MODULE 2.6.4. PHARMACOKINETICS WRITTEN SUMMARY

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List of Abbreviations

3TC	Lamivudine
DTG	Dolutegravir
GLP	Good Laboratory Practice
HEK-293	Human embryonic kidney
HPLC MS/MS	High performance liquid chromatography with tandem mass spectrometry
MATE	Multidrug and toxin extrusion transporter
OATP	Organic anion transporting polypeptide
P_{app}	Passive membrane permeability
P-gp	P-glycoprotein

1. BRIEF SUMMARY

Both dolutegravir (DTG) and lamivudine (3TC) have each undergone full development programmes as individual products and approved in the US as monotherapies [see Table 1, m2.6.1] and in combination with other antivirals. For completeness, new pharmacokinetic studies completed with dolutegravir or lamivudine which have not been previously submitted are summarized in this module.

A study was conducted with dolutegravir to determine the in vitro brain tissue binding of dolutegravir in the male mouse and rat. An in vitro study was also conducted with lamivudine to evaluate the ability of lamivudine to act as an inhibitor of the human hepatic uptake organic anion transporting polypeptide (OATP)1B1 and OATP1B3, and the multidrug and toxin extrusion transporter MATE2-K. A mechanistic kidney pharmacokinetic model was also used to assess the effect of OCT1 and 2 inhibition by lamivudine on metformin systemic exposure. In addition, a study was performed to assess the permeability of lamivudine in Caco-2 cells, alone and in combination with abacavir or dolutegravir.

A brief summary of the important findings from these studies is provided below in Sections 2 and 3. An overall assessment of the findings from these investigations is provided in Section 4, Discussion and Conclusions. 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 Good Laboratory Practice (GLP) status, is provided in Table 2.1, Table 2.2 and Table 3.1.

The structures of DTG and 3TC are given below in Figure 1.1 and Figure 1.2, respectively, with the location of the radiolabel shown for each compound.

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

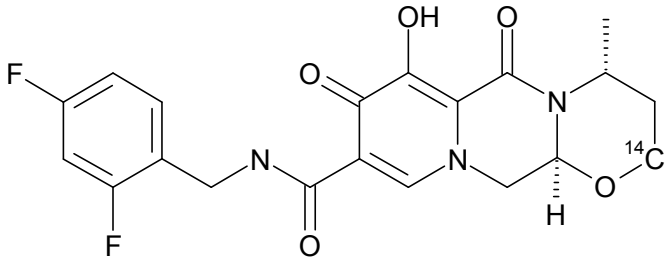
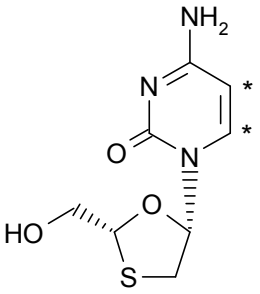


Figure 1.2 Structure of [^{14}C]-Lamivudine



* Denotes the position of the radiolabel.

Summary of Findings

Absorption

No new information.

Distribution

Dolutegravir

- In a mouse and rat in vitro brain tissue binding assay, the mean unbound fraction of dolutegravir was 5.91% and 7.21% in mouse and rat brain, respectively.

Lamivudine

- In vitro, lamivudine did not inhibit OATP1B1, OATP1B3 or MATE2-K.
- No significant changes in lamivudine passive membrane permeability (P_{app}) were observed when tested in combination with dolutegravir or abacavir.

Metabolism

No new information.

Excretion

No new information.

Drug interactions

Lamivudine

- In a mechanistic kidney pharmacokinetic model, lamivudine is not predicted to perpetrate systemic drug-drug interactions (DDI) with metformin.

Other PK studies

No new information.

2. DISTRIBUTION

2.1. Dolutegravir

2.1.1. In vitro

2.1.1.1. Brain tissue binding

The in vitro brain tissue binding of dolutegravir at 2 μM was assessed in the male mouse and rat by equilibrium dialysis [Report 2017N339387, m4.2.2.3]. Equilibrium dialysis was conducted for 4 hours at 37°C. Loperamide (2 μM) a P-glycoprotein (P-gp) substrate, was used as a reference control. Mouse and rat brain homogenate and phosphate buffered saline were analyzed for dolutegravir by a high performance liquid chromatography with tandem mass spectrometry (HPLC MS/MS) method. A tabulated summary of this study is presented in m2.6.5, Table 2.1.

The mean unbound fraction of dolutegravir was 5.91% and 7.21% in mouse and rat brain, respectively. 79.2% and 80.9% of dolutegravir was recovered after 4 hours of dialysis in mouse and rat brain, respectively.

2.2. Lamivudine

2.2.1. In vitro

2.2.1.1. Inhibition of OATP1B1, OATP1B3 and MATE2-K

An in vitro study was performed to evaluate the ability of lamivudine to act as an inhibitor of the human hepatic uptake transporters OATP1B1 and OATP1B3, and the multidrug and toxin extrusion transporter MATE2-K by measuring the transporter-mediated activity in the presence or absence of lamivudine at concentrations of 0.01, 0.03, 0.1, 1, 3, 10, 30, 100 and 300 μM [Report 2015N234461, m4.2.2.3]. Human embryonic kidney (HEK)293 cells which overexpressed OATP1B1, OATP1B3 and MATE2-K were incubated at 37°C with the varying concentrations of lamivudine. Cells were first pre-incubated and then incubated for 2 minutes for OATP1B1 and OATP1B3 and 5 minutes for MATE2-K. A tabulated summary of this study is presented in m2.6.5, Table 4.1.

Lamivudine did not inhibit OATP1B1, OATP1B3 or MATE2-K.

2.2.1.2. Membrane permeability

A study was conducted to determine the effects of changes in pH and the co-treatment with dolutegravir or abacavir sulfate (GI265235) on the permeability of lamivudine across Caco-2 cells in a transwell in vitro assay [Report 2018N376755, m4.2.2.3]. A tabulated summary of this study is presented in m2.6.5, Table 4.2.

No significant changes in lamivudine P_{app} were observed when tested in combination with dolutegravir or abacavir. Changes in lamivudine P_{app} were seen at different pH's, however, it was inconclusive whether this change was related to a pH effect on lamivudine permeability or due to the pH gradient in the apical and basolateral transwell compartments.

Table 2.1 List of Distribution Studies Performed with Dolutegravir

Type of Study	Species	No./Sex/ Group	Method of Administration	Form	Concentration	Duration of Dosing	GLP	Testing Facility	Report No.	Location in CTD
Brain tissue binding	Mouse, Rat	NA	In vitro	A	2 μM	NA	No	GSK	2017N339387	m4.2.2.3

Key:
A = GSK1349572A, the sodium salt form.
NA = Not applicable.

Testing Facility:
GSK = GlaxoSmithKline.

Table 2.2 List of Distribution Studies Performed with Lamivudine

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
Transporter inhibition assays with OATP1B1, OATP1B3 and MATE2-K	HEK293 cells expressing human transporters	NA	In vitro	X	0.01 to 300 μM	NA	No	[redacted]	2015N234461	m4.2.2.3
Membrane permeability	Caco-2 cells	NA	In vitro	X	0.006, 0.03, 0.06, 0.6, 1.2 and 2.4 mg/mL	NA	No	GSK	2018N376755 (17DMM040)	m4.2.2.3

Key:
X = Parent form.
HEK = Human embryonic kidney.
MATE = Multidrug and toxin extrusion transporter.
NA = Not applicable.
OATP = Organic anion transporting polypeptide.

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

3. PHARMACOKINETIC DRUG INTERACTIONS

3.1. Lamivudine

Mechanism-based pharmacokinetic evaluations

A mechanistic kidney pharmacokinetic model, as part of the SimCYP™ physiologically-based pharmacokinetic (PBPK) simulator, was used to assess the effect of OCT1 and OCT2 inhibition by lamivudine on metformin systemic exposure at the clinically efficacious exposures [Report 2017N352403, m4.2.2.6]. A tabulated summary of this study is presented in m2.6.5, Table 5.1.

At therapeutic exposures, no change in metformin exposure (exceeding the 1.25X bioequivalence threshold) was predicted with lamivudine. These simulations suggest that lamivudine is not predicted to perpetrate systemic DDIs with metformin.

Table 3.1 List of Pharmacokinetic Drug Interaction Studies Performed with Lamivudine

Type of Study	Species (Strain)/ Test System	No./Sex/ Group	Method of Administration	Dose	Duration of Dosing (Sampling Occasions)	GLP	Testing Facility	Report No. (Study No.)	Location in CTD
Drug interactions - Computer modelling	Human	NA	In silico	100 mg IV 100, 150 & 300 mg orally	Multiple	No	GSK	2017N352403	m4.2.2.6

Key:
IV = Intravenous.
NA = Not applicable.

Testing Facility:
GSK = GlaxoSmithKline.

4. DISCUSSION AND CONCLUSIONS TO DISTRIBUTION STUDIES

Dolutegravir

The in vitro brain tissue binding of dolutegravir (2 μM) was assessed in the male mouse and rat. The mean unbound fraction of dolutegravir was 5.91% and 7.21% in mouse and rat brain, respectively.

Lamivudine

In vitro, lamivudine (0.01 to 300 μM) did not inhibit the human hepatic uptake transporters OATP1B1 and OATP1B3 or multidrug and toxin extrusion transporter MATE2-K.

A mechanistic kidney pharmacokinetic model was used to assess the effect of OCT1 and OCT2 inhibition by lamivudine on metformin systemic exposure. The simulations suggest that lamivudine is not predicted to perpetrate systemic DDIs with metformin.

No significant changes in lamivudine P_{app} were observed when tested in combination with dolutegravir or abacavir. Changes in lamivudine P_{app} were seen at different pH's, however, it was inconclusive whether this change was related to a pH effect on lamivudine permeability or due to the pH gradient in the apical and basolateral transwell compartments.

5. REFERENCES

None.

MODULE 2.6.5. PHARMACOKINETICS TABULATED SUMMARY

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1. PHARMACOKINETICS: OVERVIEW FOR DOLUTEGRAVIR

Table 1.1 List of Distribution Studies Performed with Dolutegravir

Type of Study	Species	No./Sex/ Group	Method of Administration	Form	Concentration	Duration of Dosing	GLP	Testing Facility	Report No.	Location in CTD
Brain tissue binding	Mouse, Rat	NA	In vitro	A	2 μM	NA	No	GSK	2017N339387	m4.2.2.3

Key:
A = GSK1349572A, the sodium salt form.
NA = Not applicable.

Testing Facility:
GSK = GlaxoSmithKline.

2. PHARMACOKINETICS: DOLUTEGRAVIR - IN VITRO DISTRIBUTION

Table 2.1 Brain Tissue Binding

Test Article: Dolutegravir

Study System: In vitro

Method: Equilibrium dialysis followed by LC/MS/MS analysis

Species (Strain)	Test System	Conc. Tested (μM)	% Unbound[a]	% Recovered[a,b]	Report No.	Location in CTD
Mouse (FVB) (n=5)	Brain homogenate	2	5.91	72.9	2017N339387	m4.2.2.3
Rat (Sprague Dawley) (n=5)	Brain homogenate	2	7.21	80.9	2017N339387	m4.2.2.3

Additional Information:
Stock solutions were prepared in DMSO.
a = The % Unbound and % Recovered values are an average of all test values.
b = % recovered after 4 hours of dialysis.

3. PHARMACOKINETICS: OVERVIEW FOR LAMIVUDINE

Table 3.1 List of Distribution Studies Performed with Lamivudine

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
Transporter inhibition assays with OATP1B1, OATP1B3 and MATE2-K	HEK293 cells expressing human transporters	NA	In vitro	X	0.01 to 300 μM	NA	No	[redacted]	2015N234461	m4.2.2.3
Membrane permeability	Caco-2 cells	NA	In vitro	X	0.006, 0.03, 0.06, 0.6, 1.2 and 2.4 mg/mL	NA	No	GSK	2018N376755 (17DMM040)	m4.2.2.3

Key:
X = Parent form.
HEK = Human embryonic kidney.
MATE = Multidrug and toxin extrusion transporter.
NA = Not applicable.
OATP = Organic anion transporting polypeptide.

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

Table 3.2 List of Pharmacokinetic Drug Interaction Studies Performed with Lamivudine

Type of Study	Species (Strain)/ Test System	No./Sex/ Group	Method of Administration	Dose	Duration of Dosing (Sampling Occasions)	GLP	Testing Facility	Report No. (Study No.)	Location in CTD
Drug interactions - Computer modelling	Human	NA	In silico	100 mg IV 100, 150 & 300 mg orally	Multiple	No	GSK	2017N352403	m4.2.2.6

Key:
IV = Intravenous.
NA = Not applicable.

Testing Facility:
GSK = GlaxoSmithKline.

4. PHARMACOKINETICS: LAMIVUDINE - IN VITRO DISTRIBUTION

Table 4.1 In Vitro Evaluation as an Inhibitor of Human OATP1B1, OATP1B3 and MATE2-K Transporters

Name of Company: [redacted] **Name of Finished Product:** 3TC **Name of Active Ingredient:** Lamivudine (GR109714X)	**Tabulated Study Report**	**Location in CTD:** m4.2.2.3
Report Date: May 2015 Number: 2015N234461 Study Period (Years): 2014-2015		
Test Article: GR109714X **Test System:** HEK293 cells expressing OATP1B1, OATP1B3 or MATE2-K **Method of Administration:** In vitro		
	OATP1B1, OATP1B3	MATE2-K
Test Article (μM)	0.01, 0.03, 0.1, 0.3, 1, 3, 10, 30, 100 and 300	0.01, 0.03, 0.1, 0.3, 1, 3, 10, 30, 100 and 300
Probe Substrate	[^{3}H]E2G	[^{14}C]Metformin
Positive Control Inhibitor	Rifampicin & Cyclosporine A	Cimetidine
Nominal Cell Number Per Well	3.0 to 3.1 × 10^{5}	3.5 to 3.6 × 10^{5}
Volume Per Well (μL)	300	500
Pre-Incubation Time (min)	15	15
Test Article Incubation Time (min)	2	5
Positive Control Incubation Time (min)	2	5
Incubation Temperature (°C)	37	37
Probe Substrate Analysis Method	LSC	LSC
Additional Information: In vitro, lamivudine (0.01 to 300 μM) did not inhibit the human hepatic uptake transporters OATP1B1 and OATP1B3 or multidrug and toxin extrusion transporter MATE2-K.		
Study Conducted by the Applicant: **No** Study in Compliance with GLP: **No**		

Table 4.2 Summary of Lamivudine Passive Permeability in Caco-2 Cells

Passive Permeability in Caco-2 Cells

Test Article: Lamivudine (GR109714) **Report No.:** 2018N376755 **Location in CTD:** m4.2.2.3

Study System:	In vitro human epithelial colorectal adenocarcinoma Caco-2 cells (Sigma-Aldrich® Catalogue Number MTOX1000P24, C2BBe1 cells, a subclone of WT Caco-2 cells).
Method:	Cells were pre-incubated for 30 minutes in Hank's Balanced Salt Solution (HBSS) in both the apical and basolateral compartments. The test compound (at various concentrations) in HBSS was dosed into the apical side of triplicate wells for apical-to-basolateral (A→B) assessment. Samples were removed from the donor and receiver compartments after a 120 minutes incubation (37°C) and analysed for test compound concentration by UPLC-MS/MS.
Analysis:	UHPLC-MS/MS

The Effect of pH on the Passive Permeability of Lamivudine in Caco-2 Cells

Compound	Rate A→B (nmoles/h/cm²)	A→B Mass Balance (%)	P_{app} (nm/s) A>B
Lamivudine 1.2 mg/mL pH 7.4	36 ± 12	110 ± 9.4	19 ± 5.5
Lamivudine 1.2 mg/mL pH 6.5	75 ± 10	110 ± 3.4	42 ± 6.8
Lamivudine 1.2 mg/mL pH 5.5	110 ± 3.7	110 ± 5.1	61 ± 2.0
Atenolol 10 μM	0.047 ± 0.028	110 ± 4.6	12 ± 6.8
Metoprolol 10 μM	0.84 ± 0.049	92 ± 4.2	340 ± 27

Additional Information:
Data presented are the mean of 3 replicates unless otherwise noted.

The Effect of Co-Dosing Abacavir and Lamivudine on the Passive Permeability of Lamivudine in Caco-2 Cells

Compound	Rate A→B (nmoles/h/cm²)	A→B Mass Balance (%)	P_{app} (nm/s) A>B
Lamivudine 0.6 mg/mL + abacavir 1.2 mg/mL (0.5 x clinical dose)	5.8 ± 1.1	110 ± 7.4	5.8 ± 1.5
Lamivudine 1.2 mg/mL + abacavir 2.4 mg/mL (1 x clinical dose)	7.0 ± 2.6	100 ± 5.8	3.5 ± 1.4
Lamivudine 2.4 mg/mL + abacavir 4.8 mg/mL (2 x clinical dose)	9.8 ± 7.2	100 ± 3.3	2.9 ± 2.1
Atenolol 10 μM	0.0095 ± 0.0013	110 ± 4.3	2.4 ± 0.38
Metoprolol 10 μM	0.79 ± 0.040	91 ± 4.2	330 ± 28

Additional Information:
Data presented are the mean of 3 replicates unless otherwise noted.

Summary of Lamivudine Passive Permeability in Caco-2 Cells (Continued)

The Effect of Co-Dosing Dolutegravir and Lamivudine on the Passive Permeability of Lamivudine in Caco-2 Cells			
Compound	**Rate A→B (nmoles/h/cm²)**	**A→B Mass Balance (%)**	**P_{app} (nm/s) A>B**
Lamivudine alone 0.06 mg/mL (0.05 x clinical dose)	0.83 ± 0.74	100 ± 3.6	10 ± 8.7
Lamivudine 0.006 mg/mL + dolutegravir 0.01 mg/mL	0.04[a] ± 0.0069	100[a] ± 12	4.2[a] ± 0.96
Lamivudine 0.03 mg/mL + dolutegravir 0.01 mg/mL	0.35 ± 0.068	98 ± 8.4	7.6 ± 1.7
Lamivudine 0.06 mg/mL + dolutegravir 0.01 mg/mL (0.05 x clinical dose; mass ratio retained)	0.50 ± 0.044	110 ± 6.4	5.4 ± 0.62
Lamivudine 0.06 mg/mL + dolutegravir 0.005 mg/mL	0.41 ± 0.12	110 ± 5.6	4.1 ± 1.1
Lamivudine 0.06 mg/mL + dolutegravir 0.001 mg/mL	0.46[b] ± 0.21	120[b] ± 3.5	4.5[b] ± 1.9
Atenolol 10 μM	0.013[c] ± 0.0075	100[c] ± 2.8	3.7[c] ± 2.12
Metoprolol 10 μM	0.76[c] ± 0.11	82[c] ± 5.9	350[c] ± 39

Additional Information:
Data presented are the mean of 6 replicates unless otherwise noted.
a = 4 replicates.
b = 5 replicates.
c = 3 replicates

5. PHARMACOKINETICS: LAMIVUDINE - DRUG-DRUG INTERACTIONS

Table 5.1 Drug-Drug Interactions - Mechanistic Kidney PK Model

Test Article: Lamivudine
Location in CTD: m4.2.2.6

Type of Study (Report No. [Study No.])	Method of Administration	Species (Strain) Test System	Dose (mg/kg)	Noteworthy Findings
Other Pharmacokinetic Studies				
Drug interactions - Mechanistic kidney PK model (SimCYP PBPK simulator) (2017N352403)	In silico	Human	100 mg IV 100, 150 & 300 mg orally	At therapeutic exposures, no change in metformin exposure (exceeding the 1.25X bioequivalence threshold) was predicted with lamivudine. These simulations suggest that lamivudine is not predicted to perpetrate systemic DDIs with metformin.

Key:
DDIs = Drug-Drug Interactions.
IV = Intravenous.
PK = Pharmacokinetic.
PKPB = Physiological based pharmacokinetic.