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1. Overview of the Nonclinical Testing Strategy

MK-0518 is a potent inhibitor of the Human Immunodeficiency Virus type 1 (HIV-1)-encoded enzyme integrase and is in development as an antiretroviral agent for the treatment of HIV-1 infection.

Currently there are no drugs targeting HIV-1 integrase approved for treating HIV-1 infection. Integrase is responsible for covalently inserting the HIV-1 proviral DNA into the genomic DNA of a host cell, a step that is critical for sustained high-level HIV-1 replication [Ref. 4.3: 123]. To accomplish integration, integrase first binds to specific sites at each viral DNA end (assembly), then cleaves two nucleotides from each viral DNA 3' end (3' processing). Integrase then catalyzes the covalent insertion of the proviral DNA (strand transfer) using the newly-exposed 3' hydroxyl groups as nucleophiles to attack phosphodiester bonds on opposite strands of the host cell DNA, simultaneously breaking the target DNA strands and joining them to the viral DNA 3' ends [Ref. 4.3: 118, 119]. The resulting ragged ends are repaired, presumably by host cell enzymes, to yield the fully integrated proviral DNA. MK-0518 inhibits the third step of the integration process, strand transfer, and is therefore designated an Integrase Strand Transfer Inhibitor (InSTI). In biochemical studies, MK-0518 potently inhibited IN strand transfer activity, but did not significantly inhibit several distinct human or viral DNA polymerases or nucleases [Sec. 2.6.3.2; 2.6.3.3].

To support the clinical evaluation of MK-0518 an extensive number of nonclinical toxicity studies were conducted prior to and in parallel with the clinical program. These studies included a battery of both in vitro and in vivo genotoxicity studies, safety pharmacology studies, acute and repeated dose oral studies and developmental and reproductive toxicity studies. Rodent carcinogenicity studies are currently ongoing. All pivotal nonclinical toxicity studies were conducted consistent with ICH Nonclinical Testing Guidelines and in compliance with the Good Laboratory Practice (GLP) Regulations. The rat and dog were chosen as the preclinical toxicology species for MK-0518 development. Choice of the dog as the non-rodent species for the nonclinical safety evaluation was based upon good oral bioavailability and similar clearance mechanisms in dogs and humans. The absorption and metabolism of MK-0518 are similar in rats, dogs, and humans. The major metabolite in all three species is the glucuronide of the parent compound. A pharmacokinetic assessment of the glucuronide metabolite was conducted in repeat dose toxicity studies in both rats and dogs and demonstrated good systemic exposure in these species. In rats and dogs exposure to the glucuronide was 3.3-fold and 3.6-fold, respectively, above the projected exposure in patients receiving 400 mg/BID MK-0518. Repeated dose toxicity studies with MK-0518 were conducted for up to 6 months in rats and 12 months in dogs. Developmental and reproductive toxicity was determined in rats and rabbits and an ongoing assessment of carcinogenicity conducted in both mice and rats, species all recommended in the ICH Nonclinical Testing Guidelines. All pivotal studies were supported by toxicokinetic

measurements either within the study or from separate studies conducted under the same conditions and utilizing identical doses administered in the same vehicle. In addition, separate preclinical pharmacokinetic and drug metabolism studies were conducted in both rats and dogs to assist in the comparison of the toxicity profiles of the drug in these species with humans. These studies demonstrated very similar metabolism and excretion profiles of MK-0518 in the toxicology species. Finally, a battery of in vitro and in vivo ocular and dermal irritation and sensitization studies were conducted with MK-0518.

The potassium salt of MK-0518 was used in all nonclinical GLP studies and clinical studies and is the form proposed for registration. The potassium salt was selected as the most stable crystalline salt form which yielded the most optimal pharmacokinetics. The impurity profile of MK-0518 is listed in [Sec. 3.2.S.4.5]. The final manufacturing specifications are shown in Module 3 [Sec. 3.2.S.4.5]. It can be seen that each of the specified impurities has been qualified by virtue of having been present in lots tested in sub chronic or chronic toxicity studies in rats and/or dogs or by levels allowable under ICH Threshold Guidelines for Impurities in Drug Substances [Sec. 2.6.7.4].

2. Pharmacology

2.1 Introduction

2.1.1 Rationale for Role of Integrase and its Blockade in Treatment of HIV-1 Infection

Agents currently approved for the treatment of HIV-1 infection target two of the three virally encoded enzymes as well as the entry pathway [Ref. 4.3: 67]. HIV enzyme inhibitors belong to three distinct mechanistic classes known as nucleoside reverse transcriptase inhibitors (NRTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs), and protease inhibitors (PIs). Integrase is a third HIV-1 encoded enzyme required for viral replication [Ref. 4.3: 123]. Integrase is the enzyme responsible for catalyzing the insertion of the HIV-1 DNA into the genome of the host cell (reviewed in [Ref. 4.3: 118, 119]). During HIV-1 infection, integration occurs as series of staged reactions which include assembly of a complex with the viral DNA, 3' endonucleolytic processing of the viral DNA ends, and strand transfer or joining of the viral and cellular DNAs. Integration into cellular DNA is required for stable maintenance of the viral genome as well as efficient HIV-1 gene expression [Ref. 4.3: 118]. Small molecule integrase inhibitors block HIV-1 replication in cell culture [Ref. 4.3: 120] and SHIV replication in rhesus macaques [Ref. 4.3: 2], and these observations support the view that integrase inhibitors could be effective for treatment of HIV-1 infection.

2.1.2 Scope of the Nonclinical Pharmacology Studies with MK-0518

MK-0518 was investigated as an inhibitor of recombinant HIV-1 integrase in an enzymatic assay [Sec. 2.6.3.2]. Activity against other HIV-1-encoded phosphoryltransferase activities (the RNA-dependent DNA polymerase activity and the RNase H activity of reverse transcriptase) were also evaluated [Sec. 2.6.3.9; 2.6.3.10]. The biochemical activities of MK-0518 have also been studied in an extensive range of enzyme, transporter, and receptor counterscreens, including the human DNA polymerases α, β, and γ [Sec. 2.6.3.11; 2.6.3.12]. The antiviral activity of MK-0518 was evaluated in cell culture HIV-1 infection studies. These studies included effects on replication of a laboratory HIV-1 isolate in the T cell line MT4, in the presence of either 10% fetal bovine serum or 50% normal human serum, the latter to evaluate the effect of human serum proteins on MK-0518's potency. Additional studies to assess the breadth of MK-0518's activity were done using a panel of primary HIV-1 isolates replicating in primary human peripheral blood mononuclear cells [Sec. 2.6.3.3]. The antiviral mechanism-of-action of MK-0518 was evaluated in a cell culture infection model using a series of quantitative PCR assays to detect 1) late HIV-1 reverse transcription products; 2) a subset of HIV-1 reverse transcription products that integrated into the cellular genome, specifically those HIV-1 DNAs that integrated near a copy of the human repetitive DNA element, Alu; and 3) 2-LTR circular forms of HIV-1 reverse transcription products [Sec. 2.6.3.4]. The potential for HIV-1 to evolve resistance to MK-0518 was evaluated by culturing a laboratory HIV-1 isolate in MT4 cells in increasing concentrations of MK-0518 over a period of months, and mutations in integrase putatively conferring resistance to MK-0518 were identified by nucleotide sequence analysis of the HIV-1 species present at various points during the process [Sec. 2.6.3.5; 2.6.3.6]. These mutations, as well as other integrase mutations identified in preclinical studies with other integrase inhibitors or in clinical studies with MK-0518, were evaluated for their effects on MK-0518 potency. These mutations were introduced into a wild-type laboratory HIV-1 isolate, and the susceptibility of the resulting viruses to inhibition by MK-0518 was evaluated in a single-cycle HIV-1 infectivity assay. [Sec. 2.6.3.7; 2.6.3.8]. The effects of combining MK-0518 with other approved antiviral agents were assessed in a cell culture HIV-1 infection assay [Sec. 2.6.3.13]. The nonclinical safety pharmacology profile of MK-0518 was established using in vivo pharmacodynamic animal assays with an emphasis on cardiovascular, respiratory, gastrointestinal, renal, and behavioral functions [Sec. 2.6.3.14; 2.6.3.15; 2.6.3.16].

2.2 Primary Pharmacodynamics

2.2.1 MK-0518 Activity Against HIV-1 Integrase In Vitro

MK-0518 was tested for the ability to inhibit the enzymatic activity of purified recombinant HIV-1 integrase in an in vitro reaction. The in vitro assay used a pre-assembled complex of HIV-1 integrase with a double-stranded "donor" oligonucleotide

mimicking one end of the mature HIV-1 cDNA, and measured the ability of integrase to catalyze the covalent joining, or strand transfer, of the donor DNA into a target DNA. MK-0518 inhibited this reaction with an apparent IC_{50} of approximately 10 nM [Sec. 2.6.3.2]. This observation shows that MK-0518 is a potent inhibitor of the strand transfer activity of purified recombinant HIV-1 integrase enzyme tested in vitro.

2.2.2 Antiviral Activity of MK-0518

2.2.2.1 Activity of MK-0518 Against Laboratory and Primary HIV-1 Isolates

MK-0518 was tested for antiviral activity against both laboratory and primary HIV-1 isolates, as well as a laboratory HIV-2 isolate, in cell culture. All antiviral assays were multiple-cycle replication assays, with replication detected by measuring the accumulation of HIV-1 and -2 gag proteins (p24 and p27) in culture supernatant fluids. MK-0518 inhibited replication of the laboratory HIV-1 isolate, H9IIIB, in MT4 cells with a 95% cell inhibitory concentration (CIC_{95}) of 18.7 ± 14 nM (n=77) in the presence of 10% fetal bovine serum (FBS) and 31 ± 20 nM (n=90) in the presence of 50% normal human serum. The presence of human serum proteins therefore reduced the apparent antiviral potency of MK-0518 by less than two-fold. The fifteen primary HIV-1 isolates tested included isolates from six subtypes representing both syncytium-inducing and non-syncytium-inducing isolates. MK-0518 inhibited replication of these isolates in primary human peripheral blood mononuclear cells in the presence of 20% fetal bovine serum with CIC_{95} ranging from 6 to 50 nM. MK-0518 inhibited replication of a laboratory HIV-2 isolate in CEMx174 cells and 10% FBS with an average CIC_{95} 6.3 nM. Collectively, these studies indicate that MK-0518 has comparable antiviral activity against an array of diverse HIV-1 isolates, and that the addition of 50% human serum only modestly reduces the potency of MK-0518 [Sec. 2.6.3.3].

2.2.2.2 MK-0518's Antiviral Mechanism of Action

The mechanism by which MK-0518 inhibits HIV-1 replication was evaluated in an acute HIV-1 infection cell culture model. Cells from the human T cell line SupT1 were infected with a laboratory HIV-1 isolate that had been pseudotyped with the vesicular stomatitis virus G protein to increase infectivity. Cells were infected in the presence or absence of 1 μM MK-0518, and the progress of infection evaluated at different times using a series of quantitative PCR assays to measure the abundance of various HIV-1 DNA forms. MK-0518 had no significant effect on the abundance of late HIV-1 reverse transcription products present at either 6 hr or 48 hr after initiating infection [Sec. 2.6.3.3]. In contrast, the presence of MK-0518 resulted in a significant increase at 48 hr in the abundance of 2-LTR circular forms of the HIV-1 genome, a form of the genome that cannot sustain HIV-1 replication and that is known to increase in abundance when integrase is genetically absent [Ref. 4.3: 124] or pharmacologically inhibited [Ref. 4.3: 120]. Direct measurement of all integrated HIV DNA forms is not possible

owing to the observation that HIV-1 DNA can integrate into most sites in the human genome. However, it is possible to detect integration of HIV-1 DNA at sites near the abundant human DNA repetitive element, Alu, using one primer binding to an HIV-1 LTR and one primer binding to the Alu element [Ref. 4.3: 117]. MK-0518 significantly inhibited the abundance of Alu-LTR DNA detectable at 48 hr after infection [Sec. 2.6.3.4]. The results of these studies show that MK-0518 does not inhibit the process of viral entry or reverse transcription. Rather, these studies support the view that MK-0518's antiviral activity is attributable to inhibiting the HIV-1 integrase enzyme during the early phase of the HIV-1 lifecycle [Sec. 2.6.3.4]. Additional evidence for integrase as the physiologic target of MK-0518 is provided in the following sections, which show that mutations in integrase affect sensitivity to MK-0518.

2.2.2.3 Mutations in HIV-1 Integrase that Reduce Susceptibility to MK-0518

HIV-1 is typically able to develop resistance to direct antiretroviral agents through a process of mutation and selection. The potential for HIV-1 to develop resistance to MK-0518 was evaluated by culturing a laboratory HIV-1 isolate in human T lymphoid H9 cells in increasing concentrations of MK-0518 over a period of months. This selection process produced viral variants that were able to replicate in higher concentrations of MK-0518 [Sec. 2.6.3.5]. Viruses emerging at the end of most passages were characterized in detail by amplifying and molecularly cloning their integrase genes and determining the nucleotide sequences [Sec. 2.6.3.6]. These analyses showed that a series of specific amino acid changes occurred over time during culture in increasing MK-0518 concentrations. The first change observed was Q148K, which arose during growth in 25 to 50 nM MK-0518, concentrations exceeding MK-0518's CIC_{95}. The Q148K mutation persisted during growth in increasing MK-0518 concentrations up to 500nM. After passage in 1 μM MK-0518, mutations E138A and G140A were sequentially incorporated, and growth in still higher concentrations resulted in the appearance of additional mutations in the integrase gene. Thus, the ability of HIV variants to replicate in higher concentrations of MK-0518 was correlated with the appearance of mutations in the integrase gene [Sec. 2.6.3.6].

To determine which integrase mutations resulted in reduced MK-0518 susceptibility, some of the observed mutations were introduced into a wild-type HIV-1 isolate and the mutant viruses tested in a single-cycle HIV-1 infectivity assay. The Q148K, E138A/Q148, and E138A/G140A/Q148K mutations resulted in average fold-shift IC_{50} values of 46-fold, 90-fold, and 508-fold, respectively [Sec. 2.6.3.7]. These results demonstrate that specific mutations arising in the integrase gene during viral growth in increasing MK-0518 concentrations confer reduced susceptibility to MK-0518.

Viruses containing other integrase mutations were also tested to determine whether these mutations affect MK-0518 susceptibility. Some of these mutations were observed during cell culture resistance selection experiments with integrase inhibitors distinct from

MK-0518 [Ref. 4.3: 16]. In general, these mutations conferred only low-level (less than 10-fold) resistance to MK-0518, though the N155S mutation resulted in ~19-fold increase in MK-0518 IC_{50} [Sec. 2.6.3.7]. Other mutations were observed in the integrase genes of viruses isolated from HIV-1-infected subjects failing antiviral treatment regimens including MK-0518. These mutations, including the primary mutations N155H, Q148H, and Q148R, each conferred a greater than 10-fold increase in the MK-0518 IC_{50} [Sec. 2.6.3.7].

The observation that mutations in the HIV-1 integrase gene can alter the susceptibility of HIV-1 to MK-0518 constitutes additional evidence that integrase is the physiologic target of MK-0518.

2.3 Secondary Pharmacodynamics

A series of biochemical studies suggest that the potential for secondary effects of MK-0518 is limited. MK-0518 did not inhibit the DNA polymerase [Sec. 2.6.3.9] or RNaseH [Sec. 2.6.3.10] activities of HIV-1 reverse transcriptase at concentrations of 25 μM or higher. MK-0518 exhibited >1,000-fold selectively for integrase with respect to human DNA polymerases α, β, and γ, with IC_{50} > 50 μM for each of these polymerases [Sec. 2.6.3.11]. MK-0518 showed no significant findings in the MDS Pharma Services Merck Screen in 166 assays when tested at 10 μM or greater [Sec. 2.6.3.11]. These data suggest that MK-0518 is highly selective for HIV integrase.

2.4 Safety Pharmacology

MK-0518 was studied in a range of tests at doses of up to 10 mg/kg IV or 120 mg/kg P.O. to assess its potential for effects on cardiovascular/autonomic or respiratory function in anesthetized dogs and renal or gastric acid secretion functional assays in conscious dogs. The tests were previously validated with reference compounds. MK-0518 was also examined in conscious mice at up to 30 mg/kg P.O. to determine its potential effect on gastrointestinal motility and at 100 mg/kg P.O. to evaluate behavioral and other central nervous system effects. MK-0518 was also tested for activity against the human HERG ion channel.

Overall, there were only two significant findings in these studies. MK-0518 caused an increase in gastric motility in mice when administered at a concentration of 30 mg/kg, but not when administered at 10 mg/kg. A slight increase in body temperature was also observed in mice receiving a dose of 100 mg/kg MK-0518. The safety pharmacology studies thus showed that MK-0518 has minimal effects on a diverse range of physiological functions [Sec. 2.6.3.14; 2.6.3.15; 2.6.3.16; 2.6.3.17; 2.6.3.18; 2.6.3.19; 2.6.3.20; 2.6.3.21; 2.6.3.22].

2.5 Pharmacodynamic Drug Interactions

MK-0518, like other antiretroviral agents, is expected to be used in combination with other antiretroviral drugs for treating HIV-1 infection [Ref. 4.3: 67]. The potential for interaction of MK-0518 with other approved antiretroviral agents was assessed by testing the antiviral activity of MK-0518 in combination with each of 18 licensed agents in a cell culture HIV-1 replication assay. These 18 agents included seven nucleoside analog reverse transcriptase inhibitors (zidovudine, zalcitabine, stavudine, abacavir, tenofovir, didanosine, lamivudine), three non-nucleoside reverse transcriptase inhibitors (efavirenz, nevirapine, delavirdine), seven protease inhibitors (indinavir, saquinavir, ritonavir, amprenavir, lopinavir, nelfinavir, atazanavir), and the entry inhibitor enfuvirtide. Each of these agents was tested in combination with MK-0518 over a wide range of concentrations for the ability to inhibit HIV-1 replication in cell culture, and the results analyzed using an independent joint action model. Depending on the specific concentrations being tested, MK-0518 was either additive or synergistic with each of the approved antiretroviral agents. The cell culture system was able to detect a known antagonistic drug-drug interaction, zidovudine with ribavirin, but there was no indication of any antagonistic interaction with MK-0518 [Sec. 2.6.3.13].

2.6 Discussion and Conclusions

The sponsor has shown that MK-0518 potently inhibits the integrase enzyme of HIV-1 in vitro, and that the antiviral activity of MK-0518 in cell culture is consistent with inhibiting the integrase enzyme in the early phase of the viral lifecycle. Mutations in the HIV-1 integrase gene can confer resistance to MK-0518, further supporting the view that integrase is the physiologic target of MK-0518. MK-0518 displayed comparable antiviral activity against a diverse panel of primary HIV-1 isolates, suggesting that MK-0518 will likely have broad activity against HIV-1 clinical isolates. MK-0518's potency shifted less than 2-fold upon addition of human serum, and MK-0518's potency in human serum is comparable to that of other licensed antiretroviral agents. Studies of MK-0518 in combination with 18 licensed antiretroviral agents in 4 mechanistic classes showed no evidence of antagonistic interactions, supporting the view that it will be possible to use MK-0518 in combination with other approved agents for the treatment of HIV-1 infection.

Since MK-0518's target enzyme is of viral rather than host origin, there is no potential for mechanism-based toxicity. Counterscreening against an extensive panel of other targets, including human DNA polymerases and other enzymes, transporters, and receptors, did not reveal any significant off-target activities. MK-0518 is well tolerated in vivo and evoked no significant ancillary pharmacological or behavioral effects.

The pharmacodynamic studies have therefore adequately addressed the mechanism of action, potency, breadth of antiviral activity, and potential liabilities associated with the administration of MK-0518.

3. Pharmacokinetics

3.1 Introduction

The studies discussed in this section represent a comprehensive evaluation of the absorption, distribution, metabolism, and excretion (ADME) of MK-0518 in rats and dogs, the species used for the toxicological evaluation of the compound. The ADME properties of MK-0518 have also been studied in humans and interspecies similarities/differences have been addressed. The sponsor has evaluated MK-0518 as a substrate and inhibitor of cytochrome P450 enzymes and has conducted extensive studies to identify the UDP-glucuronosyltransferases responsible for the metabolism of MK-0518. Information from these studies is essential to assess the drug-drug interaction potential of the compound and is useful for interpreting data from clinical studies. All animal models, in vitro evaluations and analytical methods used are appropriate to support the sponsor's conclusions.

The nonclinical ADME studies conducted are listed in the Pharmacokinetics Overview Table [Sec. 2.6.5.1].

3.2 Absorption

The plasma clearance was 45.5 and 7.4 mL/min/kg following IV administration of MK-0518 to rats and dogs, respectively [Sec. 2.6.5.2]. The terminal half-life was short (≤1.6 hr) in both species, although the steady-state volume of distribution was higher in rats (2.2 L/kg) than dogs (0.4 L/kg). Following oral administration of a solution of the potassium salt (6 mg/kg), absorption was rapid (T_{max} ≤0.6 hr) and the bioavailability was high (≥61.6%). The dose-dependence of the pharmacokinetics of MK-0518 was also evaluated after single oral dose administration to rats (40 to 240 mg/kg), and dogs (5 to 135 mg/kg) [Sec. 2.6.5.3]. In rats, plasma AUC was nearly linear over the dose range of 40 to 120 mg/kg, but there was no further increase in exposure with increased dose. In dogs, AUC and C_{max} increased proportionally with dose over the dose range of 5 to 45 mg/kg; the increase in either parameter was less than dose-proportional when the dose was increased to 135 mg/kg. Thus, neither absorption nor first-pass extraction was saturable up to doses of 120 and 45 mg/kg in rats and dogs, respectively. Overall, the experiments conducted and results obtained support the sponsor's conclusion that MK-0518 is well absorbed and is not subject to high first-pass extraction.

3.3 Distribution

3.3.1 Tissue Distribution in Rats

[^{14}C]MK-0518-derived radioactivity was rapidly and widely distributed throughout the body following oral administration of radiolabeled MK-0518 at 6 mg/kg. Maximum concentrations of radioactivity (μg MK-0518 radioequivalents/g tissue) were observed 0.5 hr postdose (first sampling time) for blood (0.34 μg/g), plasma (0.60 μg/g), and most tissues [Sec. 2.6.5.4]. The highest concentrations were observed in the gastrointestinal tract and organs of excretion, with levels of 30.9 μg/g in stomach, 8.97 μg/g in small intestine, 3.91 μg/g in liver, 1.72 μg/g in kidney, and 0.44 μg/g in the urinary bladder at 0.5 hr postdose. Maximum mean concentrations in all other tissues ranged from 0.00971 μg/g (brain) to 0.221 μg/g (lungs) at 0.5 hr postdose. Levels of radioactivity declined rapidly in all tissues such that by 24 hr only 0.76% of the dose was recovered in the collected tissues. Based on the observed levels of radioactivity, it is reasonable to conclude that [^{14}C]MK-0518-derived radioactivity partitions preferentially into the extracellular fraction of blood. In addition, it can be concluded that the tissue distribution and subsequent elimination of [^{14}C]MK-0518-derived radioactivity is a fairly rapid process.

3.3.2 Placental Transfer in Rats and Rabbits

Placental transfer of MK-0518 was investigated in pregnant rats and rabbits by measuring concentrations of unchanged drug in maternal and fetal plasma on Gestation Day (GD) 20 following daily oral administration of MK-0518 at 300 or 600 mg/kg in rats on GD 6 through 20, and at 1000 mg/kg in rabbits on GD 7 through 20 [Sec. 2.6.5.5]. The ratios of fetal to maternal plasma concentration in rats ranged from 1.2 to 2.7, while in rabbits, the corresponding ratio was 0.02. The results suggest that MK-0518 readily crosses the placenta in rats and the drug also transfers to the placenta in rabbits although to a lesser extent than observed in rats.

3.3.3 In Vitro Plasma Protein Binding and Blood-to-Plasma Ratio

The sponsor has demonstrated that the binding of MK-0518 to plasma proteins from rat (74%), dog (70%), and human (83%) is modest [Sec. 2.6.5.7]. In addition, binding was independent of MK-0518 concentration. Binding was also modest (70%) in the mouse, the species used in carcinogenicity testing, and the binding in this species also did not vary as a function of MK-0518 concentration.

The partitioning of MK-0518 (0.9-18 μM) into blood cells was studied and the blood-to-plasma concentration ratios were determined to be 0.7, 0.9, and 0.6 for rat, dog, and human, respectively [Sec. 2.6.5.8]. These results indicate that total blood clearance will

be somewhat higher than plasma clearance in humans and rats, while these values will be similar in dogs.

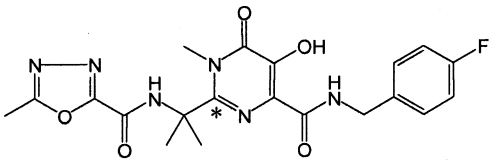
3.4 Metabolism

3.4.1 In Vivo Metabolism in Mice, Rats, Dogs, and Humans

The metabolism of [^{14}C]MK-0518 was assessed in mice (20 mg/kg P.O.), rats (3 mg/kg IV), dogs (1.5 mg/kg IV), and humans (200 mg P.O.) [Sec. 2.6.5.11]. The glucuronide of MK-0518 (M2, [Figure 2.4: 1]) was identified as the major radioactive entity in bile of mice. Similarly, the major metabolite in bile and urine of rats and dogs was M2, accounting for 62 and 31% of the administered dose, respectively. The glucose conjugate of the parent compound (M1; rat urine, dog urine and bile) and the acetyl hydrazine derivative (M3, rat urine) were the only other metabolites detected in rats and dogs and each metabolite represented <2% of the dose. The fraction of the dose excreted in urine and bile as unchanged parent compound was 10% in rats and 30% in dogs indicating that metabolism (via glucuronidation) is the major mechanism of clearance of MK-0518 in preclinical species. The only metabolite detected in humans was M2 which accounted for 72% of the dose recovered in urine. It is reasonable to conclude that metabolism via glucuronidation is also the principal mode of clearance of MK-0518 in humans since the majority of the dose recovered in urine was due to M2, and since a significant fraction of the parent compound observed in feces is likely derived from the hydrolysis of M2 secreted in bile as observed in preclinical species. The major circulating metabolite in all species (mouse, rat, dog, and human) was M2. Although M2 circulates at significant levels in humans the pharmacological activity can be attributed to the parent compound since M2 has no activity against HIV-1. Overall, these results demonstrate that the preclinical species used in the toxicological evaluation of the compound were appropriate as the only metabolite detected in humans (M2, observed in urine and plasma) was also observed in plasma and excreta of the preclinical species used in toxicological evaluation.

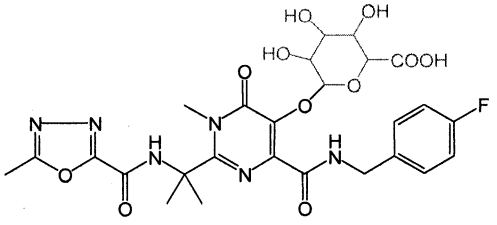
Figure 2.4: 1

Structures of [^{14}C]MK-0518 and its Major Metabolite (MK-0518 Glucuronide) in Mice, Rats, Dogs, and Humans



MK-0518

* indicates the position of the ^{14}C label



MK-0518 glucuronide (M2)

3.4.2 In Vitro Metabolism in the Rat, Dog, and Human

No turnover of MK-0518 was observed in NADPH-fortified liver microsomal incubations from any of the species studied indicating that MK-0518 is not a substrate of cytochrome P450 enzymes [Sec. 2.6.5.12]. In contrast, MK-0518 readily underwent metabolism in hepatocytes, with the major metabolite in all three species being the glucuronide of the parent compound (M2), consistent with the in vivo results. The sponsor has conducted a number of studies which collectively demonstrate that the glucuronidation of MK-0518 is mainly catalyzed by UDP-glucuronosyltransferases (UGT)-1A1 with a minor contribution from UGT1A9 and UGT1A3 [Sec. 2.6.5.15]. Although the tools for phenotyping UGT reactions are not as well established as those for cytochrome P450 enzymes [Ref. 4.3: 116], the sponsor believes the conclusions regarding the identity of the major UGT involved in the metabolism of MK-0518 is reasonable based on the following: of the tested 12 cDNA-expressed UGTs only UGT1A1 (highest activity), UGT1A3 and UGT1A9 metabolized MK-0518; bilirubin which is known to be a selective UGT1A1 substrate inhibited MK-0518 glucuronidation with an IC_{50} of 7.1 μM; the potent UGT1A1 inhibitor atazanavir inhibited formation of M2 with an IC_{50} of 0.5 μM; formation of M2 was highly correlated with UGT1A1 activity, but not UGT1A4 or UGT1A9, in a bank of human liver microsomes characterized with respect to their activities of these three UGTs.

3.4.3 MK-0518 as an Inhibitor/Inducer of Human Cytochromes P450

The sponsor has performed well-designed experiments to evaluate MK-0518 as an inhibitor of CYP activity (CYP1A2, 2B6, 2C8, 2C9, 2C19, 2D6, and 3A4) in human liver microsomes [Sec. 2.6.5.13]. At concentrations up to 100 μM, MK-0518 was found not to be a potent inhibitor (IC_{50}>100 μM) of any CYP activities. Based on this result, MK-0518 is unlikely to be an inhibitor of CYP enzymes in vivo. The potential of

MK-0518 to induce CYP3A4 was evaluated in cultures of primary human hepatocytes (n=2 donors). MK-0518 (up to 10 μM) did not induce CYP3A4 RNA expression or CYP3A4-dependent testosterone 6β-hydroxylase activity [Sec. 2.6.5.14]. As expected, the positive control rifampicin (10 μM) induced CYP3A4 RNA expression (5.2 - 14.2 fold) and enzyme activity (1.7 – 3.4 fold). Thus, the data indicate that MK-0518 has no potential to induce CYP3A4 in humans.

3.4.4 MK-0518 as an Inhibitor of Human UDP-Glucuronosyltransferases

The potential for MK-0518 to inhibit UGT1A1 or UGT2B7 was evaluated in vitro using human liver microsomes. The drug was added at various concentrations (0.07-50 μM) to a reaction mixture containing human liver microsomes, UDPGA and a UGT marker substrate. The marker substrates were estradiol (UGT1A1) and AZT (UGT2B7) and both were used at a concentration of 100 μM [Sec. 2.6.5.16]. The IC_{50} values were >50 μM for both UGT activities. Therefore, MK-0518 would not be anticipated to inhibit these enzymes at therapeutically relevant concentrations.

3.4.5 Interaction of MK-0518 With Human P-Glycoprotein

The sponsor evaluated MK-0518 as a substrate and inhibitor of P-glycoprotein (P-gp) using validated in vitro models. Directional transport studies of MK-0518 yielded transport ratios (B-A/A-B ratio) of 11.1, 7.1 and 9.4 in LLC-PK1 cells expressing human (L-MDR1), mouse (L-mdr1a), and rat (L-Rmdr1a) P-gp, respectively indicating that MK-0518 is a substrate of P-gp in the three species [Sec. 2.6.5.9]. MK-0518 was evaluated as a potential inhibitor of P-gp in human MDR1-transfected LLC-PK1 cells as well as in human P-gp-overexpressing KB-V1 cells. Over a concentration range of 1 to 100 μM MK-0518 did not affect [^{3}H]-vinblastine (VBL) accumulation in L-MDR1 and KB-V1 cell lines [Sec. 2.6.5.10]. In contrast, in the presence of a typical P-gp inhibitor, cyclosporin A, cellular accumulation of [^{3}H]-VBL was increased 10- and 12-fold in L-MDR1 and KB-V1 cells, respectively. These results indicate that MK-0518 is not an inhibitor of human P-gp and thus would not be expected to affect the pharmacokinetics of drugs whose disposition is dependent on P-gp. Although MK-0518 is a P-gp substrate, P-gp inhibitors will not have a significant effect on the elimination of MK-0518 since the compound is cleared principally by metabolism. In addition, P-gp is unlikely to have a significant effect on the absorption of MK-0518 based on the following considerations: 1) Modulation of P-gp is believed to mainly affect the rate rather than the extent of absorption and the effect of P-gp inhibition/induction appears to be more important for compounds that are substrates for both P-gp and CYP3A (MK-0518 is not a substrate of CYP3A) [Ref. 4.3: 113, 114], 2) The impact of P-gp on the absorption of high dose drugs such as MK-0518 is not expected to be significant as high drug concentration in the intestinal lumen will likely saturate P-gp-mediated efflux (MK-0518 exhibited nearly dose-proportional pharmacokinetics across a wide oral dose (10 to 1200 mg) when administered to healthy volunteers; Protocol 001) [Ref. 4.3: 115], and 3) The absorption

of MK-0518 was nearly complete over the dose range of 6 to 120 mg/kg in a preclinical species (rat) in which MK-0518 proved to be a substrate of P-gp.

3.5 Excretion

Recovery of radioactivity in the excreta exceeded 70% following administration of single IV and P.O. doses of [^{14}C]MK-0518 to rats and dogs [Sec. 2.6.5.17]. The majority of the dose (50 to 74%) was excreted in the feces with the remaining appearing in urine. The high recovery of the dose in feces after IV administration indicates that biliary secretion is the primary mode of elimination of MK-0518-related radioactivity consistent with results observed in bile-duct cannulated rats. A similar pattern of excretion was observed in humans following an oral dose of MK-0518 (32% in urine; 51% in feces). In rats, the recovery of the dose in urine was independent of the route of administration suggesting that absorption of the drug was complete in this species. In dogs, the lower recovery of dose in urine after P.O. administration relative to the IV route suggests that absorption of MK-0518 is incomplete; however, the high bioavailability (70%) indicates that absorption is nonetheless good in the dog as well.

The excretion of MK-0518 into the milk of lactating rats was investigated by measuring concentrations of the parent compound in maternal plasma and milk on Lactational Day (LD) 14 following daily oral administration of MK-0518 at 300 or 600 mg/kg/day from Gestational Day (GD) 6 to LD 14 [Sec. 2.6.5.18]. The milk-to-plasma concentration ratios were ~3 at both doses. Thus, the results demonstrated significant excretion of circulating drug into the milk of lactating rats.

3.6 Discussion and Conclusions

The ADME properties of MK-0518 have been evaluated in detail in the animal species (rat and dog) used for the Safety Assessment studies. MK-0518 is a low (dog) to intermediate (rat) clearance compound, with a short plasma half-life (≤1.6 hr), and a volume of distribution ranging from ~0.4 to 2 L/kg. After oral dosing, absorption is rapid (T_{max} ≤0.6 hr), nearly complete (≥70%), and bioavailability is high (≥61.6%). Following oral administration [^{14}C]MK-0518 to rats, radioactivity is distributed rapidly and widely throughout the body and is eliminated within 24 hr. In addition to plasma, the highest concentrations of radioactivity are observed in the gastrointestinal tract and organs of excretion, while the lowest concentration is exhibited by the brain. The drug crosses the rat and rabbit placenta and is excreted extensively in milk of lactating rats. MK-0518 shows modest binding (70 to 83%) to plasma proteins in all species examined (mouse, rat, dog, and human). In preclinical species, MK-0518 is cleared primarily by metabolism (glucuronidation), and based on urinary data MK-0518 is also eliminated principally by metabolism in humans. The glucuronide derivative of the parent compound is the only metabolite detected in humans and is the major in vivo and in vitro metabolite in preclinical species. The glucuronidation of MK-0518 is catalyzed mainly by UGT1A1 with minor

contribution from UGT1A9 and UGT1A3. Therefore, MK-0518 may be subject to drug-drug interactions when co-administered with drugs that are known to be UGT1A1 inducers (e.g., rifampin) or inhibitors (e.g., atazanavir, an HIV-protease inhibitor). However, MK-0518 is not likely to affect the metabolic clearance of drugs metabolized by UGT1A1 given its low UGT1A1 inhibitory and induction potential. Since MK-0518 is neither a substrate nor an inhibitor of cytochrome P450 enzymes and is not an inducer of CYP3A4, MK-0518 is not expected to exhibit metabolic drug interactions with substrates of cytochromes P450.

4. Toxicology

4.1 Introduction

The toxicity profile of MK-0518 was assessed in a series of acute toxicity studies in rodents and in subchronic and chronic toxicity studies in rats and dogs of up to 6 and 12 months, respectively. The reproductive and developmental toxicity profile was determined in rats and rabbits. The potential genotoxicity was assessed in vitro in bacterial and mammalian cell assays and in vivo in the mouse micronucleus assay. Finally, assessment of the carcinogenic potential of MK-0518 is ongoing in 2-year rat and mouse carcinogenicity studies conducted at the maximum-tolerated dose in mice and at the maximum feasible dose based on drug formulation in rats. A listing of the nonclinical toxicity studies conducted with MK-0518 is shown in the Toxicology Overview Table [Sec. 2.6.7.1].

4.2 Single-Dose Toxicity

Acute oral toxicity studies were conducted with MK-0518 in mice and rats to determine the approximate lethal dose following administration of a single dose and in dogs to determine its acute toxicity following a single oral dose.

In mice, MK-0518 was administered to males and females as a single oral dose at 1000, 1500, and 2000 mg/kg and observed for 3 days to determine the potential toxicity and approximate lethal $dose_{50}$ (TT #03-2616) [Sec. 2.6.7.5]. Additionally, this study supported dose selection for an in vivo micronucleus assay. Treatment-related mortality occurred in 1 out of 3 male mice at 2000 mg/kg only. No treatment-related mortality occurred in female mice at 2000 mg/kg or at 1500 or 1000 mg/kg. Treatment-related decreased activity, bradypnea, and ptosis were noted in female and male mice at 2000 mg/kg and 1500 mg/kg within approximately 2 hours postdose and resolved on Day 2. No treatment-related physical signs were noted at 1000 mg/kg. The approximate oral lethal $dose_{50}$ of MK-0518 following a 3-day observation period was >2000 mg/kg in both female and male mice.

In rats, MK-0518 was administered to female rats by oral gavage as a single dose with a 14-day observation period to determine the approximate lethal $dose_{50}$ (TT #03-2619) [Sec. 2.6.7.5]. MK-0518 produced no treatment-related mortality, physical signs, or effects on body weight at 2000 mg/kg. In conclusion, the approximate oral lethal $dose_{50}$ of MK-0518 in female rats following a 14-day observation period was >2000 mg/kg.

In dogs, administration of single-rising doses of 100, 250, 500, and 1000 mg/kg of MK-0518 produced treatment-related changes at doses ≥500 mg/kg (TT #04-0080) [Sec. 2.6.7.5]. Treatment-related physical signs consisted of emesis in 3 of 4 dogs administered 500 mg/kg and 4 of 4 dogs treated with 1000 mg/kg of MK-0518. Systemic exposures ($AUC_{0\text{-}24\ hr}$; μM•hr) at 100, 250, 500, 1000 mg/kg were 148, 277, 353, 285, respectively and C_{max} (μM) 49.6, 64.7, 75.2, 94.8, respectively. These study results have identified 250 mg/kg to be both the highest dose tolerated by dogs and a dose determined to be within the plateau of exposure to MK-0518 following a single administration.

4.3 Repeat-Dose Toxicity

Repeat-dose toxicity studies with MK-0518 were conducted in mice, rats and dogs.

Prior to initiation of toxicity studies in mice, vehicle optimization experiments were conducted with MK-0518. Mice were administered MK-0518 by oral gavage once daily for approximately 3 weeks to determine its toxicokinetic profile using 3 separate vehicles (TT #05-0079) [Sec. 2.6.7.6]. One group of 22 female and 22 male mice received 500 mg/kg/day of MK-0518 suspended in 0.5% (w/v) methylcellulose in deionized water (0.5% methylcel), and 2 groups of 24 female and 24 male mice (that included 2 spares/sex/group) received this dose of the test article (500 mg/kg/day) in polyethylene glycol 400 in deionized water 80:20 (w/w) (PEG400/DI water [80:20 w/w]) or in 20% (w/w) sucrose/4% (w/w) hydroxypropyl cellulose-super low/0.19% (w/w) sodium lauryl sulfate in deionized water (20% sucrose/4% HPC/0.19% SLS). A factor of 1.09 was used in all dose calculations since MK-0518 was supplied as the potassium salt. The dosing volume for all animals was 10 mL/kg, except for animals that received MK-0518 suspended in PEG400/DI water (80:20 w/w) for which the dosing volume was 2.5 mL/kg. Plasma drug concentration analysis was conducted in Drug Week 3.

The toxicokinetic parameters are presented in [Table 2.4: 1].

Table 2.4: 1

Plasma MK-0518 Toxicokinetic Parameters in Mice – Drug Week 3

	MK-0518 (mg/kg/day)		
	Females		
	500[b]	500[c]	500[d]
$AUC_{0\text{-}24\ hr}$ (μM•hr)[a]	75.5 ± 8.83	63.3 ± 5.46	143 ± 52.2
C_{max} (μM)[a]	35.1 ± 5.06	26.3 ± 2.37	48.1 ± 40.6
T_{max} (hr)	0.5	0.5	1.0
	Males		
	500[b]	500[c]	500[d]
$AUC_{0\text{-}24\ hr}$ (μM•hr)[a]	80.1 ± 7.56	48.3 ± 5.32	83.3 ± 20.6
C_{max} (μM)[a]	31.8 ± 3.29	18.4 ± 3.32	50.8 ± 32.7
T_{max} (hr)	0.5	0.5	0.5
	Sexes Combined		
	500[b]	500[c]	500[d]
$AUC_{0\text{-}24\ hr}$ (μM•hr)[a]	77.9 ± 5.42	55.8 ± 4.19	113 ± 27.8
C_{max} (μM)[a]	33.4 ± 2.87	22.4 ± 2.41	46.3 ± 17.2
T_{max} (hr)	0.5	0.5	0.5

[a] Mean ± SEM.
[b] 500 mg/kg/day in 0.5% Methylcellulose.
[c] 500 mg/kg/day in PEG 400/DI Water (80/20 w/w).
[d] 500 mg/kg/day in 20% Sucrose/4% HPC/0.19% SLS.

(TT #05-0079) [Sec. 2.6.7.6]

For the 3 formulations there were no substantial sex-related differences in systemic exposure ($AUC_{0\text{-}24\ hr}$) or C_{max} and absorption of MK-0518 was rapid with C_{max} occurring 0.5 hour after dosing. Although systemic exposure and C_{max} values for MK-0518 when dosed in the 20% Sucrose/4% HPC/0.19% SLS vehicle were slightly greater than for the other formulations, when considering interanimal variability, toxicokinetic parameters of MK-0518 from all 3 formulations were considered similar. Based on a similar performance of all 3 vehicles, 0.5% methylcellulose was selected as the vehicle for mice since a higher dose volume could be safely administered and the lower viscosity of methylcellulose should minimize dosing accidents.

A 5-week toxicokinetic study in mice was conducted to determine if repeat dosing at higher dose levels than previously tested affected the toxicokinetic parameters in this species [Sec. 2.6.7.7B]. MK-0518 was administered to mice by oral gavage once daily for approximately 5 weeks. Four groups of 34 female mice each received 50, 500, 1000,

or 2500 mg/kg/day of MK-0518. Since mortality was observed at 2000 mg/kg in males and not females in the previous single dose toxicity study, three groups of 34 male mice each received 50, 500, or 1000 mg/kg/day of MK-0518. Plasma samples for analyses of drug concentration were taken at various times after dosing in Drug Week 5.

One male and 1 female each died at 500 mg/kg/day although a higher incidence of mortality occurred in the 2500-mg/kg/day and 1000-mg/kg/day dose group resulting in early termination of these dose levels on Drug Day 15 (females) and Drug Day 16 (males).

The toxicokinetic parameters are presented in [Table 2.4: 2].

Table 2.4: 2

Plasma MK-0518 Toxicokinetic Parameters in Mice – Drug Week 5

	MK-0518 (mg/kg/day)	
	Females	
	50	500
$AUC_{0\text{-}24\ hr}$ (μM•hr)[a]	14.7 ± 1.12	50.4 ± 3.46
C_{max} (μM)[a]	10.6 ± 0.684	17.5 ± 1.28
T_{max} (r)	0.5	0.5
	Males	
	50	500
$AUC_{0\text{-}24\ hr}$ (μM•hr)[a]	14.6 ± 2.60	40.0 ± 7.01
C_{max} (μM)[a]	8.14 ± 1.13	25.5 ± 12.7
T_{max} (r)	0.5	0.5
	Sexes Combined	
	50	500
$AUC_{0\text{-}24\ hr}$ (μM•hr)[a]	14.5 ± 1.20	45.2 ± 4.02
C_{max} (μM)[a]	9.38 ± 0.770	21.5 ± 6.11
T_{max} (hr)	0.5	0.5
[a] Mean ± SEM.		

[Sec. 2.6.7.7B]

The results of this study indicate that after repeat oral dosing in mice the minimum lethal dose is 500 mg/kg/day. At this dose, the systemic exposure to the parent drug is approximately 2-fold greater than the AUC (54 μM•hr) at the 400-mg/BID MRD when adjusted for 30% and 17% unbound drug in mice and humans, respectively. Based on these data, 500 mg/kg/day exceeds the MTD in this species. This was confirmed in the 14-week oral range-finding study in mice discussed below.

In a 14-week oral range-finding study, MK-0518 was administered at doses of 50, 500, 1000, 2500, or 5000 mg/kg/day to groups of 15 mice/sex/group suspended in 0.5% (w/v) methylcellulose in deionized water [Sec. 2.6.7.7A]. A complete assessment of toxicity including measurements of body weights, ophthalmology, clinical pathology, and histopathology were conducted.

In general, similar types of treatment-related findings were observed in animals at dosages ≥1000 mg/kg/day. These treatment-related changes consisted of mortality, a variety of physical signs (typically distended abdomen, labored breathing, audible respiratory noises, decreased activity, and eye partially closed), and body weight loss and/or decreases in body weight gain. As a result, these dose groups were terminated early. At 500 mg/kg/day, treatment-related mortality, physical signs (consisting of distended abdomen, labored breathing, audible respiratory noises, hunched posture, decreased activity, and/or eyes partially closed), and decreases in body weight gain were observed. Histomorphologic observations were limited to changes in the stomach of animals that died early, were consistent with the clinical signs of gastrointestinal bloating and were suggestive of an irritant effect from the compound.

Based upon the physical signs observed and decreases in body weight gains (-30%) and on the histomorphologic changes in the stomach and esophagus at 500 mg/kg/day, the no-effect level for the study is 50 mg/kg/day. Furthermore, the changes observed at 500 mg/kg/day are not considered to be compatible with long term survival of mice. Considering the mortality and decreases in body weight gain in males and females at 500 mg/kg/day, and the potential for these changes to adversely affect morbidity and survivorship in long-term studies, the maximum tolerated dose for a 2-year evaluation of carcinogenic potential of this drug is considered to be 400 mg/kg/day. Doses of 50, 250, and 400 mg/kg/day in females and 50, 100 and 250 mg/kg/day in males were selected for the carcinogenicity study in mice. These doses were reviewed and approved by the FDA Executive Carcinogenicity Assessment Committee.

In rats, MK-0518 was studied in GLP oral toxicity studies of 5, 14, and 27 weeks duration (TT #04-0079, TT #03-119-0, TT #04-6022) [Sec. 2.6.7.7C; 2.6.7.7D; 2.6.7.7E]. Prior to initiating toxicology studies in rats a series of exploratory experiments were conducted to increase systemic exposure to MK-0518 in rats. Vehicles tested for MK-0518 solubility and/or in vivo exposure performance, included 0.5% methylcellulose, 100% PEG400, 80% PEG400, methylcellulose in 10% Tween 80, and distilled water [Table 2.4: 3]. Additional experiments compared once daily to twice daily dosing. The solubility of MK-0518 potassium salt was 14 mg/mL in PEG400 alone, 70 mg/mL in an aqueous vehicle (distilled water) alone and 84 mg/mL in PEG400/water (80/20). PEG400/water (80/20) yielded reasonably good and linear exposures over the 40 to 120 mg/kg dose range and evidence of a plateau of exposure between 120 and 240 mg/kg/day. The PEG400/water (80/20) vehicle produced a reasonably high AUC

value during these pharmacokinetic experiments and comparable the highest achieved AUC seen with the twice daily dosing in an aqueous vehicle (0.5% methylcellulose); approximately 65 μM•hr.

Table 2.4: 3

Pharmacokinetic Parameters After Oral Administration of the Potassium Salt of MK-0518 to Rats

Dose Paradigm	Dose (mg/kg)	AUC[a] (μM•hr)	C_{max} (μM)	T_{max} (hr)
Once Daily in 0.5% methylcellulose & 10% Tween 80	160	13.9 ± 1.3	9.0 ± 1.7	0.3 ± 0.1
Twice Daily in 0.5% methylcellulose	20	10.1 ± 2.6	6.1 ± 1.1	0.5 ± 0.0
	40	18.9 ± 7.3	5.0 ± 0.7	0.6 ± 0.3
	120	63.3 ± 8.4	16.8 ± 5.0	2.1 ± 3.3
Once Daily in PEG400/water (80/20)	40	19.7 ± 4.9	10.5 ± 5.1	0.8 ± 0.3
	80	25.4 ± 2.1	12.8 ± 3.9	0.5 ± 0.0
	120	64.6 ± 6.5	29.8 ± 2.8	0.9 ± 0.3
	240	65.9 ± 12.0	28.9 ± 5.3	0.6 ± 0.3

[a] Mean ± SD (n=4).

(PK004) [Sec. 2.6.5.3]

PEG400/water (80/20) produced an AUC equivalent to that of twice daily dosing of 0.5% methylcellulose and offered a more convenient dosing protocol therefore it was concluded that 80/20 PEG400/water administered once daily would be used in rat toxicology studies.

MK-0518 was evaluated initially at up to 120 mg/kg/day for 14 weeks. The only drug related effect observed was postdose salivation which was considered to be related to poor palatability of MK-0518.

Since dose limiting toxicity was not identified in this 14-week study, a mixing experiment was conducted to determine the highest dose possible based on formulation limitations of viscosity and mixing accuracy (maximum feasible dose). A 5-week oral toxicity study was then conducted in rats at 150, 300, 450 and 600 mg/kg/day (maximum feasible dose). Increased exposure (mean $AUC_{0\text{-}24\ hr}$ = 178 +/- 42 μM•hr) of 2-8 fold above that achieved in the previous 14-week study were seen, however, target organ toxicity was not observed and only minor increases in ALT and evidence of drug irritation to the stomach mucosa were evident. Twice daily dosing of MK-0518 at

600 mg/kg/day in 80/20 PEG400/water was investigated in an 8-day study (TT #05-6030) [Sec. 2.6.7.6]. In this study, twice daily dosing did not provide a significant increase in systemic exposure of MK-0518 to rats (once daily dosing mean $AUC_{0\text{-}24\ hr} = 231 \pm 44.6$ μM•hr; twice daily dosing mean $AUC_{0\text{-}24\ hr} = 267 \pm 34.1$ μM•hr). As the 27-week oral toxicity study was ongoing, dose levels were revised in approximately Drug Week 9 to include 600 mg/kg/day as the top dose for the remainder of the 27-week study. MK-0518 caused mortality at 600 mg/kg/day (3 male; 1 female) in the 27-week study that was associated with body weight loss, urine staining and/or decreased food consumption prior to death. Up to 17% decreases in mean body weight gain were observed in surviving male rats at 600 mg/kg/day. There were no other significant findings in the antemortem parameters of these studies including hematology, serum biochemistry, and urinalysis evaluations. Degeneration of the glandular mucosa of the stomach was seen at 600 mg/kg/day and 120 mg/kg/day upon histomorphologic evaluation of the tissues. Additional observations in rats that were likely due to poor palatability, aspiration and/or irritation of the drug included stomach inflammation in the 5-week study and postdosing salivation audible respiratory sounds, and inflammation in the nose and nasopharynx in the 27-week study.

Exposure margins based on plasma AUC values for rats relative to a therapeutic AUC in patients of about 54 μM•hr at the MRD of 400 mg/BID are shown in [Table 2.4: 4].

Table 2.4: 4

Exposure Margins Based on 27-Week Oral Toxicity Study in Rats (Drug Week 26) (TT #04-6022)

Dose (mg/kg/day)	Mean AUC (μM•hr, Sexes Combined)	Exposure Margin[a]
30	29	-
120	57.5	1.6
600	167	4.8

[a] Ratio of AUC in animals/Maximum AUC in patients (54 μM•hr) receiving 400 mg/BID; corrected for plasma protein binding (unbound fraction) of 26% in rats and 17% in humans.
- = Exposure margin is <1.

[Sec. 2.6.7.7E]

In rats, mortality and body weight decrements were seen at 600 mg/kg/day. However, effects at 120 mg/kg/day were local and considered to be related to MK-0518 irritation of the stomach mucosa. Therefore the no-adverse-effect level is 120 mg/kg/day which provides an exposure margin of 1.6-fold above the expected exposure of the 400 mg/BID dose in patients.

Considering the mortality and decreases in body weight gain seen prominently in males and not females at 600 mg/kg/day, and the potential for these changes to adversely affect morbidity and survivorship in long-term studies, the maximum tolerated dose for a 2-year evaluation of carcinogenic potential of this drug is considered to be 300 mg/kg/day in males and up to the maximum feasible dose of 600 mg/kg/day in females. Based on these results, doses of 50, 150, and 300 mg/kg/day in males and 50, 300 and 600 mg/kg/day in females were selected for the carcinogenicity study in rats. These doses were reviewed and approved by the FDA Executive Carcinogenicity Assessment Committee.

In dogs, MK-0518 was studied in GLP oral toxicity studies of 5, 14, 27 and 53 weeks duration (TT #04-9811, TT #03-118-0, TT #04-9001) [Sec. 2.6.7.7F; 2.6.7.7G; 2.6.7.7H]. MK-0518 was evaluated initially at up to 45 mg/kg/day for 14 weeks. Since dose limiting toxicity was not identified in this study, a subsequent exploratory single dose range-finding study was conducted in dogs across a broad dose range (100 to 1000 mg/kg) as described above [Sec. 2.4.4.2]. To confirm that 250 mg/kg/day should be the highest dose administered to dogs based on tolerability and less than dose proportional exposures, a GLP 5-week oral toxicity study was conducted at 125, 250, 500 mg/kg/day. In this study, mean $AUC_{0\text{-}24\ hr}$ value increases were again less than dose proportional across the dose range; two-fold increases in dose resulted in a 1.3 fold increase in mean $AUC_{0\text{-}24\ hr}$. Individual $AUC_{0\text{-}24\ hr}$ values from 3 out of 6 dogs at 500 mg/kg/day overlapped with $AUC_{0\text{-}24\ hr}$ values of several dogs at 250 mg/kg/day suggesting that there was a trend towards a plateau around 250 mg/kg/day in Drug Week 4. Dose limiting toxicity was not seen as transient emesis was the only drug-related effect. Dose levels on the 53-week oral toxicity study were modified in Drug Week 14 to include a top dose of 360 mg/kg/day. There were no deaths associated with drug treatment during any of the dog studies. With the exception of emesis with or without body weight loss, there were no treatment-related changes during the antemortem phase of the studies. Emesis was transient and/or intermittent and likely due to poor palatability of the drug. There were no treatment-related changes in the gross or histomorphologic appearance of tissues examined from these studies in dogs. Therefore, the no-effect level in dogs, with the exception of emesis, following 53 weeks of treatment was $\geq$360 mg/kg/day.

Exposure margins based on plasma AUC values for dogs relative to a therapeutic AUC in patients of about 54 μM•hr at the MRD of 400 mg/BID are shown in [Table 2.4: 5].

Table 2.4: 5

Exposure Margins Based on 53-Week Oral Toxicity Study in Dogs (Drug Week 52) (TT #04-9001)

Dose (mg/kg/day)	Mean AUC (μM•hr, Sexes Combined)	Exposure Margin[a]
15	32	1
90	158	5.1
360	273	9.1

[a] Ratio of AUC in animals/Maximum AUC in patients (54 μM•hr) receiving 400 mg/BID; corrected for plasma protein binding (unbound fraction) of 30% in dogs and 17% in humans.

[Sec. 2.6.7.7H]

In dogs, since meaningful toxicity was not observed in the 1-year study conducted at a dose which maximized systemic exposure, the safety margin based on plasma drug concentrations is 9.1-fold above the expected exposure of the 400 mg/BID dose in patients.

4.4 Genotoxicity

The mutagenic potential of MK-0518 was assessed in a microbial mutagenicity (Ames) assay using *Salmonella typhimurium* tester strains TA1535, TA97a, TA98, and TA100 and *Escherichia coli* tester strain WP2 uvrA pKM101. One exploratory (TT #03-8029) and 3 GLP (TT #03-8059, TT #03-8063, TT #03-8068) assays were conducted [Sec. 2.6.7.8A]. The assays used the base compound (early exploratory assay) or the potassium salt for subsequent preclinical and clinical development and were tested with and without a rat liver metabolic activation system (S-9) from rats pretreated with xenobiotics to induce metabolic activity. The results of these studies showed that neither form of MK-0518 induced a 2-fold or greater increase in revertants relative to the solvent control in any of the tester strains evaluated at the maximum tested concentration of 6000 μg/plate. The positive control agents gave the expected increase in revertants, indicating that the assay was working properly. Therefore, it is concluded that MK-0518 is not mutagenic in bacteria and is considered negative in this assay.

The potential for MK-0518 to induce DNA strand breaks was assessed in the in vitro rat hepatocyte DNA alkaline elution assay (TT #03-8381, TT #03-8394-GLP) [Sec. 2.6.7.8B]. This assay measures the ability of a compound to induce single strand breaks in DNA that are measured via the rate of elution of DNA through a filter. The presence of single strand breaks under alkaline conditions allows DNA to more readily pass through the filter. In this assay, MK-0518 was assessed at concentrations up to 400 μM, as greater concentrations were insoluble in culture medium. The results of this

assay showed that MK-0518 did not induce a significant increase in elution slope relative to the vehicle control group. The positive control agents gave the expected increases in elution slope indicating the assay was working properly. Therefore, it is concluded that MK-0518 is negative in this assay.

MK-0518 was tested for clastogenic activity in the Chinese hamster ovary cell (CHO cell) chromosome aberration assay (TT #03-8681 and TT #03-8687) [Sec. 2.6.7.8C]. MK-0518 was tested both with and without the addition of rat S-9 liver metabolic activation system after a 3-hour incubation using drug concentrations up to 600 μM (with S-9) and 400 μM (without S-9). These concentrations were based upon the criterion of no greater than approximately 50% inhibition of cell growth. In addition, a 20-hour incubation was done without S-9 up to a concentration of 550 μM that reduced cell growth by 49% compared to the solvent control. There were no statistically significant increases in the frequency of chromosome aberrations found in any of the MK-0518 treatment groups. The positive control agents gave the expected increases in aberration frequency. Therefore, it is concluded that MK-0518 is not clastogenic in this assay at the maximum testable concentrations based on cytotoxicity.

MK-0518 was also tested for its potential to induce chromosomal damage in an in vivo mouse micronucleus assay (TT #04-8619) [Sec. 2.6.7.9]. Single oral doses up to 1500 mg/kg were administered in aqueous suspension to male mice. After 24 or 48 hours following dosing, groups of mice were sacrificed and bone marrow smears prepared for analysis. There was no evidence of an increase in micronuclei or on proportion of polychromatophilic erythrocytes at any of the doses tested. The positive control, mitomycin C, gave the expected increase in micronuclei. Therefore, it was concluded that MK-0518 was negative in this assay at the maximum dose tested of 1500 mg/kg.

4.5 Carcinogenicity

Carcinogenicity studies in mice and rats are currently ongoing. The in-life phase of these studies is scheduled to be completed in 4Q2007 with final study reports planned to be completed in 3Q-4Q2008. MK-0518 was negative in all genetic toxicology studies. In mice, doses of 50, 250, and 400 mg/kg/day in females and 50, 100 and 250 mg/kg/day in males were selected for the carcinogenicity study. In rats, doses of 50, 150, and 300 mg/kg/day in males and 50, 300 and 600 mg/kg/day in females were selected for the carcinogenicity study. These doses were reviewed and approved by the FDA Executive Carcinogenicity Assessment Committee. Mortality of up to 38% in high dose mice (female) and 26% in high dose rats (female) has been observed. In many early death animals, mortality was caused by aspiration of dosing material into the nose/nasopharynx; similar to what was previously described in chronic toxicology studies. Expected effects of drug irritation to the nose/nasopharynx were seen and include chronic inflammation and epithelial hyperplasia and metaplasia. Additionally, in

the rat, 3 neoplasms of the nose/nasopharynx were observed and are considered to represent the expected consequence of chronic irritation and inflammation as discussed further in [Sec. 2.6.6.5.1.1]. Since these neoplasms likely resulted from continuous local deposition of the drug formulation on the nasal mucosa, the relevance of this observation to patients is expected to be minimal.

A 27-week toxicokinetic study in mice was conducted in parallel with the ongoing main carcinogenicity study (TT #05-1119) [Sec. 2.6.7.10A]. Results from this study indicate that at the high dose, 400 mg/kg/day in females and 250 mg/kg/day in males, systemic exposure is approximately 2-fold greater (females) or equal to (males) the AUC (54 μM•hr) at the 400-mg/BID MRD when adjusted for 30% and 17% unbound drug in mice and humans, respectively.

A 26-week toxicokinetic study in rats was conducted in parallel with the ongoing main carcinogenicity study (TT #05-6041) [Sec. 2.6.7.10B]. Results from this study indicate that at 300-600 mg/kg/day in females and 150 to 300 mg/kg/day in males, systemic exposure is approximately 10.3-fold greater (females) or 1.7-fold greater (males) the AUC (54 μM•hr) at the 400-mg/BID MRD when adjusted for 26% and 17% unbound drug in rats and humans, respectively.

No nonclinical short- or medium-term studies or other studies pertaining to carcinogenicity were conducted. As discussed in [Sec. 2.6.6.5.1.1], long-term carcinogenicity studies are currently ongoing and will form the basis of an assessment of carcinogenicity potential of MK-0518.

4.6 Reproductive and Developmental Toxicity

MK-0518 was administered orally once daily at doses 150, 300, 450 or 600 mg/kg/day to pregnant rats from GD 6 through LD 20 in an oral reproduction range-finding study (TT #04-7095) [Sec. 2.6.7.11]. There were no treatment-related findings in this range finding study. Therefore, in the GLP study MK-0518 was administered orally to pregnant female rats once daily at doses of 100, 300 or 600 mg/kg/day from GD 6 through 20 or through Lactation Day (LD) 20 to determine its potential developmental toxicity or its potential to induce postnatal toxicity following in utero and/or lactational exposure in rats (TT #04-7090) [Sec. 2.6.7.13A]. In the F_0 generation, there were no treatment-related deaths, physical signs of toxicity, or effects on maternal body weight gain, food consumption, or the mean length of gestation. There were no treatment-related gross findings in the thoracic and abdominal viscera. In the F_1 generation, there were no treatment-related effects on embryonic/fetal survival in the 100-, 300-, and 600-mg/kg/day groups relative to control as assessed by the mean numbers of corpora lutea, implantations, and live fetuses per pregnant female and the derived peri- and postimplantation loss calculations. There were no treatment-related effects on gross

placental morphology, fetal sex ratios, or fetal body weights. There were no treatment-related fetal external, coronal, or visceral alterations. There was a treatment-related increase in the incidence of supernumerary ribs in the 600-mg/kg/day group (percent litter mean = 15) as compared to concurrent control (percent litter mean = 5.7) and to historical control (mean = 7.4; range = 2.6 to 13.5). There were no treatment-related fetal skeletal malformations or effects on ossification in the 100-, 300-, and 600-mg/kg/day groups. In the F_1 generation (preweaning period), there were no treatment-related effects on postimplantation survival, PND 0 pup external morphology, pup survival, or pup body weight. No treatment-related physical signs were observed. In the F_1 generation (postweaning period), there were no deaths, treatment-related physical signs, or effects on body weight gain, developmental signs (vaginal opening and preputial separation), behavioral test paradigms (passive avoidance, auditory startle, and open-field motor activity), or reproductive performance. No treatment-related ophthalmic findings were noted. In the F_2 generation, there were no treatment-related effects on postimplantation survival, on PND 0 pup weights, or on PND 0 pup external morphology. Based on these results, the NOELs for maternal and developmental toxicity in rats were 600 mg/kg/day and 300 mg/kg/day, respectively. At the 300-mg/kg/day NOEL for developmental toxicity, the systemic exposure ($AUC_{0\text{-}24\ hr}$) was approximately 3.4-fold compared to expected exposures in patients receiving the 400-mg/BID MRD.

To assess potential effects on fertility in female rats, MK-0518 was administered orally at dose levels of 150, 300 or 600 mg/kg/day for 14 days prior to cohabitation, during cohabitation, and through GD 7 (TT #04-7420) [Sec. 2.6.7.12A]. There were no treatment-related effects on mating performance as assessed by time to mating and the mating index (percent of mated females/females cohabited) or on fertility parameters as assessed by the fecundity index (percent of pregnant females/mated females) and the fertility index (percent of pregnant females/females cohabited). There were no treatment-related effects on F_1 embryonic/fetal survival as assessed by the mean numbers of corpora lutea, implants, and live fetuses per pregnant female, and the derived peri-implantation and postimplantation loss values. Based on these data, the no-effect level for effects of MK-0518 on fertility in F_0 female rats was ≥600 mg/kg/day.

The potential of MK-0518 to affect fertility in male rats was assessed at oral doses of 100, 300 or 600 mg/kg/day administered for 4 weeks prior to mating and throughout mating until the day prior to necropsy (approximately 8 weeks total) (TT #05-7180) [Sec. 2.6.7.12B]. There were no treatment-related effects on mortality, physical signs, body weights, food consumption, mating performance, fertility, embryonic/fetal survival, sperm count, or sperm motility. There were no treatment-related gross changes in the thoracic or abdominal cavities. There were no treatment-related alterations in testicular weights nor were there gross or microscopic changes in the testes or epididymides. Based on these findings, the no-effect level for routine antemortem and postmortem

parameters in male rats was ≥600 mg/kg/day. The no-effect level for male fertility was ≥600 mg/kg/day.

To assess the potential for developmental toxicity in rabbits, MK-0518 was administered once daily to pregnant does at doses of 100, 500 or 1000 mg/kg/day beginning on GD 7 through GD 20 (TT #04-7220) [Sec. 2.6.7.13C]. These doses were based on a prior range-finding study (TT #04-7225) [Sec. 2.6.7.11] in pregnant rabbits showing an absence of treatment-related effects at up to 1000 mg/kg/day. There were no deaths or treatment-related physical signs observed during the study. There were no treatment-related effects on mean maternal body weight gain or food consumption. There were no treatment-related gross changes of the thoracic and abdominal viscera at scheduled necropsy of the F_0 females. No treatment-related effects on placental morphology were observed. There were no treatment-related effects on embryonic/fetal survival as assessed by the numbers of corpora lutea, implants, and live fetuses per pregnant female and the derived peri- and postimplantation loss values. There were no treatment-related effects on fetal sex ratios and live fetal weights in any dose group. Finally, there were no treatment-related fetal external, visceral, coronal, or skeletal malformations or variations in any dose group. Based on these results, the no-effect level of MK-0518 for maternal and developmental toxicity was ≥1000 mg/kg/day. At the 1000-mg/kg/day NOEL for developmental toxicity, the systemic exposure ($AUC_{0\text{-}24\ hr}$) was approximately 3.7-fold compared to expected exposures in patients receiving the 400-mg/BID MRD.

Studies to evaluate the potential effects of MK-0518 on prenatal and postnatal development including maternal function were conducted. These assessments were conducted in a combined study in rats and are described above and in [Sec. 2.6.6.6.2.2].

The potential toxicity of MK-0518 on growth and behavior in juvenile rats, including histomorphology, following oral administration from Postnatal Day (PND) 5 to Postnatal Week (PNW) 8 was determined in a range finding and GLP study (TT #05-7305, TT #05-7300) [Sec. 2.6.7.11; 2.6.7.14]. MK-0518 was orally administered to juvenile rats at 50, 200, or 600 mg/kg/day from PND 5 to PNW 8. There was no evidence of toxicity based on antemortem parameters of mortality, physical signs, body weights, developmental signs, hematology, serum biochemistry, ophthalmologic examination, behavioral assessments, and reproductive performance, including embryonic/fetal survival. Treatment-related histomorphologic findings consisted of vacuolation of the nonglandular mucosa at the limiting ridge at ≥200 mg/kg/day as well as inflammation which occurred at ≥200 mg/kg/day in males and at 600 mg/kg/day in females. Both vacuolar and inflammatory changes recovered following cessation of treatment for approximately 6 weeks. The mucosal epithelial vacuolation and associated increased inflammation were consistent with MK-0518 causing very slight irritation to the limiting ridge of the nonglandular stomach of orally gavaged rats. Based on the histomorphologic

results, the no-effect level for treatment-related changes in juvenile rats was 50 mg/kg/day. These findings in juvenile rats were consistent with the stomach irritation effects seen in adult rats.

4.7 Local Tolerance

The dermal irritation potential of MK-0518 (potassium salt) was determined using an in vitro human epidermal skin culture system (EpiDerm™) (TT #05-5509) [Sec. 2.6.7.15]. In this assay, human epidermal cells in culture are exposed to the test article and after a suitable post-exposure expression time, the cells are assayed for viability using a tetrazolium reduction assay. The results of this assay demonstrate that MK-0518 is non-irritating based on 104.2% cell viability.

The ocular irritation potential of MK-0518 as free base or a potassium salt was determined using the in vitro bovine corneal opacity (BCOP) assay (TT #04-5551, TT #05-5510) [Sec. 2.6.7.15]. This assay determines the ocular irritation potential following exposure of freshly isolated bovine corneas to the test agent by assessment of the induction of opacity and changes in permeability to fluorescein. Based upon these measures, an in vitro score is determined and the test compound classified as either a mild, moderate, or severe irritant. Based on the irritation score with MK-0518 tested as a 20% solution, the MK-0518-free base is classified as a mild irritant and the MK-0518-potassium salt is classified as a severe irritant in the BCOP in vitro assay.

The potential of MK-0518 as a free base or a potassium salt to produce a dermal sensitization response was determined in mice using the local lymph node assay (LLNA) (TT #04-5545, TT #05-5541) [Sec. 2.6.7.15]. In this assay, groups of mice were treated topically on both ears with MK-0518, vehicle, or a positive control agent for 3 consecutive days. On Day 5, mice were injected with tritiated thymidine or BrdU and 5 hours later were sacrificed and incorporation of radioactivity in the auricular lymph node measured as an index of sensitization potential. Since no significant increase in cell proliferation in the lymph node was detected, MK-0518, as either free base or potassium salt, is not considered a dermal sensitizer.

The dermal irritation potential of MK-0518 as a free base or potassium salt was determined following application of the drug to the intact skin of New Zealand White rabbits (TT #04-5550, TT #04-5546) [Sec. 2.6.7.15]. The application site was covered with a semi-occlusive dressing for 4 hours and the test and control sites evaluated at approximately 1, 24, 48, and 72 hours after treatment. No evidence of dermal irritation was found in any of the treated rabbits. Therefore, MK-0518, as either a free base or potassium salt, is not considered a dermal irritant based on the results of this study.

4.8 Other Toxicity Studies

Since UV absorption was detected by MK-0518 between 200 to 350 nM as described in [Sec. 3.1.1.1], the phototoxicity potential of MK-0518 was determined in mice. MK-0518 was administered by oral gavage to female mice at 1000, 1500, or 2000 mg/kg as a single dose with a 7-day observation period. Subsequently, mice were exposed to UVB light (approximately 280 to 320 nm) for 5 minutes and UVA light (approximately 300 to 400 nm) and visible light (approximately 400 to 900 nm) for 4 hours. Additional groups of mice were placed in the dark during the 4-hour exposure period to serve as negative controls. Observations for physical signs and the degree of erythema, sloughing, and/or necrosis of the ears were recorded as evidence of phototoxic response in each mouse beginning 3 hours after the end of the ultraviolet and visible light-exposure period or the dark period (negative controls) and daily thereafter for a total of 7 days. No treatment-related mortality, body weight effects, or phototoxic effects were noted in any dose group. Treatment-related decreased activity was observed at 2000 mg/kg in mice exposed to the ultraviolet and visible lights. Based on these findings, MK-0518 was considered non-phototoxic at doses up to 2000 mg/kg.

The toxicity of MK-0518 following intravenous administration to rats and dogs was conducted to support the [redacted] of a single dose to determine the absolute bioavailability of the drug in humans. An assessment of the potential for MK-0518 to cause in vitro hemolysis of human, dog or rat erythrocytes was conducted prior to initiation of the toxicity studies (TT #06-4903; TT #06-4905) [Sec. 2.6.7.16]. MK-0518 was negative for in vitro hemolysis in this assay. MK-0518 was administered as a single intravenous injection to female rats at 100, 200, 400, 800, or 1600 mg/kg with a 7-day observation period (TT #06-2521) [Sec. 2.6.7.16]. Treatment-related mortality occurred at ≥200 mg/kg and was associated with physical signs including recumbency, labored breathing, decreased activity. At ≥100 mg/kg evidence of drug irritation at the injection site was observed. Based on these results the highest tolerated dose was 100 mg/kg/day. A no-observed-effect-level (NOEL) was not determined. In dogs, a range finding study was conducted which included 3 rising consecutive intravenous doses of 40, 100, and 400 mg/kg/day were planned (TT #06-1036) [Sec. 2.6.7.16]. Dogs received rising doses of 40 and 100 mg/kg/day for 3 consecutive days each. However, one dog died on Day 1 of administration of the 400 mg/kg dose. The death was likely due to cardiac arrhythmia secondary to the amount of administered potassium as opposed to a direct effect of the MK-0518 molecule. The remaining dog was not dosed at the 400 mg/kg dose level. Because of the mortality and the increases in alanine aminotransferase (female and male), aspartate aminotransferase (male only), and alkaline phosphatase (male only) at 100 mg/kg/day which were not associated with histomorphologic changes in the liver, a subsequent 7-day study was conducted to further evaluate these observations and to also include a toxicokinetic assessment. In this study MK-0518 was administered to dogs intravenously once daily at doses of 30 mg/kg/day or

100 mg/kg/day for 7 days (TT #06-6030) [Sec. 2.6.7.16]. Primary changes at both doses included transient postdosing emesis/retching and local changes at the injection sites. Changes limited to the 100 mg/kg/day group consisted of body weight loss, minimal increases in serum urea nitrogen, increases in alanine aminotransferase (ALT) (up to +1967% compared to concurrent control), alkaline phosphatase (ALP) (up to +374% compared to concurrent control), and cholesterol, and very slight, multifocal tubular dilatation in the cortex of the kidneys. There was no histomorphologic change in the liver to correlate with the increases in ALT or ALP. Exposures at 100 mg/kg/day dose level represent approximately 23-fold greater than the AUC (54 μM•hr) at the 400-mg/BID MRD when adjusted for 30% and 17% unbound drug in dogs and humans, respectively. Importantly also, the plasma concentration at 100 mg/kg/day at approximately C_{max} ($C_{0.25h}$) is 71-fold greater than the C_{max} (7.4 uM) at the 400-mg/BID MRD when adjusted for 30% and 17% unbound drug in dogs and humans, respectively. At the NOEL safety margins were 6.5-fold or 24-fold the AUC and C_{max} of the 400-mg/BID MRD.

During lead optimization screening of MK-0518, MK-0518 was administered by oral gavage once daily for approximately 4 days to determine the biliary copper secretion and toxicokinetic profile, and to evaluate recovery of potential drug effects on biliary copper after 5 days (TT #03-146-0) [Sec. 2.6.7.16]. Bile was collected from all animals twice pretest (6 and 3 days prior to study start), on Drug Days 1 and 4, and on Recovery Days 1 and 4 for determination of biliary copper levels. The biliary copper concentrations were determined by inductively coupled plasma-mass spectrometry. There was no treatment-related mortality, and there were no treatment-related physical signs or changes in food consumption or in hematological or serum biochemical parameters. There was no apparent effect of MK-0518 on biliary copper secretion. The systemic exposure of MK-0518 was good, achieving 17.8 and 92.7 μM•hr ($AUC_{0\text{-}24\ hr}$) at 5 and 45 mg/kg/day, respectively. Although large variability in biliary copper secretion values were observed in control animals, it is important to note that significantly higher systemic exposure to MK-0518 was achieved on the subsequent 53-week toxicity study in dogs (273 μM•hr, $AUC_{0\text{-}24\ hr}$). The absence of any evidence of altered copper homeostasis (e.g. liver toxicity) on the 53-week dog study confirms the negative result of this early screening study conducted during lead optimization.

類縁物質B* is expected to be present in the MK-0518 drug substance at up to [redacted]%. The same 類縁物質B* is known to form as a degradate of MK-0518 within the drug product.

To support a drug product specification limit of [redacted]%, MK-0518 was administered by oral gavage once daily for approximately 5 weeks to rats (TT #06-6055) [Sec. 2.6.7.17A]. The drug substance administered to rats contained [redacted]% HPLC of 類縁物質B*. The doses used on the 5-week oral toxicity study were 30, 120, 600 mg/kg/day. The

design of this study was essentially the same as described for the 5-, 14-, and 27-week toxicity studies including histopathology, however a toxicokinetic assessment of MK-0518 was not conducted since the small amount of 類縁物質B* in the tested lot ([redacted]%) was not expected to alter the exposure parameters of MK-0518. Exposure margins for the MK-0518 類縁物質B*, based on multiples of dose administration (mg/kg basis), are presented in [Table 2.4: 6].

Table 2.4: 6

Exposure Margins Based on 5-Week Oral Toxicity Study in Rat (TT #06-6055)

MK-0518 Dose (mg/kg/day)	Dose of 類縁物質B* in Rats[a] (mg/kg/day)	Exposure Margin in Patients[b]
30	[redacted]	0.3-fold
120		1.1-fold
600		5.3-fold

[a] Drug substance contained [redacted]% of 類縁物質B*
[b] Ratio of dose (mg/kg) in animals/400 mg/BID dose in patients
Assumption: Projected highest dose of 類縁物質B* to patients = [redacted] mg/kg/day; assumes 400 mg/BID dose, [redacted]% Drug Product specification limit, 70 kg patient weight

[Sec. 2.6.7.17A]

MK-0518 was administered daily at 30, 120, and 600 mg/kg/day to rats for approximately 5 weeks. Treatment-related antemortem findings were limited to salivation at the mid- and high-dose levels, which was attributed to the poor palatability of the dosing formulation. MK-0518 caused histomorphologic changes consistent with very slight irritation to the limiting ridge of the nonglandular stomach of orally gavaged rats often accompanied by a very slight increase in inflammation at 600 mg/kg/day. Except for salivation reported also at 120 mg/kg/day but unaccompanied by histomorphological changes, the no-effect level (NOEL) for the local effect on the stomach in this study was 120 mg/kg/day.

In conclusion, administration of MK-0518 containing 類縁物質B* to rats for 1 month at > 5-fold higher than expected levels in humans (mg/kg basis) did not cause any unique toxicities relative to MK-0518 alone, nor was the NOEL for the gastric irritation significantly altered. Results from this study support a Drug Product Specification limit for 類縁物質B* up to [redacted]% which administered to patients at 400 mg/BID results in an oral dose of approximately 1.1-fold (rat NOEL) to

5.3-fold (gastric irritation) below doses of 類縁物質B* administered to rats (mg/kg/basis).

No nonclinical toxicity studies were conducted to assess antigenicity. However, the Guideline for Immunotoxicity Studies for Human Pharmaceuticals (ICH S8 Guidance) and Repeated Dose Toxicity (CPMP/SWP/1042/99) guidelines were consulted during the conduct of the nonclinical program for MK-0518. Accordingly, MK-0518 was assessed in multiple-dose studies in rats and dogs. Animals were monitored for clinical signs, by evaluation of hematological (including differential white blood cell counting) and serum biochemical parameters, and by gross and histopathological examination of lymphoid tissues (lymph nodes, spleen, and thymus). There was no evidence of antigenicity in these studies.

No specific in vivo nonclinical toxicity studies were conducted to assess immunotoxicity. However, in accordance with the tiered approach beginning with a screening phase incorporated within at least one standard repeat-dose toxicity study in rats or mice recommended in the Guideline for Immunotoxicity Studies for Human Pharmaceuticals (ICH S8 Guidance) and Repeated Dose Toxicity (CPMP/SWP/1042/99), the potential immunotoxicity of MK-0518 was assessed in multiple-dose studies in rats. This was accomplished by monitoring clinical signs, by evaluation of hematological (including differential white blood cell counting) and serum biochemical parameters, and by gross and histopathological examination of lymphoid tissues (lymph nodes, spleen, and thymus). There was no evidence of immunotoxicity in these studies.

No nonclinical dependence studies were conducted to support an antiviral indication. Tissue distribution studies indicate that MK-0518 is poorly brain penetrate, yielding the lowest detectable concentrations of all tissues tested (0.00971 μg/g of a 6 mg radiolabled oral dose of MK-0518). Further, MK-0518 was shown to be a substrate for human P-glycoprotein.

No nonclinical studies were conducted to evaluate specific metabolites. The principal metabolite of MK-0518 observed in humans and in rats and dogs is a glucuronide (L-001277512). In the rat and dog toxicity studies exposure to the glucuronide was 3.3-fold and 3.6-fold, respectively, above the projected exposure in patients receiving 400 mg/BID MK-0518.

5. Integrated Overview and Conclusions

MK-0518 is a potent inhibitor of the Human Immunodeficiency Virus type 1 (HIV-1)-encoded enzyme integrase (IN).

More than 20 drugs have been approved to treat HIV infection. All but one of these drugs are administered orally and belong to one of 3 mechanistic classes: protease inhibitors (PIs), nucleoside analog reverse transcriptase inhibitors (NRTIs), and non-nucleoside reverse transcriptase inhibitors (NNRTIs). One drug, enfuvirtide, inhibits a fourth mechanism, viral entry, but must be administered by injection. Because the prolonged administration of a single antiretroviral agent typically gives rise to viral variants that resistant to that agent, the standard of care treating HIV infection is now combination therapy with typically 3 or more drugs from 2 or more classes. The coadministration of multiple antiretroviral drugs in so-called "Highly Active Anti-Retroviral Therapy" (HAART) regimens results in greater suppression of viral replication, which in turn reduces the probability that resistant viruses will emerge and prolongs the duration of viral suppression.

Though the advent of HAART regimens proved a tremendous advance in HIV therapy, antiviral drug resistance is prevalent both in treatment-experienced patients and in primary HIV-1 infection. Drug resistance represents a significant impediment to durable antiviral therapy. Furthermore, some mutations confer cross-resistance to multiple drugs in a class, so the problem of drug resistance is not easily addressed by developing new antiviral drugs in established mechanistic classes.

MK-0518 inhibits replication of laboratory and clinical HIV-1 isolates in cell culture. When tested in the presence of 50% normal human serum to account for compound binding to serum proteins, MK-0518 inhibits replication of a laboratory HIV-1 isolate with a 95% cell inhibitory concentration (CIC_{95}) of 31 ± 20 nM. As with all direct antiretroviral agents, HIV-1 is able to evolve resistance to MK-0518. Cell culture resistance selection studies done with MK-0518 and with other Integrase Strand Transfer Inhibitors (InSTIs) have shown that amino acid substitutions at specific positions in HIV-1 integrase can confer reduced susceptibility to MK-0518. A mutation at position 148 (Q148H/K/R) or 155 (N155H/S) constitutes a primary MK-0518 resistance mutation, any of which confer phenotypic resistance to MK-0518 in cell culture. These primary mutations also tend to reduce the infectivity of HIV-1, consistent with a reduction in viral fitness. These primary mutations are almost always accompanied by secondary mutations at positions such as 74 (e.g., L74M), 92 (e.g., E92Q), 138 (e.g., E138A/K), 140 (e.g., G140S), and 151 (e.g., V151I). Though these secondary mutations confer no or low-level resistance by themselves, they substantially increase resistance to MK-0518 conferred by a primary resistance mutation, and in some cases also increase viral infectivity. Thus, generation of high-level MK-0518 resistance, along with the highest infectivity (fitness), seems to require multiple mutations in the integrase gene.

MK-0518 was tested for antiviral activity in cell culture in combination with 18 licensed antiviral drugs from all 4 classes. Each compound in each combination was tested over a wide range of concentrations. MK-0518 showed additive or synergistic activity with

each licensed compound tested. From an antiviral activity perspective, these observations support the view that MK-0518 may be used in combination with licensed antiretroviral drugs of any class.

MK-0518 is a low (dog) to intermediate (rat) clearance compound, with a short plasma half-life (≤1.6 hr). MK-0518 is cleared primarily by metabolism (glucuronidation), in both preclinical species and in humans. The glucuronide derivative of the parent compound is the only metabolite detected in humans and is the major in vivo and in vitro metabolite in preclinical species. In rats and dogs exposure to the glucuronide was 3.3-fold and 3.6-fold, respectively, above the projected exposure in patients receiving 400 mg/BID MK-0518. The glucuronidation of MK-0518 is catalyzed mainly by UGT1A1 with minor contribution from UGT1A9 and UGT1A3. Therefore, MK-0518 may be subject to drug-drug interactions when co-administered with drugs that are known to be UGT1A1 inducers or inhibitors. However, MK-0518 is not likely to affect the metabolic clearance of drugs metabolized by UGT1A1 given its low UGT1A1 inhibitory and induction potential. Since MK-0518 is neither a substrate nor an inhibitor of cytochrome P450 enzymes and is not an inducer of CYP3A4, MK-0518 is not expected to exhibit metabolic drug interactions with substrates of cytochromes P450.

MK-0518 is not genotoxic in a battery of in vitro assays in bacteria and mammalian cells or in vivo in mice designed to detect mutagenicity, direct DNA damage, or clastogenicity. Acute oral toxicity studies were conducted with MK-0518 in mice, rats and dogs to determine the approximate lethal dose and/or to measure toxicokinetics over a broad dose range following administration of a single dose. Acute toxicity (mortality) was only evident in male mice administered MK-0518 orally at 2000 mg/kg. Mortality was not evident in female mice or in males at 1500 mg/kg. In dogs, mortality was not seen following up to 1000 mg/kg of a single oral dose. In mice mortality was evident at ≥500 mg/kg/day such that the high dose level selected for the ongoing carcinogenicity study in mice is approximately 2-fold greater than the AUC (54 μM•hr) at the 400-mg/BID MRD when adjusted for 30% and 17% unbound drug in mice and humans, respectively. In rats, mortality was observed at 600 mg/kg/day. Effects at 120 mg/kg/day were local and considered to be related to MK-0518 irritation of the stomach mucosa. Therefore the no adverse effect level is 120 mg/kg/day which provides an exposure margin of 1.6-fold above the expected exposure of the 400 mg/BID dose in patients. Oral dog toxicity studies ranged from single dose to 12 months duration. In the chronic study, systemic exposure to MK-0518 was maximized; resulting in a safety margin of approximately 9-fold based on AUC values relative the MRD. There were no significant toxicologic observations at these exposures on the chronic dog study. However, when MK-0518 was administered intravenously, mortality was observed following a single high dose and was considered to result from administration of excessive amounts of potassium likely inducing fatal arrhythmias. In a subsequent 1 week intravenous study, mortality did not occur, however, increases in ALT and ALP

were observed at exposures approximately 23-fold greater than the AUC and 71-fold greater than the C_{max} [based on the approximate C_{max} ($C_{0.25h}$)] at the 400-mg/BID MRD when adjusted for 30% and 17% unbound drug in dogs and humans, respectively. At the NOAEL, the exposures were approximately 6.5-fold greater than the AUC and 24-fold greater than the C_{max} [based on the approximate C_{max} ($C_{0.25h}$)] at the 400-mg/BID MRD when adjusted for 30% and 17% unbound drug in dogs and humans, respectively. In reproductive toxicity studies, MK-0518 did not affect fertility in either male or female rats at 600 mg/kg/day. In a toxicokinetic study in pregnant and lactating rats, MK-0518 was shown to cross the placental barrier with fetal exposure values up to 1.5- to 2.5-fold greater than in maternal plasma mean drug concentrations and was also concentrated in milk about 3-fold compared to plasma. In developmental toxicity studies in rats, a slight increase in the incidence of supernumerary ribs relative to control was found at the top dose of 600 mg/kg/day. There were no external or visceral abnormalities and no other fetal or postnatal developmental effects at this dose. Based on these results, the safety margin at the NOEL for developmental toxicity is approximately 3.4-fold the value at the MRD. In rabbits no developmental toxicity was found at the maximum dose of 1000 mg/kg/day, resulting in a safety margin of about 3.7-fold relative the AUC in patients at the MRD. An assessment of the carcinogenic potential of MK-0518 is currently ongoing in a 2-year study in mice and rats.

In conclusion, the nonclinical evaluation has demonstrated that MK-0518 is well tolerated. The toxicity profile of MK-0518 after repeated oral dosing has been characterized in rats, mice, and dogs. Maximum oral and/or intravenous doses based on tolerability, formulation feasibility and/or systemic exposure were used in toxicity studies to define the toxicity profile of MK-0518. Margins of safety have been determined for each of the toxicities identified. An assessment of the carcinogenic potential of MK-0518 is ongoing, although there was no evidence of increased cellular proliferation in any tissue in the completed chronic toxicity studies. In developmental and reproductive toxicity studies, MK-0518 has been shown not to pose a significant hazard to reproduction or to the developing fetus based on studies in rats and rabbits. Therefore, the results of these nonclinical toxicity studies support the registration of MK-0518 for the treatment of HIV-1 infection.

6. List of Literature References

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