# MODULE 2.4 NONCLINICAL OVERVIEW

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## List of Abbreviations

FC = fold change
RAL = raltegravir
EVG = elvitegravir
HIV = human immunodeficiency virus
IN = integrase
INI = integrase inhibitor
HCV = hepatitis C virus
TK = toxicokinetics
RT = reverse transcriptase
QD = once daily
BID = twice daily
GI = gastrointestinal
NOAEL = no observed adverse effect level
CFR = Code of Federal Regulations (US)
NNRTI = non-nucleoside reverse transcriptase inhibitor

# 1. OVERVIEW OF THE NONCLINICAL TESTING STRATEGY

## 1.1. Introduction

Dolutegravir is a novel, potent and selective orally bioavailable integrase inhibitor. It is currently under development for the treatment of human immunodeficiency virus (HIV) infected patients, both as a single entity and as a fixed-dose combination therapy.

The present application is for the use of dolutegravir sodium tablets for oral administration in combination with other antiretroviral agents for the treatment of HIV infection in adults and pediatric patients (aged 12 to 18 years and weighing at least 40 kg). The proposed treatment regimen of dolutegravir sodium is 50 mg administered orally once daily (QD) or 50 mg twice daily (BID).

## 1.2. Rationale for the Use of Dolutegravir in the Treatment of HIV

The antiviral activity of integrase inhibitors has been demonstrated in short-term monotherapy studies for raltegravir (MK-0518, Merck; RAL) and elvitegravir (GS-9137, Gilead; EVG) with an ~2 log drop in HIV RNA-1 [Markowitz, 2006; DeJesus, 2006]. Longer-term data with RAL demonstrated a significant antiviral effect in treatment experienced patients when added to an optimized background regimen of antiretroviral therapy [Grinsztejn, 2007] and in treatment naïve patients when co-administered with a nucleoside backbone [Merck Research Laboratories, 2007]. Clinical resistance to both RAL and EVG has been reported in treatment experienced patients [Hazuda, 2007; McColl, 2007]. Therefore, the development of new integrase inhibitors (INI) with different resistance profiles is desirable, and in the case of many treatment-experienced patients with clinical resistance to RAL and EVG, is essential for providing HIV-infected individuals an option for constructing an effective antiretroviral regimen.

Dolutegravir is a potent, low nanomolar inhibitor of HIV integrase, which provides the excellent antiviral activity and tolerability demonstrated for the INI class, while also offering once-daily dosing and no requirement for pharmacokinetic boosters.

Nonclinical studies and the clinical experience of dolutegravir demonstrate that it is well tolerated, with a potentially improved safety profile compared to RAL or EVG, and thus offers another safe and effective option to these patients.

Taken together, these data provide support to the therapeutic benefit of dolutegravir sodium in the treatment of HIV.

## 1.3. Nonclinical Development Program

Nonclinical studies carried out to support the development of dolutegravir include primary pharmacology studies demonstrating inhibition of integrase activity and HIV-1 replication in vitro as well as studies to determine the potential for HIV resistance to develop via mutations. Secondary pharmacologic activity was assessed and safety

pharmacology studies were conducted to investigate any untoward pharmacologic actions of dolutegravir on the respiratory, cardiovascular, central and peripheral nervous systems.

The absorption, distribution, metabolism and excretion of dolutegravir were investigated to characterize the disposition of dolutegravir in the toxicology test species, and a full toxicological evaluation was performed which included the toxicokinetics of dolutegravir.

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

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

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

Throughout this overview, nonclinical systemic exposure comparisons (end of study, gender mean) will be made to the maximum proposed human oral therapeutic dose of 50 mg/day QD and 50 mg/day BID (100 mg total dose per day) dolutegravir sodium, which produced a steady state $C_{max}$ of 3.7 μg/mL and AUC of 53.6 μg.h/mL (QD) based on pooled data from SPRING-1 and SPRING-2, or $C_{max}$ of 4.2 μg/mL and AUC of 75.1 μg.h/mL (BID) based on pooled data from VIKING and SAILING [m2.5, Section 3.2].

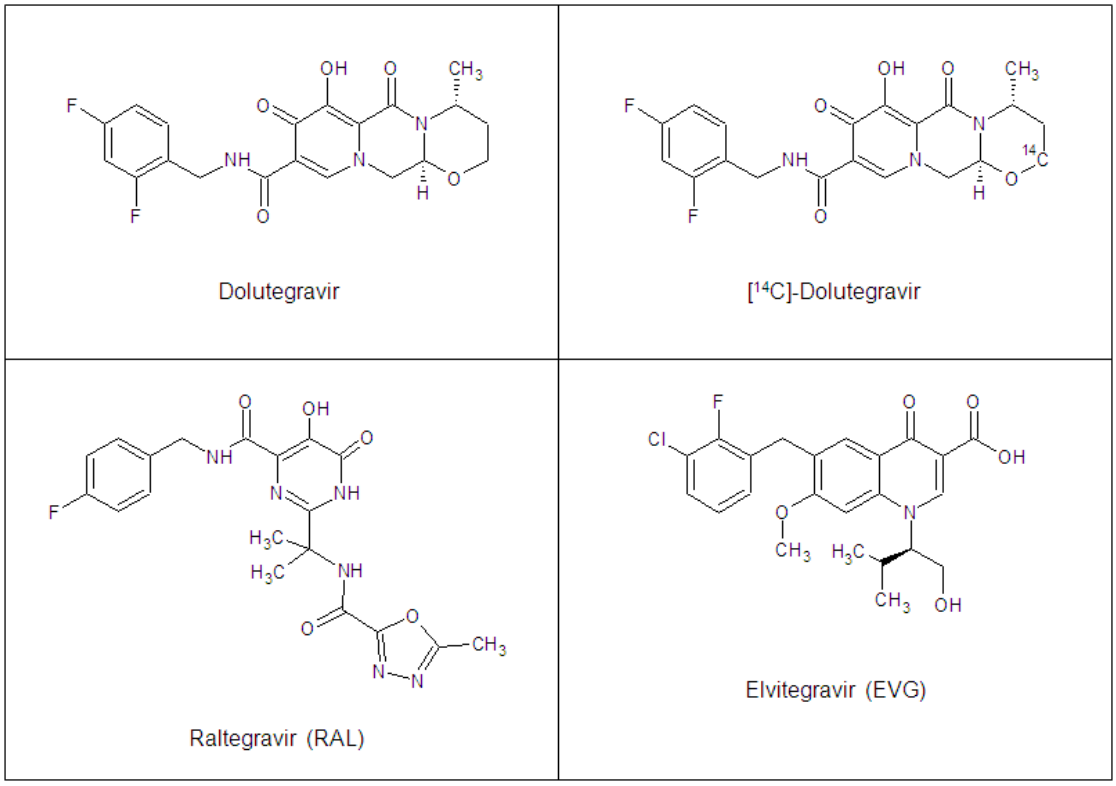
## 1.4. Test Material

Most nonclinical studies were conducted using the sodium salt of dolutegravir (GSK1349572A, referred to simply as dolutegravir throughout m2.4 Nonclinical Overview). In study reports, the parent was designated as ERC-349572A, ERC-349572 free acid or GSK1349572B, and the sodium salt was designated as ERC-349572B, ERC-349572 sodium, MTS-0297994B or GSK1349572A. The [$^{14}$C]-radiolabelled dolutegravir free acid is also designated as GSK1349572C and the [$^{14}$C]-radiolabelled dolutegravir sodium salt as GSK1349572D.

The batches of dolutegravir used in the definitive nonclinical safety studies [see m2.6.6 for a comprehensive list of toxicology batches] had impurity profiles that were consistent with the material proposed for clinical use and were made using the same synthetic route [see m2.3.S.2.2]. All doses and concentrations in this Nonclinical Overview (including analyte concentrations in biological fluids and tissues) are expressed in terms of the parent compound. See Figure 1 below for the structure of dolutegravir, the C-14 radiolabel, and the comparators raltegravir and elvitegravir.

The excipients used in the formulation for Dolutegravir Tablets, 50 mg are conventional and the amounts per tablet fall within typical ranges used. The specifications for the inactive ingredients comply with the United States Pharmacopeia/National Formulary (USP/USNF) and the European Pharmacopoeia (PhEur) (except the yellow iron oxide colorant in the [redacted] film coat). The film coating material comprises ingredients that are registered in the USP/USNF or comply with the CFR [m3.2.P.1, Description and Composition of the Drug Product]. All colorants used conform to EC Commission regulation 231/2012.

**Figure 1 Structure of Dolutegravir, $^{14}$C-Dolutegravir and the Comparators Raltegravir and Elvitegravir**




An overview of the nonclinical studies for dolutegravir is presented below and the nonclinical study reports are provided in Module 4.2. Written and tabulated summaries are provided in Module 2.6.

## 1.5. Module 4 XML Backbone Validation

The justification for the Module 4 sections for which no nonclinical study reports are available is discussed in appendix 1.

# 2. PHARMACOLOGY

## 2.1. Virology (Primary Pharmacodynamics)

A range of in vitro virology studies have been conducted to determine the mechanism of action, antiviral activity, and the potential for development of drug-resistance via mutations. An overview of these studies is provided within the Clinical Overview [m2.5, Section 4.1]. However, for the reviewer's convenience, a brief overview of the key findings from these studies is also provided below.

### 2.1.1. Mechanism of action

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

### 2.1.2. In vitro antiviral activity and potential resistance

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

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

Comparative susceptibilities to dolutegravir and RAL were obtained from 60 RAL-resistant site directed HIV-1 mutants and 6 site directed HIV-2 mutants. Dolutegravir

retained activity against a vast majority of these mutants. Additionally, susceptibilities to dolutegravir and RAL were determined for over 700 RAL-resistant clinical isolates, with dolutegravir retaining activity (<10 FC) against >90% of them.

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

## 2.2. Secondary Pharmacology

Dolutegravir was generally inactive against a panel of enzymes, receptors, ion channels, transporters, and functional tissue assays [Report RH2007/00072]. Therefore, dolutegravir is considered a potent and selective integrase inhibitor (see Section 2.1) and is unlikely to have any significant off-target pharmacological activity.

## 2.3. Safety Pharmacology

No treatment-related behavioral or overt pharmacological effects were noted in conscious male rats at ≤500 mg/kg (the highest dose tested) [m2.6.3 Table 4.1, Report RD2007/01038]. Systemic exposure at 500 mg/kg is estimated to be ~24X or 21X above the expected human $C_{max}$ of dolutegravir administered 50 mg QD or BID, respectively, based on extrapolation from Day 1 exposure in the rat 14 day toxicity study (87.1 μg/mL).

Single oral doses of dolutegravir at ≤500 mg/kg did not produce any effect on respiratory functional parameters in male rats when monitored for up to 6 hours following dosing [m2.6.3 Table 4.1, Report RD2007/01037]. Systemic exposure at 500 mg/kg is estimated to be approximately 25X or 18X above the expected human $AUC_{0-24}$ of dolutegravir administered 50 mg QD (53.6 μg.h/mL) or 50 mg BID (75.1 μg.h/mL), based on extrapolation from Day 1 exposure in the rat 14 day toxicity study (1360 μg.h/mL).

In male monkeys, single oral doses of dolutegravir at doses up to 1000 mg/kg ($C_{max}$ = 20.1 μg/mL; $AUC_{0-24}$ = 259 μg.h/mL) had no effect on arterial blood pressures, heart rate or electrocardiographic (ECG) parameters when monitored for 24 hours after dosing at a $C_{max}$ ~5X above the expected human $C_{max}$ of dolutegravir when administered 50 mg QD or BID [m2.6.3 Table 4.1, Report RD2007/01141]. Additionally, there were no treatment related effects in ECG parameters measured during the repeat dose monkey toxicity studies up to 38 weeks at doses ≤1000 mg/kg/day [see Section 4.3].

The effect of a series of dolutegravir concentrations (≤8.38 μg/mL) on hERG tail current was studied [m2.6.3 Table 4.1, Report RD2007/01039]. An $IC_{50}$ could not be determined as only 16.1% inhibition of hERG channel tail current occurred at the highest concentration, 20 μM. The high dose (20 μM or 8.4 μg/mL) is approximately 227X and 200X, respectively, above the free $C_{max}$ obtained with a 50 mg QD or 50 mg BID oral dose of dolutegravir (0.037 μg/mL for 50 mg QD, 0.042 μg/mL for 50 mg BID; based on 99% protein binding).

There were no findings from safety pharmacology studies that would indicate an unacceptable risk for oral administration of dolutegravir to patients in accordance with the proposed indication. Additionally, a supratherapeutic dose of dolutegravir (250 mg as a suspension, which achieved exposures ~3X higher than a 50 mg QD dose and ~2X higher than a 50 mg BID dose) was well tolerated and had no effect on cardiac repolarization [see m2.5, Section 5.4.6].

## 2.4. Pharmacodynamic Drug Interactions

A number of in vitro studies have been conducted with dolutegravir in combination with approved agents from all anti-HIV therapy classes (e.g. nucleoside/nucleotide reverse transcriptase [RT] inhibitors, non-nucleoside RT-inhibitors and protease inhibitors) and was shown to be additive or synergistic in all cases. These studies are discussed as part of the virology discussion [see m2.5, Section 4.1].

# 3. PHARMACOKINETICS

An extensive program of absorption, distribution, metabolism, and excretion studies has been carried out with dolutegravir in animals used in toxicity studies. In general, the systemic exposure and metabolism defined in the animal species used for toxicological assessment indicates that the species used were appropriate for predicting the safety of dolutegravir and its metabolites in humans.

## 3.1. Analytical Methods and Validation

In pharmacokinetic and toxicity studies, plasma dolutegravir concentrations were measured following protein precipitation with chiral or achiral liquid chromatographic tandem mass spectrometric (LC/MS/MS) methods. For toxicity and human studies, the chiral and achiral methods used for analysis were fully validated across each calibration range. All methods and limits of quantification were sufficiently adequate with regard to specificity and sensitivity to support the kinetic analyses of dolutegravir. Descriptions of the validated methods are presented in m2.6.4 (Appendix 1).

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

## 3.2. Pharmacokinetics and Absorption

The nonclinical pharmacokinetics of dolutegravir are characterized by low plasma clearance and low volume of distribution. Absorption is rapid with high oral bioavailability that is solubility limited. After repeat oral administration, systemic exposure to dolutegravir increased less than proportionately with dose.

### 3.2.1. Single dose

**Intravenous:** Following a single intravenous administration, dolutegravir exhibited low plasma clearance (<15% liver plasma flow) in the rat, dog and monkey [m2.6.5, Table 3.1; Reports RH2007/00101, RH2007/00102, RH2007/00103]. The low steady-state volume of distribution is consistent with the high protein binding of the compound (Section 3.3.1). The terminal half-life of 5.2 to 6.2 hours in nonclinical species was notably shorter than the apparent oral half-life in humans (~14 hours) [m2.5, Section 3.2].

**Oral:** Dolutegravir absorption from oral solution was rapid reaching peak plasma concentrations within 2 hours with high oral bioavailability (76 to 87%) in fasted rats and monkeys [m2.6.5, Table 3.2; Reports RH2007/00101, RH2007/00103]. When dolutegravir was administered as a suspension, the increase in systemic exposure ($C_{max}$ and $AUC_{0-t}$) was less than proportional to the increase in dose. The oral absorption of dolutegravir from a suspension formulation was lower and suggests that the bioavailability is limited by dissolution rate or solubility. In contrast to humans, administration of dolutegravir with food to rats reduced exposure [see m2.5, Section 3.2 for human effects].

### 3.2.2. Repeat dose toxicokinetics

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

The increase in systemic exposure ($C_{max}$ and $AUC_{0-24}$) to dolutegravir was less than proportional with the increase in repeated doses to mice, non-pregnant rats, rabbits, and monkeys. Differences (>2-fold) in systemic exposure between single and repeated administration, regardless of pregnancy status, or between the sexes were generally not observed. The high permeability and low clearance of dolutegravir provided sufficient systemic exposure in the toxicological species to assess its safety in humans.

Higher systemic exposure to dolutegravir was observed in pre-weaning rat pups (Day 13 post partum) compared to juvenile rats on Day 32 post partum and reflects the early differential expression of uridine glucuronosyl transferase (UGT), the primary drug metabolizing enzymes in the rat [Section 3.4; Kishi, 2008; Saghir, 2012; de Zwart, 2008]. No apparent differences (>2-fold) in systemic exposure between the sexes of juvenile rats were observed.

## 3.3. Distribution

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

### 3.3.1. Protein binding and blood cell association

The in vitro protein binding of dolutegravir was high (≥99%) across species (rat, monkey and human) and similar to an ex vivo assessment (>99%) in plasma from healthy human

subjects [m2.6.5, Table 6.1; Reports RH2007/00106 and 2011N119355; m2.5, Section 3.1.2]. The association of dolutegravir-related material with blood cellular components was minimal [m2.6.5, Table 8.4; Reports RD2009/00562, RD2008/00108, CD2008/00195 and RD2008/01300; m2.5, Section 3.1.2].

### 3.3.2. Efflux mediated transport and cell membrane permeability

In vitro, dolutegravir was a substrate for the human efflux transporters P-glycoprotein (P-gp) and human breast cancer resistance protein (BCRP). Dolutegravir was determined to have high passive membrane permeability (333 nm/s at pH 7.4). The absorptive membrane permeabilities also were high in the presence of FaSSIF at pH 7.4 and pH 5.5 ($P_{7.4[abs]}$ value of 253 nm/s and a $P_{5.5[abs]}$ value of 265 nm/s, respectively) [m2.6.5, Table 8.3; Report RD2008/00360]. Based on solubility and permeability determinations, dolutegravir sodium is classified as a Biopharmaceutics Classification System (BCS) Class 2 drug.

### 3.3.3. Tissue distribution

After a single oral dose of [$^{14}$C]-dolutegravir, radioactivity was widely distributed in a similar pattern between male Lister-Hooded partially pigmented rats and pregnant Sprague-Dawley rats. Radioactivity in tissues generally peaked 4 to 6 hours post dose with concentrations typically less than those in blood [m2.6.5, Table 5.1 and Table 7.1; Reports CD2008/00195 and 2012N137348]. The concentration of radioactivity in the brain was low (~2% of the blood radiocarbon concentration) due in part to restrictive protein binding. By 28 days post-dose, only bone and pigmented skin contained quantifiable concentrations of radioactivity but radioactivity was not associated with melanin in the uveal tract, lowering the concern for a phototoxicity liability (Section 4.8.2). Dolutegravir crossed the placental barrier but appeared to exert no adverse effects on fetal development (Section 4.6; [m1.14.1 (US) or m1.3 (EU)]). Radioactivity rapidly equilibrated to fetal tissue with fetal tissue to fetal blood ratios generally higher than maternal tissue to blood ratios. Dolutegravir concentrations in fetal bone marrow exceeded those in fetal blood.

In lactating rats at 10 days postpartum, radioactivity was detected in milk at concentrations typically higher than blood, with unchanged dolutegravir constituting most (97% to 83%) of the drug-related material [m2.6.5, Table 9.2; Report 2012N132387]. Pre-weaned pups were exposed to dolutegravir (Section 4.6) by nursing in the pre- and post-natal development study.

## 3.4. Metabolism

The comparative biotransformation pathways between animals and humans are presented in Figure 2. The predominant circulating component in the nonclinical species and humans is unchanged dolutegravir with no disproportionate human metabolites observed. The main metabolic route in each species and humans was by conjugation to form the ether glucuronide (M3). These studies confirm the suitability of the mouse, rat, and monkey for the toxicological assessment of dolutegravir.

### 3.4.1. In vitro biotransformation

The in vitro metabolic turnover of dolutegravir was low (<10%) indicating low intrinsic clearance consistent with the low plasma clearance. The primary biotransformation common to all species was glucuronidation to form the ether glucuronide (M3), also observed in vivo. Other metabolic products included a glucose conjugate (M2) and an N-dealkylated product (M1) [m2.6.5, Table 10.1, Table 10.3, Table 10.4 and Table 10.6; Reports RH2007/00076, RD2007/01557, RD2007/01496, RH2007/00058]. The generation of a glutathione or cysteine conjugate through oxidative defluorination with rat, monkey, and human microsomal binding suggested evidence for the formation of an electrophilic metabolic intermediate by bioactivation in vitro [m2.6.5, Table 10.6 and Table 10.3; Reports RH2007/00058, RD2007/01557]. However, in vivo these metabolites have represented only a small fraction of the metabolic clearance in these species and no microscopic liver findings were observed in mice, rats, or monkeys after repeat administration of doses at or below the NOAEL.

No notable metabolic conversion of dolutegravir to any of its possible stereoisomers occurred in vitro following incubations of dolutegravir with cryopreserved rat, dog, monkey and human hepatocytes [m2.6.5, Table 10.5; Report RH2007/00105].

### 3.4.2. In vivo

In vivo, absorbed [$^{14}$C]-dolutegravir was extensively metabolized in male and female mice, rats, and monkeys. A comparative metabolic summary of the products identified in the nonclinical species with products identified in humans is presented in Figure 2 [m2.6.5, Table 9.1; Reports RD2008/00220, RD2008/00899, RD2009/00723; m2.5, Section 3.1.3]. Metabolic profiles in nonclinical species were qualitatively similar to humans with adequate coverage for the circulating human metabolites in at least one nonclinical species.

#### 3.4.2.1. Plasma metabolic profile

Dolutegravir was the predominant component in plasma of mice, rats, monkeys, and humans with the glucuronide as the principal metabolite. No metabolite was present in the plasma at concentrations greater than 10% of parent or drug-related material. The steady-state plasma metabolic profile of dolutegravir was similar to the single dose metabolic profile, indicating data obtained after single dose administration was an adequate predictor of the profile at steady-state [see m2.7.2, Section 3.1.5]. Exposures in the nonclinical metabolism studies adequately reflected exposures in the toxicity studies. No disproportionate human metabolites were noted.

#### 3.4.2.2. In vivo biotransformation

The predominant biotransformation product in mice, rats, and humans was an ether glucuronide (M3), which was formed in approximately equal proportions with a glucose conjugate (M2) in monkeys. These conjugated metabolites, M2 and M3, are not pharmacologically active because they disrupt the two-metal binding capability of the carbamoyl pyridone motif of dolutegravir thereby completely abrogating any antiviral

activity resulting from the active site binding to the integrase enzyme. Although these conjugates were the primary constituents of the drug-related material in bile of animals, they were not observed in the feces of animals or humans. Thus, these dolutegravir conjugates are deconjugated in the intestine by host or bacterial enzymes after secretion in the bile, to reform dolutegravir. Fecal metabolites were not quantifiable in animals but an N-dealkylation product (M1) and a product of oxidative defluorination with cysteine addition (M13) was quantified in human fecal samples.

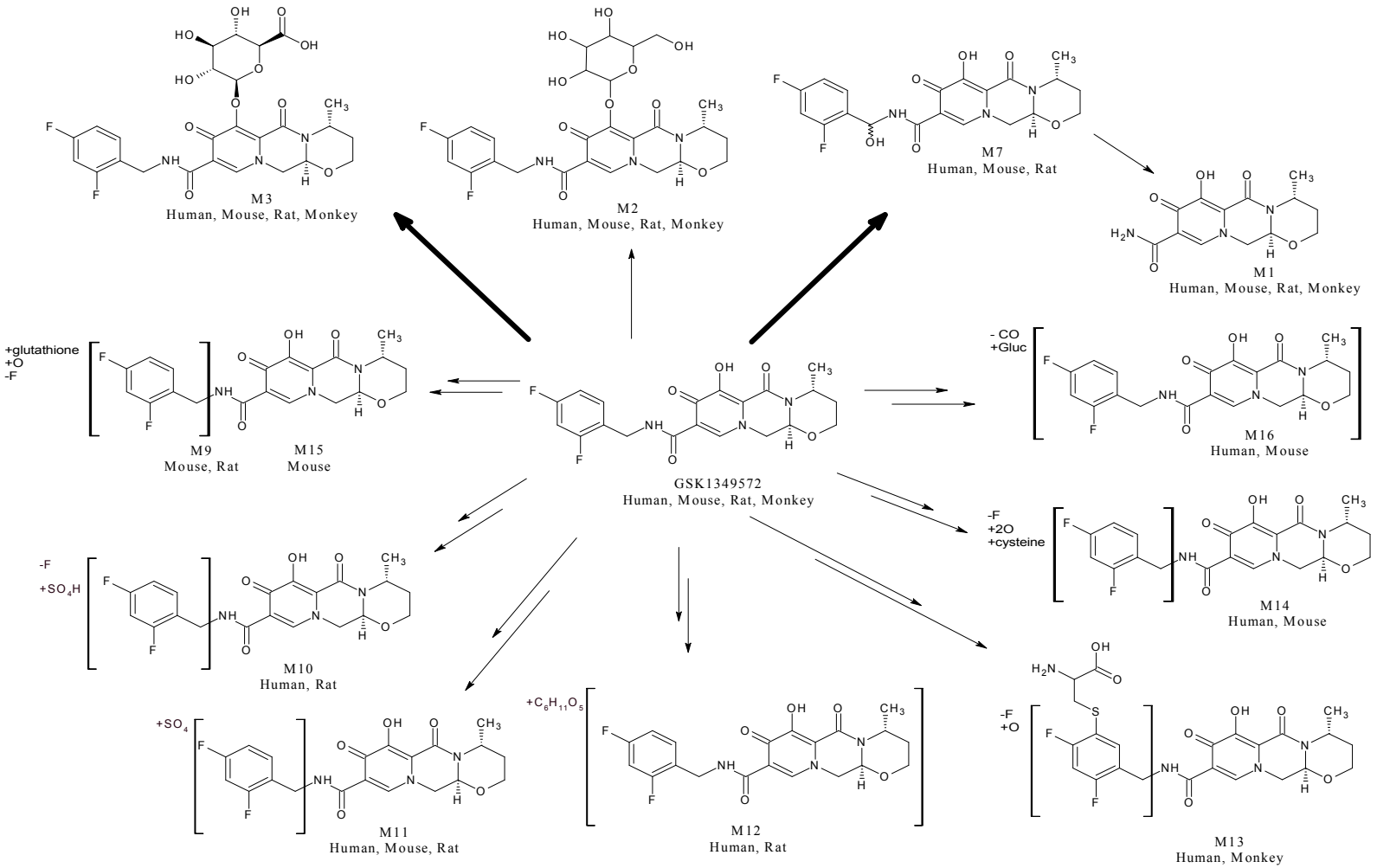
Dolutegravir constituted a very small percentage of drug-related material in the urine and bile in mice, rats, and monkeys or in urine of humans. The primary components of rat urine but representing minor components in mouse and monkey urine were products of oxidation at the benzylic carbon (M7) and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

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

Following repeat oral administration of dolutegravir for 10 days to male and female juvenile rats or to healthy human volunteers, no evidence for the in vivo metabolic conversion of dolutegravir to any of its stereoisomers was observed [m2.6.5, Table 9.3, Report RD2010/00173; and Clinical Study Report RD2008/00860].

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

**Figure 2 Comparative Metabolic Profile of Dolutegravir Between Nonclinical Species and Humans**




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

## 3.5. Excretion

Fecal excretion consisted primarily of unchanged dolutegravir and was the predominant route of elimination of dolutegravir in all species [m2.6.5, Table 13.1; Reports RD2008/00108, RD2008/01299, RD2008/01300 and RD2009/00562]. Urinary excretion of radioactivity was greater in humans [m2.5, Section 3.1.3], which is consistent with the hypothesis of a higher molecular weight threshold for biliary secretion in humans.

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

## 3.6. Pharmacokinetic Drug Interactions

No nonclinical studies have been performed specifically to evaluate potential interactions with drugs that may be co-administered with dolutegravir. However, a series of in vitro studies has been conducted to help evaluate the mechanisms and drug interaction potential of dolutegravir.

### 3.6.1. Potential effect of co-administered agents on dolutegravir

In vitro and in vivo, dolutegravir is primarily metabolized by UGT1A1 with a notable contribution from CYP3A4. UGT1A3 and 1A9 were minor pathways of metabolism [m2.6.5, Table 10.8 and Table 10.9, Reports RD2008/00373 and RD2008/01339]. Therefore, drugs that are strong inducers of UGT1A1 or CYP3A4 may decrease dolutegravir plasma concentrations. Drugs that inhibit UGT1A1 and CYP3A4 may increase dolutegravir plasma concentrations, but based on the clinical interaction study with atazanavir, a potent UGT1A1 and CYP3A4 inhibitor, any increases in dolutegravir concentrations are not expected to be clinically meaningful. Although dolutegravir is a substrate for efflux transporters, no notable effect on dolutegravir pharmacokinetics was observed in humans following coadministration with the efflux transport inhibitors lopinavir/ritonavir [m2.5, Section 3.3.2].

### 3.6.2. Effect of dolutegravir on co-administered agents

In vitro, dolutegravir was noted to have little or no inductive effects on the human Pregnane X Receptor (PXR), on CYP1A2, 2B6 or 3A4 mRNA (as determined by the increase in mRNA relative to vehicle control), or in vivo by autoinduction, or in humans with the CYP3A4 probe, midazolam. Dolutegravir demonstrated little or no inhibition ($IC_{50}$ values > 30 μM) in vitro on the transporters BCRP, multi-drug resistance protein (MRP) 2, organic anion transporting polypeptide (OATP) 1B1, 1B3, organic cation transporter (OCT) 1, and P-gp, or the enzymes CYP1A2, 2A6, 2B6, 2C8, 2C9, 2C19,

2D6, 3A4, UGT1A1 or 2B7 [m2.6.5, Table 8.1 and Tables 12.1 to 12.4]. No inhibition was noted in vivo on the CYP3A4 probe midazolam. Dolutegravir did not notably alter the pharmacokinetics of tenofovir, an organic anion transporter (OAT) and MRP4 substrate [Ray, 2006]. Drug interaction studies conducted in humans indicate that dolutegravir has a low propensity for drug interactions that result in dosage adjustments [m2.5, Section 6.1]. Dolutegravir glucuronide (M3) did not inhibit MRP2, thus inhibition of biliary clearance of bilirubin glucuronides or glucuronide conjugates of co-administered drugs is not expected.

In vitro, dolutegravir inhibited the renal OCT 2 transporter ($IC_{50}$ = 1.9 μM), which provides a mechanistic basis for the non-pathological mild serum creatinine increases observed in clinical studies. Caution should be used when considering co-administration of narrow therapeutic index drugs in which a significant part of their clearance is by renal proximal tubule secretion by OCT2. Dolutegravir is contraindicated for coadministration with the OCT2 substrate dofetilide because of the potential for toxicity due to higher exposure [m2.5, Section 6.2]. Because dolutegravir inhibits OCT2, but only weakly OCT1, and both OCT1 and OCT2 are equally expressed in rat proximal tubules [Tahara, 2005], this effect on creatinine was not observed in rats. In contrast, humans primarily express OCT2 renally and OCT1 in the liver and intestine, which provides one nonpathological explanation for the increase in creatinine noted in humans which was not observed in rats.

As a weak inhibitor of UGT1A1, dolutegravir has the potential to interfere with the conjugation of bilirubin which could result in a mild increase in total or unconjugated bilirubin on prolonged treatment with dolutegravir. Because bilirubin has low solubility and low permeability, it is transported to the UGT1A1 enzymatic site by glutathione-S-transferase. Since dolutegravir has high permeability, this favors dolutegravir access to UGT1A1, although the affinity of bilirubin for UGT1A1 is higher than that of dolutegravir.

# 4. TOXICOLOGY

Dolutegravir has undergone a comprehensive nonclinical toxicological evaluation in studies of appropriate design consistent with ICH requirements in the rat, monkey, mouse, and rabbit to support the clinical use of dolutegravir for the treatment of HIV infection.

Dolutegravir has been evaluated in oral repeat dose toxicity studies for up to 6 months duration in rats and 9 months duration in monkeys at doses selected to identify target organ toxicity and define no-observed adverse effect levels (NOAELs). Dose range finding studies in the mouse were conducted to support dose selection for the mouse carcinogenicity study. Two definitive in vitro and one in vivo genotoxicity study were conducted to assess the mutagenic and clastogenic potential of dolutegravir in bacterial and mammalian systems. The carcinogenic potential of dolutegravir was assessed in mice and rats following oral administration for 104 weeks. In addition, reproductive toxicology studies to assess the effect on reproductive performance, as well as embryofetal and post-natal development, pre- and post-natal development and juvenile

toxicity of dolutegravir were conducted. Immunotoxicity, irritancy and impurities studies were also conducted.

All definitive studies were conducted in accordance with GLP regulations. The toxicokinetic profile of dolutegravir was evaluated in all definitive repeat dose toxicology studies (see Section 3.2.2), which included appropriate analysis of samples from control animals. The impurity profiles of batches used for toxicity studies were representative of those being used in clinical studies.

Throughout this section exposure margins are presented based upon comparison of the animal systemic exposure (end of study gender mean) with that reported for patients receiving 50 mg QD ($C_{max}$ = 3.7 μg/mL; AUC = 53.6 μg.h/mL) or 50 mg BID ($C_{max}$ = 4.2 μg/mL; AUC = 75.1 μg.h/mL) dolutegravir.

The principal nonclinical toxicology finding associated with dolutegravir treatment was primarily gastrointestinal toxicity in rats and monkeys. This finding is discussed below, together with relationships to clinical exposure presented in Table 4.1. A summary of systemic exposure ($C_{max}$ and AUC) to dolutegravir achieved in these studies is presented in Table 4.2.

## 4.1. Choice of Species

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

## 4.2. Single Dose Toxicity

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

A single dose TK study in dogs was conducted at doses up to 500 mg/kg. Dolutegravir was not tolerated and resulted in vomiting at doses ≥150 mg/kg.

## 4.3. Repeat Dose Toxicity

The toxicity of repeated oral gavage doses of dolutegravir has been assessed in rats and monkeys in studies of up to 26 and 38 weeks, respectively [m2.6.7, Table 7.4 and Table 7.7, Reports RD2009/00410 and RD2009/00036].

Principal treatment related effects of dolutegravir in rats and monkeys were related to gastrointestinal toxicity. The NOAEL in the 26 week rat toxicity study was 50 mg/kg/day (Day 180 gender mean $C_{max}$ = 47 μg/mL, $AUC_{0-24}$ = 765 μg.h/mL). Systemic exposure at the NOAEL is ~14X or ~10X above the expected human exposure for a 50 mg QD or BID dose, respectively. The NOAEL in the 38 week monkey toxicity study was 15 mg/kg/day (Day 270 gender mean $C_{max}$ = 5.1 μg/mL, $AUC_{0-24}$ = 39 μg.h/mL). Systemic exposure (AUC) at the NOAEL is ~0.7X or ~0.5X the expected human exposure for a 50 mg QD or BID dose, respectively. However, it should be noted that the exposure margins at the NOAEL in the monkey are greater when compared on a $mg/m^2$ basis [see Section 4.3.2].

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

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

### 4.3.1. Treatment related mortality/morbidity

In a 14 day study, one female monkey given 1000 mg/kg/day died on Day 13 after experiencing daily emesis and diarrhea. This animal's condition deteriorated over the dosing phase and the moribund condition was considered secondary to treatment-related effects on the digestive tract (emesis, diarrhea, ulcer in colon) and resultant significant changes in blood electrolytes. This animal's systemic exposure ($AUC_{0-24}$) on Day 1 was 277 μg.h/mL. Gender-mean Day 14 exposure ($AUC_{0-24}$) at 1000 mg/kg/day was 360 μg.h/mL, which corresponds to ~7X or ~5X above the expected human exposure for a 50 mg QD or BID dose, respectively.

In a 38 week monkey toxicity study, two males in the high dose group (50 mg/kg/day) died or were euthanized on Days 59/55 after signs of gastrointestinal intolerance which consisted of diarrhea and emesis and subsequent body weight loss [m2.6.7, Table 7.7; Report RD2009/00036]. These effects and appropriate safety metrics are described further in the discussion of gastrointestinal effects in Section 4.3.2.

### 4.3.2. Gastrointestinal effects

The primary finding from repeat dose toxicity studies with dolutegravir up to 26 weeks in rats and 38 weeks in monkeys was gastrointestinal (GI) toxicity. In monkeys, the most sensitive species, GI toxicity was characterized primarily by vomiting, diarrhea, and

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

In rats, hemorrhage was observed in the lamina propria of the mucosa at 1000 mg/kg/day in the 4 week toxicity study and was reversible following a 4 week recovery period [m2.6.7, Table 7.3, Report RD2008/01628]. The NOAEL was 100 mg/kg/day. Exposure (end of study, gender mean) at 100 mg/kg/day was 752 μg.h/mL, which corresponds to ~14X or ~10X above the expected human exposure for a 50 mg QD or BID dose, respectively. The NOAEL (100 mg/kg/day) is 100X and 50X the human mg/kg equivalent dose (based on 50 kg human), and 18X and 9X the human $mg/m^2$ equivalent dose for a total daily clinical dose of 50 mg QD or BID, respectively. In the 26 week rat toxicity study, hemorrhage in the glandular stomach mucosa occurred in 1 male at the end of the 17 week dosing period and 1 male at the end of the 26 week dosing period in the 500 mg/kg/day group. No adverse findings were observed at the end of a 4 week recovery period. The NOAEL was 50 mg/kg/day. Exposure (end of study, gender mean) at 50 mg/kg/day was 765 μg.h/mL, which corresponds to ~14X or ~10X above the expected human exposure for a 50 mg QD or BID dose, respectively. The NOAEL (50 mg/kg/day) is 50X and 25X the human mg/kg equivalent dose (based on 50 kg human), and 9X and 4X the human $mg/m^2$ equivalent dose for a total daily clinical dose of 50 mg QD and BID, respectively.

Irritation of the gastrointestinal tract consisting of epithelial atrophy and mucosal hemorrhage in the stomach and lower GI tract (cecum, colon and/or rectum) was noted in monkeys given ≥300 mg/kg/day in the 14 day toxicity study. The NOAEL was 100 mg/kg/day. Exposure (end of study, gender mean) at 100 mg/kg/day was 190 μg.h/mL, which corresponds to ~4X or 3X above the expected human exposure for a 50 mg QD or BID dose, respectively. The NOAEL (100 mg/kg/day) is 100X and 50X the human mg/kg equivalent dose (based on 50 kg human), and 35X and 18X the human $mg/m^2$ equivalent dose for a clinical dose of 50 mg QD and 50 mg BID, respectively.

In the 4 week monkey toxicity study, histopathological changes of the GI tract occurred at 100 mg/kg/day and consisted of slight inflammatory cell infiltration in the lamina propria of the cecum, colon and rectum in both sexes; slight cell debris from the crypts of the cecum and colon in males; and atrophy of the mucosal epithelium of the cecum and colon [m2.6.7, Table 7.6, Report RD2008/00107]. This dose was associated with clinical

signs of vomiting, diarrhea, and body weight loss. The NOAEL was 50 mg/kg/day. Exposure (end of study, gender mean) at 50 mg/kg/day was 132 μg.h/mL, which corresponds to ~2X above the expected human exposure for a 50 mg QD or BID dose. The NOAEL (50 mg/kg/day) is 50X and 25X the human mg/kg equivalent dose (based on 50 kg human), and 18X and 9X the human $mg/m^2$ equivalent dose for a clinical dose of 50 mg QD and BID, respectively.

In the 38 week monkey toxicity study, the 50 mg/kg/day dose was reduced to 30 mg/kg/day on Day 70 for the remainder of the study due to GI intolerance [see Section 4.3.1]. In the 17 week evaluation of the 38 week monkey study, slight mononuclear cell infiltration and hemorrhage in the lamina propria in the cecum and colon were noted in the animal that was euthanized on Day 55. Abnormal feces (observed through Day 131) associated with decreased food consumption and decreased body weight was noted in the 50/30 mg/kg/day group. At the end of the 38 week dosing period, 1 female in the 50/30 mg/kg/day group had adverse findings in the stomach consisting of multifocal mononuclear cell infiltration and slight hemorrhage in the lamina propria, very slight multifocal erosions, and multifocal epithelial regeneration. At the end of a 4 week recovery period, multifocal mononuclear cell infiltration and very slight hemorrhage in the lamina propria and multifocal epithelial regeneration in the stomach were observed in one female. However, the changes in this animal were of lesser severity and there were no active erosions, suggesting recovery of changes upon cessation of treatment. Both animals with stomach lesions had diarrhea/vomiting prior to the dose reduction (50/30 mg/kg/day), but did not have clinical observations of toxicity following the dose reduction. Exposures at end of study for the two affected females were lower compared to the other animals in this dose group ($AUC_{0-24}$ = 43.5 to 48.8 μg.h/mL versus gender mean for 50/30 mg/kg/day group of 61.7 μg.h/mL) and overlapped with exposures at 15 mg/kg/day ($AUC_{0-24}$ range = 25.8 to 54.0 μg.h/mL). This observation is consistent with a local GI toxicity as opposed to a systemic effect.

The NOAEL for the 38 week dosing period was 15 mg/kg/day (Day 270 gender mean $AUC_{0-24}$ and $C_{max}$ of 39 μg.h/mL and 5.1 μg/mL, respectively), which corresponds to 0.7X and 1.4X the human AUC and $C_{max}$ exposure, respectively, for a 50 mg QD dose and corresponds to 0.5X and 1.2X the human AUC and $C_{max}$ exposure, respectively, for a 50 mg BID dose. The NOAEL for the 38 week dosing period (15 mg/kg/day) is 15X and 8X the human mg/kg equivalent dose (based on 50 kg human), and 5X and 3X the human $mg/m^2$ equivalent dose for a 50 mg QD and BID dose, respectively. The NOAEL for the 17 week interim evaluation was also 15 mg/kg/day; thus, there was not a decrease in the NOAEL from 17 weeks of dosing to 38 weeks of dosing.

Nonclinical evidence for GI toxicity with dolutegravir (including vomiting, diarrhea and gastric/colonic erosions) did not translate into significant findings for dolutegravir in double blinded randomized clinical trials, with a similar rate and nature of events reported for dolutegravir compared to raltegravir and efavirenz/tenofovir/emtricitabine (Atripla). Similar rates of nausea, vomiting and diarrhea were reported in studies of subjects with integrase resistance who received dolutegravir 50 mg BID compared to studies of subjects who were integrase naive and received dolutegravir 50 mg once daily [m2.5, Section 6.2]. Therefore, there does not appear to be an increased risk for GI events with this higher dose of dolutegravir.

### 4.3.3. Hepatic effects

Hepatocellular single cell necrosis and diffuse hepatocellular hypertrophy and/or vacuolation occurred in male monkeys given 1000 mg/kg/day in the 14 day study. Additional changes included transient ALT increases at ≥300 mg/kg/day, increased AST, bilirubin, γGTP, and triglycerides at 1000 mg/kg/day and decreased total cholesterol at 1000 mg/kg/day. The NOAEL was 100 mg/kg/day. Exposure (end of study, gender mean) at 100 mg/kg/day was 190 μg.h/mL, which corresponds to ~4X or ~3X above the expected human exposure for a 50 mg QD or BID dose, respectively. In the 38 week monkey toxicity study, liver findings were restricted to increased AST (2.5X) and bilirubin (2.8X) in the moribund animal in the 50 mg/kg/day group (euthanized on Day 55). The findings in the 38 week study were considered secondary to the moribund condition. Exposure (end of study, gender mean) at the NOAEL (15 mg/kg/day) was 39 μg.h/mL, which corresponds to ~0.7X or ~0.5X the expected human exposure for a 50 mg QD or BID dose, respectively. No treatment related adverse effects on liver were observed in rats in studies up to 26 weeks.

Human subjects were carefully monitored for liver effects and cumulative data to date suggests a hepatic safety profile for dolutegravir that is comparable to raltegravir and efavirenz, the comparators used in the Phase III studies [see m2.5, Section 6.2].

### 4.3.4. Renal effects

In the 14 day rat study, there were statistically significant increases in urine specific gravity in males given 500 mg/kg/day and in females given ≥50 mg/kg/day [m2.6.7, Table 7.2, Report RD2007/01140]. Because no treatment-related microscopic findings were observed in the kidneys, the change was not considered toxicologically significant. In the 4 week rat study there was an increased incidence of urine protein and increased urine specific gravity in animals given 1000 mg/kg/day, however, there were no related changes in blood chemistry or microscopic findings, and none of these changes occurred in the rat 26 week study at up to 500 mg/kg/day (Day 180 AUC at 500 mg/kg/day = 1558 μg.h/mL, which corresponds to ~29X or ~21X above the expected human exposure for a 50 mg QD or BID dose, respectively).

Renal tubule dilatation occurred in monkeys given 1000 mg/kg/day in the 14 day study. BUN and creatinine were increased while serum sodium and chloride were decreased in these monkeys. In the 38 week monkey toxicity study, renal findings were restricted to increased BUN (12.5X) and creatinine (3.7X), and slight kidney dilatation of distal renal tubules and cellular and hyaline casts in the moribund animal in the 50 mg/kg/day group (euthanized on Day 55). These findings (in both the 14 day and 38 week monkey toxicity studies) were considered secondary to the moribund condition related to GI toxicity. The NOAEL in the 38 week toxicity study was 15 mg/kg/day. Exposure (end of study, gender mean) at 15 mg/kg/day was 39 μg.h/mL, which corresponds to ~0.7X or ~0.5X the expected human exposure for a 50 mg QD or BID dose, respectively.

Analyses of adverse events from the Renal Systems Organ Class in clinical studies do not suggest that dolutegravir has an adverse effect on renal function [see m2.7.4, Section 3.1.2]. There was a low incidence of renal impairment or failure, and these events were

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

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

### 4.3.5. Bone marrow and lymph node effects

In the 14 day monkey study, hypocellular and/or gelatinous bone marrow and atrophy of the white pulp in the spleen occurred in monkeys given 1000 mg/kg/day and a decrease in the paracortical lymphocytes of the submandibular and/or mesenteric lymph nodes and decreased reticulocytes occurred in monkeys given ≥300 mg/kg/day [m2.6.7, Table 7.5, Report RD2007/01142]. Decreased reticulocytes, RBCs, and platelets and increased APTT occurred in monkeys given 1000 mg/kg/day and are believed to correlate with the microscopic bone marrow changes. The NOAEL was 100 mg/kg/day. Exposure (end of study, gender mean) at 100 mg/kg/day was 190 μg.h/mL, which corresponds to ~4X or ~3X above the expected human exposure for a 50 mg QD or BID dose, respectively. In the 4 week monkey study, decreased RBCs (0.91X) were observed in females given 100 mg/kg/day, with no correlating histopathology findings. The NOAEL was 50 mg/kg/day. Exposure (end of study, gender mean) at 50 mg/kg/day was 132 μg.h/mL, which corresponds to ~2X above the expected human exposure for a 50 mg QD or BID dose. No treatment related adverse effects on bone marrow and lymph nodes were observed in non-moribund animals in the 9 month monkey toxicity study at doses ≤50/30 mg/kg/day. No treatment related adverse effects on bone marrow or lymph nodes were observed in rats in studies up to 26 weeks.

A review of hematology laboratory data from clinical trials revealed no signal for bone marrow or lymph node toxicity caused by dolutegravir [see m2.7.4, Section 3.2].

## 4.4. Genotoxicity

Dolutegravir did not cause gene mutations or chromosomal damage in two definitive in vitro tests (bacterial mutation assay and mouse lymphoma L5178Y cell assay), or in an in vivo oral rat micronucleus test [m2.6.7, Table 8.1, Report WD2007/00514, Table 8.3, Report WD2007/00515 and Table 9.1, Report WD2007/00513]. Therefore, based on these data, dolutegravir does not pose a genetic toxicity risk to humans.

## 4.5. Carcinogenicity

The carcinogenic potential of dolutegravir was assessed in mice and rats following oral administration for 2 years [m2.6.7, Table 10.1, Report 2012N152419 and Table 10.2, Report 2012N152418]. Based on recommendations from the FDA Executive Carcinogenicity Assessment Committee [FDA, 2010], the doses studied were 7.5, 25, or

500 mg/kg/day in CD-1 mice and 2, 10, or 50 mg/kg/day in Sprague Dawley rats, administered via oral gavage once daily in a vehicle of 0.5% HPMC and 0.1% Tween 80 (a water control group was also included). The high dose in each study was based on saturation of absorption and concern for GI effects over the course of 2 years. Dose spacing was based on AUC.

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

The NOAEL for non-neoplastic findings after chronic oral administration was the high dose of 500 mg/kg/day for mice and 50 mg/kg/day for rats. When compared to the expected human exposure for a 50 mg QD or BID dose, the systemic exposures were ~20X or ~14X higher for mice and ~17X or ~12X higher rats.

## 4.6. Reproductive and Developmental Toxicity

There were no effects on fertility or early embryonic development in rats orally administered dolutegravir at ≤1000 mg/kg/day in males or females [m2.6.7, Table 12.1; Report XD2009/00368]. The NOAEL was 1000 mg/kg/day, which corresponds to ~33X or ~24X above the expected human exposure for a 50 mg QD or BID dose, respectively, based on gender averaged mean exposures achieved in the 4 week rat toxicity study.

No adverse effects on fetal development were observed in pregnant rats orally administered dolutegravir at ≤1000 mg/kg/day [m2.6.7, Table 13.1; Report XD2009/00367]. The NOAEL for maternal and fetal toxicity was 1000 mg/kg/day, which corresponds to ~38X or ~27X above the expected human exposure for a 50 mg QD or BID dose, respectively.

In an embryofetal development study in rabbits, dolutegravir was orally administered at 40, 200, or 1000 mg/kg/day to pregnant rabbits [m2.6.7, Table 13.2; Report XD2009/00366]. Suppressed body weight gain (13.6% on gestation Day 19), decreased food consumption (up to 53%) and scant or no feces/urine associated with the decreased food consumption were noted in the 1000 mg/kg/day dams. The NOAEL was 200 mg/kg/day for maternal general toxicity (~0.27X or ~0.19X the expected human exposure for a 50 mg QD or BID dose, respectively) and 1000 mg/kg/day for maternal reproductive function and embryofetal development (0.56X or ~0.40X the expected human exposure for a 50 mg QD or BID dose, respectively).

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

In a pre-and post-natal development study, dolutegravir was administered to female rats at doses of 5, 50 or 1000 mg/kg/day from Day 6 of gestation to Day 20 of lactation [m2.6.7, Table 14.1; Report 2011N121663]. Suppressed body weight gain and decreased food consumption were noted in dams (F0) in the 1000 mg/kg/day group during the lactation period, which were associated with mild decreases in body weights in the

offspring in the 1000 mg/kg/day group from pre-weaning until adolescence. There were no adverse effects on maternal pregnancy, parturition, lactation or offspring (F1) survival, behavioral or reproductive function. The NOAEL for maternal reproductive function was 1000 mg/kg/day (~32X or ~23X above the expected human exposure for a 50 mg QD or BID dose, respectively, based on exposures achieved in female rats in the 4 week toxicity study). Due to the decreased body weights of the offspring observed at higher doses, the NOAEL for pre- and postnatal development of the offspring (F1) was 50 mg/kg/day. At this dose, the expected human exposure is ~25X or 18X above a 50 mg QD or BID dose, respectively (extrapolated from gender mean exposures achieved in the rat 14 day toxicity study). Based on the fact that effects on offspring body weights were noted at doses where maternal toxicity was observed, and the presence of considerable safety margins expected at the proposed clinical doses, there is minimal risk for adverse effects on postnatal development in offspring of mothers receiving dolutegravir.

Dolutegravir is excreted in the milk of lactating rats. Following oral administration (50 mg/kg) to lactating rats on Day 10 post partum, total radiocarbon concentrations in milk were up to 2-fold greater than those in maternal blood. The metabolite profile of milk indicated that parent dolutegravir represented more than 95% of the total radiocarbon, consistent with the findings in plasma from female rats in an earlier study [m2.6.5, Table 9.2; Report 2012N132387]. These data suggest that F1 offspring in the pre- and postnatal toxicity study were exposed to the drug via the milk [m2.6.7, Table 14.1, Report 2011N121663]. Following oral administration of dolutegravir (50 mg/kg) to pregnant rats on Day 18 post conception, dolutegravir-related material was found, by QWBA analysis, to be widely distributed to the fetuses over the 24-hour sampling period. These data indicate that dolutegravir is able to cross the placental barrier [see above Section 3.3.3 and m1.14.1 (US) or m1.3 (EU)].

## 4.7. Juvenile Toxicity Studies

The definitive juvenile rat toxicity study has been completed in accordance with the agreed EU Paediatric Investigational Plan (PIP) [EMEA-000409-PIP01-08] to support clinical trials in paediatric patients.

A juvenile toxicity study in rats was conducted with dolutegravir at oral doses of 0.5, 2 or 75 mg/kg/day from Day 4 to 66 postpartum (pp) [m2.6.7, Table 15.1; Report CD2010/00023]. Two preweanling deaths were considered test article related at 75 mg/kg/day. Over the preweaning treatment period (Day 4 to 21 pp), mean body weight gain was decreased (0.86X control mean gain) for males and females in the 75 mg/kg/day group and the decrease persisted throughout the entire study for females during the postweaning period. There were no test article-related differences among the groups for the age at which offspring attained physical signs of sexual maturation (vaginal opening or balano-preputial skinfold separation). There were no changes considered related to dolutegravir administration in stage-dependent evaluation of spermatogenesis. There were no test article-related effects on T cell dependent antibody response (TDAR) measured on Day 67, and no effects on lymphocyte subsets (T cells, both CD4 and CD8 subsets, and B cells) and CD4 or CD8 T cell receptor Vβ usage in peripheral blood [see also Section 4.8.1.2]. Therefore, the NOAEL in juvenile rats was 2 mg/kg/day (Day 32 pp gender mean $AUC_{0-24}$ = 90 μg.h/mL and $C_{max}$ = 7.6 μg/mL).

Clinical studies in pediatric patients conducted to date have not revealed any safety issues specific to this population [m2.5, Section 6.2].

## 4.8. Other Studies

### 4.8.1. Immunotoxicity

#### 4.8.1.1. Immunotoxicity assessment in adults

Considering that the intended patient population is immunocompromised HIV infected patients, a T cell dependent antibody response (TDAR) study was conducted in rats to evaluate immunotoxicity potential. Oral administration of dolutegravir at doses up to 1000 mg/kg/day for 1 month had no effect on keyhole limpet hemocyanin (KLH) antibody titers in rats, thereby demonstrating no immunosuppressive effect of dolutegravir on a TDAR (exposure ~33X or ~24X above the expected human exposure for a 50 mg QD or BID dose, respectively, based on exposures achieved in the rat 4 week toxicity study) [m2.6.7, Table 17.1, Report RD2009/00751]. Furthermore, there were no signs of immunotoxicity from general toxicology study findings or clinical safety data. Therefore, there is a negligible risk of immunotoxicity potential to adult patient populations treated with dolutegravir.

#### 4.8.1.2. Immunotoxicity assessment in juveniles

A concern for immunotoxicity potential was theorized for juveniles based on a publication demonstrating that two HIV integrase inhibitor compounds (p8 [5CITEP] and p10 [L-708,906]) have activity on recombination activating gene (RAG1/2) and therefore may affect T and B cell repertoire development [Melek, 2002]. To address the potential effects of dolutegravir on RAG1/2, immunotoxicity endpoints (TDAR, immunophenotyping and TCRVβ usage) were added to the definitive rat juvenile toxicity study (see above Section 4.7). There were no test article-related effects on immunologic competence as measured by TDAR, and no effects on lymphocyte subset counts (T cells, both CD4 and CD8 subsets, and B cells) and CD4 or CD8 T cell receptor Vβ usage in peripheral blood. Histopathology of immunologic organs (spleen, thymus, lymph nodes) and hematology evaluation revealed no effects. The NOAEL for immunotoxicity endpoints was 75 mg/kg/day. These results provided a robust nonclinical assessment of potential developmental immunotoxicologic effects and suggest no unusual drug-specific risk of developmental immunotoxicity in juvenile animals.

### 4.8.2. Phototoxicity

In the absorption spectrum for dolutegravir there are minor peaks at 310, 325 and 340 nm, with a tail extension to 395 nm, in the region of concern for photosafety [see CMC m2.3.S.1.3]. A whole body autoradiography (WBA) study in rats following a single oral administration of [$^{14}$C]-dolutegravir (sodium salt) demonstrated wide tissue distribution of drug-related material [see Section 3.3.3]. The concentration of dolutegravir-related material in pigmented skin and uveal tract peaked at 4 to 6 hours post-dose and declined in parallel with blood levels. The tissue concentrations were

below or at the limit of detection by 7 days post-dose in the uveal tract and by 28 days in pigmented skin.

No drug-related toxicity has been identified in the eye or skin during repeat dose oral toxicity studies of up to 26 weeks in the Sprague Dawley rat or 38 weeks in the cynomolgus monkey [see Section 4.3]. Potential toxic effects on the eye and skin were assessed during these studies by ophthalmoscopy, macroscopic and microscopic examination. A review of data from clinical studies revealed no signal for photosafety caused by dolutegravir. Therefore, the risk for photosensitivity reactions in patients treated with dolutegravir is considered minimal.

### 4.8.3. Irritancy

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

### 4.8.4. Impurities

The proposed drug substance specifications [see m3.2.S.4.5] for dolutegravir indicate that the specified impurities, gsk006*, gsk007*, gsk008*, gsk009*, gsk010*, gsk011* (enantiomer) and gsk012* (diastereomer) do not exceed the 0.15% w/w ICH qualification threshold [ICH Q3A (R2)]. Based on an in silico assessment that included use of the DEREK software program (v13 Lhasa Ltd), all 7 impurities were considered to not be potentially genotoxic.

An assessment of the route of synthesis for dolutegravir has been conducted to determine whether any impurities might be present in final drug product which are known or suspected DNA-reactive mutagens [m2.6.6, Section 4.4]. Clinically, where dolutegravir will be administered in tablets at a dose of 100 mg/day, there are no impurities of mutagenic concern present at a level that would exceed the threshold of toxicological concern (TTC) as defined by the CHMP guidelines on the limits of genotoxic impurities (i.e., >1.5 µg/day [EMEA CHMP/SWP/5199/02]).

* 新薬承認情報提供時に置き換え

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

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

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

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

## Table 4.2 Comparative Assessment of Mean Systemic Exposure Following Oral Administration of Dolutegravir

| Species (Duration) | Dose (mg/kg/day) | Sex | Cmax (µg/mL) | | AUC0-24 (µg.h/mL) | | Animal to Human AUC Ratio[a,b] (50mg QD) | Animal to Human AUC Ratio[a,c] (50mg BID) |
|---|---|---|---|---|---|---|---|---|
| | | | Day 1 | End of Study | Day 1 | End of Study | | |
| Rat[d] (14 days) | 50 | M | 58.5 | 65.7 | 881 | 1040 | 19.4 | 13.8 |
| | | F | 75.4 | 95.6 | 1110 | 1610 | 30.0 | 21.4 |
| | 150 | M | 82.7 | 74.1 | 994 | 1150 | 21.5 | 15.3 |
| | | F | 83.3 | 106 | 1050 | 1740 | 32.5 | 23.2 |
| | **500 (NOAEL)** | **M** | **87.1** | **108** | **1360** | **1710** | **31.9** | **22.8** |
| | | **F** | **117** | **124** | **1350** | **1950** | **36.4** | **30.0** |
| Rat[d] (4 weeks) | 2 | M | 3.5 | 4.7 | 39.3 | 53.0 | 0.99 | 0.71 |
| | | F | 4.6 | 7.8 | 60.0 | 81.7 | 1.5 | 1.2 |
| | 10 | M | 15.2 | 23.7 | 220 | 274 | 5.1 | 3.6 |
| | | F | 21.2 | 34.6 | 278 | 378 | 7.1 | 5.0 |
| | **100 (NOAEL)** | **M** | **43.7** | **49.2** | **693** | **722** | **13.5** | **9.6** |
| | | **F** | **54.4** | **61.6** | **775** | **781** | **14.6** | **10.4** |
| | 1000 | M | 95.1 | 119 | 1678 | 1837 | 34.3 | 24.5 |
| | | F | 116 | 112 | 1615 | 1737 | 32.4 | 23.1 |
| Rat (26 weeks)[d] | 5 | M | 9.4 | 11.9 | 88.8 | 116 | 2.2 | 1.5 |
| | | F | 12.0 | 20.1 | 138 | 290 | 5.4 | 3.9 |
| | **50 (NOAEL)** | **M** | **47.3** | **38.0** | **637** | **607** | **11.3** | **8.1** |
| | | **F** | **56.5** | **56.6** | **731** | **922** | **17.2** | **12.3** |
| | 500 | M | 95.3 | 85.1 | 1450 | 1338 | 30.0 | 17.8 |
| | | F | 103.5 | 107 | 1450 | 1777 | 33.2 | 23.7 |
| Monkey[d] (14 days) | **100 (NOAEL)** | **M** | **21.3** | **24.0** | **172** | **192** | **3.6** | **2.6** |
| | | **F** | **18.9** | **23.0** | **150** | **187** | **3.5** | **2.5** |
| | 300 | M | 30.9 | 21.7 | 324 | 199 | 3.7 | 2.6 |
| | | F | 17.5 | 23.3 | 142 | 271 | 5.1 | 3.6 |
| | 1000 | M | 27.5 | 26.2 | 358 | 364 | 6.8 | 4.8 |
| | | F | 20.9 | 30.3 | 237 | 354 | 6.6 | 4.7 |
| Monkey[d] (4 weeks) | 25 | M | 8.7 | 13.9 | 60.3 | 108 | 2.0 | 1.4 |
| | | F | 11.2 | 15.4 | 67.9 | 83.9 | 1.6 | 1.1 |
| | **50 (NOAEL)** | **M** | **9.6** | **14.5** | **72.9** | **111** | **2.1** | **1.5** |
| | | **F** | **10.7** | **20.4** | **68.7** | **153** | **2.9** | **2.0** |
| | 100 | M | 12.1 | 16.0 | 99.0 | 148 | 2.8 | 2.0 |
| | | F | 12.2 | 13.4 | 90.2 | 92.0 | 1.7 | 1.2 |
| Monkey (38 weeks)[e] | 3 | M | 2.9 | 3.0 | 15.2 | 18.9 | 0.35 | 0.25 |
| | | F | 3.1 | 2.3 | 15.3 | 15.5 | 0.29 | 0.21 |
| | 10 | M | 4.7 | 4.4 | 30.7 | 32.3 | 0.60 | 0.43 |
| | | F | 6.1 | 5.1 | 34.5 | 37.7 | 0.70 | 0.50 |
| | **15 (NOAEL)** | **M** | **7.7** | **5.3** | **46.4** | **36.7** | **0.68** | **0.49** |
| | | **F** | **5.5** | **4.8** | **30.6** | **40.9** | **0.76** | **0.54** |
| | 50/30 | M | 9.0 | 7.5 | 62.9 | 61.7 | 1.2 | 0.82 |
| | | F | 10.5 | 7.8 | 63.4 | 61.7 | 1.2 | 0.82 |

**Table 4.2 (Continued) Comparative Assessment of Mean Systemic Exposure Following Oral Administration of Dolutegravir**

| Species (Duration) | Dose (mg/kg/day) | Sex | Cmax (μg/mL) | | AUC0-24 (μg.h/mL) | | Animal to Human AUC Ratio[a,b] (50mg QD) | Animal to Human AUC Ratio[a,c] (50mg BID) |
|---|---|---|---|---|---|---|---|---|
| | | | Day 1 | End of Study | Day 1 | End of Study | | |
| Mouse (14 day)[f] | 10 | M | 16.4 | 14.9 | 188 | 218 | 4.1 | 2.9 |
| | | F | 18.3 | 18.7 | 195 | 188 | 3.5 | 2.5 |
| | 100 | M | 62.7 | 60.6 | 921 | 801 | 14.9 | 10.7 |
| | | F | 62.0 | 73.4 | 980 | 1170 | 21.8 | 15.6 |
| | 500 | M | 96.5 | 77.0 | 1140 | 1090 | 20.3 | 14.5 |
| | | F | 93.2 | 90.7 | 1100 | 1190 | 22.2 | 15.8 |
| | **1500 (NOAEL)** | **M** | **106** | **104** | **1210** | **1240** | **23.1** | **16.5** |
| | | **F** | **115** | **123** | **1520** | **1630** | **30.4** | **21.7** |
| Mouse (13-week)[f] | 10 | M | 16.0 | 18.5 | 211 | 257 | 4.8 | 3.4 |
| | | F | 19.3 | 28.0 | 212 | 256 | 4.8 | 3.4 |
| | 50 | M | 43.9 | 52.7 | 477 | 653 | 12.2 | 8.7 |
| | | F | 53.7 | 62.6 | 528 | 740 | 13.8 | 9.9 |
| | 500 | M | 77.3 | 82.1 | 923 | 1010 | 18.8 | 13.4 |
| | | F | 88.3 | 109 | 1110 | 1300 | 24.3 | 17.3 |
| | **1500 (NOAEL)** | **M** | **109** | **103** | **1440** | **1320** | **24.6** | **17.6** |
| | | **F** | **114** | **118** | **1420** | **1350** | **25.2** | **18.0** |
| Rat (embryofetal development) | 100 | F | 64.1 | 78.0 | 949 | 1252 | 23.4 | 16.7 |
| | 300 | F | 74.0 | 82.2 | 1096 | 1409 | 26.3 | 18.8 |
| | **1000 (NOAEL)[g]** | **F** | **139** | **109** | **1841** | **2032** | **37.9** | **27.1** |
| Rabbit (embryofetal development) | 40 | F | 0.8 | 1.3 | 2.1 | 2.6 | 0.049 | 0.035 |
| | 200[h] | F | 1.5 | 1.7 | 15.6 | 14.5 | 0.27 | 0.19 |
| | 1000[h] | F | 2.3 | 2.1 | 36.8 | 30.1 | 0.56 | 0.40 |
| Rat (Juvenile)[i] | 0.5 | M | 4.6 | 1.5 | 92.0 | 9.9 | 0.18 | 0.13 |
| | | F | 4.7 | 2.4 | 86.5 | 27.1 | 0.51 | 0.36 |
| | **2 (NOAEL)** | **M** | **15.2** | **7.7** | **303** | **85.7** | **1.6** | **1.1** |
| | | **F** | **16.4** | **7.5** | **316** | **93.3** | **1.7** | **1.2** |
| | 75 | M | 88.0 | 69.9 | 1540 | 917 | 17.1 | 12.2 |
| | | F | 85.4 | 77.4 | 1549 | 1044 | 19.5 | 13.9 |
| Mouse (Carcino-genicity)[f, j] | 7.5 | M | 13.4 | 14.5 | 176 | 148 | 2.8 | 2.0 |
| | | F | 20.3 | 16.6 | 235 | 157 | 2.9 | 2.1 |
| | 25 | M | 40.8 | 27.4 | 579 | 327 | 6.1 | 4.4 |
| | | F | 44.4 | 43.3 | 565 | 494 | 9.2 | 6.6 |
| | **500 (NOAEL)** | **M** | **77.7** | **71.9** | **1180** | **953** | **17.8** | **12.7** |
| | | **F** | **94.7** | **94.5** | **1300** | **1210** | **24.3** | **16.1** |
| Rat (Carcino-genicity)[k] | 2 | M | 7.3 | 7.7 | 101 | 100 | 1.9 | 1.3 |
| | | F | 8.8 | 20.4 | 114 | 279 | 5.2 | 3.7 |
| | 10 | M | 24.7 | 21.3 | 348 | 340 | 6.3 | 4.5 |
| | | F | 34.3 | 40.4 | 501 | 731 | 13.6 | 9.7 |
| | **50 (NOAEL)** | **M** | **57.6** | **40.9** | **841** | **713** | **13.3** | **9.5** |
| | | **F** | **87.0** | **68.5** | **1150** | **1140** | **21.3** | **15.2** |

**Table 4.2 (Continued) Comparative Assessment of Mean Systemic Exposure Following Oral Administration of Dolutegravir**

| Species (Duration) | Dose (mg/kg/day) | Sex | Cmax (μg/mL) | | AUC0-24 (μg.h/mL) | | Animal to Human AUC Ratio[a,b] (50mg QD) | Animal to Human AUC Ratio[a,c] (50mg BID) |
|---|---|---|---|---|---|---|---|---|
| | | | Day 1 | End of Study | Day 1 | End of Study | | |
| Human[b] | 50 mg | M/F | 3.7 | | 53.6 | | NA | NA |
| Human[c] | 100 mg | M/F | 4.2 | | 75.1 | | NA | NA |

**Key:** The systemic exposure margins within the main body text of m2.4 are presented as gender averaged means.

a. Calculated for AUC based on end of treatment values
b. Based on the geometric mean of systemic human exposure AUC and Cmax values at a total daily dose of 50 mg from pooled data of Spring-1 and Spring-2
c. Based on the geometric mean of systemic human exposure AUC and Cmax values at a total daily dose of 100 mg (50 mg BID) from pooled data of Viking and SAILING.
d. Values are the mean of n=3 to 5.
e. Values are the mean of n= 7 to 9.
f. Composite plasma toxicokinetic parameters from mice, n=3/sex/group/time point.
g. The NOAEL was 1000 mg/kg/day for dams and embryo-fetal development.
h. The NOAEL was 200 mg/kg/day for maternal general toxicity and 1000 mg/kg/day for maternal reproductive function and embryofetal development.
i. Composite plasma toxicokinetic parameters for juvenile rats were examined on Day 13pp and Day 32pp. Composite parameters were derived from mean plasma concentration data. n=3/timepoint/dose, with the exception of the 8 hour timepoint on Day 13pp following 75 mg/kg/day, for which n=4.
j. Toxicokinetics conducted on Day 26 and Day 182 instead of Day 1 and End of Study, respectively.
k. Values are the mean of n=4/sex/group. Toxicokinetics conducted on Day 28 and Day 182 instead of Day 1 and End of Study, respectively.

**Note:** No observed adverse effect levels (NOAEL) are bolded. Values in parenthesis represent the range. QD = once daily. BID = twice daily.

**Table 4.3 Comparative Assessment of Mean Animal to Human Exposure Ratios (AUC, Mg/Kg and Mg/M$^2$) Following Oral Administration of Dolutegravir in the 4 and 26 week Rat Toxicology Studies**

| Species (Duration) | Dose (mg/kg/day) | Sex | Cmax (μg/mL) | | $AUC_{0-24}$ (μg.h/mL) | | ANIMAL TO HUMAN RATIOS Based on AUC, MG/KG and MG/M$^2$ (gender averaged means) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Day 1 | End of Study | Day 1 | End of Study | AUC (50mg QD) | AUC (50mg BID) | Mg/Kg (50mg QD) | Mg/Kg (50mg BID) | Mg/M$^2$ (50mg QD) | Mg/M$^2$ (50mg BID) |
| Rat (4 weeks) | 2 | M | 3.5 | 4.7 | 39.3 | 53.0 | 1.25 | 0.96 | 2 | 1 | 0.35 | 0.18 |
| | | F | 4.6 | 7.8 | 60.0 | 81.7 | | | | | | |
| | 10 | M | 15.2 | 23.7 | 220 | 274 | 6.1 | 4.3 | 10 | 5 | 1.8 | 0.88 |
| | | F | 21.2 | 34.6 | 278 | 378 | | | | | | |
| | **100 (NOAEL)** | **M** | **43.7** | **49.2** | **693** | **722** | **14.1** | **10.0** | **100** | **50** | **17.6** | **8.8** |
| | | **F** | **54.4** | **61.6** | **775** | **781** | | | | | | |
| | 1000 | M | 95.1 | 119 | 1678 | 1837 | 33.4 | 23.8 | 1000 | 500 | 176 | 88 |
| | | F | 116 | 112 | 1615 | 1737 | | | | | | |
| Rat (26 weeks) | 5 | M | 9.4 | 11.9 | 88.8 | 116 | 3.8 | 2.7 | 5 | 2.5 | 0.88 | 0.44 |
| | | F | 12.0 | 20.1 | 138 | 290 | | | | | | |
| | **50 (NOAEL)** | **M** | **47.3** | **38.0** | **637** | **607** | **14.3** | **10.2** | **50** | **25** | **8.8** | **4.5** |
| | | **F** | **56.5** | **56.6** | **731** | **922** | | | | | | |
| | 500 | M | 95.3 | 85.1 | 1450 | 1338 | 31.6 | 20.8 | 500 | 250 | 88 | 45 |
| | | F | 103.5 | 107 | 1450 | 1777 | | | | | | |
| Human | 50 mg | M/F | 3.7 | | 53.6 | | NA | NA | NA | NA | NA | NA |
| Human | 100 mg | M/F | 4.2 | | 75.1 | | NA | NA | NA | NA | NA | NA |

**Key:**
Calculations are based on 50 kg human.
For conversion of animal doses in mg/kg to dose in mg/m$^2$ , multiply by Km (Km rat = 6; Km human = 34).

**Table 4.4 Comparative Assessment of Mean Animal to Human Exposure Ratios (AUC, Mg/Kg and Mg/M$^2$) Following Oral Administration of Dolutegravir in the 14 Day and 4 and 38 Week Monkey Toxicology Studies**

| Species (Duration) | Dose (mg/kg/day) | Sex | Cmax (μg/mL) | | AUC0-24 (μg.h/mL) | | ANIMAL TO HUMAN RATIOS Based on AUC, MG/KG and MG/M$^2$ (gender averaged means) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Day 1 | End of Study | Day 1 | End of Study | AUC (50mg QD) | AUC (50mg BID) | Mg/Kg (50mg QD) | Mg/Kg (50mg BID) | Mg/M$^2$ (50mg QD) | Mg/M$^2$ (50mg BID) |
| Monkey (14 days) | **100 (NOAEL)** | **M** | **21.3** | **24.0** | **172** | **192** | **3.6** | **2.6** | **100** | **50** | **35** | **17.6** |
| | | **F** | **18.9** | **23.0** | **150** | **187** | | | | | | |
| | 300 | M | 30.9 | 21.7 | 324 | 199 | 4.4 | 3.1 | 300 | 150 | 105 | 53 |
| | | F | 17.5 | 23.3 | 142 | 271 | | | | | | |
| | 1000 | M | 27.5 | 26.2 | 358 | 364 | 6.7 | 4.8 | 1000 | 500 | 353 | 176 |
| | | F | 20.9 | 30.3 | 237 | 354 | | | | | | |
| Monkey (4 weeks) | 25 | M | 8.7 | 13.9 | 60.3 | 108 | 1.8 | 1.3 | 25 | 12.5 | 8.8 | 4.4 |
| | | F | 11.2 | 15.4 | 67.9 | 83.9 | | | | | | |
| | **50 (NOAEL)** | **M** | **9.6** | **14.5** | **72.9** | **111** | **2.5** | **1.8** | **50** | **25** | **17.6** | **8.8** |
| | | **F** | **10.7** | **20.4** | **68.7** | **153** | | | | | | |
| | 100 | M | 12.1 | 16.0 | 99.0 | 148 | 2.3 | 1.6 | 100 | 50 | 35 | 17.6 |
| | | F | 12.2 | 13.4 | 90.2 | 92.0 | | | | | | |
| Monkey (38 weeks) | 3 | M | 2.9 | 3.0 | 15.2 | 18.9 | 0.32 | 0.23 | 3 | 1.5 | 1.1 | 0.53 |
| | | F | 3.1 | 2.3 | 15.3 | 15.5 | | | | | | |
| | 10 | M | 4.7 | 4.4 | 30.7 | 32.3 | 0.65 | 0.47 | 10 | 5 | 3.5 | 1.8 |
| | | F | 6.1 | 5.1 | 34.5 | 37.7 | | | | | | |
| | **15 (NOAEL)** | **M** | **7.7** | **5.3** | **46.4** | **36.7** | **0.72** | **0.52** | **15** | **7.5** | **5.3** | **2.6** |
| | | **F** | **5.5** | **4.8** | **30.6** | **40.9** | | | | | | |
| | 50/30 | M | 9.0 | 7.5 | 62.9 | 61.7 | 1.2 | 0.82 | 50/30 | 25/15 | 17.6/10.6 | 8.8/5.3 |
| | | F | 10.5 | 7.8 | 63.4 | 61.7 | | | | | | |
| Human | 50 mg | M/F | 3.7 | | 53.6 | | NA | NA | NA | NA | NA | NA |
| Human | 100 mg | M/F | 4.2 | | 75.1 | | NA | NA | NA | NA | NA | NA |

**Key:**
Calculations are based on 50 kg human.
For conversion of animal doses in mg/kg to dose in mg/m$^2$ , multiply by Km (Km monkey = 12; Km human = 34).

# 5. INTEGRATED OVERVIEW AND CONCLUSIONS

Nonclinical studies have demonstrated that dolutegravir has low nanomolar activity against wild type HIV-1 in a variety of cells lines, regardless of subtype, and is additive or synergistic when assayed in combination with other antiretroviral agents. Passage of various HIV-1 strains and subtypes led to isolation of mutations with fold-change increases in $IC_{50}$ of 3.1 (E92Q), 3.2 (G193E), and ≤4.1 (S153Y or S153F). Comparative susceptibilities to dolutegravir and RAL were obtained from 60 RAL-resistant site directed HIV-1 mutants and 6 site directed HIV-2 mutants. Dolutegravir retained activity against a vast majority of these mutants. Additionally, susceptibilities to dolutegravir and RAL were determined for over 700 RAL-resistant clinical isolates, with dolutegravir retaining activity (<10 FC) against >90% of them. To obtain a better understanding of INI dissociation kinetics and assist in understanding dolutegravir's distinct resistance profile and resistance mechanisms, the dissociation of dolutegravir, RAL, and EVG from wild type and mutant IN proteins complexed with DNA was investigated. Dolutegravir demonstrated overall substantially slower dissociation from IN-DNA complexes tested, including those with single and double residue IN substitutions.

The nonclinical pharmacokinetics of dolutegravir were similar across the primary species (mouse, rat and monkey) used for pharmacological and toxicological evaluation. In each species, plasma clearance was low, relative to liver plasma flow. The nonclinical protein binding of dolutegravir across species was high (>99%) and not different from the in vitro and ex vivo assessment in human plasma. Absorption was rapid and extensive from solution, but was limited by solubility from suspensions and tablet formulations. The increase in exposure to dolutegravir generally was less than proportional to the increase in dose in toxicological evaluations. The absorbed dose was extensively metabolized in each species, with dolutegravir the predominant drug-related component in plasma which is also consistent with observations in humans [see m2.5 Clinical Overview, Section 3.2].

Dolutegravir is highly permeable and widely distributed to tissues of the rat, including crossing the placental barrier and secretion into milk of nursing rats. After an oral dose, dolutegravir was extensively metabolized, secreted in bile and eliminated in feces. The major metabolites were an ether glucuronide (M3) and a product of oxidation (M7) which was converted to an N-dealkylated product (M1). In humans, the biotransformation of dolutegravir was mediated predominantly by UGT1A1 with CYP3A4 contributing to a lesser extent. No disproportionate human metabolites were observed and the selection of the species for toxicity testing was validated.

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

coadministration with the OCT2 substrate dofetilide because of the potential for toxicity due to higher exposure [m2.5, Section 6.2].

Following oral administration of dolutegravir to rats and monkeys, toxicologic effects were noted in the gastrointestinal tract and liver. The principal dose-limiting toxicity in animals was gastrointestinal effects, which are clinically monitorable. There were no significant findings in safety pharmacology studies in animals evaluating the cardiovascular and respiratory systems and in a general behavioral study conducted with dolutegravir.

The primary finding from repeat dose toxicity studies up to 26 weeks in rats and 38 weeks in monkeys with dolutegravir was GI toxicity. In monkeys, the most sensitive species, GI toxicity was characterized primarily by vomiting, diarrhea, and associated mortality as well as gastrointestinal lesions, and by gastric lesions in the rat. In both species, these effects were observed at progressively lower doses with increased study duration. The GI toxicity is believed to be the result of local drug administration at the mucosal surface of the gut following oral dosing, rather than systemic toxicity. The fact that affected animals had comparable exposures to animals at dose levels which were not affected is supportive of the conclusion that the GI toxicity is due to the larger local exposure in the GI tract in those dose groups. Therefore, mg/kg or $mg/m^2$ metrics are appropriate determinates of safety cover for this toxicity because it is not based on systemic exposure. The NOAEL for the 38 week monkey toxicity study (15 mg/kg/day) is 15X and 7.5X the human mg/kg equivalent dose (based on 50 kg human), and 5X and 3X the human $mg/m^2$ equivalent dose for a 50 mg QD and BID dose, respectively. GI toxicity in animals did not translate to an increased risk for clinical adverse events at dolutegravir doses of 50 mg QD or 50 mg BID.

Liver effects were seen in the 14-day monkey study in male monkeys that received a dose that exceeded the maximum tolerated dose (1000 mg/kg/day) and consisted of hepatocellular single cell necrosis and diffuse hepatocellular hypertrophy and/or vacuolation. Additional changes included transient ALT increases at ≥300 mg/kg/day, increased AST, bilirubin, γGTP, and triglycerides at 1000 mg/kg/day and decreased total cholesterol at 1000 mg/kg/day. The NOAEL was 100 mg/kg/day (exposure corresponds to ~4X or ~3X above the expected human exposure for a 50 mg QD or BID dose, respectively). Liver findings in the 38 week toxicity study were considered secondary to dehydration-induced moribundity, a conclusion that is supported by the fact that there were no significant liver effects in any other toxicity studies. Human subjects were carefully monitored for liver effects and cumulative data to date suggests a hepatic safety profile for dolutegravir that is comparable to raltegravir and efavirenz, the comparators used in the Phase III studies [see m2.5, Section 6.2].

Dolutegravir was negative in in vitro and in vivo genetic toxicology assessments and there are no impurities of mutagenic concern, indicating that dolutegravir does not pose a genotoxic risk in humans.

Dolutegravir was not carcinogenic in 2 year rat or mouse carcinogenicity studies.

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

Dolutegravir administration resulted in suppressed body weight gain and decreased food consumption in a pre- and postnatal development study in rat dams (F0) receiving 1000 mg/k/g/day. Decreased body weights were noted in the subsequent generation (F1) in the 1000 mg/kg group from pre-weaning until adolescence. The NOAEL for maternal general toxicity (F0) and pre- and postnatal development of the offspring (F1) was 50 mg/kg/day due to the decreased body weights of the offspring (~25X or ~18X above the expected human exposure for a 50 mg QD or BID dose, respectively, extrapolated from gender mean exposures achieved in the rat 14 day toxicity study). Based on the fact that effects on offspring body weights were noted at doses where maternal toxicity was observed, and the presence of considerable safety margins expected at the proposed clinical doses, there is minimal risk for adverse effects on postnatal development in offspring of mothers receiving dolutegravir.

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

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

In conclusion, the toxic potential of dolutegravir has been well characterized in a comprehensive battery of nonclinical studies. The principal treatment related effect was GI toxicity observed in repeat dose toxicity studies in rats and monkeys. Data from these nonclinical studies support the clinical use of dolutegravir for the treatment of HIV-1.

# 6. LIST OF LITERATURE CITATIONS

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## Guidelines (not provided)

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Guideline on the Limits of Genotox Impurities.

# APPENDIX 1 STUDY REPORTS NOT INCLUDED IN MODULE 4

The following provides brief justification for the absence of studies in certain sections of m4.

Note that when study reports address more than one objective, they have been placed in a single module location in accordance with the primary objective of the study.

| Module 4 Sections Without Study Reports | Rationale for Omission |
|---|---|
| **m4.2.1 Pharmacology** | |
| m4.2.1.1 Primary Pharmacodynamics | Primary pharmacology studies (virology studies) reside in module 5.3.5.4, in agreement with ICH M4S Q&A (R4). |
| m4.2.1.4 Pharmacodynamic Drug Interactions | Some in vitro studies have been performed with dolutegravir in combination with various other drugs. These are discussed with the virology studies and reports are located in module 5.3.5.4. |
| **m4.2.2 Pharmacokinetics** | |
| m4.2.2.6 Pharmacokinetic Drug Interactions | No specific pharmacokinetic drug interaction studies were conducted. However, studies relating to pharmacokinetic drug interactions, based on distribution and metabolism studies, are provided in m4.2.2.3 and m4.2.2.4. |
| m4.2.2.7 Other Pharmacokinetic Studies | No other pharmacokinetic studies were performed beyond the study reports included in m4.2.2.1, m4.2.2.2, m4.2.2.3, m4.2.2.4 and m4.2.2.5; therefore, this section is not applicable. |
| **m4.2.3 Toxicology** | |
| m4.2.3.4.1 Carcinogenicity: Short- or medium-term studies | Short-term, dose range finding studies were conducted in mice and these reports are located in m4.2.3.2. Otherwise, only long-term carcinogenicity studies were conducted. |
| m4.2.3.4.3 Carcinogenicity: Other Studies | Only long-term carcinogenicity studies were conducted and there were no other studies performed which might be applicable to this section. |
| m4.2.3.7.1 Antigenicity | As a small molecule dolutegravir would not be expected to evoke antigenicity and due to the lack of signals in repeat dose toxicity studies, antigenicity studies were not performed. |
| m4.2.3.7.3 Mechanistic | Mechanistic studies were not conducted because there were no toxicologic findings which were considered to require further investigation. |
| m4.2.3.7.4 Dependence | Based on the absence of signals in repeat dose toxicity studies, dependence studies were not considered necessary. |
| m4.2.3.7.5 Metabolites | No metabolites occurred which would require further toxicologic characterization. |
| m4.2.3.7.7 Other | There were no other studies performed which might be applicable to this section. |