5 NONCLINICAL PHARMACOLOGY AND TOXICOLOGY SUMMARY

5.1 Pharmacodynamics

5.1.1 Pharmacodynamic Effects Relating to Proposed Indication

Atazanavir was evaluated in a series of *in vitro* assays and cell culture systems to confirm its mode of action and compare its efficacy with other clinically available protease inhibitors. The results of these studies demonstrate that atazanavir is a potent HIV-1 protease inhibitor with a distinct resistance profile and suggest that atazanavir will be an effective treatment against HIV infection.

Atazanavir inhibited the activity of HIV-RF protease in an *in vitro* processing assay with a K_i of 0.75 nM, which is comparable to the inhibitory activity of marketed protease inhibitors. In a chronically-infected cell line, atazanavir showed inhibition of gag protein (p55) cleavage by HIV protease with an EC_{50} of 47 nM. These results demonstrate that atazanavir acts on the HIV protease enzyme and blocks processing and maturation of HIV particles.

Antiviral evaluation of atazanavir using a variety of HIV-1 strains and several host-cell types resulted in EC_{50} values of 2 to 5 nM. Comparative studies using other HIV protease inhibitors revealed that atazanavir is 2- to 20-fold more potent than indinavir, saquinavir, ritonavir, nelfinavir, or amprenavir. Further, atazanavir exhibits cytotoxicity only at concentrations 6,500- to 23,000-fold higher than that required for anti-HIV activity; thus,

the selectivity index of atazanavir compares favorably with other marketed protease inhibitors.

Due to the hydrophobic nature of the binding pocket of HIV protease, all HIV protease inhibitors have reduced aqueous solubility and bind to serum proteins. The antiviral activity of atazanavir is only modestly reduced (5-fold) in the presence of human serum proteins. When compared to the other marketed HIV protease inhibitors, atazanavir retains its superior antiviral activity even in the presence of 40% human serum.

Resistance to anti-HIV drugs is commonly encountered with clinical use. The development of resistance to atazanavir *in vitro* was studied by passaging three strains of HIV-1 in MT-2 cells in the presence of increasing concentrations of atazanavir. After several months of drug selection, breakthrough viruses ranging from 93- to 183-fold resistant to atazanavir were isolated. Resistant viruses contained different mutational patterns within the viral protease, and amino acid substitutions N88S, I84V, and I50L were found to potentially play an important role in the development of atazanavir resistance. Changes were also observed at the protease cleavage sites following drug selection. The evolution to resistance seemed distinct for each of the three strains used, suggesting multiple pathways to resistance and the importance of viral genetic background.

The current group of marketed protease inhibitors select for distinct but overlapping sets of amino acid substitutions within the viral protease. To better assess the sensitivity of clinical isolates to atazanavir, a diverse panel of 551 clinical HIV-1 isolates resistant to one or more PIs (from subjects never exposed to ATV) was used to evaluate atazanavir and six marketed PIs (amprenavir, indinavir, lopinavir, nelfinavir, ritonavir, saquinavir). In general, reductions in atazanavir susceptibility required several amino acid changes and were relatively modest in degree, and susceptibility to atazanavir was retained among isolates resistant to one or two of the currently approved PIs. There was a clear trend toward loss of susceptibility to atazanavir as isolates exhibited increasing levels of cross-resistance to multiple PIs. Atazanavir appeared to have a distinct resistance profile relative to each of the other six PIs tested based on susceptibility comparisons against this panel of resistant isolates. Analysis of the genotypic profiles of 943 PI susceptible and resistant clinical isolates identified a correlation between the presence of the specific amino acid changes 10I/V/F, 20R/M/I, 24I, 33I/F/V, 36I/L/V, 46I/L, 48V, 54V/L, 63P, 71V/T/I, 73C/S/T/A, 82A/F/S/T, 84V, and 90M and decreased susceptibility to

atazanavir. While no single substitution or combination of substitutions is predictive of atazanavir resistance, the presence of at least five of these substitutions was predictive of decreased atazanavir susceptibility.

To identify amino acid substitutions that emerge on atazanavir-containing regimens, an analysis was conducted on 24 isolates from patients designated as virologic failures and displaying phenotypic resistance to atazanavir (5- to 141-fold). Twenty-one resistant isolates from treatment-naive studies AI424007/041, AI424008/044, AI424034, and AI424020 all had an I50L substitution emerge on atazanavir therapy and 11 of the 21 had a combination of I50L and A71V. These changes occurred in a variety of genetic backgrounds. Apart from a potential correlation with K45R and G73S, none of the other observed amino acid changes appeared to correlate with atazanavir resistance. Phenotypic data on these isolates indicated that emergence of resistance was generally modest in degree; specific for atazanavir; and, importantly, coincided with increased susceptibility to the other protease inhibitors tested. Phenotypic and genotypic evaluation of 10 patient isolates from treatment-experienced patients treated with the combination of atazanavir/saquinavir in Study AI424009 showed no evidence of the I50L substitution emerging in any of the on-treatment isolates. In addition, nearly all isolates displayed a loss of susceptibility to the other PIs as they became resistant to atazanavir and saquinavir. Atazanavir resistance in these patient isolates coincided with the accumulation of several additional amino acid substitutions, including I84V. The I50L substitution, sometimes in combination with an A71V change, appears to be the signature resistance substitution for atazanavir. This conclusion was supported with the observation that an atazanavir-resistance phenotype is expressed in all recombinant viral clones containing the I50L substitution in a variety of genetic backgrounds. Recombinant viruses containing either I50L or I50L/A71V combination displayed decreased susceptibility to atazanavir, were growth impaired, and showed increased susceptibility to the other protease inhibitors tested. The I50L substitution was not found in any of the on-treatment isolates from patients in any of the aforementioned studies who remained susceptible to atazanavir. Lastly, there was no evidence of cross-resistance between atazanavir and amprenavir, despite the known relationship between the 150V substitution and amprenavir resistance.

Two metabolites of atazanavir, M 2 and M 14 , were identified in the systemic circulation following administration of atazanavir to humans. These metabolites were analyzed for anti-HIV activity and cytotoxicity in cell culture assays. Three

different laboratory strains of HIV were tested and two different cell lines were used. Neither M 2 nor M 14 showed any antiviral effects up to the highest concentrations tested, 200 nM and 1000 nM, respectively. The CC_{50} for M 2 in MT-2 and PM1 cells was 312 µM and 254 µM, respectively. The CC_{50} for M 14 in MT-2 and PM1 cells was 201 µM and 122 µM, respectively. Both metabolites had higher CC_{50} values in MT-2 cells as compared with atazanavir.

5.1.2 General Pharmacodynamics

Atazanavir was evaluated in general pharmacodynamic and safety pharmacology studies to assess effects other than intended therapeutic activity. Effects on cardiovascular, CNS, and respiratory system functions were evaluated in a series of *in vitro* and *in vivo* studies. Two identified circulating human metabolites of atazanavir, M 2 and M 14 , were also evaluated *in vitro* for potential effects on cardiac action potential and ion currents. In addition, atazanavir was evaluated *in vitro* for effects on glucose and lipid metabolism and regulation to investigate mechanisms for its apparent superior metabolic profile in humans compared to those of other PIs.

The specificity of atazanavir for the HIV protease was assessed *in vitro* by determining its inhibition of human aspartyl proteases renin, cathepsin D, cathepsin E, pepsin, and gastricsin. Atazanavir failed to inhibit these enzymes at concentrations up to 10,000 nM. In contrast, atazanavir inhibited the HIV protease with an IC_{50} value of 1 nM. Based on these results, atazanavir was considered highly selective for the HIV protease enzyme.

The initial safety pharmacology assessment of atazanavir, prior to clinical dosing, was conducted as a component of the 2-week toxicity studies in rats and dogs. Concurrent with clinical development, the potential for adverse pharmacodynamic effects continued to be evaluated in the chronic toxicity studies. In all of these assessments, there were no atazanavir-related adverse effects on cardiovascular, respiratory, or CNS function in rats (≤ 1200 mg/kg/day) or dogs (≤ 360 mg/kg/day). Although ECG changes were noted in dogs at all doses (90, 180, and 360 mg/kg/day) in the initial 2-week toxicity study, these were considered secondary to the drug-induced marked clinical toxicity (emesis, reduced food consumption, body weight loss, and humane euthanasia) rather than a direct effect of atazanavir. Moreover, in the 9-month toxicity study in dogs, there was no clinical toxicity and no ECG changes at exposures generally comparable to those in the initial 2-week study at common doses of 90 and 180 mg/kg/day. In the 9-month study, plasma

concentrations of atazanavir at the high dose of 180 mg/kg/day were up to three times the Cmax and seven times the AUC in humans given the recommended dose of 400 mg/day.

In addition to the *in vivo* cardiovascular assessments, atazanavir was also evaluated in the *in vitro* rabbit Purkinje fiber assay for its potential to prolong action potential duration in compliance with the CPMP guideline for noncardiovascular drugs (Appendix 3, Table 1). Atazanavir minimally increased action potential duration (13% increase at 30 μM which is approximately four times the Cmax and 17 times the Css in humans at 400 mg/day). To evaluate the clinical relevance of this *in vitro* signal, a carefully designed clinical program was initiated in accordance with the CPMP guideline. In clinical studies, low incidences of clinically insignificant prolongation in QTc interval and/or dose-related asymptomatic prolongation in PR interval occurred in healthy and HIV-infected subjects treated with atazanavir.

In an attempt to elucidate the cellular mechanisms contributing to these cardiac electrophysiologic changes, atazanavir-related effects on sodium, potassium (I_{Kr} and I_{Ks}), and calcium currents were evaluated *in vitro* (Appendix 3, Table 1). Atazanavir weakly inhibited sodium and I_{Kr} and I_{Ks} potassium currents (IC_{50}s > 30 μM) while moderately inhibiting calcium currents (IC_{50} of 10.4 μM). Based on these *in vitro* results, the moderate inhibitory effect of atazanavir on calcium current was considered to contribute, at least in part, to the increased PR interval seen in humans. However, there was no clear cellular mechanism(s) identified to explain the prolonged action potentials observed *in vitro* or the prolonged QTc interval seen in clinical studies. Although atazanavir interacted to varying degrees with all four of the ion channels tested, it is possible that atazanavir interacts with additional ion transport pathways not evaluated directly since drug-induced altered repolarization in Purkinje fibers reflects effects across multiple ion transport systems. Importantly, no serious adverse events in clinical studies with atazanavir have been attributed to QTc or PR interval prolongation.

In addition to the cardiovascular safety pharmacology testing of atazanavir, *in vitro* cardiovascular safety pharmacology studies were conducted with two minor human metabolites of atazanavir, M 2 and M 14 . The potential effects of these metabolites on cardiac action potential and sodium, potassium (I_{Kr} and I_{Ks}), and calcium currents were assessed (Appendix 3, Table 1). Neither metabolite prolonged Purkinje fiber action potential duration, but M 2 produced a minimal reduction in action potential maximal upstroke velocity (15% at 30 μM). Both metabolites had weak to no

inhibitory effects on potassium (I_{Kr} and I_{Ks}), sodium, or calcium currents (IC_{50}s > 30 μM). Importantly, neither metabolite was more potent than atazanavir in prolonging action potential duration or inhibiting cardiac ion currents.

Other protease inhibitors (indinavir, lopinavir, nelfinavir, ritonavir, and saquinavir) were also evaluated in the rabbit Purkinje fiber and ion channel assays (Appendix 3, Table 1). These investigations showed that the other protease inhibitors also altered action potential duration or ion currents with equivalent or greater potency than atazanavir. However, only lopinavir/ritonavir and nelfinavir have been reported to cause rare ECG abnormalities in humans.

HIV protease inhibitors are implicated in the lipodystrophy, hyperlipidemia, and insulin resistance syndrome associated with antiretroviral therapy in HIV infection. In the course of clinical development, atazanavir has shown antiviral efficacy without the plasma lipid elevations or insulinemia seen with several other protease inhibitors. To determine the possible mechanisms for its apparent superior metabolic profile, atazanavir was evaluated in a series of *in vitro* studies for potential effects on glucose and lipid metabolism and regulation.

Atazanavir had little to no effect on insulin-stimulated glucose transport in murine 3T3-L1 adipocytes, rat L6 myoctes and primary myoblasts, or in mouse primary adipocytes at concentrations up to 50 μM. In comparative studies, the PIs lopinavir, ritonavir, nelfinavir, and saquinavir significantly inhibited glucose uptake at concentrations as low as 3 μM. Lopinavir and ritonavir were particularly potent in inhibiting glucose uptake. Atazanavir, ritonavir, and nelfinavir were tested for effects on accumulation of intracellular triacylglycerol (TG) in an established adipocyte model, murine 3T3-L1 cells. In these assays, atazanavir was less potent than nelfinavir and ritonavir in suppressing TG accumulation at concentrations between 3 and 30 μM. In studies comparing the potential effects of atazanavir and other PIs on lipid homeostasis in mammalian cells, atazanavir was the least potent (IC_{50} = 26 μM) protease inhibitor in inhibiting *in vitro* proteasome activity (controls turnover rate of many proteins, including apolipoprotein B in liver) when compared to lopinavir, ritonavir, nelfinavir, and saquinavir (IC_{50}s = 3 to 11 μM). In other studies, atazanavir had no effect on adipocyte SREBP-1c isoform levels (regulates lipogenic enzymes in adipocytes and liver) or on hepatic TG synthesis in HepG2 cells at concentrations up to 30 μM. In summary, atazanavir produced the least disruption in glucose and lipid regulation and metabolism

compared to other PIs. This differentiation may offer a potential explanation for the improved plasma lipid profile seen in atazanavir-treated patients.

5.1.3 Drug Interactions

Atazanavir was evaluated for anti-HIV activity in two-drug interaction studies with other anti-retrovirals. Combinations of atazanavir with either stavudine, didanosine, lamivudine, zidovudine, nelfinavir, indinavir, ritonavir, saquinavir, or amprenavir in HIV-infected peripheral blood mononuclear cells yielded additive antiviral effects. Importantly, the drug combinations did not result in antagonistic anti-HIV activity or enhanced cytotoxic effects at the highest concentrations used. These results suggest that atazanavir is an effective HIV-1 inhibitor that may be utilized in a variety of different drug combinations.

5.2 PHARMACOKINETICS

Preclinical pharmacokinetic studies were conducted with atazanavir in the mouse, rat, and dog, which are the principal species selected for the safety evaluation of atazanavir. The exposure to atazanavir was verified in mice, rats (including pregnant rats), pregnant rabbits, and dogs either in conjunction with toxicology studies or as separate toxicokinetic investigations.

5.2.1 Absorption

Atazanavir exhibited modest absolute oral bioavailability in rats (15.2% from a PEG-400 suspension) and dogs (36.3% from a capsule). The absolute oral bioavailability of atazanavir was not determined in humans; however, the bioavailability of the clinical formulation of atazanavir relative to an oral solution of atazanavir was 68% in humans. The high Caco-cell permeability of atazanavir, which is comparable to that of completely absorbed drugs, suggests that atazanavir has good oral absorption in humans. P-glycoprotein (P-gp) inhibition by atazanavir is unlikely since steady-state peak and average plasma concentrations over 24 h in humans given the recommended dose of 400 mg/day are several fold lower than the values required for P-gp inhibition. The recovery of a significant amount of unchanged drug in the rat (39%), dog (79%), and human (15%) feces after oral administration suggested incomplete absorption and/or biliary elimination of atazanavir in all three species. When compared to the decline in plasma concentrations after intravenous dosing, the slow decline in plasma levels after achieving peak plasma

levels (2 h) in non-fasted rats and dogs following oral administration suggested prolonged absorption. In comparison, peak concentrations of atazanavir in humans were achieved in 1.5 h after a single oral capsule dose in the fed state, with no evidence of a plateau effect after peak levels, indicating that the absorption is rapid.

5.2.2 Distribution

The steady state volume of distribution of atazanavir in rats (1.62 L/kg) and dogs (0.76 L/kg) is greater than the total body water (0.67 L/kg in the rat and 0.60 L/kg in the dog), indicating extravascular distribution and/or tissue protein binding. Accordingly, drug-related radioactivity was extensively distributed into and decayed from all tissues of the rat following oral administration of a 100 mg/kg dose of [^{14}C]-atazanavir, with no sequestration in any particular tissue. The highest levels of radioactivity were associated with the dosing/absorption site(s) along the gastrointestinal tract and liver. Appreciable levels of radioactivity were noted in tissues known to be reservoirs of HIV such as lymph nodes and testes. There were no detectable levels in the CSF; however, drug-related radioactivity was detected in cerebrum and cerebellum indicating some brain penetration in rats. These data suggest that a similar wide tissue distribution, with possibly low brain penetration, of atazanavir and/or its metabolite(s) should be expected in humans. The CSF levels of atazanavir were determined to be lower than plasma levels in humans (CSF:plasma ratios = 0.002 to 0.02). In comparison, undetectable CSF levels of at least one HIV protease inhibitor (nelfinavir) have been reported in HIV patients.

Tissue and plasma levels of radioactivity were generally higher in female than in male rats following [^{14}C]-atazanavir. In humans, females also had modestly higher exposures to atazanavir compared to males but the difference (< 25%) is considered not to be of clinical relevance. Pharmacokinetics of other HIV protease inhibitors, amprenavir, lopinavir (available as lopinavir/ritonavir fixed-dose combination), and nelfinavir, appear to be comparable between men and women. Indinavir exhibited differences in the pharmacokinetics between male and female rats and dogs, but no gender-dependent differences in humans. Following oral administration of a 100 mg/kg dose of [^{14}C]-atazanavir to pregnant rats, drug-related radioactivity was widely distributed in maternal tissues whereas distribution to the fetus was low. The concentrations of radioactivity in maternal and fetal tissues generally reached a maximum at 4 h after dosing and were detectable until 8 h postdose in maternal tissues and up to 12 h postdose in fetal tissues, except the fetal kidney (detectable radioactivity for 1 h). The maternal tissue

concentrations were highest in the liver, kidneys, lungs, placenta, ovaries, plasma, and uterus and were lowest in the cerebrum. Fetal tissue concentrations were highest in the kidneys, blood, and amniotic fluid and lowest in the brain. The concentrations of radioactivity in the fetus and amniotic fluid were low relative to the equivalent maternal tissues in all cases, except the fetal brain which exceeded maternal cerebrum. The mean tissue:maternal plasma ratios were < 1 at all time points for maternal blood and cerebrum; amniotic fluid; and fetal blood, brain, liver, and carcass. Drug-derived radioactivity was detectable in milk within 1 h after oral administration of 100 mg/kg of [^{14}C]-atazanavir to lactating rats and reached a maximum at 4 h postdose before declining with time. The milk:plasma concentration ratios were generally > 1, with ratios ranging from 0.93 to 3.46. These data suggest that there is a potential for fetal and neonatal exposure to atazanavir if administered to pregnant or lactating women. Amprenavir, indinavir, lopinavir, and nelfinavir are also secreted in rat milk. Furthermore, indinavir is known to cross the placental barrier into the rat fetus.

The animal and human serum protein binding and human albumin and α-1-acid glycoprotein binding of atazanavir were comparable and not extensive (86.5 to 92.8%). Atazanavir has similar binding affinity to both human albumin and α-1-acid glycoprotein. Serum protein binding and RBC distribution in animals were independent of concentration, over a 100- to 1000-fold range of therapeutic and toxicologic relevant concentrations, and were similar to the values in humans. The *in vitro* human protein binding value (86.5%) indicates that there is minimal potential for drug-drug interactions due to displacement of protein-bound drugs when coadministered with atazanavir. The human serum protein binding of atazanavir is appreciably lower than that for amprenavir (90%), lopinavir (98 to 99%), and nelfinavir (> 98%) but higher than the value for indinavir (60%).

5.2.3 Biotransformation

The metabolic pathways of atazanavir in rats, dogs, and humans are similar and involve monooxygenation, dioxygenation, glucuronidation, N-dealkylation, hydrolysis, and oxygenation with dehydrogenation. The multiple monohydroxylated, dihydroxylated, and trihydroxylated metabolites demonstrate that the oxygenation of atazanavir can occur on the phenylmethyl ring, the pyridinylphenylmethyl ring system, and at different positions on the pentaazatetradecanedioic acid dimethyl ester part of the molecule. The major elimination pathway appears to be CYP3A4-mediated conversion to these oxygenated

metabolites and excretion in the bile as either free or glucuronidated metabolites. Additional minor metabolic pathways observed consist of N-dealkylation to generate metabolites and hydrolysis to generate carbamate hydrolyzed metabolites. See Section 6.2.1.3 for a depiction of the biotransformation pathway.

In rat and dog plasma, the major circulating components were atazanavir and 4-(2-pyridyl) benzoic acid (M 2), along with small amounts of a keto metabolite (M41). In addition, traces of an N-dealkylated metabolite, M 14 , were observed in dog plasma. Atazanavir was the major circulating component in human plasma. Additionally, M 2 , M 14 and a keto metabolite (M41), each constituting approximately 10% of the plasma radioactivity, were present in human plasma. This indicates that all metabolites present in human plasma were also present in rat and/or dog plasma. M 2 and M 14 were shown to possess no anti-HIV activity.

Rat urine contained atazanavir (16% of the urinary radioactivity); at least three monohydroxylated metabolites; M 2 and its glucuronide; two N-dealkylated metabolites; two carbamate hydrolyzed metabolites; and one dihydroxylated metabolite. Besides all of the major urinary metabolites identified in the rat urine, dog urine contained six glucuronides of monohydroxylated, dihydroxylated, trihydroxylated, N-dealkylated, and unknown metabolites which were not identified in rat urine. Rat feces contained atazanavir (39% of the fecal radioactivity), multiple monohydroxylated and dihydroxylated metabolites, and minor amounts of metabolites formed from oxygenation and dehydrogenation of atazanavir. Rat biliary profiles were similar to the fecal profiles, except that the glucuronides of atazanavir metabolites excreted in the bile appeared to have been hydrolyzed by the intestinal microflora to their respective aglycones. Dog feces contained atazanavir (79% of the fecal radioactivity) and multiple monohydroxylated and dihydroxylated metabolites. Human urinary and fecal profiles were generally similar to those of the rat and dog. The overall similarity between biotransformation in rats and humans suggests human bile might also contain glucuronides that may be hydrolyzed by intestinal microflora and therefore, were not present in human feces.

In addition, atazanavir was incubated with aroclor-induced rat liver S-9 fraction, under conditions similar to that used in the *in vitro* genetic toxicity studies, to determine if metabolites detected in human plasma were present. The three human metabolites,

M 2 , M 14 , and a keto metabolite (M41), were all identified in the rat liver S-9 fraction (5 to 11% of total radioactivity).

Atazanavir was determined to be a substrate of human CYP3A4 *in vitro*. In agreement with these *in vitro* data, ritonavir (a potent CYP3A4 inhibitor) increased and efavirenz (a potent CYP3A4 inducer) decreased the exposure to atazanavir significantly following concomitant administration to humans. Interestingly, coadministration of ketoconazole (a potent CYP3A4 inhibitor) did not significantly affect the pharmacokinetics of atazanavir in humans. Although the reason for this discrepancy between the *in vitro* and *in vivo* results is not clear, it is to be noted that the plasma levels of lopinavir, which is also metabolized exclusively by CYP3A4, actually decrease by ~13% upon concomitant administration of ketoconazole. Overall, appropriate dose adjustments of atazanavir may be necessary in humans when coadministered with potent inhibitors or inducers of CYP3A4. Other HIV protease inhibitors have similar dose adjustment considerations for concomitant administration with CYP3A4 inhibitors and inducers, as they are all predominantly metabolized by CYP3A4.

Atazanavir inhibited, but did not induce, human CYP3A4 *in vitro*; it did not inhibit human CYP1A2, CYP2A6, CYP2D6, CYP2E1, or CYP4A9/11 at clinically relevant concentrations. The extent of inhibition of CYP2C9 and CYP2C19 could not be accurately determined due to interference of the inhibitors with some of the metabolite peaks. However, there was no interference with other metabolite peaks suggesting that CYP2C9 and CYP2C19 isozymes are probably not involved in the metabolism of atazanavir. Accordingly, coadministration of atazanavir caused a significant increase in the exposure to saquinavir (a CYP3A substrate) in humans. These data suggest that a dose reduction of CYP3A4 substrates may need to be considered in humans when administered concomitantly with atazanavir. Dose reductions are generally recommended for simultaneous administration of CYP3A4 substrates with amprenavir, indinavir, lopinavir, and nelfinavir. Additionally, lopinavir/ritonavir are known to inhibit CYP2D6, induce their own metabolism, as well as induce the biotransformation of drugs metabolized by CYP3A4 and glucuronidation, making it difficult to predict the potential interaction with concomitantly administered drugs. Nelfinavir has also been reported to induce its own CYP3A4-mediated metabolism in humans.

The pharmacologically inactive, minor circulating human metabolites of atazanavir, M 2 and M 14 , were also tested for CYP P450 inhibition. With the

exception of the inhibitory potential of M 14 on CYP2C19 (IC_{50} = 4.9 μM), the IC_{50} values were > 80 μM for M 14 and M 2 for CYP1A2, CYP2C9, CYP3A4, CYP2D6, and CYP2C19. Therefore, no CYP P450 inhibition would be expected at clinically relevant concentrations. With M 14 , the peak plasma concentration (Cmax = 0.54 μM) and average plasma concentration over 24 h ($Cavg_{(0-24)}$ = 0.45 μM) in humans following 6 days of dosing with atazanavir were at least 9-fold lower than the IC_{50} value for CYP2C19; therefore, an inhibitory effect of M 14 on CYP2C19 is unlikely. Further, CYP2C19 is not a major isozyme involved in the metabolism of many drugs, and few drug interactions (mostly CNS drugs) due to inhibition would be expected.

Atazanavir was found to be a moderate inhibitor of UGT1A1 *in vitro* suggesting that there may be a potential for drug interactions due to inhibition of this isozyme. In clinical studies, elevations in endogenous serum bilirubin, a UGT1A1 substrate, were observed. It is to be noted that the IC_{50} values for the inhibition of bilirubin glucuronidation by atazanavir were similar to those for nelfinavir and saquinavir, but were appreciably lower than the value for indinavir. However, increases in bilirubin levels have been observed upon administration of indinavir, but not with either nelfinavir or saquinavir. Taking serum protein binding into consideration, the IC_{50} values for nelfinavir and saquinavir were > 20- and 625-fold above the respective unbound peak plasma concentrations at the therapeutic doses compared to 2.4- and 13-fold above the unbound peak plasma levels for atazanavir and indinavir, respectively. Concentrations of atazanavir following clinically relevant doses may be high enough to lead to a significant inhibition of UGT1A1. A dose reduction of UGT1A1 substrates may need to be considered in humans when administered concomitantly with atazanavir.

5.2.4 Elimination

Atazanavir is an intermediate- to high-extraction drug in animals; the elimination half-life in the rat, dog, and human is 0.94, 0.45, and approximately 7 h, respectively. The minimal recovery of radioactivity in urine (< 7%) and substantial recovery in feces (> 67%) after intravenous administration in rats and dogs suggest that the major route of excretion of atazanavir and its metabolite(s) is via the bile. Consistent with these findings in animals, approximately 79% of the administered radioactivity was recovered in the feces of humans, suggesting biliary elimination and/or incomplete absorption. Renal elimination was a minor pathway in humans, since only 13% of the administered

radioactivity was recovered in the urine after a single 400 mg oral dose, with unchanged drug accounting for approximately half of that radioactivity. Similar to atazanavir, high fecal recovery of unchanged drug and/or metabolites and low urinary recovery of unchanged drug have been reported for other HIV protease inhibitors.

5.2.5 Comparative Toxicokinetics

5.2.5.1 Atazanavir

Dose-related systemic exposures to atazanavir were observed in the species used in the nonclinical toxicology studies (mice, rats, pregnant rats and rabbits, and dogs). The increases in exposure were generally less than dose proportional in the rodents at higher doses, probably due to reduced absorption and/or greater presystemic clearance, whereas the increases were mostly greater than dose proportional in dogs. There were consistent sex-related differences in the exposure of mice and rats to atazanavir, with females having higher exposure than males. Since atazanavir is a substrate for CYP3A4, this difference is presumed to be due to the difference in hepatic enzyme activity between male and female rodents.

There was a decrease in the exposure of atazanavir in rats following repeated doses, but exposure appeared to reach a steady state with long-term dosing. This decrease in exposure may be attributed to enhanced metabolism of atazanavir due to hepatic enzyme induction, as suggested by increased liver weights and hepatocellular hypertrophy after repeated doses in several toxicity studies in rats. In dogs, increased systemic exposure upon repeated oral administration was not apparent, indicating no accumulation of atazanavir after long-term dosing. In humans, exposure to atazanavir is generally greater than dose proportional over the dose range of 200-800 mg daily, with a 1.11- to 6.95-fold accumulation at steady-state. A modestly higher exposure (< 25%) was observed in females compared to that in males, but is considered not to be clinically relevant. Overall, the animal species used for toxicology studies were exposed to atazanavir to a comparable or greater extent than humans following 400 mg doses of atazanavir (Appendix 3, Table 2).

5.2.5.2 Atazanavir Metabolites

In supporting toxicokinetic studies, systemic exposures to M 2 and M 14 , two pharmacologically inactive minor metabolites identified in human

plasma, were verified in the animal species used in the toxicology studies (Appendix 3, Tables 3 - 4). At doses of atazanavir similar to those administered in the toxicology studies, the exposures (AUC) to M 2 in mice, pregnant rabbits, and dogs, when corrected for molecular weights, were ≤ 7, 76, and ≤ 26% of the atazanavir AUC values, respectively. In rats, the AUC values for M 2 were approximately 3 to 20 times greater than the corresponding AUC values for atazanavir. When compared to humans, exposures (AUC) to M 2 at atazanavir doses given in the toxicology studies were 0.5 to 127 times the M 2 exposure in humans given 400 mg per day atazanavir (Appendix 3, Table 3).

Exposure to M 14 was observed following atazanavir administration in mice, rats, and dogs (Appendix 3, Table 4). Exposure to M 14 in rabbits given 60 mg/kg/day, the highest dose given in the embryo-fetal development study, was below the level of detection. The AUC values for M 14 when corrected for molecular weight, in mice, rats, and dogs were ≤ 0.4, ≤ 1, and ≤ 12% of atazanavir AUC values, respectively. The exposures (AUC) of mice and rats to M 14 at these doses of atazanavir achieved levels up to approximately 10 and 4%, respectively, of that seen in humans given 400 mg/day doses. In dogs, the exposures (AUC) of M 14 at 180 mg/kg/day of atazanavir were approximately 40% of that seen in humans given 400 mg/day of atazanavir.

In addition to M 2 and M 14 , a keto metabolite of atazanavir (M41) was also detected in rat, dog, and human plasma in distribution studies (2.8 to 12.5% of plasma radioactivity). Plasma concentrations of M41 have not been determined in animals or humans in toxicokinetic or pharmacokinetic evaluations as its structure has only recently been tentatively identified.

In summary, three metabolites of atazanavir present in human plasma, M 2 , M 14 , and M41, were also identified in two or more toxicology animal species. Exposures to M 2 were higher and exposures to M 14 were lower in the animal species compared to relative exposures in humans. Systemic exposures (AUC) to M 2 and M 14 were approximately 10 and 20%, respectively, relative to known circulating drug-related material (atazanavir + M 2 + M 14) and therefore are considered minor metabolites.