MODULE 2.4. NONCLINICAL OVERVIEW

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List of Abbreviations

ART	Antiretroviral therapy
AUC	Area under the curve
BDC	Bile duct-cannulated
CAB	Cabotegravir
C_{max}	Maximum observed concentration
FC	Fold change
FDC	Fixed-dose combination
GI	Gastrointestinal
HIV	Human immunodeficiency virus
IM	Intramuscular
INSTI	Integrase strand transfer inhibitor
ISR	Injection site reactions
KLH	Keyhole limpet hemocyanin
LA	Long acting
M1	Cabotegravir glucuronide or GSK3388352
MC4	Melanocortin 4
MRHD	Maximum recommended human dose
NMR	Nuclear magnetic resonance
NNRTI	Non-nucleoside reverse transcriptase inhibitor
NOAEL	No observed adverse effect level
NOEL	No observed effect level
$PAIC_{90}$	Protein-adjusted 90% inhibitory concentration
PBMC	Peripheral bone marrow cells
PBPK	Physiologically-based pharmacokinetics
PICs	Pre-integration complexes
PK	Pharmacokinetics
PND	Postnatal day
PO	Per os (oral)
PPN	Peri- and postnatal
PopPK	Population-based pharmacokinetics
RPV	Rilpivirine
SC	Subcutaneous
TDAR	T cell dependent antibody response

1. OVERVIEW OF THE NONCLINICAL TESTING STRATEGY

1.1. Introduction

Cabotegravir (CAB, GSK1265744) is a novel, potent and selective HIV Integrase Strand Transfer Inhibitor (INSTI) being developed by ViiV Healthcare (ViiV) for the treatment of HIV-1 infection. CAB is being developed as the sodium salt for oral (tablet) administration and as the free acid for a long acting (LA) injectable formulation, to be co-administered with rilpivirine.

Rilpivirine (RPV, TMC278, GSK1329758) is a potent non-nucleoside reverse-transcriptase inhibitor (NNRTI) treatment for HIV-1 infection in antiretroviral treatment-naïve adult patients and is already approved in multiple markets including the United States of America, Europe, Canada and Japan as EDURANT®. An assessment of the nonclinical development program for RPV LA formulation is given in the RPV Nonclinical Overview.

The overall objective of the CAB + RPV clinical program was to develop a novel, highly effective, well tolerated 2-drug LA regimen administered by intramuscular (IM) injection.

1.1.1. Dosing Regimens

1.1.1.1. Every Month Dosing

The proposed CAB + RPV dosing regimen for HIV-1 infected subjects is oral CAB 30 mg once daily + oral RPV 25 mg once daily for approximately 1 month (at least 28 days) followed by initial injections of CAB LA 600 mg IM + RPV LA 900 mg IM and subsequent monthly injections of CAB LA 400 mg IM + RPV LA 600 mg IM. In HIV-1 infected patients, the modeled C_{max} and AUC_{0-24} values for a 30 mg oral CAB dose at steady state were 8.1 μg/mL and 146 μg.h/mL, respectively, using the population pharmacokinetic (PopPK) model and the C_{max} and AUC_{0-t} values for a 400 mg IM monthly CAB LA dose were 4.2 μg/mL and 2461 μg.h/mL, respectively. Exposures achieved in nonclinical toxicity studies were compared to the clinical exposure values (Table 1.1) to determine exposure margins (see Table 4.2 and Table 4.3). For CAB, the clinical exposure (AUC) from the dosing regimen of 1 injection every 2 months was similar to the exposure from the dosing regimen of 1 injection every month (see Table 1.2. Therefore, the toxicology exposure margins for CAB every monthly dosing were comparable when using the clinical exposures from CAB every 2 months dosing.

Table 1.1 CAB Exposure Values Following Administration of Proposed CAB + RPV Regimen in HIV-1-infected Subjects used for Comparison to Nonclinical Exposures

Clinical Study Report	Method of Administration	C_{max} (μg/mL)	AUC_{0-t} (μg.h/mL)
2018N384611	Oral Lead-in [a]	8.1	146
2018N384611	Monthly Injection [b]	4.2	2461

Key:
Mean exposure (C_{max} and AUC_{0-24}) following CAB 30 mg PO once daily (POPPK analysis).
Mean exposure (C_{max} and AUC_{0-t}) month 3 onward following a 400 mg IM monthly dose (POPPK analysis).

Table 1.2 CAB Exposure Values at Steady State Following Administration of Proposed CAB + RPV Regimen in HIV-1-infected Subjects: Comparison Between Injections Every Month and Injections Every 2 Months

Clinical Study Report	Dose Regimen	C_{max} (μg/mL)	AUC_{0-t} (μg.h/mL)
2019N421460	400 mg every month injection	4.32	2484.9[a]
	600 mg every 2 months injection	4.02	3764.1[b]
Key: a = (t=28 days) b = (t=2 months)			

1.1.1.2. Every 2 Months Dosing

The proposed CAB + RPV dosing regimen for HIV-1 infected subjects is oral CAB 30 mg once daily + oral RPV 25 mg once daily for approximately 1 month (at least 28 days) followed by IM injections (one of each) according to the following dosing regimen in Table 1.3.

Table 1.3 Proposed CAB Commercial LA Every 2 Months Dosing Regimen, When Used with RPV

Drug	OLI	Initiation Injection	Second Injection	Subsequent Injections
	1 Month (at least 28 days)	Month 2	Month 3	Month 5
CAB	30 mg once daily	3 mL (600 mg)	3 mL (600 mg)	3 mL (600 mg) every 2 months
RPV	25 mg once daily	3 mL (900 mg)	3 mL (900 mg)	3 mL (900 mg) every 2 months

Key:
OLI = Oral lead in.

1.2. Rationale for the Use of CAB in the Treatment of HIV

Advances in antiretroviral therapies (ART) have led to significant improvements in morbidity and mortality among HIV-infected persons and this has transformed HIV into a chronic manageable disease. Suppression of viral replication is the cornerstone of ART's success and this, in turn, is dependent on a subject's ability to adhere to the prescribed dosing regimen.

Fixed-dose combinations (FDCs) have greatly advanced HIV treatment by allowing simplification of dosing and reducing pill burden. However, incomplete adherence may lead to the emergence of drug-resistant virus that can lead to the loss of virologic control and reverse the benefits of ART. Different HIV treatment modalities are being developed to help improve adherence and subject outcomes, and to prevent the emergence of resistance and transmission of the virus.

There is also an increasing desire to develop NRTI-sparing regimens that avoid long-term NRTI-associated adverse drug reactions, including renal, hepatic, bone, and cardiovascular toxicities. In addition, simplifying treatment has long been a goal to increase treatment compliance and improve the quality of life for subjects with HIV.

1.3. Nonclinical Development Program

Nonclinical studies conducted to support the development of CAB include primary pharmacology (virology) studies which demonstrated the inhibition of integrase activity and HIV-1 replication in vitro as well as studies to determine the potential for HIV resistance to develop via mutations. Secondary pharmacologic activity was assessed, and safety pharmacology studies were conducted to investigate any untoward pharmacologic actions of CAB on the respiratory, cardiovascular, central and peripheral nervous systems.

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

The CAB clinical route of administration is intended to be both oral (by tablet) and parenteral (by IM injection). To support oral administration, CAB was evaluated in repeat dose oral toxicity studies of up to 26 weeks duration in Sprague Dawley rats, 39 weeks duration in cynomolgus monkeys, 13 weeks in CD-1 mice, and 13 days in Dutch-belted rabbits. Reproductive and developmental toxicity studies were conducted using oral administration to Sprague Dawley rats and Dutch-belted rabbits. Genotoxicity, carcinogenicity, and immunotoxicity studies were also conducted as well as skin and ocular irritancy and skin sensitization studies. To support parenteral administration, subcutaneous (SC) and IM studies of up to 13 weeks duration in the rat were also performed.

Two additional single dose IM combination studies were conducted in rats and monkeys to investigate the co-administration of CAB + RPV.

All pivotal safety pharmacology and toxicology studies were conducted in an Organisation for Economic and Cooperation and Development (OECD) member country in accordance with the OECD Test Guidelines and the Principles of Good Laboratory Practice (GLP).

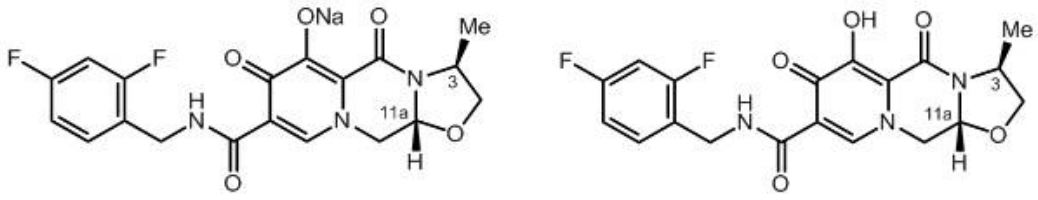
The CAB overview and conclusions are presented in Section 5, and an integrated nonclinical assessment for the co-administration of CAB + RPV is in Section 6.

1.4. Test Material

Except for a 13-week rat SC and IM study, all definitive toxicology, safety pharmacology and genetic toxicology studies were conducted with CAB sodium salt, the form intended for oral administration. The 13-week rat injection study was conducted with CAB free acid, the form intended for parenteral administration. Nonclinical pharmacokinetics studies were conducted using both CAB sodium salt and CAB free acid. The structure of CAB is shown below in Figure 1.1.

Throughout m2.4, reference is made to CAB whether it is administered orally as the sodium salt or administered parenterally as the free acid. Where appropriate, distinction is indicated.

Figure 1.1 Structure of CAB (sodium salt and free acid)



1.5. Nonclinical Studies not Included in Module 4

An overview of the nonclinical studies for CAB is presented below and the nonclinical study reports are provided in Module 4. The justification for the Module 4 sections for which no nonclinical study reports are available is discussed in Appendix 1.

2. PHARMACOLOGY

2.1. Virology (Primary Pharmacodynamics)

A range of nonclinical virology studies have been conducted with CAB to determine the mechanism of action, antiviral activity, and the potential for development of drug-resistance via mutations. These studies are described in more detail in m2.7.2.4. A brief overview of the key findings from these studies is also provided below.

2.1.1. Mechanism of action

Integration of viral DNA into the host chromosome is a necessary process in the HIV replication cycle. The key steps of DNA integration are carried out by the viral integrase, which, along with protease and reverse transcriptase (RT), is 1 of 3 enzymes encoded by HIV. Integrase is an attractive target for HIV therapy because it is essential for HIV replication [De Clercq, 2004]. The primary role of integrase is to catalyze the insertion of the viral cDNA into the chromosome of infected cells. Integration, catalyzed via integrase, requires 2 metal-dependent consecutive steps in the viral replication cycle: 3'-processing and strand transfer. Viral cDNA is primed for integration in the cytoplasm by integrase-mediated trimming of the 3'-ends of the viral DNA. Integrase remains bound to the viral cDNA ends in the pre-integration complexes (PICs). Following nuclear translocation of the PICs, integrase catalyzes the insertion of the viral cDNA ends into the host chromosomes. Following integration of viral cDNA into a chromosome, viral genome is transcribed, and viral proteins are produced. HIV integrase strand transfer inhibitors (INSTI), such as CAB, preferentially block the strand transfer step [Pommier, 2005].

2.1.2. Antiviral activity and potential resistance

CAB is a potent in vitro inhibitor of HIV integrase and inhibits the integrase catalyzed viral DNA strand transfer with IC_{50} values in the nanomolar range (3.0 to 13 nM). CAB is a potent antiviral agent when tested in a variety of in vitro assays against the HIV-1 strains Ba-L and NL432 in human peripheral blood mononuclear cells (PBMC; IC_{50} = 0.22 and 0.53 nM, respectively), and against the HIV-1 strain IIIB in MT-4 cells (IC_{50} = 0.57 and 2.1 nM, respectively) and in the PHIV assay (IC_{50} = 0.74 nM). Additionally, the IC_{50} values of CAB for viral replication of National Institute of Health (NIH) reference strains consisting of 24 strains of HIV-1 and 4 strains of HIV-2 in PBMC assays and for 3 HIV-1 strains in monocyte-derived macrophage assays were also in the low nanomolar concentration range, like that seen with HIV-1 strains Ba-L and NL432. In vitro studies suggested an approximate average of 408-fold shift in IC_{50} of CAB in the presence of 100% human serum (by method of extrapolation), and the PA-IC_{50} was estimated to be 102 nM in HIV-1 IIIB infected MT4 cells. In vitro cytotoxicity studies provided a selectivity index of ≥10,000 for CAB compared with the HIV-1 antiviral potency in PBMCs. In a viral integrase susceptibility assay using the integrase coding region from 13 clinically diverse subtype B isolates, CAB demonstrated antiviral potency like that observed for laboratory strains, with a mean IC_{50} of 1.3 nM.

CAB showed anti-HIV activity (susceptibility) equivalent to wild type virus (fold change [FC] <5) against 22 of 25 INI-resistant mutant viruses with single mutations. Of the 17 INI-resistant mutant viruses with 2 or more mutations, 8 showed susceptibility to CAB (FC<5). Exposure of MT-2 cells infected with HIV-1 IIIB to CAB for up to 112 days did not produce any highly resistant mutants (>10-fold increase in IC_{50}). No amino acid substitutions in the integrase region were selected when passaging the wild type HIV-1 NL-432 in the presence of 6.4 nM CAB through 56 days [see m2.7.2.4 for a complete summary].

2.2. Secondary Pharmacology

CAB was generally inactive against a panel of enzymes, receptors, ion channels, transporters, and functional tissue assays [Report RH2007/00209]. The only effect greater than 50% was a 53% inhibition of binding in the melanocortin-4 (MC4) receptor binding assay. There were no abnormalities in body weights or food consumption in monkey toxicity studies up to 39 weeks except in animals with severe GI intolerability at the highest dose (1000 mg/kg/day). No abnormalities in body weights or food consumption were noted in rat toxicity studies up to 26 weeks. No other findings associated with MC4 receptor agonism or antagonism have been observed in toxicity studies with CAB, indicating a lack of apparent biological activity at the MC4 receptor.

Therefore, CAB is considered a potent and selective integrase inhibitor (see Section 2.1) and is unlikely to have any significant off-target pharmacological activity.

2.3. Safety Pharmacology

As part of a rat 14 day oral toxicity study [m2.6.3 Table 4.1, Report RD2006/01741], the first 5 male rats from each dosing group given 30, 100 or 300 mg/kg/day of CAB were assessed for effects on neurobehavioral function (functional observational battery) reflecting central and peripheral nervous system activity. No effects occurred throughout the assessment interval of up to 25 hours following dosing on Day 5. Systemic exposure, C_{max} and AUC_{0-24}, in male rats given a single oral dose of 300 mg/kg was 35.8 μg/mL and 708 μg.h/mL, respectively (Day 1 systemic exposure data in males from the rat 14 day oral toxicity study).

In a respiratory safety pharmacology study, single oral doses of CAB at 30, 100 or 300 mg/kg were administered to male rats (Latin square crossover design with 7 days between doses) [m2.6.3 Table 4.1, Report CD2007/00268]. CAB did not produce any effect on respiratory functional parameters (respiratory rate, tidal volume, minute volume, pulmonary resistance) or body temperature when monitored throughout the day of dosing and up to 7 days after dosing. A dose of 300 mg/kg was associated with a mean C_{max} and AUC_{0-24} of 35.8 μg/mL and 708 μg.h/mL, respectively (Day 1 systemic exposure data in males from the rat 14 day oral toxicity study).

The effect of CAB at concentrations of 5.28 μM and the maximum soluble concentration of 17.61 μM (2.14 and 7.14 μg/mL, respectively) on hERG tail current was studied in HEK-293 cells which had been stably transfected with hERG cDNA [m2.6.3 Table 4.1,

Report FD2007/00242]. No difference from vehicle was seen at either concentration of CAB indicating that there was no effect on hERG channel tail current.

In a cardiovascular study, single oral doses of CAB were administered at either 8, 25, or 1000 mg/kg to conscious, non-restrained male monkeys (Latin square crossover design with 7 days between doses) [m2.6.3 Table 4.1, Report CD2007/00707]. A single oral dose of CAB at 1000 mg/kg (for exposure comparison, C_{max} was 67.0 µg/mL and AUC_{0-24} was 1051 µg.h/mL on Day 1 in males in the monkey 14 day oral toxicity study) produced a mild, transient increase in mean arterial pressure (3.7 to 8.6%) and a transient increase in heart rate (16 to 23%) during the first 2 hours after dosing. No ECG waveform or interval changes were associated with these pressure and heart rate changes and no effects occurred at doses of 8 and 25 mg/kg (for exposure comparison at 25 mg/kg, C_{max} was 20.8 µg/mL and AUC_{0-24} was 233 µg.h/mL on Day 1 in males in the monkey 14 day oral toxicity study). In addition, following repeat dosing in male and female monkeys at the same doses in the 14 day toxicity study, CAB produced no effects on ECG tracings [see m2.6.7 Table 7.6].

There were no findings from safety pharmacology studies that would indicate an unacceptable risk for oral or IM administration of CAB to patients when administered in accordance with the proposed indication. Additionally, a supratherapeutic dose of CAB (150 mg once every 12 hours for a total of 3 doses) was well tolerated and had no effect on cardiac repolarization [see m2.5].

3. PHARMACOKINETICS

An extensive program of studies investigating the absorption, distribution, metabolism and excretion was conducted with CAB in the same species used in toxicity studies. The systemic exposure and metabolism defined in the animal species used for toxicological assessment indicates that the species used were appropriate for predicting the safety of CAB and its metabolites in humans. A summary of each pharmacokinetics study and its findings is presented in m2.6.4, Pharmacokinetics Written Summary and tabular summaries, along with data, are presented in m2.6.5, Pharmacokinetics Tabulated Summary. A table listing of all pharmacokinetics studies is provided in both m2.6.4 and m2.6.5. All pharmacokinetics study reports are available in m4.2.2, Nonclinical Study Reports.

3.1.1. Analytical methods and validation

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

In investigations where [^{14}C]-CAB was used, determination of the radioactivity in in vitro or in vivo biological samples was carried out by either direct liquid scintillation counting (LSC) or by LSC following combustion of the sample. For analysis of radioactivity concentrations in tissues, quantitative whole-body autoradiography was used. The profiling and identification of metabolites of CAB was performed using LC-MS^n and nuclear magnetic resonance (NMR).

3.1.2. Absorption and pharmacokinetics

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

3.1.2.1. Single dose

Intravenous: Following a single intravenous administration, CAB (free acid) exhibited low plasma clearance in the rat, dog and monkey (<2% liver plasma flow in dog and monkey) [m2.6.5 Table 3.1, Reports RH2007/00168, RH2007/00169 and RH2007/00171]. The low steady state volume of distribution is consistent with the high protein binding of the compound (Section 3.1.3.1). The terminal half-life in dogs and monkeys was 4.0 to 5.7 hours [m2.6.5 Table 3.1]. The sampling regimen in the rat was insufficient to the characterize the pharmacokinetic parameters; however, the CAB concentration time profile was indicative of a lower clearance and longer half-life than in dogs and monkeys.

Subcutaneous and Intramuscular: Following subcutaneous (SC) or intramuscular (IM) administration of CAB (free acid) to rats [m2.6.5 Table 3.6, Reports RH2009/00012, RH2009/00013] and monkeys [m2.6.5 Table 3.9, Reports CD2009/00373, CD2009/00513], the compound was slowly released from the injection sites. In general, there was a trend for higher C_{max} values following IM injection and longer apparent half-life values following SC injection. The time above the $PAIC_{90}$ (protein binding adjusted 90% inhibitory concentration) was generally longer following SC injection than following IM injection. In general, no notable differences in the AUC values between the two routes of administration were observed.

A series of pharmacokinetic and toxicokinetic studies have been conducted with CAB (free acid) to evaluate the effects of particle size, vehicle, and route of administration of the long acting formulation to rats and monkeys [m2.6.5 Table 3.6 and Table 3.9, Reports RH2009/00013, CD2009/00373 and CD2009/00513]. In these studies, the systemic exposure to CAB was evaluated following SC and IM administration using suspension formulations in which the particle size x90 ranged from 1.05 to 68.9 microns. In rats, no consistent notable (>2-fold) differences in the dose normalized C_{max} or T_{max} values between the small and large particle sizes were observed. In monkeys, the larger particle size formulations resulted in consistently lower C_{max} values after SC injection at doses up

to 10 mg/kg (no notable difference in the C_{max} values after IM injection) and longer T_{max} values after SC and IM injections.

CAB and RPV were administered IM to rats and monkeys alone and in combination. In rats, the pharmacokinetic parameters of each agent were similar when co-administered compared with the individual agent alone. In monkeys, the exposure to RPV was 65% higher when administered in combination with CAB but this was attributed to a faster initial release rate from the RPV formulation. The C_{max} of CAB was higher (less than 2-fold) following administration of the combination compared to administration alone whereas the AUC_{0-t} was not notably different [m2.6.5 Table 3.12, Reports 2011N127517 and 2010N105579].

Oral: CAB (free acid) absorption from an oral solution was rapid, reaching peak plasma concentrations within 4 hours with a moderate to high oral bioavailability (44 to 83%) in fasted rats, dogs and monkeys [m2.6.5 Table 3.2, Reports RH2007/00168, RH2007/00169 and RH2007/00171]. When CAB was administered as a suspension, the increase in systemic exposure (C_{max} and AUC_{0-t}) was less than proportional to the increase in dose. The oral bioavailability of CAB following suspension or capsule formulation of free acid or sodium salt was lower (bioavailability range of 3% to 27%) and suggested that the absorption is limited by dissolution rate or solubility. At least 2% of dosed CAB was absorbed in mouse and rat (dosed at 10 mg/kg) and 29% in monkey (dosed at 30 mg/kg), as determined by the amount of [^{14}C]-drug-related material recovered in bile and urine in the BDC animals [m2.6.5 Table 13.1, Reports 2012N145873, 2012N143605 and 2013N174861].

3.1.2.2. Repeat dose toxicokinetics

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

The increase in systemic exposure (C_{max} and AUC_{0-24}) to CAB was less than proportional with the increase in dose during repeated oral administration toxicity studies in all species. Differences (>2-fold) in systemic exposure between single and repeated administration, regardless of pregnancy status [m2.6.7 Table 14.3, Report 2017N311115], or between the sexes were generally not observed. In mice and monkeys, no notable difference (>2-fold) in systemic exposure was observed between single and repeated administration, consistent with expectations based on the apparent half-life. However, in rats, exposures following repeat administration were generally greater than those after single administration consistent with the apparent lower clearance.

Subcutaneous and Intramuscular Administration: In a 13 week SC and IM injection toxicity study [m2.6.7 Table 7.2, Report 2010N104820], male and female rats were administered CAB (free acid) by SC injection at 5, 30, or 100 mg/kg/dose, or by IM injection at 2.5, 10, or 75 mg/kg/dose, on Days 1, 31, and 61. The C_{max} and AUC values along with the comparative animal to human exposure ratios are presented in Table 4.3. The systemic exposure during the third monthly SC dose interval increased less than proportionally to the increase in dose. The systemic exposure during the third monthly

IM dose interval increased in approximate proportion from the low to the mid dose and less than proportionally at the high dose.

The mean AUC values during the third monthly SC dose intervals ranged from 2- to 3-fold higher than those during the first monthly dose intervals, whereas the increase in AUC values over this timeframe following monthly IM injections was minimal (1.1- to 1.4-fold). This suggests that the apparent elimination half-life was shorter from the IM injection site than from the SC site.

No notable (>2-fold) differences in the CAB mean C_{max} or AUC values between the sexes were observed.

3.1.2.3. In Vitro Absorption

HIV INSTIs are known to chelate with polyvalent cations, resulting in decreased absorption. As CAB has also demonstrated chelation with metal cations [see m2.6.5 Table 3.13, Report 2019N401615], dose separation is required when CAB is dosed with cation antacids [see m2.7.2].

3.1.3. Distribution

CAB has high passive membrane permeability, is highly protein bound and is widely distributed and crosses the placental barrier. The glucuronide metabolite of CAB (also referred to as CAB glucuronide, M1 or GSK3388352) exhibited low plasma protein binding.

3.1.3.1. Protein binding and blood cell association

The in vitro protein binding of CAB in serum or plasma was high (>99%) across species (rat, monkey and human [m2.6.5 Table 6.1, Reports RH2007/00222, RH2007/00193 and 2015N235936]) consistent with the ex vivo unbound fraction of <1% in plasma from healthy and renally or hepatically impaired human subjects [Clinical Study Reports 2016N273991 and 2016N274024]. In vitro protein binding in human was independent of CAB concentrations over the range of 1000 to 20000 ng/mL, but a lower unbound fraction of 0.47% (99.5% bound) was observed at 500 ng/mL. The association of CAB-related material with blood cellular components was minimal in both nonclinical species and human ranging from 0.44 to 0.57 [m2.6.5 Table 8.4, Reports 2012N145873 2012N143605, 2013N179556]. The in vitro protein binding of CAB glucuronide was low (≤33%) in rat and human plasma [m2.6.5 Table 6.2, Report 2017N333514].

3.1.3.2. Permeability and transport

CAB was determined to have high passive membrane permeability (256 nm/s at pH 7.4). The absorptive membrane permeabilities also were high in the presence of fasted state simulated intestinal fluid (FaSSIF) at pH 7.4 and pH 5.5 ($P_{7.4[abs]}$ value of 1088 nm/s and a $P_{5.5[abs]}$ value of 1374 nm/s, respectively). In vitro, CAB was a substrate for the human efflux transporters P-glycoprotein (Pgp) and human breast cancer resistance protein [see m2.6.5 Table 8.3, Reports 2012N146040 and 2012N155942]. CAB was not a substrate

of hepatic uptake transporters such as OATP1B1, OATP1B3, OATP2B1 or OCT1 based on studies in cryo-preserved pooled human hepatocytes [m2.6.5 Table 8.4, Report 2018N391028].

CAB glucuronide was not a substrate for Pgp or BCRP [m2.6.5 Table 8.6, Report 2016N288197]. The canalicular and basolateral clearance of CAB glucuronide were comparable and found to be 0.16 and 0.21 mL/min/kg, respectively [m2.6.5 Table 10.10, Report 2017N320633]. In vitro, CAB glucuronide was shown to be a substrate for multiple hepatic and renal transporters (OATP1B1, OATP1B3, OAT3, MRP2, MRP3 and MRP4), but was not a substrate for OAT1 or OAT4 [m2.6.5 Table 8.6, Report 2016N298553].

Based on in vitro data, circulating CAB permeates passively into hepatocytes and is metabolized to the glucuronide (see Metabolism Section 3.1.4) which undergoes both biliary and sinusoidal excretion. Biliary excretion of CAB glucuronide is mediated by MRP2, while hepatic basolateral excretion into sinusoidal blood was via both MRP3 and MRP4. Although CAB glucuronide is a substrate of the hepatic uptake transporters OATP1B1 and OATP1B3, metabolite clearance by hepatic uptake from the systemic circulation is low (2.7% of hepatic blood flow) [m2.6.5 Table 8.8, Report 2017N320631]. Instead, circulating CAB glucuronide undergoes efficient renal clearance, where uptake into the proximal tubule is mediated by OAT3 and subsequent secretion into urine by MRP2 and MRP4, which would explain the minimal systemic exposure in human.

3.1.3.3. Tissue distribution

After a single oral dose of [^{14}C]-CAB to Lister Hooded male rats, radioactivity was slowly absorbed and widely distributed with most tissues exhibiting their highest concentrations at 1 day post-dose. Except for the gastrointestinal tract, tissue concentrations were less than those in blood at all sampling times [m2.6.5 Table 5.1, Report RD2008/00840]. The concentration of radioactivity in the brain was low (~2% of the blood radiocarbon concentration) due in part to restrictive protein binding. Elimination of radioactivity was slow with most tissues containing measurable levels of radioactivity at 28 days post dose. Radioactivity was not associated with melanin in the uveal tract. In pre- and post-natal rat studies CAB crossed the placental barrier and the concentrations observed in F_1 pups on post-natal day 10 suggested CAB was secreted in the maternal milk. Additionally, fetal CAB concentrations increased proportionally with the increase in maternal CAB plasma concentrations and there was no evidence for the preferential accumulation of CAB within the fetuses following repeated oral administration of CAB to the dams [m2.6.7 Table 14.2 and Table 14.3, Reports 2016N281797 and 2017N311115].

3.1.4. Metabolism

The comparative biotransformation pathways between humans and the nonclinical species are presented in Figure 3.1. The predominant (representing >90% of the plasma radioactivity) circulating component in the nonclinical species was unchanged CAB. In humans [see m2.7.2], CAB was the only radiolabelled component observed in the plasma, however, subsequent analysis of samples from a clinical study where

non-radiolabelled CAB was administered orally detected very low circulating concentrations of CAB glucuronide. The main metabolic clearance route in the nonclinical species and humans was by conjugation to form CAB glucuronide. The metabolic pathways in animal species used for toxicology testing were relevant to the safety assessment of CAB for human use.

3.1.4.1. In vitro biotransformation

The in vitro metabolic stability of CAB was high, indicating low intrinsic clearance consistent with the low plasma clearance [m2.6.5 Table 10.1, Report RH2007/00183]. Following 24 hours incubation of CAB (10 μM) with cryopreserved rat, dog, monkey and human hepatocytes no metabolites were observed [m2.6.5 Table 10.3, Report RH2007/00188]. A subsequent investigation with cryopreserved rat, monkey and human hepatocytes using a CAB concentration of 50 μM generated a single metabolite, CAB glucuronide [m2.6.5 Table 10.4, Report RD2008/00073]. CAB was shown to form CAB glucuronide using pooled human liver, kidney and intestinal microsomes and recombinant hUGT1A1 and hUGT1A9 in vitro [m2.6.5 Table 10.7 and Table 10.9, Reports RH2007/00166 and 2014N222268]. Scaling the in vitro data indicated that liver was the primary organ responsible for the formation of CAB glucuronide.

The generation of a glutathione conjugate through oxidative defluorination with rat, monkey and human (but not dog) liver microsomes suggested evidence for the formation of an electrophilic metabolic intermediate by bioactivation in vitro [m2.6.5 Table 10.6, Report RH2007/00164]. This was supported by the in vitro covalent binding of CAB associated radioactivity to liver microsomes, which was moderate in human and high in rat and monkey (180, 984 and 794 pmol-eq/mg of protein/h, respectively) [m2.6.5 Table 10.2, Report RD2007/01629]. However, in vivo in mice, rats, monkeys and humans, metabolic products produced via this pathway represented only a very small fraction of CAB metabolic clearance [m2.6.5 Table 8.10 and Table 9.1, Reports RD2008/00021, 2012N146427, 2012N146480 and 2013N171159]. In addition, no microscopic liver findings were observed in mice, rats, or monkeys after repeat administration of doses of CAB at or below the NOAEL. Based upon these data and the premise that in vitro levels of covalent binding to human liver microsomes ≥200 pmol-eq/mg of protein/h are considered an indicator of the potential for hepatotoxicity when compounds are dosed at ≥100 mg/day [Sakatis, 2012], the risk of hepatotoxicity in humans caused by metabolic activation of CAB, when administered at clinically relevant doses, is considered to be low.

No notable metabolic conversion of CAB to any of its possible stereoisomers occurred in vitro following incubations of CAB with cryopreserved rat, dog, monkey and human hepatocytes [m2.6.5 Table 10.5, Report RH2007/00207].

3.1.4.2. In vivo biotransformation

In vivo, absorbed [^{14}C]-CAB underwent minimal metabolism in male mice, rats and monkeys. A comparative metabolic summary of the products identified in the nonclinical species with products identified in humans is presented in Figure 3.1 [m2.6.5 Table 9.1, Reports 2012N146480, RD2008/00021, 2012N146427 and 2013N171159; see m2.7.2,

Reports 2011N115920, 2012N143472 and 2013N167401]. Metabolic profiles in nonclinical species were qualitatively similar to humans.

Metabolic profile

CAB was the predominant component in plasma of mice, rats, monkeys and humans with CAB glucuronide being the only metabolite observed [see m2.6.5 Table 9.1]. No metabolite was present in the plasma at concentrations above the quantifiable limit (i.e. 5% of parent or drug related material). In humans at steady state, CAB was the only quantifiable drug related material in the plasma; this was the same following single oral administration (and that following intramuscular or subcutaneous administration), indicating data obtained after single dose administration was an adequate predictor of the profile at steady state. Following the oral administration of CAB in combination with rifampin, plasma concentrations of CAB-glucuronide increased by a mean of 1.5-fold (maximum 2.1-fold) as compared to the levels when CAB was administered alone [see m2.7.2].

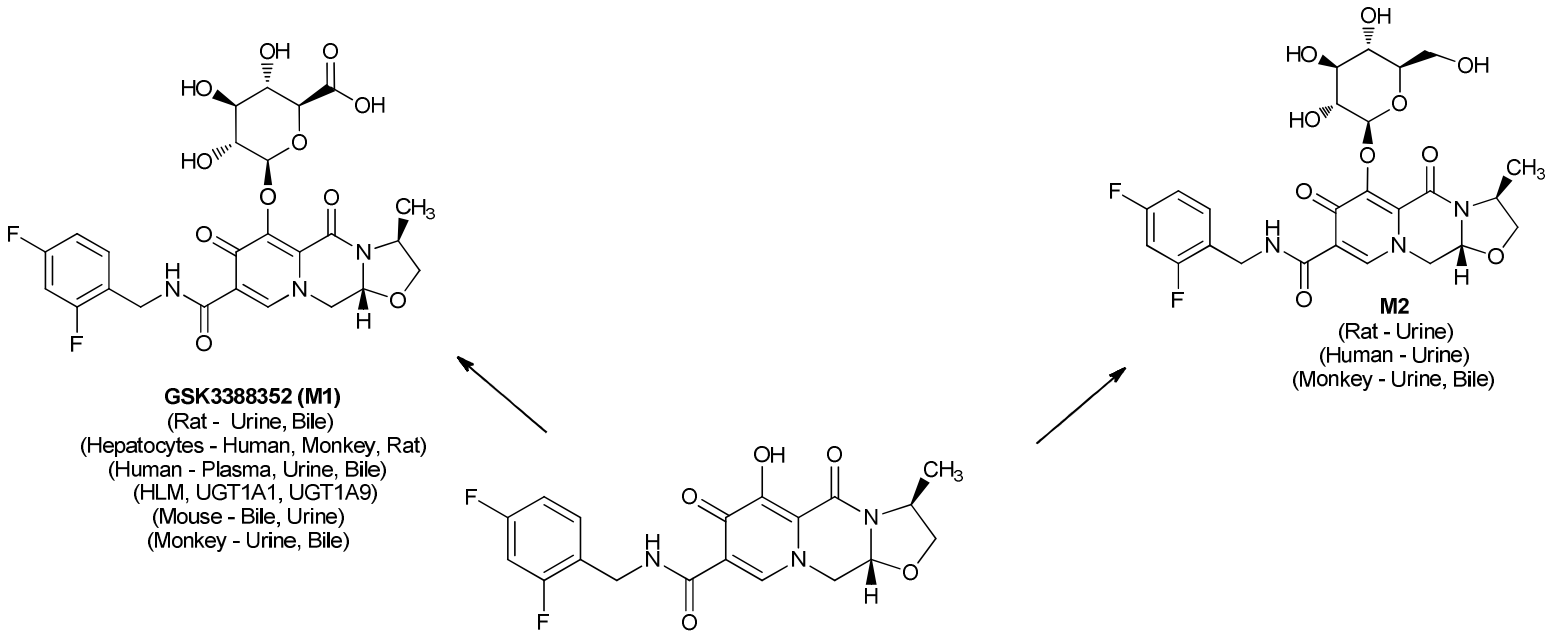
The predominant biotransformation product in mice, rats, monkey and humans was CAB glucuronide (M1), and a glucose conjugate (M2) was also observed (except mouse). These conjugated metabolites, CAB glucuronide and M2, are not pharmacologically active because they disrupt the two-metal binding capability of the carbamoyl pyridone motif of CAB thereby completely abrogating any antiviral activity resulting from the active site binding to the integrase enzyme. Although conjugates were the primary constituents of the drug-related material in bile of the nonclinical species and observed in human bile, only unchanged CAB was observed in the feces of all species. Thus, these CAB conjugates are likely deconjugated in the intestine by host or bacterial enzymes after secretion in the bile to reform CAB.

CAB was not quantifiable in the bile collected from any species investigated, or the urine from mice, monkeys or humans, while on average CAB accounted for <10% of the drug-related material recovered in rat urine [see m2.6.5 Table 9.1]. The only quantifiable metabolite observed in human urine was CAB glucuronide and this was also the predominant metabolite observed in the urine of the nonclinical species. Other minor metabolites that were observed in urine from humans and the nonclinical species were formed by glucose conjugation (M2), oxidative defluorination and cysteine conjugation (M3), pentose conjugation (M4) and oxidation (M6). Metabolite M2 and a metabolite formed by oxidative defluorination and glutathione conjugation (M5) were also observed in mouse and monkey bile.

In mice, monkeys and humans, oxidative defluorination with glutathione or cysteine addition was present, indicating the formation of an electrophilic arene oxide intermediate. In all species, these products were a small fractional part (<5%) of the overall clearance [m2.6.5 Table 9.1].

Following repeat oral administration of CAB for 14 days to healthy human volunteers, no evidence for the in vivo epimerization of CAB to any of its stereoisomers was observed [m2.6.5 Table 9.2, Report RD2008/01340].

Figure 3.1 Metabolic Profile of CAB (GSK1265744)



M2 is the preferred structure based on metabolic precedence and comparison with M1.
M3 (+cysteine, +O, -F) and M4 (+pentose) were also detected in human urine (<1% dose).
Two other minor metabolites: M5 (+glutathione, +O, -F) and M6 (+ O) were observed that were specific to the nonclinical species.
HLM = Human liver microsomes.

3.1.5. Excretion

Following oral administration of [^{14}C]-CAB, only unchanged CAB was observed in the feces and was the predominant route of elimination of administered radioactivity in all species [(see Table 3.1 below), also m2.6.5 Table 13.1, Reports 2012N146480, 2012N146427, 2013N171159 and 2013N167401]. Following oral administration of [^{14}C]-CAB, urinary excretion of radioactivity was greater in humans (approximately 27%) than in the nonclinical species (≤11.1%).

Excretion of administered radioactivity was essentially complete in all species. The radiolabel location was metabolically stable with no notable sequestration or covalent binding of CAB to plasma or excreta. Biliary excretion in mice and rats accounted for 1.60 to 1.79% of the dose and urine accounted for 0.33 to 0.42% of the dose while in the monkey 14.5% of the dose was eliminated in bile and 14.7 % in urine [see m2.6.5 Table 13.1, Reports 2012N145873, 2012N143605 and 2013N174861]. The metabolite, CAB glucuronide, was the major drug-related component observed in both the urine and the bile but was not observed in the feces. Therefore, CAB glucuronide is deconjugated in the intestine, after secretion in the bile, to reform CAB.

Table 3.1 Elimination of Drug-Related Material (Mean % Dose) Following Oral Administration of [^{14}C]-CAB

Matrix	Mouse		Rat		Monkey		Human
	Intact	BDC	Intact	BDC	Intact	BDC [b]	Intact
Urine	0.81	0.42	0.31	0.33	11.1	14.7	26.8
Feces	94.0	93.5	94.5	89.1	78.7	54.6	58.5
Bile	NA	1.79	NA	1.6	NA	14.5	NA
Total [a]	94.8	96.4	94.9	91.1	90.1	84.1	85.3

Key: Data shown are means, except for BDC monkeys as noted.
a = Includes, as appropriate, radioactivity recovered in cage rinses/washes/wipes/debris GI tract and residual carcass.
b = Values are from a single animal.
BDC = Bile duct cannulated.
NA = Not applicable.

3.1.6. Pharmacokinetic drug interactions

No nonclinical studies have been performed specifically to evaluate potential interactions with drugs that may be co-administered with CAB. However, a series of in vitro studies conducted to evaluate the clearance mechanisms and drug interaction potential of CAB have shown a low propensity for victim or perpetrator of drug interactions.

3.1.6.1. Potential effect of co-administered agents on CAB

In vitro and in vivo, CAB is primarily metabolized by UGT1A1 (fraction of total clearance in vitro 0.67) with a contribution from UGT1A9 (fraction of total clearance in vitro 0.33) [m2.6.5 Table 10.8, Report 2012N145430]. In clinical studies, co-administration of CAB and the weak to moderate UGT inducers rifabutin [Study 205712, Report 2017N334381] and etravirine [Study ITZ111839, Report RM2009/00483] to healthy human subjects did not reduce plasma CAB concentrations to a clinically relevant extent [see m2.7.2]. However, rifampicin, a known strong inducer of UGT, caused a significant reduction in the exposure of CAB [m2.7.2, Study LAI117010, Report 2015N263476]. Co-administration of CAB with strong inducers of UGT is therefore contraindicated. Oxidation of CAB accounted for <1% dose in human therefore CAB is not subject to CYP-mediated interactions.

No clinical studies have been performed to measure the impact of inhibitors of UGT1A1 and UGT1A9 on the exposure of CAB, however, a CAB PBPK model [see m2.7.2, Report 2018N389974] was developed to predict the extent of drug interaction with UGT1A1 or UGT1A9 inhibitors. The predicted mean systemic increase in CAB exposure was less than 11% when co-administered with atazanavir or mefenamic acid, UGT1A1 or UGT1A9 inhibitors, respectively. These predicted changes are considered well within the safe exposure range based on the reported safety margins for CAB therefore no exclusion of UGT inhibitors is required when dosing CAB. These predictions are supported by clinical studies in poor metabolizers of UGT1A1 (a surrogate for UGT inhibition) which resulted in a recommendation that dose adjustment is not required [m2.7.2].

CAB was a substrate for the efflux transporters BCRP and Pgp; however, its high intrinsic permeability suggests a low potential for drug interactions with BCRP and Pgp inhibitors that would result in clinically significant changes to CAB exposure. In vitro, the hepatic uptake of CAB was not mediated by the transporters OATP1B1, OATP1B3, OATP2B1 or OCT1 in cryo-preserved pooled human hepatocytes, therefore, there is low potential for drug interactions with inhibitors of these transporters (see Section 3.1.3.2).

In vitro, the metabolite CAB glucuronide was not a substrate for Pgp or BCRP and therefore its disposition is unlikely to be affected by co-administration of either Pgp or BCRP inhibitors. In vitro, CAB glucuronide was shown to be a substrate for multiple hepatic and renal transporters (OATP1B1, OATP1B3, OAT3, MRP2, MRP3 and MRP4), but was not a substrate for OAT1 or OAT4 (see Section 3.1.3.2). Because there are multiple pathways for the disposition of CAB glucuronide the potential for a clinically relevant drug interaction via co-administered compounds that might inhibit one or more of these transporters is reduced.

3.1.6.2. Effect of CAB on co-administered agents

In vitro, CAB was a weak activator of human Pregnane X receptor [m2.6.5 Table 12.4, Report RR2007/00046] and there was no notable induction of CYP1A2, 2B6 or 3A4 in human hepatocytes [m2.6.5 Table 12.5, 2013N166279]. CAB (sodium salt) demonstrated little or no direct or metabolism-dependent inhibition (IC_{50} values >100 μM) in vitro of the enzymes CYP1A2, 2A6, 2B6, 2C8, 2C9, 2C19 and 2D6, but was a weak direct inhibitor of CYP3A4/5 (IC_{50} = 84 μM) and a metabolism-dependent inhibitor of CYP3A4/5 [m2.6.5 Table 12.3, Report 2012N151766]). However, a clinical drug interaction study has demonstrated that CAB does not affect the pharmacokinetics of midazolam, a sensitive substrate of CYP3A [m2.7.2, Report 2014N208332].

In vitro, CAB is not a notable UGT inhibitor, except for UGT1A3 (IC_{50} 12 μM, [m2.6.5 Table 12.2, Report 2013N159049]), therefore a mechanistic static mathematical model was used to predict the effect of CAB on the exposure of substrates of UGT1A3. The results of the model showed CAB has low risk of being a perpetrator of drug interactions with substrates of UGT1A3 (predicted AUC change 1.0-fold) [m2.7.2, Report 2015N258625]. Based on these data, there is low potential for CAB to affect the pharmacokinetics of co-administered drugs that are substrates of UGT enzymes.

In vitro studies have shown that CAB is not an inhibitor (IC_{50} of >30 μM) of the hepatic or intestinal drug transporters Pgp, BCRP, BSEP, MRP2, MRP4, OCT1, OCT2, OATP1B1, and OATP1B3 [m2.6.5 Table 8.1, Reports 2012N146041, 2012N146057, 2012N150360, 2013N164529, 2013N174589, 2013N174474]. In vitro, CAB inhibits the renal transporters MATE1 or MATE2-K (IC_{50} values of 18 and 14 μM, respectively) and OAT1 and OAT3 with IC_{50} values of 0.81 μM and 0.41 μM, respectively [m2.6.5 Table 8.1, Report 2013N174474]. A mechanistic static mathematical model indicated that CAB has low risk of being a perpetrator of drug interactions with MATE and OAT substrates (predicted AUC change 1.01 and <1.26-fold, respectively) [m2.7.2, Report 2015N258625]. In addition, PBPK modelling simulations predict no risk of clinically relevant drug interactions (predicted AUC change 1.18) when OAT1/OAT3 substrates are co-administered with CAB and there were no renal safety concerns when oral CAB was dosed in combination with the sensitive OAT1/3 substrate tenofovir [see m2.7.2, Report 2018N389974]. Therefore, CAB has a low potential for causing clinically significant drug interactions involving drug transporters.

In vitro, CAB glucuronide is not an inhibitor (IC_{50} of > 300 μM) of Pgp, BCRP, BSEP, MRP2, OATP1B1, OATP1B3, OAT1, OAT3, OCT2, MATE1 and MATE2-K [m2.6.5 Table 8.2, Report 2015N253914] therefore confirming the low risk of perpetrator drug interactions with these transporters.

3.1.7. Effect of CAB on folate transporters or folate receptor

An in vitro study that included CAB was conducted to assess integrase inhibitors as potential inhibitors of human folate transport pathways. In vitro, CAB did not inhibit proton-coupled folate transport or reduced folate carrier activity up to the highest concentration tested (100 μM). In the folate receptor α assay, CAB demonstrated 36.7%

inhibition at 25.8 μM; this observed in vitro inhibition was not projected to be clinically relevant [m2.6.5 Table 8.9, Report 2019N396076].

4. TOXICOLOGY

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

CAB (sodium salt) has been evaluated in oral repeat dose toxicity studies for up to 26 weeks duration in rats and 39 weeks duration in monkeys at doses selected to identify target organ toxicity and define no-observed adverse effect levels (NOAELs). Two GLP toxicity studies were conducted in rats to characterize the toxicity and toxicokinetics of a CAB (free acid) injectable suspension: i) a single dose study by SC and IM administration followed by a 74 to 86 day non-treatment period and ii) a 3 month (weekly and monthly administration) SC and IM study followed by a 14 day (SC, weekly administration), 75 day (SC, monthly administration) or 51 day (IM, monthly administration) non-treatment period. Dose range finding studies in the mouse were conducted to support dose selection for the mouse carcinogenicity study. Two definitive in vitro and one in vivo genotoxicity study were conducted to assess the mutagenic and clastogenic potential of CAB in bacterial and mammalian systems. The carcinogenic potential of CAB was assessed in mice and rats following oral administration for up to 104 weeks. In addition, reproductive toxicology studies were conducted to assess the effect on reproductive performance, as well as embryofetal development, and pre- and post-natal development. Immunotoxicity, irritancy and impurities studies were also conducted.

All definitive studies were conducted in accordance with GLP regulations. The toxicokinetic profile of CAB was evaluated in all definitive repeat dose toxicology studies (see Section 4.3), which included appropriate analysis of samples from control animals. The impurity profiles of batches used for toxicity studies were representative of those being used in clinical studies [see m2.6.7, Table 4.1].

Throughout this section exposure margins are presented based upon comparison of the animal systemic exposure (end of study gender mean) with that reported for patients receiving 30 mg/daily for one month (C_{max} = 8.1 μg/mL; AUC = 146 μg.h/mL), unless otherwise stated.

The principal nonclinical toxicology finding associated with CAB treatment was primarily gastrointestinal (GI) toxicity in monkeys. This finding is discussed below and presented in Table 4.1 along with other noteworthy toxicology findings. A summary of systemic exposure (C_{max} and AUC) to oral CAB achieved in the pivotal toxicology studies is presented in Table 4.2 and the exposure comparison for parenteral CAB is presented in Table 4.3.

4.1. Choice of Species

The species studied in the definitive toxicology evaluations (rats and monkeys) were selected based on similarities in their pharmacokinetic and metabolic profiles to humans and extensive historical background data available for these species. The monkey was chosen as the non-rodent species because the dog provided less systemic exposure to CAB following oral administration.

4.2. Single Dose Toxicity

Single dose oral acute toxicity studies have not been conducted in rats or monkeys with CAB; however, the potential for acute toxicity was assessed in repeat dose studies at the highest possible systemic exposure based on saturation of absorption (rat) or highest tolerable dose (monkey). No adverse clinical observations were noted following administration of CAB to rats at ≤1000 mg/kg/day in the 4 week toxicity study. CAB was not tolerated at a dose of 1000 mg/kg/day in the 14 day monkey toxicity study and resulted in morbidity associated with clinical signs suggestive of GI effects including body weight loss, emesis, loose/watery feces, inappetence and moderate to severe dehydration.

A series of single dose toxicity studies were performed to assess the effects of administration of oral, subcutaneous and intramuscular doses of CAB, and to compare the toxicokinetics for the different routes of administration; results were consistent with findings from repeat dose toxicity studies [see m2.6.7, Table 5.1 and Table 5.2].

4.3. Repeat Dose Toxicity

The toxicity of repeated oral gavage doses of CAB has been assessed in rats and monkeys in studies of up to 26 and 39 weeks, respectively [m2.6.7 Table 7.5 and 7.8, Reports RD2009/00031 and RD2009/00027].

The main findings from all repeat dose toxicity studies are discussed below and summarized together with their effect and no effect doses in Table 4.1.

4.3.1. Moribundity

In the 14 day monkey toxicity study, all male monkeys given 1000 mg/kg/day were euthanized in moribund condition on Day 14 [see m2.6.7 Table 7.6]. Body weight loss, repeated emesis mostly during Week 2, repeated loose/watery feces, inappetence, salivation and discolored feces preceded morbidity which was generally characterized by moderate to severe dehydration, decreased activity, hunched posture and/or reluctance to move and was believed to be secondary to the GI effects discussed below. Bone marrow depletion with corresponding decreases in peripheral leukocyte, reticulocyte and platelet counts occurred in male monkeys given 1000 mg/kg/day and was characterized by a generalized decrease in cellularity of all precursor populations. No effects on bone marrow were observed in monkeys given 8 or 25 mg/kg/day or in females given 1000 mg/kg/day. No adverse effects on bone marrow were observed in monkeys given ≤500 mg/kg/day in the 4 week or 39 week toxicity studies. Additional findings

considered secondary to the debilitated condition of the male monkeys given 1000 mg/kg/day in the 14 day study included dilation of the distal convoluted tubules in one male with associated minimal increase in serum urea and increased serum urea in one other male given 1000 mg/kg/day in the absence of microscopic renal findings. Given the severity of dehydration noted in male monkeys in this group, increased urea values were attributed to prerenal azotemia/dehydration rather than renal functional impairment, and the tubular dilation noted in the one monkey may have been associated with morbidity rather than a direct test article effect. In addition, parotid and mandibular salivary gland atrophy noted in males given 1000 mg/kg/day were also likely secondary to weight loss and debilitation and were not considered direct effects of the test article.

Because the 1000 mg/kg/day males were euthanized on Day 14 following the 4 hour toxicokinetic time point, systemic exposure (C_{max} and AUC_{0-24}) are given for Day 1 in males (67.0 µg/mL and 1051 µg.h/mL, respectively). For comparison, in male monkeys given 1000 mg/kg/day, the AUC_{0-4} was 132 µg.h/mL on Day 1 and 224 µg.h/mL on Day 14. There were no adverse effects at doses up to 500 mg/kg/day (highest dose tested) in the 4 week toxicity study and the 39 week toxicity study. Day 28 exposure in the 4 week study at 500 mg/kg/day was similar to that achieved in the 14 day study at 1000 mg/kg/day (gender mean AUC_{0-24} of 902.5 µg.h/mL at 500 mg/kg/day compared to 1051 µg.h/mL in males on Day 1, and 961 µg.h/mL in females on Day 1, and 946 µg.h/mL in females on Day 14).

4.3.2. Findings considered associated with stress

In the 14-day monkey toxicity study, minimal to moderate thymic lymphoid atrophy was noted in all male monkeys given 25 or 1000 mg/kg/day but was also noted in one control male and in one male given 8 mg/kg/day [see m2.6.7 Table 7.6]. Minimal to mild, diffuse hypertrophy of cells in the adrenal cortex were present in males given ≥25 mg/kg/day and females given ≥8 mg/kg/day. These thymic and adrenal changes were not associated with a change in organ weights and were considered non-adverse and likely a stress response.

4.3.3. Gastrointestinal effects

In the 14 day monkey toxicity study, the morbidity in males given 1000 mg/kg/day, euthanized on Day 14, was considered a consequence of test article-related effects on the GI tract [see m2.6.7 Table 7.6]. Treatment-related effects included: microscopic findings of degeneration/ regeneration, glandular dilation and mucous depletion in the glandular and fundic regions of the stomach; degeneration/regeneration, glandular dilation, goblet cell hypertrophy and increased thickness of the lamina propria of the cecum and colon; and villous atrophy in the small intestine. GI findings were associated with body weight loss, emesis, loose/watery feces, salivation, lethargy and dehydration. No adverse effects on the GI system were observed in monkeys given 8 or 25 mg/kg/day or in females given 1000 mg/kg/day. Regenerative gastric changes were noted in a single female given 1000 mg/kg/day but were not considered adverse due to their minimal severity, limited distribution within the GI tract and lack of accompanying clinical signs or weight loss. No adverse effects on the GI system were observed in monkeys given ≤500 mg/kg/day in the 4 week or 39 week toxicity studies. Day 28 exposure at 500 mg/kg/day (gender

averaged AUC_{0-24} of 902.5 µg.h/mL) was similar to that achieved in the 14-day study at 1000 mg/kg/day (1051 µg.h/mL in males on Day 1, and 961 µg.h/mL in females on Day 1, and 946 µg.h/mL in females on Day 14). This suggests that GI intolerance observed in the 14 day study was the result of local drug administration and not systemic toxicity.

4.3.4. Cardiovascular effects

In a cardiovascular study in conscious, non-restrained male monkeys [m2.6.3 Table 4.1, also see Section 2.3 above], a single oral dose of CAB at 1000 mg/kg produced a mild, transient increase in mean arterial pressure (3.7 to 8.6%) and a transient increase in heart rate (16 to 23%) during the first 2 hours after dosing. No blood pressure or heart rate changes were observed in monkeys given single oral doses of 8 or 25 mg/kg. There were no adverse effects on the heart (organ weight or histopathology) and no ECG waveform or interval changes occurred after single or 14 consecutive oral doses of CAB of up to 1000 mg/kg/day, after approximately 3 weeks at ≤500 mg/kg/day (C_{max} 61.6 µg/mL, AUC_{0-24} 902.5 µg.h/mL, Day 28 systemic exposure data in males from the monkey 4 week oral toxicity study), or after approximately 38 weeks of dosing in monkeys at ≤500 mg/kg/day.

4.3.5. Studies to support parenteral administration

CAB was administered by monthly SC injection (5, 30 and 100 mg/kg/dose), monthly IM injection (2.5, 10 and 75 mg/kg/dose), or by weekly SC injection (100 mg/kg/dose) to rats for up to 3 months, followed by non-dose periods of up to 75 days to characterize toxicity and toxicokinetics of a parenteral (LA) formulation of CAB in order to support clinical studies of CAB injectable suspension. There were no adverse effects noted and no new target organ toxicities identified via these routes of administration [see m2.6.7 Table 7.2, Report 2010N104820]. Dose-proportional signs of redness and swelling were present following SC or IM injections at all dose levels [for clinical relevance, see m2.7.4]. These were accompanied by local inflammatory reactions (erythema and edema graded very slight to severe) in animals given 75 mg/kg/dose (monthly IM injections), at all doses in female animals given monthly SC injections (≥5 mg/kg/month) and in males given ≥30 mg/kg/month, and at a higher incidence in animals given 100 mg/kg/dose monthly and weekly SC injections. Treatment-related histology findings were limited to granulomatous inflammation and mixed inflammatory cell infiltration at the corresponding injection sites, with correlating macroscopic changes. These changes were dose-dependent and were most severe in animals receiving 100 mg/kg/dose SC once weekly and having the shortest non-dose period. The NOAELs for monthly administration were 100 mg/kg/dose SC and 75 mg/kg/dose IM. Exposures at the NOAELs ($AUC_{1440-2160h} = AUC_{60-90days}$) correspond to ~38X the human $AUC_{(0-t)}$ for a 400 mg IM dose (2461 µg.h/mL).

In a single dose IM combination study in monkeys given CAB (free acid; 10 mg/kg) and RPV (60 mg/kg), both CAB and RPV were well tolerated after a single dose administration and were quantifiable through 61 days after dosing when given alone and in combination.

4.4. Genotoxicity

CAB did not cause gene mutations or chromosomal damage in two definitive in vitro tests (Ames test and mouse lymphoma assay), or in an in vivo oral rat micronucleus test [m2.6.7, Table 8.1, Report WD2007/00787, Table 8.2, Report WD2007/00788 and Table 9.1, Report WD2007/00789]. Therefore, based on these data, CAB does not pose a genetic toxicity risk to humans.

4.5. Carcinogenicity

The carcinogenic potential of CAB was assessed in mice and rats following oral administration for 2 years [m2.6.7, Table 10.1, Report 2017n310750 and Table 10.2, Report 2017N310751]. Based on recommendations from the FDA Executive Carcinogenicity Assessment Committee [FDA, 2014], the doses studied in mice were 2.5, 10, and 75 mg/kg/day for males, and 2.5, 5, and 35 mg/kg/day for females, and the doses studied in rats were 0.25, 2.5, and 75 mg/kg/day.

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

When compared to the expected human exposure for a 30 mg oral dose, the systemic exposures (AUC) for CAB at the highest doses tested were >7 times higher for mice (either sex) and >30 times higher in female rats and >19 times higher in male rats.

4.6. Reproductive and Developmental Toxicity

CAB did not affect male mating and fertility parameters in the rat or embryofetal development of offspring of untreated females mated to CAB-treated males at doses up to 1000 mg/kg/day (highest dose tested) [m2.6.7, Table 12.2, Report 2014N207479]. In a rat oral female fertility, early embryonic and embryofetal development study at 0.5, 5 or 1000 mg/kg/day, CAB administration at 1000 mg/kg/day resulted in decreases in mean male and female fetal body weights (up to 6%), but no test article-related fetal malformations or variations at any dose. The NOAEL for female fertility was 1000 mg/kg/day and the NOAEL for rat embryofetal development was 5 mg/kg/day [m2.6.7, Table 12.1, Report CD2009/00105]. In a rabbit oral embryofetal development study, there were no test article-related effects at any dose and the NOAEL was 2000 mg/kg/day ($AUC_{0\,24}$ 96.1 μg.h/mL and C_{max} of 7.5 μg/mL) [m2.6.7, Table 13.1, Report CD2009/00842].

In a rat pre- and postnatal (PPN) development study, there was no test article-related effect on F_0 female body weight, food consumption, parturition, or lactation at doses up to 1000 mg/kg/day. However, decreases in F_1 pup survival and viability resulting in reduced litter sizes during the first 4 days of life were observed at doses of 1000 mg/kg/day (viability index of 87.4% versus 98.9% in control) [m2.6.7, Table 14.1, Report 2015N236973]. There was no test article-related effect on F_1 growth and development, including pre- and post-weaning body weight, sexual maturation, neurobehavioral function, reproductive performance, or survival, growth, and development of F_2 generation offspring. The NOAEL for maternal (F_0) reproductive function was 1000 mg/kg/day and for the pre- and post-natal development of the

offspring in rats (F_1) was 5 mg/kg/day (C_{max} in F1 pups on post-natal Day 10 (PND 10) was 58.4 µg/mL in males and 52.6 µg/mL in females, suggesting the presence of CAB in maternal milk). Additional data showed that fetal concentrations increased proportionally with an increase in maternal plasma levels and that there was no evidence of preferential accumulation of CAB within individual fetal compartments [see m2.6.7 Table 14.3, Report 2017N311115].

In a follow up study, CAB (up to 1000 mg/kg/day) was administered to pregnant rats during the period of organogenesis through Lactation Day 7 [m2.6.7 Table 14.2, Report 2016N281797]. Pups were cross-fostered at birth and nursed by control mothers and a similar incidence of stillbirths and neonatal mortalities were observed. There was no effect on neonatal survival of control pups nursed from birth by CAB-treated mothers. There were adverse effects in which CAB delayed the onset of parturition and which led to increased fetal mortality (stillbirths) and neonatal deaths immediately after birth (at >30 times the systemic exposure at the maximum recommended human dose [MRHD] of 30 mg/day orally, or 400 mg IM dose (exposure data from 26 week rat study) [see proposed drug label]. There was no fetal mortality when rat fetuses were delivered by caesarean section [see m2.6.7, Table 12.1, Report CD2009/00105].

4.7. Other Studies

4.7.1. Immunotoxicity

CAB was given once daily by oral gavage to male and female rats at doses of 0 (vehicle control), 0.5, 5 or 1000 mg/kg/day for 28 days [see m2.6.7 Table 17.1, Report 2013N179070]. Rats were immunized with a single IV dose of the T cell-dependent antigen, keyhole limpet hemocyanin (KLH), following 12 daily doses of CAB. A CAB-related decrease in the anti-KLH IgG antibody response was observed in males given 1000 mg/kg/day. Since the effect in males at 1000 mg/kg/day was minimal, and no effects were observed on the female anti-KLH IgG response or on the anti-KLH IgM response in either sex, CAB is not considered immunosuppressive under the conditions of this study. Based on the T cell-dependent antibody response (TDAR) assessment, the no observed effect level (NOEL), under the conditions of this study, is 5 mg/kg/day in males and 1000 mg/kg/day in females.

In a further investigative TDAR study, CAB was given orally to rats (10/sex/group) at 5 or 1000 mg/kg/day for 39 days with additional recovery animals added at 1000 mg/kg/day group [see m2.6.7 Table 17.2, Report 2018N367799]. Rats were immunized intravenously with 300 µg of KLH on Days 12 and 26 (3 hours post dosing with CAB) and following a 4-week off-dose period on Days 68 and 82 (recovery animals) for evaluation of TDAR. No CAB-related effects on anti-KLH IgM or IgG antibody response were observed during the dosing period; therefore, TDAR samples collected during the off-dose period were not evaluated. Based on the TDAR assessment, the no observed effect level (NOEL) under the conditions of this study is 1000 mg/kg/day.

Overall, the data indicate there was no immunotoxicity in rats treated with CAB.

4.7.2. Phototoxicity

In the absorption spectrum for CAB (sodium salt and free acid), there are peaks at the boundary of the ultraviolet (UV) light (UVA/UVB) region with a lambda max at 257 nm and others at 306-307, 318-319 and 335 nm (and one other at 366 nm for sodium salt only) with a tail extension to approximately 390 nm [see CMC General Properties, Section 3.2.S.1.3, CAB free acid, and m3.2.S.1.3, CAB sodium salt)]. Quantitative whole body autoradiography studies in pigmented rats following oral administration of CAB demonstrated a wide tissue distribution of drug-related material with over 50% of the tissues still containing low but quantifiable radioactivity 28 days following a single dose [see Section 3.1.3.3 above]. There was little evidence to suggest any binding of drug-related material to the melanin containing tissues in the eye and skin. No toxicities have been identified in the eye or skin during routine repeat dose toxicity studies of up to 26 weeks duration in the rat (albino) or 39 weeks duration in monkeys. In these studies, potential toxic effects on the eye and the skin were assessed at the end of the study by macroscopic and microscopic examination. Additionally, biomicroscopic (slit lamp) ophthalmoscopy was conducted in rat and monkey toxicity studies.

4.7.3. Irritancy

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

4.7.4. Impurities

The proposed drug substance specifications [see m3.2.S.4.1 CAB free acid or m3.2.S.4.1 CAB sodium salt] for CAB indicate that the specified impurities, gsk006*, gsk007*, gsk005*, gsk002*, gsk001* and gsk003*
(each impurity also occurs in the drug substance of CAB sodium salt, and is designated with the suffix “B”) do not exceed the 0.15% w/w ICH qualification threshold [ICH Q3A (R2)]. All 6 impurities were considered not genotoxic based on in silico assessments (DEREK and Leadscope software programs).

An assessment of the route of synthesis for CAB has also been conducted to determine the likelihood of mutagenic impurities being present in the drug substance [m2.6.6, Section 4.6]. This indicated that there are no impurities of known or potential mutagenic concern (as determined by Ames testing and/or DEREK and/or Leadscope) that are considered likely to be present in final drug substance at a level that would exceed the appropriate threshold of toxicological concern as per ICH M7 guidance at the planned clinical doses.

*新薬承認情報提供時に置き換え

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

Finding	Rat		Monkey	
	Effect Dose (mg/kg/day)	No Effect Dose (mg/kg/day)	Effect Dose (mg/kg/day)	No Effect Dose (mg/kg/day)
Moribund Condition and Clinical Observations Secondary to GI Effects Emesis, loose/watery feces, inappetence, salivation and discolored feces, dehydration, decreased activity, lethargy	NO	1000	1000[a]	500
Body Weight Loss	NO	1000	1000[a]	500
GI Effects: Stomach - Degeneration/regeneration of glandular and fundic regions with mucous depletion, glandular dilation and/or inflammation	NO	1000	1000[a]	500
Cecum and colon - Degeneration/regeneration of epithelial cells, goblet cell hypertrophy, dilated glands and increased thickness of the lamina propria	NO	1000	1000[a]	500
Small intestine - Villous atrophy in the duodenum, jejunum and/or ileum	NO	1000	1000[a]	500
Cardiovascular Effects: Mild, transient increase in mean arterial pressure (3.7 to 8.6%) and a transient increase in heart rate (16 to 23%) during the first 2 hours after dosing	ND	ND	1000[b]	500

Key:
a. Finding occurred only in male monkeys. See 14 day oral repeat dose toxicity study, m2.6.7, Table 7.6.
b. Only male monkeys were used in this study. See cardiovascular study in monkeys, m2.6.3, Table 4.1.
ND = Not determined.
NO = Not observed.

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

Species (Duration)	Dose (mg/kg/day)	Sex	C_{max} (μg/mL)		AUC_{0-24} (μg.h/mL)		Animal to human ratio for AUC[a]
			Day 1	End of Study	Day 1	End of Study	
Mouse[h] (104 weeks)	2.5	M	13.5	12.3	250	230	1.58
	2.5	F	18.8	17.1	306	296	2.03
	10	M	33.3	32.6	583	574	3.93
	5	F	31.8	28.8	538	532	3.64
	75	M	67.5	65.4	1290	1140	7.81
	35	F	70.9	70.2	1370	1060	7.26
Rat[b] (14 days)	30	M	14.4	82.1	294	1849	12.66
		F	14.4	73.8	308	1605	10.99
	100	M	22.9	98.4	490	2223	15.23
		F	24.0	111	472	2435	16.68
	300 (NOAEL)	**M**	**35.8 [33.9 to 37.9]**	**114 [112 to 115]**	**708 [668 to 761]**	**2510 [2422 to 2640]**	**17.19**
		F	**41.0 [39.6 to 43.6]**	**147 [131 to 171]**	**799 [760 to 842]**	**3277 [2952 to 3828]**	**22.45**
Rat[b] (4 weeks)	1	M	4.23	34.3	70.7	739	5.06
		F	4.48	38.8	78.3	852	5.84
	75	M	66.1	143	1309	3000	20.55
		F	72.8	176	1484	3832	26.25
	1000 (NOAEL)	**M**	**161 [150 to 180]**	**150 [149 to 151]**	**3368 [3266 to 2519]**	**3345 [3214 to 3432]**	**22.91**
		F	**181 [161 to 200]**	**193 [172 to 214]**	**3732 [3451 to 3993]**	**4125 [3659 to 4229]**	**28.25**
Rat[b] (26 weeks)	0.5	M	16.2	23.1	329	451	3.09
		F	19.4	31.6	404	675	4.62
	5	M	93.2	91.4	1961	1861	12.75
		F	85.1	102	1672	2083	14.27
	1000 (NOAEL)	**M**	**174 [167 to 179]**	**148 [146 to 150]**	**3753 [3656 to 3896]**	**3203 [3005 to 3313]**	**21.94**
		F	**210 [200 to 220]**	**224 [215 to 233]**	**4403 [4101 to 4627]**	**4781 [4711 to 4907]**	**32.75**
Rat[h] (104 weeks)	0.25	M	9.97	13.1	206	277	1.90
		F	14.2	19.7	295	428	2.93
	2.5	M	59.6	73.1	1260	1470	10.07
		F	76.3	92.0	1620	1980	13.56
	75	M	160	137	3360	2840	19.45
		F	181	230	4010	4810	32.95
Monkey[b] (14 days)	8	M	12.2	14.6	128	144[d]	0.99
		F	11.8	15.5	104	124	0.85
	25 (NOAEL)	**M**	**20.8 [20.4 to 21.5]**	**22.7 [20.1 to 24.6]**	**233 [190 to 287]**	**233 [198 to 257]**	**1.60**
		F	**23.6 [19.2 to 30.9]**	**22.2 [15.0 to 29.9]**	**234[d] [159 to 321]**	**231 [192 to 286]**	**1.58**
	1000	M	67.0	61.9	1051	224[c,d]	1.53
		F	59.2	65.9	961[d]	946	6.48

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

Species (Duration)	Dose (mg/kg/day)	Sex	C_{max} (μg/mL)		AUC_{0-24} (μg.h/mL)		Animal to human ratio for AUC[a]
			Day 1	End of Study	Day 1	End of Study	
Monkey[b] (4 weeks)	5	M	12.6	10.0	115	100	0.68
		F	10.4	9.07	85.3	80.9	0.55
	50	M	20.8	17.4	311	276	1.89
		F	23.9	20.0	311	279	1.91
	500 (NOAEL)	**M**	**38.2 [33.1 to 43.3]**	**58.1 [50.3 to 63.3]**	**697 [616 to 773]**	**901 [860 to 945]**	**6.17**
		F	**39.1 [33.3 to 43.1]**	**65.0 [53.7 to 79.1]**	**664 [577 to 732]**	**904 [792 to 1114]**	**6.19**
Monkey[b] (39 weeks)	5	M	7.15	3.37	62.1	37.7	0.26
		F	8.77	6.33	69.6	67.8	0.46
	50	M	22.1	21.1	251	229	1.57
		F	27.3	17.4	303	254	1.74
	500 (NOAEL)	**M**	**45.3 [37.4 to 61.7]**	**36.8 [23.9 to 57.2]**	**644 [505 to 826]**	**542 [359 to 781]**	**3.71**
		F	**56.4 [43.5 to 66.7]**	**32.4 [29.8 to 35.1]**	**807 [557 to 1008]**	**552 [447to 623]**	**3.78**
Rabbit (Embryofetal development)[ef]	30	F	NA	0.95	NA	10.5	0.072
	500	F	NA	3.4	NA	47.4	0.32
	2000 (NOAEL)	**F**	**NA**	**7.5**	**NA**	**96.1**	**0.66**
Human[g]	30 mg	M/F	-	8.1	-	146	NA

Key: NOAELs are in **bold text**. Values in brackets represent the range.

a. Calculated for AUC based on end of treatment values.
b. Values are the mean of n=3 to 4/sex/group.
c. AUC_{0-4}. All males given 1000 mg/kg/day were euthanized in moribund condition on Day 14 following the 4 hour toxicokinetic time point. Therefore, the AUC_{0-24} could not be calculated for males on Day 14.
d. Emesis was observed in one animal in this dose group.
e. Composite TK results from n=3/group.
f. Values in end of study column were from Day 11 postcoitum or dosing Day 5.
g. Mean exposure (C_{max} and AUC_{0-24}) following CAB 30 mg PO once daily (POPPK analysis), report 2018N384611.
h. AUC and C_{max} values given for Week 4 and Week 26.

Table 4.3 Estimated Margins of CAB Relative to Clinical Exposure Following Administration of CAB Injectable Suspension

Species	Dose (mg/kg)	Sex	Route of administration	Cmax (μg/mL)[a,b]	AUC (μg.h/mL)[a,c]	Animal to human ratio for AUC
Rat (Single dose)	5	M	SC	8.36	4346	1.77
		F	SC	9,04	5367	2.18
	30	M	SC	38.7	19978	8.12
		F	SC	36.9	19218	7.81
	100[d] (NOAEL)	**M**	**SC**	**98.3 [78.4 to 117]**	**47912 [38207 to 56466]**	**19.47**
		F	**SC**	**104 [80.9 to 133]**	**51104 [40209 to 64474]**	**20.77**
	2.5	M	IM	12.6	4321	1.76
		F	IM	14.2	4525	1.84
	10	M	IM	32.4	15926	6.47
		F	IM	40.6	16464	6.69
	75 (NOAEL)	**M**	**IM**	**105 [95.3 to 119]**	**60071 [53582 to 64941]**	**24.41**
		F	**IM**	**124 [107 to 141]**	**64765 [60919 to 70666]**	**26.32**
Rat (3-month)	5	M	SC monthly	19.2	11204	4.55
		F	SC monthly	26.8	15238	6.19
	30	M	SC monthly	84.8	48082	19.54
		F	SC monthly	96.8	55956	22.74
	100 (NOAEL)	**M**	**SC monthly**	**137 [107 to 166]**	**70494 [59895 to 85122]**	**28.64**
		F	**SC monthly**	**195 [172 to 208]**	**116602 [105082 to 124604]**	**47.38**
	2.5	M	IM monthly	16.9	7031	2.86
		F	IM monthly	15.9	5500	2.23
	10	M	IM monthly	49.6	26001	10.57
		F	IM monthly	55.2	24934	10.13
	75 (NOAEL)	**M**	**IM monthly**	**135 [129 to 142]**	**78051 [74734 to 80570]**	**31.72**
		F	**IM monthly**	**181 [179 to 183]**	**107080 [92466 to 115252]**	**43.51**
	100 (NOAEL)	**M**	**SC weekly**	**166 [154 to 184]**	**22291 [21631 to 22907]**	**NA**
		F	**SC weekly**	**226 [221 to 235]**	**34315 [32842 to 35565]**	**NA**
Human[e]	400 mg monthly	M/F	IM	4.2	2461	NA

Key: n=3 animals/sex/dose, except where noted. NOAELs are in **bold text**

a. Results are reported as mean and [range].
b. For rat 3-month, C_{max} reported as overall study C_{max}
c. For rat single dose, AUC reported as $AUC_{0\text{-}720}$, AUC through 30 days (morning of Day 31). For rat 3-month, AUC for SC or IM monthly groups reported for the 3rd monthly interval, $AUC_{1440\text{-}2160h}$. For rat 3-month, AUC for SC weekly groups is reported as AUC during Week 13 (from predose on Day 85 to Day 92), $AUC_{2016\text{-}2184}$.
d. n = 2 males for AUC0-t in the 100 mg/kg dose group due to unscheduled death on Day 39.
e. Mean exposure (C_{max} and $AUC_{0\text{-}t}$) month 3 onward following a 400 mg IM monthly dose (POPPK analysis), report 2018N384611.

5. OVERVIEW AND CONCLUSIONS

CAB is a potent in vitro inhibitor of HIV integrase and inhibits the integrase catalyzed viral DNA strand transfer with IC_{50} values in the nanomolar range (3.0 to 13 nM). CAB is a potent antiviral agent when tested in a variety of in vitro assays against the HIV-1 strains Ba-L and NL432 in human PBMC (IC_{50} = 0.22 and 0.53 nM, respectively), and against the HIV-1 strain IIIB in MT-4 cells (IC_{50} = 0.57 and 2.1 nM, respectively) and in the PHIV assay (IC_{50} = 0.74 nM). Additionally, the IC_{50} values of CAB for viral replication of NIH reference strains consisting of 24 strains of HIV-1 and 4 strains of HIV-2 in PBMC assays and for 3 HIV-1 strains in monocyte-derived macrophage assays were also in the low nanomolar concentration range, like that seen with HIV-1 strains Ba-L and NL432. In vitro studies suggested an approximate average of 408-fold shift in IC_{50} of CAB in the presence of 100% human serum (by method of extrapolation), and the PA-IC_{50} was estimated to be 102 nM in HIV-1 IIIB infected MT4 cells. In vitro cytotoxicity studies provided a selectivity index of ≥10,000 for CAB compared with the HIV-1 antiviral potency in PBMCs. In a viral integrase susceptibility assay using the integrase coding region from 13 clinically diverse subtype B isolates, CAB demonstrated antiviral potency like that observed for laboratory strains, with a mean IC_{50} of 1.3 nM.

CAB showed anti-HIV activity (susceptibility) equivalent to wild type virus (fold change [FC] <5) against 22 of 25 INI-resistant mutant viruses with single mutations. Of the 17 INI-resistant mutant viruses with 2 or more mutations, 8 showed susceptibility to CAB (FC<5). Exposure of MT-2 cells infected with HIV-1 IIIB to CAB for up to 112 days did not produce any highly resistant mutants (>10-fold increase in IC_{50}). No amino acid substitutions in the integrase region were selected when passaging the wild type HIV-1 NL-432 in the presence of 6.4 nM CAB through 56 days.

Following single IV administration of CAB to dogs and monkeys, the plasma clearance (<2% of hepatic plasma flow) and steady-state volume of distribution (<0.35 L/kg) were low, with half-life values of 4 to 6 hours. Following oral administration as a solution, the oral bioavailability of CAB was good (44 to 83%) and consistent with its high passive permeability. However, when administered as a suspension, or in solid dosage forms, the bioavailability appeared limited by dissolution rate or solubility which resulted in a less than proportional increase in systemic exposure of CAB relative to dose. In mice, rats and monkeys, no consistent notable (>2-fold) difference in oral systemic exposure between sexes was observed.

In rats and monkeys given a single SC or IM injection, CAB was slowly released from the injection site with a mean apparent plasma half-life ranging from 12 to 29 days (SC) or from 8 to 12 days (IM).

The protein binding of CAB in rat, dog, monkey and human plasma and serum was high (>99%). CAB is a substrate for Pgp and BCRP, but due to its high permeability, no alteration in absorption would be expected by co-administration of either Pgp or BCRP inhibitors. After oral administration of [^{14}C]-CAB to rats, radioactivity was slowly absorbed and then largely confined to the systemic circulation albeit widely distributed to other tissues. Radiolabelled drug-related material was minimally associated with cellular components of blood. Elimination of radioactivity was slow with most tissues containing

low but quantifiable radioactivity at 28 days. Association of radioactivity to the melanin-containing tissues in the eye and skin was not observed.

In general, the metabolism of CAB in the nonclinical species reflects that observed in humans, with CAB being the principal component circulating in plasma. The major metabolite of CAB in all species was CAB glucuronide, which was formed primarily by UGT1A1 (with some involvement from UGT1A9) and was eliminated in the urine and bile. Additional studies in human (IM, SC and PO) confirmed that the metabolism and excretion of CAB is independent of route of administration. Metabolic conversion of CAB to its stereoisomers was not detected in rat, dog, monkey or human hepatocytes, or in human plasma following repeat oral administration for 14 days.

Across all species, elimination of drug-related material occurred predominantly via the feces (58.5 to 94.5% of the dose). In rodents, absorbed radioactivity as determined by the amount of drug-related material recovered in the urine and bile (limited to approx. 2% dose), was predominantly secreted into the bile while renal excretion was minimal. In monkeys, the absorbed radioactivity (approximately 30% dose) was eliminated via both the biliary and renal routes.

In vitro data indicates that circulating CAB permeates passively into hepatocytes and is metabolized to CAB glucuronide which undergoes both biliary and sinusoidal excretion. Biliary excretion of CAB glucuronide is mediated by MRP2, while hepatic basolateral excretion into sinusoidal blood was via both MRP3 and MRP4. Circulating CAB glucuronide undergoes efficient renal clearance, where uptake into the proximal tubule is mediated by OAT3 and subsequent secretion into urine by MRP2 and MRP4, which would explain the minimal systemic exposure of CAB glucuronide in human.

In vitro studies have been performed to assess the risk of perpetrator PK drug interactions with CAB. No clinical drug interaction risk was identified for co-administered substrates of CYP1A2, 2A6, 2B6, 2C8, 2C9, 2C19, 2D6, 3A4, UGT1A1, 1A3, 1A4, 1A6, 1A9, 2B4, 2B7, 2B15, and 2B17, Pgp, BCRP, BSEP, OAT1, OAT3, OCT1, OCT2, OATP1B1, OATP1B3, MATE1, MATE 2-K, MRP2 or MRP4 at a clinical oral dose of 30 mg CAB. As CAB is metabolized by UGT1A1 and a clinical study has confirmed a drug interaction with the UGT1A1 inducer rifampin, the co-administration of CAB with strong inducers of UGT is contraindicated.

There was no observed PK interaction between CAB and RPV when co-administered IM to rats or monkeys. In vitro, there was no evidence of clinically relevant inhibition of folate transport or folate receptor with CAB.

In toxicology studies, there were no drug-related adverse effects in rats given CAB orally at doses up to 1000 mg/kg/day for 26 weeks. In the 14 day monkey toxicity study, a dose of 1000 mg/kg/day was not tolerated by male monkeys and resulted in morbidity associated with clinical signs suggestive of GI effects including body weight loss, emesis, loose/watery feces, and moderate to severe dehydration. There were no adverse effects in monkeys given CAB up to 500 mg/kg/day in the 4 week or 39 week toxicity studies. Day 28 exposure at 500 mg/kg/day (gender averaged AUC_{0-24} of 902.5 μg.h/mL) was similar to that achieved in the 14-day study at 1000 mg/kg/day (1051 μg.h/mL in males

on Day 1 and 961 μg.h/mL in females on Day 1 and 946 μg.h/mL in females on Day 14). This suggests that GI intolerance observed in the 14 day study was the result of local drug administration and not systemic toxicity.

When CAB was administered by monthly SC injection (5, 30 and 100 mg/kg/dose) and monthly IM injection (2.5, 10 and 75 mg/kg/dose) to rats for up to 3 months, the NOAEL values were 100 mg/kg/dose SC and 75 mg/kg/dose IM. Exposures at the NOAELs ($AUC_{1440\text{-}2160h} = AUC_{60\text{-}90days}$) correspond to ~38X the human $AUC_{(0\text{-}t)}$ for a 400 mg IM dose (2461 μg.h/mL).

CAB had no effects on male or female fertility in rats. In PPN studies in rats, CAB at 1000 mg/kg/day (>30 times the systemic exposure at the maximum recommended human dose [MRHD] of 30 mg oral or 400 mg IM dose) delayed the onset of parturition, and in some rats, this delay was associated with an increased number of stillbirths and neonatal mortalities immediately after birth [see proposed drug label]. There were no alterations to growth and development of surviving offspring. When rat pups born to CAB-treated dams (1000 mg/kg/day) were cross-fostered at birth and nursed by control mothers, a similar incidence of stillbirths and neonatal mortalities was observed. There was no effect on neonatal survival of control pups nursed from birth by CAB-treated mothers, suggesting effects were related to in utero exposure not lactational exposure. A lower dose of 5 mg/kg/day CAB (>10 times the exposure in humans at the MRHD of 30 mg oral or 400 mg IM dose) was not associated with delayed parturition or neonatal mortality in rats. When CAB (1000 or 2000 mg/kg/day) was administered orally to pregnant rats and rabbits during organogenesis, there was no effect on survival when fetuses were delivered by caesarean section. CAB crosses the placenta and can be detected in fetal tissue. No adverse effects on embryofetal development were observed in rabbit fetuses up to 2000 mg/kg/day. In rats, alterations in fetal growth (decreased body weights) were observed at 1000 mg/kg/day but no test article-related fetal malformations or variations at any dose were observed.

Data suggest that CAB does not present a genotoxic hazard to humans nor were there any safety pharmacology findings of concern for clinical use. Overall, the data indicate there was no immunotoxicity in rats treated with CAB. CAB was not carcinogenic in the 2 year rat or mouse carcinogenicity studies.

The safety assessment of the parenteral route of administration for the proposed drug product is considered “bridged” to the overall oral nonclinical development program through the conduct of the definitive 13-week rat injection study.

Based on the clinical experience with CAB and RPV (both oral and parenteral formulations), and the absence of nonclinical toxicology and pharmacokinetic signals that might indicate the potential for additive or synergistic interaction (see Section 6 for considerations related to the co-administration of CAB + RPV), the nonclinical data support the registration of both oral and parenteral formulations of CAB, co-administered with RPV, when used in accordance with the proposed labelling [see proposed drug label].

6. INTEGRATED NONCLINICAL ASSESSMENT FOR THE CO-ADMINISTRATION OF CAB AND RPV

A comprehensive assessment of the nonclinical safety of CAB and RPV was completed in support of treatment of patients with HIV. ICH Guidance M3 (R2) on Non-Clinical Safety Studies for the Conduct of Human Clinical Trials and Marketing Authorisation for Pharmaceuticals states that, "For most combinations which involve two late stage entities and for which there is adequate clinical experience with co-administration, combination toxicity studies would generally not be recommended to support clinical studies or marketing unless there is significant toxicological concern (e.g., similar target organ toxicity)" [ICH M3 (R2), 2009]. In addition, according to Question 9 in the Questions and Answers document written by the M3 (R2) Implementation Working Group for M3 (R2) Guideline "Non-Clinical Safety Studies for the Conduct of Human Clinical Trials and Marketing Authorization for Pharmaceuticals" regarding the scope of ICH M3 (R2), it is accepted that combination toxicity studies are not generally warranted for HIV products unless there is a specific cause for concern under clinically relevant conditions. However, two combination studies were conducted: a single dose rat IM study and a single dose monkey IM combination (CAB + RPV) study were conducted to investigate the effects of co-administration.

Based on this guidance, together with data from the Phase 3 clinical studies [see m2.5], the Sponsor considers that combination toxicity studies would provide no additional safety information and are not necessary to support the proposed marketing applications for CAB oral and parenteral formulations used in combination with RPV.

Further consideration of the nonclinical data for co-administration of CAB and RPV also shows:

Possibility of pharmacodynamic interactions: The concomitant administration of CAB with RPV is intended to provide two different mechanisms of inhibition of viral growth and so ensure that viral breakthrough does not occur in HIV-infected patients with undetectable viral load. Thus, pharmacodynamic interaction is an intended component of the therapeutic activity of this combination, and no adverse interaction is anticipated.

Possibility of pharmacokinetic interactions: A clinical drug interaction study [m2.7.2, Study LAI116181] was conducted to compare steady-state plasma CAB pharmacokinetics (PK) following administration of CAB 30 mg once daily with and without RPV 25 mg once daily and to compare steady-state plasma RPV PK following administration of RPV 25 mg once daily with and without CAB 30 mg once daily. The PK parameters of each drug alone were similar to those when given in combination demonstrating a lack of interaction between RPV and CAB. This clinical outcome was expected based on the different clearance mechanisms of CAB and RPV (metabolism by UGT1A1 and CYP3A4, respectively) and the low perpetrator liability for both CAB and RPV with clinically relevant enzymes and transporters.

CAB and RPV were administered IM to rats and monkeys alone and in combination. In rats, the pharmacokinetic parameters of each agent were similar when co-administered compared with the individual agent alone. In monkeys, the exposure to RPV was 65%

higher when administered in combination with CAB but this was attributed to a faster initial release rate from the RPV formulation. The C_{max} of CAB was higher (less than 2-fold) following administration of the combination compared to administration alone whereas the AUC_{0-t} was not notably different.

The systemic clearance of CAB and RPV in nonclinical species and in humans is low relative to hepatic plasma flow. Both compounds are highly protein bound (>99%) but any displacement between the two or by another agent would not be expected to result in a clinically significant alteration in the pharmacokinetics of either agent.

In summary, the in vivo drug interaction data for CAB and RPV in human and nonclinical species, together with the supporting in vitro data indicating different clearance pathways for CAB and RPV, indicate low potential for a clinically significant interaction based on pharmacokinetic mechanisms.

Possibility of toxicology interactions: Synergistic or additive toxicity is not expected from the co-administration of CAB with RPV.

The adverse findings noted in repeat dose toxicity studies with CAB (GI intolerance/ toxicity) are considered due to local irritation of the compound as opposed to a systemic effect and have not been consistently observed in clinical studies to date.

The targets relevant to human risk assessment in repeat dose toxicity studies with RPV were liver (hepatocellular hypertrophy in mouse and rat, pigmented macrophages, hepatocytes, canaliculi and gall bladder in dog), kidneys (nephropathy in mouse, nephritis and mineralization in dog), adrenals (rat, dog, NHP; partial inhibitor of CYP21 with potential downstream effects on ovaries and testes in dogs) and RBC (small reversible reduction of RBC parameters in mice, rats and dogs). Clinical pathology parameters to monitor potential effects were part of the clinical studies.

Parenteral formulations of both CAB and RPV caused injection site reactions (ISRs) in animals. Clinical monitoring assessments of ISRs, including pain, tenderness, pruritis, warmth, erythema, skin changes and secondary infections at injections sites continued throughout development [see m2.7.4].

An assessment of the nonclinical development program for RPV LA formulation is given in the RPV Nonclinical Overview.

There are no nonclinical findings which preclude the safe co-administration of CAB and RPV, as both oral and parenteral formulations, when used in accordance with the proposed dosing regimen and labelling [see proposed drug label].

7. LIST OF LITERATURE CITATIONS

Key Literature References

De Clercq E. HIV-chemotherapy and -prophylaxis: New drugs, leads and approaches. *The International Journal of Biochemistry & Cell Biology*. 2004; 36:1800-22.

Pommier Y, Johnson AA and Marchand C. Integrase inhibitors to treat HIV/AIDS. *Nature Reviews Drug Discovery.* 2005; 4:236-248.

Sakatis MZ, Reese MJ, Harrell AW et al. Preclinical strategy to reduce clinical hepatotoxicity using in vitro bioactivation data for >200 compounds. *Chem. Res. Toxicol*. 2012; 25:2067-2082.

References (Available Upon Request)

FDA 7 May 2014 correspondence: Special Protocol Assessment, Carcinogenicity Assessment Committee.

Guidelines (not provided)

ICH S2 (R1) (CHMP/ICH/126642/08)
Guidance on genotoxicity testing and data interpretation for pharmaceuticals intended for human use (2008).

ICH Q3A (R2): Impurities in New Drug Substances. ICH Harmonised Tripartite Guideline. 25 October 2006.

ICH M4S Q&A (R4): CTD Safety (M4S) Questions and Answers (R4). 11 November 2003.

ICH Topic M3 (R2): Non-Clinical Safety Studies for the Conduct of Human Clinical Trials and Marketing Authorization for Pharmaceuticals. CPMP/ICH/286/95, June 2009.

CHMP/SWP/5199/02 (2006). European Medicines Agency (EMA)
Guideline on the Limits of Genotox Impurities.

APPENDIX 1 MODULE 4 SECTIONS WITHOUT STUDY REPORTS

The following provides a brief justification for the absence of studies in certain sections of m4. Note that when study reports address more than one objective, they have been placed in a single module location in accordance with the primary objective of the study.

Module 4 Sections Without Study Reports	Rationale for Omission
m4.2.1 Pharmacology	
m4.2.1.1 Primary Pharmacodynamics	Primary pharmacology studies (virology studies) reside in module 5.3.5.4, in agreement with ICH M4S Q&A (R4).
m4.2.1.4. Pharmacodynamic Drug Interactions	In vitro studies performed with CAB in combination with other drugs will be discussed with the virology studies and the reports are in module 5.3.5.4.
m4.2.2 Pharmacokinetics	
m4.2.2.6 Pharmacokinetic Drug Interactions	No specific pharmacokinetic drug interaction studies were conducted. However, studies relating to pharmacokinetic drug interactions, based on absorption, distribution and metabolism studies, are provided in m4.2.2.2, m4.2.2.3 and m4.2.2.4.
m4.2.2.7 Other Pharmacokinetic Studies	No other pharmacokinetic studies were performed, therefore, this section is not applicable.
m4.2.3 Toxicology	
m4.2.3.4.2 Carcinogenicity: Short- or medium-term studies	Short-term, dose range finding studies were conducted in mice and these reports are in m4.2.3.2. Otherwise, only long-term carcinogenicity studies were conducted.
m4.2.3.4.3 Carcinogenicity: Other Studies	There were no studies performed that are applicable to this section.
m4.2.3.5.4 Juvenile Toxicity	No studies have been conducted in which juvenile animals were dosed with CAB.
m4.2.3.7.1 Antigenicity	As a small molecule CAB would not be expected to evoke antigenicity and due to the lack of signals in repeat dose toxicity studies, antigenicity studies were not performed.
m4.2.3.7.3 Mechanistic	There were no studies performed that are applicable to this section.
m4.2.3.7.4 Dependence	Based on the absence of signals in repeat dose toxicity studies, dependence studies were not considered necessary.
m4.2.3.7.5 Metabolites	No metabolites occurred which would require further toxicologic characterization.
m4.2.3.7.6 Impurities	Several Ames Tests were performed with impurities and the reports are in m4.2.3.3.1.
m4.2.3.7.7 Other	There were no other studies performed which might be applicable to this section.