected human exposure for a 50 mg QD dose.

In a 38 week monkey toxicity study, two males in the high dose group (50 mg/kg/day) died or were euthanized on Days 59/55 after signs of gastrointestinal intolerance which consisted of diarrhea and emesis and subsequent body weight loss [m2.6.7, DTG NDA, Report RD2009/00036]. These effects and appropriate safety metrics are described further in the discussion of gastrointestinal effects in Section 4.2.3.2.

4.2.2.2. Gastrointestinal effects

The primary finding from repeat dose toxicity studies with DTG up to 26 weeks in rats and 38 weeks in monkeys was gastrointestinal (GI) toxicity. In monkeys, the most sensitive species, GI toxicity was characterized primarily by vomiting, diarrhea and associated mortality as well as gastrointestinal lesions, and by gastric lesions in the rat. In both species, these effects were observed at progressively lower doses with increased study duration. The GI toxicity is believed to be the result of local drug administration at the mucosal surface of the gut following oral dosing rather than systemic toxicity. The fact that affected animals had comparable exposures to animals at dose levels which were not affected is supportive of the conclusion that the GI toxicity is due to the larger local exposure in the GI tract in those dose groups. Therefore, mg/kg or mg/m^2 metrics are appropriate determinates of safety cover for this toxicity because it is not based on systemic exposure. These estimates are provided below in addition to animal:human exposure comparisons based on AUC. Dermal and ocular irritancy studies in rabbits indicate DTG is a mild irritant, and GI toxicity may be a class effect of integrase inhibitors, as raltegravir (a marketed integrase inhibitor) caused irritation to GI mucosal surfaces in rodents [Merck, 2007]. A comparison of DTG animal to human exposure ratios (AUC_{0-24}, mg/kg and mg/m^2) in the definitive rat and monkey studies is presented in Table 4.2, Table 4.3 and Table 4.4.

In rats, hemorrhage was observed in the lamina propria of the mucosa at 1000 mg/kg/day in the 4 week toxicity study and was reversible following a 4 week recovery period [m2.6.7, DTG NDA, Report RD2008/01628]. The NOAEL was 100 mg/kg/day. Exposure (end of study, gender mean) at 100 mg/kg/day was 752 μg.h/mL, which corresponds to ~14X above the expected human exposure for a 50 mg QD dose. The NOAEL (100 mg/kg/day) is 100X the human mg/kg equivalent dose (based on 50 kg human) and ~18X the human mg/m^2 equivalent dose for a clinical dose of 50 mg QD. In the 26 week rat toxicity study, hemorrhage in the glandular stomach mucosa occurred in one male at the end of the 17 week dosing period and one male at the end of the 26 week dosing period in the 500 mg/kg/day group. No adverse findings were observed at the end of a 4 week recovery period. The NOAEL was 50 mg/kg/day. Exposure (end of study, gender mean) at 50 mg/kg/day was 765 μg.h/mL, which corresponds to ~14X above the expected human exposure for a 50 mg QD dose. The NOAEL (50 mg/kg/day) is 50X the human mg/kg equivalent dose (based on 50 kg human) and 9X the human mg/m^2 equivalent dose for a clinical dose of 50 mg QD.

Irritation of the gastrointestinal tract consisting of epithelial atrophy and mucosal hemorrhage in the stomach and lower GI tract (cecum, colon and/or rectum) was noted in monkeys given ≥300 mg/kg/day in the 14 day toxicity study. The NOAEL was 100 mg/kg/day. Exposure (end of study, gender mean) at 100 mg/kg/day was 190 μg.h/mL which corresponds to ~4X above the expected human exposure for a 50 mg QD dose. The NOAEL (100 mg/kg/day) is 100X the human mg/kg equivalent dose (based on 50 kg human) and 35X the human mg/m^2 equivalent dose for a clinical dose of 50 mg QD.

In the 4 week monkey toxicity study, histopathological changes of the GI tract occurred at 100 mg/kg/day and consisted of slight inflammatory cell infiltration in the lamina propria of the cecum, colon and rectum in both sexes; slight cell debris from the crypts of the cecum and colon in males; and atrophy of the mucosal epithelium of the cecum and colon [m2.6.7, DTG NDA, Report RD2008/00107]. This dose was associated with clinical signs of vomiting, diarrhea and body weight loss. The NOAEL was 50 mg/kg/day. Exposure (end of study, gender mean) at 50 mg/kg/day was 132 μg.h/mL which corresponds to ~2X above the expected human exposure for a 50 mg QD dose. The NOAEL (50 mg/kg/day) is 50X the human mg/kg equivalent dose (based on 50 kg human) and 18X the human mg/m^2 equivalent dose for a clinical dose of 50 mg QD.

In the 38 week monkey toxicity study, the 50 mg/kg/day dose was reduced to 30 mg/kg/day on Day 70 for the remainder of the study due to GI intolerance. In the 17 week evaluation of the 38 week monkey study, slight mononuclear cell infiltration and hemorrhage in the lamina propria in the cecum and colon were noted in the animal that was euthanized on Day 55. Abnormal feces (observed through Day 131) associated with decreased food consumption and decreased body weight was noted in the 50/30 mg/kg/day group. At the end of the 38 week dosing period, one female in the 50/30 mg/kg/day group had adverse findings in the stomach consisting of multifocal mononuclear cell infiltration and slight hemorrhage in the lamina propria, very slight multifocal erosions and multifocal epithelial regeneration. At the end of a 4 week recovery period, multifocal mononuclear cell infiltration and very slight hemorrhage in the lamina propria and multifocal epithelial regeneration in the stomach were observed in one female. However, the changes in this animal were of lesser severity and there were no active erosions, suggesting recovery of changes upon cessation of treatment. Both animals with stomach lesions had diarrhea/vomiting prior to the dose reduction (50/30 mg/kg/day), but did not have clinical observations of toxicity following the dose reduction. Exposures at end of study for the two affected females were lower compared to the other animals in this dose group (AUC_{0-24} = 43.5 to 48.8 μg.h/mL versus gender mean for 50/30 mg/kg/day group of 61.7 μg.h/mL) and overlapped with exposures at 15 mg/kg/day (AUC_{0-24} range = 25.8 to 54.0 μg.h/mL). This observation is consistent with a local GI toxicity as opposed to a systemic effect.

The NOAEL for the 38 week dosing period was 15 mg/kg/day (Day 270 gender mean AUC_{0-24} and C_{max} of 39 μg.h/mL and 5.1 μg/mL, respectively), which corresponds to 0.7X and 1.4X the human AUC and C_{max} exposure, respectively, for a 50 mg QD dose. The NOAEL for the 38 week dosing period (15 mg/kg/day) is 15X the human mg/kg equivalent dose (based on 50 kg human) and 5X the human mg/m^2 equivalent dose for a 50 mg QD dose. The NOAEL for the 17 week interim evaluation was also 15 mg/kg/day; thus, there was not a decrease in the NOAEL from 17 weeks of dosing to 38 weeks of dosing.

Nonclinical evidence for GI toxicity with DTG (including vomiting, diarrhea and gastric/colonic erosions) did not translate into significant findings for DTG in double blinded randomized clinical trials, with a similar rate and nature of events reported for DTG compared to raltegravir and efavirenz/tenofovir/emtricitabine (Atripla) [see m2.5].

4.2.2.3. Hepatic effects

Hepatocellular single cell necrosis and diffuse hepatocellular hypertrophy and/or vacuolation occurred in male monkeys given 1000 mg/kg/day in the 14 day study. Additional changes included transient ALT increases at ≥300 mg/kg/day, increased AST, bilirubin, γGTP and triglycerides at 1000 mg/kg/day, and decreased total cholesterol at 1000 mg/kg/day. The NOAEL was 100 mg/kg/day. Exposure (end of study, gender mean) at 100 mg/kg/day was 190 μg.h/mL which corresponds to ~4X above the expected human exposure for a 50 mg QD dose. In the 38 week monkey toxicity study, liver findings were restricted to increased AST (2.5X) and bilirubin (2.8X) in the moribund animal in the 50 mg/kg/day group (euthanized on Day 55) [m2.6.7, DTG NDA, Report RD2009/00036]. The findings in the 38 week study were considered secondary to the moribund condition. Exposure (end of study, gender mean) at the NOAEL (15 mg/kg/day) was 39 μg.h/mL which corresponds to ~0.7X the expected human exposure for a 50 mg QD dose. No treatment-related adverse effects on liver were observed in rats in studies up to 26 weeks.

Human subjects were carefully monitored for liver effects, and cumulative data to date suggests a hepatic safety profile for DTG that is comparable to raltegravir and efavirenz, the comparators used in the Phase III studies [see m2.5, Section 5.4.1.1].

4.2.2.4. Renal effects

In the 14 day rat study, there were statistically significant increases in urine specific gravity in males given 500 mg/kg/day and in females given ≥50 mg/kg/day [m2.6.7, DTG NDA, Report RD2007/01140]. Because no treatment-related microscopic findings were observed in the kidneys, the change was not considered toxicologically significant. In the 4 week rat study there was an increased incidence of urine protein and increased urine specific gravity in animals given 1000 mg/kg/day, however, there were no related changes in blood chemistry or microscopic findings, and none of these changes occurred in the rat 26 week study at up to 500 mg/kg/day (Day 180 AUC at 500 mg/kg/day = 1558 μg.h/mL which corresponds to ~29X above the expected human exposure for a 50 mg QD dose).

Renal tubule dilatation occurred in monkeys given 1000 mg/kg/day in the 14 day study. BUN and creatinine were increased while serum sodium and chloride were decreased in these monkeys. In the 38 week monkey toxicity study, renal findings were restricted to increased BUN (12.5X) and creatinine (3.7X), and slight kidney dilatation of distal renal tubules and cellular and hyaline casts in the moribund animal in the 50 mg/kg/day group (euthanized on Day 55). These findings (in both the 14 day and 38 week monkey toxicity studies) were considered secondary to the moribund condition related to GI toxicity. The NOAEL in the 38 week toxicity study was 15 mg/kg/day. Exposure (end of study, gender mean) at 15 mg/kg/day was 39 μg.h/mL which corresponds to ~0.7X the expected human exposure for a 50 mg QD dose.

Analyses of adverse events from the Renal Systems Organ Class in clinical studies do not suggest that DTG has an adverse effect on renal function [see m2.5, Section 5.4.1.2]. There was a low incidence of renal impairment or failure, and these events were more

likely a consequence of underlying disease, co-morbid conditions and concurrent drugs, and were not thought to be related to DTG treatment.

Mild elevations of creatinine are expected for DTG. These are related to a likely benign effect on creatinine secretion with blockade of the OCT2 receptor, and do not progress on continued treatment with DTG. A higher incidence of dipstick proteinuria was noted in efavirenz controlled studies but not in a raltegravir controlled study. However, quantitative measures of proteinuria showed no difference between DTG and either efavirenz or raltegravir based combination antiretroviral therapy.

4.2.2.5. Bone marrow and lymph node effects

In the 14 day monkey study, hematologic changes included mild changes in reticulocyte counts (males given ≥300 mg/kg/day) and red cell counts (females given 1000 mg/kg/day) that were statistically but not biologically significant. In males given 300 mg/kg/day, mean reticulocyte counts were increased during Week 1 and were minimally decreased in the absence of an effect on red cell mass parameters during Week 2. In females given 1000 mg/kg/day, a mild transient decrease in the RBC count (0.90X control mean in Week 1 and 0.97X control mean in Week 2) was associated with minimal changes in reticulocyte counts that included increases in 2 of 3 females during Week 2. These hematologic changes were not associated with a change in bone marrow nucleated cell count. There was microscopic evidence of gelatinous bone marrow and atrophy of the white pulp in the spleen in monkeys given 1000 mg/kg/day, and a decrease in the paracortical lymphocytes of the submandibular and/or mesenteric lymph nodes in monkeys given ≥300 mg/kg/day [m2.6.7, DTG NDA,, Report RD2007/01142]. The NOAEL was 100 mg/kg/day. Exposure (end of study, gender mean) at 100 mg/kg/day was 190 μg.h/mL which corresponds to ~4X above the expected human exposure for a 50 mg QD dose.

The hematology changes in the 14 day study were associated with moribundity, but were also relatively mild (RBC count ~0.91X in Week 1 and 0.88X in Week 4) and were associated with regenerative responses indicated by the increased reticulocyte counts in most animals. Importantly, these changes were not associated with changes in marrow total nucleated cell counts because the only decrease was observed in one male given 1000 mg/kg/day who had no change in red cell mass at any time point but a decrease in total WBC (~0.43X) in Week 2 that associated with a neutrophilic leukocytosis and increased fibrinogen in that animal. The peripheral regenerative response (reticulocytosis), combined with the lack of an effect on total nucleated cell counts in animals with red cell mass effects indicate the absence of an effect on erythropoiesis in the 14 day study. The microscopic diagnoses in bone marrow (gelatinous bone marrow) identified in two females and one male were only associated with a decreased total nucleated cell count in the male. Furthermore, this effect was limited to a non-tolerated dose and was not observed in studies of longer duration.

In the 4 week monkey study, decreased RBCs (0.91X) were observed in females given 100 mg/kg/day, with no correlating histopathology findings [m2.6.7, DTG NDA,, Report RD2008/00107]. The NOAEL was 50 mg/kg/day. Exposure (end of study, gender mean) at 50 mg/kg/day was 132 μg.h/mL which corresponds to ~2X above the

expected human exposure for a 50 mg QD dose. No treatment-related adverse effects on bone marrow and lymph nodes were observed in non-moribund animals in the 9 month monkey toxicity study at doses ≤50/30 mg/kg/day [m2.6.7, DTG NDA,, Report RD2009/00036].

No treatment-related adverse effects on bone marrow or lymph nodes were observed in rats in studies up to 26 weeks [m2.6.7, DTG NDA, Report RD2009/00410].

In summary, the hematology changes observed in monkey toxicity studies are confined to those dose groups that had evidence of GI illness (including GI atrophy and hemorrhage), body weight loss and/or anorexia, and are secondary rather than direct effects of DTG. Bone marrow changes in the most affected animals are secondary to moribundity as well. The changes in the affected animals are consistent with inflammation, blood loss and protein/nutrient deficits caused by the gastrointestinal effects. There were no consistent hematology changes at doses below the NOAELs and there were no bone marrow effects in any animals, except those with gastrointestinal effects.

A review of hematology laboratory data from clinical trials revealed no signal for bone marrow or lymph node toxicity caused by DTG [see m2.5, 5.4.2].

4.2.3. Genotoxicity

DTG did not cause gene mutations or chromosomal damage in two definitive in vitro tests (bacterial mutation assay and mouse lymphoma L5178Y cell assay), or in an in vivo oral rat micronucleus test [m2.6.7, DTG NDA, Report WD2007/00514; Report WD2007/00515; Report WD2007/00513]. Therefore, based on these data, DTG does not pose a genetic toxicity risk to humans.

4.2.4. Carcinogenicity

The carcinogenic potential of DTG was assessed in mice and rats following oral administration for 2 years [m2.6.7, DTG NDA, Report 2012N152419 and, Report 2012N152418]. Based on recommendations from the FDA Executive Carcinogenicity Assessment Committee [FDA, 2010], the doses studied were 7.5, 25 or 500 mg/kg/day in CD-1 mice and 2, 10 or 50 mg/kg/day in Sprague Dawley rats, administered via oral gavage once daily in a vehicle of 0.5% HPMC and 0.1% Tween 80 (a water control group was also included). The high dose in each study was based on saturation of absorption and concern for GI effects over the course of 2 years. Dose spacing was based on AUC.

DTG was not carcinogenic to mice at doses up to 500 mg/kg/day or rats at doses up to 50 mg/kg/day following oral administration for 104 consecutive weeks. In both species, DTG administration had no effect on survival, there were no treatment-related clinical signs, and there were no neoplastic or non-neoplastic findings attributed to DTG.

The NOAEL for non-neoplastic findings after chronic oral administration was the high dose of 500 mg/kg/day for mice and 50 mg/kg/day for rats. When compared to the expected human exposure for a 50 mg QD dose, the systemic exposures were ~20X higher for mice and ~17X higher for rats.

4.2.5. Reproductive and developmental toxicity

4.2.5.1. Fertility and embryo-fetal development

There were no effects on fertility or early embryonic development in rats orally administered DTG at ≤1000 mg/kg/day in males or females [m2.6.7, DTG NDA, Report XD2009/00368]. The NOAEL was 1000 mg/kg/day, which corresponds to ~33X above the expected human exposure for a 50 mg QD, based on gender averaged mean exposures achieved in the 4 week rat toxicity study.

No adverse effects on fetal development were observed in pregnant rats orally administered DTG at ≤1000 mg/kg/day [m2.6.7, DTG NDA, Report XD2009/00367]. The NOAEL for maternal and fetal toxicity was 1000 mg/kg/day, which corresponds to ~38X above the expected human exposure for a 50 mg QD dose.

In an embryofetal development study in rabbits, DTG was orally administered at 40, 200 or 1000 mg/kg/day to pregnant rabbits [m2.6.7, DTG NDA, Report XD2009/00366]. Suppressed body weight gain (13.6% on gestation Day 19), decreased food consumption (up to 53%) and scant or no feces/urine associated with the decreased food consumption were noted in the 1000 mg/kg/day dams. The NOAEL was 200 mg/kg/day for maternal general toxicity (~0.27X the expected human exposure for a 50 mg QD dose) and 1000 mg/kg/day for maternal reproductive function and embryofetal development (0.56X the expected human exposure for a 50 mg QD dose). [m1.14.1 (US) or m1.3 (EU)]

In summary, based on animal data, DTG is not anticipated to increase the risk of adverse developmental (or reproductive) outcomes in humans when used in accordance with dosing information in the product label [m1.14.1 (US) or m1.3 (EU)].

4.2.5.2. Pre- and postnatal development

In a pre- and post-natal development study, DTG was administered to female rats at doses of 5, 50 or 1000 mg/kg/day from Day 6 of gestation to Day 20 of lactation [m2.6.7, DTG NDA, Report 2011N121663]. Suppressed body weight gain and decreased food consumption were noted in dams (F_0) in the 1000 mg/kg/day group during the lactation period, which were associated with mild decreases in body weights in the offspring in the 1000 mg/kg/day group from pre-weaning until adolescence. There were no adverse effects on maternal pregnancy, parturition, lactation or offspring (F_1) survival, behavioral or reproductive function. The NOAEL for maternal reproductive function was 1000 mg/kg/day (~32X above the expected human exposure for a 50 mg QD dose based on exposures achieved in female rats in the 4 week toxicity study). Due to the decreased body weights of the offspring observed at higher doses, the NOAEL for pre- and post-natal development of the offspring (F_1) was 50 mg/kg/day. At this dose, the expected human exposure is ~25X above a 50 mg QD dose (extrapolated from gender mean

exposures achieved in the rat 14 day toxicity study). Based on the fact that effects on offspring body weights were noted at doses where maternal toxicity was observed, and the presence of considerable safety margins expected at the proposed clinical doses, there is minimal risk for adverse effects on post-natal development in offspring of mothers receiving DTG.

DTG is excreted in the milk of lactating rats. Following oral administration (50 mg/kg) to lactating rats on Day 10 post partum, total radiocarbon concentrations in milk were up to 2-fold greater than those in maternal blood. The metabolite profile of milk indicated that parent DTG represented more than 95% of the total radiocarbon, consistent with the findings in plasma from female rats in an earlier study [m2.6.7, DTG NDA, Report 2012N132387]. These data suggest that F_1 offspring in the pre- and post-natal toxicity study were exposed to the drug via the milk [m2.6.7, DTG NDA,, Report 2011N121663]. Following oral administration of DTG (50 mg/kg) to pregnant rats on Day 18 post-conception, DTG-related material was found, by QWBA analysis, to be widely distributed to the fetuses over the 24 hour sampling period. These data indicate that DTG is able to cross the placental barrier [see above, Section 3.2.3.3 and m1.14.1 (US) or m1.3 (EU)].

4.2.6. Juvenile toxicity

A juvenile toxicity study in rats was conducted with DTG at oral doses of 0.5, 2 or 75 mg/kg/day from Days 4 to 66 post partum (pp) [m2.6.7, DTG NDA, Report CD2010/00023]. Two pre-weanling deaths were considered test article-related at 75 mg/kg/day. Over the pre-weaning treatment period (Days 4 to 21 pp), mean body weight gain was decreased (0.86X control mean gain) for males and females in the 75 mg/kg/day group and the decrease persisted throughout the entire study for females during the post-weaning period. There were no test article-related differences among the groups for the age at which offspring attained physical signs of sexual maturation (vaginal opening or balano-preputial skinfold separation). There were no changes considered related to DTG administration in stage-dependent evaluation of spermatogenesis. There were no test article-related effects on T-cell-dependent antibody response (TDAR) measured on Day 67, and no effects on lymphocyte subsets (T-cells, both CD4 and CD8 subsets, and B-cells) and CD4 or CD8 T-cell receptor Vβ usage in peripheral blood. Therefore, the NOAEL in juvenile rats was 2 mg/kg/day (Day 32 pp gender mean $AUC_{0\text{-}24}$ = 90 μg.h/mL and C_{max} = 7.6 μg/mL). Clinical studies in pediatric patients conducted to date have not revealed any safety issues specific to this population [see m2.5, Section 1].

4.2.7. Local tolerance

Local tolerance studies have been performed for worker health and safety purposes. In vitro, DTG is slightly/mildly irritating to skin and ocular model systems. There was no indication of contact sensitization in a mouse local lymph node assay when DTG was administered topically.

4.2.8. Other toxicity

4.2.8.1. Immunotoxicity

Immunotoxicity assessment in adults

Because the intended patient population is HIV-infected patients, a T-cell-dependent antibody response (TDAR) study was conducted in rats to evaluate immunotoxicity potential. Oral administration of DTG at doses up to 1000 mg/kg/day for 1 month had no effect on keyhole limpet hemocyanin (KLH) antibody titers in rats, thereby demonstrating no immunosuppressive effect of DTG on a TDAR (exposure ~33X above the expected human exposure for a 50 mg QD dose based on exposures achieved in the rat 4 week toxicity study) [m2.6.7, DTG NDA,, Report RD2009/00751]. Furthermore, there were no signs of immunotoxicity from general toxicology study findings or clinical safety data. Therefore, there is a negligible risk of immunotoxicity potential to adult patient populations treated with DTG.

Immunotoxicity assessment in juveniles

A concern for immunotoxicity potential was theorized for juveniles based on a publication demonstrating that two HIV integrase inhibitor compounds (p8 [5CITEP] and p10 [L-708,906]) have activity on recombination activating gene (RAG1/2) and therefore may affect T- and B-cell repertoire development [Melek, 2002]. To address the potential effects of DTG on RAG1/2, immunotoxicity endpoints (TDAR, immunophenotyping and TCRVβ usage) were added to the definitive rat juvenile toxicity study (see above, Section 4.2.7). There were no test article-related effects on immunologic competence as measured by TDAR, and no effects on lymphocyte subset counts (T-cells, both CD4 and CD8 subsets, and B-cells) and CD4 or CD8 T-cell receptor Vβ usage in peripheral blood. Histopathology of immunologic organs (spleen, thymus, lymph nodes) and hematology evaluation revealed no effects. The NOAEL for immunotoxicity endpoints was 75 mg/kg/day. These results provided a robust nonclinical assessment of potential developmental immunotoxicologic effects and suggest no unusual drug-specific risk of developmental immunotoxicity in juvenile animals.

4.2.8.2. Phototoxicity

In the absorption spectrum for DTG, the main peak in the UVA/UVB region is at 258 nm (outside the range of concern), and there are minor peaks at 310, 325 and 340 nm, with a tail extension to 395 nm [see m3.2.S.1.3, General Properties]. A whole body autoradiography (WBA) study in pigmented rats following a single oral administration of [^{14}C]-DTG (sodium salt) demonstrated wide tissue distribution of drug-related material without significant retention in skin or eyes.

In the Neutral Red Uptake assay using Balb/c 3T3 fibroblasts, DTG was phototoxic when tested up to the limit of solubility in primary vehicle (7.93 mg/mL, the maximum dose formulated in DMSO). However, in an in vivo study using Long-Evans pigmented rats, DTG showed no evidence of cutaneous phototoxicity at doses up to 1000 mg/kg/day for 3 days.

No drug-related toxicity has been identified in the eye or skin during repeat dose oral toxicity studies of up to 6 months in the Sprague Dawley rat or 38 weeks in the cynomolgus monkey. Potential toxic effects on the eye and skin were assessed during these studies by ophthalmoscopy, macroscopic and microscopic examination. The Sponsor has conducted an extensive review of the clinical safety data and concluded that DTG is not phototoxic in human patients taking therapeutic doses for extended durations. The extent of exposure of DTG in the combined key Phase IIb and Phase III clinical studies is equal to approximately 1595.9 patient years. Out of a database of 1572 subjects (ISS safety population), only 2 subjects (82 and 55), both from the USA and both from Phase IIb Study ING112276, SPRING-1 who received DTG, reported photosensitivity as an adverse reaction. Both of the affected subjects were white males and the reactions were not thought related to DTG, which was continued in both cases. The time to onset of 688 days or 107 makes a relationship to DTG unlikely, especially as the sites were in areas that would have had exposure to sunlight. There were no reports of photosensitivity from any subjects treated with DTG in the Phase III program. Therefore, there is minimal risk for phototoxicity from oral treatment with DTG.

Table 4.1 Principal Toxicological Findings in Rats and Monkeys Following Oral Administration of DTG

Finding	Rat		Monkey	
	Effect Dose (mg/kg/day)	No Effect Dose (mg/kg/day)	Effect Dose (mg/kg/day)	No Effect Dose (mg/kg/day)
Mortality/Morbidity:				
Adult animals: Death preceded by repeated emesis, diarrhea with significant weight loss	NO	NO	50	30
Juvenile animals: Mortality preceded by decreased body weight gain	75	2	NA	NA
Clinical Observation: Emesis, diarrhea	NO	NO	50	10[a]
Body Weight Loss	NO	NO	50	15
Gastrointestinal Effects:				
Stomach: Gastric mucosal hemorrhage, mononuclear cell infiltration and/or multifocal epithelial regeneration	500	50	50/30	15
Stomach: Multifocal erosions	NO	NO	50/30	15
Cecum, colon, rectum: Mucosal atrophy and/or hemorrhage	NO	NO	50	15
Hepatic Effects:	NO	NO		
Hepatocellular single cell necrosis and vacuolation with AST, and γGTP elevations and/or increased bilirubin and triglycerides			1000	300
ALT elevations without corresponding anatomic pathology changes			300	100
AST & bilirubin elevations secondary to moribundity			50	15
Renal Effects Considered Associated with Moribund Condition: Renal tubule dilatation, increased BUN and CRE, and/or decreased serum sodium and chloride	NO	NO	50	15
Bone Marrow and Lymphoid Changes Considered Associated with Moribund Condition and/or Stress: Gelatinous or hypocellular bone marrow, thymic atrophy, spleenic lymphoid atrophy, decreased lymphocytes of the submandibular and mesesenteric lymph nodes, adrenal hypertrophy/increased weight, decreased retics, platelets, increased fibrinogen and/or prolonged APTT	NO	NO	300	100
Decrease in RBCs (females)	NO	NO	100	50

Key:

a = One male monkey in the 15 mg/kg/day group had non-adverse transient diarrhea with no effect on body weight that recovered during the dosing period.

ALT = Alanine aminotransferase. APTT = Activated partial thromboplastin time. AST = Aspartate aminotransferase. BUN = Blood urea nitrogen. BW = Body weight. CRE = Creatinine. FC = Food consumption. NA = Not Applicable. NO = Not observed. RBC = Red blood cell.

Table 4.2 Comparative Assessment of Mean Systemic Exposure Following Oral Administration of DTG

Species (Duration)	Dose (mg/kg/day)	Sex	C_{max} (μg/mL)		AUC_{0-24} (μg.h/mL)		Animal to Human AUC Ratio[a,b] (50 mg QD)
			Day 1	End of Study	Day 1	End of Study	
Rat[c] (14 days)	50	M F	58.5 75.4	65.7 95.6	881 1110	1040 1610	19.4 30.0
	150	M F	82.7 83.3	74.1 106	994 1050	1150 1740	21.5 32.5
	500 (NOAEL)	**M F**	**87.1 117**	**108 124**	**1360 1350**	**1710 1950**	**31.9 36.4**
Rat[c] (4 weeks)	2	M F	3.5 4.6	4.7 7.8	39.3 60.0	53.0 81.7	1.0 1.5
	10	M F	15.2 21.2	23.7 34.6	220 278	274 378	5.1 7.1
	100 (NOAEL)	**M F**	**43.7 54.4**	**49.2 61.6**	**693 775**	**722 781**	**13.5 14.6**
	1000	M F	95.1 116	119 112	1678 1615	1837 1737	34.3 32.4
Rat (26 weeks)[c]	5	M F	9.4 12.0	11.9 20.1	88.8 138	116 290	2.2 5.4
	50 (NOAEL)	**M F**	**47.3 56.5**	**38.0 56.6**	**637 731**	**607 922**	**11.3 17.2**
	500	M F	95.3 103.5	85.1 107	1450 1450	1338 1777	25.0 33.2
Monkey[c] (14 days)	**100 (NOAEL)**	**M F**	**21.3 18.9**	**24.0 23.0**	**172 150**	**192 187**	**3.6 3.5**
	300	M F	30.9 17.5	21.7 23.3	324 142	199 271	3.7 5.1
	1000	M F	27.5 20.9	26.2 30.3	358 237	364 354	6.8 6.6
Monkey[c] (4 weeks)	25	M F	8.7 11.2	13.9 15.4	60.3 67.9	108 83.9	2.1 1.6
	50 (NOAEL)	**M F**	**9.6 10.7**	**14.5 20.4**	**72.9 68.7**	**111 153**	**2.1 2.8**
	100	M F	12.1 12.2	16.0 13.4	99.0 90.2	148 92.0	2.8 1.7
Monkey (38 weeks)[d]	3	M F	2.9 3.1	3.0 2.3	15.2 15.3	18.9 15.5	0.35 0.29
	10	M F	4.7 6.1	4.4 5.1	30.7 34.5	32.3 37.7	0.60 0.70
	15 (NOAEL)	**M F**	**7.7 5.5**	**5.3 4.8**	**46.4 30.6**	**36.7 40.9**	**0.68 0.76**
	50/30	M F	9.0 10.5	7.5 7.8	62.9 63.4	61.7 61.7	1.2 1.2

Comparative Assessment of Mean Systemic Exposure Following Oral Administration of DTG (Continued)

Species (Duration)	Dose (mg/kg/day)	Sex	C_{max} (µg/mL)		AUC_{0-24} (µg.h/mL)		Animal to Human AUC Ratio[a,b] (50 mg QD)
			Day 1	End of Study	Day 1	End of Study	
Mouse (14 day)[e]	10	M F	16.4 18.3	14.9 18.7	188 195	218 188	4.1 3.5
	100	M F	62.7 62.0	60.6 73.4	921 980	801 1170	14.9 21.8
	500	M F	96.5 93.2	77.0 90.7	1140 1100	1090 1190	20.3 22.2
	1500 (NOAEL)	**M F**	**106 115**	**104 123**	**1210 1520**	**1240 1630**	**23.1 30.4**
Mouse (13 week)[e]	10	M F	16.0 19.3	18.5 28.0	211 212	257 256	4.8 4.8
	50	M F	43.9 53.7	52.7 62.6	477 528	653 740	12.2 13.8
	500	M F	77.3 88.3	82.1 109	923 1110	1010 1300	18.8 24.3
	1500 (NOAEL)	**M F**	**109 114**	**103 118**	**1440 1420**	**1320 1350**	**24.6 25.2**
Rat (embryofetal development)	100	F	64.1	78.0	949	1252	23.4
	300	F	74.0	82.2	1096	1409	26.3
	1000 (NOAEL)[f]	**F**	**139**	**109**	**1841**	**2032**	**37.9**
Rabbit (embryofetal development)	40	F	0.8	1.3	2.1	2.6	0.049
	200[g]	F	1.5	1.7	15.6	14.5	0.27
	1000[g]	F	2.3	2.1	36.8	30.1	0.56
Rat (juvenile)[h]	0.5	M F	4.6 4.7	1.5 2.4	92.0 86.5	9.9 27.1	0.18 0.51
	2 (NOAEL)	**M F**	**15.2 16.4**	**7.7 7.5**	**303 316**	**85.7 93.3**	**1.6 1.7**
	75	M F	88.0 85.4	69.9 77.4	1540 1549	917 1044	17.1 19.5
Mouse (carcino-genicity)[e,i]	7.5	M F	13.4 20.3	14.5 16.6	176 235	148 157	2.8 2.9
	25	M F	40.8 44.4	27.4 43.3	579 565	327 494	6.1 9.2
	500 (NOAEL)	**M F**	**77.7 94.7**	**71.9 94.5**	**1180 1300**	**953 1210**	**17.8 22.6**
Rat (carcino-genicity)[j]	2	M F	7.3 8.8	7.7 20.4	101 114	100 279	1.9 5.2
	10	M F	24.7 34.3	21.3 40.4	348 501	340 731	6.3 13.6
	50 (NOAEL)	**M F**	**57.6 87.0**	**40.9 68.5**	**841 1150**	**713 1140**	**13.3 21.3**

Comparative Assessment of Mean Systemic Exposure Following Oral Administration of DTG (Continued)

Species (Duration)	Dose (mg/kg/day)	Sex	C_{max} (µg/mL)		AUC_{0-24} (µg.h/mL)		Animal to Human AUC Ratio[a,b] (50 mg QD)
			Day 1	End of Study	Day 1	End of Study	
Human[b]	50 mg	M/F	3.7		53.6		NA

Key: The systemic exposure margins within the main body text of m2.4 are presented as gender averaged means.
a = Calculated for AUC based on end of treatment values.
b = Based on exposure (C_{max} and AUC_{0-t}) values from pooled data from clinical studies ING112276 and ING113086.
c = Values are the mean of n=3 to 5.
d = Values are the mean of n=7 to 9.
e = Composite plasma toxicokinetic parameters from mice, n=3/sex/group/time point.
f = The NOAEL was 1000 mg/kg/day for dams and embryofetal development.
g = The NOAEL was 200 mg/kg/day for maternal general toxicity and 1000 mg/kg/day for maternal reproductive function and embryofetal development.
h = Composite plasma toxicokinetic parameters for juvenile rats were examined on Day 13 post partum (pp) and Day 32 pp. Composite parameters were derived from mean plasma concentration data. n=3/time point/dose, with the exception of the 8 hour time point on Day 13 pp following 75 mg/kg/day, for which n=4.
i = Toxicokinetics conducted on Day 26 and Day 182 instead of Day 1 and End of Study, respectively.
j = Values are the mean of n=4/sex/group. Toxicokinetics conducted on Day 28 and Day 182 instead of Day 1 and End of Study, respectively.
QD = Once daily.
Note: No observed adverse effect levels (NOAEL) are bolded. Values in parenthesis represent the range.

Table 4.3 Comparative Assessment of Animal to Human Dose Ratios (mg/kg and mg/m^2) Following Oral Administration of DTG in the 4 and 26 Week Rat Toxicology Studies

Species (Duration)	Dose (mg/kg/day)	Sex	Cmax (μg/mL)		AUC_{0-24} (μg.h/mL)		ANIMAL TO HUMAN RATIOS Based on mg/kg and mg/m^2	
			Day 1	End of Study	Day 1	End of Study	mg/kg (50mg QD)	mg/m^2 (50mg QD)
Rat (4 weeks)	2	M	3.5	4.7	39.3	53.0	2	0.35
		F	4.6	7.8	60.0	81.7		
	10	M	15.2	23.7	220	274	10	1.8
		F	21.2	34.6	278	378		
	100 (NOAEL)	**M**	**43.7**	**49.2**	**693**	**722**	**100**	**17.6**
		F	**54.4**	**61.6**	**775**	**781**		
	1000	M	95.1	119	1678	1837	1000	176
		F	116	112	1615	1737		
Rat (26 weeks)	5	M	9.4	11.9	88.8	116	5	0.88
		F	12.0	20.1	138	290		
	50 (NOAEL)	**M**	**47.3**	**38.0**	**637**	**607**	**50**	**8.8**
		F	**56.5**	**56.6**	**731**	**922**		
	500	M	95.3	85.1	1450	1338	500	88
		F	103.5	107	1450	1777		
Human	50 mg[a]	M/F	3.7		53.6		NA	NA

Key:
Calculations are based on 50 kg human.
For conversion of animal doses in mg/kg to dose in mg/m^2, multiply by Km (Km rat = 6; Km human = 34).
a = Based on exposure (Cmax and AUC0-t) values from pooled data from clinical studies ING112276 and ING113086

Table 4.4 Comparative Assessment of Animal to Human Dose Ratios (mg/kg and mg/m^2) Following Oral Administration of DTG in the 14 Day and 4 and 38 Week Monkey Toxicology Studies

Species (Duration)	Dose (mg/kg/day)	Sex	Cmax (μg/mL)		AUC_{0-24} (μg.h/mL)		ANIMAL TO HUMAN RATIOS Based on mg/kg and mg/m^2	
			Day 1	End of Study	Day 1	End of Study	mg/kg (50mg QD)	mg/m^2 (50mg QD)
Monkey (14 days)	**100 (NOAEL)**	**M**	**21.3**	**24.0**	**172**	**192**	**100**	**35**
		F	**18.9**	**23.0**	**150**	**187**		
	300	M	30.9	21.7	324	199	300	105
		F	17.5	23.3	142	271		
	1000	M	27.5	26.2	358	364	1000	353
		F	20.9	30.3	237	354		
Monkey (4 weeks)	25	M	8.7	13.9	60.3	108	25	8.8
		F	11.2	15.4	67.9	83.9		
	50 (NOAEL)	**M**	**9.6**	**14.5**	**72.9**	**111**	**50**	**17.6**
		F	**10.7**	**20.4**	**68.7**	**153**		
	100	M	12.1	16.0	99.0	148	100	35
		F	12.2	13.4	90.2	92.0		
Monkey (38 weeks)	3	M	2.9	3.0	15.2	18.9	3	1.1
		F	3.1	2.3	15.3	15.5		
	10	M	4.7	4.4	30.7	32.3	10	3.5
		F	6.1	5.1	34.5	37.7		
	15 (NOAEL)	**M**	**7.7**	**5.3**	**46.4**	**36.7**	**15**	**5.3**
		F	**5.5**	**4.8**	**30.6**	**40.9**		
	50/30	M	9.0	7.5	62.9	61.7	50/30	17.6/10.6
		F	10.5	7.8	63.4	61.7		
Human	50 mg[a]	M/F	3.7		53.6		NA	NA

Key:
Calculations are based on 50 kg human.
For conversion of animal doses in mg/kg to dose in mg/m^2 , multiply by Km (Km rat = 6; Km human = 34).
a = Based on exposure (C_{max} and AUC_{0-t}) values from pooled data from clinical studies ING112276 and ING113086

4.3. RPV

The toxicology of RPV was evaluated in a series of in vitro and in vivo studies and is described in detail in m2.6.6, RPV NDA. The studies are listed in m2.6.7 RPV NDA.

The species studied in the definitive toxicology evaluations (rats and dogs) were selected on the basis of similarities in their pharmacokinetic and metabolic profiles to humans and extensive historical background data available for these species.

4.3.1. Single dose toxicity

No formal single dose studies were done as single dose evaluations were part of the initial oral dose range finding studies or, in the case of mice, part of the bone marrow micronucleus test.

In mice, no relevant effects were noted following an oral single dose of up to 1600 mg/kg RPV base in PEG400 + CA, the maximum feasible dose in this vehicle for this species. Exposures at 1600 mg/kg were similar as those at 400 mg/kg indicating saturation of absorption [see m2.6.7, RPV NDA].

Rats dosed with an oral maximum feasible single dose of 800 mg/kg RPV base in PEG400 showed no treatment-related effects [see m2.6.7, RPV NDA].

Dogs that received an oral maximum feasible dose of 80 mg/kg RPV base in PEG400 or PEG400 + CA vomited more frequently and had softer stool than dogs treated with the vehicle. No other effects were noted [see m2.6.7, RPV NDA].

4.3.2. Repeat toxicity

Systemic toxicity of RPV base or RPV after repeat dosing was studied in mice, rats, rabbits, dogs and cynomolgus monkeys. Pivotal repeat dose studies were conducted in mice (3 months), rats (1 and 6 months), dogs (1, 3, 6, and 12 months), and cynomolgus monkeys (8 weeks). The C_{max} and AUC values of RPV after repeated oral administration in various animal species used in the toxicology studies along with ratios for C_{max} and AUC values in comparison with humans are presented in Table 4.5.

The mouse study served as dose range finder for the carcinogenicity study in that species. The 5-day rabbit study was a pilot for the rabbit embryo-fetal toxicity study. The studies in rats and dogs were designed to investigate the toxicity profile of RPV and to support clinical studies and marketing authorization. A further mechanistic juvenile toxicity study was done in immature female cynomolgus monkeys. The reversibility upon repeat dosing was investigated in rats and dogs.

All studies in rats and dogs were conducted with RPV base dissolved in PEG400, usually with CA. In the rabbit study, RPV base was suspended in 0.5% (m/v) HPMC in water as rabbits do not tolerate PEG400. Following the selection of the HCl salt as the chemical form to be marketed, 1-month studies in rats and dogs compared the kinetics and toxicity of RPV base and RPV. The studies in mice and the studies in cynomolgus monkeys were conducted with RPV suspended in aqueous HPMC. For an overview of all repeat dose toxicity studies see [see m2.6.7, RPV NDA]. In this section only pivotal studies are summarized.

Table 4.5 RPV Exposure in Animals Relative to Human After Oral Administration

Species	RPV formulation	Sampling Period	Sex/n	Dose (mg/kg/day)	C_{max} (µg/mL)	AUC_{0-24h} (µg.h/mL)	C_{max} ratio	AUC_{0-24h} ratio
Mouse	RPV in HPMC (0.5% w/v)	Week 28	M/9	20	9.8	76	75	32
				60	22	230	169	96
				160	36	505	277	210
			F/9	20	9.9	51	76	21
				60	29	278	223	116
				160	58	766	446	319
Rat	RPV base in PEG400/CA (10%)	Day 175[a]	M/3	40	1.7	12	13	5.0
				120	3.0	35	23	15
				400	6.2	73	48	30
			F/5	40	6.6	50	51	21
			F/5	120	8.8	116	68	48
			F/6	400	16	244	123	102
	RPV in HPMC (0.5% w/v)	Week 39	M/9	40	0.82	6.3	6.3	2.6
				200	1.3	8.2	10	3.4
				500	1.8	14	14	5.8
				1500	2.2	18	17	7.5
			F/9	40	2.1	14	16	5.8
				200	4.7	41	36	17
			F/8	500	8.5	46	65	19
			F/9	1500	9.4	84	72	35
Pregnant rat	RPV base in PEG400/CA (10%)	Day 11 (GD 16)	F/4	<u>40</u>	5.6	37	43	15
				120	7.2	63	55	26
			F/6	400	13	152	100	63
Juvenile rat (aged 25 days)	RPV in HPMC (0.5% w/v)	Day 14	M/8	40	2.6	12	20	5
			M/7	120	3.7	34	28	14
			M/7	400	9.1	50	70	21
			F/8	40	5.8	18	45	8
			F/8	120	3.6	28	28	12
			F/7	400	7.3	53	56	22
Pregnant rabbit	RPV base in HPMC (0.5% w/v)	Day 14 (GD19)	F/3	5	6.7	105	52	44
				10	10	170	77	71
				20	15	232	115	97
Dog	RPV base in PEG400/CA (10%)	Day 363	M/4	5	1.1	17	8.5	7.1
			M/2	10	1.3	24	10	10
			M/4	40	4.1	65	32	27
			F/4	5	1.5	19	12	7.9
				10	2.2	36	17	15
			F/3	40	5.5	61	42	25
Monkey	RPV in HPMC (1%)/Tween 20	Day 55	F/8	100 b.i.d	0.14[b]	2.7	1.1	1.1
			F/7	250 b.i.d	0.31[b]	4.6	2.4	1.9

[a] : total dosing volume of 10 mL/kg was changed after Day 83 to two administrations of 5 mL/kg with 1.5 hours between the two administrations. The underlined dose is the NOAEL dose when it is determined, CA: citric acid; HPMC: hydroxypropyl-methylcellulose

[b] : after the first dosing**;** CA : citric acid ; HPMC: hydroxypropyl-methylcellulose.

4.3.2.1. Mice

The doses of RPV in the 3-month oral gavage study with CD-1 mice were 20, 80, or 320 mg/kg/day [see m2.6.7, RPV NDA]. There were no mortalities. Effects occurred almost exclusively at 320 mg/kg/day. No clinical signs were noted except abdominal distention, from week 6 of treatment onwards. Body weight and body weight gain were increased in line with higher food consumption throughout the dosing period. Red blood cell count, hemoglobin, and hematocrit were lower; in females, associated with an increase in reticulocyte count. In males, leukocyte count was decreased. Serum alkaline phosphatase (ALP) and alanine aminotransferase (ALT) activities and calcium and inorganic phosphate concentrations were increased. Females showed an increase in serum concentrations of urea and cholesterol, total protein, and albumin. Liver weight was increased in a dose-related fashion and showed hepatocellular hypertrophy with a dose-related increase in incidence and severity, at 80 and 320 mg/kg/day. At 320 mg/kg/day, this effect was associated with hepatocellular vacuolization, single cell necrosis, and pigmentation and proliferation of Kupffer cells, all to a slight to moderate degree. Moreover, electron microscopy revealed peroxisome proliferation. In kidney, female mice showed minimal to moderate nephropathy characterized by slight to marked multifocal tubular basophilia, minimal to slight glomerulopathy (atrophic glomeruli with thickened Bowman's capsule amidst basophilic tubules), minimal to moderate mononuclear cell infiltration, minimal to slight interstitial fibrosis, minimal tubular dilatation, and slight cortical mineralization. In adrenal glands, a marginally increased incidence was noted of swollen cells and/or cells with dense cytoplasm in the zona fasciculata in males ,whereas females showed a marginal decrease of a clear X-zone with increased brown degeneration in that zone. Ovaries showed a marginal decrease of the number and generations of corpora lutea. Also, granulocyte infiltration in the endometrium was marginally decreased. It cannot be excluded that these gonadal effects indicate a reduced cyclic activity. Moreover, extramedullar hematopoiesis in liver (marginal) and spleen (slight to moderate), and slight to moderate increase of the myeloid/erythroid ratio in bone marrow was noted. These effects are probably associated with the effects on red blood cell parameters.

Based on the findings in animals with 80 mg/kg/day in liver, the No Observed Adverse Effect Level (NOAEL) was considered to be 20 mg/kg/day associated with AUC values of 80 and 61 µg.h/mL for respectively males and females.

4.3.2.2. Rats

RPV base formulated in PEG400 + CA was administered once daily, by oral gavage, for 1 month to Sprague Dawley rats at 0 (water, negative control), 0 (vehicle), 10, 40 or 160 mg/kg/day in 10 mL/kg with satellite animals per group for the plaque forming cells (PFC) assay with sheep red blood cells [see m2.6.7, RPV NDA]. There were no mortalities associated with RPV, relevant clinical signs, or effects on body weight or food consumption, no treatment-related ophthalmic effects and no effects on the PFC assay. Higher thyroid gland weight and liver weights compared to the vehicle group were recorded in the groups dosed with 40 and 160 mg/kg/day. The increase in thyroid gland weight was associated with minimal follicular hypertrophy. The weight of the pituitary gland was slightly increased in animals dosed with 160 mg/kg/day.

The NOAEL was established at 10 mg/kg/day associated with AUC values of 7.2 and 14 µg.h/mL for males and females, respectively. Moreover, the absence of effects in the PFC assay indicated that RPV has no immunotoxic potential.

In the 6-month study, RPV base formulated in PEG400 + CA was administered once daily, by oral gavage, to Sprague Dawley rats at 0 (vehicle), 40, 120 or 400 mg/kg/day in 10 mL/kg [see m2.6.7, RPV NDA]. Increasing difficulties with the daily administration by gavage, morbidity and deaths started to occur after the first 2 months of dosing with comparable incidences in all groups including controls. For this reason, the dosing regimen was changed from once daily to twice daily dosing from Day 84 onwards. There were no relevant effects on ophthalmic examinations, body weight or food consumption.

In hematology, increases in activated partial thromboplastin time (APTT) and prothrombin time (PT) were noted in males of all groups without a dose-related trend, not showing recovery. Red blood cell count, hemoglobin, and hematocrit of males at 400 mg/kg/day were reduced slightly. The eosinophil count in females of all groups was decreased without a clear dose-related trend. Red blood cell parameters and eosinophil counts showed complete recovery after termination of dosing.

Serum chemistry showed increases in concentrations of total protein (females at 400 mg/kg/day), albumin (120 and 400 mg/kg/day), inorganic phosphate in females of all groups and males at 120 and 400 mg/kg/day, urea (males at 120 and 400 mg/kg/day), and creatinine (males of all groups). ALP activities were increased in males of all groups. Decreases were recorded in concentrations of triglycerides and total bilirubin in all groups. All serum chemistry changes showed complete reversibility at the end of the recovery period. Urinalysis showed no effects.

Serum thyroid stimulating hormone (TSH) concentrations were increased in all dose groups, associated with a decrease of serum thyroxine (T_4) concentrations. In contrast, triiodothyronine (T_3) concentrations showed lesser and equivocal effects: decrease in males at 40 mg/kg/day and increase in males at 400 mg/kg/day. At the end of the post-dosing period, all parameters, except T_4 showed recovery. Hormone concentrations showed an overall trend indicated a decrease of corticosterone levels and an increase in adrenocorticotropic hormone (ACTH) and progesterone concentrations at 120 and 400 mg/kg/day. No trend of any effect was observed at the end of the post-dosing period.

At necropsy, liver weight was increased associated with hepatocellular hypertrophy at 120 and 400 mg/kg/day. Thyroid gland weight was increased in all groups associated with a dose-related increase of diffuse follicular hypertrophy. Reversibility of the effect in females was not complete at 1 month after dosing. In the pituitary gland of males from all groups, the number of swollen/vacuolated cells in the pars distalis was increased. These cells are known to produce TSH [MacEnzie, 1990]. This effect was not noted in animals killed at the end of the post-dosing period, indicating complete recovery. In animals treated with 400 mg/kg/day, the macrophages that spontaneously form aggregates in the mesenteric lymph nodes had a swollen-vacuolated appearance. This effect showed no recovery.

In view of the effects on coagulation parameters and the thyroid and pituitary glands observed at the lowest dose of 40 mg/kg/day associated with AUC values of 12 and

50 µg.h/mL in males and females, respectively, a NOAEL could not be established in this study.

4.3.2.3. Dogs

RPV base formulated in PEG400 + CA was administered once daily, by oral gavage, for 1 month to beagle dogs at 0 (water, negative control), 0 (vehicle), 5, 10, or 40 mg/kg/day in 1 mL/kg [see m2.6.7, RPV NDA]. Reversibility of the effects was evaluated in a 1-month post-dosing period. No mortalities occurred in this study. At 40 mg/kg/day, body weight loss and reduced body weight gain were noted associated with a reduced food intake.

Red blood cell count, hemoglobin, and hematocrit were lower and white blood cell count was higher at 40 mg/kg/day. Albumin, total protein and triglycerides concentrations at 10 and 40 mg/kg/day were lower. The concentrations of cholesterol and total bilirubin as well as the activities of ALP and ALT at 40 mg/kg/day were higher. Progesterone concentrations were increased in a more or less dose-related fashion, in all groups. The AUCs of ACTH were increased at the end of the dosing period. The AUCs of cortisol showed a tendency for decrease at 10 and 40 mg/kg/day.

In the adrenal cortex, the number of swollen cells with dense cytoplasm and reduced Oil red O-staining was increased at 10 and 40 mg/kg/day. Weight of ovaries was increased in all groups in a dose-related way. The female genital tract and mammary glands showed increased activation at 10 and 40 mg/kg/day. In the ovaries, corpora lutea were detected in 2 animals at 10 mg/kg/day and in one animal at 40 mg/kg/day. More prominent tertiary follicles were noted in all test article-treated animals that had not ovulated at the end of dosing period. In liver, minimal to moderate centrilobular perivascular inflammatory reaction was observed in males. Minimal increase in the number of multifocally dispersed centrilobular hepatocytes with a clear appearance was noted at 10 and 40 mg/kg/day. Moreover, a slight to moderate increase of mononuclear phagocytic system (MPS)-aggregates, slight to minimal centrilobular hepatocellular single cell necrosis and multifocal centrilobular perivascular fibrosis and minimal multifocal bile duct proliferation occurred at 10 and 40 mg/kg/day. All adverse effects were completely reversible within a 1-month recovery period, except for the changes in the liver and the increased level of ALP in the serum.

Given the dose–related trend in endocrinology results and ovaries weight already evident in the group treated with the low dose of 5 mg/kg/day associated with AUC values of 27 and 37 µg.h/mL in males and females, respectively, a No Observed Effect Level (NOEL) was not established.

In the 6-month study, RPV base formulated in PEG400 + CA was administered once daily, by oral gavage to beagle dogs at 0 (vehicle), 5, 10, or 40 mg/kg/day in 1 mL/kg [see m2.6.7, RPV NDA]. One third of the animals were killed for an ad interim evaluation after 3 months. No mortalities occurred in this study. Animals at 40 mg/kg/day lost body weight associated with reduction of food consumption. There were no relevant effects on heart rate, ECG, ophthalmology, hematology or urinalysis.

At 40 mg/kg/day, serum chemistry showed increase in cholesterol and total bilirubin (also females at 10 mg/kg/day) concentrations and ALP activity (also in females at 5 and in males and females at 10 mg/kg/day).

The cortisol precursor 17α-hydroxyprogesterone was increased in all groups. AUCs of cortisol showed a dose-related decrease in males of all groups. AUCs of ACTH showed clear increases in males of all groups. Females showed similar effects as males on cortisol and ACTH, but to a lower extent.

Histopathology showed effects on the female genital tract and adrenal glands similar to those seen in the 1-month study. In addition, testes, liver and gall bladder were affected by RPV. In liver, minimal number of macrophages laden with presumably lipogenic (Perl's negative) pigment was noted perivascularly in some animals at 10 and 40 mg/kg/day. Minimal brown pigmentation of the gall bladder epithelium was noted at 40 mg/kg/day and incidentally at 10 mg/kg/day. In testes, minimal to slight Leydig cell hypertrophy occurred in one animal dosed with 10 mg/kg/day and in the two animals dosed with 40 mg/kg/day, after 3 months of treatment, and in two animals (minimal) treated for 6 months with 40 mg/kg/day. Ovaries weight was increased in all groups after 3 months and at 10 and 40 mg/kg/day after 6 months of dosing. At 40 mg/kg/day after 6 months, ovaries, uterus, and vagina had a swollen aspect. Histopathology showed a slight increase in the number of atretic follicles at 10 and 40 mg/kg/day and of regressive corpora lutea at 40 mg/kg/day whereas the number of tertiary follicles was increased in all groups.

Given the changes seen in adrenals and ovaries at the low dose of 5 mg/kg/day associated with AUC values of 21 and 17 µg.h/mL in males and females, respectively, no NOAEL was established.

For a 12-month toxicity evaluation, RPV base formulated in PEG400 + CA was administered once daily, by oral gavage to beagle dogs at 0 (vehicle), 5, 10, or 40 mg/kg in 1 mL/kg [see m2.6.7, RPV NDA]. No test article-related mortalities occurred. Body weight gain reduction was noted in all groups.

Hematology, serum chemistry, and urinalysis parameters were affected only at 40 mg/kg/day. Red blood cell count, hemoglobin, and hematocrit in males were lower. Serum calcium and total bilirubin concentrations were decreased and those of inorganic phosphate in females and of creatinine in males were increased. ALP activity in serum was increased.

Hormone analyses showed basically the same results as in the 6-month study. The additional hormones determined LH and testosterone showed no treatment-related effects. Estradiol concentrations in males were undetectable and in females were highly variable due to the estrous cycle.

Post-mortem evaluations showed basically the same effects as in the 6 month study with exceptions for liver and gall bladder, testes, and kidneys. In liver, yellow pigmentation in hepatocytes and canaliculi was noted at 40 mg/kg/day and incidentally at 10 mg/kg/day. Prominent brown pigment in the epithelium of the gall bladder was noted at 40 mg/kg/day. In testes, minimal hypertrophy of the Leydig cells was recorded in two males given 40 mg/kg/day. However, this effect had no impact on Sertoli cell functioning or spermatogenesis. In kidney at 40 mg/kg/day, acute interstitial nephritis in two males and minimal to slight corticomedullary mineralization in all terminally killed females were noted.

As a consequence of the body weight and adrenal changes at the low dose group of 5 mg/kg/day associated with AUC values of 17 and 19 µg.h/mL in males and females, respectively, no NOAEL was established.

4.3.2.4. Monkeys

RPV suspended in 1% m/v aqueous HPMC with 0.5% Tween 20 was administered for 8 weeks to immature female cynomolgus monkeys at 0 (vehicle), 200 or 500 mg/kg/day. Animals were dosed 0, 100, or 250 mg/kg twice daily (b.i.d.), with a 6-hour interval, at a volume of 5 mL/kg [see m2.6.7, RPV NDA]. No mortalities occurred in this study. No adverse or relevant effects were observed on body weight, clinical pathology, organ weights, or gross lesions.

In (trough level) samples taken prior to the daily dosing, increased levels of 17α-hydroxyprogesterone and progesterone were noted throughout the dosing period in a more or less dose-related fashion. Reduced concentrations of androstenedione were evident. Decreased dehydroepiandrosterone (DHEA) concentrations were noted only in the group dosed with 500 mg/kg/day. Upon ACTH challenge, serum concentrations of 17α-hydroxyprogesterone showed a strong dose-related increase. A similar pattern was visible for progesterone upon challenge. Androstenedione and DHEA levels in the vehicle group showed only limited response to challenge. The C_{max} values of androstenedione and DHEA at 500 mg/kg/day were lower. Cortisol C_{max} values at 500 mg/kg/day were decreased at the end of the dosing period.

Vaginal swabs were not indicative of menses. Moreover, no follicular or ovulatory effects were noted on serum levels of progesterone, estradiol, or LH. Microscopic evaluation of ovaries did not show any indication of activation. Minimal follicular cell hypertrophy in the thyroid gland was scored in one control animal, three animals dosed with 200 mg/kg/day, and four animals dosed with 500 mg/kg/day.

Since at the lowest dose of 200 mg/kg/day associated with an AUC value of 2.7 µg.h/mL several hormonal effects were evident, a NOAEL was not established.

4.3.2.5. General toxicity assessment

Mortality

No RPV-related mortality occurred in any of the single or repeat dose studies with rats, rabbits, dogs, and cynomolgus monkeys. Incidental mortalities occurred or humane killings were necessary as result of gavage incidents notably in studies with the PEG400 + CA vehicle, due to its viscosity.

In the pilot 2-week CD-1 mouse study, doses of 2000 mg/kg/day by oral gavage and 5000 mg/kg/day via diet were not tolerated. In the 4-week study with transgenic CB6F1-nonTgrasH2 mice, two RPV-related mortalities occurred at 320 mg/kg/day. The cause of these deaths could not be established [see m2.6.7, RPV NDA].

The trend test for mortality with dose, when all treated groups were included, was statistically significant for male mice in the mouse carcinogenicity study. For females, the trend test for mortality with dose was not statistically significant. In male and female mice dosed with 160 mg/kg/day, hepatic tumors caused death more frequently than in males and females of the control group [see m2.6.7, RPV NDA]. In the rat carcinogenicity study, there was no trend for mortality with dose and none of the tumors contributed to the deaths of the animals [see m2.6.7, RPV NDA].

In the 6 month rat study, increasing difficulties with the daily administration by gavage, morbidity and deaths started to occur after the first 2 months of dosing with comparable incidences in all groups including controls. For this reason, the dosing regimen was changed from once daily to twice daily dosing (5 mL/kg with an interval of 1.5 hour) from Day 84 onwards. The cause of death (reaching 50% in some of the groups) was concluded to be associated with the relatively high volume and viscosity of the formulation, necessitating a long gavage time during which the animals physically opposed the manual restraint. This led to gavage errors, regurgitation, and restrain trauma. In the 1-month post-dosing period, no further mortalities occurred [see m2.6.7, RPV NDA].

Exposure Margin

The exposure margin of RPV is expressed as the ratio of the AUC-value at the No Observed Adverse Effect Level (NOAEL) or Lowest Observed Adverse Effect Level (LOAEL) in animals and that in man at the recommended dose of 25 mg RPV q.d.. At this dose, the C_{max} of RPV in patients was 0.13 µg/mL and the $AUC_{0\text{-}24h}$ was 2.4 µg.h/mL. The AUC-values and the NOAELs and LOAELs are presented in Table 4.6.

Table 4.6 Ratio Animal/Man Exposures at NOAEL or LOAEL

Species	Type study	NOAEL (mg/kg/day)		LOAEL (mg/kg/day)		$AUC_{0\text{-}24h}$ (µg.h/mL)		Ratio	
		M	F	M	F	M	F	M	F
Mouse	3-mo Tox	20	20			80	61	33	25
	Carcinogenicity			*20*	*20*	*76*	*51*	*32*	*21*
Rat	1-mo Tox	10	10			7.2	14	3	6
	Teratogenicity		40 [b]				37		15
	Carcinogenicity			*40*	*40*	*6.3*	*14*	*3*	*6*
Rabbit	Teratogenicity		20 [b]				232		97
Dog	12-mo Tox			5	5	17	19	7	8
Cynomolgus monkey	8-wk Tox				200		2.7		1.1
Man	CPIII 25 mg q.d. [a]					2.4			

[a]: Pooled data Clinical Study C215 C209; [b] fetal NOAEL;
NOAEL: No Observed Adverse Effect Level; LOAEL: Lowest Observed Adverse Effect Level; AUC: area under the time vs concentration curve; Ratio: AUC animal/AUC man; M: male; F: female; mo: month; wk: week; CPIII: clinical phase III; q.d.: once daily; *Italic*: effect observed not relevant for man

In the dog and the cynomolgus monkey, a NOAEL could not be established as effects pertaining to inhibition of key enzymes of the adrenal steroidogenesis were still present at the lowest dose tested. However, upon close monitoring in man no indications of interference of RPV with adrenal steroidogenesis could be found in long term safety data for RPV with a dose of up to 150 mg q.d. In the carcinogenicity studies, a NOAEL could not be established as neoplastic lesions occurred in the lowest doses tested. In Section 4.3.4, the results of the carcinogenicity studies are evaluated and the relevance for man is assessed. It is concluded that RPV induced tumors in liver of mice and rats and in thyroid gland of rats by an epigenetic mechanism involving liver enzyme induction. This mechanism bears little or no relevance for man.

General Toxicity

The targets of toxicity of RPV identified in the repeat dose studies were: red blood cells (mouse, rat, and dog), coagulation (rat), liver (rat and dog), kidneys (mouse and dog), thyroid gland with secondary effects on the pituitary gland (rat), adrenal glands (mouse, rat, dog, and cynomolgus monkey), testes (dog), and ovaries (dog, in immature females with secondary effects on other tissues of the genital tract and on mammary glands). The majority of the induced effects appeared to be completely reversible after a 1-month post-dosing period. The effects on thyroid gland and coagulation in rats and on liver and serum ALP in dogs showed signs of recovery but this was not complete at the end of the 1-month post-dosing period. A number of targets were affected at the low dose tested in dogs and cynomolgus monkeys preventing establishment of a NOAEL in these species.

Liver

Liver effects in rats and mice differed qualitatively from those in dogs. In the rodent species, predominantly hepatocellular hypertrophy occurred accompanied by effects on liver-associated serum parameters. Liver enzyme activities in serum, ALT and ALP, were more consistently increased in the mouse studies than in the studies in rats. Moreover, serum total protein concentrations mainly due to serum albumin were increased. However, serum triglycerides and total bilirubin (rats, only) concentrations were reduced

Mice showed some additional or different effects compared to rats. These differences may be associated with the much higher exposures in mice than in rats. The hepatocellular hypertrophy in mice was accompanied by an increase in vacuolization and single cell necrosis. Electron microscopy indicated that this hypertrophy was associated with peroxisome proliferation. By contrast, the incidence of these additional effects was lower in rats. In addition, mice showed pigmentation and proliferation of Kupffer cells and increased serum concentrations of cholesterol, calcium, and inorganic phosphorus. The interrelationship of the serum chemistry effects is doubtful and an association with the Kupffer cell effects is not very likely.

In dogs, the liver effects changed with the duration of treatment. In the 1-month study, perivascular inflammatory reactions together with multifocal perivascular fibrosis and single cell necrosis were noted in the central part of the lobules. Moreover, increased MPS aggregates occurred and multifocal bile duct proliferation. These effects seem to be associated with increased serum concentrations of cholesterol and total bilirubin and increased activities of ALP and ALT. In the 6-month study, pigment-laden perivascular macrophages were noted and in males only, prominent brown pigment in the gall bladder epithelium. The pigment in the macrophages was not stainable with Perl's stain indicating that it probably was of lipogenic origin. This pigmentation was the only liver effect observed after 3 months of dosing (in high dose males only) indicating the relatively slow development of this effect which also applies for the pigmentation in the gall bladder. In the 12-month dog study, pigmentation of hepatocytes and canaliculi was noted together with prominent brown pigment in gall bladder epithelium, both in males and females. The histopathological effects were associated with an increased serum concentration of total bilirubin and with an increase in serum ALP activity.

The nature and degree of the observed effects in serum chemistry, liver histopathology, and liver enzyme induction as well as the absence of significant bioaccumulation, generation of reactive metabolites, and immune-related hepatic effects support the conclusion that RPV has no potential for inducing hepatotoxicity [EMEA/CHMP/SWP/150115/2006].

In the studied species, liver effects did not occur at the lowest dose tested indicating that the exposure in animal studies without liver effects is at least 3-fold higher than the exposure at the intended human dose of 25 mg RPV q.d. (Table 4.6). Long term safety data for RPV do not indicate an adverse effect of RPV on liver [see Section 5.4.1.1, m2.5].

Thyroid Gland

Effects on the thyroid gland in rats were characterized by an increased organ weight, hypertrophy of follicular epithelium, and reduced serum concentrations of thyroxine. The effects occurred similarly in males and females and were noticed already after 7 days of treatment. Upon longer duration of treatment, increased serum concentrations of TSH were recorded and also a differential effect on T_3. The serum concentrations of this thyroid hormone were less reduced than those of thyroxine and in some studies not affected or even increased. The effects on thyroid gland are thought to be caused by an increased clearance of thyroxine. A well-known and frequent cause of the enhanced thyroxine clearance in rats is induction of T_4-uridine diphosphate glucuronosyltransferase (UDPGT) in liver. Ex vivo enzyme induction determinations in liver homogenate from rats treated with RPV for 6 months showed only a 20% higher UDPGT activity of the high dose group dosed with 400 mg/kg/day compared to controls. There are no indications that RPV has a direct effect on thyroid gland or a particular affinity to thyroidal tissues. The NOAEL for the thyroid gland effects is 10 mg/kg/day associated with AUC levels of 7.2 and 14 µg.h/mL in male and female rats, respectively.

The minimal follicular cell hypertrophy that was noted in thyroid gland of immature female cynomolgus monkeys in the 8-week study is considered associated with the young age of these animals at the start of the study. In this study, no levels of thyroid hormones or TSH were determined. Microscopical evaluation of other organs did not show any cause for the thyroidal effect.

The majority of circulating thyroxine in rodents is unbound to plasma proteins due to low expression of thyroid binding globulin (TBG). Primarily, the unbound fraction of this hormone is metabolized by UDPGT. Consequently, even a small increase in metabolic clearance has a significant impact on the plasma concentrations of thyroxine, in rodents. In contrast, thyroxine in man is almost completely bound primarily to TBG and to a lesser extent to transthyretin and albumin. Should UDPGT induction occur in man, this will have only a small effect on the total thyroxine concentration in plasma. For this reason, the thyroid effect seen in rats bears no relevance for man.

Pituitary Gland

Effects on the pituitary gland occurred only in rats and were characterized by an increase of swollen and vacuolated cells in the pars distalis. These effects are considered secondary to the effects on thyroxine clearance. The indicated cells produce TSH. The low concentration of thyroxine in the circulation is the feedback signal for the pituitary gland to produce more TSH. The NOAEL for effects on the pituitary gland or serum concentrations of TSH is 10 mg/kg/day associated with AUC levels of 7.2 and 14 µg.h/mL in male and female rats, respectively. As the effects on pituitary gland and TSH are secondary to the effects of RPV on rodent-specific thyroxine clearance, they bear no relevance for man.

Kidneys

Transgenic mice treated with 320 mg/kg/day, and a few treated with 80 mg/kg/day, and wild type mice at 320 mg/kg/day showed minimal to moderate degenerative or necrotic nephropathy. In the carcinogenicity study with CD-1 mice, no increased incidence of nephropathy, of mortality associated with renal effects, or of renal neoplasia was noted. The NOEL for this effect is 20 mg/kg/day. At this dose, the exposure is at least 25 times higher than the exposure at the recommended dose of 25 mg RPV q.d. in man (Table 4.6).

Dogs treated for 12 months with 40 mg/kg/day showed nephritis in males and mineralization in the corticomedullary region in females. The NOEL for these effects is 10 mg/kg/day. At this dose, the exposure is at least 10 times higher than the exposure at the recommended dose of 25 mg RPV q.d. in man (Table 4.5).

The mechanism of these kidney effects in mice and dogs is not clear taken into account the low percentage of the dose excreted via urine. Long term safety data of RPV-C204 indicate a small but consistent increase of the serum creatinine concentration. The kidney effects seen in mice and dogs are not indicative of an effect on glomerular filtration or proximal tublar resorption. For these reasons and in view of the exposure margins, the non-clinical kidney effects are considered not relevant for man.

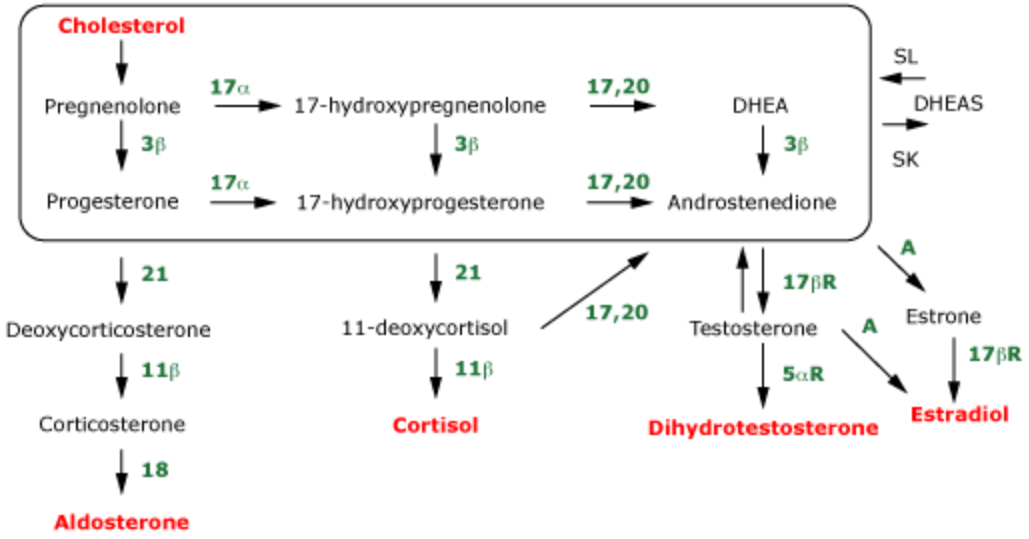
Adrenal Glands

In all species involved in non-clinical safety studies with RPV except in rabbits, an effect on adrenal gland has been demonstrated. The levels of adrenal hormones or their precursors and ACTH in serum of rats and dogs, and the results of the in vitro studies with Guinea pig primary cell cultures and homogenate of adrenal gland cortex of dog indicate that RPV partially inhibits 21-hydroxylase also known as cytochrome P450 isozyme 21 (CYP21) (see Figure 5). CYP21 catalyzes the conversion from progesterone and 17α-hydroxyprogesterone to 11-deoxycorticosterone and 11-deoxycortisol, respectively, the direct precursors of respectively corticosterone and cortisol. A partial block of this pathway will lead to a reduced production of downstream hormones of which cortisol and corticosterone are the most important. Moreover, an accumulation of the substrates of CYP21, progesterone and 17α-hydroxyprogesterone and more upstream hormones may occur. In vivo, the reduced serum levels of cortisol and corticosterone due to inhibition of CYP21 will trigger an ACTH- response from the pituitary. The increased stimulus of the adrenal cortex by ACTH will partially counteract the reduction of the cortisol and corticosterone synthesis but at the same time will increase the accumulation of the CYP21 substrates.

An additional effect of the CYP21 inhibition, notably in species that have a significant androgenic adrenal pathway, is that the increased ACTH stimulus and subsequent accumulation of progesterone and 17α-hydroxyprogesterone may lead to an increased androgen production. 17α-hydroxypregnenolone and 17α-hydroxyprogesterone are converted to DHEA and androstenedione, respectively, catalyzed by 17, 20 lyase (CYP17). CYP17 in the zona fasciculata catalyzes the hydroxylation of pregnenolone and progesterone and is also known as 17-hydroxylase. To investigate the impact of the

stimulation of the androgenic pathway, a study was conducted in cynomolgus monkeys. This monkey strain and all non-human primates share with man a significant androgenic adrenal pathway. The 8-week study in cynomolgus monkeys showed that RPV inhibits both CYP21 and CYP17. This dual effect led to a clear accumulation of progesterone and 17α-hydroxyprogesterone, both being substrates for both CYP21 and CYP17, and a reduction of the unchallenged serum levels of androstenedione and DHEA and of the response of these androgens to ACTH challenge in this species.

Figure 5 Synthetic Pathways for Adrenal Steroid Synthesis in Non-human Primates and Man



The first step in adrenal steroid synthesis is the combination of acetyl CoA and squalene to form cholesterol, which is then converted into pregnenolone. The enclosed area contains the core steroidogenic pathway utilized by the adrenal glands and gonads.
17α: 17α-hydroxylase (CYP17, P450c17); 17,20: 17,20 lyase (also mediated by CYP17); 3β: 3β-hydroxysteroid dehydrogenase; 21: 21-hydroxylase (CYP21A2, P450c21); 11β: 11β-hydroxylase; (CYP11B1, P450c11); 18 refers to the two-step process of aldosterone synthase (CYP11B2, P450c11as), resulting in the addition of an hydroxyl group that is then oxidized to an aldehyde group at the 18-carbon position; 17βR: 17β-reductase; 5αR: 5α-reductase; DHEA: dehydroepiandrostenedione; DHEAS: DHEA sulfate; A: aromatase (CYP19).

The effects of RPV on adrenal gland and its hormonal biosynthetic pathways are more prominent than the effects on gonads and their pathways (see below in more detail). This may be explained by the affinity of RPV for adrenal gland in repeat dose studies in dogs and of radiolabel in the rat single dose quantitative whole body autoradiography (QWBA) studies with ^{14}C-RPV. In the QWBA studies, no particular affinity for gonads was noted.

Effects on adrenal hormones were noted in all repeat dose dog studies with treatment duration of at least 7 days. Apparently, the effect needs some time to become manifest. This may explain why no effects were noted in the single oral dog study with a corticotrophin releasing factor (CRF) challenge 24 hours after dosing.

The fact that effects on adrenal pathways were already detected at the lowest dose levels in the dog and cynomolgus studies prevented establishment of a NOAEL in these species. Long term safety data from RPV-C204 showed no clinically relevant effects of inhibition of CYP21 and CYP17 in adults.

Ovaries

The effects in ovaries of dogs after at least 1 month of treatment with RPV were characterized by increased numbers of tertiary follicles, (cystic) luteinized follicles, and in some dogs by corpora lutea. These effects did not intensify over time after 3 months of dosing. The age of the dogs at the start of dosing in the 1-month studies was between 6.5 and 8 months. In the 1-month studies, the ovarian effects were associated with changes in the other parts of the female genital tract and with the prominent activation of the mammary glands. It is conceivable that the mechanism of action of the effects on the ovaries in dogs is associated with the inhibition of CYP21 and CYP17 by RPV. But the exact mechanism of action of the observed early maturation (1-month studies) or activation of ovaries in the dog could not be evaluated from the general toxicity studies. Effects of RPV at the level of serum concentrations of progesterone and estradiol were difficult to evaluate due to the estrous cycle of the dogs.

The absence of ovarian effects in rats and immature cynomolgus monkeys provided no contribution to the establishment of the mechanism of the ovarian effect in dogs. The absence of the ovarian effects in the cynomolgus monkeys cannot be explained by lack of sensitivity of that species for the endocrine effects of RPV. The compound had a clear effect on adrenal CYP21 and CYP17 in that species as shown by the increases in serum progesterone and 17α-hydroxyprogesterone and from the reduction of DHEA and androstenedione. The more early stage of development of the monkeys (approximately 20 months of age at necropsy, at least one year prior to the onset of puberty) compared to the dogs (approximately 7 months of age at necropsy, a few months prior to the onset of puberty) may have contributed to the difference in response. However, a dog-specific effect cannot be excluded. A NOAEL could not be established as the ovarian effects occurred also in the lowest dose tested in dogs.

Testes

The Leydig cell changes seen in dogs only were characterized by bilateral diffuse swelling or hypertrophy noted at the end of the 1-month and 12-month treatment and hyperplasia and hypertrophy after 3 and 6 months of treatment. The effects were minimal in the 1-month study, most prominent after 3 months of treatment and again minimal after 6 and 12 months. It cannot be excluded that the minimal Leydig cell hypertrophy is associated with the effects of RPV on CYP21 and CYP17. However, the exact mechanism of action could not be evaluated in these general toxicity studies in which there were no effects on serum testosterone or luteinizing hormone (LH). Moreover, long term safety data from TMC278-C204 showed no clinically relevant effects.

In addition to the Leydig cell effects, marginal findings were noted in the seminiferous tubules and secondarily in the epididymides. The marginally increased focal unilateral atrophic tubules seen at the high dose after 3 and 6 months of treatment were associated

with marginal cellular debris in the epididymides, in the mid and the high dose groups. Reviewing all the data on testes after completion of the development program, this effect and the reduced spermiogenesis leading to reduced numbers of spermatozoa in the epididymides of a single animal in the mid and high dose groups after 6 months of treatment are not considered of toxicological relevance. The main reasons for this conclusion are the similarity with background data and the lack of effects on spermiogenesis in the 12-month study upon staging of the spermatogenic cycles.

Red Blood Cells

In mice, rats, and dogs, RPV caused a small (never exceeding 10%) reversible reduction of red blood parameters in combination with regenerative signs. The effects usually occurred only in the high dose groups in each of the studies with the indicated species. There were no clinical signs or symptoms of anemia noted in any of the species. Since exposures at the high doses in the various species differed considerably, it seems that the effect on red blood cells is not directly associated with exposure. Regeneration was shown by extramedullar hematopoiesis leading to increases in reticulocyte counts. The mechanism of action of the effect on red blood parameters could not be established in any species. However, there were no signs indicative of bone marrow suppression. The increased cellularity in bone marrow of female mice at the high dose is likely due to an increase of erythroid elements compensatory to the decrease of red blood cells in the circulation. The increased myeloid/erythroid ratio in bone marrow of male mice is assumed due to an increase in myeloid precursor cells compensating the decrease of lymphocytes in the circulation. Moreover, in the mouse and rat carcinogenicity studies, no effects were noted on bone marrow and hematopoietic cells.

The NOEL for this effect with the lowest exposure in terms of AUC was established in rats at 120 mg/kg/day (Table 4.6). The associated AUC value of 35 µg.h/mL is approximately 15 times higher than the exposure in man at the recommended dose of 25 mg RPV q.d. For this reason, it is not likely that the small effect on red blood cells in the non-clinical species is associated with the decrease of red blood cell count in Clinical Phase III studies TMC278-C209 and TMC278-C215.

Coagulation

In male rats, increased values for APTT and PT were noted in the 2-week pilot study with RPV at doses of 1500 mg/kg/day and higher and in the 6-month study at all doses of RPV from 40 up to 400 mg/kg/day. Increased values of intrinsic (APTT) and extrinsic (PT) coagulation times indicate a reduced efficacy of these major coagulation pathways. The relevance of this limited effect (less than 30% increase) is questionable since it was not associated with any bleedings, did not demonstrate a dose effect relationship, and occurred in males only.

The effect did not occur in the lowest dose tested, 10 mg/kg/day, associated with an AUC value in males that was 3 times higher than the exposure at the recommended dose of 25 mg RPV q.d. (Table 4.6).

4.3.3. Genotoxicity

Genotoxicity testing of RPV base or RPV comprised 4 non-mammalian (bacterial) reverse gene mutation (Ames) tests, 2 mammalian (mouse lymphoma) forward mutation assays and 1 in vivo mouse bone marrow micronucleus assay.

RPV was tested up to the maximum concentration that allowed scoring due to precipitation. No increased mutation frequency [see m2.6.7, RPV NDA] or increased frequency of structural chromosomal aberrations [see m2.6.7, RPV NDA] was noted. In the Ames test with human S9 [see m2.6.7, RPV NDA], metabolites M30/M31, that are formed following Michael addition at the unsaturated nitrile moiety of RPV, were present at the same level as determined in human feces. Also this Ames test did not show an increased mutation frequency.

In the in vivo mouse bone marrow micronucleus test, the maximum feasible dose of 1600 mg/kg RPV base did not induce an increase of micronuclei (C_{1h} was 60 and 58 μg/mL and AUC_{0-6h} was 307 and 287 μg.h/mL for respectively males and females) [see m2.6.7, RPV NDA].

4.3.4. Carcinogenicity

4.3.4.1. Mice

RPV formulated in 0.5% m/v aqueous HPMC was administered once daily by oral gavage for 24 months. Groups of 60 male and 60 female CD-1 mice were given 0 (vehicle), 20, 60 or 160 mg/kg/day at a dose volume of 10 mL/kg [see m2.6.7, RPV NDA]. In the liver, a statistically significant dose-related increase in total hepatocellular tumors (adenomas and carcinomas combined) was seen in males dosed with RPV from 20 mg/kg/day onwards. The incidences of carcinomas and of adenomas for all groups treated with RPV and for the groups dosed with 60 and 160 mg/kg/day, respectively, were above the ranges expected from background data of the testing facility. In female mice treated with 60 and 160 mg/kg/day, the incidences of liver adenomas and of adenomas and carcinomas combined were statistically significantly increased and above the background data range.

It was concluded that oral administration of RPV to CD-1 mice produced a dose-related increase in total hepatocellular tumors, adenoma and carcinoma), in males dosed with 20 and in males and females that received 60 and 160 mg/kg/day. For that reason, a NOAEL could not be determined. The associated systemic exposure expressed as AUC values determined in Week 28 in animals dosed with 20 mg/kg/day was 76 and 51 μg.h/mL in males and females, respectively.

4.3.4.2. Rat

RPV formulated in 0.5% m/v aqueous HPMC was administered once daily by oral gavage for 24 months. Groups of 65 male and 65 female Sprague Dawley rats were given 0 (vehicle), 40, 200, 500 or 1500 mg/kg/day at a dose volume of 10 mL/kg [see m2.6.7, RPV NDA]. There was no effect of treatment on mortality. No treatment-related

pathologies contributing to death and no adverse clinical signs were observed. Hematology did not show treatment-related effects. Serum chemistry showed mainly at 500 and 1500 mg/kg/day, liver-associated effects.

In liver, an increase in hepatocellular adenomas was seen in animals given 40, 200, 500, or 1500 mg/kg/day. There was no apparent dose-related trend and the differences from control values did not reach statistical significance. When compared with the historical background data, the incidence of tumors in females was above the background level in all groups treated with RPV, whereas in the males the incidence of tumors was equal to the maximum historical background incidence recorded.

In thyroid gland, a statistically significant increase of the number of follicular cell adenomas and of adenomas and carcinomas combined was seen in all groups with only a marginally apparent dose-related trend. The incidence of follicular cell adenomas was above background levels at 200, 500, or 1500 mg/kg/day. The incidence of the carcinomas was above background levels in males only at 200 and 1500 mg/kg/day. None of the liver or thyroid gland tumors was considered contributory to death in any rat.

It is concluded that the oral administration of RPV to Sprague Dawley rats at doses of 40, 200, 500, or 1500 mg/kg/day produced neoplastic changes in liver and thyroid gland. For that reason, a NOAEL could not be determined. The associated systemic exposure expressed as AUC values determined in Week 39 in animals dosed with 40 mg/kg/day was 6.3 and 14 µg.h/mL in males and females, respectively.

4.3.4.3. Genotoxicity and carcinogenicity assessment

Genotoxicity tests, in vitro and in vivo, have shown RPV to be free of a genotoxic potential . Nevertheless, the carcinogenicity studies with RPV in mice and rats induced hepatocellular adenomas and carcinomas and in rats follicular adenomas and carcinomas in the thyroid.

Hepatocellular adenomas and carcinomas are common spontaneous liver neoplasms in rodents [Greaves, 2007]. In general, hepatocarcinogens are divided into genotoxic and non-genotoxic agents [Grasso, 1991]. Since RPV is not genotoxic in a battery of in vitro and in vivo assays, the neoplastic lesions in liver observed in the mouse and rat carcinogenicity studies are considered a consequence of a non-genotoxic mechanism of the action of RPV rather than an expression of a direct carcinogenic potential of the compound. For non-genotoxic carcinogens, several mechanisms of action have been reported for rodent liver neoplasm development including phenobarbital-like cytochrome P450 induction [Holsapple, 2006]. The increased liver weight associated with signs of enzyme induction caused by RPV was already evident in the repeat dose general toxicity studies. Ex vivo hepatic enzyme activity evaluated in the 3-month mouse study and the 6-month rats study showed that RPV caused strong induction of the CYP4A family in male and female mice and male rats and induction of the CYP3A family in female rats. Electron microscopy of mouse liver noted peroxisome proliferation, a lesion commonly associated with CYP4A induction [Lake, 2009]. This pattern of liver enzyme induction correlates well with the incidence of hepatocellular adenomas and carcinomas [Lake, 2009].

The repeat dose toxicity studies in rats have demonstrated, without exception, effects on the thyroid gland considered due to increased clearance of thyroxine by UDPGT induction. UDPGT induction is a well-known cause of increased thyroid hormone clearance and, if it occurs life-long, such induction, is associated with the development of follicular adenomas and carcinomas [Capen, 1999].

No neoplastic lesions were observed in adrenal glands in spite of the high affinity of RPV and/or its metabolites for this tissue and the observed indications of inhibition of CYP21. The latter caused a reduced output of cortisol and corticosterone in several species including the rat. The decreased serum levels of these corticosteroids lead to increased stimulation of the adrenal gland by ACTH to compensate for the reduced output. The absence of neoplasia in adrenal tissue for which RPV has a high affinity indicates the absence of a direct genotoxic potential of the compound.

Taking into account the results of the genotoxicity and the carcinogenicity studies, it is concluded that RPV has no potential to induce direct DNA-related effects.

The epigenetic carcinogenic effects on mouse and rat liver and rat thyroid gland are associated with induction of liver enzymes CYP3A, CYP4A, and UDPGT. A similar association between liver enzyme induction and carcinogenesis does not exist for man [Grasso, 1991 and Holsapple, 2006].

For these reasons, it is concluded that the epigenetic carcinogenic effects of RPV in mouse and rat bear no relevance for man.

4.3.5. Reproductive and developmental toxicity

4.3.5.1. Fertility

For a male fertility study, RPV suspended in 0.5% (m/v) aqueous HPMC was administered once daily, by oral gavage to Sprague Dawley rats at 0 (vehicle), 100, 400, or 1600 mg/kg/day in a dose volume of 10 mL/kg [see m2.6.7, RPV NDA]. Treatment started 10 weeks prior to mating, during mating and for 3-4 weeks after the mating period. The males were paired 1:1 for mating with untreated females. There were no mortalities associated with RPV. There were no relevant clinical signs and no relevant effects on body weight, food consumption, gross or histopathological lesions, weights of epididymides or testes, and no adverse effects on the motility, concentration, or morphology of the sperm. There was no effect on fertility up to 1600 mg/kg/day. Weights of liver and thyroid gland showed a dose-related increase in all groups receiving RPV.

The NOAEL for male fertility is at least 1600 mg/kg/day

Female Sprague Dawley rats were dosed with RPV suspended in 0.5% (m/v) aqueous HPMC once daily, by oral gavage at 0 (vehicle), 40, 120, or 400 mg/kg/day in a dose volume of 10 mL/kg [see m2.6.7, RPV NDA]. Treatment started 2 weeks prior to mating, during mating and till Day 7 of pregnancy. The females were paired 1:1 for mating with untreated males. No mortalities were noted. Moreover, there were no relevant clinical signs and no relevant effects on body weight, food consumption, gross pathology, estrous

cycle, mating or pregnancy rate, the number of corpora lutea, implantations or live fetuses, or early embryonic development indices. It was concluded that there was no effect on female fertility, fecundity, or early embryonic development up to 400 mg/kg.

Therefore, the NOAEL for female fertility, fecundity, and early embryonic development was at least 400 mg/kg/day.

4.3.5.2. Embryo-fetal development

Rat

RPV base dissolved in PEG400 + CA was administered once daily, by oral gavage, from Gestation Day (GD); day of sperm detection is GD 0) 6 to 17 to pregnant Sprague Dawley rats at 0 (vehicle, 24 animals), 40, 120, or 400 mg/kg/day at a dose volume of 10 mL/kg [see m2.6.7, RPV NDA]. The dams were necropsied on GD 21. There were no mortalities associated with RPV. Reduced body weight gain and food consumption were noted in dams given 120 and 400 mg/kg/day. Weight of thyroid gland showed a dose-related increase at 120 and 400 mg/kg/day. Visceral examinations showed a slight increase in a minor variation, dilated renal pelvis, in 5 out of 149 and 7 out of 149 fetuses from the groups treated with 120 and 400 mg/kg/day, respectively.

The maternal and embryo fetal NOAEL was 40 mg/kg/day based on the changes on the body weight, food consumption and the increase of dilated renal pelvis seen at higher doses. Systemic exposure expressed as AUC value determined at the end of the dosing period in animals dosed with the NOAEL was 37 µg.h/mL.

Rabbit

RPV base suspended in aqueous 0.5% (m/v) HPMC was administered once daily, by oral gavage, from GD 6 to 19 to pregnant New Zealand white rabbits at 0 (vehicle), 5, 10, or 20 mg/kg/day in a dose volume of 5 mL/kg [see m2.6.7, RPV NDA]. The females were killed on GD 28 and a necropsy was performed. There was a slight increase in numbers of fetuses exhibiting changes commonly seen in rabbits that are considered to have little or no biological significance.

The maternal NOAEL was at least 20 mg/kg/day and the fetal NOAEL was 10 mg/kg/day. Systemic exposure of the dams expressed as AUC values determined at the end of the dosing period at the fetal NOAEL was 170 µg.h/mL.

4.3.5.3. Peri- and postnatal development

RPV suspended in 0.5% (m/v) aqueous HPMC was administered once daily by oral gavage to time-mated female Sprague Dawley rats from GD 6 to lactation day (LD) 20 at 0 (vehicle), 40, 120, or 400 mg/kg/day in a dose volume of 10 mL/kg [see m2.6.7, RPV NDA]. Developmental landmarks of the pups were recorded on LD 3, 5, 15, and 21. At necropsy of the dams after weaning of their litters, the number of implantation scars in uterus was determined. From the offspring (F1-generation), 20 males and 20 females per group were kept and their growth, post-weaning development and behavior and

reproductive performance were assessed. There were no effects on any of the determined parameters. The NOAEL for both the F0 and F1 generation was at least 400 mg/kg/day.

4.3.5.4. Reproductive and developmental assessment

The reproductive and developmental toxicity studies did not demonstrate any effects on male or female fertility or fecundity. The NOAEL in the male fertility study was at least 1600 mg/kg/day, associated with an $AUC_{0\text{-}24h}$ of approximately 85 µg.h/mL, obtained from 2 week oral rat studies with RPV [see m2.6.7, RPV NDA]. The NOAEL in the female fertility study is at least 400 mg/kg/day, associated with an $AUC_{0\text{-}24h}$ of approximately 100 µg.h/mL, obtained from 2 week oral rat studies with RPV [see m2.6.7, RPV NDA]. The exposures at NOAEL in rats are at least 35 times higher than the exposure at the recommended dose of 25 mg RPV q.d., in man.

The results of the embryo-fetal developmental studies in rats and rabbits with the highest feasible exposures to RPV demonstrated the absence of a potential for teratogenicity [see m2.6.7, RPV NDA]. The maternal and fetal NOAELs in rats were established at 40 mg/kg/day, associated with a maternal $AUC_{0\text{-}24h}$ of 37 µg.h/mL. In rabbits, the fetal NOAEL was established at 10 mg/kg/day, associated with a maternal $AUC_{0\text{-}24h}$ of 170 µg.h/mL. The exposures at NOAEL for embryo-fetal toxicity are at least 15 times higher than the exposure at the recommended dose of 25 mg RPV q.d., in man [m1.14.1 (US) or m1.3 (EU)]. The safety of RPV has not been assessed in pregnant women in well controlled clinical trials. Therefore, women of child bearing potential should only use RPV if the potential benefit justifies the potential risk.

In the peri- and postnatal developmental study in rats, no effects were observed on maternal behavior after parturition and during weaning, or on development of offspring from dams treated with RPV during pregnancy and lactation. The NOAEL in the peri- and postnatal developmental study is at least 400 mg/kg/day, associated with a maternal $AUC_{0\text{-}24h}$ of approximately 100 µg.h/mL, obtained from 2-week oral rat studies with RPV [see m2.6.7, RPV NDA]. This exposure is at least 40 times higher than the exposure at the recommended dose of 25 mg RPV q.d., in man. In animals, no studies have been conducted to assess directly the excretion of RPV into the milk. In humans it is not known if RPV is excreted in milk. Because of the potential for HIV transmission to nursing infant, mothers should be instructed not to breastfeed if they are receiving RPV [see Section 5.8, m2.5].

4.3.6. Juvenile Toxicity

4.3.6.1. Rat

RPV suspended in 0.5% m/v aqueous HPMC was administered once daily by oral gavage to time-mated female Sprague Dawley rats from GD 6 to Lactation Day 7 (LD, day of delivery is LD 0) at 0 (vehicle, 2 groups), 40, 120 and 400 mg/kg/day in a dose volume of 10 mL/kg [see m2.6.7, RPV NDA]. On LD 7, 8 male and 8 female pups of each group were selected for oral dosing by gavage with the same dose and at the same dose volume as their mothers. The selected pups from the second vehicle group were to be dosed with 400 mg/kg/day. The selected pups were dosed from LD 12 up to and including LD 25.

Blood samples for toxicokinetics were taken on LD 25. Necropsy was performed after the last sampling. Two pups from the group treated with 400 mg/kg/day were killed for humane reasons. The cause of death could not be established. There were no effects on the pups dosed by gavage with RPV during the dosing period or at necropsy. Systemic exposures for control pups dosed with 400 mg/kg/day and pups from dams treated with 400 mg/kg/day and subsequently dosed with the same dose expressed as AUC values determined at the end of the dosing period were 40 to 50 µg.h/mL in male and female pups.

4.3.6.2. Dog

No specific dog juvenile toxicity studies were done. However, in all dog studies, the animals were at the start of dosing 6 to 8 months old, i.e. immature. In the 1-month dog study, the negative control female animals were still immature (no signs of ovarian maturation or ovulation) at necropsy. RPV induced more prominent tertiary follicles in the ovaries of animals that did not ovulate and induced ovulation in 3 animals. The effects by RPV in the dogs that ovulated in the 1-month study were basically similar to those observed in mature dogs at the end of the 6- and 12-month dog studies.

4.3.6.3. Monkeys

In the 8-week immature female cynomolgus monkey study (see Section 4.3.2.4), RPV caused only effects indicative of inhibition of adrenal CYP21 and CYP17. These effects comprised increased serum concentrations of progesterone and 17-hydroxyprogesterone and decreased serum concentrations of androstenedione and DHEA. RPV did not induce any effects on ovaries such as those observed in immature dogs.

4.3.6.4. Juvenile toxicity assessment

In the rat juvenile toxicity study with pups from RPV-treated mothers dosed by gavage from LD12 to 25, no effects were noted [see m2.6.7, RPV NDA]. The exposure of these pups was similar to that of adult rats dosed with the same dose of RPV. Studies with immature dogs and cynomolgus monkeys [see m2.6.7, RPV NDA] showed no effects different from those in adult animals, in the case of dogs, and no effects apart from those associated with inhibition of adrenal CYP21 and CYP17 in the immature female cynomolgus monkeys. These adrenal effects in cynomolgus monkeys are considered independent of the age of development and are similar (with respect to CYP21) to those seen in immature and adult dogs and adult rats. The ovarian effects in immature dogs did not occur in immature cynomolgus monkeys and are considered dog-specific and not relevant for man. Therefore, it is concluded that RPV will not induce different effects in children and adolescents from those it has in adults.

4.3.7. Local tolerance

RPV tested negative for the potential to cause phototoxicity in vitro, skin irritation in rabbits, and delayed-type hypersensitivity in the mouse local lymph node assay [see m2.6.7, RPV NDA]. The compound did not induce an immunotoxic effect on the challenge of rats with sheep red blood cells. RPV was classified as a moderate eye irritant in an in vitro test.

4.3.8. Other toxicity

4.3.8.1. Impurities

The drug substance contains 3 impurities that needed to be qualified according to Guideline Q3A of the International Conference on Harmonization (ICH), entitled: "Impurities in New Drug Substances". Two of these, gsk001* and gsk002* , have been evaluated upon spiking into the drug substance at a level of 4%. Qualification comprised a bacterial reverse mutation Ames test, a mouse lymphoma assay, and a 1-month oral rat study. The presence of the impurities at 4% did not modify the effects of RPV in any of the tests. The third impurity, gsk003*, is the [redacted] of RPV. This [redacted] was present in all drug substance batches involved in pivotal non-clinical studies at the level of minimally 0.61%. In view of the close structural relationship with RPV and the overage between the lowest non-clinical dose (5 mg/kg/day) and the recommended dose of 25 mg q.d., separate qualification of gsk003* is not considered relevant.

Three further impurities, gsk004*, gsk005*, and gsk006*, that contain moieties with a mutagenic alert, are present in the drug substance at levels that do not warrant qualification according to ICH Q3A. The mutagenic potential of the HCl salt of gsk004*, namely gsk007*, and of gsk005* and gsk006* was tested in an Ames test. Only gsk007* showed a mutagenic potential. It is therefore concluded that gsk004* is a genotoxic impurity.

4.3.8.2. Impurity assessment

gsk004* appeared to be genotoxic whereas the potential impurities gsk008* and gsk009* with structural analogy to gsk004* did not induce an increased mutation frequency in the Ames test. Probably, the primary aromatic amine moiety of gsk004* caused the genotoxic effect. The maximum allowable level of genotoxic impurity gsk004* in a daily dose of 25 mg RPV was calculated to be [redacted] ppm, on the basis of the Threshold of Toxicological Concern (TTC) approach [EMEA/CHMP/QWP/251344/2006] with daily treatment for longer than 12 months. The levels of gsk004* in representative RPV drug substance batches manufactured according to the proposed commercial synthesis method is less than [redacted] ppm [see m3.2.S.3.2, for RPV].

* 新薬承認情報提供時に置き換え

The impurities qualified at the level of [redacted]% according to ICH Q3A occurred in representative RPV drug substance batches manufactured according to the proposed commercial synthesis method for less than [redacted]% for gsk002*, less than [redacted]% for gsk001*, and less than [redacted]% for the non-qualified [redacted] of RPV, gsk003*.

Three impurities present in the drug substance have been qualified according to ICH Q3A. A number of trace or theoretical impurities of the drug substance have screened for genotoxicity in the Ames test. gsk004* appeared to be genotoxic and its presence in drug substance and drug product has been managed on the basis of the Threshold for TTC. The maximum concentration of gsk004* on the basis of TTC is [redacted] ppm. The impurity is controlled at less than [redacted] ppm.

4.4. Combination drug product

4.4.1. Impurity Assessment

For DTG and RPV, there are no specified impurities in the proposed Drug Substance Specification that exceed the [redacted]% w/w ICH qualification threshold [m3.2.S.4.1 DTG and m3.2.S.4.1 RPV].

DTG and RPV are established active pharmaceutical ingredients (API) which have been used in registered products since their introduction in the 2014 and 2011, respectively. Specified impurities in each drug substance have been qualified on the basis of the levels of the impurities present in batches used, and the profiles of impurities, which were tested in pivotal nonclinical toxicity studies. DTG and RPV drug substance are manufactured by an approved supplier using an approved route.

A genotoxic risk assessment of the DTG drug substance impurities is provided in m2.6.6. DTG NDA and for RPV is provided in m2.6.6 RPV NDA. A further genotoxic risk assessment was conducted to determine whether any additional items of concern would result from the combination of DTG and RPV. This risk assessment, in addition to forced degradation studies on the combination tablet [m3.2.P.8.3 DTG], did not result in any new impurities and the degradation profiles were comparable to TIVICAY (dolutegravir) and EDURANT (rilpivirine). No additional risks were identified through an evaluation of the manufacturing process.

* 新薬承認情報提供時に置き換え

5. INTEGRATED ASSESSMENT OF THE FIXED DOSE COMBINATION PRODUCT

A comprehensive assessment of the nonclinical safety of DTG and RPV was completed in support of treatment of patients with HIV and registration of the single entities. DTG has been evaluated in toxicity studies of up to 26 weeks in duration in Sprague Dawley rats, 38 weeks in cynomolgus monkeys and 13 weeks in Crl:CD-1 (ICR) BR mice. RPV has been evaluated in toxicity studies in mice for 12 weeks, rats for up to 26 months, in dogs up to 52 weeks and in immature female cynomolgus monkeys up to 8 weeks as part of the assessment of the effects of RPV on juvenile animals. The carcinogenicity of DTG and RPV were evaluated in 2-year studies in mice and rats. Both are approved products and routinely prescribed in clinical practice [see Section 6.1, m2.5]. The pharmacology, pharmacokinetics, toxicology and safety profile in clinical use of these two compounds are well known.

ICH Guidance M3 (R2) on Non-Clinical Safety Studies for the Conduct of Human Clinical Trials and Marketing Authorisation for Pharmaceuticals states that, "For most combinations which involve two late stage entities and for which there is adequate clinical experience with co-administration, combination toxicity studies would generally not be recommended to support clinical studies or marketing unless there is significant toxicological concern (e.g., similar target organ toxicity)" [ICH M3 (R2), 2009]. In addition, according to Question 9 in the Questions and Answers document written by the M3 (R2) Implementation Working Group for M3 (R2) Guideline "Non-Clinical Safety Studies for the Conduct of Human Clinical Trials and Marketing Authorization for Pharmaceuticals" regarding the scope of ICH M3 (R2), it is accepted that combination toxicity studies are not generally warranted for HIV products unless there is a specific cause for concern under clinically relevant conditions. An extensive review of the nonclinical data for DTG and RPV has shown:

- No anticipated adverse pharmacodynamic interactions and no anticipated impact on established exposure margins as a consequence of pharmacokinetic interactions.
- No nonclinical signals that might indicate the potential for additive or synergistic toxicity.
- No increased genotoxic or carcinogenic risk.

Based on this, together with data from the Phase 3 clinical studies 201636 and 201637 [see Section 3 m2.5] the Sponsor considers that combination toxicity studies would provide no additional safety information and are not necessary to support the proposed marketing application for the DTG/RPV FDC.

A summary of the nonclinical information on which this conclusion is based is provided below:

5.1. Secondary and safety pharmacology

DTG: Dolutegravir was generally inactive against a panel of enzymes, receptors, ion channels, transporters, and functional tissue assays and is unlikely to have any significant off-target pharmacological activity. There were no findings from safety pharmacology studies that would indicate an unacceptable risk for oral administration of dolutegravir to patients in accordance with the proposed indication.

RPV: No relevant effects of RPV on a variety of receptors, on mechanisms controlling gastric acidity, or on human polymerase. Moreover, no relevant effects were noted on vital body functions except cardiovascular electrophysiological parameters. RPV inhibited three potassium currents involved in the repolarization phase of the cardiac action potential, I_{Kr}, I_{Ks}, and I_{to}. These effects became relevant at 0.3 μM (0.11 μg/mL) of unbound RPV. In addition, RPV showed relevant inhibition of trafficking of the hERG channel. However, only in the perfused ventricular rabbit wedge model, these inhibitions led to a small but significant QT prolongation without affecting transmural dispersion or inducing early after depolarizations. In none of the in vivo animal models, was any effect on electrophysiological cardiovascular or hemodynamic parameters noted. These effects partially explain the mechanism of the delayed-onset QT prolongation observed in clinical TQT study TMC278-C131 with 75 or 300 mg RPV q.d. [see Section 2.4.4, m2.7.2]. In particular, the results of the rabbit wedge study demonstrate that RPV has only a marginal potential for inducing TdPs, only because of minor QT prolongation in the wedge model.

5.2. Pharmacokinetics

DTG: The nonclinical pharmacokinetics of dolutegravir were similar across the primary species (mouse, rat and monkey) used for pharmacological and toxicological evaluation. In each species, plasma clearance was low, relative to liver plasma flow. The nonclinical protein binding of dolutegravir across species was high (>99%) and not different from the in vitro and ex vivo assessment in human plasma. Absorption was rapid and extensive from solution, but was limited by solubility from suspensions and tablet formulations. The increase in exposure to dolutegravir generally was less than proportional to the increase in dose in toxicological evaluations. The absorbed dose was extensively metabolized in each species, with dolutegravir the predominant drug-related component in plasma which is also consistent with observations in humans [see Section 3.1, m2.7.2].

DTG was highly permeable and widely distributed to tissues of the rat, including crossing the placental barrier and secretion into milk of nursing rats. After an oral dose, dolutegravir was extensively metabolized, secreted in bile and eliminated in feces. The major metabolites were an ether glucuronide and a product of oxidation which was converted to an N-dealkylated product. In humans, the biotransformation of dolutegravir was mediated predominantly by UGT1A1 with CYP3A4 contributing to a lesser extent. No disproportionate human metabolites were observed and the selection of the species for toxicity testing was validated.

DTG demonstrated weak or no induction potential and inhibition potential of UGT (UGT1A1 and 2B7) or CYP (CYP1A2, 2A6, 2B6, 2C8, 2C9, 2C19, 2D6 and 3A4) enzymes following in vitro incubations. Dolutegravir was a substrate for efflux transporters (P-gp and BCRP) but due to its high permeability, absorption is not expected to be affected by efflux transporter inhibitors. Dolutegravir inhibited the renal transporter OCT2, but no notable inhibition of other transporters was observed. Other than sensitive OCT2 substrates with narrow therapeutic ranges (e.g. dofetilide), inhibition of metabolism of co-administered drugs by these mechanisms is not expected. Caution should be exercised with sensitive OCT2 substrates. Dolutegravir is contraindicated for coadministration with the OCT2 substrate dofetilide because of the potential for toxicity due to higher exposure [see Section 6.3, m2.5].

RPV: In pharmacokinetic studies, RPV demonstrated intermediate transepithelial intestinal permeability. Passive transcellular diffusion was proposed as a mechanism for rilpivirine absorption. The solubility, and in the case of suspensions and solid dosage forms, possibly also the dissolution seem to limit the rate and extent of absorption, resulting in saturation of absorption and less than dose-proportional exposure at high dose levels. After oral administration of RPV (base and HCl forms), peak plasma concentrations were generally reached rapidly, followed by a decline at lower dose levels whereas at higher dose levels, the plasma profiles showed a plateau until at least 8 hours in all species. RPV was extensively bound to plasma proteins in all species and the plasma protein binding was found to be concentration independent. Mainly human albumin and to a much lesser extent to α_1-acid glycoprotein were involved in the plasma protein binding. Tissue distribution in rats was rapid and extensive. In pregnant rats, the placenta presents a partial barrier for ^{14}C-RPV. No unique human metabolites were observed and the human profile of metabolites was reflected in the species tested. The animal pharmacokinetic data in general reflected the clinical data. For most of the species (except monkeys), plasma exposures across species were much higher than the human exposure at the recommended dose (25 mg q.d.). In all species including human, RPV was more abundant than any metabolite in plasma. The elimination of RPV is mainly metabolic by the liver, with fecal excretion of the metabolites.

5.2.1. Potential for pharmacokinetic interactions

Based on the current knowledge of the comparative absorption, distribution, metabolism, and excretion (ADME) profiles of DTG and RPV and more importantly the results of the clinical drug-drug interaction study [see m2.5, section 3, Clinical Study LAI116181] clinically relevant PK changes are not anticipated for either DTG or RPV when the two agents are co-administered.

5.3. Toxicology

5.3.1. Repeat dose toxicity studies

DTG: The primary finding from repeat dose toxicity studies with DTG up to 26 weeks in rats and 38 weeks in monkeys was GI toxicity. In monkeys, the most sensitive species, GI toxicity was characterized primarily by vomiting, diarrhea and associated mortality as well as gastrointestinal lesions, and by gastric lesions in the rat. In both species, these effects were observed at progressively lower doses with increased study duration. The GI toxicity is believed to be the result of local drug administration at the mucosal surface of the gut following oral dosing, rather than systemic toxicity. The fact that affected animals had comparable exposures to animals at dose levels which were not affected is supportive of the conclusion that the GI toxicity is due to the larger local exposure in the GI tract in those dose groups. Therefore, mg/kg or mg/m^2 metrics are appropriate determinates of safety cover for this toxicity because it is not based on systemic exposure. The NOAEL for the 38 week monkey toxicity study (15 mg/kg/day) is 15X and 7.5X the human mg/kg equivalent dose (based on 50 kg human) and 5X the human mg/m^2 equivalent dose for a 50 mg QD dose. GI toxicity in animals did not translate to an increased risk for clinical adverse events at DTG doses of 50 mg QD.

RPV: In single dose and repeat dose toxicity studies with RPV, up to 12 weeks in mice, 26 weeks in rats, 52 weeks in dogs, and 8 weeks in immature female cynomolgus monkeys, only relatively mild effects were demonstrated. The target organs/systems identified were liver in mice, rats, and dogs, the thyroid gland and pituitary gland in rats, kidneys in mice and dogs, steroidogenesis in adrenal glands in mice, rats, dogs, and cynomolgus monkeys and probably in testes in dogs and ovaries in mice and dogs, red blood cells in mice, rats, and dogs, and coagulation in male rats.

The effects in liver of mice and rats, and in thyroid gland and pituitary gland in rats were associated with liver enzyme induction and comprised an increase of the organ weight caused by hepatocellular hypertrophy. In mice, electron microscopy revealed also peroxisome proliferation. In rats and to a lesser extent the CYP3A family of isozymes was induced. In addition, induction of the CYP4A family was noted, more prominent in mice than in rats. The effects on thyroid gland and pituitary gland in rats were associated with induction of UDP-GT. This induction caused an increased clearance of thyroid hormones and subsequently increased release and synthesis of TSH by the pituitary gland. The liver effects in dogs were associated with the increased serum concentrations of cholesterol and bilirubin as well as increased activity of serum ALP and ALT observed in this species. The effects comprised mild to moderate perivascular inflammatory reactions together with fibrosis and single hepatic cell necrosis in the central part of the lobules. Moreover, MPS aggregates were noted and multifocal bile duct proliferation. These liver effects are not considered relevant for man. Moreover, no increased levels of ALP or ALT were noted in man whereas increased serum activities of these enzymes were associated with the liver effects in dogs.

The kidney effects in mice and dogs were different in nature and onset. In mice, minimal to moderate degenerative nephropathy was noted in half the number of animals dosed with 320 mg/kg/day. In the dogs, 2 animals dosed with 40 mg/kg/day showed signs of acute nephritis, only in the 12-month study. Clinical pathology or urinalysis provided no indications of a mechanism of action of these effects. In particular, no signs of interaction with renal transporters were noted in these species. A dose relationship of these effects cannot be excluded but is rather unlikely in view of the low clearance of RPV in the urine of these species. These effects do not bear any relationship to the small increases of serum creatinine and concomitant decrease of eGFR observed in the Clinical Phase II study TMC278-C204.

The effects in adrenal glands of all the species involved in general toxicity studies except rabbits and likely the effects in dog testes and ovaries of mice and dogs are due to the apparent inhibition of CYP21 and CYP17, key enzymes in steroidogenesis. Even at the highest doses tested, this inhibition was partial and non-persistent showing dynamics associated with the kinetics of RPV in the circulation. A decrease of serum cortisol levels or increase of serum 17-hydroxy progesterone levels characteristic for inhibition of CYP21 has not been observed in clinical studies with adult men and women, neither at baseline nor after ACTH stimulation. Similarly, no inhibition of CYP17 has been demonstrated in these clinical studies. The partial and non-persistent inhibitory effects of RPV on CYP21 and CYP17 are not expected to be relevant for children and adolescents. Children or adolescents are not known to be more sensitive to inhibition of the enzymes. The clinical manifestations of partial deficiencies of these enzymes are not relevant as the inhibition of these enzymes by RPV, in case this would be significant in children or adolescents, which is not expected, is minor with the consequences of any enzyme deficiencies. The increased ACTH feedback on the adrenal gland due to inhibition of CYP21 may lead in pre-adolescents to increased serum levels of adrenal androgens as a consequence of the stimulation of the adrenal androgenic pathway. As a result of the concomitant inhibition of CYP17, serum levels of adrenal androgens would be decreased rather than increased in pre-adolescents, should the adrenal enzyme inhibition by RPV in these subjects be significant. A subnormal level of androgens in pre-adolescents is not considered to affect the onset or development of puberty.

The reducing effects on red blood cell parameters occurred almost exclusively in the highest dose tested in mice, rats, and dogs. The effects were small and led in no case to clinical manifestations of anemia in these species. Signs of regeneration were noted and no signs pointed toward bone marrow suppression. In man, no significant signs of anemia have been reported.

The mild to moderate increase of coagulation times of both the intrinsic and extrinsic pathways occurred only in male rats. There were no clinical manifestations of affected coagulation in this species. In clinical studies, no effects on coagulation have been reported.

In the dog and the cynomolgus monkey, a NOAEL could not be established as effects pertaining to inhibition of key enzymes of the adrenal steroidogenesis were still present at the lowest dose tested. However, upon close monitoring in man no indications of interference of RPV with adrenal steroidogenesis could be found in clinical studies with a

duration of up to 96 weeks and a dose of up to 150 mg q.d. In the carcinogenicity studies, a NOAEL could not be established as neoplastic lesions occurred in the lowest doses tested. In Section 4.3.4.3, the results of the carcinogenicity studies are evaluated and the relevance for man is assessed. It is concluded that RPV induced tumors in liver of mice and rats and in thyroid gland of rats by an epigenetic mechanism that bears no relevance for man.

The AUC ratios presented in Table 4.2 and Table 4.5 indicate a sufficient exposure margin for the effects observed in animals.

5.3.1.1. Genotoxicity and carcinogenicity

DTG: DTG was not genotoxic in a battery of in vitro tests and an in vivo rat micronucleus assay and was not carcinogenic in 2 year rat and mouse carcinogenicity studies.

RPV: Genotoxicity studies with RPV have shown the absence of a potential to induce mutations and chromosomal damage. Carcinogenicity studies in mice and rats resulted in hepatocellular adenomas and carcinomas in mice associated with induction of CYP4A and peroxisome proliferation. In rats, hepatocellular adenomas and follicular adenomas and carcinomas in thyroid gland occurred as a result of induction of CYP3A and UDPGT. It is concluded that the tumors are not the result of interaction of RPV with DNA but rather are due to an epigenetic mechanism. As the liver enzyme inductions underlying the carcinogenetic effects in rodents do not occur in man, the neoplastic effects bear no relevance for man.

The combination of the 2 components is not expected to have an altered genotoxicity or carcinogenicity profile as compared with that of the individual agents.

5.3.1.2. Fertility, pregnancy and lactation

DTG: Dolutegravir had no effects on male or female fertility in rats and no effect on embryofetal development in pregnant rats or rabbits. Based on animal data, dolutegravir is not anticipated to increase the risk of adverse developmental (or reproductive) outcomes in humans when used in accordance with dosing information in the product label [m1.14.1 (US) or m1.3 (EU)].

Dolutegravir administration resulted in suppressed body weight gain and decreased food consumption in a pre- and postnatal development study in rat dams (F0) receiving 1000 mg/k/g/day. Decreased body weights were noted in the subsequent generation (F1) in the 1000 mg/kg group from pre-weaning until adolescence. The NOAEL for maternal general toxicity (F0) and pre- and postnatal development of the offspring (F1) was 50 mg/kg/day due to the decreased body weights of the offspring (~25X above the expected human exposure for a 50 mg QD), extrapolated from gender mean exposures achieved in the rat 14 day toxicity study). Based on the fact that effects on offspring body weights were noted at doses where maternal toxicity was observed, and the presence of considerable exposure margins expected at the proposed clinical doses, there

is minimal risk for adverse effects on postnatal development in offspring of mothers receiving dolutegravir.

In a juvenile toxicity study in rats dolutegravir administration resulted in two preweanling deaths at 75 mg/kg/day. Over the preweaning treatment period, mean body weight gain was decreased in this group and the decrease persisted throughout the entire study for females during the postweaning period. There were no test article-related differences among the groups for the age at which offspring attained physical signs of sexual maturation (vaginal opening or balano-preputial skinfold separation) and no treatment related changes in stage-dependent evaluation of spermatogenesis. There were no new target organs identified in juveniles compared to adults and the NOAEL in juvenile rats was 2 mg/kg/day. This study supports the clinical use of dolutegravir in pediatric populations.

RPV: RPV had no effects on fertility or fecundity and did not affect parturition, maternal behaviour or pre- and postnatal development. RPV had no teratogenic potential, RPV has not demonstrated different or more severe effects in juvenile and immature rats, dogs, and cynomolgus monkeys than in adult animals [m1.14.1 (US) or m1.3 (EU)].

5.3.1.3. Other Toxicity Studies

DTG: DTG was not immunotoxic, as assessed by TDAR, in adult rats at doses ≤1000 mg/kg/day. In juvenile rats, there were no test article-related effects on TDAR, and no effects on lymphocyte subsets (T cells, both CD4 and CD8 subsets, and B cells) and CD4 or CD8 T cell receptor Vβ usage in peripheral blood.

RPV: RPV did not elicit antigenicity, has no immunotoxic or phototoxic effects, does not cause skin irritation, and is a moderate eye irritant, on the basis of the results of an in vitro test.

5.3.1.4. FDC impurities

For DTG and RPV, there are no specified impurities in the proposed Drug Substance Specification that exceed the 0.50% w/w ICH qualification threshold [m3.2.S.4.5 for DTG and m3.2.S.4.5 for RPV].

DTG and RPV are established active pharmaceutical ingredients (API) which have been used in registered products since their introduction in the 2014 and 2011, respectively. Specified impurities in each drug substance have been qualified on the basis of the levels of the impurities present in batches used, and the profiles of impurities, which were tested in pivotal nonclinical toxicity studies. DTG and RPV drug substance are manufactured by an approved supplier using an approved route.

A genotoxic risk assessment of the DTG drug substance impurities is provided in m2.6.6, DTG NDA and for RPV is provided in m2.6.6 RPV NDA. A further genotoxic risk assessment was conducted to determine whether any additional items of concern would result from the combination of DTG and RPV. This risk assessment, in addition to forced degradation studies on the combination tablet [m3.2.P.8.3], did not result in any

new impurities and the degradation profiles were comparable to TIVICAY (dolutegravir) and EDURANT (rilpivirine). No additional risks were identified through an evaluation of the manufacturing process.

5.4. Conclusions

The toxic potential of DTG and RPV has been well characterized in a comprehensive battery of nonclinical studies. The principal treatment related effect of DTG was GI toxicity observed in repeat dose toxicity studies in rats and monkeys. The targets of toxicity of RPV identified in the repeat dose studies were: red blood cells (mouse, rat, and dog), coagulation (rat), liver (rat and dog), kidneys (mouse and dog), thyroid gland with secondary effects on the pituitary gland (rat), adrenal glands (mouse, rat, dog, and cynomolgus monkey), testes (dog), and ovaries (dog, in immature females with secondary effects on other tissues of the genital tract and on mammary glands). The majority of the induced effects appeared to be completely reversible after a 1-month post-dosing period. The effects on thyroid gland and coagulation in rats and on liver and serum ALP in dogs showed signs of recovery but this was not complete at the end of the 1-month post-dosing period. A number of targets were affected at the low dose tested in dogs and cynomolgus monkeys preventing establishment of a NOAEL in these species.

Data from these nonclinical studies support the clinical use of DTG and RPV for the treatment of HIV-1 in accordance with the proposed dose regimen.

The safe use of DTG/RPV FDC in human pregnancy has not been established. The effect of DTG on human pregnancy is unknown. In reproductive toxicity studies in animals, DTG was shown to cross the placenta. Therefore, administration of DTG/RPV FDC in pregnancy should be considered only if the benefit to the mother outweighs the possible risk to the fetus.

It is recommended that HIV-infected women do not breast-feed their infants under any circumstances in order to avoid transmission of HIV. Furthermore, DTG is excreted in the milk of lactating rats. It will therefore be recommended that mothers do not breast-feed their babies while receiving treatment with the DTG/RPV [see m1.14.1 (US) or m1.3 (EU)].

6. LIST OF LITERATURE CITATIONS

Key Literature References

Capen CC. Thyroid and Parathyroid Toxicology, Mechanisms of Toxicity: Thyroid Follicular Cells. In: Endocrine and Hormonal Toxicology, New York: John Wiley & Sons; 1999: 42-47.

de Zwart L, Scholten M, Monbaliu JG, Annaert PP, Van Houdt JM, Van den Wyngaert I et al. The ontogeny of drug metabolizing enzymes and transporters in the rat. *Reproductive Toxicology,* 2008;26:220-30.

Dennis A., Wang L., Wan X., Ficker E. hERG-channel trafficking: novel targets in drug-induced long QT syndrome. Biochemical Society Transactions. 35, 1060 – 1063, 2007.

Grasso P, Hinton RH. Evidence for and possible mechanisms of non-genotoxic carcinogenesis in rodent liver. Mutation Research 1991; 248: 271-290.

Greaves P. Liver and Pancreas. In: Histopathology of Preclinical Toxicity Studies, Interpretation and Relevance in Drug Safety Evaluation, 3rd ed. Academic Press Elsevier; 2007: 457-569.

Holsapple MP, Pitot HC, Cohen SH, et al. Mode of action in relevance of rodent liver tumors to human cancer risk. Toxicological Sciences 2006; 89(1): 51-56.

Kiser JJ, Carten ML, Aquilante CL, Anderson PL, Wolfe P, King TM et al. The effect of lopinavir/ritonavir on the renal clearance of tenofovir in HIV-infected patients. *Clinical Pharmacology & Therapeutics,* 2008;83:265-72.

Kishi M, Emi Y, Sakaguchi M, Ikushiro S and Iyanagi T. Ontogenic isoform switching of UDP-glucuronosyltransferase Family 1 in rat liver. *Biochemical and Biophysical Research Communications,* 2008;377:815-9.

Liu T, Brown B, Wu Y, et al. Blinded validation of the isolated arterially perfused rabbit ventricular wedge in preclinical assessment of drug-induced proarrhythmias. Heart Rhythm 3, 948-956, 2006.

Lake BG. Species differences in the hepatic effects of inducers of CYP2B and CYP4A subfamily forms: relationship to rodent liver tumor formation. Xenobiotica 2009, 39: 582-96.

MacEnzie W.F., Boorman, G.A. Pituitary gland. Hypertrophy/hyperplasia of thyrotrophs in the pituitary gland is a well-known histological finding related to a compensatory increased TSH demand in the rat In: Pathology of the Fischer rat (Boorman G.A. et al.), Academic Press, Inc., San Diego, 1990, p. 498

Melek M, Jones JM, O'Dea MH, Pais G, Burke TR Jr, Pommier Y et al. Effect of HIV integrase inhibitors on the RAG1/2 recombinase. *Proceedings of the National Academy of Sciences,* 2002;99:134-7.

Pommier Y, Johnson AA and Marchand C. Integrase inhibitors to treat HIV/AIDS. *Nature Reviews Drug Discovery,* 2005;4:236-48.

Saghir SA, Khan SA and McCoy AT. Ontogeny of mammalian metabolizing enzymes in humans and animals used in toxicological studies. *Critical Reviews in Toxicology,* 2012;42:323-57.

Tahara H, Kusuhara H, Endou H, Koepsell H, Imaoka T, Fuse E et al. A species difference in the transport activities of H_2 receptor antagonists by rat and human renal organic anion and cation transporters. *The Journal of Pharmacology and Experimental Therapeutics,* 2005;315:337-45.

Yang X, Gandhi YA, Duignan DB and Morris ME. Prediction of biliary excretion in rats and humans using molecular weight and quantitative structure-pharmacokinetic relationships. *AAPS Journal,* 2009;11:511-25.

References (available upon request)

FDA 4 February 2010 correspondence: Special Protocol Assessment, Carcinogenicity Assessment Committee.

Merck Research Laboratories. Raltegravir_ACM_BGPK_1.doc, Version 9.2, August 2007. FDA Advisory Committee, September 5, 2007. http://www.fda.gov/ohrms/dockets/ac/07/briefing/2007-4314b1-00-index.htm.

Guidelines (not provided)

ICH Q3A (R2): Impurities in New Drug Substances. ICH Harmonised Tripartite Guideline. 25 October 2006.

ICH M4S Q&A (R4): CTD Safety (M4S) Questions and Answers (R4). 11 November 2003.

ICH Topic M3 (R2): Non-Clinical Safety Studies for the Conduct of Human Clinical Trials and Marketing Authorization for Pharmaceuticals. CPMP/ICH/286/95, June 2009.

Draft Non-Clinical Guideline for Drug-Induced Hepatotoxicity. European Medicines Agency. Committee for Medicinal Products for Human Use (CHMP). Doc. Ref. EMEA/CHMP/SWP/150115/2006. London, 24 January 2008.

Committee for medicinal product for human use (CHMP). Guideline on the limits of genotoxic impurities. CPMP/SWP/5199/02. EMEA/CHMP/QWP/251344/2006 28 June 2006.