SCIENTIFIC DISCUSSION

This module reflects the initial scientific discussion and scientific discussion on procedures which have been finalised before 1 October 2004. For scientific information on procedures after this date please refer to module 8B.

1. Introduction

Pulmonary arterial hypertension (PAH) is a rare, progressive disease involving vasoconstriction, vascular remodelling, and thrombosis *in situ* resulting in a progressive increase in pulmonary vascular resistances (PVR) and right ventricular hypertrophy leading to right ventricular failure. It can occur without an obvious cause, as inprimary pulmonary hypertension (PPH), or secondary to systemic disease or congenital heart disease. Functional status is a predictor of survival. Patients who are in functional class II and III have a mean survival of 3.5 years compared with a mean survival of 6 months for those who are in functional class IV.

The conventional therapy for patients with PAH includes vasodilators, such as high doses of calcium antagonists, anticoagulant agents and oxygen. Diuretics and supplementary oxygen are used to relieve symptoms, such as dyspnoea and peripheral oedema. In patients responding to testing with nitric oxide (approximately 1/3 of the patients), symptoms are improved and survival prolonged by the combined treatment of high doses of calcium channel blockers (CCB) and anticoagulants. Epoprostenol (prostacyclin), delivered via a portable pump system into an in-dwelling central vein catheter, has been shown to improve haemodynamic parameters, exercise capacity and life expectancy in patients with severe PPH (NYHA Class III and IV). Pharmacological tolerance with need for dose increments during long-term treatment is commonly observed.

The active substance of Tracleer, bosentan, is a non-peptide antagonist of human endothelin receptors (ET_A and ET_B). Endothelin (ET) levels are elevated in patients with PAH and correlate with disease severity and prognosis, suggesting a causal role. Bosentan is intended to affect vasoconstricting, hypertrophic and fibrotic effects by blocking the actions of receptors ET_A and ET_B.

The approved indication: “Treatment of pulmonary arterial hypertension (PAH) to improve exercise capacity and symptoms in patients with grade III functional status. Efficicacy has been shown in:

- Primary PAH
- PAH secondary to scleroderma without significant interstitial pulmonary disease.”

The recommended maintenance dose is 125 mg twice daily.

2. Chemical, pharmaceutical and biological aspects

Composition

Tracleer is presented as immediate release film-coated tablets containing 62.5 mg or 125 mg bosentan as the monohydrate. The 62.5 mg tablets are orange-white, round, biconvex film-coated tablets embossed with "62,5"on one side. The 125 mg tablets are orange-white, oval, biconvex film-coated tablets embossed with “125” on one side.

The composition of the cores for the 62.5 mg and the 125 mg tablets are proportional (common blend), and the ingredients are bosentan monohydrate, maize starch, pregelatinised starch, sodium starch glycollate, povidone K90, glycerol dibehenate and magnesium stearate, while the coating is composed of hypromellose, glycerol triacetate, talc, titanium dioxide [E171], yellow [E172] and red [E172] iron oxides, and ethylcellulose aqueous dispersion (solid part).

The tablets are presented in PVC/PE/PVDC/aluminium blisters, each containing 14 tablets. Secondary packaging consists of cartons containing 14 (62.5 mg strength only), 56 or 112 tablets. Not all pack sizes may be marketed.

Active substance

The active substance, bosentan monohydrate, is synthesised in 7 steps from 2-chloropyrimidine. Satisfactory control specifications and associated methods are provided for the starting materials, key intermediates, reagents and solvents. No metal catalysts are used. The active ingredient is milled after manufacture.

Bosentan has no asymmetric atoms and is therefore achiral.

Twenty-seven possible impurities from the route of synthesis are discussed but only 3 impurities, Ro 47-0005, Ro 47-4056 and Ro 47-9931, are regularly formed. The other possible impurities have not been observed in significant amounts. The specified limits set for Ro 47-0005, Ro 47-4056 and Ro 47-9931 are < 0.2 %, < 0.3 % and < 0.3 %, respectively. The < 0.3 % limits set for the levels present in the batch used in toxicological studies qualify the 2 latter impurities. The level of Ro 47-0005 in the toxicological batch was below the specified limit set but the limit is nevertheless considered qualified in view of the maximum daily dose of bosentan (250 mg). The organic solvents used in the last 3 synthesis steps are dimethylformamide, denatured ethanol, methanol, isopropyl acetate and cyclohexane. However, the levels of all solvents except ethanol were below the detection limits in the batches presented. The specified limit set for ethanol (≤ 0.2 %) is below the ≤ 0.5 % limit generally accepted for class 3 solvents.

The structural and physico-chemical characterisations of bosentan are satisfactory. The partition co-efficient for bosentan in octanol/buffer is as follows: at pH 4: log P= 3.1, at pH 7.4: log D= 1.3. Bosentan has a pKa of 5.46. Bosentan monohydrate is freely soluble in acetone and dichloromethane, soluble in ethanol and ethyl acetate, slightly soluble in methanol and isopropanol, and very slightly soluble in hexane. Bosentan monohydrate is non-hygroscopic and polymorphism has not been observed.

The active substance specification includes tests for identity and tests and limits for assay, related substances, residual solvents, sulphated ash, heavy metals, and particle size distribution. All analytical methods have been submitted, as well as validation data, in accordance with the relevant Note for Guidance.

Batch analysis results are presented for 3 commercial batches manufactured by the commercial manufacturer and the results confirm satisfactory uniformity of results and compliance with the specification. The levels of Ro 47-0005, Ro 47-4056, Ro 47-9931 and residual ethanol were < 0.10 %, 0.11-0.14 %, 0.11-0.15 % and 0.02-0.03 %, respectively.

The stability of bosentan has been examined under a variety of stress testing conditions. Real-time and accelerated stability studies have been performed on bosentan powder in accordance with the ICH Guideline. No significant changes in any parameter were observed. The proposed retest period of 3 years (and no special storage conditions) is justified.

Other ingredients

The excipients all comply with the respective current PhEur monographs, except the red and yellow iron oxides, which are claimed to comply with EEC requirements, and ethylcellulose aqueous dispersion, which complies with its USP/NF monograph.

Regarding TSE compliance, the applicant declares that none of the excipients used for commercial manufacture of Tracleer will be of animal origin. Although magnesium stearate of bovine origin has been used for manufacture of the registration batches, it is stated that the magnesium stearate used for manufacture of future clinical and commercial batches will be of vegetable origin only.

Satisfactory control specifications are provided for the primary packaging materials.

Product development and finished product

The original development of bosentan tablets was initiated by F. Hoffmann-La Roche, but in 1999 all activities except on-going stability studies were discontinued. Later Actelion Ltd licensed the right to bosentan and contracted out the manufacture to Patheon Inc., Canada. The product development was focussed on selection of dosage form, choice of excipients, compatibility of excipients and active ingredient, optimisation of the manufacturing process, transfer of the manufacturing process from Roche to Patheon, and development of the dissolution test method.

The finished product is manufactured using a typical wet granulation method as follows: bosentan monohydrate, maize starch, pregelatinised starch, sodium starch glycollate, and povidone are dry blended, purified water is sprayed on the blend, and the mixture is kneaded to granules in a high shear granulator. The granules are then dried (fluid bed), sieved, mixed with glycerol dibehenate and magnesium stearate, and compressed to tablets and coated. The applicant commits to perform process validation on the first commercial batches produced. Reference is also made to the batch analysis certificates presented for 3 pilot batches. However the manufacturing parameters of the different steps of the process are satisfactorily validated.

The specification for the finished products at release and end of shelf-life includes tests for shape, colour, average mass, uniformity of mass, identity, assay (95-105 %), related substances (Ro 47-4056: max. 0.3 % and others: each max. 0.2 % and total max.0.5 %), dissolution (paddle, 50 rpm, 1 % SLS; 75 % after 30 minutes), and microbial contamination. The limits for degradation products are qualified. All the methods of analysis are satisfactorily described and validated.

Batch analysis results are presented for 3 batches of each tablet strength manufactured at the commercial manufacturing site. The scale of these batches is approximately one third of the indicated maximum scale. All batches complied with the finished product specification and demonstrated consistency of manufacture. The content of Ro 47-4056 and other related substances in the batches of both strengths were 0.14-0.17 % and < 0.1 %, respectively. The amount of bosentan dissolved was over 99 % after 30 minutes for all batches.

Stability of the Product

Stability results have been presented for 3 batches of each strength manufactured in one third of maximum scale at the commercial manufacturing site. The batches were packaged in PVC/PE/PVDC/aluminium blisters. The data for these commercial batches include results from 9 months accelerated and 18 months long-term storage (at 25°C/60%RH). Also presented are supplementary data for one batch of each strength manufactured at a previous site and packaged in amber glass bottles. The supplementary data, which are of limited value since the packaging materials differ, include 24 months accelerated data and up to 24 months long-term stability data. All batches are more or less unchanged during storage at all conditions. The data provided by the applicant confirmed a slight decrease in bosentan content for the commercial batches, but the product is remaining within its specifications for each storage condition.

In general the stability studies support the shelf life and storage conditions as defined in the SPC (Do not store above 30°C). Whilst a 24-month shelf-life for Tracleer 62.5 mg and 125 mg film coated tablets is justified, this should be confirmed with real-time data, and the applicant has committed to provide this as soon as it becomes available. As a post-authorisation Follow up Measure, the MAH has submitted real-time stability data for Tracleer 62.5 mg and 125 mg film coated tablets and a 36-month shelf life is justified.

3. Toxico-pharmacological aspects

All safety studies were performed according to good laboratory practice (GLP) with the exception of the safety pharmacology studies and the studies of phototoxic potential.

Pharmacodynamics

Endothelin (ET) is a neurohormone secreted by the endothelium. It is a very potent vasoconstrictor, as well as a stimulator of cell proliferation, fibrosis and inflammation. The two receptors ET_A and ET_B are involved in the contractile effect of ET-1 in human pulmonary arteries. Recent publications suggest a beneficial effect of ET_A blockade in cardiovascular and renal disease; whereas ET_B mediated effects may be protective in animals.

- *In vitro* studies

In vitro functional experiments performed on animal and human tissues show that bosentan behaves as a competitive antagonist on ET receptors. Bosentan competes with the binding of ET-1 and other ET peptides to both ET_A and ET_B receptors, with a slightly higher affinity for ET_A receptors (K_i = 4.1–43 nM) than for ET_B receptors (K_i = 38-730 nM).
ET is a potent growth factor in combination with other growth factors such as platelet-derived growth factor. Bosentan inhibits the growth-inducing effects of ET and in various models; bosentan significantly reduces vascular hypertrophy, neointima formation and cardiac hypertrophy.
ET also stimulates collagen synthesis. This fibrotic effect is essentially mediated via ET_B receptors. Bosentan prevents the cardiac fibrosis induced not only by ET, but also by aldosterone or angiotensin II infusion in rats.

- *In vivo* studies

Bosentan increases ET plasma concentrations by a factor of 2-3 fold. When bosentan is given chronically, the increase in ET levels is less marked after prolonged treatment than early on. Bosentan was tested in several animal models of chronic PAH, and was either given as a preventive treatment or as a curative treatment after the establishment of PAH. In the chronic hypoxic rat model, bosentan not only prevented the development of pulmonary hypertension but also reversed established pulmonary hypertension and vascular remodelling. Bosentan has been shown to have an effect both on pulmonary vascular remodelling and the development of right ventricular hypertrophy. Bosentan inhibits the pressor effects of ET peptides on ET_A and ET_B receptors, and decreases blood pressure and peripheral vascular resistance in various rat models of hypertension without inducing tachyphylaxis. In contrast to these pathological situations, bosentan has no significant blood pressure-lowering effect in normotensive animals, with the exception of normotensive guinea pigs. The lowering of blood pressure induced by bosentan is not associated with an increase in heart rate.

- Pharmacodynamic drug interactions

The potential cardiovascular interaction between sumatriptan and bosentan on heart rate and blood pressure was evaluated in rats. Sumatriptan was injected intravenously in increasing doses (0.1 to 3.0 mg/kg) 10 min after i.v. injection of saline (control) or 30 mg/kg of bosentan. The combination of sumatriptan and bosentan did not have any significant effect on blood pressure or heart rate.

- General and safety pharmacology programme

Bosentan has choleretic effects and increases bile flow in dogs. However, bosentan also induces functional cholestasis and competes in a concentration-dependent fashion with bile salt elimination through liver canalicular membranes by inhibiting the bile salt export pump (Bsep). Intravenous injection of high doses of bosentan in rats leads to a dose-dependent increase in plasma bile salt concentrations. Cyclosporine A and glibenclamide also increase bile salt concentration in rats. The combination of bosentan and glibenclamide leads to an additive effect. The inhibition of bile salt elimination by bosentan results in a concentration-dependent accumulation of bile salts, which are cytotoxic to hepatocytes at high concentrations.

Bosentan decreases vascular permeability, increases plasma volume and decreases haematocrit in normal rats and in models of increased plasma extravasation. The explanation might be that ET has pro-inflammatory effects, enhancing microvascular permeability, thereby decreasing plasma volume and increasing haematocrit.

Bosentan was studied in a number of safety pharmacology studies in vivo after single oral doses up to 300 mg/kg and single i.v. doses up to 50 mg/kg and in some in vitro experiments. In these tests, bosentan showed:

- no effect on the central nervous system in mice,
- no effect on gastro-intestinal motility in mice,
- no effect on respiratory or cardiac function in anesthetised dogs,
- a transient dose-dependent decrease in urine volume and electrolyte excretion in rats (It might be secondary to a decrease in perfusion pressure due to the vasodilatory effect of bosentan.),
- no effect on smooth muscle contraction in vitro,
- no effect in vitro on cardiac repolarization in rabbit Purkinje fibres.

In the cardiovascular study in anaesthetised dogs no adverse effects on the ECG were reported after intravenous administration of bosentan. There was no data on systemic exposure of the three main human metabolites presented, however, extrapolation from other studies indicate that exposure was somewhat higher than that anticipated in human after therapeutic dose. In vitro electrophysiology testing with bosentan in rabbit Purkinje fibres did not show any significant changes. The main metabolites were not tested, but this was considered acceptable considering the chemical structure of the metabolites similar to the parent compound and the lack of effect on cardiovascular parameters in the clinical setting.

No studies were performed on autonomic nervous system, on platelet aggregation, on endocrine system. However, results obtained in primary pharmacological studies, toxicological studies and clinical studies do not suggest any concern.

The *in vivo* effect of bosentan on mitochondrial function is unknown.

- *Summary of salient findings*

Bosentan has been shown to be a competitive antagonist of ET binding to both ET_A and ET_B receptors. Bosentan has been demonstrated to exert the expected vasodilatory response. Pharmacodynamic studies have shown that bosentan reduces pulmonary artery pressure and improves other pulmonary parameters in various in vivo animal models. However, the selectivity for pulmonary vessels as opposed to other vascular systems, such as cerebral, coronary, renal and extremities, has not been demonstrated experimentally.

Bosentan exerts a concentration-dependent functional cholestatic effect by competing with bile salt elimination via the Bsep.

Pharmacokinetics

A number of assay methods using HPLC/UV and LC/MS/MS were developed over several years in various analytical laboratories and applied to non-clinical and clinical pharmacology, kinetic and toxicokinetic studies in order to determine the concentrations of bosentan (Ro 47-0203) and its 3 major metabolites (Ro 48-5033, Ro 47-8634, and Ro 64-1056) in biological fluids.
Validation of methods used in toxicokinetics and clinical pharmacokinetic is satisfactory.

The pharmacokinetics of bosentan has been characterised in various animal species following both intravenous and oral administration. The pharmacokinetics of bosentan differs among animal species. The dog was shown to be the most representative species for man. Bosentan showed good bioavailability (50 to 70%) when administered orally to rats, mice and dogs. Over the higher dose ranges of bosentan, the plasma levels were less than dose proportional. In the rat and mouse, but not the other species, exposure in females was higher than in males. Bosentan is highly bound to plasma proteins *in vitro* (98.1 % in humans, 95.9 % in dog, and 98.5 % in rat), mainly to albumin. The metabolism of bosentan is characterised by phase I transformation and metabolic pathways are similar in all species including man. The following three main metabolites are formed: the hydroxylation product of the t-butyl group (Ro 48-5033), the free phenol metabolite (Ro 47-8634), and the secondary metabolite, Ro 64-1056, which is the result of both metabolic reactions. Rats selectively formed an additional metabolite, Ro 47-8279, which was identified as an oxidation product of the ethylene glycol side chain to the corresponding aldehyde. Bosentan metabolites are excreted almost exclusively in the bile with very little unchanged drug detected. Renal excretion is negligible. Bosentan is a microsomal enzyme inducer in mice and dog. An increase of liver cytochrome P450 concentrations/activities is observed after subchronic oral treatment at high dose levels (up to 4500 mg/kg/d) in the mouse and dog, but not in the rat. This likely contributes to the time-dependent decrease in plasma concentrations observed in the animal toxicology studies. Following administration of ^{14}C bosentan, concentrations of drug-related material greatly exceeding those in plasma are found in liver and intestinal content, which

is consistent with excretion of bosentan by the biliary route. In most other organs, levels of radioactivity were below those in plasma. Levels in the brain are below the limit of detection. The majority of bosentan-related material is excreted within 3–5 days in the rat, marmoset and dog.

Toxicology

Single dose toxicity

The acute toxicity of bosentan was determined by the oral, i.v., s.c. and i.p. routes of administration in mice and rats and by the oral route of administration in dogs. In mice and rats the highest non-lethal doses were in the 125 to 250-mg/kg ranges by the i.v. and i.p. routes of administration, 1000 mg/kg or more by the s.c. route and 2000 to > 4000 mg/kg by the oral route. Signs of mild central nervous system (CNS) depression were observed on the first day after dosing and convulsions were observed at lethal doses. After i.v. administration red coloured urine was also observed. The acute oral toxicity in the dog was also low where the lethal dose was estimated to be greater than 2000 mg/kg.

Repeat dose toxicity

The choice of the rat as rodent species is considered acceptable since alternative species, such as the mouse, did not show any kinetic/metabolic advantages. The pharmacokinetic profile in dogs was very close to that in humans and since the metabolic patterns also were similar, the dog is the most appropriate non-rodent species. The cynomolgus monkey and the marmoset are considered inappropriate due to insufficient systemic exposure to the drug.

Oral administration

Oral repeated dose toxicity studies were conducted in rats, dogs, and marmosets. Three studies were conducted in rats including a 4-week gavage study; a 4-week dose range finding study by dietary admixes and a 6-month study by dietary admix. Four studies were conducted in beagle dogs with durations of 4 weeks (2 studies), 6 months, and 12 months. One 4-week repeated-dose study was conducted in marmosets. Each of the studies included toxicokinetic evaluations.

In the 4-week oral gavage study (20, 200, and 2000 mg/kg/day) in rats, bosentan was generally well tolerated at doses up to 200 mg/kg/day. Evidence for altered thyroid function (mild thyroid hormone imbalance in male rats in which there was a significant increase in T4 levels and thyroid gland weights and a trend for increased TSH) and slightly decreased red blood cell (RBC) parameters were observed. In the 4-week oral (admix) dose range finding study (200, 600, and 1500 mg/kg/day), bosentan was generally well tolerated. In the 6-month oral (admix) study in rats (40, 200, and 1000 mg/kg/day), bosentan was well tolerated. Clinical signs were limited to stertorous breathing for female rats at the mid dose and for both sexes at the high dose. Body weight gain was decreased for female rats at the mid and high doses and food consumption was slightly increased for both sexes at the high dose. In bone marrow smears of high-dose rats of both sexes, lymphocytes were slightly decreased and eosinophils and basophils were slightly increased. Liver weights of male and female rats were increased at the high dose, and adrenal gland weights were increased for high-dose female rats.

In most of the dog studies, a mild decrease in red blood cell (RBC) parameters was observed that may be due to the pharmacodynamic effects of bosentan. In addition, mild increases in alkaline phosphatase (AP) along with increased liver weights and hepatocellular hypertrophy are compatible with the microsomal enzyme inducing properties of the drug in the dog. In a 4-week study conducted at very high doses (500 and 1000 mg/kg) increased serum liver enzymes and, histologically, bile duct proliferation and single cell necrosis were observed. In the 6-month dog study, no significant toxicity was observed at doses up to 400 mg/kg. In the 12-month oral (capsule) study in dogs, bosentan treatment (60, 180, and 500 mg/kg/day) was also generally well tolerated. A mild transient decrease in partial thromboplastin times in mid- and high-dose dogs and slightly decreased RBC parameters in high-dose dogs were observed. A minimal increase in alkaline phosphatase (AP) at the mid and high dose and a marked increase in serum bile salts at the high dose were observed. The AP isozyme from bone decreased in parallel with the increase in AP, indicating another source such as liver as opposed to cholestasis. At necropsy, liver and kidney weights were increased in mid- and high-dose male dogs. Histopathologically, mid-dose dogs showed an increased yellow pigment and vacuolation in the gallbladder epithelium and at the high dose increased marginal signs of cholestasis, increased mucus

secretion, yellow pigment and vacuolation in gallbladder epithelium, and increased yellow pigment in the kidney were observed. One female had interstitial fibrosis in the kidney. Bosentan and its metabolites were determined in bile and liver specimens of high-dose (500 mg/kg/day) dogs 24 hours after dosing. Bosentan did not accumulate with less than 0.1% of a daily dose retained in the liver; however, high concentrations of bosentan and its three major metabolites were observed in bile. In summary, histological changes indicative of a mild cholestasis and increased serum bile salts were observed at the high dose, most likely as a result of a competitive inhibition of bile salt transport.

In a 4-week oral toxicity study in marmosets, the administration of bosentan (10, 80, and 500 mg/kg/day) was generally well tolerated. At the high dose, a slight increase in the level of isocitrate dehydrogenase was observed in females and increased liver weights were noted in male marmosets. Following a 4-week recovery period, the isocitrate dehydrogenase levels returned towards normal, but the liver weights remained slightly elevated.

Intravenous administration

Repeat-dose toxicity studies conducted by the i.v. route with bosentan consisted of a 4-week rat study (5, 20, and 40 mg/kg), a 7-day continuous infusion study in dogs (20, 80, and 200 mg/kg), and a 4-week i.v. study in marmosets (5, 20, or 40 mg/kg). In the rat study, gross and microscopic findings were limited to local damage at the injection site. Other than a mild decrease in RBC parameters, as noted in the other dog studies, no systemic toxicity was observed with bosentan in the dog or marmoset.

Genotoxicity

Bosentan has no mutagenic effect (Ames, Gene mutation test in Chinese Hamster V79 cells, Unscheduled DNA Synthesis Assay) and no clastogenic effect (Micronucleus test in Fu-moro mice, Chromosomal aberration test in human lymphocytes).

Carcinogenicity

Two 2-year carcinogenicity studies were conducted with bosentan in CD-1 mice and Wistar rats along with range-finding and toxicokinetic studies.

An initial carcinogenicity study was conducted in CD-1 mice, however due to problems with recording of feed consumption the study was aborted after 29 weeks of treatment.
A second 2-year oral (dietary admix) carcinogenicity study was conducted with bosentan in CD-1 mice at doses of 100, 450, 2000, and 4500 mg/kg/day. The high dose of 4500 mg/kg/day was selected because there was a small increase in exposure to bosentan and its metabolites at this dose, which represents essentially the maximum feasible dose. There was no effect on survival. At necropsy, there was a dose- and treatment-related increase in liver weights in male and female mice at all dose levels. An increased incidence of hepatic masses was observed in male mice treated with bosentan at dose levels of 450, 2000, and 4500 mg/kg/day. Histopathologically, a statistically significant moderate increase in the combined incidence of hepatocellular adenomas and carcinomas was observed in male mice at dose levels of 450, 2000 and 4500 mg/kg/day. There was no increase in the incidence of tumours in female mice or at sites other than liver in male mice. Toxicokinetic monitoring indicated that a moderately high systemic exposure to bosentan was achieved. Exposure increased less than dose-proportionally and was higher in females.
A two-year oral (pelleted admix) carcinogenicity study was conducted in Wistar rats with bosentan administered at dosages of 0 (control 1), 0 (control 2), 125, 500, 2000 and 3000 mg/kg/day. Although partial saturation of absorption was observed at doses greater than 500 mg/kg/day, the high dose of 3000 mg/kg was selected, which represents the maximum feasible dose. Although there was no increase in mortality, there were an increased number of females without a known cause of death. After 104 weeks, there was a treatment-related significant increase in the combined incidence of thyroid follicular cell adenomas and carcinomas in male rats at the high dose of 3000 mg/kg/day. There was no significant increase in the incidence of tumours in female rats or at sites other than thyroid gland in male rats. Systemic exposure to bosentan increased less than dose-proportionally and exposure was higher in females.

Reproduction Toxicity

Fertility studies were conducted in rats with bosentan at doses up to 1500 mg/kg/day (orally) or 40 mg/kg/day (i.v.) no effects were observed on mating performance or fertility, nor was there any adverse effect on the development of the preimplantation embryo or on implantation. Sperm parameters were assessed in both studies and there were no changes in sperm count, motility or viability or on testes weights.

Three modified embryofoetal-toxicity studies in rats were conducted. In an embryofoetal-toxicity study in rats, bosentan showed clear dose-dependent teratogenic effects that included variations in the arteries originating from the aorta, agenesis of the soft palate and craniofacial bone abnormalities; effects similar to malformations observed in endothelin knockout mice. In addition, poor postnatal survival was observed in pups from treated dams. A second embryofoetal-toxicity study, conducted with a batch containing a higher level of impurities, did not differ indicating that impurities are not involved in the response. A third study conducted with a litter exchange design indicated that *in utero* effects were responsible for the poor survival of pups.

In contrast to the rat, bosentan was not teratogenic in the pregnant rabbit at doses up to 1500 mg/kg/day, or in an additional high-dose embryofoetal-toxicity study. A toxicokinetic study in rabbits revealed that the exposure to bosentan in the rabbit is much lower as compared to rats, which may explain the lack of teratogenicity in the rabbit.

Bosentan was also evaluated in two *in vitro* test systems; one involving explanted mouse palate culture and the other the micro-mass LBCC. In the mouse palate culture system, bosentan resulted in a concentration-dependent increase in cleft palate. In the micro-mass LBCC, 4 endothelin antagonists, including bosentan, were tested. None of the compounds were active in this system.

No good practice segment III study was performed.

Local Tolerance

Evaluation of intravascular tolerability included two local intra-arterial tolerability studies in NZW rabbits, a single-dose haemolysis study in dogs, and an *in vitro* haemolysis test in blood from dogs and human volunteers. Bosentan formulations produced severe and irreversible arterial damage following i.a. administration to NZW rabbits. A single i.v. dose to dogs (30 mg/kg 6% solution) did not produce haemolysis. *In vitro*, no haemolysis was observed in canine or human blood at a concentration of 0.6% or lower, partial haemolysis at 1.2%, and total haemolysis at 2.4% or more.

Primary skin and eye irritation were evaluated in NZW rabbits. Bosentan or Ro 47-0005, an impurity, were not irritant to rabbit skin. Bosentan was considered non-irritant for the rabbit eye.

Immunotoxicity studies

Antigenicity studies conducted in guinea pigs showed a weak immunogenicity in a first study (1/10 animal positive for ASA and 5/10 animals positive for IgG antibodies directed to bosentan) when bosentan was injected alone ID with Freund's adjuvant (30 mg/animal x 3). In addition, when animals were immunized with bosentan linked to guinea pig serum albumin and challenged with bosentan alone, positive ASA was observed and anti-bosentan antibodies were present. In the same study, penicillin alone or penicillin conjugated to albumine was strongly positive. These results were not confirmed in a second study using the same protocol and in which ASA, PCA and IgG antibodies directed to bosentan were evaluated. The same type of study was conducted in mice and showed negative results. Determination of delayed contact hypersensitivity was evaluated in guinea pigs by the Maurer optimization test. Results showed that bosentan was positive in this test when injected ID, however no positive reactions were found after epicutaneous challenge. One impurity has sensitizing properties when it is used at a 50% concentration (a non irritant concentration) in the guinea pig sensitization test. This impurity is present at 0,2% in bosentan batches. Finally, treatment of dogs for 6-months with bosentan did not induce antibody production directed to bosentan. These studies were well conducted and do not raise concerns.

Other studies

The phototoxicity of bosentan was evaluated in an *in vitro* 3T3 murine fibroblast neutral red uptake assay and in three *in vivo* studies in hairless rats, one by single i.v. administration, another by single oral administration, and a third by single and 14-day oral administration. *In vitro*, bosentan exhibited phototoxic a low potential with UVA, but not UVB irradiation. *In vivo,* there was no evidence for phototoxicity in hairless rats following single oral bosentan doses up to 2000 mg/kg, single i.v. doses of 40 mg/kg, or multiple oral doses of 2000 mg/kg/day for 14 days and subsequent UVA irradiation. In the human safety database, the data do not suggest any phototoxic potential. No warnings are required regarding potential phototoxicity.

Impurities/Metabolites

The main impurities (and % maximum specifications) are Ro 47-4056 (0.3%), Ro 47-0005 (0.2%), Ro 47-9931 (0.3%). A batch with higher levels of impurities than batches normally used was tested in a 4-week rat study, a 13-week mouse study, a teratology study in rats, a mutagenicity study in bacteria and a clastogenicity study in mammalian lymphocytes. Based on the results from these studies the impurities are considered qualified at the proposed specification levels.

Discussion on toxico-pharmacological aspects

Although no pharmacokinetic studies were performed with the therapeutic recommended dosage in PAH patients, the kinetic studies provided allow an acceptable estimation of safety margins. It should be noticed that in spite of high doses, exposure to the animal are low, clearances being high. This leads to small safety margins, especially if one considers that exposure in patients with PAH is expected to be higher than in healthy subjects.
While exposure to Ro 64-1056 metabolite overall seemed low in repeated dose toxicity studies, this metabolite was present and exposure was likely continuous up to two years in the carcinogenicity studies.

After one year of oral treatment, the NOAEL in dog (the more representative species) has been evaluated at 180 mg/kg/day. In dog, the major target organ is liver. Bosentan produces mild clinical manifestations on hepatic functions such as an increase of alkaline phosphatase, hepatocellular hypertrophy bile duct proliferation. In rat there were two major target organs, liver (increased liver weights) and thyroid (increased T4 levels). Bosentan and its main biliary metabolite seem to be competitive inhibitors of bile salt excretion at the hepatocyte canalicular membrane, resulting in increased serum bile salts in rats, dogs and humans. Cholestasis is seen in the rat and dog at high exposures, and is associated with histopathological evidence of hepatocellular damage with increased aminotransferase. Preclinical data have not evidenced intrahepatocyte accumulation of bosentan in animals but these results cannot be strictly extrapolated to human. Due to the potential hepatotoxicity, liver enzymes should be periodically monitored during treatment.

Other drugs that also compete for bile acid excretion could show an additive effect in combination with bosentan (e.g. oestrogen). An *in vivo* study to evaluate the effect of each compound alone and of the combination of bosentan and oestrogens on bile salts in rats is planned and would contribute to address the potential effect of their concomitant use on bile salt pump. Results of this study will be submitted as a follow-up measure.

Red blood cell parameters were decreased in both the non-clinical and clinical studies. The changes were small in magnitude and might possibly be related to the pharmacodynamic properties of the drug in decreasing vascular permeability resulting in haemodilution.

In rodent carcinogenicity studies, bosentan treatment resulted in a statistically significant increase in the incidence of hepatocellular tumours in male mice (adenoma, carcinoma) at doses from 450 mg/kg/day and a statistically significant increase in the incidence of thyroid follicular tumours in male rats at the high dose (3000 mg/kg/day). There was no increase in tumours in female mice or rats despite higher exposures as compared to males. In view of lack of any evidence for mutagenic or clastogenic effects of bosentan in a comprehensive battery of tests for genotoxicity, it is reasonable to assume that the species and sex specific tumours observed are caused by non-genotoxic mechanisms, but no endocrine studies or hormone measurements have been performed in animals. In human, there was no change in thyroid hormone levels or TSH after 8 days treatment in one placebo-controlled study including 24 healthy volunteers. The liver tumours observed in male mice and the thyroid gland

tumours in male rats may be related to an epigenetic phenomenon without dose/effect relationship. In summary, bosentan showed carcinogenic potential. Extrapolation to human remains uncertain.

No teratogenic effect was observed in rabbit, but a teratogenic effect (agenesis of soft palate, variations in the arteries originating from the aorta, and craniofacial malformations) has been observed in rats for all doses up to 30 mg/kg/day at exposures that could be achieved in humans. The similarity of the pattern of malformations in endothelin receptor knockout mice and other endothelin receptor antagonists, indicate that this is a class effect. The lack of response in the rabbit is likely due to much lower exposures in rabbits as compared to rats since the bioavailability was lower. This precludes valid conclusions from the rabbit study. No conclusions can be drawn on the effect of bosentan on development and lactation since no good practice segment III study was performed.
In view of these findings, bosentan should be contraindicated in pregnancy and appropriate precautions should be taken for women of childbearing potential. Milk excretion has not been studied. Bosentan is a lipophilic substance for which excretion or even accumulation seems to be highly likely. Breast-feeding should be contraindicated during treatment.

4. Clinical aspects

The clinical pharmacology programme for bosentan consisted of 26 studies (including a total of 596 subjects). Of these studies, nine were of an exploratory nature in various indications, the other studies investigated the potential for drug–drug interactions or were conducted to establish the pharmacodynamic, pharmacokinetic and safety profile of bosentan in healthy volunteers or in special patient populations.

The clinical documentation provided to assess the efficacy and safety of bosentan by the oral route in the claimed indication (PAH) consisted of two placebo-controlled studies. Study AC-052-351 included 21 patients who received bosentan 125 mg b.i.d. (11 patients receiving placebo). Study AC-052-352 (BREATH-1) included 145 patients who received bosentan at 125 mg b.i.d. (N= 74 patients) or 250 mg b.i.d. (N=70 patients) and 69 patients who received placebo. Both studies were followed by a non-controlled open label extension study. The IV route PAH performed a supportive study in patients with.

Seven studies were performed in other indications (5 in severe chronic heart failure including the two

Most studies were performed according to GCP. However, for 3 pharmacokinetic studies performed at VanTx, Switzerland, GCP compliance cannot be confirmed. These 3 studies were drug-drug interaction studies with ketoconazole and losartan (AC-052-101), simvastatin (AC-052-102) and glibenclamide (AC-052-103). The studies were therefore considered as only informative and Actelion has performed repeated interaction studies with ketoconacole and simvastatin.

Clinical pharmacology

Pharmacodynamics

Three studies were performed in healthy volunteers including 167 young males receiving ascending IV or oral doses up to 2400 mg. Bosentan modestly reduced blood pressure and increased pulse rate, but only at high doses. An increase in ET-1 levels (up to 3-fold) was seen at higher doses. Big ET-1 and ET-3 were unaffected, indicating that ET production was not increased and that ET-1 levels were increased, possibly due to reduced receptor binding. Bosentan was generally well tolerated. Headache was the most common adverse event (AE) and nausea and vomiting occurred at IV doses > 500 mg. Elevations of liver enzymes were also reported at the therapeutic doses. No effects on ECG were reported.
Study BD14884 in patients with PAH was initially designed to assess ENABLE trials, 1 in systemic hypertension and 1 in subarachnoid haemorrhage). safety and, as a secondary objective, the efficacy, pharmacokinetics and pharmacodynamics of single intravenous and multiple oral doses of bosentan. Single ascending doses of bosentan were administered intravenously (50 mg/5 min, 150 mg/10 min and 300 mg/15 min) at 2-hour intervals. Patients were then randomised in a 1:1 ratio to receive in a double-blind design, oral doses of bosentan (1000 mg b.i.d) or placebo during 8-weeks. The study was

prematurely terminated after 7 patients had been entered, due to 2 deaths. A small, sustained fall in PAP and PVR was shown, but there was no selectivity for the pulmonary vasculature at doses tested. Dose-dependent decreases in blood pressure and an increase in heart rate were observed in association with bosentan infusion. Mean baseline levels of ET-1 were approx. 2-fold higher compared with healthy controls and bosentan administration was associated with an increase in plasma ET-1 (2-3 fold). Two patients died on day 3. Both were severely ill and had received IV bosentan followed by oral placebo. They were non-responders to inhaled nitric oxide. Both deaths were assessed as possibly related to bosentan; the cause of death was not clear in one patient, whereas in the other patient it was given as cardiac failure due to PAH. A rebound effect cannot be strictly excluded to explain these two deaths after high doses of infusion.

The effects of long-term treatment with Tracleer on ET-1 levels in patients with PAH are unknown.

New pharmacodynamic and pharmacokinetic data in children have become available post-authorisation as a result of the finalisation of the BREATHE-3 Study (AC-052-356) – see "post-authorisation" changes later on.

Pharmacokinetics

Bosentan was studied at oral doses of 3 to 2400 mg and intravenous doses of 10 to 750 mg. A large part of the pharmacokinetic documentation was obtained with doses higher than the doses applied for the indication PAH.

Different formulations were used in the clinical programme (oral solution early tablet formulation, ETF, and the formulation intended for marketing, IMF). Bosentan was fairly well absorbed in the dose range applied for. Maximum plasma concentrations are reached after about 3 h. Food has a small effect on the rate and extent of absorption of the IMF formulation (C_{max} increased with 22% and AUC with 10%). Bioequivalence was demonstrated between the IMF and the oral solution, while ETF had a lower relative bioavailability. The absolute bioavailability at a 600 mg dose of the oral suspension was 46%.

Bosentan has a relatively small volume of distribution, about 18 l, and is highly bound to plasma proteins, mainly albumin. Protein binding of bosentan was>98%.

Bosentan is eliminated by metabolism; less than 1% of an i.v. dose was excreted unchanged in the urine and less than 4% in faeces. Excretion of bosentan and its metabolites is primarily (94.5%) in faeces. Bosentan is metabolised by CYP2C9 and CYP3A4 to two primary metabolites Ro 48-5033 (hydroxylation) and Ro 47-8634 (demethylation). Both these metabolites are further metabolised by CYP2C9 and/or CYP3A4 to Ro 64-1056. In plasma bosentan, Ro 48-5033 and Ro 47-8634 accounted for 67%, 8.3% and 3.1% of drug-related radioactivity, respectively. The major component excreted in faeces was Ro 48-5033, accounting for 65% of the radioactivity. Ro 48-5033 may contribute to the pharmacological effects of bosentan to some extent (up to 20%), but not the other metabolites.

Bosentan displays dose-and time dependent pharmacokinetics. Clearance decreases with increased doses and increases with time. After a single i.v. dose of 250 mg CL was about 8.5 l/h. The terminal half-life was about 5 h. After oral administration the AUC seems to increase proportional to dose up to about 500 mg. CL/F thereafter increases, which most likely is due to a reduced bioavailability as the dose is increased. Thus, there is a dose dependency in clearance, which seems to be of limited importance as exposure is proportional to dose in the therapeutic range after oral administration. Upon repeated administration bosentan induces its own metabolism resulting in a reduction of the AUC of about 35-50%. The autoinduction is dose dependent and maximum autoinduction seems to have been reached after 4-5 days.

In vitro data suggest that CYP3A4 accounts for 2/3 of the metabolism of bosentan and CYP2C9 1/3, but the relative contribution of CYP2C9 and CYP3A4 in the metabolism of bosentan *in vivo* is unknown. Concomitant administration of the CYP3A4 inhibitor ketoconazole resulted in a 2.2-fold increase in AUC and 1.9-fold increase in C_{max}. No interaction study has been performed with a CYP2C9 inhibitor. A more pronounced interaction between a CYP2C9 inhibitor and bosentan than for ketokonazole cannot be excluded. Concomitant administration simultaneously with an inhibitor of CYP3A4 and an inhibitor of CYP2C9 could lead to very large increases in plasma exposure of bosentan. The same applies for concurrent use of CYP3A4 inhibitors in poor metabolisers of CYP2C9.

A major increase in bosentan exposure (30-fold increase in trough concentration) was observed after the first dose of concomitant CsA administration. Upon repeated administration the bosentan levels decreased and were approximately 3-4-fold increased at steady state. The mechanism for this interaction is unclear and the pharmacokinetic information after the first dose is limited to trough concentrations. The co-administration of bosentan and cyclosporine A is contraindicated.

In vitro studies suggest that bosentan has a low potential to inhibit cytochrome P450 isoenzymes. A small inhibitory effect could be observed on CYP2C9 (Ki 22 μM), CYP3A4 (Ki ≈34 μM) and CYP2C8 (Ki ≈46 μM) and no effect on CYP1A2, 2A6, 2B6, 2D6, 2C19 and 2E1.

In vivo interaction studies showed induction of CYP2C9 and CYP3A4 after multiple dosing for 4.5-8 days. AUC of CYP3A4 substrates (simvastatine, cyclosporin A (CsA), R-warfarin) decreased 40-50% and of the CYP2C9 substrate S-warfarin about 30%. *In vitro* studies indicate a possible induction of CYP2C19. A small effect on digoxin pharmacokinetics was also observed with reductions of AUC, C_{max} and C_{min} of 12, 9 and 23%, respectively. This suggests that bosentan may induce P-glycoprotein.

Bosentan has been shown to inhibit the bile salt export pump (Bsep) *in vitro*. Bosentan and glibenclamide have synergistic effects on Bsep inhibition *in vitro*. In a clinical trial high dose of bosentan given concomitantly with glibenclamide resulted in an increased incidence of elevated aminotransferase levels. Tracleer and glibenclamide should not be used concomitantly. An alternative antidiabetic medicinal product should be used in patients in whom an antidiabetic treatment is indicated.

Concomitant administration with oestrogen as known to inhibit the bile salt pump has not been addressed. No interaction study with any kind of contraceptive pill has been performed. Tracleer may render hormonal contraceptives ineffective. Bearing in mind that pregnancy may cause life-threatening complications in women and that bosentan is teratogenic, hormonal contraceptives cannot be used as the sole contraceptive method and an additional or an alternative reliable method of contraception must be used. In women of childbearing potential, treatment with Tracleer should not be initiated unless result of a pre-treatment pregnancy test is negative. Also, monthly pregnancy tests are recommended during treatment with Tracleer.

Very limited pharmacokinetic information from 7 patients with PAH suggests that the exposure in these patients can be expected to be about twice as high as in healthy volunteers.. Patients with CHF had on average about 30-40% higher exposure at steady state than healthy volunteers. At the time of the initial Marketing Authorisation Application, additional pharmacokinetic data were being collected in ongoing studies in PAH patients (AC-052-357 and AC-052-356 -BREATH-3 in children). These data have subsequently been provided as FUMs - see “post-authorisation” changes section.

In patients with severe renal impairment (creatinine clearance 17-27 ml/min), C_{max} was about 35% lower and AUC about 10% lower than in healthy volunteers. Plasma concentrations of bosentan metabolites increased about 2-fold in these patients as compared to subjects with normal renal function. No dose adjustment is required in patients with renal impairment. There is no specific clinical experience in patients undergoing dialysis. Based on physicochemical properties and the high degree of protein binding, bosentan is not expected to be removed from the circulation by dialysis.

A study (AC-052-107) to assess the effect of *mild hepatic impairment* (Child-Pugh class A) on the pharmacokinetics of bosentan after single dose and multiple dose administration was conducted. Eight patients were included. Pharmacokinetics in patients with mild hepatic impairment (Child-Pugh class A) are similar to those in healthy volunteers. Steady state AUC of bosentan was 9% higher and AUC of Ro 48-5033 was 33% higher in mild hepatic impairment than in healthy volunteers. No dose adjustment is needed in patients with mild hepatic impairment. (Tracleer is contraindicated in patients with moderate to severe hepatic impairment.)

No specific studies were performed in order to evaluate the effect of gender, age, weight and race on the pharmacokinetics of bosentan. Pooled data from several studies suggested no effect of age (in adults), weight or race on bosentan pharmacokinetics, but a slightly higher C_{max} and AUC in females than in males (C_{max} was 42% higher and AUC 26%, most likely due to differences in weight).

Clinical efficacy

Two clinical studies were provided to support the efficacy and safety of bosentan by oral route in the claimed indication (PAH):

- A multicenter placebo-controlled trial (AC-052-351) including 21 patients who received bosentan 125 mg b.i.d. (11 patients receiving placebo) for 12 weeks to 28 weeks.
- A larger pivotal multicenter placebo-controlled trial (study AC-052-352; BREATH-1: Bosentan randomized trial of endothelin antagonist therapy for pulmonary hypertension) including 145 patients receiving bosentan at 125 mg b.i.d. (N= 74 patients) or 250 mg b.i.d. (N=70 patients), for 16 weeks to 28 weeks.

In both trials, the study drugs were added to the patient's existing PAH therapy, but patients treated with IV prostacycline within 3 months were not included.

After 4 weeks with 62.5 mg bid, the posology was increased to the maintenance dose (i.e. 125 mg b.i.d. in study AC-052-351; 125 mg b.i.d. or 250 mg b.i.d; in study AC-052-352). Both studies were followed by a non-controlled open label extension study.

On behalf of the EMEA, a GCP inspection of the multicentre, phase III, clinical trial AC-052-352 was performed. Compliance with the protocol and GCP appeared to be satisfactory and the data quality was acceptable.

Dose-response studies and main clinical studies

Dose response study

Pharmacodynamic and dose-finding studies in patients with PAH are virtually lacking and the proposed starting dose, titration scheme and maintenance dose are based on experience gained from studies of bosentan in CHF and hypertension. No formal dose-response study has been conducted with oral bosentan in patients with pulmonary hypertension (PH).

Main studies

Study AC-052-351

This study was a multicenter, double blind, randomized, placebo-controlled study to evaluate the effects of bosentan given orally twice daily for at least 12 weeks to ambulatory adult outpatients with PAH whether or not secondary to scleroderma (SSc/PHT), but without moderate to severe interstitial disease.

Patients were randomised in a 2:1 ratio to bosentan 62.5 mg b.i.d. or placebo. After 4 weeks, dosages were up titrated to bosentan 125 mg b.i.d. or placebo. The study was completed when the last enrolled patient, not prematurely withdrawn, had completed week 12 assessments. Since the study continued until that time, included patients go on receiving double- blind study treatment (bosentan 125 mg b.i.d. or placebo) after 12 weeks for varying lengths of time (period II) until the study was completed.

The *primary endpoint* was the effect of bosentan on exercise capacity assessed by change from baseline to Week 12 in the distance walked by patients in 6 minutes (6-minute walk test). Comparison between placebo and bosentan was performed using a one-sided Student's t-test at the 5% level of significance. The study sample size was calculated under the assumption of an expected difference of 50 m (SD= 50m) on the primary outcome. Thirty patients in total were estimated to be sufficient.

Secondary efficacy criteria included:

- Central haemodynamic parameters (Pulmonary arterial pressures (PAP), Pulmonary vascular resistance (PVR), Mean right atrial pressure (RAP), Pulmonary capillary wedge pressure (PCWP), Cardiac index (CI), Systemic vascular resistance (SVR)),
- Time from randomization to clinical worsening (clinical worsening was defined as death from all causes, lung trans-plantation or discontinuation of therapy due to clinical deterioration),
- Clinical parameters *(Borg Dyspnea Index, WHO Functional Class),*
- Change from baseline to week 12 in Raynaud's Symptoms (in the sub-population of patients with Raynaud's phenomenon secondary to systemic sclerosis),
- And safety parameters.

Demographics and baseline characteristics of patients

Thirty-two patients were enrolled. 21 patients received bosentan and 11 received placebo. All patients were in WHO functional Class III of pulmonary hypertension. Patients had mainly primary PAH (only 4 patients with systemic sclerosis received bosentan, one patient received placebo). As expected in a PAH trial more females than males were included and all 4 males were assigned to bosentan group.

Results

Three placebo patients were prematurely withdrawn because of clinical worsening of PAH and were transferred to epoprostenol. No bosentan patients were withdrawn, died, underwent lung transplantation or discontinued study medications during the period of the trial.
At the time the study was completed, the duration of bosentan treatment ranged from 51 to 202 days. 20 patients among the 21 bosentan-treated patients had reached week-20 assessment, 6 of them reached the Week-28 assessment.

A significant improvement of exercise capacity (assessed by the 6-minute walk test as primary end point) has been shown after 12 weeks treatment at the posology of 125 mg bid (See table below).

Study AC-052-351 - Change in walk test from baseline to Week 12 (ITT population)

Total walk distance (m)	**Bosentan (n = 21)**	**Placebo (n = 11)**
Baseline Value (Visit 2)		
Mean ± SD	360.5 ± 86.1	355.5 ± 81.8
Median (Min, Max)	380.0 (218, 483)	405.5 (218.5, 437)
Week 12 Value[a]		
Mean ± SD	430.5 ± 66.4	349.6 ± 147.1
Median (Min, Max)	431.0 (294, 535)	399.0 (0, 497)
Change from BL at Week 12		
Mean ± SD	70.1 ± 56.2	-5.8 ± 120.5
95% CL of mean	44.5, 95.6	-86.8, 75.2
Median (Min, Max)	51.0 (-24.5, 196)	-6.0 (-267.5, 224.5)
95% CL of median	35.0, 113.5	-83.5, 60.0
Absolute Difference from Placebo (bosentan effect)		
Mean ± SEM	75.9 ± 31.0	
95% CL of mean	12.5, 139.2	
Median (95% CL of median)	59.0 (13.2, 130.3)	
p-value[b]	0.0205 (0.0190)	

[a] For one bosentan (patient n°10110) and one placebo patient (n°10503), a missing Week-12 assessment was replaced using the last value carried forward (day 74 and 34 respecyively) .
One placebo patient (n°20101) withdrew from the study without a valid end-of-study assessment, and a "worst case" (0 m) was substituted for the Week-12 assessment.

[b] Determined by Student's t-test, two-sided (Wilcoxon-Mann-Whitney U-test).

BL= baseline; CL = confidence limit; SD = standard deviation; SE = standard error.

Haemodynamics improved at 12 weeks in the bosentan treated patients (see table below). There were no cases of clinical worsening in patients receiving bosentan treatment during the course of the study whereas three placebo-patients were transferred to epoprostenol for clinical worsening. After 12 weeks of double-blind treatment, mean scores assessed with the Borg dyspnoea index (0-10 scale) for maximal dyspnea during the 6-minute walk test showed a trend to amelioration in the bosentan group. No difference in mortality rate was observed. The short duration of the study as compared to the life expectancy in patients with functional status Class III that is expected to be longer than 12 weeks, precludes any conclusion on effect of bosentan on survival.

Change from Baseline to Week 12 in Haemodynamic Parameters in Study AC-052-351 (ITT Population)

Haemodynamic Parameter	Bosentan (n = 20)	Placebo (n = 10)	Treatment Difference	
			Mean (95% CL)	p-value
Mean PAP (mmHg)				
Baseline Value	53.7 ± 3.0	55.7 ± 3.3		
Change from BL	–1.6 ± 1.2	5.1 ± 2.8	–6.7 (–11.9, -.5)	0.0134
% Change from BL	–2.5 ± 2.4	9.4 ± 5.0		
PVR (dyn·sec/cm^5)				
Baseline Value	896 ± 97	942 ± 136		
Change from BL	–223 ± 56	191 ± 74	–415 (-608, -221)	0.0002
% Change from BL	–19.9 ± 4.8	27.1 ± 10.7		
Mean RAP (mmHg)				
Baseline Value	9.7 ± 1.3 [b]	9.9 ± 1.3		
Change from BL	–1.3 ± 0.9	4.9 ± 1.5	–6.2 (-9.6, -2.7)	0.0010
PCWP (mmHg)				
Baseline Value	9.3 ± 0.6 [b]	8.3 ± 1.1		
Change from BL	0.1 ± 0.8	3.9 ± 1.8	–3.8 (-7.3, -0.3)	0.0353
Cardiac index (l/min/m^2)				
Baseline	2.35 ± 0.16	2.48 ± 0.33		
Change from BL	0.50 ± 0.10	–0.52 ± 0.15	1.02 (0.65, 1.39)	<0.0001
% Change from BL	26.4 ± 5.7	–-19.9 ± 5.9		

Values are mean ± standard error.
BL = baseline; CL = confidence limit; PAP = pulmonary arterial pressure; PCWP = pulmonary capillary wedge pressure; RAP = right atrial pressure; PVR = pulmonary vascular resistance.

Study AC-052-352

This study was a multicentre, randomised, double blind, placebo-controlled study with 3 parallel groups (placebo, bosentan 125 mg, bosentan 250 mg). It was designed to evaluate the effects of bosentan 125 mg or 250 mg on change in walking distance when given for at least 16 weeks up to 28 weeks to ambulatory outpatients aged 12 years or more, with PAH either as PPH, or as a complication of connective tissue diseases (e.g., scleroderma, systemic lupus erythematosus). The design was similar to the previous study. 144 patients received bosentan at 125 mg b.i.d. (N= 74 patients) or 250 mg b.i.d. (N=70 patients) and 69 patients received placebo. All bosentan patients received 62.5 mg b.i.d. during the first 4 weeks of the study and then were up titrated to the target dose (125 mg b.i.d. or 250 mg b.i.d.). The primary efficacy criteria were change from baseline to Week 16 in exercise capacity as assessed by the 6-minute walk test.

Baseline characteristics:

Most of the included patients (92 %) were in WHO class III (130 in the bosentan groups and 65 in the placebo group). 18 patients were in WHO class IV (bosentan 14 and placebo 4).
Most of the included patients had PAH (102 receiving bosentan; 48 receiving placebo): 33 patients of the bosentan groups had PAH related to scleroderma (14 patients in the placebo group), 16 patients were coded with etiology as “other” and8 patients (5 in the bosentan group and 3 in the placebo group) were assigned systemic lupus erythematosus as primary aetiology. Other connective tissue diseases were represented by mixed (4 patients), unclassified (2 patients), and polyarteritis nodosa (1 patient). In one patient, PAH was secondary to chronic thromboembolic disease.

As expected in PAH most patients were female (79.2% and 78.3% in bosentan and placebo groups, respectively).

Results

An improvement of exercise capacity (6 minute-walk test) from baseline was shown after 16 weeks in bosentan groups; the mean increase from baseline was 36.4 meters [95% CI 24.9 meters, 47.8 meters]. A significant treatment effect as compared to placebo was shown; + 44.2 meter [95% CI: 21.4 meters, 67.0 meters] (p=0.0002). Results are detailed in the table below. The mean treatment effect was lower than observed in the previous trial (AC-052-351) but remained significant.

The dose-response relation (125 mg b.i.d vs 250 mg b.i.d.) has been analysed only descriptively. The increase in walk distance appeared to be dose related, since the mean increased in the 6-minute walk distance observed in the 250 mg b.i.d. group was larger than in the 125 mg b.i.d. group but the confidence interval limits overlapped at Week 8 and week 16 time-points. The posology of 125 mg b.i.d. is therefore recommended as the maintenance dose. Nevertheless, although statistical significance was not demonstrated between the two doses, it cannot be excluded that some patients not responding well to 125 mg may slightly improve their exercise capacity with 250 mg twice daily.

Study AC-052-352

Total Walk test (m)	Bosentan 125 mg N=74	Bosentan 250 mg N=70	All Bosentan N=144	Placebo N=69
Baseline Value (m)				
Mean ± SD	326.3 ± 73.2	333.0 ± 75.4	329.6 ± 74.1	344.3 ± 76.4
95% CL of mean	309.3 , 343.2	315.0 , 351.0	317.4 , 341.8	326.0 , 362.7
Median	333.0	338.8	337.3	359.0
95% CL of median	306.5 , 357.5	316.0 , 369.0	320.0 , 357.0	344.0 , 382.5
Min , Max	159.0 , 464.5	173.5 , 440.0	159.0 , 464.5	150.0 , 448.5
Week 16 value (m)				
Mean ± SD	353.1 ± 115.0	379.5 ± 101.2	365.9 ± 109	336.5 ± 129.2
CL of mean	326.4 , 379.7	355.3 , 403.6	348.0 , 383.9	305.4 , 367.5
Median	376.5	384.5	379.5	355.0
95% CL of median	338.0 , 396.0	363.0 , 417.0	363.0 , 396.0	333.0 , 378.0
Min , Max	0.0 , 602.0	57.0 , 555.0	0.0 , 602.0	0.0 , 585.0
Change from baseline at Week 16				
Mean ± SD	26.8 ± 75.3	46.5 ± 69.5	36.4 ± 61.7	-7.8 ± 96.1
95% CL of mean	9.3 , 44.2	31.7 , 61.2	24.9 , 47.8	-30.9 , 15.2
Median	32.8	49.8	4.5	9.0
95% CL of median	19.5 , 40.0	19.5 , 66.0	26.0 , 48.5	-18.0 , 26.0
Min , Max	-205.0 , 214.0	-131.0 , 257.5	-205.0 , 257.5	-383.0 , 227.5
Bosentan EFFECT (absolute difference from placebo)				
Mean	34.6	54.3	44.2	
95% CL of mean	6.2 , 63.1	27.3 , 81.4	21.4 , 67.0	
Median	28.2	45.0	36.7	
95% CL of median	7.5 , 51.5	23.1 , 67.1	17.9 , 55.9	
p-value Mann-Whitney U-test	(0.0107)*	(0.0001)*	0.0002	

* Exploratory analysis.
CL=confidence limits, ITT=intent to treat.

In a retrospective responder analysis based on change in walking distance, WHO functional class and dyspnoea of the 95 patients randomised to Tracleer 125 mg bid in the placebo controlled trials it was found that at week 8, 66 patients had improved, 22 were stable and 7 had deteriorated. Of the 22 patients stable at week 8, 6 improved at week 12/16 and 4 deteriorated as compared to baseline value. Of the 7 patients that deteriorated at week 8, 3 improved at week 12/16 and 4 deteriorated compared with baseline.

Secondary efficacy parameters were descriptively analysed. Patients on bosentan showed a slight decrease in maximal dyspnoea on exercise (Borg Dyspnoea Index), Central haemodynamic parameters were not assessed during this study. Treatment with Tracleer led to a WHO functional class improvement in 42.4% of patients (placebo 30.4%). A significant reduction in the rate of clinical worsening was observed as compared with placebo at 28 weeks (10.7% vs 37.1%, respectively; p = 0.0015).

Only 14 patients with grade IV functional status received bosentan among the 130 patients (11%) included in study AC-052-352. The sub-population analysis performed by the applicant with respect to

the severity of patients' functional status showed a weak effect in patients with grade IV. Change from baseline in the 6 minute walk test was weak (mean of – 2.0 meters [95 % CL: – 46.2 and 42.2 meters]. Most of them were stabilised or deteriorated; of the 14 class IV patients at baseline, 5 remained in class IV, 6 improved to class III and 3 to class II.

A sub analysis with respect to aetiology showed that absolute values in change from baseline of the primary end point were higher in PAH patients than in patients with PAH due to scleroderma but the placebo-corrected treatment effect remained significant. Bosentan cannot be recommended to improve patients with moderate to severe interstitial disease.

Clinical studies in special populations

At the time of the initial MAA no studies were available in children less than 12 years. New pharmacodynamic and pharmacokinetic data in children have become available post-authorisation as a result of the finalisation of the BREATHE-3 Study (AC-052-356) – see "post-authorisation" changes later on.

Long-term data

The two placebo-controlled studies previously described were followed by open label extension studies (AC-052-353 and AC-052-354) in which 29 and 198 patients respectively received bosentan. Only interim reports were provided since these studies are ongoing.

At the clinical cut-off date of study (AC-052-353) (31 March 2001), the exposure to bosentan ranged from 15 weeks to 85 weeks (21 months). No patients have died during the reported period. 28 patients have been treated with bosentan for about 1 year or more and 5 patients for more than 1.5 years. Among the 29 patients included, 3 patients have been up-titrated to 250 mg because of clinical deterioration after more than 16 months treatment, one patient has been transferred to epoprostenol after about 3.5 months because of deterioration and non compliance to bosentan treatment. After 6 months of treatment in the open-label trial, 13/29 patients were considered improved (i.e., 12 grade III patients at baseline was improved to grade II and one to grade I functional status). 5 of the 29 patients had a decrease in their 6-minute walk test distance after about 1 year of treatment with the 125 mg b.i.d. dose of Tracleer. In those 5 patients, an increase in the dose of Tracleer to 250 mg b.i.d. resulted in an improvement in the walk distance in all 5 patients at 1–2 months after increasing the dose. According to the applicant, after 7–9 months of treatment with the 250 mg b.i.d dose no increase in liver aminotransferases has been observed in these patients.

At the clinical cut-off date of the second Study AC-052-354 (31 May 2001), the exposure to bosentan ranged from 3.5 months to 11 months (i.e. 13 weeks to 44 weeks). 100 patients have been treated for more than 6 months, and 12 patients have been treated for more than 9 months. 3 patients have died (pulmonary haemorrhage, multi-organ failure, and haemoptysis/hypovolaemic shock). 8 more patients have had treatment discontinued for adverse events (6 for elevated liver aminotransferases, one because of anaemia and one because of gastrointestinal haemorrhage). Six patients have been put on concomitant epoprostenol and bosentan; five of these patients have improved, and one died (multi-organ failure). 66/195 grade III patients at baseline were improved (60 to grade II, 6 to grade II). 11/18 grade IV patients at baseline were improved (7 to grade III, 3 to grade II, 1 to grade 1).

Supportive studies

Study AC-052-355 (BREATHE-2) has been submitted post-authorisation (see see "post-authorisation changes").

Discussion on clinical efficacy

Based on the results of the two trials provided in PAH, bosentan showed a significant improvement in exercise capacity and symptoms in patients with primary PAH and secondary pulmonary hypertension related to scleroderma with grade III functional status (WHO classification). The results support that bosentan beneficially affects quality of life by improving exercise capacity and symptoms in these patients but the available data are not sufficient to support survival improvement. The duration of both randomised studies is too short to support a benefit on survival. Indeed, the life expectancy of included patients with grade III functional status is longer than 16 weeks. No difference in mortality rate was shown as compared to placebo groups.

Data support a maintenance dose of 125 mg twice daily. Based on the (non statistically significant) dose-response relation (125 mg bid vs 250 mg bid) in study AC-052-352 and data from open-label extension study, although the safety margin of bosentan is small, the benefit/risk ratio of 250 mg bid may remain favourable in patients showing a decrease of their clinical response to bosentan 125 mg b.i.d. Taking into account restricted availability of other medicinal products in PAH, the benefit/risk ratio of 250 mg may be acceptable in patients with late deterioration despite treatment with Tracleer at 125 mg bid, since it may improve their quality of life, provided that appropriate warning are stated in the summary of product characteristics and to ensure that patients will be adequately monitored.

There is limited experience in patients with a body weight below 40 kg (only 2 patients were included in the clinical trials).

Most responders will be detected at week 8 after initiation of bosentan. However, some patients not responding at week 8 might improve at week 16. In the case of clinical deterioration (e.g. decrease in 6-minute walk test distance by at least 10% compared with pre-treatment measurement) after 8 weeks of treatment (i.e. target dose for at least 4 weeks), alternative therapies should be considered.

No data are available in those patients with PAH secondary to scleroderma with moderate to severe interstitial disease since those patients were excluded at the screening visit. The benefit in those patients could indeed be expected to be limited. Regarding PAH secondary to scleroderma the indication should be restricted to patients without interstitial disease. Moreover, the SPC should mention that no studies have been performed in secondary PAH other than related to connective tissue disease i.e. primarily scleroderma (e.g. AIDS).

No grade I or II patients were included. There is no evidence of benefit of bosentan when it is started at early stages of the disease as compared to conventional therapy.

Only 14 patients with grade IV functional status received bosentan in study AC-052-352. The sub-population analysis performed by the applicant with respect to the severity of patients' functional status showed a weak effect in patients with grade IV. It is emphasised that patients requiring epoprostenol at short term have been excluded at the screening visit. Epoprostenol has demonstrated benefit on survival in patients with grade IV functional status. They will be improved to a larger extent with epoprostenol. Bosentan may not be appropriate in the larger proportion of grade IV patients. Overall, in those patients with severe conditions and short life expectancy, there is no reason to delay epoprostenol treatment which will provide a larger improvement in symptoms and which has demonstrated effect on survival. Data do not support the use of bosentan in patients with grade IV functional status.

No relevant data are available on the combination with epoprostenol. The applicant foresees to perform 2 studies (AC-052-355/BREATH-2 and AC-052-356/BREATH-3) designed to provide further information in this context (these studies have subsequently been submitted and are discussed in the "post-authorisation" section). Therefore, the SPC should state there is no evidence at this time to recommend this combination.

Clinical safety

Patient exposure

The bosentan safety database includes a mixture of patients with 4 different diseases (PAH, CHF, systemic hypertension and subarachnoid haemorrhage). Data from these studies related to over 2400 patients and 300 healthy volunteers.

In the claimed indication, PAH, data from 2 randomised studies including 29 and 145 patients treated with bosentan respectively were available.

Adverse events and serious adverse event/deaths

None of the reported deaths have been considered related to study medication but rather to progression of the treated or concomitant disease.

All placebo-controlled trials

In eight placebo-controlled studies, six of which were for indications other than PAH, a total of 677 patients were treated with bosentan at daily doses ranging from 100 mg to 2000 mg and 288 patients were treated with placebo. The foreseen duration of treatment ranged from 2 weeks to 6 months. The adverse drug reactions (ADRs) that occurred more frequently on bosentan than on placebo (≥ 3% of bosentan-treated patients, with ≥ 2% difference) were headache (15.8% vs 12.8%), flushing (6.6% vs 1.7%), abnormal hepatic function (5.9% vs 2.1%), leg oedema (4.7% vs 1.4%), and anaemia (3.4% vs 1.0%), all of which were dose related.

Placebo-controlled trials in pulmonary arterial hypertension

The table below shows the ADRs that occurred in ≥ 3% of patients treated with Tracleer (125 and 250 mg twice daily) in placebo-controlled trials in PAH, and which were more frequent in these patients:

Adverse drug reactions occurring in ≥ 3% of patients, and more frequently in patients on Tracleer (125 and 250 mg twice daily), in placebo-controlled trials in pulmonary arterial hypertension

Body system / Adverse event	**Placebo N = 80**		**Tracleer (all) N = 165**	
	No.	**%**	**No.**	**%**
Respiratory, thoracic and mediastinal disorders				
Upper respiratory tract infection	9	11%	20	12%
Nasopharyngitis	6	8%	18	11%
Pneumonia	1	1%	5	3%
Cardiac disorders				
Oedema lower limb	4	5%	13	8%
Palpitations	1	1%	8	5%
Oedema	2	3%	7	4%
Gastrointestinal disorders				
Dyspepsia		–	7	4%
Dry mouth	1	1%	5	3%
Nervous system disorders				
Headache	16	20%	36	22%
Vascular disorders				
Flushing	4	5%	15	9%
Hypotension	3	4%	11	7%
Skin & subcutaneous tissue disorders				
Pruritus		–	6	4%
General disorders and administration site conditions				
Fatigue	1	1%	6	4%
Hepato-biliary disorders				
Hepatic function abnormal	2	3%	14	8%

Note: only AEs with onset from start of treatment to 1 calendar day after end of treatment are included. One
patient could have had more than one AE.

At the recommended maintenance dose or double the dose (i.e., 125 or 250 mg twice daily) the ADRs that occurred more frequently with Tracleer than with placebo (in ≥ 3% of Tracleer-treated patients, with ≥ 2% difference) were nasopharyngitis, flushing, abnormal hepatic function, leg oedema, hypotension, palpitations, dyspepsia, fatigue and pruritus. ADRs that occurred in ≥ 1% and < 3% of these patients and more frequently on Tracleer than on placebo (≥ 2% difference) were anaemia, gastro-oesophageal reflux disease and rectal haemorrhage, all 2.4% on Tracleer versus 0% on placebo.

Discontinuation due to adverse events

Treatment discontinuations due to AEs, during the clinical trials in patients with PAH, at doses of 125 and 250 mg twice daily, were less frequent in Tracleer than in placebo-treated patients (5.5% vs 10%, respectively). In study AC-052-351, no patients treated with bonsentan discontinued prematurely. In study AC-052-352, premature discontinuations due to AEs were more frequently due to abnormal hepatic function in the bosentan group (2.1% vs. none with placebo), and aggravated pulmonary hypertension (1.4% vs. 5.8%, respectively) and to syncope (0.0% vs.2.9%) in the placebo group.

Laboratory findings

Decrease in haemoglobin was frequent (more than 50% of bosentan-patients experienced a decrease of at least 1 g/dl) but these changes are usually mild to moderate in intensity since haemoglobin concentration remained within normal limits in most patients and a marked decrease (≥ 15% from baseline to < 11 g/dl) was observed only in few bosentan-patients (5.6%). In the trials, decrease in haemoglobin occurred during the first weeks of treatment and then remained stable. Although the outcomes were mainly favourable, some patients needed blood transfusions (12/36 bosentan-patients who experienced a marked decrease in haemoglobin concentration). Based on the integrated safety summary, marked decrease in haematocrit was reported in 41/492 (8.3%) bosentan-patients and 6/204 (2.9%) placebo-patients in placebo-controlled studies. Decrease in hemoglobin was not paralleled by changes in leucocytes, platelets, bilirubin or reticulocytes, indicating the absence of hemolysis or bone marrow depression. No risk of bleeding was found. Nevertheless, few investigations were performed: only 2 biopsies performed in CHF patients, no results of reticulocyte count, no erythropoietin values. The most likely cause is vasodilatation and subsequent and fluid shift producing hemodilution. This is well matched with the mechanism of action of bosentan and consistent with preclinical data (i.e. rats).

Hepatotoxicity: A major safety concern is the dose related increase in liver transaminases: bosentan has been associated with frequent dose-related elevations in liver aminotransferases, i.e., aspartate and alanine aminotransferase.

In the pool of placebo-controlled studies, 74/658 patients experienced these laboratory abnormalities (11.2% versus 1.8% among placebo-patients). Elevations in liver transaminases appeared to be dose-related until 250-500 mg/day. 6.8% of bosentan-patients experienced increase ≥ 5×ULN and 3.2% of them experienced increase ≥ 10×ULN. In addition, in an ongoing blinded study in patients with CHF (ENABLE, AC-052-301/302), 0.3% of patients experienced increase ≥ 20×ULN. The REACH-1 trial (in CHF) which was conducted with high doses, was prematurely stopped because of the high incidence of elevated liver transaminases > 3×ULN (15.6% of bosentan-patients including 5.7% of patients with an increase ≥ 8×ULN). The time to onset was delayed: in about 90% of cases, the first elevation in transaminases was detected during the first 16 weeks of bosentan treatment and most cases occurred between the 4th and the 12th week of treatment. In all cases during the clinical programme, enzyme returned to pre-treatment levels without sequelae, within a few days to 9 weeks, either spontaneously or after dose reduction or discontinuation. In most cases observed during the placebo-controlled studies, liver laboratory abnormalities were asymptomatic, no case of liver failure, liver transplant or death due to fulminant hepatitis were reported. However, associated symptoms that could indicate liver dysfunction were temporarily reported in 9 patients: abdominal pain, nausea/vomiting or fever. In addition, elevated bilirubin and/or jaundice were reported in 2 patients and pruritic rash in another patient (considered related to atorvastatin).

The mechanism of hepatotoxicity remains unclear as few experimental data are available. Bosentan has been shown to inhibit *in vitro* the canalicular bile salt export pump (Bsep). Hepatocellular liver injuries without cholestatic features were however observed in about 50 % of cases with elevated liver amino transferases. As proposed by the applicant, some cases could be explained by the bile salt pump inhibition (i.e. when bile acids is accumulated in hepatocytes), but other mechanisms of hepatotoxicity cannot be excluded; namely immunological reactions or intrahepatocyte accumulation of bosentan and/or metabolites (as suggested by the delayed time to onset). Clinical investigations are insufficient to allow reliable conclusions; antibodies have only been measured in three patients, only one liver biopsy is available (showing granuloma and mild to moderate hepatocellular necrosis), and no direct human intrahepatocyte measurements of bosentan or metabolites have been performed to exclude intrahepatocyte accumulation. Effect of bosentan on mitochondria is unknown. Hepatic events cannot be definitively explained only by the bile salt pump inhibition. Moreover, liver injury risk may be increased when Tracleer is used with product that inhibit bile salt pump, e.g. rifampicine.

As a consequence, recommendations for monitoring of the hepatic function during treatment with Tracleer have been defined (in accordance with the protocol of the clinical studies performed). Aminotransferases should be measured prior to initiation of treatment and monitored at least every month. Bosentan should not be initiated in patients with liver aminotransferase (AST/ALT) greater than 3 times the upper limit of normal. The dose should be reduced in case of increase between 3 and 5 times the upper limit of normal. The treatment should be discontinued when enzyme increase over 5

time the normal limit. In case of re-introduction of bosentan is considered, the advice of a hepatologist is recommended. After discontinuation for liver dysfunction, re-introduction of bosentan is not recommended when ALT/AST levels elevation were higher than 8 time upper limit of normal. In case of clinical symptoms of liver injury i.e. nausea, vomiting, fever, abdominal pain, jaundice, unusual lethargy or fatigue, flue like syndrome (arthralgia, myalgia, fever) treatment must be stopped and re-introduction is not to be considered.

Safety in special populations

Pregnancy: in the context of pulmonary arterial hypertension, *pregnancy* must be avoided since it leads to cardiovascular stress with harmful consequences to pregnant women. Moreover, a teratogenic effect has been shown in animal studies, therefore bosentan is contraindicated during pregnancy and in *women of childbearing potential* not using reliable contraception. Bosentan should not be initiated in women of childbearing unless the result of the pre-treatment pregnancy test is negative. Bosentan is an inducer of CYP450 and may render oral contraceptives ineffective. Consequently, oral contraceptive should not be used as the sole method of contraception in women. Additional or an alternative reliable method of contraception is recommended. Repeated monthly pregnancy tests are recommended.

Little experience is available in patients with systolic blood pressure (SBP) lower than 100 mmHg since patients with SBP < 85 mmHg at baseline were excluded from the studies. As a precaution, it must be recommended that blood pressure should be higher than 85 mmHg before initiation of bosentan. Moreover, based on kinetic data, the initiation period should never be shorter than one week since steady state is never reached before this period.

Discussion on clinical safety

Based on the integrated safety database and the study AC-052-352, the adverse reactions in patients with PAH receiving bosentan were mostly related to vasodilatory effects such as flush, headache, syncope and lower limb oedema. Most of all these adverse effects were mild or moderate in intensity. Based on the adverse effects reported in clinical trials with PAH, no first dose effect with pronounced harmful vasodilatory effects has been reported during the initiation period of 4 weeks at half dose (62.5 mg) in patients with PAH.

Treatment with bosentan was associated with a dose-related, modest decrease in haemoglobin concentration. Bosentan-related decreases in haemoglobin concentration were not progressive, and stabilised after the first 4–12 weeks of treatment. It is recommended that haemoglobin concentrations be checked prior to initiation of treatment, every month during the first 4 months, and quarterly thereafter.

A substantial safety issue is related to the hepatotoxicity for which the mechanisms have not been clearly established. Liver aminotransferase levels will have to be measure prior to initiation of treatment and subsequently at monthly intervals, at least. Recommendations in case of ALT/AST elevations and in case of clinical symptoms of liver injury have been provided in the summary of product characteristics. The post-marketing programme should ensure safety data collection especially regarding liver function monitoring for at least two years after approval to validate the guideline for monitoring as recommended in the SPC.

The risk of rebound effect when bosentan is discontinued is unknown since few patients experienced abrupt discontinuation of the vasodilatory treatments in the PAH studies provided. The effect of bosentan on plasmatic ET levels has not been assessed in patients with PAH. Due the occurrence of severe deleterious and fatal outcome related to increase in pulmonary arterial pressure (PAP) and pulmonary vascular resistance (PVR) described after withdrawals of other vasodilatory agents the applicant recommends gradual dose reduction (halving the dose for 3 to 7 days). Although no experimental data support this guidance, the proposed scheme to gradually reduce the dose before discontinuation is acceptable.

Due to the disease itself and the teratogenic effect of bosentan, Tracleer is contraindicated during pregnancy and in women of childbearing potential not using reliable contraception. As bosentan is an inducer of CYP450, oral contraceptives should not be used as the sole method of contraception in women. Additional or an alternative reliable method of contraception is recommended. Repeated monthly pregnancy tests are recommended.

5. Overall conclusions, benefit/risk assessment and recommendation

Quality

Tracleer is presented as film-coated tablets (two strengths). The quality dossier indicates that the active substance and finished product are manufactured and controlled in a relevant manner, in compliance with current EU and ICH guidelines. Satisfactory information has been provided to demonstrate that the manufacture and control processes routinely and consistently generate a product of uniform quality when used in accordance with the conditions defined in the SPC.

At the time of the Opinion, the CPMP concluded that one minor quality issue, which had no impact on the risk/benefit balance of the product when used in accordance with the SPC, remained to be resolved and it was agreed that this would be resolved as a follow-up measure to be submitted post-authorisation.

Preclinical pharmacology and toxicology

The primary pharmacodynamic studies provided adequate evidence that bosentan is a competitive antagonist of ET binding to both ET_A and ET_B receptors, reducing pulmonary artery pressure. However, the selectivity for pulmonary vessels has not been demonstrated experimentally.

Bosentan exerts a concentration-dependent functional cholestatic effect by competing with bile salt elimination via the Bsep. Cholestasis was seen in the rat and dog and was associated with histopathological evidence of hepatocellular damage with increased aminotransferase.

Red blood cell parameters were decreased in both non-clinical and clinical studies. The changes were small in magnitude and might possibly be related to the pharmacodynamic properties of the product in decreasing vascular permeability resulting in haemodilution.

In rodent carcinogenicity studies, bosentan treatment resulted in a statistically significant increase in the incidence of hepatocellular tumours in male mice (adenoma, carcinoma) and a statistically significant increase in the incidence of thyroid follicular tumours in male rats. The extrapolation of this carcinogenic potential to human remains uncertain.

A teratogenic effect has been observed in rats at exposures that could be achieved in humans. Bosentan should be contraindicated in pregnancy and appropriate precautions should be taken for women of childbearing potential. Milk excretion has not been studied. Bosentan is a lipophilic substance for which excretion or even accumulation seems to be highly likely. Nursing women taking Tracleer should be advised to discontinue breast-feeding.

Information has been included in the summary of product characteristics accordingly.

The Company should provide as follow-up measure, the results of the *in vivo* study planned to evaluate the effect of each compound and of the combination of bosentan and oestrogens on bile salts in rats.

Efficacy

Based on the results of the two trials provided in PAH, bosentan showed a significant improvement in exercise capacity and symptoms in patients with primary PAH and secondary pulmonary hypertension related to scleroderma with grade III functional status (WHO classification). No difference in mortality rate was shown as compared to placebo groups.

Data support a maintenance dose of 125 mg twice daily. The benefit/risk ratio of 250 mg is acceptable in case of late deterioration despite treatment with Tracleer at 125 mg bid since it may slightly improve their exercise capacity and provided that patients are adequately monitored.

The benefit/risk balance of bosentan has not been established at early stage of the disease e.g. patients with grade I and grade II functional status. Regarding PAH secondary to scleroderma the indication should be restricted to patients without significant interstitial disease. Moreover, the SPC should mention that no studies have been performed in secondary PAH other than related to connective tissue

primarily scleroderma (e.g. AIDS). Data do not support the use of bosentan in patients with grade IV functional status.

Safety and efficacy have not been established in children under the age of 12 years. Pharmacokinetic data in children should be submitted as follow-up measure (these data have subsequently been submitted and are presented in the "post-authorisation" section).

Insufficient data are available to address the concomitant use of epoprostenol and Tracleer. The SPC should state that there is no evidence at this time to recommend this combination. The applicant should provide, as a follow-up measure, the report on study AC-052-355 (BREATH-2) designed to provide further information in this context (these data have subsequently been submitted and are presented in the "post-authorisation" section).

Bosentan is metabolized by CYP2C9 and CYP3A4 and is an inducer of the cytochrome P450 (CYP) isoenzymes CYP2C9 and CYP3A4. Consequently, specific recommendations have to be taken for administrations of drugs metabolized by these isoenzymes or inhibiting these isoenzymes. In addition, co-administration of Tracleer and cyclosporine A is contraindicated and glibenclamide should not be co-administered.

The final report of the kinetic study performed in PAH patients should be provided as follow-up measure (these data have subsequently been submitted and are presented in the "post-authorisation" section).

Safety

The adverse reactions in patients with PAH receiving bosentan were mostly related to vasodilatory effects such as flush, headache, syncope and lower limb oedema. Nevertheless, bosentan carries safety risks with respect to medicinal products interactions, teratogenicity, hepatotoxicity and decrease in haemoglobin with unclear mechanisms, for which appropriate information should be included in the summary of product characteristics and the package leaflet.

Hepatotoxicity is a safety issue since the mechanism remains unclear. Further experience is required to clarify the mechanism of liver injury and the subsequent optimal monitoring of liver function. However, it is a fact that no irreversible damage or fatal event related to liver injury induced by bosentan has been reported until today since the product has been used with close monitoring of liver function in clinical trials or through compassionate use. The proposed guideline that was the one used in clinical trials seems to be appropriate to avoid irreversible harmful injury.

Tracleer is contraindicated during pregnancy and in women of childbearing potential not using reliable contraception. Repeated monthly pregnancy tests are recommended.

When used in the acceptable indication, a progressive short-term fatal disease, the efficiency of bosentan to improve exercise capacity might offset its potential deleterious effect provided that physician is well informed of the risk carried by bosentan and appropriate management and monitoring are ensured.

Benefit/risk assessment

Tracleer has shown efficacy in improving exercise capacity and symptoms in patients with pulmonary arterial hypertension (PAH) grade III, primary or secondary to scleroderma without significant interstitial pulmonary disease. Data do not support the use of bosentan in patients with grade IV functional status.

The benefit/risk balance of bosentan has not been established at early stage of the disease i.e. patients with grade I and grade II functional status. Although results are promising, further experience is required to address the effect of bosentan on survival and on the natural history of the disease.

Treatment with Tracleer carries safety risks with respect to medicinal products interactions, teratogenicity, hepatotoxicity and decrease in haemoglobin with unclear mechanisms, for which appropriate precautions and monitoring are necessary.

Based on the review of the data on quality, safety and efficacy, the benefit risk of Tracleer is positive when used in accordance with the conditions defined in the summary of product characteristics, in the following indication:

"Treatment of pulmonary arterial hypertension (PAH) to improve exercise capacity and symptoms in patients with grade III functional status. Efficicacy has been shown in:

- Primary PAH
- PAH secondary to scleroderma without significant interstitial pulmonary disease."

Tracleer should only be initiated and monitored by a physician experienced in the treatment of pulmonary arterial hypertension.

A post-marketing programme should ensure firstly that the prescribing physician will be aware of potential safety issues and recommendations for regular monitoring as stated in the SPC, and secondly that safety information will be collected including detailed clarifications when the drug is discontinued. The data should be analysed in order to help to further clarify the mechanism of hepatotoxicity in humans and to provide sufficient reassurance to validate the optimal guideline for monitoring of the product especially with regard to liver function concerns.

The indications for which the medicinal product is intended are encountered so rarely that the applicant cannot reasonably be expected to provide comprehensive data on the clinical safety of the medicinal product. Therefore, the marketing authorisation should be granted under exceptional circumstances.

Recommendation

Based on the CPMP review of data on quality, safety and efficacy, the CPMP considered by consensus that the benefit/risk profile of Tracleer in the treatment of Primary pulmonary arterial hypertension (PAH) and PAH secondary to scleroderma without significant interstitial pulmonary disease, to improve exercise capacity and symptoms in patients with grade III functional status was favourable and therefore recommended the granting of the marketing authorisation under exceptional circumstances.

6. Post-Authosisation data submitted as Follow-Up Measures and related changes in the SPC (Sections 4.2, 4.4, 4.5, 5.1 and 5.2) and Package Leaflet to reflect the new clinical findings

The MAH has submitted substantial new clinical data in order to fulfil the follow up measures.

6.1 Clinical Data

6.1.1 A comparative effect of bosentan and epoprostenol on survival in PAH (Report B-02.014)

The objective of this study was to compare survival with Tracleer vs epoprostenol as first-line treatment for patients with either PPH or pulmonary hypertension secondary to connective tissue diseases (PH/CTD). Data on patients treated with bosentan were collected from September 1999 to March 2002 and obtained from the case report forms of each patient from the 2 pivotal studies (AC-052-351 and AC-052-352), already assessed in the original MAA, and their respective open-label extensions (AC-052-353 and AC-052-354). Data on patients treated with epoprostenol were obtained from uncontrolled, long-term surveillance studies collected from 6 different centres involved in PAH follow-up studies from April 1987 to March 2002. The data included general demographic information as well as baseline haemodynamics. Patients with aetiology other than PPH or PH/CTD were excluded from the analysis. The observed survival of the epoprostenol- and bosentan-treated patients was compared with the predicted survival as calculated according to the formula derived from the National Institutes of Health (NIH) Registry on patients with PPH on conventional therapy.

Results

A total of 901 patients were included in the study; 219 patients received bosentan in a blinded manner for 12 or 16 weeks prior to entering the open-label extension study, and 682 patients were on epoprostenol. Although epoprostenol patients were followed for a longer period, only the first 30 months (the longest time available for bosentan therapy) were used for the analysis. Patients with PPH (714) or CTD/PH (187) were included from the start of treatment until death, transfer to commercial bosentan, or the cut-off date if on bosentan, and to death, loss to follow-up, or lung transplantation if

on epoprostenol. Patient demographics and PAH characteristics at baseline in the epoprostenol and bosentan groups (ITT population) are shown in the following table:

Characteristic / Parameter	Epoprostenol (n = 682)	Bosentan (n = 219)
Gender [n (%)]		
Male	147 (21%)	43 (20%)
Female	535 (78%)	176 (80%)
Age (years)	43.3 ± 14.1 (n = 666)	49.3 ± 15.8 (n = 219)
Min, Max	0.1, 79	13, 80
Aetiology of PAH [n (%)]		
PPH	545 (80%)	169 (77%)
PH/CTD	137 (20%)	50 (23%)
NYHA/WHO[1] functional class [n (%)]	(n = 659)	(n = 219)
I	0 (0%)	1 (0%)
II	5 (1%)	16 (7%)
III	416 (64%)	184 (84%)
IV	238 (36%)	18 (8%)
Cardiac index (l/min/m^2)	1.9 ± 0.6 (n = 625)	2.4 ± 0.8 (n = 218)
Pulmonary vascular resistance (Wood)	18.4 ± 10.1 (n = 422)	12.1 ± 7.9 (n = 206)
Mean pulmonary artery pressure (mmHg)	64.6 ± 17.4 (n =646)	54.5 ± 16.0 (n = 219)
Right atrial pressure (mmHg)	12.5 ± 6.0 (n = 642)	9.7 ± 5.8 (n = 215)
6-minute walking distance (m)	300 ± 121 (n=232)	340 ± 88 (n=219)

Values are given as mean ± standard deviation.
[1]NYHA / WHO: New York Heart Association / World Health Organization.

Baseline characteristics indicate that, overall, the epoprostenol-treated patients had more severe PAH (i.e., lower cardiac index, larger proportion of patients in WHO class IV) and had been treated for longer than the bosentan-treated patients. The average exposure time to treatment was 2.7 ± 2.2 years and 1.2 ± 0.5 years in the epoprostenol and bosentan-treated patients, respectively.

There were 182/682 and 18/219 deaths among epoprostenol- and bosentan-treated patients, respectively (crude death rates 26.7% and 8.22%, respectively; p = 0.0001). The effects of several prognostic variables were investigated using Cox proportional hazard regression with gender, age, haemodynamics, WHO class, and walk distance as covariates to control for known confounding factors. The baseline 6-minute walking distance was not available for a number of patients.

The hazard ratios for epoprostenol relative to bosentan from 3 regressions are shown below:

Patients	Hazard ratio (95% confidence intervals)		
	Regression 1 controlling for haemodynamics[1]	Regression 2 controlling for functional class[2]	Regression 3 controlling for walking distance[3]
PPH and PH/CTD	2.4 (1.4 – 4.1) (n = 836)	2.0 (1.2 – 3.3) (n = 886)	1.9 (1.1 – 3.3) (n = 501)
PPH	2.8 (1.4 – 5.5) (n = 662)	2.1 (1.1 – 4.1) (n = 701)	1.9 (< 1 – 3.9) (n = 399)
PH/CTD	1.7 (0.7 – 3.8) (n = 174)	1.8 (0.8 – 4.0) (n = 185)	1.8 (0.7 – 4.7) (n = 102)

[1]Variables in regression 1 include gender, age, cardiac index, mean right atrial pressure and mean pulmonary artery pressure.
[2]Variables in regression 2 include gender, age, and NYHA/WHO functional class.
[3]Variables in regression 3 include gender, age, and 6-minute walking distance.

The hazard ratio was higher for PPH than for PH/CTD patients in all regressions. The results were not significant for the smaller group of PH/CTD patients. Moreover, the results in PPH were not significant when controlling for walking distance.

6.1.2. BREATHE-2 Study (AC-052-355)

This was a multicentre, double blind, randomised, placebo-controlled study in patients with severe PAH in need of epoprostenol initiation treatment, conducted in the US and Europe in 2002. The aim was to assess the effects of Tracleer when combined with epoprostenol on cardiopulmonary haemodynamics (primary) and on exercise capacity, dyspnoea-fatigue rating, and functional status

classification (secondaries). A further objective was to evaluate the safety and tolerability of the epoprostenol+bosentan combination. The MAH has provided data from the 16-week double-blind phase of the study. An open-label extension phase will be separately reported when this phase is completed. The study design is shown in figure 1.

Patients had to be ≥ 12 years of age presenting PPH, SSc/PH, or scleroderma spectrum disorders (including mixed CTD) in WHO function class III or IV and scheduled to receive epoprostenol treatment within 2 months after screening. They were to have life expectancy > 4 months as best assessed by the investigator and not requiring more aggressive up-titration of epoprostenol than that used in the protocol (2 ng/kg/min at initiation to a maximum dose of 16 ng/kg/min at Week 16).

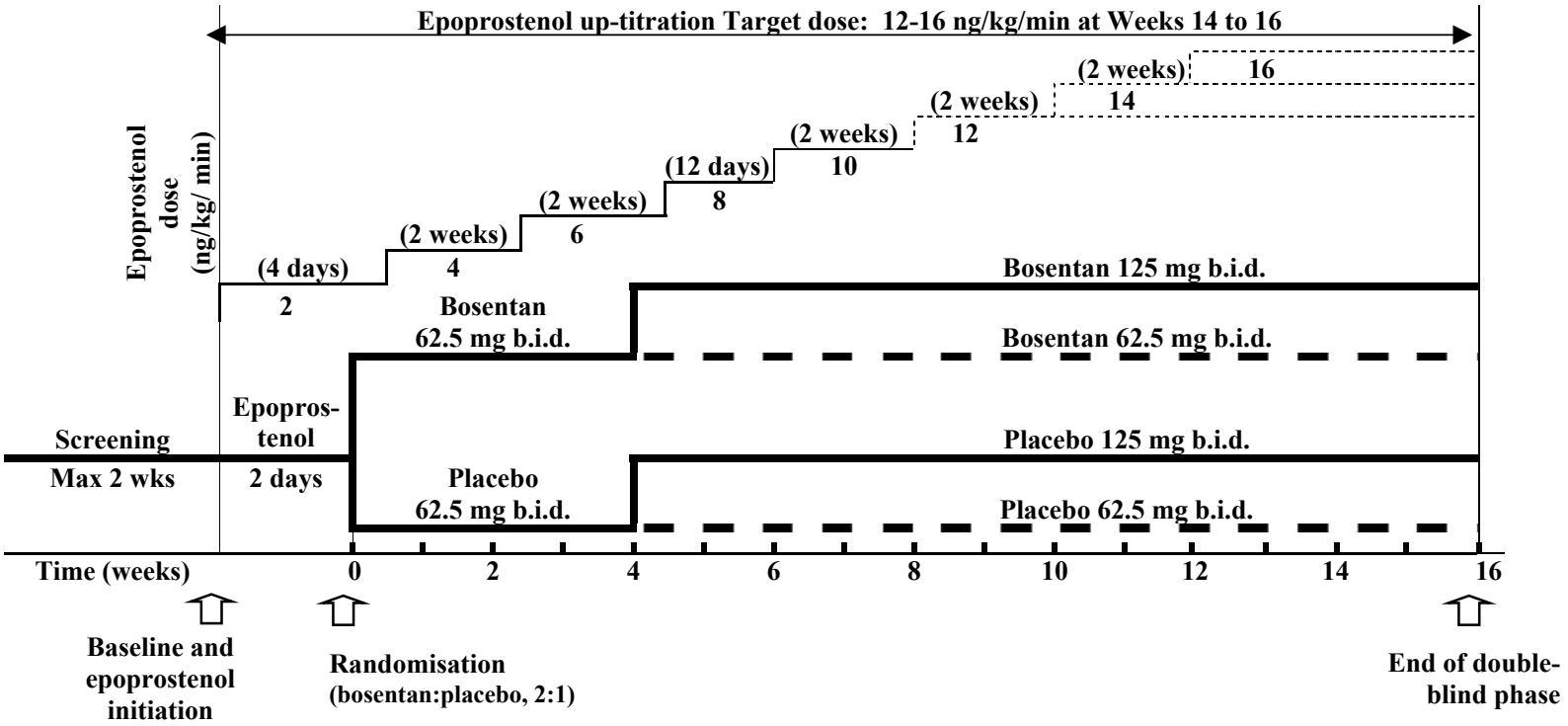
Patients were treated for a total of 16 weeks and were evaluated at baseline and after 1, 4, 6 (Europe only), 8, 12, and 16 weeks of treatment, or at premature withdrawal. If treatment was prematurely discontinued because of clinical deterioration (criteria not pre-defined in the protocol), the patient was to be permanently withdrawn from the study and followed up for 28 days after the end of treatment to collect information on ongoing AEs and serious adverse events (SAEs).

The primary *endpoint* was a change from baseline to Week 16 in total pulmonary resistance (TPR), expressed as a percentage of the baseline value. Secondary endpoints were changes from baseline to Week 16 in the following parameters:
- cardiac index, cardiac output, PVR, PVR index, mean PAP, and mean RAP.
- walk distance (6-minute walk test)
- dyspnoea-fatigue rating, consisting of 3 components that measured the magnitude of the task that evokes dyspnoea or fatigue, the magnitude of the pace (or effort) with which the task was performed, and the associated functional impairment in general activities. All 3 components were to be rated using a 5-point scale (0 - 4) resulting in a total score ranging from 0 (severe dyspnoea/fatigue) to 12 (symptomatic only with extraordinary activity).
- WHO functional class

Safety endpoints included AEs, concomitant treatments, clinical laboratory tests, vital signs, physical examination 12-lead electrocardiogram (ECG) and body weight assessed at screening/baseline and Week 16 or premature withdrawal.

Sample size was calculated assuming a 2:1 randomisation, a two-sided $\alpha = 0.05$ using the Student's t-test for treatment-group comparisons, a type II error of $\beta = 0.20$ (80% power), and an expected treatment difference in the mean change in TPR from baseline to Week 16 of 28%, with a standard deviation of 25%. Given these assumptions, a sample size of 20 evaluable patients for the bosentan group and 10 evaluable patients for the placebo group was required.

Figure 1. Study design



Note : at Week 4, the dose increase to 8 ng/kg/min was to be made 2 days following the up-titration of randomised treatment so that both medications were not increased on the same day.

Thirty-three patients were enrolled. Four patients discontinued Tracleer and 1 discontinued placebo and were not replaced.

The study population was largely Caucasian women with PPH, but also included two Blacks, two Hispanics, and one Asian.

Results
The results of the *primary endpoint* are shown below:

Change from baseline in TPR (dyn*sec/cm^5) to Week 16, ITT population

	Placebo N=11	Bosentan N=22
Baseline		
Mean	1628	1697
Standard deviation	511	666
Median	1697	1597
Min, Max	817, 2720	718, 3629
Week 16		
Mean	1242	1016
Standard deviation	509	367
Median	1051	971
Min, Max	700, 2246	549, 1943
Absolute change from baseline		
Mean	-386	-681
Standard deviation	403	569
Median	-191	-648
Min, Max	-1281, 0	-2459,0
Percent change from baseline		
Mean	-22.6	-36.3
Standard deviation	20.4	20.1
Median	-14.3	-39.6
Min, Max	-64.5, 0.0	-67.8,0.0
TREATMENT EFFECT		
Absolute change		
p-value Mann-Whitney U-test		0.1269
p-value t-test		0.1357
Percent change		
p-value Mann-Whitney U-test		0.0759
p-value t-test		0.0758

Note: All patients in the study are treated with epoprostenol in addition to randomised treatment (placebo or bosentan).
TPR = total pulmonary resistance.

All evaluable patients in both treatment groups had an improvement in TPR from baseline. The mean treatment difference obtained in the primary endpoint was approximately 14%, and not the expected 28% used to calculate the sample size. A trend to a greater mean decrease in TPR, although not statistically significant (p = 0.0758 Student's t-test), was obtained with bosentan+epoprostenol as compared to placebo+epoprostenol (–36.3% vs –22.6%, respectively).

Regarding *secondary efficacy parameters*, the differences in cardio-pulmonary haemodynamics (PVR, PVRI, and mean PAP) between the two groups were not significant, although a trend for improvement was observed in the bosentan group. Such trend was not observed in the percentage change in mean RAP. No statistical difference was observed between the two groups in the 6-minute Walk Test; in fact, the mean change was higher in the placebo group [72.4m or 27.6% (95% CL: 8.4, 46.9)] as compared to the bosentan group [43.1m or 15.6% (95% CL: -9.6, 40.7)]. Similar small improvements in dyspnoea-fatigue ratings were obtained in both treatment groups. No treatment effect was shown in absolute change from baseline (p = 0.6378 Mann-Whitney U-test). The improvement in WHO functional class was slightly greater in the bosentan group as compared to placebo (59.1% vs 45.5%, respectively). However, this study was underpowered to detect a difference between the two groups.

Safety data were compared between treatment groups by inspection, as no formal statistical tests were applied. Exposure to randomised medications and to epoprostenol was similar between the 2 treatment groups. Most AEs were mild or moderate in intensity in both treatment groups and were considered unrelated to randomised treatment by the investigators. Jaw pain and other common AEs associated with epoprostenol therapy (diarrhoea, flushing, headache) occurred in both treatment groups. Anaemia

occurred in 2 patients on bosentan (vs none on placebo). Leg oedema was more frequent in the bosentan group (6/22= 27.3%) than in the placebo group (1/11=9.1%). Oedema and leg oedema among patients on bosentan+epoprostenol occurred within the first 38 days of treatment, and in one case was associated with a report of weight gain. Most cases of oedema and/or leg oedema were considered unrelated to treatment, and none required a change in randomised treatment. SAEs occurred in 3 patients (27.3%) on placebo+epoprostenol and in 10 (45.5%) on bosentan+epoprostenol. No meaningful change in clinical chemistry variables was observed in either treatment group.

6.1.3. Study AC-032-357

This was a multicentre, open-label, single-arm study of oral bosentan in 115 PAH patients designed to collect long-term safety data. Patients were enrolled and treated at an initial dose of 62.5 mg b.i.d. for 4 weeks followed by the maintenance dose of 125 mg b.i.d. until Tracleer became commercially available or the sponsor terminated the study.

Patients *included* were prostacyclin- and bosentan-naive. Patients with unstable PAH disease (*i.e.* who had started or stopped treatment for PAH within 1 month of screening, excluding anticoagulation with warfarin) were *excluded*. Visits were scheduled each month (±5 days) for the first 6 months, and every 3 months thereafter until the end of the study.

The *safety parameters* in this trial were deaths, other SAEs, AEs that led to dose reduction or temporary/permanent discontinuation of study medication, and marked laboratory abnormalities.

Regarding *demographic characteristics*, patients were predominantly Caucasian (78%), female (77%), with a median age of 50 years (range 10 - 80 years; Mean age 50.4 years; only one patient <12 years). The mean weight was 79.6 kg (min: 36.3; max: 139; median 74.8 kg). About 70% of patients had PAH; PAH was associated with scleroderma in 24 patients, systemic lupus erythematosus in 4, HIV infection in 4, and a congenital heart defect in 2. Seven patients were grade II, 101 were grade III and 7 were grade IV. About 20% had a history of appetite suppressant use.

Five males and 8 females were included in a *pharmacokinetic sub-study*, where multiple-dose pharmacokinetic evaluations were scheduled after at least 2 weeks of treatment at each dose level. The 13 patients had a mean age of 45 years (range 24 – 48 years) and mean weight 67 kg (range 46-108 kg). Eight were Caucasian, 3 Asian, 1 Indian and 1 Hispanic. PPH was the most common aetiology (8 patients); 4 patients had PAH related to scleroderma, and 1 had PAH related to a congenital heart defect. Two patients were treated for less than 2 weeks at the 125 mg level and in 1 subject an incomplete plasma profile was obtained at the 62.5 mg level. Thus, 12 and 11 patients were included in the pharmacokinetic analysis at the 62.5 mg and 125 mg dose levels, respectively.

Results

A summary of pharmacokinetic parameters of bosentan and its metabolites in PAH patients after multiple-dose administration of bosentan 62.5 and 125 mg b.i.d. is shown below:

Bosentan			
Treatment	**C_{max} (ng/ml)**	**t_{max} (h)**	**AUC_{τ} (ng·h/ml)**
62.5 mg twice daily	1187 (814, 1560)	3.0 (1.0 - 4.0)	6232 (4582, 7881)
125 mg twice daily	2286 (1234, 3337)	2.3 (1.0 - 6.0)	8912 (6296, 11531)
Ro 47-8634			
Treatment	**C_{max} (ng/ml)**	**t_{max} (h)**	**AUC_{τ} (ng·h/ml)**
62.5 mg twice daily	34.4 (17.4, 51.4)	3.0 (2.2 - 6.0)	238 (79.3, 397)
125 mg twice daily	58.9 (35.1, 82.6)	3.0 (2.0 - 6.0)	295 (176, 415)
Ro 48-5033			
Treatment	**C_{max} (ng/ml)**	**t_{max} (h)**	**AUC_{τ} (ng·h/ml)**
62.5 mg twice daily	356 (85.2, 627)	4.0 (0.0 - 9.0)	2460 (613, 4307)
125 mg twice daily	429 (49.3, 808)	2.3 (0.0 - 6.0)	2573 (93, 5053)
Ro 64-1056			
Treatment	**C_{max} (ng/ml)**	**t_{max} (h)**	**AUC_{τ} (ng·h/ml)**
62.5 mg twice daily	196 (61.5, 330)	4.0 (0.0 - 9.0)	1683 (429, 2937)
125 mg twice daily	242 (52.3, 433)	4.0 (0.0 - 6.0)	1833 (17.0, 3649)

Data are expressed as arithmetic mean (and 95% confidence limits) or, for t_{max}, as median (and range). N = 12 for the 62.5-mg group, and N = 11 for the 125-mg group.

The exposure to bosentan was greater after 125 mg b.i.d. than after 62.5 mg b.i.d., but was less than dose-proportional. The exposure to the metabolites Ro 47-8634, Ro 48-5033 and Ro 64-1056 relative to the exposure to bosentan was 3.8%, 39%, and 27% after 62.5 mg b.i.d., and 3.3%, 29%, and 21%, after 125 mg b.i.d, respectively. The exposure to bosentan in patients with PAH was similar to that in patients with PAH secondary to scleroderma, and also between male and female patients. The design of the study (i.e. no collection of blood samples beyond 12 hours after drug administration) did not allow for the estimation of $t_{1/2}$.

The C_{max} and AUC values observed were approximately 2-fold greater in patients with PAH than has been observed in healthy subjects (see Table below).

Comparison of the arithmetic mean C_{max} and AUC for bosentan obtained for PAH patients in this study (AC-052-357) with data from healthy adult subjects in previously reported studies

Population	**Dose**	**AUC_{τ} (ng·h/ml)**	**C_{max} (ng/ml)**
Healthy subjects*	62.5 mg twice daily	2857	544
PAH patients	62.5 mg twice daily	6232	1187
Healthy subjects†	125 mg twice daily	4804	1083
PAH patients	125 mg twice daily	8912	2286

Values are arithmetic means.
* From Study AC-052-108 (n = 10).
† From Study AC-052-109 (n = 9).

Ro 48-5033 and Ro 64-1056 showed plasma concentrations greater than those of Ro 47-8634, which was similar to observations in healthy subjects. The overall exposure to the metabolites relative to the exposure to bosentan was 70% and 53% after dosing regimens of 62.5 and 125 mg b.i.d., respectively. In healthy subjects, the overall exposure to the metabolites after multiple dosing usually did not exceed 25% that to bosentan. The increased exposure to the metabolites after a dose of 62.5 mg b.i.d. was mainly due to the high exposures measured in 3 patients. For two of these patients,

pharmacokinetic data for the 125-mg dose were not available, which explains the decrease in relative exposure to the metabolites at the higher dose. These 3 patients were not different from the other substudy patients in terms of demographics and co-medications. However, only in these 3 patients were levels of alkaline phosphatase above the ULN observed in association with levels of bilirubin above the ULN. Both abnormalities were already present at baseline in all 3 patients, and in 2 patients clinically relevant elevations in ALT occurred during the study. The association of high alkaline phosphatase and high bilirubin may indicate the presence of cholestasis, possibly resulting in decreased biliary excretion of the metabolites.

Regarding *Safety*, the mean treatment duration during this study was 15.8 weeks, 85% of patients had at least 12 weeks of treatment, 40% of patients had at least 16 weeks of treatment, and some patients were treated for up to 31 weeks. Twenty patients (17.4%) were prematurely discontinued from the study, 3 of which transferred early to commercial Tracleer. Four patients died related to progression of PAH; another death, also due to progression of PAH, occurred 16 days after the end of the study. None of these deaths were considered related to bosentan treatment. Ten patients had treatment discontinued because of an AE, including 3 for worsening of the patient's condition and seven for other AEs. One patient was discontinued because of a lack of improvement and 2 patients were lost to follow-up.

Bosentan was discontinued because of an AE in 10 patients, with or without a previous dose reduction or temporary interruption in treatment; 3 patients were discontinued due to worsening of their condition (aggravated PAH), 2 patients discontinued due to elevated liver aminotransferases (abnormal hepatic function), and discontinuation in other patients was due to cardiac/renal failure, fluid overload, hepatitis, nausea/vomiting, and renal failure.

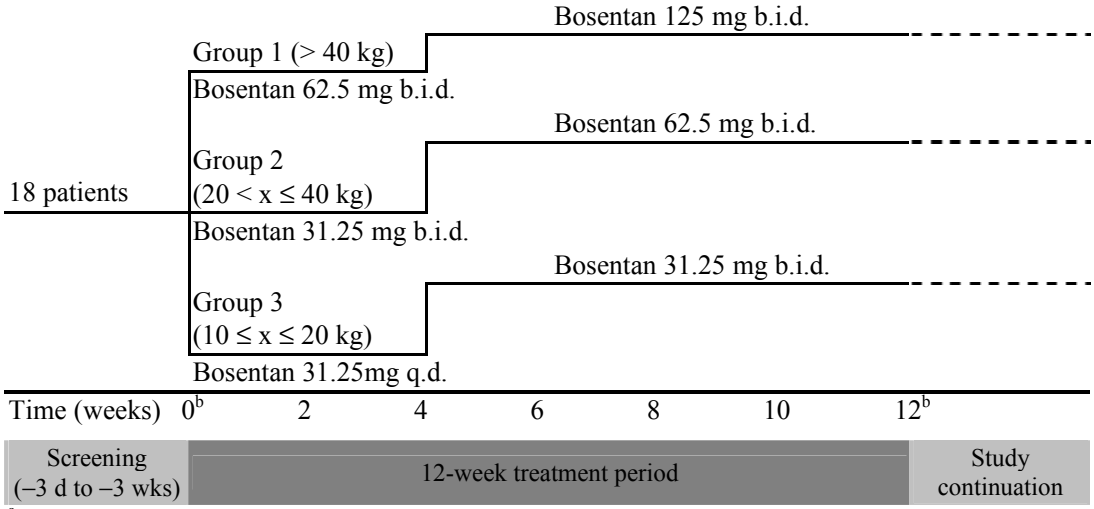
The most frequent AEs reported leading to changes in study medication were abnormal hepatic function (4.3%) and aggravated PAH (3.5%). Two patients had a marked decrease from baseline in haemoglobin concentration and/or haematocrit. Marked increases in AST and ALT were observed in 10 and 11 patients, respectively; 3 of these patients had peak values $\leq 5 \times$ ULN, 3 had values 5 - 8 $\times$ ULN, and 2 patients had values $> 8 \times$ ULN (a marked increase in bilirubin was associated). Treatment was discontinued in these two patients and values returned to baseline within 19 and 45 days, respectively. In the other 6 patients with increases in aminotransferases (between 3 and 8 $\times$ ULN), the bosentan dose was down titrated or in one case not up titrated as scheduled because of low body weight, and the aminotransferase levels decreased. An associated marked increase in alkaline phosphatase was seen in 2 patients, one of who was among those discontinued.

6.1.4. BREATHE-3 STUDY (AC-052-356)

This was a multicentre, open-label, non-controlled, parallel-group single- and multiple-dose trial in *paediatric patients* with PAH, with stratification for weight and epoprostenol use. The primary objective was to investigate the pharmacokinetics of bosentan given as single and multiple oral doses in pediatric patients with PAH. Secondary objectives were to evaluate the safety and tolerability of single and multiple oral doses of Tracleer and to obtain preliminary data on changes in exercise capacity, Borg dyspnoea index, haemodynamics, and WHO functional class after 12 weeks of treatment.

Patients were assigned to one of 3 parallel bosentan treatment arms on the basis of body weight. On Day 1, patients were treated with a single dose of Tracleer and blood samples were taken. On Day 2, patients began daily treatment with the initial dose (62.5 mg b.i.d., 31.25 mg b.i.d., or 31.25 mg once daily [q.d.]) for 4 weeks, after which the dosage was up-titrated to twice the initial dose (target dose). At the Week-12 visit, patients were again hospitalised for tests, and on the third morning were treated with a single Tracleer dose (125 mg, 62.5 mg, or 31.25 mg) after which blood samples for multiple-dose pharmacokinetic profiles were taken.

Study design



[a] Each group included 3 patients on conventional vasodilator/anticoagulant therapy and 3 on epoprostenol therapy.
[b] Single-dose pharmacokinetic profiles were obtained on Day 1 and multiple-dose profiles at the Week-12 visit. Single doses given on Day 1 were 125 mg (Group 1), 62.5 mg (Group 2), and 31.25 mg (Group 3).

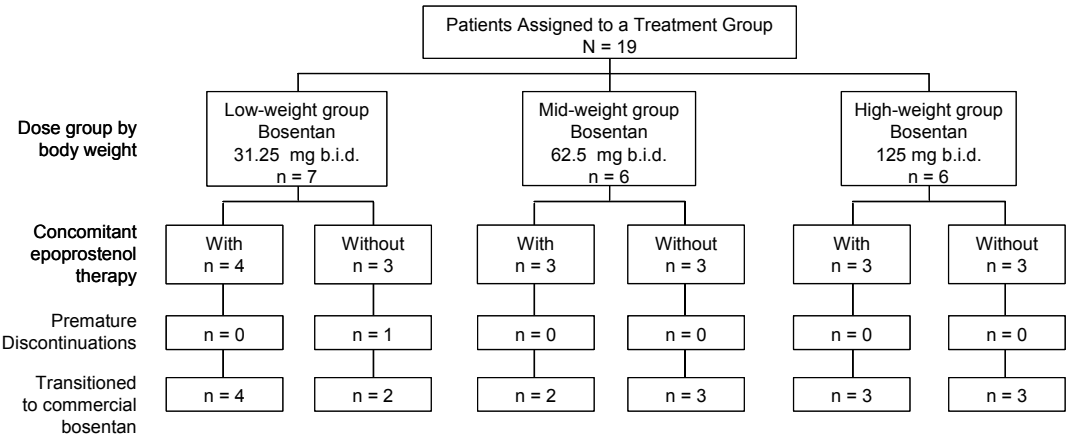
Patients who completed the 12-week pharmacokinetic evaluation were eligible to continue treatment, with visits to study centres scheduled at Months 6 and 12, and every 3 months thereafter until the end of the study. Safety parameters and WHO functional class were assessed regularly until the end of the study. Pharmacodynamic assessments were performed at baseline and Week 12 prior to pharmacokinetic assessments.

Pharmacokinetic profiles of Tracleer and its 3 main metabolites obtained at baseline/initiation (single dose) and Week 12 (multiple dose) were based on blood samples drawn immediately predose and at subsequent intervals postdose over a 24-hour period.

The following assessed and derived pharmacodynamic parameters were measured at baseline/ initiation (Visit 2) and after 12 weeks of bosentan treatment (visit 5):

+ Cardiopulmonary haemodynamic variables [systolic, diastolic, and mean PAP and sytemic arterial pressure (SAP)]; mean pulmonary capillary wedge pressure (PCWP), mean (RAP), cardiac output, pulmonary blood flow (PBF), pulmonary arterial oxygenation saturation (Pasat), (pulmonary venous saturation (Pvsat), measured O_2 consumption, heart rate, SaO_2, mixed venous saturation (Mvsat), cardiac index, stroke index, PVR, PVRI, systemic vascular resistance (SVR), systemic vascular resistance index (SVRI), PVR/SVR.
+ Walk distance during the 6-minute walk test
+ Borg dyspnoea index score
+ Cycle ergometry test variables (respiratory O_2 and CO_2 exchange (VO_2 and VCO_2), and ventilation (VE), heart rate, blood pressure, work rate, and exercise time) measured at rest and at peak exercise.
+ Anaerobic threshold values were determined graphically from V-slope plots.
+ WHO functional class

No formal *sample size* calculation was performed since there was no information available on pharmacokinetics of bosentan in paediatric patients with PAH. For pragmatic reasons, 18 patients were proposed and were considered sufficient to reach the study objectives. Patients withdrawn from the assessments were to be replaced. The distribution of patients was as follows:



One patient in the low-weight dose group was prematurely discontinued from the study on Day 7 because of abnormal hepatic enzymes. The remaining 18 patients completed the Week-12 assessments. An additional patient had bosentan discontinued (Day 197) because of abnormal liver function test results, and 17 patients transitioned to commercial Tracleer at the end of the study.

All 19 enrolled patients were included in the safety and pharmacodynamic analyses; however, the patient who was prematurely discontinued had no Week-12 assessment and was therefore omitted from the evaluation of all parameters that had no other post-baseline assessment (*i.e.*, all but WHO functional class). Pharmacokinetic data were analysed in 18 patients.

Regarding *demographics and baseline characteristics*, 80% of patients were Caucasian and 9 were male. The mean age was 9.7 years old (range 3.0 - 15.0 years) and 6 patients were < 8 years. The mean weight was 30.8 kg (range 13.9 - 54.0 kg). All 19 patients suffered from either PPH (n = 10) or PAH related to congenital systemic-to-pulmonary communications (n = 9). Congenital heart defects associated with PAH included atrial septal defect (ASD) (n=4), ventricular septal defect (VSD) (n=2), and patent *ductus arteriosus* (n=2). In addition, one patient was reported to have had ASD, VSD, and anomalous pulmonary venous return. All patients were either WHO functional class II (78.9%) or III (21.1%) at baseline. Antithrombotics were reported for all patients (warfarin 84.2% and heparin 52.6%). About half the patients reported using supplemental oxygen. Other medications for PAH included high-ceiling (loop) diuretics (52.6%, all on furosemide), cardiac glycosides (42.1%, all on digoxin), and calcium channel blockers (26.3%).

Analyses of variance (ANOVA) and covariance (ANCOVA) were calculated. Explanatory variables were the following: dose (continuous), weight (continuous), age (continuous), gender (categorical) and use of Flolan (categorical).

Results:

Pharmacokinetic

Ro 48-5033 was the most prominent metabolite, followed by Ro 64-1056 and Ro 47-8634 with both single- and multiple-dose administration of bosentan. The overall exposure to the metabolites after both single (19%) and multiple dosing (24% - 30%) was small relative to bosentan.

After *single-dose* administration, the exposure to bosentan (geometric mean values) varied from 5,453 ng.h/ml in the 31.25-mg group to 10,777 ng.h/ml in the 125-mg group. The elimination $t_{1/2}$ was comparable in the 3 different weight groups, and there was a trend for t_{max} to increase with dose. Upon *multiple-dose* administration, exposure to bosentan was reduced in a manner consistent with the known induction properties of bosentan, and was most notable in the 125 mg b.i.d. (–43%) and 31.25-mg b.i.d. (–36%) groups, and least pronounced in the 62.5-mg b.i.d. group (–11%; 4/6 children had no reduction in exposure). When compared to single-dose administration the trend for t_{max} to increase with dose disappeared and there was a tendency towards shorter t_{max} values.

A greater exposure to bosentan was measured in children when compared to healthy adult volunteers, and this was most pronounced in the 31.25-mg group. However, after correction for differences in body weight and dose of the different groups, the exposure tended to be greater in healthy adults than

in children. Further to a request from CPMP, the MAH provided a comparison between children and adults patients. The results are shown in the table below and show that the exposure in children was also lower than in adult patients.

Comparison of bosentan C_{max} and AUC data obtained in Study AC-052-356 with data from previously reported studies in healthy adult volunteers and patients:

Population	Dose	$AUC_{0-\infty}$ SD (ng•h/ml)	AUC_{τ} MD (ng•h/ml)	$AUC_{0-\infty}$ SD* (ng•h/ml)	AUC_{τ} MD* (ng•h/ml)
Healthy volunteers	31.25 mg	2049[1]	NA	4616	NA
Paediatric patients	31.25 mg	5453	3496	2879	1846
Healthy volunteers	62.5 mg	4234[2]	2744[2]	5303	3437
Paediatric patients	62.5 mg	6118	5428	3035	2692
Healthy volunteers	125 mg	8791[3]	4586[4]	5486	2862
Adult patients	125 mg	NA	8149[5]		
Paediatric patients	125 mg	10777	6124	4009	2278

Population	Dose	C_{max} SD (ng/ml)	C_{max} MD (ng/ml)	C_{max} SD* (ng/ml)	C_{max} MD* (ng/ml)
Healthy volunteers	31.25 mg	333[1]	NA	750	—
Paediatric patients	31.25 mg	959	685	506	362
Healthy volunteers	62.5 mg	617[2]	516[2]	769	643
Adult patients	62.5 mg	NA	1062[5]		
Paediatric patients	62.5 mg	815	1136	404	563
Healthy volunteers	125 mg	1612[3]	1006[4]	1006	616
Adult patients	125 mg	NA	1878[5]		
Paediatric patients	125 mg	1709	1200	635	446

* = Corrected for body weight and dose (value divided by dose/mean weight).
[1] Study AC-052-110 (n = 10); [2] Study AC-052-108 (n = 10); [3] Study AC-052-106 (n = 16); [4] Study AC-052-109 (n = 9).
[5] Study AC-052-357 (n = 11–12)
MD = multiple dose, NA = not available, SD = single dose.

Similar mean of C_{max} and AUC values of bosentan were observed after both single- and multiple-dose administration in the presence and absence of epoprostenol, as shown in the table below:

Arithmetic mean values and 95% confidence intervals of C_{max} and AUC of bosentan in the presence and absence of concomitant epoprostenol administration

	Single-dose administration			
	No epoprostenol C_{max} (ng/ml)	+ epoprostenol C_{max} (ng/ml)	No epoprostenol $AUC_{0-\infty}$ (ng·h/ml)	+ epoprostenol $AUC_{0-\infty}$ (ng·h/ml)
Mean (95% CI)	7.7 (5.1, 10.3)	9.1 (5.8, 12.4)	46.2 (32.6, 59.8)	54.0 (38.4, 69.6)
Median	7.2	8.0	39.5	54.2
	Multiple-dose administration			
	No epoprostenol C_{max} (ng/ml)	+ epoprostenol C_{max} (ng/ml)	No epoprostenol AUC_{τ} (ng·h/ml)	+ epoprostenol AUC_{τ} (ng·h/ml)
Mean (95% CI)	8.9 (4.1, 13.7)	6.6 (4.3, 8.9)	37.3 (22.3, 52.3)	33.4 (24.9, 41.9)
Median	6.2	5.5	36.8	31.1

Data are body weight and dose-corrected values.

Efficacy

Haemodynamics were improved after 12 weeks of Tracleer treatment; a decrease in mean PAP was observed in 15/18 patients and an increase in cardiac index in 11/17 patients with measurements. In the group as a whole, statistically significant decreases were observed in the following parameters:
- mean PAP (mean and median changes of –8.0 and –7.0 mmHg, respectively, p = 0.0003),
- PVR (–389 and –266 dyn·sec/cm^5, p = 0.0021),
- PVRI (–300 and –274 dyn·sec·m^2/cm^5, p = 0.0026),
- mean SAP (–8.6 and –7.0 mmHg, p = 0.0003),
- SVR (–517 and –322 dyn·sec/cm^5, p = 0.0032),
- SVRI (–426 and –384 dyn·sec·m^2/cm^5, p = 0.0067).

Statistically significant increases were obtained in cardiac output (0.61 and 0.50 L/min, p = 0.0490) and stroke index (0.006 and 0.009 ml/m^2, p = 0.0267). There was no overall effect on heart rate (–2.4 and 0.3 bpm, p = 0.6935).

Haemodynamic variables were generally similar between subpopulations treated with bosentan alone and bosentan+epoprostenol. Patterns between the two subpopulations were generally consistent, but there was a larger percentage decrease in the PVRI compared to the SVRI and a larger percentage increase in cardiac index among patients on bosentan alone. Although not significant because of the small number of patients, this suggests that there may have been a more selective pulmonary vasodilation with bosentan alone than with the combination therapy. Additionally, an increase in pulmonary arterial oxygen saturation was observed in patients on bosentan alone.

Exercise parameters were evaluated for exploratory purposes in 12 patients who were at least 8 years of age. Changes in exercise test parameters at week 12 from baseline were highly variable and none were significant, although some patients showed beneficial trends. The mean and median changes from baseline in peak VO_2 were 53.0 and –3.5 ml/min, respectively (p = 0.4697).

The investigator based on knowledge of the patient and parental input assessed WHO Functional Class. During the 12 weeks of treatment 3 patients improved from class III to II, and 2 from class II to I. One patient deteriorated (class II to III). The other 13 remained in the same functional class (12 class II, 1 class III). At one site the investigator chose to reduce the concomitant epoprostenol dose in 3 patients with stable class II, starting at Week 13 or 14 of bosentan treatment (not per protocol). Epoprostenol dosages were gradually reduced from 97 to 83, from 72 to 40 and from 146.5 to 100 ng/kg/min/day in the 3 patients, while the protocol-stipulated Tracleer dose was continued. Follow-up assessments in these patients showed no deterioration in their clinical status (stable WHO class II) during or following epoprostenol reduction.

Safety

Patients were exposed to bosentan for a mean and median of 23.9 weeks; 13 patients had ≥ 16 weeks of treatment, and 9 patients had 6 months of bosentan treatment in the study.

The following AEs were considered probably or possibly related to treatment: abnormal hepatic function (3 patients), flushing, headache, oedema, nausea, hypotension (1 patient each), and the moderate tachycardia, hypertension, tremor, and dizziness in one patient (reported as SAEs because they prolonged hospitalisation). Flushing was noted only in patients also on epoprostenol. Aggravated PAH, pyrexia, and a variety of infections occurred in 2 patients each. Mild fluid retention was reported for 2 patients and moderate oedema for 1; however, unlike in previous studies, it did not occur early in treatment but rather after at least 79 days of treatment. The incidences of these and other AEs events did not appear to have any relationship to weight group, nor did they particularly occur in the patients with the highest exposures ($AUC_{0-\infty}$ and C_{max}). No clinically relevant change in mean heart rate or PQ, QRS, QT, or QTc interval was observed.

In addition, further to the CHMP assessment of the ENABLE studies the MAH was requested to include information for prescribers warning them of the dangers of fluid retention especially in patients with severe systolic dysfunction. The MAH was also requested to state that no experience is

available with Tracleer in patients with pulmonary veno-occlusive disease and that frequent deterioration with massive pulmonary oedema has been reported in response to vasodilators in patients with veno-occlusive disease.

6.2 Discussion

6.2.1. Efficacy

Study AC-052-356 (BREATHE-3)
A small number of children with heterogeneous PAH aetiology have been studied in an open-labelled trial with no control group. The data are too limited to conclude on the appropriate dosing in children. The dosage regimen was chosen in order to obtain an exposure comparable to adult PAH patients treated with 125 mg bid, where efficacy and safety have been demonstrated. This aim has clearly not been achieved since the steady state systemic exposure in paediatric patients weighing 10-20 kg, 20-40 kg and >40kg was 43%, 67% and 75%, respectively, of the adult patient systemic exposure. Exposure to bosentan also tended to be lower compared to adult healthy volunteers. Therefore, the pharmacokinetic data suggest that children may receive sub-optimal doses with the investigated dose regimen, especially younger children. These findings are reflected in sections 4.2 and 5.2 of the SPC.

No effect of epoprostenol on the pharmacokinetics of bosentan was expected given the different metabolic pathways, routes of administration and that epoprostenol is not known to inhibit cytochrome P450 enzymes. Steady state data indicate that the pharmacokinetics in patients with or without epoprostenol is similar. This has been reflected in section 4.5 of the SPC.

Although an improvement from baseline in haemodynamic parameters has been observed based on very limited measurements, changes in exercise test parameters were highly variable and none were significant. These findings are reflected in section 5.1 of the SPC.

The conclusions of the MAH stating that the results of BREATH-3 indicate that the doses of bosentan used were appropriate for children with PAH are questionable. The results only suggest that tolerance seems to be satisfactory at the experimental dosing schedule, but the rationale for this dosing regimen to ensure optimal efficacy has not been demonstrated. In conclusion, based on the kinetic findings in children and the limited efficacy data in this population, it cannot be excluded that patients will receive sub-optimal doses. The lower AUC in children may be related to increased hepatic metabolism and excretion. However, bearing in mind the hepatotoxicity of bosentan (biliary pump mechanism) it is not excluded that higher doses will increase liver injury. Thus, whilst the CPMP ackowledges that the information available in children is useful, the SPC has been revised to clearly reflect the uncertainty in the current knowledge on the appropriate recommendations to be provided.

Study AC-032-357

The kinetic data demonstrate that the exposure of bosentan in PAH patients was about two-fold higher than in healthy volunteers and is in line with the very limited previous data after iv administration of high doses in PAH patients. The exposure to metabolites - other than Ro 47-8634 - relative to the exposure to bosentan was also higher than that observed in healthy volunteers and displayed large inter-subject variability, with a relative exposure to Ro 48-5033 and Ro 64-1056 ranging from 9-130% and 6-97%, respectively. This is likely to be of limited relevance for Ro 64-1056 given its low affinity for the ET receptor compared with bosentan. On the other hand, Ro 48-5033 has an affinity half that of bosentan and is less protein bound (the free fraction is 3 fold higher). Thus, the contribution of Ro 48-5033 to efficacy in PAH patients may be higher than what was estimated in healthy volunteers. On average the contribution was 37% and 29% at the 62.5 mg and 125 mg dose levels respectively, but individual data was as high as 163% and 196% at the two dose levels, respectively. Excluding the 3 patients with highest exposure to metabolites (and with high pre-dose levels of alkaline phosphatase and bilirubin indicating cholestasis), the contribution of Ro 48-5033 to effect was on average about 25% and ranged up to 49%. Thus, in specific individuals a large part of the effect may be attributed to this metabolite. Ro 48-5033 is mainly eliminated by biliary excretion (65% of an oral dose and 48% of an i.v. dose is recovered as Ro 48-5033 in faeces), but also to some extent by subsequent metabolism

by CYP3A4 and CYP2C9 to Ro 64-1056 (13% of an oral dose and 18% of an i.v. dose is recovered as Ro 64-1056 in faeces). It seems likely that cholestasis could decrease the elimination of Ro 48-5033 resulting in unexpected high exposure to this active metabolite.

Section 5.2 of the SPC has been revised to include the additional information on the active metabolite, including the relative exposure of patients compared to healthy volunteers, details on its elimination, the possibility of increased exposure in patients with cholestasis, and the contribution to efficacy in PAH patients.

6.2.2. Safety

No new safety concern has emerged in PAH patients. Most SAEs were expected in this patient population, and the deaths are likely to be a consequence of disease progression.

Regarding the *combination of Tracleer and epoprostenol,* it has been investigated in 2 studies, AC-052-355 (BREATHE-2) and AC-052-356 (BREATHE-3). The combination therapy was well tolerated in children and adults, although its clinical benefit has not been demonstrated. This information has been included in section 5.1 of the SPC.

7. Safety Updates of the SPC and Package Leaflet

7.1 Changes requested by CPMP following the assessment of the ENABLE studies

The ENABLE program consists of two independent multicentre studies designed to evaluate the long-term effects of bosentan 125 mg b.i.d. on clinical status, morbidity/mortality, and safety in patients with chronic heart failure (CHF). They are not disccused in this EPAR since the applicant has not requested the indication in CHF. Nonetheless, these studies enlarge the safety database of bosentan in the context of the current Marketing Authorisation.

An increased risk of hospitalisation for CHF, observed mainly during the first 4 - 8 weeks of bosentan treatment, questioned the tolerability of bosentan in patients with CHF. The MAH considers that it is the result of fluid retention early in treatment, as reflected in weight gain, decreased haemoglobin concentration and increased incidence of peripheral/leg oedema among bosentan-treated patients during this period. In addition, a greater proportion of bosentan-treated patients experienced chest pain and unstable angina as SAEs or reasons for hospitalisation, and a slight proportion of bosentan-treated patients experienced myocardial infarction.

It is debatable whether the deleterious effects observed in ENABLE can be extrapolated to the target population of Tracleer i.e. PAH. The haemodynamic conditions in PAH are not similar since PPH induces right ventricular impairment but not left ventricular dysfunction. However, left dysfunction and coronary disease can be associated with PAH (e.g. patients with scleroderma are predisposed to the occurrence of coronary disease and potential myocardial infarction). The 2 controlled studies performed to address safety and efficacy of bosentan in patients with PAH (AC-052-351 and AC-052-353) provide no information in patients with post-capillary pulmonary hypertension since subjects with left ventricular dysfunction (PCWP measurement or echocardiogram) and those with PAH associated with pulmonary venous hypertension (e.g. left –sided heart disease) or congenital heart disease were excluded.

The safety findings of the ENABLE studies have been summarised in section 4.4 of the SPC and an appropriate warning and/or precaution for prescribers regarding fluid retention has been included.

Finally, a review of the published literature reflecting the experience in post-capillary pulmonary hypertension shows that frequent deterioration with massive pulmonary oedema has been reported in response to vasodilators in patients with veno-occlusive disease. Thus, a warning has been added in case of veno-occlusive disease in section 4.4. of the SmPC informing prescribers that no experience is available with Tracleer in patients with pulmonary veno-occlusive disease.

7.2 Other safety updates following the assessment of PSURs

A statement regarding the co-administration of tacrolimus or sirolimus and bosentan warning of a possible kinetic interaction resulting in increased plasma concentrations of bosentan and decreased levels of tacrolimus/sirolimus has been included in section 4.5 of the SPC. Patients in need of the combination should be closely monitored for adverse events related to bosentan and for tacrolimus and sirolimus blood concentrations.

As of 13 February 2004, a total of 17 pregnancies have been reported since bosentan was launched, including 15 cases associated with maternal exposure. Despite the fact that 3 pregnancies resulted in normal babies, the potential risk to humans of taking Tracleer during pregnancy is still unknown and Tracleer must therefore still be considered a human teratogen and must not be taken during pregnancy. Section 4.6 of the SPC has been updated to reflect that there are minimal data on the use of Tracleer in pregnant women from very few cases received in the post-marketing period.

The following adverse drug reactions (ADRs) have been included in section 4.8 of the SPC and the PL to reflect the post marketing experience with the product:

- Nausea, vomiting, abdominal pain and diarrhoea under "Gastrointestinal disorders"
- Aminostransferase elevations associated with hepatitis and/or jaundice under "Hepato-biliary disorders"
- hypersensitivity reactions including dermatitis, pruritus and rash under "Skin and subcutaneous tissue disorders"
- anaphylaxis and/or angioedema under "Immune system disorders".