CTD 第 2 部

2.4　非臨床試験の概括評価

MSD 株式会社

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LIST OF ABBREVIATIONS, ACRONYMS AND DEFINITION OF TERMS

Abbreviation/Acronym	Definition
3TC	Lamivudine
5-HT2B	5-hydroxytryptamine (serotonin) receptor 2B
AhR	Arylhydrocarbon Receptor
API	Active Pharmaceutical Ingredient
AUC	Area Under the Curve
AUC_{0-24}	Area Under the Curve (0 to 24 hours)
BCRP	Breast Cancer Resistance Protein
BDC	Bile Duct Cannulated
b.i.d.	Twice-daily Dosing
BSEP	Bile Salt Extrusion Pump/Bile Salt Extrusion Protein
CAR	Constitutive Androstane Receptor
CHO	Chinese Hamster Ovary
CLp	Plasma Clearance
C_{max}	Maximal Concentration
CNS	Central Nervous System
CYP	Cytochrome P450
DNA	Deoxyribonucleic Acid
DOR	Doravirine
EC_{50}	Concentration at which 50% Maximal Effect is Achieved
ECG	Electrocardiogram
EFV	Efavirenz
ETR	Etravirine
EU	European Union
FDC	Fixed-dose Combination
FOB	Functional Observation Battery
GD	Gestation Day
GLP	Good Laboratory Practice
hERG	Human Ether-à-go-go Related Gene
HIV-1	Human Immunodeficiency Virus Type 1

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Abbreviation/Acronym	Definition
HPMCAS	Hydroxypropyl methylcellulose-acetate succinate
hr	Hour(s)
IC_{20}	Concentration at which 20% Inhibition is Achieved
IC_{50}	Concentration at which 50% Inhibition is Achieved
ICH	International Council on Harmonisation
IV	Intravenous
LAP	Long Acting Parenteral
LE	Long Evans
LLC-PK1	Lilly Laboratory Cell – Porcine Kidney 1
MATE	Multidrug and Toxin Extrusion Protein
MDCK-II	Madin-Darby Canine Kidney-II
MDR1	Multidrug Resistance Protein 1
MN	Micronuclei
MOI	Multiplicity of Infection
NCE	Normochromatic Erythrocytes
NNRTI	Non-nucleoside Reverse Transcriptase Inhibitor
NOAEL	No-Observed-Adverse-Effect Level
NOEL	No-Observed-Effect Level
NRTI	Nucleos(t)ide Reverse Transcriptase Inhibitor
OAT	Organic Anion Transporter
OATP	Organic Anion-transporting Polypeptide
OCT	Organic Cation Transporter
Papp	Apparent Permeability
PCE	Polychromatic Erythrocytes
PD	Pharmacodynamic
PND	Postnatal Day
PNW	Postnatal Week
P-gp	P-glycoprotein
PK	Pharmacokinetic
QD	Once-daily Dosing

Abbreviation/Acronym	Definition
RNA	Ribonucleic Acid
RPV	Rilpivirine
RT	Reverse Transcriptase
$T_{1/2}$	Half-life
TDF	Tenofovir Disoproxil Fumarate
UDPGA	uridine 5'-diphospho-glucuronic acid
UGT	UDP Glucuronosyl Transferase
Vd	Volume of Distribution
Vdss	Steady State Volume of Distribution
WH	Wistar-Hannover

1 OVERVIEW OF THE NONCLINICAL TESTING STRATEGY

Doravirine (also referred to as DOR or MK-1439) is a potent non-nucleoside reverse transcriptase inhibitor (NNRTI) for the treatment of human immunodeficiency virus type 1 (HIV-1), developed as a single entity to be part of anti-retroviral therapy (ART) or as part of a fixed dose combination (FDC) tablet with two nucleoside reverse transcriptase inhibitors, lamivudine (also referred to as 3TC) and tenofovir disoproxil fumarate (also referred to as TDF). The FDC (also referred as MK-1439A and DOR/3TC/TDF) is targeted for use as a complete regimen for the treatment of HIV-1 infection in treatment-naive adult patients and offers advantages of both compliance and ease of administration. The recommended human dose of DOR is 100 mg as a single entity and in the FDC [DOR-100 mg/3TC-300 mg /TDF-300 mg] tablet. Nonclinical exposure margins of DOR to humans were based on population pharmacokinetics analysis from the Phase 3 trials (P018 and 021), where a 100 mg QD DOR clinical dose resulted in systemic geometric mean exposures of $AUC_{0\text{-}24hr}$ of 37.8 µM•hr and a C_{max} of 2.26 µM. Nonclinical comparisons for 3TC and TDF are based on information from the US Prescribing Information and the EU Marketing Authorization of the respective marketed agents.

Doravirine

DOR is an allosteric inhibitor of HIV-1 reverse transcriptase (RT) that binds to a hydrophobic pocket in the p66 subunit of the p66/p51 heterodimer near the polymerase active site. Five NNRTIs, efavirenz (EFV), delavirdine, nevirapine, etravirine (ETR), and rilpivirine (RPV) have been licensed for clinical use. However, the low barrier to resistance development and tolerability issues related to these NNRTIs can limit their clinical utility. Nonclinical data, including in vitro inhibition assays and safety assessment studies, indicate that DOR has the potential to fulfill a significant and growing medical need. Therefore, DOR has been brought forward as a potential next generation NNRTI.

DOR displayed potent antiviral activity when tested in vitro against wild-type (WT) HIV-1 and variants harboring prevalent mutations (such as K103N and Y181C mutants). DOR exhibited similar antiviral activities against 10 different HIV-1 subtype viruses. Based on the susceptibility of the mutants to NNRTIs, DOR displayed an overall resistance profile superior to that of EFV and comparable to those of ETR and RPV, when tested against a broad array of clinical NNRTI-associated mutant viruses. In the in vitro resistance selection studies, DOR displayed unique mutation development pathways where virus containing V106A substitution was the major mutant selected in the study, whereas E138K and L100I (followed by K103N) mutants represented the major viruses selected by RPV and EFV, respectively. In two-drug combination studies, no antagonistic effects on antiviral activity were observed within the concentration ranges examined between DOR and any of the 18 licensed HIV-1 antiviral agents tested [Sec. 2.6.2].

DOR, and its major circulating metabolite M9, were evaluated in vitro for potential for off-target activity. When tested in a panel of 110 human enzyme and receptor assays, no off-target activity was observed with M9 (IC_{50} >10 µM) and DOR only showed moderate affinity to the 5-HT2B receptor (IC_{50} of 2.5 µM) in a ligand binding assay, with no activity

detected in a follow-up functional assay in cells expressing 5-HT2B at DOR concentrations ≥100 µM. When DOR was tested against the human DNA polymerases α, ß, and γ, the IC_{50} values were determined to be greater than 100 µM. DOR was also tested for cytotoxicity in various cell types including proliferating (MT4, HL60, and SupT1), stationary, and activated cells (CD4, PBMC, macrophages, and monocytes), with no toxicity observed at maximal concentrations tested (CC_{50} >100 µM).

DOR was evaluated for its potential effects on cardiovascular, neurological, and respiratory function in several in vivo and in vitro safety pharmacology models, consistent with International Council on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) S7A and S7B guidances. There were no significant safety pharmacology findings identified for DOR. An overview of nonclinical pharmacology studies conducted with DOR is provided in [Sec. 2.6.3.1].

The absorption, distribution, metabolism and excretion (ADME) properties of DOR were extensively studied in preclinical species. An overview of nonclinical pharmacokinetic studies conducted with DOR is provided in [Sec. 2.6.5.1].

Studies were conducted in male CD-1 mice, male Wistar-Hannover rats, female Dutch-belted rabbits and male Beagle dogs to establish pharmacokinetic properties and routes of elimination. Evaluations of the performance of various nonclinical formulations were conducted in rats and dogs for the purpose of formulation selection for pivotal nonclinical safety studies. Interspecies comparison of metabolism, excretion, plasma protein binding and blood-to-plasma distribution ratios was also conducted. In vitro studies investigating the involvement of enzymes and transporters in the disposition of DOR were conducted using expression systems for major cytochrome P450 (CYP) enzymes and major drug transporters. DOR was also evaluated in vitro for its potential to elicit drug interactions via inhibition of major CYP enzymes, uridine glucuronosyltransferase (UGT) 1A1, and the transporters breast cancer resistance protein (BCRP), bile-salt extrusion protein (BSEP), P-glycoprotein (P-gp), organic anion transporting polypeptides (OATP) 1B1 and1B3, organic anion transporters (OAT) 1 and 3, organic cation transporter (OCT) 2, and multidrug and toxin extrusion transporters (MATE) 1 and 2K.

DOR was evaluated in a comprehensive nonclinical safety assessment program that included in vitro and in vivo genetic toxicity assays and repeat-dose oral tolerability and toxicity studies in mice up to 3 months duration, in rats up to 6 months duration, and in dogs up to 9 months duration to support oral administration to humans. Developmental and reproductive toxicology studies conducted with DOR included a male and female rat fertility study, embryo-fetal developmental toxicity studies in rats and rabbits, a pre- and post-natal toxicity study in rats, and juvenile toxicity studies in rats. To assess the carcinogenic potential of DOR, a six-month oral carcinogenicity study in rasH2 transgenic mice and a two-year oral carcinogenicity study in rats was conducted. In addition, a rat phototoxicity study, local tolerance bioassays, a one month oral combination toxicity study in rats and three month-oral rat toxicity studies evaluating potential drug substance and drug product impurities were completed. An overview of the toxicology program for DOR is provided in [Sec. 2.6.7.1].

Studies evaluating Long Acting Parenteral (LAP) micro-particulate suspensions of DOR administered in intramuscular and subcutaneous routes are included in this application to provide the collective nonclinical experience with DOR; however, results from these studies using non-relevant routes of administration for clinical oral dosing and utilizing exploratory feasibility LAP formulations do not impact the nonclinical risk assessment of the oral DOR program. DOR systemic exposures achieved in LAP studies were far lower than those achieved in the oral program.

All pivotal safety pharmacology and toxicity studies of DOR were conducted in accordance with the principles of Good Laboratory Practice (GLP) and were consistent with ICH guidances. These studies were conducted at Merck Research Laboratories, West Point, Pennsylvania, U.S.A.; Laboratoires Merck Sharp & Dohme-Chibret, Centre de Recherche, Riom, France, or [redacted], U.S.A. The U.S.A. and France are members of the Organisation for Economic Co-operation and Development (OECD) and therefore part of the OECD Mutual Acceptance of Data system. Compliance statements are included in all GLP study reports. All pivotal nonclinical studies were supported by toxicokinetic evaluation of DOR within the study or from studies conducted under the same conditions. Test article batches used in the nonclinical studies are provided in [Sec. 2.6.7.4].

The rat and dog were selected as the rodent and non-rodent species for DOR toxicity assessment based on in vitro and in vivo metabolic profiles and the demonstration of satisfactory exposure. In the pivotal GLP studies in rats, mice and rabbits, animals were orally administered DOR as a [redacted] formulation [consisting of [redacted]% DOR base form and [redacted]% of Hydroxypropylmethylcellulose-acetate succinate (HPMCAS), suspended in 0.5% methylcellulose with 5 mM hydrochloric acid in deionized water] or control article [consisting of HPMCAS at concentrations of up to 180 mg/mL in 0.5% methylcellulose with 5 mM hydrochloric acid in deionized water] once-daily at a dosing volume of 10 mL/kg. This enabled formulation of DOR was selected to maximize plasma exposures compared to other evaluated formulations. HPMCAS is an inert cellulose-based polymer extensively used in solid dispersion formulations clinically and nonclinically [Ref. 4.3: 04NSFN]. In all pivotal toxicity studies using [redacted] formulation, the HPMCAS polymer was present in control article at the same concentration as present in the highest tested dose level of DOR [redacted] formulation. The DOR high dose of 450 mg/kg/day was the maximum feasible oral dose as a [redacted] formulation under the conditions of the pivotal GLP studies in adult rats, mice and rabbits. In dogs, oral administration of jet-milled DOR suspended in 10% polysorbate 80 gave superior exposures in comparison to other tested formulations, with additional up to approximately two-fold DOR accumulation (AUC) occurring in dogs after repeat-dose administration attributed to a long half-life for DOR in dogs. DOR doses were orally administered to dogs on pivotal studies as suspensions of the base compound in 10% polysorbate 80 or vehicle at a dosing volume of 5 mL/kg. The high dose of the three- and nine-month dog studies was the limit dose, 1000 mg/kg/day, per ICH M3(R2). The M9 major human metabolite was assessed in all nonclinical animal species, including in rat and dog plasma from the chronic oral toxicity studies [Sec. 2.4.4].

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The specifications of the drug substance and product are presented in [Sec. 3.2.S.4.1] and [Sec. 3.2.P.5.1]. All impurities/degradation products were either assessed in toxicology studies or were below the ICH [ICH Q3A(R2); ICH Q3B(R2)] qualification threshold of 0.15%, and have been assessed for mutagenic potential according to ICH M7 guidance.

3TC and TDF

No nonclinical studies were conducted with 3TC and TDF alone, or in combination. For supporting nonclinical data for 3TC collected from ICH-compliant safety assessment studies, the Sponsor refers to EPIVIR US Prescribing Information [Ref. 4.3: 04P95N], the EPIVIR US New Drug Application (NDA 20-564) [Ref. 4.3: 03TKG7] and the EU Marketing Authorization (MA) for EPIVIR EU/1/96/015 [Ref. 4.3: 03RRT8] and scientific literature [Ref. 4.3: 03RL6N], [Ref. 4.3: 03W0QN], [Ref. 4.3: 03W0QQ], [Ref. 4.3: 03W0QP], [Ref. 4.3: 03X0YB], [Ref. 4.3: 03X24P]. For supporting nonclinical data for TDF collected from ICH-compliant safety assessment studies, the Sponsor refers to the VIREAD US Prescribing Information [Ref. 4.3: 04P95P], VIREAD US NDA 21-356 [Ref. 4.3: 0438FY] and the EU Marketing Authorization (MA) for VIREAD EU/1/01/200 [Ref. 4.3: 0438G4], and scientific literature [Ref. 4.3: 043B7S], [Ref. 4.3: 043B7Z].

3TC is a dideoxy analog of the nucleoside cytidine which, after phosphorylation, inhibits HIV-1 reverse transcriptase and causes premature DNA chain termination. 3TC is approved by the FDA, European Commission, and other health authorities for use in combination with other antiretroviral therapies for the treatment of HIV-1 infected individuals, with over 22 years of clinical experience. 3TC has good bioavailability in preclinical species. It is excreted in urine by glomerular filtration and active tubular secretion. Metabolism plays a minor role in the elimination of 3TC in rat but it is balanced with renal excretion in dog. Based on available clinical and preclinical data, 3TC is not likely to perpetrate drug interactions via CYP enzymes or transporters. The pharmacokinetics of 3TC may be affected by OCT inhibitors in kidney but this interaction is of little clinical significance. The recommended clinical dose of 3TC is 150 mg b.i.d, or 300 mg QD in adults and adolescents >16 years of age (>12 years of age in the EU). The dose in pediatric patients aged 3 months to 16 years of age (and 3 months to 12 years of age in the EU) is weight-based.

The nonclinical safety of 3TC has been evaluated to determine its genotoxicity, developmental and reproductive toxicity, juvenile toxicity, general toxicity profile following acute and repeated dose studies in rats and dogs of up to 12 months duration, and carcinogenic potential in rat and mouse carcinogenicity studies of 105 weeks duration. In animals treated with 3TC, reported changes at supratherapeutic exposures were restricted to reductions in red cell counts, occasionally associated with reductions in total lymphocyte counts, but with no effect on bone marrow cytology.

TDF is an acyclic nucleoside phosphonate diester analog of adenosine monophosphate that requires initial diester hydrolysis for conversion to tenofovir and subsequent phosphorylations by cellular enzymes to form tenofovir diphosphate. Tenofovir diphosphate inhibits the activity of HIV-1 reverse transcriptase and HBV reverse transcriptase by competing with the natural substrate deoxyadenosine 5'-triphosphate and, after incorporation

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into DNA, by DNA chain termination. TDF is approved by the FDA, European Commission, and other health authorities for use in combination with other antiretroviral therapies for the treatment of HIV-1 infection in adults and pediatric patients 2 years of age and older. TDF is readily converted to tenofovir presystemically in preclinical species and humans, so that tenofovir is the major species circulating in plasma. Tenofovir is primarily excreted via glomerular filtration with a small contribution from active tubular secretion. In vitro, tenofovir is a substrate of OAT1/3 and multidrug resistant protein 4 (MRP4). Available preclinical and clinical data indicate that TDF is unlikely to perpetrate or be a victim of drug interactions. The recommended clinical dose of TDF is 300 mg QD in adults and children weighing at least 35 kg (and aged 12 years or older, in the EU). The dose in children below 35 kg (and at least 2 years of age) is weight based.

The nonclinical safety of TDF has been evaluated to determine its genotoxicity, developmental and reproductive toxicity, juvenile toxicity, general toxicity profile following acute and repeated doses in dogs, rats, and monkeys, and carcinogenic potential in rat and mouse carcinogenicity studies of 105 weeks duration. TDF was administered in toxicology studies to rats and dogs up to 42 weeks, and in monkey studies up to 24 months. Target organs described in animals were kidney (observed in all species), bone (reduced bone mineral density and osteomalacia seen in rats and monkeys), and gastrointestinal tract (seen in rats only), and were well characterized.

DOR/3TC/TDF

In vitro antiviral studies evaluating two-drugs indicated no antagonistic effects are expected when combining DOR with licensed HIV-1 antiviral agents, including combinations of DOR with 3TC or TDF. Nonclinical pharmacology, pharmacokinetic and safety assessment studies evaluating the DOR/3TC/TDF FDC have not been performed. Both 3TC and TDF are marketed, well characterized compounds. An assessment of the nonclinical ADME profile of 3TC and TDF was conducted using data in the public domain and is provided in [Sec. 2.4.3] and [Sec. 2.6.4.1]. The existing knowledge of the individual components and clinical assessment of the pharmacokinetics of the components administered concomitantly [Sec. 2.7.2.3.1.2] and [Sec. 2.7.2.3.1.3] indicate no risk for pharmacokinetic drug interactions between the FDC components. Significant changes in the pharmacokinetics of the DOR/3TC/TDF components are anticipated for DOR when DOR/3TC/TDF is co-administered with CYP3A4 inducers or inhibitors [Sec. 2.7.2.1.6].

No significant toxicologic concern, similar target organ toxicity, or potential for synergistic toxicity was identified concerning DOR, 3TC and TDF co-administration. Therefore, no combination toxicity studies were conducted for the DOR/3TC/TDF FDC, in accordance with regulatory guidance (ICH M3 [R2]: *Nonclinical Safety Studies for the Conduct of Human Clinical Trials and Marketing Authorization for Pharmaceuticals; Human Immunodeficiency Virus-1 Infection: Developing Antiretroviral Drugs for Treatment Guidance for Industry, Center for Drug Evaluation and Research, November 2015, Revision 1*), which states for combinations involving marketed entities, nonclinical combination toxicity studies would generally not be recommended to support clinical studies

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or registration unless data from nonclinical studies of an investigational agent suggest a potential for serious synergistic toxicity with an approved therapeutic drug combination.

Overall, the nonclinical profile supports the safe use of DOR, both alone and in combination with 3TC and TDF, at the proposed clinical doses and regimens for the treatment of HIV-1 infection.

The nonclinical studies conducted with DOR are detailed below. As reviewed above, no nonclinical studies were conducted with 3TC, TDF, or with the DOR/3TC/TDF combination, with the exception of the evaluation of DOR antiviral activity in two-drug combination studies.

2 PHARMACOLOGY

2.1 Primary Pharmacodynamics

2.1.1 Primary Pharmacodynamics In Vitro

DOR is a potent HIV-1 NNRTI. In biochemical assays, DOR displays IC_{50}s <15 nM in the inhibition of the ribonucleic acid (RNA)-dependent deoxyribonucleic acid (DNA) polymerization catalyzed by HIV-1 RT variants (WT, K103N, and Y181C). In cell-based assays, DOR showed EC_{50}s of 12, 21, 31, 55 and 33 nM against the WT virus, K103N, Y181C, G190A, and K103N/Y181C mutants, respectively, in the presence of 100% human serum. Based on these results, DOR is 4.7-, 2.7-, 5.5-, 1.6- and 9.6-fold more potent than RPV and 2.5-, 56-, 2.9-, 16- and 95-fold more potent than EFV, respectively against this panel of viruses.

The resistance profile of DOR is superior to EFV and similar to ETR and RPV based on susceptibility of a broad set of NNRTI-associated mutant viruses. In the test with 96 NNRTI-associated mutant viruses, more than 60% of viruses exhibited greater than 10-fold resistance to EFV, whereas more than 60% of viruses had a ≤ 5 fold-change when tested with DOR, ETR, and RPV. Most mutants that confer > 10-fold resistance to DOR contain more than two mutations, with the exception of the Y188L mutant, which requires two base changes. Although viruses containing Y188L mutation exhibited >100-fold resistance to DOR, this mutation was not selected by DOR in the in vitro resistance selection study.

DOR exhibited similar antiviral activities (in the range from 1.2 nM to 10 nM) against a panel of 93 wild-type viruses comprised of several isolates from each of 10 different HIV-1 subtypes.

Resistance selection of subtype B virus with DOR was conducted under both high and low multiplicity-of-infection (MOI) conditions in 10% fetal bovine serum. Viruses containing RT with V106A, V108I, and L234I substitutions were most often selected under both conditions. The V106A, V108I, and L234I mutant viruses conferred approximately 12-, 4-, and 11-fold resistance to DOR, respectively. Additional mutations such as F227 variants emerged at elevated concentrations of DOR under high and low MOI conditions. Emergence of the additional mutations further reduces the susceptibility of the mutant viruses to DOR.

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For example, the V106A/F227L double mutant conferred greater than 500-fold resistance to DOR. In the resistance selection with subtype A, B, and C viruses using MT4-GFP cells, viruses containing substitutions at the position of V106, V108, F227 and L234 also accounted for the majority of mutants identified in the breakthrough viruses under low MOI conditions. In the resistance selection with subtypes A and C viruses, the V106M mutant was also selected in addition to the V106A mutant. Mutations selected under high MOI conditions with subtype B virus using MT4-GFP cells were consistent with the mutations selected under low MOI conditions.

In two-drug combination studies DOR exhibited no antagonistic effects on the antiviral activity with of any of the 18 licensed antiretroviral drugs from different mechanistic classes within the concentration ranges examined, including combinations of DOR with 3TC or TDF.

2.1.2 Primary Pharmacodynamics In Vivo

Consistent with the FDA *Guidance for Industry Human Immunodeficiency Virus-1 Infection: Developing Antiretroviral Drugs for Treatment, November 2015, Revision 1*, demonstration of anti-HIV activity in an animal model is not required; therefore no in vivo primary pharmacodynamic studies were conducted.

2.2 Secondary Pharmacodynamics

2.2.1 Secondary Pharmacodynamics In Vitro

In an in vitro screening panel [ ] evaluating over 110 receptors and enzymes, binding only to the 5-HT2B receptor was observed, while in a subsequent 5-HT2B receptor functional assay, no agonist or antagonist activity was observed at concentrations up to 100 µM. The major circulating metabolite in human plasma (M9) was also evaluated in the in vitro screening panel and there was no significant activity at concentrations up to 10 µM. At 10 µM, there is an approximately 18-fold margin to the DOR clinical C_{max} (based on the DOR clinical C_{max} of 2.26 µM.hr and unbound human protein binding of 0.24); see [Sec. 2.4.3.3.3].

2.2.2 Secondary Pharmacodynamics In Vivo

Specific studies evaluating in vivo secondary pharmacodynamics have not been conducted.

2.3 Safety Pharmacology

Safety pharmacology studies consistent with ICH S7A and S7B guidances were performed in both in vitro and in vivo test systems to assess the potential cardiovascular, nervous system, and respiratory effects of DOR.

DOR inhibited hERG current with an IC_{50} value of 88 µM and an IC_{20} value of 19 µM, approximately 162-fold and 35-fold respectively the unbound DOR levels at the human C_{max} exposure at the therapeutic dose of 100 mg (TT # -4716) [Sec. 2.6.3.4]. There were no

test-article related effects on cardiovascular parameters in anesthetized, vagotomized dogs up to 10 mg/kg of cumulative dose (plasma concentrations up to 14 µM, approximately 6-fold DOR levels at the human C_{max} exposure) (TT #[redacted]-5006) [Sec. 2.6.3.4]. In conscious dogs (TT #[redacted]-5606) [Sec. 2.6.3.4], the no-observed-effect level (NOEL) for cardiovascular and respiratory function was ≥10 mg/kg, highest dose tested (expected C_{max} ≥ 8.24 µM, approximately 3.5-fold DOR levels at the human C_{max} exposure) based on C_{max} attained at 10 mg/kg in an oral dog toxicity study (TT #[redacted]-6030) [Sec. 2.6.7.6]. Repeat dose toxicity studies in dogs of up to 9 month duration were conducted at DOR doses up to 1000 mg/kg/day [Sec. 2.6.7.7E], [Sec. 2.6.7.7F] and were without findings upon ECG assessment (C_{max} of 32.7 µM, approximately 14-fold DOR levels at the human C_{max} exposure). There were no effects on neurological function in mice (TT #[redacted]-5262) and rats (TT #[redacted]-6029) at single doses up to 750 and 450 mg/kg (C_{max} not determined in mice as blood for toxicokinetics was not collected; C_{max} of 15.2 µM in rats at 450 mg/kg, approximately 6.5-fold DOR levels at the human C_{max} exposure), respectively [Sec. 2.6.3.4].

Overall, there were no DOR-related effects of concern on cardiovascular, nervous system, and respiratory functions observed in well characterized safety pharmacology models [Sec. 2.6.3.4].

2.4 Pharmacodynamic Drug Interactions

Based on the absence of changes of concern in the DOR safety pharmacology studies, the previously characterized pharmacology profiles of 3TC and TDF, and in accordance with ICH M3(R2) guidance which states that combination safety pharmacology studies generally are not recommended to support marketing if the individual agents have been tested according to current standards, no safety pharmacology studies were conducted where DOR, 3TC and TDF were co-administered. In two-drug in vitro antiviral combination studies, DOR exhibited no antagonistic effects on the antiviral activity of any of the 18 licensed antiretroviral drugs from different mechanistic classes within the concentration ranges examined, including combinations of DOR with 3TC or TDF.

3 PHARMACOKINETICS

The non-clinical ADME studies are listed in [Sec. 2.6.5.1].

3.1 Analytical Methods and Validation Reports

Bioanalytical methods using liquid chromatography (LC) coupled with tandem mass spectrometry (MS/MS) were used for the DOR analysis in plasma, urine, muscle, and milk samples collected during preclinical studies [Sec. 2.6.5]. Sample analysis for GLP studies was conducted in compliance with GLP. The bioanalytical methods used in support of GLP studies for the analysis of mouse, rat and dog plasma, and rat milk were validated in accordance with regulatory guidances in effect at the time the studies were conducted. All assays had good accuracy and precision and attained a dynamic range from 12 to 4,800 ng/mL (0.028 – 11.3 µM). In mouse plasma, an LC-MS/MS assay with a dynamic range of 4 to 4800 ng/mL (0.0094 - 11.3 µM) was also validated. In some instances, a

bioanalytical method, validated according to laboratory procedures for exploratory work, was used according to the study purpose to quantify DOR in rabbit plasma, rat urine and rat muscle. These assays had a concentration range of 12 to 4,900 ng/mL in rabbit plasma, 25 (0.0564 µM) to 10,000 ng/mL (23.5 µM) in rat urine, and 400 (0.940 µM) to 200,000 (470 µM) ng/mL in rat muscle [Sec. 2.6.5.2].

3.2 Absorption

There were no significant differences in the pharmacokinetics of DOR across preclinical species, with low plasma clearance (CLp), moderate volume of distribution at steady state (Vdss) and moderate oral bioavailability in CD-1 mice, Wistar Hannover (WH) rats, Dutch-belted rabbits and Beagle dogs. Following intravenous (IV) administration, the CL_p was 6.05, 2.6, 4.0 and 0.44 mL/min/kg and the volume of distribution at steady state (Vdss) was 0.99, 1.4, 2.67 and 0.9 L/kg in mice, rats, rabbits and dogs, respectively. Half-life values ($t_{½}$) were 2.7, 6.4, 9, and 21.7 hr in mice, rats, rabbits, and dogs, respectively. DOR, administered in an enabled formulation ([redacted] drug over a HPMCAS polymer at a drug load of [redacted]%), had oral bioavailability of 39 to 47% in preclinical species. DOR is a BCS Class II compound [Sec. 2.7.1.3] with good permeability but poor solubility. This, in conjunction with its low clearance, suggested that bioavailability is not limited by permeability or first-pass effect. Thus, solubility-limited absorption is considered the major determinant of bioavailability [Sec. 2.6.4.3].

Studies conducted in rats to evaluate the performance of different formulations at doses up to 100 mg/kg indicated less than dose-proportional exposures with several formulations, including suspensions of [redacted] and [redacted] drug. Better exposures were obtained with the [redacted] formulation (consisting of [redacted]% DOR and [redacted]% HPMCAS) and this was selected for use on GLP toxicology studies. A summary of the exposures in rats dosed at 100 mg/kg with various formulations of DOR is shown in [Table 2.4: 1].

Given the demonstrated low aqueous solubility and evidence of poor performance of certain formulations when evaluated in rats related to drug form stability, assessment in dog focused on evaluating the impact of [redacted] state and particle size on DOR exposures following oral administration at high doses. A suspension of jet-milled drug in 10% polysorbate 80 and a nanosuspension were assessed in male dogs at the respective maximal feasible doses. The suspension of jet-milled drug demonstrated better exposures and was selected for use in the GLP toxicology studies [Table 2.4: 1].

Table 2.4: 1 Comparison of Pharmacokinetic Parameters for DOR Administered Orally to Male Wistar-Hannover Rats and Beagle Dogs

Species, (Gender)	Form	Vehicle	Dose (mg/kg)	Cmax (µM)	AUC_{0-24h} (µM•hr)
Rat (Male)	[redacted]	10% Polysorbate 80	100	2.7	30.4
	Ball Milled	10% Polysorbate 80	100	9.9	102
	[redacted]	Emulsifying lipids[a]	100	4.9	74.1
	Nanosuspension	HPC-dextrose in water[b]	100	4.5	60.0
	[redacted][c]	0.5% MC[d]	100	8.83	136
Dog (Male)	Jet Milled	10% Polysorbate 80	1000	13.6	283
	Nanosuspension	HPC-DOSS in water[e]	450	7.3	147

Data represent mean values of n=2.
[a] Emulsifying lipids: Labrasol (79%) Capryol 90 (13%) and Labrafil M 1944 CS (8%)
[b] Hydroxypropylcellulose-SL (0.25% w:v) and dextrose (5% w:v) in water
[c] [redacted] DOR, at a [redacted]% drug load with HPMCAS
[d] MC = methylcellulose acidified with 10 mM citrate
[e] Hydroxypropylcellulose-SL (10% w:v) and dioctyl sulfosuccinate sodium salt (5% w:v) in water

3.3 Distribution

3.3.1 Tissue Distribution in Rat

Oral administration of a solution of [^{14}C]-labeled DOR in 10% polysorbate 80 resulted in widespread distribution of the radioactivity throughout a wide range of tissues in both WH albino rats and Long Evans (LE) partially pigmented rats. The similarity between pigmented tissue (e.g. eye uvea and skin) concentrations in WH and LE rats, suggested no specific association of DOR or its metabolites with melanin. The highest concentrations of radioactivity in blood and most tissues were observed at 2 hr postdose in both strains. The highest overall concentrations were observed in the contents of the alimentary canal, bile, and urine, consistent with movement through the alimentary canal and the major routes of elimination. Most tissues, except central nervous system tissues, eye lens, and bone, had concentrations that were greater than the concentration in blood throughout the study. Elimination of radioactivity was complete in most tissues by 48 hr (WH rats) or 168 hr (LE rats) postdose [Sec. 2.6.5.4].

3.3.2 Placental Transfer in Rat and Rabbit

Placental transfer of DOR was investigated in pregnant WH rats following daily oral administration of DOR at 5 or 450 mg/kg on Gestation Day (GD) 6 through 20 and in pregnant Dutch Belted rabbits dosed with DOR at 300 mg/kg once a day on GD 7 through 20. Concentrations of DOR in maternal and fetal plasma were measured on GD 20 at 2 and 24 hr for rats and 4 and 24 hr for rabbits. The DOR fetal to maternal plasma concentration ratio ranged from 0.48 to 0.52 in rats and from 0.36 to 0.40 in rabbits. These data demonstrated that DOR was able to cross the placental barrier in both species. All animals survived until scheduled study termination [Sec. 2.6.5.4].

3.3.3 In Vitro Plasma Protein Binding and Blood-to-Plasma Partitioning

The binding of DOR and its major circulating metabolite, M9, to proteins in plasma from nonclinical species and humans was determined using equilibrium dialysis [Sec. 2.6.5.6].

The plasma protein binding of DOR was moderate in all species tested and did not change significantly within a concentration range (0.1 to 5 µM) that reflects clinical exposures at the therapeutic dose of 100 mg. At 5 µM, the unbound fraction was 0.262, 0.272, 0.279, 0.192 and 0.251 in mouse, rat, rabbit, dog and human plasma, respectively. The mean unbound fraction in human plasma over the concentration range of 0.1 to 5 µM was 0.24.

The plasma protein binding of M9 was also moderate and did not vary significantly within the concentration range tested (0.1 to 1 µM). In dog, the extent of binding was comparable to DOR binding. In rat, binding was within two-fold of that for DOR; however, in human, the unbound fraction of M9 was three-fold lower than that of DOR. In addition, the unbound fraction of M9 in rat and dog plasma was respectively 2.2 and 2.8-fold higher than that observed in human plasma (0.09 at 1 µM).

The blood-to-plasma concentration ratio was independent of the DOR concentration (0.1 to 10 µM) and it was approximately 1 across species, except in the mouse, for which the ratio varied from 0.65 to 0.71. These results indicate no preferential distribution of DOR into blood cells. The blood-to-plasma partitioning ratio for metabolite M9 in rat, dog and humans was in general lower across species compared to DOR blood-to-plasma partitioning, with values ranging from 0.7 to 0.9 in rat, dog and human [Sec. 2.6.5.4].

3.4 Metabolism

3.4.1 In Vivo Metabolism in CD-1 Mice, Wistar-Hannover Rats, Dutch-belted Rabbits, Beagle Dogs, and Humans

The metabolism of DOR was investigated in intact male CD-1 mice, intact female Dutch-belted rabbits, and bile duct-cannulated (BDC) male WH rats and male Beagle dogs orally administered 5 mg/kg radiolabeled DOR (either [^{3}H] or [^{14}C]). Results from these as well as the in vitro studies supported the selection of the rat and the dog as nonclinical toxicology species.

The metabolism of DOR in humans was evaluated in healthy subjects (P008) [Sec. 2.7.2.2.1.3].

The proposed metabolic pathways of DOR are shown in [Figure 2.4: 1].

Elimination of DOR in mice, rabbits and humans was mediated primarily via oxidative metabolism, with renal excretion of unchanged DOR as a secondary pathway of elimination. In dogs, elimination via metabolism was balanced with renal excretion of unchanged DOR. Metabolism was primarily oxidative, via formation of M9. Conjugation of DOR was minor relative to oxidation.

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In mice, the most abundant metabolite identified in urine and feces was the oxidative metabolite M9. Additional metabolites eliminated in urine were products of subsequent oxidations (M12) or combinations of oxidation and glucuronidation (M20). Approximately 23.7% of the administered dose was recovered in feces as unchanged, presumably unabsorbed DOR, within 24 hr. Excretion of unchanged DOR in urine was minor (2.6%). In plasma, M9 was identified as the major circulating metabolite and was present at levels comparable to DOR [Sec. 2.6.5.5].

In BDC rats, metabolism was extensive and the majority of the metabolites in excreta corresponded to secondary oxidation products or conjugates of oxidative metabolites. The major metabolite in rat, M7, was identified as a glucuronide of M9. It accounted for 19.5% of the administered dose and was excreted predominantly in bile. Other metabolites excreted in bile and/or urine included M2 (glucuronide of an oxidative metabolite), M8 (de-methylated product) and various minor primary and secondary oxidative products and conjugates (M1, M3, M4, M6, M10). Although M9 was a relatively minor metabolite in rats, formation of M9 is a major metabolic pathway and further glucuronidation of M9 to form M7, rather than direct excretion, is predominant in this species [Sec. 2.6.5.5.1]. Excretion of unchanged DOR in urine was an alternate mechanism of elimination (18.5% of the administered dose). Biliary excretion was a minor pathway of elimination (4.7% of the administered dose). Absorption of the radioactive dose was high. Only 13% of the dose was recovered in feces and no profiling was conducted in this matrix. In plasma, unchanged parent was the major circulating drug-related component. Additional metabolites (M1, M8, M11, M12 and M13) were observed only in trace amounts [Sec. 2.6.5.5].

Studies in rabbits indicated that the majority of the radioactivity (60.7%) was recovered as unchanged DOR in feces, presumably as unabsorbed drug, while 28.5% was recovered in the urine, primarily as M9 and DOR (19.5 and 6.9% of the dose, respectively). Additional metabolites eliminated in urine included glucuronides of DOR (M6, M6a, M6b), and an *N*-acetyl-cysteine conjugate of DOR (M15). Therefore, metabolism appears to be the major route of elimination, with oxidation, predominantly via formation of M9, as the predominant metabolic pathway [Figure 2.4: 1]. The main circulating species in plasma was DOR, and several metabolites, including M6, M9, M11 and M17, were detected in trace amounts [Sec. 2.6.5.5].

In BDC dogs, recovery of the radioactive dose was low, and with this in consideration, elimination of DOR was balanced between metabolism (predominantly oxidative) and renal excretion of unchanged drug. The metabolite M9 was present in significant amounts in both urine and feces. Other significant metabolites, recovered predominantly in bile, were a glucose conjugate of an oxidative metabolite (M14) and M15. Unchanged DOR was recovered in feces, presumably as unabsorbed dose (24.5% of the dose), and in urine (approximately 40% of the radioactivity in bile and urine). DOR was the major circulating species identified in plasma and some metabolites were observed in trace amounts (M15, M16 and M17) [Sec. 2.6.5.5].

The metabolism and excretion of DOR in humans were studied following administration of 350 mg [^{14}C] DOR sodium pentahydrate salt (approximately 200 µCi, equivalent to 277 mg

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free acid DOR) to six healthy male subjects. Recovery was complete (approximately 100%) within 8 days. Pharmacokinetic data indicated a bioavailability of approximately 16% for the fit for purpose radiolabeled formulation used in this trial, which was significantly lower than the bioavailability of the final market image (FMI) (approximately 64%) but resulted in comparable systemic exposures to those observed with the FMI at the clinical dose of 100 mg. In agreement with the systemic bioavailability, DOR in feces accounted for 84.1% of the administered dose, indicating that the majority of the radioactivity in feces corresponded to unabsorbed dose. Consequently, the relative amounts of metabolites in excreta and DOR in urine, were estimated based on the absorbed, rather than the administered dose. The predominant mechanism of elimination of the absorbed dose was metabolism, primarily via oxidation to M9, which was the major metabolite excreted in urine and feces. Amounts of major metabolites relative to the total and the absorbed dose are listed in [Table 2.4: 2]. Renal excretion of unchanged DOR was low. Based on the data obtained from BDC rats and dogs, and the fact that the calculated systemic bioavailability in humans is consistent with the amount of DOR recovered in feces, biliary excretion of unchanged drug is not expected to be a major route of elimination for DOR in humans [Sec. 2.6.4.5.5].

Table 2.4: 2 Relative Amounts of DOR and its Major Metabolites in Excreta from Humans After Oral Administration of 350 mg [^{14}C]DOR Sodium Pentahydrate

Component	Relative Amount as % of Total Dose		Relative Amount as % of Absorbed Dose [a]
	Feces	Urine	
DOR	84.1	2.2	12.9[b]
M8	Trace	0.2	1.2
M9	2.7	6.7	55.3
M10	Trace	0.1	0.6
M18	Trace	0.2	1.2

[a] Estimated as 17% based on systemic bioavailability of 16% and anticipated low first-pass effect.
[b] Estimated using radioactivity in urine only. The amount in feces was considered to be unabsorbed dose
ND = Not determined

[Sec. 2.6.4.5.5]

DOR was the main circulating component in plasma, accounting for 75% of the total drug-related material. The major metabolite in plasma was the oxidative product M9 (12.9%). Various metabolites were identified in plasma in trace amounts. All of the metabolites detected in the excreta and in circulation have also been observed in mice, rats, rabbits or dogs [Sec. 2.6.5.5]. In line with these results, analysis of plasma samples from subjects that had received multiple 240 mg daily doses of the [redacted] drug in oral compressed tablets (P001) [2.7.2.2.1.1], indicated that M9 was the most abundant metabolite, present at levels that were within 2-fold of levels observed in the above study (approximately 6.7% of the

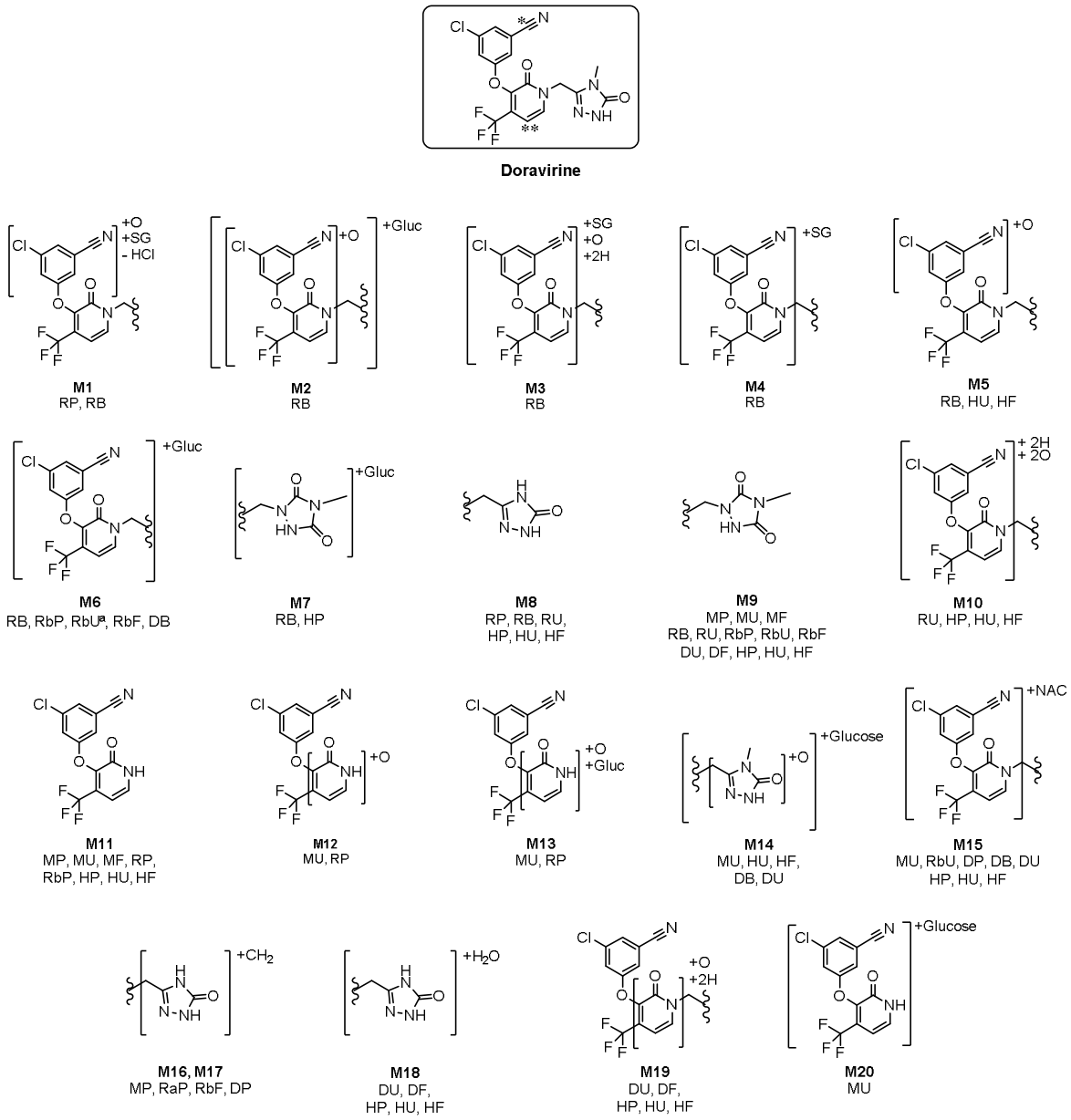
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total parent-related components while DOR represented 79.4% of the total parent-related component in plasma) [Sec. 2.6.4.5.5].

Additional studies were conducted to determine exposures of M9 in the chronic rat and dog toxicity studies. Plasma obtained from the chronic toxicity studies was analyzed and compared against plasma from human subjects administered repeated daily doses of 240 mg DOR. Results from these studies indicated that M9 levels in plasma from rats and dogs administered chronic daily oral doses of 450 and 1000 mg/kg DOR respectively [the no-observed-adverse-effect level (NOAEL) doses] were within two-fold (0.52 and 0.51 for rat and dog, respectively) of DOR levels in plasma from subjects that received multiple daily doses of 240 mg DOR. In addition, and as indicated earlier, the unbound fraction of M9 is approximately 2.2 and 2.8-fold higher in rat and dog plasma, respectively, relative to that in human plasma. Therefore, unbound concentrations of M9 in rat and dog plasma are approximately similar or slightly above to those expected in humans at the clinical dose of 100 mg. Thus, M9 is considered to be adequately tested in chronic safety studies [Sec. 2.6.4.5.6].

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Figure 2.4: 1 Proposed Metabolites of [^{14}C]DOR in Mice, Rats, Rabbits, Dogs and Humans



* Indicates the position of ^{14}C and ** indicates the position of ^{3}H.

a Two metabolites identified in rabbit urine as doravirine glucuronides appeared to be different from M6 and were designated as M6a and M6b to differentiate from M6 observed in other species.

MP, RP, RbP, DP, HP = mouse, rat, rabbit, dog, human plasma; RB, DB = rat, dog bile; MU, RU, RbU, DU, HU = mouse, rat, rabbit, dog, human urine; MF, RF, RbF, DF, HF = mouse, rat, rabbit, dog, human feces; Gluc = glucuronide; NAC = *N*-acetyl-cysteine; SG = glutathione.

[Sec. 2.6.5.5.5]

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3.4.2 In Vitro Metabolism in Sprague-Dawley Rat, Beagle Dog and Human

In vitro metabolic turnover of DOR (10 μM) in NADPH supplemented rat, dog and human liver microsomes was very low. M9 was generated in all three species. Several other metabolites (M5, M8, and M10) were observed in rat liver microsomes while only M10 was observed in human liver microsomes, but no other metabolites were observed in dog liver microsomes. DOR was not a substrate for glucuronidation in human liver microsomes supplemented with UDPGA as the cofactor, and no metabolites were detected in incubations with hepatocytes from rats, dogs or humans [Sec. 2.6.5.5].

M9 was not metabolized further when incubated with dog or human hepatocytes but was glucuronidated to M7 in rat hepatocytes. These results are consistent with in vivo data which indicated that M9 is extensively metabolized in rats to M7, the main metabolite in rat excreta, while in dogs and humans, M9 is excreted, predominantly in urine, without significant further metabolism [Sec. 2.6.5.5].

3.5 Excretion

3.5.1 Excretion in Mouse, Rat, Rabbit and Dog

Excretion studies were conducted with [^{3}H] or [^{14}C]DOR administered orally to intact male CD-1 mice, male BDC WH rats, female intact Dutch-belted Rabbits and male BDC Beagle dogs [Table 2.4: 3].

Following administration of [^{14}C]DOR to intact mice, approximately 70.1 % of the radioactive dose was recovered over a 72-hr period. Excretion was balanced between urine and feces. In BDC rats, 93.4% of the dose of [^{3}H]DOR was recovered in a 72-hr period. The majority of the radioactivity was recovered within the first 24 hr, predominantly in bile. In rabbits, approximately 89.2% of the radioactivity was recovered over a 72-hr period, predominantly in feces. Following administration of [^{3}H]DOR to BDC dogs, the average total radioactivity recovered in the excreta over a 72-hr collection period represented 73.6% of the dose and was balanced between bile, urine and feces.

Table 2.4: 3 Excretion of DOR in Preclinical Species

Species	Dose Recovery (%)			
	Bile	Urine[a]	Feces	Total
CD-1 mice[b]	N/A	32.7	35.3	70.1
BDC Wistar-Hannover Rats[c]	56.7	22.9	13.0	93.4
Dutch-Belted Rabbits[b]	N/A	26.4	60.7	89.2
BDC Beagle Dogs[b]	14.3	29.7	29.3	73.6

All animal studies were conducted in males, except for studies in rabbits, which were conducted in females.
Values represent averages of measurements from 3 (rats, rabbits) or 2 (dogs) animals.
BDC = Bile duct-cannulated

N/A: Not available. Samples not collected in the study
[a] Includes amounts recovered from cage rinses.
[b] Administered as a solution of 5 mg/kg [^{14}C] or [^{3}H]DOR in 10% polysorbate 80.
[c] Administered as a solution of 5 mg/kg [^{3}H]DOR in PEG-400.

3.5.2 Excretion in Milk of Rats

The excretion of DOR into the milk of lactating rats was investigated by measuring concentrations in maternal plasma and milk on Lactational Day (LD) 14 following daily oral administration of DOR at 5 or 450 mg/kg/day from Gestational Day (GD) 6 to LD 14. Concentrations of DOR in milk were about 1.47 and 1.32 fold higher than the maternal plasma concentrations, for the 5 and 450 mg/kg/day doses, respectively. Thus, the results demonstrated excretion of circulating DOR into the milk of lactating rats at 2 hr post-dose [Sec. 2.6.5.6.2].

3.6 Pharmacokinetic Drug Interactions

3.6.1 Drug Metabolism Enzymes Involved in the Elimination of DOR

The metabolism of DOR was evaluated in incubation with recombinant human CYPs 1A1, 1A2, 1B1, 2A6, 2B6, 2C8, 2C9*1, 2C9*2, 2C9*3, 2C18, 2C19, 2D6*1, 2E1, 2J2, 3A4, 3A5, and 3A7. Only CYPs 3A4 and 3A5 carried out the oxidation of DOR. Additional studies demonstrated near complete inhibition of oxidative metabolism in human liver microsomes in the presence of an anti-CYP3A inhibitory antibody. Thus, it was concluded that the oxidative metabolism of DOR was mainly catalyzed by CYP3A4 and CYP3A5 [Sec. 2.6.5.7.1].

The intrinsic clearance (CLint) for the metabolism of DOR by recombinant (r)CYP3A4 and rCYP3A5 was determined. Results indicated an approximate 20-fold higher catalytic efficiency for CYP3A4 (0.110-0.142 µL/min/pmol CYP) relative to CYP3A5 (0.0048-0.0049 µL/min/pmol CYP). This finding, added to the predominant expression of CYP3A4 in intestine and liver suggests that although both CYP3A4 and 3A5 catalyze the oxidation of DOR, CYP3A4 is the major enzyme responsible for the elimination of DOR in humans [Sec. 2.6.5.7.2].

3.6.2 Transporters Involved in Disposition of DOR

DOR displayed high permeability in LLC-PK1 cells, with a mean apparent permeability coefficient (P_{app}) of 25 x 10^{-6} cm/sec. Thus, transporters are not anticipated to have a significant involvement in the absorption and disposition of DOR [Sec. 2.6.5.7.4].

- *Efflux of DOR by BCRP and P-gp*

The ratio of basolateral-to-apical/apical-to-basolateral (B-A/A-B) uptake for DOR was 1.1 in control cells and 1.9 in BCRP-expressing cells. The B-A/A-B ratio in the BCRP-expressing cells was not decreased in the presence of a BCRP inhibitor. These results indicated that DOR is not a substrate of BCRP [Sec. 2.6.5.7.3].

In LLC-PK1 cells transfected with human Multidrug Resistance Protein 1 (MDR1, P-gp), a ratio of 4.2 for B-A/A-B uptake in P-gp transfected cells indicated that DOR is a substrate of P-gp. However, drug interactions with P-gp inhibitors are not anticipated to be significant due to the good passive permeability of DOR [Sec. 2.6.5.7.4].

- *Uptake by OATP1B1 and OATP1B3*

DOR was evaluated as a substrate of OATP1B1 and OATP1B3 in MDCKII cells stably expressing OATP1B1 or OATP1B3. Rapid, time-dependent uptake of DOR into control cells was observed, likely as a result of good passive permeability. A small increase in uptake into OATP1B1-, but not into OATP1B3-expressing cells, relative to control cells, was observed. This uptake was not inhibited by an OATP1B1 and 1B3 inhibitor. Similar studies conducted in HEK-293 cells demonstrated that uptake into OATP1B1 or OATP1B3 expressing cells was similar to that in control cells, indicating that DOR is not a substrate of either transporter [Sec. 2.6.5.7.5] [Sec 2.6.5.7.6].

Follow-up uptake studies in cryopreserved human hepatocytes demonstrated time-dependent uptake of DOR that was partially inhibited by the addition of an inhibitor cocktail (10 µM cyclosporin A, 10 µM rifamycin SV, 100 µM rifampin and 50 µM quinidine) designed to fully inhibit OATP1B1, OATP1B3, NTCP and OCT1. In the same studies, the cocktail inhibited the uptake of probe substrates of these transporters. As observed in experiments with transfected cells, good passive permeability appeared to be the major driver for the uptake of DOR in hepatocytes [Sec. 2.6.5.7.7].

3.6.3 Evaluation of DOR as an Inhibitor of Human Liver Microsomal Cytochromes P450 and UGT1A1

DOR was a weak inhibitor or did not inhibit the activities of CYPs 1A2, 2B6, 2C8, 2C9, 2C19, 2D6, 3A4 and UGT1A1 in human liver microsomes at concentrations up to 100 µM. Furthermore, DOR did not cause time-dependent inhibition of CYP3A4 activity at up to 50 µM. These results indicated that DOR is not likely to cause drug interactions via inhibition of major CYPs or UGT1A1 at the concentrations associated with the clinical dose (unbound C_{max} < 1 µM) [Sec. 2.6.5.7.8].

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3.6.4 Evaluation of DOR as an Inhibitor of Drug Transporters

BCRP, P-gp and BSEP

DOR's therapeutic concentrations are anticipated to be below 4 µM, with a corresponding unbound concentration below 1 µM. The in vitro IC_{50} value for BCRP was 51 µM, more than 50-fold above DOR unbound concentrations in plasma. Therefore, systemic drug interactions via this transporter are unlikely. However, DOR could inhibit the intestinal efflux of substrates of this transporter and increase their exposure. The theoretical calculations of gut lumen concentrations of DOR (400 µg/mL or 940 µM) at the therapeutic dose exceed the in vitro IC_{50}; however, due to the limited solubility of DOR, concentrations are not anticipated to exceed 300 µM based on the DOR aqueous solubility of [redacted] µg/mL ([redacted] µM). In clinical studies, DOR co-administration resulted in a 30% increase in the exposure of the BCRP substrate dolutegravir. This effect, however, was observed at a dose of 200 mg and was of no clinical significance [Sec. 2.7.2.2.3.2.3].

DOR did not inhibit P-gp or BSEP at concentrations of up to 100 and 50 µM, respectively, indicating that DOR is unlikely to mediate systemic drug interactions by inhibiting these transporters [Sec. 2.6.5.7.9.3]. Although inhibition of P-gp in the gut was not adequately characterized in vitro due to limited solubility of DOR in the assay media, its clinical significance may be limited due to limited solubility of DOR in the gastrointestinal fluid. As described above, theoretical concentrations are unlikely to be reached.

OATP1B1 and OATP1B3

DOR inhibited OATP1B1 and 1B3 in stably transfected MDCK-II cells, with IC_{50} values of 39 and 31 µM, respectively. Based on the approximate bioavailability of 64% for the final market image (FMI), a mean unbound C_{max} of 0.7 µM or lower and an unbound hepatic inlet concentration of approximately 4 µM, DOR is unlikely to inhibit OATP1B1 or 1B3 in vivo and has a low risk to perpetrate drug interactions via these transporters [Sec. 2.6.5.7.9.4] [Sec. 2.6.5.7.9.5].

OAT1, OAT3 and OCT2

DOR inhibited OAT1, OAT3 and OCT2 in vitro with IC_{50} values of >75, 16 and 67 µM, respectively. Based on mean unbound plasma concentrations of 0.7 µM or lower for DOR at the therapeutic dose, systemic drug interactions via these transporters are unlikely [Sec. 2.6.5.7.9.6] to [Sec. 2.6.5.7.9.8].

MATE1 and MATE2K

DOR inhibited MATE1 and MATE2K-mediated efflux by 28% and 39% respectively, at 50 µM, the highest concentration tested in these experiments. This weak inhibition, added to an unbound C_{max} of 0.7 µM or lower at the therapeutic dose, indicates that the risk for interaction of DOR with substrates of these transporters is very low [Sec. 2.6.5.7.9.9] [Sec. 2.6.5.7.9.10].

3.6.5 Evaluation of DOR as an Inducer of Cytochrome P450 1A2, 2B6 and 3A4

DOR (0.1 to 20 µM) was evaluated as an inducer of CYP1A2, 2B6 and 3A4 in cryopreserved plated human hepatocytes from three donors by determining concentration dependent increases in messenger RNA (mRNA) and activity of these enzymes.

DOR did not increase CYP1A2 and CYP2B6 mRNA or enzyme activity. DOR caused an increase in CYP3A4 mRNA at 10 µM (1.2- to 4.0-fold) and 20 µM (1.4- to 4.2-fold). This effect was small (3.1 to 20.3% across hepatocytes lots, relative to the positive control rifampicin at 10 µM) and had no corresponding effect on CYP3A4 enzyme activity, indicating that the change in mRNA expression did not affect changes in protein expression [Sec. 2.6.5.7.10].

Results from these experiments indicate that DOR is not likely to cause drug interactions via activation of the aryl hydrocarbon receptor (AhR) or the constitutive androstane receptor (CAR). Although DOR increased CYP3A4 mRNA at 10 and 20 µM by 1.4- to 4.2-fold, the maximal unbound concentrations achieved at the clinical dose (100 mg) does not exceed 1 µM. Additionally, in clinical studies, 120 mg DOR administered for 14 days did not have a clinically meaningful effect on the pharmacokinetics of midazolam [Sec. 2.7.2.2.3.2.1].

3.7 Other Pharmacokinetic Studies

No other pharmacokinetic studies were performed. For 3TC and TDF, preclinical pharmacokinetic data is available in the public domain.

4 TOXICOLOGY

DOR has been evaluated in a comprehensive nonclinical safety assessment program that included in vitro and in vivo genetic toxicity assays and repeat-dose oral tolerability and toxicity studies in mice up to three months duration, in rats up to six months duration, and in dogs up to nine months duration to support oral administration to humans [Table 2.4: 4]. Developmental and reproductive toxicology studies conducted with DOR included a male and female rat fertility study, embryo-fetal developmental toxicity studies in rats and rabbits, a pre- and post-natal toxicity study in rats, and juvenile toxicity studies in rats. In addition, a six-month oral carcinogenicity study in rasH2 transgenic mice, a two-year oral carcinogenicity study in rats, a rat phototoxicity study, a one-month combination toxicity study, and three month-oral rat toxicity studies evaluating potential impurities were completed.

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Table 2.4: 4 DOR Oral Toxicology Program: GLP Studies

Study Type/Duration	Route	Species	Dose Levels (mg/kg/day, unless otherwise specified)	NOAEL (mg/kg/day, unless otherwise specified)	Study No. (GLP Status)
Repeat-dose Toxicity					
1-Month Range-Finding with TK	Oral (gavage)	rasH2 Wild-Type Mice (Hybrid)	0, 30, 100, 300, 450	≥450	TT #[redacted]-6010 (GLP)[a]
3-Month Range-Finding with TK	Oral (gavage)	Mice CRL:CD1(ICR)	0, 3, 30, 150, 300, 450	≥450	TT #[redacted]-6013 (GLP)
7-Day TK	Oral (gavage)	Rats Crl:WI(Han)	1.5, 15, 175, 225 (given as b.i.d. doses) 30, 175, 225 (QD)	N/A	TT #[redacted]-0019 (GLP)
2-Week Toxicity with FOB & TK	Oral (gavage)	RatsCrl:WI(Han)	0, 5, 75, 450	≥450	TT #[redacted]-6029 (GLP)
3-Month Toxicity with TK	Oral (gavage)	Rats Crl:WI(Han)	0, 3, 30, 450	≥450	TT #[redacted]-6040 (GLP)
6-Month Toxicity with TK	Oral (gavage)	Rats Crl:WI(Han)	0, 3, 30, 450	≥450	TT #[redacted]-6019 (GLP)[b]
14-Day Toxicity with TK	Oral (gavage)	Dogs (Beagle)	10	≥10	TT #[redacted]-6030 (GLP)
3-Month Toxicity with TK	Oral (gavage)	Dogs (Beagle)	0, 1,10, 1000	≥1000	TT #[redacted]-6039 (GLP)
9-Month Toxicity with TK	Oral (gavage)	Dogs (Beagle)	0, 1, 10, 1000	≥1000	TT #[redacted]-6018 (GLP)[c]
Genotoxicity – In Vitro					
Microbial Mutagenesis Assay	In vitro	*Salmonella typhimurium* TA97a, TA98, TA100, and TA1535 and *Escherichia coli* WP2 uvrA pKM101	100 to 5000 µg/plate	Negative	TT #[redacted]-8099 (GLP) (reported with TT #[redacted]-8021)
Microbial Mutagenesis Assay	In vitro	TA100	100 to 5000 µg/plate	Negative	TT #[redacted]-8113 (GLP) (reported with TT #[redacted]-8021)
In Vitro Assay for Chromosomal Aberration	In Vitro	Chinese hamster ovary cells	75 to 500 µM	Negative	TT #[redacted]-8666 (GLP) (reported with TT #[redacted]-8662)

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Study Type/Duration	Route	Species	Dose Levels (mg/kg/day, unless otherwise specified)	NOAEL (mg/kg/day, unless otherwise specified)	Study No. (GLP Status)
Genotoxicity - In Vivo					
Assay for Micronucleus Induction in Rat Bone Marrow	Oral (gavage)	Rats Crl:WI(Han)	0, 5, 75, 450	Negative	TT #[redacted]-8725 (GLP) (In conjunction with 2-WK Oral Toxicity Study TT #[redacted]-6029)
Carcinogenicity					
6-Month Carcinogenicity with TK	Oral (gavage)	rasH2 Transgenic Mice	0, 10, 60, 300	≥300 No evidence of carcinogenic potential	TT #[redacted]-6005 (GLP)
2-Year Carcinogenicity with TK	Oral (gavage)	Rat Crl:WI(HAN)	0, 3, 30, or 450	≥450 for neoplastic & non-neoplastic findings	TT #[redacted]-6029 (GLP)[d]
Reproductive & Developmental Toxicity					
Fertility	Oral (gavage)	Rats Crl:WI(Han)	0, 5, 30, 450	≥450	TT #[redacted]-7300 (GLP)[e]
Embryo-Fetal Developmental Toxicity with TK	Oral (gavage)	Rats Crl:WI(Han)	0, 5, 45, 450	≥450 (for both maternal and developmental toxicity)	TT #[redacted]-7040 (GLP)[f]
Embryo-Fetal Developmental Toxicity with TK	Oral (gavage)	Rabbits (Dutch Belted)	0, 2, 15, 300	15 (maternal toxicity); ≥300 (developmental toxicity)	TT #[redacted]-7050 (GLP)[f]
Pre- and Postnatal Development	Oral (gavage)	Rats Crl:WI(Han)	0, 5, 45, 450	≥450 (for both maternal and F_1 generation toxicity)	TT #[redacted]-7010 (GLP)[e]
Toxicity with TK in Juveniles	Oral (gavage)	Rats Crl:WI(Han)	0, 10, 45, 300	≥300	TT #[redacted]-7140[e] (GLP)
Local Tolerance					
Bovine Corneal Opacity and Permeability Test	In vitro	Bovine Corneas	0.75 mL ([redacted]% DOR solution)	N/A	TT #[redacted]-7857 (GLP)
MatTek EpiDerm™ MTT Viability Assay	In vitro	MatTek EpiDerm™ tissue samples	100 mg	N/A	TT #[redacted]-7858 (GLP)
Other Toxicity Studies					
- Immunotoxicity					
Local Lymph Node Assay	Topical	Mouse (CBA/J)	5, 10 and 25% (w/v) DOR in DMF	N/A	TT #[redacted]-7859 (GLP)

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Study Type/Duration	Route	Species	Dose Levels (mg/kg/day, unless otherwise specified)	NOAEL (mg/kg/day, unless otherwise specified)	Study No. (GLP Status)
- Impurity Studies					
3-Month Toxicity (Batch Study)	Oral (gavage)	Rats Crl:WI(HAN)	0, 115	≥115	TT #[redacted]-6035 (GLP)
3-Month Toxicity (Phenol Degradate)	Oral (gavage)	Rats Crl:WI(HAN)	30	≥30 (0.12 of DOR phenol degradate)	TT #[redacted]-6017 (GLP)
3-Month Toxicity with a Micronucleus Assay (DOR or TDF Degradate G)	Oral (gavage)	Rats Crl:WI(HAN)	Group 1: DOR & TDF Degradate G Control Group 2: DOR at 30 mg/kg/day Group 3: TDF Degradate G [fumaric acid ester] 0.3 mg/kg/day	≥30 DOR (with impurities of interest) ≥0.3 TDF Degradate G [fumaric acid ester]	TT #[redacted]-6010 (GLP)
- Other Toxicity Studies – Other					
Phototoxicity	Oral (gavage)	Rats Crl:LE (Long-Evans)	0, 30, 450	≥450	TT #[redacted]-9011 (GLP)
One-Month Combination Toxicity Study *(with MK-8591)*	Oral (gavage)	Rats Crl:WI(Han)	0, 0+3 MK-8591; 30+1 MK-8591, 30+3 MK-8591	≥30 +3 MK-8591	TT #[redacted]-1012 (GLP)

NA = Not Applicable

a With the exception of confirmatory genotyping of female study animals.

b With the exception of urinary crystal identification and metabolite profiling.

c With the exception of metabolite profiling.

d With the exceptions of physical stability of the [redacted] formulation of DOR in the vehicle and the exploratory metabolite profiling.

e With the exception of determination of the physical stability of the formulation.

f With the exception of the skeletal evaluation by micro-CT and determination of the physical stability of the formulation.

Nonclinical exposure margins of DOR to humans were based on population pharmacokinetics analysis from Phase 3 DOR trials (P018 and P021), where a 100 mg QD DOR clinical dose resulted in systemic geometric mean exposure of 37.8 µM•hr $AUC_{0\text{-}24hr}$ and a C_{max} of 2.26 µM.

4.1 Single-Dose Toxicity

No formal acute toxicity studies have been conducted with DOR. However, the toxicity after a single dose can be assessed from the repeat-dose oral toxicity studies in mice (up to 450 mg/kg/day), rats (up to 750 mg/kg/day) and dogs (up to 1000 mg/kg/day), as discussed below in [Sec. 2.4.4.2]. A dog oral pharmacology study testing single doses of DOR ranging from 2 mg/kg to 750 mg/kg was performed as described in the pharmacology written summary [Sec. 2.6.2], and single dose toxicokinetic studies in rabbits to support embryo-fetal

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toxicity studies were performed as described in [Sec. 2.4.4.5]. Overall, there was no evidence of acute toxicity in these species.

4.2 Repeat-Dose Toxicity

4.2.1 Mice

In mice, there were no adverse antemortem or postmortem findings when DOR was administered in one- (TT #[redacted]-6010) [Sec. 2.6.7.6] and three-month (TT #[redacted]-6013) [Sec. 2.6.7.7A] toxicity studies in TgRasH2 wild type mice and CD1 mice, respectively, at exposures ($AUC_{0\text{-}24\ hr}$ exposures achieved of 220 and 201 µM•hr respectively) approximately 5.5-fold above the DOR exposure at the human 100 mg daily dose.

4.2.2 Rats

In a two-week toxicity and toxicokinetic study in rats administered doses of 5, 75, or 450 mg/kg/day of DOR by oral gavage, (TT #[redacted]-6029) [Sec. 2.6.7.7B] test article-related antemortem findings seen at 450 mg/kg/day only were limited to postdosing salivation in both sexes and the presence of ex vivo urinary crystals in the urine of females. There were no postmortem test article-related findings. Based on the lack of postmortem findings or adverse antemortem findings, the NOAEL for this study was ≥450 mg/kg/day ($AUC_{0\text{-}24\ hr}$: 233 µM•hr, approximately 6-fold above the DOR exposure at the human 100 mg daily dose).

In a three-month toxicity and toxicokinetic study in rats administered doses of 3, 30, or 450 mg/kg/day of DOR by oral gavage (TT #[redacted]-6040) [Sec. 2.6.7.7C], test article-related antemortem changes were noted at doses ≥30 mg/kg/day and were limited to post-dose salivation in both sexes at 450 mg/kg/day, and the presence of ex vivo urinary crystals in the urine of the majority of females at 450 mg/kg/day and in a single female at 30 mg/kg/day. There were no test article-related postmortem findings. Based on the lack of postmortem findings or adverse antemortem findings, the NOAEL for this study was ≥450 mg/kg/day ($AUC_{0\text{-}24\ hr}$: 253 µM•hr, approximately 6.5-fold above the DOR exposure at the human 100 mg daily dose).

In the six-month (chronic) rat toxicity study (TT #[redacted]-6019) [Sec. 2.6.7.7D], test article-related antemortem findings were observed in males at ≥30 mg/kg/day and in females at 450 mg/kg/day. Very slight to slight increases in prothrombin time and activated partial thromboplastin (5-10%) were noted. Urinalysis findings observed consisted of very slight decreases in urinary volume (-56%), very slight increases in urine specific gravity, and low individual pH values in females (pH values of 5.5 observed in a minority of animals), and presence of urinary crystals. There were no test article-related postmortem findings. Based on the absence of postmortem findings and lack of adverse antemortem findings, the NOAEL was ≥450 mg/kg/day ($AUC_{0\text{-}24\ hr}$: 279 µM•hr, approximately 7.5-fold above the DOR exposure at the human 100 mg daily dose).

An exploratory study in rats were conducted to explore the nature and the origin of the urinary crystals sporadically observed in in the urine of female and male rats predominantly at doses of 450 mg/kg/day DOR in all repeat-dose rat toxicity studies after overnight ex vivo

urine collection. Exploratory analyses identified the composition of urinary crystals to be DOR; presence of crystals in rat urine therefore reflect excreted drug product and apparent DOR supersaturation in urine, consistent with very low DOR solubility in aqueous solutions [Sec. 3.2.S.1.3.3]. In addition, these urinary crystals were demonstrated to be formed ex vivo during overnight urine collection but were not observed when collected in vivo. There were no crystals in urine from DOR-treated animals when urine was collected by cystocentesis in vivo, while there were crystals in urine observed from the same DOR-treated animals after ex vivo urine collection following collection using an overnight procedure similar to that used in the toxicity studies. These data demonstrate that urinary crystal formation in rats was associated with ex vivo exposure to environmental conditions during overnight urine collection, with no evidence of in vivo urinary crystal formation (TT #[redacted]-1095) [Sec. 2.6.7.17].

4.2.3 Dogs

No test article-related antemortem or postmortem findings were seen in a fourteen-day toxicity and toxicokinetic study in dogs given DOR at an oral gavage dose of 10 mg/kg/day (TT #[redacted]-6030) [Sec. 2.6.7.6]. Based on the absence of antemortem or postmortem findings, the NOEL was ≥10 mg/kg/day (Study Day 14 mean $AUC_{0\text{-}24\ hr}$: 301 µM•hr, approximately 8-fold above the DOR exposure at the human 100 mg daily dose).

In a three-month toxicity study in dogs administered DOR at doses of 1, 10, and 1000 mg/kg/day, test article-related antemortem findings were limited to a discoloration (pale brown or white) of the feces in both sexes at 1000 mg/kg/day, most likely attributed to the non-absorbed whitish DOR formulation (TT #[redacted]-6039) [Sec. 2.6.7.7E]. There were no test article-related postmortem changes. Based on the lack of postmortem findings and adverse antemortem findings, the NOAEL for this study was ≥1000 mg/kg/day ($AUC_{0\text{-}24\ hr}$: 683 µM•hr, approximately 18-fold above the DOR exposure at the human 100 mg daily dose).

In the nine-month toxicity study in dogs administered 1, 10, and 1000 mg/kg/day of DOR, test article-related clinical observations at all doses consisted of discoloration of feces (resembling test article) and increased incidence of unformed stools (TT #[redacted]-6018) [Sec. 2.6.7.7F]. Sporadic salivation was observed at 1000 mg/kg/day in both sexes and in males at 1 mg/kg/day and 10 mg/kg/day. At doses ≥ 10 mg/kg/day, sporadic and transient unilateral or bilateral clear eye discharge was observed during the study. There were no test article-related gross, organ weight or histomorphologic changes at final necropsy upon postmortem evaluation. Clear eye discharge, salivation, and presence of discolored (resembling administered test article) and/or unformed stool were not considered adverse based on the sporadic incidence, lack of correlative or associated clinical or anatomic pathology findings, and absence of biologic and toxicologic impact. Based on the absence of postmortem findings and lack of adverse antemortem findings, the NOAEL was ≥1000 mg/kg/day ($AUC_{0\text{-}24\ hr}$: 673 µM•hr, approximately 18-fold above the DOR exposure at the human 100 mg daily dose).

4.3 Genotoxicity

4.3.1 In vitro

DOR was not genotoxic in microbial mutagenesis assays (TT #[redacted]-8021, TT #[redacted]-8099, and TT #[redacted]-8113) [Sec. 2.6.7.8A] and was negative in chromosomal aberration assays in Chinese Hamster Ovary Cells (TT #[redacted]-8662 and TT #[redacted]-8666), [Sec. 2.6.7.8B]). These studies were conducted in accordance with the ICH S2(R1) guideline. The top concentrations used in these studies were the limit concentration or limited by solubility, respectively.

4.3.2 In vivo

DOR was negative for the induction of micronuclei in bone marrow polychromatic erythrocytes (TT #[redacted]-8725) [Sec. 2.6.7.9B] of female and male rats from the two-week oral toxicity study (TT #[redacted]-6029). The dose levels of DOR scored for micronuclei were 5, 75, and 450 mg/kg/day for both males and females. The frequencies of micronucleated PCE (MN-PCE) for each DOR dose group were similar to the concurrent controls and within the range for historical vehicle controls. There was also no apparent effect on the proportions of PCE among total erythrocytes in the bone marrow. This study was conducted in accordance with the ICH S2(R1) guideline and the high dose evaluated was the maximum feasible dose.

4.4 Carcinogenicity

4.4.1 Long-term studies

A study to evaluate the carcinogenic potential of DOR when administered by oral gavage to rasH2 transgenic mice for approximately 6 months was conducted using doses of 10, 60, and 300 mg/kg/day (TT #[redacted]-6005) [Sec. 2.6.7.10A]. There were no test article-related antemortem findings, no test article-related increase in mortality, and no evidence of a carcinogenic potential after 6 months of daily oral administration of DOR of up to 300 mg/kg/day ($AUC_{0\text{-}24\ hr}$: 228 µM•hr, approximately 6-fold above the DOR exposure at the human 100 mg daily dose).

A study to evaluate the carcinogenicity potential of DOR when administered by oral gavage to rats for two years was conducted using doses of 3, 30, or 450 mg/kg/day (TT #[redacted]-6029) [Sec. 2.6.7.10B]. There was no statistically significant effect on unscheduled death rate. Test article-related antemortem findings were limited to intermittent salivation in both sexes at 450 mg/kg/day and were considered of minimal toxicologic significance. There was no DOR-related tumorigenicity. Test article-related non-neoplastic changes were limited to very slight centrilobular hypertrophy of hepatocytes in the livers from males at 450 mg/kg/day which, in the absence of associated degenerative, neoplastic or pre-neoplastic liver changes, were considered of minimal toxicologic significance. Based on assessment of C_{min} and C_{max} exposures in this study, the projected $AUC_{0\text{-}24hr}$ exposures would have equaled or exceeded that achieved in the chronic rat toxicity study (≥7.5-fold above the DOR exposure at the human 100 mg daily dose). The NOAEL was ≥450 mg/kg/day (≥7.5-fold above the DOR exposure at the human 100 mg daily dose) for non-neoplastic changes in this

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study, and there was no DOR-related tumorigenicity or evidence of carcinogenic potential in this study.

4.4.2 Short-term or medium-term studies

No short-term or medium-term studies were conducted in association with the mouse and rat carcinogenicity studies discussed above.

4.4.3 Other studies

There were no tumor findings observed in the routine carcinogenicity studies that suggested significant risk to humans. Therefore, no other studies were conducted.

4.5 Reproductive and Developmental Toxicity

Developmental and reproductive toxicology studies conducted with DOR included a male and female rat fertility study, embryo-fetal developmental toxicity studies in rats and rabbits, a pre- and post-natal toxicity study in rats, and juvenile toxicity studies in rats. The toxicokinetic profile of DOR in rabbits was evaluated in exploratory oral single-dose and multiple dose toxicokinetic studies preceding the pivotal rabbit embryo-fetal studies. In rabbits, the DOR [redacted] formulation showed superior exposures (AUC) at lower dose levels when compared to the DOR base compound conventional formulations. A plateau in exposure was determined to occur at [redacted] formulation doses ≥300 mg/kg/day DOR in rabbit.

4.5.1 Fertility and early embryonic development

No effects on female and male fertility and early embryonic development were observed in the oral male and female rat fertility study, where doses up to 450 mg/kg/day were evaluated (TT #[redacted]-7300) [Sec. 2.6.7.12]. DOR exposures were projected to be approximately 7.5-fold above the DOR exposure at the human 100 mg daily dose, based on exposures observed in the repeat-dose oral rat toxicity studies at similar doses.

4.5.2 Embryo-fetal development

There were no developmental toxicities observed in rat or rabbits at any dose levels tested. In the rabbit embryo-fetal developmental toxicity and toxicokinetic study, the top dose of 300 mg/kg/day (TT #[redacted]-7050) [Sec. 2.6.7.13B] resulted in DOR AUC exposures of 315 µM•hr, approximately 8.5-fold above the DOR exposure at the human 100 mg daily dose. In the rat embryo-fetal developmental toxicity and toxicokinetic study (TT #[redacted]-7040) [Sec. 2.6.7.13A], the AUC exposure at the top dose of 450 mg/kg/day DOR was 345 µM•hr, approximately 9-fold above the DOR exposure at the human 100 mg daily dose.

4.5.3 Prenatal and postnatal development, including maternal function

No toxicities on development, growth, behavior, reproductive performance, and fertility of the F_1 generation in rats following oral administration (5, 45 or 450 mg/kg/day) of DOR to

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F_0 females from Gestation Days (GD) 6 through Lactation Day (LD) 20 were seen (TT #[redacted]-7010) [Sec. 2.6.7.14]. The NOAEL for F_0 maternal toxicity and for F1 generation developmental toxicity was ≥450 mg/kg/day.

4.5.4 Studies in which the offspring (juvenile animals) are dosed and/or further evaluated

DOR was orally administered to juvenile rats once daily at doses of 0, 10, 45, or 300 mg/kg/day from Postnatal Day (PND) 14 to 55 (TT [redacted]-7140) [Sec. 2.6.7.15]. DOR-related findings were limited to a dose-dependent increase in the incidence of rats with ex vivo urine crystals in Postnatal Week 8 (PNW 8) that were not correlated with other clinical or anatomic pathology observations, and that were no longer present following a 4-week treatment-free period. These urinary crystal observations were also observed in the subchronic and chronic rat toxicity studies, and considered to be formed ex vivo related to DOR supersaturation in urine and overnight urine collection. In juvenile rats, there was no evidence of DOR-related toxicity based on mortality, clinical signs, body weights, food consumption, developmental landmarks, open field motor activity, hematology and serum biochemistry parameters, gross observations, organ weight changes, or histomorphologic changes. Based on the results of this study, the NOAEL was ≥300 mg/kg/day ($AUC_{0\text{-}24hr}$: 333 µM.hr, approximately 9-fold above the DOR exposure at the human 100 mg daily dose)

4.6 Local Tolerance

Local tolerance studies were conducted in support of the occupational safety program and consisted of the bovine corneal opacity and permeability assay [BCOP] (TT #[redacted]-7857, [Sec. 2.6.7.16]) and the MatTek EpiDerm™ MIT Viability assay (TT #[redacted]-7858, [Sec. 2.6.7.16]) to assess the ocular and dermal irritation potential of DOR, respectively. DOR was classified as a non-irritant.

4.7 Other Toxicity Studies

4.7.1 Antigenicity

There were no observations or changes considered to be due to potential antigenicity induced by DOR in the routine repeat-dose toxicity studies [Sec. 2.6.6.3]. Therefore, no antigenicity evaluations were conducted.

4.7.2 Immunotoxicity

There was no indication of immunotoxicity observed for DOR in the routine oral repeat-dose toxicity studies [Sec. 2.6.6.3] and there was no evidence of immunotoxicological risk. As such, in accordance with ICH Guidance S8 *Guidance on Immunotoxicity Studies for Human Pharmaceuticals*, no additional immunotoxicity studies were conducted. Results in the repeat dose oral studies in mice, rat, and dogs showed no evidence of autoimmune or inflammatory adverse effects, nor any adverse effects on lymphoid tissues. In these studies, the combination of hematologic, organ weight, and macroscopic and histopathologic

evaluation provided compelling weight of evidence that DOR was not associated with cause for immunotoxicity concern.

A local lymph node assay (LLNA) in mice was conducted in support of the occupational safety program (TT #[redacted]-7859, [Sec. 2.6.7.17]). In this study, DOR was not a dermal sensitizer.

4.7.3 Mechanistic studies

The mechanism of action was assessed as part of pharmacodynamic studies [Sec. 2.6.2]. DOR targets a non-mammalian enzyme with high specificity and no relevant off-target activity was observed. No additional mechanistic studies were conducted.

4.7.4 Dependence

There was no indication that DOR or its major metabolite(s) have a pharmacologic profile consistent with drug abuse liability potential. In addition, there was no evidence in the central nervous system (CNS) safety pharmacology study or in the routine repeat-dose toxicity studies of psychoactive effects (i.e., sedative or stimulant effects). As such, no animal abuse potential studies were conducted.

4.7.5 Metabolites

All circulating metabolites that were detectable in human plasma or human excreta were also present in plasma and/or excreta of nonclinical species. The only metabolite observed at exposures greater than 10% of total drug-related exposure in a single dose radiolabeled clinical study was the oxidative metabolite M9, which represented 13% of total drug-related human exposure [Sec. 2.7.2.2.1.3]. Formation of M9 was also the main metabolic pathway in nonclinical species, and was detected in plasma and excreta (urine and/or feces) of all nonclinical species [Sec. 2.6.4.5].

Exploratory analyses were performed to assess levels of the M9 metabolite in plasma from rat and dog chronic safety studies relative to levels of M9 in clinical samples collected in healthy subjects that had received daily doses of 240 mg DOR for 10 days (P008) [Sec. 2.7.2.2.1.3]. Plasma samples collected in a 24 hr period from the animals dosed chronically with DOR were pooled in an $AUC_{0\text{-}24hr}$ proportional manner such that the resulting peak area of M9, determined by UPLC-HRMS analysis, was directly proportional to the exposure of M9. A similar AUC-pooled sample was prepared from healthy subjects that had received daily doses of 240 mg DOR for 10 days. Comparison of the M9 peak area in rat and dog plasma to the peak area of M9 in the human plasma sample enabled the estimation of the abundance of M9 in the safety samples relative to its abundance in the clinical sample. The data indicated that M9 (total M9, consisting of unbound and bound levels) was present in rat and dog plasma at levels that were approximately 52 and 51% relative to levels observed in human plasma after 10 daily administrations of 240 mg DOR. Consequently, the total M9 exposure in rat and dog chronic toxicity studies was at least 50% of the total M9 exposure in humans at the recommended DOR dose of 100 mg. As the unbound fraction of M9 in rat and dog plasma was approximately 2.2 and 2.8-fold higher

than in humans [Sec. 2.4.3.3.3], the unbound M9 exposure in rat and dog plasma was approximately similar to the unbound plasma exposure of M9 in humans after 10 daily administrations of 240 mg DOR. Therefore, the unbound systemic exposure of M9 in the chronic rat and dog studies is similar or slightly greater to the M9 exposure in humans at the recommended DOR dose of 100 mg. As such, characterization of M9 toxicity is considered adequate, according to I*CH M3(R2) guidance, Questions and Answers (R2), Question 5 in "Metabolites"* section which states that characterization of metabolite toxicity would generally be considered adequate when animal exposure is at least 50% the exposure seen in humans. M9 levels were not measured directly in mouse or rabbit safety assessment studies, however data generated from single dose studies indicated M9 to be the major circulating metabolite in mice and the predominant metabolite in excreta for both mice and rabbits [Sec. 2.6.4.5].

4.7.6 Impurities

DOR impurities and degradation products were qualified according to ICH Q3A(R2) and ICH Q3B(R2) guidances by virtue of their presence in the batches evaluated in the repeat-dose toxicity studies of ≥3-months in duration. In addition, TDF degradate G (also known as fumaric acid ester), which is specified at a level above the qualification threshold in the DOR/3TC/TDF FDC tablet, was qualified in a three-month oral rat toxicity study with micronucleus evaluation, (TT #[redacted]-6010) [Sec. 2.6.7.17]. The dose multiples for each DOR-related impurity or degradation product relative to the dose of impurity in a 100 mg human daily dose of DOR, or TDF degradate G relative to the dose of degradate in a 300 mg daily dose of TDF as part of the DOR/3TC/TDF fixed dose combination, are presented in [Table 2.4: 5]. Based on body surface area comparisons, dose multiples for each impurity were achieved compared to human exposures, and each impurity is therefore qualified at the acceptance limits found in the active pharmaceutical ingredient (API).

In addition, potential and actual impurities, including identified impurities in each starting material, intermediate, and drug substance/product, have been assessed for mutagenic potential according to ICH M7 guidance, as described in [Sec. 3.2.S.3.2] (Drug Substance) and [Sec. 3.2.P.5.5] (Drug Product).

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Table 2.4: 5 DOR Impurities and Dose Multiples

Impurity	Specification Limits	Impurity Level %	Rodent Total Daily Intake of Impurity (mg/kg)	Human Total Daily Intake of Impurity (mg/kg)[a]	Dose Multiple based on mg/kg[c]	Rodent Total Daily Intake of Impurity (mg/m^2)[d]	Human Total Daily Intake of Impurity (mg/m^2)[d]	Dose Multiple based on mg/m^2[e]
Drug Substance Impurities								
[redacted]	≤ [redacted] %	[redacted][f]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	≤ [redacted] %	[redacted][f]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	≤ [redacted] %	[redacted][f]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	≤ [redacted] %	[redacted][f]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	≤ [redacted] %	[redacted][f]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
MK-1439 [redacted]	≤ [redacted] %	[redacted][g]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
Drug Product Impurities								
Tenofovir Deg G (also known as fumaric acid ester)	≤ [redacted] %	[redacted][g]	[redacted]	[redacted][b]	[redacted]	[redacted]	[redacted]	[redacted]
MK-1439 [redacted]	≤ [redacted] %	[redacted][g]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	≤ [redacted] %	[redacted][h]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	≤ [redacted] %	[redacted][h]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]

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a Based upon a total daily DOR dose of 100 mg and presuming a 60 kg patient

b Based upon a total daily TDF dose of 300 mg and presuming a 60 kg patient

c Dose multiple was calculated as a ratio of total daily intake in rats (mg/kg) divided by the total daily intake in humans (mg/kg).

d Based upon a mg/kg to mg/m^2 conversion factor of 6 for rat, 3 for mouse, and 37 for an adult human e.g. mg/kg X 37 equals adult human dose on basis of mg/m^2.

e Dose multiple was calculated as a ratio of total daily intake in rodents (mg/m^2) divided by the total daily intake in humans (mg/m^2).

f Based on the nonclinical Batch/Lot # [redacted] (consisting of [redacted]% [redacted] and [redacted]% of hydroxypropyl methylcellulose acetate succinate polymer) used in the GLP 3-month oral toxicity study in rats TT #[redacted]-6035 [Sec. 2.6.7.17].

g Impurities tested in DOR batch # [redacted] and Deg G (fumaric acid ester) Lot # [redacted] used in the GLP 3-month oral toxicity study in rats TT#[redacted]-6010 [Sec. 2.6.7.17].

h Impurities tested in DOR batch # [redacted] (consisting of [redacted]% [redacted] and [redacted]% of hydroxypropyl methylcellulose acetate succinate polymer) used in the GLP 3-month oral range-finding and toxicokinetic study in CD1 mice TT #[redacted]-6013 [Sec. 2.6.7.7A].

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4.7.7 Other

4.7.7.1 Phototoxicity

Spectroscopic analysis performed indicates that DOR absorbs at approximately 327 nM, between 290 and 700 nM of the UV-visible wavelength range, and its molecular extinction coefficient has been calculated as 1323.4 L $mol^{-1}cm^{-1}$ [Sec. 3.2.S.3.1.1], above the threshold of 1000 L $mol^{-1}cm^{-1}$ for phototoxicity assessment as per ICH S10 Guidance. Hence, to evaluate the potential phototoxic effects of DOR, Long-Evans pigmented male rats were administered oral (gavage) doses of DOR (0, 30 or 450 mg/kg/day) for three consecutive days followed approximately 60 minutes after the final administration by a single exposure to solar-simulated ultraviolet radiation (UVR exposure) (TT #[redacted]-9011) [Sec. 2.6.7.17]. There were no skin reactions or ocular observations (confirmed by histopathology of the eye tissues) indicative of phototoxicity. The NOEL for phototoxicity was ≥450 mg/kg/day.

4.7.7.2 Oral Combination Toxicity study in Rats

A one-month combination oral toxicity was conducted in rats with DOR and MK-8591, an investigational nucleoside reverse transcriptase translocation inhibitor (NRTTI) for the treatment of HIV-1 (TT #[redacted]-1012) [Sec. 2.6.7.17]. DOR was evaluated at a 30 mg/kg/day dose level in the study, either alone or in combination with two different MK-8591 dose levels (1 and 3 mg/kg/day MK-8591). There were no findings identified when DOR was administered alone or in combination with MK-8591. Based on the lack of antemortem or postmortem findings in the study, the NOEL was ≥ 30 mg/kg/day DOR, and ≥ 30/3 mg/kg/day of DOR/MK-8591.

4.7.7.3 Long-Acting Parenteral (LAP) Formulation Studies

Long acting parenteral formulations of DOR were intramuscularly or subcutaneously administered as single or repeated doses in rats and dogs to support potential clinical dosing of DOR formulations by these routes [Sec. 2.6.7.17]. These studies are included in this application to provide the collective nonclinical experience with DOR, however, the exploratory results from these LAP studies using non-relevant routes of administration for oral clinical dosing and exploratory DOR microparticulate suspension formulations do not impact the nonclinical risk assessment of the oral DOR program. DOR systemic exposures achieved in LAP studies were far lower than those achieved in the oral program. There were no toxicities directly attributed to DOR when administered as microparticulate suspension LAP formulations. There was no relationship between DOR systemic exposures and local or systemic LAP formulation-related findings.

5 INTEGRATED OVERVIEW AND CONCLUSIONS

DOR is a potent NNRTI for the treatment of HIV-1 infection. In biochemical assays, DOR showed IC_{50}s <15 nM in the inhibition of the ribonucleic acid (RNA)-dependent Deoxyribonucleic acid (DNA) polymerization catalyzed by HIV-1 RTs (WT, K103N, or Y181C RT). Antiviral activity of DOR was tested in cell culture in the presence of 100% human serum. DOR displayed an EC_{50} of 12, 21, 31, and 33 nM against the WT virus,

K103N, Y181C, and K103N/Y181C mutant viruses, respectively. The resistance profile of DOR was evaluated against a broad panel of prevalent NNRTI-associated mutant viruses using EFV as a comparator; based on the susceptibility of the mutants to NNRTIs, DOR exhibited superior resistance profile to EFV. DOR also exhibited similar antiviral activities against WT isolates from 10 different HIV-1 subtypes.

Resistance selection was conducted with DOR and EFV in subtype B HIV-1 under high and low MOI conditions in cell culture. Resistance selection was also conducted with subtypes A and C viruses under low MOI conditions. Mutants containing V106A, V108I, F227L/C, and L234I mutations accounted for the majority of mutants selected despite different conditions. F227C or F227L containing mutants often emerged with other mutation(s) at higher concentrations of DOR and conferred high level of resistance to DOR (>500-fold).

DOR and its major metabolite M9 did not display notable off-target activities when evaluated in a large panel of standard enzyme and other receptor assays. DOR was tested against the human DNA polymerases α, ß, and γ, and IC_{50}s were determined to be greater than 100 µM. DOR exhibited CC50 >100 µM when test in various cell types including proliferating, stationary, and activated cells. In a two-drug antiviral activity combination study, DOR did not show antagonistic effects in cell culture when combined with any of 18 licensed antiviral agents (including 3TC and TDF) within the range of drug concentrations examined.

DOR, when evaluated for its potential effects on cardiovascular, neurological, and respiratory function in several safety pharmacology models, was without notable findings at exposures exceeding those achieved in humans at the clinical 100 mg daily dose. DOR inhibited hERG current with an IC_{50} value of 88 µM and an IC_{20} value of 19 µM, approximately 162-fold and 35-fold, respectively of the unbound DOR levels at the human C_{max} exposure. There were no test article-related effects on cardiovascular parameters in anesthetized, vagotomized dogs up to a 10 mg/kg cumulative dose (plasma concentrations up to 14 µM, approximately 6-fold DOR levels at the human C_{max} exposure). In conscious dogs orally administered DOR, there were no DOR-related effects on the cardiovascular and respiratory functions up to the highest dose tested of 10 mg/kg (3.5-fold DOR levels at the human C_{max} exposure); repeat dose oral toxicity studies of up to 9 month duration at doses up to 1000 mg/kg/day were without ECG findings (C_{max} of 32.7 µM, approximately 14-fold DOR levels at the human C_{max} exposure). There were no effects on neurological function in mice and rats at single oral doses up to 750 mg/kg and 450 mg/kg (C_{max} of 15.2 µM at 450 mg/kg in rats, approximately 6.5-fold DOR levels at the human C_{max} exposure), respectively.

DOR had a low clearance in all preclinical species studied (mouse, rat, rabbit, and dog) with a moderate volume of distribution and moderate oral bioavailability due to its good permeability and minimal first-pass effect. Low DOR solubility however, limits systemic exposures, and therefore multiple nonclinical formulations were evaluated to maximize DOR AUC and C_{max} levels in animals. Use of an enabled [redacted] formulation provided exposures in rodent and rabbit studies above those exposures expected at the clinical 100 mg dose that were acceptable for nonclinical safety assessment. In dogs, a conventional formulation of jet-milled DOR achieved exposures several fold above the clinical dose

exposures; additionally a long half-life in this species resulted in favorable accumulation for higher exposures (18-fold above clinical exposures) with chronic administration.

DOR binding to plasma proteins was moderate and was similar across preclinical species and humans and did not change within the concentration range expected at therapeutic doses (0.1 to 5 μM). Consistent with its good permeability, DOR distributed freely into blood cells and tissues. However, the distribution of DOR into the central nervous system was limited.

The elimination of DOR in preclinical species and humans occurred primarily via CYP-mediated oxidation. Renal excretion of unchanged drug was a secondary pathway of elimination. Conjugation pathways were only significant in dogs but not in other species, including humans. Following administration with [^{3}H] or [^{14}C]-labeled DOR, the oxidative metabolite M9 was the major radioactive component in excreta from preclinical species and humans and was observed circulating in significant amounts in mouse (~50% of total radioactivity) and human (~13% of total radioactivity) plasma, but it was not detected in rat or dog plasma and was present in trace amounts only in rabbit plasma at pharmacologically relevant doses (5 mg/kg). Follow up analysis of plasma from rats and dogs administered DOR chronically at 450 and 1000 mg/kg/day, respectively, demonstrated that exposures of M9 in both species were within 2-fold of that observed in plasma from healthy subjects that received multiple 240 mg once-daily doses of DOR. Thus, the safety of M9 at the therapeutic dose (100 mg) was adequately characterized in chronic safety studies. Other metabolites observed in humans were also generated in preclinical species.

In vitro data suggested that metabolism of DOR, including formation of M9, is mediated by CYP3A4, with very minor contribution from CYP3A5, so that DOR is anticipated to be a victim of drug interaction when co-administered with inhibitors or inducers of CYP3A enzymes. This is consistent with results from clinical studies with ritonavir, ketoconazole, and multiple doses of rifampin, which demonstrated an effect of these drugs on the exposure of DOR [Sec. 2.7.2.2.3.1].

DOR was a substrate for P-gp; however, based on preclinical and clinical data, the role of P-gp on the systemic clearance of DOR is anticipated to be minor. At the absorption level, clinical drug interactions with P-gp inhibitors may be small due to good passive permeability of DOR. This was confirmed in a single dose interaction study with rifampin, a P-gp inhibitor, where the C_{max} of DOR increased only by 40%. Similarly, DOR C_{max} increased by only 25-30% after co-administration with ritonavir and ketoconazole, which inhibit P-gp as well as CYP3A [Sec. 2.7.2.2.3.1].

DOR was not a substrate for the liver uptake transporters OATP1B1 or 1B3 or the efflux transporter BCRP. Furthermore, excretion studies in preclinical species did not indicate involvement of active renal or hepatic uptake or biliary secretion on the disposition of DOR. Thus, involvement of drug transporters in the disposition of DOR in humans is unlikely.

DOR is not expected to be a perpetrator of drug interactions via inhibition of major drug metabolism enzymes, including CYP1A2, 2B6, 2C8, 2C9, 2C19, 2D6, 3A4, or UGT1A1. DOR was not an inducer of CYP1A2, 2B6 or 3A4 at clinically relevant concentrations. In

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clinical studies, co-administration of DOR with midazolam, a sensitive CYP3A4 substrate, had no significant impact on the pharmacokinetics of midazolam [Sec. 2.7.2.2.3.2.1]. Furthermore, although DOR was a weak inhibitor of OATP1B1, OATP1B3, OAT1, OAT3, OCT2, BCRP, MATE1 and MATE2K in vitro, no clinically relevant interactions with substrates of these transporters are anticipated at the therapeutic doses. DOR did not inhibit BSEP or P-gp at concentrations up to 50 and 100 µM respectively; therefore, it is unlikely to perpetrate interactions via these transporters. In summary, DOR is not a perpetrator of clinically significant drug interactions but its pharmacokinetic profile may be affected by CYP3A inhibitors and inducers.

DOR has been evaluated in a comprehensive nonclinical safety assessment program that included in vitro and in vivo genetic toxicity assays and repeat-dose oral tolerability and toxicity studies in mice up to three months duration, in rats up to six months duration, and in dogs up to nine months duration to support oral administration to humans [Table 2.4: 4]. Developmental and reproductive toxicology studies conducted with DOR included a male and female rat fertility study, embryo-fetal developmental toxicity studies in rats and rabbits, a pre- and post-natal toxicity study in rats, and juvenile toxicity studies in rats. In addition, a six-month oral carcinogenicity study in rasH2 transgenic mice, a two-year oral carcinogenicity study in rats, a rat phototoxicity study, and three month-oral rat toxicity studies evaluating potential impurities were completed.

DOR repeat-dose oral administration to rats, mice and dogs up to either maximal feasible doses or a toxicity limit dose of 1000 mg/kg/day was well tolerated and did not result in adverse findings in general toxicity studies. Assessment of the M9 metabolite indicated adequate exposure to M9 in the general toxicity, embryofetal development toxicity and carcinogenicity studies in rats and in the dog general toxicity studies. In rodents, there were no adverse antemortem or postmortem findings when administered in one- and three-month toxicity studies in TgRas wild type mice and CD1 mice, respectively ($AUC_{0\text{-}24\ hr}$ range : 201-220 µM•hr, approximately 5.5-fold above the DOR exposure at the human 100 mg daily dose). In the chronic six-month rat toxicity study, test article-related non-adverse antemortem findings observed in males at ≥30 mg/kg/day and in females at 450 mg/kg/day consisted of very slight to slight increases in prothrombin time and activated partial thromboplastin (5-10%) coagulation parameters, and urinary findings of very slight decreases in urinary volume (-56%), very slight increases in urine specific gravity, and low individual pH values in females (pH values of 5.5 observed in a minority of animals). There were no test article-related postmortem findings observed. These findings were considered non-adverse due to the slight magnitude of change, lack of correlating postmortem effects, and absence of toxicologic significance. Based on the absence of postmortem findings and lack of adverse antemortem findings, the NOAEL in rats was ≥450 mg/kg/day ($AUC_{0\text{-}24\ hr}$: 279 µM•hr, approximately 7.5-fold above the DOR exposure at the human 100 mg daily dose). In the two year rat carcinogenicity study, test article-related changes were limited to very slight centrilobular hypertrophy of hepatocytes in the livers from males at 450 mg/kg/day which, in the absence of associated degenerative, neoplastic or preneoplastic liver changes, were considered of minimal toxicologic significance.

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Urinary crystals, described as yellow-brown, needle-like, were also sporadically observed in the urine of rats after overnight ex vivo urine collection predominantly at doses of 450 mg/kg/day in all repeat-dose rat toxicity studies after overnight ex vivo urine collection. Exploratory analyses identified the urinary crystals as consisting of DOR only due to apparent supersaturation of DOR in urine. These crystals were demonstrated to be formed ex vivo during overnight urine collection at room temperature but were not present in the same animals when urine was collected in vivo by cystocentesis in an investigative study in rats.

In the pivotal nine-month (chronic) dog study, test article-related clinical observations at all dose levels consisted of discoloration of feces (resembling test article) and increased incidence of unformed stools. Sporadic salivation was observed at 1000 mg/kg/day in both sexes and in males at 1 mg/kg/day and 10 mg/kg/day. At doses ≥ 10 mg/kg/day, sporadic and transient unilateral or bilateral clear eye discharge was observed during the study. There were no test article-related gross, organ weight or histomorphologic changes at final necropsy upon postmortem evaluation. Clear eye discharge, salivation, and presence of discolored (resembling administered test article) and/or unformed stool was not considered adverse based on its sporadic incidence, lack of correlative or associated clinical or anatomic pathology findings, and limited biologic and toxicologic impact. Preceding two-week and three-month toxicity studies in dogs also did not indicate any postmortem or adverse antemortem findings. Based on the absence of postmortem findings and lack of adverse antemortem findings, the NOAEL for the chronic dog toxicity was ≥1000 mg/kg/day ($AUC_{0\text{-}24\ hr}$: 673 μM•hr, approximately 18-fold above the DOR exposure at the human 100 mg daily dose).

In summary, the findings observed in the repeat-dose studies in mice, rats, and dogs were limited to non-adverse antermortem findings of minimal amplitude which did not impact the physical conditions of the animals and did not correlate with postmortem observations. In the two year rat carcinogenicity study, very slight centrilobular hypertrophy of hepatocytes in the livers from males at 450 mg/kg/day was observed which, in the absence of associated degenerative, neoplastic or preneoplastic liver changes, were considered of minimal toxicologic significance. As such, the NOAELs were the highest dose tested in all nonclinical repeat-dose studies.

DOR was not mutagenic or genotoxic when assessed in a battery of in vitro or in vivo studies carried out according to ICH guidance that included a microbial mutagenesis assay (“Ames” test), a chromosomal aberrations assay, and an in vivo assay for micronucleus induction in rat bone marrow. The top doses tested were the limit dose or were limited by solubility in the in vitro genetic toxicity studies, and was the maximum feasible dose in the in vivo genetic toxicity study. No in vivo or in vitro genetic toxicity was identified, and no carcinogenic potential was identified in a six-month mouse rasH2 transgenic mice carcinogenicity study or a two-year rat carcinogenicity study.

There were no effects on male and female fertility in rats, or adverse findings on embryo-fetal development in rats or rabbits, on pre- and post-natal development study in rats, or on juvenile development in rats.

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There was no evidence of phototoxicity in pigmented rats following repeat-dose oral DOR administration followed by exposure to solar-simulated ultraviolet radiation. DOR was classified as a non-irritant and as a non-dermal sensitizer in local tolerance assays.

Impurities and degradation products were assessed according to ICH Q3A(R2), ICH Q3B(R2) and ICH M7 guidances.

Exposure comparisons in animals versus humans (based on the human DOR clinical dose of 100 mg QD associated with a systemic DOR $AUC_{0\text{-}24hr,ss}$ of 37.8 µM•hr) indicates DOR systemic NOAEL exposure multiples in mouse, rat and dog to be approximately 5.5X, 7.5X and 18X, respectively. Exposure comparisons in animals versus humans are presented in the following table [Table 2.4: 6]. Based on assessment of the M9 metabolite in animal species, there was adequate exposure to M9 in rats in the general toxicity, embryofetal development toxicity and carcinogenicity studies and in the dog general toxicity studies.

Table 2.4: 6 DOR Systemic Exposure Multiples

Species	Study	Dose Levels (mg/kg/day)	$AUC_{0\text{-}24\ hr}$ (µM•hr)	Exposure Multiples[†]
Mouse (CD1)	Three Month Toxicity Study	3 30 150 300 (NOEL) 450 (NOAEL)	19.8 79.5 170 201 196	<1X 2X 4.5X 5.5X 5.5X
Rat	Six Month Toxicity Study	3 (NOEL) 30 450 (NOAEL)	19.1 87.3 279	<1X 2X 7.5X
Dog	Nine Month Toxicity Study	1 10 1000 (NOAEL)	82.2 383 673	2X 10X 18X
Rat	Male and Female Fertility	5 30 450 (NOEL)	NA[#]	~1X[#] 2X[#] 7.5X[#]
Rat	Embryo-Fetal Developmental Toxicity Study	5 45 450 (NOEL – Maternal and Developmental)	61.2 243 345	1.5X 6.5X 9.5X
Rat	Pre- & Post-Natal Development	5 45 450 (NOAEL – Maternal and F_1 Generation)	NA^	1.5X 6.5X 9.5X
Rat	Juvenile Toxicity	10 45 300	48.5 137 333	1X 3.5X 9X
Rabbit	Embryo-Fetal Developmental	2 15 (NOEL-Maternal)	13.8 80.7	<1X 2X

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Species	Study	Dose Levels (mg/kg/day)	$AUC_{0\text{-}24\ hr}$ (µM•hr)	Exposure Multiples[†]
	Toxicity Study	300 (NOEL-Developmental) (NOAEL-Maternal)	315	8.5X

450 mg/kg/day was the maximum feasible oral dose (MFD) of the DOR [redacted] formulation in adult mouse, rat, and rabbit studies, and 300 mg/kg/day was the MFD in juvenile-aged rats. Saturation of exposure was demonstrated in mouse and rabbit at a 300 mg/kg/day dose. In dogs, a limit dose of 1000 mg/kg/day (DOR suspended in 10% polysorbate 80 formulation) was used.

[†]Exposure multiples were based on population pharmacokinetics analysis from the Phase 3 trials (P018 and 021), where a 100 mg QD DOR clinical dose resulted in a systemic geometric mean exposure of 37.8 µM•hr $AUC_{0\text{-}24hr}$.

[#]In the rat male and female fertility study, no toxicokinetic evaluation was performed; exposures were determined to those measured in the rat repeat-dose toxicity study.

[^]In the rat pre- & post-natal development study, no toxicokinetics were performed; exposures were determined to those measured in the rat embryo-fetal developmental toxicity study.

Nonclinical studies evaluating the DOR/3TC/TDF FDC have not been performed. Both 3TC and TDF are marketed, well characterized therapeutic agents used as part of an antiretroviral regimen for the treatment of HIV-1. An assessment of the nonclinical pharmacology, ADME, and safety assessment profile of 3TC and TDF was conducted using data in the public domain. In vitro antiviral cell based assays evaluating 2 drugs indicated no antagonistic effects are expected when combining DOR with licensed HIV-1 antiviral agents, including 3TC and TDF. The drug interaction profiles for the individual drugs indicate that they are not likely to cause pharmacokinetic drug interactions between the FDC components. Significant changes in the pharmacokinetics of the DOR/3TC/TDF components are only anticipated for DOR when DOR/3TC/TDF is co-administered with CYP3A4 inducers or inhibitors.

No significant toxicologic concern (e.g., similar target organ toxicity) or potential for synergistic toxicity was identified concerning the co-administration of DOR with the well-characterized marketed approved compounds, 3TC and TDF. In animals treated with 3TC, reported changes at supratherapeutic exposures were restricted to reductions in red cell counts with reductions in total lymphocyte counts on occasion, but with no effect on bone marrow cytology. For TDF, target organs in animals were the gastrointestinal tract (seen in rats only), kidney (tubular dilatation and karyomegaly, interstitial nephritis), and bone (osteomalacia and reduced bone mineral density) and were well characterized. No target organ toxicities are identified with DOR in animals at any tested dose level.

Overall, the nonclinical pharmacology, pharmacokinetic, and toxicologic profile supports the safe use of DOR (100 mg) as a single entity or in the DOR/3TC/TDF FDC tablet for the treatment of HIV-1 infection.

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