MODULE 2.4. NONCLINICAL OVERVIEW

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List of Abbreviations

ABC	Abacavir
ADME	Absorption, distribution, metabolism, and excretion
AIDS	Acquired immunodeficiency syndrome
ALT	Alanine aminotransferase
API	Active pharmaceutical ingredient
AST	Aspartate aminotransferase
AUC	Area under the concentration vs time curve
BCRP	Breast cancer resistance protein
BID	Twice daily
BSEP	Bile salt export pump
CFR	Code of Federal Regulations (US)
C_{max}	Maximum observed plasma drug concentration
CYP	Cytochrome P450
DTG	Dolutegravir
ENT	Equilibrative nucleoside transporter
EVG	Elvitegravir
FC	Fold change
FDC	Fixed dose combination
GI	Gastrointestinal
GLP	Good Laboratory Practice
HCV	Hepatitis C virus
HIV	Human immunodeficiency virus
HIV-1	Human immunodeficiency virus type-1
HPLC	High Performance Liquid Chromatography
IN	Integrase
INI	Integrase inhibitor
INSTI	Integrase strand transfer inhibitor
LC/MS/MS	Liquid chromatographic tandem mass spectrometric
MATE	Multidrug and toxin extrusion transporter
MRP	Multidrug resistance protein
NRTI	Nucleoside reverse transcriptase inhibitor
NNRTI	Non-nucleoside reverse transcriptase inhibitor
NOAEL	No observed adverse effect level
NTD	Neural tube defect
OAT	Organic anion transporter
OATP	Organic anion transporting polypeptide
OCT	Organic cation transporter
OECD	Organisation for Economic Co-operation and Development
PICs	Pre-integration complexes
P-gp	P-glycoprotein
PBPK	Physiological based pharmacokinetic
PXR	Pregnane X receptor
QD	Once daily
QWBA	Quantitative whole body autoradiography
RAL	Raltegravir
RT	Reverse transcriptase
RTV	Ritonavir
TK	Toxicokinetics
UGT	Uridine glucuronosyl transferase
3TC	Lamivudine

1. OVERVIEW OF THE NONCLINICAL TESTING STRATEGY

1.1. Introduction

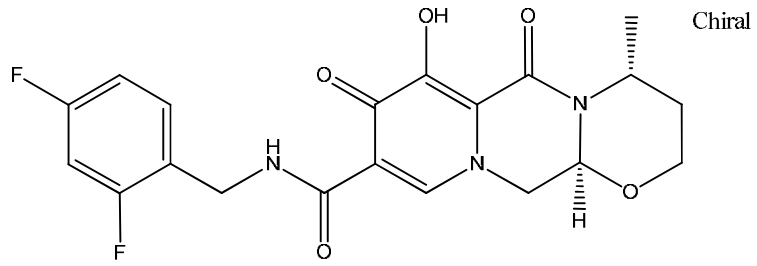
Dolutegravir (DTG) is a potent, selective and novel integrase strand transfer inhibitor (INSTI) and lamivudine (3TC) is a potent cytidine nucleoside reverse transcriptase inhibitor (NRTI), see Figure 1.

DTG is approved for the treatment of Human immunodeficiency virus type-1(HIV-1) infection. It is licensed in the European Union (EU; EMEA/H/C/002753; approved January 2014) and United States (US; NDA 204790; approved August 2013) as a single entity under the trade name TIVICAY, and as a component of the 2 fixed dose combinations (FDCs) TRIUMEQ and JULUCA. 3TC is also approved for treatment of HIV-1 infection in combination with other antiretroviral therapy. 3TC is licensed as a single entity in the EU (EMEA/H/C/000107; approved August 1996) and US (NDA 020564, approved November 1995) under the trade name EPIVIR, and is a component of the FDCs TRIUMEQ, KIVEXA/EPZICOM, COMBIVIR and TRIZIVIR.

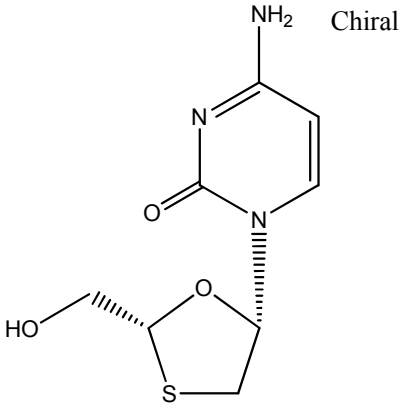
This current application is for DTG/3TC FDC as a two-drug regimen for use as a complete regimen for the treatment of HIV-1 infection.

The dose will be orally administered as an FDC tablet. Each film coated tablet contains 50 mg of dolutegravir (as the sodium salt) and 300 mg of lamivudine.

Figure 1 Structure of DTG and 3TC



Dolutegravir (DTG)



Lamivudine (3TC)

1.2. Nonclinical Development Programme

1.2.1. DTG/3TC in combination

In agreement with ICH Guidance M3 (R2), nonclinical combination studies with DTG/3TC were not considered warranted to support the current application.

Refer to Section 5 for a complete discussion of the nonclinical considerations regarding the combined use of DTG/3TC.

The key nonclinical information relating to the virology of DTG and 3TC, 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].

1.2.2. DTG and 3TC as monotherapies

Full packages of nonclinical safety studies have been completed with DTG and 3TC individually to support their development as monotherapies.

Experiments undertaken to determine the virology of DTG and 3TC and preliminary nonclinical toxicity studies to establish suitable dose levels for use in repeat dose toxicity and pharmacokinetics studies were conducted in line with Company Divisional Standard Operating Procedures and Policies and in general accordance with the principles of Good Laboratory Practice (GLP). All definitive toxicity studies were carried out in full compliance with GLP regulations in an Organisation for Economic Co-operation and Development (OECD) member country in accordance with the OECD Test Guidelines.

Except where indicated, all studies described in this section were performed using either the sodium salt of DTG or the base (parent) of 3TC. All dosages and concentrations quoted in this summary are expressed in terms of the parent compounds (referred to simply as DTG and 3TC).

An overview of the nonclinical studies for each of the individual actives, DTG and 3TC, is presented below, Nonclinical pharmacokinetic study summaries presented in m2.6.4 and m2.6.5 are new monotherapy nonclinical studies which have not been previously submitted. An integrated assessment of nonclinical considerations for the DTG/3TC FDC is also presented in Section 5 and supports the conclusion that DTG/3TC FDC can be used safely in patients for the treatment of HIV.

Throughout this section, exposure margins for DTG are presented based upon comparison of the animal systemic exposure (end of study gender averaged) with that reported for patients receiving 50 mg DTG once daily (QD) (C_{max} = 3.7 μg/mL; AUC = 53.6 μg.h/mL) [pooled data from Clinical Studies ING112276 and ING113086]. For 3TC exposure margins are presented based upon comparison of the animal systemic exposure with that reported for patients receiving 300 mg 3TC QD in combination with DTG and abacavir (ABC) (C_{max} = 2.1 μg/mL; AUC = 12.3 μg.h/mL) [Clinical Study ING114580].

1.2.2.1. DTG

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

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

To assess the nonclinical safety of DTG, repeat dose toxicity was studied in rats and monkeys following oral administration for up to 26 weeks and 38 weeks, respectively. A series of genotoxicity studies were performed to determine the mutagenic and clastogenic potential of DTG. Toxicology studies were also conducted to assess potential carcinogenicity in mice and rats. Fertility and early embryonic development studies were conducted in male and female rats, and embryofetal development studies in pregnant rats and rabbits. Juvenile toxicity studies and a pre- and post-natal development toxicity study were conducted in rats. Other studies were performed to assess the local tolerance and potential immunotoxicity of DTG, and the genotoxicity of potential impurities. In addition, investigations are ongoing to determine if nonclinical models can be utilised to detect neural tube defects (NTD) (see Section 4.2.6.3).

1.2.2.2. 3TC

A range of in vitro and in vivo nonclinical studies were conducted to characterise the pharmacodynamic properties of 3TC. The Primary Pharmacodynamics/Virology studies are listed in m2.7.2.4, Special Studies.

Absorption, distribution, metabolism and excretion studies were performed with 3TC. Although the programme was initiated using the racemate, gsk001* , in most cases the pharmacokinetic studies were subsequently repeated with 3TC itself, except for the distribution profile in the rat which was obtained using the racemate.

3TC has undergone a comprehensive toxicological evaluation. Acute oral studies in the mouse and rat and repeat oral dose studies for durations of up to 52 weeks in the dog and 26 weeks in the rat have been performed. The genetic toxicity of 3TC has been investigated in a battery of in vitro and in vivo assays, and its tumorigenic potential has been studied in oral carcinogenicity studies in the mouse and the rat. A range of reproductive toxicity studies have been performed in the rat and rabbit following oral administration. Additionally, studies investigating haematotoxicity have been conducted, and impurities studies and local tolerance studies have also been performed.

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2. PHARMACOLOGY

2.1. Presentation Order of Pharmacology Summaries

Pharmacology Summary	Section
Pharmacology for DTG and 3TC in combination	Not applicable.
Pharmacology of DTG alone	Section 2.2
Pharmacology of 3TC alone	Section 2.3
Overall summary of all pharmacological studies performed with DTG or 3TC	Section 5, Integrated Assessment of the Fixed Dose Combination Product

2.2. DTG

A summary of each secondary and safety pharmacology study and its findings is presented in m2.6.2, DTG NDA. A listing of each secondary and safety pharmacology study conducted, and a tabular summary of each safety pharmacology study and its findings, is presented in m2.6.3, DTG NDA.

2.2.1. Virology (primary pharmacodynamics)

A range of in vitro virology studies have been conducted to determine the mechanism of action, antiviral activity and the potential for development of drug resistance via mutations. In accordance with ICH M4S Q&A (R4), a summary of these studies is provided within m2.7.2.4. However, for the reviewer's convenience, a brief summary of the key findings from these studies is also provided below.

2.2.1.1. Mechanism of action

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

2.2.1.2. In vitro antiviral activity and potential resistance

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

When HIV-1 Strain IIIB was passaged in the presence of DTG for 112 days, viruses with a 4.1-fold maximum increase in IC_{50} and S153Y or S153F substitutions in integrase polymorphic sites were observed. Passage of the wildtype HIV-1 NL432 in the presence of 6.4 nM DTG selected for E92Q (FC=3.1) and G193E (FC=3.2) substitutions in the IN region on Day 56. Passage of HIV-1 NL432 with Q148H, Q148K or Q148R RAL-resistant mutations resulted in selection of additional mutations and an increase in DTG FC. Passage of HIV-1 subtypes B and A/G in TZM-bl cells selected for integrase mutations G118R (FC=10) and R263K (FC=1.5).

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

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

2.2.2. Secondary pharmacology

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

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

2.2.3. Safety pharmacology

2.2.3.1. Neurobehavioral and pulmonary safety

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

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

2.2.3.2. Cardiovascular safety

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

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

There were no findings from safety pharmacology studies that indicate an unacceptable risk for oral administration of DTG to patients in accordance with the proposed indication. Additionally, a supratherapeutic clinical dose of DTG (250 mg as a suspension, which achieved exposures ~3X higher than a 50 mg QD dose) was well tolerated and had no effect on cardiac repolarization [see m2.7.2 Section 2.2.5.1].

2.2.4. Pharmacodynamic drug interactions

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

2.3. 3TC

A summary of safety pharmacology studies and pharmacodynamic interaction studies conducted is presented in m2.6.2, 3TC NDA. A listing of each study conducted, and a tabular summary of each safety pharmacology study and its findings, is presented in m2.6.3, 3TC NDA.

2.3.1. Virology (primary pharmacodynamics)

A range of in vitro virology studies have been conducted to determine the mechanism of action, antiviral activity and the potential for development of drug resistance via mutations. A detailed summary of these studies is provided in m2.7.2.4. However, for the reviewer's convenience, a brief overview of the key findings from these studies is also provided below.

2.3.1.1. Mechanism of action

Intracellularly, 3TC is phosphorylated to its active 5'-triphosphate metabolite, 3TC triphosphate (3TC-TP). The principal mode of action of 3TC-TP is the inhibition of HIV-1 reverse transcriptase (RT) via DNA chain termination after incorporation of the nucleotide analogue into viral DNA 3TC-TP is a weak inhibitor of mammalian DNA polymerases α, β and γ.

2.3.1.2. In vitro antiviral activity and potential resistance

3TC has low μM activity against wildtype HIV-1 in a variety of cell lines, regardless of subtype. 3TC has little activity against non-HIV viruses, displaying the highest antiviral activity against Hepatitis B virus. 3TC is additive or synergistic when assayed in combination with other antiretroviral agents.

3TC resistant variants have been isolated in cell culture. Genotypic analysis showed that the resistance was due to a specific amino acid substitution in the HIV-1 reverse transcriptase at codon 184 changing the methionine to either isoleucine or valine (M184V/I). The substitution at M184I/V causes almost complete resistance to 3TC without the additional substitutions K65R, L74M and Y115F.

Studies demonstrate that although 3TC 5'-triphosphate is a substrate for the DNA dependent DNA polymerase activity of DNA polymerase γ, the product of this reaction is also a substrate for the 3'-5' exonuclease activity of DNA polymerase γ. This may explain the low levels of mitochondrial toxicity observed with 3TC.

2.3.2. Secondary pharmacology

Specific nonclinical secondary pharmacology studies have not been performed with 3TC. No off-target pharmacological activity has been reported during the extensive clinical use of 3TC.

2.3.3. Safety pharmacology

There were no significant overt pharmacodynamic actions of 3TC following acute oral administration in the rat and dog at doses up to 600 mg/kg [Report WBA/91/005]. Similarly, there were no effects on intestinal transport (up to 300 mg/kg oral in mice), or on respiration, blood pressure, heart rate or the electrocardiogram (up to 100 mg/kg IV in anaesthetised cats and 600 mg/kg oral in dogs) [Reports NPY/96/006, WBA/91/003 and WPT/94/218].

In saline loaded rats, a single oral dose of 3TC at 300 mg/kg slightly increased the urinary excretion of potassium ions with an accompanying small increase in urine osmolarity [Report NPY/96/006]. This slight effect on urinary potassium excretion has also been observed, though not consistently, at the high dose levels in repeat dose toxicity studies in rats and dogs. The possibility that this reflects a transient change in potassium excretion in rats cannot be discounted.

2.3.4. Pharmacodynamic drug interactions

gsk001* (the racemate of 3TC) had no effect on the duration of pentobarbitone-induced sleeping time, suggesting that it does not interact with the enzymes responsible for pentobarbitone metabolism. The antiviral activity of 3TC was evaluated in vitro in combination with 7 commercially available antibacterial agents, 3 antifungal agents, 2 antiviral agents and. None of the anti-infective compounds tested affected the antiviral activity of 3TC.

3. PHARMACOKINETICS

Extensive programs of studies investigating the absorption, distribution, metabolism and excretion have been carried out individually with DTG and 3TC in animals used in toxicity studies. In general, the systemic exposure and metabolism defined in the animal species used for toxicological assessment indicates that the species used were appropriate for predicting the safety of DTG and 3TC, and their metabolites in humans.

3.1. Presentation Order of Pharmacokinetics Summaries

Pharmacokinetics Summary	Section
Pharmacokinetics for DTG and 3TC in combination	Not applicable.
Pharmacokinetics of DTG alone	Section 3.2
Pharmacokinetics of 3TC alone	Section 3.3
Overall summary of all pharmacokinetics studies performed with DTG or 3TC	Section 5, Integrated Assessment of the Fixed Dose Combination Product

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3.2. DTG

A summary of each pharmacokinetics study and its findings is presented in m2.6.4, DTG NDA. A listing of each pharmacokinetics study conducted, tabular summaries and the findings are presented in m2.6.5, DTG NDA. A summary of a new pharmacokinetic study conducted with DTG and its findings is presented in m2.6.4, Pharmacokinetics Written Summary, Section 2. A tabular summary and the findings are presented in m2.6.5, Pharmacokinetics Tabulated Summary, Section 2. The new pharmacokinetic study report is available in m4.2.2, Nonclinical Study Reports.

3.2.1. Analytical methods and validation

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

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

3.2.2. Absorption and pharmacokinetics

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

3.2.2.1. Single dose

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

Oral: DTG absorption from an oral solution was rapid, reaching peak plasma concentrations within 2 hours with high oral bioavailability (76 to 87%) in fasted rats and monkeys [Reports RH2007/00101 and RH2007/00103]. When DTG was administered as a suspension, the increase in systemic exposure (C_{max} and AUC_{0-t}) was less than

proportional to the increase in dose. The oral bioavailability of DTG from a suspension formulation was lower (bioavailability range of 25% to 52%) and suggested that the absorption is limited by dissolution rate or solubility. Administration of DTG with food to rats reduced exposure whereas in humans food effect is not clinically significant [see m2.7.1, Section 2.4.1].

3.2.2.2. Repeat dose toxicokinetics

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

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

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

3.2.3. Distribution

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

3.2.3.1. Protein binding and blood cell association

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

3.2.3.2. Efflux-mediated transport and cell membrane permeability

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

3.2.3.3. Tissue distribution

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

The in vitro brain tissue binding of dolutegravir at 2 μM was assessed in the male mouse and rat by equilibrium dialysis [Report 2017N339387, m2.6.5, Table 2.1]. The mean unbound fraction of dolutegravir was 5.91% and 7.21% in mouse and rat brain, respectively with 79.2% and 80.9% of dolutegravir recovered after 4 hours of dialysis in mouse and rat brain, respectively.

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

3.2.4. 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 DTG and there were no human plasma metabolites representing >10% total drug-related material - the threshold for further consideration in nonclinical species [ICH M3 (R2)]. The main metabolic route in each species and humans was by conjugation to form the ether glucuronide (M3). The drug metabolism in animal species used for toxicology testing was relevant to the safety assessment of DTG for human use.

3.2.4.1. In vitro biotransformation

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

metabolic intermediate by bioactivation in vitro [Reports RH2007/00058 and RD2007/01557]. However, in vivo in mice, rats, monkeys and humans, these metabolic products have represented only a small fraction of the metabolic clearance.

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

3.2.4.2. In vivo studies

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

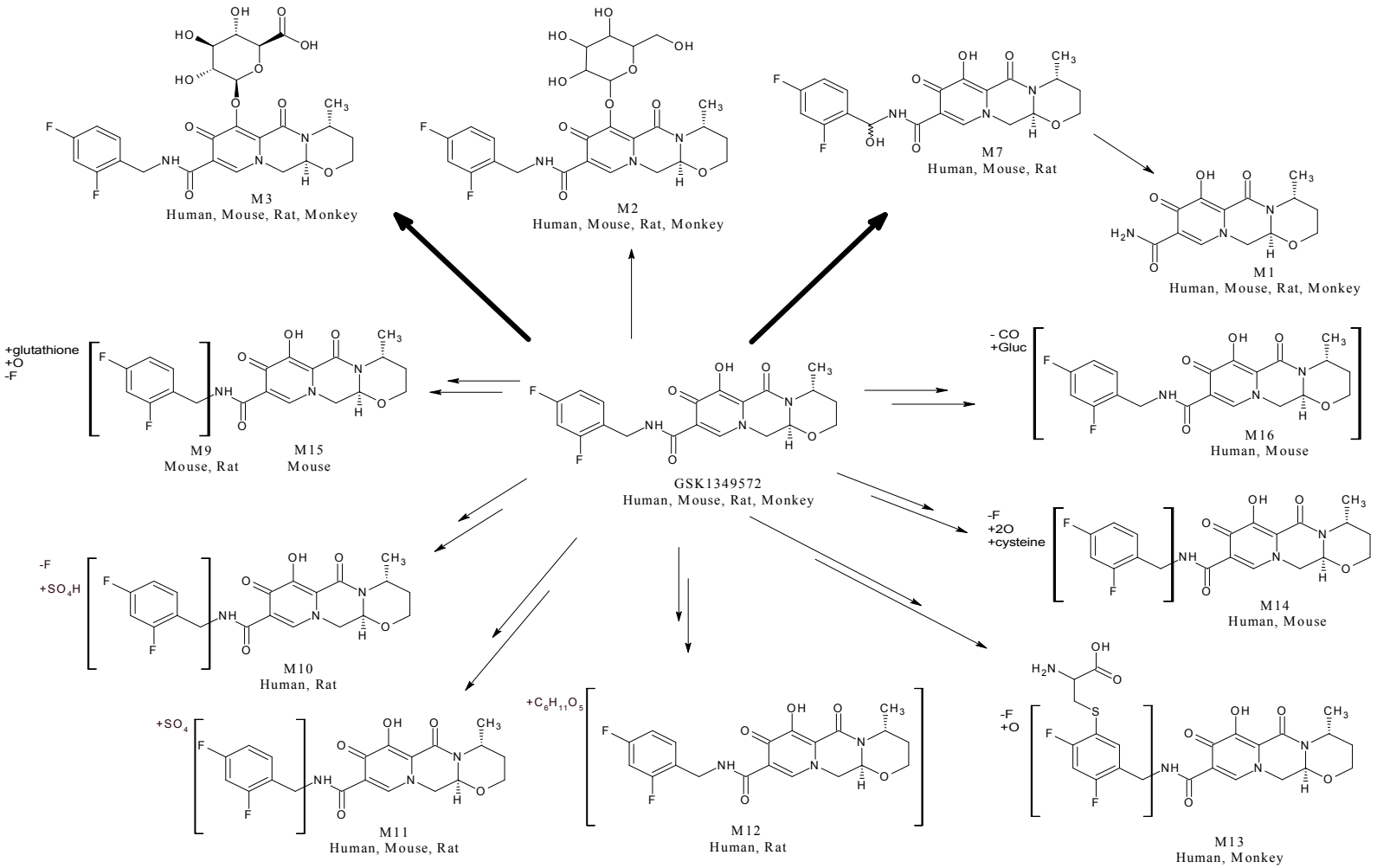
Plasma metabolic profile

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

Biotransformation

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

Figure 2 Comparative Metabolic Profile of DTG Between Nonclinical Species and Humans



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

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

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

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

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

3.2.5. Excretion

Fecal excretion of radioactivity consisted primarily of unchanged DTG and was the predominant route of elimination of administered radioactivity in all species [Reports RD2008/00108, RD2008/01299, RD2008/01300 and RD2009/00562]. Following oral administration of [^{14}C]-DTG, urinary excretion of radioactivity was greater in humans (approximately 32%) than in animals (≤6%) [see m2.7.2, section 3.1.4], which is consistent with the hypothesis of a higher molecular weight threshold for biliary secretion in humans [Yang, 2009].

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

3.2.6. Pharmacokinetic drug interactions

No nonclinical studies have been performed specifically to evaluate potential interactions with drugs that may be co-administered with DTG. However, a series of in vitro studies have been conducted to help evaluate the mechanisms and drug interaction potential of DTG, and, except for sensitive organic cation transporter (OCT)2 substrates (e.g., dofetilide, pilsicainide), dolutegravir has a low propensity to cause drug interactions based on in vitro results. DTG is a substrate of P-gp and BCRP, but inhibition of these transporters is unlikely to affect absorption of dolutegravir to a clinically relevant extent.

3.2.6.1. Potential effect of co-administered agents on DTG

In vitro and in vivo, DTG is primarily metabolized by UGT1A1 with a notable contribution from cytochrome P450 (CYP)3A4. UGT1A3 and 1A9 were minor glucuronidation pathways [Reports RD2008/00373 and RD2008/01339]. Therefore, drugs that are strong inducers of UGT1A1 or CYP3A4 may decrease DTG plasma concentrations. Although drugs that inhibit UGT1A1 and CYP3A4 may increase DTG plasma concentrations, based on the clinical interaction study with atazanavir, a potent UGT1A1 and CYP3A4 inhibitor, any increases in DTG concentrations are not expected to be clinically meaningful. Although DTG is a substrate for efflux transporters, no notable effect on DTG pharmacokinetics was observed in humans following co-administration with lopinavir/ritonavir, inhibitors of the efflux transporters P-gp and BCRP [see m2.7.2, Table 3]. These data, together with the rapid absorption in humans, low to moderate pharmacokinetic variability and high intrinsic permeability suggests a low potential for drug interactions with BCRP and P-gp inhibitors that would result in clinically significant changes to DTG exposure.

3.2.6.2. Effect of DTG on co-administered agents

In vitro, DTG was noted to have little or no inductive effects on the human Pregnane X receptor (PXR) on CYP1A2, 2B6 or 3A4 mRNA (as determined by the increase in mRNA relative to vehicle control). DTG demonstrated little or no inhibition (IC_{50} values >30 μM) in vitro on the transporters BCRP, bile salt export pump (BSEP), multidrug resistance protein (MRP) 2, MRP4, organic anion transporting polypeptide (OATP) 1B1, OATP1B3, OCT1, OCT3, equilibrative nucleoside transporter (ENT) 4 and P-gp, or the enzymes CYP1A2, 2A6, 2B6, 2C8, 2C9, 2C19, 2D6, 3A4, UGT1A1 or 2B7, demonstrating a low propensity to cause drug interactions through modulation of these systems. DTG glucuronide (M3) did not inhibit MRP2, thus, inhibition of biliary clearance of bilirubin glucuronides or glucuronide conjugates of co-administered drugs is not expected. In vitro DTG did not alter the paracellular permeability of the probe substrate Lucifer yellow, nor did it alter transport of metformin across the Caco2 cells up to concentrations of 100 μM, suggesting that DTG is unlikely to alter the permeability of these compounds across the intestinal lumen.

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

In vitro, DTG inhibited OAT1 and OAT3 with IC_{50} values of 2.12 μM and 1.97 μM, respectively; however, in vivo, no notable changes in plasma concentrations of the OAT substrates tenofovir [see m2.7.2, Table 3] or p-aminohippurate [see ING114819] were observed in healthy subject Phase 1 studies. MRP2 and MRP4 are anion transporters responsible for the transport of anions (e.g., tenofovir) from the renal tubule to the urine with MRP4 as the predominate transporter for tenofovir excretion. DTG did not inhibit MRP2 and weakly inhibited MRP4 (IC_{50} value of 84 μM) with a 50-fold unbound C_{max} value less than the K_i estimate. Furthermore, polymorphic MRP4 that decreased tenofovir renal clearance by 15% also increased plasma tenofovir concentrations by 32% [Kiser, 2008], which was not observed in the drug interaction study assessing the impact of DTG on tenofovir PK. In addition, a physiological based pharmacokinetic (PBPK) mechanistic kidney model (Simcyp v,12 R1) developed for steady state concentrations of tenofovir (300 mg once daily) predicts that co-administration of DTG at 50 mg once daily would result in a minimal decrease in tenofovir renal clearance with no notable change in tenofovir exposure within the proximal tubule cells of the kidney. Based on these collective data, no clinically significant interaction with tenofovir by DTG at the renal tubule is expected.

In vitro, DTG inhibited the basolateral renal OCT2 (IC_{50} = 1.9 μM) and the renal apical transporters, multidrug and toxin extrusion transporter (MATE) 1 (IC_{50} = 6.34 μM) and MATE2-K (IC_{50} = 24.8 μM) [Reports 2010N104937 and 2013N161621] which provides a mechanistic basis for the non-pathological mild serum creatinine increases observed in clinical studies. Because DTG inhibits OCT2, but only weakly OCT1, and both OCT1 and OCT2 are equally expressed in rat proximal tubules [Tahara, 2005], this effect on creatinine was not observed in rats. In a clinical drug interaction study with metformin, a substrate of OCT2 and MATE1, DTG increased metformin AUC exposure by 79% and 145% after QD and BID dosing, respectively [see m2.7.2 Section 3.1.6.2]. These data indicate caution should be used due to the potential for a drug interaction in vivo when DTG is co-administered with cationic compounds that have a narrow therapeutic index drugs and in which a significant part of their clearance is by renal proximal tubule secretion by OCT2. DTG is contraindicated for co-administration with the OCT2 substrates dofetilide and pilsicainide because they possess narrow therapeutic indices that present the potential for toxicity due to higher exposure [m2.7.2, Section 2]. DTG has a low potential to affect the transport of MATE2-K substrates with a 50-fold unbound C_{max} value less than the K_i estimate.

3.3. 3TC

A summary of each pharmacokinetics study and its findings is presented in m2.6.4, 3TC NDA. A listing of each pharmacokinetics study conducted, tabular summaries and the findings are presented in m2.6.5, 3TC NDA. A summary of a new pharmacokinetic studies conducted with 3TC and findings is presented in m2.6.4, Pharmacokinetics Written Summary, Section 2 and 3. A tabular summary and the findings are presented in m2.6.5, Pharmacokinetics Tabulated Summary, Section 4 and 5. The new pharmacokinetic study reports are available in m4.2.2, Nonclinical Study Reports.

3.3.1. Analytical methods and validation

In pharmacokinetic and toxicity studies, plasma 3TC concentrations were measured following protein precipitation with trichloroacetic acid or solid phase extraction (for lower limits of quantification) which was then separated using reversed phase HPLC with ultraviolet (UV) absorbance detection for studies during early development and by tandem mass spectrometric (MS/MS) detection for later studies. All methods and limits of quantification were adequate with regard to specificity and sensitivity to support the kinetic analyses of 3TC.

Chiral analysis was performed on plasma or serum samples by collecting the eluate fraction of parent 3TC from a reversed phase C18 column and analyzing it with a chiral Cyclobond 1 Acetyl column and UV detection. This methodology was sufficiently sensitive to detect 2 ng of the enantiomer in the presence of 2 μg/mL of 3TC.

3.3.2. Absorption and pharmacokinetics

3TC has moderate to high plasma clearance, dose-proportional systemic exposure and high bioavailability in animals and humans.

3.3.2.1. Single dose

Intravenous: Following a single intravenous administration, 3TC was rapidly cleared from the systemic circulation with a terminal elimination half-life in the rat and dog of approximately 1.2 to 1.7 hours. In rats, the renal clearance (3.3 mL/min) exceeded the glomerular filtration rate (approximately 1.8 mL/min), indicating that tubular secretion played a significant role in the elimination of 3TC. In dogs, metabolism was a more significant contributor to the total clearance of 3TC with renal and metabolic clearance of approximately equal importance. In humans, 3TC was excreted primarily unchanged in the urine with tubular secretion playing a significant role [m2.7.2, Section 3.2.6.1]. The volume of distribution was moderate in rats and dogs and was equal to or greater than the total body water volume [Reports GDM/91/015, GDM/91/077, GDM/91/008 and GDM/91/080]. No notable sex-related differences in the pharmacokinetics were observed.

Oral: In rat and dog, 3TC was rapidly and extensively absorbed following oral administration with oral bioavailability ranging from 60% to 69% in the rat and from 67% to 83% in the dog. At higher dose levels (600 to 2000 mg/kg), systemic exposures were linear with dose in the rat but the terminal phase half-life ranged 3.7 to 4.6 hours, indicating flip flop kinetics. In contrast, in the dog no notable change in half-life was observed but exposures generally increased less than proportionally at higher doses [Reports GDM/91/003 and GDM/91/036].

The pharmacokinetics of 3TC in human is more like the pharmacokinetics in rats than in dogs.

3.3.2.2. Repeat dose toxicokinetics

Oral: The repeat dose toxicokinetics of 3TC 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 3TC is presented in Table 4.5.

Following repeat dose oral administration to the rat, the relationship between dose and concentration of 3TC in plasma was linear, and in general there was no change in systemic exposure over a dosing period of up to 6 months. Repeated administration of 3TC to the dog for up to 12 months caused an increase in systemic exposure with an increase in dose but the increase was less than proportional at higher doses. The systemic exposure to 3TC in juvenile rats (Day 16) was at least 2 times greater than the corresponding single dose adult values.

3.3.3. Distribution

The binding of 3TC to plasma proteins at therapeutic concentrations was low. After oral administration to rats, the distribution of 3TC was rapid and widespread throughout most tissues. 3TC crosses the placenta in pregnant rats and rabbits and is excreted into the milk of nursing rats.

3.3.3.1. Protein binding and blood cell association

The in vitro protein binding of 3TC was low and concentration-dependent. At clinical concentrations and above, binding was <17%. At 0.1 μg/mL, protein binding ranged 35% to 50%. The low protein binding indicates that 3TC does not influence in vitro viral inhibition potency measurements. Binding to blood cellular components was moderate (40% to 56%) and similar across species in rats, dogs and humans [Reports GDM/91/010 and GDM/91/011].

3.3.3.2. Efflux-mediated transport and cell membrane permeability

In vitro, 3TC was assessed as undergoing passive transcellular and paracellular diffusion across Caco-2 cell monolayers [Report UCP/92/028].

In vitro in Caco-2 and MDCK-MDR1 monolayers, 3TC was assessed as having a moderate permeability both alone and in the presence of DTG [m2.6.5, Table 4.2, Report 2018N376755]. 3TC also was shown to be a P-gp substrate but the low transport ratio indicates 3TC was not a good substrate and as a result is unlikely that P-gp would significantly affect its absorption [de Souza, 2009].

3.3.3.3. Tissue distribution

The pharmacokinetics of 3TC were determined with the chiral molecule, however, the program was initiated using the racemate, gsk001* , and the oral distribution profile of the racemate was studied by whole body autoradiography in the rat [Report GDM/90/040].

Distribution of radiolabelled gsk001* was rapid and widespread throughout most tissues including the brain parenchyma with rapid clearance from tissues. There was no evidence of any sex-related difference in the distribution of gsk001*-related material nor was there evidence for any association with melanin in pigmented rats.

Following oral administration of [^{3}H]-3TC to pregnant rats and rabbits, radioactivity crossed the placenta and was widely distributed in maternal tissues and in fetal tissues at low concentrations relative to maternal tissues. In rats, the pattern of distribution of radioactivity in maternal tissues was similar to that found in non-pregnant rats administered radiolabelled racemic gsk001* , indicating no impact of pregnancy or stereochemistry on the distribution of 3TC-related material. These studies indicate that during reproductive toxicity testing the fetuses received exposure to drug-related material [Reports GDM/91/050 and GDM/91/058].

Following oral administration of 3TC to lactating rats, concentrations of 3TC generally were higher in milk than in plasma. Thus, suckling pups would have been exposed to drug-related material during the peri- and post-natal toxicity studies [Report GDM/92/017].

3.3.4. Metabolism

3TC is cleared almost entirely by renal elimination of unmetabolized drug in the rat, with significant active tubular secretion. In the dog, renal and metabolic clearance are equally important. Two metabolites are present in dogs, the principal metabolite being the trans-sulphoxide metabolite and the minor metabolite being cytosine.

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3.3.4.1. In vivo biotransformation

A comparison of the primary characterized metabolites in animals and humans are presented in Figure 3. The primary metabolic pathway is designated with the bolded arrow.

Systemic (plasma or serum) metabolic profile

The systemic profile in rats following oral and intravenous administration show close agreement between plasma concentration of 3TC and total radioactivity, suggesting little systemic exposure to metabolites [Reports GDM/91/015 and GDM/91/077]. Following oral administration of radiolabelled 3TC to pregnant rabbits, a comparison of the serum AUC values indicated that 3TC accounted for 69% of the serum radiolabel [Report GDM/91/058] with a large portion attributed to tritiated water formed by intermolecular exchange. In dogs, 3TC accounted for 27% to 58% of the plasma radioactivity with the remaining radioactivity attributed to systemic exposure to metabolites [Reports GDM/91/008 and GDM/91/080]. Tritium exchange was small in these dog studies.

Chiral analysis of dog and human plasma or serum samples indicated no evidence for the presence of the (+)-enantiomer, gsk002* , confirming that no inversion of the single enantiomeric form occurs in vivo [Reports GDM/91/008 and WD1997/00356].

Biotransformation

In humans, approximately 70% of the dose following oral or intravenous administration 3TC was excreted unchanged in the urine. A notable metabolite, the trans-sulphoxide metabolite of 3TC, was observed in the urine accounting for approximately 10% of the dose. This metabolite was also observed in the nonclinical species used to investigate the safety of 3TC.

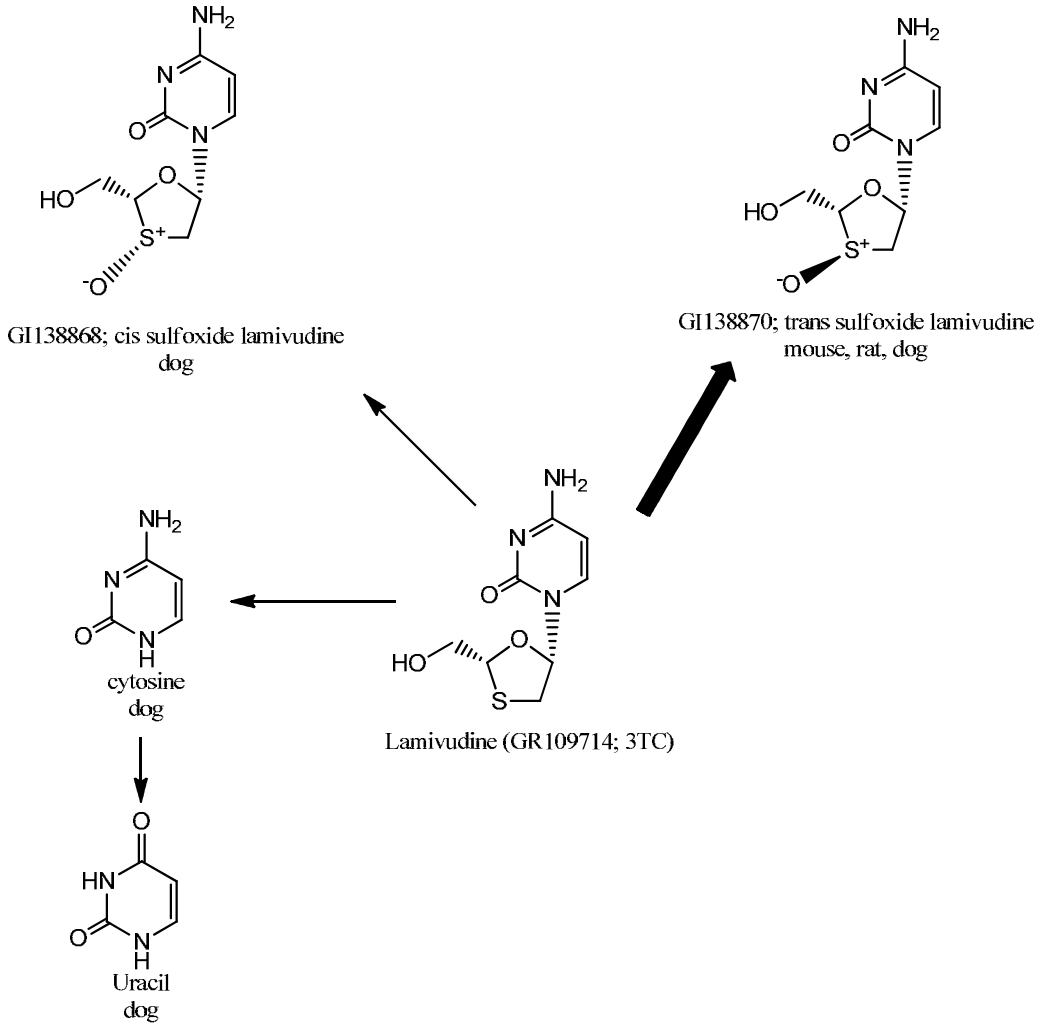
3TC represented ≥90% of the urinary radioactivity in mice and rats and approximately 80% of the urinary radioactivity in rabbits [Reports GDM/92/045, GDM/91/014, GDM/91/015, GDM/91/077 and GDM/91/059]. In these 3 species, two additional products of metabolism were observed. In mice and rats one was identified as the trans-sulfoxide [Report GDM/94/123]. 3TC accounted for greater than 94% of the fecal radioactivity in rats and rabbits [Reports GDM/91/050, GDM/91/014, GDM/91/017 and GDM/94/076].

In dogs, 3TC represented 39% to 48% with two metabolites representing 41% to 57% of the urinary radioactivity [Report GDM/91/008]. The principal metabolite identified was the trans-sulphoxide metabolite of 3TC and the minor metabolites as cis-sulphoxide, cytosine and uracil [Reports GDM/91/080, GDM/94/123, NME/97/010 and NME/97/014].

No evidence of sex-related differences, pregnancy status, dose level, or between Han Wistar and AHA strains of rat in the metabolic profile of 3TC was observed.

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Figure 3 Comparative Metabolic Profile of 3TC Metabolites Between Nonclinical Species and Humans



3.3.5. Excretion

3TC was cleared almost entirely by renal elimination of unmetabolized drug in the rat, with active tubular secretion playing a significant role. In contrast, tubular secretion does not play a significant role in the elimination of 3TC in the dog, and renal and metabolic clearance are of equal importance.

Following oral and intravenous administration of [^{3}H]-labelled 3TC to the mouse, rat, rabbit and dog, the majority of drug-related material was excreted in the urine, mainly as unchanged 3TC in the mouse, rat and rabbit [Reports GDM/92/045, GDM/91/014, GDM/91/015, GDM/91/077, GDM/91/059, GDM/91/008 and GDM/91/080] with most of the excretion occurring within the first 24 hours. The mechanism of the renal excretion of 3TC was investigated in the anesthetized rat model of renal clearance. Administration of trimethoprim or cimetidine significantly reduced the renal clearance of 3TC but not the renal cortex concentration, indicating that secretion in the renal proximal tubule by cationic transporter was involved.

3.3.6. Pharmacokinetic drug-drug interactions

The potential for 3TC to be a victim of, or perpetrate, drug interactions is discussed in this section and is based on data available from published literature, prescribing information and Report 2015N234461 [m2.6.5, Table 4.1].

3.3.6.1. Potential effect of co-administered agents on 3TC

3TC is predominantly (~70% of the absorbed dose) cleared by the kidney via glomerular filtration and active tubular secretion of unchanged drug. As a result, any effect of CYP inhibition or induction by other drugs is unlikely to have a large effect on the systemic exposure of 3TC. OCT2, MATE1 and MATE2-K have been shown to be clinically important for the active renal secretion of 3TC. OCT2 facilitates 3TC uptake from the blood into the kidney as demonstrated in vitro in transfected human embryonic kidney (HEK) cells [Km = 248 μM; Jung, 2008]. The apical kidney transporters, MATE1 and MATE2-K, mediate 3TC efflux out of the kidney into the urine [Muller, 2011].

Trimethoprim is an inhibitor of OCT2 (IC_{50} = 13 μM), MATE1 (IC_{50} = 6.2 μM) and MATE2-K (IC_{50} = 0.66 μM) (Muller, 2013), and has been shown to increase 3TC plasma concentrations by 43% in HIV patients [Moore, 1996]; however, this interaction is not considered clinically significant, as no dose adjustment of 3TC is needed. No clinical drug interaction studies have been conducted between 3TC and cimetidine, which is also a MATE1/2-K and OCT2 inhibitor. Cimetidine unbound plasma concentrations are 3.6-7.8 μM, which are of similar magnitude to trimethoprim unbound plasma concentrations (4.66 μM). Cimetidine, however, is a less potent OCT2 and MATE2-K inhibitor than trimethoprim, with Ki values of 95-146 μM (OCT2), 1.1-3.8 μM (MATE1), and 2.1-6.9 μM (MATE2-K) [Ito, 2012]. Although a cimetidine-mediated PK interaction with 3TC is possible, a more marked and clinically significant drug interaction compared to trimethoprim is unlikely to occur. Although dolutegravir inhibits OCT2 (IC_{50} = 1.9 μM) a dolutegravir-mediated drug interaction with 3TC is also not expected based on dolutegravir's significantly lower unbound systemic concentrations (0.088 μM) and weaker MATE inhibition [IC_{50} = 6.3 μM (MATE1) and 25 μM (MATE2-K)]. This is consistent with clinical studies showing no meaningful change in 3TC exposure on co-administration with dolutegravir.

3TC has been shown to be a weak substrate of P-gp [efflux ratios = 1.9-2.6; de Souza, 2009], and BCRP [efflux ratios =3; Kim, 2007]. Others have shown, however, that 3TC is not a P-gp substrate [Janneh, 2011; Shiraki, 2000], nor is 3TC disposition significantly influenced by functional BCRP variants in healthy subjects [Kim, 2007]. Thus, it is unlikely that P-gp or BCRP play a significant role in the absorption or excretion of 3TC. These data are consistent with 3TC being a Class 3 Biopharmaceutics Drug Disposition Classification System (BDDCS) drug, in which the contribution of efflux transporters to its drug disposition is minimal [Minuesa, 2011]. Thus, co-administration of drugs that are inhibitors of these efflux transporters is unlikely to affect the disposition and elimination of 3TC.

3TC is a substrate of the hepatic uptake transporter OCT1 [Jung, 2008]. However, based on in vitro and in vivo data, hepatic elimination plays a minor role in the clearance of 3TC and therefore, drug interactions due to inhibition of OCT1, or other hepatic uptake transporters, such as OATP1B1/3, are unlikely to be of clinical significance.

There are no definitive reports of 3TC being a substrate of OCT3, a low-affinity, high-capacity transporter expressed in the central nervous system and other organs, however it is unclear what clinical relevance this transporter may have on the disposition and elimination of renally cleared molecules, such as 3TC.

Jung et al [Jung, 2013] have demonstrated that OCT1 and OCT2 are expressed in CD4 cells of HIV-infected patients and that these transporters play a role in the cellular accumulation of 3TC in HIV-infected individuals. Although it could be argued that the potential for drug interactions with co-administered drugs that inhibit OCT may occur, clinical studies with DTG, a known OCT2 inhibitor, showed no effect on the exposure, safety or efficacy of 3TC.

Results from these investigations indicate that no notable effect on 3TC absorption is expected and there is a low potential for interactions to affect 3TC disposition based on inhibition or induction of hepatic enzymes or transporters. Co-administration of agents that strongly inhibit OCT2 (such as trimethoprim) may result in increased systemic concentrations of 3TC, but based on the accumulated safety data any increase is unlikely to be clinically significant [see m2.7.2, Table 3].

3.3.6.2. Effect of 3TC on co-administered agents

In vitro, 3-TC did not inhibit or induce CYP enzymes (such as CYP3A4, CYP2C9 or CYP2D6) and demonstrated no or weak inhibition of OATP1B1, OATP1B3, OCT3, BCRP, P-gp, MATE1 or MATE2-K. 3-TC is therefore not expected to affect the plasma concentrations of drugs that are substrates of these enzymes or transporters.

In vitro, 3TC inhibited OCT1 and OCT2, with IC_{50} values of 17 and 33 µM, respectively [Jung, 2008]. However, at therapeutic exposures, no change in metformin exposure (exceeding the 1.25X bioequivalence threshold) was predicted with 3TC in a PBPK mechanistic kidney model (Simcyp v16 R1) [Report 2017N352403, m2.6.5, Table 5.1] suggesting that 3TC is not predicted to perpetrate systemic DDIs with metformin [m2.7.2, Table 3].

The combination of DTG with 3TC should not alter the perpetrator risk potential for 3TC when compared to monotherapy or other approved 3TC combinations.

4. TOXICOLOGY

4.1. Presentation Order of Toxicology Summaries

Toxicology Summary	Section
Toxicology for DTG and 3TC in combination	Not applicable.
Toxicology of DTG alone	Section 4.2
Toxicology of 3TC alone	Section 4.3
Overall summary of all toxicology studies performed with DTG or 3TC	Section 5, Integrated Assessment of the Fixed Dose Combination Product

4.2. DTG

A summary of toxicology studies conducted is presented in m2.6.6, DTG NDA. A listing of each study conducted, and a tabular summary of each safety pharmacology study and its findings, is presented in m2.6.7, DTG NDA.

4.2.1. 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 DTG due to intolerance vomiting) observed after a single dose at ≥150 mg/kg. Exposure in this single dose TK study at 100 mg/kg/day was ~44 μg.h/mL, which is approximately one-eighth that achieved in the monkey 14 day study at the high dose of 1000 mg/kg/day.

4.2.2. Single dose toxicity

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

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

4.2.3. Repeat dose toxicity

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

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

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

A tabular summary of the principal treatment-related findings and effect/no effect doses in repeat dose studies in rats and monkeys is presented in Table 4.1 , and comparisons of systemic exposure to DTG are presented in Table 4.2. A comparison of DTG animal to human exposure ratios (AUC_{0-24}, mg/kg and mg/m^2) in the definitive rat and monkey studies is presented in Table 4.3 and Table 4.4 and respectively.

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

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

4.2.3.2. Gastrointestinal effects

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

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

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

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

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

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

Nonclinical evidence for GI toxicity with DTG (including vomiting, diarrhea and gastric/colonic erosions) did not translate into significant findings for DTG in double blinded randomized clinical trials [see m2.7.4 Section 2.1.2].

4.2.3.3. 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 above the expected human exposure for a 50 mg QD dose. In the 38 week monkey toxicity study, liver findings were restricted to increased AST (2.5X) and bilirubin (2.8X) in the moribund animal in the 50 mg/kg/day group (euthanized on Day 55) [Report RD2009/00036]. The findings in the 38 week study were considered secondary to the moribund condition. Exposure (end of study, gender mean) at the NOAEL (15 mg/kg/day) was 39 μg.h/mL which corresponds to ~0.7X the expected human exposure for a 50 mg QD dose. No treatment-related adverse effects on liver were observed in rats in studies up to 26 weeks.

Human subjects were carefully monitored for liver effects, and cumulative data to date suggests an acceptable hepatic safety profile for DTG similar to that observed for other antiretroviral anchor drugs.

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

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

Analyses of adverse events from the Renal Systems Organ Class in clinical studies do not suggest that DTG has an adverse effect on renal function [see m2.7.4 Section 3.3]. 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 (e.g., tenofovir disoproxil fumarate), and were not thought to be related to DTG treatment.

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

4.2.3.5. Bone marrow and lymph node effects

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

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

In the 4 week monkey study, decreased RBCs (0.91X) were observed in females given 100 mg/kg/day, with no correlating histopathology findings [Report RD2008/00107]. The NOAEL was 50 mg/kg/day. Exposure (end of study, gender mean) at 50 mg/kg/day was 132 μg.h/mL which corresponds to ~2X above the expected human exposure for a 50 mg QD dose. No treatment-related adverse effects on bone marrow and lymph nodes were observed in non-moribund animals in the 9 month monkey toxicity study at doses ≤50/30 mg/kg/day [Report RD2009/00036].

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

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

A review of hematology laboratory data from clinical trials revealed no signal for bone marrow or lymph node toxicity caused by DTG [see m2.7.4 Section 3.7].

4.2.4. Genetic toxicology

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

4.2.5. Carcinogenicity

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

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

The NOAEL for non-neoplastic findings after chronic oral administration was the high dose of 500 mg/kg/day for mice and 50 mg/kg/day for rats. When compared to the expected human exposure for a 50 mg QD dose, the systemic exposures were ~20X higher for mice and ~17X higher for rats. Comparisons of systemic exposure to DTG in the mice and rat carcinogenicity studies is presented in Table 4.2.

4.2.6. Reproductive toxicology

4.2.6.1. Fertility and embryofetal development

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

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

In an embryofetal development study in rabbits, DTG was orally administered at 40, 200 or 1000 mg/kg/day to pregnant rabbits [Report XD2009/00366]. Suppressed body weight gain (13.6% on gestation Day 19), decreased food consumption (up to 53%) and scant or no feces/urine associated with the decreased food consumption were noted in the 1000 mg/kg/day dams. The NOAEL was 200 mg/kg/day for maternal general toxicity (~0.27X the expected human exposure for a 50 mg QD dose) and 1000 mg/kg/day for maternal reproductive function and embryofetal development (0.56X the expected human exposure for a 50 mg QD dose). Comparisons of systemic exposure to DTG in the embryofetal development studies is presented in Table 4.2.

In summary, animal data does not indicate direct or indirect harmful effects with respect to reproductive toxicity when DTG is used in accordance with dosing information in the product label [m1.14.1].

4.2.6.2. Pre- and post-natal development

In a pre- and post-natal development study, DTG was administered to female rats at doses of 5, 50 or 1000 mg/kg/day from Day 6 of gestation to Day 20 of lactation [Report 2011N121663]. Suppressed body weight gain and decreased food consumption were noted in dams (F_0) in the 1000 mg/kg/day group during the lactation period, which were associated with mild decreases in body weights in the offspring in the 1000 mg/kg/day group from pre-weaning until adolescence. There were no adverse effects on maternal pregnancy, parturition, lactation or offspring (F_1) survival, behavioural or reproductive function. The NOAEL for maternal reproductive function was 1000 mg/kg/day (~32X above the expected human exposure for a 50 mg QD dose based on exposures achieved in female rats in the 4 week toxicity study). Due to the decreased body weights of the offspring observed at higher doses, the NOAEL for pre- and post-natal development of the offspring (F_1) was 50 mg/kg/day. At this dose, the expected human exposure is ~25X above a 50 mg QD dose (extrapolated from gender mean exposures achieved in the rat 14 day toxicity study). Based on the fact that effects on offspring body weights were noted at doses where maternal toxicity was observed, and the presence of considerable safety margins expected at the proposed clinical doses, there is minimal risk for adverse effects on post-natal development in offspring of mothers receiving DTG.

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

4.2.6.3. Ongoing Investigations

A review of the existing nonclinical data and a search of the scientific literature for DTG has been conducted to ensure that there are no signals that should be reconsidered based on a potential signal for NTDs observed in an ongoing birth outcomes surveillance study (see m2.7.4, Section 6.1). The review did not identify any evidence of adverse effects on neural tube formation or embryonic toxicity, nor did this review suggest a plausible mechanism for the safety signal.

To address the lack of an experimental model investigations are ongoing into the utility of the rat whole embryo-culture model to detect NTDs and therefore, if a signal was detected, possibly due to more direct exposure of the embryo to DTG, it would provide a model system for further evaluations. In addition, the potential of integrase inhibitors to disrupt folate transport is being investigated. This includes exploring the feasibility of assays to investigate potential effects on folate transporters (proton-coupled folate transporter [PCFT]) and the reduced folate carrier [RFC]).

4.2.7. Juvenile toxicology

A juvenile toxicity study in rats was conducted with DTG at oral doses of 0.5, 2 or 75 mg/kg/day from Days 4 to 66 post-partum (pp) [Report CD2010/00023]. Two pre-weanling deaths were considered test article-related at 75 mg/kg/day. Over the pre-weaning treatment period (Days 4 to 21 pp), mean body weight gain was decreased (0.86X control mean gain) for males and females in the 75 mg/kg/day group and the decrease persisted throughout the entire study for females during the post-weaning period. There were no test article-related differences among the groups for the age at which offspring attained physical signs of sexual maturation (vaginal opening or balano-preputial skinfold separation). There were no changes considered related to DTG administration in stage-dependent evaluation of spermatogenesis. There were no test article-related effects on T-cell-dependent antibody response (TDAR) measured on Day 67, and no effects on lymphocyte subsets (T-cells, both CD4 and CD8 subsets, and B-cells) and CD4 or CD8 T-cell receptor Vβ usage in peripheral blood. Therefore, the NOAEL in juvenile rats was 2 mg/kg/day (Day 32 pp gender mean $AUC_{0\text{-}24}$ = 90 μg.h/mL and C_{max} = 7.6 μg/mL). Comparisons of systemic exposure to DTG in the rat juvenile toxicity study is presented in Table 4.2.

4.2.8. Local tolerance

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

4.2.9. Other toxicity

4.2.9.1. Immunotoxicity

Immunotoxicity assessment in adults

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

Immunotoxicity assessment in juveniles

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

4.2.9.2. Phototoxicity

In the absorption spectrum for DTG, the main peak in the UVA/UVB region is at 258 nm (outside the range of concern), and there are minor peaks at 309, 321 and 335 nm, with a tail extension to 395 nm [see m3.2.S.1.3, General Properties]. A whole body autoradiography (WBA) study in pigmented rats following a single oral administration of [^{14}C]-DTG (sodium salt) demonstrated wide tissue distribution of drug-related material without significant retention in skin or eyes.

In the Neutral Red Uptake assay using Balb/c 3T3 fibroblasts, DTG was phototoxic when tested up to the limit of solubility in primary vehicle (7.93 mg/mL, the maximum dose formulated in DMSO) [Report 2014N193936]. However, in an in vivo study using Long-Evans pigmented rats, DTG showed no evidence of cutaneous phototoxicity at doses up to 1000 mg/kg/day for 3 days [Report 2014N213488].

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

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

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

Key:

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

4.3. 3TC

A summary of toxicology studies conducted is presented in m2.6.6, 3TC NDA. A listing of each study conducted, and a tabular summary of each safety pharmacology study and its findings, is presented in m2.6.7, 3TC NDA.

4.3.1. Choice of species

In general, the similarities between the pharmacokinetics of 3TC in man and the animal species used in the toxicity studies indicate that these species were appropriate for predicting the safety of the drug and its metabolites.

4.3.2. Single dose toxicity

3TC has a low acute toxicity by the oral and intravenous routes. Acute intravenous administration of a single 2000 mg/kg dose of 3TC was well tolerated in both mice and rats and was not associated with any target organ toxicity [Reports WPT/91/110 and WPT/91/088]. Acute oral administration of 4000 mg/kg 3TC (two doses of 2000 mg/kg, 4 hours apart) to mice resulted in no deaths and no evidence of target organ toxicity [Report WPT/91/098]. An acute oral toxicity study in rats was not performed since very large doses (up to 4000 mg/kg/day) were used for repeat dose studies.

4.3.3. Repeat dose toxicity

The repeated administration of either 3TC or the racemate, gsk001* , was very well tolerated. Treatment-related effects were largely confined to the highest dose group following oral administration for up to 6 months in the rat (4000 mg/kg/day) and 12 months in the dog (3000 mg/kg/day for males; 2000 mg/kg/day for females [Reports WPT/93/361 and WPT/92/407].

In rats, changes observed in clinical pathology parameters were minor and reversible. A chronic low grade irritation of the cecum/anus was seen with the highest dose of 3TC (4000 mg/kg/day. However, there was a clear no effect level for the cecal changes at the intermediate dose (850 mg/kg/day, which is approximately 142 times higher than the therapeutic dose (300 mg or 6 mg/kg for a 50 kg human on a mg/kg basis. While approximately 35% of an orally administered dose of 3TC is unabsorbed and excreted in the feces of the rat, only 15 to 20% is unabsorbed in humans. Combining these safety margins, the finding in the cecum was not considered to represent a safety hazard for humans.

In a 3 month oral study in dogs, one female receiving a dose of 3000 mg/kg/day died during Week 5 and, over the following 2 weeks, 2 further animals from this group were killed for humane reasons [Report WPT/92/132]. While these deaths were considered to be treatment-related, the precise cause of death was not established and many of the effects seen in these animals were considered to reflect a general decline in condition after a prolonged period of inappetence. No deaths occurred in the 12 month dog study where the high dose in females was 2000 mg/kg/day.

In the chronic toxicity studies, hematological changes in the dog were more pronounced than in the rat. They consisted of mild to moderate decreases in erythrocyte, total leukocyte, neutrophil and lymphocyte counts, and increases in mean corpuscular volume (MCV, mean corpuscular haemoglobin (MCH and mean activated partial thromboplastin time (APTT. White cell changes were restricted to the high dose group throughout, but minor red cell changes were evident in the low dose group (90 mg/kg/day in the latter part of the 12 months study. All changes showed evidence of regression in recovery animals.

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In the 12 month dog study, plasma folate and Vitamin B_{12} levels were measured, as reductions in these are a potential cause of macrocytic anaemia. Surprisingly, treatment-related increases in plasma folate and Vitamin B_{12} were seen, with values in the high dose males being approximately twice those of the control animals. The reason for this is unknown, but as both folate and Vitamin B_{12} are involved in nucleotide metabolism, it may be a biochemical response to the large doses of 3TC (a cytidine analogue. All changes showed evidence of regression in recovery animals.

In dogs, excluding changes noted in 3 females that died or were euthanized in moribund condition in the 3 month study, histopathological findings were limited to slight centrilobular lipid deposition in the liver and moderate thymic atrophy in high dose animals (3000 mg/kg/day in the 3 month study, and minimal hemosiderosis in the spleen in the high dose group of the 12 month study [Report WPT/92/407]. There was no alteration in bone marrow cytology. All changes ameliorated after a 4 week recovery period.

Preliminary studies in the marmoset were conducted on both the racemate, gsk001* , and 3TC using intravenous administration. gsk001* was administered at 600 mg/kg/day for 7 days and 3TC at doses up to 1200 mg/kg/day for 14 days. These studies revealed no unexpected toxicities and any findings were consistent with the responses seen in the rat and dog.

Comparisons of systemic exposure to 3TC in the definitive rat and dog toxicity studies is presented in Table 4.5.

4.3.4. Genetic toxicology

In vitro studies

3TC was negative in the Ames assay at concentrations up to 5000 μg/plate and in the fluctuation test using *E. coli* tester strains [Reports WPT/90/240 and WPT/93/246]. In an in vitro cytogenetic test in human peripheral lymphocytes, 3TC showed clastogenic activity at the highest concentrations evaluated in the absence of S9-mix (300 μg/mL) and in the presence of S9-mix (2292.5 μg/mL; 10 mM) [Report WPT/93/120]. In the mouse lymphoma assay, 3TC induced small increases in mutation frequency at concentrations of 1000 μg/mL and above when a 24 hour exposure period was used [Report WPT/90/271], but was not mutagenic in the same test system at concentrations up to 5000 μg/mL using a 3 hour treatment period. 3TC did not induce morphological transformation of mouse embryo cells at concentrations up to 320 μg/mL in the absence of S9-mix or up to 5000 μg/mL in the presence of S9-mix [Report UTX/92/018].

In vivo studies

Mitochondrial toxicity is considered to be a class effect of NRTIs; therefore, studies investigating the potential effects of 3TC on mitochondrial DNA have been carried out in pregnant mice and their offspring.

＊ 新薬承認情報提供時に置き換え

B6C3F$_1$/Tk$^{+/-}$ mice were administered daily intraperitoneal injections of 200 mg/kg/day 3TC from Days 1 to 8 of age. 3TC was found not to cause an increase in micronuclei, did not induce mutations in the Hprt gene and did not cause a significant increase in the Tk mutant frequency. Therefore, 3TC was not genotoxic in B6C3F$_1$/Tk$^{+/-}$ mice [Von Tungeln, 2002]. For a more complete discussion of NRTI-related mitochondrial toxicity, refer to Section 4.3.7.

When pregnant CD-1 mice were treated with 100 mg/kg/day 3TC from Day 12 to Day 18 of gestation there were no increases in the Hprt mutant frequency in male offspring on post-natal Days 13, 15 and 21 following treatment with 3TC compared with control [Torres, 2007]. A further study evaluated mutagenic effects in pregnant C57BL/6N mice treated with 120 mg/kg/day 3TC, from Day 12 to Day 18 of gestation, alone or in combination with zidovudine. No effects were detected in neonates from pregnant mice treated with 3TC alone, except for a weak increase in chromosomal damage measured on Day 1, and an increase in Tk mutant frequency in 5 week old female mice [Von Tungeln, 2007].

No in vivo genotoxic activity was evident following oral doses of 3TC of up to 2000 mg/kg in either the rat bone marrow metaphase analysis assay, the rat bone marrow micronucleus test (3 daily doses) or the rat liver unscheduled DNA synthesis assay [Reports WPT/90/275, WPT/93/195 and WPT/90/408].

4.3.5. Carcinogenicity

3TC showed no evidence of carcinogenic potential in a 24 month study in mice receiving doses up to 2000 mg/kg/day in the diet (~12X above human exposure for 300 mg dose, 12.3 μg.h/mL) [Report WPT/95/297]. A dose-related reduction in body weight gain was seen at all dose levels as were minor hematological changes. Histopathological examination showed a slightly higher incidence of histiocytic sarcomas in the 180 and 2000 mg/kg/day female groups. However, there was no trend with dose and the increases were only significant in one out of the two statistical methods employed. Furthermore, as the incidences were similar to that of historical controls, the occurrence of histiocytic sarcoma was considered to be incidental to treatment with 3TC. There was a slight but non-dose-related increase in the incidence and degree of mineralisation of the cortex of the kidney of all treated male mice. The femur of females treated with 600 or 2000 mg/kg/day showed a non-dose-related increase in bone deposition. Both of these findings were considered to be exacerbations of normal age-related changes in mice.

3TC showed no evidence of carcinogenic potential in a 24 month study in rats receiving doses up to 2000 mg/kg/day for males (~43X above human exposure for 300 mg dose, 12.3 μg.h/mL) and 3000 mg/kg/day for females (~72X above human exposure for 300 mg dose, 12.3 μg.h/mL) by dietary administration [Report WPT/95/298]. There was no effect of treatment on survival. Final body weight was 8 to 18% lower than that of the controls in groups treated with doses of 1000 mg/kg/day or more. There was no evidence of the expected decrease in leucocyte or erythrocyte counts in 3TC-treated groups at the end of the study. There was a slight increase in the incidence of endometrial epithelial tumors in females dosed at 3000 mg/kg/day. However, this was only slightly higher than historical control values but achieved statistical significance (p=0.017). Furthermore,

there was no increase in the incidence of pre-neoplastic findings in the endometrium (e.g., proliferative changes) in the 3TC dosed groups. For these reasons the occurrence of endometrial tumors was considered to be unrelated to treatment. The only non-neoplastic finding of note was an increase in the incidence and severity of mineralization in the kidney, seen at doses of 1000 mg/kg/day and above, with accompanying suburothelial capillary dilatation in males at 2000 mg/kg/day. This was considered to be an exacerbation of a normal age-related change commonly seen in rat kidney.

Comparisons of systemic exposure to 3TC in the mice and rat carcinogenicity studies is presented in Table 4.5.

4.3.6. Reproductive and developmental toxicity

In a rat fertility study, with the exception of a few minor changes at the high dose level, the overall reproductive performance of the F_0 and F_1 generation animals, and the development of the F_1 and F_2 generation, was unaffected by treatment with 3TC at doses up to 4000 mg/kg/day [Report WPT/93/210].

In oral organogenesis studies in rats, there were no treatment-related effects on gestation length and index, the incidence of pre- and post-implantation loss, the number of implantations and live fetuses, fetal sex ratio, fetal body weight or embryofetal development at doses up to 4000 mg/kg/day [Report WPT/91/305].

Rabbits were generally more sensitive to 3TC than rats and therefore lower doses had to be used for the rabbit organogenesis studies. In the main study, one intermediate dose (300 mg/kg/day) animal died on Day 17 (Day 1 = day of mating) [Report WPT/91/333]. One low dose (90 mg/kg/day), one intermediate dose (300 mg/kg/day) and 3 high dose (1000 mg/kg/day) animals aborted. A reduction in the amount of feces excreted, thin appearance and orange colored urine were noted in all groups, including the controls, although these findings were observed more frequently at the high dose level. Body weight gain was transiently reduced in the control, low and intermediate dose groups. At the high dose level, body weight loss and a reduction in food and water consumption were observed throughout the treatment period. There were no post mortem abnormalities attributable to 3TC in an animal found dead, the animals which aborted or the dams that were killed at term.

Fetal examination in the rabbit study revealed total in utero litter loss in 2 intermediate dose animals and in one high dose animal. Pre-implantation loss was slightly increased at all dose levels and, consequently, the mean number of viable fetuses per litter was decreased compared with the control group, although these findings were not dose-related. Since pre=implantation loss, as assessed here, may include very early post-implantation deaths which could coincide with the onset of dosing, this finding was considered to represent a possible treatment-related embryolethal effect. Post-implantation loss and the proportion of male fetuses were unaffected by treatment.

A further oral organogenesis study was performed to establish a no effect level for the increased incidence of pre-implantation loss seen in the previous study. Pre-implantation loss was slightly, but not significantly, increased at 40 and 90 mg/kg/day, with consequent reductions in the number of implantations and fetuses. The no effect level was 15 mg/kg/day.

At the lowest dose level at which the slight increase in pre-implantation loss was observed in rabbits (20 mg/kg BID), systemic exposure on Day 20 of pregnancy based on the C_{max} and AUC (1.05 μg/mL and 5.9 μg/h/mL) was 0.48X the estimated exposure in humans at 300 mg. There was no evidence of any effect on pre-implantation loss in the rat at plasma C_{max} concentrations approximately 32 times the anticipated human exposure for a 300 mg/day dose.

No treatment-related external or visceral fetal abnormalities were apparent during organogenesis studies in rabbits following administration of 3TC at doses up to 1000 mg/kg/day. An indirect effect on fetal skeletal development (reduction in epiphysial ossification and increased incidence of unilateral supernumerary ribs) was observed at the highest dose only. In a subsequent study, skeletal examination revealed increases in the incidence of supernumerary ribs at doses up to 90 mg/kg/day. The occurrence of supernumerary ribs in the fetus has been shown in some species to be an indirect effect of maternal toxicity or stress [Beck, 1989; Branch, 1992], and appears to be a condition which is reversible post-natally and has no permanent structural effect on the skeleton [Wickramaratne, 1988]. This was not, therefore, regarded as a significant finding.

In a combined peri-/post-natal and juvenile toxicity study in rats, mated females received 3TC at 90, 450 or 2000 mg/kg twice daily from Day 17 of pregnancy until post-natal Day 22. Two male and female offspring from each dam received a single daily dose of 3TC orally at 90, 450 or 2000 mg/kg/day from Litter Day 3 until Litter Day 43. No treatment-related effects were observed on gestation length, gestation index, the number of offspring born, the proportion of male offspring, birth index, viability index or weaning/lactation index [Report WPT/93/165]. The majority of dams receiving 4000 mg/kg/day showed a swollen/reddened anus/rectum during the lactation period, which correlated with histological inflammatory changes at the ano-rectal junction. An increased incidence of swollen/reddened anus/rectum was also observed in some offspring receiving 2000 mg/kg/day, with similar histological changes to those seen in the dams. These changes, together with slight diffuse epithelial hyperplasia in the cecum of several dams and offspring at the high dose levels, were attributed to the prolonged exposure to large concentrations of unchanged 3TC in the gastrointestinal tract, resulting in typical responses to an irritant material. Similar cecal changes were also recorded in the 6 month oral toxicity study in the rat.

Hematological changes consistent with a mild macrocytic anaemia, as seen in the adult repeat dose rat studies, were noted in the juvenile rats treated with up to 2000 mg/kg/day 3TC. A statistically significant reduction in testis weight was observed in juveniles at this dose level. Histological examination of the testes revealed slight to moderate dilatation of the seminiferous tubules in a number of high dose males (8/25). There was no evidence of morphological abnormality other than slight compression of the germinal

epithelium. None of the other toxicity studies conducted with 3TC using adult rats and dogs have revealed testicular effects. Similarly, no effects were noted in a fertility study in the rat. The toxicity would therefore appear specific to the developing/immature testis. The no effect level for the testicular effects was considered to be the intermediate dose level of 450 mg/kg/day (~80X above human exposure based on C_{max}).

Pregnant CD-1 mice were administered 500 mg/kg BID 3TC orally from Day 10 of gestation to delivery. Offspring locomotion and nociceptive sensitivity were examined on post-natal Days 8, 14 and 28 after administration of two doses of GABAergic agonist muscimol. A 30 minute locomotor activity test and 60 second hot plate test were used. Pre-natal 3TC exposure had a limited effect on locomotor activity development and no effect on nociception [Ricceri, 2001].

3TC was administered orally to pregnant CD-1 mice at doses of 125, 250 or 500 mg/kg BID from Day 10 of gestation to delivery. Data on reproductive performance were collected and offspring were examined for a series of different somatic and behavioral end points. Learning and retention performances of a passive avoidance task on post-natal Day 20/21 were unaffected by 3TC treatment, while decreased habituation in an automated locomotor activity test was evident in male offspring exposed to 250 and 500 mg/kg [Calamandrei, 1999]. In another study in mice at the same doses, offspring behavior was examined on post-natal Day 35 in a 20 minute social interaction test. At adulthood, different behavioral endpoints were analyzed. Findings confirmed the low neurotoxicity of 3TC, however, some significant behavioural alterations were noted. These were a decrease in immobility in the open field test, an increase in the responsiveness to scopolamine shown by the 500 mg/kg 3TC treated mice in the open field and a longer escape latency in the first day of the reversal phase in the water maze. No significant changes in either pain sensitivity, social/affiliative or maternal behavior were found, although a higher occurrence of aggressive behavior toward foster pups was noted in both the 125 and 500 mg/kg females [Calamandrei, 2000].

Comparisons of systemic exposure to 3TC in the definitive rat and rabbit embryofetal development studies is presented in Table 4.5.

4.3.7. Mitochondrial toxicity

Mitochondrial toxicity is a class effect of NRTIs [Lewis, 2005; Moyle, 2005]. Studies investigating the effects of in utero exposure to NRTIs, conducted in mice and monkeys, have focused on the potential to cause mitochondrial toxicity in the heart. There is relatively little data on the effects of 3TC alone; however, in one study, CD-1 mice were administered 75 mg/kg/day 3TC throughout gestation and for 28 days post-natally prior to cardiomyocyte examination for mitochondrial damage. There was a depletion in mtDNA copy number and an increase in mtDNA lesions in 3TC-treated 4 week old mice of both sexes; however, these parameters had recovered to control levels by 10 weeks of age. There were no significant mtDNA deletions or changes in cytochrome c oxidase (COX) staining intensity [Chan, 2007].

In order to assess the occurrence and persistence of cardiac changes, a study was conducted in which CD-1 mice were exposed in utero to 3TC (40 mg), zidovudine (80 mg) or 3TC plus zidovudine (40/80 mg) during Days 12 to 18 of gestation [Torres, 2010]. Hearts from female mouse offspring were examined at 13 and 26 weeks post-partum (pp) and ECG measurements indicated progressive thinning of the left ventricular posterior wall. The induction of cardiac effects by 3TC and zidovudine were additive compared with either drug alone.

Mitochondrial morphology and mtDNA content have also been investigated in the umbilical cord and cord blood of *Erythrocebus patas* monkeys exposed in utero to 24 mg/day 3TC during the last 4 weeks of gestation [Divi, 2007a]. Umbilical cord endothelial cells contained some swollen mitochondria with pale matrices and disorganised cristae. This was accompanied by a small (<35%) decrease in mtDNA content, which remained unaffected in cord blood leukocytes. In the same study, samples were evaluated from HIV-infected pregnant humans treated with 3TC (and/or other antiretrovirals): transplacental exposures induced similar mitochondrial damage in cord blood and umbilical cords from uninfected monkey infants and from human infants born to HIV-infected women.

Mitochondrial damage was also investigated in skeletal muscle from the offspring of *E. patas* monkeys administered 3TC alone, or with zidovudine or stavudine, for the last 10 weeks of gestation, or for 10 weeks of gestation and 6 weeks after birth [Divi, 2007b]. Skeletal muscle mitochondrial compromise occurred at birth and was found to persist at 1 year of age, although the effect in monkeys which had been treated with 3TC alone was much less extensive than in skeletal muscle from monkeys treated with the combinations.

The significance for humans of the nonclinical neurobehavioural and mitochondrial effects described above is uncertain. There are currently no clinical data assessing possible mitochondrial dysfunction following peri-natal exposure to 3TC alone, the limited available data being derived from infants exposed to 3TC combined with zidovudine. Evidence of mitochondrial dysfunction was reported in 8 of 1754 HIV uninfected children exposed peri-natally to NRTIs, with neurological symptoms prevalent among the affected individuals. Two of the 8 children, both of whom had been exposed peri-natally to 3TC and zidovudine, died at 11 and 13 months of age [Blanche, 1999]. Subsequently, the Peri-Natal Safety Review Working Group has systematically reviewed the cases of more than 20000 children born to HIV-infected mothers, among whom 1459 (6%) were exposed peri-natally to 3TC and zidovudine. Cases were reviewed for deaths at less than 5 years of age and evidence of mitochondrial disease among the living. The authors concluded that there was no indication that antiretroviral therapy was associated with deaths related to mitochondrial dysfunction [PSRWG, 2000]. In a further study in 1798 African infants exposed peri-natally to 3TC and zidovudine, there was no evidence of increased neurologic events after 18 months follow up [PETRA, 2002]. A review of the data from the Paediatric AIDS Clinical Trials Group (PACTG) cohorts from protocols 219 and 219C concerning children peri-natally infected with HIV found a higher risk of possible mitochondrial dysfunction amongst children exposed to stavudine, 3TC, 3TC-stavudine combination and stavudine-didanosine combinations compared to other antiretrovirals. The authors deployed two sets of case definition criteria (Enquête Périnatal Française and the Mitochondrial Disease Classification) to identify

children exhibiting clinical signs and symptoms consistent with possible mitochondrial dysfunction. Overall, 33.5% of 2931 HIV-infected children met at least one published case definition for 'possible mitochondrial dysfunction' and 16.4% met both sets of criteria. The case definitions were based on clinical signs since appropriate biochemical, histopathological, molecular and genetic data on which to confirm the diagnosis were not available, limiting the precision with which odds ratios could be calculated.

In addition, the Antiviral Pregnancy Registry [APR, 2018] contains sufficient numbers of first trimester exposures to 3TC in humans to be able to detect at least a 1.5-fold increase in overall birth defects, and a 2-fold increase in defects in the more common classes, cardiovascular and genitourinary systems, but no increase associated with mitochondrial dysfunction has been observed to date.

4.3.8. Phototoxicity

3TC does not absorb light in the 290 to 700 nm wavelength range. Photosensitivity reactions have not been a clinical issue with 3TC, for which there is significant clinical experience. Thus, nonclinical phototoxicity studies have not been performed on 3TC.

Table 4.5 Comparative Assessment of Mean Systemic Exposure Following Oral Administration of 3TC

Species (Duration)	Dose (mg/kg BID, Unless Stated)	C_{max} (µg/mL)[a]	AUC_{0-t} (µg.h/mL)	Animal to Human AUC Ratio (300 mg)[b]
		End of Study	End of Study	
Rat (3 month)	45	4.7	20.9	3.4
	300	30.9	145	23.5
	2000	94.6	690	112
Rat (6 month)	90	11.8	69.3	11.3
	425	31.7	133	21.6
	2000	113	624	101
Dog (3 month)	45	21.5	66.2	10.8
	260	89.8	367	59.7
	1500	331	1582	257
Dog (12 month)	45	22.9	74.5	12.1
	260	85.9	343	55.8
	1500 (M)	213	872	142
	1000 (F)	216	948	154
Rat (embryofetal development)	45	3.1	ND	ND
	300	23.8	ND	ND
	2000	66.9	ND	ND
Rabbit (embryofetal development)	7.5	0.45	2.3	0.37
	20	1.05	5.9	1.0
	45	2.55	14.8	2.4
	150	8.0	ND	ND
	500	68.4	ND	ND
Rat (juvenile)	90 (males)	18.1	ND	ND
	90 (females)	21.1	ND	ND
	450 (males)	167	ND	ND
	450 (females)	176	ND	ND
	2000 (males)	406	ND	ND
	2000 (females)	472	ND	ND
Mouse carcinogenicity[c]	180 mg/kg/day	1.1	16.4	1.3
	600 mg/kg/day	3.4	47.6	3.9
	2000 mg/kg/day	9.7	151	12.3

Comparative Assessment of Mean Systemic Exposure Following Oral Administration of 3TC (Continued)

Species (Duration)	Dose (mg/kg BID, Unless Stated)	C_{max} (µg/mL)[a]	AUC_{0-t} (µg.h/mL)	Animal to Human AUC Ratio (300 mg)[b]
		End of Study	End of Study	
Rat carcinogenicity[c]	300 mg/kg/day	4.5	75	6.1
	1000 mg/kg/day	15	242	19.7
	2000 mg/kg/day	28	533	43.3
	3000 mg/kg/day	38	880	71.5
Human[d]	300 mg/day	2.1	12.3	NA

Key:
a = Approximate C_{max} values are at either 1 or 2 hours post dose. Value presented is that obtained following the first daily administration in the last week of the study, except for carcinogenicity studies where determinations were made in Week 5.
b = In the case of twice daily dosing in animal toxicity studies, the AUC_{tau} for a 12 hour dosing interval is doubled before calculating the approximate multiple of the AUC_{tau} in man for a 24 hour dosing interval.
c = Carcinogenicity studies were dosed QD as opposed to other toxicity studies which were dosed BID.
d = Based on exposure (C_{max} and AUC_{0-t}) achieved in the single dose pivotal bioequivalence study of DTG/ABC/3TC, Study ING114580.

4.4. Impurities and Excipients

For DTG there are no specified impurities in the proposed Drug Substance Specification that exceed the 0.15% w/w ICH qualification threshold [m3.2.S.4.1].

3TC is an established active pharmaceutical ingredient (API) which has an extensive history of use in registered products since its first introduction in the 1990's. Specified impurities in the 3TC drug substance were previously considered qualified on the basis of the levels of the impurities present in batches used, and the profiles of impurities, which were tested in pivotal nonclinical toxicity studies. 3TC drug substance is manufactured by approved suppliers using an approved route.

A genotoxic risk assessment was conducted to determine whether any additional items of concern would result from the combination of DTG with 3TC in the drug product. This risk assessment did not identify any new degradation products that are not already present in established commercial products containing DTG or 3TC e.g. TIVICAY (DTG) and EPIVIR (3TC). No additional risks were identified through an evaluation of the drug product manufacturing process.

5. INTEGRATED ASSESSMENT OF THE FIXED DOSE COMBINATION PRODUCT

The Sponsor has completed a comprehensive assessment of the nonclinical safety of DTG in support of treatment of patients with HIV. DTG has been evaluated in toxicity studies of up to 26 weeks in duration in Sprague Dawley rats, 38 weeks in cynomolgus monkeys and 13 weeks in Crl:CD-1 (ICR) BR mice. The carcinogenicity of DTG has been evaluated in 2-year studies in mice and rats. 3TC is an established product that has been in use worldwide for an extended period of time. It is routinely prescribed in clinical practice. The pharmacology, pharmacokinetics, toxicology and safety profile in clinical use of 3TC is well known. 3TC has been evaluated in toxicity studies of up to 26 weeks in duration in rats and 52 weeks in dogs. The carcinogenicity of 3TC has been evaluated in 2-year studies in mice and rats.

ICH Guidance M3 (R2) on Non-Clinical Safety Studies for the Conduct of Human Clinical Trials and Marketing Authorisation for Pharmaceuticals states that, "For most combinations which involve two late stage entities and for which there is adequate clinical experience with co-administration, combination toxicity studies would generally not be recommended to support clinical studies or marketing unless there is significant toxicological concern (e.g., similar target organ toxicity)" [ICH M3 (R2), 2009]. In addition, according to Question 9 in the Questions and Answers document written by the M3 (R2) Implementation Working Group for M3 (R2) Guideline "Non-Clinical Safety Studies for the Conduct of Human Clinical Trials and Marketing Authorization for Pharmaceuticals" regarding the scope of ICH M3 (R2), it is accepted that combination toxicity studies are not generally warranted for HIV products unless there is a specific cause for concern under clinically relevant conditions. An extensive review of the nonclinical data for DTG and 3TC has shown:

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

Based on this, together with data from the Phase 3 clinical studies, GEMINI-1 Study 204861 and GEMINI-2 Study 205543 [m2.5, Section 4], the Sponsor considers that combination toxicity studies would provide no additional safety information and are not necessary to support the proposed marketing application for the DTG/3TC FDC.

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

5.1. Secondary and safety pharmacology

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

3TC: No specific nonclinical secondary pharmacology studies have been performed with 3TC. There were no findings from safety pharmacology studies that would indicate an unacceptable risk for oral administration of 3TC to patients in accordance with the proposed indication.

5.2. Pharmacokinetics

DTG: The nonclinical pharmacokinetics of dolutegravir were similar across the primary species (mouse, rat and monkey) used for pharmacological and toxicological evaluation. In each species, plasma clearance was low, relative to liver plasma flow. The nonclinical protein binding of dolutegravir across species was high (>99%) and not different from the in vitro and ex vivo assessment in human plasma. Absorption was rapid and extensive from solution, but was limited by solubility from suspensions and tablet formulations. The increase in exposure to dolutegravir generally was less than proportional to the increase in dose in toxicological evaluations. The absorbed dose was extensively metabolized in each species, with dolutegravir the predominant drug-related component in plasma which is also consistent with observations in humans [see m2.7.2, Section 3.1.3].

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

DTG demonstrated weak or no induction potential and inhibition potential of UGT (UGT1A1 and 2B7) or CYP (CYP1A2, 2A6, 2B6, 2C8, 2C9, 2C19, 2D6 and 3A4) enzymes following in vitro incubations. Dolutegravir was a substrate for efflux transporters (P-gp and BCRP) but due to its high permeability, absorption is not expected to be affected by efflux transporter inhibitors. In the clinic dolutegravir inhibited the renal transporter OCT2 as determined by an increase in exposure of co-administered metformin, but no notable inhibition of other transporters was observed. Dolutegravir is contraindicated for co-administration with the OCT2 substrate dofetilide because of the potential for toxicity due to higher exposure [see m2.7.2, Table 3]. Other than sensitive OCT2 substrates with narrow therapeutic ranges, inhibition of metabolism or transporters of co-administered drugs by these aforementioned mechanisms is not expected.

3TC: 3TC has moderate to high plasma clearance, dose-proportional systemic exposure and high bioavailability in animals and humans.

The binding of 3TC to plasma proteins at therapeutic concentrations was low (<17%). After oral administration to rats, the distribution of 3TC was rapid and widespread throughout most tissues. 3TC crosses the placenta in pregnant rats and rabbits and is excreted into the milk of nursing rats.

3TC is cleared almost entirely by renal elimination of unmetabolized drug in the rat, with active tubular secretion playing a significant role. In contrast, tubular secretion does not play a significant role in the elimination of 3TC in the dog, and renal and metabolic clearance are of equal importance.

Following oral and intravenous administration of [^{3}H]-labelled 3TC to the mouse, rat, rabbit and dog, the majority of drug-related material was excreted in the urine, mainly as unchanged 3TC in the mouse, rat and rabbit.

3TC has a low propensity to alter systemic concentrations based on lack of or low inhibition of transporters P-gp, BCRP, OATP1B1, OATP1B3, OCT3, MATE1 and MATE2-K. In vitro, 3TC inhibited OCT1 and OCT2; the clinical consequences are not known, however, a risk assessment conducted using PBPK modelling suggests the risk of a clinically significant interaction is low [see m2.7.2, Section 3.2.6.1].

5.2.1. Potential for pharmacokinetic interactions

Based on the current knowledge of the comparative absorption, distribution, metabolism, and excretion (ADME) profiles of DTG and 3TC, and the results of the pivotal clinical bioequivalence study [see m2.7.2, Section 1.2 Study 204994], clinically relevant PK changes are not anticipated for either DTG or 3TC when the two agents are co-administered.

5.3. Toxicology

5.3.1. Repeat dose toxicity studies

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

human mg/m^2 equivalent dose for a 50 mg QD dose. GI toxicity in animals did not translate to an increased risk for clinical adverse events at DTG doses of 50 mg QD.

3TC: The main treatment-related effects of 3TC in rats and dogs were hematological reductions in red cell counts. In dogs, these findings were associated with increased MCV and MCH, and reductions in total leukocyte, neutrophil and lymphocyte counts. A chronic low grade irritation of the cecum/anus was observed in the rat 26 week toxicity study with the highest dose of 3TC (4000 mg/kg/day). However, there was a clear no effect level for the cecal changes at the intermediate dose (850 mg/kg/day), which is approximately 150 times higher than the recommended therapeutic dose based on a mg/kg basis.

5.3.1.1. Genotoxicity and carcinogenicity

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

3TC: 3TC was not mutagenic in bacterial tests, but like many other nucleoside analogues showed activity in in vitro mammalian tests. 3TC was not carcinogenic in 2 year rat and mouse carcinogenicity studies.

5.3.1.2. Fertility, pregnancy and lactation

DTG: Dolutegravir had no effects on male or female fertility in rats and no effect on embryofetal development in pregnant rats or rabbits. Animal data does not indicate direct or indirect harmful effects with respect to reproductive toxicity when used in accordance with dosing information in the product label [m1.14.1].

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

In a juvenile toxicity study in rats dolutegravir administration resulted in two preweanling deaths at 75 mg/kg/day. Over the preweaning treatment period, mean body weight gain was decreased in this group and the decrease persisted throughout the entire study for females during the postweaning period. There were no test article-related differences among the groups for the age at which offspring attained physical signs of sexual maturation (vaginal opening or balano-preputial skinfold separation) and no treatment

related changes in stage-dependent evaluation of spermatogenesis. There were no new target organs identified in juveniles compared to adults and the NOAEL in juvenile rats was 2 mg/kg/day. This study supports the clinical use of dolutegravir in pediatric populations.

3TC: 3TC had no effects on male or female fertility in rats. It was not teratogenic in animal studies, but a small increase in early embryonic loss was seen when administered to pregnant rabbits at exposure levels comparable to those achieved in humans. However, there is no indication of this effect in the rat at exposure levels up to 35 times that in humans.

The safety of DTG + 3TC as a 2-drug regimen has not been evaluated in clinical studies enrolling adolescent subjects. However, a summary of the existing safety data in adolescents or pediatric subjects from the single-entity components and other FDCs containing DTG and/or 3TC is presented in the Adolescent Extrapolation Report (see m2.7.2 Section 3.3.4).

5.3.1.3. Other toxicity studies

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

3TC: No immunotoxicity studies were performed with 3TC as there were no signals of concern for potential immunotoxicity.

5.3.1.4. Impurities

For DTG and 3TC, there are no specified impurities in the proposed Drug Substance Specification that exceed the 0.15% w/w ICH qualification threshold [m3.2.S.4.5].

3TC is an established active pharmaceutical ingredients (API) which has an extensive history of use in registered products since its first introduction in the 1990's. Specified impurities in the 3TC drug substance were previously considered qualified on the basis of the levels of the impurities present in batches used, and the profiles of impurities, which were tested in pivotal nonclinical toxicity studies. 3TC drug substance is manufactured by approved suppliers using an approved route.

A genotoxic risk assessment was conducted to determine whether any additional items of concern would result from the combination of DTG with 3TC in the drug product. This risk assessment did not identify any new degradation products that are not already present in established commercial products containing DTG or 3TC e.g. TIVICAY (DTG) and EPIVIR (3TC). No additional risks were identified through an evaluation of the drug product manufacturing process.

6. OVERALL CONCLUSIONS

The toxic potential of DTG and 3TC has been well characterized in a comprehensive battery of nonclinical studies. The principal treatment related effect of DTG was GI toxicity observed in repeat dose toxicity studies in rats and monkeys. For 3TC treatment related effects were restricted to minor haematological (mainly red cell parameters). In general, the systemic exposures at the NOAEL in animal toxicity studies are approximately at parity with (DTG), or significantly higher (3TC) than, the expected clinical exposures.

Data from these nonclinical studies support the clinical use of DTG and 3TC for the treatment of HIV-1 in accordance with the proposed dose regimen.

The safe use of DTG/3TC FDC in human pregnancy has not been established. The effect of DTG on human pregnancy is unknown. In reproductive toxicity studies in animals, DTG was shown to cross the placenta. Therefore, administration of DTG/3TC FDC in pregnancy should be considered only if the benefit to the mother outweighs the possible risk to the fetus.

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

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