MODULE 2.6.4. PHARMACOKINETICS WRITTEN SUMMARY

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$AUC_{(0-24)}$	Area under the plasma concentration verses time curve (from time 0 to 24 hours)
$AUC_{(0-t)}$	Area under the plasma concentration verses time curve (from time 0 to last time point)
C_{max}	Maximum observed plasma drug concentration
CL_b	Blood clearance
CL_p	Plasma clearance
CL_i	Intrinsic clearance
equiv	Equivalents
F	Absolute bioavailability
t_{max}	Time at which C_{max} occurred
$t_{½}$	Half-life
V_{SS}	Volume of distribution at steady state
$PAIC_{90}$	Protein-adjusted IC_{90}

1. BRIEF SUMMARY

Studies have been performed to characterize the absorption, pharmacokinetics, distribution, metabolism and excretion of dolutegravir. Most of the data were derived from specific single dose studies performed at doses within the range of those used in repeat dose toxicity testing of the compound. Work was carried out by the oral route of administration as this is the proposed therapeutic route in humans and also by the intravenous route to assess the pharmacokinetics and bioavailability. Investigative studies using the subcutaneous and intramuscular routes were performed to assess potential long-acting parenteral formulations. A number of in vitro investigations have also been conducted to determine the binding of dolutegravir to serum or plasma proteins, its interaction with transporters, and its metabolism by or interaction with cytochrome P450 enzymes. All of these studies have been conducted in compliance with Company Divisional Standard Operating Procedures and Policies and in general accordance with the principles of Good Laboratory Practice (GLP). Analysis in support of the pivotal repeat dose toxicity studies, and select other in vivo studies, was performed in full compliance with GLP regulations. Nothing occurred to affect adversely the quality or integrity of the experimental data.

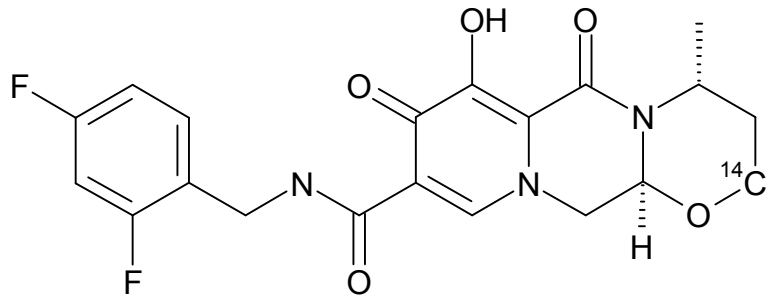
The species and strains used in the present studies reflected those employed in the toxicological testing of dolutegravir, to enable meaningful assessment of the exposure levels in the toxicity studies and provide confidence in the conclusions drawn regarding the safety of dolutegravir in humans. The species and strains used were the CD-1 mouse, the Sprague Dawley rat and the Lister-Hooded rat, the Japanese white rabbit, the beagle dog, and the cynomolgus monkey.

A brief summary of the important findings from the pharmacokinetic studies is provided below. In Sections 3 to 8, a discussion of the design and findings from these studies is presented. An overall assessment of the findings from these investigations is provided in Section 9, Discussion and Conclusion. Tabulations of these studies are provided in m2.6.5. A listing of the studies conducted, together with the location of the reports within Module 4 and their GLP status, is provided in Table 3.1, Table 4.1, Table 5.1, and Table 6.1. A list of studies that were undertaken with dolutegravir but are not included in this submission is presented in Appendix 2.

1.1. Test Substance

A selection of single dose pharmacokinetic studies and those investigating the distribution, metabolism and excretion of dolutegravir were performed with [^{14}C]-dolutegravir (see Figure 1.1 for structure). The desired specific activity of radioactivity was achieved by diluting radiolabelled drug with unlabelled dolutegravir.

Figure 1.1 Structure of [^{14}C]-dolutegravir



Repeat dose toxicokinetic analyses were carried out by measuring non-radiolabelled dolutegravir in plasma samples taken during repeat dose toxicity studies, and a number of other studies were also performed using non-radiolabelled dolutegravir.

Reference standards of the stereoisomers of dolutegravir (gsk013*, gsk014* or gsk015*) were used to evaluate the potential for epimerization and to test chiral methods of separation.

All studies described in this section were performed using the sodium salt of dolutegravir, unless otherwise specified. The sodium salt is the form proposed for use in humans; however, all doses and concentrations quoted in this summary are expressed in terms of the parent compound (referred to simply as dolutegravir).

Summary of Findings

Absorption

- Dolutegravir is rapidly absorbed, with high solution bioavailability. Absorption of dolutegravir from an oral suspension was solubility-limited.
- Following repeated administration in rats and monkeys, the increase in systemic exposure to dolutegravir was less than proportional with the increase in dose. Differences (>2-fold) in systemic exposure between the sexes or among sampling days were generally not observed.
- Higher systemic exposure to dolutegravir in pre-weaning rat pups than in juvenile rats reflects the early differential expression of uridine glucuronosyl transferase, the primary metabolic pathway for dolutegravir.

Distribution

- Dolutegravir is a highly permeable molecule and is widely distributed. Dolutegravir-related components were selectively associated with melanin of the skin but not with melanin of the uveal tract. Dolutegravir-related components were associated only with pigmented skin and bone at 28 days post-dose.
- Protein binding of dolutegravir is high in all species tested (>99%).
- Placental and milk transfer of dolutegravir was observed in rats.

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- Dolutegravir is a substrate of P-gp and BCRP but these transporters are unlikely to affect absorption of dolutegravir.
- Dolutegravir inhibited OCT2 with no notable inhibition of other transporters.

Metabolism

- The primary metabolism of dolutegravir in rats and monkeys is by conjugation to form an inactive glucuronide.
- No disproportionate human metabolites were observed and dolutegravir is the predominant plasma drug-related component with no metabolite >10% of parent or drug-related material.

Excretion

- Following single administration, fecal excretion was the primary route of elimination (67 to 94%) in all species tested. In animals, urine was a minor elimination pathway (1 to 6%).
- The absorbed dose is extensively metabolized with low pre-systemic clearance and is primarily secreted into the bile.

Drug Interactions

- Nonclinical studies have not been performed in vivo to specifically investigate potential pharmacokinetic interactions with drugs that are likely to be co-administered with dolutegravir. However, in vitro studies on enzyme inhibition / induction, protein binding and transporter interactions have been conducted and, except for sensitive OCT2 substrates (e.g. dofetilide), dolutegravir has a low propensity to cause drug interactions based on in vitro results.

2. METHODS OF ANALYSIS

In pharmacokinetic and toxicity studies, plasma dolutegravir concentrations were measured by methods based on protein precipitation followed by chiral or achiral liquid chromatographic separation and tandem mass spectrometric (LC/MS/MS) detection. The lower limit of quantification (LLQ) ranged between 4.75 and 500 ng/mL, depending on study requirements.

For toxicity studies, the chiral and achiral methods used for analysis were validated across the calibration range with respect to specificity, recovery, accuracy, precision, and stability under a variety of conditions. Summaries of the assay methodologies and validation are provided in Appendix 1. The reports are provided in m4.2.2.1, Analytical Methods and Validation Reports. The methods and limits of quantification were sufficiently adequate with regard to specificity and sensitivity to support the kinetic analyses of dolutegravir.

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

Non-compartmental methods were used for pharmacokinetic and toxicokinetic data analysis.

3. ABSORPTION

Studies investigating the absorption and pharmacokinetics of dolutegravir after single and repeated administration have been performed in the mouse, rat, rabbit, dog and monkey and are listed in Table 3.1. An inter-species comparison of dolutegravir plasma concentrations following its oral administration in the definitive toxicity studies is presented in Table 9.1, Subsection 9, Discussion and Conclusions to Pharmacokinetics Studies. A tabular summary of the repeat dose toxicokinetic data derived from the toxicity studies is presented in m2.6.7, Table 3.

3.1. Mouse

3.1.1. Oral administration

3.1.1.1. Pharmacokinetics/Toxicokinetics after repeated administration

14 day study

The toxicokinetics of dolutegravir were investigated in a 14 day repeat dose toxicity study in mice [Report RD2009/01546, m4.2.3.2]. Groups of CD-1 mice (n=18/sex/group) were orally administered dolutegravir at dose levels of 10, 100, 500 and 1500 mg/kg/day for 14 days. Plasma samples were taken on Days 1 and 14 for toxicokinetic analysis. The toxicokinetic parameters of dolutegravir derived from this study are presented in Table 9.1, and a summary of the toxicology data is presented in m2.6.6, Section 3.2.

In general, male and female mice were exposed to quantifiable concentrations of dolutegravir at all dose levels. The systemic exposure (AUC_{0-24} and C_{max}) of mice to dolutegravir increased with increasing dose levels, however, the increases were less than dose proportional for both Days 1 and 14. There was no evidence of accumulation of dolutegravir after repeat dosing and, in general, there were no sex differences in exposure to dolutegravir.

13 week study

The toxicokinetics of dolutegravir were investigated in a 13 week repeat dose toxicity study in mice [Report RD2009/00028, m4.2.3.2]. Groups of CD-1 mice (n=54/sex/group except control group which was 27/sex) were orally administered dolutegravir at dose levels of 10, 50, 500 and 1500 mg/kg/day for 13 weeks. Plasma samples were taken on Days 1, 28 and 85 for toxicokinetic analysis. The toxicokinetic parameters of dolutegravir derived from this study are presented in Table 9.1, and a summary of the toxicology data is presented in m2.6.6, Section 3.2.

Systemic exposure to dolutegravir (AUC_{0-24} and C_{max}) increased less than dose proportionally with increasing doses on Days 1, 28 and 91. No notable difference in

systemic exposure to dolutegravir was observed among the sampling days or between the sexes at any dose level.

104 week study (carcinogenicity)

The toxicokinetics of dolutegravir were investigated during a 104-week carcinogenicity study in mice [Report 2012N152419, m4.2.3.4.1]. Satellite groups of 45 male and female CD-1 mice were orally gavaged once daily with dolutegravir sodium at dose levels of 0 (water control), 0 (vehicle control; 0.5% w/w hydroxypropyl methylcellulose with 0.1% w/w Tween 80), 7.5, 25, or 500 mg/kg/day. Plasma was collected (n = 3/sex/timepoint; one sample per animal) on Day 26 and Day 182 from the control groups at 1, 6, and 24 hours postdose, and from dolutegravir-treated animals prior to and up through 24 hours postdose. The plasma toxicokinetic parameters were estimated based on mean plasma concentration-time profiles and are presented in Table 9.1. A summary of the toxicology data is presented in m2.6.6 Section 5.2.

The plasma concentrations from all samples collected from the control groups were below the limit of quantification indicating that the animals were not exposed to dolutegravir. The systemic exposure (C_{max} and AUC_{0-24}) to dolutegravir increased with increasing dose on Day 26 and Day 182 with the increases equal to or less than proportional to the increase in dose. Systemic exposure values on Day 26 and Day 182 were not notably different (>2-fold). No marked differences (>2-fold) in exposure values were observed between the sexes on Day 26 or Day 182.

3.2. Rat

3.2.1. Intravenous administration

3.2.1.1. Pharmacokinetics/Toxicokinetics after single doses

A study was conducted to characterize the single dose pharmacokinetics of dolutegravir in the rat after intravenous (1 mg/kg, injection) and oral administration (5 mg/kg) [Report RH2007/00101, m4.2.2.2]. Data from the oral part of the study are presented below in Section 3.2.2. A group of male Sprague Dawley rats (n=3) was administered a single intravenous dose of dolutegravir as a solution by dissolving in N,N-dimethyl acetamide and diluting with 50 mM N-methylglucamine in 3% aqueous mannitol. Plasma samples were collected at various times through 24 hours post-dose and analyzed for dolutegravir. The pharmacokinetic parameters of dolutegravir derived from this study are presented in m2.6.5, Table 3.1.

Following intravenous administration of dolutegravir to male Sprague Dawley rats, the total body plasma clearance and steady state volume of distribution were low at 0.229 mL/min/kg and 103 mL/kg, respectively, with a terminal half-life of dolutegravir of 6.18 hours.

3.2.2. Oral administration

3.2.2.1. Pharmacokinetics/Toxicokinetics after single doses

A series of studies were conducted to characterize the single dose pharmacokinetics and relative bioavailability of dolutegravir in male Sprague Dawley rats after oral administration (5 mg/kg) as a solution, and as a 0.5% methylcellulose suspension of dolutegravir sodium salt in fasted and non-fasted rats. The relative bioavailability of dolutegravir sodium salt and free acid powder in a capsule (7.06 to 8.00 mg/kg) also was investigated. The systemic exposure to dolutegravir was assessed after giving increasing oral doses of dolutegravir sodium as a 0.5% hydroxypropyl methylcellulose suspension (50, 100, 250, 500 and 1000 mg/kg) [Report RH2007/00101, m4.2.2.2]. Groups of male Sprague Dawley rats (n=2 or 3) were administered single oral doses of dolutegravir and plasma samples were collected at various time through 24 hours post-dose and analyzed for dolutegravir. The key pharmacokinetic parameters of dolutegravir derived from this study are presented in m2.6.5, Table 3.2.

The oral bioavailability of dolutegravir solution at 5 mg/kg in fasted rats was 75.6%. The bioavailability of a suspension of dolutegravir (5 mg/kg) was 34.2% in non-fasted rats and 51.5% in fasted rats. In fasted rats, the oral bioavailability after capsule administration of dolutegravir sodium (7.06 to 7.42 mg/kg) and free acid dolutegravir (7.31 to 8.00 mg/kg) was 47.6% and 34.7%, respectively. These results suggested that the absorption of free acid dolutegravir could be limited by the solubility and dissolution rate. The systemic exposure after oral administration of dolutegravir in suspension increased with dose from 50 to 500 mg/kg, although not proportionally.

3.2.2.2. Pharmacokinetics/Toxicokinetics after repeated administration

14 day study

The toxicokinetics of dolutegravir were investigated in a 14 day repeat dose toxicity study in rats [Report RD2007/01140, m4.2.3.2]. Groups of Sprague Dawley rats (n=4/sex/group) were orally administered dolutegravir at dose levels of 50, 150 and 500 mg/kg/day for 14 days. To evaluate toxicokinetics, plasma samples were taken on Day 1 and Day 14 at intervals up to 24 hours post-dose and plasma was analyzed for concentrations of dolutegravir. The toxicokinetic parameters of dolutegravir derived from this study are presented in Table 9.1, and a summary of the toxicology data is presented in m2.6.6, Section 3.3.

Systemic exposure (C_{max} and AUC_{0-24}) generally increased with increased dose, although increases were less than dose-proportional. No notable (>2-fold) sex-related differences were observed. On Day 14, systemic exposure to dolutegravir was similar (<2-fold difference) to Day 1.

4 week study

The toxicokinetics of dolutegravir were investigated in a 4 week repeat dose toxicity study in rats [Report RD2008/01628, m4.2.3.2]. Groups of Sprague Dawley rats

(n=4/sex/group) were orally administered dolutegravir at dose levels of 2, 10, 100 and 1000 mg/kg/day for 4 weeks. The plasma concentration of dolutegravir was measured at various times up through 24 hours post-dose on Days 1, 14 and 29. The toxicokinetic parameters of dolutegravir derived from this study are presented in Table 9.1, and a summary of the toxicology data is presented in m2.6.6, Section 3.3.

The toxicokinetics parameters (C_{max} and AUC_{0-24} values) increased with increasing dose although less than dose proportionally at ≥10 mg/kg/day. There were no obvious sex differences and no effect on exposure to dolutegravir due to repeat dosing.

26 week study

The toxicokinetics of dolutegravir were also investigated in a 26 week repeat dose toxicity study in rats [Report RD2009/00410, m4.2.3.2]. Groups of Sprague Dawley rats (n=6/sex/group) were orally administered dolutegravir at dose levels of 5, 50 and 500 mg/kg/day for up to 26 weeks. The plasma concentration of dolutegravir was measured at various times through 24 hours post-dose on Days 1, 30, 120 and 180. The toxicokinetic parameters of dolutegravir derived from this study are presented in Table 9.1, and a summary of the toxicology data is presented in m2.6.6, Section 3.3.

Systemic exposure to dolutegravir was confirmed in all dolutegravir-treated groups and no quantifiable concentrations of dolutegravir were observed in the control group at any time-point measured. Mean C_{max} and AUC_{0-24} values for dolutegravir on Days 1, 30, 120 and 180 of dosing generally increased less than dose proportionally in both males and females, and the values of females tended to be greater than those of males. There was a slight increase noted in exposure with repeat dosing in females in the 5 mg/kg/day group. There were no other obvious sex differences nor any significant accumulation of dolutegravir noted after repeat dosing.

104 week study (carcinogenicity)

The toxicokinetics of dolutegravir were investigated during a 104 week carcinogenicity study in rats [Report 2012N152418, m4.2.3.4.1]. Satellite groups of 12 male and female Sprague Dawley rats were orally gavaged once daily with dolutegravir sodium at dose levels of 0 (water control), 0 (vehicle control; 0.5% w/w hydroxypropyl methylcellulose with 0.1% w/w Tween 80), 2, 10, or 50 mg/kg/day. Plasma was collected (n = 4/sex/timepoint) in a composite design on Day 28 and Day 182 from the control groups at 1, 6, and 24 hours post dose, and from dolutegravir-treated animals prior to and up through 24 hours post-dose. The plasma toxicokinetic parameters were estimated based on mean plasma concentration-time profiles and are presented in Table 9.1. A summary of the toxicology data is presented in m2.6.6 Section 5.3.

The plasma concentrations from all samples collected from the control groups were below the limit of quantification indicating that the animals were not exposed to dolutegravir. The systemic exposure (C_{max} and AUC_{0-24}) to dolutegravir increased with increasing dose on Day 28 and 182 but the increases were generally less than dose proportional. Systemic exposure values on Day 26 and Day 182 were not notably

different (>2 fold). In general, the AUC_{0-24} and C_{max} values in males were comparable (on Day 28) to or less (on Day 182) than those values in females.

Juvenile rats

Juvenile male and female Sprague Dawley rats (n=4) were given oral doses of dolutegravir at dose levels of 5, 50, 100, 500 and 1000 mg/kg/day from Day 4 to 21 postpartum (pp) [Report CD2009/00409, m4.2.3.5.4]. Plasma samples were taken at intervals up to 24 hours post-dose from groups of juvenile rats (n= 1 to 2 animals/timepoint) on Day 21 pp for toxicokinetic analysis. A summary of the toxicology data is presented in m2.6.6, Section 6.5.1.

There were no marked sex differences (≥2-fold) observed in the plasma systemic exposure (C_{max} or AUC_{0-24}) of dolutegravir. The systemic exposure increased much less than in proportion to the increase in dose. Systemic exposure following 500 mg/kg/day was essentially not different from that following 100 mg/kg/day in either sex.

Juvenile male and female Sprague Dawley rats (n=12) were given oral doses of dolutegravir at dose levels of 2, 25, 75 and 300 mg/kg/day from Day 4 to 31 pp [Report CD2009/00770, m4.2.3.5.4]. Plasma samples were taken at intervals up to 24 hours post-dose from groups of juvenile rats (n=2 to 3 animals/timepoint) on Day 13 pp for toxicokinetic analysis. A summary of the toxicology data is presented in m2.6.6, Section 6.5.1.

On Day 13 pp, there were no marked sex differences observed in the plasma systemic exposure of dolutegravir. The exposure on Day 13 pp increased much less than in proportion to the increase in dose. In both males and females, the systemic exposure following 300 mg/kg/day was essentially not different or lower than that following 75 mg/kg/day.

Juvenile male and female Sprague Dawley rats (n=30) were given oral doses of dolutegravir at dose levels of 0.5, 2 and 75 mg/kg/day from Day 4 to 66 pp [Report CD2010/00023, m4.2.3.5.4]. Plasma samples were taken at intervals up to 24 hours post-dose from groups of juvenile rats (3 animals/ timepoint, except the 8 hour timepoint, which was n=4) on Day 13 pp and on Day 32 pp (3 animals/ timepoint) for toxicokinetic analysis. The toxicokinetic parameters of dolutegravir derived from this study are presented in Table 9.1, and a summary of the toxicology data is presented in m2.6.6, Section 6.5.1.

The systemic exposure (C_{max} and AUC_{0-24}) to dolutegravir was generally lower on Day 32 pp compared to that on Day 13 pp. There was no apparent difference in exposure between sexes.

Pregnant rats

A study was performed in pregnant Sprague Dawley rats in which dolutegravir was administered orally at dose levels of 100, 300 and 1000 mg/kg/day from Gestation Days 6 to 17 [Report XD2009/00367, m4.2.3.5.2]. Plasma samples were taken at

intervals up to 24 hours post-dose from groups of pregnant rats (n=5/group) on Gestation Days 6 and 17 for toxicokinetic analysis. The toxicokinetic parameters of dolutegravir derived from this study are presented in Table 9.1, and a summary of the toxicology data is presented in m2.6.6, Section 6.3.1.

The mean systemic exposure values (C_{max} and AUC_{0-24}) in the 300 mg/kg group were almost the same as those of the 100 mg/kg group, while those values increased less than dose proportionally between the 300 and 1000 mg/kg groups on Gestation Days 6 and 17. Repeat dosing did not cause any obvious effect on systemic exposure.

3.2.3. Subcutaneous and intramuscular administration

An investigative study was conducted to characterize the toxicokinetics of dolutegravir following a single subcutaneous (SC) or intramuscular (IM) injection (2.5 mg/kg) in male Sprague Dawley rats [Report RD2009/00921, m4.2.3.1]. Groups of male rats (n=6/group) were administered a single SC or IM injection of dolutegravir as long acting parenteral (LAP) wet bead-milled suspensions in an aqueous 2.0 % (w/w) pluronic F127/0.2% (w/v) polysorbate 80/0.18 % (w/v) vehicle. The final particle size was 0.67 micron at x50 and 2.93 microns at x90. Plasma samples were collected at various times through 336 hours post-dose and analyzed for dolutegravir. The pharmacokinetic parameters of dolutegravir derived from this study are presented in m2.6.5, Table 3.4a.

Plasma concentrations of dolutegravir were quantifiable through 336 (Day 15) hours following the SC dose and through 168 hours following the IM dose. Mean C_{max}, AUC_{0-24}, and AUC_{0-t} values for dolutegravir were similar between the SC and IM routes of administration.

A second investigative study was conducted to characterize the toxicokinetics of dolutegravir at a larger particle size than used in the first study. Dolutegravir was formulated as a homogenized suspension, in the same 2% pluronic F127 (w/v) vehicle used for the first study, and was given by single SC or IM injection to male Sprague Dawley rats (n=3/group) at doses of 2.5 or 5 mg/kg [Report RD2009/00959, m4.2.3.1]. The particle size of the 2.5 mg/kg dose solution was 40.7 microns at x90 and for the 5 mg/kg dose suspension was 35.8 microns at x90. Plasma samples were collected at various times through Day 43 post-dose and analyzed for dolutegravir. The pharmacokinetic parameters of dolutegravir derived from this study are presented in m2.6.5, Table 3.4a.

Mean dolutegravir C_{max}, AUC_{0-24}, and AUC_{0-t} values for the 2.5 or 5 mg/kg doses were similar between SC and IM administration. Following an increase in dose from 2.5 to 5 mg/kg, the mean AUC_{0-t} increased 2.3- to 3.2-fold after SC or IM administration. The duration that dolutegravir plasma concentrations remained above the protein adjusted IC_{90} was up to Day 15 SC or Day 22 IM for the 2.5 mg/kg dose and up to Day 43 SC or IM for the 5 mg/kg dose.

Another long acting parenteral formulation study was conducted to characterize the toxicokinetics of dolutegeravir following administration as a single intramuscular dose to evaluate further formulations [Report 2011N123574, m4.2.3.1]. Dolutegravir was

formulated as suspensions in a polysorbate 20 pluspolyethylene glycol 3350, mannitol in sterile water vehicle (Formulation 1); polysorbate 20, polyethylene glycol 3350, mannitol and sodium carboxymethylcellulose in sterile water for injection vehicle (Formulation 2); or sesame oil (Formulation 3). Particle size for Formulation 1 was d50 33.9 μm and d90 73.4 μm, Formulation 2 was d50 47.1 μm and d90 994.4 μm, and Formulation 3 was d50 61.6 μm and d90 117 μm. Doses were administered to male Sprague Dawley rats (n=3/group) at a dose of 9.4 mg/kg once by intramuscular injection. The actual doses administered ranged from 4 to 8.7 mg/kg. Plasma samples were collected at various times through Day 43 post-dose and analyzed for dolutegravir. The pharmacokinetic parameters of dolutegravir derived from this study are presented in m2.6.5, Table 3.4a.

None of these three formulations provided sustained concentrations of dolutegravir >60 ng/mL for at least 43 days. The highest dose-normalized (to 4 mg/kg) systemic exposure (C_{max} and $AUC_{0-\infty}$) and the least amount of variability tended to be provided by the sesame oil formulation (Formulation 3). The results suggest that the rate of dolutegravir absorption was lower when dosed in Formulation 2.

A fourth investigative long acting parenteral formulation study was conducted to characterize the toxicokinetics of dolutegeravir following administration as a single intramuscular dose (10 mg/kg) to male Sprague Dawley rats (n=3/group) using multiple vehicles [Report 2012N136936, m4.2.3.1]. Dolutegravir was formulated as suspensions in various vehicles. For the microparticle and micronized free acid formulations (Groups 3-7), the vehicle was polysorbate 20, mannitol, and sodium carboxymethylcellulose (CMC) in sterile water for injection. For the micronized free acid suspension formulations (Groups 2 and 10), the vehicle was polysorbate 20, polyethylene glycol 3350, and mannitol in sterile water for injection. For the in situ gel slow release formulation (Groups 1 and 9), the vehicle was 33.3% 75/25 poly(lactic-co-glycolic acid) (PLGA) and 66.7% N-methylpyrrolidone (NMP). For the in situ gel fast release formulation (Group 8), the vehicle was 20% 75/25 PLGA and 80% NMP. Plasma samples were collected at various times through Day 43 post-dose and analyzed for dolutegravir. The pharmacokinetic parameters of dolutegravir derived from this study are presented in m2.6.5, Table 3.4b.

Dosing dolutegravir in the in situ gel with slow release provided the longest period of plasma concentrations above 0.064 μg/mL ($PAIC_{90}$). The higher systemic exposure (mean C_{max} and AUC_{0-t}) values were achieved with the in situ gel with fast release, the in situ gel with slow release, or micronized free acid suspension.

3.3. Rabbit

3.3.1. Oral administration

3.3.1.1. Pharmacokinetics/Toxicokinetics after repeated administration

Non-pregnant rabbits

Non-pregnant Japanese white rabbits were administered oral doses of dolutegravir at 30, 100, 300 and 1000 mg/kg/day for 14 days [Report RD2008/01760, m4.2.3.5.2]. Plasma samples were taken at intervals up to 24 hours post-dose from groups of non-pregnant female rabbits (n=3/group) on Days 1 and 14 for toxicokinetic analysis. A summary of the toxicology data is presented in m2.6.6, Section 6.3.2.

Mean C_{max} values increased less than dose proportionally between 30 and 300 mg/kg/day while C_{max} at 1000 mg/kg/day was approximately the same as at 300 mg/kg/day. Mean AUC_{0-24} values increased dose proportionally between 30 and 300 mg/kg/day while those values at 1000 mg/kg/day were approximately the same as at 300 mg/kg/day. There were no obvious effects of repeat dosing on the systemic exposure values.

Pregnant rabbits

Pregnant Japanese white rabbits were administered dolutegravir orally at doses of 40, 200 and 1000 mg/kg/day on Days 6 to 18 of gestation [Report XD2009/0366, m4.2.3.5.2]. Plasma samples were taken at intervals up to 24 hours post-dose from groups of pregnant rabbits (n=5/group) on gestation Days 6 and 18 for toxicokinetic analysis. The toxicokinetic parameters of dolutegravir derived from this study are presented in Table 9.1, and a summary of the toxicology data is presented in m2.6.6, Section 6.3.2.

The mean C_{max} values increased less than dose proportionally between 40 and 1000 mg/kg/day. The mean AUC_{0-24} values increased dose proportionally between 40 and 200 mg/kg/day while the increase was less than dose proportional between 200 and 1000 mg/kg/day. There was no obvious effect of repeat dosing on the systemic exposure values.

3.4. Dog

3.4.1. Intravenous administration

3.4.1.1. Pharmacokinetics/Toxicokinetics after single doses

A study was conducted to characterize the single dose pharmacokinetics of dolutegravir in the dog after intravenous (1 mg/kg, injection) and oral administration (5 mg/kg) [Report RH2007/00102, m4.2.2.2]. Data from the oral part of the study are presented below in Section 3.4.2. Two non-fasted male beagle dogs were administered a single intravenous dose of dolutegravir as a solution in N,N-dimethyl acetamide which was

diluted with 50 mM N-methylglucamine in 3% aqueous mannitol. Blood samples were collected at intervals up to 24 hours post-dose and analyzed for dolutegravir. The pharmacokinetic parameters of dolutegravir derived from this study are presented in m2.6.5, Table 3.1.

Following intravenous administration of dolutegravir to non-fasted male beagle dogs, the total body plasma clearance and steady state volume of distribution were low at 2.17 mL/min/kg and 352 mL/kg, respectively, with a terminal half-life of dolutegravir of 5.24 hours.

3.4.2. Oral administration

3.4.2.1. Pharmacokinetics/Toxicokinetics after single doses

A study was conducted to characterize the single dose pharmacokinetics of dolutegravir in the dog after oral administration (5 mg/kg) [Report RH2007/00102, m4.2.2.2]. Two non-fasted male beagle dogs were administered a single oral dose of dolutegravir as a suspension in 0.5% methyl cellulose. Blood samples were collected at intervals up to 24 hours post-dose and analyzed for dolutegravir.

After oral administration of dolutegravir (5 mg/kg) to non-fasted male beagle dogs, the C_{max} and AUC_{0-24} were 2210 ng/mL and 15100 ng.h/mL, respectively, with a calculated oral bioavailability of 39%.

In another study conducted in female beagle dogs (n=1/group), dolutegravir was administered as single oral doses of 30, 100, 150, 250 and 500 mg/kg [Report RD2009/00963, m4.2.3.1].

Plasma exposure increased with increasing dose but the increase was less than proportional. It was speculated that vomiting observed in the animals given 150, 250 or 500 mg/kg influenced the exposure level seen in these animals, thus it was difficult to achieve an appropriate dose relationship.

3.4.2.2. Evaluation of Pediatric [redacted] Formulation

A study was conducted in male dogs to evaluate two pediatric [redacted] formulations [Report 2012N137977, m4.2.2.2]. Two groups of fasted male beagle dogs (n=6/group) received one of the two [redacted] formulations (53 mg dolutegravir/dog) as a suspension in mineral water (containing [redacted] and [redacted]) and in water for injection.

The [redacted] formulation which contained [redacted] and [redacted] in the composition (and for which pH is controlled with [redacted] and [redacted]) was determined to be the formulation best suitable for further investigations because the C_{max} and AUC_{0-24h} were most comparable when reconstituted with either mineral water or water for injection.

3.5. Monkey

3.5.1. Intravenous administration

3.5.1.1. Pharmacokinetics/Toxicokinetics after single doses

A study was conducted to characterize the single dose pharmacokinetics of dolutegravir in the monkey after intravenous (1 mg/kg, injection) and oral administration (5 mg/kg) [Report RH2007/00103, m4.2.2.2]. Data from the oral part of the study are presented below in Section 3.5.2. Two male cynomolgus monkeys were administered a single intravenous dose of dolutegravir as a solution in N,N-dimethyl acetamide diluted with 50 mM N-methylglucamine in 3% aqueous mannitol. Blood samples were collected at intervals up to 24 hours post-dose and analyzed for dolutegravir. The pharmacokinetic parameters of dolutegravir derived from this study are presented in m2.6.5, Table 3.1.

Following intravenous administration of dolutegravir to male cynomolgus monkeys, the total body plasma clearance and steady state volume of distribution were low at 2.12 mL/min/kg and 279 mL/kg, respectively, with a terminal half-life of dolutegravir of 6.00 hours.

3.5.2. Oral administration

3.5.2.1. Pharmacokinetics/Toxicokinetics after single doses

A study was conducted to characterize the single dose pharmacokinetics of dolutegravir in the monkey after oral administration (5 mg/kg) [Report RH2007/00103, m4.2.2.2]. One fasted male cynomolgus monkey was administered a single oral dose of free acid dolutegravir as a solution in dimethyl sulfoxide/solutol/50 mM N-methylglucamine in 3% mannitol. Two non-fasted male cynomolgus monkeys were administered a single oral dose of dolutegravir sodium as a suspension in 0.5% methylcellulose. Blood samples were collected at intervals up to 24 hours post-dose and analyzed for dolutegravir. The pharmacokinetic parameters of dolutegravir derived from this study are presented in m2.6.5, Table 3.2.

Following oral administration of free acid dolutegravir to the fasted male cynomolgus monkey, the C_{max}, T_{max} and bioavailability of dolutegravir were 8210 ng/mL, 2 hours, and 87%, respectively. Following oral administration of dolutegravir sodium to non-fasted male cynomolgus monkeys, the C_{max}, T_{max} and bioavailability of dolutegravir were 785 ng/mL, 5 hours, and 24.9%, respectively.

In a study designed to determine the toxicokinetic profile of dolutegravir, dose levels of 50, 125, 250 and 500 mg/kg were administered as single oral doses. Dolutegravir was administered as a suspension in 0.5% hydroxypropylmethylcellulose/0.1% Tween 80 to 1 fasted female cynomolgus monkey per dose group [Report 2007/01184, m4.2.3.1]. Blood samples were collected at various times through 24 hours post-dose to determine plasma concentrations of dolutegravir. The toxicokinetic parameters of dolutegravir derived from this study are presented in m2.6.5, Table 3.3.

The C_{max} values were 15.0, 24.8, 14.9, and 25.3 μg/mL at 50, 125, 250 and 500 mg/kg, respectively. The AUC_{0-24} values were 116, 182, 136 and 262 μg.h/mL at 50, 125, 250 and 500 mg/kg, respectively. No dose relationship was noted in the exposure values. There were no apparent differences in the C_{max} values between 125 and 500 mg/kg and the AUC_{0-24} value at 500 mg/kg was only approximately 1.4-fold the AUC_{0-24} value at 125 mg/kg. Therefore, the systemic exposure was considered to have attained the steady state at 125 mg/kg.

A supplemental toxicokinetic study was performed in which three fasted female cynomolgus monkeys were administered dolutegravir as single oral doses of 1, 3, 10 and 50 mg/kg [Report RD2008/01762, m4.2.3.1]. Blood samples were collected at various intervals through 24 hours post-dose. The toxicokinetic parameters of dolutegravir derived from this study are presented in m2.6.5, Table 3.3.

The systemic exposure to dolutegravir in the female monkeys was dose-related in nature, over the range of oral doses given. However, large inter-animal differences were noted at dose levels ≥10 mg/kg.

3.5.2.2. Pharmacokinetics/Toxicokinetics after repeated administration

14 day study

Dolutegravir was administered orally to cynomolgus monkeys for 14 days at doses of 100, 300 and 1000 mg/kg/day [Report RD2007/01142, m4.2.3.2]. Plasma samples were collected from monkeys (n=3/sex/group) at intervals up to 24 hours post-dose on Days 1, 7 and 14 for toxicokinetic analysis. The toxicokinetic parameters of dolutegravir derived from this study are presented in Table 9.1 and a summary of the toxicology data is presented in m2.6.6, Section 3.4.

Some animals exhibited emesis and were excluded from toxicokinetic evaluations. In animals not exhibiting emesis, increases in systemic exposure (mean C_{max} and AUC_{0-24}) to dolutegravir were less than dose-proportional and no notable sex-related differences (>2-fold) in exposure were observed on any sampling day or at any dose. Dolutegravir was detectable in all plasma samples at 24 hours post dose. On Day 14, systemic exposure to dolutegravir was generally similar to Day 1.

4 week study

Cynomolgus monkeys were given dolutegravir orally at doses of 25, 50 and 100 mg/kg/day for 4 weeks [Report RD2008/00107, m4.2.3.2]. Plasma samples were collected from the monkeys (5/sex/group for control and high dose, and 3/sex/group for low and mid dose) at intervals up to 24 hours post-dose on Days 1, 15 and 30 for toxicokinetic analysis. The toxicokinetic parameters of dolutegravir derived from this study are presented in Table 9.1 and a summary of the toxicology data is presented in m2.6.6, Section 3.4.

The absorption and elimination of dolutegravir were considered to be relatively fast and there was no accumulation of dolutegravir in monkeys based on the profile of plasma

concentrations and the mean T_{max} and C_{24hr} values of dolutegravir. In both sexes, the mean C_{max} and AUC_{0-24} values were nearly the same levels in all treated groups on all sampling days. Therefore, dose-linearity was not confirmed at the dose levels used in this study. No obvious sex differences were noted in any TK parameter.

38 week study

Dolutegravir was administered orally to cynomolgus monkeys at dose levels of 3, 10, 15 and 50/30 mg/kg/day for 17 weeks (n=7 or 9/sex/group) or 38 weeks (n=4 to 6/sex/group) [Report RD2009/00036/01, m4.2.3.2]. In animals given 50 mg/kg/day, the dose level was dropped to 30 mg/kg/day on Day 70 due to the moribund condition of some animals. Plasma samples were collected from the monkeys at intervals up to 24 hours post-dose on Days 1, 30, 69, 120, 180 and 270 for toxicokinetic analysis. The toxicokinetic parameters of dolutegravir derived from this study are presented in Table 9.1 and a summary of the toxicology data is presented in m2.6.6, Section 3.4.

The systemic exposure to dolutegravir was confirmed in all test article-treated groups and no peaks were detected in the control group at any timepoint. As a whole, the mean C_{max} and AUC_{0-24} values increased almost with dose in both sexes throughout the study. However, the differences were small and there were some cases of reversed dose-relationship between 10 and 15 mg/kg/day or between 15 and 50/30 mg/kg/day. Individual animals also showed similar findings. The short T_{max} values (within 3 hours) indicated that absorption was relatively rapid and was not significantly different among dose groups, sampling days, or sexes. No accumulation due to repeat dosing occurred in males. The C_{max} and AUC_{0-24} in females slightly increased with repeat dosing in the 10, 15, and 50/30 mg/kg/day groups until Day 120 and specifically, when compared with Day 1 of dosing, Days 69 and 120 were approximately 2-fold higher in the females at 15 mg/kg/day; however, C_{max} and AUC_{0-24} in females on Day 270 were similar to those on Day 1 of dosing. No sex difference was noted on any sampling day.

3.5.3. Subcutaneous and intramuscular administration

An investigative study was conducted to characterize the toxicokinetics of dolutegravir following single oral (3 mg/kg), subcutaneous (1 mg/kg), or intramuscular (1 mg/kg) administration to two groups of four female cynomolgus monkeys [Report CD2009/00647, m4.2.3.1]. Dolutegravir was formulated as a suspension in 0.5% hydroxypropylmethylcellulose (HPMC) / 0.1% Tween 80™ for oral administration, and as a long acting parenteral (LAP) wet bead-milled suspension in an aqueous 2.0 % (w/w) pluronic F127/0.2% (w/v) polysorbate 80/0.18 % (w/v) for SC and IM injection. Plasma samples were collected at various times through 24 hours following oral administration and through 336 hours post-dose following SC and IM injection and analyzed for dolutegravir. The pharmacokinetic parameters of dolutegravir derived from this study are presented in m2.6.5, Table 3.5.

All plasma concentrations of dolutegravir in one monkey administered an SC injection were not quantifiable, therefore, this monkey was not included in the calculation of mean values. The mean dose–normalized C_{max} values following oral administration were higher than that following SC administration; however, the mean dose–normalized

AUC_{0-24} following oral administration was similar to the dose-normalized AUC_{0-t} following SC administration. There were no marked differences (≥2-fold) in the mean C_{max}, AUC_{0-24} or AUC_{0-t} values following SC or IM administration. Concentrations following SC or IM injection were only above the protein adjusted IC_{90} value (0.064 μg/mL) for 10 hours post-dose.

Table 3.1 List of Single and Repeat Dose Pharmacokinetic/Toxicokinetic Studies Performed with Dolutegravir

Type of Study	Species (Strain)/ Test System	No./Sex/ Group	Method of Administration	Form	Dose (mg/kg/day) or Concentration	Duration of Dosing (Sampling Occasions)	GLP	Testing Facility	Report No. (Study No.)	Location in CTD
Single dose										
Pharmacokinetics	Rat (Sprague Dawley)	3M	IV (bolus) Oral (gavage)[a] Oral (capsule)	A A A B	1 5 7.06 to 7.42 7.31 to 8	Single	No	[redacted]	RH2007/00101	m4.2.2.2
Pharmacokinetics	Rat (Sprague Dawley)	2M	Oral (gavage)	A	50, 100, 250, 500, 1000	Single	No	[redacted]	RH2007/00101	m4.2.2.2
Toxicokinetics	Rat (Sprague Dawley)	6M 6M	SC IM	B	2.5	Single	No	GSK	RD2009/00921 (R42470)	m4.2.3.1
Toxicokinetics	Rat (Sprague Dawley)	3M 3M	SC IM	B	2.5, 5	Single	No	GSK	RD2009/00959 (R42475)	m4.2.3.1
Toxicokinetics	Rat (Sprague Dawley)	3M	IM	B	4.0, 7.3, 8.7	Single	No	GSK	2011N123574 (R42826)	m4.2.3.1
Toxicokinetics	Rat (Sprague Dawley)	3M	IM	B	10	Single	No	GSK	2012N136936	m4.2.3.1
Pharmacokinetics	Dog (beagle)	2M	IV (bolus) Oral (gavage)	A A	1 5	Single	No	[redacted]	RH2007/00102	m4.2.2.2
Toxicokinetics	Dog (beagle)	1F	Oral (gavage)	A	30, 100, 150, 250, 500	Single	No	[redacted]	RD2009/00963	m4.2.3.1

Table 3.1 (Continued) List of Single and Repeat Dose Pharmacokinetic/Toxicokinetic Studies Performed with Dolutegravir

Type of Study	Species (Strain)/ Test System	No./Sex/ Group	Method of Administration	Form	Dose (mg/kg/day)	Duration of Dosing (Sampling Occasions)	GLP	Testing Facility	Report No. (Study No.)	Location in CTD
Pharmacokinetics	Dog (beagle)	6M	Oral (gavage)	A	53 mg	Single	No	GSK	2012N137977	m4.2.2.2
Pharmacokinetics	Monkey (cynomolgus)	1 to 2M	IV (bolus) Oral (gavage)	A B A	1 5 5	Single	No	[redacted]	RH2007/00103	m4.2.2.2
Toxicokinetics	Monkey (cynomolgus)	1F	Oral (gavage)	A	50, 125, 250, 500	Single	No	[redacted]	RD2007/01184	m4.2.3.1
Toxicokinetics	Monkey (cynomolgus)	3F	Oral (gavage)	A	1, 3, 10, 50	Single	No	[redacted]	RD2008/01762 (S-349572-TB-44-R)	m4.2.3.1
Toxicokinetics	Monkey (cynomolgus)	4F	Oral SC IM	B A A	3 1 1	Single	No	GSK	CD2009/00647 (D09113)	m4.2.3.1
Repeat Dose										
Toxicokinetics	Mouse (CD-1)	18M/18F	Oral (gavage)	A	10, 100, 500 1500	14 days (Days 1 & 14)	No	[redacted]	RD2009/01546 (S-349572-TF-066-R)	m4.2.3.2
Toxicokinetics	Mouse (CD-1)	54M/54F	Oral (gavage)	A	10, 50, 500, 1500	13 weeks (Days 1, 28 & 85)	Yes	[redacted]	RD2009/00028 (S-349572-TF-068-L)	m4.2.3.2
Toxicokinetics	Mouse (CD-1)	45M/45F	Oral (gavage)	A	7.5, 25, 500	104 weeks	Yes	[redacted]	2012N152419	m4.2.3.4.1
Toxicokinetics	Rat (Sprague Dawley)	4M/4F	Oral (gavage)	A	50, 150, 500	14 days (Days 1 & 14)	Yes	[redacted]	RD2007/01140 (E-349572-TB-012-L)	m4.2.3.2

Table 3.1 (Continued) List of Single and Repeat Dose Pharmacokinetic/Toxicokinetic Studies Performed with Dolutegravir

Type of Study	Species (Strain)/ Test System	No./Sex/ Group	Method of Administration	Form	Dose (mg/kg/day) or Concentration	Duration of Dosing (Sampling Occasions)	GLP	Testing Facility	Report No. (Study No.)	Location in CTD
Toxicokinetics	Rat (Sprague Dawley)	4M/4F	Oral (gavage)	A	2, 10, 100, 1000	4 weeks (Days 1, 14 & 29)	Yes	[redacted]	RD2008/01628 (E-34572-TB-043-L)	m4.2.3.2
Toxicokinetics	Rat (Sprague Dawley)	6M/6F	Oral (gavage)	A	5, 50, 500	26 weeks (Days 1, 30, 120 & 180)	Yes	[redacted]	RD2009/00410 (S-349572-TF-055-L)	m4.2.3.2
Toxicokinetics	Rat (Sprague Dawley)	12M/12F	Oral (gavage)	A	2, 10, 50	104 weeks	Yes	[redacted]	2012N152418	m4.2.3.4.1
Toxicokinetics	Rat (pregnant) (Sprague Dawley)	5F	Oral (gavage)	A	100, 300, 1000	12 days (Days 1 & 12)	Yes	[redacted]	XD2009/00367 (S-349572-TB-062-L)	m4.2.3.5.2
Toxicokinetics	Rat (juvenile) (Sprague Dawley)	4M/4F	Oral (gavage)	A	5, 50, 100, 500, 1000	18 days (Days 4 to 21 pp) (Day 21 pp)	No	GSK	CD2009/00409 (D09072)	m4.2.3.5.4
Toxicokinetics	Rat (juvenile) (Sprague Dawley)	12M/12F	Oral (gavage)	A	2, 25, 75, 300	28 days (Days 4 to 31 pp) (Days 13 and 32 pp)	No	GSK	CD2009/00770 (D09126)	m4.2.3.5.4
Toxicokinetics	Rat (juvenile) (Sprague Dawley)	30M/30F	Oral (gavage)	A	0.5, 2, 75	63 days (Days 4 to 66 pp) (Days 13 and 32 pp)	Yes	GSK	CD2010/00023 (G09229)	m4.2.3.5.4

Table 3.1 (Continued) List of Single and Repeat Dose Pharmacokinetic/Toxicokinetic Studies Performed with Dolutegravir

Type of Study	Species (Strain)/ Test System	No./Sex/ Group	Method of Administration	Form	Dose (mg/kg/day) or Concentration	Duration of Dosing (Sampling Occasions)	GLP	Testing Facility	Report No. (Study No.)	Location in CTD
Toxicokinetics	Rabbit (non-pregnant) (Japanese white)	3F	Oral (gavage)	A	30, 100, 300, 1000	14 days (Days 1 & 14)	Yes	[redacted]	RD2008/01760 (S-349572-TF-052-L)	m4.2.3.5.2
Toxicokinetics	Rabbit (pregnant) (Japanese white)	5F	Oral (gavage)	A	40, 200, 1000	13 days (Days 1 & 13)	Yes	[redacted]	XD2009/00366 (S-349572-TF-065-L)	m4.2.3.5.2
Toxicokinetics	Monkey (cynomolgus)	3M/3F	Oral (gavage)	A	100, 300, 1000	14 days (Days 1, 7 & 14)	Yes	[redacted]	RD2007/01142 (E-349572-TB-012-L)	m4.2.3.2
Toxicokinetics	Monkey (cynomolgus)	3M/3F[b]	Oral (gavage)	A	25, 50, 100	4 weeks (Days 1, 15 & 30)	Yes	[redacted]	RD2008/00107 (E-349572-TF-036-L)	m4.2.3.2
Toxicokinetics	Monkey (cynomolgus)	4 to 6 (for 38 weeks)	Oral (gavage)	A	3, 10, 15, 50/30[c]	38 weeks (Days 1, 30, 69, 120, 180 & 270)	Yes	[redacted]	RD2009/00036 (S-349572-TF-047-L)	m4.2.3.2

Key:
a = Administered as a solution and a suspension.
b = An additional 2 animals/sex added to the 100 mg/kg/day dose and used for TK evaluation.
c = From Day 70 the dose of 50 mg/kg/day was reduced to 30 mg/kg/day because of 2 male deaths.
A = GSK1349572A, the sodium salt form.
B = GSK1349572B, the parent form.
pp = Post partum.

Testing Facility:
GSK = GlaxoSmithKline.
[redacted] = [redacted]
[redacted] = [redacted]
[redacted] = [redacted]
[redacted] = [redacted]

4. DISTRIBUTION

A range of in vitro studies have been performed to investigate the binding of dolutegravir to serum and plasma proteins and to investigate dolutegravir interactions with a range of cellular transporters. In addition, in vivo studies have been performed to investigate dolutegravir distribution in whole blood, liver to blood ratios, lacteal secretion, placental transfer, and tissue distribution of radioactivity following oral administration of [^{14}C]-dolutegravir. These studies are listed in Table 4.1.

4.1. In Vitro Distribution Studies

4.1.1. Serum and plasma protein binding studies

In a preliminary study, the in vitro protein binding of dolutegravir at 10 μM (4.19 μg/mL) was assessed in the male rat, dog, monkey and human serum by equilibrium dialysis [Report RH2007/00106, m4.2.2.3]. Equilibrium dialysis was conducted for 24 hours at 37°C. Serum and phosphate buffered saline were analyzed for dolutegravir by an HPLC-MS/MS method. A tabulated summary of this study is presented in m2.6.5, Table 6.1.

The percentage of dolutegravir bound to serum proteins was 99.9% in rats, 95.4% in dogs, 99.1% in monkeys and 99.3% in human.

A pilot study was conducted to evaluate the in vitro protein binding of dolutegravir (0.21 to 21 μg/mL or 0.5 to 50 μM) in human plasma to encompass observed concentrations in clinical studies [Report 2010N104947, m4.2.2.3]. Plasma (with EDTA anticoagulant) was taken from a single male human and protein binding was determined using a 96-well plate equilibrium dialysis device. The concentration of dolutegravir was determined by using LC-MS/MS. A tabulated summary of this study is presented in m2.6.5, Table 6.1.

The plasma protein binding of dolutegravir was high (91.5 to 93.8%) and tended to decrease with increased concentration of dolutegravir in plasma.

A second in vitro human plasma protein binding study was conducted to further investigate the results of the above pilot study; the objective was to confirm whether or not binding to plasma proteins was different from binding to serum proteins. Dolutegravir (0.5 to 25 μM) binding was tested in pooled fresh and frozen human plasma containing EDTA or heparin anticoagulants by using a 96-well plate equilibrium dialysis device [Report 2011N119355, m4.2.2.3]. Concentrations of dolutegravir were determined by HPLC-MS/MS. This study superseded the pilot study in plasma from a single donor obtained through a commercial source. A tabulated summary of this study is presented in m2.6.5, Table 6.1.

The plasma protein binding of dolutegravir in pooled human plasma containing EDTA or heparin anticoagulants in fresh and frozen plasma was high at an average of 99.3%. There was no evidence of concentration dependence, anticoagulant dependence or the use of fresh versus frozen human plasma on the protein binding of dolutegravir.

4.1.2. P-glycoprotein transport and membrane permeability

In a preliminary study, the transport of dolutegravir (3 μM) was investigated in stably transfected human multidrug resistance 1-Madin Darby canine kidney (hMDR1-MDCK II) cells in both the presence or the absence of the P-gp inhibitor GF120918 (2 μM) [Report RH2007/00067, m4.2.2.3]. The transport studies were conducted at 37°C in a humidified incubator with shaking for 60 minutes.

In the presence of GF120918, the apparent passive permeability value (P_{app}) for dolutegravir averaged 577 nm/sec, suggesting that dolutegravir has high permeability. In the absence of GF120918, P-gp attenuated the apical to basolateral (A→B) flux of dolutegravir across hMDR1-MDCK II cells by approximately 40%.

A follow up study was designed to determine the passive membrane permeability of [^{14}C]-dolutegravir (3 μM) at pH 7.4 and the absorptive membrane permeability at pH 5.5 and 7.4 in the presence of a bio-relevant buffer FaSSIF (fasted State simulated intestinal fluid) to simulate conditions in the intestine in vitro using MDCKII-hMDR1 cells transfected with the human MDR gene (which expresses the P-gp protein) [Report RD2008/00360, m4.2.2.3]. A tabulated summary of this study is presented in m2.6.5, Table 8.3.

[^{14}C]-dolutegravir was determined to have high passive membrane permeability of 333 nm/s at pH 7.4. The absorptive membrane permeabilities of [^{14}C]-dolutegravir in the presence of FaSSIF were both high at pH 7.4 and pH 5.5 with a $P_{7.4[abs]}$ value of 253 nm/s and a $P_{5.5[abs]}$ value of 265 nm/s. These high permeability values are consistent with good absorption of dolutegravir when administered as a solution and the high permeability values and the low solubility of dolutegravir classify it as a BCS II compound [see m2.7.1].

A study was performed to determine the potential for dolutegravir to be a substrate of the P-gp transporter in vitro [Report RD2008/00361, m4.2.2.3]. The bi-directional transport of [^{14}C]-dolutegravir (3 μM) was investigated using MDCKII-MDR1 cells transfected with the human MDR1 gene, which produces the P-gp protein. Cell monolayers were pre-incubated at 37°C in transport medium, with and without 2 μM GF120918, for 15 to 30 minutes prior to addition of dolutegravir. After adding test article, incubation continued for 90 minutes. A tabulated summary of this study is presented in m2.6.5, Table 8.2.

The data demonstrate that [^{14}C]-dolutegravir at 3 μM was a substrate for human P-gp with a moderate efflux ratio of 3.8, which together with the high protein binding can contribute to low CNS penetration.

4.1.3. Inhibition of P-glycoprotein

The potential inhibitory effect of dolutegravir (0.3 to 100 μM) against digoxin transport via P-gp was evaluated using an MDCKII-MDR1 cell line which heterologously expressed human P-gp [Report RD2008/00292, m4.2.2.3]. After pre-incubation, the

plates were incubated at 37°C with shaking for a further 90 minutes. A tabulated summary of this study is presented in m2.6.5, Table 8.1.

Dolutegravir inhibited transport of digoxin via human P-gp in vitro with an IC_{50} value >100 μM.

4.1.4. Human breast cancer resistance protein-mediated transport

An in vitro study was conducted to evaluate [^{14}C]-dolutegravir (3 μM) for the potential to be a substrate of the human Breast Cancer Resistance Protein (BCRP) transporter in MDCKII-BCRP cells [Report 2011N112380, m4.2.2.3]. The bi-directional transport of [^{14}C]-dolutegravir was determined and [^{14}C]-cimetidine (3 μM) was used as a positive control. Plates were incubated at 37°C with shaking for 90 minutes. A tabulated summary of this study is presented in m2.6.5, Table 8.2.

The results showed that [^{14}C]-dolutegravir was a substrate for human BCRP, with an efflux ratio of 3.1 at a concentration of 3 μM.

4.1.5. Inhibition of BCRP transport

Dolutegravir was evaluated as a potential inhibitor of the human BCRP in vitro [Report 2010N110625, m4.2.2.3]. Inhibition of BCRP-mediated transport of [^{14}C]-cimetidine (100 nM) by [^{14}C]-dolutegravir (concentration range 0.3 to 100 μM) was investigated by determining the basolateral to apical ([B→A]) transport of [^{14}C]-cimetidine by MDCKII-BCRP cells. Plates were incubated at 37°C with shaking for 90 minutes. A tabulated summary of this study is presented in m2.6.5, Table 8.1.

Dolutegravir inhibited transport of cimetidine (50% of control) via human BCRP in vitro at a concentration of 100 μM. However, the data were insufficient to calculate an IC_{50} value.

4.1.6. Inhibition of multidrug resistance associated protein-2 transporter

Dolutegravir was tested for the potential to inhibit the human multidrug resistance associated protein-2 transporter (MRP2) in vitro [Report 2010N109746, m4.2.2.3]. Inhibition of uptake of the probe substrate [H^3] estradiol 17-β-D-glucuronide (EG) was determined for dolutegravir at a concentration range from 0.1 to 100 μM. Membrane vesicles prepared from recombinant baculovirus infected Sf9 cells were used to express the human MRP2 transporter. The vesicles were incubated for 5 minutes at 37°C with each concentration of dolutegravir. A tabulated summary of this study is presented in m2.6.5, Table 8.1.

Dolutegravir (up to 100 μM) did not inhibit human MRP2 under these test conditions.

Dolutegravir glucuronide (GSK2832500; M3) was tested for the potential to inhibit the human multidrug resistance associated protein-2 transporter (MRP2) in vitro [Report

2011N120047, m4.2.2.3]. Inhibition of uptake of the probe substrate [H^3] estradiol 17-β-D-glucuronide (EG) was determined for dolutegravir glucuronide at a concentration range from 0.1 to 100 μM. Membrane vesicles prepared from recombinant baculovirus infected Sf9 cells were used to express the human MRP2 transporter. The vesicles were incubated for 5 minutes at 37°C with each concentration of dolutegravir. A tabulated summary of this study is presented in m2.6.5, Table 8.1.

Dolutegravir glucuronide (up to 100 μM) did not inhibit human MRP2 under these test conditions.

4.1.7. Inhibition of OATP1B1 and OATP1B3

In an in vitro study to assess potential inhibition of the human hepatic uptake transporters OATP1B1 and OATP1B3, dolutegravir was tested at concentrations of 0.1, 1, 3, 10, 30 and 100 μM [Report RD2008/00216, m4.2.2.3]. CHO and HEK MSRII cells which overexpressed OATP1B1 and OATP1B3, respectively, were incubated at 37°C with the varying concentrations of dolutegravir. Cells were first pre-incubated and then incubated for 5 minutes for OATP1B1 and for 10 minutes for OATP1B3. A tabulated summary of this study is presented in m2.6.5, Table 8.1.

Dolutegravir did not inhibit OATP1B1 or OATP1B3 in vitro.

4.1.8. Inhibition of OCT1 and OCT2 mediated transport

A study was performed to assess any inhibitory potential of dolutegravir on the hepatic uptake transporter, organic cation transporter 1 (OCT1), using the human embryonic kidney epithelial cell system (HEK293) [Report 2012N132572, m4.2.2.3]. A single concentration of dolutegravir (10 μM) was used to investigate the OCT1-mediated transport of 10 μM [^{14}C]-metformin in HEK293 cells expressing OCT1. The positive control inhibitors, repaglinide and quinidine, and a cell tolerability assessment, were used to confirm the test system function. A tabulated summary of this study is presented in m2.6.5, Table 8.1.

Dolutegravir was classified as not having a significant inhibitory potential on OCT1 (weak inhibition noted at 22% of control).

A pilot study was performed to assess the inhibitory effect of dolutegravir alone, or in combination with the dual nucleoside agents abacavir and lamivudine, or tenofovir disoproxil and emtricitabine, on the OCT2-mediated transport of 10 μM [^{14}C]-metformin [Report RD2010/00555, m4.2.2.3]. A single high concentration of dolutegravir (25 μM) was used with clinically relevant concentrations of the co-incubated nucleosides. MDCK-II cells expressing OCT2 were used in this assay. Cells were first pre-incubated at 37°C and then incubated for 5 minutes with dolutegravir or with the combination of test articles. The inhibition of [^{14}C]-metformin transport was measured.

At a concentration of 25 μM, dolutegravir inhibited OCT2-mediated transport of metformin by 91%. OCT2 inhibition results for abacavir and lamivudine or tenofovir

disoproxil and emtricitabine, co-administered with dolutegravir, were similar to dolutegravir alone. The effect of the additional compounds co-administered with dolutegravir was difficult to discern given the substantial inhibition of OCT2-mediated metformin transport by dolutegravir.

A further study was conducted with dolutegravir (0.1 to 30 μM) to determine an IC_{50} value against human OCT2-mediated transport of 10 μM [^{14}C]-metformin [Report 2010N104937, m4.2.2.3]. MDCK-II cells expressing OCT2 were used in this assay. Cells were first pre-incubated at 37°C and then incubated for 5 minutes with the varying concentrations of dolutegravir. A tabulated summary of this study is presented in m2.6.5, Table 8.1.

Dolutegravir inhibited OCT2 with an IC_{50} of 1.93 μM.

Additionally, a study was performed to assess the potential additive inhibitory effects of the nucleoside combinations of tenofovir disoproxil and emtricitabine, or abacavir and lamivudine, on OCT2-mediated transport of 10 μM [^{14}C]-metformin when incubated with dolutegravir at a concentration of 1 μM [Report 2010N104937, m4.2.2.3]. The study methodology was the same as described above.

The co-administration of dolutegravir with abacavir and lamivudine, or with tenofovir disoproxil and emtricitabine, inhibited OCT2 transport by 56% and 54%, respectively, and is considered a nominal increase over dolutegravir alone.

4.2. In Vivo Distribution Studies

4.2.1. Mouse

4.2.1.1. Blood:plasma and liver:blood ratios

As part of an excretion study (see Section 6), liver concentrations of dolutegravir-related radioactivity and blood cell association of dolutegravir-related material were determined following a single oral administration of [^{14}C]-dolutegravir (100 mg/kg) to intact male and female mice [Report RD2009/00562, m4.2.2.5]. Blood and liver samples were taken from 10 animals/sex/time point at 2, 10 and 24 hours. A tabulated summary of this study is presented in m2.6.5, Table 8.4.

The highest pooled concentrations of radioactivity in blood (26078 ng-equiv/g) and plasma (50679 ng-equiv/g) were observed at 2 hours post-dose and decreased through 24 hours. No association of dolutegravir-related material with the cellular components of mouse blood was detected through 24 hours. The blood to plasma ratios of radioactivity ranged from 0.49 to 0.54.

Mean liver to blood concentration ratios ranged from 0.34 to 0.46 through 24 hours post-dose.

4.2.2. Rat

4.2.2.1. Whole body distribution

A whole body autoradiography study was conducted using partially pigmented Lister-Hooded male rats (n=7; 1 per timepoint) administered a single oral dose of [^{14}C]-dolutegravir (50 mg/kg) [Report CD2008/00195, m4.2.2.3]. Specific tissue concentrations of radioactivity were quantified in a single animal at each of the following post-dose intervals: 2, 4, 6 and 10 hours and 1, 7 and 28 days. A tabulated summary of this study is presented in m2.6.5, Table 5.1.

Radioactivity was widely distributed with most tissues containing peak levels at 6 hours. Peak blood concentrations of radioactivity also occurred at 6 hours and declined to below the limit of quantification at 28 days. Concentrations of radioactivity in the brain were low (~2% of the blood radiocarbon concentration) and not quantifiable after 10 hours post-dose. By 28 days post-dose, only bone and pigmented skin contained quantifiable levels of radioactivity. [^{14}C]-dolutegravir-related radioactivity was selectively associated with melanin in skin but not with melanin in the uveal tract. The elimination of low levels of dolutegravir-related material in bone was notably slow, with similar concentrations present at 7 and 28 days post-dose.

4.2.2.2. Blood:plasma and liver:blood ratios

In conjunction with an excretion study (see Section 6), liver concentrations of radioactivity and blood cell association of dolutegravir-related material were determined following a single oral administration of [^{14}C]-dolutegravir (50 mg/kg) to intact male and female rats [Report RD2008/00108, m4.2.2.5]. Blood and liver samples were taken from 3 animals/sex/time point at 2, 6 and 24 hours. A tabulated summary of this study is presented in m2.6.5, Table 8.4.

The highest mean concentrations of radioactivity in blood and plasma were observed at 6 hours post-dose. The blood to plasma ratios of total radioactivity ranged from 0.51 to 0.53, indicating that radioactivity was largely associated with plasma components of blood.

Mean liver to blood concentration ratios were 0.47 and 0.30 in males and females, respectively, through 24 hours post-dose.

4.2.2.3. Placental transfer

The distribution and placental transfer of radioactivity were determined following administration of a single oral dose of [^{14}C]-dolutegravir (50 mg/kg) to timed-pregnant rats (n=5) on gestation day 18 [Report 2012N137348, m4.2.2.3]. One animal/time point was prepared for quantitative whole body autoradiography at 2, 4, 6, 10 and 24 hours post-dose. A tabulated summary of this study is presented in m2.6.5, Table 7.1.

Placental transfer of radioactivity was evident. In timed-pregnant rats, radioactivity was rapidly and widely distributed to most dam and fetal tissues, with the highest values

obtained from 2 to 10 hours post-dose. The fetus and fetal organs had measurable concentrations of radioactivity through 24 hours post-dose, with the exception of the fetal brain and fetal spinal cord, which had the last measurable concentrations at 10 hours post-dose. Drug-related radioactivity was quantifiable at low levels through 10 hours in the maternal organs protected by the blood:brain barrier, and additionally at 24 hours in brain cerebrum.

The concentration in the whole fetus was 2410 ng equiv/g at 2 hours post-dose, and steadily increased to 3950 ng equiv/g at 10 hours post-dose before decreasing to 1250 ng equiv/g at 24 hours post-dose.

The dam matrices with the highest concentrations of radioactivity at 2 hours post-dose were blood, bile, placenta, kidney medulla, uterus, and lungs, with respective values of 33200, 25900, 22000, 20300, 20300, and 19100 ng equiv/g. The fetal matrices with the highest concentrations of radioactivity at 2 hours post-dose were fetal blood, fetal myocardium, and fetal muscle, with values of 3490, 2910, and 2770 ng equiv/g, respectively.

By 24 hours post-dose, notable decreases in radioactive concentrations were apparent. The dam matrices with the highest concentrations of radioactivity at 24 hours post-dose were blood, uterus, urinary bladder, amniotic sac, and placenta, with values of 9790, 9070, 7520, 7280, and 6860 ng equiv/g, respectively. The fetal matrices with the highest concentrations of radioactivity at 24 hours post-dose were fetal bone marrow, fetal blood, and fetal muscle, with values of 5230, 1530 and 1430 ng equiv/g, respectively.

4.2.2.4. Lacteal secretion

Lacteal excretion of radioactivity was determined following administration of a single oral dose of [^{14}C]-dolutegravir (50 mg/kg) to lactating rats (n=12) at 10 days postpartum [Report 2012N137348, m4.2.2.3]. Milk was collected from 3 animals/time point at 2 and 4 hours post-dose and from 2 animals/time point at 1, 8 and 24 hours post-dose. Blood samples were collected from each animal following milk collection. A tabulated summary of this study is presented in m2.6.5, Table 7.2.

Radioactivity was detected in milk at the first time point of 1 hour post-dose, with a mean concentration of 10000 ng equiv/g. The concentration of radioactivity in milk steadily increased to a mean Cmax value of 47300 ng equiv/g at 8 hours post-dose, and then decreased to a mean of 1800 ng equiv/g at 24 hours post-dose. Mean milk to blood concentration ratios were 0.452 at 1 hour post-dose, steadily increased to a maximum of 2.3 at 8 hours post-dose, and then decreased to 1.28 at 24 hours post-dose.

Mean blood to plasma concentrations ratios ranged from 0.542 to 0.566 through 24 hours, indicating low association of drug-derived radioactivity with blood cells. Mean milk to plasma concentration ratios were 0.245 at 1 hour post-dose, and steadily increased to a maximum of 1.25 at 8 hours post-dose.

4.2.3. Monkey

4.2.3.1. Blood:plasma ratio

As a part of an excretion study (see Section 6), blood cell association of dolutegravir-related material was determined following a single oral administration of [^{14}C]-dolutegravir (10 mg/kg) to intact male and female cynomolgus monkeys (n=3/sex) [Report RD2008/01300, m4.2.2.5]. Blood samples were taken at 2, 6, 12 and 24 hours post-dose. A tabulated summary of this study is presented in m2.6.5, Table 8.4.

The highest mean blood and plasma concentrations were observed at 2 hours post-dose. The mean ratios of the concentration of radioactivity in blood to plasma ranged from 0.643 to 0.728 through 24 hours post-dose for both sexes, indicating that association of radioactivity with blood cells was low.

In conjunction with another excretion study, blood cell association of dolutegravir-related material was determined following a single oral administration of [^{14}C]-dolutegravir (10 mg/kg) to male bile duct-cannulated cynomolgus monkeys (n=2) [Report RD2008/01299, m4.2.2.5]. Blood samples were taken at 2, 6, 12 and 24 hours post-dose. A tabulated summary of this study is presented in m2.6.5, Table 8.4.

The highest mean concentration of radioactivity for both blood and plasma was observed at 2 hours post-dose. The blood to plasma ratio ranged between mean values of 0.74 to 0.79 through 24 hours post-dose, indicating low association of radioactivity with blood cells.

Table 4.1 List of Distribution Studies Performed with Dolutegravir

Type of Study	Species (Strain)/ Test System	No./Sex/ Group	Method of Administration	Form	Dose (mg/kg/day) or Concentration	Duration of Dosing (Sampling Occasions)	GLP	Testing Facility	Report No. (Study No.)	Location in CTD
Plasma protein binding	Rat, Dog, Monkey, Human	NA	In vitro	B	10 μM	NA	No	[redacted]	RH2007/00106	m4.2.2.3
Plasma protein binding	Human	NA	In vitro	B	0.5 to 50 μM (0.21 to 21 μg/mL)	NA	No	GSK	2010N104947 (10DMR021)	m4.2.2.3
Plasma protein binding	Human	NA	In vitro	A	0.5 to 25 μM	NA	No	GSK	2011N119355	m4.2.2.3
Membrane permeability and transport by Pgp	Human (transfected MDCK II cells)	NA	In vitro	B C C	3 μM	NA	No	GSK	RH2007/00067 RD2008/00360 RD2008/00361	m4.2.2.3
Inhibition of digoxin transport by Pgp	Human (transfected MDCK II cells)	NA	In vitro	A	0.3 to 100 μM	NA	No	GSK	RD2008/00292 (08DMR021)	m4.2.2.3
OATP1B1 and OATP1B3 transport	Human (transfected CHO or MSRII cells)	NA	In vitro	A	0.1 to 100 μM	NA	No	GSK	RD2008/00216 (08DMR016)	m4.2.2.3
BCRP transport	Human (transfected MDCK II cells)	NA	In vitro	D	3 μM	NA	No	GSK	2011N112380 (11DMR004)	m4.2.2.3

Table 4.1 (Continued) List of Distribution Studies Performed with Dolutegravir

Type of Study	Species (Strain)/ Test System	No./Sex/ Group	Method of Administration	Form	Dose (mg/kg/day) or Concentration	Duration of Dosing (Sampling Occasions)	GLP	Testing Facility	Report No. (Study No.)	Location in CTD
Inhibition of OCT2-mediated transport	Human	NA	In vitro	A	25 μM	NA	No	[redacted]	RD2010/00555 (OPT-2010-104)	m4.2.2.3
Inhibition of OCT2	Human	NA	In vitro	A	0.1 to 30 μM	NA	No	[redacted]	2010N104937 (OPT-2010-119)	m4.2.2.3
Inhibition of OCT1	Human	NA	In vitro	A	10 μM	NA	No	[redacted]	2012N132572	m4.2.2.3
Inhibition of BCRP	Human	NA	In vitro	A	0.3 to 100 μM	NA	No	GSK	2010N110625 (10DMR033)	m4.2.2.3
Inhibition of MRP2	Human	NA	In vitro	A	0.1 to 100 μM	NA	No	GSK	2010N109746 (10DMR031)	m4.2.2.3
Inhibition of MRP2	Human	NA	In vitro	*	0.1 to 100 μM	NA	No	GSK	2011N120047	m4.2.2.3
Quantitative whole body auto-radiography	Rat (Lister Hooded)	7M	Oral (gavage)	D	50	Single	Yes	[redacted]	CD2008/00195 (2990/253)	m4.2.2.3
Blood to plasma ratio	Mouse (CD-1)	30M/ 30F	Oral (gavage)	D	100	Single	No	GSK	RD2009/00562 (09DMR021)	m4.2.2.5
Blood to plasma ratio	Rat (Sprague Dawley)	9M/9F	Oral (gavage)	D	50	Single	No	GSK	RD2008/00108 (08MDR008)	m4.2.2.5

Table 4.1 (Continued) List of Distribution Studies Performed with Dolutegravir

Type of Study	Species (Strain)/ Test System	No./Sex/ Group	Method of Administration	Form	Dose (mg/kg/day) or Concentration	Duration of Dosing (Sampling Occasions)	GLP	Testing Facility	Report No. (Study No.)	Location in CTD
Blood to liver concentrations	Mouse (CD-1)	30M/ 30F	Oral (gavage)	D	100	Single	No	GSK	RD2009/00562 (09DMR021)	m4.2.2.5
Blood to liver concentrations	Rat (Sprague Dawley)	9M/9F	Oral (gavage)	D	50	Single	No	GSK	RD2008/00108 (08MDR008)	m4.2.2.5
Placental transfer Lacteal secretion	Rat (Sprague Dawley)	5F 12F	Oral (gavage)	D	50	Single	No	GSK	2012N137348	m4.2.2.3
Blood to plasma ratio	Monkey (cynomolgus)	3M/3F 2M BDC	Oral (gavage)	D	10	Single	Yes	[redacted] [redacted]	RD2008/01299 (650-098) RD2008/01300 (7717-703)	m4.2.2.5

Key:
A = GSK1349572A, the sodium salt form. B = GSK1349572B, the parent form.
C = ^{14}C-labelled GSK1349572B. D = ^{14}C-labelled GSK1349572A.
NA = Not applicable.
BCRP = Breast cancer resistance protein.
OCT2 = Organic cation transporter 2.
Pgp = P-glycoprotein
* The test material for this study was GSK2832500 (a GSK1349572 glucuronide metabolite)

Testing Facility:
[redacted] = [redacted]
GSK = GlaxoSmithKline.
[redacted] = [redacted]
[redacted] = [redacted]
[redacted] = [redacted]
[redacted] = [redacted]

5. METABOLISM

In vitro and in vivo studies have been performed to investigate the metabolic fate of dolutegravir. These studies are listed in Table 5.1. The metabolic flow diagram for dolutegravir is located in m2.6.5 Section 11.

5.1. In Vitro Studies

5.1.1. Metabolism studies with microsomes, hepatocytes or recombinant enzymes derived from animals and humans

5.1.1.1. Metabolic stability in liver S9 and in hepatocytes

Preliminary investigations were conducted to evaluate the metabolic stability of dolutegravir (1 μM) in rat, dog, monkey and human liver S9 preparations and at 0.5 μM in rat and human fresh and cryopreserved hepatocytes [Report RH2007/00076, m4.2.2.4]. Incubations were performed at 37°C at intervals up to 60 or 120 minutes for S9 or hepatocytes, respectively. The supernatant from each reaction was analyzed using LC-MS/MS analysis. A tabulated summary of this study is provided in m2.6.5, Table 10.1.

Dolutegravir was stable in rat, monkey and human S9 ($t_{½}$ ≥90 minutes). No conclusion could be drawn from dog S9 incubations (n=2 independent assays) due to high inter-assay variability. Dolutegravir was stable in rat cryopreserved hepatocytes and in both fresh and cryopreserved human hepatocytes ($t_{½}$ >360 minutes). In fresh rat hepatocytes, the intrinsic clearance (25 mL/min/kg body weight) and $t_{½}$ (268 minutes) were determined.

5.1.1.2. Formation of glutathione adducts

Dolutegravir (100 μM) was tested in a glutathione reactive metabolite trapping assay using rat and pooled human liver microsomes with a NADPH regenerating system [Report RH2007/00058, m4.2.2.4]. Dolutegravir was incubated with the mixture at 37°C for 30 minutes and samples were analyzed by LC-MS/MS. A tabulated summary of this study is provided in m2.6.5, Table 10.6.

The results showed in vitro evidence for formation of a metabolite consistent with addition of glutathione through oxidative defluorination, in both rat and human liver microsomes.

5.1.1.3. Metabolic microsomal binding

A study was performed to evaluate the potential for metabolic activation when [^{14}C]-dolutegravir (10 μM) was incubated with rat, monkey and human liver microsomes using acetaminophen as a positive control [Report RD2007/01557, m4.2.2.4]. Incubations were performed at 37°C for 30 or 60 minutes and samples were analyzed using liquid scintillation counting. A tabulated summary of this study is provided in m2.6.5, Table 10.3.

The level of non-extracted radioactivity from rat, monkey and human microsomes following exhaustive solvent extraction was 1026, 2413 and 452 pmol eq/mg protein/hour, respectively. The non-extracted radioactivity observed was predominantly dependent on NADPH co-factor. Total non-extracted radioactivity from the positive control, acetaminophen, in rat, monkey and human microsomes was lower at 101 to 178 pmol eq/mg protein/hour.

5.1.1.4. Metabolism by hepatocytes

Information on the likely routes of metabolism of dolutegravir across species was investigated in vitro by incubating dolutegravir (10 μM) with cryopreserved rat, dog, monkey and human hepatocytes [Report RH2007/00060, m4.2.2.4]. Incubations were performed at 37°C for 0, 4 and 24 hours and samples were analyzed using LC/MS/MS. A tabulated summary of this study is provided in m2.6.5, Table 10.2.

No metabolites were detected by MS when dolutegravir was incubated up to 24 hours with cryopreserved rat, dog, monkey and human hepatocytes.

In a follow up study, the metabolism of [^{14}C]-dolutegravir (50 μM) was investigated in male rat, monkey and pooled (mixed gender) human cryopreserved hepatocytes [Report RD2007/01496, m4.2.2.4]. Incubations were performed at 37°C with [^{14}C]-dolutegravir in the presence of hepatocytes for up to 24 hours. Samples were analyzed using liquid scintillation counting. A tabulated summary of this study is provided in m2.6.5, Table 10.4.

The metabolic turnover of [^{14}C]-dolutegravir in rat and monkey hepatocytes was low and similar to human hepatocytes (approximately 3.5 to 9.4% turnover). In human hepatocytes, the notable route of metabolism for [^{14}C]-dolutegravir was glucuronidation. Metabolite profiles of the nonclinical species and human were qualitatively similar. The human metabolite (glucuronidation) was observed in hepatocytes from the two nonclinical species.

5.1.1.5. Recombinant CYP enzymes and liver microsomes

The oxidative enzymology of dolutegravir was investigated using pooled human liver microsomes and recombinant cytochrome P450 (CYP) enzymes [Report RD2008/00373/00, m4.2.2.4]. Incubations were performed at 37°C with [^{14}C]-dolutegravir (5 μM) in the presence of liver microsomes or recombinant CYP enzymes for up to 120 minutes and samples were analyzed using liquid scintillation counting. Azamulin, a selective CYP3A4 inhibitor, was used to detect potential inhibition of dolutegravir metabolism. A tabulated summary of this study is provided in m2.6.5, Table 10.8.

[^{14}C]-dolutegravir was metabolized in human liver microsomes to M7 (oxidation). M7 formation, which constituted 14 % of the metabolites generated by human liver microsomes, was completely inhibited by azamulin. Metabolites M1 (N-dealkylation) and M7 were formed in recombinant CYP3A4 incubations. Incubations with recombinant CYP1A2, 2B6, 2C8, 2C9, 2C19 and 2D6 showed no metabolism. The data

suggest that CYP3A4 is the primary CYP enzyme involved in the metabolism of dolutegravir in vitro.

5.1.1.6. Recombinant UGT enzymes and liver microsomes

An exploratory study to evaluate the potential of dolutegravir (0.5, 5 and 50 μM) to undergo in vitro glucuronidation by UDP glucuronosyltransferase 1A1 (UGT1A1) was investigated in incubations with pooled human liver microsomes (PHLM) and UGT1A1 supersomes (recombinantly expressed human UGT1A1 in baculovirus infected insect cells) [Report RH2007/00104, m4.2.2.4]. Incubations were performed at 37°C for up to 120 minutes and samples were analyzed using LC/MS/MS. A tabulated summary of this study is provided in m2.6.5, Table 10.7.

Under optimized study conditions using recombinant UGT1A1 supersomes, 30% of dolutegravir was metabolized to an ether glucuronide (M3) at the end of the 120 minute incubation with recombinant UGT1A1.

In a follow up study, the human UDP glucuronosyltransferase (UGT) enzymology of dolutegravir was investigated in vitro using pooled human liver microsomes and recombinant human UGT enzymes [Report RD2008/01339, m4.2.2.4]. Incubations were performed with [^{14}C]-dolutegravir (16 μM) at 37°C for up to 4 hours. Select samples were analyzed by liquid scintillation counting to determine the recovery of radiolabeled material, and supernatant samples from reaction mixtures were analyzed by radio-HPLC. β-glucuronidase digestions were performed to confirm glucuronide formation in the [^{14}C]-dolutegravir microsomal incubations. Atazanavir, a UGT1A1 inhibitor, was used to determine the potential to inhibit glucuronidation of dolutegravir. A tabulated summary of this study is provided in m2.6.5, Table 10.9.

[^{14}C]-dolutegravir was metabolized in human liver microsomes to a single UDPGA-dependent metabolite. β-glucuronidase digestions were used to identify this metabolite as a glucuronide. The estimated kinetic parameters, Km and V_{max}, for its formation in human liver microsomes were 149 μM and 409 pmol/min/mg, respectively. Atazanavir inhibited [^{14}C]-dolutegravir glucuronidation in human liver microsomes with a calculated IC_{50} value of 0.39 μM. [^{14}C]-dolutegravir was metabolized in recombinant UGT1A1 enzymes, resulting in calculated Km and V_{max} values of 21 μM and 67 pmol/min/mg, respectively. [^{14}C]-dolutegravir glucuronidation was also observed in recombinant UGT1A3 and 1A9 incubations but to a lesser extent in comparison to UGT1A1. Glucuronidation was not observed in recombinant UGT1A4, 1A6, 2B4, 2B7, 2B15 or control UGT incubations. These data suggest that UGT1A1 is the primary UGT enzyme involved in the glucuronidation of dolutegravir in vitro, with contribution from UGT1A3 and 1A9.

5.1.1.7. Potential metabolic formation of stereoisomers

Dolutegravir has two chiral centers, so the potential for metabolism of dolutegravir (10 μM) to its respective enantiomer and two diastereomers was investigated in incubations with cryopreserved rat, dog, monkey and human hepatocytes [Report

RH2007/00105, m4.2.2.4]. Incubations were performed at 37°C for 0, 1, 4, 6 and 24 hours and samples were analyzed using LC/MS/MS. A tabulated summary of this study is provided in m2.6.5, Table 10.5.

Although low amounts (~0.4% of the peak area of dolutegravir) of the enantiomer (gsk015*, also referred to as gsk016*) were detected at all incubation times, concentrations of gsk015* did not increase with time and no peak that corresponded the diastereomer (gsk013*, also referred to as gsk017*) was detected. Because dolutegravir and the diastereomer of its enantiomer (gsk014*, also referred to as gsk018*) could not be separated chromatographically, formation of this metabolite in the incubation could not be determined. These data indicate that no significant metabolic conversion of dolutegravir to its enantiomer or one of two possible diastereomers occurs in rat, dog, monkey or human hepatocytes.

5.1.2. In vitro inhibition and induction potential in animals and humans

5.1.2.1. Induction of rat PXR

An in vitro study was performed to determine the potential for dolutegravir to activate rat Pregnane X receptor (PXR), thereby causing induction of CYP3A and other target genes [Report RR2007/00024, m4.2.2.4]. HepG2 cells were transfected with rat PXR and were treated with dolutegravir (0.2 nM to 10 μM). Transcription of the reporter gene (luciferase) due to PXR activation was measured by a luminescence assay. The rat PXR activator, PCN (5-pregnan-3β-OL-20-ONE-16α carbonitrile), was included as a positive control. A tabulated summary of this study is provided in m2.6.5, Table 12.3.

Treatment of rat PXR transfected HepG2 cells with dolutegravir resulted in PXR activation with a maximum response of 1.3 to 4.8% compared to that observed with the rat PXR activator PCN. Dolutegravir was a weak activator of rat PXR in vitro and is therefore unlikely to cause induction of PXR target genes, including CYP3A, in this species in vivo.

5.1.2.2. Induction of human PXR

An in vitro study was performed to determine the potential for dolutegravir to activate human PXR [Report RR2007/00025, m4.2.2.4]. HepG2 cells were transfected with human PXR and were treated with dolutegravir (0.2 nM to 10 μM). Transcription of the reporter gene (luciferase) due to PXR activation was measured by a luminescence assay. The human PXR activator, rifampicin, was included as a positive control. A tabulated summary of this study is provided in m2.6.5, Table 12.3.

Treatment of human PXR transfected HepG2 cells with dolutegravir resulted in PXR activation with a maximum response of 41.6 to 58.1% compared to that observed with the human PXR activator, rifampicin. Dolutegravir was a moderate activator of human PXR in vitro and therefore may cause induction of the PXR target gene (e.g., CYP3A4) in vivo.

* 新薬承認情報提供時に置き換え

5.1.2.3. Inductive potential in human hepatocytes

A study was conducted to examine the potential for induction of select nuclear receptor target genes by dolutegravir when tested in vitro at concentrations of 1 to 40 μM [Report RH2007/00074, m4.2.2.4]. After an acclimatization period, cultured human hepatocytes were treated with dolutegravir at 37°C once daily for 2 days and quantitative real time PCR was used to determine the mRNA levels of CYP1A2, 2B6 and 3A4. Prototypical inducers were included as positive controls. A tabulated summary of this study is provided in m2.6.5, Table 12.2.

Following 48 hour incubations of dolutegravir, little to no effect on the mRNA levels of CYP1A2, 2B6 and 3A4 in human hepatocytes was observed. Fold changes from control for dolutegravir were <15% of the fold changes from control for the prototypical inducers.

5.1.2.4. Inhibition of CYP enzymes

Preliminary studies were conducted to assess the in vitro potential of dolutegravir (0.033 to 33 μM) to inhibit various human CYP enzymes [Report RH2007/00077, m4.2.2.4]. Recombinant human CYPs (CYP1A2, 2C9, 2C19, 2D6 and 3A4) and pooled human liver microsome (PHLM) assays were performed using selective probe substrates and inhibition of CYPs was assessed by IC_{50} determinations using both methods. Time-dependent inhibition (TDI) of CYP3A4 was assessed by measuring IC_{50} shifts with pre-incubation in PHLM only.

No direct inhibition (IC_{50} >33 μM) was observed for CYP2C9, 2C19, 2D6 or 3A4 using either the recombinant human CYPs or PHLM methods. For recombinant human CYP1A2, dolutegravir showed a moderate inhibition with an IC_{50} of 12.5 μM, while no inhibition (IC_{50} >33 μM) was observed with PHLM. No TDI of CYP3A4 was observed.

A follow up study was performed to determine the direct and metabolism-dependent inhibition potential of dolutegravir on various human CYP enzymes [Report WD2010/00908, m4.2.2.4]. The effects of dolutegravir (up to 100 μM) on CYP1A2, 2A6, 2B6, 2C8, 2C9, 2C19, 2D6 and 3A4 were determined using pooled human liver microsomes. The assays were performed using selective probe substrates and inhibition of CYPs was assessed by IC_{50} determinations. A tabulated summary of this study is provided in m2.6.5, Table 12.1.

Dolutegravir inhibited CYP3A4 with IC_{50} values of >54 μM. Dolutegravir inhibited CYP2B6, 2C9, 2C19 and 2D6 with IC_{50} values of >100 μM. Dolutegravir did not inhibit CYP1A2, 2A6 or 2C8 at concentrations up to 100 μM. Metabolism-dependent inhibition of CYP3A4 by dolutegravir was observed in this in vitro study, shifting IC_{50} values to >33 μM. There was no metabolism-dependent inhibition of CYP1A2, 2A6, 2B6, 2C8, 2C9, 2C19 or 2D6.

5.1.2.5. Inhibition of UDP-glucuronosyltransferase

An in vitro study was conducted to investigate the inhibition of human recombinant UDP-glucuronosyltransferease 1A1 (UGT1A1) and 2B7 (UGT2B7) by dolutegravir [Report RD2009/00862, m4.2.2.4]. The enzyme activities of scopoletin (UGT1A1) and 7-hydroxy-4-(trifluoromethyl) coumarin (UGT2B7) glucuronidation were measured by using recombinant UGT1A1 and 2B7 enzymes in the presence and absence of dolutegravir (0.1 to 100 μM). The production of scopoletin or 7-hydroxy-4-(trifluoromethyl) coumarin glucuronide in each incubation was quantified via fluorescence detection and the inhibition of glucuronide formation determined. A tabulated summary of this study is provided in m2.6.5, Table 12.1.

Dolutegravir was not an inhibitor of UGT2B7 at concentrations up to 100 μM. Inhibition of UGT1A1 was observed, however, inhibition at the highest concentration tested was insufficient to calculate an IC_{50} value (IC_{50}>100 μM).

5.1.2.6. Isolated perfused rat liver

The metabolism of dolutegravir was investigated in the bile, perfusate and liver extract from a male isolated perfused rat liver [Report RD2007/01493, m4.2.2.4]. [^{14}C]-dolutegravir was added to the perfusate at an amount equivalent to 15 mg free acid/kg of donor rat body weight. Methods were developed for the separation of notable metabolites in bile, perfusate and liver extract. A tabulated summary of this study is provided in m2.6.5, Table 10.10.

Notable routes of metabolism observed were N-dealkylation (M1), oxidation (M7), hexose conjugation (M2), glucuronidation (M3), and hexose or glucuronide conjugation in combination with N-dealkylation (M4 and M5) or with oxidation (M6 and M8).

Table 5.1 List of Metabolism Studies Performed with Dolutegravir

Type of Study	Species (Strain)/ Test System	No./Sex/ Group	Method of Administration	Form	Dose (mg/kg/day) or Concentration	Duration of Dosing (Sampling Occasions)	GLP	Testing Facility	Report No. (Study No.)	Location in CTD
Metabolic stability in liver S9	Rat, Dog, Monkey, Human	NA	In vitro	B	1 μM	NA	No	GSK	RH2007/00076 (07APK019)	m4.2.2.4
Bioactivation in liver microsomes	Rat, Monkey, Human	NA	In vitro	C	10 μM	NA	No	GSK	RD2007/01557 (07DMR124)	m4.2.2.4
Metabolic stability in hepatocytes	Rat, Human	NA	In vitro	A	0.5 μM	NA	No	GSK	RH2007/00076 (07APK019)	m4.2.2.4
Metabolism in hepatocytes	Rat, Dog, Monkey, Human	NA	In vitro	B	10 μM	NA	No	GSK	RH2007/00060 (07APK014)	m4.2.2.4
Metabolism in hepatocytes	Rat, Monkey, Human	NA	In vitro	C	50 μM	NA	No	GSK	RD2007/01496 (07DMR121)	m4.2.2.4
Metabolism in Supersomes and PHLM	Human	NA	In vitro	D	5 μM	NA	No	GSK	RD2008/00373 (08DMR033)	m4.2.2.4
Potential metabolic formation of stereoisomers	Rat, Dog, Monkey, Human	NA	In vitro	B	10 μM	NA	No	GSK	RH2007/00105 (07RCD8654)	m4.2.2.4

Table 5.1 (Continued) List of Metabolism Studies Performed with Dolutegravir

Type of Study	Species (Strain)/ Test System	No./Sex/ Group	Method of Administration	Form	Dose (mg/kg/day) or Concentration	Duration of Dosing (Sampling Occasions)	GLP	Testing Facility	Report No. (Study No.)	Location in CTD
Formation of glucuronide metabolite	Human	NA	In vitro	A	0.5, 5, 50 μM	NA	No	GSK	RH2007/00104 (07APK024)	m4.2.2.4
Formation of glutathione adducts in microsomes	Rat, Human	NA	In vitro	B	100 μM	NA	No	GSK	RH2007/00058 (06RCM8059)	m4.2.2.4
UDP-glucuronosyltrans-ferase enzymology	Human	NA	In vitro	C	16 μM	NA	No	GSK	RD2008/01339 (08DMR067)	m4.2.2.4
Inhibition of UDP-glucuronosyl-transferase 1A1 and 2B7	Human	NA	In vitro	A	0.1 to 100 μM	NA	No	GSK	RD2009/00862 (09DMR031)	m4.2.2.4
CYP induction in hepatocytes	Human	NA	In vitro	B	1 to 40 μM	NA	No	GSK	RH2007/00074 (07APK018)	m4.2.2.4
PXR activation assays	Rat Human	NA	In vitro	B	0.2 nM to 10 μM	NA	No	GSK	RR2007/00024 (Rat) RR2007/00025 (Human)	m4.2.2.4
Metabolite identification in IPRL	Rat	1M	Ex-vivo	C	15[a]	NA	No	GSK	RD2007/01493 (07DMR120)	m4.2.2.4

Table 5.1 (Continued) List of Metabolism Studies Performed with Dolutegravir

Type of Study	Species (Strain)/ Test System	No./Sex/ Group	Method of Administration	Form	Dose (mg/kg/day) or Concentration	Duration of Dosing (Sampling Occasions)	GLP	Testing Facility	Report No. (Study No.)	Location in CTD
CYP inhibition in recombinant enzymes and liver microsomes	Human	NA	In vitro	B	0.033 to 33 μM	NA	No	GSK	RH2007/00077 (07APK020)	m4.2.2.4
Direct and metabolism-dependent CYP inhibition	Human	NA	In vitro	A	Up to 100 μM	NA	No	GSK	WD2010/00908 (10DMW013)	m4.2.2.4
Metabolism in vivo	Mouse (CD-1)	30M/30F 4M BDC	Oral (gavage)	D	100	Single	No	GSK	RD2009/00723 (09DMR028)	m4.2.2.4
Metabolism in vivo	Rat (Sprague Dawley)	3M/3F 3M BDC	Oral (gavage)	D	50	Single	No	GSK	RD2008/00220 (08DMR017)	m4.2.2.4
Potential for epimerization in juvenile plasma	Rat (Sprague Dawley)	2M/2F	Oral	A	2, 25, 75, 300	10 days	No	GSK	RD2010/00173 (10DMR005)	m4.2.2.4
Metabolites in rat milk	Rat (Sprague Dawley)	3F	Oral (gavage)	D	50	Single	No	GSK	2012N132387	m4.2.2.4
Effect on hepatic drug metabolizing enzymes	Rat	6M/6F	In vivo	A	50 150, 500	14 days	Yes	[redacted]	RD2007/01034	m4.2.2.4

Table 5.1 (Continued) List of Metabolism Studies Performed with Dolutegravir

Type of Study	Species (Strain)/ Test System	No./Sex/ Group	Method of Administration	Form	Dose (mg/kg/day) or Concentration	Duration of Dosing (Sampling Occasions)	GLP	Testing Facility	Report No. (Study No.)	Location in CTD
Metabolism in vivo	Monkey (cynomolgus)	3M/3F 2M BDC	Oral (gavage)	D	10	Single	No	GSK	RD2008/00899 (08DMR054)	m4.2.2.4
Effect on hepatic drug metabolizing enzymes	Monkey (cynomolgus)	3M/ 2 to 3F	In vivo	A	100, 300, 1000	14 days	Yes	[redacted]	RD2007/01142	m4.2.3.2

Key:
a = Equivalent dose compared to body weight of the animal whose liver was used in testing.
A = GSK1349572A, the sodium salt form. B = GSK1349572B, the parent form.
C = ^{14}C-labelled GSK1349572B. D = ^{14}C-labelled GSK1349572A.
IRPL= Isolated perfused rat liver.
NA = Not applicable.
PXR = Pregnane X receptor.

Testing Facility:
GSK = GlaxoSmithKline.
[redacted] = [redacted]

5.2. In Vivo Studies

5.2.1. Mouse

5.2.1.1. Metabolic profile following oral administration

The in vivo metabolism of dolutegravir was investigated in male and female mice following a single oral dose of [^{14}C]-dolutegravir at 100 mg/kg [Report RD2009/00723, m4.2.2.4]. Samples of plasma and homogenates of liver (2, 10 and 24 hours), urine and feces (0 to 168 hours), from intact male and female animals, and bile, urine and feces from male bile duct-cannulated mice (0 to 72 hours), were obtained from an excretion study in mice [Report RD2009/00562], with the elimination results discussed in Section 6.1.1. A tabulated summary of the male data from this study is provided in m2.6.5, Table 9.1.

Dolutegravir was the principal radiolabeled component in male and female mouse plasma at the 2 and 10 hour post-dose sampling times (≥90.2% of the plasma radiocarbon) through the last sampling time of 24 hours post-dose (≥78% of the plasma radiocarbon). Two unknown metabolites were present in mouse plasma through 24 hours post-dose and represented 1.3 to 6.6% of the plasma radiocarbon.

Dolutegravir was the principal radiolabeled component in male and female mouse liver homogenate at each sampling time of 2, 10, and 24 hours post-dose, and represented 55.1 to 89.5% of the liver radiocarbon. An ether glucuronide of parent (M3, GSK2832500) was present at low concentrations (3.8% of liver radiocarbon) at 10 hours post-dose in male and female mouse liver homogenate, but was not detected at 24 hours post-dose. An unknown metabolite, observed at 10 hours post-dose in male and female mouse liver homogenate, represented 3.7 and 3.0% of the radiocarbon, respectively.

Elimination of the absorbed dose in the mouse was through metabolism with 2.5% excreted in the bile and less than 2.0% in the urine. Dolutegravir glucuronide (M3) and a metabolite resulting from fluorine loss and the addition of glutathione and oxidation were the principal components in mouse bile accounting for 24.3 and 20.8% of the sample radiocarbon (0.7 and 0.5% of the dose), respectively. Other identified metabolites in the bile each represented ≤10% of the sample radiocarbon (≤0.2% of the dose). Dolutegravir glucuronide (M3) was the major component in the urine accounting for 53.0 and 46.8% of the radiocarbon (0.6 and 0.9% of the dose) in males and females, respectively. Metabolites resulting from hexose conjugation of parent, N-dealkylation and an oxidation at the benzylic carbon were also observed as minor components in urine (≤0.1% of the dose). Dolutegravir represented a mean of 8.7 and 1.6% of the urinary radiocarbon (≤0.1% of the dose) in male and female mice, respectively. Dolutegravir represented a mean of 6.1% of the biliary radiocarbon (0.2% of the dose).

Dolutegravir was the only component detected in feces and represented a mean of 96.7% of the fecal radiocarbon (89.1% of the dose) in male and female mice.

5.2.2. Rat

5.2.2.1. Metabolic profile following oral administration

The in vivo metabolism of dolutegravir was investigated in male and female rats following a single oral dose of [^{14}C]-dolutegravir at a dose level of 50 mg/kg [Report RD2008/00220, m4.2.2.4]. Samples from intact male and female rats (plasma and liver at 2, 6 and 24 hours, and urine and feces up to 168 hours post-dose), and from male bile duct-cannulated rats (bile, urine and feces collected up to 96 hours post-dose) were obtained from an excretion study in rats [Section 6.2.1, Report RD2008/00108]. A tabulated summary of the male data from this study is provided in m2.6.5, Table 9.1.

The only quantifiable radiolabeled component in male and female rat plasma at 2, 6 and 24 hours post-dosing was unchanged dolutegravir which represented 96.3%, 96.9% and 90.3% of the plasma radiocarbon in males and 97.1%, 96.5% and 98.3% of plasma radiocarbon in females at those timepoints, respectively.

The principal radiolabeled component in male and female rat liver homogenate at 2, 6 and 24 hours post-dosing was unchanged dolutegravir which represented 90.9%, 86.9% and 62.4% of the liver radiocarbon in males and 93.2%, 91.2% and 90.2% of liver radiocarbon in females at those timepoints, respectively.

An average of 95.2% of the fecal radiocarbon (86.2% of the dose) in intact male and female rats was eliminated as unchanged dolutegravir. The predominant metabolites in rat bile resulted from glucuronidation (M3) and hexose conjugation accounting for a combined total of 63% of biliary radiocarbon (4.0% of the dose). Other notable metabolites in bile included loss of fluorine in combination with oxidation and glutathione addition representing 11.7% of the radiocarbon (0.8% of the dose) and pentose conjugation representing 1.7% of the radiocarbon (0.1% of the dose). Oxidation and N-dealkylation were the major biotransformation products in the urine of male and female rats accounting for an average of 40.6 and 24.3% of the radiocarbon (1.2 and 0.7% of the dose), respectively. Other notable metabolites observed in rat urine resulted from glucuronidation (M3) and hexose conjugation, each representing 12.5% of the radiocarbon (0.4% of the dose). Biliary and renal elimination of unchanged dolutegravir was very low over the time period examined (≤1.5% of the sample radiocarbon or ≤0.1% of the dose).

5.2.2.2. Potential epimerization in juvenile rats

The potential for epimerization of dolutegravir to occur in vivo was assessed in juvenile rats following repeat oral dosing of dolutegravir at 2, 25, 75 and 300 mg/kg/day [Report RD2010/00173, m4.2.2.4]. Plasma samples were obtained from a reproductive toxicity study in which male and female juvenile rats were administered doses of dolutegravir for 10 days from Day 4 to Day 13 post partum [Report CD2009/00770]. A tabulated summary of this study is provided in m2.6.5, Table 9.3 and a summary of the toxicology data is presented in m2.6.6, Section 6.5.

There was no evidence for the in vivo epimerization of dolutegravir to any of its stereoisomers, gsk013*, gsk014* or gsk015*, in juvenile rat plasma samples following repeat oral administration of dolutegravir.

5.2.2.3. Metabolites in milk from lactating rats

Lactating rats were administered a single oral dose of [^{14}C]-dolutegravir (50 mg/kg) and milk samples were collected and examined for metabolites of dolutegravir [Report 2012N132387, m4.2.2.4]. Milk samples were obtained from a separate study [Report 2012N137348] and were collected from 3 animals/time point at 2 and 4 hours post-dose and from 2 animals/time point at 1, 8 and 24 hours post-dose. Identification of dolutegravir-related material in samples was accomplished by radio-HPLC/MS/MS. A tabulated summary of this study is provided in m2.6.5, Table 9.2.

Dolutegravir was the predominant component in the rat milk and represented 82.9% to 97.0% of the radiocarbon over the 24 hours sampling period. In addition, minor uncharacterized components were observed that were below the quantifiable limit (LLQ).

The C_{max} was observed at 8 hours post-dose for dolutegravir (45.6 μg-eq/g) and total radiocarbon (51.1 μg-eq/g). The AUC_{0-24} for dolutegravir (412 h μg-eq/g) and total radiocarbon (459 h μg-eq/g) were similar (ratio = 0.90), indicating that dolutegravir comprised nearly all of the drug-related material in milk.

5.2.2.4. Cytochrome P450 induction

In a study to evaluate the effects of dolutegravir on hepatic drug metabolizing enzymes using rat liver samples obtained from a repeat dose toxicity study, hepatic liver microsomes were prepared from rats (n=6/sex/group) treated with dolutegravir at 50, 150 and 500 mg/kg/day for 14 days [Report RD2007/01034, m4.2.2.4]. Assays were performed for specific enzyme activities of known inducible CYPs. A tabulated summary of this study is provided in m2.6.5, Table 12.4 and a summary of the toxicology data is presented in m2.6.6, Section 3.3.

In females, the only statistically significant effect was a decrease in the total CYP content at the 50 mg/kg/day dose. In males, there were dose-dependent statistically significant decreases for CYP3A activities at the 150 mg/kg/day dose and in total CYP content and for CYP3A, CYP2C11 and CYP2B1 activities at the 500 mg/kg/day dose.

5.2.3. Monkey

5.2.3.1. Metabolic profile following oral administration

The in vivo metabolism of dolutegravir was investigated in male and female monkeys following a single oral dose of [^{14}C]-dolutegravir at a dose level of 10 mg/kg [Report RD2008/00899, m4.2.2.4]. Samples from intact male and female monkeys (plasma at 2, 6, 12 and 24 hours, and urine and feces up to 168 hours post-dose), and from male bile duct-cannulated monkeys (bile, urine and feces collected up to 96 hours post-dose) were

obtained from excretion studies in monkeys [Reports RD2008/01299 and RD2008/01300/00], with the elimination results discussed in Section 6.3.1. A tabulated summary of the male data from this study is provided in m2.6.5, Table 9.1.

Through 24 hours post-dose, dolutegravir was the principal radiolabeled component in male and female monkey plasma and represented 85.0 to 99.1% of the plasma radioactivity. Dolutegravir glucuronide (M3) was present at low concentrations (≤3.5% of plasma radiocarbon) through 12 hours post-dose.

Conjugation was the primary biotransformation process for the formation of biliary and urinary metabolites, representing greater than 65% of the sample radiocarbon. Glucuronide (M3) and hexose conjugates were the principal components in bile accounting for 36.5 and 28.7% of the sample radioactivity, respectively. A metabolite, resulting from fluorine loss and the addition of cysteine and oxygen, was a notable radioactive component in bile representing 16% of the radioactivity (<2.0% of the dose) but was not measurable in urine. Dolutegravir glucuronide (M3) was the major component in the urine accounting for approximately 68% of the radiocarbon (3.1% of the dose). A hexose conjugate and N-dealkylated metabolite were also observed as minor components in urine (<0.5% of the dose). Biliary and renal elimination of dolutegravir was very low (≤2.5% of the sample radiocarbon or ≤0.3% of the dose).

Dolutegravir was the only notable component in feces and represented 90.0% of the fecal radiocarbon (64.2% of the dose) in male and female monkeys. The presence of dolutegravir in the feces appears to be due to a contribution from deconjugation of biliary metabolites in addition to lack of absorption.

No notable qualitative or quantitative differences in the metabolic profile between males and females were observed.

5.2.3.2. Cytochrome P450 induction

In a study to evaluate the effects of dolutegravir on hepatic drug metabolizing enzymes using monkey liver samples obtained from a repeat dose toxicity study, hepatic liver microsomes were prepared from monkeys (n=2 to 3/sex/group) treated with dolutegravir at 100, 300 and 1000 mg/kg/day for 14 days [Report RD2007/01142, m4.2.3.2]. Assays were performed for specific enzyme activities of known inducible CYPs. A tabulated summary of this study is provided in m2.6.5, Table 12.5 and a summary of the toxicology data is presented in m2.6.6, Section 3.4.

A statistically significant increase in the total liver protein and a statistically significant decrease in the CYP content were noted for males in the 1000 mg/kg/day group only.

6. EXCRETION

A range of studies has been performed in which the excretion balance of [^{14}C]-dolutegravir was investigated. These studies are listed in Table 6.1.

6.1. Mouse

6.1.1. Excretion following oral administration

The elimination of radioactivity was investigated following a single oral administration of [^{14}C]-dolutegravir (100 mg/kg) to intact male and female mice, and bile duct cannulated (BDC) male mice (n=4/sex/group) [Report RD2009/00562, m4.2.2.5]. [^{14}C]-dolutegravir was formulated as a suspension in 0.5% HPMC and 0.1% Tween 80 in water. All samples were analyzed by liquid scintillation counting (LSC) for total radioactivity. A tabulated summary of this study is presented in m2.6.5, Table 13.1.

In male and female intact mice, fecal excretion was the primary route for elimination of radioactivity accounting for means of 93.0 and 94.1% of the administered dose, while urinary elimination accounted for less than 2% of the administered dose. Elimination of radioactivity was rapid with the majority of the dose (>92%) being recovered within 24 hours post-dose. The mean total recovery (including cage wash) of the administered dose in intact male and female mice was 94.5 and 96.2%, respectively, at 168 hours. No sex-related differences were observed in the rate or extent of radioactivity elimination.

In male BDC mice, biliary secretion accounted for a mean of 2.51% of the dose, while means of 86.3 and 1.83% were eliminated via feces and urine, respectively. The sum of the mean biliary and urinary recoveries of radioactivity indicated that at least 4.34% of the oral dose was absorbed. The mean total recovery of the administered dose was 91.0% at 72 hours.

6.2. Rat

6.2.1. Excretion following oral administration

The elimination of radioactivity was investigated following a single oral administration of [^{14}C]-dolutegravir (50 mg/kg) to intact male and female rats, and bile duct cannulated (BDC) male rats (n=3/sex/group) [Report RD2008/00108, m4.2.2.5]. [^{14}C]-dolutegravir was formulated as a suspension in 0.5% HPMC and 0.1% Tween 80 in water. All samples were analyzed by LSC for total radioactivity. A tabulated summary of this study is presented in m2.6.5, Table 13.1.

The major route of elimination of drug-related material in intact rats was via the feces with a mean of 92.6% and 90.7% of the dose in males and females, respectively, over 168 hours after dosing. Urinary elimination accounted for less than 4% of the dose in males and females. Elimination of radioactivity was rapid with the majority of the dose (>83%) being recovered within 24 hours post-dose. The mean total recovery (including

cage washings) was 96.1% and 94.6% of the dose in males and females, respectively, at 168 hours post-dose.

In male BDC rats, means of 6.99%, 86.2% and 2.51% of the dose were eliminated via the bile, feces and urine, respectively. The mean total recovery (including cage washings) was 95.9% of the dose at 96 hours post-dose. A mean of at least 9.5% of the dose was absorbed by these animals, as judged by the amounts of drug-related material in bile and urine.

6.3. Monkey

6.3.1. Excretion following oral administration

The rate and extent of elimination of radioactivity were investigated in three male and three female intact monkeys after a single oral administration of [^{14}C]-dolutegravir (10 mg/kg) [Report RD2008/01300, m4.2.2.5]. The rate and extent of elimination of radioactivity were also investigated in two male bile duct-cannulated monkeys after a single oral administration of [^{14}C]-dolutegravir (10 mg/kg) [Report RD2008/01299, m4.2.2.5]. In both studies, [^{14}C]-dolutegravir was formulated as a suspension in 0.5% HPMC and 0.1% Tween 80 in water. All samples were analyzed by LSC for total radioactivity. Tabulated summaries of these studies are presented in m2.6.5, Table 13.1.

The predominant route of elimination of [^{14}C]-dolutegravir related material in intact monkeys was via fecal excretion, accounting for mean recoveries of 66.9% and 77.5% of the dose in males and females, respectively. Urinary excretion accounted for means of 6.00% and 4.42% of the recovered dose in males and females, respectively. Mean total recoveries of radioactivity from all matrices at 168 hours post-dose were 79.7% for males and 85.0% for females. No sex-related differences in excretion patterns were observed.

In BDC monkeys, biliary secretion accounted for a mean of 11.8% of the administered dose. Mean fecal and urinary recoveries of radioactivity were 70.0% and 7.2%, respectively. The combined biliary and urinary radioactivity recoveries suggest that approximately 19.0% of the oral dose was absorbed. The mean total recovery of the radioactivity dose from all matrices collected through 96 hours post-dose was 91.6%.

Table 6.1 List of Excretion Studies Performed With Dolutegravir

Type of Study	Species (Strain)/ Test System	No./Sex/ Group	Method of Administration	Dose (mg/kg/day)	Duration of Dosing (Sampling Occasions)	GLP	Testing Facility	Report No. (Study No.)	Location in CTD
Elimination	Mouse (CD-1)	4M/4F 4M BDC	Oral (gavage)	100	Single	No	GSK	RD2009/00562 (09DMR021)	m4.2.2.5
Elimination	Rat (Sprague Dawley)	3M/3F 3M BDC	Oral (gavage)	50	Single	No	GSK	RD2008/00108 (08MDR008)	m4.2.2.5
Elimination	Monkey (cynomolgus)	3M/3F 2M BDC	Oral (gavage)	10	Single	Yes	[redacted] [redacted]	RD2008/01299 (650-098) RD2008/01300 (7717-703)	m4.2.2.5

Key:

^{14}C-labelled dolutegravir was used in these studies.
NA = Not applicable.

Testing Facility:
[redacted] = [redacted]
GSK = GlaxoSmithKline.
[redacted] = [redacted]

7. PHARMACOKINETIC DRUG INTERACTIONS

Nonclinical studies have not been performed in vivo to specifically investigate potential pharmacokinetic interactions with drugs that are likely to be co-administered with dolutegravir. However, relevant data from cytochrome P450 inhibition/ induction, protein binding and transporter studies are available.

Dolutegravir is primarily metabolized by UGT1A1 with a notable contribution from CYP3A4. The enzymes UGT1A3 and 1A9 were minor pathways. Consistent with the in vitro mechanistic studies, the clinical drug interaction studies have confirmed the low propensity of dolutegravir to alter the pharmacokinetics of co-administered agents that are substrates of CYP enzymes, UGT enzymes, or major drug transporters (except for OCT2). Based on the clinical drug interaction studies with potent UGT1A1and CYP3A4 inhibitors, co-administration of enzyme inhibitors are not expected to alter dolutegravir pharmacokinetics in a clinically meaningful manner. Clinical studies with moderate and strong inducers showed varied effects on dolutegravir pharmacokinetics and dosing recommendations are made based on the drug regimen used [see m2.7.2, Section 3.4].

8. OTHER PHARMACOKINETIC STUDIES

No studies appropriate to this category have been performed with dolutegravir.

9. DISCUSSION AND CONCLUSIONS TO PHARMACOKINETICS

Pharmacokinetic, absorption, distribution, metabolism and elimination studies with dolutegravir have been performed in mice, rats, rabbits, dogs and monkeys. A comparison of the systemic exposure of dolutegravir achieved in the nonclinical species during toxicity studies and in humans at the planned therapeutic dose is presented below in Table 9.1.

After single doses of dolutegravir to rats, dogs, and monkeys, the steady-state volume of distribution and the plasma clearance were low with half-life values of 5 to 6 hours. The oral bioavailability of dolutegravir as a solution was high (75 to 87%), consistent with its high passive and absorptive permeability. The oral bioavailability at higher doses in suspensions appeared limited by dissolution rate or solubility, which resulted in a less than proportional increase in systemic exposure to dolutegravir with an increase in dose. In rats and monkeys, no consistent notable difference in systemic exposure between sexes was observed.

In vitro the protein binding of dolutegravir in rat and monkey sera, and in human serum and plasma, was high (>99%); low association to blood cellular components was noted in vivo. In rats, dolutegravir-related material was widely distributed to tissues. Longer term association of dolutegravir-related material was observed with bone and with melanin in the skin but not with melanin in the uveal tract.

Structural information on dolutegravir metabolites in humans following a single oral 20 mg dose of [^{14}C]-dolutegravir indicated that the principal metabolites resulted from glucuronic acid conjugation (M3), benzylic carbon oxidation, and N-dealkylation [see m2.7.2, Section 2.1.1.5]. These biotransformations are consistent with those observed in vivo in mice, rats, and monkeys in which all notable human metabolites were observed in at least one nonclinical species. There was no evidence for the in vivo epimerization of dolutegravir to any of its stereoisomers in juvenile rat plasma or in humans. The qualitatively similar metabolic results support the selection of the species used in the safety assessment of dolutegravir for clinical use.

Elimination of drug-related material occurred predominantly via the feces in animals, with a major portion of the absorbed compound being secreted into the bile. Over 24 hours following oral administration to rodents, mean liver to blood concentration ratios ranged from 0.3 to 0.47, indicating low extraction by the liver, consistent with the low metabolic clearance observed.

In vitro, UGT1A1 and CYP3A4 appeared to be the primary enzymes involved in the metabolism of dolutegravir. Co-administration with known UGT1A1 and CYP3A4 inducers (such as etravirine and efavirenz) lowered the exposure to dolutegravir. As a result of the parallel clearance pathways, alterations in the pharmacokinetics of dolutegravir were not clinically meaningful following co-administration with UGT1A1 and CYP3A4 inhibitors (such as atazanavir and lopinavir/ritonavir). Dolutegravir is a substrate for P-gp but because of its high permeability, no alteration in absorption would

be expected by coadministration of P-gp inhibitors (such as ritonavir and saquinavir). Dolutegravir is also a substrate for the efflux transporter, BCRP.

In vitro, dolutegravir was not a direct inhibitor of the transporters MRP2, OATP1B1 or OATP1B3 or of the enzymes UGT2B7, CYP1A2, CYP2A6, or CYP2C8. In addition, dolutegravir glucuronide did not inhibit MRP2. Dolutegravir was a weak inhibitor of the human efflux transporters P-gp and BCRP and of the enzymes UGT1A1, CYP2B6, CYP2C9, CYP2C19, CYP2D6, and CYP3A4. Dolutegravir did not affect the pharmacokinetics of efavirenz (CYP2B6 substrate) or midazolam (CYP3A4 substrate), when co-administered to humans. Based on these data and the likely dolutegravir therapeutic plasma concentrations, dolutegravir has a low propensity to alter pharmacokinetics of co-administered agents through interaction with these transporters or enzymes.

In vitro, dolutegravir was an inhibitor of the renal organic cation transporter 2 (OCT2). The inhibition of OCT2 by dolutegravir in the presence of abacavir and lamivudine, or tenofovir disoproxil and emtricitabine, was similar to that of dolutegravir alone. In vitro incubation with dolutegravir (at concentrations observed in humans after a 50 mg oral dose) produced a 90% inhibition of the OCT2. These in vitro results indicate the potential for a drug interaction in vivo with cationic compounds that are renally cleared by this transporter such as the endogenous substrate, creatinine, and the antiarrhythmic drug, dofetilide. Dofetilide should not be used with dolutegravir, because dolutegravir may inhibit its renal tubular secretion resulting in increased dofetilide concentrations that may be toxic.

These data, taken together, support the selection of the toxicology species for the evaluation of safe use of dolutegravir in the proposed patient population when prescribed according to the proposed dosing regimen.

Table 9.1 Comparative Assessment of Mean Systemic Exposure Following Oral Administration of Dolutegravir

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

Table 9.1 (Continued) Comparative Assessment of Mean Systemic Exposure Following Oral Administration of Dolutegravir

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

Table 9.1 (Continued) Comparative Assessment of Mean Systemic Exposure Following Oral Administration of Dolutegravir

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

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

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

Note: No observed adverse effect levels (NOAEL) are bolded.
QD = once daily. BID = twice daily.

APPENDIX 1 ANALYTICAL METHODS USED FOR THE DETERMINATION OF DOLUTEGRAVIR IN BIOLOGICAL FLUIDS

Validation Report	Nonclinical Studies Supported	Method Description and Performance
Mouse (CD-1) Plasma (Heparin) (Original chiral method) [redacted] **Title:** Validation of an Analytical Method for Determination of S-349572 in Mouse Plasma by LC/MS/MS for TK analysis Validation Number: 08-8596 Document Number: 2012N142266	Mouse 13-week preliminary carcinogenicity 09-2119 (S-349572-TF-068-L) Document: RD2009/00028 Mouse carcinogenicity 09-2177 (S-349572-TF-083-L) Document: 2012N152419	Dolutegravir was extracted from 30 μL mouse plasma by protein precipitation using methanol:acetonitrile (1:1) with 0.3% phosphoric acid containing [$^{2}H_{7}{}^{15}N$]-dolutegravir as an internal standard. Extracts were separated by chiral HPLC with a Chiralcel OJ-RH column (150mm x 2.1mm; 5μm) and analyzed by MS/MS detection using a Turbo IonSpray interface in the positive ion mode and multiple reaction monitoring (m/z 420>277 and m/z 428>283). **Validated Range:** 0.5 to 500 μg/mL **Lower limit of quantification (LLQ)** 0.5 μg/mL Within-run Precision (%CV): 3.1% to 16.2% Accuracy (% Bias): -4.2 to 13.2% **QC levels:** 1.5, 75, 375 μg/mL Within-run Precision (%CV): 2.1 to 8.1% Accuracy (% Bias): -8.0 to 7.2% **Dilution Effects:** 5-fold and 10-fold Within-run Precision (%CV): 9.5 and 2.6% Accuracy (% Bias): 0% and 1.0% **Recovery:** 101 to 118% dolutegravir 95.4 to 98.2% internal standard **Stability in Mouse Plasma**: 5 freeze-thaw cycles from -20°C at least 28 days at -20°C at least 6 hours at ambient temperature **Processed Extract Stability:** At least 4 days at 4°C

Validation Report	Nonclinical Studies Supported	Method Description and Performance
Rat [Sprague Dawley] Plasma (Heparin) (Original chiral method – full validation) [redacted], Japan **Title:** Validation of an Analytical Method for Determination of ERC-349572 in Rat Plasma by LC/MS/MS Validation Number: SG06304 (E-349572-TF-010-N) Document Number: RD2007/01185	Rat two week oral toxicity E-349572-TB-012-L Document: RD2007/01140	Dolutegravir was extracted from 30 μL rat plasma by protein precipitation using methanol:acetonitrile (1:1 v/v) containing MTS-0297889 as an internal standard. Extracts were separated by chiral HPLC with a Chiralcel OJ-RH column (150mm x 4.6mm; 5μm) and analyzed by MS/MS detection using a Turbo IonSpray interface in the positive ion mode and multiple reaction monitoring (m/z 420>277 and m/z 434>291). **Lower limit of quantification (LLQ)**: 0.03 μg/mL **Validated Range:** 0.03 to 30 μg/mL **QC levels:** 0.03, 0.1, 3, 30 μg/mL Within-run Precision (%CV): 5.8 to 7.4% Inter-day Precision (%CV) 5.9 to 12% Accuracy (% Bias): 2 to 11% **Dilution Effects:** 100-fold Within-run Precision (%CV): 8.7%% Accuracy (% Bias): 5.8% **Recovery:** 103 to 107% dolutegravir 102% internal standard **Stability in Rat Plasma**: 3 freeze-thaw cycles from -30°C at least 4 Weeks at -30°C at least 4 hours at ambient temperature **Processed Extract Stability**: At least 48 hours at 10°C

Validation Report	Nonclinical Studies Supported	Method Description and Performance
Rat (Sprague Dawley) Plasma (Heparin) **(Re-validation (full) of chiral assay method using a stable isotopic lable as the internal standard)** [redacted], Japan **Title:** Validation of an Analytical Method for Determination of ERC-349572 in Rat Plasma by LC/MS/MS (2) Validation Number: S-349572-TB-041-N Document Number: RD2009/00444	Rat one month oral toxicity S-349572-TB-043-L Document: RD2008/01628 Rat six month oral toxicity SBL055-082 (S-349572-TF-055-L) Document: RD2009/00410 Rat oral embryofetal development S-349572-TB-062-L Document: XD2009/00367 Rat carcinogenicity 09-2178 (S-349572-TF-084-L) Document: 2012N152418	Dolutegravir was extracted from 30 μL rat plasma by protein precipitation using methanol:acetonitrile (1:1) with 0.3 vol% phosphoric acid containing [$^{2}H_{7}{}^{15}N$]-dolutegravir as an internal standard. Extracts were separated by chiral HPLC with a Chiralcel OJ-RH column (150mm x 2.0mm; 5μm) and analyzed by MS/MS detection using a Turbo IonSpray interface in the positive ion mode and multiple reaction monitoring (m/z 420/277 and m/z 428>283). **Lower limit of quantification (LLQ)**: 0.5 μg/mL **Validated Range:** 0.5 to 500 μg/mL **QC levels:** 0.5, 15, 500 μg/mL Within-run Precision (%CV): 0.7 to 5.4% Between-run Precision (%CV) 4.9 to 6.0% Accuracy (% Bias): -10.7 to 7.4% **Dilution Effects:** 100-fold Within-run Precision (%CV): 8.7% Accuracy (% Bias): 5.8% **Recovery:** 81.0 to 98.3% **Stability in Rat Plasma**: 3 freeze-thaw cycles from -20°C at least 56 days at -20°C at least 4 hours at ambient temperature Processed Extract Stability: At least 48 hours at 10°C

Validation Report	Nonclinical Studies Supported	Method Description and Performance
Rat (Sprague Dawley) Plasma (EDTA) (Achiral method – full validation) GlaxoSmithKline Five Moore Driver Research Triangle Park, NC 27709 USA **Title:** The Validation of a Method for the Determination of GSK1349572 in Rat Plasma (range 250 to 10,000 ng/mL) using HPLC-MS/MS Validation Number: GSK1349572RTPLVALC Document Number: RD2010/00176	Rat juvenile toxicity 09-2119 (S-349572-TF-068-L) Document: RD2009/00028	Dolutegravir was extracted from 25 μL rat plasma by protein precipitation using acetonitrile containing [$^{2}H_{7}{}^{15}N$]-dolutegravir as an internal standard. Extracts were separated by reversed-phase HPLC on a XBridge C18 column (50mm x 2.1mm; 3.5μm) and analyzed by MS/MS using a Turbo IonSpray interface in the positive ion mode and multiple reaction monitoring (m/z 420>277 and m/z 428>283). **Lower limit of quantification (LLQ)**: 0.25 μg/mL **Validated Range:** 0.25 to 100 μg/mL **QC levels:** 0.25, 0.75, 8, 80, 100 μg/mL Within-run Precision (%CV): 1 to 4.4% Between-run 1.6 to 5.7% Accuracy (% Bias): -13.4 to 7.2% **Dilution Effects:** 5-fold and 10-fold Within-run Precision (%CV): 1.6% Accuracy (% Bias): -2.6% **Recovery:** 95.6 to 106% **Stability in Rat Plasma**: 3 freeze-thaw cycles from -20°C at least 28 days at -20°C at least 24 hours at ambient temperature **Whole Blood Stability:** at least 4 hours at 37°C **Processed Extract Stability**: At least 3 days at ambient temperature

Validation Report	Nonclinical Studies Supported	Method Description and Performance
Rabbit (Kbl:JW) Plasma (heparin) (Original chiral method – full validation) [redacted], Japan **Title:** Validation of an Analytical Method for Determination of ERC-349572 in Rabbit Plasma by LC/MS/MS Validation Number: (S-349572-TB-048-N) Document Number: RD2009/00442	Rabbit 2-week oral toxicity non-pregnant rabbits SG08062 (S-349572-TF-052-L) Document: RD2008/01760/00 Rabbit oral embryo-fetal development SG08064 (S-349572-TF-065-L) Document: XD2009/0366	Dolutegravir was extracted from 30 μL rabbit plasma by protein precipitation using methanol:acetonitrile (1:1 v/v) with 0.3 vol% phosphoric acid containing [$^{2}H_{7}{}^{15}N$]-dolutegravir as an internal standard. Extracts were separated by chiral HPLC with a Chiralcel OJ-RH column (150mm x 2.0mm; 5μm) and analyzed by MS/MS detection using a Turbo IonSpray interface in the positive ion mode and multiple reaction monitoring (m/z 420>277 and m/z 428>283). **Lower limit of quantification (LLQ)**: 0.5 μg/mL **Validated Range:** 0.5 to 500 μg/mL **QC levels:** 0.5, 15, 500 μg/mL Intra-day Precision (%CV): 0.7 to 6.5% Inter-day Precision (%CV): 3.5 to 4.8% Accuracy (% Bias): -10.6 to 3.4% **Dilution Effects:** 100-fold Within-run Precision (%CV): 2.9%% Accuracy (% Bias): -4.6% **Recovery:** 97.1 to 108% dolutegravir 99.3 internal standard **Stability in Rabbit Plasma**: 3 freeze-thaw cycles from -20°C at least 65 days at -20°C at least 4 hours at ambient temperature **Processed Extract Stability**: At least 48 hours at 10°C

Validation Report	Nonclinical Studies Supported	Method Description and Performance
Monkey (cynomolgus) Plasma (heparin) (Original chiral method – full validation) [redacted], Japan **Title:** Validation of an Analytical Method for Determination of ERC-349572 in Monkey Plasma by LC/MS/MS Validation Number: SG07029 (E-349572-TF-027-N) Document Number: RD2007/01187	Monkey 2-week oral toxicity SG07030 (E-349572-TF-029-L) Document: RD2007/01142	Dolutegravir was extracted from 30 μL monkey plasma by protein precipitation using methanol:acetonitrile (1:1 v/v) containing MTS-0297889 as an internal standard. Extracts were separated by chiral HPLC with a Chiralcel OJ-RH column (150mm x 4.6mm; 5μm) and analyzed by MS/MS detection using a Turbo IonSpray interface in the positive ion mode and multiple reaction monitoring (m/z 420 >277 and m/z 434 > 291). **Lower limit of quantification (LLQ)**: .03 μg/mL **Validated Range:** 0.03 to 30 μg/mL **QC levels:** 0.1, 3, 30 μg/mL Intra-day Precision (%CV): 1.9 to 4.9% Inter-day Precision (%CV): 5.5 to 6.9% Accuracy (% Bias): -5.3 to 6.0% **Dilution Effects:** 100-fold Within-run Precision (%CV): 2.9%% Accuracy (% Bias): -4.6% **Recovery:** 55.9 to 63.0% **Stability in Monkey Plasma**: 3 freeze-thaw cycles from -30°C at least 8 weeks at -30°C at least 4 hours at ambient temperature **Processed Extract Stability**: At least 48 hours at 10°C

Validation Report	Nonclinical Studies Supported	Method Description and Performance
Monkey (cynomolgus) Plasma (Heparin) **(Re-validation (full) of chiral assay method using a stable isotopic lable as the internal standard)** [redacted], Japan **Title:** Validation of an Analytical Method for Determination of ERC-349572 in Monkey Plasma by LC/MS/MS (2) Validation Number: E-349572-TB-035-N Document Number: RD2009/00443	Monkey one month oral toxicity SG07224 (E-349572-TF-036-L) Document: RD2008/00107 Monkey nine month oral toxicity SBL055-074 (S-349572-TF-047-L) Document: RD2009/00036	Dolutegravir was extracted from 30 μL monkey plasma by protein precipitation using methanol:acetonitrile (1:1 v/v) 0.3 vol% phosphoric acid containing [$^{2}H_{7}{}^{15}N$]-dolutegravir as an internal standard. Extracts were separated by chiral HPLC with a Chiralcel OJ-RH column (150mm x 2.0mm; 5μm) and analyzed by MS/MS detection using a Turbo IonSpray interface in the positive ion mode and multiple reaction monitoring (m/z 420>277 and m/z 428>283). **Lower limit of quantification (LLQ)**: 0.01 μg/mL **Validated Range:** 0.01 to 30 μg/mL **QC levels:** 0.01, 0.3, 30 μg/mL Within-run Precision (%CV): 0.7 to 6.4% Between-run Precision (%CV) 2.5 to 6.0% Accuracy (% Bias): -12.2 to -4.3% **Dilution Effects:** 10-fold Within-run Precision (%CV): 1.7%% Accuracy (% Bias): -4.0% **Recovery:** 99.6 to 101% dolutegravir 99.1% internal standard **Stability in Monkey Plasma**: 3 freeze-thaw cycles from -40°C at least 27 days at -40°C at least 6 hours at ambient temperature **Processed Extract Stability:** At least 48 hours at 10°C

APPENDIX 2 ADDITIONAL INFORMATION

Studies summarized in this sectional summary (m2.6.4) comprise those conducted during the formal research and development of dolutegravir. Early screening studies completed on multiple compounds during the candidate selection phase have not been included. However, this information has been reviewed within GlaxoSmithKline (GSK) and the information is considered to have no bearing on safety.

MODULE 2.6.5. PHARMACOKINETICS TABULATED SUMMARY

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1. PHARMACOKINETICS: OVERVIEW FOR DOLUTEGRAVIR

Table 1.1 List of Single and Repeat Dose Pharmacokinetic/Toxicokinetic Studies Performed with Dolutegravir

Type of Study	Species (Strain)/ Test System	No./Sex/ Group	Method of Administration	Form	Dose (mg/kg/day) or Concentration	Duration of Dosing (Sampling Occasions)	GLP	Testing Facility	Report No. (Study No.)	Location in CTD
Single dose										
Pharmacokinetics	Rat (Sprague Dawley)	3M	IV (bolus) Oral (gavage)[a] Oral (capsule)	A A A B	1 5 7.06 to 7.42 7.31 to 8	Single	No	[redacted]	RH2007/00101	m4.2.2.2
Pharmacokinetics	Rat (Sprague Dawley)	2M	Oral (gavage)	A	50, 100, 250, 500, 1000	Single	No	[redacted]	RH2007/00101	m4.2.2.2
Toxicokinetics	Rat (Sprague Dawley)	6M 6M	SC IM	B	2.5	Single	No	GSK	RD2009/00921 (R42470)	m4.2.3.1
Toxicokinetics	Rat (Sprague Dawley)	3M 3M	SC IM	B	2.5, 5	Single	No	GSK	RD2009/00959 (R42475)	m4.2.3.1
Toxicokinetics	Rat (Sprague Dawley)	3M	IM	B	4.0, 7.3, 8.7	Single	No	GSK	2011N123574 (R42826)	m4.2.3.1
Toxicokinetics	Rat (Sprague Dawley)	3M	IM	B	10	Single	No	GSK	2012N136936	m4.2.3.1
Pharmacokinetics	Dog (beagle)	2M	IV (bolus) Oral (gavage)	A A	1 5	Single	No	[redacted]	RH2007/00102	m4.2.2.2
Toxicokinetics	Dog (beagle)	1F	Oral (gavage)	A	30, 100, 150, 250, 500	Single	No	[redacted]	RD2009/00963	m4.2.3.1

Table 1.1 (Continued) List of Single and Repeat Dose Pharmacokinetic/Toxicokinetic Studies Performed with Dolutegravir

Type of Study	Species (Strain)/ Test System	No./Sex/ Group	Method of Administration	Form	Dose (mg/kg/day)	Duration of Dosing (Sampling Occasions)	GLP	Testing Facility	Report No. (Study No.)	Location in CTD
Pharmacokinetics	Dog (beagle)	6M	Oral (gavage)	A	53 mg	Single	No	GSK	2012N137977	m4.2.2.2
Pharmacokinetics	Monkey (cynomolgus)	1 to 2M	IV (bolus) Oral (gavage)	A B A	1 5 5	Single	No	[redacted]	RH2007/00103	m4.2.2.2
Toxicokinetics	Monkey (cynomolgus)	1F	Oral (gavage)	A	50, 125, 250, 500	Single	No	[redacted]	RD2007/01184	m4.2.3.1
Toxicokinetics	Monkey (cynomolgus)	3F	Oral (gavage)	A	1, 3, 10, 50	Single	No	[redacted]	RD2008/01762 (S-349572-TB-44-R)	m4.2.3.1
Toxicokinetics	Monkey (cynomolgus)	4F	Oral SC IM	B A A	3 1 1	Single	No	GSK	CD2009/00647 (D09113)	m4.2.3.1
Repeat Dose										
Toxicokinetics	Mouse (CD-1)	18M/18F	Oral (gavage)	A	10, 100, 500 1500	14 days (Days 1 & 14)	No	[redacted]	RD2009/01546 (S-349572-TF-066-R)	m4.2.3.2
Toxicokinetics	Mouse (CD-1)	54M/54F	Oral (gavage)	A	10, 50, 500, 1500	13 weeks (Days 1, 28 & 85)	Yes	[redacted]	RD2009/00028 (S-349572-TF-068-L)	m4.2.3.2
Toxicokinetics	Mouse (CD-1)	45M/45F	Oral (gavage)	A	7.5, 25, 500	104 weeks	Yes	[redacted]	2012N152419	m4.2.3.4.1
Toxicokinetics	Rat (Sprague Dawley)	4M/4F	Oral (gavage)	A	50, 150, 500	14 days (Days 1 & 14)	Yes	[redacted]	RD2007/01140 (E-349572-TB-012-L)	m4.2.3.2

Table 1.1 (Continued) List of Single and Repeat Dose Pharmacokinetic/Toxicokinetic Studies Performed with Dolutegravir

Type of Study	Species (Strain)/ Test System	No./Sex/ Group	Method of Administration	Form	Dose (mg/kg/day) or Concentration	Duration of Dosing (Sampling Occasions)	GLP	Testing Facility	Report No. (Study No.)	Location in CTD
Toxicokinetics	Rat (Sprague Dawley)	4M/4F	Oral (gavage)	A	2, 10, 100, 1000	4 weeks (Days 1, 14 & 29)	Yes	[redacted]	RD2008/01628 (E-34572-TB-043-L)	m4.2.3.2
Toxicokinetics	Rat (Sprague Dawley)	6M/6F	Oral (gavage)	A	5, 50, 500	26 weeks (Days 1, 30, 120 & 180)	Yes	[redacted]	RD2009/00410 (S-349572-TF-055-L)	m4.2.3.2
Toxicokinetics	Rat (Sprague Dawley)	12M/12F	Oral (gavage)	A	2, 10, 50	104 weeks	Yes	[redacted]	2012N152418	m4.2.3.4.1
Toxicokinetics	Rat (pregnant) (Sprague Dawley)	5F	Oral (gavage)	A	100, 300, 1000	12 days (Days 1 & 12)	Yes	[redacted]	XD2009/00367 (S-349572-TB-062-L)	m4.2.3.5.2
Toxicokinetics	Rat (juvenile) (Sprague Dawley)	4M/4F	Oral (gavage)	A	5, 50, 100, 500, 1000	18 days (Days 4 to 21 pp) (Day 21 pp)	No	GSK	CD2009/00409 (D09072)	m4.2.3.5.4
Toxicokinetics	Rat (juvenile) (Sprague Dawley)	12M/12F	Oral (gavage)	A	2, 25, 75, 300	28 days (Days 4 to 31 pp) (Days 13 and 32 pp)	No	GSK	CD2009/00770 (D09126)	m4.2.3.5.4
Toxicokinetics	Rat (juvenile) (Sprague Dawley)	30M/30F	Oral (gavage)	A	0.5, 2, 75	63 days (Days 4 to 66 pp) (Days 13 and 32 pp)	Yes	GSK	CD2010/00023 (G09229)	m4.2.3.5.4

Table 1.1 (Continued) List of Single and Repeat Dose Pharmacokinetic/Toxicokinetic Studies Performed with Dolutegravir

Type of Study	Species (Strain)/ Test System	No./Sex/ Group	Method of Administration	Form	Dose (mg/kg/day) or Concentration	Duration of Dosing (Sampling Occasions)	GLP	Testing Facility	Report No. (Study No.)	Location in CTD
Toxicokinetics	Rabbit (non-pregnant) (Japanese white)	3F	Oral (gavage)	A	30, 100, 300, 1000	14 days (Days 1 & 14)	Yes	[redacted]	RD2008/01760 (S-349572-TF-052-L)	m4.2.3.5.2
Toxicokinetics	Rabbit (pregnant) (Japanese white)	5F	Oral (gavage)	A	40, 200, 1000	13 days (Days 1 & 13)	Yes	[redacted]	XD2009/00366 (S-349572-TF-065-L)	m4.2.3.5.2
Toxicokinetics	Monkey (cynomolgus)	3M/3F	Oral (gavage)	A	100, 300, 1000	14 days (Days 1, 7 & 14)	Yes	[redacted]	RD2007/01142 (E-349572-TB-012-L)	m4.2.3.2
Toxicokinetics	Monkey (cynomolgus)	3M/3F[b]	Oral (gavage)	A	25, 50, 100	4 weeks (Days 1, 15 & 30)	Yes	[redacted]	RD2008/00107 (E-349572-TF-036-L)	m4.2.3.2
Toxicokinetics	Monkey (cynomolgus)	4 to 6 (for 38 weeks)	Oral (gavage)	A	3, 10, 15, 50/30[c]	38 weeks (Days 1, 30, 69, 120, 180 & 270)	Yes	[redacted]	RD2009/00036 (S-349572-TF-047-L)	m4.2.3.2

Key:
a = Administered as a solution and a suspension.
b = An additional 2 animals/sex added to the 100 mg/kg/day dose and used for TK evaluation.
c = From Day 70 the dose of 50 mg/kg/day was reduced to 30 mg/kg/day because of 2 male deaths.
A = GSK1349572A, the sodium salt form.
B = GSK1349572B, the parent form.
pp = Post partum.

Testing Facility:
GSK = GlaxoSmithKline.
[redacted] = [redacted]
[redacted] = [redacted]
[redacted] = [redacted]
[redacted] = [redacted]

Table 1.2 Listing of Distribution Studies with Dolutegravir

Type of Study	Species (Strain)/ Test System	No./Sex/ Group	Method of Administration	Form	Dose (mg/kg/day) or Concentration	Duration of Dosing (Sampling Occasions)	GLP	Testing Facility	Report No. (Study No.)	Location in CTD
Plasma protein binding	Rat, Dog, Monkey, Human	NA	In vitro	B	10 μM	NA	No	[redacted]	RH2007/00106	m4.2.2.3
Plasma protein binding	Human	NA	In vitro	B	0.5 to 50 μM (0.21 to 21 μg/mL)	NA	No	GSK	2010N104947 (10DMR021)	m4.2.2.3
Plasma protein binding	Human	NA	In vitro	A	0.5 to 25 μM	NA	No	GSK	2011N119355	m4.2.2.3
Membrane permeability and transport by Pgp	Human (transfected MDCK II cells)	NA	In vitro	B C C	3 μM	NA	No	GSK	RH2007/00067 RD2008/00360 RD2008/00361	m4.2.2.3
Inhibition of digoxin transport by Pgp	Human (transfected MDCK II cells)	NA	In vitro	A	0.3 to 100 μM	NA	No	GSK	RD2008/00292 (08DMR021)	m4.2.2.3
OATP1B1 and OATP1B3 transport	Human (transfected CHO or MSRII cells)	NA	In vitro	A	0.1 to 100 μM	NA	No	GSK	RD2008/00216 (08DMR016)	m4.2.2.3
BCRP transport	Human (transfected MDCK II cells)	NA	In vitro	D	3 μM	NA	No	GSK	2011N112380 (11DMR004)	m4.2.2.3

Table 1.2 (Continued) List of Distribution Studies Performed with Dolutegravir

Type of Study	Species (Strain)/ Test System	No./Sex/ Group	Method of Administration	Form	Dose (mg/kg/day) or Concentration	Duration of Dosing (Sampling Occasions)	GLP	Testing Facility	Report No. (Study No.)	Location in CTD
Inhibition of OCT2-mediated transport	Human	NA	In vitro	A	25 μM	NA	No	[redacted]	RD2010/00555 (OPT-2010-104)	m4.2.2.3
Inhibition of OCT2	Human	NA	In vitro	A	0.1 to 30 μM	NA	No	[redacted]	2010N104937 (OPT-2010-119)	m4.2.2.3
Inhibition of OCT1	Human	NA	In vitro	A	10 μM	NA	No	[redacted]	2012N132572	m4.2.2.3
Inhibition of BCRP	Human	NA	In vitro	A	0.3 to 100 μM	NA	No	GSK	2010N110625 (10DMR033)	m4.2.2.3
Inhibition of MRP2	Human	NA	In vitro	A	0.1 to 100 μM	NA	No	GSK	2010N109746 (10DMR031)	m4.2.2.3
Inhibition of MRP2	Human	NA	In vitro	*	0.1 to 100 μM	NA	No	GSK	2011N120047	m4.2.2.3
Quantitative whole body auto-radiography	Rat (Lister Hooded)	7M	Oral (gavage)	D	50	Single	Yes	[redacted]	CD2008/00195 (2990/253)	m4.2.2.3
Blood to plasma ratio	Mouse (CD-1)	30M/ 30F	Oral (gavage)	D	100	Single	No	GSK	RD2009/00562 (09DMR021)	m4.2.2.5
Blood to plasma ratio	Rat (Sprague Dawley)	9M/9F	Oral (gavage)	D	50	Single	No	GSK	RD2008/00108 (08MDR008)	m4.2.2.5

Table 1.2 (Continued) List of Distribution Studies Performed with Dolutegravir

Type of Study	Species (Strain)/ Test System	No./Sex/ Group	Method of Administration	Form	Dose (mg/kg/day) or Concentration	Duration of Dosing (Sampling Occasions)	GLP	Testing Facility	Report No. (Study No.)	Location in CTD
Blood to liver concentrations	Mouse (CD-1)	30M/ 30F	Oral (gavage)	D	100	Single	No	GSK	RD2009/00562 (09DMR021)	m4.2.2.5
Blood to liver concentrations	Rat (Sprague Dawley)	9M/9F	Oral (gavage)	D	50	Single	No	GSK	RD2008/00108 (08MDR008)	m4.2.2.5
Placental transfer Lacteal secretion	Rat (Sprague Dawley)	5F 12F	Oral (gavage)	D	50	Single	No	GSK	2012N137348	m4.2.2.3
Blood to plasma ratio	Monkey (cynomolgus)	3M/3F 2M BDC	Oral (gavage)	D	10	Single	Yes	[redacted] [redacted]	RD2008/01299 (650-098) RD2008/01300 (7717-703)	m4.2.2.5

Key:
A = GSK1349572A, the sodium salt form. B = GSK1349572B, the parent form.
C = ^{14}C-labelled GSK1349572B. D = ^{14}C-labelled GSK1349572A.
NA = Not applicable.
BCRP = Breast cancer resistance protein.
OCT2 = Organic cation transporter 2.
Pgp = P-glycoprotein
* The test material for this study was GSK2832500 (M3: a GSK1349572 glucuronide metabolite)

Testing Facility:
[redacted] = [redacted]
GSK = GlaxoSmithKline.
[redacted] = [redacted]
[redacted] = [redacted]
[redacted] = [redacted]
[redacted] = [redacted]

Table 1.3 Listing of Metabolism Studies with Dolutegravir

Type of Study	Species (Strain)/ Test System	No./Sex/ Group	Method of Administration	Form	Dose (mg/kg/day) or Concentration	Duration of Dosing (Sampling Occasions)	GLP	Testing Facility	Report No. (Study No.)	Location in CTD
Metabolic stability in liver S9	Rat, Dog, Monkey, Human	NA	In vitro	B	1 μM	NA	No	GSK	RH2007/00076 (07APK019)	m4.2.2.4
Bioactivation in liver microsomes	Rat, Monkey, Human	NA	In vitro	C	10 μM	NA	No	GSK	RD2007/01557 (07DMR124)	m4.2.2.4
Metabolic stability in hepatocytes	Rat, Human	NA	In vitro	A	0.5 μM	NA	No	GSK	RH2007/00076 (07APK019)	m4.2.2.4
Metabolism in hepatocytes	Rat, Dog, Monkey, Human	NA	In vitro	B	10 μM	NA	No	GSK	RH2007/00060 (07APK014)	m4.2.2.4
Metabolism in hepatocytes	Rat, Monkey, Human	NA	In vitro	C	50 μM	NA	No	GSK	RD2007/01496 (07DMR121)	m4.2.2.4
Metabolism in Supersomes and PHLM	Human	NA	In vitro	D	5 μM	NA	No	GSK	RD2008/00373 (08DMR033)	m4.2.2.4
Potential metabolic formation of stereoisomers	Rat, Dog, Monkey, Human	NA	In vitro	B	10 μM	NA	No	GSK	RH2007/00105 (07RCD8654)	m4.2.2.4

Table 1.3 (Continued) List of Metabolism Studies Performed with Dolutegravir

Type of Study	Species (Strain)/ Test System	No./Sex/ Group	Method of Administration	Form	Dose (mg/kg/day) or Concentration	Duration of Dosing (Sampling Occasions)	GLP	Testing Facility	Report No. (Study No.)	Location in CTD
Formation of glucuronide metabolite	Human	NA	In vitro	A	0.5, 5, 50 μM	NA	No	GSK	RH2007/00104 (07APK024)	m4.2.2.4
Formation of glutathione adducts in microsomes	Rat, Human	NA	In vitro	B	100 μM	NA	No	GSK	RH2007/00058 (06RCM8059)	m4.2.2.4
UDP-glucuronosyltrans-ferase enzymology	Human	NA	In vitro	C	16 μM	NA	No	GSK	RD2008/01339 (08DMR067)	m4.2.2.4
Inhibition of UDP-glucuronosyl-transferase 1A1 and 2B7	Human	NA	In vitro	A	0.1 to 100 μM	NA	No	GSK	RD2009/00862 (09DMR031)	m4.2.2.4
CYP induction in hepatocytes	Human	NA	In vitro	B	1 to 40 μM	NA	No	GSK	RH2007/00074 (07APK018)	m4.2.2.4
PXR activation assays	Rat Human	NA	In vitro	B	0.2 to 10 μM	NA	No	GSK	RR2007/00024 (Rat) RR2007/00025 (Human)	m4.2.2.4
Metabolite identification in IPRL	Rat	1M	Ex-vivo	C	15[a]	NA	No	GSK	RD2007/01493 (07DMR120)	m4.2.2.4

Table 1.3 (Continued) List of Metabolism Studies Performed with Dolutegravir

Type of Study	Species (Strain)/ Test System	No./Sex/ Group	Method of Administration	Form	Dose (mg/kg/day) or Concentration	Duration of Dosing (Sampling Occasions)	GLP	Testing Facility	Report No. (Study No.)	Location in CTD
CYP inhibition in recombinant enzymes and liver microsomes	Human	NA	In vitro	B	0.033 to 33 μM	NA	No	GSK	RH2007/00077 (07APK020)	m4.2.2.4
Direct and metabolism-dependent CYP inhibition	Human	NA	In vitro	A	Up to 100 μM	NA	No	GSK	WD2010/00908 (10DMW013)	m4.2.2.4
Metabolism in vivo	Mouse (CD-1)	30M/30F 4M BDC	Oral (gavage)	D	100	Single	No	GSK	RD2009/00723 (09DMR028)	m4.2.2.4
Metabolism in vivo	Rat (Sprague Dawley)	3M/3F 3M BDC	Oral (gavage)	D	50	Single	No	GSK	RD2008/00220 (08DMR017)	m4.2.2.4
Potential for epimerization in juvenile plasma	Rat (Sprague Dawley)	2M/2F	Oral	A	2, 25, 75, 300	10 days	No	GSK	RD2010/00173 (10DMR005)	m4.2.2.4
Metabolites in rat milk	Rat (Sprague Dawley)	3F	Oral (gavage)	D	50	Single	No	GSK	2012N132387	m4.2.2.4
Effect on hepatic drug metabolizing enzymes	Rat	6M/6F	In vivo	A	50 150, 500	14 days	Yes	[redacted]	RD2007/01034	m4.2.2.4

Table 1.3 (Continued) List of Metabolism Studies Performed with Dolutegravir

Type of Study	Species (Strain)/ Test System	No./Sex/ Group	Method of Administration	Form	Dose (mg/kg/day) or Concentration	Duration of Dosing (Sampling Occasions)	GLP	Testing Facility	Report No. (Study No.)	Location in CTD
Metabolism in vivo	Monkey (cynomolgus)	3M/3F 2M BDC	Oral (gavage)	D	10	Single	No	GSK	RD2008/00899 (08DMR054)	m4.2.2.4
Effect on hepatic drug metabolizing enzymes	Monkey (cynomolgus)	3M/ 2 to 3F	In vivo	A	100, 300, 1000	14 days	Yes	[redacted]	RD2007/01142	m4.2.3.2

Key:
a = Equivalent dose compared to body weight of the animal whose liver was used in testing.
A = GSK1349572A, the sodium salt form. B = GSK1349572B, the parent form.
C = ^{14}C-labelled GSK1349572B. D = ^{14}C-labelled GSK1349572A.
IRPL= Isolated perfused rat liver.
NA = Not applicable.
PXR = Pregnane X receptor.

Testing Facility:
GSK = GlaxoSmithKline.
[redacted] = [redacted]

Table 1.4 Listing of Excretion Studies with Dolutegravir

Type of Study	Species (Strain)/ Test System	No./Sex/ Group	Method of Administration	Dose (mg/kg/day)	Duration of Dosing (Sampling Occasions)	GLP	Testing Facility	Report No. (Study No.)	Location in CTD
Elimination	Mouse (CD-1)	4M/4F 4M BDC	Oral (gavage)	100	Single	No	GSK	RD2009/00562 (09DMR021)	m4.2.2.5
Elimination	Rat (Sprague Dawley)	3M/3F 3M BDC	Oral (gavage)	50	Single	No	GSK	RD2008/00108 (08MDR008)	m4.2.2.5
Elimination	Monkey (cynomolgus)	3M/3F 2M BDC	Oral (gavage)	10	Single	Yes	[redacted] [redacted]	RD2008/01299 (650-098) RD2008/01300 (7717-703)	m4.2.2.5

Key:
^{14}C-labelled dolutegravir was used in these studies.
NA = Not applicable.

Testing Facility:
[redacted] = [redacted]
GSK = GlaxoSmithKline.
[redacted] = [redacted]

2. ANALYTICAL METHODS AND VALIDATION REPORTS

Table 2.1 Pharmacokinetics: Analytical Methods and Validation Reports

Type of Study	Species	Quantification Limits	Report No. (Method Reference)	Location in CTD
Validation of an analytical method for determination of S-349572 in mouse plasma by LC/MS/MS.	Mouse	0.5 to 500 μg/mL	2012N142266	m4.2.2.1
Method for the determination of GSK1349572 in rat plasma by LC/MS/MS.	Rat	30 to 30000 ng/mL	RD2007/01185 (E-349572-TF-010-N)	m4.2.2.1
Method for the determination of GSK1349572 in rat plasma by LC/MS/MS.	Rat	500 to 500000 ng/mL	RD2009/00444 (S-349572-TB-041-N)	m4.2.2.1
Validation of a method for the determination of GSK1349572 in rat plasma using HPLC-MS/MS.	Rat	250 to 100000 ng/mL	RD2010/00176 (GSK1349572RTPLVALC)	m4.2.2.1
Method for the determination of GSK1349572 in rabbit plasma by LC/MS/MS.	Rabbit	500 to 500000 ng/mL	RD2009/00442 (S-349572-TB-048-N)	m4.2.2.1
Method for the determination of GSK1349572 in monkey plasma by LC/MS/MS.	Monkey	30 to 30000 ng/mL	RD2007/01187 (E-349572-TB-027-N)	m4.2.2.1
Method for the determination of GSK1349572 in monkey plasma by LC/MS/MS.	Monkey	10 to 30000 ng/mL	RD2009/00443 (E-349572-TB-035-N)	m4.2.2.1

3. PHARMACOKINETICS: ABSORPTION AFTER A SINGLE DOSE

Table 3.1 Plasma Pharmacokinetic Parameters for Dolutegravir Following Intravenous Administration

Test Article: Dolutegravir	**Location in CTD:** m4.2.2.2		
Species (Strain):	**Rat (Sprague Dawley)**	**Dog (beagle)**	**Monkey (cynomolgus)**
Report No.	RH2007/00101	RH2007/00102	RH2007/00103
Gender (M/F)/Number of Animals:	M/3	M/2	M/2
Feeding Condition:	Non-fasted	Non-fasted	Non-fasted
Vehicle/Formulation:[a]	a	a	a
Method of Administration:	Intravenous	Intravenous	Intravenous
Dose (mg/kg):	1	1	1
Sample:	Plasma	Plasma	Plasma
Analyte:	GSK1349572	GSK1349572	GSK1349572
Assay:	HPLC/MS/MS	HPLC/MS/MS	HPLC/MS/MS
PK Parameters:			
V_{ss} (L/kg)	0.10	0.35	0.28
CLp (mL/min/kg)	0.23	2.2	2.1
$t_{½}$ (h)	6.2	5.2	6.0
$AUC_{0-\infty}$ (µg.h/mL)	74.3	7.72	7.91

Additional Information:
a = Dolutegravir was dissolved in N, N-dimethylacetamide and then diluted with 50 mM N-methylglucamine in 3% Mannitol.
CLp = Plasma clearance.
$t_{½}$ = Half-life.
V_{ss} = Steady state volume of distribution.

Table 3.2 Estimates of the Bioavailability in Male Rats and Monkeys After Dolutegravir is Administered as a Single Oral Dose in a Solution or a Suspension Formulation

Test Article: Dolutegravir

Location in CTD: m4.2.2.2

Analyte: GSK1349572
Assay: HPLC/MS/MS
Sample: Plasma
Vehicle/Formulation:[a]

Rat (Male, n=3) Report RH2007/00101			Monkey (Male) Report RH2007/00103		
Dose (mg/kg/day)	**Formulation (Fasting State)**	**%F**	**Dose (mg/kg)**	**Formulation (Fasting State)**	**%F**
5	Solution (fasted)	75.6	5	Solution (fasted; n=1)	87.0
5	Suspension (fasted)	51.5	5	Suspension (non-fasted; n=2)	24.9
5	Suspension (non-fasted)	34.2			

Additional Information:
a = Solutions of dolutegravir were formulated in DMSO, Solutol and 50 mM N-methylglucamine in 3% Mannitol (1/1/8 v/w/v). Suspensions of dolutegravir were formulated in 0.5% HPMC and 0.1% Tween 80.
%F = Oral bioavailability.

Table 3.3 Exposure of Monkeys to Single Oral Escalating Doses of Dolutegravir

Test Article: Dolutegravir

Location in CTD: m4.2.2.2

Analyte: GSK1349572
Assay: HPLC/MS/MS
Sample: Plasma
Vehicle/Formulation: 0.5% HPMC with 0.1% Tween 80

Species:	**Monkey (female, n=1)**				**Monkey (female, n=3)**			
Report No.:	RD2007/01184				RD2008/01762			
Feeding Condition:	Fasted				Fasted until 8 hours post dose			
Sample Collection Intervals (Hour):	0.5, 1, 2, 4, 8, 24				0.5, 1, 2, 4, 8, 24			
Dose Level (mg/kg):	**50**	**125**	**250**	**500**	**1**	**3**	**10**	**50**
PK Parameters								
AUC_{0-24} (μg.h/mL)	116	182	136	262	5.16	16.20	45.73	80.34
C_{max} (μg/mL)	15.0	24.8	14.9	25.3	1.01	2.97	6.79	9.33
T_{max} (h)	2	4	4	2	1.3	2.2	2.3	5.3

Additional Information:
Data presented are mean values.

Table 3.4a Plasma Pharmacokinetic Parameters for Dolutegravir Following Single Intramuscular or Subcutaneous Administration to Rats

Test Article: Dolutegravir
Species (Strain): Rat (Sprague Dawley) **Location in CTD:** m4.2.3.1

Report No.:	RD2009/00921		RD2009/00959			2011N123574		
Gender (M/F)/Number of Animals:	M/6		M/3			M/3		
Feeding Condition:	Non-fasted		Non-fasted			Non-fasted		
Vehicle/Formulation:	[a]		[a, b]		[d]	[e]	[f]	
Analyte:	GSK1349572		GSK1349572			GSK1349572		
Assay:	HPLC/MS/MS		HPLC/MS/MS			HPLC/MS/MS		
Sample:	Plasma		Plasma			Plasma		
Sample Collection Intervals (Day):	1, 2, 3, 4, 6, 8, 15		1[c], 2, 3, 4, 6, 8, 15, 22, 29, 36, 43			1[c], 2, 3, 4, 6, 8, 15, 22, 29, 36, 43		
Method of Administration:	Intramuscular	Subcutaneous	Intramuscular		Subcutaneous	Intramuscular (3 different formulations)		
Dose (mg/kg):	2.5	2.5	2.5	5	2.5	5	4	7.3	8.7
PK Parameters:									
AUC_{0-t} (μg.h/mL)	196	242	174	400	182	581	193[g]	364[g]	726[g]
AUC_{0-24} (μg.h/mL)	104	91.8	41.4	55.2	43.7	73.5	193[h]	199[h]	334[h]
C_{max} (μg/mL)	6.35	4.61	2.34	2.97	2.34	3.69	1.34[h]	0.94[h]	4.32[h]
Median T_{max} (h)	2.0	8.0	2.0	4.0	8.0	8.0	24	336	12

Additional Information:
a = 2% pluronic F127 (w/w), 0.2% Polysorbate 80 (w/v), 0.18% methylparaben (w/v), 0.02% propylparaben (w/v), 0.004M NaH_2PO_4 H_2O, 0.006M Na_2HPO_4 with NaCl.
b = Larger particle size of GSK1349572 compared to that used in Study RD2009/00921.
c = Samples collected at multiple times on Day 1.
d = 20 mg/mL Polysorbate 20, 20 mg/mL polyethylene glycol 3350, 45 mg/mL Mannitol.
e = 20 mg/mL Polysorbate 20, 20 mg/mL polyethylene glycol 3350, 45 mg/mL Mannitol, 10 mg/mL carboxymethylcellulose.
f = Sesame oil.
g = $AUC_{0-\infty}$ (μg.h/mL).
h = $AUC_{0-\infty}$ and C_{max} values normalized to 4.0 mg/kg dose.

Table 3.4b Plasma Pharmacokinetic Parameters for Dolutegravir Following Single Intramuscular Administration to Rats

Test Article: Dolutegravir
Species (Strain): Rat (Sprague Dawley) **Location in CTD:** m4.2.3.1

Report No. (Study Number):	2012N136936 (R42920)					
Gender (M/F)/Number of Animals:	M/3					
Feeding Condition:	Non-fasted					
Analyte:	GSK1349572					
Assay:	HPLC/MS/MS					
Sample:	Plasma					
Sample Collection Intervals (Day):	1, 2, 3, 4, 6, 8, 15, 22, 29, 36, 43					
Method of Administration:	Intramuscular					
Dose (mg/kg):	10					
Vehicle/Formulation:	a	b	c	d	e	f
PK Parameters:						
AUC_{0-t} (μg.h/mL)	69.7	532	482	828	825	797
C_{max} (μg/mL)	0.876	1.86	3.03	8.19	5.79	3.04
Median T_{max} (h)	24	12	24	24	24	24

Additional Information:
a = Microparticles (low drug loading of 40%), 1 mg/mL Polysorbate 20, 30 mg/mL Mannitol and 10 mg/mL sodium carboxymethylcellulose (CMC) in sterile water.
b = Microparticles (high drug loading of 70%), 1 mg/mL Polysorbate 20, 30 mg/mL Mannitol and 10 mg/mL CMC in sterile water.
c = Microparticles (low drug loading of 40% and micronized free acid), 1 mg/mL Polysorbate 20, 30 mg/mL Mannitol and 10 mg/mL CMC in sterile water.
d = In situ gel (fast release), 20% 75/25 poly(lactic-co-glycolic acid) (PLGA), 80% N-methylpyrrolidone (NMP).
e = In situ gel (slow release), 33% 75/25 PLGA, 66.7% NMP.
f = Micronized free acid suspension, 20 mg/mL Polysorbate 20, 20 mg/mL polyethylene glycol 3350, 45 mg/mL Mannitol in sterile water.

Table 3.5 Plasma Pharmacokinetic Parameters for Dolutegravir Following Single Oral, Intramuscular or Subcutaneous Administration to Monkeys

Test Article: Dolutegravir
Report No.: CD2009/00647 **Location in CTD:** m4.2.3.1
Species (Strain): Monkey (cynomolgus)

Gender (M/F)/Number of Animals:	F/4	F/4	
Feeding Condition:	Fasted (fed within 4 h post dose)	Fasted (fed within 1 h post dose)	
Vehicle/Formulation:	[a]	[b]	
Analyte:	GSK1349572	GSK1349572	
Assay:	HPLC/MS/MS	HPLC/MS/MS	
Sample:	Plasma	Plasma	
Day Samples Collected Post Dose:	0.5, 1, 2, 4, 8, 10, 24	0.5, 1, 2, 4, 8, 10, 24, 48, 72, 120, 168, 336	
Method of Administration:	Oral	Intramuscular	Subcutaneous[c]
Dose (mg/kg):	3	1	1
PK Parameters:			
AUC_{0-t} (μg.h/mL)	13.3	7.01	5.82
AUC_{0-24} (μg.h/mL)	13.3	1.85	1.76
C_{max} (μg/mL)	2.83	0.20	0.15
Median T_{max} (h)	1.5	2.0	4.0

Additional Information:
a = 0.5% HPMC with 0.1% Tween 80.
b = 2% pluronic F127 (w/w), 0.2% Polysorbate 80 (w/v), 0.18% methylparaben (w/v), 0.02% propylparaben (w/v), 0.004M NaH_2PO_4 H_2O, 0.006M Na_2HPO_4 with NaCl.
c = One animal administered dolutegravir subcutaneously was not included in the calculation of the mean parameter values because all concentrations at all time points were not quantifiable.

4. PHARMACOKINETICS: ABSORPTION AFTER REPEATED DOSES

Table 4.1 Absorption after Repeated Doses

The pharmacokinetic parameters determined following repeated administration during the toxicology studies are summarized in m2.6.7, Table 3, Overview of Toxicokinetic Data, and in the individual report Tables in m2.6.7.

Table 4.2 Estimates of the Bioavailability in Male Rats and Monkeys After Dolutegravir is Administered as Repeat Oral Doses in a Suspension Formulation

Test Article: Dolutegravir

Location in CTD: m4.2.3.2

Analyte: GSK1349572
Assay: HPLC/MS/MS

Rat (Male)			Monkey (Male)		
Report Number Dose (mg/kg/day)	**Formulation (Fasting State)**	**%F**	**Report Number Dose (mg/kg/day)**	**Formulation (Fasting State)**	**%F**
Report RD2009/00410 5 (26 week study)[1]	Suspension (non-fasted)	31.2	Report 2009/00036 10 (38 week study)[1]	Suspension (fasted)	40.8
Report RD2008/01628 10 (4 week study)[1]	Suspension (non-fasted)	36.9	Report 2009/00036 15 (NOAEL; 38 week study)[1]	Suspension (fasted)	30.9
Report RD2009/00410 50 (NOAEL; 26 week study)[1]	Suspension (non-fasted)	16.3	Report RD2008/00107 50 (NOAEL; 4 week study)[1]	Suspension (fasted)	28.1
Report RD2008/01628 100 (NOAEL; 4 week study)[1]	Suspension (non-fasted)	9.72			

Additional Information:
1 = Oral exposure at the end of each study.
%F = Oral bioavailability [Calculated by using the intravenous data in Reports RH2007/00101 (rat) and RH2007/00103 (monkey)].
Suspensions of dolutegravir were formulated in 0.5% HPMC and 0.1% Tween 80 aqueous solution.

5. PHARMACOKINETICS: ORGAN DISTRIBUTION

Table 5.1 Organ Distribution

Test Article: Dolutegravir sodium

Location in CTD: m4.2.2.3
Report No.: CD2008/00195 **Study No.:** 2990/253

Species (Strain): Rat (Lister Hooded) - partially pigmented
Gender (M/F)/Number of Animals: Male
Feeding Condition: Fasted
Vehicle/Formulation: 0.5% HPMC with 0.1% Tween 80
Method of Administration: Oral gavage
Dose (mg/kg): 50
Radionuclide: ^{14}C
Specific Activity: 4.27 μCi/mg
Sampling Times (h): 2, 4, 6 and 10 hours and Days 1, 7 and 28

Concentrations of Test Substance-Related Radioactivity in the Tissues of Male Partially Pigmented Rats After a Single Oral Administration of [^{14}C]-Dolutegravir at 50 mg/kg (μg equivalents of dolutegravir/g of tissue)

Tissue Type	Tissue	Animal Number and Sex / Sampling Time	60M 2 Hours	67M 4 Hours	62M 6 Hours	68M 10 Hours	63M 1 Day	65M 7 Days	66M 28 Days
Vascular/ Lymphatic	Blood (cardiac)		23.2	20.4	26.2	19.7	3.80	0.146	BLQ
	Aorta		9.22	7.12	14.1	10.2	0.734	BLQ	BLQ
	Bone marrow		5.45	4.82	4.99	4.19	0.728	BLQ	BLQ
	Mandibular lymph nodes		6.91	2.56	8.23	5.43	0.854	BLQ	BLQ
	Spleen		3.96	2.87	3.49	2.57	0.390	BLQ	BLQ
	Thymus		2.96	1.83	2.16	2.34	0.341	BLQ	BLQ
Metabolic/ Excretory	Liver		13.7	11.3	15.3	9.35	1.60	0.113	BLQ
	Renal cortex		7.49	7.72	9.39	7.33	1.20	0.106	BLQ
	Renal medulla		8.89	9.30	12.9	9.73	1.49	0.116	BLQ

Table 5.1 (Continued) Organ Distribution

		µg equivalents of dolutegravir/g of tissue						
	Animal Number and Sex	60M	67M	62M	68M	63M	65M	66M
Tissue Type	Tissue Sampling Time	2 Hours	4 Hours	6 Hours	10 Hours	1 Day	7 Days	28 Days
Central Nervous System	Brain	0.506	0.446	0.458	0.380	BLQ	BLQ	BLQ
	Choroid plexus	2.63	3.41	5.82	4.13	BLQ	BLQ	BLQ
	Meninges	4.18	4.23	4.51	3.40	1.30	BLQ	BLQ
	Pineal body	NS	6.72	7.09	6.80	0.810	BLQ	BLQ
Endocrine	Adrenal cortex	6.11	4.65	8.58	4.84	0.867	BLQ	BLQ
	Adrenal medulla	9.47	7.32	14.4	8.94	1.33	BLQ	BLQ
	Pituitary	5.62	5.03	6.87	5.13	0.97	BLQ	BLQ
	Thyroid	6.92	4.96	7.80	5.31	1.30	BLQ	BLQ
Secretory	Exorbital lachrymal gland	4.92	2.96	5.10	3.66	0.585	BLQ	BLQ
	Harderian gland	5.30	3.16	6.86	2.72	0.357	BLQ	BLQ
	Intra-orbital lachrymal gland	4.84	6.20	6.03	4.10	0.829	BLQ	BLQ
	Pancreas	5.09	4.25	6.65	4.42	0.696	BLQ	BLQ
	Salivary glands	7.43	4.94	6.58	4.89	0.715	BLQ	BLQ
Fatty	Brown fat	5.13	2.65	7.31	3.53	0.753	BLQ	BLQ
	White fat	3.46	1.89	1.43	1.02	0.239	BLQ	BLQ
Reproductive	Bulbo-urethral gland	NS	NS	19.3	10.3	NS	BLQ	BLQ
	Epididymis	2.66	4.39	5.54	4.75	0.842	BLQ	BLQ
	Preputial gland	4.88	2.86	4.56	2.64	0.377	BLQ	BLQ
	Prostate	4.26	3.53	3.77	5.51	0.548	BLQ	BLQ
	Seminal vesicles	2.13	0.639	0.772	3.82	0.165	BLQ	BLQ
	Testis	3.00	2.94	3.90	3.73	0.504	BLQ	BLQ
Muscular	Muscle (skeletal)	2.24	1.87	2.20	1.86	0.261	BLQ	BLQ
	Myocardium (heart)	10.9	6.49	10.5	7.84	1.17	BLQ	BLQ
Dermal	Non-pigmented skin	3.24	3.83	5.90	4.49	0.873	0.246	BLQ
	Pigmented skin	5.65	3.87	12.1	7.64	1.43	5.17	0.105

Table 5.1 (Continued) Organ Distribution

		µg equivalents of dolutegravir/g of tissue						
	Animal Number and Sex	60M	67M	62M	68M	63M	65M	66M
Tissue Type	Tissue Sampling Time	2 Hours	4 Hours	6 Hours	10 Hours	1 Day	7 Days	28 Days
Ocular	Lens of the eye	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
	Uveal tract	6.10	8.65	8.58	7.71	1.70	BLQ	BLQ
Respiratory	Lung	21.9	17.7	23.1	15.7	2.42	0.132	BLQ
Tract	Nasal mucosa	5.60	2.62	2.46	2.77	0.511	0.136	BLQ
Skeletal	Bone[1]	1.13	1.72	0.816	1.19	0.527	0.221	0.223
Alimentary	Oesophagus	23.8	6.99	15.4	NS	1.49	BLQ	BLQ
Canal	Stomach mucosa	18.4	26.5	7.44	6.54	1.07	BLQ	BLQ
	Small intestine contents	758	928	241	28.7	4.64	0.492	BLQ
	Small intestine mucosa	33.2	36.3	23.9	9.76	1.45	0.258	BLQ
	Caecum mucosa	4.00	18.8	22.7	9.75	1.13	BLQ	BLQ
	Large intestine contents	BLQ	35.1	646	388	15.4	2.61	BLQ
	Large intestine mucosa	11.9	100	21.6	46.5	3.08	0.202	BLQ
	Rectum mucosa	4.13	11.2	5.08	4.88	1.94	BLQ	BLQ
Upper Limit of Quantification	866	for all measurements						
Lower Limit of Quantification	0.100	for all measurements						

Additional Information:
1 = Concentration should be considered the minimum present in the tissue.
BLQ = Tissue radioactivity concentration below the lower limit of quantification.
NS = Tissue not sectioned.

6. PHARMACOKINETICS: PLASMA PROTEIN BINDING

Table 6.1 Protein Binding

Test Article: Dolutegravir

Study System: In vitro
Method: Equilibrium dialysis followed by LC/MS/MS analysis

Species	Test System	Conc. Tested (μM)	% Bound	Report No. (Study No.)	Location in CTD
Rat (n=2)	Pooled serum	10	99.9	RH2007/00106 (07DMPK-ERC-349572-004)	m4.2.2.3
Dog (n=3)	Pooled serum	10	95.4	RH2007/00106 (07DMPK-ERC-349572-004)	m4.2.2.3
Monkey (n=5)	Pooled serum	10	99.1	RH2007/00106 (07DMPK-ERC-349572-004)	m4.2.2.3
Human (n=5)	Pooled serum	10	99.3	RH2007/00106 (07DMPK-ERC-349572-004)	m4.2.2.3
Human (n=1)[a]	Plasma	0.5 to 50	91.5 to 93.8	2010N104947 (10DMR021)	m4.2.2.3
Human (n=5)	Plasma (pooled fresh and frozen)	0.5, 5 and 25	99.3[b]	2011N119355 (11DMR030)	m4.2.2.3

Additional Information:
Stock solutions of dolutegravir were prepared in DMSO for studies in serum and solutions were prepared in 1:1 water:acetonitrile for tests conducted in plasma.
a = Single donor.
b = The % bound value is an average of all test values. There was no evidence on this study of concentration dependence, EDTA or heparin anticoagulant dependence, or the use of fresh versus frozen human plasma on the protein binding of dolutegravir.

7. PHARMACOKINETICS: STUDY IN PREGNANT OR NURSING ANIMALS

Table 7.1 Study in Pregnant or Nursing Animals - Placental Transfer

Test Article: Dolutegravir

Location in CTD: m4.2.2.3

Placental Transfer: **Report No.:** 2012N137348 **Study No.:** 8257653

Species (Strain): Rat (Sprague Dawley)
Gestation Day/Number of Animals: Gestation Day 18, n=1 dam/time point (Test Group 1)
Feeding Condition: Non-fasted
Vehicle/Formulation: 0.5% HPMC with 0.1% Tween 80
Route of Administration: Oral
Dose (mg/kg): 50
Analyte: ^{14}C
Specific Activity: 3.06 μCi/mg
Assay: Quantitative whole body autoradiography

Time Interval Post Dose:	2 Hours	4 Hours	6 Hours	10 Hours	24 Hours
Sample Concentration μg equiv [^{14}C]-dolutegravir/g:					
Fetal blood	3.490	4.210	4.620	4.760	1.530
Fetal bone marrow	2.130	5.060	6.390	8.400	5.230
Fetal brain	0.588	0.619	0.855	0.895	BLQ
Fetal eye	1.510	2.010	3.380	2.750	0.836
Fetal gastrointestinal tract	2.350	2.570	3.830	4.740	1.250
Fetal kidney	2.410	2.370	3.970	3.930	1.060
Fetal liver	2.240	2.550	3.320	3.410	1.080
Fetal lung(s)	1.820	2.150	2.500	2.320	0.769
Fetal muscle	2.770	2.940	4.470	4.320	1.430
Fetal myocardium	2.910	2.200	2.730	2.630	1.060
Fetal spinal cord	0.554	0.570	0.952	0.847	BLQ
Fetus	2.410	2.590	3.610	3.950	1.250

Additional Information:
BLQ = Below limit of quantitation (0.318 μg equiv [^{14}C]-dolutegravir/g).

Table 7.2 Study in Pregnant or Nursing Animals - Excretion into Milk

Test Article: Dolutegravir

Location in CTD: m4.2.2.3

Excretion into Milk: **Report No.:** 2012N137348 **Study No.:** 8257653

Species:	Rat
Lactating Date:	Post partum Day 10, n=2 or 3/time point (Test Group 2)
Feeding Condition:	Non-fasted
Vehicle/Formulation:	0.5% HPMC with 0.1% Tween 80
Route of Administration:	Oral
Dose (mg/kg):	50
Analyte:	^{14}C
Specific Activity:	3.06 μCi/mg
Assay:	LSC

Time Interval Post Dose: **Sample Concentration:**	**1 Hour**	**2 Hours**	**4 Hours**	**8 Hours**	**24 Hours**
Milk (μg equiv [^{14}C]-dolutegravir/g):	10.0	18.2	25.9	47.3	1.8
Blood (μg equiv [^{14}C]-dolutegravir/g):	22.2	27.4	23.1	20.6	1.4
Milk/blood ratio:	0.452	0.662	1.09	2.30	1.28

Additional Information:
LSC = Liquid scintillation counting.

8. PHARMACOKINETICS: OTHER DISTRIBUTION STUDIES

Table 8.1 Summary of the In Vitro Inhibition of Human Transporters by Dolutegravir

Test Article: Dolutegravir

Target	Inhibition IC_{50} (μM)	Report No.	Location in CTD
P-gp	>100	RD2008/00292	m4.2.2.3
BCRP	IS	2010N110625	m4.2.2.3
MRP2	None	2010N109746	m4.2.2.3
MRP2[a]	None	2011N120047	m4.2.2.3
OATP1B1	None	RD2008/00216	m4.2.2.3
OATP1B3	None	RD2008/00216	m4.2.2.3
OCT1	>10	2012N132572	m4.2.2.3
OCT2	1.93	2010N104937	m4.2.2.3

Additional Information:
a = Dolutegravir glucuronide (GSK2832500 or M3) was the test material in this study.
BCRP = Breast Cancer Resistance Protein; tested with MDCKII-BCRP cells.
IS = Insufficient data to calculate an IC_{50}; observed 50% inhibition of control at 100 μM.
MRP2 = Multidrug resistance associated protein-2 transporter tested with membrane vesicles prepared from recombinant baculovirus infected Sf9 cells expressing the human MRP2 transporter.
None = No inhibition detected.
OATP = Organic anion-transporting polypeptide.
OATP1B1 tested with Chinese Hamster Ovary cells expressing OATP1B1.
OATP1B3 tested with human embryonic kidney MSRII cells expressing OATP1B3.
OCT = Organic cation transporter.
OCT1 tested with HEK293 cells expressing the OCT1 transporter.
OCT2 tested with MDCKII cells expressing the OCT2 transporter.
P-gp = P-glycoprotein; tested with MDCKII-hMDR1 cells.

Table 8.2 In Vitro Human Transporter Substrate Studies for Dolutegravir

Test Article: Dolutegravir

Transporter	Concentration (μM)	Efflux Ratio	Report No.	Location in CTD
P-gp	3	3.8	RD2008/00361	m4.2.2.3
BCRP	3	3.1	2011N112380	m4.2.2.3

Additional Information:
[^{14}C]-dolutegravir was the test material.
The results of these studies indicate that dolutegravir is a substrate for these transporters in vitro.

Table 8.3 In Vitro Human Cell Membrane Permeability for Dolutegravir

Test Article: Dolutegravir

P-glycoprotein (P-gp) Permeability					
Transporter	**Cells**	**Concentration (μM)**	**Permeabilities (nm/s)**	**Report No.**	**Location in CTD**
P-gp	hMDR1-MDCK	3	Passive (Papp) = 333	RD2008/00360	m4.2.2.3
P-gp	hMDR1-MDCK[a]	3	$P_{7.4[abs]}$ and $P_{5.5[abs]}$ = 253 and 265	RD2008/00360	m4.2.2.3

Additional Information:
[^{14}C]-dolutegravir was the test material.
a = These data are the results from using a biorelevant buffer (FaSSIF, fasted state simulated intestinal fluid) to simulate conditions in the gastrointestinal tract.
Papp = Apparent passive permeability.
$P_{7.4[abs]}$ and $P_{5.5[abs]}$ = The absorptive membrane permeabilities at pH 7.4 and pH 5.5.
These studies were conducted using 2 μM GF120918, a potent P-gp inhibitor, as a control. The results of these studies indicate that dolutegravir has high membrane permeability.

Table 8.4 Blood:Plasma and Liver:Blood Ratios

Test Article: Dolutegravir

Gender (M/F): Male + Female
Feeding Condition: Non-fasted
Vehicle/Formulation: 0.5% HPMC w/ 0.1% Tween 80
Route of Administration: Oral
Radionuclide: [^{14}C]
Specific Activity: 5.7, 4.29 and 7.8 μCi/mg in mouse, rat and monkey, respectively.

Species: Mouse (n=10) **Dose (mg/kg):** 100 **Report No.:** RD2009/00562 **Location in CTD:** m4.2.2.5
Species: Rat (n=3) **Dose (mg/kg):** 50 **Report No.:** RD2008/00108 **Location in CTD:** m4.2.2.5
Species: Monkey (n=3) **Dose (mg/kg):** 10 **Report No.:** RD2008/01300 **Location in CTD:** m4.2.2.5
Species: BDC Monkey (n=2) **Dose (mg/kg):** 10 **Report No.:** RD2008/01299 **Location in CTD:** m4.2.2.5

		Blood : Plasma Ratio		Liver : Blood Ratio	
Species	**Time (Hour)**	**Male**	**Female**	**Male**	**Female**
Mouse	2	0.51	0.49	0.39	0.34
	10	0.54	0.49	0.36	0.34
	24	0.49	0.50	0.42	0.46
Rat	2	0.53	0.52	0.31	0.29
	6	0.53	0.51	0.29	0.26
	24	0.51	0.51	0.47	0.30
Monkey	2	0.66 (BDC = 0.74)	0.64	NT	NT
	6	0.67 (BDC = 0.74)	0.65	NT	NT
	12	0.72 (BDC = 0.75	0.69	NT	NT
	24	0.73 (BDC = 0.79)	0.66	NT	NT

Additional Information:
NT = Not tested
BDC = bile duct cannulated - mean from 2 animals.

9. PHARMACOKINETICS: METABOLISM IN VIVO

Table 9.1 Metabolism In Vivo

Test Article: Dolutegravir

Gender (M/F): Male + Female (only male shown below)
Feeding Condition: Non-fasted
Vehicle/Formulation: 0.5% HPMC w/ 0.1% Tween 80 for all nonclinical studies; sodium laurel sulphate and hypromellose in human (mass balance). 50 mg tablet in human repeat dose.
Route of Administration: Oral
Radionuclide: [^{14}C]
Specific Activity: 5.7, 4.29, 7.8 and 3.63 μCi/mg in mouse, rat, monkey and human, respectively.

Species: Mouse	**Dose (mg/kg):** 100	**Report No.:** RD2009/00723	**Location in CTD:** m4.2.2.4
Species: Rat	**Dose (mg/kg):** 50	**Report No.:** RD2008/00220	**Location in CTD:** m4.2.2.4
Species: Monkey	**Dose (mg/kg):** 10	**Report No.:** RD2008/00899	**Location in CTD:** m4.2.2.4
Species: Human	**Dose (mg):** 20	**Report No.:** RD2009/00356	**Location in CTD:** m5.3
Species: Human	**Dose (mg/day):** 50	**Report No.:** RD2010/00413	**Location in CTD:** m5.3

Relative Abundance of Dolutegravir and Metabolites in Males

Peak ID	Species	Urine		Feces		Bile	
		%Matrix	%Dose	%Matrix	%Dose	%Matrix	%Dose
Dolutegravir	Mouse	8.7	0.1	96.7	88.7	6.1	0.2
	Rat	NQ	---	95.7	88.0	0.5	0.0
	Monkey	NQ	---	89.1	59.0	2.5	0.3
	Human	2.2	0.7	89.1	53.1	---	---
M2	Mouse	2.2	0.1	NQ	---	4.3	0.1
	Rat	14.9	0.4	NQ	---	17.5	1.1
	Monkey	7.5	0.4	NQ	---	36.5	4.0
	Human	1.4	---	---	---	---	---
M3	Mouse	53.0	0.6	NQ	---	24.3	0.7
	Rat	13.2	0.4	NQ	---	45.4	2.9
	Monkey	72.8	3.8	NQ	---	28.7	3.4
	Human	62.5	18.9	NQ	---	---	---

Table 9.1 (Continued) Metabolism In Vivo

Relative Abundance of Dolutegravir and Metabolites in Males

Peak ID	Species	Urine		Feces		Bile	
		%Matrix	%Dose	%Matrix	%Dose	%Matrix	%Dose
M1+M7	Mouse	4.6	0.1	NQ	---	NQ	---
	Rat	57.8	1.6	NQ	---	NQ	---
	Monkey	1.2	0.1	NQ	---	NQ	---
	Human	21.9	6.6	2.2	1.3	---	---
M10+M11	Mouse	NQ	---	NQ	---	7.4	0.2
	Rat	NQ	---	NQ	---	3.4	0.2
	Human	2.3	---	---	---	---	---
M12	Rat	NQ	---	NQ	---	1.7	0.1
	Human	0.2	---	---	---	---	---
M13	Monkey	NQ	---	NQ	---	16.1	1.9
	Human	NQ	---	3.1	1.8	---	---
M14	Mouse	NQ	---	NQ	---	5.5	0.1
	Human	0.1	---	---	---	---	---
M16	Mouse	NQ	---	NQ	---	4.1	0.1
	Human	0.1	---	---	---	---	---

Additional Information:
M1 and M7 combined because M1 is formed from M7 via hydrolysis.
M10 and M11 combined because in the rodent radiochromatogram they co-eluted.
NQ = Not quantifiable.
--- = Not determined.

Table 9.2 Metabolites in Milk of Lactating Rats

Test Article: Dolutegravir

Gender (M/F): Female for all samples
Feeding Condition: Non-fasted
Vehicle/Formulation: 0.5% HPMC w/ 0.1% Tween 80
Route of Administration: Oral
Radionuclide: [^{14}C]
Specific Activity: 3.06 μCi/mg **Days of Dosing:** Single day
Species: Rat **Dose (mg/kg):** 50 **Report No.:** 2012N132387 **Location in CTD:** m4.2.2.4

Tabulated Results:

Time Interval	1 Hour	2 Hours	4 Hours	8 Hours	24 Hours
% Radioactivity in Milk Represented as Dolutegravir (μg equivalents [^{14}C]-GSK1349572/g)	96.7 (10.2)	97.0 (17.7)	94.4 (25.6)	89.1 (45.6)	82.9 (1.53)
Total Radioactivity in Pooled Milk (μg equivalents/g)	10.5	18.3	27.1	51.1	1.84
% Overall Recovery[a]	96.9	98.1	94.5	91.5	85.5[b]

Additional Information:
a = Percentages are based on the radioactivity present in sample after extraction and reconstitution.
b = Due to the low radioactivity, the third extract (8.4%) was not included for profiling.
Assumption for calculation purposes in the milk matrix, 1 mL = 1 gram.

Conclusion: The results indicate that dolutegravir comprised nearly all of the drug-related material in milk.

Table 9.3 Potential for Epimerization of Dolutegravir in Juvenile Rat Plasma

Test Article: Dolutegravir

Gender (M/F):	Male and female				
Feeding Condition:	Non-fasted				
Vehicle/Formulation:	0.5% HPMC w/ 0.1% Tween 80				
Route of Administration:	Oral	**Days of Dosing:**	10		
Species: Rat	**Dose (mg/kg):** 2, 25, 75, 300	**Report No.:** RD2010/00173		**Location in CTD:** m4.2.2.4	

Tabulated Results:

The potential for GSK1349572 to epimerize in vivo was assessed in juvenile rat plasma following repeat oral administration of GSK1349572A for 10 days to male and female juvenile Crl:CD(SD) rats from Day 4 to Day 13 post partum.

There was no evidence for the in vivo epimerization of GSK1349572 to any of its stereoisomers, gsk013*, gsk014* or gsk015*, in juvenile rat plasma samples following oral administration of GSK1349572.

Additional Information:
There was no evidence for the in vivo epimerization of dolutegravir to any of its stereoisomers in juvenile rats.

* 新薬承認情報提供時に置き換え

10. PHARMACOKINETICS: METABOLISM IN VITRO

Table 10.1 Metabolic Stability in S9 and Hepatocytes In Vitro

Test Article: Dolutegravir

Study System: S9 and hepatocytes

Location in CTD: m4.2.2.4
Report No.: RH2007/00076
Study No.: 07APK019

Percent Parent Drug Remaining (GSK1349572)								
	S9				Rat Hepatocytes		Human Hepatocytes	
Time (min)	Rat	Dog	Monkey	Human	Fresh	Cryopreserved	Fresh	Cryopreserved
0	100	100	100	100	100	100	100	100
15	97	93	99	95	98	105	93	103
30	96	95	97	93	98	100	94	104
60	94	107	98	89	89	100	91	92
90	NT	NT	NT	NT	85	98	90	92
120	NT	NT	NT	NT	73	84	87	83
$T_{½}$ (min)	>180	>180	>180	>180	268	>360	>360	>360
0	100	100	100	100				
15	101	68	88	97				
30	86	78	79	79				
60	NR	30	83	NR				
$T_{½}$ (min)	141	37	>180	90				

Additional Information:
NR = No result due to signal below detection limit.
NT = Not tested.

Table 10.2 In Vitro Metabolite Characterization Using Cryopreserved Hepatocytes

Test Article: Dolutegravir

Location in CTD: m4.2.2.4

Report No.: RH2007/00060

Study System: Hepatocytes

Species: Rat, dog, monkey, human

Method: Cryopreserved hepatocytes were pre-incubated at 37°C for 10 minutes prior to addition of GSK1349572. At 0, 4 and 24 hours, an organic mixture was added to quench the incubation reaction. The samples were analyzed by LC/MS/MS.

Results:
No metabolites were detected in any species at 4 or 24 hours.

Table 10.3 In Vitro Metabolic Activation in Liver Microsomes

Test Article: Dolutegravir

Location in CTD: m4.2.2.4
Report No.: RD2007/01557

Study System: Liver microsomes
Species: Rat, monkey, human

Method: The non-extracted binding of [^{14}C]-GSK1349572 (10 μM) and [^{14}C]-acetaminophen was determined in the presence and the absence of NADPH co-factor in rat, monkey and human liver microsomes. After termination of the reactions, samples were filtered and washed and analyzed with liquid scintillation counting.

Results:	**Non-Extracted Radioactivity (pmol eq/mg protein/hour)**	
Species	**[^{14}C]-GSK1349572**	**[^{14}C]-Acetaminophen**
Rat	1026	101
Monkey	2413	154
Human	452	178

Additional Information:
The non-extracted radioactivity observed was predominantly co-factor-dependent.

Table 10.4 In Vitro Metabolic Turnover in Hepatocytes

Test Article: Dolutegravir

Location in CTD: m4.2.2.4
Report No.: RD2007/01496

Study System: Hepatocytes
Species: Male rat and monkey, and pooled (mixed gender) human

Method: Hepatocytes were incubated at 37°C with [^{14}C]-GSK1349572 (50 μM). At 0, 4 and 24 hours, an organic mixture was added to quench the incubation reaction. The samples were analyzed by using liquid scintillation counting. Metabolic viability of the cells was evaluated with the probe substrate, 7-ethoxycoumarin.

Results:
The metabolic turnover of [^{14}C]-GSK1349572 in rat and monkey hepatocytes was similar to human hepatocytes (approximately 3.5 to 9.4% turnover). In human hepatocytes, the notable route of metabolism for [^{14}C]-GSK1349572 was glucuronidation. Metabolite profiles of the nonclinical species and human were qualitatively similar. The human metabolite (glucuronidation) was observed in hepatocytes from the two nonclinical species.

Table 10.5 In Vitro Metabolism of Dolutegravir to its Enantiomer and Diastereomers

Test Article: Dolutegravir

Location in CTD: m4.2.2.4
Report No.: RH2007/00105

Study System: Cryopreserved hepatocytes
Species: Rat, dog, monkey, human

Method: Cryopreserved hepatocytes were pre-incubated at 37°C for 10 minutes prior to addition of GSK1349572. At 0, 1, 4, 6 and 24 hours, an organic mixture was added to quench the incubation reaction. The samples were analyzed using a chiral LC/MS/MS assay.

Results:
While low amounts (~0.4% of the peak area of GSK1349572) of the enantiomer were detected at all incubation time points, those concentrations did not increase with time; no peaks that corresponded to the retention time for the diastereomer were detected. Because GSK1349572 and the diastereomer of its enantiomer (gsk014*) could not be separated chromatographically, production of gsk014* in the incubation could not be observed. The data indicate that no significant metabolic conversion of GSK1349572 to its diastereomer or enantiomer was observed.

* 新薬承認情報提供時に置き換え

Table 10.6 In Vitro Potential to Form Glutathione Adducts

Test Article: Dolutegravir

Location in CTD: m4.2.2.4
Report No.: RH2007/00058

Study System: Pooled liver microsomes
Species: Rat and human

Method: Microsomes were incubated with GSK1349572 (5 μL of a 10 mM solution per incubation mix) at 37°C for 30 minutes and then the incubation mixture was quenched. Samples were analyzed by LC/MS/MS.

Results:
GSK1349572 showed evidence for formation of a common glutathione metabolite in both rat and pooled human liver microsomes.

Table 10.7 In Vitro Metabolic Enzymology

Test Article: Dolutegravir

Study System: Pooled human liver microsomes and recombinant enzymes
Species: Human
Method: Incubation at 37°C for 20, 60 or 120 minutes

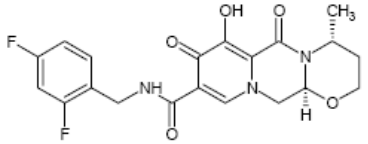
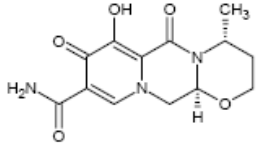
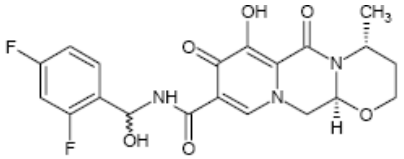
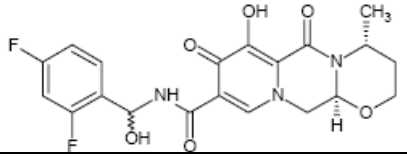
Location in CTD: m4.2.2.4
Report No.: RH2007/00104

Metabolic Stability With Recombinant Human UGT1A1 and PHLM (Dolutegravir 5 μM; UGT1A1 0.25 mg/mL)				Metabolic Stability With and Without Recombinant Human UGT1A1 (Dolutegravir 0.5 μM; UGT1A1 2.5 mg/mL)			
		% Parent Remaining		With UGT1A1		Without UGT1A1	
Compound	Time (min)	UGT1A1	PHLM[c]	% Parent Remaining	St Dev	% Parent Remaining	St Dev
GSK1349572A	0	100	100	100	NA	100	NA
	20	92	95	NT	NA	NT	NA
	60	99	93	NT	NA	NT	NA
	120	NT	NT	70	1.2	105	2.5
β-estradiol[a]	0	100	100	NT	NA	NT	NA
	20	88	96	NT	NA	NT	NA
	60	99	63	NT	NA	NT	NA
7hydroxy4TFMC[b]	0	100	100	100	NA	100	NA
	20	96	1	NT	NA	NT	NA
	60	95	0	NT	NA	NT	NA
	120	NT	NT	25	1.0	107	7.6

Additional Information:
a = Metabolic stability of β-estradiol was determined for 50 μM incubations instead of 5 μM incubations because of poor MS sensitivity for this compound.
b = 7hydroxy4TFMC = 7-hydroxy-4-trifluoromethylcoumarin.
c = PHLM is pooled human liver microsomes.
NA = Not applicable.
NT = Not tested.

Table 10.8 In Vitro Metabolic Enzymology

Test Article: Dolutegravir
Study System: Pooled human liver microsomes and recombinant CYP enzymes
Species: Human
Radionuclide: [^{14}C] **Location in CTD:** m4.2.2.4
Specific Activity: 58.6 mCi/mmol **Report No.:** RD2008/00373

Peak ID	RT (min)[a]	Proposed Structure[b]	Mean % Total Radioactivity		Biotransformation
			CYP3A4	HLM	
GSK1349572	64		32*	73*	NA
M1	16.5		1.8*	Not detected*	N-dealkylation
M7[c]	49		7.3*	1.2*	Oxidation
M7[c]	53		33*,[d]	13*	Oxidation

Additional information: Incubations with recombinant CYP1A2, 2B6, 2C8, 2C9, 2C19 and 2D6 showed no metabolism.
HLM and recombinant and 3A4 samples were the source for metabolite structural identification (50 μM concentration only).
* = Metabolite structural characterization performed on these samples.
Values indicate % total radioactivity from 5 μM incubations.
a = MS retention time.
b = The structure of GSK1349572 is represented by the major tautomeric form.
c = Represents two diastereomers identified in Table 10.10.
d = MS and MS/MS confirmed in collected sample, accurate mass information obtained from LC/MS data.

Table 10.9 In Vitro Metabolic Enzymology

Test Article: Dolutegravir

Study System: Pooled human liver microsomes and recombinant UGT enzymes
Species: Human
Radionuclide: [^{14}C] **Location in CTD:** m4.2.2.4
Specific Activity: 58.6 mCi/mmol **Report No.:** RD2008/01339

Method: Incubations with UGT enzymes were performed at 37°C in duplicate for up to 4 hours. Each sample contained 16 μM [^{14}C]-GSK1349572 and 1 mg/mL protein.

Summary of [^{14}C]-GSK1349572 Glucuronide Formation

UGT Enzyme	Mean % Total Radioactivity[a,b]	Mean Rate of Metabolite Formation (pmol/min/mg)[b]	Metabolite Formation after β-Glucuronidase Digestion (pmol/min/mg)
HLM	20	27	0
UGT1A1[c]	23	31	0
UGT1A3[c]	2.1	2.7	0
UGT1A4	0	0	ND
UGT1A6	0	0	ND
UGT1A9[c]	4.2	5.5	0
UGT2B4	0	0	ND
UGT2B7	0	0	ND
UGT2B15	0	0	ND
Control UGT	0	0	ND

Additional Information:
a = A single glucuronide formed in these incubations was confirmed by β-glucuronidase digestions from this study and retention time comparison of HPLC radioprofiles from a previous [^{14}C]-GSK1349572 metabolism study [Report RD2008/00899/00].
b = Values indicate % total radioactivity or metabolite rates from 4 hour incubations (UGT1A4, 1A6, 2B4, 2B7, 2B15 and control UGT enzymes) or 2 hour incubations (HLM, UGT1A1, 1A3 and 1A9) with 16 μM [^{14}C]-GSK1349572.
c = Metabolite was not detected in the no UDPGA controls (HLM, UGT1A1, 1A3 and 1A9).
ND = β-glucuronidase digestions were not performed.

Table 10.10 Metabolism In Isolated Perfused Rat Liver

Test Article: Dolutegravir

Location in CTD: m4.2.2.4
Report No.: RD2007/01493

Study System: Isolated perfused rat liver (IPRL)

Summary of [^{14}C]-GSK1349572 Metabolites in IPRL Bile, Liver and Perfusate Following Addition of [^{14}C]-GSK1349572 at 15 mg/kg Body Weight (259 μCi)

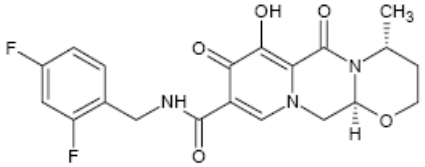
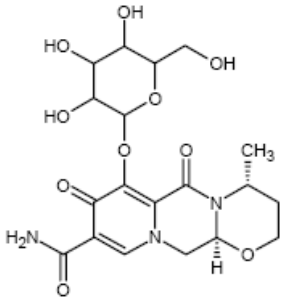
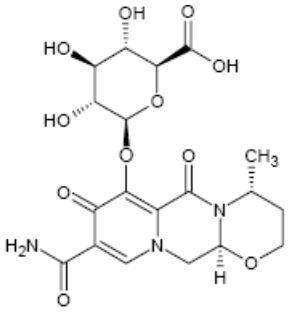
ID	Biotransformation	Retention Time (Minutes)	Proposed Structure
GSK1349572	Parent	63.0	
M4	N-dealkylation, hexose conjugation	6.0	
M5	N-dealkylation, glucuronidation	6.0	

Table 10.10 (Continued) Metabolism In Isolated Perfused Rat Liver

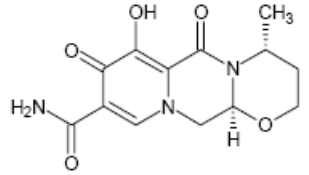
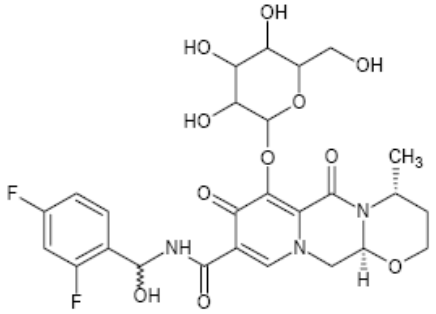
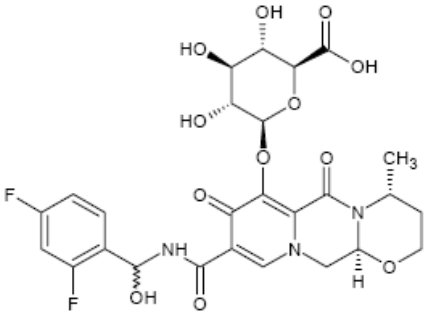
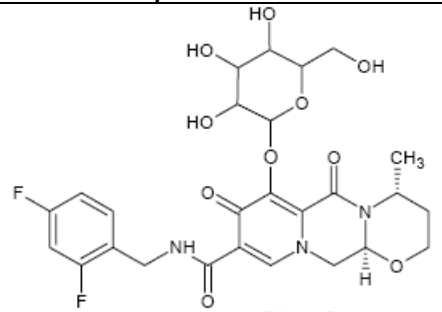
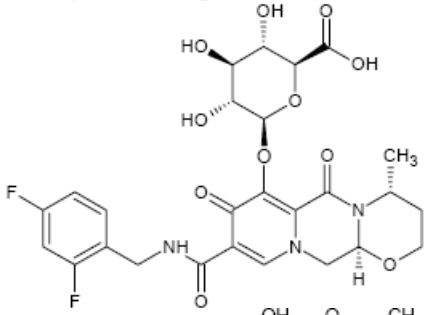
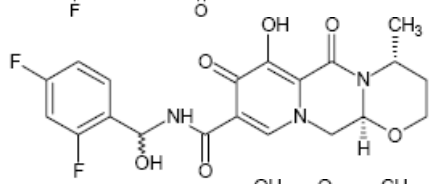
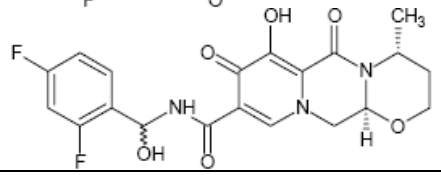
ID	Biotransformation	Retention Time (Minutes)	Proposed Structure
M1	N-dealkylation	15.5 to 15.8	
M6	Oxidation, hexose conjugation	31.8	
M8	Oxidation, glucuronidation	31.8	

Table 10.10 (Continued) Metabolism In Isolated Perfused Rat Liver

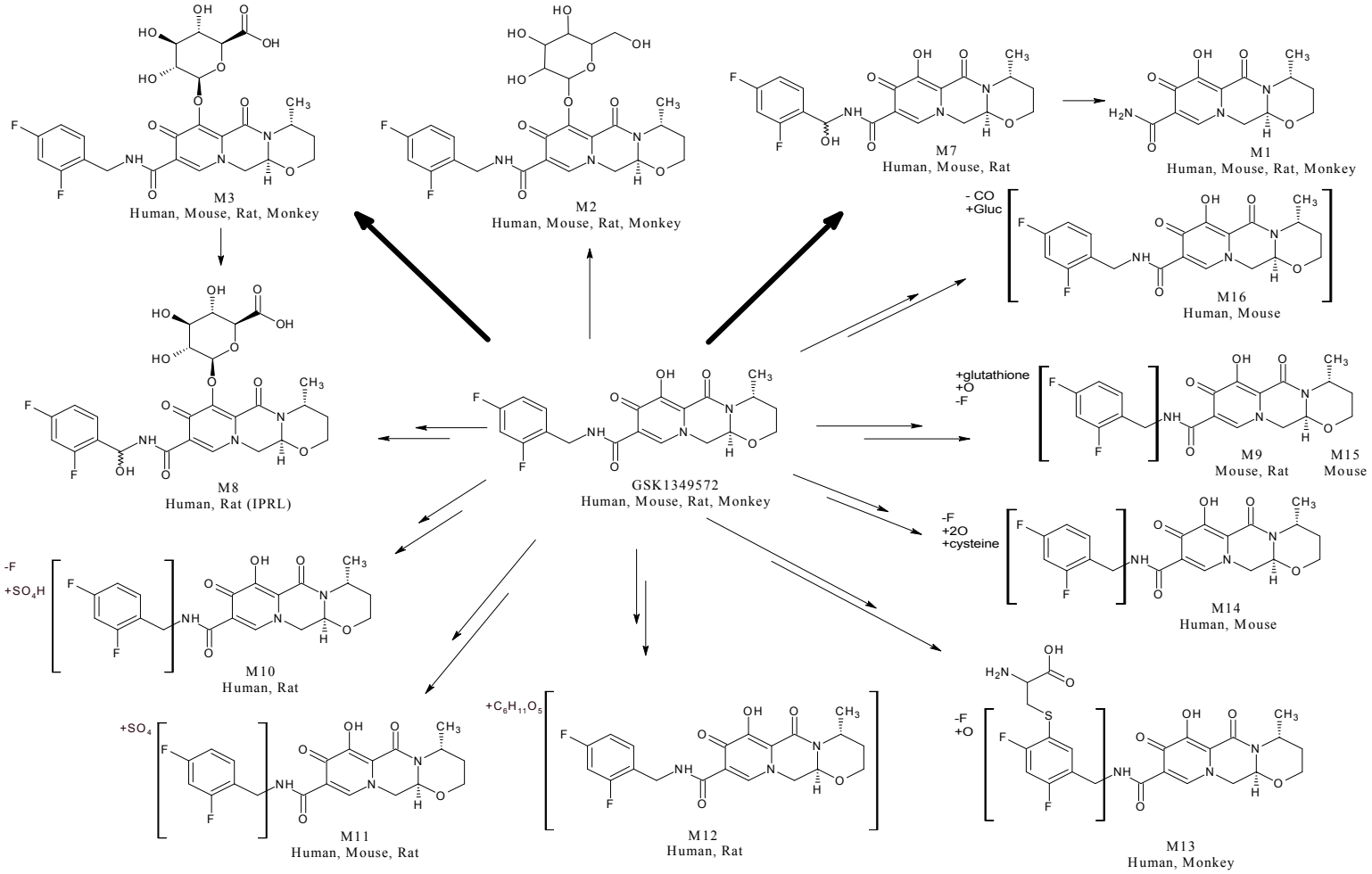
ID	Biotransformation	Retention Time (Minutes)	Proposed Structure
M2	Hexose conjugation	39.8	
M3	Glucuronidation	42.5	
M7[a]	Oxidation	52.0 to 52.3	
M7[a]	Oxidation	56.5	

Additional Information:

a = Peaks could not be isolated independently for NMR analysis and had identical MS spectra. LC and spectroscopic data suggest that the two observed LC peaks may be due to two diastereomers.

11. PHARMACOKINETICS: POSSIBLE METABOLIC PATHWAYS

Table 11.1 Comparison of Dolutegravir Metabolism Between Nonclincial Species and Humans



Key: IPRL = Isolated perfused rat liver. Bolded arrows indicate the primary products in human.

12. PHARMACOKINETICS: INDUCTION/INHIBITION OF DRUG METABOLISING ENZYMES

Table 12.1 Inhibition of Drug Metabolising Enzymes

Test Article: Dolutegravir

Type of Study: Potential In Vitro Inhibition of Human Drug Metabolising Enzymes by Dolutegravir

Target	Inhibition IC_{50} (μM)	Metabolism-Dependent Inhibition IC_{50} (μM)	Report No.	Location in CTD
CYP1A2	None	None	WD2010/00908	m4.2.2.4
CYP2A6	None	None		
CYP2B6	>100	None		
CYP2C8	None	None		
CYP2C9	>100	None		
CYP2C19	>100	None		
CYP2D6	>100	None		
CYP3A4 (atorvastatin)	>54	33		
CYP3A4 (nifedipine)	>100	65		
CYP3A4 (midazolam)[a]	[a]	[a]		
UGT1A1	>100		RD2009/00862	m4.2.2.4
UGT2B7	None			

Additional Information:
a = Activation was noted.
CYP = Cytochrome P450; CYP enzymes were tested in pooled human liver microsomes.
IC_{50} = Concentration giving 50% of the maximum inhibition.
None = No inhibition detected.
UGT = Uridine diphosphate glucuronyltransferase.
UGT Enzymes were tested using recombinant human enzymes.

Table 12.2 Induction of Drug Metabolising Enzymes

Test Article: Dolutegravir

Type of Study: Potential In Vitro Induction of Human Drug Metabolising Enzymes (CYP1A2, CYP2B6 and CYP3A4)

Report No.: RH2007/00074 **Location in CTD:** m4.2.2.4

Study System:	Human hepatocytes were obtained from CellzDirect in a sandwich configuration on a collagen substratum with a Matrigel overlay.
Method:	Pre-warmed duplicate culture medium solutions containing GSK1349572 (1, 5, 10, 20, 30 and 40 μM), β-naphthoflavone (BNF, 35 μM), phenobarbital (PB, 1 mM) or rifampin (RIF, 20 μM) were added to human hepatocytes. All cells were treated with drug/control once daily for 48 hours. At the end of the incubation period the culture medium was removed, cells were lysed and total RNA was extracted. RNA was converted to double stranded cDNA and then PCR amplified. The specific mRNA level was quantitatively detected for the following genes: CYP1A2, 2B6, 3A4 and the housekeeping gene, glyceraldehyde 3-phosphate dehydrogenase (GAPDH).

Effects of Dolutegravir and Prototypical CYP Inducers on the mRNA Levels of Cytochrome P450s (Ratio of Treated Over Control*)

Gene	CYP1A2	CYP2B6	CYP3A4
Treatment			
1 μM GSK1349572	0.27	1.04	0.58
5 μM GSK1349572	0.50	1.27	0.74
10 μM GSK1349572	0.20	1.01	0.43
20 μM GSK1349572	0.24	1.55	0.53
30 μM GSK1349572	0.33	1.33	0.50
40 μM GSK1349572	0.26	1.00	0.86
Prototypical Inducer	6.23 (BNF)	11.34 (PB)	6.64 (RIF)

Additional Information:
* = Controls are defined as 0.1% v/v DMSO.

Table 12.3 Induction of Pregnane X Receptor (PXR) Target Genes (e.g., CYP3A4)

Test Article: Dolutegravir

Type of Study: **Potential In Vitro Transactivation of PXR**
Test System: HepG2 cells transfected with rat or human PXR
Number of Replicates: n=3 for rat and human

Target	Maximum Response	pEC_{50}	Maximum Response Concentration of GSK1349572B	Response	CYP3A4 Induction Potential	Report No. (Location in CTD)
Rat PXR	1.3	<5	1.52 nM	Weak	Unlikely	RR2007/00024 (m4.2.2.4)
Rat PXR	1.5	<5	3.33 μM	Weak	Unlikely	
Rat PXR	4.8	<5	4.57 nM	Weak	Unlikely	
Human PXR	58.1	<5	10 μM	Moderate	Possible	RR2007/00025 (m4.2.2.4)
Human PXR	52.4	<5	10 μM	Moderate	Possible	
Human PXR	41.6	<5	10 μM	Moderate	Possible	

Additional Information:
Positive controls for PXR activation were rifampicin for human PXR and 5-Pregnan-3β−OL-20-ONE-16α carbonitrile for rat PXR.

Table 12.4 Induction of Drug Metabolising Enzymes

Test Article: Dolutegravir

Type of Study: **Effects of Dolutegravir on Hepatic Drug Metabolizing Enzymes in 2 Week Oral Toxicity Study in Rats**

Species/Strain: Rat/Crl:CD(SD)
Initial Age: 11 weeks
Date of First Dose: November 22 and 23, 2006 **Method of Administration:** Gavage **Special Features:** None
Vehicle/Formulation: Aqueous 0.5% HPMC with 0.1% Tween 80
GLP Compliance: Yes **Report No.:** RD2007/01034 **Location in CTD:** m4.2.2.4

Daily Dose (mg/kg)	0 (Control)		50		150		500	
(M/F):Number of Animals	M:10	F:10	M:10	F:10	M:10	F:10	M:10	F:10
Died or Sacrificed Moribund	0	0	0	0	0	0	0	0
Number Examined	6	6	6	6	6	6	6	6
Protein (mg/g liver)	50.5	39.8	54.4	43.9	58.1	39.2	53.5	40.7
Cytochrome P450 (nmol/mg protein)	0.481	0.449	0.492	0.368*	0.424	0.408	0.388**	0.420
T6β-OHase[a] (nmol/min/mg protein)	0.907	0.119	0.751	0.126	0.632*	0.120	0.489**	0.126
T16α-OHase[a] (nmol/min/mg protein)	3.086	NT	2.585	NT	2.458	NT	1.975**	NT
T16β-OHase[a] (nmol/min/mg protein)	0.022	0.051	0.020	0.050	0.020	0.050	0.017**	0.049
T2α-OHase[a] (nmol/min/mg protein)	2.151	NT	1.835	NT	1.746	NT	1.439**	NT
EROD[a] (nmol/min/mg protein)	0.080	0.110	0.076	0.101	0.069	0.094	0.074	0.106

Additional Information:
Group means are shown.
Dunnett's multiple comparison test: * = $p<0.05$. ** = $p<0.01$.
a = T6β-OHase = testosterone 6β-hydroxylase; T16β-OHase, testosterone 16β-hydroxylase; T2α-OHase, testosterone 2α-hydroxylase; T16α-OHase, testosterone 16α-hydroxylase; EROD, ethoxyresorufin *O*-deethylase.
NT indicates not tested.

Table 12.5 Induction of Drug Metabolising Enzymes

Test Article: Dolutegravir

Type of Study: **Effects of Dolutegravir on Hepatic Drug Metabolizing Enzymes in 2 Week Oral Toxicity Study in Monkeys**

Species/Strain: Monkey/cynomolgus
Age at First Dose: 2 years and 6 to 9 months
Date of First Dose: March 22 and 23, 2007 **Method of Administration:** Gavage **Special Features:** None
Vehicle/Formulation: Aqueous 0.5% HPMC with 0.1% Tween 80
GLP Compliance: Yes **Report No.:** RD2007/01142 **Location in CTD:** m4.2.3.2

Daily Dose (mg/kg)	0 (Control)		100		300		1000	
(M/F):Number of Animals	M:3	F:3	M:3	F:3	M:3	F:3	M:3	F:3
Died or Sacrificed Moribund	0	0	0	0	0	0	0	1
Number Examined	3	3	3	3	3	3	3	2
Protein (mg/g liver)	17.4 [R]	19.7	23.2 [R]	21.2	22.0 [R]	24.9	22.6* [R]	21.7
Cytochrome P450 (nmol/mg protein)	0.732	0.681	0.754	0.721	0.725	0.721	0.524*	0.637
EROD[a] (pmol/min/mg protein)	59.4	66.5	61.7	76.8	48.1	33.8	23.2	22.7
T6β-OHase[a] (nmol/min/mg protein)	4.71	4.17	5.59	4.55	5.38	4.43	6.22	4.92
T16α-OHase[a] (nmol/min/mg protein)	0.120	0.106 [R]	0.149	0.101 [R]	0.157	0.108 [R]	0.149	0.104 [R]
T16β-OHase[a] (nmol/min/mg protein)	0.249	0.226	0.262	0.236	0.255	0.226	0.219	0.210

Additional Information:
Group means are shown.
Method of statistical test: Dunnett's test unless indicated by [R] where mean rank test of Dunnett type was used
* = $p<0.05$ significantly different from control
a = EROD, ethoxyresorufin *O*-deethylase, T6β-OHase = testosterone 6β-hydroxylase; T16α-OHase, testosterone 16α-hydroxylase; 3T16β-OHase, testosterone 16β-hydroxylase;
NT indicates not tested.

13. PHARMACOKINETICS: EXCRETION

Table 13.1 Excretion

Test Article: Dolutegravir

Species:	Mouse			Rat			Monkey			Human
Feeding Condition:	Non-fasted			Non-fasted			Fasted			Fasted
Vehicle:	0.5% HPMC / 0.1% Tween 80			0.5% HPMC / 0.1% Tween 80			0.5% HPMC / 0.1% Tween 80			Sodium laurel sulphate and hypromellose
Method of Administration:	Oral			Oral			Oral			Oral
Dose (mg/kg):	100			50			10			20 mg/subject
Radionuclide:	[^{14}C]			[^{14}C]			[^{14}C]			[^{14}C]
Specific Activity (μCi/mg):	5.7			4.29			7.8			3.63
Assay:	Liquid scintillation counting			Liquid scintillation counting			Liquid scintillation counting			Liquid scintillation counting
Report No.:	RD2009/00562			RD2008/00108			RD2008/01300		RD2008/01299	RD2009/01002
Location in CTD (Module):	m4.2.2.5			m4.2.2.5			m4.2.2.5		m4.2.2.5	M5.3
Number of Animals/(M/F):	4M	4F	4M (BDC)	3M	3F	3M (BDC)	3M	3F	2M (BDC)	6M
Sample	**Mean Percent of Administered Dose Recovered**									
Urine	1.23	1.95	1.83	3.43	3.88	2.51	6.00	4.42	7.20	31.6
Feces	93.0	94.1	86.3	92.6	90.7	86.2	66.9	77.5	70.0	64.0
Bile	NA	NA	2.51	NA	NA	6.99	NA	NA	11.8	NA
Cage wash	0.22	0.12	0.37	0.06	0.07	0.20	6.72	3.1	2.6	NA
Total[a]	94.5	96.2	91.0	96.1	94.6	95.9	79.6	85.0	91.6	95.6

Key: Data shown are means.
a = Includes, as appropriate, radioactivity recovered in cage rinses/washes/wipes/debris GI tract and residual carcass.
BDC = Bile duct cannulated. NA = Not applicable.

14. PHARMACOKINETICS: EXCRETION INTO BILE

Data pertaining to excretion of dolutegravir in bile are presented in Table 13.1 above.

15. PHARMACOKINETICS: DRUG-DRUG INTERACTIONS

No studies appropriate to this category have been performed with dolutegravir.

16. PHARMACOKINETICS: OTHER

No studies appropriate to this category have been performed with dolutegravir.