Janssen Research & Development

Nonclinical Overview

MODULE 2.4

Rilpivirine Long-Acting

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＊新薬承認情報提供時に置換

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LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

Abbreviations

3TC	lamivudine
ACTH	adrenocorticotropic hormone
ALARP	as low as reasonably possible
ALP	alkaline phosphatase
ALT	alanine aminotransferase
APTT	activated partial thromboplastin time
ARV	antiretroviral
ATPase	adenosine triphosphatase
AZT	zidovudine
BCOP	bovine corneal opacity and permeability
b.i.d.	bis in diem = twice daily
CA	citric acid
CAB	cabotegravir
CHO	Chinese hamster ovary
CNS	central nervous system
[redacted]	[redacted]
CRP	C-reactive protein
CYP	cytochrome P450
DDI	drug-drug interaction
DHEA	dehydroepiandrosterone
DNA	deoxyribonucleic acid
EADs	early afterdepolarizations
ECG	electrocardiography
EDTA	ethylenediamine tetra-acetic acid
EFV	efavirenz
ETR	etravirine
F	female
F0	parents
F1	offspring
FDA	Food and Drug Administration
GD	gestation day
GI	gastro-intestinal
GLP	Good Laboratory Practices
HCl	hydrochloride
hERG	human ether-à-go-go
HET-CAM	Hen's Egg Test Chorioallantoic Membrane
HIV-1	human immunodeficiency virus type 1
HPMC	hydroxypropyl-methyl-cellulose
ICH	International Conference on Harmonization
IM	intramuscular(ly)
IV	intravenous(ly)
LA	long-acting
LC-MS/MS	liquid chromatography with tandem mass spectrometry
LD	lactation day
LH	luteinizing hormone
LLNA	local lymph node assay
LLOQ	lower limit of quantification
m/v	mass per volume
M	male
MAA	Marketing Authorization Application
Mx	metabolite number x
MATE	multidrug and toxic compound extrusion
MPS	mononuclear phagocytic system
N(t)RTI	nucleoside or nucleotide reverse transcriptase inhibitor
NNRTI	non-nucleoside reverse transcriptase inhibitor
NO(A)EL	no observed (adverse) effect level

NMP	N-methylpyrrolidone
NVP	nevirapine
OCT	organic cation transporter
OECD	Organization for Economic Cooperation and Development
P188	Poloxamer 188
P338	Poloxamer 338
PEG400	polyethylene glycol 400
PFC	plaque forming cells
P-gp	P-glycoprotein
PI	protease inhibitor
PK	pharmacokinetic(s)
PopPK	population PK
PS80	polysorbate 80
PT	prothrombin time
q.d.	quaque die = 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
QWBA	quantitative whole-body autoradiography
RAL	raltegravir
RAM	resistance associated mutation
RBC	red blood cell
RPV	rilpivirine
RT	reverse transcriptase
S9	metabolic activation system
SC	subcutaneous(ly)
SDM	site-directed mutant
SIV	simian immunodeficiency virus
T_3	triiodothyronine
T_4	thyroxine
TEA	^{14}C-tetra ethyl ammonium
TdP	Torsade de Pointes
TMC	Tibotec Medicinal Compound
TR	total radioactivity
TSH	thyroid stimulating hormone
TTC	threshold of toxicological concern
tQT	thorough QT prolongation study according to ICH E14
UDP-GT	uridine diphosphate-glucuronosyltransferase
Vd_{ss}	volume of distribution at steady-state
UK	United Kingdom
USA	United States of America

Definitions of Terms

AUC	area under the concentration-time curve
AUC_{0-x}	area under the concentration-time curve from time zero to time x
Cl_{bl}	blood clearance
C_{1h}	plasma concentration at 1 hour
C_{max}	maximum plasma concentration
EC_{50}	median effective concentration
IC_{50}	half maximal inhibitory concentration
F_{abs}	absolute bioavailability
$I_{Ca,L}$	high threshold L calcium current
I_{Ki}	inward rectifying potassium current
I_{Kr}	rapidly activating rectifying potassium current
I_{Ks}	slowly activating rectifying potassium current
I_{Na}	fast sodium current
I_{to}	transient outward potassium current
K_i	inhibitor constant
T_{last}	time of the last measurable concentration
V_{dss}	apparent volume of distribution at steady-state

1. OVERVIEW OF THE NONCLINICAL TESTING STRATEGY

Rilpivirine (RPV, previously known as TMC278, JNJ-16150108 or R278474), a diarylpyrimidine derivative, is a potent non-nucleoside reverse transcriptase inhibitor (NNRTI) with in vitro activity against wild-type human immunodeficiency virus type 1 (HIV-1) and NNRTI resistant mutants. Rilpivirine is available as a 25 mg oral tablet, which has been approved for the treatment of HIV-1 infection in antiretroviral treatment-naïve adult patients in multiple countries including the United States of America (USA), Europe (EMEA/H/C/002264), Canada, and Japan as EDURANT®.

Janssen Sciences Ireland UC in partnership with ViiV Healthcare Company are developing the RPV long-acting (LA) + cabotegravir (CAB) LA injectable regimen for the treatment of HIV-1 infection. The overall objective of the CAB + RPV clinical program is to develop a novel, highly effective, well tolerated 2-drug intramuscular (IM) injectable regimen LA administration for the treatment of HIV-1 infection. This Marketing Authorization Application (MAA) focuses on the RPV LA component of this 2-drug regimen. CAB will be the subject of a separate, parallel, MAA, submitted by ViiV Healthcare. Janssen is the sponsor of the RPV LA development and manufacturing program; ViiV Healthcare is the sponsor of the CAB + RPV clinical program.

The proposed CAB + RPV regimen for HIV-1-infected subjects is oral CAB 30 mg once daily + oral RPV 25 mg once daily for approximately 1 month (at least 28 days) followed by initial injections (one of each) of CAB LA 600 mg IM + RPV LA 900 mg IM and subsequent monthly injections of CAB LA 400 mg IM + RPV LA 600 mg IM. As the predicted C_{max} values for RPV LA (0.17 µg/mL after an initial IM dose of 900 mg on the day of the last oral dose, or 0.14 µg/mL following subsequent IM doses of 600 mg) is similar to that of the oral formulation (0.13 µg/mL), conclusions made for the oral RPV formulation can be applied to the RPV LA formulation as well.

This document is a critical assessment of the nonclinical program specifically for the RPV LA injectable formulation, in support of its use in HIV-infected subjects.

The nonclinical strategy adhered to the requirements of the International Conference on Harmonization (ICH) M3 guideline “Guidance on Nonclinical Safety Studies for the Conduct of Human Clinical Trials and Marketing Authorization for Pharmaceuticals” [9] and took into account the USA Food and Drug Administration (FDA) guidance on “Nonclinical Safety Evaluation of Reformulated Drug Products and Products Intended for Administration by an Alternate Route” [3].

The RPV LA formulation (G001, final clinical formulation; 300 mg RPV base/mL suspension and poloxamer 338 (P338)) was comprehensively reviewed for its pharmacokinetic properties, local tolerability and comparative toxicology profile relative to the oral product. Pharmacokinetics of RPV after G001 formulation was studied in rabbit, dog and minipig and also a distribution study was performed in rats. Local tolerability of the IM RPV LA formulation was evaluated in minipigs and a bridging toxicology study was performed in dogs. Minipigs were chosen for evaluation of local tolerability to allow injection of a full human dose with the intended formulation. Dogs were chosen for the bridging toxicology study to evaluate potential

differences in systemic toxicity as in the RPV oral program dogs proved to be more sensitive to the systemic effects of RPV compared to rodents and their metabolism profile resembled more that of humans. The excipient P338 is currently not registered for IM administration, and to complete available data, P338 was additionally evaluated in genotoxicity, general toxicity and reproductive toxicity studies. Available bridging data from non-GLP P338 studies conducted by the supplier, the general toxicity profile of poloxamers, as well as data from a similar poloxamer, poloxamer 188 (P188), are discussed in Section 4.8.9.

All studies were conducted in accordance with best scientific principles. Pivotal safety pharmacology and toxicology studies were performed in compliance with the USA FDA Good Laboratory Practice (GLP) regulations (21 CFR Part 58) and Organization for Economic Co-operation and Development (OECD) principles of GLP (Directive 2004/10/EC) in OECD-adherent countries.

In Appendix 1 a justification is given for the absence of certain nonclinical studies, conducted with RPV LA formulation, in the current submission.

The following convention is applied throughout this Module: reference is made to ‘RPV’ when the hydrochloride (HCl) salt was administered and to 'RPV base' when the free base was administered. The RPV LA formulation contains the RPV base form.

(Safety) Pharmacology/Virology

Primary pharmacology included an assessment of the activity of RPV against laboratory strains and recombinant clinical isolates of wild-type HIV-1 in T-cell lines, wild-type recombinant HIV-1 group M and against HIV-1/IIIB. RPV was also tested for additive or synergistic antiviral activity in combination with commonly prescribed nucleoside or nucleotide reverse transcriptase inhibitors (N(t)RTIs); protease inhibitors (PIs); NNRTIs; INIs; the fusion inhibitor enfuvirtide; and the entry inhibitor maraviroc. Resistance to RPV was assessed in cell cultures and in samples from antiretroviral (ARV) treatment-naïve subjects in Clinical Phase III trials TMC278-C209 and TMC278-C215. Cross-resistance to efavirenz (EFV), nevirapine (NVP) and etravirine (ETR) was assessed in vitro. In samples from ARV treatment-naïve subjects in Clinical Phase III trials TMC278-C209 and TMC278-C215 as well as in the pivotal Phase 3 trials ATLAS and FLAIR, cross-resistance between NNRTIs was assessed. The primary pharmacodynamics/virology studies are listed in Mod2.7.2.4/Sec2.

To assess its secondary pharmacodynamics, RPV was screened in vitro for interaction with a series of α- or β-adrenergic, dopaminergic, muscarinergic, serotonergic, opioid, interleukin, or chemokine receptors. Moreover, its potential to inhibit human deoxyribonucleic acid (DNA) polymerase α, β, or γ was evaluated. To investigate the possible mechanism of the drug-drug interaction between omeprazole and RPV as noted in clinical study TMC278-C114 (Mod5.3.3.4/TMC278-C114-CRR), the interactions of RPV with histamine H2 receptors in the isolated guinea pig right atrium, with adenosine triphosphatase (ATPase) in isolated pig stomach, and with pentagastrin-induced gastric acidity in rats were evaluated.

Safety pharmacology evaluations including cardiovascular, pulmonary and central nervous system (CNS) evaluations are based upon studies performed as part of the oral RPV formulation development. The RPV maximum plasma concentration (C_{max}) in HIV-infected patients after administering the RPV LA formulation at an initial dose of 900 mg (followed by a once monthly 600 mg continuation dose), is comparable with the C_{max} value after administering an oral daily dosing at 25 mg. Therefore, assessments for the oral RPV formulation also apply to the LA formulation given IM.

The pharmacology studies conducted are listed in Mod2.6.3.1/Pharmacology Overview Table.

Pharmacokinetics

For nonclinical pharmacokinetics of RPV after IM injection of RPV LA, studies in different species were performed using different formulations administered IM or subcutaneously (SC). Studies conducted to investigate the pharmacokinetics of RPV LA are listed in the Mod2.6.5.1/Pharmacokinetics Overview Table. The present nonclinical overview is focusing on the final clinical formulation G001 containing 300 mg RPV base/mL suspension and poloxamer 338 (P338; 50 mg/mL). A distribution study in rats was performed after IM administration of the G001 formulation. Protein binding, distribution, in vitro and in vivo metabolism, and excretion of RPV are based upon studies supporting the registration of oral RPV (EDURANT®), as these are independent of the administration route. In addition, as the clinical RPV C_{max} value after IM RPV LA administration at 600 mg every 4 weeks is comparable to the C_{max} value after oral daily dosing at 25 mg, the potential for systemic drug-drug interactions (DDIs) remains the same.

Toxicology

Rilpivirine studies supporting the registration of oral RPV (EDURANT®)

No formal **single oral dose studies** were done for RPV 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 oral 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. 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 **genotoxicity 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 postnatal 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. Additional **mechanistic studies** to evaluate the adrenal and gonadal effects observed in repeat-dose studies in mice, rats, and dogs included an in vitro study with dog adrenal cortex homogenate and in vivo guinea pig, dog, and immature female cynomolgus monkey studies. 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. The toxicity studies conducted are listed in Mod2.6.7.1/Toxicology Overview Table.

Rilpivirine LA

Toxicology studies for IM RPV LA focus mainly on the local tolerability of the G001 formulation and comparability of the toxicology profile with the oral RPV formulation. To this purpose, repeat-dose toxicity studies were conducted with the G001 formulation in minipigs at a dose and volume comparable to the clinical schedule and a toxicology bridging study to compare RPV LA with orally dosed RPV was performed in dogs with the highest feasible dose and volume. Local tolerability was evaluated in an in vitro Hen's Egg Chorioallantoic Membrane Irritation Test (HET-CAM) with RPV base as a nanosuspension and in single dose irritation studies in rabbits and minipigs with RPV LA, which were conducted before the onset of the repeat-dose studies. In all pivotal studies, the toxicokinetics of RPV was determined.

As the toxicology profile of RPV is well known from the RPV oral development program, no additional single dose studies or reproductive studies (including fertility, embryo-fetal development and pre- and postnatal development) were conducted. No new genotoxicity or carcinogenicity studies were performed. Impurities of the drug substance are discussed in view of the new route of administration but based on data available from the oral RPV formulation. Impurities related to the RPV LA drug product are discussed as well.

Studies conducted in support of the registered EDURANT® product as well as for the current LA application are listed in Mod2.6.7.1/Toxicology Overview Table. The dates of study conduct, and location of the raw data are provided in the individual reports provided in Module 4.

2. PHARMACOLOGY

2.1. Primary Pharmacodynamics

Mechanism of action

Rilpivirine is an NNRTI that showed sub-nanomolar median effective concentration (EC_{50}) values against wild-type HIV-1 group M isolates A, B, C, D, E, F, and G (0.07–1.01 nM or 0.00003–0.00037 µg/mL), HIV-1/IIIB (0.73 nM or 0.00027 µg/mL) and nanomolar EC_{50} values against HIV-1 group O isolates (2.88–8.45 nM or 0.00106– 0.0031 µg/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. Rilpivirine had antiviral

activity in the micromolar range against HIV-2 and simian immunodeficiency virus (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 reverse transcriptase (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).

Resistance

Rilpivirine retained antiviral activity against 63.0% (136 of 216) of the HIV-1/HXB2 site-directed mutants (SDMs) carrying single, double, triple and quadruple RT mutations; as compared to 57.7% for ETR, 45.8% for EFV and 36.2% for 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 wild-type HIV-1 of different origins and subtypes as well as NNRTI resistant HIV-1. At high virus inoculum, emergence of resistant strains from wild-type 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 (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.

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 in vitro cross-

resistance 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.

2.2. Secondary Pharmacodynamics

With regard to the secondary pharmacology, potential effects of RPV base were investigated in several in vitro and in vivo studies (Mod2.6.2/Sec3). The studies conducted are listed in Mod2.6.3.1 and individual study details are given in Mod2.6.3.3.

At unbound concentrations greatly (at least 20-fold) exceeding the C_{max} in man following an oral dose of 25 mg once daily (q.d.), RPV base did not cause any inhibition in vitro of α- or β-adrenergic, dopaminergic, muscarinergic, serotonergic, opioid, interleukin, or chemokine receptors or human DNA polymerase α, β, or γ and showed no agonistic or antagonistic activity on histamine H2 receptors in the isolated guinea pig right atrium. Also, no inhibition of ATPase was noted in isolated pig stomach and in vivo, RPV did not cause any significant inhibition of pentagastrin-induced gastric acidity in rats.

No additional studies were conducted for RPV LA, as the active pharmaceutical ingredient for the oral and the LA formulation is the same (HCl salt for the oral RPV formulation versus base for the LA formulation). As the C_{max} value for RPV LA (0.17 µg/mL after an initial IM dose of 900 mg on the day of the last oral dose, or 0.14 µg/mL following subsequent IM doses of 600 mg) is similar to that of the oral formulation, conclusions made for the oral RPV formulation can be applied to the RPV LA formulation as well.

2.3. Safety Pharmacology

Cardiovascular, respiratory and nervous system safety has been thoroughly evaluated for RPV in several in vitro and in vivo safety pharmacology studies (Mod2.6.2/Sec4). The studies conducted are listed in Mod2.6.3.1 and individual study details are given in Mod2.6.3.4.

Cardiovascular System Assessment

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

In follow-up of a delayed-onset prolongation of the QT interval reported in a clinical thorough QT (tQT) study, additional nonclinical studies were done. An inhibition of the slowly activating rectifying potassium current (I_{Ks}) was noted with a half maximal inhibitory concentration (IC_{50}) of 3.1 µM (1.15 µg/mL), as well as a reduction of the transient outward potassium current (I_{to}), however without effects on the inward rectifying potassium current (I_{Ki}), the fast sodium current (I_{Na}) or the high threshold L-calcium current ($I_{Ca,L}$). In vitro, a concentration-dependent inhibition of trafficking of the human ether à go-go (hERG) channel by RPV base was observed

from 1 µM (0.37 µg/mL) onwards. However, no signs of trafficking, determined as delayed onset of QT prolongation, were noted in telemetered guinea pigs orally dosed for 16 days at 10 mg/kg/day (C_{max} 0.6 to 0.9 µg/mL). In a rabbit arterially perfused left ventricular wedge model, RPV showed only a marginal potential to induce proarrhythmic effects and a marginal Torsade de Pointes (TdP) score (0.5) at 10 µM (3.7 µg/mL). Careful clinical monitoring has shown no safety concern for humans and no QT prolongation was observed with the marketed 25 mg once daily dose of EDURANT®.

The margin between the nominal concentration of RPV causing a marginal TdP score of 0.5 in the rabbit ventricular wedge model (3.7 µg/mL) and the recommended oral dose of 25 mg once daily (0.2 µg/mL) is 20. As the C_{max} values with the current RPV LA formulation are very similar to the C_{max} values after repeat oral administration at 25 mg, a similar safety margin can be calculated, and the conclusions of the oral RPV are applicable to RPV LA as well.

Respiratory and Central Nervous System Safety

Rilpivirine tested up to a maximum plasma concentration of 2.6 µg/mL in anesthetized dogs dosed with a single 1-hour IV infusion of 5 mg/kg or conscious instrumented dogs dosed with a single oral dose of 20 mg/kg showed no potential to effect on respiratory parameters. The safety margin was calculated as 13 for the clinical dose of 25 mg q.d.

At the level of the CNS, 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. Incidental effects in rats were noted at an estimated maximum plasma concentration of 10 µg/mL, but no indications for effects on the CNS were noted in general toxicity studies. The margin of exposure for effects of RPV on the CNS at a clinical dose of 25 mg q.d. is approximately 7.

As the C_{max} values with the current RPV LA formulation are very similar to the C_{max} values after repeat oral administration at 25 mg, the safety margins for pulmonary and CNS safety as well as the general conclusions of oral RPV are applicable for the proposed dose and regimen for RPV LA.

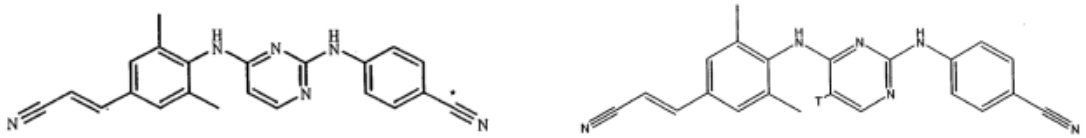
3. PHARMACOKINETICS

3.1. Method of Analysis

Rilpivirine studies supporting the registration of oral RPV (EDURANT®),

Rilpivirine and/or its metabolites were determined in various in vitro and biological samples (plasma, urine, bile and feces) (Mod2.6.4/Sec2.1). Several studies were conducted with radiolabeled RPV, which was labeled with ^{14}C or ^{3}H as outlined in Figure 1.

Figure 1: Structural Formula of ^{14}C-RPV Base (Left) and ^{3}H-RPV Base (Right)



*: ^{14}C-label; T: ^{3}H-label

The metabolites were identified by co-chromatography with authentic substances using different high-performance liquid chromatography methods and detection systems such as radio detection and tandem mass spectrometry (LC-MS/MS). An LC-MS/MS method was validated for the determination of RPV in mouse, rat, rabbit, dog and monkey ethylenediamine tetra-acetic acid (EDTA) plasma and in dog heparin plasma.

For RPV LA development, in addition, an LC-MS/MS method was validated for the determination of RPV in minipigs to support the GLP studies, with a lower limit of quantification (LLOQ) of 0.001 µg/mL.

In non-GLP studies, plasma samples were analyzed with a qualified research method based on the validated plasma method.

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 (Mod2.6.4/Sec2.1).

Poloxamer 338

For P338, bioanalytical methods were developed to support the P338 toxicokinetic and pharmacokinetic studies. The analyte is a polymer that undergoes in-source fragmentation and multiple transitions are monitored and summed to obtain the required sensitivity, which increases assay variability. An LC-MS/MS method was validated for the determination of P338 in rat and rabbit EDTA plasma at [redacted] United Kingdom [UK]). For non-GLP studies, qualified LC-MS/MS methods were used for the determination of P338 at Janssen's internal Bioanalysis laboratory. The LLOQ in plasma ranged between 0.075 – 1.00 µg/mL. A qualified method was also used for tissues and the LLOQ ranged between 1.00 - 5.00 µg/g (Mod2.6.4/Sec2.2).

3.2. Absorption

In minipigs and rabbits, after a single IM administration of RPV LA as the final P338-containing formulation (G001), the RPV release was fast, with a C_{max} reached within 24 h, after which mean plasma concentrations declined, remained fairly constant thereafter and were still quantifiable after 3 months. The absolute bioavailability (F_{abs} at 3 months) was 67% in rabbits at 150 mg/kg and ranged between 35 and 62% in minipigs at 600 mg, indicating the release from the depot was still incomplete after 3 months (Mod2.6.4/Sec3).

Several studies were performed in rabbits and minipigs, mainly comparing different formulations containing P338 to the G001 formulation. No relevant changes in plasma profiles across studies were observed (Mod2.6.4/Sec3).

3.3. Tissue Distribution

A single IM dose of RPV LA (as the G001 formulation) was administered in rabbits and rats to determine the distribution of RPV in certain tissues/organs. For the EDURANT® submission, tissue distribution of RPV has been studied in pigmented Long Evans rats and pregnant female Sprague-Dawley rats after a single oral administration of ^{14}C-RPV by means of quantitative whole-body autoradiography (QWBA), as well as in a single oral dose pharmacokinetic study administering RPV in rats (Mod2.6.4/Sec4.1).

In rabbits, after a single IM dose of RPV LA (G001; 150 mg/kg; 0.5 mL/kg; n=3) at the end of a 1-month follow-up period, the RPV concentrations were 1456-fold higher at the administration site, compared those in the contralateral side. In the lymph nodes, the RPV concentrations were similar between the injection and contralateral side except for one rabbit for which higher concentrations were seen in the accessory popliteal lymph nodes of the injection site (Mod2.6.4/Sec4.1.1.2).

In rats, after a single IM administration of RPV LA (60 mg/kg; G001), the highest exposures of RPV were measured in the left popliteal and medial iliac lymph nodes adjacent to the injection site with tissue/plasma $AUC_{0\text{-day}42}$ ratios of 12,203 and 2256, respectively. In the contralateral right popliteal and medial iliac lymph nodes, the tissue/plasma $AUC_{0\text{-day}42}$ ratios were 6.7 and 2.6, respectively. In the kidney, adrenal glands, lungs, liver, and pancreas, the tissue/plasma $AUC_{0\text{-day}42}$ ratios were 3.7, 3.2, 1.5, 1.5 and 1.2, respectively. The tissue/plasma $AUC_{0\text{-day}42}$ ratios were lower than 1 in brain (0.4), heart (0.8), spleen (0.97) and thymus (0.87) (Mod2.6.4/Sec4.1.1.1).

Comparing to the distribution after oral administration of RPV at 40 mg/kg the tissue/plasma exposure (AUC) ratios of RPV in adrenal gland and brain were in the same order of magnitude and the liver ratio was higher after oral, in line with the route of administration. In addition, the tissue/blood exposure (AUC) ratios of RPV after RPV LA were in the same ranking as after oral administration of ^{14}C-RPV (Mod2.6.4/Sec4.1.2), except again in the liver (Table 1).

Table 1: Tissue/plasma or blood ratios of RPV after oral administration of RPV or RPV LA in rats

Organs/tissue	Tissue/plasma ratio after RPV LA at 60 mg/kg ADME_58575	Tissue/plasma ratio after oral RPV at 40 mg/kg FK4195	Tissue/blood ratio after RPV LA at 60 mg/kg ADME_58575	Tissue/blood ratio after oral ^{14}C-RPV at 40 mg/kg TMC278-NC108-FK4951
Adrenal gland	3.2	2.6	4.9	4.9
Brain	0.29	0.49	0.4	0.66
Heart	0.69	-	1.0	1.9
kidney	3.7	-	5.6	3.6
Liver	1.4	3.4	2.1	12
Lung	1.5	-	2.2	2
Pancreas	1.2	-	1.9	2.9
Spleen	0.97	-	1.5	-
Thymus	0.87		1.3	-

In addition, after oral administration of ^{14}C-RPV in rats, 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.

In human, recent clinical study results (substudy of LATTE 2) (Mod2.7.2/Sec2.1.2.4) indicated that the ratio cerebrospinal fluid:plasma of RPV after IM RPV LA administration is comparable to this ratio after oral administration of COMPLERA® (RPV, emtricitabine, and tenofovir disoproxil fumarate) described by Mora-Peris et al [16].

3.4. Plasma Protein Binding

As the data for RPV submitted for EDURANT® can be extrapolated to RPV LA, no additional studies were performed nor warranted. For the EDURANT® submission, 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 (Mod2.6.4/Sec4.2).

Rilpivirine was highly 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%. Rilpivirine was highly 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 at an 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 (RBC) is limited in all species.

3.5. Metabolism

As the data for RPV submitted for EDURANT® can be extrapolated to RPV LA, no additional studies were performed nor warranted.

Rilpivirine 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 2 and Table 2). In plasma of animals and human, unchanged RPV was more abundant than all metabolites combined. After repeated oral 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 (Mod2.6.4/Sec5).

Figure 2: In Vivo Metabolic Pathways of RPV in Animals and Humans (Excluding Rat Bile)

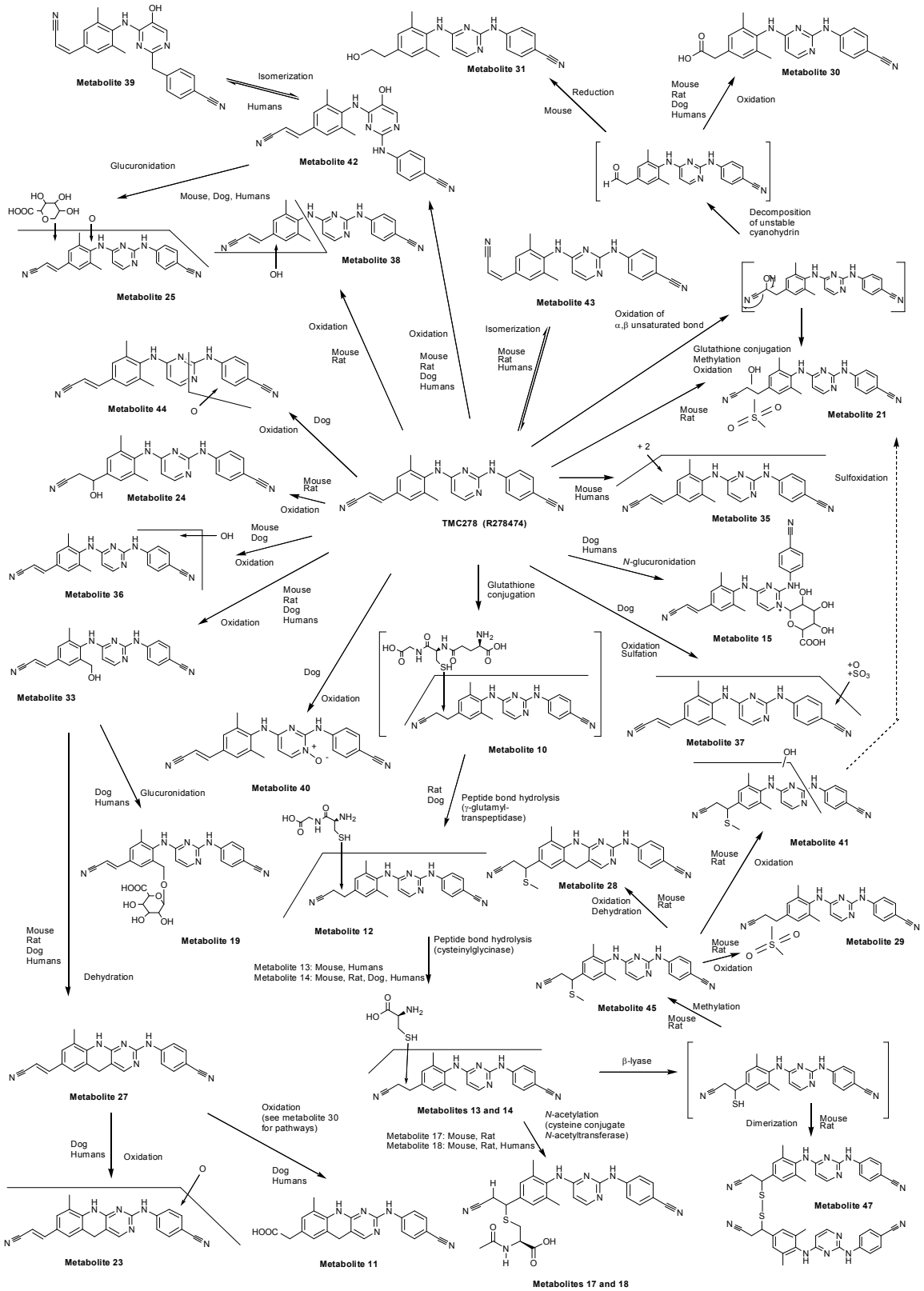


Table 2: Total Percentage of the Administered Dose Metabolized per Major Pathways in Human and its Corresponding Percentages in Mice, Rats and Dogs After Oral Administration of ^{14}C-RPV Base

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

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 was 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 (Mod2.6.4/Sec5).

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.

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 DDI 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.

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 considering the mean C_{max} of 0.13-0.14 µg/mL obtained in HIV-infected treatment-naïve patients at 25 mg q.d and at steady-state after 600 mg of RPV LA (G001) given every 4 weeks. In vivo, some DDI studies were performed using substrates of CYP3A4 (atorvastatin, omeprazole (omeprazole sulfone pathway) and norethindrone) and substrate of CYP2C19 (omeprazole (hydroxyomeprazole pathway)). No increase in exposure of these 3 compounds with RPV was observed.

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 oral dose of RPV (150 mg q.d); RPV at the recommended oral dose of 25 mg q.d. or 600 mg monthly of RPV LA 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 DDI 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 an oral dose of 150 mg q.d was seen on the chlorzoxazone exposure.

In vitro, RPV is an inhibitor of CYP2C8 and CYP2C9 with an inhibitor constant (K_i) value of 10 µM (3.7 µg/mL) and 1.7 µM (0.62 µg/mL), respectively. Taking into account the mean C_{max} of 0.13-0.14 µg/mL obtained in HIV-infected subjects after 25 mg after oral administration or 600 mg after RPV LA, inhibition of CYP2C8 and CYP2C9 by RPV is not expected. The limited mechanism-based inhibition of CYP2C9 by RPV is unlikely to have clinical relevance at therapeutic doses of RPV.

For other CYPs (CYP1A1, CYP2A6 and CYP2D6), considering the mean C_{max} of 0.13-0.14 µg/mL, inhibition by RPV is unlikely. RPV might be a very weak inducer of CYP1A2 and CYP2B6.

The clinical DDI studies are described in detail in the Clinical Pharmacology Summary (Mod2.7.2/Sec3.2.8).

3.6. Excretion

As the data for RPV submitted for EDURANT® can be extrapolated to RPV LA, no additional studies were performed nor warranted.

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 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 IV administration. In humans, the terminal elimination 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), beagle 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 non-restrained animals, respectively). The amount of unchanged RPV excreted in bile was negligible (about 0.2 % within 24 hours) (Mod2.6.4/Sec6).

In rats, there was indication that RPV was excreted in milk.

3.7. Drug Interaction Study

3.7.1. Transporters

As the data for RPV submitted for EDURANT® can be extrapolated to RPV LA, no additional studies were performed nor warranted.

Inhibition of the organic cation transporter (OCT) 2 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) (Mod2.6.4/Sec7.1). Given the fact that RPV plasma concentrations are low as compared to this IC_{50} (C_{max}/IC_{50} = 0.07), the clinical relevance of this finding is not clear. The inhibition of multidrug and toxic compound extrusion (MATE)-mediated transport by RPV was investigated in vitro in CHO cells overexpressing MATE-1 and MATE-2K. 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 (Mod2.6.4/Sec7.1). Based on the respective IC_{50} for the in vitro inhibition of these transporters, inhibition of MATE-2 by RPV is more likely to be relevant in vivo, while it is less likely for MATE-1 and OCT2. As such, the small mean increases from baseline in serum creatinine by RPV seem to represent changes in the disposition of creatinine, without reflecting a change in renal function.

Given the effect of RPV on OCT2 and MATE-1/-2K *in vitro*, the effect of RPV on the pharmacokinetics of a typical substrate, metformin, was evaluated in a clinical DDI trial, in order to provide further guidance for co-administration of RPV with metformin (Mod2.7.2/Sec3.2.8).

Rilpivirine did not affect the plasma or urine pharmacokinetic parameters of metformin when coadministered.

3.7.2. In Vivo

In rats following single IM administration at 60 mg/kg of RPV LA (G001) alone or in combination with a LA injectable suspension of GSK1265744A (CAB) at 10 mg/kg, the plasma concentrations of RPV were comparable for the 2 groups and the mean C_{max} and $AUC_{0\text{-}1444h \text{ or } 2months}$ values of RPV were similar (see Mod2.6.4/Sec7.3).

3.8. Comparison of Exposure in Animals and Man

The C_{max} and AUC values of RPV after repeated oral administration in various animal species used in the toxicology studies and after repeated IM administration of RPV LA in dogs and minipigs are summarized in Table 3. These exposures were compared with the human predicted exposure (C_{max} = 0.14 µg/mL and $AUC_{0\text{-}day28}$ = 83 µg.h/mL) in HIV-1 infected patients at steady-state after 600 mg of RPV LA given every 4 weeks (G001) (Mod5.3.5.3/RPV LA PopPK report) using the population PK (PopPK) model.

After repeated oral administration of RPV in various animal species used in the toxicology studies compared to IM administration of RPV LA in patients, the highest C_{max} ratio (animal/human) of RPV was around 406 in female mice and 252 in male mice, 82 in female rats and 39 male rats, 123 in pregnant rabbits, 33 in dogs, and 2 in female monkeys. The highest $AUC_{0\text{-}day28}$ ratio (animal/human) of RPV was around 258 in female mice, 170 in male mice, 82 in female rats, 25 in male rats, 78 in pregnant rabbits, 22 in dogs, and 2 in female monkeys. After IM administration of RPV LA in minipigs and dogs compared to IM administration of RPV LA in patients, the C_{max} ratios (animal/human) of RPV were around 2 and 10 and $AUC_{0\text{-}day28}$ (animal/human) ratios of RPV were around 0.6 and 5, respectively.

Table 3: RPV Exposure in Animals (After Oral or IM Administration) Relative to Human (After IM Administration)

Species	RPV formulation	Sampling Time	Sex/n	Dose (mg/kg/day)	C_{max} (µg/mL)	C_{max} ratio	AUC_{0-24h} (µg.h/mL)	$AUC_{0-day28}$ ratio[b]
Mouse	PO: RPV in HPMC (0.5% w/v)	Week 28	M/9	20	9.8	69	76	26
			M/9	60	22	154	230	78
			M/9	160	36	252	505	170
			F/9	20	9.9	69	51	17
			F/9	60	29	203	278	94
			F/9	160	58	406	766	258
Rat	PO: RPV base in PEG400/CA (10%	Day 28	M/4	10	0.88	6.3	7.2	2.4
			M/4	40	2.6	19	27	9.1
			M/4	160	6.7	48	51	17
			F/4	10	1.6	11	14	4.7
			F/4	40	5.8	41	42	14
			F/4	160	8.8	62	89	30
Rat	PO: RPV base in PEG400/CA (10%)	Day 175[a]	M/3	40	1.7	12	12	4
			M/3	120	3.0	21	35	12
			M/3	400	6.2	43	73	25
			F/5	40	6.6	46	50	17
			F/5	120	8.8	62	116	39
			F/6	400	16	112	244	82
	PO: RPV in HPMC (0.5% w/v)	Week 39	M/9	40	0.82	6	6.3	2
			M/9	200	1.3	9	8.2	3
			M/9	500	1.8	13	14	5
			M/9	1500	2.2	15	18	6
			F/9	40	2.1	15	14	5
			F/9	200	4.7	33	41	14
			F/8	500	8.5	59	46	16
			F/9	1500	9.4	66	84	28
Pregnant rat	PO: RPV base in PEG400/CA (10%)	Day 11 (GD 16)	F/4	40	5.6	39	37	12
			F/4	120	7.2	50	63	21
			F/6	400	13	91	152	51
Juvenile rat (aged 25 days)	PO: RPV in HPMC (0.5% w/v)	Day 14	M/8	40	2.6	18	12	4
			M/7	120	3.7	26	34	11
			M/7	400	9.1	64	50	17
			F/8	40	5.8	41	18	6
			F/8	120	3.6	25	28	9
			F/7	400	7.3	51	53	18
Pregnant rabbit	PO: RPV base in HPMC (0.5% w/v)	Day 14 (GD 19)	F/3	5	6.7	47	105	35
			F/3	10	10	70	170	57
			F/3	20	15	105	232	78
Dog	PO: RPV base in PEG400/CA (10%)	Day 363	M/4	5	1.1	8	17	6
			M/2	10	1.3	9	24	8
			M/4	40	4.1	29	65	22
			F/4	5	1.5	10	19	6
			F/4	10	2.2	15	36	12
			F/3	40	5.5	38	61	21
Monkey	PO: RPV in HPMC (1%)/Tween 20	Day 55	F/8	100 b.i.d	0.14	1	2.7	0.9
			F/7	250 b.i.d	0.31	2	4.6	1.6
Minipig	IM: RPV LA (G001)	Day 224 (after 8 injections)	M/3	600 mg/ 4weeks	0.35	2	50[c]	0.6
			F/3	600 mg/ 4weeks	0.41	3	44[c]	0.5
Dog	IM: RPV LA (G001)	Day 224 (after 8 injections)	M/3	1200 mg/ 2 weeks	1.4	10	435[d]	5
			F/3	1200 mg/ 2 weeks	1.2	9	410[d]	5

[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 no observed adverse effect level (NOAEL) dose when it is determined;

[b] animal/human AUC ratio was calculated as follows: $AUC_{0\text{-}24h}$ at steady-state multiplied by 28 days in animals divided by $AUC_{0\text{-}day28}$ obtained in HIV-infected patients at steady-state after 600 mg every 4 weeks; [c] $AUC_{0\text{-}672h\ (28days)}$; [d] $AUC_{0\text{-}600h\ (25days)}$ b.i.d.: twice daily; CA: citric acid; F: female; HPMC: hydroxypropyl-methylcellulose; IM: intramuscular; M: male; PEG400: polyethylene glycol 400; PO: oral.

3.9. Other Studies

3.9.1. After Administration of Other non-G001 Rilpivirine LA Formulations

Other studies were performed after administration of RPV LA at lower concentration of RPV containing poloxamer 388 (P338) or with polysorbate 80 (PS 80) after IM or SC in different species. A faster increase of the RPV concentrations were observed after administration of P338 containing formulation compared to PS80 containing formulation (Mod2.6.4/Sec8.1).

3.9.2. Rilpivirine Impurity related substance D*

Some studies were performed in vitro or after administration of related substance D* (the only genotoxic impurity of RPV), as single IM injection of RPV LA, in rats. It could be concluded that in the rat, related substance D* is rapidly metabolically cleared and that the main metabolite pathways are sulfate conjugation and loss of the nitrile function (Mod2.6.4/Sec8.2). This impurity is controlled below acceptable levels, as discussed in Section 4.8.8.

3.9.3. Poloxamer 338

After Single Dose

The plasma concentrations of P338 were measured after a single oral administration of P338 (solution in water) at 1600 mg/kg to female Sprague-Dawley rats and to female New Zealand white rabbits, using the same experimental conditions as the GLP embryo-fetal development studies (Section 4.7). The plasma concentrations of P338 were below the quantification limit in all samples, i.e., below 0.1 µg/mL in female rats. In female rabbits, P338 concentrations were quantifiable, i.e., above the LLOQ of 0.075 µg/mL, starting from 2 h to 8 h. C_{max} values were reached between 48 to 72 h after dosing, followed by a mono-phasic declined. Concentrations were still above the LLOQ at 336 h in 3 out of 4 rabbits (Mod2.6.4/Sec8.3).

After single IM injection of P338 in male rats at 10 mg/kg, P338 concentrations were measured in plasma, liver, kidney, feces and urine. The samples were collected up to Day 22. The C_{max} of P338 (61 µg/mL) was achieved at 7 h and the corresponding $AUC_{0\text{-}529h\ (\sim 22\ days)}$ was 2170 µg.h/mL. The tissue to plasma $AUC_{0\text{-}529h}$ ratios were 1.1 in kidneys and 5 in the liver. In urine, 9.41% of the dose was excreted as unchanged P338 in the 0-144h interval. Since all concentrations were below quantification limit in feces, only ~10% of the administered dose was eliminated at the end of the experiment, and via the urine. At the last time point 529h after dosing, P338 is still detected in plasma, liver and kidney, showing the slow elimination of P338 in rats.

In female Göttingen minipigs, after single IM administration of RPV LA at 600 mg using the same experimental conditions as in the 9-month GLP study (Section 4.7), concentrations of

P338, were measured. The corresponding dose of P338 was 100 mg (4.6 mg/kg). The minipig plasma pharmacokinetic parameters of P338 are described in Table 4.

P338 plasma concentrations were also measured in human samples from a clinical study (Mod5.3.1.2/TMC278LAHTX1001) in healthy volunteers after single IM administration of RPV LA (300 mg/mL in 50 mg/mL P338; 2-mL injection). The C_{max} and $AUC_{0\text{-}672h\ (28\ days)}$ values of P338 (Mod2.6.4/Sec8.3.1) were 3.91 µg/mL and 717 µg.h/mL respectively.

After Repeat Administration

In rats, after repeated IM administration from 2.5 to 10 mg/kg every 3 days up to 63 days, in general, exposure (C_{max} and $AUC_{0\text{-}72h}$) values of P338 increased less than dose-proportional or close to dose-proportional (Mod2.6.4/Sec8.3.2.1). Comparing the first and last day of dosing, C_{max} and $AUC_{0\text{-}72h}$ values were similar or slightly higher (up to 1.6-fold) after repeated IM administration every 3 days or every week. No clear difference in exposure (C_{max} and $AUC_{0\text{-}72h}$) was seen between males and females.

In rabbits, after repeated IM administration for 2 weeks from 2.5 mg/kg every 3 days or every week to 5 mg/kg every week, in general, exposure (C_{max} and $AUC_{0\text{-}tlast}$) values of P338 increased in close to dose-proportional (Mod2.6.4/Sec8.3.2.2). Comparing the first and last day of dosing, C_{max} and $AUC_{0\text{-}72h}$ values were similar or slightly higher (up to 1.4-fold) after repeated IM administration every 4 or 6 days or every week.

The C_{max} and AUC values of P338 after IM administration in various animal species are summarized in Table 4. These exposures were compared with the human exposure (C_{max} = 3.91 µg/mL, $AUC_{0\text{-}336h}$ (day 14) = 580 µg.h/mL and $AUC_{0\text{-}672h\ (day28)}$ = 717 µg.h/mL) of P338 obtained in human after single dose of RPV LA.

Table 4: P338 Exposure in Animals Relative to Human After IM Administration

Species	Formulation	Dose of P338	Number per group/ Sex	C_{max} (µg/mL)	C_{max} ratio	$AUC_{0\text{-tlast}}$ (µg.h/mL)	$AUC_{0\text{-tlast}}$ ratio
Minipig (FK13161)	RPV LA	100 mg	4/F	52.3	13	13,600 (28 days)	19
Rat (fertility and embryo-fetal study; TOX13391)	P338	2.5 E3d mg/kg	3/M	10	2.6	7870[b] (cum over 63 days)	5 [a]
			3/F	-	-	-	-
		5 E3d mg/kg	3/M	23	6	16,000[b] (cum over 63 days)	11 [a]
			3/F	17	4	4960[b] (cum over 33 days)	7[b]
		10 E3d mg/kg	3/M	34	9	26,000 (cum over 63 days)	18 [a]
			3/F	26	7	6220 (cum over 33 days)	9[b]
Rat (pre- and postnatal development Study TOX13546)	P338	2.5 E3d mg/kg	3/F	7.8	2	-	
		5 E3d mg/kg	3/F	14	4	4860 (cum over 33 days)	7[b]
		10 E3d mg/kg	3/F	25	6	8410 (cum over 33 days)	12[b]
Rabbit (embryo-fetal study; TOX13376)	P338	2.5 every week	4/F	25	6	2820 (cum over 12 days)	5[c]
		5 every week	4/F	49	13	5080 (cum over 12 days)	9[c]

[a] rat/human AUC ratio was calculated as follows: cumulative $AUC_{0\text{-}1512h\ (63\ days)}$ of P338 in rats divided by $AUC_{0\text{-}672h\ (28\ days)}$ of P338 multiplied by 2 obtained in human

[b] rat/human AUC ratio was calculated as follows: cumulative $AUC_{0\text{-}792h\ (33\ days)}$ of P338 in rats divided by $AUC_{0\text{-}672h\ (28\ days)}$ of P338 obtained in human

[c] rabbit/human AUC ratio was calculated as follows: cumulative $AUC_{0\text{-}288h\ (12\ days)}$ of P338 in rabbits divided by $AUC_{0\text{-}336h\ (14\ days)}$ of P338 obtained in human

F: female; IM: intramuscular; E3d: every 3 days; cum: cumulative; M: male

4. TOXICOLOGY

4.1. Single Dose Toxicity

Rilpivirine studies supporting the registration of oral RPV (EDURANT®)

No formal single dose studies were done following oral administration of RPV (base) 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 polyethylene glycol (PEG) 400 + citric acid (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 (Mod2.6.6/Sec2.1.1).

Rats dosed with an oral maximum feasible single dose of 800 mg/kg RPV base in PEG400 showed no treatment-related effects (Mod2.6.6/Sec2.1.2).

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 stools than dogs treated with the vehicle. No other effects were noted (Mod2.6.6/Sec2.1.3).

Rilpivirine LA

Also, with the final RPV LA formulation, no separate single dose toxicity studies were performed, however, in vivo evaluation after each dose administration was included in the repeat-dose studies. Findings were limited to minimal local injection site reactions.

During development, single dose studies with other formulations than the current G001 formulation were conducted in mice, rats, dogs and minipigs and are described in Mod2.6.6/Sec8.7.1.

4.2. Repeat-Dose Toxicity

For RPV, supporting the registration of oral RPV (EDURANT®), systemic toxicity after repeated oral 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 mouse study served as dose range finder for the carcinogenicity study in that species. The reversibility upon repeat dosing was investigated in rats and dogs. Systemic toxicity of RPV LA was compared to the known toxicity profile of RPV in a bridging study in dogs.
All orally dosed 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% (mass/volume [m/v]) hydroxypropyl-methyl cellulose (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 oral gavage 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 the RPV LA formulation, pivotal studies evaluating the final G001 formulation were conducted in dogs (4-week bridging study) and minipigs (6-week and 9-month studies). Additional fertility, embryo-fetal development and peri-and postnatal development studies were conducted with IM administration of the excipient P338 and are further discussed in Section 4.7.

In this and following sections, only pivotal studies of RPV are summarized for the oral and LA application. Non-pivotal studies and studies with exploratory LA formulations, are described in Mod2.6.6/Sec8.7.2 and are not further discussed here.

4.2.1. Mice (Rilpivirine studies supporting the registration of oral RPV (EDURANT®)

The doses of RPV in the 3-month oral gavage study with CD-1 mice were 20, 80, or 320 mg/kg/day (Mod2.6.6/Sec3.1.1.2). 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 were noted. These effects are probably associated with the effects on RBC 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.

4.2.2. Rats (Rilpivirine studies supporting the registration of oral RPV (EDURANT®))

Rilpivirine 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 RBC (Mod2.6.6/Sec3.1.2.2.1).

There were no mortalities associated with RPV base, 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 (Mod2.6.6/Sec3.1.2.2.2). 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. 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 6-month study.

4.2.3. Cynomolgus Monkeys (Rilpivirine studies supporting the registration of oral RPV (EDURANT®))

Rilpivirine suspended in 1% (m/v) aqueous HPMC with 0.5% Tween 20 was administered by oral gavage 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 (Mod2.6.6/Sec3.1.5.1). 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-hydroxy-progesterone and progesterone were. 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 luteinizing hormone (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.

4.2.4. Dogs (Rilpivirine studies supporting the registration of oral RPV (EDURANT®))

Rilpivirine 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 (Mod2.6.6/Sec3.1.4.2.1). 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 (Mod2.6.6/Sec3.1.4.2.2). 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, electrocardiography (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 (Mod2.6.6/Sec3.1.4.2.3). 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.

Rilpivirine LA

For the RPV LA application, a 4-week dog study (Mod2.6.6/Sec3.2.1) was performed to serve as a **bridging study** between RPV oral and RPV LA. The dog was chosen for this study as from the studies conducted with oral RPV it was evident that the dog is the most sensitive species; targets of toxicity identified after oral administration were generally more often seen in dogs compared to rats. While some effects were seen in dogs and not in other species (e.g. testes and ovary effects; kidney effects were seen in dogs and mice but not in rats), other effects were only seen in rat (thyroid and pituitary) but are known to be typically rodent specific (UDP-GT induction-related). In addition, in terms of metabolism, in dog and man, oxidation of RPV is the principal pathway, whereas in rats, the glutathione conjugation pathway is predominant. One month of dosing was considered sufficient as relevant findings could already be detected in the 1-month oral dog study.

In this bridging study, dogs were dosed IM at 150 or 1200 mg/dog (300 mg/mL) every two weeks for 4 weeks, followed by a treatment free period of 2 weeks. The high dose level tested was considered the maximum feasible for application as four injections of 1 mL each. Effects related to local administration consisted of swelling and edema in all dogs dosed at 1200 mg/dog and in 1 dog dosed at 150 mg/dog. These effects persisted until the end of the recovery period in the high dose group. At necropsy, white deposits were noted at the

administration site related to macrophage aggregation and mononuclear cell infiltration, similar to what has been seen in the minipig studies.

Effects related to systemic exposure to RPV LA were consistent with those seen after oral administration at the low dose in dogs (5 mg/kg/day): increases in progesterone and 17α-hydroxyprogesterone were noted up to the end of the study, however without changes in cortisol.

The exposure (C_{max} and $AUC_{0\text{-}25\ days}$) values after IM administration of RPV LA at 1200 mg/dog, the maximum feasible dose, were similar to those obtained in the 12-month dog study after oral administration at 5 mg/kg/day (Table 5) and covered exposures at 600 mg IM dosing both with regard to C_{max} (0.14 µg/mL) and AUC ($AUC_{0\text{-}day28}$=83 µg.h/mL; Table 3).

Table 5: Exposure obtained after oral administration of RPV (12-month toxicity study) versus RPV LA in dogs (1-month toxicity study)

Species	RPV formulation	Sampling Time	Sex/n	Dose (mg/kg/day)	C_{max} (ng/mL)	$AUC_{0\text{-}24h}$ (ng.h/mL)	$AUC_{0\text{-}day25}$ (ng.h/mL)
Dog	PO: RPV base in PEG400/CA (10%)	Day 363	M/4	5	1100	17,000	425,000[a]
			F/4	5	1500	19,000	475,000[a]
	IM: RPV LA (G001)	Day 224 (injections on Days 1 and 15)	M/3	1200 mg/ 2 weeks	1440	-	435,000
			F/3	1200 mg/ 2 weeks	1230	-	410, 000

[a]: $AUC_{0\text{-}24h}$ at steady-state multiplied by 25 days to be able to compare with $AUC_{0\text{-}25\ days}$ after IM RPV LA. CA: citric acid; F: female; IM: intramuscular; M: male; PO: oral.

4.2.5. Minipigs (RPV LA)

Intramuscular administration of RPV LA was evaluated in a 6-week and a 9-month minipig study where RPV LA was generally well tolerated when dosed IM at 600 mg RPV LA per injection (300 mg/mL) every two weeks or once monthly, respectively. There was no mortality and no effects on body weight or weight gain, food consumption, clinical chemistry urinalysis or organ weights (Mod2.6.6/Sec3.2.2).

Increases in C-reactive protein, noted in all animals within 24 h after injection, tended to be higher in RPV LA-dosed animals compared to control or vehicle groups, with minimal increases in white blood cells and fibrinogen only noted after the last dose in single animals in the 9-month study. In both studies clinical observations were limited to the injection site and consisted of short-lasting erythema (1 to 4 days after injection). These findings can be related to white deposits present at the injection sites at necropsy and swollen draining lymph nodes with a white discoloration. Although not confirmed via chemical analysis, the content of these white deposits and discolorations is considered to be RPV. Histologically extracellular eosinophilic amorphous material was present next to macrophage infiltration and multinucleated macrophages containing the same material in the injection site and regional lymph nodes.

In conclusion, the RPV LA formulation (G001) was well tolerated when injected every two weeks for 6 weeks or once monthly for 9 months (see Table 3 for exposures).

4.3. Genotoxicity (Rilpivirine studies supporting the registration of oral RPV (EDURANT®))

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 (Mod2.6.6/Sec4).

RPV was tested up to the maximum concentration that allowed scoring due to precipitation. No increased mutation frequency with or without metabolic activation (rat S9) or increased frequency of structural chromosomal aberrations was noted. In the Ames test with human S9, metabolites M30/M31, that are formed following Michael addition at the unsaturated nitrile moiety of RPV (Mod2.6.4/Sec5), were present at the same level as determined in human feces (Mod2.6.4/Sec5). 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).

4.4. Carcinogenicity (Rilpivirine studies supporting the registration of oral RPV (EDURANT®))

No carcinogenicity studies were performed with RPV LA. Carcinogenicity studies with RPV oral formulation were performed in rats and mice and are summarized below.

4.4.1. Mice

Rilpivirine formulated in 0.5% (mass per volume (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 (Mod2.6.6/Sec5.1.1). 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 mg/kg/day 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_{0-24h} 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.

4.4.2. Rats

Rilpivirine 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 (Mod2.6.6/Sec5.1.2). 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 in 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 $AUC_{0\text{-}24h}$ 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.

4.5. Reproductive and developmental toxicity (Rilpivirine studies supporting the registration of oral RPV (EDURANT®))

Male and female fertility studies and peri- and postnatal development studies were conducted in rats. Embryo-fetal development was evaluated in rats and rabbits. A further mechanistic juvenile toxicity study was done in immature female cynomolgus monkeys.

4.5.1. 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 (Mod2.6.6/Sec6.1.1). 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 (Mod2.6.6/Sec6.1.2). 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.

4.5.2. Embryo-Fetal Development

Rats

Rilpivirine base dissolved in PEG400 + CA was administered once daily, by oral gavage, from Gestation Day (GD) 6 to 17 (day of sperm detection is GD 0) 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 (Mod2.6.6/Sec6.2.1). The dams were necropsied on GD 21. There were no mortalities associated with RPV base. 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 and food consumption and the increase in incidence 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.

Rabbits

Rilpivirine 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 (Mod2.6.6/Sec6.2.2). 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 $AUC_{0\text{-}24h}$ values determined at the end of the dosing period at the fetal NOAEL was 170 µg.h/mL.

4.5.3. Peri-and Postnatal Development

Rilpivirine 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 (Mod2.6.6/Sec6.3). 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 parental (F0) and F1 generation was at least 400 mg/kg/day.

4.5.4. Juvenile Toxicity

Rats

Rilpivirine 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 LD 7 (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 (Mod2.6.6/Sec6.3.1). 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 $AUC_{0\text{-}24h}$ values determined at the end of the dosing period were 40 to 50 µg.h/mL in male and female pups.

Dogs

No specific dog juvenile toxicity studies were done. However, in all dog studies, the animals were at the start of dosing 6 – 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 (see Mod2.6.6/Sec3).

Cynomolgus Monkeys

In the 8-week immature female cynomolgus monkey study (see Section 4.2.3), 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.

4.6. Phototoxicity and Local Tolerance

Rilpivirine studies supporting the registration of oral RPV (EDURANT®)

Rilpivirine tested negative for the potential to cause phototoxicity in vitro, skin irritation in rabbits, and delayed-type hypersensitivity in the mouse LLNA. The compound did not induce an immunotoxic effect on the challenge of rats with sheep RBC. RPV was classified as a moderate eye irritant in an in vitro test (Mod2.6.6/Sec7.1.).

RPV LA

In support of the LA formulation, RPV base was evaluated in different vehicle suspensions in an in vitro HET-CAM test and found to have no significant irritation potential. In an in vivo LLNA, RPV in acetone:olive oil (4:1) and RPV base in 5% vitamin E-D-α-tocopheryl polyethyleneglycol 1000 succinate (VitE-TPGS), Tween 80 or P338 did not show skin sensitization potential (Mod2.6.6/Sec7.2).

Single dose local tolerability studies were performed in rabbits and minipigs with experimental formulations as well as formulations containing most or all of the excipients of the G001 formulation. All these formulations were well tolerated with minimal injection site reactions. In the rabbits, the formulation close to the G001 showed the most favorable characteristics with regard to injection site reactions and in minipigs the formulation containing 600 mg RPV base plus P338 showed the highest plasma exposures (Mod 2.6.6/Sec7.2).

4.7. Other Toxicity Studies: Poloxamer 338

Genotoxicity

To support the use of P338 in the RPV LA formulation, genotoxicity of P338 was evaluated in vitro in bacterial and mammalian cell systems and was concluded non-mutagenic and without potential for chromosome aberrations, respectively, both in presence and absence of metabolic activation through S9 mix (Mod2.6.6/Sec8.9.1).

General Toxicity and Local Tolerability

P338 was included as a vehicle (2 mL IM administration) in the 6-week minipig study at 50 and 160 mg/mL every two weeks and in the 9-month study at 50 mg/mL (100 mg total) once monthly.

No mortality occurred in either study and no effects were noted on body weight, weight gain, food consumption, hematology, coagulation, clinical chemistry, urinalysis or organ weights or at macroscopic or histopathologic evaluation. Marked increases in C-reactive protein (CRP) were seen in P338 as well as RPV LA treated groups within 24 hours of injection but returned to baseline within 3 days.

In conclusion, IM injections every 2 or 4 weeks of P338 to minipigs for a period of 6 weeks or 9 months, respectively were well tolerated with no indication for any local or systemic toxicity. The 100 mg dosed once monthly in the 9-month minipig study can therefore be considered equivalent to a NOAEL for long term administration.

Exposure was evaluated in a separate PK study where P338 was administered as a single IM dose to minipigs at 50 mg/mL (2 mL injection; Table 4).

Reproductive and Developmental Toxicity

The developmental toxicity of P338 was assessed in rats and rabbits upon oral dosing at the maximum feasible dose of 1,600 mg/kg/day (Mod2.6.6/Sec8.9.2). There were no relevant findings noted in these studies on maternal or fetal parameters except for minor effects on weight gain and soft feces in the pregnant rabbits. However, based on two pharmacokinetics studies the exposure to P338 after oral administration in both species proved very limited. Therefore, the potential for reproductive and developmental toxicity was evaluated after IM administration, including determination of systemic exposure to P338. Exposure was evaluated in a separate PK study (Section 3.9.3)

In a combined male and female rat fertility and embryo-fetal development study, P338 was administered by IM injection at dose levels of 2.5, 5 and 10 mg/kg/occasion to male and female Sprague-Dawley rats, every three calendar days before and during mating and up to 3 days before necropsy of the males or until GD 15 for the females. There was no indication for effects on fertility (estrous cycles, mating performance, mean pre-coital interval) on gonadal organ weights in males or females, on implantation, embryo-fetal survival, mean fetal weight or sex ratio in any group. There were no external, visceral or skeletal abnormalities attributed to P338. Exposure, without an indication for differences according to sex, was confirmed in all P338-treated animals. A safety margin of at least 9-fold (at 10 mg/kg/occasion) over the anticipate clinical exposure was calculated (Table 4).

Embryo-fetal development was also evaluated in pregnant rabbits, where animals were dosed IM at 2.5 and 5 mg/kg/occasion of P338 on GD 6 and 12. There were no indications for maternal toxicity and no relevant effects on embryo-fetal survival, mean fetal weight or sex ratio in any group and no external, visceral or skeletal abnormalities were attributed to P338. Exposure was confirmed in all P338 dosed rabbits, and a safety margin of at least 9-fold (at 5mg/kg/occasion) was calculated relative to the clinical exposure (Table 4).

Lastly, a peri- and postnatal development study was conducted in time-mated female rats dosed at 2.5, 5 and 10 mg/kg/occasion, every three calendar days, from G6 till weaning. There were no indications for maternal toxicity and no relevant effects on gestation, parturition, pup number, sex ration, survival or physical condition. The pups (F1) did not show any effects on body weight, weight gain, general or sexual development, fertility, mating performance or pregnancy parameters. No relevant findings were present at the necropsy of the maternal or F1 generation. Exposure was confirmed in all P338 dosed animals and exceeded the anticipated clinical exposure by at least 12-fold (at 10 mg/kg/occasion) (Table 4).

4.8. Toxicology evaluation

As the active moiety for the oral and LA formulation is essentially the same (HCl salt for the oral RPV formulation versus base for the LA formulation), no genotoxicity, carcinogenicity or reproductive, developmental or juvenile studies were done with RPV LA. The conclusion on these studies can be applied to the RPV LA formulation as well.

4.8.1. Mortality

Rilpivirine studies supporting the registration of oral RPV (EDURANT®)

No RPV-related mortality occurred in any of the single or repeat-dose oral 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.

RPV LA

No mortality occurred in any of the RPV LA studies after IM administration in dogs or minipigs at doses up to 1200 and 600 mg, respectively.

4.8.2. Assessment of General Toxicity

Rilpivirine studies supporting the registration of oral RPV (EDURANT®)

The targets of toxicity of RPV identified in the oral repeated dose studies were: RBCs (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 effects in rats and mice (Mod2.6.6/Sec3.1.1 and Sec3.1.2) differed qualitatively from those in dogs. In rodents, hepatocellular hypertrophy was associated with liver enzyme (i.e. UDP-GT) induction, an increase of the organ weight, and in mice an increase in vacuolization and single cell necrosis. The differences between rats and mice may be explained by the much higher exposures in mice than in rats. In dogs (Mod2.6.6/Sec3.1.4.2), in the 1-month study mild to moderate perivascular inflammatory reactions together with fibrosis and single hepatic cell necrosis in the central part of the lobules were seen in association with serum changes in cholesterol, bilirubin, ALP and ALT. In the 6-month dog study, pigmentation in liver and gallbladder was seen indicating a slower onset of these changes. These findings were noted at exposures approximately 8-times the clinical exposure at 25 mg orally once daily or 600 mg IM

once monthly. No indication for hepatotoxicity has been noted in clinical trials (also see Mod2.7.4).

Thyroid related effects seen in the 6-month rat study (Mod2.6.6/Sec3.1.2.2.2) were considered related to rodent specific thyroxine (T_4) clearance due to induction of T_4- UDP-GT in the liver. The changes mainly included an increased organ weight, hypertrophy of follicular epithelium, and reduced serum concentrations of T_4. Long term clinical safety data do not indicate any effect of RPV on plasma T_4 concentrations in man (Mod2.7.4). Also, the histopathological changes in the **pituitary gland** (which only occurred in rats, Mod2.6.6/Sec3.1.2) are considered related to this mechanism.

In mice, minimal to moderate degenerative or necrotic nephropathy (Mod2.6.6/Sec3.1.1) and in dogs, moderate acute nephritis (Mod2.6.6/Sec3.1.1.1.1) were noted in the **kidneys** at exposures far exceeding the clinical exposure at 25 mg orally once daily or 600 mg IM once monthly. The NOELs were 20 mg/kg/day for mice (exposure at least 25 times clinical exposure) and 10 mg/kg/day for dogs (exposure at least 8 times clinical exposure). Given these changes are not indicative of an effect on glomerular filtration or proximal tubular resorption and the safety margins for each of the effects, they are considered not relevant for man.

Effects on the **adrenal glands** (including increased weight, hypertrophy in the zona fasciculata), and serum changes in adrenal hormones (or precursors) and ACTH in nonclinical species are considered related to a partial inhibition of 21-hydroxylase, also known as CYP21 (Mod2.6.6/Sec9.1). Due to this partial block, the conversion from progesterone and 17α-hydroxyprogesterone to 11-deoxycorticosterone and 11-deoxycortisol, respectively is hampered and a reduced production of downstream hormones (mainly cortisol and corticosterone) and accumulation of progesterone and 17α-hydroxyprogesterone and more upstream hormones can be seen. In species that have a significant androgenic adrenal pathway the accumulation of the adrenal precursors may contribute to an increased androgen production via the CYP17 pathway. The **ovarian** effects in mice (decreased number of corpora lutea) and dogs (increased numbers of tertiary follicles, [cystic] luteinized follicles, and in some dogs by corpora lutea) and the **testicular** effects in dogs (Leydig cell hypertrophy) (Mod2.6.6/Sec3.1.1 and Sec3.1.4) are likely also related to this mechanism. In the dog and the cynomolgus monkey studies, 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 of the adrenal and gonadal hormones, no indications of interference of RPV with adrenal steroidogenesis could be found in long term safety and pharmacovigilance data (Mod2.7.4).

A small reversible reduction of **red blood parameters** without clinical sequelae and in combination with regenerative signs was seen in mice, rats and dogs, usually only in the high dose groups in each of the studies. Although the mechanism could not be established, there were no signs indicative of bone marrow suppression. Exposure (AUC) at the NOEL of this effect (120 mg in 6-month rat; Mod2.6.6/Sec3.1.2.2.2) was approximately 12-times higher than the exposure in man at the recommended doses of 25 mg orally q.d. or 600 mg IM once monthly.

Non-dose-related increased **coagulation** times (APTT and PT) were only noted in male rats and were not associated with any bleedings (Mod2.6.6/Sec3.1.2.2.2). Considering the absence of clinical manifestations, this effect in rats only is likely not relevant for humans.

As the active moiety for the oral and LA formulation is essentially the same (HCl salt for the oral RPV formulation versus base for the LA formulation), the conclusion on these findings can be applied to the RPV LA formulation as well

Rilpivirine LA

Intramuscular administration of RPV LA to dogs once every 2 weeks for 4 weeks at 150 and 1200 mg/dog was well tolerated. There were no new target organ toxicities identified due to the change in kinetic profile (oral versus LA), or due to the change in route of administration versus what was seen in the RPV oral toxicity studies. The increases seen in progesterone and 17α-hydroxyprogesterone are consistent with the known toxicity profile of RPV. The highest dose level of IM administration (1200 mg/dog) gave a systemic exposure similar to that obtained with the low dose level of oral administration (5 mg/kg/day).

4.8.3. Assessment of RPV LA Local Tolerability

Rilpivirine base in different formulations, including one containing P338 in the mouse LLNA, did not show any irritating or sensitizing potential (Mod2.6.6/Sec7.2.1 and 7.2.2). When injected IM in single dose tolerability studies in rabbits and minipigs with a formulation close to the G001 or in repeat-dose studies in dog and minipig with the final clinical G001 formulation, RPV LA was well tolerated with only minimal injection site reactions (Mod2.6.6/Sec7.2.3 and Sec7.2.4). Local reactions after repeated administration included moderate swelling/slight edema in the dog (2-week interval) (Mod2.6.6/Sec3.2.1) which was still evident 2 weeks after the last injection, and slight, short-lasting erythema (up to 4 days after injection) in the minipig. In the minipigs (Mod2.6.6/Sec3.2.2), where CRP was measured, an increase was noted 24h after injection, returning to baseline within 3 days. Minimal increases in white blood cells and fibrinogen relative to controls were also noted (at the end of the dosing period). These hematological changes are considered consistent with the minimal macrophage infiltration at the injection site, in response to the IM injection and the presence of amorphous eosinophilic deposits (likely RPV) at the site.

4.8.4. Assessment of Genotoxicity and Carcinogenicity (Rilpivirine studies supporting the registration of oral RPV (EDURANT®))

Genotoxicity tests, in vitro and in vivo, have shown RPV to be free of a genotoxic potential (Mod2.6.6/Sec4). 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 (Mod2.6.6/Sec5).

Hepatocellular adenomas and carcinomas are common spontaneous liver neoplasms in rodents [6]. In general, hepatocarcinogens are divided into genotoxic and non-genotoxic agents [5]. 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 CYP induction [8]. The increased liver weight associated with signs of enzyme induction caused by RPV was already evident in the repeat-dose general toxicity studies (Mod2.6.6/Sec3). 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 (Mod2.6.4/Sec5.5.3). Electron microscopy of mouse liver noted peroxisome proliferation, a lesion commonly associated with CYP4A induction [15].This pattern of liver enzyme induction correlates well with the incidence of hepatocellular adenomas and carcinomas [15].

The repeat-dose toxicity studies in rats (Mod2.6.6/Sec3.2) have demonstrated, without exception, effects on the thyroid gland considered due to increased clearance of thyroxine by UDP-GT induction. UDP-GT 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 [1].

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 UDP-GT. A similar association between liver enzyme induction and carcinogenesis does not exist for man [5,8].

For these reasons, it is concluded that, although a NOAEL could not be established (as neoplastic lesions occurred in the lowest doses tested), the epigenetic carcinogenic effects of RPV in mouse and rat bear no relevance for man.

4.8.5. Assessment of reproductive and developmental toxicity (Rilpivirine studies supporting the registration of oral RPV (EDURANT®))

The reproductive and developmental toxicity studies did not demonstrate any effects on male or female fertility or fecundity (Mod2.6.6/Sec6.1). 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. 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. The exposures at NOAEL in rats are at least 28-times higher than the exposure at the oral dose of 25 mg RPV q.d. or IM at 600 mg RPV LA, 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 (Mod2.6.6/Sec6.2). 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 50 times higher than the exposure at the oral dose of 25 mg RPV q.d. or IM at 600mg RPV LA, in man. Although RPV has not been assessed in pregnant women in well-controlled clinical trials, data is available from the ARV Pregnancy Registry (APR) where no increases in risk of overall birth defects (at least 2-fold) have been detected after first trimester exposures to oral EDURANT®.

In the pre- 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 (Mod2.6.6/Sec6.3). 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. This exposure is at least 40 times higher than the exposure at the oral dose of 25 mg RPV q.d. or IM at 600mg RPV LA, in man. In animals, no studies have been conducted to assess directly the excretion of RPV into the milk, although indirect data on presence of RPV in pups and mammary glands suggest potential for excretion in milk (Section 3.6). 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.

4.8.6. Assessment of juvenile toxicity (Rilpivirine studies supporting the registration of oral RPV (EDURANT®))

In the rat juvenile toxicity study with pups from RPV-treated mothers dosed by oral gavage from LD12 to 25, no effects were noted (Mod2.6.6/Sec6.3.1). 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 (Mod2.6.6/Sec3) 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.

4.8.7. RPV LA Safety Margins

The C_{max} and AUC values of RPV after repeated oral administration in various animal species used in the toxicology studies and after repeated IM administration of RPV LA in dogs and minipigs are summarized in Table 3. These exposures were compared with the human modeled

exposure (C_{max} = 0.14 µg/mL and $AUC_{0\text{-day}28}$ = 83 µg.h/mL) obtained in patients at steady-state after 600 mg monthly of RPV LA given every 4 weeks (G001).

Safety margins for toxicological changes noted in the studies with oral administration are discussed in the previous sections. In summary for each of the general toxicity effects deemed potentially relevant for man, a safety margin of at least 8 was calculated. For reproductive toxicity studies, the margin on exposure was at least 28-times the human exposure. As exposures after repeated administration of RPV LA are very similar to those after repeated oral administration, the same safety margins can be used for RPV LA.

The NOAEL in the 4-week dog study with RPV LA was 1200 mg/dog dosed every 2 weeks, as the changes noted on progesterone were not considered adverse. At this dose systemic exposures were 5 times higher than the exposure in patients. The RPV LA injections were well tolerated in minipigs in the selected clinical RPV LA formulation, containing 300 mg/mL RPV when injected at 2mL/injection every two weeks for 6 weeks or once monthly for 9 months.

4.8.8. Assessment of impurities

Rilpivirine studies supporting the registration of oral RPV (EDURANT®)

One potential and two actual impurities have been identified (related substance I*, related substance H*, and related substance C*) for RPV drug substance which needed to be qualified according to ICH Q3A guideline "Impurities in New Drug Substances" [11].

The first two (related substance I* and related substance H*) have been toxicologically qualified based on absence of genotoxic potential and absence of relevant toxicologic effects in a 1-month oral toxicity study in rats, where the impurities were spiked to the drug substance at 4%. The two impurities spiked at 4% showed no genotoxic potential. The three batches tested in the one-month rat study showed no relevant differences in toxicological profile. Consequently, for both impurities the qualified dose level is 0.4 mg/kg/day. For the oral application at the recommended daily dose of 25 mg (or 0.5 mg/kg/day for a 50 kg person this translates to a qualified level of up to 80%. For the LA formulation, the highest potential dose of 900 mg/injection (initial dose) was taken into account as a worst-case, although it is followed by a once monthly continuation dose of 600 mg. The 900mg dose translates to a daily dose of RPV LA of 30 mg/day (taking into account an average of 30 days a month) or 0.6 mg/kg/day (assuming 50 kg human body weight). Consequently, the impurities related substance H* and related substance I* are qualified up to 67% each. These impurities occur in commercially representative drug substance batches at less than 0.05% (Mod3.2.S.3.2).

The third impurity (related substance C*, the [redacted]-isomer of RPV base) was present in all drug substance batches involved in pivotal nonclinical studies and was toxicologically qualified based on its presence (0.61%) in a drug substance batch used in a 1-month dog study where the lowest dose was 5 mg/kg/day RPV. A NOAEL could not be established as effects pertaining to reduced adrenal steroidogenesis and increased ovarian activity were still present at 5 mg/kg/day. However, upon close monitoring in man no indications of oral RPV-related effects on adrenal steroidogenesis or ovarian cycling were found. The 5 mg/kg/day dose level was therefore considered suitable for impurity qualification purposes. Consequently, the qualified dose level of

related substance C* is 0.03 mg/kg/day. For the oral application it is concluded that related substance C* up to 6.2% of the daily dose of RPV is unlikely to incur untoward effects in man, and for the LA application, taking into account a similar deduction as above, a level of 5% is considered qualified. The representative commercial drug substance batches contain on average 0.10% of this impurity (Mod3.2.S.3.2).

The only genotoxic impurity (related substance B) present in the drug substance, was considered to have a mutagenic and clastogenic potential in an in vitro bacterial reverse mutation test (Ames) and mammalian chromosomal aberration assay, respectively. The impurity is therefore controlled below the Threshold of Toxicological Concern (TTC) (ICH M7 [R1] guideline [9]) which was calculated as 60 ppm for a daily dose of 25 mg and daily treatment for longer than 12 months. For the LA application, the worst-case approach with 900 mg as a maximum dose as described above was used with a TTC of 10 µg/day over a 10-year dosing period (as a monthly dosing schedule leads up to a total of 912 dosing days or 2.5 years over a life time period) and the allowable level was therefore set at 11.1 ppm.

The actually measured levels of related substance D in batches representative of the commercial process are below 1 ppm, and therefore well below the TTC-based allowable limit for both oral and LA applications (Mod3.2.S.3.2).

RPV LA

N-Methylpyrrolidone (NMP) is a specified residual solvent in the RPV LA drug product. It is an ICH Q3C (R7) Class 2 solvent [12]; its permitted daily intake is 5.3 mg/day. Using the same worst-case approach as above (initiation dose of 900 mg followed by a once monthly continuation dose), with a presumed daily dose of 30 mg RPV, NMP is qualified up to 176,666 ppm. Following the as low as reasonably possible (ALARP) principle, the specification limit is set at 2000 ppm (Mod3.2.S.3.2). The actually measured concentration of NMP is 458 ppm in drug product.

4.8.9. Assessment of Poloxamer 338

Poloxamer 338 is added as wetting agent to the RPV LA formulation. P338 is mainly registered for topical and oral applications, but currently not for IM administration. Several safety related studies have been conducted by the manufacturer, including genotoxicity, local irritation and orally dosed chronic toxicity and developmental toxicity studies, however these were conducted under non-GLP conditions, and without evaluation of systemic exposures. The results indicated non-irritating, non-mutagenic properties and a low potential for general and reproductive toxicity [13,19]. Amongst commonly used poloxamer excipients, P338 has the highest molecular weight and is relatively hydrophilic as expressed by its high Hydrophilic-Lipophilic-Balance value and polyethyleneoxide fraction.

A similar poloxamer, with the same molecular composition but of a lower molecular weight, poloxamer 188 is registered for oral, IM, SC and IV administration in the FDA Inactive ingredients database [4]. It is recognized that data from other large poloxamers with similar properties and which differ only in chain length, can provide additional information on the safety

of less characterized poloxamers (e.g., FDA Guidance Nonclinical Studies for the Safety Evaluation of Pharmaceutical Excipients, [2]). A complete safety package acute toxicity studies (oral in rats, dermal in rabbits and IV in rat, mouse, rabbit and dogs), skin and eye irritation, sensitization in rabbit and dog, 2-week (IV) study in rabbit and dog, 6-month (oral, diet) in rats and dogs, 2-year (oral, diet) carcinogenicity in rats and Ames test were performed for the poloxamer 188 [14]. There were no indications of notable adverse effects, however all these studies were performed under non-GLP conditions, and without evaluation of systemic exposure. More recently as part of the Norditropin SimpleXx NDA application [17,18], poloxamer 188 was evaluated after SC administration in repeated dose rat and dog studies, reproductive and developmental toxicity studies in rats and rabbits and in genetic toxicology studies. General toxicity was evaluated in a daily SC 4-week repeated dose toxicity study in the rat with a 2 week follow-up and in a 13-week study with a 4 week follow-up each at 4 dose levels: 0, 10, 100 and 500 mg/kg/day. An increase in incidence and severity of tubular vacuolation correlating with increases in kidney weight was seen at 100 and 500 mg/kg/day in both studies and was at least partially recovered at the end of the treatment free period. The low dose of 10 mg/kg/day was considered the NOAEL and was 41-fold the expected human load of poloxamer 188 of 2.25 mg/patient/day based on a body surface area comparison. No reproductive or genotoxic findings were noted. Systemic exposures were not mentioned.

Although poloxamers are considered to generally have a low-grade toxicity profile [19], systemic exposure in relation to the presence or absence of toxicity and full genotoxic evaluation were considered missing from available data and, therefore additional studies, including evaluation of P338 pharmacokinetics, where applicable, have been performed under GLP conditions.

Poloxamer 338 was evaluated for its potential to induce point and/or gene mutations in mammalian and non-mammalian cell systems and was considered devoid of genotoxic properties.

Poloxamer 338 was included as a vehicle (50 mg/mL at 2 mL/injection) in the 6-week and 9-month minipig studies, in addition to a control (buffer without P338). No relevant systemic or local toxicity related to P338 was noted and there were no indications for cardiovascular, respiratory or CNS effects in these studies and the dose of 100 mg/month can be considered equivalent to a NOEL. Systemic exposure to P338 was not included in these studies but was evaluated in a separate pharmacokinetic study under identical conditions (Mod2.6.6/Sec8.3.1). Poloxamer 338 C_{max} and $AUC_{0\text{-}672h}$ values were found to be 13-fold and 19-fold higher after single IM administration, respectively, than those in human after a single IM dose of 600 mg (Table 4).

No adverse findings on male or female fertility, embryo-fetal development or pre- and postnatal development were noted in the rat up to doses of 10 mg/kg P338 every three days. At this dose systemic exposures (C_{max} and AUC) were found to be 6-9-fold and 9-18-fold higher compared to human exposures (Table 4). Also, in rabbits no effect on embryo-fetal development was present up to 5 mg/kg P338 administered on GD 6 and 12. Systemic exposures in rabbits at 5 mg/kg

(C_{max} and AUC) were found to be 13-fold and 9-fold higher compared to human exposures (Table 4).

5. INTEGRATED OVERVIEW AND CONCLUSIONS

5.1. Pharmacology

The primary pharmacodynamics are discussed in Mod2.7.2/Virology Summary

The secondary pharmacodynamics and safety pharmacology studies revealed 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. In vitro, RPV inhibited three potassium currents involved in the repolarization phase of the cardiac action potential, IK_r, IK_s, 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 afterdepolarizations (EADs). In none of the in vivo animal models was any effect on electrophysiological cardiovascular or hemodynamic parameters noted. A delayed onset heart rate-corrected QT (QTc) interval prolongation observed at steady-state was observed with the oral dose of 75 mg (median C_{max} = 636 ng/mL) and 300 mg in the thorough QT study C131. No QT prolongation was observed with the marketed 25-mg-dose RPV once daily (EDURANT®). As the C_{max} from 900 mg RPV LA as an initial dose followed by 600 mg-dose every 4 weeks is similar to the C_{max} after oral administration of 25 mg once daily, QT interval prolongation is not considered to be a safety concern for RPV LA.

5.2. Pharmacokinetics

In minipigs and rabbits, after a single IM administration of RPV LA as the P338-containing formulation (G001), the RPV release was fast, with a C_{max} reached within 24 h, after which mean plasma concentrations declined, remained fairly constant thereafter and were still quantifiable after 3 months. After 3 months, the F_{abs} is 67% in rabbits at 150 mg/kg and ranges between 35 and 62% in minipigs at 600 mg, indicating the release from the depot was still incomplete after 3 months. Comparing the distribution of RPV in rats after IM administration of RPV LA at 60 mg/kg and oral administration of RPV at 40 mg/kg the tissue/plasma exposure (AUC) ratios of RPV in adrenal gland and brain were in the same order of magnitude and the liver ratio was higher after oral, in line with the route of administration. In addition, the tissue/blood exposure (AUC) ratios of RPV after RPV LA were in the same ranking as after oral administration of ^{14}C-RPV, except again in the liver. Therefore, after IM administration of RPV LA in rats, no undue retention of RPV in tissues was observed including a filter organ like the spleen and the distribution in different tissues was in the same order of magnitude or ranking as after oral administration. However, adjacent to the injection site, high concentrations were observed in rats and rabbits. This can be related to the local “white deposits” noted at necropsy in the single dose local tolerability study in the rabbit, however this did not lead to adverse clinical or post mortem observations. In rats, following single IM administration at 60 mg/kg of RPV LA (G001) alone or in combination with a LA injectable suspension of GSK1265744A (CAB) at 10 mg/kg, the

plasma concentrations, the mean C_{max} and $AUC_{0\text{-}1444h \text{ or } 2months}$ values of RPV were similar for the 2 groups.

Rilpivirine was highly 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 α1-acid glycoprotein were involved in the plasma protein binding. 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. 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. In rats, there was indication that RPV was excreted in milk.

In vitro, the CYP3A4 isoenzyme plays a major role in the biotransformation of RPV. Rilpivirine might be a very weak inducer of CYP1A2 and CYP2B6 and a moderate inducer of CYP2C19 and CYP3A4. Rilpivirine is an inhibitor of CYP2C8 (Ki = 10μM) and CYP2C9 (Ki = 1.7 μM) in vitro whereas no inhibition is expected in vivo. In human liver microsomes, the limited mechanism-based inhibition of CYP2C9 is unlikely to have clinical relevance at therapeutic doses of RPV.

Rilpivirine was shown to have P-glycoprotein (P-gp) inhibitor properties with an apparent IC_{50} value of 9.2 μM (3.4 μg/mL ie much higher then RPV concentrations observed in humans). Inhibition of the OCT2 transporter by RPV was evaluated in vitro. The in vitro IC_{50} for inhibition of OCT2 by RPV was 5.46 μM (2.0 μg/mL). The inhibition of MATE-mediated transport by RPV was investigated in vitro in CHO cells overexpressing MATE-1 and MATE-2K. The uptake of ^{14}C-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 (<0.018 μg/mL) for MATE-2K. 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. In a DDI study, there was no impact of RPV on the pharmacokinetics of metformin.

After oral administration in rats and rabbits, no or very limited absorption of P338 was observed. After IM administration of RPV LA in minipigs and human, the P338 release was fast, after which plasma concentrations declined, remained fairly constant thereafter and were still quantifiable after at least 672 h. In rats, the tissue to plasma $AUC_{0\text{-}529h}$ ratios were 1.1 in kidneys and 5 in the liver. In urine, 9.41% of the dose was excreted as unchanged P338 in the 0-144h interval. Since all concentrations were below quantification limit in feces, only ~10% of the administered dose was eliminated at the end of the experiment, and via the urine. At the last time point 529h after dosing, P338 is still detected in plasma, liver and kidney, showing the slow elimination of P338 in rats, probably explaining the low recovery in urine. As P338 only differs from poloxamer 188 by chain length, the metabolism of P338 is likely similar to poloxamer 118 with a limited metabolism [7].

5.3. Toxicology

As the active moiety for the oral and LA formulation is essentially the same (HCl salt for the oral RPV formulation versus base for the LA formulation), conclusions on target organs/systems,

genotoxicity, carcinogenicity and reproductive toxicity of the oral RPV formulation can be applied to the RPV LA formulation. In addition, a repeated dose bridging study in the dog demonstrated a similar toxicological profile and no additional target organs with RPV LA compared to oral dosed RPV at similar exposures.

In oral single dose and repeat-dose toxicity studies with RPV, up to 3 months in mice, 6 months in rats, 12 months 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, RBCs 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 (UDP-GT). They included serum related changes, increased organ weights, hypertrophy, and in mice livers, vacuolization and single cell necrosis. These effects are not considered relevant for man. In the dog, mild to moderate perivascular inflammatory reactions in the liver in the 1-month study, and pigmentation in liver and gallbladder in the 6-month study were noted at exposures approximately 8-times the clinical exposure at 25 mg orally once daily or 600 mg IM once monthly. Also, the kidney effects in mice and dogs were noted at exposures far exceeding (25 times for mice, 10 times for dog) the clinical exposures. In mice, the minimal to moderate degenerative or necrotic nephropathy and in dogs, the moderate acute nephritis are not indicative of an effect on glomerular filtration or proximal tubular resorption. Based on this, together with the safety margins, the effects are not considered relevant for man. 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 in serum cortisol levels or increase in serum 17-hydroxy progesterone levels characteristic for inhibition of CYP21 has not been observed in clinical studies with adult men and women or with adolescents (12 to less than 18 years of age), neither at baseline nor after ACTH stimulation. Similarly, no inhibition of CYP17 has been demonstrated in these clinical studies. The reducing effects on RBC 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.

Long term administration of RPV LA showed it was well tolerated in dogs at 1200 mg once weekly for 4 weeks and in minipigs at 600 mg once monthly for 9 months. Main findings comprised local effects noted at the injection sites. Slight, short-lasting erythema was observed, and white deposits were noted at the injection sites at necropsy, accompanied by swelling and discoloration of draining lymph nodes. Microscopic examination showed macrophage infiltration and eosinophilic deposits of/at the injection sites. A macrophage infiltration response was also noted in the draining/regional lymph nodes. These findings were considered to be a reaction to

the deposited material rather than a manifestation of local irritation. Effects related to systemic exposure to RPV LA in the dog (bridging) study were consisted with those seen after oral administration at similar exposures: increases in progesterone and 17α- hydroxyprogesterone were noted up to the end of the study, however without changes in cortisol.

Genotoxicity studies with RPV have shown the absence of a potential to induce mutations and chromosomal damage. Carcinogenicity studies after oral administration 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 UDP-GT. 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.

Rilpivirine has no effects on fertility or fecundity and does not affect parturition, maternal behavior or pre- and postnatal development. RPV has no teratogenic potential, does 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. RPV base did not show any irritating or skin sensitizing potential. RPV has not demonstrated different or more severe effects in juvenile and immature rats, dogs, and cynomolgus monkeys than in adult animals.

The RPV LA formulation was well tolerated as a single dose in rabbits and minipigs and at repeated administration in dogs and minipigs. Local (inflammatory) reactions were observed at the injection sites but were generally mild and transient.

Three specified impurities present in the drug substance (related substance H*, related substance I* and related substance C*) have been qualified according to ICH Q3A [11] and one genotoxic impurity related substance D*is controlled below the TTC (ICH M7; [10]). Each has been re-evaluated with regard to their qualification limit in the current proposed regimen. One residual solvent in the RPV LA drug product (NMP) is controlled according to ICH Q3C and the ALARP principle. Levels of each of these impurities in representative clinical batches are far below the set limits.

Poloxamers are generally considered of low-grade toxicity, and literature data available on both P338 and a similar lower grade poloxamer P188 indicate only limited toxicity after oral, dermal or SC administration. However, as studies were generally not done according to GLP guidelines and as no systemic exposure is available in relation to these data, additional studies were conducted to further evaluate the safety of P338. No relevant general or local toxicity was present after repeated administration at the dose of P338 used in the RPV LA formulations, and no effects on fertility embryo-fetal development or per- and postnatal development were noted. Systemic exposures in these studies far exceed the exposure seen after a single IM administration in humans.

In conclusion, studies included in the EDURANT® oral development program together with specific nonclinical studies with the LA formulation as described in this summary are considered

adequate to support the use of RPV LA in combination of CAB LA for the treatment of subjects with HIV-1 infections.

5.4. Combination with Cabotegravir

The intended therapeutic indication for RPV LA will be in combination with CAB LA. Synergistic or additive toxicity is not expected from the co-administration of CAB with RPV and no combination toxicity studies were conducted.

The adverse findings noted in repeat-dose toxicity studies with CAB (GI intolerance/toxicity) are considered due to local irritation of the compound as opposed to a systemic effect and have not been consistently observed in clinical studies to date.

The main targets relevant to human risk assessment in repeat-dose toxicity studies with RPV were: liver (hepatocellular hypertrophy in mouse and rat, pigmented macrophages, hepatocytes, canaliculi and gall bladder in dog), kidneys (nephropathy in mouse, nephritis and mineralization in dog), adrenals (rat, dog, monkey; partial inhibitor of CYP21 with potential downstream effects on ovaries and testes in dogs) and RBC (small reversible reduction of RBC parameters in mice, rats and dogs). Clinical pathology parameters to monitor potential effects were part of the clinical studies.

Based on the current knowledge of the comparative ADME profiles of CAB and RPV, clinically relevant changes in the pharmacokinetics are not expected for either CAB or RPV following co-administration. The lack of interaction between CAB and RPV was confirmed in clinical drug interaction studies following co-administration (Mod2.7.2/Sec2.1.1 and 2.5.3).

6. LIST OF LITERATURE CITATIONS

Literature citations are located in Mod4.3.

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7. APPENDIX

Appendix 1: Justification for Absence of Documents in Module 4 for Rilpivirine Long-Acting, and the Combination with Cabotegravir

Module	Module Title	Justification
Rilpivirine Long-Acting (IM)		
4.2.1.2	Secondary Pharmacodynamics	No secondary pharmacodynamics studies were conducted with RPV LA. As the active moiety for the LA formulation is essentially the same as for the oral formulation (hydrochloride (HCl) salt for the oral formulation versus base for the LA formulation), the secondary pharmacology studies conducted for the development of oral RPV are considered relevant for RPV LA.
4.2.1.3	Safety Pharmacology	No safety pharmacology studies were conducted with RPV LA as the active moiety for the oral and LA formulation is the same.
4.2.1.4	Pharmacodynamic Drug Interactions	Studies on pharmacodynamic drug interactions are included in Mod2.7.2/Summary of Virology.
4.2.2.4	Metabolism	No specific metabolism studies were conducted with RPV LA as the active moiety for the oral and LA formulation is the same.
4.2.2.5	Excretion	As the data for RPV submitted for oral RPV (EDURANT®) can be extrapolated to RPV LA, no additional studies were performed nor warranted. No specific excretion studies have been conducted with RPV oral formulation. Excretion is investigated as part of the metabolism studies listed under Mod4.2.2.4.
4.2.3.1	Single Dose Toxicity	No separate single dose toxicity studies were conducted with RPV or the clinical formulation G001 (300 mg/mL RPV LA in 50 mg/mL P338). Local tolerability studies with other surfactants in the LA formulations, or single dose studies examining varying concentrations and particle sizes of RPV, and various routes of administration have been conducted.
4.2.3.2	Repeated Dose Toxicity	A 1-month bridging toxicology study in dogs and up to 9-month IM toxicity studies in minipigs were conducted with the clinical formulation G001. No other repeated dose toxicity studies were conducted with RPV LA. Multiple oral repeat-dose toxicity studies were conducted with RPV in mouse, rat, rabbit, dog, and non-human primate that are considered relevant for the toxicity profile of the compound as the active moiety for the LA formulation is essentially the same as for the oral formulation (hydrochloride (HCl) salt for the oral formulation versus base for the LA formulation).
4.2.3.3	Genotoxicity	Genotoxicity testing with RPV LA was not performed. Genotoxicity studies performed with RPV were considered relevant as the active moiety for the LA formulation is essentially the same as for the oral formulation (hydrochloride (HCl) salt for the oral formulation versus base for the LA formulation).
4.2.3.4	Carcinogenicity	No carcinogenicity studies were performed with RPV LA. Carcinogenicity (2-year) studies with the oral formulation of RPV were performed in mouse and rat and were considered relevant as the active moiety for the LA formulation is essentially the same as for the oral formulation (hydrochloride (HCl) salt for the oral formulation versus base for the LA formulation).
4.2.3.5	Reproductive and Developmental Toxicity	No reproductive and developmental toxicity studies were conducted with RPV LA. Reproductive and developmental toxicity studies performed with RPV were considered relevant as the active moiety for the LA formulation is essentially the same as for the oral formulation (hydrochloride (HCl) salt for the oral formulation versus base for the LA formulation).

4.2.3.7.1	Antigenicity	In accordance with the ICH guideline, data from the general toxicology studies are considered sufficient to evaluate antigenic potential of RPV. Based on these data, it was concluded that there are no indications that RPV (base) might elicit allergic reactions and therefore no additional studies were performed.
4.2.3.7.2	Immunotoxicity	Immunotoxicity was assessed as part of a 4-week repeat-dose toxicity study in the rat (Mod.4.2.3.2/TMC278-Exp. 5692).
4.2.3.7.3	Mechanistic Studies	No specific mechanistic studies were conducted with RPV LA.
4.2.3.7.4	Dependence	In view of the primary pharmacodynamics of RPV, as NNRTI active against HIV-1, and the absence of relevant indications in the secondary pharmacodynamics profile, no dependence studies have been conducted.
4.2.3.7.5	Metabolites	In view of the absence of a unique human metabolite and of structural alerts in human metabolites identified, no specific safety studies on metabolites have been conducted.
Rilpivirine Long-Acting + Cabotegravir		
No combination studies have been conducted as no synergistic or additive toxicities are expected.		