5.3 Nonclinical toxicology

The toxicity of atazanavir was well characterized in a comprehensive nonclinical toxicology program that included single- and repeat-dose oral toxicity studies in mice, rats, and/or dogs; reproductive and developmental toxicity studies in rats and rabbits; a battery of *in vitro* and *in vivo* genotoxicity studies; *in vitro* and *in vivo* ocular and dermal irritation studies; a skin sensitization study in guinea pigs; and an immunotoxicity study in rats.

5.3.1 Single-Dose Toxicity

Single-dose oral toxicity studies were conducted in mice and rats at doses of 200 to 1600 mg/kg. The high dose for both studies was limited to 1600 mg/kg, based on a maximum feasible dose (concentration and dose volume limited) established at the time the studies were conducted. Atazanavir demonstrated a minimal order of acute toxicity in both species. In mice, atazanavir was well tolerated up to 400 mg/kg. Minimal lethal doses were 1600 and 800 mg/kg in males and females, respectively. Deaths were not associated with any drug-related gross or histopathologic changes. Clinical signs, considered to be agonal (loss of righting reflex, recumbency, and labored respiration), were present just prior to or concomitant with death. Other clinical signs (scant stool, tremors, hypoactivity, urogenital staining, and ptosis) were observed in surviving animals at high doses. In rats, no drug-related changes were observed up to and including 1600 mg/kg. The greater sensitivity of mice to the acute effects of atazanavir compared to rats may be related to higher systemic exposure to atazanavir as demonstrated in subsequent toxicity studies. It is not certain if the observations in animal studies accurately predict the reactions that may be seen in a human overdose, but patients should be closely monitored and provided supportive therapy as needed.

5.3.2 Repeat-Dose Toxicity

Repeat-dose oral toxicity studies were conducted in rats for up to 6 months and in dogs for up to 9 months. In rats, the toxicity of atazanavir was evaluated at doses up to 1200 mg/kg/day in the 2-week study and up to 900 mg/kg/day in the 6-month study with a 3-month interim evaluation and a 2-month postdose recovery period. In dogs, the toxicity of atazanavir was evaluated at doses up to 360 mg/kg/day in 2-week studies and up to 180 mg/kg/day in the 9-month study. Because of marked clinical toxicity (90 to

360 mg/kg/day) in the initial 2-week dog study, a second 2-week study was conducted at lower doses (10 to 75 mg/kg/day). In the 9-month study in dogs, the low dose (10 mg/kg/day) was increased to 180 mg/kg/day after 3 months due to the absence of drug-related findings at 10 mg/kg/day. Although ICH guidelines recommend dosing for 9 months in the nonrodent, 6 months of dosing in dogs with 180 mg/kg/day atazanavir was considered sufficient to evaluate chronic toxicity in the absence of any unexpected drug-related changes at 30 and 90 mg/kg/day between 3 and 9 months of dosing in the 9-month study and in the absence of additional toxicity in rats between 3 and 6 months of dosing. In addition to rats and dogs, the repeat-dose oral toxicity of atazanavir was evaluated in a range-finding study in mice at doses up to 80 mg/kg/day in males and 640 mg/kg/day in females for 3 months. This study was designed to assist in dose selection for the carcinogenicity study.

In repeat-dose toxicity studies, the liver was identified as the principal target organ in rats, dogs, and mice (Appendix 3, Table 5). In rats, atazanavir was generally well tolerated at doses up to 600 mg/kg/day in the 2-week study and 900 mg/kg/day in the 6-month study. Liver changes observed consisted of: increased serum total bilirubin (≥ 300 mg/kg/day); increased liver weights (≥ 100 mg/kg/day) and associated minimal to mild hepatocellular hypertrophy (≥ 100 mg/kg/day), which were considered an adaptive response consistent with hepatic enzyme induction; pale livers (900 mg/kg/day); and minimal to moderate hepatocellular cytoplasmic vacuolation (lipid) (≥ 100 mg/kg/day). None of the hepatic changes were accompanied by elevations in serum transaminases or microscopic evidence of cholestasis or degenerative liver changes. Female rats were more affected than males, which corresponded to higher systemic exposures to atazanavir. The hepatic alterations generally did not progress between 3 and 6 months of dosing and, with the exception of increased liver weights (900 mg/kg/day), were reversible. Systemic exposures (AUC) in male and female rats at the well-tolerated dose of 900 mg/kg/day for 6 months are equivalent to and four times, respectively, the exposure at the recommended clinical dose of 400 mg/day, supporting that adequate doses were administered to assess potential human risk. Other clinicopathologic and organ weight changes observed in the rat studies were considered to be toxicologically insignificant since they were generally minimal in severity or were unaccompanied by microscopic tissue changes.

In dogs, atazanavir was poorly tolerated in the initial 2-week study due to marked clinical toxicity (emesis, body weight loss, and reductions in food consumption) at all doses (90, 180, and 360 mg/kg/day); this necessitated humane euthanasia in some mid- and

high-dose dogs. In contrast, atazanavir was well tolerated in subsequent 2-week (10, 30, and 75 mg/kg/day) and 9-month (30, 90, and 10/180 mg/kg/day) toxicity studies. The clinical relevance of the toxicity observed in the initial 2-week study is unclear since common doses (90 and 180 mg/kg/day) producing generally comparable systemic exposures in the 9-month study failed to result in similar clinical toxicity.

In the initial 2-week study, hepatic changes consisted of minimal to moderate increases in serum total bilirubin, liver enzymes (alanine and aspartate aminotransferases, gamma glutamyltransferase, and alkaline phosphatase), cholesterol, and triglycerides and decreases in protein and/or albumin at all doses. In the second 2-week study, there were no drug-related changes. In the 9-month study, hepatic changes were limited to minimally increased serum total bilirubin and gamma glutamyltransferase (≥ 30 mg/kg/day), minimally to moderately increased serum alkaline phosphatase (90 mg/kg/day) in individual animals, and minimally increased liver weights (90 mg/kg/day). No drug-related gross or microscopic liver changes or microscopic evidence of cholestasis were observed in any of the studies in dogs. At the well-tolerated dose of 180 mg/kg/day for 6 months, systemic exposures (AUC) in male and female dogs were approximately two and seven times, respectively, human exposure at 400 mg/day, supporting that adequate doses were administered to assess potential human risk.

A spectrum of additional findings in the initial 2-week study were considered secondary to the observed clinical toxicity and/or stress associated with atazanavir administration. These findings included clinical signs, changes in ECGs (including prolongation of QT and PR intervals) and various clinicopathologic parameters, decreased adrenal weights, and gross and/or microscopic findings in various organs and tissues. In the 9-month study, additional findings of minimally altered water balance and minimally decreased heart weights were not associated with any functional impairment or gross or microscopic organ changes and were considered to be toxicologically insignificant.

In mice, mid-doses of 40 (M) and 160 (F) mg/kg/day were generally well tolerated after 3 months with no effects seen at the low doses of 20 (M) and 40 (F) mg/kg/day, respectively. Similar to the liver changes noted in rats and/or dogs, a mild increase in serum total bilirubin [640 (F) mg/kg/day], minimal to marked increases in liver weights [≥ 40 (M) and ≥ 160 (F) mg/kg/day], hepatocellular hypertrophy and vacuolation (lipid) [≥ 40 (M) and ≥ 160 (F) mg/kg/day], and pale livers [80 (M), ≥ 160 (F) mg/kg/day] were also observed in mice. In addition, clinical and microscopic evidence of hepatotoxicity

[80 (M) and 640 (F) mg/kg/day], characterized by minimal to moderate elevations in serum transaminases and a low incidence of minimal hepatocellular single-cell necrosis in females, was seen in mice. Increased cellular glycogen [80 (M) and 640 (F) mg/kg/day] was also noted only in mice. Systemic exposures (AUC) in male and female mice at the well-tolerated doses [40 (M) and 160 (F) mg/kg/day] are slightly below to four times, respectively, the exposure in humans given 400 mg/day. The highest doses resulting in hepatotoxicity produced exposures equivalent to (M) and 12 (F) times that seen in humans. This high exposure to atazanavir in female mice may account in part for the increased hepatotoxicity.

Other drug-related findings in female mice included mild to moderate decreases in platelet counts (≥ 160 mg/kg/day); minimal to mild increases in leukocytes, neutrophils, and lymphocytes (640 mg/kg/day); and morphologic changes in erythrocytes (640 mg/kg/day). Increased spleen weight and size and histologic evidence of increased splenic and hepatic extramedullary hematopoiesis in females (≥ 160 mg/kg/day) were interpreted as secondary effects related to the reduced platelet counts rather than direct drug effects. However, a clinical or morphologic basis for the reduction in platelets was not determined. All of these changes, which were limited to female mice, have no established clinical relevance as systemic exposures at doses producing these alterations were high (four to 12 times human exposure at the recommended efficacious dose) and similar consistent changes were not observed in rats and dogs treated chronically with atazanavir. Importantly, there have been no reports of thrombocytopenia in patients treated with atazanavir for over 1 year.

The nonclinical repeat-dose toxicities of atazanavir and all marketed HIV protease inhibitors were compared and evaluated in relationship to the recommended clinical dose for each drug (Appendix 3, Table 6). Overall, the nonclinical toxicity profile of atazanavir compared favorably with the HIV protease inhibitors amprenavir, indinavir, lopinavir/ritonavir, ritonavir, and saquinavir. Nelfinavir had a slightly more benign toxicity profile; however, the highest doses evaluated in the repeat-dose toxicity studies represented lower multiples of the respective recommended dose when compared to atazanavir. Importantly, the hepatic changes seen with atazanavir, including findings of hepatotoxicity, were also noted with all marketed HIV protease inhibitors except nelfinavir. The no-adverse-effect doses of atazanavir in mice, rats, and dogs varied from slightly below to slightly above the recommended dose in humans on a mg/m^2 basis.

However, these low dose multiples were not unexpected as similar low dose multiples were noted with most of the marketed HIV protease inhibitors.

In clinical trials to date, reversible dose-related hyperbilirubinemia (predominantly indirect bilirubin without associated changes in liver function tests) has occurred with atazanavir treatment, and this was predicted by the animal studies. Treatment with the marketed HIV protease inhibitor indinavir also results in indirect hyperbilirubinemia in humans. *In vitro* mechanistic studies demonstrated that the atazanavir- and indinavir-related increased bilirubin levels were essentially due to the competitive inhibition of human UGT1A1 isoform, which is the sole bilirubin glucuronidating enzyme. Importantly, there has been no clinical evidence of cholestatic liver disease in patients treated with atazanavir for greater than 3 years.

Increases in serum transaminases have also been observed in clinical trials with atazanavir and reported in humans treated with the marketed HIV protease inhibitors indinavir, ritonavir, saquinavir, and lopinavir/ritonavir. Elevated liver enzymes (transaminases, alkaline phosphatase, gamma glutamyltransferase) were also seen in dogs and mice with atazanavir treatment, but progressed to morphologic evidence of hepatocellular necrosis only in mice at high exposures, 12 times that seen in humans at the recommended daily dose of 400 mg. Moreover, the risk of liver injury in humans is considered low at efficacious exposures since chronic atazanavir treatment in rats and dogs at exposures up to seven times clinical exposure did not result in hepatocellular degeneration or necrosis.

Increases in serum total cholesterol, triglycerides, and glucose were observed in rats and dogs; however, similar alterations have not been seen clinically. In fact, atazanavir appears to be unique among the other marketed protease inhibitors in that lipodystrophy, hyperlipidemia, and insulin resistance syndrome have not occurred with atazanavir as a component of antiretroviral therapy. As previously mentioned, a variety of *in vitro* evaluations confirmed that atazanavir produced the least disruption in glucose and lipid regulation and metabolism when compared with lopinavir, ritonavir, nelfinavir, and/or saquinavir. Thus, the changes in glucose and lipid levels in animals appear to have no established clinical relevance at this time and have not been predictive of similar alterations in humans.

5.3.3 Genetic Toxicity

The genotoxic potential of atazanavir was evaluated in a comprehensive battery of *in vitro* and *in vivo* test systems. Atazanavir was not mutagenic in either the bacterial mutagenicity screening assay or in the definitive Ames reverse-mutation study. In the definitive Ames assay, cytotoxicity was observed in each of the Salmonella and *E. coli* strains at the highest concentration evaluated (2500 µg/plate), both with and without metabolic activation. In the *in vitro* cytogenetics test in primary human lymphocytes, atazanavir produced an increased frequency of chromosome aberrations at 30 µg/ml in the absence of metabolic activation and 240 µg/ml in the presence of metabolic activation. Moreover, atazanavir was not clastogenic in the *in vivo* micronucleus test in rats and did not cause DNA damage in the *in vivo-in vitro* hepatocyte DNA repair (UDS) study in rats at doses up to 2000 mg/kg (maximum dose recommended by international regulatory guidelines). The maximum nongenotoxic concentrations in the primary human lymphocyte study in the absence (15 µg/ml) and presence (120 µg/ml) of metabolic activation were three and 22 times Cmax, respectively, and 12 and 98 times Css, respectively, at the recommended clinical dose of 400 mg/day. Plasma exposures (AUC and Cmax) in rats at the nongenotoxic dose of 2000 mg/kg in the *in vivo* tests were approximately 1.5 to 2 (M) and 2.3 to 2.5 (F) times human exposures at 400 mg/day. Based on the weight of evidence from the battery of genotoxicity studies completed at this time, atazanavir is considered to pose no genotoxic risk to humans.

5.3.4 Carcinogenicity

The carcinogenic potential of atazanavir is currently under investigation. Two-year oral (gavage) studies in ICR mice and SD rats were initiated in 日付 , respectively, and are ongoing.

Doses administered in the oral carcinogenicity study in mice are 20, 40, and 80 mg/kg/day in males and 40, 120, and 360 mg/kg/day in females. These doses were selected based on the results of the 2-week oral toxicokinetic and 3-month oral range-finding toxicity studies and were approved by the Executive Carcinogenicity Assessment Committee of the FDA. High doses of 80 mg/kg/day for males and 360 mg/kg/day for females were based on deaths observed in the 2-week toxicokinetic study in males at doses ≥ 100 mg/kg/day and in females at 700 mg/kg/day and on the hepatotoxicity observed in the subsequent 3-month oral range-finding study in males at

80 mg/kg/day and females at 640 mg/kg/day. The high doses administered in the carcinogenicity study provide plasma exposures (AUC) to atazanavir that are approximately 4 (M) and 7 (F) times that seen in humans at the recommended dose of 400 mg/day.

Doses administered in the oral carcinogenicity study in rats are 100, 350, and 1200 mg/kg/day. Selection of these doses, which took into account recommendations of the Executive Carcinogenicity Assessment Committee of the FDA, were based on the maximum concentration (160 mg/ml) of atazanavir that can be suspended in the test article carrier PEG-400 and the maximum dose volume (7.5 ml/kg) of atazanavir in PEG-400 that can be administered chronically. The high dose of 1200 mg/kg/day is considered the maximum feasible dose and produces plasma exposures (AUC) to atazanavir approximately 2 (M) and 6 (F) times the exposure in humans given 400 mg/day.

5.3.5 Reproductive and Developmental Toxicity

A complete battery of reproductive toxicity studies was conducted with atazanavir to assess potential effects on fertility, reproductive function, gestation, parturition, and lactation of the parental generation in rats; on embryonic and fetal development in rats and rabbits; and on growth, development, and reproductive performance of progeny in rats. In the study of fertility and reproductive performance in rats, drug-related effects were limited to prolonged diestrus with abbreviated estrus and metestrus in females at doses ≥ 100 mg/kg/day. Minimally lower fertility was seen in female rats at the high dose of 1400 mg/kg/day; however, fertility was only marginally below that observed historically in control rats. This finding was not duplicated in a second fertility study, and therefore was considered not to be drug related. There were no effects of atazanavir on early embryonic development or reproductive performance, including mating, at doses up to and including 1400 mg/kg/day. In the absence of any adverse effects on mating, fertility, or reproductive performance or microscopic changes in the ovaries and female reproductive tract in toxicity studies, the perturbation of estrous cyclicity in female rats is considered to be of limited toxicologic significance. Systemic exposures (AUC) in male and female rats at 1400 mg/kg/day, which had no effect on fertility, are at least comparable to (M) and three (F) times that observed in humans at 400 mg/day (based on data from the 3-month rat study at 900 mg/kg/day). Based on data at 1000 mg/kg/day, systemic exposure in pregnant rats at 1400 mg/kg/day, which produced no effects on

reproductive performance or early embryonic development, is at least twice that observed in humans given 400 mg/day.

Similar to atazanavir, the marketed HIV protease inhibitors produced no adverse effects on fertility, reproductive performance, or embryonic development in rats, except for lopinavir/ritonavir and ritonavir. Both drugs were embryotoxic (pregnancy loss, decreased fetal viability and weights) at maternally toxic doses resulting in exposures comparable to or below exposures in humans at therapeutic doses. In fertility and early embryonic development studies with the marketed HIV protease inhibitors, systemic exposures in animals ranged from below to slightly above the exposures seen in humans at recommended clinical doses.

In the embryo-fetal development studies, atazanavir produced no adverse embryonic or fetal effects at maternally toxic doses (up to 1920 mg/kg/day in rats and 60 mg/kg/day in rabbits). Maternal plasma exposures (AUC) at the fetal no-effect doses in rats and rabbits are two times and comparable to, respectively, that observed in humans at 400 mg/day. In comparison, other marketed HIV protease inhibitors have been associated with retarded fetal growth and development; increased embryo-fetal resorptions; and increased incidences of fetal skeletal variations, malformations and/or anomalies. Moreover, when compared to atazanavir, comparable to lower safety margins, based on systemic exposures, were observed at fetal no-effect doses in developmental toxicity studies with the marketed HIV protease inhibitors.

In the study of pre- and postnatal development in rats with atazanavir, findings were limited to mean body weight gain suppression in the F_1 generation from 4 days of age through the early postweaning growth period at the maternally toxic dose of 1000 mg/kg/day, with no effects on developmental or reproductive parameters. This finding was considered likely to be secondary to the maternal body weight reductions rather than a direct effect of atazanavir. At the highest no-effect dose (220 mg/kg/day), systemic exposure to atazanavir is comparable to that observed in humans at the recommended daily dose of 400 mg. In comparison to atazanavir, indinavir and amprenavir similarly decreased pup body weights during lactation, whereas lopinavir/ritonavir caused deaths in offspring during lactation. Nelfinavir, ritonavir, and saquinavir had no effects on offspring development, growth, and survival. Atazanavir, like the other HIV protease inhibitors, had no effect on the reproductive performance in the F_1 generation.

In conclusion, the reproductive and developmental toxicity of atazanavir was adequately qualified in appropriately designed studies. Importantly, atazanavir demonstrated no selective developmental toxicity and no effects on reproductive function or fertility at exposures equivalent to three times that in humans at the recommended daily dose of 400 mg. Overall, the reproductive toxicity profile of and/or associated systemic exposures to atazanavir were superior in comparison with the marketed HIV protease inhibitors.

5.3.6 Local Tolerance

Studies were conducted to determine the dermal and ocular irritation potential of atazanavir. Atazanavir (neat material) was non-irritating to rabbit skin. In the bovine corneal opacity assay, atazanavir markedly increased corneal opacity indicating it may be severely irritating to eyes. Appropriate precautions should therefore be taken to protect eyes from contact with atazanavir.

5.3.7 Special Toxicity Studies

The potential for atazanavir to produce delayed contact hypersensitivity was evaluated in a skin sensitivity study in guinea pigs. Guinea pigs sensitized intradermally and then topically with atazanavir showed no dermal reactions following topical challenge with atazanavir.

A 1-month oral study of T-cell dependent antibody response in rats was conducted to determine the potential immunotoxicity of atazanavir. Additionally, the immunotoxic potential of atazanavir was assessed by standard hematologic and morphologic endpoints in repeat-dose toxicity studies. In the immunotoxicity study, no significant changes in the T-cell dependent antibody response to sheep RBC antigen were noted at doses of 100, 300, or 900 mg/kg/day, indicating the humoral response was unaltered by treatment with atazanavir. Plasma exposures (AUC) in rats at the nonimmunotoxic dose of 900 mg/kg/day are equivalent to (M) and three (F) times the exposure in humans at 400 mg/day (based on data from the 3-month study). Moreover, there were no direct drug-related morphologic changes in lymphoid and hematopoietic tissues of rodents and dogs in repeat-dose toxicity studies of atazanavir. Moderate to marked thymic involution, minimal lymphoid depletion of the spleen and intestine, and mild bone marrow hyperplasia or depletion that occurred in dogs in the initial 2-week toxicity study were considered secondary to the marked clinical toxicity of atazanavir and associated stress

and not the result of direct drug-related effects. Based on the lack of evidence for adverse drug-related changes in immune system function in the nonclinical studies, additional immunotoxicity testing is considered unnecessary.

5.4 Discussion and Conclusions

The scope of the nonclinical virology and general pharmacodynamic testing was sufficient to fully characterize atazanavir with regard to its proposed indication and to assess its selectivity and potential for adverse pharmacodynamic effects on vital organ systems. Based on results of *in vitro* and cell culture studies, atazanavir is a potent HIV-1 protease inhibitor that is highly effective against HIV replication. The antiviral activity of atazanavir is selective since cytotoxic concentrations are 6,500 - to 23,000-fold higher than that required for anti-HIV activity. *In vitro* drug interaction studies suggest that atazanavir can be combined with reverse transcriptase inhibitors or other protease inhibitors to produce additive antiviral effects without antagonistic anti-HIV activity or enhanced cytotoxicity. Susceptibility profiling of 950 clinical isolates showed that atazanavir resistance requires several amino acid substitutions and is generally modest in degree. Atazanavir has a distinct resistance profile relative to other marketed protease inhibitors, with susceptibility retained among 86% of isolates resistant to one or two of the currently approved protease inhibitors. There is a clear trend toward loss of susceptibility to atazanavir as isolates displayed increasing levels of cross-resistance to other protease inhibitors. A unique I50L substitution appears to be the key signature resistance substitution for atazanavir and emerged in isolates obtained from patients who have failed on-treatment regimens containing atazanavir as the sole protease inhibitor. The appearance of I50L substitution is frequently accompanied by A71V. Viruses containing either I50L or I50L/A71V conferred atazanavir-specific resistance; impaired viral growth; and, most importantly, increased susceptibility to other protease inhibitors.

The general pharmacodynamic and safety pharmacology profile of atazanavir indicated that it is highly specific for the HIV-1 protease enzyme and exerts no adverse effects on mammalian respiratory or CNS function. Results of *in vitro* action potential duration and ionic current assays signaled the need for carefully designed clinical studies to assess potential cardiovascular risk. Since dose-dependent increases in PR intervals were observed in healthy subjects primarily at high doses, the Phase III program was designed to include extensive electrocardiographic evaluations to further define the level of risk. To date, no serious adverse events in clinical studies have been attributed to QTc or PR

interval prolongation. The frequency of QTc or PR interval prolongation was low and comparable with comparator regimens. In a series of *in vitro* studies evaluating the potential for drug-related disruption of glucose and lipid metabolism and regulation, atazanavir produced little to no effect as compared to other PIs. This may, in part, explain its apparent superior metabolic profile clinically.

The pharmacokinetics and metabolism of atazanavir were extensively evaluated in a series of studies that utilized appropriate *in vitro* systems and species routinely used for toxicologic assessment (ie, mouse, rat, rabbit, and dog). These studies demonstrated that rats and dogs were acceptable species for the safety evaluation of atazanavir, since its elimination is mainly by metabolic clearance and biotransformation profiles were qualitatively similar across species. The less than dose-proportional increase in atazanavir exposures in rats compared to humans may have been due to reduced absorption and/or greater presystemic clearance at higher doses; however, this did not preclude achieving clinically relevant systemic exposures to atazanavir in pivotal toxicity studies. Although pharmacokinetic and distribution studies suggested extravascular distribution of atazanavir and/or its metabolites, the only toxicologically significant tissue changes were limited to the liver.

The toxicity of atazanavir was adequately evaluated in animals at up to multiples of the therapeutic exposure in a comprehensive battery of GLP-compliant nonclinical toxicology studies. Based on the results of these studies, only the liver was identified as a target organ of atazanavir. The liver was also a target organ of the marketed HIV protease inhibitors, although several of these drugs had additional target organs (eg, kidney, thyroid, eyes, gastrointestinal tract). Microscopic evidence of hepatotoxicity was observed in female mice at high systemic exposure to atazanavir. In contrast, minimal to moderate increases in serum bilirubin in rats and dogs and in hepatic enzymes in dogs were not accompanied by microscopic evidence of hepatocellular degeneration/necrosis or cholestasis. Atazanavir demonstrated no selective developmental toxicity and no effects on reproductive function or fertility at exposures equivalent to three times that in humans at the recommended clinical dose of 400 mg/day. Although carcinogenicity studies in mice and rats are not complete, the weight of evidence in the battery of genotoxicity studies completed at this time indicates that atazanavir is not genotoxic, thereby supporting the safe administration of atazanavir to humans.

Systemic exposure to the three minor metabolites of atazanavir present in human plasma, M 2 , M 14 , and M41, were verified in the toxicology species utilized in the safety assessment of atazanavir. Exposures to M 2 and M 14 in the toxicology animal species were higher and lower, respectively, than those in humans. M41 plasma radioactivity in rats and dogs was comparable to that in humans confirming exposure to this human metabolite in both animal species. The presence of all three minor metabolites was also confirmed in aroclor-induced rat liver S-9 fraction thereby confirming exposure in *in vitro* genotoxicity studies. Additionally, M 2 and M 14 were shown to have no pharmacologic activity against the HIV virus *in vitro* and little to no potential for drug interactions due to CYP 450 inhibition. The antiviral activity or potential for pharmacokinetic interaction of M41 has not been determined since only a tentative structure has been postulated for this metabolite.

In conclusion, results of the nonclinical virology and safety testing program were predictive of atazanavir's demonstrated efficacy against HIV infection and low potential for adverse effects in clinical trials. There were no nonclinical findings that preclude the marketing approval of atazanavir for treatment of humans with HIV infection.

6 HUMAN BIOAVAILABILITY AND PHARMACOKINETIC SUMMARY

6.1 Biopharmaceutic Studies

The biopharmaceutic evaluation of ATV was performed in several crossover studies in healthy subjects in order to select a solid dosage form for adults and a formulation for children and others who might have difficulty swallowing the solid dosage form. Comparative bioavailability was assessed with four solid dosage forms (powder filled prototype capsule, Gelucire® prototype capsule, and two film coated prototype tablets), and two oral powder formulations (powder prototype I and powder prototype II). The bioavailability of ATV from a capsule formulation, relative to an oral solution, was determined and a food effect study was performed.

ATV was rapidly absorbed following single doses of selected formulations in the fasted state, with median Tmax values ranging from 1 to 2.5 h. After Tmax, the plasma profiles declined in a biexponential manner and the terminal half-life ranged from 2.81 to 6.91 h. Bioavailability of ATV from the capsule was 68% relative to the solution but was either