SCIENTIFIC DISCUSSION

This module reflects the initial scientific discussion for the approval of Reyataz. This scientific discussion has been updated until 1 August 2004. For information on changes after this date please refer to module 8B.

1. Chemical, pharmaceutical and biological aspects

Composition

REYATAZ is available in two pharmaceutical forms (hard capsule and powder for oral use) containing as active substance atazanavir in the form of the sulphate salt.

Hard capsule

REYATAZ is presented as hard capsules, containing 100 mg, 150 mg and 200 mg of atazanavir expressed as free base. The different strengths can be distinguished by the size, colour and imprinting of the capsules.

The other ingredients include lactose monohydrate, crospovidone, magnesium stearate, imprinting ink and hard gelatine capsule shell.

The capsules are packed in HPDE bottle with child resistant polypropylene closures or in aluminium/aluminium blister.

Powder for oral use

REYATAZ is also formulated as an oral powder containing 1.5 g of powder per levelled measuring spoon, equivalent to 50 mg of atazanavir expressed as free base.

The other ingredients include aspartame, sucrose and orange vanilla flavour.

The oral powder is presented in a multidose HPDE bottle with child resistant polypropylene closures. A polypropylene measuring spoon is supplied. The oral powder is intended to be dispersed in an appropriate vehicle such as water, milk, and applesauce or yoghurt immediately prior administration to patients.

Active substance

Atazanavir sulphate is an azapeptide HIV-1 protease inhibitor. Information on its synthesis and control has been provided by the way of an active substance master file ("EDMF").

The active substance is a white to pale yellow powder, freely soluble in organic solvents and slightly soluble in water (4-5 mg/ml). The solubility of the substance is pH dependant (maximum solubility at pH 1.9). The substance contains four chiral centres however the manufacturing process leads, in a consistent way, to the single enantiomer *S, S, S, S.* The absolute configuration has been confirmed by single X-ray analysis on a triethanol solvate crystal.

Atazanavir sulphate shows also the phenomenon of polymorphism. It has been demonstrated that the commercial process produces exclusively a non-solvated, highly crystalline form designed as form A. The substance is not hygroscopic up to 75% relative humidity (RH), but undergoes solid-state modifications to a predominantly amorphous form in aqueous suspension and when exposed to 95% RH.
A number of synthetic processes (A, C, D and E) have been developed and used to produce batches used in non-clinical/clinical studies. However, for future commercialisation atazanavir sulphate will be manufactured through process F at two different manufacturing sites.

Satisfactory specifications and associated methods have been provided for the starting materials, key intermediate, reagents and solvents in the ASM Restricted part of the "EDMF".

Specification

The active substance specification includes tests for appearance, identity (IR and HPLC), assay (HPLC), impurity content (HPLC), sulphuric acid, sulphated ash (Ph Eur), water content (KF), residual solvents (GC), heavy metals and particle size.

The formation of the desired enantiomer of atazanavir is ensured through the route of synthesis and adequate controls performed on the starting materials and the intermediate of appropriate isomeric purity. Therefore, the omission of a chiral assay in the specifications has been supported in this particular case.

Potential impurities have been well discussed. The suitability of the HPLC method for control of impurities including 4 significant stereoisomers has been demonstrated. The substance manufactured from the processes E and F is obtained in a high degree of purity.

Considering the consistency of results obtained by X-ray diffraction for a great number of batches, control of polymorphism is not part of the specification of the active substance.
Specifications of the active substance are in adequacy with the route of synthesis and considered as appropriate for the declared source of active substance in the dossier. The analytical methods used are well validated and suitable for their intended use.

Batch analysis data provided for 79 lots produced according to processes A, C, D, E and F confirm satisfactory compliance and uniformity with the proposed specifications. No significant differences between lots obtained by processes have been noted, especially in term of impurity profile.

Stability

Stability data have been obtained for 9 batches synthesised at the intended sites using either process E or F and stored under long term and accelerated ICH conditions. A photostability study has been performed and indicates that the active substance is not light sensitive. A retest period of 12 months with precaution storage of "Do not store above 30°C" and "protect from moisture" is supported by the presented data when the substance is stored in double polyethylene anti-static bags placed in fibre drums with secure fitting lids.

Other ingredients

Hard capsule

The excipients are of Ph Eur quality. Regarding the TSE risk, satisfactory certificates of suitability have been provided for the gelatine capsule shells. The lactose from milk of bovine origin has been considered in compliance with the current TSE requirements. The magnesium stearate is derived from vegetable fatty acids.

The HPDE bottle and cap meet the Ph Eur requirements for plastic primary packaging material and are in compliance with European regulation on foodstuffs.
Powder for oral use

All the excipients comply with the Ph Eur requirements except the orange vanilla flavour, which is adequately controlled according to an in-house standard.

The HPDE bottle and polypropylene closures meet the Ph Eur requirements for plastic primary packaging material and are in compliance with European regulation on foodstuffs. The measuring

spoon is CE marked and has been approved for its intended use. The accuracy and reproducibility of the dose delivered by this medical device has been satisfactorily demonstrated.

Product development and finished product

Hard capsule

This oral dosage form is of standard formulation and has been developed to release the active substance rapidly prior to absorption.

All the excipients selected are commonly used for this type of dosage form. A wet granulation formulation has been chosen based on rapid and complete in-vitro dissolution profiles obtained. During granulation, the dissolution rate of the finished product is further improved by transformation of atazanavir sulphate crystalline form A into a predominantly amorphous form (see active substance). The amount of water for granulation being a critical parameter for complete conversion of the crystalline form, a suitable range of water has been selected based on X-ray diffraction pattern of development batches. Studies have confirmed that this transition occurs in a reproducible way and is under control. In any case, *in vivo* studies have shown that the bioavailability of the amorphous form and of the crystalline form is not significantly different. As the qualitative composition of the capsules remained similar after phase I studies, no bioequivalence study has been performed to compare clinical formulations and the commercial formulation.

The method of manufacture involves the following operations: mixing of the active substance with lactose monohydrate and crospovidone, low shear wet granulation, drying, milling, addition of the remaining crospovidone/magnesium stearate final mixing and encapsulation.

The four strengths of capsules are manufactured from common stock granulation sublots (55.5% w/w drug substance as free base), which are assembled during the final mixing steps. In order to permit appropriate adjustment of the capsule fill weight, atazanavir sulphate assay on the stock granulation prior to encapsulation is part of the IPCs. Validation data have been provided for five pilot-scale stock granulation batches (3 using process E drug substance and 2 using process F drug substance) and for 3 full-scale stock granulation batches, as well as for the corresponding capsule batches. Blend uniformity for the different batch sizes has been satisfactorily demonstrated.

These validation data together with the results obtained during process and formulation optimisation studies show the robustness of the formulation and that the identified critical parameters are under control. No differences are evident between batches manufactured with process E/process F active substance.

Specification

The product specification includes tests controlled by validated methods for appearance, identity (HPLC, IR), assay (HPLC), impurity content (HPLC), dissolution, uniformity of mass (Ph Eur) and microbial limit (Ph Eur).

The conditions of the dissolution test selected are discriminatory, and can detect undergranulated (incomplete conversion of form A to amorphous) finished product batches.

Batch analysis data have been provided for 33 batches manufactured at various facilities from pilot to commercial scale. Results from product of each strength manufactured at the commercial facility are included. All data comply with the specifications and indicate consistent and reproducible manufacture.

Powder for oral use

This pharmaceutical form was primarily developed for children and adults, who encounter difficulty in swallowing capsules. It is intended to be dispersed in an appropriate vehicle such as water, milk, apple sauce or yoghurt immediately prior administration to patients.

Originally, the development of an oral liquid suspension was discontinued due to the conversion of the sulphate salt to the free base (pH around 3). A powder for oral use containing polysorbate 80 was also discontinued because of its poor bioavailability compared to the capsules. This was due to polysorbate 80 enhancing conversion of the soluble salt form to the insoluble free base in aqueous environment with pH>2. The formulation without polysorbate 80 was shown to have similar bioavailability to the capsules and was consequently selected as final formulation.

Based on satisfactory development studies, suitable mixing equipment and processing conditions have been selected to manufacture a powder with satisfactory blend uniformity and resistance to segregation. The fine powder blend obtained tends to be cohesive and poorly flowable, thus decreasing possibility of de-mixing and segregation during processing and shipping. In order to ensure control of the finished product performance, particle size is part the specification of atazanavir sulphate (see active substance).

A bulk powder blend containing 3.33% of atazanavir sulphate expressed as free base is prepared by a standard direct milling/mixing process and subsequently filled into HPDE bottles. At the beginning of the manufacturing process, the active substance is screened as it may contain agglomerates and the excipients are milled in order to prevent segregation induced by particle size differences.

Validation data are provided for one full-scale batch manufactured at the commercial site using process F active substance. The process validation will be completed at commercial scale on an ongoing basis.

The formulation used clinical studies was of the same composition as that proposed for marketing.

The capsule and the oral powder formulation have similar bioavailability.

The finished product specification includes tests controlled by validated methods for appearance, identity (HPLC, IR), assay (HPLC), impurities (HPLC), uniformity of dose (Ph Eur), dissolution, moisture content and microbial limit (Ph Eur).
The conditions of the dissolution test selected enhance to detect potential slow down in dissolution of finished product batches due to atazanavir sulphate free base formation.

Batch analysis data have been provided for 4 production-scale and 1 pilot-scale batches manufactured at a development site and for 2 production-scale batches manufactured at the commercial site. All data comply with the specifications and confirm the robustness and the reproducibility of the manufacturing process. Comparable results were obtained for batches manufacturing with process E/process F active substance.

<u>Stability of the Product</u>

Hard capsule

12-month data long term stability data (25°C/60% RH – intended packaging) have been provided for three batches (one batch of 50 mg, 100 mg and 200 mg strength) containing atazanavir sulphate synthesised by process F. 18-month supportive long term stability data have been provided for seven batches (3 batches of 50 mg strength, 1 batch of 100 mg strength and 3 batches of 200 mg strength) containing atazanavir sulphate synthesised by process E. Accelerated stability studies were performed (40°C/75% RH) over a 6-month duration for the product in the commercial packaging and 3 months in open dishes. Data are also available under intermediate conditions (30°C/70% RH).

Samples have also been stored under stress conditions (at 5°C, 50°C and in a temperature cycling protocol (-15°C/30°C 12 hours every 24 hours for 2 weeks). Photostability studies have shown that the drug product is non-light sensitive.

The data provided support the proposed shelf life and storage conditions as defined in the SPC.

Powder for oral use

Stability of the Product before constitution

Stability data are available for four batches containing atazanavir sulphate synthesised either by process E or F. Up to 24-month long-term stability data (25°C/60% RH – packaging intended for commercialisation – open/closed bottle) are available. Accelerated stability studies were performed (40°C/75% RH packaging intended for commercialisation) over a 9 weeks in open bottles and 6 months in closed bottles. Data are also available under intermediate conditions (30°C/70% RH).

Samples have also been stored under stress conditions (at 5°C, 50°C and in a temperature cycling protocol (-15°C/30°C 12 hours every 24 hours for 2 weeks)). Photostability studies have shown that the finished product is non-light sensitive.

The data provided support the proposed shelf life, in-use shelf life and storage as defined in the SPC.

In-use stability after constitution

The stability of the finished product when constituted was determined by performing dissolution tests immediately after constitution in water, milk, applesauce or yoghurt after 3 and 6 hours storage. Chemical stability was also evaluated as a function of pH and temperature.

The data provided support the proposed in-use shelf life and storage conditions as defined in the SPC.

Discussion on chemical, pharmaceutical and biological aspects

The active substance is well characterised and documented. The pharmaceutical forms selected are adequate taken into account the properties and stability of the active substance. The polymorphism described for atazanavir is not critical. The excipients are commonly used in this kind of formulation and the packaging material is well documented. The manufacturing processes were developed and optimised to obtain reproducible finished product batches. Stability tests under ICH conditions indicate that the products are stable for the proposed shelf life.

Benefit/risk assessment

The quality of this product is considered to be acceptable when used in accordance with the conditions defined in the SPC. Physicochemical and biological aspects relevant to the uniform clinical performance of the product have been investigated and are controlled in a satisfactory way. At the time of the CPMP opinion there were some outstanding minor quality issues which had no impact on the benefit/risk profile. The applicant committed to provide the necessary information as follow-up measures within an agreed timeframe, and to submit variations if required following the evaluation of this additional information.

2. Toxico-pharmacological aspects

GLP aspects

The studies conducted to assess the pharmacodynamic effects relating to proposed indication were not in compliance with GLP regulations since they were not safety tests and compliance with GLP regulations is not required.

The ADME pharmacokinetic studies were not in compliance with GLP regulations. 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. These toxicokinetic evaluations in support of pivotal toxicology studies were conducted in compliance with GLP regulations.

Pharmacodynamics

Pharmacodynamic drug interactions
Atazanavir (ATV) was evaluated for anti-HIV activity in two-drug interaction studies with other anti-retrovirals. Combinations of atazanavir with stavudine, didanosine, lamivudine, zidovudine, nelfinavir, indinavir, ritonavir, saquinavir, or amprenavir in HIV-infected peripheral blood mononuclear cells yielded additive antiviral effects. The drug combinations did not result in antagonistic anti-HIV activity or enhanced cytotoxic effects at the highest concentrations used.

Safety pharmacodynamics
In safety pharmacology studies, there were no atazanavir-related adverse effects on, respiratory, or central nervous system function in rats (≤ 1200 mg/kg/day) or dogs (≤ 360 mg/kg/day).

- *Cardiovascular function*

In the in vitro Purkinje fibre assay, atazanavir was shown to increase action potential duration (13% increase at 30 μM which is four times the C_{max} and 17 times the C_{ss} in humans at a 400 mg dose). Furthermore, atazanavir-related effects on sodium, potassium, and calcium currents were evaluated *in vitro*. Atazanavir weakly inhibited sodium and HERG-encoded potassium currents (IC_{50}>30 μM) while moderately inhibiting calcium currents (IC_{50} of 10.4 μM). Electrocardiographic changes (sinus bradycardia, prolongation of PR interval, prolongation of QT interval, and prolongation of QRS complex) were only observed in an initial 2-week oral toxicity study and not in subsequent 2-week and 9-month oral toxicity studies performed in dogs.

Pharmacokinetics

ADME
ADME studies were conducted in the mouse, rat and dog, which are the principal species selected for the safety evaluation. The doses of atazanavir in these studies ranged from doses equivalent to the human clinical dose to doses used in the toxicology studies. 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.

The bisulphate salt (BMS-232632-05) was used in all *in vivo* studies. The free base (BMS-232632) or the bisulphate salt was used in the *in vitro* experiments. All doses and concentrations are expressed in terms of the free base equivalent. If radiolabeled material was used, the radiochemical purity of the material was generally > 99%.

HPLC/UV and LC/MS were used to determine the plasma and urine concentrations of atazanavir, respectively. The concentrations of radioactivity in plasma and urine samples from radio labelled studies were determined by direct liquid scintillation counting (LSC) of the samples mixed with the scintillation cocktail. The samples of blood and the homogenates of faces and various organs/tissues were either bleached, solubilised, or combusted prior to analysis by LSC.

- *Absorption*

Absolute bioavailability was measured in rat and dog, but not in human.
The permeability of atazanavir was evaluated *in vitro* using the Caco-2 cell model. The apical to basolateral permeability was determined to be pH independent. The permeability coefficient values at all pH tested were ~100 nm/sec which was comparable to drugs that are completely absorbed in humans after oral administration.

- *Distribution*

The *in vitro* binding of atazanavir to mouse, rat, dog, and human serum proteins was 92.4, 92.8, 92.0, and 86.5 %, respectively, and was independent of concentration. Atazanavir was bound to both human serum albumin (86.2 %) and α -1-acid glycoprotein (88.7 %). The *in vitro* distribution of atazanavir in mouse, rat, dog, and human red blood cells was 43.5, 26.5, 30.4 and 29.5 %, respectively, which was independent of concentration.

Following administration of a single 100 mg/kg oral dose of dual labelled [^{14}C]-atazanavir to rat, tissue to plasma AUC ratios were < 1 for cerebrum, cerebellum, bone, testes, eyes, bone marrow, heart, skeletal muscle, skin, spleen, thymus, epididymis, prostate, salivary glands and ovaries. The ratios were > 1 for adipose tissue, adrenals, aorta, kidneys, lungs, mesenteric lymph nodes, pancreas, pituitary, trachea and small intestine. The highest ratios (>10) were observed for liver, large intestine and stomach. By 24 h, the majority of the radioactivity was excreted and no single tissue contained more than 1 % of the dosed radioactivity. Tissue C_{max} and AUC values for females were greater than for males, ~ 2 fold, but there were no appreciable gender-related differences in the tissue T_{max} and $T_{1/2}$ values. Whole body autoradiography showed a similar profile.

Radioactivity was distributed in foetal tissues following a single oral (100 mg/kg) dose of dual labelled [^{14}C]-atazanavir to pregnant rats. Foetal tissue concentrations were highest in the kidneys, blood and amniotic fluid and lowest in the brain. The concentrations of radioactivity in the foetus and amniotic fluid were low relative to the equivalent maternal tissues except the foetal brain which exceeded maternal cerebrum at most time points. The whole body analyses (WBA) data for pregnant female rats were consistent with these results.

Radioactivity was detectable in milk within 1 h after an oral (100 mg/kg) dose of dual labelled [^{14}C]-atazanavir to lactating rats. The mean concentration of radioactivity in milk increased until it reached a maximum at 4 h postdose. The milk/plasma concentration ratios were generally greater than 1, with ratios ranging from 0.93 to 3.46.

- *Metabolism*

Metabolic pathway

Metabolism was studied *in vitro* and *in vivo*. The metabolic pathways of atazanavir involve monooxygenation, dioxygenation, glucuronidation, N-dealkylation, hydrolysis and oxygenation with dehydrogenation.

Several oxidative, but no conjugated metabolites of atazanavir were detected following incubation with mouse, rat, dog or human liver microsomes and cryopreserved hepatocytes.

CYP enzymes responsible for the metabolism

Incubation with specific inhibitors of CYP isozymes using human liver microsomes showed that the metabolism of atazanavir was inhibited 71 and 100% by CYP3A4 inhibitors, troleandomycin and ketoconazole, respectively; while extent of inhibition was only 0-16% by CYP1A2, 2A6, 2B6, 2C9, 2C19, 2D6, and 2E1 inhibitors. Furthermore, a correlation analysis between the rates of oxidation of atazanavir to that of a substrate of CYP3A4 (testosterone) was significant. These results indicate that CYP3A4 is the major isozyme responsible for the metabolism of atazanavir in human liver microsomes

CYP enzymes interactions

Atazanavir was determined to be a competitive inhibitor of CYP3A4, with a Ki value of 2.35 μM; in comparison, the Ki of ketoconazole for CYP3A4 in this system is < 0.1 μM. Atazanavir was also found to competitively inhibit CYP1A2 and CYP2C9, but the Ki values were appreciably higher (≥12.2 μM) than the steady state plasma concentrations of atazanavir observed in humans following 400 mg doses. Atazanavir was not an inhibitor of CYP2A6, CYP2C19, CYP2D6, CYP2E1, or CYP4A9/11. It showed little or no capacity to function as a mechanism-based inhibitor of CYP1A2, CYP2A6, CYP2C9, CYP2C19, CYP2D6, CYP2E1, or CYP4A9/11. In another study, atazanavir inhibited testosterone 6 β-hydroxylation (marker of CYP3A4 activity) by 61-92% in primary human hepatocytes and immortalised human hepatocytes transfected with CYP3A4. Atazanavir did not

induce testosterone 6 β-hydroxylation in primary human hepatocytes, indicating that it is not an inducer of CYP3A4 *in vitro.*

Glucuronidation inhibition
Atazanavir was found to inhibit bilirubin glucuronidation in microsomal fractions of lymphoblast cells heterologously expressing human uridine diphosphate glucuronosyl transferase 1A1 (UGT1A1) by a linear "mixed-type " mechanism, with Ki and αKi (measure of affinity of enzyme-substrate complex for the inhibitor) values of 1.9 and 16.4 μM, respectively. However, the contribution of αKi to overall inhibition was small (< 10%) suggesting that competitive inhibition is predominant. Indinavir also exhibited inhibition of bilirubin glucuronidation by a similar mechanism, but had a higher Ki (47.9 μM) and αKi (1317 μM) than atazanavir.

The IC_{50} values for the inhibition of bilirubin glucuronidation in human liver microsomes and cDNA-expressed UGT1A1 were similar for atazanavir (2.5 and 2.4 μM, respectively) and nelfinavir (2.7 and 8.4 μM, respectively) and saquinavir (5.0 and 7.3 μM, respectively), but were appreciably lower than the value for indinavir (68 and 87 μM, respectively). However, the IC_{50} values for these other drugs were > 13 fold above the respective unbound C_{max} values at the therapeutic doses compared to ~ 2.4 fold above the unbound C_{max} for atazanavir.

Excretion
Since only a small amount of radioactivity remained in rat tissues 24 h post dose the un-recovered portion of the dose was presumed to be either due to incomplete urinary/faecal collection or due to loss by radioactivity as $^{14}CO_2$ in expired air.

<u>*Toxicology*</u>

Single dose toxicity
Atazanavir demonstrated a minimal order of acute toxicity in mice and rats.

- In mice, minimal lethal doses were 1600 and 800 mg/kg in males and females, respectively and were not associated with any drug-related gross or histopathologic changes. Atazanavir was well tolerated in mice up to 400 mg/kg.
- In rats, the minimal lethal dose was greater than 1600 mg/kg; and although no drug-related changes were observed.

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.

Repeat-dose toxicity
In repeat-dose toxicity studies in rats, atazanavir was administered at dosages up to 1200 mg/kg in a two weeks study. At the high dose reduced food intake and signs of dehydration were present suggesting that this dose was close to the maximal tolerable level. Results showed that liver was the main target organ with hypertrophy, vacuolisation, increased weight and increased levels of bilirubin. No signs of cholestasis were reported. Cholesterol levels were augmented in higher than 300 mg/kg. Haematological parameters were also slightly affected with a reduction in white blood cell numbers observed above 300 mg/kg. Glucose levels were augmented at 1200 mg/kg. All these effects were mainly observed in females due to a higher exposure (1.2 to 3.2 fold compared to males).

In three and six months studies atazanavir was administered at dosages up to 900 mg/kg. Liver was the main target organ with the same signs observed precedent in the two weeks study. At six months animals showed an augmentation in water consumption and of urine volume suggesting that animals were dehydrated. NOAEL was less than 100 mg/kg.

In repeat-dose toxicity studies in dogs, atazanavir was administered at dosages up to 360 mg/kg in a two weeks study. Dosages higher than 90 mg/kg were poorly tolerated. A second study of the same duration was then undertaken with dosages up to 75 mg/kg. Unexpectedly, this high dose was well tolerated by the animals with no effects observed.

For the 9-month pivotal study conducted in dogs the applicant used 90 mg/kg for the high dose. However since the 90 mg/kg was very well tolerated the applicant decided to raise the 10-mg/kg doses to 180 mg/kg 3 months after the beginning of the study. Consequently, animals of the first group were treated for 3 months with 10 mg/kg and 6 months with 180 mg/kg. Results showed that increased levels of bilirubin, alkaline phosphatase and gamma glutamyltransferase. Not all the animals showed these effects. However, no signs of cholestasis were reported in this study. Toxicokinetic results showed a high variability rending the interpretation difficult.

Toxicokinetics
In the 3-month oral range-finding toxicity study, the exposure of female mice to atazanavir increased in a ratio less than the increment in dose following oral doses of 40-640 mg/kg/day, but the exposure in male mice increased in a ratio more than the increment in dose over the range of 20-80 mg/kg/day.

In the 6-month oral toxicity study in rats, exposure was generally less than dose proportional over the range of 100-900 mg/kg/day and was 2.4-4.9 fold higher in females compared to males. There were 13-49% decreases in exposure after repeated doses above 100 mg/kg/day compared to the exposures after the first dose, but exposure after dosing for 3 months was reasonably similar to those after 6 months of dosing.

In the 9-month oral toxicity study in dogs, greater than dose-proportional systemic drug exposure was observed over the range of 10-180 mg/kg/day, increases or decreases in systemic exposure upon repeated oral administration were not apparent.

Genotoxicity
Atazanavir was evaluated in a comprehensive battery of in vitro and in vivo genotoxicity studies. In an in vitro cytogenetics test in primary human lymphocytes, ATV increased chromosomal aberrations at concentrations =240 µg/ml in the presence of metabolic activation and =30 µg/ml in the absence of metabolic activation. These findings were reproduced in a second in vitro cytogenetics assay in primary human lymphocytes at similar to higher ATV concentrations.

In other in vitro studies, ATV was not mutagenic in a bacterial mutagenicity screening test or a definitive Ames reverse-mutation assay.

In the in- vitro cytogenetics assay in human peripheral lymphocytes, the dose response for clastogenic effects in the presence and absence of metabolic activation suggests the parent compound (rather than a metabolite) is clastogenic.

In in-vivo genotoxicity studies, ATV did not induce micronuclei in bone marrow, DNA damage in duodenum, or unscheduled DNA repair in liver at plasma and tissue concentrations exceeding those that were clastogenic in vitro.

Carcinogenicity
Two studies have been conducted to test the carcinogenic potential of atazanavir.

In rats, atazanavir has been given orally by gavage at dosages up to 1200 mg/kg (0, 100, 350 and 1200 mg/kg). Exposures at the highest dose are 1,6 and 6 fold the human exposure in males and females respectively. The results are negative. Animals showed a dose-related augmentation of lipidic hepatocellular vacuolation and hepatocellular hypertrophy. However, no hepatocellular necrosis was recorded in this study.

Mice have been treated orally at dosages up to 80 mg/kg and 360 mg/kg in males and females respectively (0, 20, 40, 80 mg/kg in males; 0, 40, 120 and 360 mg/kg in females). Exposures at the highest dose are 4,2 and 7,2 fold the human exposure in males and females respectively based on a 2-week toxicokinetic study. Results showed a significant augmentation of hepatocellular adenoma at 360 mg/kg in female mice. At this same dose, there was an increased incidence and severity of hepatocellular single-cell necrosis. In addition, hepatic foci of cellular alteration, hepatocellular

hypertrophy and cytoplasmic lipid vacuolation were also present at this high dose, suggesting a toxic effect of atazanavir on the liver. No augmentation of hepatocellular carcinoma has been recorded in this study. Results in males were negative.

Investigative studies have been also conducted to elucidate the mechanism of adenoma induced by atazanavir.

PCNA labelling was performed on livers of female and male mice from control and high-dose group that have survived to terminal necropsy. No differences were observed between control and treated animals probably due to the low proliferative potential of mice aged hepatocytes.

However, in a 2-week study, a statistical augmentation of hepatocyte proliferation was observed in female mice treated with 360 mg/kg assessed by BrdU labelling. In addition, transaminase levels were significantly augmented and single cell necrosis was also present in this group of animals. Results were negative in control and high-dose male mice groups (80 mg/kg).

Reproductive and developmental toxicity
In the first study conducted in both males and females at dosages up to 1400 mg/kg, results showed a decreased fertility rate at 1400 mg/kg and a significant reduction in the number of oestrus cycles since 375 mg/kg. Consequently, males from this study were placed in cohabitation with untreated females for 2 weeks. Mating and fertility were not affected in males at any dose. No effect on fertility was observed when treated females (1400 mg/kg) were mated with non-treated males.
In the embryo-foetal development studies, atazanavir produced no adverse embryonic or foetal effects at maternally toxic doses up to 1920 mg/kg/day in rats and 60 mg/kg/day in rabbits. Atazanavir induced a decrease in body weight at weaning at the maternally toxic doses (1000 mg/kg).

Other toxicity studies

The toxicity of atazanavir was further evaluated in *in vitro* and *in vivo* ocular and dermal irritation studies. Atazanavir is an ocular irritant.

A 1-month oral study of T-cell dependent antibody response in rats showed no evidence of immunotoxicity of atazanavir.

Ecotoxicity/environmental risk assessment

The risk of an adverse environmental impact from use of atazanavir is of no immediate concern based on the results from a Phase I environmental risk assessment. The excipients in the capsules and the other components of the oral powder have been in use for many years and are not expected to pose a significant impact to the environment. Atazanavir is poorly water-soluble and is expected to adsorb to sludge particles to some extent. The PEC for soil is less than the action limit of 10 μg/kg. Additionally, the compound is not toxic to sludge microorganisms at concentrations up to 1000 mg/L, and the PEC/PNEC ratio is less than 1 for aquatic species.

Discussion on toxico-pharmacological aspects

In safety pharmacology studies, there were no atazanavir-related adverse effects on, cardiovascular respiratory, or central nervous system function in rats (≤ 1200 mg/kg/day) or dogs (≤ 360 mg/kg/day). Atazanavir minimally increased the duration of the rabbit Purkinje fibre action potential, weakly inhibited sodium and potassium IKr (HERG-encoded), and IKs currents and moderately inhibited calcium current *in vitr*o.

NOAEL obtained in repeated toxicity studies (≥ 100 mg/kg in rats and ≥ 30 mg/kg in dogs) correspond to exposure less than the one obtained in humans. However, animals have been exposed to the highest dosages possible since augmenting the doses would not have result in a proportional increase of animal exposure.

The main target organ identified in these repeated toxicity studies was the liver with hypertrophy, vacuolisation, increased weight and increased levels of bilirubin observed in rats. In dogs, only bilirubin, alkaline phosphatase and gamma glutamyltransferase levels were augmented. No histological signs of cholestasis were observed in these studies. Augmentation of bilirubin levels may be the consequence of altered glucuronidation by atazanavir as shown in microsomal fractions of lymphoblast cells heterologously expressing human uridine diphosphate glucuronosyl transferase 1A1 (UGT1A1). The hepatic alterations observed in rats 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.

Atazanavir was the first PI to be shown clastogenic in vitro. Regarding genotoxic potential, the applicant has provided new data with the answer to the day 120 LoQ, indicating that atazanavir did not induce DNA damage in duodenum (UDS and comet assay), or unscheduled DNA repair in liver (UDS) at plasma and tissue concentrations exceeding those that were clastogenic *in vitr*o. The increase in chromosome aberrations was reproducible in the second *in vitro* primary human lymphocyte assay, thus confirming that atazanavir is clastogenic *in vitr*o. Nevertheless, the mechanism for *in vitro* clastogenicity is not known. The fact, that atazanavir did not induce micronuclei, DNA damage (comets), or UDS in a variety of rodent tissues *in vivo* at plasma and tissue concentrations exceeding those that were clastogenic *in vitro,* could suggest that the mechanism of *in vitro* clastogenicity may not be biologically relevant in animals and humans or has a threshold.

The results obtained in the carcinogenicity studies presented suggest that atazanavir induced hepatocellular adenoma only in female mice at the highest dose tested. This observation is probably due to an epigenetic mechanism with hepatocyte hyperproliferation after cell necrosis, which is a well-known mechanism, currently observed in rodents. Furthermore, it is well recognised by the scientific community that mice present an increased sensitivity to hepatocellular tumours. In this mice study, liver carcinomas were not present, and in rats, all the results were negative. All together, these results suggest that atazanavir will not increase the carcinogenic risk in humans.

A complete battery of reproductive and developmental toxicity studies was conducted in rats and rabbits to assess potential effects of atazanavir on fertility and reproductive performance, gestation, parturition, and lactation of the parental generation; embryonic and foetal development; and growth, development, and reproductive performance of the offspring. Although atazanavir altered oestrous cyclus in female rats at all doses, reproductive performance, including mating, was not adversely affected at any dose.

3. Clinical aspects

GCP aspects

The clinical studies were conducted in accordance with good clinical practice.

Clinical pharmacology

Pharmacodynamics

Atazanavir (ATV) is a human immunodeficiency virus (HIV) protease inhibitor. It is an azapeptide that blocks the processing of viral gag-pol proteins in HIV-1 infected cells, thus preventing formation of mature virions.

Primary pharmacology

- *Inhibition of HIV protease by ATV*

Atazanavir inhibited the cleavage activity of HIV-RF protease with a K_i of 0.75 nM, which is comparable to the inhibitory activity of other protease inhibitors. Indinavir, nelfinavir, saquinavir, and ritonavir gave K_i values of 0.73 nM, 1.05 nM, 0.39 nM, and 1.01 nM, respectively, in this assay.

Atazanavir inhibited the cleavage of gag (p55) by HIV protease with an IC_{50} of 47 nM.

The cytotoxic concentration of ATV is 6500-23000 fold higher than IC and revealed a selective index comparable to other approved HIV protease inhibitor.

- *Antiviral activity of ATV in vitro*

Experiments with wild type HIV laboratory strains have been performed in the absence and in the presence of 40 % of human serum to assess the antiviral activity of atazanavir.

In the absence of serum, comparative studies revealed that atazanavir (with an EC50 value of 2 – 5 nM) is 2- to 20-fold more active than other protease inhibitors including indinavir (IDV), nelfinavir (NFV), ritonavir (RTV), saquinavir (SQV), and amprenavir (APV). The activity of atazanavir (EC_{50} values) against seven subtypes of group M and against a group O isolate varied by more than 10-fold. Activity against other subtypes of HIV-1 or against HIV-2 has not been explored.

In 40% human serum the atazanavir EC_{50} value increased from 1.5 nM to 7.8 nM (5-fold)**,** similar to the other protease inhibitors but significantly less than nelfinavir (≥ 17-fold). The resulting atazanavir EC_{50} value is 3-to 19-fold lower than the EC_{50} of each of the approved protease inhibitors. Lopinavir/ritonavir was not tested. In 50% human serum the antiviral activity of lopinavir against HIV IIB in MT4 cells was 102±44 nM.

The two metabolites of ATV identified in the systemic circulation following administration of atazanavir to humans have no antiviral activity.

- *Resistance data in vitro*

According to *in vitro* characterisation of resistance profile of atazanavir, it appears that:

- After *in vitro* passages in presence of atazanavir, 3ATV-resistant strains of HIV with multiple mutational patterns were selected. Of note, N88S substitution appeared in 2 of 3 strains and I84V in the third strain.

- According to cross-resistance analyses, viruses resistant to atazanavir remained sensitive to SQV and APV. Conversely, viruses resistant to NFV, SQV or APV remained sensitive to ATV while IDV or RTV resistant isolates displayed a cross resistance to ATV.

Resistance data in vivo

Phenotypic analysis of 115 isolates from naïve and experienced patients undergoing virologic failure identified 27 isolates (24%) with decreased susceptibility to ATV and genotypic analyses were performed in a subgroup of these isolates. ATV was also profiled against a panel of 399 susceptible and 551 PI resistant clinical isolates (Virologic, VIRCO, pre-screening enrolment process of ATV clinical studies 009, 043 and 045).

Based on the analyses of these clinical isolates, two distinct resistance patterns are confirmed:

- In antiretroviral naïve patients, the I50L substitution, sometimes in combination with an A71V change is emerging as the signature mutation for atazanavir. This resistance profile did not result in a loss of sensitivity to the other protease inhibitors tested and there is no evidence of specific cross-resistance between atazanavir and amprenavir while an I50V substitution has been previously shown to reduce sensitivity to amprenavir.

- In antiretroviral pre-treated patients, the results of the cross resistance study suggest that ATV is expected to remain poorly effective against isolates resistant to more than 2 of the currently marketed PIs (only 25% of isolates resistant to 3 - 4 PIs remain susceptible to ATV). The

accumulation of mutations (>5 at residues 10, 20 24, 33, 36, 46, 48, 54, 63, 71, 73, 82, 84 or 90) shortly leads to atazanavir resistance. Of note, these mutations are known to play a part in amprenavir and lopinavir/ritonavir resistance.

Further virological data were requested by the CPMP to expand on the initial observations and better characterised the pathway to resistance to atazanavir. Data are now derived from 23 clinical isolates in atazanavir treatment naïve patients and in 74 PI experienced patients treated with ATV, ATV/RTV and ATV/SQV. In naïve patients, data confirmed that the I50L substitution, previously described as the signature mutation for atazanavir, resulted in decreased susceptibility to atazanavir. Resistance levels ranged from 3.5 to 29-fold. This resistance appears to be specific for atazanavir. Within the 74 isolates from experienced patients who developed resistance to atazanavir on therapy that included either ATV, ATV/r and ATV/SQV 9 isolates from patients treated with either ATV and ATV/r display the I50L phenotype previously described in naïve patients. The remaining isolates showed no evidence of the I50L substitution and no obvious pattern of changes beyond accumulation of the primary and secondary resistance substitutions described previously to be involved in PI resistance. These isolates developed higher levels of resistance to the other PIs. All isolates from experienced patients who were treated with the PI combination of ATV/SQV become resistant to both ATV and SQV.

Pharmacokinetics

The major shortcoming of the pharmacokinetic programme is that it is almost exclusively based on the use of atazanavir unboosted at 400 mg QD dose, whereas it is recommended to use atazanavir only boosted with ritonavir at 300/100 mg QD. This is explained by the fact that the 400 mg dose of atazanavir was mainly used in the clinical development. The use of atazanavir boosted with ritonavir at 300/100 mg QD, considered as an optimisation of the schedule regimen, was only explored later in the clinical development programme.
Therefore, the pharmacokinetics parameters and especially the interaction profile of atazanavir/ritonavir at 300/100 mg QD will be further substantiated within the frame of post approval commitments.

Healthy volunteers Studies	**AI424028**	**AI424056**	**Ratio**
	ATV 400	ATV 300 + RTV 100	
AUC_{TAU} (ng/h/ml) Geometric Mean (C.V.%)	30495 (21)	57039 (37)	Approx. 2
C_{max} (ng/ml) Geometric Mean (C.V.%)	5690 (19)	6129 (31)	Approx 1.5
C_{min} (ng/ml) Mean (S.D.)	186 (118)	1441 (757)	Approx 7-8

Co-administration of ATV 300 mg and RTV 100 mg both QD was selected for further clinical development since it resulted in a substantial increase in the mean C_{min} value of ATV relative to ATV 400 mg alone.

In patients, the impact of ritonavir on the pharmacokinetics of atazanavir could be observed within the comparison of C_{min} values from study AI424008 in antiretroviral naïve patients (atazanavir unboosted at 400 mg QD dose) and preliminary C_{min} values in studyAI424045 (atazanavir boosted with ritonavir at 300/100 mg QD dose). A 2-3-fold increase of C_{min} was observed when boosted with ritonavir:

Atazanavir 400 mg QD: C_{min} (ng/ml) 273$\pm$298

Atazanavir/ritonavir 300/100 mg QD: C_{min} (ng/ml) 627+343

The applicant committed to submit the pharmacokinetic report of the study AI424045. This report will further substantiate the pharmacokinetics parameters achieved with the recommended posology of atazanavir/ritonavir 300/100 mg QD.

Atazanavir pharmacokinetics exhibits a nonlinear disposition and a high interindividual variability (CV>90%). There was a greater than dose-proportional increase in AUC and C_{max} in fasted and fed Healthy Volunteers after 200-800 mg atazanavir. Also, accumulation on multiple dosing was greater than predicted from single dose data and was about 3-fold after 400 mg dosing after food.

Bioequivalence has been demonstrated between the oral powder and the capsule. Considering the lack of data currently available in paediatric patients, this oral powder will be proposed for adults not able to swallow the capsule

- *Absorption*

In a pharmacokinetic study in HIV-positive patients (n=10), multiple dosing of REYATAZ 300 mg once daily with ritonavir 100 mg once daily with a light meal for 2 weeks produced a mean steady-state C_{max} of 5,233 ng/ml, occurring approximately 3.0 hours (T_{max}) after administration, and a mean steady-state trough concentration of 862 ng/ml. The mean steady-state plasma AUC of atazanavir was 53,761 ng·hr/ml.

- *Food effect*

Administration of atazanavir with either a light meal or a high fat meal decreased the coefficient of variation of AUC and C_{max} approximately one-half compared to the fasting state. A similar decrease in the coefficient of variation was noted when REYATAZ 300 mg once daily with ritonavir 100 mg once daily was administered with a light meal in healthy subjects. To enhance bioavailability and minimise variability, atazanavir should be taken with food.

- *Distribution*

Atazanavir was approximately 86% bound to human serum proteins over a concentration range of 100 to 10,000 ng/ml. Atazanavir binds to both alpha-1-acid glycoprotein (AAG) and albumin to a similar extent (89% and 86%, respectively, at 1,000 ng/ml).

- *Metabolism*

Studies in humans and *in vitro* studies using human liver microsomes have demonstrated that atazanavir is mainly metabolised by CYP3A4 isozyme to oxygenated metabolites. Metabolites are then excreted in the bile as either free or glucuronidated metabolites. Additional minor metabolic pathways consist of N-dealkylation and hydrolysis. Two minor metabolites of atazanavir in plasma have been characterised. Neither metabolite demonstrated *in vitro* antiviral activity.

- *Elimination*

The mean elimination half-life of atazanavir in HIV-infected adult patients (n= 10) was 8.6 hours at steady state following a dose of 300 mg daily with ritonavir 100 mg once daily with a light meal.

Special populations

- *Impaired renal function*

In healthy subjects, the renal elimination of unchanged atazanavir was approximately 7% of the administered dose. There are no pharmacokinetic data available on patients with renal insufficiency, however the impact of renal impairment on atazanavir elimination is anticipated to be minimal.

- *Impaired hepatic function*

Atazanavir is metabolised and eliminated primarily by the liver. Atazanavir has been studied in adult patients with moderate to severe hepatic impairment after a single 400-mg dose. The mean $AUC_{(0-\infty)}$ was 42% greater in patients with impaired hepatic function than in healthy volunteers. The mean half-life of atazanavir in hepatically impaired patients was 12.1 hours compared to 6.4 hours in healthy volunteers. The effects of hepatic impairment on the pharmacokinetics of atazanavir after a 300 mg dose with ritonavir have not been studied. Concentrations of atazanavir with or without ritonavir are expected to be increased in patients with moderately or severely impaired hepatic function. The applicant is requested to further explore this issue.

Pharmacokinetic/pharmacodynamic

A pharmacokinetic population sub study (n=96) was derived from study AI424007 in antiretroviral naïve patients exposed to atazanavir 200, 400 and 500 mg QD. This study demonstrated the relationship between AUC and C_{min} and both safety (hyperbilirubinemia) and efficacy (log drop in viral load). However, the final report of the phase II population pharmacokinetic study based on data collected in patients of studies AI424007 and also AI424008 did not demonstrate a strong relationship between AUC or C_{min} atazanavir and drop from baseline in log10 HIV RNA. Nevertheless, based on this modelling, the applicant concluded that the 400 mg QD dose would achieve an acceptable balance between antiviral activity and hyperbilirubinemia. However, a significant inter-subject variability was observed in phase I studies, C_{min} =273$\pm$298 and AUC=22262$\pm$20158.

- *Interaction studies*

The pharmacokinetic programme included assessments of interactions with other antiretroviral drugs (NRTIs: ddI, d4T, ZDV, 3TC; NNRTIs: EFV; and PI: RTV, SQV). Also, with drugs used for the treatment of opportunistic infections (rifabutin, clarithromycin, ketoconazole), other drugs known to be metabolised by CYP3A4 (diltiazem) and drugs known to affect QT or PR interval (atenolol, diltiazem, clarithromycin).
At the time of the original submission, the following results of multiple dose studies performed in healthy subjects with atazanavir were submitted. These interaction studies were mainly performed with atazanavir unboosted at 400 mg QD regimen. This is regarded as a major shortcoming with regard to the potential extrapolation to the recommended posology (atazanavir/ritonavir 300/100 mg QD).

Combined drug	**Effect on Atazanavir**	**Effect of Atazanavir on Combined drug**
stavudine lamivudine zidovudine	no change	no change
Didanosine (with antiacid) chewable tablet	decrease, 90% exposure (concomitant administration) no change (1 hour interval)	no change
Efavirenz	Decrease, 75% exposure	
Efavirenz **ATV/RTV 300/100 QD**	Increase 39% (AUC)	no change
Ritonavir ATV/RTV schedule regimens studied: 200/100 QD 200/200 QD 400/100 QD 400/200 QD 300/100 QD	5 fold (200/100) versus 200 mg ATV alone 3 fold (300/100) versus 300 mg ATV alone ≈2 fold for other combinations	

Saquinavir (ATV 400 /SQV 800, 1200 and 1600 mg QD)	no change	5-10 fold increase
Diltiazem	no change	2 fold increase
Ketoconazole	no change	
Rifabutin	no change	2 fold (AUC) increase
Oral contraceptives **Norethindrone** **Ethinyl estradiol**	no change	 2 fold (AUC) increase 50% (AUC) increase
Clarithromycin	30% increase	2 fold increase

- Interaction study with atazanavir and tenofovir

During the procedure, the applicant provided the results of the drug interaction study (AI454181).

Co administration of atazanavir and tenofovir leads to a decrease in atazanavir mean AUC and C_{max} values by approximately 20-25% and a decrease in the mean C_{min} value by approximately 40% in comparison to ATV 400 mg QD alone while lamivudine, stavudine or zidovudine did not appear to interact with ATV.

The results of study AI424045, suggest that the effect of tenofovir is compensated when atazanavir is boosted with ritonavir (300/100 mg QD). The results are in line with a report from Taburet et al. (CROI, 2003).

However, the applicant should further investigate this drug-drug interaction, in particular to investigate the mechanisms underlying the decrease in ATV blood levels when it is given with tenofovir.

In this study the interaction between atazanavir (400 mg QD) and ddI was also explored. However, the results of the co-administration were compared to the non-validated use of ddI-EC 500 mg with food. Therefore, the study design does not allow for any conclusions regarding co-administration of ddI-EC and atazanavir.

Clinical efficacy

Three clinical studies in experienced patients (AI424009, AI424043, AI424045) were submitted. Moreover, some clinical studies were performed in antiretroviral naïve patients with atazanavir unboosted (400mg QD). However, only an indication in treatment-experienced patients was finally claimed by the MAH.

The dose selection was based on phase I studies and PK/PD studies (see above).

Main studies

- *Description of the studies*

Three studies in antiretroviral-experienced patients (AI424009, AI424043, AI424045) were submitted.

In two studies (AI424009, AI424043) studies, atazanavir was used unboosted at the 400 mg QD dose. In study AI424045, atazanavir was used boosted with ritonavir at the 300/100 mg QD dose.

The studies were designed as non-inferiority with previously accepted predefined margins for head to head comparisons.

The comparators used were nelfinavir (studies AI424007, AI424008) and efavirenz (study AI424034) in antiretroviral naïve patients, in antiretroviral experienced patients saquinavir/ritonavir (study AI424009) and lopinavir/ritonavir (studies AI424043 and AI424045) were used as comparators.

- **Study AI424009**

At the time of the initial submission, study AI424009 was the only study provided to substantiate the efficacy and safety of atazanavir in antiretroviral-experienced patients.

Study AI424009 was a randomised, active controlled multi-centre three-arm study designed to compare the safety and antiviral activity of atazanavir 400/600 QD/SQV 1200 QD RTV 400 BID/SQV 400 BID.

Antiretroviral experienced patients (≥ 18 years) with plasma HIV RNA viral load ≥ 1,000 c/ml and a CD4 cell count ≥ 100 cells/mm^3 (or ≥ 75 cells/mm^3 with no prior history of any AIDS-defining diagnoses) obtained within 4 weeks prior to randomisation were enrolled.

The Study was blinded only with respect to the dose level of atazanavir (400 mg or 600 mg).

- Regime I: ATV 400 mg QD +SQV 1200 mg QD + 2NRTIs**
- Regime II: ATV 600 mg QD +SQV 1200 mg QD + 2NRTIs**
- Regime III: RTV 400 mg + SQV 400 mg + 2NRTIs

***Subjects received two NRTIs to which their screening viral isolate was sensitive (≤ 2.5 times the EC_{50}). The NRTI combinations allowed were ddI + d4T, d4T + 3TC, ddI + ZDV, or 3TC + ZDV. The Investigators selected NRTIs based on the phenotypic susceptibility results. If phenotypic resistance testing could not be performed, and the subject was otherwise eligible for the study, the subject was assigned to two NRTIs never previously taken.*

Eighty-five subjects were randomised (34 ATV400/SQV; 28 ATV600/SQV, 23 RTV/SQV).

No reliable interpretation of this study could be made since a high rate of premature discontinuation was observed (52% in the RTV/SQV control arm).

In response to the major objection pertaining to the insufficiency of the efficacy demonstration in antiretroviral experienced patients t, the applicant has provided the results of two new studies:

- Study AI424043: atazanavir unboosted
- Study AI424045: atazanavir boosted with ritonavir low dose

In both studies lopinavir/ritonavir was used as comparator.

- **Study AI424043**

This was a randomised, multinational, open-label, active-controlled, two-arm study to compare the antiviral activity, metabolic changes, safety, and tolerability of ATV vs. LPV/RTV, each in combination with two nucleosides (ZDV + 3TC, d4T + 3TC, ZDV + ddI, d4T + ddI, or ABC + appropriate NRTI (ddI, d4T, or 3TC)) in HIV-infected subjects who had failed prior antiretroviral treatment(s) which included one PI.

The median baseline HIVRNA level was 4.17 log10 c/ml (only 15% of patients had viral load >5 $\log_{10}$ c/ml) and the median baseline CD4 cell count was 273 cells/mm3 (only 30% had a CD4 cell count < 200/mm^3). 25% were classified as AIDS.

Results on the primary efficacy endpoint TAD (Time-Averaged-Difference) estimate (ATV-LPV/RTV) for the change from baseline in HIV RNA level through Week 24 (0.30 log10 c/ml (97.5% CI: 0.09, 0.51) was not in accordance with the predefined non inferiority margin (an upper confidence limit boundary on the difference (ATV - LPV/RTV) of 0.5 $\log_{10}$ c/mL. Moreover, the positive TAD

estimate and the lower limit of the 97.5% CI was greater than zero demonstrating the superiority of the LPV/RTV regimen versus ATV.

- **Study AI424045**

This open label study was designed during 2000 and aimed at comparing atazanavir (boosted with low dose of ritonavir or in combination with saquinavir) with lopinavir/ritonavir.

Antiretroviral experienced patients (>16 years) with plasma HIV RNA viral load ≥1,000 c/ml and a CD4 cell count ≥ 50 cells/mm^3 were enrolled in the study. Patients were enrolled who had had a virological failure on two or more HAART regimens that included at least one drug from all approved classes (PI, NNRTI, NRTI). They also had to have had a prior virological response to at least one HAART regimen, defined as a 1.0 $\log_{10}$ decline or a decline in viral load to < 400 c/ml.

Subjects co-infected with HBV or HCV were not excluded.

In response to questions raised by the CPMP, the applicant first submitted the preliminary 16 weeks data and later the interim 24 weeks data . In addition, a synopsis of the 48 weeks data was submitted. A final report on the 48 week data, confirming the results was submitted later, following authorisation of atazanavir.

Primary Objective:

- To compare the magnitude of reduction of plasma HIV RNA from baseline, expressed in log10, (as assessed by the TAD) through 24 and 48 weeks.

Secondary Objectives:

- To describe and correlate the proportions of subjects who have a ≥0.5 log10 decrease in HIV RNA from baseline or HIV RNA < 50 c/ml (or < 400 c/ml) at Week 48 with their baseline phenotypic sensitivity to their randomised PI
- To assess the proportion of subjects with HIV RNA levels less than the limit of quantification ([LOQ] equals 400 c/mL and LOQ equals 50 c/ml) through Week 48.
- To assess the magnitude of changes in CD4 cell counts through Week 48.
- To assess the change in HIV RNA from baseline through Week 2
- To assess the magnitude of changes in total cholesterol, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, glucose, and triglycerides through Week 48.
- To assess the safety and tolerability of atazanavir
- To assess the effect on PR and QTc intervals at baseline and steady state.

Sample size

The planned sample size of 330 randomised subjects (110 per regimen) provided 99% power to demonstrate that the TAD in reduction in log10 HIV RNA levels from baseline through Week 24 was similar between two regimens (each ATV regimen compared to LPV/RTV regimen) assuming:

- Two-sided 97.5% confidence interval, 0.05 significance level adjusted for two pairwise comparisons
- Upper confidence limit boundary on the difference (ATV - LPV/RTV) of 0.5 log10 c/ml
- Standard deviation in the HIV RNA change of 0.8 log10 c/m

The planned sample size of 110 randomised subjects per regimen provided 32% power for the pairwise comparisons (each ATV regimen to the LPV/RTV regimen) of the secondary objective showing that the proportion of subjects with HIV RNA < 400 c/ml at Week 48 was similar assuming:

- A two-sided 95% confidence interval
- 50% of subjects have Week 48 HIV RNA < 400 c/ml
- Lower confidence limit for non-inferiority of -10%.

Results

- Patient Disposition

Overall, 8% discontinued prior to week 24, the reasons were balanced between the treatment arms.

When considering the 48 weeks data, it appears that overall 19% of patients discontinued prior to week 48. It is worth noting that more discontinuations occurred in the ATV/RTV treatment arm (22 % versus 11% in the LPV/RTV arm). This is mainly due to treatment failure or lack of efficacy: only 6 patients in the LPV/RTV arm discontinued because of treatment failure/lack of efficacy compared with 17 (14%) and 11 (10%) in the two ATV arms.

- Baseline Characteristics

The median number of weeks on prior PI was 131 (0.1-469.7), 265.6 (0.1-782.4) on NRTI and 77.09 (0.1-304.0) on NNRTI.

The population enrolled had a median viral load inferior to 5 $\log_{10}$ copies/ml (only 25% of patients had viral load >5 $\log_{10}$ c/ml) and a median CD4 cell count of approximately 300/mm^3 (only 30% had a CD4 cell count < 200/mm^3). Of these patients 30% were classified as AIDS. In view of the duration on prior PI and the phenotypic sensitivity of baseline isolates, it appears that the population enrolled mainly consisted of patients with limited PI experience.

Baseline isolates showed a limited decrease in phenotypic sensitivity. Less than a 2.5x IC50-difference vs the control strain was observed in 83% of isolates for amprenavir and saquinavir, 74% for atazanavir, 75% for lopinavir, 56% for nelfinavir (with 23% harbouring >10x IC50 of control strain) and 65% for ritonavir (with 21% harbouring >10x IC50 of control strain).

GENOTYPIC MUTATIONS AT BASELINE – RANDOMIZED SUBJECTS				
	NUMBER OF SUBJECTS (%)			
	TREATMENT REGIMEN			
	ATV 300/RTV N = 120	ATV 400/SQV N = 115	LPV/RTV N = 123	Total N = 358
PI Mutations				
0	18 (15)	11 (10)	13 (11)	42 (22)
1	28 (23)	35 (30)	27 (22)	90 (25)
2	23 (19)	19 (17)	27 (22)	69 (19)
3	12 (10)	12 (10)	11 (9)	35 (10)
4	10 (8)	13 (11)	15 (12)	38 (11)
5	8 (7)	12 (10)	8 (7)	28 (8)
6	7 (6)	5 (4)	6 (5)	18 (5)
7	5 (4)	5 (4)	12 (10)	22 (6)
8	5 (4)	2 (2)	3 (2)	10 (3)
9	2 (2)	1 (<1)	1 (<1)	4 (1)
10	2 (2)	0	0	2 (<1)
NRTI Mutations				
0	18 (15)	22 (19)	17 (14)	57 (16)
1	23 (19)	20 (17)	15 (12)	58 (16)
2	9 (8)	7 (6)	18 (15)	34 (9)
3	23 (19)	14 (12)	20 (16)	57 (16)
4	18 (15)	26 (23)	16 (13)	60 (17)
5	14 (12)	16 (14)	21 (17)	51 (14)
6	11 (9)	9 (8)	12 (10)	32 (9)
7	4 (3)	1 (<1)	4 (3)	9 (3)

Efficacy Results

Primary endpoint:

Virologic Suppression Through Week 16, 24 and 48- Randomized Subjects

	HIV RNA Level Change From Baseline (log10 c/ml)	
	Time-Averaged Difference (TAD) Estimate (97.5% CI)	
	ATV 300/RTV - LPV/RTV	ATV 400/SQV - LPV/RTV
Through Week 16 – Subjects Randomized		
Overall	0.15 (-0.06, 0.37)	0.29 (0.06, 0.52)
Last observation carried forward	0.11 (-0.11, 0.32)	0.31 (0.08, 0.54)
Overall adjusted by region	0.16 (-0.06, 0.38)	0.29 (0.06, 0.52)
Through Week 24 – Subjects Randomized		
Overall	0.14 (-0.09, 0.37)	0.31 (0.07, 0.55)
Last observation carried forward	0.10 (-0.13, 0.33)	0.34 (0.10, 0.58)
Overall adjusted by region	0.15 (-0.08, 0.38)	0.31 (0.07, 0.55)
Through Week 48 – Subjects Randomized (synopsis)		
Overall	0.13 (-0.12, 0.39)	0.33 (0.07, 0.60)
Last observation carried forward	0.11 (-0.15, 0.36)	0.38 (0.11, 0.64)
Overall adjusted by region	0.14 (-0.12, 0.39)	0.34 (0.07, 0.61)

At week 16, results for the comparison ATV/RTV versus LPV/RTV were in accordance with the predefined non-inferiority margin (an upper confidence limit boundary on the difference (ATV - LPV/RTV) of 0.5 $\log_{10}$ c/ml). According to the primary efficacy endpoint, non-inferiority between ATV/RTV and LPV/RTV was also demonstrated in the week 24 and week 48 analyses.
However, non-inferiority was not demonstrated between atazanavir/saquinavir and lopinavir/ritonavir at any timepoint.

Secondary endpoints

Virologic suppression through week 16, 24 and 48 stratified by PI sensitivity- randomized subjects:

	HIV RNA Level Change From Baseline (log10 c/ml)	
	Time-Averaged Difference (TAD) Estimate (95% CI)	
	ATV 300/RTV - LPV/RTV	ATV 400/SQV - LPV/RTV
Through Week 16 – Subjects Randomized		
Overall stratified by PI sensitivity	0.15 (-0.02, 0.33)	0.28 (0.09, 0.48)
PI Sensitive	0.03 (-0.17, 0.22)	0.25 (0.03, 0.48)
PI resistant	**0.50 (0.11, 0.88)**	0.36 (-0.04, 0.76)
Through Week 24 – Subjects Randomized		
Overall stratified by PI sensitivity	0.14 (-0.84, 0.32)	0.30 (0.10, 0.51)
PI Sensitive	0.02 (-0.18, 0.23)	0.27 (0.03, 0.51)
PI resistant	**0.48 (0.08, 0.87)**	0.38 (-0.03, 0.78)
Through Week 48 – Subjects Randomized		
Overall stratified by PI sensitivity (N= 355)	**0.14 (-0.07, 0.34)**	**0.34 (0.12, 0.56)**
PI Sensitive (N= 260)	**0.03 (-0.21, 0.26)**	**0.30 (0.04, 0.56)**
PI resistant (N= 95)	**0.45 (0.03, 0.86)**	**0.46 (0.04, 0.87)**

Virologic suppression through week 16, 24 and 48 stratified by number of baseline PI Mutations*- randomized subjects

	HIV RNA Level Change From Baseline (log10 c/ml)	
	Time-Averaged Difference (TAD) Estimate (95% CI)	
	ATV 300/RTV - LPV/RTV	ATV 400/SQV - LPV/RTV
Through Week 16 – Subjects Randomized		
Overall stratified by Baseline PI Mutations	0.19 (0.01, 0.37)	0.32 (0.13, 0.51)
Less than Four	0.06 (-0.15, 0.27)	0.19 (-0.04, 0.42)
Four or More	**0.42 (0.10, 0.74)**	0.56 (0.23, 0.88)
Through Week 24 – Subjects Randomized		
Overall stratified by Baseline PI Mutations	0.18 (-0.01, 0.36)	0.33 (0.13, 0.52)
Less than Four	0.03 (-0.18, 0.24)	0.22 (-0.01, 0.46)
Four or More	**0.46 (0.12, 0.80)**	0.53 (0.18, 0.87)
Through Week 48 – Subjects Randomized		
Overall stratified by Baseline PI Mutations	0.17 (-0.04, 0.38)	0.37 (0.15, 0.58)
Less than Four	0.03 (-0.23, 0.28)	0.21 (-0.07, 0.48)
Four or More	0.44 (0.07, 0.81)	0.66 (0.31, 1.02)

**PI mutations/site: 10-20-24-32-33-36-46-48-50-54-63-71-73-82-84-90*

The results significantly favoured the lopinavir/ritonavir arm when considering the subset of patients with 4 or more PI mutations among the following: 10, 20, 24, 32, 33, 36, 46, 48, 50, 54, 63, 71, 73, 82, 84 and 90.

Proportions in Response at Week 48 (LOQ Equals 400 c/ml) - Randomized Subjects

	Responder/Evaluable (%) Treatment Regimen			Difference Estimate (95% CI)	
Analysis	**ATV 300/RTV N = 120**	**ATV 400/SQV N = 115**	**LPV/RTV N = 123**	**ATV 300/RTV - LPV/RTV**	**ATV 400/SQV - LPV/RTV**
VHLS-R	77/120 (64)	60/115 (52)	84/123 (68)	-4.1 (-16.0, 7.8)	-16.1 (-28.5, -3.7)
VR-OC	66/93 (71)	43/81 (53)	69/105 (66)	5.3 (-7.7, 18.3)	-12.6 (-26.8, 1.6)
TRWPF	64/120 (53)	42/115 (37)	67/123 (54)	-1.1 (-13.7, 11.4)	-17.9 (-30.6, -5.3)

Proportions in Response at Week 48 (LOQ Equals 50 c/ml) - Randomized Subjects

VHLS-R	*76/120 (63)*	*60/115 (52)*	*84/123 (68)*	*-5.0 (-16.9, 7.0)*	*-16.1 (-28.5, -3.7)*
VR-OC	*48/93 (52)*	*31/81 (38)*	*57/105 (54)*	*-2.7 (-16.6, 11.3)*	*-16.0 (-30.5, -1.5)*
TRWPF	*43/120 (36)*	*28/115 (24)*	*52/123 (42)*	*-6.4 (-18.7, 5.8)*	*-17.9 (-29.9, -5.9)*

The results of the HIV RNA < 50 c/ml TRWPF (ITT) and VR-OC (AT) and HIV RNA < 400 c/ml VR-OC (AT) analyses show little change for either ATV/RTV or LPV/RTV between Week 24 and Week 48. The Week 48 mean change from baseline HIV RNA is similar, ATV/RTV (-1.93) and LPV/RTV (-1.87). At 48 weeks, based on TRWPF definition (**LOQ=400c/ml**), the hazard ratio and 95% CI for ATV 300/RTV:LPV/RTV was 1.09 (0.76, 1.57), indicating the time to treatment failure for ATV300/RTV was comparable to that for LPV/RTV. Based on TRWPF definition **(LOQ=50c/ml)** through Week 48, the hazard ratio and 95% CI for ATV 300/RTV:LPV/RTV was 1.24 (0.91, 1.70) favoring LPV/RTV CD4 cell increases were comparable with both regimens demonstrating further increases between 24 and 48 weeks.

Among the PI mutations selected for the subgroup analysis (i.e. as above) some are considered to be more critical for the emergence of resistance (i.e. mutations at positions 10, 46, 54, 82, 84 and 90). Therefore, the applicant was requested to perform further sub-analyses with these more critical PI-mutations:

Virological suppression through week 48 by Selected Baseline PI Mutations at position 10, 46, 54, 82, 84, 90.

HIV RNA Level Change From Baseline (log10 c/ml)					
	Mean change from baseline (SE			**Time-Averaged Difference (TAD) Estimate (95% CI)**	
	ATV 300/RTV	**ATV /SQV**	**LPV/RTV**	**ATV300/RTV-LPV/RTV**	**ATV400/SQV-LPV/RTV**
Overall stratified by B/L PI Mutations*				0.14 (-0.07, 0.35)	0.35 (0.13, 0.58)
Less than Four	N=84 -2.01 (0.13)	N=72 -1.66 (0.15	N=88 1.93 (0.13)	0.06 (-0.17, 0.28)	0.33 (0.08, 0.57)
Four or More	N=6 -0.79 (0.30)	N=7 -0.40 (0.30)	N=11 -1.35 (0.43)	0.71 (0.13, 1.30)	0.59 (-0.10, 1.28)

**PI mutations/site : 10-46-54-82-84-90*

For patients with less than four of the protease gene mutations 10, 46, 54, 82, 84, and 90, the proportion with HIV RNA <400 copies/ml (<50 copies/ml) was 59% (41%) for atazanavir + ritonavir and 58%(44%) for lopinavir + ritonavir at Week 48. There were too few patients with 4 of these mutations to assess the comparability of the REYATAZ + ritonavir and lopinavir + ritonavir regimens, but reduced virologic activity may be anticipated among patients with this resistance profile.

Discussion on clinical efficacy

Study AI424043

This study further confirmed the concerns regarding the adequacy of 400 mg atazanavir once daily.

As expected from PK/PD considerations, atazanavir 400 mg QD was shown to be inadequate for the treatment of antiretroviral-experienced patients even with limited PI experience. That is, 400 mg QD would not be expected to produce a sufficient C_{min} to overcome reduced susceptibility (in strains with increased IC50).

Study AI424045

This open label study was aimed at comparing atazanavir (boosted with low dose ritonavir or in combination with saquinavir) with LPV/RTV.
Results have been made available for weeks 16, 24 and 48 (primary endpoint = magnitude of reduction of plasma HIV RNA from baseline, expressed in log10, (as assessed by the TAD) through 24 and 48 weeks) .

At week 24, the rate of premature discontinuation was 8%, increasing to 19% at 48 weeks.

The population enrolled mainly had a median viral load inferior to 5 log_{10} copies/ml (only 25% of patients had viral load >5 log_{10} c/ml) and a median CD4 cell count of approximately 300/mm^3 (only 30% had a CD4 cell count < 200/mm^3). 30% were classified as AIDS. The population mainly consisted of patients with limited PI experience (approx. 2 years). It is worth noting that, as a result of the inclusion criteria, only a limited number of patients had PI therapy at baseline (only 35% had recent PI use compared to 62% with recent NNRTI). Also, only a limited number of patients had viruses with four or more PI mutations at baseline and approximately 40% of patients had viruses with <2 NRTI mutations.

At 24 weeks, results for the ATV/RTV arm were compatible with the predefined hypothesis of non-inferiority (upper limit of the confidence interval of the TAD between both arms <0.5 log copies/ml) with respect to LPV/RTV. This finding is supported by the 48 weeks data (primary analysis). However, the point estimate favours the LPV/RTV arm. This was also the case for the secondary endpoint of the percentage of patients with undetectable viral load (400 and 50 c/ml thresholds). When considering the post-hoc subgroup analysis performed with patients with viral stains harbouring less or more than 4 PI mutations results were significantly in favour of LPV/RTV.

Therefore, based on the virological and clinical data, no benefit is expected in patients with multi-PI resistant strains.

Although atazanavir/ritonavir appears to be of lesser potency than lopinavir/ritonavir, the overall risk benefit relationship was considered to be acceptable when taking into account once daily dosing, the low risk of dyslipidemia and the better gastro-intestinal tolerance observed in comparison to LPV/RTV.

However, it is clear that atazanavir/ritonavir is not appropriate for salvage therapy.

Clinical safety

Although involving a different schedule regimen than the currently recommended boosted regimen with ritonavir 300/100mg OAD, the safety data derived from the use of atazanavir unboosted (mainly from studies performed in antiretroviral naïve patients), were taken into account for the overall assessment of the safety profile of the drug.

Patient exposure

In pharmacological studies, 648 healthy subjects have been exposed to atazanavir, among these 398 (61%) experienced AEs.

REYATAZ has been evaluated for safety and tolerability in combination therapy with other antiretroviral medicinal products in Phase II and III trials in 1,596 adult patients. The majority of patients (1,046) received REYATAZ 400 mg once daily without ritonavir. The median duration of treatment was 102 weeks in Phase II trials and 31 weeks in the Phase III trials..

- **Phase II-III studies (except study AI424043 and AI424045)**

Adverse events and serious adverse event/deaths

On pooling all data for the 400 mg dose (715 patients), the commonest AEs considered to be drug-related have been nausea (30%), headache and rash (both 12%), clinical jaundice and abdominal pain (both 10%), vomiting (9%), fatigue (8%), diarrhoea and dizziness (all 8%).

Discontinuations due to adverse events were reported in 5% of atazanavir treated patients and in 7% of patients treated with comparators. In all atazanavir groups, discontinuations increased with the dose of atazanavir, and most frequent related AEs were lactic acidosis, hyperbilirubinemia and liver enzyme increased. Preclinical and in vitro studies draw the attention also to cardiotoxic profile of the product.

Deaths were reported in ≤1% of treated patients and were in majority due to HIV disease or lactic acidosis (in all fatal lactic acidosis cases, atazanavir was in combination with stavudine).

- **Cardiotoxicity**

Atazanavir minimally increased rabbit Purkinje fibre action potential duration (13% at 30μM), inhibited calcium currents (IC_{50} of 10.4μM) and had a minimal effect on sodium current (IC_{50} >30μM). Moreover, atazanavir produced weak inhibition on Ikr current (HERG) (15% at 30μM).

In clinical pharmacology studies, Atazanavir dose-dependently increased the QT interval (by ~14 ms at 400 mg QD). Atazanavir per se seems to have comparable effect on the QT as other protease inhibitors. Atazanavir dose-dependently increased the PR interval in healthy volunteers. First-degree heart block was very common. No clinical signal was reported even if some palpitation and syncope were described in atazanavir group regimens in Phase II/III studies.

- **Hyperbilirubinemia**

Dose related hyperbilirubinemia that is reversible after discontinuation or on dose reduction was very common in atazanavir treated patients.

Jaundice and scleral icterus were very commonly reported in clinical trials.

Atazanavir inhibits bilirubin glucuronidation by uridine diphosphate-glucuronosyl transferase (UDP-GT) 1A1 and so produces clinical jaundice in humans. The mechanism appears to be predominantly competitive enzyme inhibition by atazanavir. Other factors affecting bilirubin production (haemolysis), transport (albumin), uptake to, transport in and export from hepatocytes were ruled out.

- **Hepatic events**

Moderate elevations in AST and/or ALT were more common in atazanavir groups than in nelfinavir groups, although rates were comparable with the efavirenz group in study AI424034 and lower than seen in the ritonavir group in AI424009. These transaminase elevations were associated with slight elevations in alkaline phosphatase that were similar in atazanavir and comparator groups. Discontinuations due to transaminase elevations were uncommon, occurring in 7/742 (0.9%) subjects who received atazanavir in Phase II and one subject who received atazanavir in AI424034.

Hyperbilirubinemia induced by atazanavir does not seem to be associated with hepatotoxicity, particularly cholestatic, since no correlation between Grade 3 - 4 transaminase elevations and elevations in bilirubin have been observed.

- **Lactic acidosis**

Seventeen cases of lactic acidosis syndrome (LAS) and symptomatic hyperlactatemia (SHL) occurred in two clinical trials (AI424007 and AI424008)**.** Fifteen cases (2.2%) occurred in the atazanavir arm

vs. 2 (1.0%) in the NFV arms (p=0,3904). Due to the randomisation schemes, a substantially greater number of patients were randomised to the ATV versus NFV arms, including a high number of women. All these patients received a d4T-containing ART regimen. The mean duration of ART exposure was 9.3 months (range 3.5 months to 14 months).

There were no cases of LAS in AI424009, in which patients received d4T (77%) but the number of randomised patients was small. There were also no cases reported at 24-weeks in the ongoing phase III study AI424034 in patients receiving ATV/3TC/ZDV. The 48-weeks safety data are very important in a setting where the mean time to onset was 9.3 months (around 42 weeks).

Review of these cases identified female gender and obesity as additional risk factors for LAS.
Five cases of lactic acidosis had fatal outcome (four with atazanavir and one with comparator NFV). These cases occurred in females (three from South America, one from Africa, one from Asia), and among these two were pregnant (1/ATV 500 mg; 1/NFV). Three had BMI <25 and two >25. The time to onset was about 12 months. Two cases occurred in the 500 mg dose group and two in the 600mg group.

Based on the available literature on NRTI-associated SHL and LAS, NRTI associated SHL appears to be common, and treatment with a d4T-containing regimen appears to be the strongest risk factor. In the majority of cases, patients are asymptomatic, or only mildly symptomatic. Serious sequelae, such as LAS and death due to LAS, appear to be uncommon (<2%).

Pivotal studies

- **Study AI424043**

There was one death reported on the atazanavir regimen (traumatic cardiac arrest), unrelated to study therapy.

Discontinuation of study therapy due to AEs was infrequent on both treatment regimens (ATV, 2 subjects; LPV/RTV, 4 subjects).

Adverse events, serious adverse events, and discontinuations due to adverse events were generally comparable in the ATV and LPV/RTV treatment regimens. The incidence of AEs was slightly less frequent on the ATV treatment regimen (69%) compared to the LPV/RV treatment regimen (79%). AEs that were reported more frequently on the atazanavir treatment regimen included rash (13% vs. 7%), dizziness (8% vs. 3%), extremity pain (8% vs. 2 %), jaundice (10% vs. 0%) and scleral icterus (6% vs. 0%). AEs that were reported more frequently on the LPV/RTV treatment regimen included diarrhoea (32% vs. 10%), somnolence (9% vs. 3%), pruritus (7% vs. 3%) and anorexia (5% vs. <1%).

Isolated elevations in serum bilirubin, primarily indirect, were frequent on the atazanavir regimen. However, consistent with previous findings, these changes were benign, and were not associated with elevations in hepatic transaminases. Dose reductions and discontinuation of therapy due to these changes were infrequent (2% and 1%, respectively).

At Week 24, negative estimates for the pair wise difference (ATV-LPV/RTV) in the mean percent change from baseline in lipid concentrations demonstrate a superiority of the atazanavir treatment regimen versus LPV/RTV for LDL cholesterol (co-primary endpoint).

	ATV 400 **N=144**	**LPV/RTV** **N=146**	**pairwise difference** **(ATV - LPV/RTV)**
Co-primary endpoint			
Fasting LDL cholesterol concentration			
N	123	106	
Observed values	-6% (-8.3% -3.1%)	+5% (2.3% ; 8.1%)	-10.3%(-17.8%, -2.5%) *

Secondary endpoint			
Total Cholesterol			
N	127	108	
Observed values	-2%	17%	-16.1% (-20.5%, -11.5%) **
HDL Cholesterol			
N	127	108	
Observed values	12%	18%	-5.8% (-12.1%, 0.9%)
Fasting triglycerides			
N	124	107	
Observed values	-2%	55%	-35.2% (-43.6%, -25.6%) **

Difference estimates are stratified by NRTI backbone
Confidence level is 97.5% for fasting LDL cholesterol and 95% for other lipids
** p-value <0.0001 * p-value <0.05

Minimal mean QTc interval changes from baseline were observed in both treatment groups.

- **Study AI424045**

Most frequent AEs Grade 1-4	**Treatment regimen**					
	ATV300/RTV N=119		**ATV400/SQV N=110**		**LPV/RTV N=118**	
	24 weeks	**48 weeks**	**24 weeks**	**48 weeks**	**24 weeks**	**48 weeks**
Deaths N (%)	0	0	0	1 (<1)	0	1 (<1)
AEs leading to discontinuation N (%)	5 (4)	6 (5)	6 (5)	8 (7)	4 (3)	5 (4)
Serious AEs N (%)	10 (8)	12 (10)	12 (10)	14 (12)	9 (7)	11 (9)
Most frequent AEs Grade 1-4						
Any Adverse event N (%)	89 (75)	97 (82)	89 (81)	93 (85)	96 (81)	103 (87)
Infection	31 (26)	39 (33)	25 (23)	36 (33)	39 (33)	45 (38)
Diarrhea	20 (17)	25 (21)	27 (25)	29 (26)	52 (44)	54 (46)
Nausea	16 (13)	19 (16)	22 (20)	24 (22)	13 (11)	15 (13)
Vomiting	6 (5)	8 (7)	11 (10)	14 (13)	4 (3)	5 (4)
Abdominal pain	7 (6)	10 (8)	23 (21)	26 (24)	9 (8)	12 (10)
Jaundice	18 (15)	19 (16)	5 (5)	6 (5)	0	0
Scleral icterus	12 (10)	13 (11)	4 (4)	3 (3)	0	0
Headache	18 (15)	21 (18)	21 (19)	24 (22)	15 (13)	18 (15)
Peripheral neurologic symptom	10 (8)	13 (11)	10 (9)	12 (11)	16 (14)	22 (19)
Rash	9 (8)	11 (9)	9 (8)	13 (12)	16 (14)	17 (14)
Grade 3-4 Lab abnormalities						
Neutrophil reduction	8/119 (7)	8/119 (7)	6/108 (6)	8/108 (7)	6/118 (5)	10/118 (8)
Platelet reduction	2/119 (2)	2/119 (2)	4/108 (4)	4/108 (4)	2/118 (2)	3/118 (3)
ALT elevation	4/119 (3)	4/119 (4)	4/108 (4)	4/108 (4)	3/118 (3)	4/118 (3)
AST elevation	4/119 (3)	4/119 (3)	2/108 (2)	2/108 (2)	1/118 (<1)	4/118 (3)
Total bilirubin elevation	54/119 (45)	58/119 (49)	20/108 (19)	22/108 (20)	1/118 (<1)	1/118 (<1)

Throughout the 48 weeks, the rates for the most frequent adverse events were comparable in all treatment groups with those seen at 24 weeks, with no new safety issues emerging during longer-term exposure to atazanavir.

Diarrhoea, infection, rash and peripheral neurological symptoms were more common in the LPV/RTV treatment group. Headache, nausea, vomiting and abdominal pain were more common in the ATV400/SQV treatment group.

- **Hyperbilirubinemia**

Elevations in unconjugated bilirubin, jaundice and icterus were described almost exclusively in atazanavir containing regimens. These events were not associated with increases in hepatic transaminases or particular neurological complications. Mean total bilirubin was 1.6 mg/dL and 0.83 mg/dL higher at week 24 than at baseline for respectively ATV/RTV and ATV/SQV treated subjects, compared to 0.08 mg/dL for LPV/RTV. Elevations in total bilirubin (all grades) were reported in 90% of ATV/RTV patients vs. 75% (ATV/SQV) and 8% (LPV/RTV). Grade 4 elevations in bilirubin were reported in 9% of patients on ATV300/RTV group, and in 2% of patients on ATV400/SQV group. At 24 weeks, no patients discontinued the treatment due to these events, and dose reductions due to hyperbilirubinemia were only observed on the ATV 300/RTV regimen (in 8% of subjects).

The following table shows a comparison of safety data between unboosted ATV (derived from study AI424043) and ATV/RTV:

	AI424043		**AI424045**		
	ATV400 (n=144)	**LPV/RTV (n=146)**	**ATV300/RTV (n=119)**	**ATV400/SQV (n=110)**	**LPV/RTV (n=118)**
Jaundice 24 weeks 48 weeks	14 (10%)	0	18 (15%) 19 (16%)	5 (5%) 6 (5%)	0 0
Icterus 24 weeks 48 weeks	9 (6%)	0	12 (10%) 13 (11%)	4 (4%) 3 (3%)	0 0
Grade 2-4 total bilirubin **in subjects with jaundice/scleral icterus** 24 weeks	20/22 (91%)	0	24/27 (89%)	6/8 (75%)	0
Hyperbilirubinemia (All grades) 24 weeks	108 (76%)	16 (11%)	107 (90%)	81 (75%)	10 (8%)
Hyperbilirubinemia (Grades 3-4) 24 weeks 48 weeks	31 (22%)	0	54 (45%) 58 (49%)	20 (19%) 22 (20%)	1 (<1%) 1 (<1%)
Dose Reductions 16 weeks 24 weeks 48 weeks	3 (2%)	0	3 (3%) 9 (8%) not provided	0 0 not provided	0 0 not provided
Discontinuations 24 weeks 48 weeks	2 (1%)	0	0 0	0 0	0 0

The use of ritonavir as a pharmacokinetic enhancer leads to a higher incidence (ATV300/RTV versus ATV 400 mg alone) of hyperbilirubinemia (90% versus 76%), jaundice (15% versus 10%) and icterus 10% versus 6%).

However, the fact that no patient discontinued because of hyperbilirubinemia, jaundice or icterus is reassuring in term of acceptability of these adverse events.

- **Lipids**

Atazanavir containing regimens demonstrated a better lipid profile compared to LPV/RTV treatment regimen with regard to total cholesterol, fasting LDL cholesterol and fasting triglycerides. The greater decline in mean fasting LDL cholesterol levels observed on the atazanavir-containing regimens compared with the LPV/RTV-containing regimens was not statistically significant. HDL cholesterol levels showed decreases with ATV/RTV at 24 and 48 weeks, whereas levels were stable in the other groups.

The use of lipid reducing agents was less common on the ATV-containing regimens, particularly ATV300/RTV (8%), than in the LPV/RTV group (19%).

No differences in lipid parameters were noted in the ATV/RTV group between 24 weeks and 48 weeks.

Based on the comparison between studies AI424043 and AI424045, the addition of ritonavir to 300 mg atazanavir daily did not seem to be associated with a less favourable lipid profile compared to ATV alone.

	AI424043 24 weeks		**AI424045 24 weeks**			**AI424045 48 weeks**		
	ATV400	LPV/RTV	ATV/RTV	ATV/SQV	LPV/RTV	ATV/RTV	ATV/SQV	LPV/RTV
Total Cholesterol	-2%	17%	-8%	-9%	3%	-8%	-4%	6%
LDL Cholesterol	-6%	5%	-10%	-11%	-4%	-10%	-3%	1%
HDL Cholsterol	12%	18%	-7%	-1%	0%	-7%	4%	2%
Triglycerides	-2%	55%	-2%	-14%	31%	-4%	-14%	30%

Of note, the rate of lipodystrophy was similar between treatment groups at 48 weeks (study AI424045).

- **Cardiotoxicity**

Minimal effects on the QT and PR (interval prolongations) were described, and these are comparable between all treatment groups. No signal regarding cardiovascular events is observed in atazanavir-treatment groups.

Discussion on clinical safety

Since the original MAA submission, long term data from study antiretroviral naïve patients and results from studies AI424043 and AI424045 in antiretroviral experienced patients have been provided that describe further the safety profile of atazanavir.

- Hepatotoxicity:

The concerns that elevations in AST and/or ALT were more common in atazanavir groups than in nelfinavir groups, and that there were inadequate data to fully assess the potential for atazanavir to trigger serious hepatic events were raised in the D70 assessment report. The complementary data

derived from the additional studies submitted in response to the D120 CPMP LoQ have been reassuring with regard to the hepatotoxicity of the drug. In common with other antiretrovirals, atazanavir is associated with hepatic events but does not appear to be associated with a higher risk of hepatotoxicity in comparison with the other studied PIs (nelfinavir and lopinavir/ritonavir).
As previously described for other antiretrovirals, co-infection with HCV and /or HBV, and prior transaminase elevations, are risk factors for severe hepatotoxicity following initiation of atazanavir.

A warning regarding the potential increased risk for hepatic events in patients with pre-existing liver dysfunction should be included in the SmPC, in line with the wording proposed by the CPMP for the other antiretrovirals. This population should be closely monitored.

- Hyperbilirubinemia

Hyperbilirubinemia was observed frequently in atazanavir treated patients.

The applicant was asked to discuss the potential impact of hyperbilirubinemia in the therapeutic management of patients (dose reduction and treatment discontinuation being risk factors for emergence of resistance), and the possible long-term consequences.

In response to the day 180 LOQ, data from the clinical studies as well as from the early access program (EAP) were submitted. Based upon the clinical trial experience for 1597 atazanavir-treated subjects and more than 3000 patients receiving atazanavir through the EAP, bilirubin elevation and its clinical manifestations (i.e., jaundice and scleral icterus) would be expected to result in treatment discontinuation very infrequently. Furthermore, a 5% discontinuation rate, if it is indeed observed in "real life", is well within observed discontinuation rates for other ARV drugs due to AEs.

Since dose reduction is not recommended, no impact on the efficacy of an atazanavir-containing regimen is expected.

The long-term consequences of mild and modest elevations in unconjugated bilirubin are generally believed to be inconsequential. In response to the CPMP's request, the applicant presented an examination of the clinical trial database for atazanavir for potential side effects of increased bilirubin (e.g., gallstones and gall bladder disease and neurotoxicity). Among 1597 subjects who received atazanavir, some for up to 3.5 years (reflecting greater than 2000 patient years), only 7 cases of biliary disease were identified in the clinical trial database. This rate is within the range expected for an HIV control population. No cases of neurotoxicity potentially attributable to bilirubin elevations were observed. Therefore, based upon the biology of unconjugated bilirubin elevations, the vast experience with Gilbert.s syndrome, and the entire clinical trial database, chronic hyperbilirubinemia does not appear to represent a safety concern for the use of atazanavir.

- Lactic acidosis:

The safety data are reassuring with regard to the potential (rather unexpected for a PI) of atazanavir to induce lactic acidosis. An association with D4T and/or 3TC and/or ZDV has been described in all cases and so a relationship with atazanavir has not been established.

- Cardiotoxicity/EEC changes

A signal regarding electrophysiological change was derived from the preclinical and clinical development programmes. Some clarifications were requested regarding electrophysiological data from in-vitro, pre-clinical and clinical studies in order to assess the cardiotoxicity of atazanavir.

Atazanavir is associated with plasma concentration dependent prolongation of PR interval so that first-degree heart block is common. Although the potential of atazanavir to prolong the QT interval is confirmed, the dose and concentration relations regarding this effect need to be further evaluated, particularly when atazanavir is co-administered with ritonavir. The effect was more apparent above the recommended dose. These ECG changes were asymptomatic. However associated clinical events

such as proxies (palpitations, syncopes) should be monitored and a warning in association with other drugs which have the potential to increase the QT interval and/or in patients with pre-existing risk factors (bradycardia, electrolytes disturbances) has been included in the SmPC.

Responses of the applicant on the electrophysiological studies were not totally satisfactory. Therefore as a specific obligation following Marketing Authorisation, further assessment of the cardiotoxicity potential of atazanavir, particularly in combination with ritonavir, is ongoing.

4. Overall conclusions, benefit/risk assessment and recommendation

Quality

The quality of this product is considered to be acceptable when used in accordance with the conditions defined in the SPC. Physicochemical and biological aspects relevant to the uniform clinical performance of the product have been investigated and are controlled in a satisfactory way.

Preclinical pharmacology and toxicology

Overall, safety pharmacology studies provided adequate evidence that there were no atazanavir-related adverse effects on, cardiovascular respiratory, or central nervous system function in rats or dogs.

The drug was shown to be clastogenic *in vitro* (increase in chromosome aberrations confirmed in a second in-vitro primary human lymphocyte assay) but not *in vivo*.

A particular concern was raised regarding the carcinogenicity studies presented, which suggest that atazanavir induced hepatocellular adenoma in female mice at the highest dose tested. This observation is probably due to an epigenetic mechanism with hepatocyte hyperproliferation after cell necrosis, which is a well-known mechanism, currently observed in rodents. Furthermore, it is well recognised by the scientific community that mice present an increased sensitivity to hepatocellular tumours. In this mice study, liver carcinomas were not present, and in rats, all the results were negative. All together, these results suggest that atazanavir will not increase the carcinogenic risk in humans.

Atazanavir did not alter the fertility parameters and was not teratogenic or embryotoxicity in rodents.

Efficacy

The major shortcoming of the pharmacokinetic programme is that it is almost exclusively based on the use of atazanavir unboosted at 400 mg QD dose, whereas it is recommended to use only when boosted with ritonavir at 300/100 mg QD. Co administration of ATV 300 mg and RTV 100 mg both QD has been selected since it results in a substantial increase in the mean C_{min} value of ATV relative to ATV 400 mg alone.

Therefore, the applicant has committed have to further substantiate the pharmacokinetics of atazanavir boosted with ritonavir as well as the interaction profile of this combination as part of the post-approval commitments.

There is an unexpected drug interaction between tenofovir and atazanavir while lamivudine, stavudine or zidovudine do not appear to interact with atazanavir. Co-administration of atazanavir and tenofovir leads to a decrease in atazanavir mean AUC and C_{max} values by approximately 20-25% and a decrease in the mean C_{min} value by approximately 40% in comparison to atazanavir 400 mg QD alone.

The results of study AI424045, in which ATV 300/RTV 100 mg QD was always administered in combination with tenofovir indicate that administration of ATV with RTV may compensate for the effects of tenofovir. Therefore, tenofovir may be combined with atazanavir when it is boosted with ritonavir (300/100 mg QD). However, the applicant committed to investigate this drug-drug interaction.

Bioequivalence has been demonstrated between the oral powder and the capsule. Considering the lack of data currently available in paediatric patients, this oral powder will be proposed for adults not able to swallow the capsules.

In response to the Day 180 LOQ, the applicant provided further virological data to expand on the initial observations and better characterised the pathway to resistance to atazanavir. Within the 74 isolates from experienced patients who developed resistance to atazanavir on therapy that included either ATV, ATV/r and ATV/SQV 9 isolates from patients treated with either ATV and ATV/r display the I50L phenotype previously described in naïve patients. The remaining isolates showed no evidence of the I50L substitution and no obvious pattern of changes beyond accumulation of the primary and secondary resistance substitutions described previously to be involved in PI resistance. These isolates developed higher levels of resistance to the other PIs. All isolates from experienced patients who were treated with the PI combination of ATV/SQV become resistant to both ATV and SQV.

Atazanavir boosted with ritonavir (300/100 mg QD) provided comparable virological suppression to LPV/RTV based on the primary endpoint. At 24 weeks results were compatible with the predefined hypothesis of non-inferiority (upper limit of the confidence interval of the TAD between both arms <0.5 log copies/ml). This finding was supported by the 48 weeks data. ATV/RTV was numerically inferior to LPV/RTV for several secondary endpoints. The results of the post-hoc subgroup analysis of patients with viral stains harbouring less or more than 4 PI mutations were significantly in favour to LPV/RTV in patients with viral strain of more than 4PI mutations. Therefore, based on the virological and clinical data, no benefit is expected in patients with multi-PI resistant strains.

It is clear that atazanavir/ritonavir is not appropriate for salvage therapy.

Overall, atazanavir/ritonavir appears to be of lesser potency than lopinavir/ritonavir. However, the risk benefit relationship for the combination is considered acceptable in the light of the once daily dosing regimen, the low risk of dyslipidemia and the better gastro-intestinal tolerance compared with LPV/RTV.

Safety

Particular concerns have been raised with regard to cardiotoxicity, hepatotoxicity and hyperbilirubinemia. Atazanavir is associated with a dose-concentration dependent prolongation of PR interval. Atazanavir also increase the QTc interval. Dose and concentration relationships should be further discussed. Although these ECG changes were asymptomatic, clinical events such as palpitations and syncope should be monitored. A warning regarding concomitant administration with other drugs that have the potential to increase the PR interval and/or in patients with pre-existing conduction problems is included in the SmPC. Further investigations are necessary to better assess the cardiotoxic potential of atazanavir.

In common with other antiretrovirals, atazanavir is associated with hepatic events but does not appear to be associated with a higher risk of hepatotoxicity in comparison with other PIs. A warning regarding the potential increased risk for hepatic events in patients with pre-existing liver dysfunction is included in the SmPC, in line with the wording proposed by the CPMP for the other antiretrovirals. This population should be closely monitored.

Hyperbilirubinemia was observed frequently in atazanavir treated patients. The long-term consequences of mild and modest elevations in unconjugated bilirubin are generally believed to be inconsequential. No cases of neurotoxicity potentially attributable to bilirubin elevation were observed. Therefore, based upon the biology of unconjugated bilirubin elevations, the vast experience with Gilbert's syndrome, and the entire clinical trial database, chronic hyperbilirubinemia does not appear to represent a safety concern for the use of atazanavir.

Atazanavir therapy was not associated with undesirable changes in total cholesterol, fasting LDL cholesterol and fasting triglycerides. However, an increase in HDL cholesterol was noted in patients

treated by ATV400 mg unboosted whereas a decrease was observed with ATV/RTV 300/100 (e.g. +12% in study 043 versus -7% in studyAI424045 at week 24).

A specific study assessing the impact of atazanavir/ritonavir in patients with dyslipidemia at baseline has not been performed. The applicant is requested to provide such a study within the frame of post-approval commitment. Also, the long-term clinical implications of these findings remain to be investigated and the applicant has committed to investigate this issue.

The applicant committed to monitor events regarding lipids abnormalities, hepatic events, lipodystrophy hyperbilirubinemia, jaundice, icterus, rash, lactic acidosis and depression closely during post-marketing surveillance.

Benefit/risk assessment

In a written response to the day 180 LOI and during an oral explanation, the applicant addressed CPMP's concerns regarding efficacy in antiretroviral-experienced patients. Furthermore the applicant was asked to elaborate on the potential benefit of atazanavir as a low dyslipidemia inducer.

Atazanavir boosted with ritonavir (300/100 mg QD) provided comparable virological suppression to LPV/RTV in moderately experienced patients. However, based on the virological and clinical data (subgroup analyses performed in study 045 in patients with more than 4 PI mutations significantly favoured the lopinavir/ritonavir arm), the drug was not considered suitable to treat patients with multi-PI resistant strains. This was clearly reflected in the SPC.

Overall, atazanavir/ritonavir might not be as potent as lopinavir/ritonavir. However, the risk benefit relationship was considered acceptable in moderately experienced patients.

Since the efficacy and safety profile of 300ATV/100RTV is currently limited, the CPMP considered that an **approval under exceptional circumstances** was justified. The final report of the 48 week data of study 045 as well as additional clinical studies in antiretroviral experienced patients with dyslipidemia at baseline were made specific obligations in order to further substantiate the efficacy and safety of the drug in antiretroviral experienced patients. Moreover, the applicant was requested to provide complementary pharmacokinetic data (notably, interaction studies) with the recommended regimen, since the pharmacokinetic programme was mainly based on the use of atazanavir without ritonavir at the 400 mg QD regimen. The CPMP encouraged the applicant to explore the use of atazanavir boosted with ritonavir in antiretroviral naïve patients.

Some members of the CPMP took a divergent negative view and felt that the current lack of comprehensive efficacy and safety data did not allow a recommendation of a positive opinion.

Recommendation

Based on the CHMP review of data on quality, safety and efficacy, the CHMP considered by majority decision that the benefit/risk profile of Reyataz was favourable in the treatment of HIV-1 infected, antiretroviral treatment experienced adults, in combination with other antiretroviral medicinal products.