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Long-term results from the EARLY study of bosentan in WHO functional class II pulmonary arterial hypertension patients

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ABSTRACT

Background: The double-blind phase of the EARLY study of bosentan remains the only randomized controlled trial of a PAH-targeted therapy in World Health Organization functional class (FC) II patients. We report on the efficacy, safety, disease worsening, survival and prognostic factors in mildly symptomatic pulmonary arterial hypertension (PAH) patients treated with bosentan in the open-label extension phase of the EARLY study.
Methods: Exploratory efficacy outcomes included 6-minute walk distance (6MWD) and WHO FC. Adverse events were recorded. Kaplan–Meier analysis was used to estimate time to first PAH worsening event (death, initiation of intravenous or subcutaneous prostanoids, atrial septostomy or lung transplantation) and survival. Cox regression analysis determined factors prognostic of survival.
Results: Median exposure to bosentan ($n = 173$) was 51 months. At the end of the bosentan-treatment assessment period, 77.8% of patients were in WHO FC I/II. Adverse events led to discontinuation of bosentan in 20.2% of patients. Aminotransferase elevations >3× upper limit of normal occurred in 16.8%. Four-year PAH-event-free survival and survival were 79.5% (95% confidence intervals [95% CI] 73.4, 85.6) and 84.8% [95% CI 79.4, 90.2], respectively. Low 6MWD, low mixed venous oxygenation, high N-terminal pro hormone of brain natriuretic peptide levels and PAH associated with connective tissue disease were associated with a higher risk of death.
Conclusions: The majority of patients exposed to long-term bosentan maintained or improved their functional class. Approximately 20% of the patients discontinued treatment because of adverse events, which were most commonly PAH worsening and elevated liver enzymes.

1. Introduction

Pulmonary arterial hypertension (PAH) is characterized by remodeling of the pulmonary vasculature [1]. Progressive vasculopathy results in an increase in pulmonary arterial pressure (PAP) and pulmonary vascular resistance (PVR), ultimately leading to right heart failure and death [2].

The World Health Organization functional class (WHO FC) system comprises four classes (I–IV), and characterizes patients with PAH by increasing functional compromise with symptoms such as dyspnea, fatigue, chest pain, and syncope initially on exertion and subsequently at rest. At the time of diagnosis, approximately 75% of patients are in WHO FC III/IV [3]. Functional class at the time of diagnosis as well as during follow-up is of pivotal prognostic importance [4]. Patients starting therapy in WHO FC I/II have a better prognosis than those in WHO FC III/IV [5] and patients who achieve WHO FC I/II after treatment have a better prognosis than those who remain in WHO FC III/IV [6,7]. Despite a relatively well preserved exercise capacity and mild symptoms, patients in WHO FC II are already hemodynamically compromised

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[8]. Therefore, both treating and maintaining patients in WHO FC II or better is a logical treatment strategy.

To date, EARLY (Endothelin Antagonist tRial in miLdlY symptomatic PAH patients) remains the only prospective, randomized, placebo-controlled, clinical trial of a PAH-targeted therapy in an exclusively WHO FC II PAH patient population. This multicenter study randomized 185 patients from 21 countries to receive bosentan or placebo over a 6-month double-blind treatment period. Bosentan significantly reduced PVR in comparison with placebo (-22.6%, $p < 0.0001$) and significantly delayed time to PAH worsening (hazard ratio 0.227, $p = 0.0114$) [8]. EARLY included an open-label extension (OLE) phase to provide continued treatment for those who were eligible and to collect long-term safety data on bosentan-treated patients. This article presents the final results of the EARLY study of bosentan in WHO FC II PAH patients; data from the OLE phase and long-term follow-up are reported. In addition, the collection of follow-up data for all randomized patients also provided a unique opportunity to investigate the effect of a PAH-targeted therapy on PAH worsening, survival and factors influencing survival.

2. Methods

2.1. Patients

Inclusion and exclusion criteria for WHO FC II PAH patients who entered the double-blind phase of EARLY have been described previously [8]. The etiologies of PAH enrolled into the EARLY study were idiopathic PAH (iPAH), heritable PAH, or PAH associated with human immunodeficiency virus (HIV) infection, anorexigen use, congenital heart disease (PAH-CHD; atrial septal defect of less than 2 cm in diameter, ventricular septal defect of less than 1 cm in diameter, patent ductus arteriosus), connective tissue disease (PAH-CTD) or auto-immune diseases. The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in *a priori* approval by each institutions human research committee. Written informed consent was obtained from each patient for entry into the double-blind phase and again for continuation into the OLE phase.

2.2. Study design

The EARLY study was a Phase IIIb, multicenter, clinical trial of bosentan in mildly symptomatic PAH which comprised a 6-month, double-blind, randomized, placebo-controlled phase, an open-label, single-arm extension phase and long-term follow-up of all randomized patients. The EARLY study was initiated in September 2004 and its OLE was completed in February 2011 when ≥50% of the patients had had the opportunity to be exposed to bosentan for ≥5 years (end of study). The design of the double-blind, randomized phase of EARLY has been described previously [8]. In brief, after screening, eligible patients were stratified according to sildenafil use and randomized 1:1 to bosentan 62.5 mg twice daily for the first month followed by up-titration to the target dose of 125 mg twice daily thereafter (or remained at 62.5 mg twice daily if body weight was <40 kg), or matching placebo for 6 months. The stratification of patients on sildenafil was included in an amendment to the protocol to reflect a change in clinical practice and was not intended for the purpose of assessing a combination treatment approach. Patients who completed the 6-month double-blind phase of EARLY, and who tolerated treatment, were eligible to receive bosentan therapy in the OLE phase. As treatment was still blinded at the start of the OLE phase all patients who continued in the study received an initial dose of bosentan 62.5 mg twice daily for 4 weeks followed by up-titration to the 125 mg twice-daily target dose. The dose could remain at, or be down-titrated to, the initial dose at any time for reasons of intolerability, with subsequent up-titration to the target dose.

2.3. Study assessments

All non-invasive efficacy assessments conducted in the double-blind phase continued in the OLE phase. These exploratory efficacy outcomes (6-minute walk distance [6MWD], Borg dyspnea index and WHO FC) were assessed during the OLE at 6-month clinic visits and at the end of the extension phase or premature withdrawal visit. Only adverse events (AEs) which led to premature discontinuation of bosentan, or a fatal outcome, were recorded during the OLE phase, while serious AEs (SAEs) were recorded up to 28 days post treatment. Monthly liver function tests (which included alanine and aspartate aminotransferases [ALT/AST]), quarterly hemoglobin tests, and monthly pregnancy tests in women of childbearing age were conducted throughout the OLE phase. Complete laboratory tests were performed at the end of the OLE phase or at premature withdrawal visit. Concomitant PAH medications were continuously monitored throughout the EARLY study. In all randomized patients PAH worsening events (death [all causes], initiation of intravenous (iv) or subcutaneous (sc) prostanoids, atrial septostomy and lung transplantation), and use of other PAH medications were recorded annually until the end of study, independently from bosentan treatment duration.

2.4. Data analysis of the bosentan-treated population

The bosentan-treated population comprised all patients who received ≥1 dose of bosentan during the double-blind or OLE phases. Demographics, disease and clinical characteristics at baseline, bosentan exposure, exploratory efficacy outcomes, and safety parameters were described or evaluated. The analysis of PAH worsening and survival included all events irrespective of whether or not they occurred during the bosentan treatment period. Baseline was defined as the last assessment before bosentan treatment. That is, for patients receiving placebo during the double-blind phase, baseline values were obtained at the last assessment before receiving drug in the open-label phase, and for those patients initially randomized to bosentan, baseline was the last assessment before the double-blind phase. There was no imputation for missing data, except for missing or incomplete dates in AE and concomitant medication data. Unless otherwise stated, data were summarized descriptively: mean ± standard deviation (SD) (and if informative also as median [range]) for quantitative variables; frequency and proportions for categorical variables.

For the exploratory efficacy outcomes, change from baseline at study end, and at 6-month intervals up to Month 36 (retrospectively chosen as >75% patients had data available at this time point), in patients for whom data were available at each specific time point, were reported for 6MWD and Borg dyspnea index. Changes in WHO FC from baseline were reported at study end. The proportions of patients who improved to a lower FC or deteriorated to a higher FC at the end of treatment were also provided together with 95% confidence intervals (CI).

Safety data are presented for patients who had ≥1 AE and events per 100 patient-years are provided. Elevations of ALT/AST > 3 × upper limit of normal (ULN) and marked decreases in hemoglobin ≤ 10 g/dL during the bosentan-treatment period are described. Time to first ALT/AST elevation during the same treatment period with event rate corrected to account for individual exposure times was determined by Kaplan–Meier analysis.

For PAH worsening and survival, Kaplan–Meier analysis (estimates reported as percentages and 95% CI) was used to estimate time to PAH worsening and its separate components. A post-hoc comparison of the patients on monotherapy at the start of the EARLY study who remained on monotherapy with those who had moved on to combination therapy at 4 years was performed for patients who had a) died, b) were known to be alive and c) who had been censored. Time to death was further estimated by Kaplan–Meier analysis according to PAH etiology, as was time to initiation of other PAH-targeted drugs (oral phosphodiesterase type 5 inhibitors and/or oral/inhaled prostanoids).

2.5. Cox analysis on the all-randomized population

In order to determine factors prognostic of survival, Cox regression analyses were conducted on the all-randomized population which included all randomized patients, whether they received study medication or not. Univariate (significance set at $p < 0.1$ cut-off) and multivariate (backward selection set at $p < 0.1$ cut-off, including all variables having a p-value < 0.1 in the univariate analysis) Cox analyses were employed.

3. Results

3.1. Patient disposition

One hundred and eighty-five patients were randomized in the double-blind phase of EARLY (Fig. 1); 92 to placebo and 93 to bosentan and constituted the all-randomized population. Of these, 80/92 who were randomized to placebo and 77/93 randomized to bosentan entered the OLE phase. In total, 173 patients received ≥1 dose of bosentan during the double-blind or OLE phase and constituted the bosentan-treated population.

3.2. Bosentan-treated population

3.2.1. Baseline characteristics

Baseline patient demographics as well as disease and clinical characteristics for the all-randomized population have been previously reported [8]. Table 1 reports baseline patient demographics and disease and clinical characteristics for the bosentan-treated population. Patients in the OLE phase of EARLY had a similarly mild clinical profile as the double-blind cohort, with over 90% being classified as WHO FC II, a high 6MWD and a low Borg dyspnea index. However, high baseline PAP, PVR, and right atrial pressure show that the patients were hemodynamically compromised.

3.2.2. Bosentan exposure

Due to the study design, exposure to bosentan was variable. Overall, 73.3% of patients received bosentan for at least 3 years and 61.6% for at

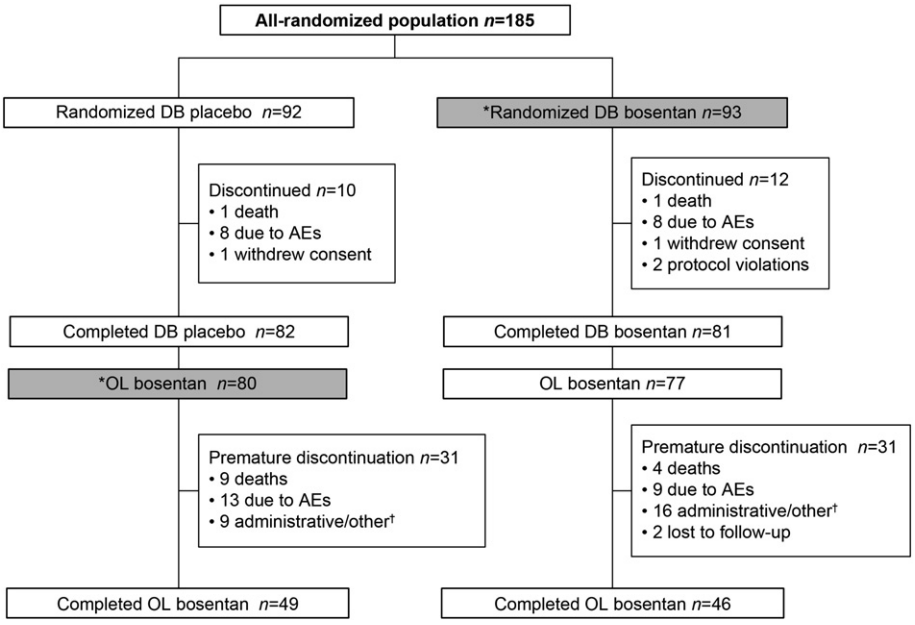


Fig. 1. Patient disposition. Patient flow and disposition in the randomized, double-blind study and open-label extension. *Patients comprising the bosentan-treated population, $n = 173$. AEs = adverse events; DB = double-blind; OL = open-label. †Administrative/other reasons included protocol violation or non-compliance, withdrawal of consent, personal reasons, investigator's decision, switch to commercial drug, switch to another study.

least 4 years (supplementary Table S1). The mean (SD) exposure to bosentan was 43.3 (SD 21.5) months and median (range) exposure was 51.4 (0.1–72.8) months ($n = 172$; 1 patient was lost to follow-up).

3.2.3. Exploratory efficacy outcomes

A total of 154 patients had baseline and at least one post-baseline assessment for 6MWD and Borg dyspnea index. Mean change from baseline to end of treatment in 6MWD was −3.7 m (95% CI −17.0, 9.5). Mean changes from baseline to 6-month intervals up to Month 36 are shown in Fig. 2 for patients who had an assessment at each point. On the Borg dyspnea index upon exercise, there was a minimal change from baseline to end of treatment (mean score 0.30 [95% CI −0.02, 0.62]). Similarly, mean changes from baseline to 6 monthly intervals up to Month 36 were minimal.

Of the 158 patients who had available post-baseline WHO FC data, 154 (97.5%) were classified as WHO FC I or II at baseline. At end of treatment, the majority of patients ($n = 123$; 77.8%) were still in WHO FC I or II, with 27 (17.1%) in WHO FC III, and 8 (5.1%) in WHO FC IV (Table 2). Overall, 18.4% (95% CI 12.7, 25.3) of patients had improved and 22.8% (95% CI 16.5, 30.1) had deteriorated.

3.2.4. Safety

Thirty-five patients (20.2%) had an AE that led to bosentan discontinuation, which equates to 5.6 events per 100 patient-years. All AEs leading to bosentan discontinuation are listed in supplementary Table S2.

For seven patients (4%) worsening of PH was recorded as an AE leading to discontinuation, in the double-blind phase (one patient) or open-label phase (six patients). However, the most common AE that led to bosentan discontinuation was coded as an abnormal liver function test in 12 patients. One further patient who was specified as having increases in ALT and AST also discontinued bosentan.

There were three additional patients with ALT/AST elevations >3× ULN but whose reason for discontinuation from bosentan was reported as hepatitis and hepatitis C (one patient), toxic hepatitis (one patient) and autoimmune hepatitis (one patient). While in the double-blind study period the patient with hepatitis C first experienced iatrogenic hepatitis, considered an adverse drug reaction to bosentan, which improved following drug discontinuation. This was followed by hepatitis C, with eventual recovery of aminotransferase levels to normal baseline values. The events were judged by the investigator to be serious, severe and related to bosentan treatment, resolving with sequalae. For the patient with toxic hepatitis the event occurred during the open label phase of EARLY. Bosentan was temporarily interrupted for 23 days and restarted at the 62.5 mg dose but permanently discontinued 9 days later. The event resolved without sequelae. The investigator considered this non-serious event to be moderate in intensity and related to bosentan treatment. For the patient with autoimmune hepatitis, the condition had been diagnosed to be pre-existing and recurrent. In this patient the elevated liver enzymes (>8 × ULN) were unresolved but had, prior to bosentan discontinuation, been treated without tolerability issues for 3.5 years. The elevations in liver enzymes were considered by the investigator not to be related to bosentan treatment. At the last safety follow-up before study end her clinical condition improved and liver function tests were being conducted twice weekly.

Over the 5-year duration of the study, 29/173 (16.8%) patients experienced elevations in ALT and/or AST > 3 × ULN, with 14 patients (8.1%) having elevations >8 × ULN. The majority of the ALT and/or AST elevations occurred by Year 1, with all 20 events actually occurring in the first 6 months, giving a crude event rate of 11.6% at Month 6. The number of additional events by Years 2, 3, 4 and 5 were 5, 2, 1 and 1 respectively. The Kaplan–Meier estimate of time to AST/ALT elevation which corrected for the shorter exposure time of bosentan for some patients provides event rates of 12.0% at Month 6 (which is the same as at Year 1) and 18.6% at Year 5 (Fig. 3). The Kaplan–Meier curve clearly shows that the majority of these cases occurred within the first 6 months of bosentan treatment, with few additional patients experiencing liver enzyme elevations—after 6 months.

All but one case of elevated liver enzymes, as described above in the patient with pre-existing, recurrent autoimmune hepatitis, resolved after either a decrease in the bosentan dose, continued treatment, or after treatment discontinuation.

A hemoglobin concentration of ≤10 g/dL was found in 26 (15.0%) patients, only one of whom had a pre-treatment baseline value of ≤10 g/dL. No patient discontinued bosentan due to anemia, two patients required dose reduction, and transfusion was reported in six cases.

Table 1
Patient demographics and baseline characteristics (bosentan-treated population).

Characteristic	Bosentan-treated population ($n = 173$)
Sex, *n* (%)	
Female	120 (69.4)
Age, yrs	45.0 ± 17.6
Weight, kg	68.5 ± 16.3
Race	
White	148 (85.5%)
Asian	11 (6.4%)
African descent	5 (2.9%)
Hispanic	1 (0.6%)
Other	8 (4.6%)
Time from diagnosis, yrs	3.4 ± 5.9
Etiology	
Idiopathic/heritable	106 (61.3%)
Congenital heart disease[a]	31 (17.9%)
Connective tissue disease/auto-immune disease	29 (16.8%)
Human immunodeficiency virus	7 (4.0%)
Concomitant treatments	
Oral anticoagulants	98 (56.6%)
Platelet aggregation inhibitors excluding heparin[b]	14 (8.1%)
Heparin	12 (6.9%)
Calcium channel blockers	58 (33.5%)
Sildenafil	29 (16.8%)
6-minute walk distance, m	435.8 ± 93.5
Borg dyspnea index	3.31 ± 2.09
WHO functional class	
I	6 (3.5%)
II	160 (92.5%)
III	7 (4.0%)
Hemodynamic parameters	
Mean pulmonary arterial pressure, mmHg	53.1 (8.3)[c]
Mean right atrial pressure, mmHg	7.5 (4.7)[c]
Pulmonary capillary wedge pressure, mmHg	9.4 (4.2)[d]
Cardiac output, L min^{-1}	4.6 (1.5)[e]
Cardiac index, L $min^{-1} \cdot m^{-2}$	2.7 (0.8)[e]
Pulmonary vascular resistance[f], dyn s cm^{-5}	853.0 (505.2)

[a] $n = 170$. Unless otherwise stated data are presented as mean (standard deviation).
[b] Platelet aggregation inhibitor was acetylsalicylic acid.
[c] $n = 172$.
[d] $n = 165$.
[e] $n = 169$.
[f] When pulmonary capillary wedge pressure was not determined, it was set to 0 in the calculation of pulmonary vascular resistance.

A total of 30 deaths occurred up to end of study in the bosentan treated-population; 21 deaths had occurred within the 28-day period after bosentan discontinuation and nine had occurred more than

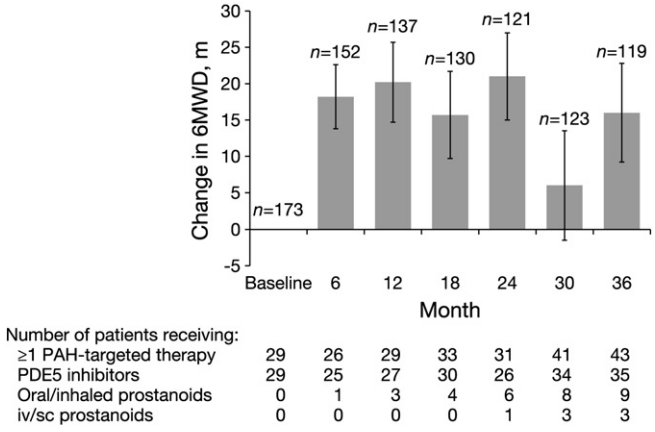


Fig. 2. 6-Minute walk distance. Change in 6-minute walk distance from baseline at 6 monthly intervals up to Month 36 in the bosentan-treated population. Data are presented as means and standard errors of the mean. PAH = pulmonary arterial hypertension; PDE5 = phosphodiesterase type 5; iv = intravenous; sc = subcutaneous.

Table 2
Change in WHO functional class from baseline to end of treatment (bosentan-treated population).

n	Baseline	End of treatment period				
		n	I *n* (%)	II *n* (%)	III *n* (%)	IV *n* (%)
158[a]	I	5	3 (1.9%)	2 (1.3%)	–	–
	II	149	27 (17.1%)	89 (56.3%)	26 (16.5%)	7(4.4%)
	III	4	1 (0.6%)	1 (0.6%)	1 (0.6%)	1 (0.6%)
	IV	0	–	–	–	–

[a] 158 patients who had post-baseline WHO functional class data were included in this analysis. Data are missing from 15 patients; 2 died and 13 patients did not have valid post-baseline data.

28 days after bosentan discontinuation. Of the patients who died during the bosentan treatment period or within 28 days after discontinuation, progression of PAH (four patients), pneumonia and sudden death (three patients each) were the most frequent causes of death. The treating physician judged death to be related to study medication in two cases (convulsions/vasculitis/worsened PAH, and antiphospholipid syndrome/sudden death/systemic lupus erythematosus).

3.2.5. PAH worsening and survival

The estimated proportion of patients who remained free of PAH worsening (event-free survival) at 1, 2, 3, and 4 years were 96.5%, 90.7%, 87.8%, and 79.5%, respectively (Fig. 4A). PAH worsening events, which occurred as first events, were death ($n = 25$) and initiation of iv or sc prostanoids ($n = 18$). Two patients underwent lung transplantation, both of whom first received iv or sc prostanoids. Survival estimates at 1, 2, 3, and 4 years were 97.1%, 92.4%, 90.1%, and 84.8%, respectively (Fig. 4B). Patients with PAH-CTD had poorer survival than those with idiopathic/heritable PAH or PAH-CHD (Fig. 4C).

It was estimated that after 4 years, 7.6% of patients had received iv or sc prostanoid therapy (Fig. 4D) and 29.6% of patients had received other PAH-targeted drugs, namely phosphodiesterase type 5 inhibitors (sildenafil or tadalafil) and/or oral/inhaled prostanoids (Fig. 5). At 4 years, among the 144 patients who had started bosentan as a monotherapy (*i.e.* excluding the 29 already on sildenafil at baseline, see Table 1), 21 were known to have died (15 remained on monotherapy, six had received combination therapy) and four were lost to follow-up and censored. Of the 119 patients known to be alive at 4 years, 75

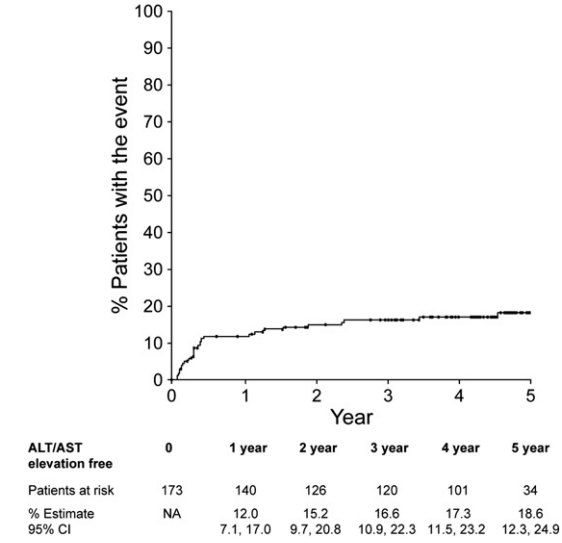
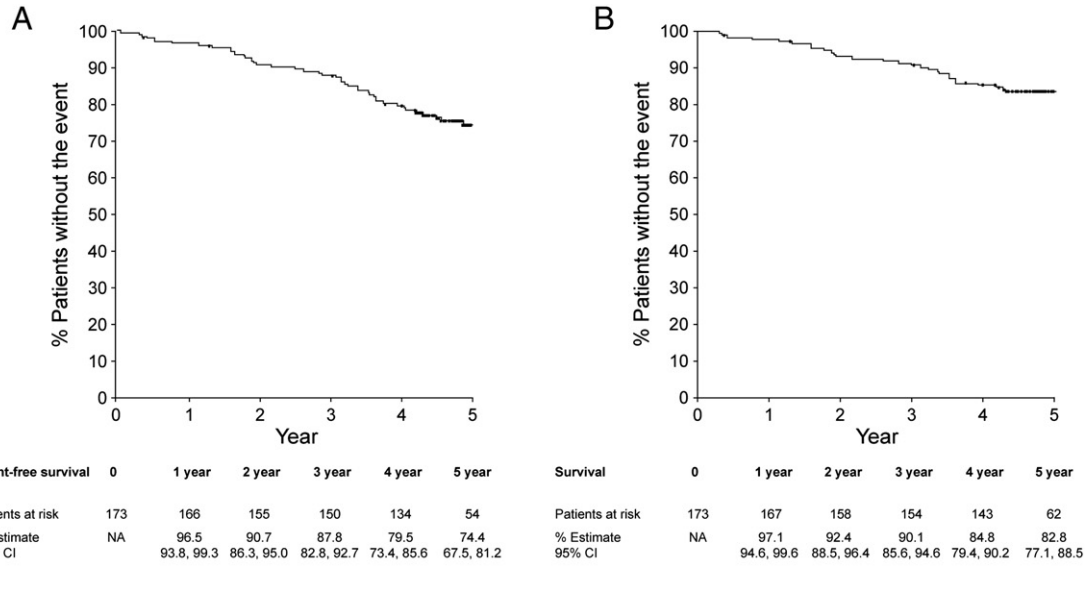
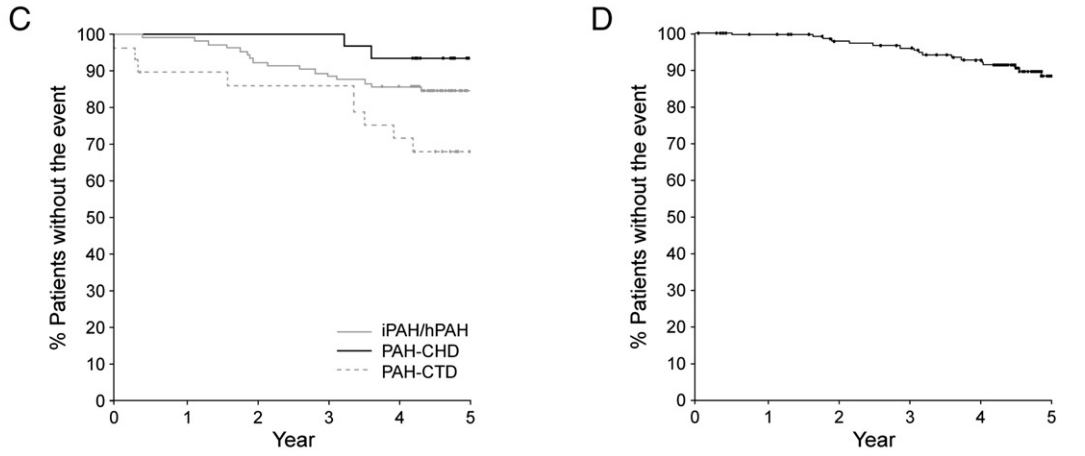


Fig. 3. Liver enzymes. Kaplan–Meier estimates of time to alanine and aspartate aminotransferase elevations in patients receiving bosentan in the bosentan-treated population. ALT = alanine aminotransferase; AST = aspartate aminotransferase; CI = confidence intervals; NA = not applicable.



Event-free survival	0	1 year	2 year	3 year	4 year	5 year
Patients at risk	173	166	155	150	134	54
% Estimate	NA	96.5	90.7	87.8	79.5	74.4
95% CI		93.8, 99.3	86.3, 95.0	82.8, 92.7	73.4, 85.6	67.5, 81.2

Survival	0	1 year	2 year	3 year	4 year	5 year
Patients at risk	173	167	158	154	143	62
% Estimate	NA	97.1	92.4	90.1	84.8	82.8
95% CI		94.6, 99.6	88.5, 96.4	85.6, 94.6	79.4, 90.2	77.1, 88.5



Survival	Etiology	0	1 year	2 year	3 year	4 year	5 year
Patients at risk		106	105	98	94	89	35
% Estimate	iPAH/hPAH	NA	91.1	92.5	88.7	85.8	84.7
95% CI			97.2, 100	87.4, 97.5	82.6, 94.7	79.2, 92.5	77.8, 91.6
Patients at risk		31	31	31	31	29	16
% Estimate	PAH-CHD	NA	100.0	100.0	100.0	93.5	93.5
95% CI			100, 100	100, 100	100, 100	84.9, 100	84.9, 100
Patients at risk		29	25	24	24	20	10
% Estimate	PAH-CTD	NA	89.7	86.1	86.1	71.7	68.1
95% CI			78.6, 100	73.4, 98.7	73.4, 98.7	55.1, 88.3	50.9, 85.3

Free of iv/sc prostanoid initiation	0	1 year	2 year	3 year	4 year	5 year
Patients at risk	173	166	155	150	134	54
% Estimate	NA	99.4	97.5	95.6	92.4	87.8
95% CI		98.2–100	95.2–99.9	92.5–98.8	88.2–96.5	82.2–93.5

Fig. 4. Time to pulmonary arterial hypertension worsening, overall and by its components. Kaplan–Meier estimates of time to A) pulmonary arterial hypertension worsening; B) death (all causes) all patients; C) death (all causes) by PAH etiology, and D) intravenous/subcutaneous prostanoid initiation in the bosentan-treated population. Pulmonary arterial hypertension (PAH) worsening was defined as first occurrence to death (all-causes), initiation of intravenous/or subcutaneous (iv/sc) prostanoids, atrial septostomy or lung transplantation. Causes of PAH worsening were death ($n = 25$; 58.1% and initiation of an iv/sc prostanoid ($n = 18$, 41.9%). CI = confidence intervals; hPAH = heritable pulmonary arterial hypertension; iPAH = idiopathic pulmonary arterial hypertension; NA = not applicable; PAH-CHD = pulmonary arterial hypertension associated with congenital heart disease; PAH-CTD = pulmonary arterial hypertension associated with connective tissue disease.

remained on monotherapy and 44 had moved to combination therapy. All four censored patients were on monotherapy at time of censoring.

3.2.6. All-randomized population—prognostic factors of survival

The univariate Cox analysis conducted in the all-randomized population, for which there were 35 deaths in this population, showed significant prognostic factors for a high risk of death were time since PAH diagnosis of up to 16 months, 6MWD ≤ 437 m, mixed venous oxygen saturation ≤68%, high levels of N-terminal pro hormone of brain natriuretic peptide (NT-proBNP) and a diagnosis of PAH-CTD (Table 3). With the exception of time since PAH diagnosis, all these factors were confirmed as significant in the multivariate analysis (Table 3).

4. Discussion

The exposure to bosentan in EARLY exceeds that of many other studies. As such, EARLY provides valuable information on the long-term safety, in particular liver safety, of bosentan in patients with PAH. The 4-year data from this study also underscore the fact that patients with WHO FC II PAH have a severe and often fatally progressive disease. PAH worsening occurred at a rate of approximately 5% annually and mortality, estimated at 15% over 4 years, remains substantial. An important finding of this study was the identification of *a priori* factors that portend a poor outcome in WHO FC II patients treated with monotherapy. This could be useful in identifying a high-risk incident

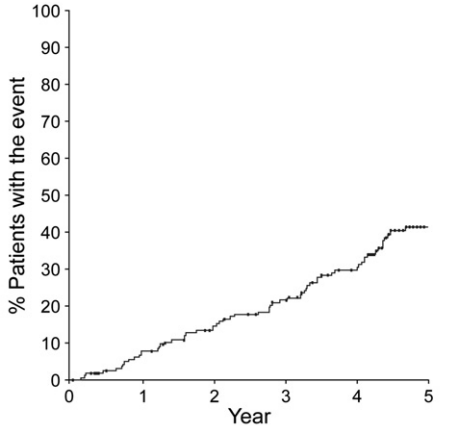


Fig. 5. Time to other drug initiation. Kaplan–Meier estimate of time to other pulmonary arterial hypertension-targeted therapy (phosphodiesterase type 5 inhibitors and oral/inhaled prostanoids) in the bosentan-treated population. The number of patients for whom phosphodiesterase type 5 inhibitors were added was 48 and the number of patients for whom oral/inhaled prostanoids were added was 13. CI = confidence intervals; NA = not applicable.

population that might benefit in particular from up-front combination therapy. Lastly, the use of standard efficacy measures in EARLY can also provide some indication of the symptomatic efficacy of bosentan over the long-term but these data need to be interpreted with caution.

4.1. Safety

Information on elevations in liver enzymes was of primary importance in the EARLY study given the long period of bosentan exposure in this study of up to 5 years. Thus we report all elevations of AST and/or ALT regardless of whether they led to bosentan discontinuation. The proportion of patients with AST and/or ALT elevations (irrespective of whether they led to bosentan discontinuation or not) in EARLY was 16.8% (crude rate) or 18.6% by Kaplan–Meier estimate. Given that most patients were treated for at least 4 years, this rate is not unexpected and is similar to that of open-label bosentan studies of up to 3 years exposure [9]. Consistent with these previous findings [9] most of the increases in AST and/or ALT associated with bosentan occurred during the first 6 months of treatment. The crude rate estimate at Month 6 of 11.6% and the Kaplan–Meier estimate of 12.0% are similar to the 6-month estimate of 11.3% reported in the Tracleer® (bosentan) Summary of Product Characteristics [10]. While it is more likely that later increases in liver enzymes are associated with the disease or other background factors or concomitant medications given as patients deteriorate, bosentan-related increases cannot be excluded from occurring later on during the course of therapy. These data highlight that monthly blood tests to monitor liver enzymes should be conducted the entire time a patient is treated with bosentan. Treatment discontinuation, dose reduction or continued treatment coupled with regular assessment should be employed in cases of liver enzyme abnormalities. As evidenced in this study, most cases of elevated liver enzymes are resolved after deployment of one of these strategies.

Of the 29 patients with elevated liver enzymes of >3 × ULN, 16 discontinued bosentan treatment. For 13 of these patients, the elevations were asymptomatic and recorded as either an AE of abnormal liver enzymes (in 12 patients) or specified as an AE of increased ALT and AST. Three patients had elevated aminotransferases with a clinical presentation of hepatitis, which was recorded as the AE leading to discontinuation. In two of these cases the investigator considered the hepatitis to be drug related, however as liver biopsy was not performed it is not possible to confirm if this was the case.

Seven patients discontinued bosentan due to worsening of their pulmonary hypertension, one in the double-blind phase of the study and six in the open-label phase. In the double-blind phase the protocol specified that patients who worsened should discontinue treatment. While in the open-label phase patients could continue bosentan treatment if another PAH-targeted therapy was added, combination therapy was not common clinical practice during some of the time the trial was being conducted.

Twenty-one patients died while taking bosentan, or within 28 days after cessation, and most deaths were due to PAH or conditions that would be expected in a population of chronically ill patients. In two cases death was linked by the treating physicians to bosentan (see above).

4.2. Estimates of PAH worsening and survival, and prognostic factors for survival

As data on PAH worsening and vital status were collected for all bosentan-treated patients, independently of treatment duration,

Table 3
Significant prognostic factors for a low and high risk of death in patients with WHO functional class II pulmonary arterial hypertension (univariate and multivariate analyses in the all-randomized population).

	Univariate analyses (n = 185)			Multivariate analysis (n = 142[a])		
Baseline parameter	Hazard ratio	95% CI	p	Hazard ratio	95% CI	p
Time since PAH diagnosis > 16 months (n = 183)	0.47	0.22–1.00	0.0493	–	–	–[b]
6MWD (>437 m) (n = 185)	0.44	0.22–0.90	0.0241	0.47	0.21–1.08	0.0739
Mixed venous oxygen saturation > 68% (n = 167)	0.26	0.11–0.61	0.0019	0.26	0.09–0.72	0.0093
NT-proBNP (10-fold increase)[c] (n = 158)	2.71	1.39–5.28	0.0034	2.28	0.99–5.26	0.0541
Etiology (n = 185)			0.0090[d]			
–CHD vs iPAH/hPAH-HIV	0.32	0.07–1.36	0.1234			
–CTD vs iPAH/hPAH-HIV	2.33	1.15–4.75	0.0192			
–CTD vs iPAH/hPAH–HIV–CHD	–	–	–	4.28	1.81–10.09	0.0009[e]

Variables included in the univariate analyses: Time from diagnosis (≤16 months, >16 months); randomization group at start of double-blind (placebo, bosentan); 6MWD (≤median, >median); NT-proBNP (log-transformed); cardiac index (≤median, >median); pulmonary vascular resistance (≤ median, > median); mixed venous oxygen saturation (≤median, >median); sex (male, female); age (≤median, >median); etiology (3 classes: iPAH/hPAH-HIV, CHD, CTD); mean blood pressure (from vital signs (≤median, >median); monotherapy/combination therapy (sildenafil) therapy at baseline.
CHD = congenital heart disease; CTD = connective tissue disease, HIV = human immunodeficiency virus; iPAH/hPAH = idiopathic/heritable pulmonary arterial hypertension; NT-proBNP = N-terminal pro hormone of brain natriuretic peptide; 6MWD = 6-minute walk distance.
[a] Multivariate analysis was conducted on patients without missing data for the variables retained (p-value < 0.1 in univariate analyses).
[b] Dropped from the multivariate analysis during backward selection.
[c] Risk of a patient with an NT-proBNP level 10-fold greater than another patient who otherwise has the same characteristics.
[d] Global test for influence of etiology.
[e] Since (CHD vs iPAH/hPAH-HIV) was not selected by univariate analyses, etiology was reduced to CTD vs iPAH/hPAH-HIV-CHD.

EARLY provides the first disease worsening and survival data for a WHO FC II PAH population. At 4 years, event-free survival and survival were approximately 80% and 85%. These data confirm that survival has improved in the era of PAH-targeted therapy *versus* historical data from the pre-drug era [11]. However, a death rate of 15% over 4 years in a mildly symptomatic PAH patient population still shows how further research into new treatments, more effective treatment strategies, and understanding the disease are still required.

Data collection on vital status for all randomized patients also enabled a Cox analysis to be conducted to identify factors that were predictive of death. We identified 6MWD of 437 m or less, a PAH diagnosis of 16 months or less, a mixed venous oxygen saturation of 68% or less, high NT-proBNP levels, and PAH-CTD as factors prognostic of a high risk of death in WHO FC II patients. Such information can alert physicians to pay particular attention to patients with risk factors and employ modified treatment strategies such as more regular assessment or more aggressive treatment including up-front combination therapy, although this is not currently supported by randomized clinical trial evidence.

Similar high risk factors have also been identified in other studies. In the Sildenafil Use in Pulmonary Hypertension (SUPER)-2 trial, Cox regression analysis showed that for patients who had a 6MWD of <325 m at baseline in SUPER-1, deterioration in 6MWD during the first 12 weeks of sildenafil treatment was associated with poor survival (hazard ratio 0.241). The association was weaker between change in 6MWD and survival in patients with a 6MWD ≥ 325 m at baseline [12]. High 6MWD (≥440 m) is also associated with a low score on The Registry to EValuate Early And Long-term pulmonary arterial hypertension disease management (REVEAL) risk score calculator [13]. Although time since diagnosis of more than 16 months was no longer predictive of better survival in the multivariate analysis, it should be noted that the multivariate analysis was underpowered compared with the univariate analysis since patients who had missing data for any the parameters included in the multivariate analysis were excluded.

B-type natriuretic peptide (BNP) and NT-proBNP are biomarkers of heart failure and high plasma levels have consistently been reported as predictors of mortality [14–16]. NT-proBNP levels > 1400 pg/mL and >1800 pg/mL, respectively, have been shown to be predictive of worse 3-year outcomes in patients with severe PAH [7,16] and prognostic analysis from REVEAL shows BNP levels > 180 pg/mL are significantly associated with death [14]. High BNP levels are part of the risk score calculator from REVEAL [13].

That the Cox regression analysis identified PAH-CTD as a high risk is congruent with the Kaplan–Meier survival analysis by etiology in this study and in other studies, such as the French PAH Registry [17], which showed PAH-CTD patients to have poorer survival than those with iPAH or PAH-CHD. REVEAL confirms a significantly lower survival rate at 1 year in PAH-CTD vs iPAH, 86% vs 93%; $p < 0.0001$ [18].

4.3. Exploratory efficacy outcomes of bosentan treatment

Over the course of the bosentan treatment period (median 51.4 months) exercise capacity was largely maintained in the majority of patients. However, care must be taken when interpreting the 6WMD and Borg dyspnea index data as the changes are a population mean and thus include data from responders and non-responders as well as patients on combination therapy or bosentan monotherapy. Other PAH-targeted therapies, namely phosphodiesterase type 5 inhibitors and oral or inhaled prostanoids, were initiated gradually over time and, after 4 years, 30% of patients were receiving combination therapy. The observed less than expected number of patients on combination therapy is a reflection of the EARLY protocol, which allowed for new drugs only to be added if patients deteriorated. Treating and maintaining patients in WHO FC II with monotherapy, if possible, and combination therapy where required, is recommended as a key treatment goal [19]. In this study, 78% of the patients remained in WHO FC II or improved to WHO FC I during a 4-year observation period.

5. Study limitations and strengths

One of the biggest limitations of this study is a lack of comparator. Ethical considerations prohibited the inclusion of a long-term placebo arm. A second limitation is the fact that some patients received additional therapy, either at baseline or during follow-up, and so the data are not from a monotherapy study. Moreover, as few patients ($n = 29$) were on sildenafil at baseline it is not possible to draw conclusions with regards to combination therapy. A further limitation of this study was that PAH worsening events were not adjudicated and only included endpoints that could reliably and realistically be collected over such a long-term study, eg initiation of iv or sc prostanoids and death. Therefore, data on disease worsening cannot be directly compared with similar data from other studies which may have included disease progression measures such as deterioration in 6MWD.

This data set is the only comprehensive one to report long-term results, with the majority of patients being treated for at least 4 years, in a primarily mildly symptomatic PAH population (97.5% in WHO FC I/II at baseline). Despite some larger OLE studies having a relatively high proportion of patients in WHO FC I/II, [12,20] in-depth analyses of this patient sub-population examining factors such as survival and clinical worsening have not been published. Patients were followed up for the entire duration of the study, irrespective of whether they remained on bosentan for events related to PAH worsening. Thus the analysis of survival and PAH worsening was akin to the intent-to-treat principle, providing robust data for these long-term results. Data from the EARLY study provide relevant clinical information on a WHO FC II PAH patient population treated with an advanced PAH therapy with regards to disease progression, patient survival, and factors associated with survival.

6. Conclusion

The tolerability and safety of an extended period of bosentan exposure in this patient population was in line with its known safety profile. PAH patients in WHO FC II had a 4-year survival rate of approximately 85%. A low 6MWD, a low mixed venous oxygen saturation, a high NT-proBNP level, and PAH-CTD were associated with a greater risk of death.

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Conflict of interest

Gérald Simonneau has served as a consultant for Actelion Pharmaceuticals Ltd, GlaxoSmithKline, Eli Lilly & Co, Novartis, Pfizer and United Therapeutics and has received speaker fees from Actelion Pharmaceuticals Ltd, GlaxoSmithKline & Co, Eli Lilly and Pfizer.

Nazzareno Galiè has acted as a consultant for Actelion Pharmaceuticals Ltd, Bayer HealthCare, Eli Lilly & Co GlaxoSmithKline and Pfizer.

Pavel Jansa has acted as a consultant for Actelion, Pharmaceuticals Ltd, AOP Orphan and United Therapeutics and lecture fees from Actelion Pharmaceuticals Ltd, Bayer HealthCare, and GlaxoSmithKline.

Gisela Martina Bohns Meyer has received fees for lectures and/or consultations from Bayer HealthCare, and Eli Lilly & Co and GlaxoSmithKline.

Hikmet Al-Hiti reports no conflict of interests.

Andjela Kusic-Pajic is an employee of Actelion Pharmaceuticals Ltd.

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Appendix A. Supplementary data

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