European Medicines Agency
Evaluation of Medicines for Human Use

Doc.Ref.:EMEA/123999/2008

ASSESSMENT REPORT

FOR

Volibris

International Nonproprietary Name: ambrisentan

Procedure No. EMEA/H/C/000839

Assessment Report as adopted by the CHMP with
all information of a commercially confidential nature deleted.

7 Westferry Circus, Canary Wharf, London, E14 4HB, UK
Tel. (44-20) 74 18 84 00 Fax (44-20) 74 18 84 16
E-mail: mail@emea.europa.eu http://www.emea.europa.eu
© European Medicines Agency, 2008. Reproduction is authorised provided the source is acknowledged

1. BACKGROUND INFORMATION ON THE PROCEDURE

1.1 Submission of the dossier

The applicant Glaxo Group Limited submitted on 2 March 2007 an application for Marketing Authorisation to the European Medicines Agency (EMEA) for Volibris, through the centralised procedure falling within the Article 3(1) and point 4 of Annex of Regulation (EC) No 726/2004. The eligibility to the centralised procedure was agreed upon by the EMEA/CHMP on 11 April 2005.

The legal basis for this application refers to:

A - Centralised / Article 8(3) / New active substance.

Article 8.3 of Directive 2001/83/EC, as amended - complete and independent application

The application submitted is a complete dossier composed of administrative information, complete quality data, non-clinical and clinical data based on applicants' own tests and studies and/or bibliographic literature substituting/supporting certain tests or studies.

The applicant Glaxo Group Limited submitted on 2 March 2007 an application for Marketing Authorisation to the European Medicines Agency (EMEA) through the centralised procedure for Volibris, which was designated as an orphan medicinal product EU/3/05/273 on 11 April 2005. Volibris was designated as an orphan medicinal product in the following indication: Treatment of pulmonary arterial hypertension and chronic thromboembolic pulmonary hypertension. The calculated prevalence of this condition was in the ranges from 0.187 to 0.26 per 100,000 EU populations.

The applicant applied for the following indication treatment of pulmonary arterial hypertension (PAH).

Similarity:
Pursuant to Article 8 of Regulation (EC) No. 141/2000 and Article 3 of Commission Regulation (EC) No 847/2000, the application contained a critical report addressing the possible similarity with authorised orphan medicinal products.

Scientific Advice:
Scientific Advice was received from the CHMP on 15 December 2005. The Scientific Advice pertained to quality and clinical aspects of the dossier.

Licensing status:
A new application was filed in the following countries: United States on 18 December 2006.
The product was not licensed in any country at the time of submission of the application.

The Rapporteur and Co-Rapporteur appointed by the CHMP were:
Rapporteur: **Concepción Prieto Yerro** Co-Rapporteur: **Martin Votava**

1.2 Steps taken for the assessment of the product

- The application was received by the EMEA on 2 March 2007.
- The procedure started on 21 March 2007.
- The Rapporteur's first Assessment Report was circulated to all CHMP members on 13 June 2007 . The Co-Rapporteur's first Assessment Report was circulated to all CHMP members on 8 June 2007.
- During the meeting on 16-19 July 2007, the CHMP agreed on the consolidated List of Questions to be sent to the applicant. The final consolidated List of Questions was sent to the applicant on 23 July 2007
- The applicant submitted the responses to the CHMP consolidated List of Questions on 5 October 2007.
- The Rapporteurs circulated the Joint Assessment Report on the applicant's responses to the List of Questions to all CHMP members on 29 November 2007.
- During the CHMP meeting on 10-14 December 2007, the CHMP agreed on a List of Outstanding Issues to be addressed in writing by the applicant.
- The applicant submitted the responses to the CHMP consolidated List of Outstanding Issues on 21 January 2008.
- The Rapporteurs circulated the Joint Assessment Report on the applicant's responses to the List of Outstanding Issues to all CHMP members on 5 February 2008.
- During the meeting on 18-21 February 2008, the CHMP, in the light of the overall data submitted and the scientific discussion within the Committee, issued a positive opinion for granting a Marketing Authorisation to Volibris on 21 February 2008. The applicant provided the letter of undertaking on the follow-up measures to be fulfilled post-authorisation on 18 February 2008.
- The CHMP adopted a report on similarity of Volibris with iloprost (Ventavis®), treprostinil (Remodulin®), bosentan (Tracleer®), sildenafil (Revatio®), and sitaxentan (Thelin®) on 19 July 2007.
- The CHMP opinions were forwarded, in all official languages of the European Union, to the European Commission, which adopted the corresponding Decisions on 21 April 2008.

2. SCIENTIFIC DISCUSSION

2.1 Introduction

Pulmonary Arterial Hypertension (PAH) is an uncommon disease of the small pulmonary arteries that is characterised by vascular proliferation and remodelling. It results in increased pulmonary artery pressure and pulmonary vascular resistance and, ultimately, right ventricular heart failure and death. The World Health Organization (WHO) functional assessment classification of PAH is as follows:

- Class I: PAH without a resulting limitation of physical activity. Ordinary physical activity does not cause undue dyspnoea or fatigue, chest pain, or near-syncope.
- Class II: PAH resulting in a slight limitation of physical activity. The patient is comfortable at rest, but ordinary physical activity causes undue dyspnoea or fatigue, chest pain, or near-syncope.
- Class III: PAH resulting a marked limitation of physical activity. The patient is comfortable at rest, but less than ordinary activity causes undue dyspnoea or fatigue, chest pain, or near-syncope.
- Class IV: PAH resulting in an inability to carry out any physical activity without symptoms. The patient has signs of right heart failure. Dyspnoea, fatigue, or both may be present even at rest, and discomfort is increased by any physical activity.

Treatment options are limited for PAH. At present, conventional treatment for patients with primary and secondary PAH includes calcium-channel blockers, anticoagulants, diuretics and oxygen. In addition, two oral endothelin-1 receptor antagonists (bosentan and sitaxentan), an intravenous prostacyclin (epoprostenol), an inhaled prostacylin (iloprost), a subcutaneous prostacyclin (treprostinil) and a phosphodiesterase-5 inhibitor (sildenafil) have also been licensed for the treatment of PAH in various European countries. Of these, bosentan (Tracleer®), iloprost (Ventavis®), sildenafil (Revatio®) and sitaxentan (Thelin®) have been authorised through the centralised procedure for orphan medicinal products. All these four medicinal products are only licensed for patients with NYHA/WHO functional class III. As a final alternative, a lung or heart/lung transplant may be offered to the patient.

The present application for marketing authorisation of Volibris is made under Article 8.3 (i) and concerns a new active substance, ambrisentan, for which a complete dossier has been submitted.

The approved indication for Volibris is: “the treatment of patients with pulmonary arterial hypertension (PAH) classified as WHO functional class II and III, to improve exercise capacity (see section 5.1). Efficacy has been shown in idiopathic PAH (IPAH) and in PAH associated with connective tissue disease”.

Volibris is to be taken orally at a dose of 5 mg once daily with or without food. Some additional efficacy has been observed with 10 mg Volibris in patients with class III symptoms, however an increase in peripheral oedema has also been observed. Patients with PAH associated with connective tissue disease may require 10 mg Volibris for optimal efficacy.

Ambrisentan was designated as an orphan medicinal product EU/3/05/273 on 11 April 2005, in the following indications: Treatment of pulmonary arterial hypertension and chronic thromboembolic pulmonary hypertension. The calculated prevalence of these conditions was in the ranges from 0.187 to 0.26 per 100,000 EU populations.

2.2 Quality aspects

Introduction

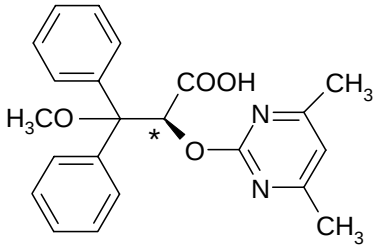
Volibris contains ambrisentan as the active substance. It is presented as immediate release film-coated tablets in two strengths of 5mg and 10 mg. To avoid confusion the two strengths have different colour, shape and markings.

Other ingredients in the tablet core include lactose monohydrate, microcrystalline cellulose, crosscaramellose sodium and magnesium stearate. The tablets are coated with an aesthetic film coating.

The tablets are packaged in opaque polyvinyl chloride/polyvinylidene chloride (PVC/PVdC) and aluminium foil blisters.

Active Substance

The chemical name of ambrisentan is (*S*)-2-(4,6-dimethylpyrimidin-2-yloxy)-3-methoxy-3,3-diphenylpropanoic acid.



* Chiral centre

The molecular structure of ambrisentan contains a single chiral centre with two possible enantiomeric forms (R- , S-enantiomers). The active substance is the resolved S-enantiomer that in non-clinical studies has been shown to be the most active.

Ambrisentan is a white to off-white crystalline substance that is practically insoluble in water (0.06 mg/ml), but soluble in alkaline buffer solutions and a range of organic solvents. There is only one crystalline form and no evidence of polymorphism.

The chemical structure of ambrisentan has been confirmed by elemental analysis, mass spectrometry, nuclear magnetic resonance spectrometry, single crystal X-ray diffraction, UV and IR spectrophotometry.

- Manufacture

The active substance is synthesised in four steps using benzophenone, methyl chloroacetate and 4, 6-dimethyl-2-methyl-sulfonyl-pyrimidine, as starting materials. The synthetic process is designed to selectively produce the S-enantiomer.

The impurity profile of ambrisentan has been extensively studied. All potential impurities have been identified and characterised and appropriate levels have been set supported by the results of toxicological studies. All solvents used in the manufacture of the active substance are controlled in compliance with ICH guidelines.

Batch analysis data from nine non-clinical, clinical and stability batches and three production scale batches demonstrate the ability of the manufacturing process to produce a constant crystalline form.

- Specification

The active substance specification includes tests for appearance, identification, water content, assay (IR, chiral HPLC), specified impurities (HPLC), enantiomeric purity (chiral HPLC), residual solvents (GC), heavy metals (Ph.Eur.), sulfated ash (Ph.Eur.) and particle size (sieving).

The analytical methods employed are either pharmacopoeial or have been adequately described and validated and are considered to be suitable to control the quality of the active substance.

Batch analysis data have been provided for six batches manufactured by the method intended for commercial manufacturing and six batches manufactured by an earlier slightly different synthetic process. In all cases the results comply with the proposed specifications.

- Stability

Stability studies have been performed in accordance with ICH requirements. Samples from three batches manufactured using the process intended for marketing have been stored at 40ºC/75%RH for 6 months and at 25ºC/60%RH for 24 months.
The parameters tested were appearance, water content, related substances, R-enantiomer and assay.
The analytical methods used were the same as those used for routine testing and have been shown to be stability indicating. In all cases the stability results presented were satisfactory and support the proposed shelf life.

Medicinal Product

- Pharmaceutical Development

Ambrisentan is formulated as immediate-release film coated tablets in 5 and 10 mg strengths for oral administration. The active substance is considered to be a "low solubility and high permeability drug" (Class II in the Biopharmaceutics Classification System, (BCS)). Since it is practically insoluble in water, dissolution is the rate eliminating step for its release. Therefore the particle size of the active substance is considered to be a critical quality attribute and a control test has been introduced in the specifications. In addition a dissolution test has been developed to control the release of the active substance. The test is using a biorelevant medium and a pH within the range, where ambrisentan is soluble, in order to avoid the use of surfactants. The discriminatory power of the dissolution test to identify formulation or manufacturing changes with an impact to product quality has been satisfactorily demonstrated.
The excipients used in the formulation development are commonly used in oral dosage forms. A non-bovine (vegetable) source of magnesium stearate is used, while lactose monohydrate is manufactured using milk sourced from healthy animals in the same conditions as milk collected for human consumption in accordance with the "Note for Guidance on Minimizing the Risk of Transmiting Animal Spongiform Encephalopathy Agents via Human and Veterinary Medicinal Products". All excipients comply with their corresponding European Pharmacopoeia monographs and there is no need for additional requirements to assure consistent product performance. The compatibility of the excipients with the active substance has been demonstrated through appropriate stability studies.
Ambrisentan tablets are packaged into blister strips, formed with opaque polyvinyl chloride (PVC) film coated with polyvinylidene chloride (PVdC). The packaging materials comply with the requirements of the Directive pertaining to suitability for direct contact with foodstuffs.

- Manufacture of the Product

The manufacturing process is a standard direct compression process and consists of the following steps: mixing, tableting and film coating.
All critical process parameters have been identified and are controlled by appropriate in process controls. The reproducibility and robustness of the manufacturing process has been sufficiently demonstrated with the manufacture of several production scale batches. At the time of approval no validation studies have been performed, however the applicant has submitted the process validation scheme for the finished product, which will be applied to three consecutive production scale batches prior to commercialisation.
Different product formulations have been used in the early studies compared to the one intended for marketing. However bioequivalence between the clinical trial formulations and the one intended for marketing has been demonstrated.

- Product Specification

The specification of the finished product at release and shelf life includes tests for appearance, identification (UV and HPLC), assay (HPLC), impurities (HPLC), content uniformity (Ph.Eur.), dissolution and microbial purity (HPLC). All tests included in the specification have been satisfactorily described and the analytical procedures employed are adequately validated.
Batch analysis data from 14 stability and clinical batches (5 and 10 mg tablets) have been presented.
All batches met the test limits as defined in the release specification and test methodology valid at the time of batch release.

- Stability of the Product

Stability studies were carried out on three primary stability batches for each strength manufactured at the commercial site and scale according to ICH requirements. Samples were stored at 25oC/60 % RH for 18 months, 30°C/65 % RH for 12 months and 40°C/75 % RH for 6 months. The parameters tested were appearance, assay, related substances, dissolution, hardness and water content.
In all cases the stability results presented were satisfactory and support the proposed shelf life for the commercially packaged product under the conditions specified in the SPC. For both the 5 and 10 mg tablets stored under accelerated conditions a considerable decrease in tablet hardness was observed. However this did not result in a decrease in ambrisentan content, formation of degradation products or change in the dissolution profile. Therefore, the observed changes in tablet hardness do not impact product quality or functionality.
Data have also been presented from forced degradation and photostability studies. The results demonstrate that the finished product does not require additional protection from light and that significant degradation is only observed under acidic or oxidative stress conditions.

Discussion on chemical, pharmaceutical and biological aspects.

The quality of Volibris is adequately established. In general, satisfactory chemical and pharmaceutical documentation has been submitted for marketing authorization. There are no major deviations from EU and ICH requirements.

The active substance is well characterised and documented. It exhibits low solubility and high permeability (Class II in BCS) and a suitable dissolution method has been developed to test its release. The excipients are commonly used in immediate release oral formulations and comply with Ph. Eur. requirements. The packaging material is commonly used and well documented. The manufacturing process of the finished product is a standard direct compression process that has been adequately described. Stability tests indicate that the product under ICH guidelines conditions is chemically stable for the proposed shelf life.

2.3 Non-clinical aspects

Introduction

Two sources of data were used for the summarized non-clinical results in this application: studies initiated by Knoll AG, Germany and studies undertaken by or for Gilead Colorado Inc., USA (formerly Myogen Inc., USA).
Investigation of the toxicological properties of ambrisentan commenced in 1999 with the last reports completed at the end of 2006.

Safety pharmacology (24 out of 27) and all pivotal toxicology studies were conducted in compliance with Good Laboratory Practice (GLP) regulations. GLP compliant studies were performed under conditions commensurate with national and international regulatory guidance that were current from years 1997 through 2006. Pharmacokinetics were not determined in the safety pharmacology studies (years 1998 to 2000), but toxicokinetics were routinely evaluated in the series of GLP toxicology studies (years 1998 to 2006).

The non-clinical studies were performed using several different batches of ambrisentan drug substance. The spectrum of impurities and degradants present in these batches was in general comparable to that proposed for the commercial specification for drug substance and drug product, unless for the R-enantiomer which was below the specification of 0.5%. The proposed specification for ambrisentan contains no impurities at levels above ICH qualification thresholds. Therefore no specific studies have been performed to investigate the non-clinical properties of impurities, degradation products or formulation excipients.
All non-clinical safety studies have been undertaken with ambrisentan, the resolved S-enantiomeric form with >99% enantiomeric purity.

In this assessment report, CHMP/ICH Non-clinical Guidelines have been considered, and mainly Note for guidance on safety pharmacology studies for human pharmaceuticals CPMP/ICH/539/00 (ICH topic S7A), Note for Guidance on the non-clinical evaluation of the potential for delayed ventricular repolarization (QT interval prolongation) by human pharmaceuticals CHMP/ICH/423/02 (ICH topic S7B), Guideline on detection of early signals of drug-induced hepatotoxicity in non-clinical studies CHMP/SWP/150115/2006 (Draft), Notes for Guidance on the limits of genotoxic impurities CPMP/SWP/5199/02 and CHMP/QWP/251344/2006, Note for Guidance on the need for carcinogenicity studies of pharmaceuticals CPMP/ICH/140/95 (ICH topic S1A), Note for Guidance on carcinogenicity: testing for carcinogenicity of pharmaceuticals CPMP/ICH/299/95 (ICH topic S1B), and Dose selection for carcinogenicity studies of pharmaceuticals & Limited Dose CPMP/ICH/383/95 (ICH topic S1 C (R1)), Note for Guidance on immunotoxicity studies for human pharmaceuticals CHMP/167235/2004 (ICH topic S8), Guidance on the environmental risk assessment of medicinal products for human use CHMP/SWP/4447/00, and the multidisciplinary Guideline on Investigation of Chiral Active Substances.

Pharmacology

The primary and secondary pharmacodynamics (PD) studies were not evaluated with respect to ambrisentan's action in males and females separately.
In 2 non-clinical studies both male and female animals were used; however, the data were averaged across gender, suggesting that ambrisentan was effective independent of sex. This approach was considered acceptable.
The lack of an animal PAH model to describe PD profile of ambrisentan was also adequately justified by the applicant.

- Primary pharmacodynamics

Ambrisentan is an orally active, non-sulfonamide, propanoic-class, endothelin receptor antagonist (ERA) that is selective for the endothelin type A (ET_A) receptor. ET_A receptor antagonists inhibit phospholipase C-mediated vasoconstriction and protein kinase C-mediated cell proliferation, while preserving nitric oxide (NO) and prostacyclin production, cyclic GMP- and cyclic AMP-mediated vasodilation, and endothelin-1 (ET-1) clearance that is associated with the endothelin type B (ET_B) receptor.

ERAs have proven therapeutic benefit in the treatment of PAH in humans. Ambrisentan has a high affinity (K_i of 16 pM) against human myocardial native ET_A receptors, with selectivity for the ET_A receptor of approximately 4000-fold relative to the ET_B receptor. Under the experimental conditions of the study MSR-0001, ambrisentan demonstrated higher affinity (lower Ki) than the prior approved ERAs, bosentan and sitaxentan. The selectivity for the ET_A receptor was in line with sitaxentan (Ki ratio ETB: ETA 4347±1237 vs 3302±365, mean ± SEM, respectively).

Oral doses of ambrisentan were demonstrated to block the pressor effects of exogenous endothelin in the intact rat, to decrease arterial blood pressure in intact rats and dogs and to inhibit neointimal proliferation after arterial damage in the pig. Ambrisentan showed a vascular antiproliferative effect in the balloon catheter injured coronary artery of the pig, an action that suggests this drug has the potential to attenuate the detrimental pulmonary vascular remodelling common to PAH patients, as consequence of proliferation of myofibroblast cells in the intima of small pulmonary arteries.

- Secondary pharmacodynamics

When tested for specificity using a battery (100) of receptors and ion channels, ambrisentan, at 10 μM was not active (<50% inhibition). The R-enantiomer and 4-hydroxymethyl metabolite of ambrisentan (BSF 379912) were also inactive in a similar specificity panel. According to these results, ambrisentan is unlikely to interact with a wide variety of receptors/ion channels at concentration similar or slightly above the clinical plasma levels. However, it is observed that the studies to investigate the binding response curves to histamine and acetylcholine of ambrisentan have been performed for affinity to

wide receptor panels, high-throughput profile and contraction fixed concentrations up to 10 μM, which means a low safety margin of approximately 3-fold the clinical plasma concentration.
The applicant conducted a study of ambrisentan effects on haemolysis and coagulation (MPF/FG9924E). Human blood samples were used in this study. Presented values of haemoglobin (Hb) concentration were different from the average values (decimal place and absolute value). This difference was adequately clarified. Hereafter there was a decrease of Hb concentration independent on the dose administered.

- Safety pharmacology programme

A complete core battery of safety pharmacology on vital functions and several supplemental studies on other organ systems have been performed. All the batches used in preclinical and clinical studies contained only up to a 0.3% of the R- enantiomer, whereas the proposed quality specifications for ambrisentan claim a content of this impurity up to 0.5%. Consequently, the safety pharmacology and toxicity preclinical studies conducted with ambrisentan support the qualification of the R-enantiomer impurity only up to 0.3 % in most cases. Up to 5% was considered qualified *in vivo* (see Impurities in Toxicology Section).

The maximum concentration of ambrisentan in the *in vitro* safety pharmacology studies reached 10 μM, with the exception of the hERG assay where ambrisentan was tested at 10 μM and 100 μM. In the *in vivo* studies, doses used were 10 to 300 mg/kg administered via intravenous (i.v.) or oral route. Animal exposure achieved was not measured. Nevertheless, the safety margins were roughly estimated where possible based on plasma concentration data from repeat dose toxicology studies. For *in vitro* studies, the safety margin based on C_{max} is low (approximately 3). For *in vivo* studies, the safety margin based on animal to human exposure ratio occasionally is also low.

Several possible central and peripheral nervous system-related toxicological findings are observed in the safety pharmacology studies reports (e.g. increased aggressive behaviour and startle response in mice Irwin's test) and in the chronic toxicity studies (e.g. hypersalivation, vomiting in dogs). Locomotor activity and motor coordination study results concluded that ambrisentan had no effect on motor activity in mice.

No relevant effects on cardiac output, ECG, blood velocity, respiratory rate, tidal volume, minute volume and minute ventilation were observed in anesthetized dogs treated with ambrisentan (MPF/FG 9910E). In this study, Q-T prolongation did not occur, but across the chronic toxicology studies in dogs, a slight Q-T interval prolongation was reported in 2 of 3 studies (7 to 8% and 9 to 11%). In primary PD study MPF/FE 9924 on conscious male normotensive dogs, QT remained basically unaffected, but QTc intervals could not be calculated since individual animal line item data were not available.

Ambrisentan had no significant effect on hERG tail current (eliciting a 6.3% and 17.5% inhibition at 10 and 100 μM, respectively) and had no effect on action potential parameters (duration of action potential (APD) repolarization, maximum upstroke velocity, and amplitude of the action potential) of isolated guinea pig papillary muscle. It is concluded that ambrisentan is unlikely to cause any relevant cardiac effect in humans.

- Pharmacodynamic drug interactions

Studies of non-clinical PD drug interactions have not been performed with ambrisentan. There is the potential for clinical drug-drug interactions with concomitantly administered vasodilators including other specific treatments for PAH (e.g., prostenoids and sildenafil). The potential for such interactions has been monitored in the clinic setting, and therefore the lack of preclinical studies is acceptable.

Pharmacokinetics

Studies characterizing the ADME properties of ambrisentan in pre-clinical species showed that the disposition characteristics were generally similar across the species tested.

Following i.v. administration, ambrisentan resulted to be a low clearance compound with low to moderate volume of distribution in all preclinical species. The elimination was characterized as triphasic, with terminal half-life of about 6-8 hours in rats and dogs. In humans, the terminal half-life following oral administration was determined to be approximately 15 hours (range 13.6 to 16.5 hours) in healthy volunteers and 9 to 15 hours in PAH patients.

Absorption

Ambrisentan was well absorbed following oral administration. It also showed high absolute oral bioavailability in preclinical species, indicating that it undergoes little or no first pass metabolism. In the toxicology studies, toxicokinetic results demonstrated that escalation in dose generally resulted in a proportional increase in systemic exposure. The exposure was generally higher in females than in males; however, the differences were generally less than 2-fold. A relationship between higher systemic exposure and a greater susceptibility to the toxicological effects of ambrisentan was seen more in female mice and dogs than in males. This was not observed in rats. Consequently, it is concluded that the occurrence of gender-related AUC variation and AUC oscillations over the time in all species cannot be ruled out. Ambrisentan exposures determined periodically in repeat dose studies in mouse, rat and dog, showed, although not consistently, a reduction in AUC over the time in a chronic treatment (beyond week 26). The reason for this exposure decrement is unknown, although it could be due to reduced absorption and/or increased elimination of ambrisentan. The population pharmacokinetic analysis did not however show any clinically significant gender difference in pharmacokinetics of ambrisentan in humans. Therefore, although change in exposure over time was observed in preclinical species, pharmacokinetics in humans has shown to be time-independent in both healthy volunteers and in PAH patients.

Distribution

In vitro results showed that ambrisentan binds to plasma proteins to a higher extent in humans (98.9%) than in preclinical species (91.8-97.2%). In human serum, it was apparent that albumin was the primary binding protein in human plasma.

The results of a rat tissue distribution study with [14C]-ambrisentan indicated a wide distribution of drug into tissues but elimination occurred relatively rapidly. There was no evidence of retention in any tissues, including melanin-containing tissues. Since ambrisentan is not anticipated to pose a phototoxic risk to humans, the lack of studies of phototoxicity is justified.

No data on milk excretion and placental transfer have been provided and no further investigations are requested since ambrisentan is considered to be teratogenic.

Metabolism

Metabolism data obtained following administration of [14C]-ambrisentan showed that metabolic pathways for ambrisentan were qualitatively similar in various species. The metabolites identified include 4,6 dimethyl-2-hydroxypryimidine (M1), ambrisentan glucuronide (M2), hydroxylated ambrisentan (M3), O-demethylated ambrisentan (M4), dihydroxylated ambrisentan (M5), dihydroxylated ambrisentan glucuronide (M6), hydroxylated ambrisentan glucuronide (M7), and O-demethylhydroxymethyl ambrisentan (M8). M7 was not detected as a circulating metabolite in pre-clinical species, however, it represented <2% of the plasma radioactivity in humans. Acyl glucuronide metabolite (M2) was a substantial metabolite in dog plasma accounting for 21-28% of radioactivity, and a minor metabolite in mouse, rat, and rabbit (5% of radioactivity). The formation of acyl glucuronide metabolites has been associated with immunogenic responses. Nevertheless, according to the applicant, the acyl glucuronide of ambrisentan (M2) is unlikely to cause allergic reactions due to the lack of covalent binding to proteins observed in an *in vitro* study with dog plasma. This result suggests that the likelihood for protein adduct formation that could act as hapten is low, but this possibility cannot be excluded, since the immunogenicity of ambrisentan and its metabolites has not been tested (see Antigenicity in Toxicology section).

Hydroxylated ambrisentan (M3), the only other major metabolite observed in human plasma was also seen in the mouse, rat, and rabbit plasma, but not in dog plasma.

Excretion

Based on disposition studies conducted with [14C]-ambrisentan, it was apparent that the primary route of excretion of drug-related material was faeces in all preclinical species as well as in humans. In

humans, about 66% of the dose was recovered in faeces and in animal species, the faecal recovery generally accounted for 69%-91% of the dose. Urinary excretion was a minor route of elimination in both animal species (7-23%) as well as in humans (23%). Studies conducted in bile duct-cannulated rats and dogs suggested that in large part, faecal radioactivity likely represented absorbed dose that was secreted in bile. Also in a rat study, results suggested the potential for a substantial enterohepatic recirculation of ambrisentan and possibly its metabolite.
All three metabolites observed in human excreta, M2, M3, and M7, were also observed in preclinical species, either in urine, or faeces, indicating that these metabolic pathways for ambrisentan observed in humans were also operative in animal species.

Pharmacokinetic drug interactions
Ambrisentan is metabolised by phase I oxidative metabolism and by phase II hepatic glucuronidation. About 30% of the administered dose undergoes oxidative metabolism mainly by CYP3A4 and to a lesser extent by CYP3A5 and CYP2C19. The induction effect of ambrisentan on hepatic phase I and II enzymes was examined *ex vivo* in both rats and dogs, following repeated oral administration during toxicology studies. There was no clear evidence from these studies that ambrisentan has the potential to induce cytochrome P450 enzymes, glutathione-S-transferase (GST) or UDP-glucuronosyltransferase (UDP-GT) concentration or activity in rodents, at clinically relevant concentrations. Upon incubation of ambrisentan with liver microsomes, the formation of glucuronide metabolite was unaffected by the presence of any of the 24 potential concomitant drugs at concentrations up to 10-times the maximal therapeutic levels expected in humans. The above results suggest that ambrisentan is unlikely to be involved in metabolism-related drug-drug interactions. The potential effect of ambrisentan on human hepatic cytochromes has not been totally clear (see Clinical PK section). Oral treatment for 4 weeks of Wistar rats produced an increase of CYP4A and a slight to moderate increase of UDP-GT in males and females, at 400 mg/kg/day (Report MPF/DT 6199).

Oral treatment for 4 weeks in dogs produced strong evidence that ambrisentan acts as a moderate to marked phenobarbital-type like P450 inducer, with slight to moderate induction of cytochrome b5 (38 to 85%) and total cytochrome P450 (22 to 97%), both in males and females (Report MPF/PT 3301). The hepatocellular centrilobular hypertrophy observed in dogs treated with 1000mg/kg/day for 4 weeks was associated with increased cytochrome P450. The mechanism underlying the same finding in mice treated with 150 mg/kg/day, and in rats given >100 mg/kg/day remains unclear (see Repeat Dose liver finding in the Toxicology section).

The effect of ambrisentan on human hepatic CYP450 was evaluated *in vitro* on cDNA-expressed human CYP isoforms (Report MPF/DDM 0025). The results showed that ambrisentan is a slight inductor of CYP1A2 (32%) and had a very week inhibitory effect on CYP3A4 (10%) at a concentration of 10 μM (approximately 3-fold the clinical C_{max}). Bosentan and sitaxentan by contrast are principally metabolized by hepatic cytochromes and are an inducer and inhibitor of CYP3A4 respectively.

Data was presented concerning influence of CYP3A4 inhibition on metabolism of ambrisentan, and also on ambrisentan effect on inhibition of CYP isoforms. Exposure of ambrisentan expressed with increased $AUC_{(0-\infty)}$ and C_{max} values after co-administration of strong CYP3A4 inhibitor is of no clinical significance.

Ambrisentan did not inhibit human Pgp-mediated transport of digoxin in MDCK cells at concentrations up to 100 μM, but it was a substrate for P-gp and its transport was inhibited a 56% by cyclosporine in a CaCo-2 monolayer model.

In studies using the rat hepatocyte sandwich cell culture model, it was found that ambrisentan had no inhibitory effect on the four transporters investigated. Under the same conditions, marked concentration-dependent inhibition was observed with two other endothelin receptor antagonists, bosentan and sitaxentan, that are structurally distinct from ambrisentan.
Results of studies conducted to evaluate the inductive effects of ambrisentan on hepatic transport suggest that the likelihood of ambrisentan having an adverse interaction with hepatic transporters is low.

Toxicology

The toxicity profile of ambrisentan has been defined in oral and i.v. single dose studies in mouse and rat, and in oral repeat dose studies of up to 13 weeks duration in mice, 6 months duration in rats and 9 months duration in dogs.

In all the definitive repeat dose studies, including carcinogenicity and embryofetal development toxicity studies, toxicokinetics were evaluated. With the exception of 2 i.v. single dose studies, all other studies used an oral administration route (gavage, capsule or diet admixture). Toxicokinetic measurements in dietary studies were comparable to those obtained in gavage studies. For therapeutic administration ambrisentan has been formulated for oral administration at dosages up to 10 mg/day (equivalent to 200 µg/kg to a 50 kg person). At the highest dose of 10 mg, given once daily for 12 weeks to subjects with PAH (two females and one male), the steady state $C_{max\ ss}$ was 1.19 µg/ml and $AUC_{ss\ 0\text{-}24h}$ was 14 µg.h/ml [Report AMB-220].

- Single dose toxicity

The maximum oral non-lethal single dose of ambrisentan is 1000 mg/kg in the mouse, and 3160 mg/kg in the rat. The maximum i.v. non-lethal single dose of ambrisentan is 511 mg/kg in the mouse, and 464 mg/kg in the rat. Clinical signs observed at lethal doses included lassitude, forced respiration, prone position, partial palpebral closure, convulsions, and injection site reactions (tail necrosis). Necropsy findings on decedents revealed congestion in the lungs, liver and kidneys.

- Repeat dose toxicity (with toxicokinetics)

Ambrisentan was well tolerated for up to 3 months in mice at a NOAEL of 60 mg/kg/day, based on findings in the nasal cavities. In male rats dosed for 6 months, the NOAEL was lower than the low dose of 5 mg/kg/day due to an increased incidence of diffuse testicular tubular atrophy. In females, the NOAEL was 5 mg/kg/day based on nasal cavity findings. Systemic exposures in rat and mouse at these dose levels were below the estimated efficacious human exposure level. In dogs, the NOAEL could not be established, due to testicular atrophy in males and rales in females treated at the lowest dose level of 30 mg/kg/day. The safety margin for these findings should be <6- fold the clinical AUC, in any case. For the rest of toxicological effects, the NOAEL was established to be 100 mg/kg/day based on clinical observations of audible breathing sounds, emesis and occasional behavioural changes. This dose corresponds to approximately 14-fold and 17-fold the estimated efficacious human exposure level, respectively.

Nasal cavity effects

Nasal inflammation and epithelial degeneration effects observed in the nasal cavity of rodents with ambrisentan occurred at exposures below the estimated human exposure. These findings are similar in nature to the degenerative changes of the olfactory epithelium reported for sitaxentan [EPAR for Thelin (sitaxentan), 2006]. Nasal changes with bosentan are limited to flushed appearance, inflammation of the throat and nasal passages [EPAR for Tracleer (bosentan), 2005].
In the dog, minimal to slight purulent recoverable inflammation was observed in the nasal cavity for animals dosed at 900 mg/kg/day for 26 weeks, corresponding to an AUC of 190.0 and 243.5 µg hr/ml in males and females, respectively.

The implication of these findings for humans is unknown, however, an elevated incidence of upper respiratory tract conditions (including nasal congestion, rhinitis and sinusitis) have been reported in clinical studies.

Nasal bone hyperplasia of the ethmoid turbinates observed in the nasal cavity of rats treated with ambrisentan, at exposure level 3- fold the clinical AUC, are not reported for other ERAs and its possible mechanism remains unexplained.

The applicant was requested to further clarify the potential mechanism causing the nasal bone hyperplasia effect of ambrisentan and its possible clinical relevance. The mechanism of this effect is not clear but an association with inflammatory process in the nasal cavity area is suggested. The possibility of relation between endothelin-1 level and incidence of nasal cavity effects is very likely. The statement regarding this finding in animals has been maintained in the SPC since similar effects in humans after long-term exposure cannot be discarded.

Testes/epididymides
Testicular tubular atrophy, occasionally associated with aspermia, not always fully recoverable, has been observed in all tested preclinical species, including dogs, without safety margin. The relevance of these findings to human males is unknown, however, no adverse effects were observed in a limited analysis of semen samples and of male fertility hormones from clinical study AMB320/321. Further clinical data however will be provided post-authorisation.

Testicular tubular atrophy has also been reported with other approved endothelin receptor antagonists.

Liver
Long-term administration of ambrisentan revealed centrilobular hypertrophy in mice treated with 150 mg/kg/day, in rats given ≥100 mg/kg/day and in dogs treated with 250 (females) or 500 mg/kg/BID (males) treated at 500 mg/kg/BID.

In mouse studies after 13 weeks of treatment at doses of 500 mg/kg/day and above, there were increases (2- to 3-fold concurrent controls) in serum AST and ALT levels in females, associated with slight fatty change, with a safety margin of 2- fold, based on clinical AUC. Increases in AST, ALT and AP have been observed in male rats receiving 500 mg/kg/day for 26 weeks, at exposure levels approximately 15- fold the human AUC. In dogs, females treated with 500 mg/kg/BID displayed a marked increase (5-fold) ALT activity, with an approximate safety margin of 40- fold the clinical AUC.

In mice and dogs some relationship was observed between the pattern of susceptibility to the toxicological liver effects of ambrisentan and the higher systemic exposure, this was seen in females more than in males, and not observed in rats, thus supporting a gender-related AUC variation.

According to the Guideline on detection of early signals of drug-induced hepatotoxicity in non-clinical studies CHMP/SWP/150115/2006 (Draft), the occurrence of histopathological liver alterations and the increase of ALT activity in the range of 2-4-fold higher than controls should be sufficient to raise concern as an indication of potential hepatic injury.

In order to address the potential hepatotoxicity of ambrisentan the applicant was requested to comment more in depth on the relationship of the content in P450, the histopathological liver alterations and the serum hepatic enzyme changes observed in the repeat dose toxicity studies in mice, rats and dogs. Further discussion on the relevance of these findings to predict human hepatotoxicity was also requested. Furthermore, the applicant was requested to justify the lack of *in vitro* mechanistic tests for potential hepatotoxicity (step II), given the known class effect and the early hepatotoxic signals occurring *in vivo* (step I). This issue was addressed, and the conclusion reached, based on the *in vivo* experience with ambrisentan in multiple preclinical species, is that ambrisentan does not present a hepatic toxicology concern (step I). Accordingly, additional studies are not considered to be warranted. This conclusion is reflected in the SPC.

- Genotoxicity

A complete genotoxicity studies battery has been conducted with ambrisentan. There are no concerns on the mutagenic or a clastogenic risk to humans.

- Carcinogenicity

Lifelong treatment with ambrisentan was not associated with an increase in the incidence of neoplasms in mice and rats.

Oral administration of ambrisentan to rats at 30/20 mg/kg (dose lowered on week 51) and mice at 100 mg/kg for 104 weeks did not result in an increased incidence of neoplasms. These doses correspond to AUC of 55.6 and 68.7 µg.h/ml in the rat (exposure <5- fold AUC at the MRHD) and 11.8 and 19.1 µg.h/ml in the mouse (exposure <2- fold AUC at the MRHD), for males and females, respectively. Nevertheless, these results might be flawed by the treatment early termination due to declining survival of the high dose group on week 69 for males and week 93 for females in rats, and on week 76 in females and week 96 in males in mice. Besides, the female mice high dose group was terminated at week 84 due to low survival while high dose males were maintained to scheduled termination. These limitations might have hampered the potency of the studies to identify carcinogenic potential. No additional studies were considered to be required. The applicant however was requested to discuss further in depth the consequences of the early treatment termination and poor survival on the interpretation of carcinogenesis results, clearly defining the mechanism of ambrisentan's pharmacological activity to which the non-neoplastic effects observed in long-term studies are attributed. It was concluded that administration of ambrisentan to rodents likely results in increased levels of endogenous endothelin-1. However, relevant increase in level of endothelin-1 was not observed in humans after administration of therapeutic dose of ambrisentan. Respiratory distress, especially nasal cavity inflammation and hyperplasia as consequence of increased level of endogenous endothelin-1 are unlikely to occur in humans and were not observed in the presented clinical studies.

- Reproduction Toxicity

Ambrisentan treatment resulted in testicular tubular atrophy, altered sperm morphology, a slight increase in pre-implantation loss, and a slight reduction in male and female fertility indices. The occurrence of testicular tubular atrophy and associated infertility did not fully reverse after a 13 week off-treatment recovery period. There were no test-item related effects on embryonic early development when ambrisentan was administered directly to pregnant female rats, or indirectly via treatment of males. The NOAEL for male fertility was less than the low dose of 10 mg/kg/day. The NOAEL for female fertility (due to the slight increase in pre-implantation loss), and early embryo-foetal development (gestation days 0-6), is 100 mg/kg/day.

In a subsequent male fertility study, dosed at 300 mg/kg/day, no effects were observed in reproduction parameters, however reductions in epididymal weight, sperm count, and decreased numbers of morphologically normal sperm were observed at the end of treatment, which reversed following a 20 week recovery period. Microscopic evaluation revealed testicular tubular atrophy at the end of treatment, which failed to fully reverse.

Ambrisentan was shown to be teratogenic to rats and rabbits when administered on gestation days 6 through 18 in embryo-foetal development studies, at exposures lower than 4-fold and 2- fold the AUC at the MRHD. In view of the current guidelines and the legal basis, and in particular due to the evidence of a non-clinical and clinical teratogenic effect, the evidence of a known direct pharmacologic effect leading to malformations, and the availability of alternative therapeutics for PAH, a strict pregnancy contraindication has been included in the SPC.

The effect of ambrisentan on pre- and post-natal development and maternal function was assessed in rats. Administration to female rats from late-pregnancy through lactation caused adverse events on maternal behaviour, reduced pup survival and impairment of the reproductive capability of the F1 (with observation of small testes at necropsy), at exposure 3- fold the AUC at the MRHD. At 150 mg/kg/day, ambrisentan caused delayed vaginal opening in the offspring. These findings are reflected in the SPC.
The male and female offspring of dams that received 150 mg/kg/day showed decreased fertility (fewer animals mated, fewer became pregnant). The NOAEL was 15 mg/kg/day.

Toxicokinetics was not investigated in pups.

The extent of ambrisentan excretion in milk was not evaluated.

Studies in which the offspring (juvenile animals) are dosed and/or further evaluated

No studies in juvenile animals have been performed, which is acceptable, since ambrisentan is not recommended for use in children under the age of 18 years due to insufficient data.

- Local tolerance

An assessment of ambrisentan local tolerance (oral, GI), based on the toxicological finding observed in the repeat dose toxicity studies as well as in the carcinogenicity study with orally administered ambrisentan was presented. Of special interest were the respiratory tract findings and whether these findings, including rales, could be attributed to local intolerance or to other causes and their relation to humans and the course of the primary disease.

The findings observed within the respiratory tract are believed to be related to inflammatory processes in the nasal cavity, consequence of the anticipated pharmacology of ambrisentan. The proposed mechanism of the nasal cavity inflammation/degenerative epithelial changes is due to ambrisentan administration resulting in elevated levels of endogenous endothelin.
In a study investigating the effects of a single dose of ambrisentan on the cardiovascular parameters of dogs, a dose related increase in the plasma level of endothelin was observed, with a 20-fold elevation evident at the highest dose of 100 mg/kg. It was concluded that a clinically relevant dose (i.e: up to 10mg/subject/day) of ambrisentan does not present a local tolerance concern as the elevation of endothelin-1 level observed in human is considered safe relating to that observed in dog. In addition, the effects observed in the nasal cavities are a consequence of anticipated pharmacology, which are exacerbated in obligate nose breathing species.

- Other toxicity studies

Antigenicity
The immunogenicity of ambrisentan and its metabolites has not been investigated, despite the formation of acyl glucuronide metabolites has been associated with immunogenic responses. Nevertheless, the acyl glucuronide of ambrisentan (M2) is unlikely to cause allergic reactions due to the lack of covalent binding to proteins observed in an in vitro study with dog plasma.
Signs of antigenic stimulation have been observed in the toxicological studies, e.g. nasal inflammatory conditions, reddening of the sclera and watery and/or mucous ocular discharge.

Immunotoxicity
In unstimulated murine spleen cells, ambrisentan showed no mitogenic activity. In mitogen-stimulated murine spleen cells, neither suppressive nor co-mitogenic effects were observed over the complete concentration range tested.
Decreased white blood cell counts (WBC) were observed at a dose of 500 mg/kg/day following 26 weeks in the rat, and at 150 mg/kg/day following 13 weeks in the mouse. No histological changes were observed in bone marrow cellularity or morphology.
Mesenteric nodes alterations compatible with chronic excessive antigenic exposure have been reported in the repeated-dose toxicity studies. Thymus atrophy was also seen in the repeat-dose toxicity studies in mice and in dogs. Although this effect could be due stress, an immunotoxic effect of ambrisentan could not be excluded, since the immunotoxic potential had not been thoroughly investigated.

The above findings raised the need to consider further immunotoxicity studies, including those to elucidate the potential for immune-mediated hepatotoxicity (as recommended in the Note for Guidance on immunotoxicity studies for human pharmaceuticals CHMP/167235/2004 (ICH topic S8)). The effects on thymus, lymph nodes and WBC occurred only at doses that produced excessive toxicity or were lethal. Potential immunotoxic signals from the general toxicity studies were generally considered secondary to poor health status and stress, or secondary to lesions in other target organs, as opposed to direct drug effects. Additionally, there is no elevated cause for concern of immunotoxicity raised by the Note for Guidance document namely: 1) pharmacological properties of ET_A receptor

antagonists, 2) intended patient population (patients with life threatening PAH), 3) structural class, 4) disposition of the drug, or 5) signs from clinical trials. Therefore further immunotoxicity studies are not considered necessary. Studies to elucidate the potential for immune-mediated hepatoxicity are not considered necessary either, as ambrisentan does not present any evidence of hepatotoxicity (see Toxicology section).

Dependence
No studies were done to assess the abuse liability/dependence of ambrisentan. However, due to the characteristics and intended use of ambrisentan, dependence issues are not expected.

Metabolites
No studies have been done to specifically assess the toxicity of ambrisentan metabolites. This is acceptable. The data from the human mass balance study does not indicate any unique metabolites in humans or any metabolites that were seen in animals at exposures less than that in humans after ambrisentan administration.

Studies on impurities
No specific studies on impurities were conducted and they are not requested. All the batches used in preclinical and clinical studies contained only up to a 0.3% of the R-enantiomer, whereas the proposed quality specifications for ambrisentan claim a content of this impurity up to 0.5%. Additionally, *in vivo* interconversion of the S- to the R-enantiomer up to 2.7% in males and 5.4% in females has been reported in dogs, and no interconversion has been observed in rodents. Consequently, the safety pharmacology and toxicity preclinical studies conducted with ambrisentan support the qualification of the R-enantiomer impurity only up to 0.3 % in most cases. Up to 5% has been considered qualified *in vivo* as it was confirmed that the *in vivo* conversion of ambrisentan S-enantiomer to R-enantiomer in humans is very low and does not exceed the threshold of 5.4% observed in dogs (see also Safety Pharmacology section).

An assessment of the route of synthesis for ambrisentan has identified three impurities which are known or suspected to be DNA reactive genotoxins: methyl p-toluenesulphonate, ethyl p-toluenesulphonate and glycidic ester 3109A. Investigations have been confined to the active ingredient, ambrisentan, and separate studies on impurities are not required.

Other studies
A study was conducted to evaluate the effect of age (relative to skeletal growth) and ambrisentan treatment on the development of nasal cavity osseous hyperplasia in the rat [Report MPF/DT 3300]. The NOAEL was reported to be <100 mg/kg/day for both young and older rats. Osseous hyperplasia in rats appears to be independent of skeletal maturation.

Ecotoxicity/environmental risk assessment

The Predicted Environmental Concentrations of ambrisentan arising from use of ambrisentan tablets 5 and 10 mg for the treatment of PAH will be below the nominal trigger value of 0.01 µg/l and no further evaluation is required. Ambrisentan is not considered to pose any adverse environmental effects.

2.4 Clinical aspects

Introduction

The clinical program of ambrisentan consists of 2 randomized, double-blind, placebo-controlled phase 3 studies (AMB-320 and AMB-321). These data are supported by two open-label phase 2 studies (AMB-220 and AMB-222). Long-term efficacy data for ambrisentan in PAH is being evaluated in 2 open label studies, Study AMB-320/321-E and Study AMB-220-E.

The approved indication for Volibris is: "the treatment of patients with pulmonary arterial hypertension (PAH) classified as WHO functional class II and III, to improve exercise capacity (see section 5.1). Efficacy has been shown in idiopathic PAH (IPAH) and in PAH associated with connective tissue disease".
Volibris is to be taken orally at a dose of 5 mg once daily with or without food. Some additional efficacy has been observed with 10 mg Volibris in patients with class III symptoms, however an increase in peripheral oedema has also been observed. Patients with PAH associated with connective tissue disease may require 10 mg Volibris for optimal efficacy.

Scientific Advice was received from the CHMP on 15 December 2005. The Scientific Advice pertained to quality and clinical aspects of the dossier.

No clinical studies were conducted in paediatric subjects, as such ambrisentan is not recommended for use in patients below 18 years of age. A proportion of patients in the phase 3 studies were above 65 years of age. There is limited experience in patients with renal and hepatic impairment; all related limitations are reflected in the SPC.

The following table shows a summary of the clinical studies with ambrisentan to support efficacy and safety.

Study	Phase	Description	Number exposed to ambrisentan	Doses of ambrisentan	Duration of treatment
AMB-220	2	Efficacy and safety dose ranging study (PAH patients)	64	1, 2.5, 5, 10 mg	12 weeks
AMB-220-E	2	Long term safety and efficacy (Extension of Study AMB-220)	54	1, 2.5, 5, 10 mg	Long term
AMB-222	2	LFT safety study in those failed on other ERA therapy (PAH patients)	36	5 mg	12 weeks + long-term extension
AMB-320	3	Efficacy and safety study (PAH patients)	134	5, 10 mg	12 weeks
AMB-321	3	Efficacy and safety study (PAH patients)	127	2.5, 5 mg	12 weeks
AMB-320/321-E	3	Long-term Efficacy and Safety (Extension of Study AMB-320 and Study AMB-321)	383	2.5, 5, 10 mg	Long term

LFT=Liver function test
* Multiple single dose exposures

GCP

The Clinical trials were performed in accordance with GCP as claimed by the applicant.

The applicant has provided a statement to the effect that clinical trials conducted outside the community were carried out in accordance with the ethical standards of Directive 2001/20/EC.

Pharmacokinetics

Seven studies were performed to assess the pharmacokinetic profile of ambrisentan in healthy volunteers. The programme consists of a single ascending and multiple ascending dose studies (EE-001 and EE-002, respectively), a mass balance study with radiolabelled ambrisentan (AMB-107), two drug-drug interaction studies (AMB-105 with sildenafil and AMB-106 with warfarin) and a bioequivalence (BE) study comparing the clinical trial and commercial tablets (AMB-103). The pharmacokinetics of ambrisentan in patients with PAH were also determined in AMB-220, AMB-222 and in the extension phase AMB-320/320E.

Pharmaceutical development: Ambrisentan has been developed as an immediate-release tablet dosage form (5 and 10 mg tablets) for oral administration. Three formulations have been used over the course

of development named as phase 1, clinical, and commercial formulations. In phase 1, randomized, open-label BE study AMB-103, three doses of ambrisentan (2.5, 5 and 10 mg) were administered to 65 healthy volunteers in a 3-arm cross-over design. The 2.5 and 5 mg commercial formulations and the 2.5 and 5 mg clinical formulations were bioequivalent. In the case of the 10 mg dose, two different lots of the commercial formulation with differences in the particle agglomeration of the drug substance were tested. Only one of these commercial formulations was bioequivalent with the clinical tablets, being the other formulation outside the limits for C_{max} (ratio commercial: clinical 87.2%, 90% CI 79.3-95.9%). This lack of equivalence has been attributed to differences in the particle agglomeration of the drug substance batches. As a result of this different performance, the applicant has included a control of the amount of agglomerated particles as part of the drug product specifications.

- Absorption

Ambrisentan is rapidly absorbed in humans following oral administration, with C_{max} occurring around 1.5 hours post dose under both fasted and fed conditions. C_{max} and AUC increase dose proportionally over the therapeutic dose range in healthy volunteers. The single-dose geometric mean C_{max} value at the proposed therapeutic dose of 5 mg once daily was 345 ng/mL (CV 21%) and the AUC(0-inf) was 2137 ng.h/mL (CV 34.5%). After repeat once daily dosing to healthy subjects the Cmax value was 344 ng/ml (CV 59.1%) and the AUC (0-24) 2500 ng.h/ml (CV 22.1%) for the 5 mg dose. The terminal elimination half-life following repeat dosing ranges from 13.6 to 16.5 hours. Steady-state is generally achieved following 4 days of repeat dosing.

- Distribution

In vitro studies show that ambrisentan is highly protein bound (98.8%) in the plasma over a wide concentration range of 0.2 to 20 ug/mL (MPF/DDK 9912). Ambrisentan is primarily bound to albumin (96.5%) and to a lesser extent to α1-acid glycoprotein. The distribution of ambrisentan into red blood cells is low.
A food-effect was observed in study EE-001 involving administration of ambrisentan to healthy volunteers under fasting conditions and with a high-fat meal. C_{max} was decreased 12% while the AUC remained unchanged. This decrease in peak concentration was not considered clinically significant by the applicant, and therefore administration of ambrisentan in the phase 2 and 3 studies was performed with or without food.

- Elimination

After administration of the radiolabeled drug in AMB-107, ambrisentan was the predominant ambrisentan-related species circulating in plasma. Ambrisentan is glucuronidated via several UGT isoenzymes to form ambrisentan glucuronide and also undergoes oxidative metabolism mainly by CYP3A4 and to a lesser extent by CYP3A5 and CYP2C19 to form 4-hydroxymethyl ambrisentan. The latter is further glucuronidated to 4-hydroxymethyl ambrisentan glucuronide. Based on the results of AMB-107, the major route of elimination for ambrisentan and its metabolites is biliary excretion in faeces (65.4%), with urinary excretion (22.1%) representing a minor route. Approximately 40% of the administered dose was excreted as unchanged in urine (3.3%) and faeces (36%). Most of the remaining excreted dose was excreted as ambrisentan glucuronide (13%), 4-hydroxymethyl ambrisentan (21%), and 4-hydroxymethyl ambrisentan glucuronide (5%).

An additional study (ABS-108) was submitted in order to clarify if inter-conversion had been investigated in humans and its possible consequences on the efficacy of ambrisentan. One of the secondary objectives of this study was to assess the *in vivo* conversion of (S)-ambrisentan to (R)-ambrisentan in healthy volunteers. The results show that the levels of (R)-ambrisentan appear to be lower than 5% and about the same level as those found in female dogs (5.4%). The little conversion together with the lower affinity of this (R)-enantiomer compared to (S)-enantiomer by the ET_A receptors, make it unlikely that this enantiomer contributes to the efficacy or safety profile of ambrisentan.

- Dose proportionality and time dependencies

Data provided in AMB-103 and EE-001 show that AUC and C_{max} increase proportionally across doses from 1 to 50 mg. From 50 to 100 mg higher doses are proportional when considering the AUC parameter, but C_{max} increases in a lesser dose proportional manner. Ambrisentan pharmacokinetics appear to be time-independent and no sign of accumulation after repeat doses has been shown.

Pharmacokinetic data in PAH patients show a 2- to 3-fold higher exposure. This increased exposure in PAH patients has also been observed for bosentan and has been attributed to a reduction in metabolic capacity of the liver as a consequence of PAH. The preliminary results of the population PK analysis also showed an oral clearance approximately 2-fold lower in PAH patients. Although the PK data in healthy volunteers seems to be lineal in the range from 1 to 50 mg, the data obtained in PAH patients do not permit to conclude dose proportionality in the therapeutic range. While the results obtained in 34 patients in AMB-320/321-E indicate a lineal trend, the results from AMB-220 show a more than proportional increase in the AUC parameter in the therapeutic range from 5 to 10 mg.

The final results of the PK population analysis show that ambrisentan concentrations following oral administration are well described by a two-compartment model with first-order absorption with lag time and first order elimination. Ambrisentan oral clearance is 38% lower in PAH patients compared to healthy subjects, probably as a result of hepatic congestion. Creatinine clearance, bilirubin and gender were identified as significant covariates on ambrisentan oral clearance, as was body weight on central volume. One unexpected covariate significantly affecting the Cl/F was the creatinine clearance since the urinary excretion of ambrisentan is low. In the worst case scenario (creatinine clearance of 30 ml/min), the model predicts that exposure to ambrisentan in patients given 5 mg would be between the 5 and 10 mg exposure for a typical patient. A 28.3% and a 63% greater exposure could be found if a 10 mg dose is administered to patients with 50 mL/min and 30 mL/min, respectively. Taking into account the inter-individual variation in exposure of approximately 32%, no dose adjustment is proposed in renal insufficiency (see section 4.2 SPC), however an advice for caution in patients with a CrCL<30 mL/min is included. Information relating to baseline renal function will be collected as part of the post marketing surveillance programme to assess the safety profile in the renal impaired population.
With regard to the gender, the differences found are lower than the inter-individual variability in CL/F and the increase in exposure is small. Therefore these differences are not likely to be of clinical relevance.

- Special populations

Pharmacokinetics of ambrisentan has not been assessed in special populations.

- Pharmacokinetic interaction studies

Results of the *in vitro* studies performed show a low potential for ambrisentan to produce PK protein binding interactions and to inhibit cytochrome enzymes CYP1A2, CYP2B6, CYP2C9, CYP2C19, CYP2D6, CYP2E1, CYP3A4 and UGT1A1, UGT1A6, UGT1A9 and UGT2B7. It also seems that PK interactions with drugs whose clearance is dependent on some hepatic and efflux transport proteins are unlikely. In Caco-2 cells ambrisentan is a weak substrate for P-gp-mediated efflux and cyclosporine A inhibited the efflux transport of ambrisentan by 56%. The applicant will perform a DDI study to evaluate the potential interaction with cyclosporine as a post-authorisation follow up measure. Information about this potential interaction is also included in Section 4.5 of the SPC.

A DDI study with ambrisentan and digoxin (ABS-109) has been conducted and its conclusions about no monitoring digoxin levels when ambrisentan is administered concomitantly are endorsed and reflected in the SPC.

An *in vitro* study with 24 drugs used concomitantly with ambrisentan in phase 2 and 3 trials has shown no effect on the formation of ambrisentan glucuronide, suggesting absence of an *in vivo* effect The results with nifedipine show a greater percentage of inhibition (near 40%) than the other drugs, although the effect was not concentration dependent. The results of nifedipine inhibitory effects on the glucuronidation of ambrisentan could be attributed to an artefact as this inhibition was not concentration-dependent. Ketoconazole is an inhibitor of some UGT enzymes, being among them UGT2B7, one of the potentially UGT involved in the metabolism of ambrisentan. Results of a DDI

study with ketoconazole (a more potent inhibitor of UGT-2B7 than nifedipine) have not shown effects on the PK of ambrisentan. In general the results with the rest drugs suggest an absence of an *in vivo* effect.

Two interaction studies have been performed to assess the possible interactions between ambrisentan with sildenafil (AMB-105) and with warfarin (AMB-106), which can potentially be coadministered with ambrisentan in PAH patients. Results of AMB-105 show that co-administration of ambrisentan with sildenafil does not affect the PK of both drugs. These results are consistent with data from EE-002, in which the potential for ambrisentan to induce CYP3A4 activity in healthy volunteers was explored.

In AMB-106 changes in the PK profile of both products were not observed. A decrease of 15% in the maximum PT and INR values following co-administration of ambrisentan and warfarin was observed, although the 95% CIs were within the predefined equivalence criteria of 80-125%. Ambrisentan and warfarin have been concomitantly administered in studies ARIES C and AMB 220E and clinically relevant changes in INR and PT when compared with the baseline were not observed. Warfarin had no clinically significant effects on the PK of ambrisentan, therefore warfarin dose correction after starting coadministration with ambrisentan is not required Data with other anticoagulant agents that potentially could be administered with ambrisentan (acenocumarol, phenprocumon) was not provided. However, as the metabolic pathway for acenocumarol and phenprocumon involves also CYP2C9, it is not expected that the clearance of these drugs are affected by ambrisentan. With regard to fluindione, although its metabolic pathway has not been determined, some authors have hypothesized that CYP2C9 is also involved, not expecting a different performance.

From the data submitted it can be concluded that ambrisentan has a lower potential to produce interactions than the other members of the ERA class. As ambrisentan is metabolized in the liver mainly by CYP3A, a DDI study with ketoconazole was performed to assess the effect on the PK of ambrisentan produced by drugs that are know to inhibit or induce this cytocrome. The results indicate that the exposure of ambrisentan is increased in a 35% when co-administered with ketoconazole, but this increase is of the same order magnitude as the CV of inter-variability in exposure. A DDI study with rifampicin to further assess the effect of an inducer on the exposure of ambrisentan will be performed as a post-authorisation follow-up measure.

Pharmacodynamics

- Mechanism of action

The mechanism of action of ambrisentan as an antagonist for the ET receptors is similar to that described for the two ERAs available, bosentan and sitaxentan. A 4000 fold selectivity of ambrisentan for the ET_A receptor subtype over the ET_B subtype is estimated by the applicant. While bosentan is a dual receptor antagonist exerting its action on both ET_A and ET_B with a slightly higher affinity for ET_A receptors; sitaxentan is a single receptor antagonist. Its selectivity is described to be approximately 6,500-fold higher for ET_A receptors than for ET_B receptors.

- Primary and Secondary pharmacology

Endothelin-1 plasma levels have been measured in healthy volunteers and in PAH patients. Endothelin-1 increased in a dose-dependent manner up to doses of 50 mg in healthy volunteers. In PAH patients the change from baseline in ET-1 concentrations was statistically significant for patients who received 5 and 10 mg of ambrisentan. However, the effect does not seem to be dose-dependent as the increase was higher in the 5 mg dose (83% vs 48% in 5 and 10 mg, respectively). In spite of this increase, there is no evidence of rebound symptoms following discontinuation; this is reflected in the SPC. The rebound effect, however, when ambrisentan is discontinued has not been studied.

Plasma B-type natriuretic peptide (BNP), an amino-acid polypeptide secreted primarily from the cardiac ventricles, has been shown to be increased in PAH patients and proposed by some authors to be a prognostic factor in patients with IPAH. A statistically significant reduction of this peptide in plasma has been shown in a subset of PAH patients treated with ambrisentan in studies AMB-220, AMB-320 and AMB-321. The effect appeared greater for patients who received the 10 mg dose.

Regarding the effect on blood pressure and heart rate no consistent effect was observed in healthy volunteers. Although no increases in heart rate were observed in AMB-105 and EE-001, a moderate increase was observed in EE-002 and AMB-104. In AMB-104, there was no evidence of a treatment-related effect on systolic and diastolic blood pressures and a modest dose related increase was observed in heart rate.

Decreases in PAP and PVR were observed in a subset of PAH patients in AMB-220. The mean cardiac index was also increased. These haemodynamic effects have also been shown with bosentan and sitaxentan, and support the therapeutic effect of ambrisentan.

A PK/PD analysis was performed with the results of clinical studies AMB-220, AMB-222, and AMB-320/321-E. Based on the results of the population PK analysis a strong correlation between exposure and clinical outcomes has not been observed

Clinical efficacy

- Dose response studies

Pharmacokinetic information from non-clinical studies was used to establish a dosing regimen for the Phase 1 clinical studies.
Early clinical studies demonstrated that daily doses of ambrisentan up to 10 mg were well tolerated on repeat dosing, that ambrisentan has a PK profile consistent with once daily dosing, and that doses in the range 1-10 mg have PD activity.
The clinical efficacy of ambrisentan in the dose range 1-10 mg over 12 weeks in subjects with PAH was evaluated in phase 2 dose ranging study AMB-220. The primary endpoint was the change in 6MWD supported by other clinically relevant assessments- the Borg dyspnoea index, WHO functional class, time to clinical worsening, and PD measures (haemodynamics, plasma endothelin). A dose response was not observed for the primary endpoint. However, the study was not powered to examine pair-wise comparisons between individual dose groups and was only 80% powered to detect a linear dose-response relationship between the 4 dose groups. This was the first study in subjects with PAH, and as such a dose-titration scheme was used to gradually increase to the higher ambrisentan doses (5 and 10 mg). This up-titration schedule resulted in a 12-week exposure for the 1 and 2.5 mg doses, a 10-week exposure for the 5 mg dose, and an 8-week exposure for the 10 mg dose. Although the numbers are small, there is some evidence of a dose response for haemodynamic parameters, especially for the 10 mg dose group. The phase 2 data provide evidence that ambrisentan over the range 1-10 mg daily has the potential to offer significant clinical benefits in patients with PAH and more limited evidence that higher doses are associated with greater benefit than the lowest ones. Three doses, 2.5 mg, 5 mg and 10 mg were taken forward for evaluation in phase 3 clinical trials.

- Main studies

Pivotal studies AMB-320 and AMB-321 had identical design: randomized, double-blind and placebo-controlled to assess the efficacy and safety of two ambrisentan dose levels QD in patients with PAH. No control arm with an active product was included. Comparisons versus placebo are considered acceptable bearing in mind the methodological difficulties in performing a non-inferiority trial in this specific population; however, it would have been desirable to have included an active control arm for descriptive purposes, especially considering that there are other orally administered medicinal products available.

Data from these studies were integrated and the results of the combined analyses were presented in an additional study report (AMB-320/321).

METHODS

Study Participants
Patients with IPAH, PAH associated with connective tissue disease (e.g., mixed CTD, CREST syndrome, systemic sclerosis [scleroderma], overlap syndrome, or systemic lupus erythematosus), anorexigen use, or HIV infection were included in these trials. Subjects who had a serum ALT or AST

lab value >1.5xULN at the Screening Visit were excluded. The administration of other ERAs, PDE-5 inhibitors used for the treatment of PAH and prostacyclin (or prostacyclin derivatives) was forbidden.

Subjects were required to have a mean PAP >25 mmHg, PVR >3 mmHg/L/min, and PCWP or left ventricle end diastolic pressure (LVEDP) of <15 mmHg, which are consistent with the accepted definition of pulmonary hypertension. Baseline cardiopulmonary haemodynamic parameters were documented prior to the initiation of study procedures, including mPAP, cardiac index, PVR, right atrial pressure (RAP), and pulmonary capillary wedge pressure (PCWP)/left ventricle end diastolic pressure (LVEDP).

Two screening 6MWT distance (at least 150 m but no more than 450 m) were needed prior to randomisation, and the criteria for stability of screening was not to vary by more than 15%.

The co-medication in the clinical development program was not standardized, and the studies were conducted in different regions.

Treatments
- Study AMB-320: placebo, 5, or 10 mg of ambrisentan,
- Study AMB-321: placebo, 2.5, or 5 mg of ambrisentan.

Study drug was administered once daily in the morning with or without food for 12 weeks (after the 2-week screening period).
One blinded dose reduction was permitted during the treatment period in the event of study drug intolerance (e.g., 10 to 5 mg, 5 to 2.5 mg, 2.5 to 1 mg, placebo to placebo). Subjects received a daily dose of 2.5 mg or 1 mg only if they had been dose reduced from the lowest dose group.

Following 12 weeks of treatment, subjects had the option of continuing (or starting if on placebo) ambrisentan treatment in the long-term extension study.

Objectives
Primary Study Objective: To determine the effect of ambrisentan on exercise capacity in subjects with PAH.
Secondary Study Objectives: To evaluate the effects of ambrisentan on other clinical measures of PAH, as well as the safety and tolerability of the study drug.

Outcomes/endpoints
The primary endpoint was the change from baseline (defined as the mean 6MWD of the last two 6MWTs prior to randomization) in 6MWD evaluated after 12 weeks of treatment compared to placebo. The 6MWT is considered a valid main endpoint in evaluating efficacy in PAH trials. It is easy to perform, safe and reproducible, correlates with several cardiopulmonary exercise testing and provides important prognosis information. In addition, this test allows comparisons with results from previous trials. The treatment period of 12 weeks is considered long enough to provide efficacy results of clinical relevance. This period, however, is not long enough to evaluate long-term effect of ambrisentan or improvement on survival.

The main secondary endpoints were Time to Clinical Worsening (TCW); change from baseline in WHO functional class, in physical functioning scale (SF-36 Health Survey completed by the subject) and in the Borg Dyspnoea Index (BDI) measured after 12 weeks of treatment compared to placebo. Other efficacy variables were changes in the concentration ET-1, cardiac troponin T [cTnT], and B-type natriuretic peptide [BNP]. These selected secondary endpoints are acceptable. Haemodynamic parameters that are considered related to the prognosis of the disease (especially RAP, PAP, cardiac output and central venous O2 saturation) were not considered as secondary endpoints in these trials. This was considered acceptable as the correlations between the haemodynamic parameters and clinical state, functional class, exercise capacity and prognosis appear weak, and it is currently not clear enough if haemodynamic studies have to be part of the development program.

Sample size

A test of the null hypothesis of no treatment group difference in change from baseline in the 6MWD with 62 subjects per group had approximately 90% power to detect an average placebo-adjusted treatment effect of 35 m based on a 2-sample t-test and a standard deviation of 55m.

Randomisation
Randomization was performed with the use of an Interactive Voice Response System (IVRS) via the telephone. Randomization occurred no more than 2 weeks after the Screening Visit.
Subjects were stratified based on the underlying aetiology of PAH (IPAH, non-IPAH) and randomized to 1 of three treatment groups in a ratio of 1:1:1.

Blinding (masking)
Study drug was provided in the form of round, biconvex oral tablets. Active treatment was indistinguishable from placebo. Subjects, the principal investigator and staff, as well as the sponsor and specified designees were blinded to the dose of study drug throughout the 12-week treatment period.

Statistical methods
Efficacy and safety analyses were performed on 2 different populations:
The intention-to-treat (ITT) population was defined as all randomized subjects who received at least 1 dose of study drug. Subjects were considered as belonging to their randomized treatment group, regardless of the actual treatment received.

The safety population was defined as all randomized subjects who received at least 1 dose of study drug. Subjects were considered as belonging to treatment group according to the highest actual treatment received.

A missing Week 12 value in 6MWD was imputed using last observation carried forward (LOCF), unless the subject withdrew prematurely due to clinical worsening of PAH without a premature withdrawal assessment. In the later case, the imputations will be 0 for 6MWD, 10 for Borg dyspnoea index, IV for WHO class.

Descriptive statistics are presented for change and percentage change from baseline in LOCF 6MWD by treatment group for Weeks 4, 8, and 12. A 95% confidence interval (CI) for mean change and mean percentage change from baseline are also provided.

Change from baseline for Weeks 4, 8, and 12 in each of the 2 ambrisentan treatment groups were compared to placebo. The mean change was reported with 2-sided 95% CIs calculated by normal theory. The primary comparison was the change from baseline at Week 12. The Wilcoxon rank sum test stratified by IPAH and non-IPAH subjects was used for inference. A fixed sequence approach was used to control the type I error rate accounting for the 2 comparisons. The higher dose was first compared to placebo. Because the p-value from the Wilcoxon rank sum test was less than 0.05 for the higher dose (10 mg dose group for AMB-320 and 5 mg dose group for AMB-321), the difference was considered significant, and the lower dose was compared to placebo, again at the full 0.05 α-level.

The two ambrisentan dose groups were also combined and compared to the placebo group. A p-value was reported, but for descriptive purposes only, with no impact on the fixed sequence procedure used for comparing the 2 individual doses to placebo. The primary variable analysis described above was repeated for observed data (non-LOCF) to explore the robustness of the results with respect to the problem of missing data, again with no impact on the fixed sequence procedure.

For the LOCF data only, consistency of the treatment effect was explored for subgroups of subjects.

Combined Analysis (AMB320-321): The analyses described in the data analysis plan (DAP) were not planned in the protocols. However, this DAP was finalized and approved by the sponsor prior to the planned database lock date of approximately November 30, 2005 for AMB-321 and May 2006 for AMB-320. Thus, all are ad hoc and may be considered to be additions to those mentioned in the protocols. This analysis mirrors the analyses for the individual studies. All doses and all significant

doses from this analysis (if more than one dose is significant) were combined into one analysis for the purpose of estimating the treatment effect with a two-sided 95% confidence interval.

The statistical analysis plan can be regarded as globally acceptable for all the studies. The applicant provided an additional analysis of the change from baseline in 6MWD using a non-parametric (Hodges-Lehmann) approach.

RESULTS

Participant flow

The following table shows the randomised subject disposition of the phase 3 pivotal trials. A total of 484 patients were screened, of which 90 were screen failures.

Treatment group	Placebo	2.5 mg ambrisentan	5 mg ambrisentan	10 mg ambrisentan	Combined ambrisentan	Total
Disposition, n (%)	(N = 132)	(N = 64)	(N = 130)	(N = 68)	(N = 262)	(N = 394)
Randomized	132 (100.0)	64 (100.0)	130 (100.0)	68 (100.0)	262 (100.0)	394 (100.0)
Subjects who took at least 1 dose of study drug	132 (100.0)	64 (100.0)	130 (100.0)	67 (98.5)	261 (99.6)	393 (99.7)
Completed	111 (84.1)	58 (90.6)	121 (93.1)	63 (92.6)	242 (92.4)	353 (89.6)
Withdrew	21 (15.9)	6 (9.4)	9 (6.9)	5 (7.4)	20 (7.6)	41 (10.4)
Reasons for withdrawal:						
Adverse event	4 (3.0)	1 (1.6)	4 (3.1)	1 (1.5)	6 (2.3)	10 (2.5)
Clinical status did not improve or deteriorated	1 (0.8)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.3)
Withdrawal of consent	2 (1.5)	3 (4.7)	2 (1.5)	1 (1.5)	6 (2.3)	8 (2.0)
Treatment with other PAH treatment agents	1 (0.8)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.3)
Non-compliance to any of the procedures	1 (0.8)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.3)
Discretion of Myogen	0 (0.0)	0 (0.0)	0 (0.0)	1 (1.5)	1 (0.4)	1 (0.3)
Lost to follow-up	0 (0.0)	0 (0.0)	1 (0.8)	0 (0.0)	1 (0.4)	1 (0.3)
Other	1 (0.8)	0 (0.0)	1 (0.8)	0 (0.0)	1 (0.4)	2 (0.5)
Met early escape criteria[1]	11 (8.3)	2 (3.1)	1 (0.8)	2 (2.9)	5 (1.9)	16 (4.1)
Decrease from baseline 6MWD of ≥20%	10 (7.6)	2 (3.1)	1 (0.8)	0 (0.0)	3 (1.1)	13 (3.3)
Increase ≥1 WHO functional class	8 (6.1)	2 (3.1)	0 (0.0)	1 (1.5)	3 (1.1)	11 (2.8)
Worsening right ventricular failure	5 (3.8)	0 (0.0)	1 (0.8)	2 (2.9)	3 (1.1)	8 (2.0)
Progressing cardiogenic, hepatic, or renal failure	1 (0.8)	0 (0.0)	0 (0.0)	1 (1.5)	1 (0.4)	2 (0.5)
Refractory systolic hypotension	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)

[1]Subjects who met 2 or more early escape criteria were eligible to prematurely discontinue the study. After all Premature Discontinuation Visit assessments were complete, the subject was unblinded to treatment, and if on placebo, the subject was eligible to enter the long-term extension study.
Source: Summary Table 14.1.1

Recruitment
The periods of recruitment for the phase 3 pivotal trials were the following:
- AMB-320 December 2003 to February 2006
- AMB-321 December 2003 to October 2005

Conduct of the study
Two global amendments to the original protocol were implemented throughout the course of the studies. The changes were identical for both studies.

Protocol deviations were summarized in the following categories:

1) procedures not performed
2) visit window deviations
3) study drug compliance deviations
4) inclusion/exclusion criteria deviations

Most deviations were categorized as a "procedure not performed". Overall, 13 subjects missed at least one 6MWDT and in most cases a justification was provided for not performing the test. As a LOCF and a non-LOCF analysis were performed for the evaluation of efficacy, missing data did not modify the efficacy results substantially. Inclusion or exclusion criteria protocol deviations were mainly related to haemodynamic assessments and were either missing tests or test results just outside the protocol specified parameters. The outcomes were not impacted by these deviations.

Baseline data
A total of 393 subjects received at least 1 dose of study drug and were included in the ITT populations and safety populations. Overall, baseline characteristics were comparable between treatment groups within each study. The majority of patients randomised were female (around 80%) and Caucasian (around 70%) with a mean age of around 50 yrs. Study AMB-321 was not open to enrolment in the US or Australia; therefore, no subjects in the 2.5 mg group were from these regions. Similarly, only a few centres from Eastern and Western Europe participated in AMB-320.

IPAH and PAH associated with CTD, were the two most frequent aetiologies. The main baseline functional class was class III followed by class II. Very few patients with functional classes IV and I were included in these trials.

The baseline 6MWD ranged from 150 to 449 metres, with a mean of 341 ± 75.8m for AMB-320 and 348.4 ± 84.46m for AMB-321. For AMB-320 the median of the baseline walking distance for functional classes II and III were 380m (min 160, max 449) and 354m (min 192, max 442) respectively. For AMB-321 the median for functional classes II and III were 398m (min 190, max 449) and 350m (min 150, max 445) respectively.

The applicant requested the use of ambrisentan in patients with functional class II, a group of patients for whom no medicinal product has been authorised so far. Patients with PAH are classified in functional classes according to clinical parameters. An overlap between functional classes is expected as the diagnosis is only based on symptomatic considerations. It is also well-known that 6MWD correlates with functional class. This correlation has been systematically scrutinised in all clinical trials submitted for the evaluation of other products previously authorised, in an attempt to clarify whether those patients classified as functional class II were representative of such a class.

The French National Registry of PAH, recently published (Humbert et al. Am J Respir Crit Care Med 2006; 173:1023–1030), included a substantial number of patients (n=674) with PAH diagnosed according to clinical criteria. In this study, a correlation between 6MWD and functional class II, III and IV was observed, being 415 (±86), 319 (±92) and 192 (±96) meters the baseline mean distance walked, respectively. These distances are similar (though lower for class II) to those previously described in the paper by Miyamoto (Am J Resp Crit Care Mad 2000; 161: 487-92) (n=43) where patients with functional class II and III reported a baseline mean walking distance of approximately 450 and 310m respectively.

The baseline 6MWD reported for AMB-320 and AMB-321 raised doubts with respect to the representativeness of the included patients for the whole range of patients with functional class II (particularly the less severe ones). At the time when other medicinal products previously authorised for PAH were evaluated, concerns were raised not only in relation to the representativeness of patients included in class II but also to the magnitude of the effect achieved, that was very limited and not statistically significant. As a result, only functional class III was considered for the indication of these medicinal products. In the ambrisentan dossier, the applicant stated that nearly half of the patients (47%) with functional class II had a 6MWD>400m compared with 26.9% of patients with functional class III (when both clinical trials are combined). This data suggests that a substantial number of less severe patients (class II) could have been included in both clinical trials. Therefore, the applicant was requested to further characterise these patients, according to the clinical and functional criteria currently used in clinical practice. The applicant provided data on 6MWD at baseline that indicates a good correlation between functional class and the expected mean 6MWD at baseline (according to the walking distance reported in the French register); data which supports the adequate classification of patients in functional classes in the pivotal trials. In addition, the applicant further characterised the patients from a clinical point of view. Haemodynamic parameters and Borg dyspnoea index at baseline show significant differences between the patients classified as functional class II and III. In addition, a lower consumption of concomitant medicines for patients classified as functional class II was reported. All this data suggests that the patients classified as class II are, at least, different from those classified as class III and, additionally, less severe from the clinical point of view.

Concomitant medication was similar between the different subgroups. It should be noted, however, that a high percentage of patients took calcium channel blockers (CCB) (AMB-320: placebo 39%, 5mg ambrisentan 24%, 10mg ambrisentan 31%; AMB-321: placebo 57%, 2.5mg ambrisentan 50%, 5mg ambrisentan 46%). These percentages, that were maintained during the duration of the studies, were higher than expected as no more than 20% of patients with PAH are usually responders to CCB (after the acute vasoreactivity test). Furthermore, they are not consistent with the percentage of vasoreactivity reported for each study (14.4% for AMB-320 and 23.4% for AMB-321). In both studies the use of CCB was higher than the rate of known vasoreactivity. Data, however, shows that the improvement in 6MWD associated with ambrisentan treatment was similar whether patients took CCBs or not.

Outcomes and estimation

Primary Endpoint:
The primary ITT analysis showed a statistically significant and clinically relevant mean placebo-corrected ambrisentan treatment effect on the 6MWD of 31m-59 meters in all active treatment groups.

A dose-response relationship for the change in 6MWD was observed for each of the placebo-controlled studies, although the effect on the 6MWD for the 5mg ambrisentan dose group in AMB-321 is double than the one shown in AMB-320. The applicant discussed all items that could explain the differences observed between clinical trials, such as factors related with the population characteristics, clinical practice, etc, however, no reasons for the different effect observed have been identified.

Secondary endpoints
Following the Statistical Plan the evaluation of the secondary efficacy endpoints was performed by combining the subjects from the 2 ambrisentan dose groups (3 in the case of the combined analysis study) compared to placebo, as each ambrisentan treatment group demonstrated a statistically significant improvement compared to placebo in the primary endpoint analysis in all studies.

In AMB-320 none of the two key secondary endpoints, TCW and change in WHO functional class, were significant versus placebo. When the weighted Hommel Test (assign 80%=T CW and 20%=WHO class) was taken into consideration, TCW and WHO functional class did not reach signification either. Thus, statistical testing was not considered on the remaining secondary efficacy variables. However, the analysis for AMB-321 showed that TCW was statistically significant, though not WHO, and the rest of secondary variables were considered for the analysis. Statistical testing was also significant for SF-36 and BDI.

The applicant provided, as part of the secondary endpoint "Clinical worsening" the deaths and hospitalization for PAH for both studies; very important parameters from the clinical point of view. A lower absolute number of deaths for both doses were reported in both trials. With respect to hospitalization for PAH a clear downward trend was observed in AMB-321 for both doses (pla: 13.8%, 2,5mg 4,7% and 5 mg 3.2%).

Results of other descriptive variables showed that the change from baseline was greater than placebo at Week 12 for the geometric mean percent change in plasma ET-1 for the 5 mg group, but not for the 2.5 mg group; while the decrease from baseline was greater than placebo for the 2.5 mg group and the 5 mg group when assessing the geometric mean plasma BNP. The changes in cTnT concentrations during the 12-week study were minor and not remarkably different from zero.

The following table shows the efficacy results on primary and secondary endpoints of the pivotal studies and the combined analyses (AMB-320/321).

Study No.	Treatment Arm	No. Enrolled[1]/ Completed	Primary Endpoint[2]		Secondary Endpoints[2]					
PHASE 3 PLACEBO-CONTROLLED STUDIES										
			6MWD, m		Time to ClinWorsening KM pvalue HR	WHO class		Borg Dyspnoea Index		SF-36® physical functioning scale
			Δbaseline mean (SD)	pl-adj Δbaseline mean, pvalue		%Imp	%Det	Δbaseline m (SD)	pl-adj Δbaseline m, pvalue	
						pvalue[3]				
Integrated AMB-320 and AMB-321	placebo	132/111	-9.0 (86.22)	NA	NA	20.5%,	17.4%	0.4 (2.46)	NA	1.07 ±7.64
	2.5 mg	64/58	+22.2 (82.67)	+31.2 p = 0.022	p = 0.030 72%	15.6%	4.7% p = 0.206	-0.2 (2.17)	-0.6 p = 0.046	3.86 ± 7.14 p = 0.023
	5 mg	130/121	+35.7 (80.18)	+44.6 p <0.001	p = 0.005 71%	21.5%	2.3% p = 0.027	-0.3 (1.96)	-0.7 p = 0.031	3.34 ± 8.30 p = 0.033
	10 mg	67/63	+43.6 (65.91)	+52.5 p <0.001	p = 0.028 72%	29.9%	4.5% p = 0.096	-0.9 (1.93)	-1.3 p =0.002	4.52 ± 7.16 p = 0.004
	combined ambrisentan	261/242	+34.4 (77.51)	+43.3 p <0.001	p <0.001 71%	22.2%	3.4% p =0.011	-0.5 (2.01)	-0.9 p <0.001	3.77 ± 7.73 p = 0.003
AMB-320	placebo	67/57	-7.8 (78.88)	NA	NA	23.9%	16.4%	0.0 (2.22)	NA	2.31 ± 7.65
	5 mg	67/63	+22.8 (82.98)	+30.6 p = 0.008	p = 0.307 50%	28.4%	1.5% p = 0.073	-0.3 (1.93)	-0.3 p = 0.316	3.86 ± 7.14 p = 0.543
	10 mg	67/63	+43.6 (65.91)	+51.4 p <0.001	p = 0.292 50%	29.9%	4.5% p = 0.096	-0.9 (1.93)	-0.9 p = 0.002	4.52 ± 7.16 p = 0.111
	combined ambrisentan	134/126	+33.2 (75.37)	+41.0 p <0.001	p = 0.214 49%	29.1 %	3.0% p = 0.043	-0.6 (1.95)	-0.6 p = 0.017	4.10 ± 8.39 p = 0.229
AMB-321	placebo	65/54	-10.1 (93.79)	NA	NA	16.9%	18.5%	0.8 (2.63)	NA	-0.20 ± 7.14
	2.5 mg	64/58	+22.2 (82.67)	+32.3 p = 0.022	p = 0.005 80%	15.6%	4.7% p = 0.206	-0.2 (2.17)	-1.0 p = 0.046	3.86 ± 7.14 p = 0.005
	5 mg	63/58	+49.4 (75.36)	+59.4 p = <0.001	p = 0.008 79%	14.3%	3.2% p = 0.187	-0.4 (1.99)	-1.2 p = 0.040	2.96 ± 6.81 p = 0.040
	combined ambrisentan	127/116	+35.7 (79.99)	+45.8 p <0.001	p <0.001 80%	15.0%	3.9% p = 0.120	-0.3 (2.08)	-1.1 p = 0.019	3.41 ± 6.96 p = 0.005

1. Number of subjects enrolled/randomized in the study and also included in the ITT population
2. Refers to the phase 3, placebo-controlled studies
3. p-value determined from 7-point scale
4. Study is ongoing; 36 subjects were enrolled, 2 discontinued prematurely and 34 subjects were still continuing the study as of 01 June 2006
5. SGA used in Study AMB-220 and SF-36 physical functioning scale used in Study AMB-222

6MWD = 6-minute walk distance, Δbaseline = change from baseline, m = meters, SD = standard deviation, pl-adj = placebo adjusted; KM = Kaplan Meyer, HR = hazard ratio, imp = improved, det = deteriorated, BDI= borg dyspnoea index, SGA=subject global assessment

Ancillary analyses

- Analysis performed across trials (pooled analyses and meta-analysis)

The applicant provided subgroup analyses for 6MWD on gender, PAH aetiology, WHO functional class, region, supplemental oxygen use, CCB use and vasoreactivity. The subgroup analyses and associated p-values are presented descriptively.

With respect to the functional classes, data shows a statistically significant change in 6MWD versus placebo in AMB-320. The effect was clinically relevant for 10 mg in functional class II (43.4m) and for both doses (2.5 and 5mg) in functional class III (34m and 57m respectively). In AMB-321 the

effect was statistically and clinically relevant for 5 mg in functional class II (68.6m) and clinically and statistically relevant for the dose of 5 mg in functional class III (53.5m).

The applicant provided additional data showing the percentage of change from baseline in 6MWD by baseline 6MWD (<400m and >400m) in order to provide more information in less severe patients (predominantly class II). This cut-off point was chosen taking into consideration the baseline 6MWD of patients published in the French registry. Results from this combined analysis suggest that less severe patients are as likely to improve as those with functional class III. With the aim of having more data on the effect of ambrisentan on less severe patients, the applicant presented data on the change from baseline (and placebo-corrected) in 6MWD by baseline 6MWD with several cut-off points (e.g., >400m and <400 m, and >415m and <415m). An ANCOVA analysis adjusted by baseline walk distance for each functional class was also presented. Overall, results for the 5 mg dose are similar regardless of the 6MWD cut analysed.

The analysis by PAH aetiology showed a statistically and clinically significant change in 6MWD for IPAH for all doses in both studies. For non-IPAH, which mainly includes CTD-associated PAH, only the 10mg dose in AMB-320 had a statistically and clinically significant change. In AMB-321 the change was not significant for any of the doses, probably due to the small population size. A beneficial effect was also observed for secondary variables (proportion of deteriorated and BDI). Given these results, the efficacy of ambrisentan in CTD-associated PAH was considered questionable and further discussion was requested. Baseline characteristics of non-IPAH patients for the 5 and 10mg doses were reviewed to see if the lack of significance for the 5mg dose could be linked to these. The analysis did not reveal any substantial differences likely to provide an explanation. Patients with PAH-CTD have a consistently smaller response to other ERAs compared to patients with IPAH. The greater efficacy associated with the 10 mg dose in patients with PAH-CTD was considered therefore to possibly be a result of a dose-response effect in this patient group.
Data from the phase 3 studies identified that the 10mg dose brings higher benefit to patients with class III symptoms and patients with PAH-CTD in comparison with 5 mg dose; as such, this has been reflected in the posology section of the SPC.

The results in 6MWTD by subgroups for the combined analysis AMB-320/AMB-321 support the ones for the individual studies: an effect is shown in females, Caucasians, IPAH, functional classes II and III.

- Clinical studies in special populations

No clinical studies were conducted in paediatric subjects, as such ambrisentan is not recommended for use in patients below 18 years of age.

In phase 3 studies the proportion of patients >65 years was 21.5% and > 75 years 4.8%. Based on the results of the population pharmacokinetic analysis in healthy volunteers and patients with PAH, the pharmacokinetics was not significantly influenced by age, and no dose adjustment is required in patients over the age of 65.

Based on the results of the PPK analysis, No dose adjustment is required in patients with renal impairment, however due to limited experience an advice for caution if therapy with ambrisentan is initiated in patients with severe renal impairment (creatinine clearance <30 ml/min) is included in the SPC.

As stated in the clinical pharmacology section, inclusion criteria were very restrictive for hepatic function. A contraindication in patients with severe hepatic impairment has been included in the SPC since in this patient population an increase in the exposure would be expected with a potential impact on safety. Sections 4.2 and 4.4 also reflect concerns relative to this population.

- Supportive study

AMB-320/321-E (Extension study)

An ongoing extension study was designed to continue the safety and efficacy assessments of ambrisentan in PAH patients who completed treatment in AMB-320 or AMB-321. A preliminary analysis has been performed using study data collected through 16 February 2006.
Data on efficacy, as reassuring as the results for the functional and clinical variables, suggest that the effect on 6MWD and other secondary variables is maintained. However, given the lack of a control arm, the data should be considered with caution.

Overall, the safety profile was similar to that observed in the AMB-320 and AMB-321. Twenty one subjects died during the studies or within 30 days of receiving the last dose of study drug. Nearly all deaths (n = 20) were considered unrelated to study drug. Nearly all these subjects had WHO class III or WHO class IV symptoms prior to initiating ambrisentan treatment. This finding can be considered consistent with the natural history of the disease.

The applicant provided an analysis on survival where historical comparison with the NIH registry was performed. The observed probability of survival at 1 year for subjects receiving ambrisentan (combined ambrisentan dose group) was 95%, without any notable effect of ambrisentan dose on long-term survival. Moreover, the observed probability of survival for IPAH subjects receiving any dose of ambrisentan was 97% at 1-year, compared to 72% as predicted by NIH estimates (for the 5mg dose the observed probability of survival was 95%, compared to 72% as predicted by NIH estimates). A downward trend in survival is observed at 2 and 2.5-years being the rates similar to that predicted by NIH estimates. In addition, and compared favourably with the estimated 1-yr survival calculated with the NIH equation (72%), the French National Registry of PAH showed an observed probability at 1-year of 88.4% in the whole incident group (n =121). These results are reassuring although historical comparisons are difficult to interpret and should be carefully analysed. It is important to remark that at the cut-off date, the combined mean exposure for subjects who received ambrisentan was 38.6 ± 26.52 weeks. The applicant has provided an update of the survival analysis for patients with functional classes II and III over a period of 3.5 years, although historical comparison with the NIH registry have not been given. According to this data the observed probability of survival at 2 years for subjects with class II was 94% compared to 78% for subjects with class III. The probability at 3 years was 94% for class II compared to 70% for class III respectively. Although no firm conclusion can be drawn from this data these results suggest that patients with functional class II treated with ambrisentan have a better prognosis than those with functional class III.

Clinical safety

- Patient exposure

The safety dossier includes data from the 2 pivotal trials (AMB-320 and AMB-321), two phase 2 studies - AMB-220 (a dose-controlled/open-label study) and AMB-222 (an open-label study) and the long-term safety is being evaluated in 2 studies: AMB-320/321-E (a dose-controlled/open-label study) and AMB-220-E (an open-label study). Data for ongoing clinical trials (AMB-222, Study AMB-220-E, and Study AMB-320/321-E) to a cut off date of 30th November 2006 is provided. A safety update up to the 31st of January 2007 for the SAEs and fatal adverse events is included in the clinical overview of the dossier.

Considering all performed studies, 725 subjects were treated with ambrisentan (AMB). Four hundred and eighty three (483) subjects with PAH were exposed to ambrisentan in the phase 2 or 3 studies with an overall mean exposure to ambrisentan of 79.5 ± 50.28 weeks and a maximum exposure of 212.3 weeks. This represents drug exposures of at least 6 months for the majority of subjects (86.5%) and at least 1 year in more than two-thirds of subjects (71.0%). Approximately one quarter of subjects have had drug exposures of 2 or more years (24.8%) and 8.7% subjects have had drug exposures of 3 or more years.

The majority of the ambrisentan treated subjects in phase 3 trials were female (79.1%) and Caucasian (76.8%). Mean BMI at baseline in the all phase 3 trials was 27.2 kg/m2. The majority (309) of 393 subjects in the phase 3 studies were younger than 65 years and 19 subjects were ≥75 years of age.

Adverse Events:
In the pivotal studies, the majority of patients experienced at least one AE. Peripheral oedema, nasal congestion, sinusitis, flushing, nasopharyngitis, abdominal pain, constipation, palpitations, dyspnoea and headache had a higher incidence in the combined ambrisentan group than in the placebo group (≥1% difference in incidence).

However, right ventricular failure, nausea, urinary tract infection, pulmonary hypertension, dizziness, ALT increased, arthralgia, dyspnoea exacerbated, and upper respiratory tract infection had a higher incidence in the placebo group (≥1% difference in incidence). The higher incidence of right ventricular failure and pulmonary hypertension in subjects receiving placebo could reflect the disease progression.

The most common AEs were those expected according to those observed with other similar medicinal products: headache, flushing, nasal symptoms (especially congestion) and peripheral oedema. A global description of the safety profile for the 10 mg dose; the most remarkable AE which is dose related is the incidence of peripheral oedema and when it occurs it may be more severe, particularly in the elderly (see also the discussion on Post-Marketing experience). For this dose the incidence of larger decreases in Hb appears more common and a greater mean decrease in blood pressure is observed but it is recognised that this has rarely been associated with adverse clinical sequelae in clinical trials.

When data from the individual pivotal trials is assessed, marked incidence differences of AEs between the 2 studies that contributed to the combined AMB-320/321 analysis population are observed. The incidence of peripheral oedema was substantially different between AMB-320 and AMB-321 for the 5 mg treatment groups (26.9% versus 9.5%, respectively). The reason for these differences is unclear The applicant argues that despite these differences among the two doses, the incidence of peripheral oedema was almost identical for the placebo groups in each study (10.4% in AMB-320 and 10.8% in AMB-321).

Treatment-related AEs were reported in similar percentage in the 5 and 10 mg treatment groups (42.3% and 43.3%, respectively) and in a similar percentage in the placebo and 2.5 mg groups (32.6% and 29.7%) respectively, in the pooled safety analysis. Common AEs (incidence ≥3%) judged as treatment-related in the combined ambrisentan group, were peripheral oedema, headache and nasal congestion. Peripheral oedema and nasal congestion were the only common TAE that appeared dose-related. No adverse events others than peripheral oedema and headache were reported as treatment-related at an incidence ≥1% in placebo-treated subjects.

- Serious adverse event/deaths/other significant events

Liver safety: Other ERAs have demonstrated dose-dependent LFT abnormalities, specifically increases in serum aminotransferases that can be associated with hepatotoxicity. Clinical studies with ambrisentan were designed to critically evaluate the incidence and severity of LFT abnormalities in order to identify any chance of DILI (Drug Induced Liver Injury). Very few reported events were confirmed with repeated testing (placebo n=2, ambrisentan 5 mg n=2, ambrisentan 10 mg n=2), however, it is acknowledged that in some cases (n=4) repeated testing was not performed as defined in the protocol. A clearer explanation for the absence of retesting within 7 days regarding laboratory liver abnormalities has been provided.

The following table shows serum aminotransferase abnormalities (ALT and/or AST) by severity in all ambrisentan PAH studies (Population: All Studies) (Cut-off 16th November 2006).

Serum Aminotransferase Abnormalities (ALT and/or AST) by Severity (Population: All Studies) ALT and/or AST	Cumulative incidence for all PAH studies (>1 day)1				
	AMB (N = 483)1 n (%)	Distribution by dose at event, n			
		1 mg	2.5 mg	5 mg	10 mg
>3xULN and ≤5xULN	13 (2.7)	1	3	5	4
>5xULN and ≤8xULN	0 (0.0)	0	0	0	0
>8xULN	4 (0.8)	0	0	1	3
All >3xULN	17 (3.5)	1	3	6	7

1Total number of subject does not include the 14 subjects ongoing in Study AMB-323 as of 30 November 2006. None of these subjects developed ALT/AST >3x at the time of data cut-off.
Source: Clinical Safety Update Table 25

Based on the information available at present no specific concerns were observed by applying the Hy´s law criteria for drug induced hepatotoxicity, (Zimmerman HF 1999).

A specific cohort of patients considered as high risk has been evaluated, those participating in the AMB-222 study (PAH subjects who have previously had significant aminotransferase abnormalities associated with the use of bosentan or sitaxentan). Data from this study are in principle reassuring: no subject had elevations in serum aminotransferases greater than >3xULN that resulted in discontinuation of ambrisentan. These results suggest a more favorable safety profile of ambrisentan, although some limitations, as the number of patients and the length of treatment, should be taken into account.

Finally, a review of the AEs that could be related to DILI is provided. One case of hepatic cirrhosis, moderate in severity and unrelated to therapy, was reported. Additionally, two cases of jaundice were notified. In the first case, which was reported as mild and unrelated to study drug, bilirubin and other LFTs were within normal ranges. In the second case, bilirubin and alkaline phosphatase increased but not AST and ALT and was reported by the investigator as mild and possibly related to AMB treatment.

One fatal event of abdominal pain has been reported for which currently there is insufficient information to definitively exclude a hepatotoxicity event associated with ambrisentan (Subject 221-003). The applicant was requested to provide all relevant information available on this issue. However, the available information does not allow for a definitive assessment of this case. At present, the overall pattern of liver safety appears better than that of other existing sulfonamide ERAs, however, a cautious approach is considered appropriate. The SPC labelling for the monitoring of liver enzyme elevations is in line with other ERAs. In addition, liver enzyme elevations are an important aspect of the RMP.

During the period between the data cut-off and the 31 January 2007 two additional cases of elevated asymptomatic LFTs were notified. In the first case an increase >4 x ULN in LFTs was reported 3.5 years after starting AMB 10 mg. The other case was an asymptomatic elevation ALT>3 x ULN reported 2 years after starting AMB 5 mg. The event was considered related probably to AMB and was temporarily discontinued. Reports for 6 subjects with SAEs of liver enzyme elevations in ongoing clinical trials with onset during the period between 30 November 2006 and 31 July 2007 are also included. This additional information does not alter the conclusion that ambrisentan appears to have a favourable hepatic safety profile.

Electrocardiography: A “Thorough QT/QTc” study was performed fulfilling the requirements of the ICH (E 14) on “The Clinical Evaluation of QT/QTc Interval Prolongation and Proarrhythmic Potential for Non-Antiarrhythmic Drugs”. The design of the study seems appropriate in terms of randomization, placebo and positive control, blinding and timing of ECGs, considering the PK profile of ambrisentan. Subject enrolment for the trial and discontinuation criteria are also deemed adequate.

The primary objective of this study was to assess whether administration of ambrisentan 10 mg/day on Days 1-5, followed by a 40 mg dose on Day 6 had the potential to cause QTc prolongation in healthy volunteers. Ambrisentan was administered at the higher intended dose (10 mg) and at a dose 4 times higher to provide a sufficient safety margin. Moxifloxacin 400 mg/day on day 1, 5 and day 6 was included so as to establish assay sensitivity.

Most of the participants were male (74.5%; nearly half of patients were Black or African American). Other demographic variables (i.e. age, weight, height...) were equally distributed.

Three types of corrections for the QT interval were initially planned; QTcS (QTc obtained using Framingham/Sagie correction formula), QTcB (QTc obtained by the Bazzet's formula) and QTcF (QTc obtained by the Fridericia's formula). A post-hoc method QTcIb (individually corrected QTcIb) was included after a non expected increase in heart rate was observed. The correction for QTcIb was derived using the slope of the actual QT-RR relationship observed prior to treatment: i.e., using the 18 ECGs recorded on day 0 and the 3 ECGs recorded prior to dosing on day 1 in each subject. Each subject's corrected QT for each ECG was then determined using the linear regression formula: QTcIb = observed QT + (baseline individualized slope x (1000 – observed RR in msec).

As previously discussed, the effect of ambrisentan on heart rate was not consistent. Although no increases in heart rate were observed in AMB-105 and EE-001, a moderate increase was observed in EE-002. Unexpectedly, AMB treatment was consistently associated with an initial decrease in time-matched heart rate at earlier time points and a subsequent increase at later time points, the maximum increase being observed on day 6, two hours after the administration of the higher dose (40 mg).

An expert report justifying this new approach emphasized that due to this unexpected moderate increase of heart rate with ambrisentan the analysis of change of QT was mistaken because of the "often misleading effects" of QT correction using the Bazett, Fridericia and linear regression (Sagie) correction formulae. This mistake is apparent in the large differences observed in the mean changes and in the outlier frequency for the 3 corrections. Some tables and figures were provided showing the adjustment in the QT-RR by different alternatives. The applicant states that since a strong inverse relationship was observed for heart rate and QT (i.e., QT values decreased with increased heart rate) on day 6 for subjects receiving 40 mg ambrisentan, QTcS appeared to overcorrect for heart rate resulting in an apparent increase in QTcS with increased heart rate, which was similar for QTcF and more apparent for QTcB.

Thus, the primary analysis and all the conclusions for the effect of AMB on change in QTc are based on this QTcIb (individually corrected QTc). Treatment effect on QTcIb was assessed using the time-matched, baseline-adjusted mean change from baseline on day 6 at all time points of the AMB group (40mg) to the time-matched, baseline-adjusted mean change from baseline on day 6 in the placebo group and its two-side 90% confidence interval (CI). No adjustment was made for type I error for the comparisons reported at multiple time-points, for p-values or for confidence intervals.

The largest increase in mean time-matched and placebo-adjusted QT for AMB at day 1 (10 mg single dose) was 1.16 ms (upper bound of the 90% CI: 6.10 ms) at predose level. For QTcIb, was 4.93 (90% CI: -0.92 to 8.94) which occurred 2 hours after dosing at day 6 (40 mg). Based on that, it is emphasized that neither the crude QT values nor the QTcIb indicated any cause for concern, according to the current ICH E14 requirements.

Considering the pre-specified primary variable, the largest increase in mean time-matched and placebo-adjusted QTcS was 10.19 ms (upper bound of the 90% CI: 14.22 ms) which occurred 2 hours after dosing at day 6 (40 mg). And for the QTcF and QTcB were 8.94 90% (CI: 4.94 to 12.95) and 16.39 (CI: 11.23 to 21.54) respectively, which occurred 2 hours after dosing at day 6 (40 mg) also, above all, the threshold of 10 ms criterion for a negative Thorough QT/QTc study. Once again, the expert's report argues that this increase was associated with (and potentially due to) the increase in heart rate, thus basing all his conclusions on the QTcIb results.

In this particular case, Fridericia correction, which is generally accepted and regarded as more appropriate in those cases where heart rate is increased, did not show the expected results.

The positive control was moxifloxacin 400 mg/day on day 1, day 5 and day 6. The largest increase in mean time-matched and placebo-adjusted was 17.24 ms (upper bound of the 90% CI: 22.69 ms) at 1h on day 6 for the QTcB adjustment. The mean change with the selected QTcIb correction exceeded 5 ms for most time points and nearly all lower bounds of the 90% CI were >0 ms and all upper bounds reached the 10 ms threshold. In principle, these treatment effects were consistent with the expected values for subjects treated with 400 mg moxifloxacin. Therefore, the applicant concludes that the study had sufficient sensitivity to provide data for the effects of ambrisentan on QTc.

Analyses were also performed to identify subjects with QTc≥ 450 ms, 480 ms and 500 ms. For QT, only moxifloxacin had a value ≥ 450 ms in one subject. For QTcB, placebo had one value ≥ 450 ms in one subject, ambrisentan had 13 values ≥ 450 ms in 4 subjects, and moxifloxacin had 19 elevations in 5 subjects. For QTcF, only moxifloxacin had a value ≥ 450 ms in one subject. For QTcS, moxifloxacin had a value ≥ 450 ms in one subject also. Only one case of QTcIb > 450 ms was found in the moxifloxacin treatment group. No cases of QTcIb > 480 ms were found in any group.

In categorical analyses, after a single dose of ambrisentan (day 1) the percentage of subjects in the ambrisentan group with a change from baseline QTcIb or QTcS >30 msec and ≤60 msec was 1.9% AMB vs 1.9 PLAC for the QTcIb and 1.9 % AMB vs 3.8 % PLAC for the QTcS adjustment. After multiple once-daily dosing of 10 mg ambrisentan (day 5) the percentage of subjects in the ambrisentan group with a change from baseline QTcIb >30 msec and ≤60 msec was greater than the placebo group (3.8% AMB vs 1.9% PLA); this difference was greater for the QTcS (7.7 % AMB vs 1.9% PLA). No subject in any treatment group had a change in the QTc (using all corrections) that exceeded 60 msec.

Due to the small number of female subjects, no clear conclusions on changes in QTc by gender can be drawn. No clinically significant effect on PR/QRS intervals, QRS axis shift or ST-T-U morphology has been noticed in this study.

The dose-response and the concentration-response relationship have been studied. A 4-fold increase in ambrisentan dose was associated with less than a 2-fold increase in the mean time-matched and placebo-adjusted QTc; therefore, these data does not support a well-defined dose-response relationship on potential QT/QTc changes.

Further clinical data from placebo-controlled phase 3 studies was provided to be more confident with the results. Electrocardiographic data obtained after 12 weeks of treatment (AMB-320 and AMB-321) indicated that similar percentage of patients in all treatment groups [placebo: 8.3%; ambrisentan 2.5 mg: 9.4%; ambrisentan 5 mg: 6.2%; ambrisentan 10 mg: 11.9%] had abnormal ECG readings considered clinically significant. The types of abnormalities were qualitatively and quantitatively similar to the abnormalities observed at baseline and were expected in patients with PAH. Few cases of sudden death and cardiac arrest have been reported during treatment with ambrisentan and placebo, but none have been linked to Torsades de Pointes.

Since AMB was not expected to produce an increase in heart rate, it is understandable the applicant's effort to find other QT correction strategies not so influenced by heart rate. As stated in the ICH Topic E14 suitable correction approach for study population with broad range of heart rates is individualized one, though the choice correction method is a subject of controversy. The justification and discussion provided by the applicant for the adequacy of the QTcIb correction method and the implications of the study results are acknowledged.

In conclusion there were no significant ambrisentan-induced QT changes observed in the AMB-104 study.

ECG: Since AMB-104 was regarded as negative, no expanded ECG safety evaluations during later stages of drug development were performed. To date, ambrisentan has not demonstrated any clinically

significant effect due to the increases in the QT/QT_C interval, regarded as below the level of regulatory concern from the AMB-104 study results.

Haemorrhagic events: As stated in the pharmacology section, the assessment of the possible interaction between ambrisentan and warfarin did not demonstrate any changes in the PK profile of either product that would translate into clinical issues (higher risk of thromboembolism or bleeding episodes). The review of the haemorrhagic events reported for the main trials and their extension periods, leads to the conclusion that there is not a clear bleeding pattern that would suggest a relationship between the ambrisentan administration and the occurrence of hemorrhagic events. Most of the bleeding episodes were not related to treatment.

Deaths: From the beginning of the first phase 2 study until 30 November 2006, 48 of 483 subjects (9.9%) have died while receiving ambrisentan or within 4 weeks after the last dose of study drug.

A total of 21 (4.3%) deaths occurred during the safety update period (17 February 2006 to 30 November 2006). At the time of death, 1, 2, 9 and 9 subjects were receiving 1 mg, 2.5 mg, 5 mg and 10 mg ambrisentan respectively. Of the 21 subjects who died during the safety update period, 14 had their ambrisentan dose increased at least once. Nearly all deaths that occurred during the safety update period were attributed to progressing right heart or respiratory failure and were considered unrelated to study drug.

Overall, the number of deaths during the study was not so much higher than those reported for other PAH treatments at standard dose. However, the low number of patients treated, the poor prognosis of the disease and the absence of a control group in the extension studies make it difficult to draw any firm conclusions about the cause of death. Once again we miss the presence of an active comparator even if only for a descriptive intention.

In order to be more confident with the data, a summary of deaths in ambrisentan phase 2 and 3 studies over the initial analysis period (24 weeks for AMB-222; 12 weeks for AMB-320 and AMB- 321) has been provided. This shows the same incidence of death (7 deaths per 100 years of exposure) in the combined ambrisentan group from both the phase 2 and 3 studies. The rate in the placebo treated group in AMB-320/321 was 20 deaths per 100 years of exposure to placebo.

SAEs: In the phase 3 placebo-controlled trials, a higher proportion of patients in the placebo group (16.7%) had at least 1 SAE compared to the combined AMB group (9.2%). The most frequent SAE for both the placebo and combined ambrisentan groups was right ventricular failure (placebo, 6.1%; ambrisentan, 1.9%), which is undoubtedly a sign of disease progression. A frequent AE such as peripheral oedema was reported as a treatment-related SAE in only 2 (0.8%) subjects receiving ambrisentan. There was no apparent relationship to ambrisentan dose in the occurrence of SAE.

- Laboratory findings

Incidence of clinically relevant laboratory changes was assessed. To date no clinically meaningful changes from baseline, others than those expected (haematological parameters and LFTs abnormalities), were detected in laboratory values.

Haemaglobin (Hb) and haematocrit changes: One of the most noticeable laboratory abnormalities, shared with other ERAs, was the decrease in Hb concentrations. Throughout the phase 3 studies, mean Hb figures and haematocrit values decreased for subjects in the ambrisentan groups as early as during the first 4 weeks of treatment (2.5 mg: 0.74 g/dL, 5 mg: 0.76 g/dL, 10 mg: 1.1 g/dL, and combined ambrisentan: -0.83 g/dL). Little change was seen for the placebo group (0.10 g/dL). They appear to be related with the ambrisentan dose which corresponds to what had been observed with sitaxentan and bosentan. The same pattern was observed for haematocrit. Furthermore, clinically significant reductions in haemoglobin and haematocrit were not observed with long-term ambrisentan treatment beyond Week 4. Of note, a similar frequency of commonly observed AEs possibly related to low haemoglobin and haematocrit (e.g., anaemia and epistaxis) was observed in the ambrisentan groups compared to placebo.

Overall, this data indicates that AMB exhibits, in principle, a moderate, non progressive and dose-related Hb reduction. As for other drugs of the same group, the initiation of treatment is not recommended in patients with clinically significant anaemia. The rationale for this Hb and haematocrit abnormalities is not fully clarified. At present, no signs of bone marrow toxicity or hemolysis have been observed which is reassuring.

LFTs: Mean baseline values for total bilirubin, serum alkaline phosphatase, and GGT were similar among treatment groups. At Week 12, total bilirubin, alkaline phosphatase, and GGT increased for the placebo group; whereas, clinically relevant decreases were observed for the combined ambrisentan group and for each ambrisentan treatment group compared to placebo. These changes probably reflect a reduction in hepatic congestion associated with improved haemodynamics.

Male Fertility: According to non-clinical data, a relationship between chronic use of ERA-class compounds and development of testicular atrophy and infertility has been suggested. In order to evaluate this potential risk, subjects were asked to provide semen and blood samples every 12 weeks for the first year (48 weeks) and every 24 weeks thereafter. Laboratory evaluations were carried out on a limited number of patients (around 27 patients). Preliminary data did not suggest that ambrisentan was associated with an adverse effect on male reproduction but the safety database on this specific aspect is considered unacceptably limited. Further data from ongoing studies included limited additional data relating to sperm counts and male hormone analysis. In the absence of an indication for a clinically relevant effect on male fertility in these seriously ill patients, a balanced description of pre-clinical findings has been included the SPC. Further clinical data will be provided post-authorisation.

Vital signs: No clinically relevant mean changes in heart rate were observed with ambrisentan in healthy subjects or in subjects with PAH. The differences of the heart rate figures pertain to the clinical differences among healthy and PAH patients, mainly relaying on the role of the neuro-hormonal activation in the PAH patients.

Only minor decreases were observed in mean systolic and diastolic blood pressures during ambrisentan treatment, with minimal fluctuations observed after Week 4. In addition, a similar frequency of AEs possibly related to low blood pressure (e.g., hypotension, dizziness, and syncope) was observed in the pivotal trials for AMB groups compared to placebo.

- Safety in special populations

Information regarding the lack of data on children and patients with renal impairment has been adequately reflected in the SPC. Based on the results of the PPK analysis, an advice for caution if therapy with ambrisentan is initiated in patients with severe renal impairment is included in the SPC.

As stated in the clinical pharmacology section, inclusion criteria were very restrictive for hepatic function. A contraindication in patients with severe hepatic impairment has been included since in this patient population an increase in the exposure would be expected with a potential impact on safety.

- Safety related to drug-drug interactions and other interactions

No significant drug-drug interactions have been observed so far in the clinical studies. The principal conclusions regarding drug interactions are provided in the PK section.

- Discontinuation due to adverse events

The most common reason for premature discontinuation in the ambrisentan group was AEs. Incidence of discontinuation due to AE was low, both in the placebo and in the ambrisentan arm. The most frequently reported AEs that resulted in discontinuation were pulmonary hypertension and right ventricular failure.

- Post marketing experience

Ambrisentan was approved for marketing in the United States (US) on 15 June 2007. The applicant states that post-marketing reports from the US of fluid retention occurring within weeks after starting ambrisentan have been received and, in some cases, have required intervention with a diuretic or hospitalisation for fluid management.

A cumulative total of 32 reports (spontaneous, n=25 and serious clinical study reports n=7) of fluid retention / oedema / weight gain were identified. Of the 32 cases, 5 were considered to provide some evidence of a causal association with ambrisentan.
Of the 32 cases, 5 reported heart failure (or pulmonary oedema). However, it is explained that of these, 2 reported a relatively long time to onset and were confounded by intercurrent pneumonia or underlying atrial fibrillation. Two further reports showed a stronger temporal relationship but one patient had a history of atrial fibrillation and the other was confounded by underlying interstitial lung disease and intercurrent upper respiratory tract infection. It is acknowledged that these confounding factors could also be risk factors for ambrisentan-associated oedema. The remaining case was poorly documented. Overall, it was considered that the currently available information was insufficient to support an association between ambrisentan and decompensate heart failure.

The remaining 27 reports were considered to be confounded (n = 13) or poorly documented (n = 12) or the event description was considered more likely indicative of peripheral oedema (n = 2).

In view of the post-marketing information it is considered appropriate to include information relating to the clinical trial experience with peripheral oedema and the post marketing experience with fluid retention in section 4.4 of the SPC.

Together with the post-marketing information, a detailed discussion examining the baseline characteristics in the pivotal trials for factors that might affect the incidence of peripheral oedema was presented. This discussion showed only that there was a slightly higher proportion of patients with less symptomatic (WHO class II) disease in AMB-321 than in AMB-320. Patients with class II symptoms at baseline have a lower risk of developing peripheral oedema with the 5mg dose compared with patients with Class III symptoms**.**
This point was considered of relevance and has been reflected in section 4.2. of the SPC.

The incidence and consequences of peripheral oedema/fluid retention will be one of the main endpoints of the proposed Post-Marketing Surveillance Program designed with the intention to collect additional safety information for ambrisentan during standard clinical care.

2.5 Pharmacovigilance

Detailed description of the Pharmacovigilance system

The CHMP considered that the Pharmacovigilance system as described by the applicant fulfils the legislative requirements.

Risk Management Plan

The MAA submitted a risk management plan, which included a risk minimisation plan

Table: Summary of the risk management plan

Safety concern	**Proposed pharmacovigilance activities**	**Proposed risk minimisation activities**
Teratogenicity	• Routine pharmacovigilance activities, including use of targeted follow-up questionnaires. • Additional information that	• Contraindication information in section 4.3 of SPC for use of ambrisentan in pregnancy and in women of childbearing potential who are not using reliable

Safety concern	Proposed pharmacovigilance activities	Proposed risk minimisation activities
	may arise from clinical trials. • Post-marketing surveillance program to collect additional safety data.	contraception. • The SPC (section 4.4, 4.6 and 5.3) includes appropriate language concerning teratogenicity. • Pregnancy must be excluded prior to initiation of treatment and monthly pregnancy testing during treatment is recommended. • Physicians, pharmacists, patients and male partners of female patients will receive supplemental educational material regarding safety concern as part of a Pregnancy Prevention Program and controlled distribution system. • Ambrisentan treatment should only be initiated by physicians experienced in the treatment of PAH. • Pack size will be limited to 30 tablets.
Anaemia (decreases in haemoglobin and/or haematocrit)	• Routine pharmacovigilance activities, including use of targeted follow-up questionnaires. • Additional information from clinical trials. • Post-marketing surveillance program to collect additional safety data.	• Appropriate warnings included in section 4.4 and appropriate labeling for adverse reactions in section 4.8 of the SPC. • Treatment should not be initiated in patients with clinically significant anaemia. • Haematocrit and/or haemoglobin concentrations are to be checked during treatment with ambrisentan, for example at 1 month, 3 months and periodically thereafter in line with clinical practice.
Oedema (peripheral oedema/ fluid retention)	• Routine pharmacovigilance activities, including use of targeted follow-up questionnaires. • Additional information from clinical trials. • Post-marketing surveillance program to collect additional safety data.	• Appropriate warnings included in sections 4.4 and appropriate labeling for adverse reactions in sections 4.2 and 4.8 of SPC. • Peripheral oedema has been observed with ERAs including ambrisentan. Most cases of peripheral oedema in clinical studies with ambrisentan were mild to moderate in severity, although it appeared to occur with greater frequency and severity in patients ≥ 65 years. Peripheral oedema was reported more frequently with 10 mg ambrisentan. • Post-marketing reports of fluid retention occurring within weeks after starting ambrisentan have been received and, in some cases, have required intervention with a diuretic or hospitalisation for fluid

Safety concern	Proposed pharmacovigilance activities	Proposed risk minimisation activities
		management. • If clinically significant fluid retention develops, with or without associated weight gain, further evaluation should be undertaken to determine the cause, such as ambrisentan or underlying heart failure, and the possible need for specific treatment or discontinuation of ambrisentan therapy.
Hypersensitivity	• Routine pharmacovigilance activities, including use of targeted follow-up questionnaires. • Additional information from clinical trials. • Post-marketing surveillance program to collect additional safety data.	• Contraindication information in section 4.3 of SPC for hypersensitivity to active substance, soya or to any of the excipients. • SPC includes a precaution in section 4.4 of SPC for potential of allergic reactions with azo colouring agent Allura red AC Aluminum Lake (E129) and appropriate labeling for adverse reactions in section 4.8.
Hepatotoxicity	• Routine pharmacovigilance activities, including use of targeted follow-up questionnaires. • Additional information from clinical trials. • Post-marketing surveillance program to collect additional safety data.	• Contraindication in patients with severe hepatic impairment (with or without cirrhosis) and in patients with baseline values of hepatic aminotransferases (AST and/or ALT) > 3X ULN in section 4.3 of SPC. • Appropriate warnings in section 4.4 of SPC. • Monthly on therapy monitoring of ALT and/or AST is recommended. • If patients develop sustained, unexplained clinically significant ALT and/or AST elevation or if ALT and/or AST elevation is accompanied by signs or symptoms of hepatic injury (e.g. jaundice), ambrisentan therapy should be discontinued. • In subjects without clinical symptoms of hepatic injury or jaundice, re-initiation of ambrisentan may be considered following resolution of hepatic enzyme abnormalities. The advice of a hepatologist is recommended. • Physicians, pharmacists and patients will receive supplemental educational material regarding this safety concern as part of a controlled distribution system. • Ambrisentan treatment should only be initiated by physicians experienced in the treatment of PAH.

Safety concern	Proposed pharmacovigilance activities	Proposed risk minimisation activities
		• Pack size will be limited to 30 tablets.
Testicular tubular atrophy	• Routine pharmacovigilance activities. • Additional information from clinical trials. • Investigation of chronic administration of ambrisentan and effect on sperm production in male patients with PAH.	• The SPC contains appropriate language concerning preclinical testicular tubular atrophy findings and fertility in sections 4.6 and 5.3.
Symptomatic hypotension	• Routine pharmacovigilance activities, including use of targeted follow-up questionnaires. • Additional information from clinical trials. • Post-marketing surveillance program to collect additional safety data.	• The SPC contains appropriate language concerning potential of hypotension with an ambrisentan overdose in sections 4.9 and 5.3.
Use in paediatric/ adolescent patients	• Routine pharmacovigilance activities. • Additional information from clinical trials.	• The SPC contains appropriate language concerning special populations in section 4.2 of SPC. • Ambrisentan is not recommended for use in children below 18 years of age due to a lack of data on safety and efficacy.
Use in patients with severe renal impairment	• Routine pharmacovigilance activities. • Additional information from clinical trials. • Post-marketing surveillance program to collect additional safety data.	• The SPC contains appropriate language concerning special populations in sections 4.2 and 5.2 of SPC. • There is limited data for use of ambrisentan in individuals with severe renal impairment (<30 mL/min) and in these patients, initiation of therapy should be undertaken cautiously.
Use in patients with severe hepatic impairment	• Routine pharmacovigilance activities.	• Contraindication in section 4.3 of the SPC in patients with severe hepatic impairment (with or without cirrhosis) and in patients with baseline values of hepatic aminotransferases (AST and/or ALT)> 3X ULN. • The SPC contains appropriate language concerning special populations in sections 4.2 and 5.2 of SPC.
Use in other PH patient groups and some patient subgroups within “group 1”	• Routine pharmacovigilance activities. • Additional information from clinical trials.	• Sections 4.1 and 5.1 of SPC will clearly define patient population where efficacy has been established.
Lactation	• Routine pharmacovigilance	• Contraindication in section 4.3 for

Safety concern	Proposed pharmacovigilance activities	Proposed risk minimisation activities
	activities.	use of ambrisentan in lactation. • The SPC contains appropriate language concerning lactation in sections 4.6 and 5.3.
Drug-drug interactions	• Routine pharmacovigilance activities. • Additional information from clinical trials for drug-drug interaction studies with oral contraceptives, cyclosporine A, rifampin and tadalafil.	• Appropriate labeling for drug-drug interactions in sections 4.5 and 5.2 of SPC.

The CHMP, having considered the data submitted in the MA application is of the opinion that the following risk minimisation activities are necessary for the safe and effective use of the medicinal product: see as detailed in section 2.3 of this CHMP Assessment Report

2.6 Overall conclusions, risk/benefit assessment and recommendation

Quality

The quality of the 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. There are no unresolved quality issues, which have a negative impact on the Benefit Risk balance of the product.

Non-clinical pharmacology and toxicology

Overall the primary PD studies provided adequate evidence that ambrisentan blocks the pressor effects of exogenous endothelin, to decrease arterial blood pressure and to inhibit neointimal proliferation after arterial damage.

From the pK point of view, the disposition characteristics were generally similar across the species tested, showing ambrisentan to be a low clearance compound with low to moderate volume of distribution in all preclinical species. The elimination was characterized as triphasic.
The PK drug interaction studies showed that ambrisentan is unlikely to be involved in metabolism-related drug-drug interactions. Results of studies conducted to evaluate the inductive effects of ambrisentan on hepatic transport suggest that the likelihood of ambrisentan having an adverse interaction with hepatic transporters is low.

Overall the general toxicology programme revealed that ambrisentan produced nasal bone hyperplasia of the ethmoid turbinates observed in the nasal cavity of rats and also testicular tubular atrophy. The mechanism of the nasal bone hyperplasia is not clear but an association with inflammatory process in the nasal cavity area is suggested. Information to this respect has been maintained in the SPC since similar effects in humans after long-term exposure cannot be discarded. Testicular tubular atrophy has been observed in all tested preclinical species, and further clinical data however will be provided post-authorisation.
Ambrisentan was shown to be teratogenic to rats and rabbits, and a strict pregnancy contraindication has been included in the SPC.

Efficacy

The primary ITT analysis showed a statistically significant and clinically relevant mean placebo-corrected ambrisentan treatment effect on the 6MWD of an increase of 31 - 59 meters in all active treatment groups.

A dose-response relationship for the change in 6MWD was observed for each of the placebo-controlled studies, although the effect for the 5mg dose group in AMB-321 is double than the one shown in AMB-320. No reasons for the different effect observed have been identified.

The conclusions from the primary outcome parameter are supported by those from secondary variables in the combined analysis, as all endpoints evaluated were statistically significant for the ambrisentan group. When the analysis was performed for each individual study, some secondary endpoints were also statistically significant for AMB-321, although none were statistically significant for AMB-320.

One of the main concerns identified in this application was the true representativeness of the patients considered as functional class II. The applicant has extensively explored the data and has provided analyses which confirmed that the patients classified as class II are different from those classified as class III and are less severe from a clinical point of view.
The available information indicates that in patients classified as class II at baseline there is a lack of dose relationship for improvement in 6MWD. However, PAH is a progressive disease and deterioration despite targeted therapy is commonly observed in the long-term. Therefore, from a clinical point of view, there may be a clinical need for the 10mg dose of ambrisentan, in patients who have tolerated the 5mg dose well but whose symptoms have progressed.

Data from the phase 3 studies identified that the 10 mg dose may bring higher benefit to patients with PAH-CTD in comparison with the 5 mg dose. The baseline characteristics of the study populations (5 and 10 mg) did not reveal any explanation for the observed difference in response to ambrisentan. Patients with PAH-CTD have, however, a consistently smaller response to other ERAs compared to patients with IPAH. The greater efficacy associated with the 10 mg dose may be the result of a dose-response effect in this patient group.

Safety

The safety profile of ambrisentan is in line with that expected for this product class. The most common AEs are headache, flushing, nasal symptoms (especially congestion) and peripheral oedema. Concerning the 10mg dose it is of importance to point out the dose-related incidence of peripheral oedema and its potential severity, particularly in the elderly. Decreases in Hb, as for other ERAs, are also a common AE with a higher incidence in the 10mg dose.

An important safety concern in the ERA class is hepatotoxicity. At present, ambrisentan appears to have a better hepatic safety profile than for other ERAs, however, a cautious approach is considered appropriate.

Effects on QTc were thoroughly investigated with at present no significant ambrisentan-induced QT changes observed.

Non-clinical data has shown a relationship between chronic use of ERA-class compounds and development of testicular atrophy and infertility has been suggested. Preliminary clinical data did not suggest that ambrisentan was associated with an adverse effect on male reproduction but the safety database on this specific aspect is considered too small to draw robust conclusions. This information is included in the SPC and further data will be collected post-authorisation.

No studies have been performed in renally impaired patients, however results from the population PK analysis have lead to an advice for caution in patients with a CrCL<30 mL/min been included in the SPC. Information relating to baseline renal function will be collected as part of the post marketing surveillance programme to assess the safety profile in the renal impaired population.

It is important to state that post-marketing reports from the US on fluid retention occurring within weeks after starting ambrisentan have been presented and assessed. The incidence and consequences of peripheral oedema/fluid retention will be one of the main endpoints of the proposed Post-Marketing Surveillance Program designed with the intention to collect additional safety information for ambrisentan during standard clinical care.

From the safety database all the adverse reactions reported in clinical trials and post-marketing have been included in the Summary of Product Characteristics.

Having considered the safety concerns in the risk management plan, the CHMP considered that the proposed activities described in section 3.5 adequately addressed these.

- User consultation

User consultation has been performed on a package leaflet. A pilot test followed by two rounds of testing was carried out and minimum requirements according to current European guidelines were achieved. The amendments made between first and second round were generally accepted, and the user consultation considered acceptable with implementation of results in the product information.

Risk-benefit assessment

The pivotal trials have shown a statistically significant effect of ambrisentan on the 6-Minute Walk Test. Results of the primary endpoint are supported by those from secondary variables in the combined analysis.

The available information indicates that the 10mg dose is generally well tolerated in patients classified as class II at baseline, however there is a lack of dose relationship for improvement in 6MWD in this class. However, due to the progressive nature of PAH, patients initially classified as class II could benefit from 10mg if during the course of the disease their symptoms progress to class III.
Data presented also supports the efficacy of ambrisentan in CTD-associated PAH especially with 10 mg dose.

In relation to safety, the AEs reported were similar to those described with other ERAs. Long-term safety data available is consistent with this observed for the pivotal studies. The safety profile from a new updated safety analysis is consistent with that observed in the original analysis period. However, safety data should be cautiously interpreted considering the relatively small number of patients as well as the lack of a control arm. Additional experience will be gained from the post-marketing experience and pharmacovigilance activities. A safety issue, however, which at present deserves further assessment, is the effect of ambrisentan on male fertility.

Data provided indicates that in principle the adverse events are manageable in clinical practice. In addition, different actions are proposed to further clarify and characterize the risk of clinically significant changes in haemoglobin, peripheral oedema and hepatic safety profile as part of the RMP.

The B/R ratio for ambrisentan is considered positive provided that the applicant commits to perform a number of post authorisation follow-up measures to be reported back to the CHMP within predefined timeframes.

A risk management plan was submitted. The CHMP, having considered the data submitted, was of the opinion that pharmacovigilance activities in addition to the use of routine pharmacovigilance were needed to investigate further some of the safety concerns. The following additional risk minimisation activities were required: see as detailed in section 2.3.

Similarity with authorised orphan medicinal products

The CHMP is of the opinion that Volibris is not similar to iloprost (Ventavis®), treprostinil (Remodulin®), bosentan (Tracleer®), sildenafil (Revatio®), and sitaxentan (Thelin®) within the meaning of Article 3 of Commission Regulation (EC) No. 847/200 (See appendix 1).

Recommendation

Based on the CHMP review of data on quality, safety and efficacy, the CHMP considered by consensus that the risk-benefit balance of Volibris in the treatment of pulmonary arterial hypertension was favourable and therefore recommended the granting of the marketing authorisation.

In addition, the CHMP, with reference to Article 8 of Regulation EC No 141/2000, considers Volibris not to be similar (as defined in Article 3 of Commission Regulation EC No. 847/2000) to iloprost (Ventavis®), treprostinil (Remodulin®), bosentan (Tracleer®), sildenafil (Revatio®), and sitaxentan (Thelin®) for the same therapeutic indication.