

MEDICAL REVIEW OF EFFICACY

NDA#21,290

Drug Name: Bosentan (Tracleer™)

Sponsor: Acetlion

Date: June 29, 2001

Medical Reviewer: Maryann Gordon, M.D.

Summary of efficacy

Bosentan has been evaluated for efficacy in pulmonary arterial hypertension (PAH) in 3 studies, 2 with the oral formulation evaluating walking distance and 1 with the iv formulation evaluating acute hemodynamics. The latter trial was stopped prematurely for safety reasons and is included in the efficacy review only for completeness.

Study no.	Design/duration	Primary efficacy parameter	No. planned/completed	doses	comments
AC 052-352	Double blind, randomized, placebo controlled for 16 weeks	6 min walk test	150/214	Oral 62.5 mg bid up titrated to 125 mg bid; 62.5 mg bid up titrated to 250 mg bid	Significant increase in walking distance compared to placebo.
AC 052-351	Double blind, randomized, placebo controlled for 12 weeks	6 min walk test	30/32	Oral 62.5 mg bid up titrated to 125 mg bid at week 4	Significant increase in walking distance compared to placebo.
BD14884	Open label iv dosing 1 day followed by oral dosing	Acute cardiac hemodynamics	30/7	iv: 50 mg, 150 mg, 300 mg. Oral: 1000 mg bid	Stopped for safety reasons

Demonstration of efficacy of bosentan

The walking distance was significantly increased in the bosentan groups compared to placebo in both study AC 052 352 (352) and study AC 052 351 (351). Compared to placebo, bosentan also delayed time clinical worsening, improved Borg dyspnea index, improved WHO functional class, and lessened the increase in therapy for PAH.

There was no evidence of loss of efficacy of bosentan up to 16 weeks of placebo controlled evaluation.

Study details

The studies were double blind, multicenter, parallel groups, placebo controlled. Bosentan doses used were 125 mg bid (both studies), 250 mg bid (352 only). Both studies included a starting dose of 62.5 mg bid for 4 weeks. Minimum duration on study drug was 12 weeks. Treatment could continue beyond the fixed efficacy timepoint¹.

Patient type

Patients were those:

- with PAH resulting from primary pulmonary hypertension (PPH) or connective tissue or autoimmune disease such as scleroderma or systemic lupus erythematosus;
- WHO functional class III-IV despite optimal therapy with vasodilators, cardiac glycosides, diuretics, and /or supplemental oxygen.
- receiving anticoagulants but not receiving prostacyclin therapy or scheduled to receive Flolan.
- walk between 150 m and 500 m, inclusive, on a 6-minute walk test. 352 reduced the maximum walk distance to 450 m.
- did not have ALT/AST greater than 3 times upper limit of normal or hemoglobin/hematocrit <30% below normal range.

Primary efficacy endpoint

The primary efficacy endpoint for both studies was change from baseline at endpoint in total walk distance (meters). The studies predefined how data would be handled for those patients who died or did not complete the study for other reasons.

Secondary endpoints

The secondary efficacy endpoints included time to clinical worsening, changes in Borg dyspnea index, changes in WHO functional class, and increase in therapy for PAH, number of days patient was known to be alive and out of hospital during first 28 weeks (study 352), and number of drop outs in the first 28 weeks.

¹ patients in study 352 who were recruited by 30 September 2000 were scheduled to be treated for up to an additional 12 weeks (Period 2). The study was completed when the last included patient completed Period 1. All patients had the opportunity to enter an open-label extension study after completion of this trial, if eligible. Patients recruited after 30 September 2000 could have entered the open-label extension upon completion of Period 1. Patients who were recruited up to and including on 30 September 2000 could have entered the open-label extension after completion of Period 2 or at any time during Period 2 if they met the predefined stopping criteria. Patients who were withdrawn during Period 1 were not eligible for the open-label extension trial.

Patients in study 351 who completed the 12 week double blind treatment phase as well as subjects who dropped out of the study prematurely had the option of entering an open label, uncontrolled phase.

Patient disposition

No. of patients

	Study 352			Study 351	
	Bos 125 mg bid	Bos 250 mg bid	Placebo bid	Bos 125 mg bid	Placebo bid
No. randomized	76	70	69	21	11
No. prematurely withdrawn	3	3	6	0	3

Reasons for premature withdrawal

No. of patients

	Study 352			Study 351	
	Bos 125 mg bid	Bos 250 mg bid	Placebo bid	Bos 125 mg bid	Placebo bid
No. prematurely withdrawn	3	3	6	0	3
Worsening of condition	2	1	3	0	1
Death	1	0	2	0	0
AE/ intercurrent illness	0	1	0	0	2
Increased LFTs	0	1	0	0	0
Lack of clinical/walk test improvement	0	0	1	0	0

Demographics

	Study 352			Study 351	
	Bos 125 mg bid	Bos 250 mg bid	Placebo bid	Bos 125 mg bid	Placebo bid
No. randomized	76	70	69	21	11
No. female	57	57	54	17	11
No. male	17	13	15	4	0
Mean age (yrs)	50.4	47.0	47.2	52.2	47.4
No. white	57	54	59	16	9
Mean time from diagnosis to randomization	898	893	843	634	1091

The most common concomitant medications in both studies were antithrombotic agents, diuretics, ca channel blocker, and cardiac glycosides.

6 minute walk test

Mean distances (m)

	Study 352			Study 351	
	Bos 125 mg bid N=74	Bos 250 mg bid N=70	Placebo bid N=69	Bos 125 mg bid N=21	Placebo bid N=11
Baseline	326.3	333.0	344.3	360.5	355.5
Endpoint [^]	353.1	379.5	336.5	430.5	349.6
Change from baseline	26.8	46.5	-7.8	70.1	-5.0
Placebo subtracted effect	34.6	54.3	-	75.9*	-

[^] week 16 for study 352, week 12 for study 351

*p=0.0205 using t-test

***p=0.0002 using Mann-Whitney U test

Dose effect

Only study 352 used more than 1 dose of bosentan. The walking distance by dose is shown in the figure below

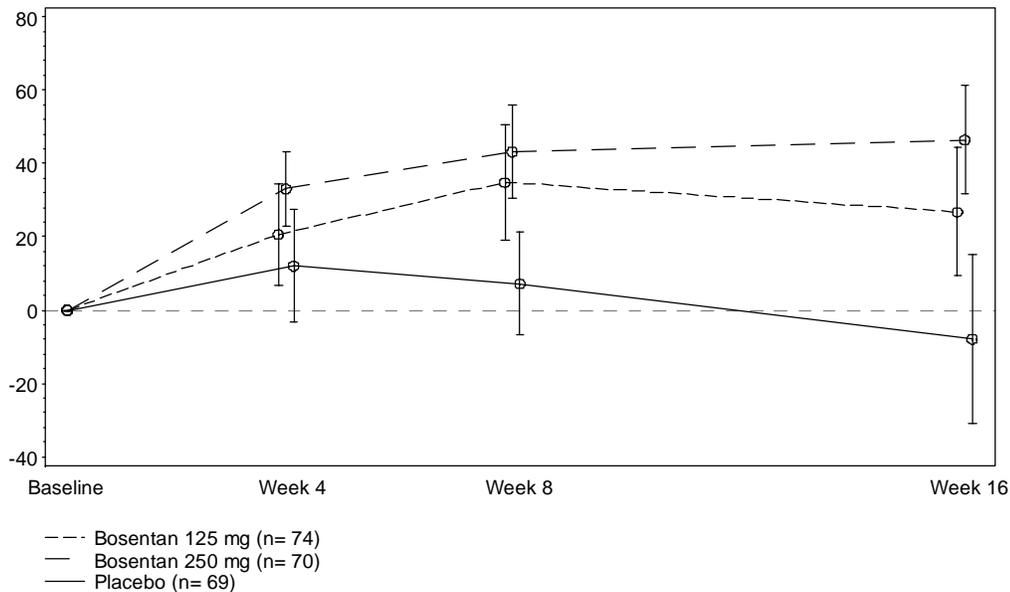
Walk distance: Change from baseline over time during Period 1 by dose, ITT population

Ro 47-0203, Protocol: AC-052-352

FIGURE 2a: Absolute change from baseline (mean +/- 95% CL)

Population: ITT

TOTAL WALK DISTANCE (m)



Note: All bosentan patients received 62.5 m bid during the first 4 weeks of the study and then were up-titrated to the target dose (125 mg bid or 250 mg bid).

The 250 mg bosentan group was numerically superior to the 125 mg group at weeks 8 and 16 but the confidence limits overlapped at both time points.

Walk distance by visit

The changes from baseline at weeks 4, 8 and 16 for the combined bosentan group and placebo in walk distance in study 352 and weeks 4, 6 and 12 for in study 351 are shown in the figures below.

**Walk distance: Change from baseline over time during Period 1,
ITT population**

Ro 47-0203, Protocol: AC-052-352
FIGURE 2b: Absolute change from baseline (mean +/- 95% CL)
Population: ITT

TOTAL WALK DISTANCE (m)

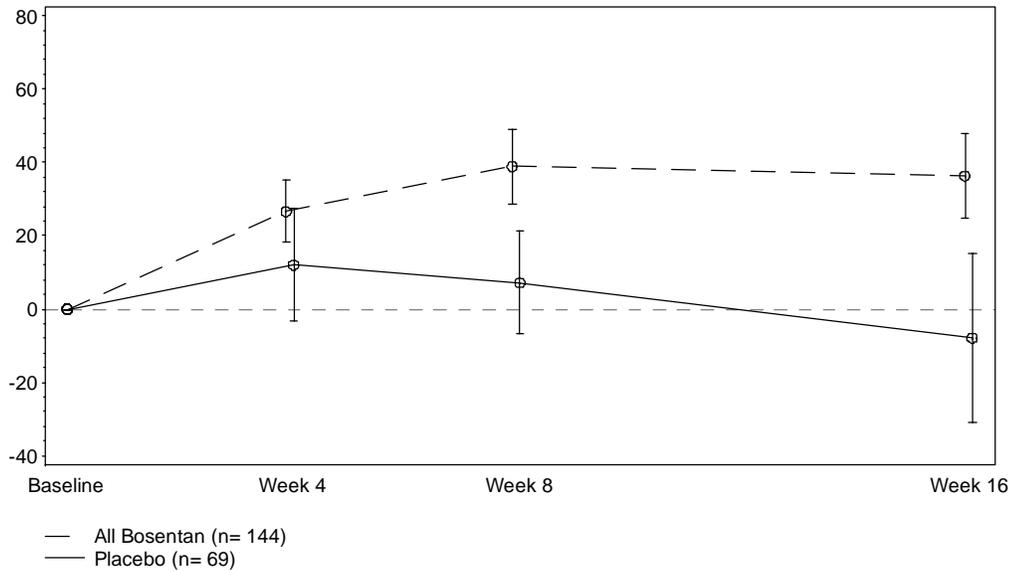
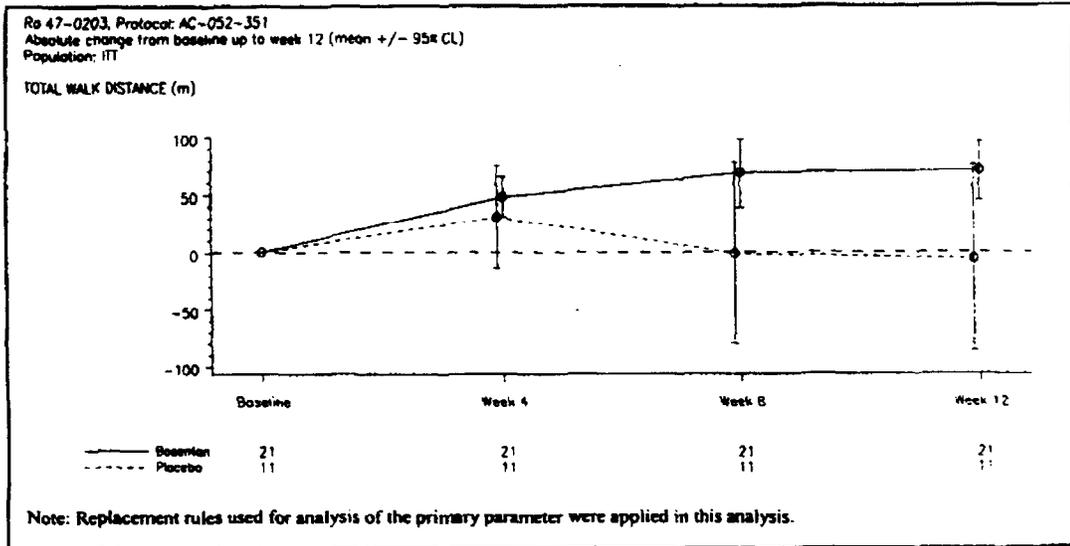


Figure 3 Walk Distance: Change from Baseline to Week 12



There is no evidence that the effect of bosentan on walk distance dissipates over 12-16 weeks of treatment.

Subgroups

Effect of bosentan by subgroups was only examined in study 352. Bosentan increase walk distance, compared to placebo, regardless of gender, age, weight, location, race, time from diagnosis, etiology (PPH vs. scleroderma), and baseline walk test. Patients who entered the study with lower mean PAP (< 50 mmHg) and/or higher cardiac index (≥ 2.3 l/min/m²) had a smaller improvement with bosentan than did those who entered with a higher mean PAP and/or lower cardiac index.

Secondary endpoints

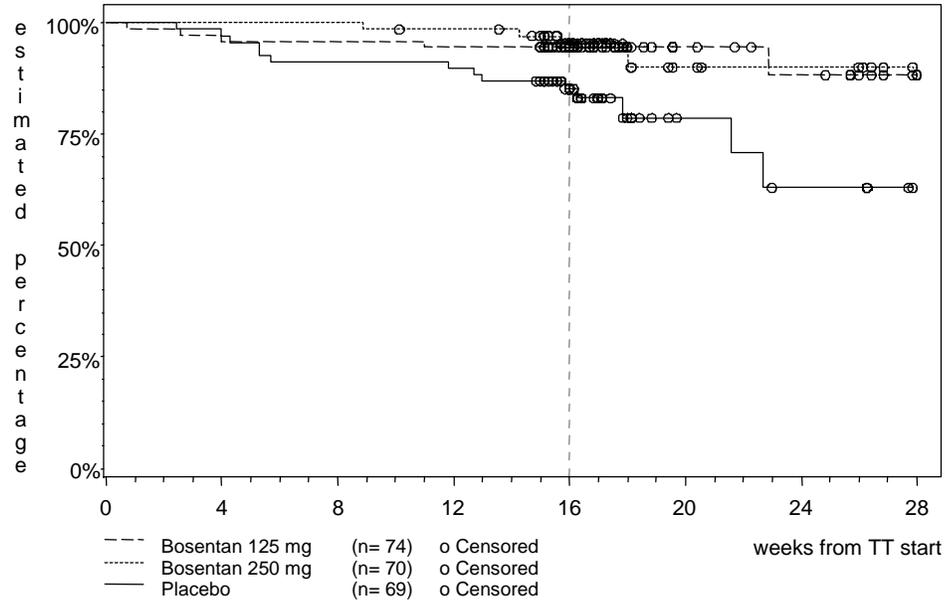
Time to clinical worsening defined as the shortest time to death, lung transplantation, hospitalization or discontinuation due to worsening pulmonary arterial hypertension, start of prostacyclin therapy or septostomy

The figure below shows the results for 352, by dose of bosentan and placebo.

Time from randomization to clinical worsening by dose, ITT population

Ro 47-0203, Protocol: AC-052-352
 FIGURE 3a Time from randomization to clinical worsening up to 28 weeks
 (Kaplan Meier estimates)

Population: ITT



There was an earlier time to deterioration by patients on placebo compared to those on either dose of bosentan.

There were 3 placebo and 0 bosentan patients who experienced clinical worsening in study 351.

Changes in Borg dyspnea index

Borg scale measures the levels of perceived exertion and the scale ranges from 0 (nothing at all) to 10 (maximal).

Means

	Study 352			Study 351	
	Bos 125 mg bid N=74	Bos 250 mg bin=70	Placebo bid N=69	Bos 125 mg bid N=21	Placebo bid N=11
Baseline	3.3	3.8	3.8	4.38	4.18
Endpoint [^]	3.3	3.3	4.2	4.19	5.55
Change from baseline	-0.1	-0.6	+0.3	-0.19	+1.36
Placebo subtracted effect	-0.4	-0.9	-	-1.55	-

[^] week 16 for study 352, week 12 for study 351

At baseline patients in study 351 had a worse perception of their exertion compared to the patients in study 352. However, the bosentan patients in both studies had an improvement as measured by the scale compared to the placebo patients. The overall treatment effect was greater in the 351 study, which may mean that sicker patients have a greater perception of improvement with bosentan. This would have to be confirmed by additional studies.

Changes in WHO functional class

Only patients WHO functional classes III or IV at baseline were enrolled into the efficacy studies.

No. and (percent) of patients who improved

	Study 352			Study 351	
	Bos 125 mg bid N=74	Bos 250 mg bid=70	Placebo bid N=69	Bos 125 mg bid N=21	Placebo bid N=11
Improved from baseline	32 (43.2)	29 (41.1)	21 (30.4)	9 (42.9)	1 (9.1)

^ week 16 for study 352, week 12 for study 351

More bosentan patients improved in their functional class compared to placebo patients.

Increased therapy for PAH

The evaluation of the incidence of increased therapy for PAH was only evaluated in study 352. The results are shown below.

Table 1 Incidence of increased therapy for pulmonary arterial hypertension, ITT population

(Table T14 / 04MAY01)

	Bosentan 125 mg N=74	Bosentan 250 mg N=70	All Bosentan N=144	Placebo N=69
Period 1 (weeks 0 to 16)				
n	74	70	144	69
Patients with increase [n(%)]	18 (24.3%)	14 (20.0%)	32 (22.2%)	20 (29.0%)
95% confidence limits (%)	15.1 , 35.7	11.4 , 31.3	15.7 , 29.9	18.7 , 41.2
Treatment effect*				
Difference	-4.7%	-9.0%	-6.8%	
95% confidence limits (%)	-20.0 , 10.6	-24.3 , 5.9	-19.5 , 7.1	
Period 1+2 (weeks 0 to 28)†				
n	19	16	35	13
Patients with increase [n(%)]	6 (31.6%)	5 (31.3%)	11 (31.4%)	5 (38.5%)
95% confidence limits (%)	12.6 , 56.6	11.0 , 58.7	16.9 , 49.3	13.9 , 68.4
Treatment effect				
Difference	-6.9%	-7.2%	-7.0%	
95% confidence limits (%)	-41.0 , 27.5	-43.1 , 27.7	-36.5 , 25.4	

† Analyzed only in those patients who were scheduled to continue to period 2.

Compared to placebo, fewer bosentan patients had an increase in therapy for PAH and this remained true for the few patients who had up to 28 weeks of treatment.

Protocol AC 052-352

Because this study was submitted at end of the NDA review, its safety is discussed fully in this section. The safety from this trial was only partially integrated with the overall safety medical review.

1.0 Introduction

The protocol was dated March 28, 2000. The first patient's first visit was July 2000 and the last patient's last visit was October 2000.

1.1 Study Design

This was a double blind, multicenter, parallel, placebo controlled, randomized study in patients with pulmonary arterial hypertension (PAH). The study was conducted in Europe, North America, Israel, and Australia. Eligible subjects were those with either symptomatic severe primary pulmonary hypertension (PPH) or pulmonary hypertension secondary to scleroderma (SSc/PH), who were ambulatory and in functional class III-IV (1998 WHO classification).

1.2 Study Objective

The objective of this study was to evaluate the efficacy of 2 doses of bosentan compared to placebo on peak exercise capacity (6 minute walk test).

1.3. Patient Type

1.3.1. Inclusion Criteria

Eligible patients had to meet all of the following inclusion criteria at the initial screening visit of the study (visit 1) and at baseline for the 6-minute walk test (visit 1 and visit 2)

1. Male or female aged 12 years or more. Amendment #2 UK only: patients had to be ≥ 18 years of age.
2. Patients must have provided written informed consent to participate in the study. For a patient under the age of consent, written informed consent must be obtained from the patient's legal guardian.
3. PAH resulting from either:
 - primary pulmonary hypertension (PPH) or
 - Connective tissue or autoimmune diseases such as scleroderma (SSc/PH) or systemic lupus erythematosus (SLE).

In patients with SSc/PH and interstitial lung disease, total lung capacity (TLC) was to be $>70\%$. However, if TLC was between 60-70%, a high resolution CT scan should have been used to confirm the mild nature of disease with a total CT score ≤ 2 . The diffusion lung capacity for carbon monoxide (DLCO) could be used to aid in the diagnosis. The CT scan was repeated at the end of the study.

4. PAH of WHO functional class III-IV despite optimal therapy with oral vasodilators, cardiac glycosides, diuretics and/or supplemental oxygen for at least one month. Patients had to be receiving oral anticoagulants unless there was a contraindication.
5. Hemodynamic documentation of PAH within 2 months prior to screening, i.e. a patient who has undergone invasive hemodynamics in the month preceding screening with the following results:
 - Mean pulmonary arterial pressure (PAP) > 25 mmHg at rest

- Pulmonary capillary wedge pressure (PCWP) < 15 mmHg
- Pulmonary vascular resistance (PVR) > 3 mmHg/l/min.

If the capillary wedge pressure was not measured, an echocardiogram was acceptable to rule out left ventricular dysfunction.

6. Baseline 6-min walk test of ≥ 150 m and ≤ 500 m (Amendment #1 reduced this to ≤ 450 m). The difference between the screening and randomization walk tests had to be $\leq 15\%$. If the two tests differed by more than 15%, the patient underwent an additional walk test at least 1 day later, the results of which had to be within 15% of the previous walk test. The definition of baseline as the average of the last 2 test results was added later (but not as an amendment).

7. women who were not pregnant and women who were postmenopausal, surgically sterile or using an acceptable method of contraception.

Exclusion Criteria

Eligible patients could not have any of the following exclusion criteria at the initial screening visit (visit 1) or at baseline (visit 1 or visit 2):

1. PAH of WHO functional class I or II.
2. PAH due to conditions other than PPH or connective tissue diseases, e.g.:
 - congenital heart diseases (Eisenmenger's syndrome)
 - pulmonary venous hypertension (e.g. left sided heart diseases)
 - associated with disorders of the respiratory system (e.g. chronic obstructive pulmonary disease (COPD), sleep apnea, and patients with moderate to severe interstitial disease even of SSc origin)
 - associated with chronic thrombotic or embolic diseases
 - condition of inflammatory origin (HIV, schistosomiasis, sarcoidosis)
 - portal pulmonary hypertension.
3. SSc/PH with moderate to severe interstitial disease or with TLC <60% or a CT scan total score (CT.Tot score) >2.
4. Patients who stopped treatment with oxygen, diuretics, oral vasodilators, cardiac glycosides within one month of screening.
5. Patients who started new treatment with oxygen, diuretics, oral vasodilators, cardiac glycosides within one month of screening.
6. Patients receiving prostacyclin therapy within 3 months of study screening. However patients who received acute prostacyclin at the time of a catheterization procedure to test pulmonary vascular reactivity could be included.
7. Patients scheduled to receive prostacyclin therapy.
8. Musculoskeletal or rheumatic disorders or any other condition that could limit his/her ability to perform the 6-minute walk tests.
9. Hypotension defined as systolic blood pressure < 85 mmHg.
10. Hemoglobin or hematocrit of less than 30% below the normal range (patients with secondary polycythemia were allowed).

11. AST and/or ALT values greater than 3 times the upper limit of normal.
12. Patients receiving cyclosporin-A, glibenclamide (glyburide), troglitazone, encainide, flecainide, disopyramide, propafenone, moricizine, pinacidil, minoxidil or oral positive inotropic agents other than digitalis at inclusion into the study or were expected to receive any of these drugs during the study period.
13. Patients who received therapy with an investigational drug in the month preceding screening.
14. Known drug or alcohol dependence or any other factors which could have interfered with conduct of the study or interpretation of the results.
15. Any illness other than PAH which might reduce life expectancy to less than 6 months.

1.4 Sample Size

Sample size, 80 patients for each of the 3 treatment arms, was based on an assumed change from baseline in walking distance of 45 m with standard deviation of 75 m.

1.5 Dose and duration

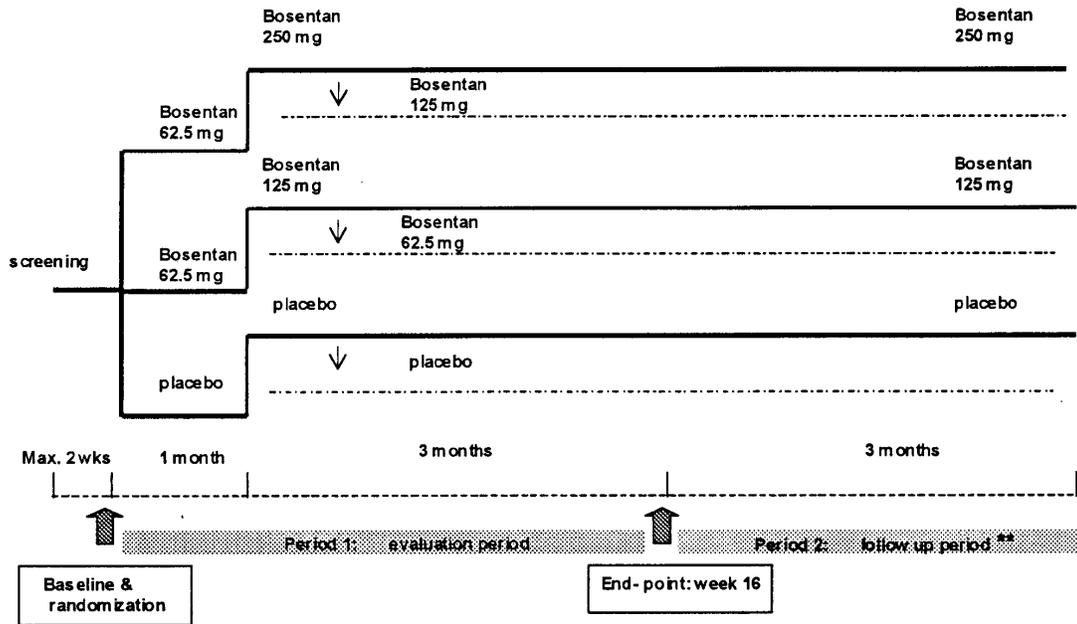
The 3 treatment arms were 1.) placebo bid for 16 weeks, 2.) bosentan 125 mg bid for 4 weeks titrated to 250 mg bid for 12 weeks, and 3.) bosentan 62.5 mg bid for 4 weeks titrated to 125 mg bid for 12 weeks. Down titrating to the starting dose was allowed if patient could not tolerate the higher dose. Amendment#1: patients with body weight < 40 kg were given half the randomized target dose. If patients had to be discontinued, they were weaned for 3-7 days.

Patients were instructed to take study drug at the time of or within 30 minutes after food intake.

Each subject received a minimum of 16 weeks of double blind treatment² and this period was used to determine efficacy. Patients recruited by 9-30-2000 took part in period 2 as well which was an additional 12 week treatment with double blind medication. This period was reviewed for efficacy but was not part of primary efficacy endpoint.

² meaning that all subjects received double blind treatment until the last enrolled subject, not prematurely withdrawn, completed week 16

All doses in the chart below are BD



Double blind period 1 and period 2/placebo controlled trial/ n= 80 patients per treatment group
 Primary variable: change from baseline in a 6 min walk test

** the follow up period is maximum 3 months for the first half of the patients

1.7 Study Procedure

The flow chart below outlines study procedures at each clinic visit.

Table 1: Schedule of Assessments

Treatment Week [Study day]	Period 1							Period 2		
	Screening [-14,-1]	Random- ization a	1 [5-9] f	4 [21-35]	8 [49-63]	12 [77-91]	16 [105-119] end of period 1	Premature withdrawal	22 [147-161]	28 [189-203] end of study
Visit	1	2	3	4	5	6	7	-	8	9
Informed consent	X									
History	X									
Physical examination	X						X	X		X
Vital signs (blood pressure and heart rate)	X	X		X	X	X	X	X	X	X
Body weight	X	X					X	X		X
ECG (12-lead)	X						X	X		X
Laboratory tests	X ^b			X	X (only LFT)	X (only LFT)	X	X	X (only LFT)	X
Right heart cath.		X ^c								
High resolution CT scan ^d	X									X
Functional class	X	X	X	X	X	X	X	X	X	X
Exercise: 6-minute walk test. Borg dyspnea index	X ^e	X ^e		X	X		X	X		X
Dispense/Return study medication		X		X	X	X	X	X	X	X
Forced up-titration of study medication				X						
Optional down-titration of study medication					X	X	X		X	
Concomitant medications	X	X	X	X	X	X	X	X	X	X
Adverse events		X	X	X	X	X	X	X	X	X

- a: Visits 1 and 2 may be performed on the same day (see section 4.2/screening (Visit 1))
- b: Includes a pregnancy test performed locally in women of childbearing potential; LFT: liver function tests
- c: Invasive hemodynamics not requested if PHT hemodynamically documented within 2 months before screening, additional follow up catheterizations are optional
- d: To be performed in patients with SSC and interstitial lung disease, see Appendix 1
- e: Pulse oximetry to be performed before walk test
- f: Visit 3 can be performed by phone

1.8 Protocol define study hypotheses and efficacy endpoints:

Primary endpoint of the study: the change from baseline (defined as the average of screening and randomization walk tests³) in exercise capacity at 16 weeks (6-minute walk test).

- Patients who died, underwent lung transplantation during the study, or discontinued study medication because of worsening of their PAH condition were analyzed using their last assessment if it was recorded at the time of premature withdrawal. If no assessment of walk distance was obtained at the time of premature withdrawal, these patients were assigned a walk distance of 0 meters at the 16-week time point.
- All other patients including those with serious adverse events not related to PAH worsening or lost to follow up without a week 16 assessment of the primary endpoint had their last assessment carried forward. If no assessment was obtained during the treatment period, the patient was assigned “no change” (zero change from baseline, equivalent to carrying forward the baseline value).

7.2 Secondary Endpoints⁴

1. Time from randomization to clinical worsening, defined as death from all causes, lung transplantation or discontinuation of therapy due to clinical deterioration due to PAH or need for prostacyclin or septostomy. Patients without documentation of any of the events listed above were

³ This definition was added to the study report. It was not in the protocol.

⁴ As defined by protocol

included in the analysis as censored observations from randomization to the last date the patient was known to be free of any of these events.

2. Changes from baseline in dyspnea index (Borg dyspnea index). Patients prematurely withdrawn from therapy and having had a “premature withdrawal” assessment will be included in the analysis using the last available assessment on treatment. Patients without any assessment during the treatment period were assigned worst rank (10) if the reason for premature withdrawal is death, lung transplantation or worsening of the patient’s PAH condition. Patients without any assessment during the treatment period for reasons other than these were excluded from the analysis.

3. Changes from baseline in WHO functional class. Patients prematurely withdrawn from therapy and having had a “premature withdrawal” assessment were included in the analysis using the last available assessment on treatment. Patients without any assessment during the treatment period were assigned worst rank if the reason for premature withdrawal is death, lung transplantation or worsening of the patient’s PAH condition. Patients without any assessment during the treatment period for reasons different than these were excluded from the analysis.

4. Increase in any therapy for PAH. Patients with missing data were analyzed as in item 3 above.

5. Number of days the patient is known to be alive and out of hospital in the first 28 weeks.

6. Number of dropouts in the first 28 weeks.

1.9 Disallowed concomitant medications

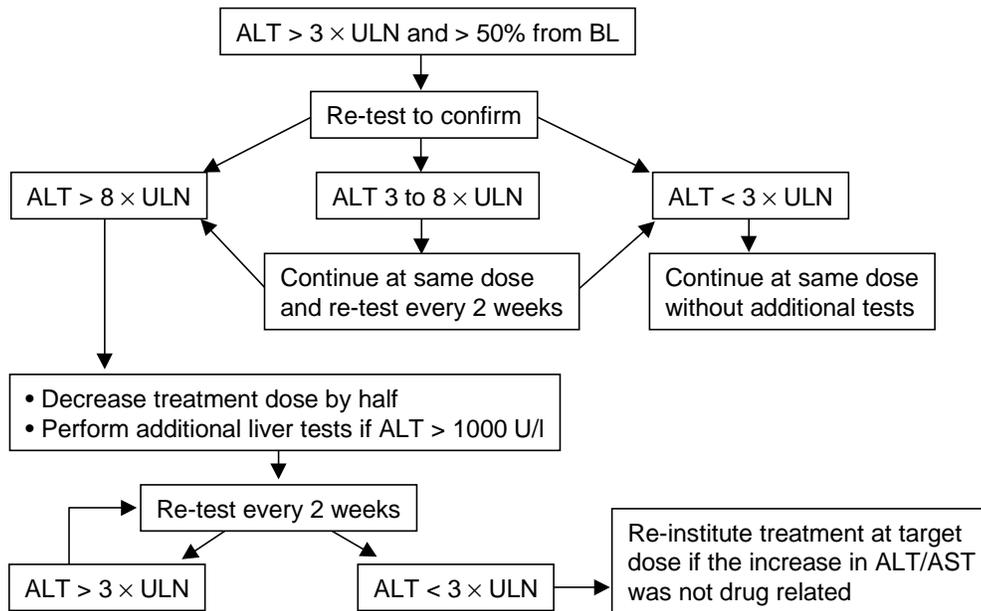
Prostacyclin and any investigational agent other than study drug intended for the treatment of PAH, cyclosporin-A, glibenclamide (glyburide), troglitazone, encainide, flecainide, disopyramide, propafenone, moricizine, pinacidil, minoxidil or oral positive inotropic agents other than digitalis.

1.10 Abnormal laboratory values

a) Liver function test (LFT)

Patients found to have abnormal LFTs during the study were treated according to the following outline.

Figure 1 Guidelines for treatment of asymptomatic increases in liver enzymes



b) Hemoglobin

- If hemoglobin decreased by at least 15% from baseline and was < 10 g/dl, and/or if hematocrit decreased by at least 15% from baseline and was < 0.30, a repeat test was performed to confirm the abnormality.
- If the abnormality was confirmed, the investigator was required to perform the following assessments using the local laboratory:
 - Complete blood count including reticulocytes, mean corpuscular volume, mean corpuscular hemoglobin concentration, mean corpuscular hemoglobin, red cell distribution width, and direct inspection of blood smears
 - Bilirubin, direct and indirect
 - Serum iron, transferrin, iron-binding capacity, and serum ferritin

1.11 Major protocol amendments

Amendment 1, May 30, 2000: the hypothesis and analyses were simplified by changing from comparisons between each bosentan arm and placebo to a single comparison between pooled bosentan arms and the placebo arm. The change in analysis allowed a reduction in the number of patients required from 240 to 150 (50 per group) without affecting the power calculation and a consequent reduction in the number of centers from 40 to 30. The Mann-Whitney U-test replaced the Student's t-test as a more appropriate test with the non-normal data distribution due to zero introduced as a substitutive value. The dose-response relationship would only be analyzed descriptively. Primary and secondary parameters were to be analyzed descriptively in four groups: the bosentan 125-mg arm, the bosentan 250-mg arm, the pool of both bosentan arms, and the placebo arm.

The upper limit of the 6-minute walk test that determined eligibility was changed from < 500 m to ≤ 450 m. To avoid potential overdosing, patients with body weights of ≤ 40 kg were to be given half of the randomized target dose (i.e., 62.5 mg bid or 125 mg bid).

Amendment 2, June 30, 2000. This amendment applied only to centers in the UK. Local Institutional Review Boards (IRBs) for centers in the UK requested that only patients ≥ 18 years of age be included in the trial.

Amendment 3, July 19, 2000. This amendment applied only to centers in Austria. Local IRBs in Austria requested that only patients > 19 years of age be included in the trial and that pregnancy tests be performed every 4 weeks in all women of childbearing potential.

Amendment 4, August 22, 2000. This amendment introduced an echo/Doppler substudy that was to be conducted at 12 of the study centers (world wide) in a subset of study patients (29 in each treatment group) who were recruited into the substudy. Echo/Doppler imaging techniques were to be used to evaluate hemodynamic changes and alterations in right heart structure and function during the trial.

Amendment 5, September 4, 2000. This amendment applied only to centers in the UK. Local IRBs for centers in the UK requested that only patients over the age of 50 years and amenorrheic for at least 1 year be considered naturally sterile with regard to inclusion criteria. In addition, medication labels had been made for patients with body weight > 40 kg and could not be changed before the end of recruitment in the UK. Therefore, an inclusion criterion was added requiring patients in the UK to have a body weight of > 40 kg.

Amendment 6, 6 October 2000. Due to the delay in recruitment and in order to complete the study within the planned timelines, a recruitment date of September 30, 2000 was set as the cut-off for patients scheduled to continue randomized treatment past the first 16 weeks (i.e., participate in Period 2). Patients enrolling after September 30, 2000 were to participate in 16 weeks of randomized treatment only (Period 1), and the trial was to end when the last entered patient completed Period 1.

Some clarifications of study procedures were added and some changes were instituted to enhance patient safety (an inclusion criterion was changed so that hormone-based contraceptives alone were not acceptable forms of contraception; this change addressed concerns that an induction of CYP3A4 by bosentan would lead to a higher rate of metabolism of these compounds and possibly result in a loss of contraceptive efficacy; a pregnancy test at the end-of-study/premature-discontinuation visit in women of childbearing potential was added as a precautionary measure; and the procedures for use of weaning treatment upon temporary or permanent discontinuation of study medication were clarified for patients not entering the extension study; and study treatment was to be re-instituted within 15 days of a temporary discontinuation.

The management of patients with an increase in serum transaminases was changed at the request of the Data Safety and Monitoring Board. The safety committee felt a reduction in the dose rather than temporary discontinuation of study medication would be more appropriate for asymptomatic patients with a confirmed transaminase concentration > 8 times the upper limit of normal. Following dose reduction or discontinuation, the patient could be re-challenged with study medication at the target dose (rather than half the target dose) provided that liver enzymes had returned to baseline values at two consecutive tests, and there was clear evidence that the reason for the increase was not related to study drug.

2.0 Results

A total of 258 patients were screened and 214 were enrolled into double blind treatment phase. There were 27 sites in Europe, North America, Israel, and Australia.

2.1 Patient disposition

Table below shows the outcome for all randomized subjects by treatment group.

	Bosentan 125 mg bid	Bosentan 250 mg bid	Placebo
No. randomized	76	70	69
No. who received study medication	75 [^]	70	69
Included in safety and efficacy evaluations	74	70	69
No. prematurely withdrawn	3	3	6

[^]1 patient (206 20604) was randomized but did not receive study medication because he had an exclusion criterion (Eisenmenger's syndrome) and difficulty in keeping appointments because he lived in a remote location)

Patients who discontinued prematurely from the trial are shown below.

Appendix 1 Summary of premature discontinuations during Period 1, safety population

Ro 47-0203, Protocol: AC-052-352
 Table T02a: Summary of premature discontinuations in period 1
 Population: Safety

Produced by maddest on 04MAY01

Reason for premature discontinuation	Bosentan 125 mg N=74		Bosentan 250 mg N=70		All Bosentan N=144		Placebo N=69	
	No.	%	No.	%	No.	%	No.	%
Total pts with at least one reason	3	4.1%	3	4.3%	6	4.2%	6	8.7%
WORSENING OF PATIENT CONDITION	2	2.7%	1	1.4%	3	2.1%	3	4.3%
DEATH	1	1.4%	-	-	1	0.7%	2	2.9%
AE/INTERCURRENT ILLNESS	-	-	1	1.4%	1	0.7%	-	-
INCREASED LIVER ENZYMES	-	-	1	1.4%	1	0.7%	-	-
LACK OF CLINICAL/WALK TEST IMPROVEMENT	-	-	-	-	-	-	1	1.4%

Note: only the discontinuations in period 1 are considered
 (Page 1/1)

There were 12 premature discontinuations in the first 16 weeks of treatment: 3 bosentan 125 mg, 3 bosentan 250 mg, and 6 placebo. Of the 6 who discontinued because of worsening condition, 3 were on bosentan and 3 were on placebo. There were 3 deaths: 1 on bosentan 125 mg and 2 on placebo. There was 1 drop out (bosentan 250 mg) for adverse event/intercurrent illness; 1 drop out (bosentan 250 mg) for increased liver enzymes, and 1 drop out (placebo) for lack of clinical/walk test improvement.

2.2 Demographics and baseline characteristics

Demographics for the study subjects, by treatment group, are shown below.

Table 2 Summary of patient demographics, ITT population

(Table T04 / 04MAY01)

	Bosentan 125 mg N=74	Bosentan 250 mg N=70	All Bosentan N=144	Placebo N=69
SEX [n (%)]				
n	74	70	144	69
Males	17 23.0%	13 18.6%	30 20.8%	15 21.7%
Females	57 77.0%	57 81.4%	114 79.2%	54 78.3%
AGE (years)				
n	74	70	144	69
Mean	50.4	47.0	48.7	47.2
Standard deviation	15.9	15.6	15.8	16.2
Median	50.5	48.0	49.0	50.0
Min , Max	15.0 , 79.0	13.0 , 80.0	13.0 , 80.0	12.0 , 80.0
AGE [n (%)]				
n	74	70	144	69
12 - 20 years	3 4.1%	4 5.7%	7 4.9%	6 8.7%
21 - 40 years	13 17.6%	18 25.7%	31 21.5%	15 21.7%
41 - 60 years	38 51.4%	33 47.1%	71 49.3%	33 47.8%
> 60 years	20 27.0%	15 21.4%	35 24.3%	15 21.7%
WEIGHT (kg)				
n	74	70	144	69
Mean	71.6	70.5	71.0	73.7
Standard deviation	21.2	17.8	19.6	18.3
Median	67.0	69.7	68.0	73.0
Min , Max	35.8 , 137.4	41.8 , 137.4	35.8 , 137.4	33.0 , 123.4
HEIGHT (cm)				
n	74	70	144	69
Mean	163.7	163.8	163.7	162.9
Standard deviation	10.2	8.3	9.3	9.0
Median	162.6	162.6	162.6	160.0
Min , Max	137.2 , 190.5	150.0 , 188.0	137.2 , 190.5	148.0 , 187.0
RACE [n (%)]				
n	74	70	144	69
Caucasian/white	57 77.0%	54 77.1%	111 77.1%	59 85.5%
Black	5 6.8%	7 10.0%	12 8.3%	1 1.4%
Asian	2 2.7%	1 1.4%	3 2.1%	-
Other	10 13.5%	8 11.4%	18 12.5%	9 13.0%
LOCATION [n (%)]				
n	74	70	144	69
US	41 55.4%	38 54.3%	79 54.9%	39 56.5%
Non-US	33 44.6%	32 45.7%	65 45.1%	30 43.5%

The majority of patients were female, in their late forties, weighed about 72 kg, and were white. More than half of the study patients resided in the U.S. The treatment groups were fairly well balanced.

2.2.1 Disease history

Summary of baseline disease characteristics is shown below.

Table 3 Summary of baseline disease characteristics, ITT population

(Table T05 / 04MAY01)

	Bosentan 125 mg N=74	Bosentan 250 mg N=70	All Bosentan N=144	Placebo N=69
Time from diagnosis of PAH (days)*				
n	73	70	143	69
Mean	898.4	892.5	895.5	843.4
Standard deviation	985.3	1144.2	1062.3	1442.4
Median	511.0	421.0	453.0	381.0
Min , Max	9.0 , 3907.0	5.0 , 5259.0	5.0 , 5259.0	7.0 , 9923.0
Etiology of PAH [n (%)]				
n	74	70	144	69
PPH	57 77.0%	45 64.3%	102 70.8%	48 69.6%
SSc/PHT	13 17.6%	20 28.6%	33 22.9%	14 20.3%
Other	4 5.4%	5 7.1%	9 6.3%	7 10.1%
Presence of Raynaud's syndrome [n (%)]				
n	74	70	144	69
Yes	20 27.0%	21 30.0%	41 28.5%	19 27.5%
No	54 73.0%	49 70.0%	103 71.5%	50 72.5%
Presence of antinuclear antibody [n (%)]				
n	74	70	144	69
Yes	22 29.7%	18 25.7%	40 27.8%	24 34.8%
No	35 47.3%	28 40.0%	63 43.8%	29 42.0%
Unknown	17 23.0%	24 34.3%	41 28.5%	16 23.2%
Presence of rheumatoid factor [n (%)]				
n	74	70	144	69
Yes	4 5.4%	6 8.6%	10 6.9%	7 10.1%
No	35 47.3%	31 44.3%	66 45.8%	28 40.6%
Unknown	35 47.3%	33 47.1%	68 47.2%	34 49.3%
WHO grade at Baseline [n (%)]				
n	74	70	144	69
III	68 91.9%	62 88.6%	130 90.3%	65 94.2%
IV	6 8.1%	8 11.4%	14 9.7%	4 5.8%
Baseline oxygen saturation (%) †				
n	74	68	142	68
Mean	94.3	93.7	94.0	94.6
Standard deviation	3.8	4.5	4.1	3.7
Median	95.0	95.0	95.0	96.0
Min , Max	77.0 , 99.9	81.0 , 99.9	77.0 , 99.9	85.0 , 100.0

(*) Reported number of days from diagnosis of pulmonary hypertension to randomization
 (†) Last valid value between the visits 1 (Screening) and 2 (Randomization)
 PAH=pulmonary arterial hypertension, PPH=primary pulmonary hypertension,
 SSc/PHT=pulmonary hypertension due to scleroderma

The mean time to from time of diagnosis to randomization was about 2.5 years. The etiology of PAH for the majority of patients was PPH and the WHO grade at baseline was class II for 89%-94% of patients; somewhat more bosentan patients than placebo patients were identified as class IV. Mean oxygen saturation at baseline was about 94%. Again, the treatment groups were well balanced.

2.2.2 Baseline hemodynamics

Summary of baseline hemodynamics is shown below.

Table 4 Summary of baseline hemodynamics, ITT population

(Table T06 / 04MAY01)

	Bosentan 125 mg N=74	Bosentan 250 mg N=70	All Bosentan N=144	Placebo N=69
Mean PAP (mmHg)				
n	74	70	144	69
Mean	52.8	56.7	54.7	53.4
Standard deviation	14.5	16.8	15.8	16.6
Median	51.8	53.5	52.0	51.0
Min , Max	28.0 , 92.0	27.0 , 114.5	27.0 , 114.5	25.5 , 108.5
PVR (dyn*sec/cm ⁵)				
n	73	62	135	65
Mean	884	1167	1014	880
Standard deviation	412	875	678	540
Median	857	962	888	800
Min , Max	196 , 2067	248 , 6467	196 , 6467	133 , 3727
Cardiac index (l/min/m ²)				
n	74	70	144	68
Mean	2.46	2.24	2.35	2.43
Standard deviation	0.82	0.81	0.82	0.69
Median	2.41	2.06	2.23	2.33
Min , Max	1.19 , 5.84	0.35 , 4.84	0.35 , 5.84	1.35 , 4.16
PCWP (mmHg)				
n	73	62	135	66
Mean	9.7	8.7	9.2	9.2
Standard deviation	4.1	3.6	3.9	4.1
Median	10.0	8.0	9.0	9.0
Min , Max	1.0 , 21.0	1.0 , 20.0	1.0 , 21.0	1.0 , 25.0
Mean RAP (mmHg)				
n	74	69	143	67
Mean	9.7	9.9	9.8	8.9
Standard deviation	5.4	6.5	5.9	5.1
Median	8.3	8.0	8.0	8.0
Min , Max	1.0 , 27.0	0.0 , 27.0	0.0 , 27.0	0.5 , 20.0

PAP = pulmonary arterial pressure, PCWP = pulmonary capillary wedge pressure, PVR = pulmonary vascular resistance, RAP = right atrial pressure.

Mean pulmonary arterial pressure, pulmonary capillary wedge pressure, and pulmonary venous resistance were approximately 53 mmHg, 9.2 mmHg, and 1000 dyn-sec/cm⁵, respectively. The treatment groups were well balanced.

2.2.3 Concomitant diseases

Summary of previous and concomitant diseases is shown below.

Table 5 Summary of previous and concomitant diseases by class, safety population

(Table T07 / 04MAY01)

	Bosentan 125 mg N=74		Bosentan 250 mg N=70		All Bosentan N=144		Placebo N=69	
	No.	%	No.	%	No.	%	No.	%
ALL DISEASE CLASSES								
Total pts with at least one disease	63	85.1%	63	90.0%	126	87.5%	59	85.5%
Total number of diseases	324		290		614		297	
GASTROINTESTINAL DISORDERS	28	37.8%	30	42.9%	58	40.3%	22	31.9%
METABOLISM AND NUTRITION DISORDERS	26	35.1%	25	35.7%	51	35.4%	30	43.5%
VASCULAR DISORDERS	27	36.5%	23	32.9%	50	34.7%	21	30.4%
MUSCULOSKELETAL, CONNECTIVE TISSUE AND BONE DISORDERS	23	31.1%	22	31.4%	45	31.3%	21	30.4%
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS	22	29.7%	18	25.7%	40	27.8%	21	30.4%
CARDIAC DISORDERS	17	23.0%	23	32.9%	40	27.8%	16	23.2%
ENDOCRINE DISORDERS	14	18.9%	19	27.1%	33	22.9%	10	14.5%
PSYCHIATRIC DISORDERS	20	27.0%	8	11.4%	28	19.4%	17	24.6%
IMMUNE SYSTEM DISORDERS	14	18.9%	13	18.6%	27	18.8%	5	7.2%
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS	13	17.6%	10	14.3%	23	16.0%	12	17.4%
REPRODUCTIVE SYSTEM AND BREAST DISORDERS	12	16.2%	9	12.9%	21	14.6%	12	17.4%
NERVOUS SYSTEM DISORDERS	10	13.5%	10	14.3%	20	13.9%	14	20.3%
BLOOD AND LYMPHATIC SYSTEM DISORDERS	10	13.5%	5	7.1%	15	10.4%	9	13.0%
SKIN & SUBCUTANEOUS TISSUE DISORDERS	5	6.8%	6	8.6%	11	7.6%	8	11.6%
HEPATO-BILIARY DISORDERS	4	5.4%	5	7.1%	9	6.3%	7	10.1%
RENAL AND URINARY DISORDERS	7	9.5%	2	2.9%	9	6.3%	6	8.7%
EYE DISORDERS	2	2.7%	2	2.9%	4	2.8%	5	7.2%
SURGICAL & MEDICAL PROCEDURES	1	1.4%	1	1.4%	2	1.4%	2	2.9%
EAR AND LABYRINTH DISORDERS	2	2.7%	-	-	2	1.4%	1	1.4%
INFECTIOUS AND INFESTATIONS	1	1.4%	-	-	1	0.7%	1	1.4%
INVESTIGATIONS	1	1.4%	-	-	1	0.7%	1	1.4%
PREGNANCY, PUERPERIUM AND PERINATAL CONDITIONS	-	-	1	1.4%	1	0.7%	1	1.4%
CONGENITAL AND FAMILIAL/GENETIC DISORDERS	-	-	1	1.4%	1	0.7%	-	-
NEOPLASMS BENIGN AND MALIGNANT (INCLUDING CYSTS AND POLYPS)	-	-	1	1.4%	1	0.7%	-	-
INJURY AND POISONING	-	-	-	-	-	-	1	1.4%

Most patients (85%-90%) had at least 1 concomitant disease. The most frequently reported diseases were GI disorders, followed by metabolism and nutritional disorders, vascular disorders, musculoskeletal/connective tissue/and bone disorders. The treatment groups were well balanced.

2.2.4 Concomitant medications

Summary of previous and concomitant treatments for PAH is shown below.

Table 6 Summary of previous and concomitant treatments for pulmonary arterial hypertension by class, safety population

(Table T08 / 04MAY01)

	Bosentan 125 mg N=74		Bosentan 250 mg N=70		All Bosentan N=144		Placebo N=69	
	No.	%	No.	%	No.	%	No.	%
ALL TREATMENT CLASSES								
Total pts with at least one TRT	65	87.8%	64	91.4%	129	89.6%	64	92.8%
Total number of TRTs	167		167		334		163	
ANTITHROMBOTIC AGENTS	51	68.9%	50	71.4%	101	70.1%	50	72.5%
HIGH-CEILING DIURETICS	34	45.9%	31	44.3%	65	45.1%	26	37.7%
CALCIUM CHANNEL BLOCKERS	33	44.6%	31	44.3%	64	44.4%	36	52.2%
POTASSIUM SPARING AGENTS	15	20.3%	16	22.9%	31	21.5%	17	24.6%
CARDIAC GLYCOSIDES	12	16.2%	16	22.9%	28	19.4%	13	18.8%
LOW CEILING DIURETICS, THIAZIDES	6	8.1%	6	8.6%	12	8.3%	5	7.2%
AGENTS ACTING ON THE RENIN-ANGIOTENSIN SYSTEM	5	6.8%	4	5.7%	9	6.3%	3	4.3%
MINERAL SUPPLEMENTS	1	1.4%	4	5.7%	5	3.5%	1	1.4%
BETA BLOCKING AGENTS	4	5.4%	-	-	4	2.8%	1	1.4%
HYDRAZINOPHTHAZINE DERIVATIVES	1	1.4%	1	1.4%	2	1.4%	2	2.9%
LOW-CEILING DIURETICS, EXCL. THIAZIDES	-	-	2	2.9%	2	1.4%	1	1.4%
ANTIARRHYTHMICS, CLASS I AND III	1	1.4%	1	1.4%	2	1.4%	-	-
ANTI-ASTHMATICS	1	1.4%	-	-	1	0.7%	1	1.4%
ORGANIC NITRATES	-	-	1	1.4%	1	0.7%	1	1.4%
ANTIADRENERGIC AGENTS, PERIPHERALLY ACTING	-	-	1	1.4%	1	0.7%	-	-
CORTICOSTEROIDS FOR SYSTEMIC USE	1	1.4%	-	-	1	0.7%	-	-
OTHER VASODILATORS USED IN CARDIAC DISEASES	-	-	1	1.4%	1	0.7%	-	-
ANTIADRENERGIC AGENTS, CENTRALLY ACTING	-	-	-	-	-	-	1	1.4%
IMMUNOSUPPRESSIVE AGENTS	-	-	-	-	-	-	1	1.4%

Note: only the medications reported during the visit 1 are included

Most patients (88%-93%) were taking at least 1 concomitant medication. The most common were antithrombotic agents followed by diuretics, calcium channel blockers, and cardiac glycosides. The treatment groups were well balanced.

2.3 Efficacy

2.3.1. Study discontinuations

The table below shows the number and percent of all premature study discontinuations in periods 1 and 2 (extension of the double blind phase).

Table 7 Summary of all premature discontinuations, safety population

(Table T02c / 04MAY01)

Reason for premature discontinuation	Bosentan 125 mg N=74		Bosentan 250 mg N=70		All Bosentan N=144		Placebo N=69	
	No.	%	No.	%	No.	%	No.	%
Total pts with at least one reason	10	13.5%	9	12.9%	19	13.2%	11	15.9%
ADMINISTRATIVE/OTHER	6	8.1%	3	4.3%	9	6.3%	3	4.3%
WORSENING OF PATIENT CONDITION	3	4.1%	2	2.9%	5	3.5%	5	7.2%
INCREASED LIVER ENZYMES	-	-	3	4.3%	3	2.1%	-	-
DEATH	1	1.4%	-	-	1	0.7%	2	2.9%
AE/INTERCURRENT ILLNESS	-	-	1	1.4%	1	0.7%	-	-
LACK OF CLINICAL/WALK TEST IMPROVEMENT	-	-	-	-	-	-	1	1.4%

Note: All the discontinuations in periods 1 and 2 are considered, including 7 patients who should not have had a period 2 and dropped for administrative reasons in period 2.

A slightly higher percentage of placebo patients (15.9%) discontinued the study compared to the all bosentan patients (13.2%). The most common reason was administrative (6.3% bosentan and 4.3% placebo) followed by worsening of the patient's condition (3.5% bosentan and 7.2% placebo). There were 3 bosentan patients (2.1%) who withdrew because of increased liver enzymes, 1 bosentan and 2 placebo patients who died, and 1 bosentan patient who withdrew because of an adverse event.

2.3.2 Primary endpoint (6 minute walk test)

The mean baseline (the average of the screening and randomization) walk test, the mean walk test at week 16, and the mean change from baseline at endpoint for the walk test for the intent to treat population are shown in the table below by treatment groups.

Table 8 Walk test: Change from baseline to Week 16, ITT population

(Table T09 / 09MAY01)

Walk test (m)	Bosentan 125 mg N=74	Bosentan 250 mg N=70	All Bosentan N=144	Placebo N=69
Use of supplemental oxygen during screening/randomization walk tests				
n	74	70	144	69
Yes	11 14.9%	12 17.1%	23 16.0%	16 23.2%
No	63 85.1%	58 82.9%	121 84.0%	53 76.8%
Baseline				
n	74	70	144	69
Mean	326.3	333.0	329.6	344.3
Standard deviation	73.2	75.4	74.1	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				
n	74	70	144	69
Mean	353.1	379.5	365.9	336.5
Standard deviation	115.0	101.2	109.0	129.2
95% 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				
n	74	70	144	69
Mean	26.8	46.5	36.4	-7.8
Standard deviation	75.3	61.7	69.5	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	34.5	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
TREATMENT EFFECT				
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			0.0002	

CL=confidence limits.

The mean baseline walk tests were between 326.3 m and 344.3 m. Compared to the bosentan groups, the placebo group walked the longest at baseline by about 15 m.

Both bosentan groups, but not placebo, had a longer mean walk test at week 16 compared to baseline. The mean absolute changes from baseline (95% confidence limits) for the 125 mg and 250 mg bosentan groups were 26.8 m (9.3, 44.2) and 46.5 m (31.7, 61.2), respectively. The mean change⁵ for the placebo group was -7.8 m (-30.9, 15.2).

The mean treatment effects (95% CL) for the 1.25 mg and 250 mg bosentan groups were 34.6 m (6.2, 63.1) and 54.3 (27.3, 81.4), respectively. The mean change in walk distance was significantly greater (p=0.0002 using the Mann-Whitney U test) for the all bosentan group (n=144) compared to the placebo group.

2.3.2.1 Mean walk distances by visit

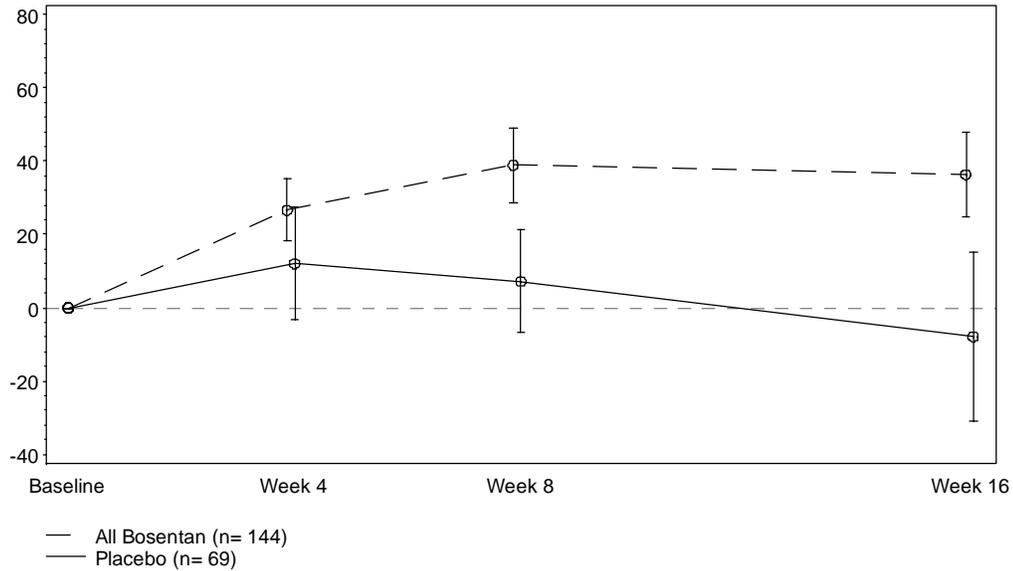
The changes from baseline for the walk distance at baseline, weeks 4, 8, and 16 for the all bosentan group and the placebo group are shown in the figure below. N.B. all bosentan patients were receiving 62.5 mg bid for the first 4 weeks of treatment.

⁵ median change was 9 m indicating that there was one really poor performer in the placebo group

Figure 2 **Walk distance: Change from baseline over time during Period 1, ITT population**

Ro 47-0203, Protocol: AC-052-352
FIGURE 2b: Absolute change from baseline (mean +/- 95% CL)
Population: ITT

TOTAL WALK DISTANCE (m)



Note: All bosentan patients received 62.5 mg bid during the first 4 weeks of the study and then were up-titrated to the target dose (125 mg bid or 250 mg bid).

The change from baseline for the bosentan group was greater at weeks 8 and 16 than at week 4 and there was no discernable difference between the change from baseline at weeks 8 and 16.

Placebo, on the other hand, showed a slight increase in the mean walking distance at week 4 compared to baseline, but there was a dramatic loss of effect at week 8 in this treatment group and it fell below baseline by week 16.

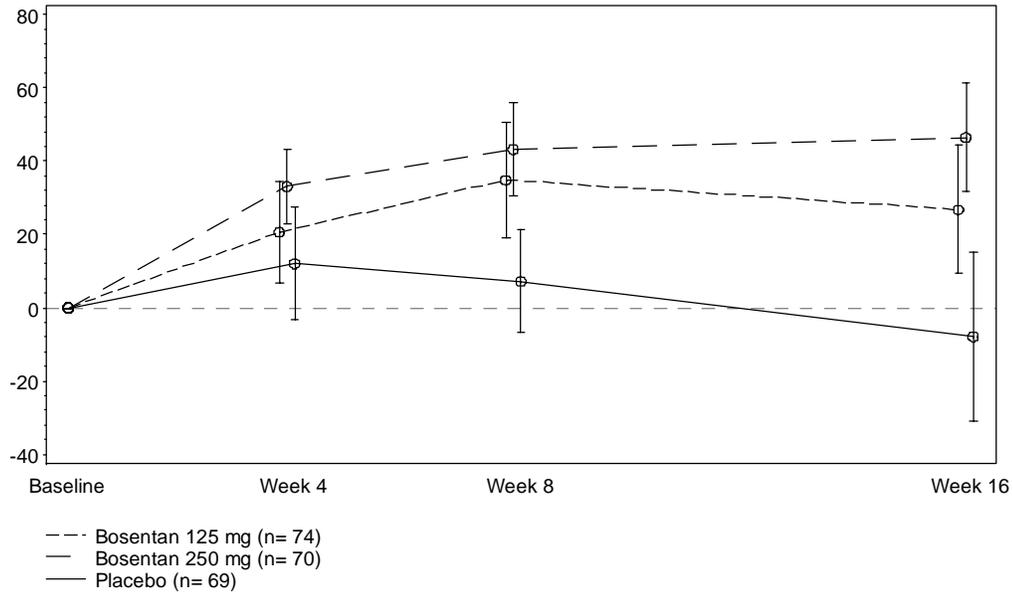
2.3.2.2 Dose effect

The change from baseline at weeks 4, 8, and 16 by dose for the 2 bosentan dose groups as well as the placebo effect are shown in the figure below. At week 4, all bosentan patients were taking 62.5 mg bid.

Figure 3 **Walk distance: Change from baseline over time during Period 1 by dose, ITT population**

Ro 47-0203, Protocol: AC-052-352
FIGURE 2a: Absolute change from baseline (mean +/- 95% CL)
Population: ITT

TOTAL WALK DISTANCE (m)



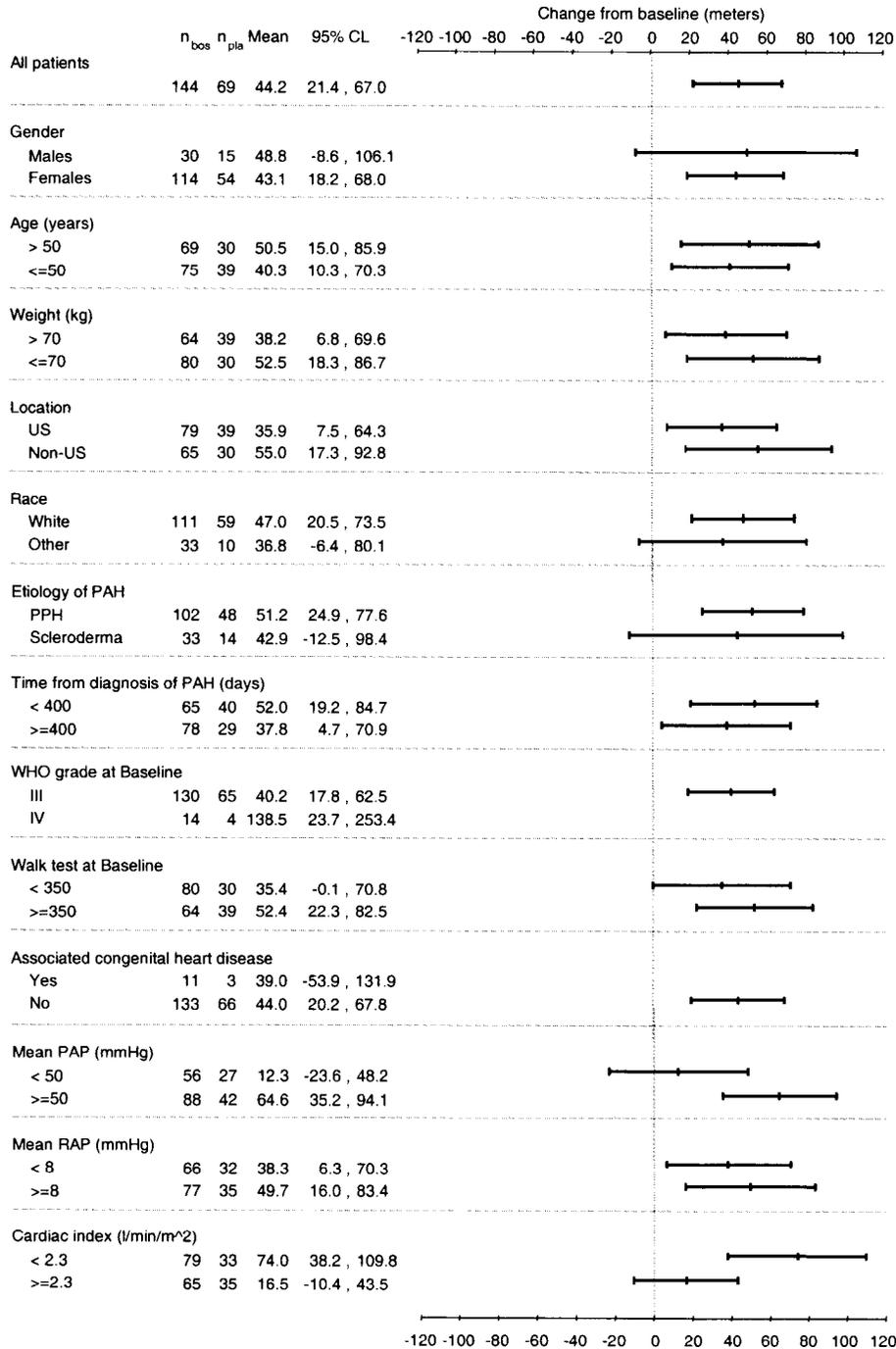
Note: All bosentan patients received 62.5 m b.i.d. during the first 4 weeks of the study and then were up-titrated to the target dose (125 mg b.i.d. or 250 mg b.i.d.).

The 250 mg bosentan group was numerically superior to the 125 mg group at weeks 8 and 16, but the confidence limits overlapped at both time points.

2.3.2.3 Subgroups

The placebo corrected change from baseline for the walk test by sub group are shown below.

Figure 8 Walk test: Placebo-corrected change from baseline to Week 16 in subgroups, ITT population



The point estimates for all subgroups were all above 0 m with most being at least 20 m improvement over baseline.

2.3.3 Secondary endpoints

2.3.3.1 Time from randomization to clinical worsening (defined as death from all causes, lung transplantation or discontinuation of therapy because of clinical deterioration)

The table and figures below shows the Kaplan Meier estimate of the event free rate (%), by treatment group.

Table 9 Time from randomization to clinical worsening up to study end, ITT population

(Table T12a / 04MAY01)

K-M estimate of the event-free rate (%)	Bosentan 125 mg N=74	Bosentan 250 mg N=70	All Bosentan N=144	Placebo N=69
At 8 weeks				
Patients at risk	71	70	141	63
Patients censored	-	-	-	-
Patients with event	3	-	3	6
K-M estimate (%)	95.9	100	97.9	91.3
95% confidence interval (%)	91.5 , 100	100.0 , 100	95.6 , 100	84.7 , 98.0
At 16 weeks				
Patients at risk	52	44	96	41
Patients censored	18	23	41	18
Patients with event	4	3	7	10
K-M estimate (%)	94.6	95.3	95.0	85.2
95% confidence interval (%)	89.4 , 99.7	90.2 , 100	91.4 , 98.6	76.7 , 93.7
At 28 weeks				
Patients at risk	5	6	11	3
Patients censored	64	60	124	52
Patients with event	5	4	9	14
K-M estimate (%)	88.3	90.0	89.3	62.9
95% confidence interval (%)	75.4 , 100	78.8 , 100	80.6 , 97.9	41.2 , 84.7
Treatment difference				
Logrank p-value vs. placebo	0.0133	0.0122	0.0015	

Patients were censored at the date of trial treatment end plus 1 day.
 K-M = Kaplan-Meier.

Figure 4 Time from randomization to clinical worsening, ITT population

Ro 47-0203, Protocol: AC-052-352
 FIGURE 3b Time from randomization to clinical worsening up to 28 weeks
 (Kaplan Meier estimates)

Population: ITT

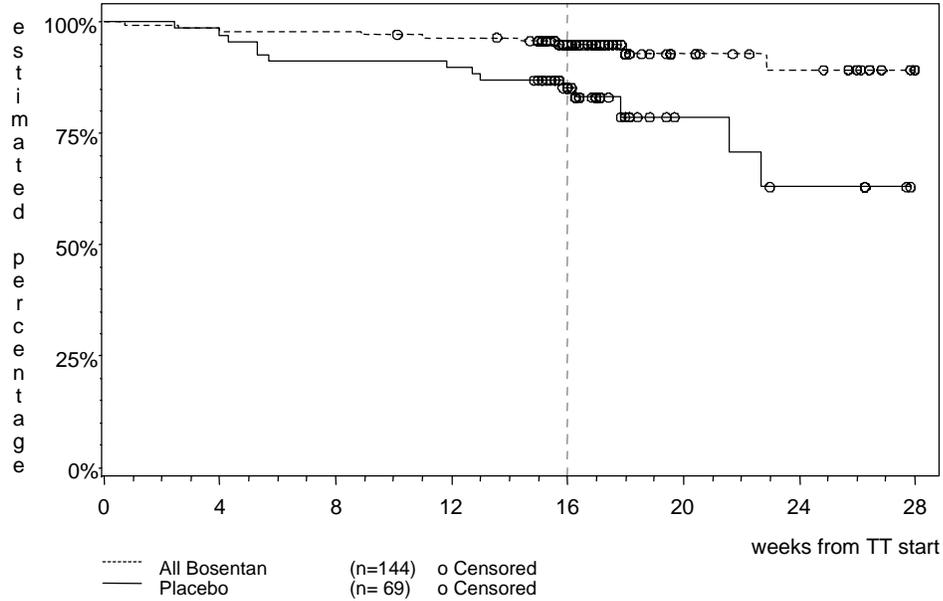
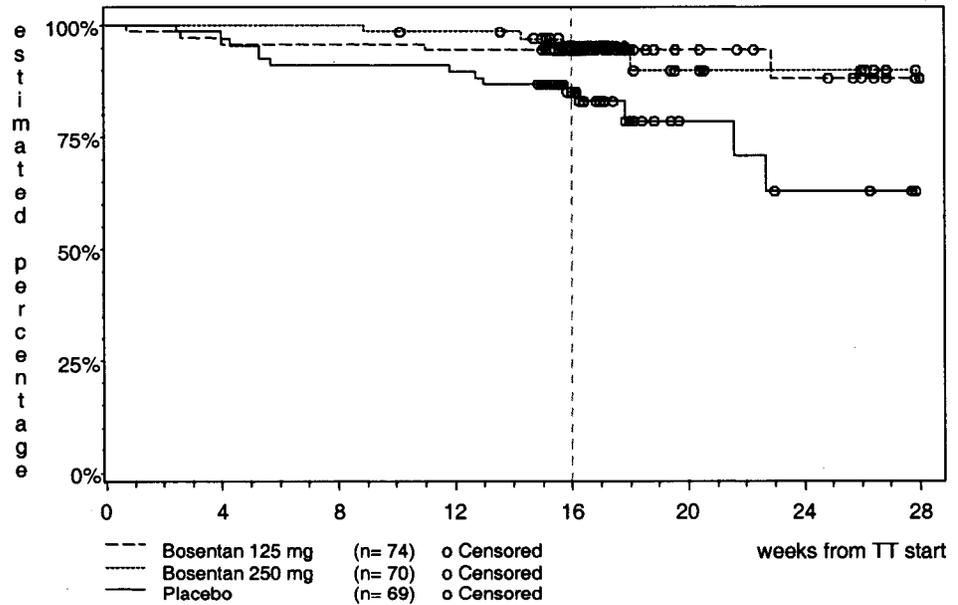


Figure 6 Time from randomization to clinical worsening by dose, ITT population

Ro 47-0203, Protocol: AC-052-352
 FIGURE 3a Time from randomization to clinical worsening up to 28 weeks
 (Kaplan Meier estimates)

Population: ITT



The placebo group was significantly worse at week 16 compared to the 2 bosentan groups. There is little difference between dose groups.

The incidence of clinical worsening by treatment group is shown below.

Table 10 Incidence of clinical worsening, ITT population

(Table T13 / 04MAY01 and T13b / 07MAY01)

	Bosentan 125 mg N=74		Bosentan 250 mg N=70		All Bosentan N=144		Placebo N=69	
	No.	%	No.	%	No.	%	No.	%
Patients with clinical worsening to week 28*	5	6.8%	4	5.7%	9	6.3%	14	20.3%
Death	1	1.4%	-	-	1	0.7%	2	2.9%
Hospitalization for PHT	3	4.1%	3	4.3%	6	4.2%	9	13.0%
Lack of clinical/walk test improvement	-	-	-	-	-	-	1	1.4%
Worsening of patient condition	3	4.1%	2	2.9%	5	3.5%	5	7.2%
Receipt of prostacyclin	2	2.7%	2	2.9%	4	2.8%	3	4.3%
Patients with clinical worsening in Period 1*	4	5.4%	3	4.3%	7	4.9%	12	17.4%
Death	1	1.4%	-	-	1	0.7%	2	2.9%
Hospitalization for PHT	3	4.1%	3	4.3%	6	4.2%	9	13.0%
Lack of clinical/walk test improvement	-	-	-	-	-	-	1	1.4%
Worsening of patient condition	2	2.7%	1	1.4%	3	2.1%	3	4.3%
Receipt of prostacyclin	2	2.7%	1	1.4%	3	2.1%	2	2.9%

* Patients may fall into more than one category.

The overall incidence rate of worsening was much greater for the placebo group at weeks 16 and 28 (17.4% and 20.3%, respectively) compared to the all bosentan group (4.9% and 6.3%, respectively). The largest difference was the result of the placebo patients having a greater tendency to be hospitalized for pulmonary hypertension.

There was no evidence of a waning of effect of bosentan over the relatively brief time of the study (28 weeks).

2.3.3.2 Changes from baseline dyspnea index (Borg dyspnea index).

Table 11 Borg dyspnea index: Change from baseline to Week 16, ITT population

(Table T10 / 04MAY01)

Borg dyspnea index	Bosentan 125 mg N=74	Bosentan 250 mg N=70	All Bosentan N=144	Placebo N=69
Baseline				
n	74	70	144	69
Mean	3.3	3.8	3.6	3.8
Standard deviation	2.2	1.9	2.0	2.0
95% CL of mean	2.8 , 3.8	3.4 , 4.3	3.2 , 3.9	3.4 , 4.3
Median	3.0	4.0	3.0	4.0
95% CL of median	3.0 , 4.0	3.0 , 4.0	3.0 , 4.0	3.0 , 4.0
Min , Max	0.0 , 10.0	0.0 , 9.0	0.0 , 10.0	0.0 , 10.0
Week 16				
n	74	70	144	69
Mean	3.3	3.3	3.3	4.2
Standard deviation	2.7	2.3	2.5	2.4
95% CL of mean	2.6 , 3.9	2.7 , 3.8	2.9 , 3.7	3.6 , 4.8
Median	3.0	3.0	3.0	4.0
95% CL of median	2.0 , 3.0	3.0 , 3.0	3.0 , 3.0	3.0 , 4.0
Min , Max	0.0 , 10.0	0.0 , 9.0	0.0 , 10.0	0.0 , 10.0
Change from baseline				
n	74	70	144	69
Mean	-0.1	-0.6	-0.3	0.3
Standard deviation	2.1	1.9	2.0	2.0
95% CL of mean	-0.5 , 0.4	-1.0 , -0.1	-0.6 , 0.0	-0.2 , 0.8
Median	0.0	-0.3	0.0	0.0
95% CL of median	0.0 , 0.0	-1.0 , 0.0	0.0 , 0.0	0.0 , 0.0
Min , Max	-6.5 , 6.0	-6.0 , 4.0	-6.5 , 6.0	-3.0 , 7.0
TREATMENT EFFECT				
Mean	-0.4	-0.9	-0.6	
95% CL of mean	-1.1 , 0.3	-1.6 , -0.2	-1.2 , -0.1	
Median	-0.2	-0.7	-0.3	
95% CL of median	-0.8 , 0.4	-1.4 , -0.0	-1.0 , 0.1	

CL=confidence limits.

Bosentan patients had an improvement in their Borg scale by week 16 while placebo patients deteriorated. The placebo subtracted improvement ranged from -0.4 for the 125 mg bid group to -0.9 for the 250 mg bid group.

2.3.3.4.Changes from baseline in WHO functional class

The incidence of improvement in WHO functional class is shown below.

Table 12 Incidence of improvement in WHO functional class during Period 1, ITT population

(Table T11b / 04MAY01)

Change at week 16	Bosentan 125 mg N=74	Bosentan 250 mg N=70	All Bosentan N=144	Placebo N=69
n	74	70	144	69
Improved [n (%)]	32 (43.2%)	29 (41.4%)	61 (42.4%)	21 (30.4%)
95% confidence limits (%)	31.8 , 55.3	29.8 , 53.8	34.2 , 50.9	19.9 , 42.7
Treatment effect*				
Difference	12.8%	11.0%	11.9%	
95% confidence limits (%)	-4.0 , 28.6	-5.9 , 27.0	-2.9 , 25.2	

* Compared with placebo

A higher incidence of improvement was seen in the bosentan group (42.2%) compared to the placebo group (30.4%). There was no difference between the 2 bosentan groups.

The percent of patients who changed WHO functional class is shown below.

Table 13 WHO functional class: Change from baseline to Week 16, ITT population

(Table T11a / 04MAY01)

Treatment	N	n (%)	Baseline WHO class	Week 16							
				I		II		III		IV	
				No.	%	No.	%	No.	%	No.	%
Bosentan 125 mg	74	68 (91.9%) 6 (8.1%)	III	2	2.7%	26	35.1%	38	51.4%	2	2.7%
			IV	-	-	2	2.7%	2	2.7%	2	2.7%
Bosentan 250 mg	70	62 (88.6%) 8 (11.4%)	III	1	1.4%	23	32.9%	37	52.9%	1	1.4%
			IV	-	-	1	1.4%	4	5.7%	3	4.3%
All Bosentan	144	130 (90.3%) 14 (9.7%)	III	3	2.1%	49	34.0%	75	52.1%	3	2.1%
			IV	-	-	3	2.1%	6	4.2%	5	3.5%
Placebo	69	65 (94.2%) 4 (5.8%)	III	-	-	19	27.5%	42	60.9%	4	5.8%
			IV	-	-	-	-	2	2.9%	2	2.9%

At week 16, the majority of patients remained in their functional class:

-All bosentan: 75 (52.1%) were class III at baseline and remained class III; 5 (3.5%) were class IV at baseline and remained class IV;

-Placebo: 42 (60.9%) were class III at baseline and remained class III; 2 (2.9%) were class IV at baseline and remained class IV.

However, more bosentan patients than placebo patients improved:

-All bosentan: 49 (34%) moved from class III at baseline to class II at week 16, 3 (2.1%) moved from class III at baseline to class I at week 16, and 6 (4.2%) moved from class IV at baseline to class III at week 16;

-Placebo: 19 (27.5%) moved from class III at baseline to class II at week 16, 0 moved from class III at baseline to class I at week 16, and 2 (2.9%) moved from class IV at baseline to class III at week 16

And fewer bosentan patients than placebo patients grew worse:

-All bosentan: 3 (2.1%) moved from class III to class IV;

-Placebo: 4 (5.8%) moved from class III to class IV.

2.3.3.5 Increased therapy for PAH

The number and percent of patients who had an increase in their therapy for PAH is shown below.

Table 14 Incidence of increased therapy for pulmonary arterial hypertension, ITT population

(Table T14 / 04MAY01)

	Bosentan 125 mg N=74	Bosentan 250 mg N=70	All Bosentan N=144	Placebo N=69
Period 1 (weeks 0 to 16)				
n	74	70	144	69
Patients with increase [n(%)]	18 (24.3%)	14 (20.0%)	32 (22.2%)	20 (29.0%)
95% confidence limits (%)	15.1 , 35.7	11.4 , 31.3	15.7 , 29.9	18.7 , 41.2
Treatment effect*				
Difference	-4.7%	-9.0%	-6.8%	
95% confidence limits (%)	-20.0 , 10.6	-24.3 , 5.9	-19.5 , 7.1	
Period 1+2 (weeks 0 to 28)†				
n	19	16	35	13
Patients with increase [n(%)]	6 (31.6%)	5 (31.3%)	11 (31.4%)	5 (38.5%)
95% confidence limits (%)	12.6 , 56.6	11.0 , 58.7	16.9 , 49.3	13.9 , 68.4
Treatment effect				
Difference	-6.9%	-7.2%	-7.0%	
95% confidence limits (%)	-41.0 , 27.5	-43.1 , 27.7	-36.5 , 25.4	

† Analyzed only in those patients who were scheduled to continue to period 2.

For the first 16 weeks, 22.2% of bosentan patients had an increase in PAH therapy compared to 29% of placebo patients. For the prolonged study period (up to 28 weeks), 31.4% bosentan patients compared to 38.5% placebo patients had an increase in PAH therapy.

2.4 Safety

2.4.1 Dose and duration of treatment

Summary of treatment duration is shown below.

Table 15 Summary of extent of exposure to trial treatment, safety population

(Table T15 / 04MAY01)

	Bosentan 125 mg N=74	Bosentan 250 mg N=70	All Bosentan N=144	Placebo N=69

During Period 1 (up to Week 16)				
Duration of treatment (days)				
n	74	70	144	69
Mean	113.4	113.8	113.6	112.8
Standard deviation	16.8	10.6	14.1	17.7
95% CL of mean	109.5 , 117.3	111.3 , 116.4	111.3 , 115.9	108.6 , 117.1
Median	114.0	114.0	114.0	114.0
95% CL of median	113.0 , 118.0	112.0 , 118.0	113.0 , 117.0	112.0 , 119.0
Min , Max	24.0 , 133.0	64.0 , 131.0	24.0 , 133.0	18.0 , 139.0
Number (%) of patient who				
n	74	70	144	69
Remained at target dose	63 85.1%	59 84.3%	122 84.7%	57 82.6%
Had >=1 reduction in dose	7 9.5%	7 10.0%	14 9.7%	6 8.7%
Were withdrawn/weaned	4 5.4%	4 5.7%	8 5.6%	6 8.7%

During the study (up to end of treatment)				
Duration of treatment (days)				
n	74	70	144	69
Mean	129.4	128.7	129.1	123.8
Standard deviation	35.8	32.4	34.1	33.8
95% CL of mean	121.1 , 137.7	121.0 , 136.5	123.5 , 134.7	115.7 , 131.9
Median	118.0	118.0	118.0	115.0
95% CL of median	114.0 , 123.0	113.0 , 121.0	115.0 , 121.0	113.0 , 119.0
Min , Max	24.0 , 207.0	64.0 , 204.0	24.0 , 207.0	18.0 , 214.0
Number (%) of patient who				
n	74	70	144	69
Remained at target dose	56 75.7%	55 78.6%	111 77.1%	52 75.4%
Had >=1 reduction in dose	7 9.5%	5 7.1%	12 8.3%	6 8.7%
Were withdrawn/weaned	11 14.9%	10 14.3%	21 14.6%	11 15.9%

Patients on treatment [n (%)]				
n	74	70	144	69
>= 1 and < 4 weeks	1 1.4%	-	1 0.7%	1 1.4%
>= 4 and < 8 weeks	1 1.4%	-	1 0.7%	1 1.4%
>= 8 and < 16 weeks	16 21.6%	20 28.6%	36 25.0%	16 23.2%
>= 16 and < 28 weeks	49 66.2%	44 62.9%	93 64.6%	47 68.1%
>= 28 weeks	7 9.5%	6 8.6%	13 9.0%	4 5.8%

Mean duration of treatment was about 113 days for all 3 treatment groups, and similar percentage of patients in each group remained at the target dose (about 83%). Dose reduction occurred in 8.7% of the placebo group compared to 9.7% in the all bosentan group. There was little difference between the 125 mg and 250 mg groups. The majority of patients (about 66%) in all 3 treatment groups remained on drug for at least 16 weeks.

2.4.2 Serious safety

2.4.2.1. Deaths

The deaths reported during treatment and up to 28 days after stop of treatment are shown below.

Table 19 Summary of deaths during study treatment or within 28 days of treatment end, safety population
 (Table T17 / 04MAY01)

Placebo Cause of death N=69	Bosentan 125 mg N=74		Bosentan 250 mg N=70		All Bosentan N=144		
	No.	%	No.	%	No.	%	
Total patients who died 2.9%	1	1.4%	3	4.3%	4	2.8%	2
CARDIAC FAILURE NOS	1	1.4%	1	1.4%	2	1.4%	-
PNEUMONIA NOS	-	-	1	1.4%	1	0.7%	-
PULMONARY HAEMORRHAGE	-	-	1	1.4%	1	0.7%	-
SEPSIS NOS	-	-	1	1.4%	1	0.7%	-
PULMONARY HYPERTENSION NOS AGGRAVATED 2.9%	-	-	-	-	-	-	2

Note: All the deaths reported up to 28 days after end of randomized treatment are included.
 Patients may have had more than one cause of death.

The incidence rates for death during the first 16 weeks of the study (and up to 28 days after the stop of study drug treatment) were similar for the placebo (2.9%) and the all bosentan group (2.8%). The incidence rate for the bosentan 125 mg group (4.3%) was twice the rate in the bosentan 125 mg group (1.4%).

The 6 deaths (4 bosentan and 2 placebo) are discussed below.

Patient #	Dose/day of study	comments
115 10117	Bos 250 mg bid/84	69 year old female patient with systemic sclerosis and connective tissue disease, Raynaud's phenomenon, Sjogren's syndrome, atrial septal defect, anemia, duodenal and gastric ulcer hemorrhage, hiatus hernia, gastro-esophageal reflux disease, drug hypersensitivity, hypertension, tobacco abuse and cataract extraction. Concomitant medication included lansoprazole, furosemide, mecamylamine, and spironolactone. The patient was hospitalized on day 70 with bilateral pneumonia, hypotension and renal insufficiency. Study medication was withdrawn. She became hypoxemic and required intubation and mechanical ventilation. She was treated with antibiotics, dobutamine, dopamine and levophed, she remained hypotensive and required mechanical ventilation. Bilateral pleural effusion consistent with legionella pneumonia and pulmonary edema were reported. Cholelithiasis was seen on ultrasound. She developed hypotension and bradycardia within the context of septic shock and died on day 84. The cause of death was given as pneumonia and sepsis.
301 30007	Bos 250 mg bid/68	49-year-old white male patient with systemic sclerosis, allergic rhinitis, gastrointestinal hemorrhage and esophagitis, thrombophlebitis and epilepsy and concomitant medication including digoxin, furosemide, spironolactone, warfarin, xylometazoline, omeprazole, phenobarbitone, carbamazepine, prednisolone eye drops, hypermellolose eye drops, paracetamol, penicillin, clarithromycin, flucloxacillin, nystatin and vitamin K was prematurely withdrawn from the study on day 64 because of worsening of his condition. Patient reported dyspnea, dizziness, esophagitis and oral candidiasis during the early part of the trial. He was hospitalized with increased shortness of breath, pitting edema, cyanosis and abdominal ascites on day 63. Bosentan was stopped and the patient was treated with iv furosemide and epoprostenol. The patient died on day 68 of renal failure, hepatic failure and right ventricular failure.
117 10105	Bos 250 mgbid/128 (open label phase)	26 year old white male with a history of hemoptysis and thromboembolic pulmonary hypertension received bosentan 250 mg bid. Concomitant medication included warfarin.. he sustained an iatrogenic urethral tear as part of

		catheterization during a workup for lung transplantation on day 70. He required suprapubic catheter placement and treatment with antibiotic through day 93 until he had corrective cystoscopic surgery. Patient reported severe headache, nausea and vomiting on day 78. He was admitted to hospital and treated with compazine for the nausea and vomiting. A lumbar puncture revealed positive sero-sanguinous fluid. CT scan ruled out SAH. He was discharged the same day with the headache resolved. Patient was re-hospitalized on day 111 because of hemoptysis and lower extremity edema following a day of shortness of breath. Chest x-ray showed pneumonia. He was treated with antibiotics and antitussives and study medication was discontinued on day 113. He was enrolled into open label study AC 052-354 and after an intensive in-hospital workup revealed both pulmonary thromboses and hemorrhage, bosentan was discontinued after 7 days. The patient died on day 128.
208 2001	Bos 125 mg bid/78	77 year old female patient with systemic sclerosis and Raynaud's syndrome and concomitant medications at time of event included furosemide, nadroparin, allopurinol and dopamine. The patient was hospitalized on day 44 of the study with worsening peripheral ischemia in both inferior limbs. Stenosis of the left popliteal artery was found and percutaneous dilation was performed on Day 59. Subsequently, her renal function deteriorated and she required increased furosemide, dopamine infusion, and oxygen. Chest X-ray showed bilateral pleural effusions, an abdominal echo revealed ascites, and an ECG showed asystole on day 78. The patient went on to develop hypotension; study medication was reduced but there was no improvement. She died the same day; the cause of death was irreversible right heart failure induced by further deterioration in renal function. Died of cardiac failure
112 10070	Placebo/18	Died of aggravated PAH
201 20614	Placebo/76	Died of aggravated PAH
105 10014^	Bos 250 mg bid/115	48-year-old white female with pulmonary hypertension secondary to systemic sclerosis had a medical history of cardiac failure, anemia, thrombocytopenia, lupus erythematosus, Raynaud's syndrome, rectal bleeding, urinary tract infection, cholelithiasis, hip arthroplasty due to aseptic necrosis, and constipation. Concomitant medication included prednisone, mometasone, acetylsalicylic acid, omeprazole, fluorazepam, paracetamol, docusate, and calcium. She was randomized to bosentan She reported nausea treated with promethazine on day 1, dyspepsia on day 6, tinnitus on day 31, and arthralgia day 87. Hepatic function test abnormalities: elevated AST, ALT, alk phos levels at 659, 554 and 275 U/l, respectively. Bilirubin (direct and total) were also increased at 22 and 36 mm/l, respectively) on day 99. Study drug was permanently discontinued on day 116. Hepatic function tests returned to normal on day 141. The patient reported to her local pneumologist with symptoms of worsening pulmonary hypertension on day 145. Epoprostenol therapy was initiated. Inotropic drugs and mechanical ventilation were started. She died 3 days later; cause of death was given as right heart failure secondary due to worsening pulmonary hypertension. The investigator assessed the study drug-event relationship as unrelated. No autopsy was performed.

^The sponsor did not report this death in the BREATHE-1 table of Deaths because the clinical worsening for PAH occurred 29 days after the study medication was discontinued.

With the understanding that this is a small sample size, there is no real indication that bosentan has an impact on survival. However, the larger number of deaths in the bosentan 250 mg is not reassuring.

2.4.2.2 Discontinuations for adverse events

The premature discontinuations of study drug because of an adverse event are shown below.

Table 16 Summary of discontinuations of study treatment due to adverse events, safety population

(Table T21c / 04MAY01)

Body system / Adverse event	Bosentan 125 mg N=74		Bosentan 250 mg N=70		All Bosentan N=144		Placebo N=69	
	No.	%	No.	%	No.	%	No.	%
ALL BODY SYSTEMS								
Total pts with at least one AE	3	4.1%	6	8.6%	9	6.3%	5	7.2%
Total number of AEs	3		9		12		7	
HEPATIC FUNCTION ABNORMAL NOS	-		3	4.3%	3	2.1%	-	
PULMONARY HYPERTENSION NOS AGGRAVATED	2	2.7%	-		2	1.4%	4	5.8%
CARDIAC FAILURE NOS	1	1.4%	1	1.4%	2	1.4%	1	1.4%
CHOLELITHIASIS	-		1	1.4%	1	0.7%	-	
JAUNDICE NOS	-		1	1.4%	1	0.7%	-	
PNEUMONIA NOS	-		1	1.4%	1	0.7%	-	
RENAL FAILURE NOS	-		1	1.4%	1	0.7%	-	
SEPSIS NOS	-		1	1.4%	1	0.7%	-	
SYNCOPE	-		-		-		2	2.9%

Note: All the discontinuations in periods 1 and 2 are considered, including 7 patients who should not have had a period 2 and dropped for administrative reasons in period 2.

The incidence rate of dropouts for adverse events was slightly higher in placebo (7.2%) compared to all bosentan (6.3%). However, the incidence rate for the higher dose of bosentan (8.6%) was more than twice the rate for either placebo or low dose bosentan (4.1%).

The most common adverse event given as a reason for placebo patients dropping out was aggravated pulmonary hypertension, another way of indicating lack of effect. On the other hand, there were 3 bosentan patients who dropped out for abnormal hepatic function and 1 for jaundice (all were receiving 250 mg dose). These patients are discussed below.

Jaundice

Patient # 110 10037. 43 year old white female with systemic sclerosis gastro-esophageal reflux disease, calcinosis, Raynaud's syndrome, sclerodactyly and telangiectasis and concomitant medication including digoxin, furosemide, omeprazole, metolazone, spironolactone, thyroid, hydrocodone, phenyltoloxamine, guaifenesin, metoclopramide, triamcinolone, warfarin, and azithromycin. During the course of the study, the patient experienced vomiting, dehydration, edema, respiratory tract infection, alopecia, drying of mucus membranes, and jaundice. On day 127, the patient's pulmonary hypertension worsened (indicated by a decrease in walking performance) and the patient was treated with i.v. epoprostenol. The study medication was

permanently discontinued on day 147 due to worsening of the condition. The LFTs are shown below. The patient had elevated levels at baseline.

Laboratory: ALT, AST, BILIRUBIN TOTAL, ALKALINE PHOSPHATASE;
 Treatment: Bosentan 250 mg

Centre	Pno	Sex	Age	Date	Day	SGOT (ASAT)		SGPT (ALAT)		Alkaline Phosphat.		Bilirubin						
						Inves value	Std. value	Inves value	Std. value	Inves value	Std. value	Inves value	Std. value					
110	10037	2	43	09AUG00	-2	U/L	32	U/L	24	U/L	42	U/L	37	H	U/L	U/L	umol/L	umol/L
				31AUG00	21	32	24	42	37	104	98	32	26.1	H				
				10OCT00	61	25	18	30	26	110	104	H	31	25.2	H			
				08NOV00	90	30	22	21	19	113	107	H	44	35.8	H			
				06DEC00	118	33	24	18	16	120	113	H	51	41.5	HH			
				05JAN01	148	30	22	15	13	120	113	H	46	37.5	H			
						23	17	14	12	127	120	H	79	64.3	HH			

Elevated LFT

Patient# 113 10050 59-year-old white male patient with primary pulmonary hypertension and systemic hypertension. Concomitant medication included potassium, felodipine, furosemide, metolazone, spironolactone, and warfarin. He was found to have elevated liver enzymes (ALT 108 U/L, AST 135 U/L, alk phos 79 U/L, total bilirubin 12.2 mmol/L) and urine discoloration on day 57. The study medication was reduced to half on day 64. Liver enzymes continued to rise (ALT 628 U/L, AST 414 U/L, alk phos 108 U/L, total bilirubin 26.1 mmol/L day 92). Study drug was stopped on day 95. LFTs gradually returned to baseline and the urine color normalized by day 127. The patient was rolled over to the open label extension study.

2.4.2.3 Serious adverse events

The sponsor stated that a number of adverse events considered by regulatory definition as serious were censored in this study because these events were expected in this patient population. Additionally, aggravated PAH that was reported for one or more of these events was also not counted as serious in this study.

A list of serious adverse events that were reported during the study or within 2 days of treatment discontinuation followed by a list of serious adverse events that were reported from 3 to 28 days after treatment discontinuations are displayed below.

Appendix 22 Summary of all serious adverse events during study treatment or within 2 days of treatment end, safety population

Produced by madesu on 04MAY01

Ro 47-0203, Protocol: AC-052-352

Table T19a: Summary of all serious adverse events reported during randomized treatment or within 2 calendar days of the end of randomized treatment, by frequency

Population: Safety

Body system / Adverse event	Bosentan 125 mg N=74		Bosentan 250 mg N=70		All Bosentan N=144		Placebo N=69	
	No.	%	No.	%	No.	%	No.	%
ALL BODY SYSTEMS								
Total pts with at least one SAE	12	16.2%	13	18.6%	25	17.4%	13	18.8%
Total number of SAEs	15		20		35		27	
ABDOMINAL PAIN NOS	2	2.7%	-		2	1.4%	1	1.4%
PNEUMONIA NOS	-		2	2.9%	2	1.4%	1	1.4%
ANAEMIA NOS	2	2.7%	-		2	1.4%	-	
INTESTINAL OBSTRUCTION NOS	2	2.7%	-		2	1.4%	-	
BRONCHITIS NOS	-		1	1.4%	1	0.7%	2	2.9%
ATRIAL FIBRILLATION	-		1	1.4%	1	0.7%	1	1.4%
CARDIAC FAILURE NOS	1	1.4%	-		1	0.7%	1	1.4%
CHEST PAIN NEC	-		1	1.4%	1	0.7%	1	1.4%
DYSPNOEA NOS	-		1	1.4%	1	0.7%	1	1.4%
UPPER RESPIRATORY TRACT INFECTION NOS	-		1	1.4%	1	0.7%	1	1.4%
VOMITING NOS	1	1.4%	-		1	0.7%	1	1.4%
ATRIOVENTRICULAR BLOCK COMPLETE	-		1	1.4%	1	0.7%	-	
BRADYCARDIA NOS	1	1.4%	-		1	0.7%	-	
CONSTIPATION	1	1.4%	-		1	0.7%	-	
DIARRHOEA NOS	1	1.4%	-		1	0.7%	-	
DIVERTICULITIS	-		1	1.4%	1	0.7%	-	
ECTOPIC PREGNANCY	-		1	1.4%	1	0.7%	-	
FEMORAL ARTERY ANEURYSM	-		1	1.4%	1	0.7%	-	
HAEMOPTYSIS	-		1	1.4%	1	0.7%	-	
HEADACHE NOS	-		1	1.4%	1	0.7%	-	
HERPES ZOSTER	-		1	1.4%	1	0.7%	-	
HYPOKALAEMIA	-		1	1.4%	1	0.7%	-	
HYPOTENSION NOS	1	1.4%	-		1	0.7%	-	
MYOCARDIAL INFARCTION	1	1.4%	-		1	0.7%	-	
PERIPHERAL ISCHAEMIA NOS	1	1.4%	-		1	0.7%	-	
PNEUMOTHORAX NOS	-		1	1.4%	1	0.7%	-	
PYREXIA	-		1	1.4%	1	0.7%	-	
RESPIRATORY TRACT INFECTION VIRAL NOS	-		1	1.4%	1	0.7%	-	
SEPSIS NOS	-		1	1.4%	1	0.7%	-	
URINARY TRACT INJURY NOS	-		1	1.4%	1	0.7%	-	
VENOUS THROMBOSIS NOS	1	1.4%	-		1	0.7%	-	
PULMONARY HYPERTENSION NOS AGGRAVATED	-		-		-		3	4.3%
ABDOMINAL DISTENSION	-		-		-		1	1.4%
ABDOMINAL PAIN UPPER	-		-		-		1	1.4%
ASTHMA AGGRAVATED	-		-		-		1	1.4%
ATRIAL FLUTTER	-		-		-		1	1.4%
COLONOSCOPY	-		-		-		1	1.4%
HEPATIC CONGESTION	-		-		-		1	1.4%
HYPERGLYCAEMIA NOS	-		-		-		1	1.4%
HYPERKALAEMIA	-		-		-		1	1.4%
LOWER RESPIRATORY TRACT INFECTION NOS	-		-		-		1	1.4%
MENORRHAGIA	-		-		-		1	1.4%
NAUSEA	-		-		-		1	1.4%
PAIN IN LIMB	-		-		-		1	1.4%
PLEURAL EFFUSION	-		-		-		1	1.4%
RENAL FAILURE NOS	-		-		-		1	1.4%

Note: only SAEs with onset from start of treatment to 2 calendar days after end of treatment are included.
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Appendix 23 Summary of all serious adverse events reported from 3 to 28 days after study treatment end, safety population

Ro 47-0203, Protocol: AC-052-352
 Table T19b: Summary of serious adverse events reported from 3 calendar days to 28 calendar days after randomized treatment end by frequency
 Population: Safety
 Produced by madesu on 04MAY01

Body system / Adverse event	Bosentan 125 mg N=74		Bosentan 250 mg N=70		All Bosentan N=144		Placebo N=69	
	No.	%	No.	%	No.	%	No.	%
ALL BODY SYSTEMS								
Total pts with at least one SAE	1	1.4%	3	4.3%	4	2.8%	4	5.8%
Total number of SAEs	1		4		5		5	
ANAEMIA NOS	1	1.4%	1	1.4%	2	1.4%	-	
CARDIAC FAILURE NOS	-		1	1.4%	1	0.7%	1	1.4%
GASTRITIS NOS	-		1	1.4%	1	0.7%	-	
PULMONARY EMBOLISM	-		1	1.4%	1	0.7%	-	
HEPATIC FUNCTION ABNORMAL NOS	-		-		-		1	1.4%
NAUSEA	-		-		-		1	1.4%
URINARY TRACT INFECTION NOS	-		-		-		1	1.4%
VOMITING NOS	-		-		-		1	1.4%

Note: only SAEs with onset from 3 days to 28 days after end of treatment are included.
 (Page 1/1)

The most commonly reported serious events (adding events from appendices 22 and 23 together) in the bosentan group were anemia (4/144, 2.8% bosentan and 0 placebo) and abdominal complaints⁶ (6/144, 4.2% bosentan and 3/69, 4.8% placebo).

The 4 anemia reports are discussed below.

Bosentan 125 mg bid. Patient# 104 10030: 67-year-old male with pulmonary hypertension due to systemic sclerosis was randomized to bosentan 125 mg bid. He had a history of systemic hypertension, hiatal hernia, ischemic heart disease with CABG, prostate cancer, GERD, CREST syndrome and borderline anemia and was receiving furosemide, spironolactone, aspirin, lisinopril, and omeprazole. On Day 28 of the study, the patient went to see his local doctor and was diagnosed with low hemoglobin. His hemoglobin and hematocrit values were as follows:

Visit Day	Hemoglobin (g/dl)	Hematocrit (%)
Screening (0)	11.6	36
Day 28	8.6	27
Day 36	6.9	23
Day 42	10.9	36

On each of Days 37 and 39 he was transfused with 2 units of red blood cells. The study medication was not discontinued during the event.

⁶ includes abdominal pain, gastritis, intestinal obstruction, diverticulitis, abdominal distension, upper abdominal pain.

Bosentan 125 mg bid. Patient #104 10139: 63 year old white female with pulmonary hypertension due to systemic sclerosis was randomized to bosentan 125 mg bid. She had a medical history of scleroderma and GERD and was receiving treatments with omeprazole, estrogen, levothyroxine, warfarin, furosemide, lorazepam, diphenhydramine, etidronate, and potassium. On day 47 of the study, the patient's hemoglobin (Hb) and hematocrit (Hct) were found to be lower than at screening. Her laboratory values were as follows:

	<u>Hgb</u>	<u>Hct</u>
Screening:	10.5 g/dL	33 %
Day 35	9.5 g/dL	30 %
Day 47	8.3 g/dL	25.7%

She was transfused with two units of RBCs. The study medication was not discontinued during the event. The patient had experienced a similar drop in Hct levels (from 28 % to 25%) one month prior to study start. At that time she had been transfused with two units of RBC.

Bosentan 125 mg bid. Patient #117 10107: 64 year old female patient with pulmonary hypertension was randomized to 125 mg bosentan bid and on day 111 was rolled over to Open Label study AC-052-354, taking 62.5 mg bosentan bid. No relevant medical history was reported. Concomitant medications included warfarin, furosemide, and omeprazole. The patient was hospitalized on day 10 of the open label study complaining of worsening dyspnea. The previous day at home she had felt dizzy and had a syncopal episode that resulted in a fall during which she had sustained trauma to her left arm. On admission clinical laboratory investigations showed Hgb 8.9. She was diagnosed with normocytic anemia, and study medication was discontinued. The patient received two units of packed red blood cells. Of note: two hemoglobin values done at screening and Day 28 of the blinded study were 16.1 and 15.0.

Bosentan 250 mg bid. Patient# 207 20713: 40 year old female patient with primary pulmonary hypertension was randomized to bosentan 250 mg bid and was rolled over to Open Label study AC-352-054 on day 107, receiving bosentan 62.5 mg bid. No additional medical history was given. Concomitant medication included digoxin, furosemide and spironolactone. The patient was hospitalized on day 8 of the open label study with progressive edema, dyspnea and increasing ascites, considered to be exacerbation of right heart failure, and anemia with no evidence of blood loss. Hgb 9.2, Hct 29 and INR 1.5. She was treated with increased furosemide, dopamine, oxygen and blood transfusion. On Day 27 the patient stopped taking bosentan. Of note: Hemoglobin at randomization: 12.0; day 34: 10.8; day 107: 10.3. WBC decreased from the initial $5.7 \times 10^9/L$ to 3.6; eosinophils remained unchanged and WNL.

2.4.3.All adverse events⁷

Adverse events by body systems are shown below.

⁷ Only adverse events with onset dating from start of treatment to 2 calendar days after the end of treatment are included.

No. and (percent) of patients with at least 1 event per body system

Body system Disorder	Bosentan			Placebo n=69	Placebo subtracted ^ (%)
	125 mg n=74	250 mg n=70	All n=144		
Vascular	18 (24.3)	17 (24.3)	35 (24.3)	9 (13.0)	11.3
Hepatobiliary	4 (5.4)	12 (17.1)	16 (11.1)	3 (4.3)	6.8
Skin, subcutaneous	14 (18.9)	10 (14.3)	24 (16.7)	8 (11.6)	5.1
Immune	1 (1.4)	1 (1.4)	2 (1.4)	0	1.4
Infections	1 (1.4)	3 (4.3)	4 (2.8)	1 (1.4)	1.4
GI	30 (40.5)	24 (34.3)	54 (37.5)	25 (36.2)	1.3
Metabolism and nutrition	2 (2.7)	6 (8.6)	8 (5.6)	3 (4.3)	1.3
Eye	5 (6.8)	5 (7.1)	10 (6.9)	4 (5.8)	1.1
Respiratory, thoracic, mediastinal	35 (47.3)	39 (55.7)	74 (51.4)	39 (56.5)	-5.1
Cardiac	34 (45.9)	23 (32.9)	57 (39.6)	31 (44.9)	-5.3
Nervous	22 (29.7)	23 (32.9)	45 (31.3)	23 (33.3)	-2.0
Musculoskeletal, connective tissue, bone	15 (20.3)	14 (20.0)	29 (20.1)	19 (27.5)	-6.9
General	5 (6.8)	10 (14.3)	15 (10.4)	9 (13.0)	-2.6
Renal and urinary	7 (9.5)	6 (8.6)	13 (9.0)	11 (15.9)	-6.9
Blood and lymph	5 (6.8)	3 (4.3)	8 (5.6)	4 (5.8)	-0.2
Reproductive and breast	1 (1.4)	5 (7.1)	6 (4.2)	3 (4.3)	-0.1
Ear	2 (2.7)	2 (2.9)	4 (2.8)	2 (2.9)	-0.1
Psychiatric	2 (2.7)	1 (1.4)	3 (2.1)	5 (7.2)	-5.1
Injury	0	2 (2.9)	2 (1.4)	1 (1.4)	0
Endocrine	0	1 (1.4)	1 (0.7)	1 (1.4)	-0.7

^from the all bosentan group

The body systems with events reported more often by the all bosentan group compared to the placebo group include vascular, hepatobiliary, skin/subcutaneous, immune, infections, GI, metabolism/endocrine, and eye. The systems with the largest placebo subtracted incidence rates were vascular (11%), hepatobiliary (6.8%), and skin/subcutaneous (5.1%).

Individual adverse events that were reported a) by more than 2 of "all bosentan" patients and b) by 1% or more of the "all bosentan" patients than placebo patients are shown below.

No. and (percent) of patients

Adverse event	bosentan			Placebo n=69	Placebo @subtracted (%)
	125 mg n=74	250 mg n=70	All n=144		
Any event	70 (94.6)	66 (94.3)	136 (94.4)	64 (92.8)	1.6
Abnormal Hepatic function	4 (5.4)	10 (14.3)	14 (9.7)	2 (2.9)	6.8
Edema#	13 (17.6)	9 (12.9)	22 (15.3)	6 (8.7)	6.6
Anemia^	5 (6.8)	2 (2.9)	7 (4.9)	0	4.9
Flushing	7 (9.5)	6 (8.6)	13 (9.0)	3 (4.3)	4.7
Fatigue+	4 (5.4)	4 (5.7)	8 (5.6)	1 (1.4)	4.2
Pruritus	3 (4.1)	2 (2.9)	5 (3.5)	0	3.5
Syncope	6 (8.1)	7 (10.0)	13 (9.0)	4 (5.8)	3.2
Dyspepsia	1 (1.4)	3 (4.3)	4 (2.8)	0	2.8
GE reflux	1 (1.4)	3 (4.3)	4 (2.8)	0	2.8
Palpitations	3 (4.1)	3 (4.3)	6 (4.2)	1 (1.4)	2.8
Hypotension	5 (6.8)	5 (7.1)	10 (6.9)	3 (4.3)	2.6
Dry mouth	3 (4.1)	2 (2.9)	5 (3.5)	1 (1.4)	2.1
Pneumonia	2 (2.7)	3 (4.3)	5 (3.5)	1 (1.4)	2.1
Mouth ulceration	3 (4.1)	0	3 (2.1)	0	2.1
Rectal hemorrhage	3 (4.1)	0	3 (2.1)	0	2.1
Blurred vision	2 (2.7)	1 (1.4)	3 (2.1)	0	2.1
Headache	14 (18.9)	16 (22.9)	30 (20.8)	13 (18.8)	2.0
Contusion	3 (4.1)	1 (1.4)	4 (2.8)	1 (1.4)	1.4
Dermatitis	0	4 (5.7)	4 (2.8)	1 (1.4)	1.4

@the placebo rate is subtracted from the all bosentan rate

^includes hematocrit decreased, hemoglobin decreased

+includes lethargy

#includes lower limb edema, peripheral edema, edema nos

The adverse events with the highest placebo subtracted reporting rates were abnormal hepatic function (6.8%), edema (6.6%), anemia (4.9%), flushing (4.7%), fatigue (4.2%), pruritus (3.5%), and syncope (3.2%).

2.5.Laboratory values

The change from baseline at endpoint for laboratory parameters and including only those patients with baseline and endpoint values are shown in the table below.

Table 23 Change from baseline to study end in laboratory parameters, safety population

(Table T23d / 04MAY01)

Parameter	Unit	Bosentan 125 mg N=74			Bosentan 250 mg N=70			All Bosentan N=144			Placebo N=69		
		N	BL	Change	N	BL	Change	N	BL	Change	N	BL	Change
HEMATOLOGY													
Hemoglobin	g/dL	70	16.1	-0.9	70	16.2	-1.1	140	16.2	-1.0	68	15.7	-0.1
Hematocrit	fraction	70	0.47	-0.02	70	0.48	-0.02	140	0.47	-0.02	68	0.47	0.01
Erythrocytes	10e12/L	70	5.0	-0.2	70	5.1	-0.3	140	5.0	-0.3	68	4.9	0.1
Leukocytes	10e9/L	70	8.8	-1.1	70	8.4	-1.2	140	8.6	-1.1	68	8.3	0.0
Neutrophils	10e9/L	70	5.85	-0.81	70	5.58	-0.92	140	5.72	-0.87	68	5.40	0.23
Lymphocytes	10e9/L	70	2.35	-0.26	70	2.23	-0.21	140	2.29	-0.24	68	2.19	-0.16
Monocytes	10e9/L	70	0.41	-0.03	70	0.44	-0.04	140	0.42	-0.03	68	0.42	-0.02
Eosinophils	10e9/L	70	0.15	0.02	70	0.13	0.01	140	0.14	0.02	68	0.16	-0.02
Basophils	10e9/L	70	0.05	0.00	70	0.06	-0.01	140	0.06	-0.00	68	0.06	-0.00
Platelets	10e9/L	69	223	-13	64	210	10	133	217	-2	67	222	6
CLINICAL CHEMISTRY													
SGOT (ASAT)	U/L	72	20	5	70	23	20	142	22	12	68	21	2
Bilirubin direct	UMOL/L	72	2.7	-0.4	70	2.8	0.2	142	2.7	-0.1	68	2.3	0.1
Bilirubin	umol/L	72	12.1	-3.1	70	11.6	-1.6	142	11.9	-2.3	68	10.4	0.5
SGPT (ALAT)	U/L	72	22	8	70	23	30	142	22	19	68	22	3
Alkaline Phosphat.	U/L	72	75	0	70	80	6	142	77	3	68	74	4
Albumin	g/L	72	40.5	-0.9	70	40.0	0.3	142	40.2	-0.3	68	40.1	-0.9
Creatinine	umol/L	72	93	-8	70	80	-4	142	87	-6	68	85	8
Sodium	mmol/L	71	140	0	70	140	0	141	140	0	68	141	-1
Potassium	mmol/L	71	4.2	0.0	68	4.2	-0.0	139	4.2	-0.0	68	4.2	0.1
Glucose	mmol/L	72	5.66	-0.43	68	5.59	-0.02	140	5.63	-0.23	68	5.97	-0.50
BUN	MMOL/L	72	6.7	-0.7	70	6.0	0.1	142	6.4	-0.3	68	6.5	0.6

Values are means.

Compared to placebo, the bosentan patients had decreased means for hemoglobin, hematocrit, erythrocyte counts. This is consistent with the increased reports of anemia as an adverse event. The bosentan group also had a decrease in mean leukocytes, neutrophils, lymphocytes, and platelets. Mean eosinophil counts are up slightly compared to placebo.

As expected, mean LFTs are increased in the bosentan group compared to placebo, but bilirubin is not. Alk phos is little changed. Changes in creatinine and BUN and serum electrolytes are similar for both treatment groups.

Number and percent of patients with marked laboratory abnormalities are shown below. N.B. Marked abnormalities in LFTs are discussed separately.

Table 17 Incidence of marked laboratory abnormalities in hematology and clinical chemistry variables, safety population

(Table T23 / 04MAY01)

Laboratory Abnormality (*)		Bosentan 125 mg N=74		Bosentan 250 mg N=70		All Bosentan N=144		Placebo N=69	
		No.	%	No.	%	No.	%	No.	%
HEMATOLOGY									
Hemoglobin	HH	0 / 72		0 / 70		0 / 142		0 / 68	
	LL	3 / 72	4.2%	2 / 70	2.9%	5 / 142	3.5%	1 / 68	1.5%
Hematocrit	HH	0 / 72		0 / 70		0 / 142		0 / 68	
	LL	2 / 72	2.8%	2 / 70	2.9%	4 / 142	2.8%	1 / 68	1.5%
Leukocytes	HH	1 / 72	1.4%	0 / 70		1 / 142	0.7%	0 / 68	
	LL	0 / 72		2 / 70	2.9%	2 / 142	1.4%	0 / 68	
Neutrophils	LL	1 / 72	1.4%	2 / 70	2.9%	3 / 142	2.1%	1 / 68	1.5%
	HH	0 / 72		0 / 70		0 / 142		0 / 68	
Platelets	HH	0 / 72		0 / 69		0 / 141		0 / 67	
	LL	0 / 72		1 / 69	1.4%	1 / 141	0.7%	0 / 67	
CLINICAL CHEMISTRY									
Albumin	HH	0 / 73		0 / 70		0 / 143		0 / 68	
	LL	1 / 73	1.4%	0 / 70		1 / 143	0.7%	0 / 68	
Creatinine	HH	0 / 73		0 / 70		0 / 143		0 / 68	
	HH	0 / 73		0 / 70		0 / 143		0 / 68	
Sodium	HH	0 / 73		0 / 70		0 / 143		0 / 68	
	LL	1 / 73	1.4%	0 / 70		1 / 143	0.7%	1 / 68	1.5%
Potassium	HH	0 / 73		0 / 70		0 / 143		0 / 68	
	LL	0 / 73		0 / 70		0 / 143		0 / 68	
Glucose	HH	0 / 73		1 / 70	1.4%	1 / 143	0.7%	0 / 68	
	LL	0 / 73		0 / 70		0 / 143		0 / 68	
BUN	HH	1 / 73	1.4%	0 / 70		1 / 143	0.7%	0 / 68	
	HH	1 / 73	1.4%	0 / 70		1 / 143	0.7%	0 / 68	

Values given are the number of patients with at least one abnormality/number of patients assessed (%)
 HH=above the marked reference range, LL=below the marked reference range

Consistent with the other findings, there were more markedly low hemoglobin (3.5%) and/or hematocrit values (2.8%) compared to placebo (1.5%). In addition, the incidence rate for abnormally low leukocytes and neutrophils were 1.4% and 2.1%, respectively, compared to 0% and 1.5%, respectively, for the placebo group. There was 1 abnormally low platelet count in the bosentan group and none in placebo.

2.5.1 Hemoglobin/hematocrit abnormalities

Patients with marked hemoglobin and/or hematocrit abnormalities are discussed below.

Bosentan 250 mg bid.

Patient 104 10027 (bosentan 250 mg bid.): 64-year-old white male with pulmonary arterial hypertension due to systemic sclerosis. He had a history of gastro-esophageal reflux, and he was treated with aspirin and warfarin. The hemoglobin concentration dropped gradually from 15.7 to 11.9 g/dl after 205 days of treatment with bosentan (hematocrit dropped from 50% to 35% during the same time period). Ankle edema was reported during the trial, and albumin levels decreased slightly (37 to 35 g/l).

Patient 105 10014 (bosentan 250 mg bid.): 48-year-old white female with pulmonary arterial hypertension due to systemic sclerosis. She had a history of lupus erythematosus, anemia, thrombocytopenia, leucopenia and rectal bleeding due to hemorrhoids. After 31 days of treatment with bosentan, hemoglobin concentration dropped from 14.0 to 11.5 g/dl. The low platelet count observed at baseline did not change and the WBC count dropped slightly from 3500 to 2900/ml. The patient was treated with prednisone and aspirin. Patient was decreased to half dose on day 101 and then discontinued on day 115 because of rising LFTs (ALT and AST > 8 x ULN and bilirubin). The lab abnormalities returned to normal after drug was stopped but the patient's underlying pulmonary disease grew worse and she died 32 days later.

Patient 202 20203 (bosentan 250 mg b.i.d.): 71-year-old white female with pulmonary arterial hypertension due to systemic sclerosis. The hemoglobin concentration dropped from 12.1 to 9.9 g/dl after 111 days of treatment with bosentan. The patient was treated with acenocoumarol, but there was no evidence for bleeding. There was no evidence for hemolysis as bilirubin levels decreased. Platelet count increased, and WBC count, which was high at baseline, decreased to normal levels. The slight increase in body weight associated with leg edema and the decrease in albumin levels from 35 to 32 g/l suggested that hemodilution could be the reason for the drop in hemoglobin.

Bosentan 125 mg b.i.d.

Patient 104 10030 (bosentan 125 mg b.i.d.): 67-year-old white male with pulmonary arterial hypertension due to systemic sclerosis. He had a history of gastro-esophageal reflux, and he was treated with aspirin. The hemoglobin concentration decreased from 11.6 g/dl at baseline to 6.9 g/dl on Day 36. He was treated with 4 units of blood (2 units on each of Days 37 and 38). Hemoglobin concentrations were 10.9 and 9.8 g/dl on Days 40 and 63, respectively, and 13.0 g/dl on Day 117 of treatment. There were no relevant changes in WBC or platelet counts, and bilirubin levels decreased. Black stools (occult gastrointestinal bleeding) were reported as an adverse event in the same month.

Patient 104 10139 (bosentan 125 mg bid.): 63-year-old white female with pulmonary arterial hypertension due to systemic sclerosis. She had a history of anemia, and she received 2 units of blood 1 month before the trial due to a significant drop in hemoglobin concentration. She had gastro-esophageal reflux disease, and she was being treated with warfarin. Her hemoglobin concentration dropped from 11.1 g/dl at baseline to 9.9 and 8.4 g/dl on Days 34 and 48 of treatment, respectively. She was treated with 2 units of blood on Day 51. Platelet count increased, and WBC count remained unchanged. Extensive diverticulosis with diverticulitis was detected during colonoscopy, but there was no evidence for bleeding. On Day 111 of treatment, hemoglobin concentration was 12.9 g/dl.

Patient 109 10157 (bosentan 125 mg bid.): 54-year-old white female with pulmonary arterial hypertension due to PPH. She had a history of epistaxis and anemia. During the trial, she continued to suffer from nose bleeds, and her hemoglobin concentration dropped from 10.5 to 8.9 g/dl (Day 30). The patient received 3 units of blood, and on Day 114 her hemoglobin concentration was 11.5 g/dl. Bilirubin, which was high at baseline, dropped; WBC and platelet counts decreased slightly but remained within normal limits. On Day 30, albumin levels were lower than at baseline (29 vs. 35 g/l), and body weight was slightly higher (69 vs 67 kg).

Placebo

Patient 101 10006 (placebo): 66-year-old white female with pulmonary arterial hypertension due to PPH. She had chronic renal failure, and she was being treated with warfarin. Hemoglobin concentration dropped from 12.3 to 8.5 g/dl, which was concomitant with worsening renal function (creatinine increased from 362 μ mol/l at baseline to 530 μ mol/l). She also had vaginal bleeding and blood in the stool, which were considered related to a hiatal hernia and gastric erosions.

2.5.2 Marked abnormalities in LFTs

Patients with marked abnormalities in LFTs are shown below.

Table 18 Incidence of marked laboratory abnormalities in liver function tests, safety population

(Table T23a / 07MAY01)

Laboratory Abnormality (*)	Bosentan 125 mg N=74		Bosentan 250 mg N=70		All Bosentan N=144		Placebo N=69	
	No.	%	No.	%	No.	%	No.	%
LIVER FUNCTION TESTS								
SGOT(ASAT)	HH 10 / 73	13.7%	12 / 70	17.1%	22 /143	15.4%	1 / 68	1.5%
SGOT(ASAT) > 3*upper std	7 / 73	9.6%	8 / 70	11.4%	15 /143	10.5%	0 / 68	
SGPT(ALAT)	HH 14 / 73	19.2%	13 / 70	18.6%	27 /143	18.9%	2 / 68	2.9%
SGPT(ALAT) > 3*upper std	9 / 73	12.3%	10 / 70	14.3%	19 /143	13.3%	0 / 68	
ALT or AST > 3*upper std	10 / 73	13.7%	10 / 70	14.3%	20 /143	14.0%	0 / 68	
Alkaline Phosphat.	HH 2 / 73	2.7%	3 / 70	4.3%	5 /143	3.5%	0 / 68	
Bilirubin	HH 0 / 73		2 / 70	2.9%	2 /143	1.4%	0 / 68	

Values given are the number of patients with at least one abnormality/number of patients assessed (%)
 HH=above the marked reference range, LL=below the marked reference range

The placebo subtracted incidence rates for AST and ALT abnormalities for the all bosentan group were 13.9 % and 16.0 %, respectively. The placebo subtracted incidence rates for AST and ALT abnormalities >3 x ULN were 10.5% and 13.3%, respectively. Incidence rates for markedly abnormal alk phos values were less common (3.5% for all bosentan and 0 for placebo). Incidence rates for markedly abnormal bilirubin values were 1.4% for all bosentan and 0 for placebo.

There were 10 bosentan patients (10 patients for each of the 2 dose groups) with ALT or AST that were > 3 x ULN and of these, 7 had values >8 ULN(5 of 10).

Table 19 Patients with ALT and/or AST > 3 x ULN

Treatment [n (%)]	Total with > 3 x ULN	Patients with ALT and/or AST values		
		> 3 and ≤ 5 x ULN	> 5 and ≤ 8 x ULN	> 8 x ULN
Bosentan 125 mg b.i.d.	10	6	2	2
Bosentan 250 mg b.i.d.	10	4	1	5

Note: The sponsor's upper limit of the normal range was 30 U/l for ALT and 25 U/l for AST.
 ALT = alanine aminotransferase, AST = aspartate aminotransferase, ULN = upper limit of normal.

The outcomes for these patients are described below.

Table 20 Outcomes of patients with ALT and/or AST > 3 × ULN

	Transient value*		Last value [†]		Continued to open-label study	
	Stable dosing	Dose reduction	Premature withdrawn	End of study	Yes	No
Bosentan 125 mg b.i.d.	6	1	0	3	10	0
Bosentan 250 mg b.i.d.	2[‡]	3	3	2	6	4

Note: The sponsor's upper limit of the normal range was 30 U/l for ALT and 25 U/l for AST.

* Transient value = liver function tests decreased to < 3 × ULN while on treatment.

[†] Last value = the last known value on drug before premature discontinuation or interruption of study drug or the end of the study.

[‡] One patient (115 10117) died due to pneumonia/sepsis.

ALT = alanine aminotransferase, AST = aspartate aminotransferase, ULN = upper limit of normal.

Of the 10 patients on bosentan 125 mg bid, 6 remained on the randomized dose and 1 had a dose reduction. There were 3 with an elevated value at the end of the study.

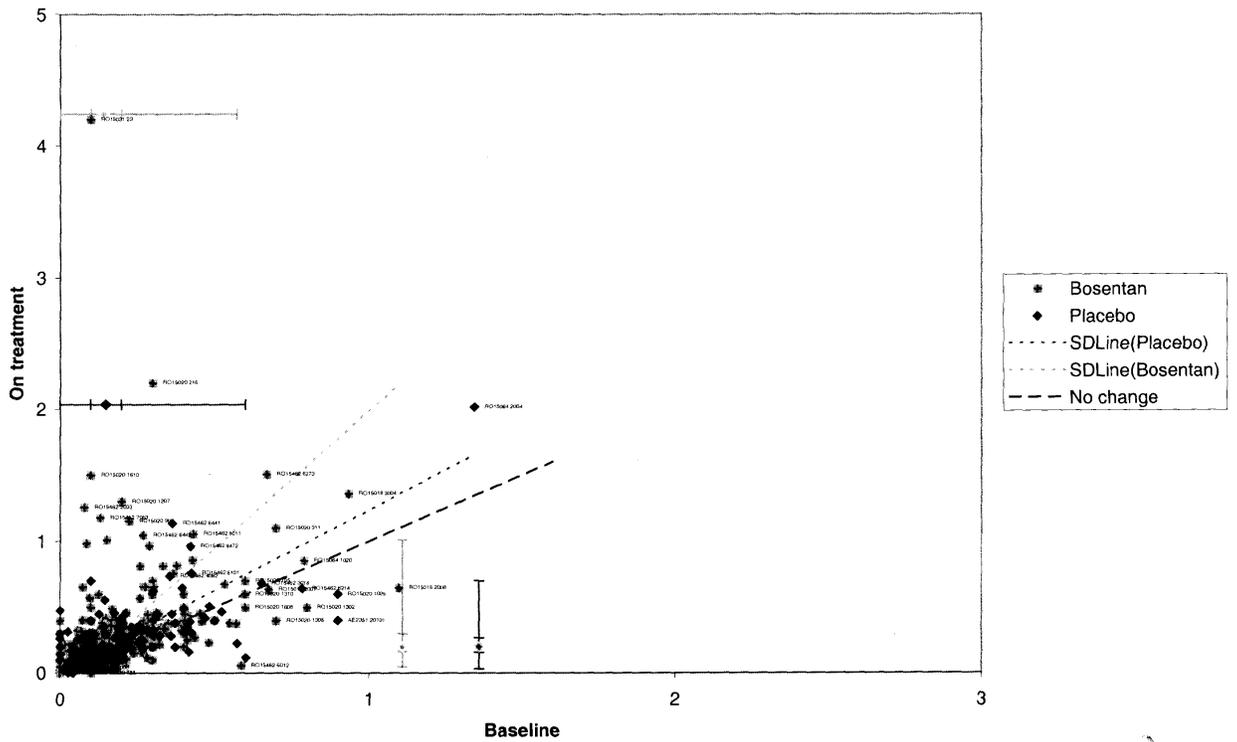
Of the 10 patients on bosentan 250 mg bid, 1 patient died of pneumonia, 2 remained on randomized dose and 3 had a dose reduction. Three patients were withdrawn early (with values > 8xULN) and 2 had dose reduction. Six patients continued into the open label study and 4 did not.

2.5.3. Eosinophils

The following figure shows a plot of individual eosinophil values at baseline and at endpoint. There is a suggestion that there are more bosentan patients with higher values at endpoint compared to placebo patients.

If this is true, this phenomenon is not well understood.

EOSINOPHILS



2.6 Vital signs

Mean values at baseline and endpoint as well as mean changes from baseline at endpoint for heart rate, blood pressure, respiratory rate and weight are shown below.

Table 21 Vital signs: Change from baseline to study end, safety population

(Table T32 / 04MAY01)

	Bosentan 125 mg N=74		Bosentan 250 mg N=70		All Bosentan N=144		Placebo N=69	
Pulse Rate (bpm)								
n	74		68		142		68	
Baseline	82.2 ±	14.0	83.8 ±	13.0	83.0 ±	13.5	80.9 ±	12.0
Last up to end of study	79.5 ±	13.3	85.1 ±	13.5	82.2 ±	13.6	84.2 ±	13.2
Change from baseline	-2.7 ±	13.3	1.4 ±	13.8	-0.8 ±	13.6	3.4 ±	13.7
% Change from baseline	-1.9 ±	17.4	3.2 ±	20.4	0.5 ±	19.0	5.6 ±	18.5
Systolic BP (mmHg)								
n	73		68		141		68	
Baseline	116.9 ±	20.5	117.1 ±	15.9	117.0 ±	18.4	121.5 ±	19.2
Last up to end of study	116.5 ±	16.1	110.6 ±	14.8	113.6 ±	15.7	117.1 ±	17.2
Change from baseline	-0.4 ±	19.0	-6.5 ±	14.9	-3.4 ±	17.4	-4.4 ±	16.4
% Change from baseline	1.3 ±	15.6	-4.8 ±	12.3	-1.7 ±	14.4	-2.7 ±	12.8
Diastolic BP (mmHg)								
n	73		68		141		68	
Baseline	72.9 ±	11.4	73.8 ±	12.4	73.3 ±	11.8	75.0 ±	10.6
Last up to end of study	70.6 ±	12.2	70.6 ±	10.2	70.6 ±	11.2	75.6 ±	11.3
Change from baseline	-2.3 ±	13.0	-3.2 ±	12.3	-2.7 ±	12.6	0.6 ±	10.4
% Change from baseline	-1.6 ±	19.4	-2.7 ±	16.3	-2.1 ±	17.9	1.7 ±	14.2
Respiration Rate (per min)								
n	67		64		131		59	
Baseline	19.6 ±	3.2	19.0 ±	3.3	19.3 ±	3.3	19.7 ±	3.8
Last up to end of study	20.1 ±	4.0	22.0 ±	10.0	21.0 ±	7.6	19.8 ±	3.1
Change from baseline	0.5 ±	4.6	3.0 ±	9.5	1.7 ±	7.5	0.1 ±	3.9
% Change from baseline	4.4 ±	21.0	16.6 ±	42.1	10.4 ±	33.5	3.0 ±	19.6
Weight (Kg)								
n	74		70		144		67	
Baseline	71.5 ±	21.0	70.2 ±	18.0	70.9 ±	19.6	74.0 ±	18.2
Last up to end of study	72.2 ±	21.2	70.8 ±	18.0	71.5 ±	19.7	74.6 ±	18.2
Change from baseline	0.7 ±	3.7	0.6 ±	3.5	0.7 ±	3.6	0.6 ±	4.0
% Change from baseline	1.2 ±	5.0	1.0 ±	4.6	1.1 ±	4.8	1.0 ±	5.7

Note: Values are mean ± standard deviation.
 BP = blood pressure.

Heart rate and weight remained basically unchanged while blood pressure decreased slightly and respiration increased.

2.7 ECG

Changes in ECG intervals are shown below.

Table 22 Change from baseline to study end in quantitative 12-lead ECG parameters, safety population
 (Table T30 / 04MAY01)

	Bosentan 125 mg N=74		Bosentan 250 mg N=70		All Bosentan N=144		Placebo N=69	
PQ (PR) Interval (ms)								
n	65		67		132		66	
Baseline	166.3 ±	23.7	172.7 ±	33.4	169.5 ±	29.1	169.5 ±	29.1
Last up to end of study	167.4 ±	25.1	170.2 ±	42.5	168.8 ±	34.9	171.3 ±	34.4
Change from baseline	1.1 ±	22.2	-2.5 ±	37.2	-0.7 ±	30.7	1.8 ±	22.1
% Change from baseline	1.5 ±	14.6	-0.5 ±	19.2	0.5 ±	17.1	1.6 ±	13.3
QRS Interval (ms)								
n	73		68		141		67	
Baseline	93.0 ±	20.6	94.3 ±	20.3	93.6 ±	20.4	90.1 ±	15.6
Last up to end of study	91.8 ±	20.6	94.6 ±	20.5	93.2 ±	20.6	92.4 ±	14.9
Change from baseline	-1.2 ±	11.8	0.3 ±	13.9	-0.5 ±	12.8	2.4 ±	15.0
% Change from baseline	-0.4 ±	12.9	1.8 ±	18.1	0.7 ±	15.6	5.2 ±	27.4
QT Interval (ms)								
n	73		68		141		67	
Baseline	384.5 ±	43.2	375.3 ±	45.9	380.0 ±	44.6	377.0 ±	44.0
Last up to end of study	386.1 ±	42.4	373.7 ±	47.8	380.1 ±	45.4	375.6 ±	44.1
Change from baseline	1.7 ±	40.1	-1.6 ±	45.1	0.1 ±	42.5	-1.4 ±	37.8
% Change from baseline	1.0 ±	11.1	0.3 ±	13.0	0.7 ±	12.0	0.1 ±	10.3
Heart Rate (bpm)								
n	73		68		141		67	
Baseline	80.2 ±	13.0	83.0 ±	16.2	81.5 ±	14.6	79.9 ±	12.0
Last up to end of study	77.5 ±	13.6	82.0 ±	14.2	79.7 ±	14.0	81.7 ±	14.0
Change from baseline	-2.7 ±	10.4	-0.9 ±	12.9	-1.8 ±	11.6	1.8 ±	11.5
% Change from baseline	-2.8 ±	12.5	0.7 ±	18.3	-1.1 ±	15.6	2.9 ±	14.6

Note: Values are mean ± standard deviation.

There are no signals that bosentan has an adverse effect on cardiac conduction.

The reported ECG abnormalities are shown below.

Table 29 Summary of treatment-emergent ECG findings, safety population

(Table T31 / 04MAY01)

ECG finding	Bosentan 125 mg N=74		Bosentan 250 mg N=70		All Bosentan N=144		Placebo N=69	
	No.	%	No.	%	No.	%	No.	%
Total pts with at least one ECG finding	21	28.4%	25	35.7%	46	31.9%	27	39.1%
Total number of ECG findings	33		34		67		40	
INTRA-VENTRICULAR CONDUCTION DEFECT	2	2.7%	5	7.1%	7	4.9%	1	1.4%
ST-T CHANGES	4	5.4%	4	5.7%	8	5.6%	6	8.7%
RIGHT AXIS DEVIATION	1	1.4%	4	5.7%	5	3.5%	6	8.7%
VENTRICULAR EXTRASYSTOLES	2	2.7%	3	4.3%	5	3.5%	1	1.4%
ATRIAL FLUTTER AND / OR FIBRILLATION	2	2.7%	3	4.3%	5	3.5%	-	
LEFT ATRIAL ENLARGEMENT	1	1.4%	3	4.3%	4	2.8%	-	
OTHER FINDINGS	-		3	4.3%	3	2.1%	-	
SINUS TACHYCARDIA	1	1.4%	2	2.9%	3	2.1%	1	1.4%
SINUS ARRHYTHMIA	-		2	2.9%	2	1.4%	1	1.4%
RIGHT VENTRICULAR HYPERTROPHY	5	6.8%	1	1.4%	6	4.2%	2	2.9%
ATRIOVENTRICULAR BLOCK FIRST DEGREE	1	1.4%	1	1.4%	2	1.4%	1	1.4%
ATRIOVENTRICULAR BLOCK SECOND DEGREE MOBITZ I	-		1	1.4%	1	0.7%	1	1.4%
SUPRAVENTRICULAR EXTRASYSTOLES	-		1	1.4%	1	0.7%	1	1.4%
PACEMAKER RHYTHM	-		1	1.4%	1	0.7%	-	
RIGHT ATRIAL ENLARGEMENT	5	6.8%	-		5	3.5%	6	8.7%
SINUS BRADYCARDIA	2	2.7%	-		2	1.4%	3	4.3%
BILATERAL ATRIAL ENLARGEMENT	3	4.1%	-		3	2.1%	-	
HIGH VOLTAGE	1	1.4%	-		1	0.7%	2	2.9%
PROLONGED QT	-		-		-		4	5.8%
EVIDENCE OF MYOCARDIAL INFARCTION	1	1.4%	-		1	0.7%	1	1.4%
LOW VOLTAGE	1	1.4%	-		1	0.7%	1	1.4%
BUNDLE BRANCH BLOCK RIGHT	1	1.4%	-		1	0.7%	-	
LEFT AXIS DEVIATION	-		-		-		1	1.4%
LEFT POSTERIOR HEMI-BLOCK	-		-		-		1	1.4%

Note: Only new ECG findings observed during randomized treatment or up 2 days after the end of randomized treatment are included.

Slightly more total ECG abnormalities were reported for placebo (39.1%) than for all bosentan (31.9%). Compared to placebo, all bosentan had more reports of intra-ventricular conduction defects, atrial fibrillation/flutter, and right ventricular hypertrophy. There is no indication that these reports are anything other than random variation.

Protocol AC 052-351

AC-052-351 was designed as a small pilot efficacy study. The original protocol is dated May 6, 1999. The study was conducted between September 8, 1999 and April 3, 2000.

3.1.1 Study Design

This was a small double-blind, multicenter, parallel, study with a 2 (drug): 1 (placebo) randomization scheme. Subjects with either symptomatic severe primary pulmonary hypertension (PPH) or pulmonary hypertension secondary to scleroderma (SSc/PH), who were ambulatory and were in functional class III-IV (1998 WHO classification) were eligible to be enrolled.

Subjects were allowed to be receiving any of the following: oral vasodilators, anticoagulants, diuretics, cardiac glycosides and supplemental oxygen provided that none of the treatments was started or stopped within 1 month of screening. Subjects could neither have received prostacyclin therapy within 3 months prior to screening nor have been scheduled to receive prostacyclin therapy for 3 months following randomization.

3.1.2 Study Objective

The objective of this study was to determine if bosentan, compared to placebo, increases peak⁸ exercise capacity in study subjects.

3.1.3. Patient Type

3.1.3.1. Inclusion Criteria

Subjects who were

- 18 years or older;
- either male or non-pregnant female (only those who were post menopausal, surgically sterile or practicing an acceptable method of contraception).
- diagnosed with PPH or SSc/PH and remained in functional class III-IV (1998 WHO classification) despite optimal therapy with oral vasodilators, cardiac glycosides, anticoagulants, diuretics and/or supplemental oxygen for at least one month.
- able to demonstrate a baseline 6-minute walk test of ≥ 150 m and ≤ 500 m.

At randomization visit (visit 2), subjects had to meet the following criteria:

- no change in nature or dosage of background medication for PAH within 7 days prior to randomization;
- the second 6-minute walk test was within 15 % of the screening walk test, or the third 6-minute walk test was within 15% of the second walk test;
- baseline mean PAP > 25 mm Hg (mean of two repeated measures during expiration) and PVR > 3 mm Hg/liter/min (mean of two repeated measures during expiration) and PCWP < 15 mm Hg (measured once only);
- Systolic blood pressure was ≥ 85 mm Hg.

3.1.3.2 Exclusion Criteria

⁸ 2-6 hours after last dose

Subjects were excluded if they had

- pulmonary hypertension as the result of conditions other than PPH or SSc/PH;
- SSc/PH with moderate to severe interstitial disease;
- stopped treatment with oxygen, diuretics, oral vasodilators, cardiac glycosides or anticoagulants within one month of screening;
- started a new treatment with oxygen, diuretics, oral vasodilators, cardiac glycosides or anticoagulants within one month of screening;
- received prostacyclin therapy within 3 months of study screening;
- been scheduled to receive proslacyclin therapy within 3 months of randomization.
- a musculoskeletal disorder or any other condition that limited his/her ability to perform the 6-minute walk tests;
- hypotension, defined as systolic blood pressure < 85 mm Hg;
- hemoglobin, hematocrit or leucocyte count of more than 30% outside the normal range. (Subjects with secondary polycythemia were permitted in the study.)
- ASAT and/or ALAT values greater than 3 times the upper limit of normal.
- received glibenclamide (glyburide) or cyclosporine-A within one month of entry into the study or expected to receive glibenclamide (glyburide) or cyclosporine A during the study period.
- received therapy with another investigational drug within one month.
- a known drug or alcohol dependence or any other factors which would interfere with the study conduct or interpretation of the results.
- any illness other than PAH which might reduce life expectancy to less than 6 months.

3.1.4 Sample Size

The sample size of 30 subjects (20 bosentan and 10 placebo) was based on an assumed change from baseline in walking distance of 50 meters with standard deviation of 50 meters. A total of 32 subjects were randomized (21 received bosentan and 11 received placebo).

3.1.5 Duration

Phase I (double blind phase)

Each subject received a minimum of 12 weeks (between 70 and 98 days) of double blind treatment. In other words, all subjects received double blind treatment until the last enrolled subject, not prematurely withdrawn, completed week 12.

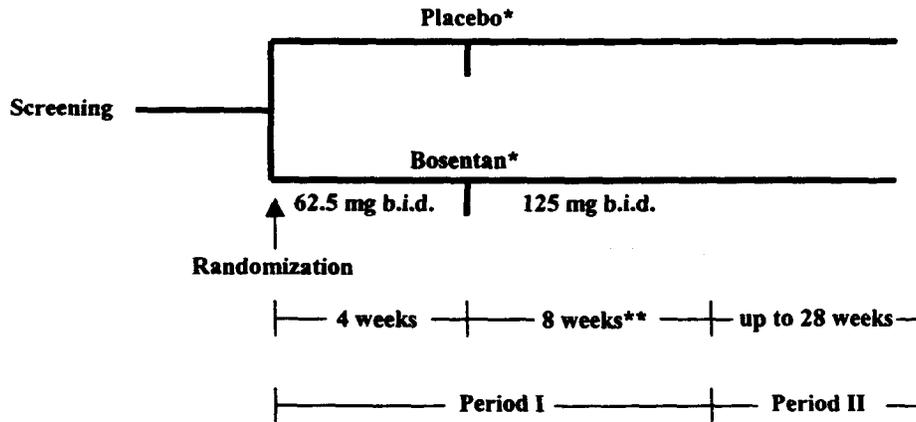
Phase II

All subjects who completed the double blind treatment phase (12 weeks) as well as subjects who dropped out of the study prematurely had the option of entering an open label, uncontrolled phase.

3.1.6 Treatments

Subjects were randomized to placebo bid or bosentan 62.5 mg bid for 4 weeks. The dose of bosentan was then increased to 125 mg bid for at least an additional 8 weeks. Subjects were down titrated to 1 tablet once daily if they experienced intolerable side effects thought to be attributed to study drug. The higher dose was reintroduced if the investigator thought it could be tolerated.

Figure 1 Study Design



The initial 12 weeks of double-blind therapy was designated "Period I" and the part of double-blind treatment following week 12 assessment to the end of the study was designated "Period II". Period I was fixed (a duration of 12 weeks) whereas the duration of Period II was variable (the duration being determined by the timepoint of the last visit of the last patient).

** The dose of study medication was increased in all patients unless the patient experienced drug-related adverse events.*

*** The study was completed when the last enrolled patient not prematurely withdrawn had completed Week 12 assessments.*

3.1.7 Study Procedure

The flow chart (includes amendments) below outlines study procedures at each clinic visit.

Changes: Table 1: Schedule of Assessments

Treatment Week [Study day]	Screening [-14,-1]	Rando- mization*	1 [5-9]	4 [21-35]	8 [49-63]	12 [70-98]	20 [126-154]	28 [182-210]	36 [238-266]	44 [294-322]	Premature withdrawal or Study end
Visit	1	2	3	4	5	6	7	8	9	10	
Informed Consent	X										
History	X										
Physical exam	X					X					X
ECG (12 lead)	X					X					X
Laboratory tests @	All#			Hem, LFT	LFT	All	LFT	LFT		LFT	All#
Functional class	X	X	X	X	X	X	X	X	X	X	X
Exercise 6-minute walk test Borg Dyspnea Index	X	X		X	X	X	X	X	X	X	X
Right heart catheterization and hemodynamics		X°				X**					X**
Vital signs (BP, HR, body weight)	X	X	X	X	X	X	X	X	X	X	X
Dispense/return study medication		X		X	X	X	X	X	X	X	X
Concomitant medications	X	X	X	X	X	X	X	X	X	X	X
Adverse events/ intercurrent illness		X	X	X	X	X	X	X	X	X	X

- * Visits 1 and 2 may be combined if the patient had documented laboratory test results meeting the screening eligibility criteria within 2 weeks prior to randomization
- # includes a pregnancy test in women
- ° Should not be repeated if the patient has had a right heart catheterization as part of his/her regular clinical assessment within 72 hours of the time of randomization
- ** In case of premature withdrawal prior to week 12, right heart catheterization should be performed whenever possible. Right heart catheterization is not required if the week 12 assessment was performed.
- @ LFT: Liver function tests (LFT) include ASAT (SGPT), ALAT (SGOT), bilirubin direct and indirect and alkaline phosphatase.
- All: All tests include hematology ((Hem): hemoglobin, hematocrit, erythrocytes, platelets, white blood cells and differential count, prothrombin time), + liver function tests + creatinine, urea, potassium, sodium, glucose, albumin and urinalysis (dipstick for blood, protein and glucose).

3.1.8 Protocol define study hypotheses and efficacy endpoints:

3.1.8.1 the primary endpoint: change from baseline in exercise capacity at 12 weeks as determined by the 6-minute walk test.

Subjects who died, underwent lung transplantation during the study, or discontinued study medications because of worsening condition were analyzed using their last assessment. In the event that no assessment of walk distance was obtained at the time of premature withdrawal, subjects were assigned a walk distance of 0 meter at the 12-week time point.

All other subjects without a week 12 assessment of the primary endpoint were to have their last assessment carried forward. If no assessment was obtained during the treatment period, the subject was assigned "no change" (0 change from baseline, equivalent to carrying forward the baseline value).

3.1.8.2 secondary endpoints:

-time from randomization to clinical worsening, defined as death from all causes, lung transplantation or discontinuation of therapy because of clinical deterioration. Subjects without documentation of these events were to be included in the analysis as censored;

-changes from baseline in mean trough (10 to 14 hours post-dose) PAP, PVR, CI and RAP at 12 weeks of therapy. Subjects prematurely withdrawn from therapy with a "premature withdrawal" assessment were included in the analysis using the last available assessment on treatment. Subjects without any assessment during the treatment period were assigned worst rank if the reason for premature withdrawal was death, lung transplantation or worsening of their clinical condition. Subjects without any assessment during the treatment period for reasons different than the above were excluded from the analysis;

-changes from baseline in dyspnea index (Borg dyspnea index);

-changes from baseline in functional class;

-changes from baseline in Raynaud's symptom assessment in patients with Raynaud syndrome at baseline.

3.1.9 Disallowed concomitant medications

Prostacyclin or any investigational agent other than bosentan intended for the treatment of PAH. Previous medications for PAH if the medications were started at least 1 month prior to screening) were to be continued. The patient was not to be started on glibenclamide or cyclosporin A during the trial because of possible drug interactions.

3.1.10 The major protocol amendments

Amendment 1, November 15, 1999

- introduce a regulatory requirement to maintain blinding for at least 2 weeks after roll-over to the open-label extension trial (AC-052-353).
- allow acceptance of routine right heart catheterization results as baseline if performed within 72 hours of randomization.
- introduce a potential requirement for patients to rest between repeat 6-minute walk tests.
- allow measurement of hemodynamic parameters at week 12 at peak rather than trough.
- add measurement of pulmonary capillary wedge pressure (PCWP) at 12 weeks.
- add hematological assessment at 4 weeks.
- allow addition of anticoagulant therapy.
- allow use of bottles of study medication for more than 3 months.
- change the reporting instructions for specific SAEs.

3.2 Results

A total of 32 patients were enrolled into double blind treatment phase. There were 6 sites (5 in US and 1 in France) that enrolled between 3 and 10 subjects per site (submission dated 10-30-00).

3.2.1 Patient disposition

Table below shows the outcome for all randomized subjects by treatment group.

Number of patients

	<u>Bosentan</u>	<u>Placebo</u>
No. enrolled	21	11
Completed entire 12 week efficacy period without withdrawing	21	8
No. prematurely withdrawn	0	3

The 3 placebo patients who withdrew did so because of adverse events:

- 1) subject 20101 had right ventricular failure (withdrew on day 50, visit 5),
- 2) subject 10404 had presyncope and increased dyspnea secondary to worsening pulmonary hypertension (withdrew on day 54, visit 5), and
- 3) subject 10105 had worsening of pulmonary hypertension (withdrew on day 77, visit 5).

All but 1 (2010) had a exercise test at the time of withdrawal.

No bosentan patient was withdrawn during the double blind phase.

3.2.2 Demographics

Demographics for the study subjects, by treatment group, are shown below.

	Bosentan	Placebo
	N=21	N=11
SEX		
n	21	11
Males	4 19.0%	-
Females	17 81.0%	11 100%
AGE (years)		
n	21	11
Mean	52.2	47.4
SD	12.2	14.0
Stderr	2.7	4.2
Median	52.0	53.0
Min , Max	33 , 73	25 , 67
WEIGHT (kg)		
n	21	11
Mean	85.9	87.1
SD	22.8	17.7
Stderr	5.0	5.3
Median	78.5	89.8
Min , Max	56.7 , 147	55 , 113.4
HEIGHT (cm)		
n	21	11
Mean	164	164
SD	14	7
Stderr	3	2
Median	164	165
Min , Max	135 , 203	152 , 173
RACE		
n	21	11
CAUCASIAN/WHITE	16 76.2%	9 81.8%
BLACK	3 14.3%	2 18.2%
OTHER	2 9.5%	-

Most subjects were white and female. There were no males in the placebo group while the bosentan group had 4. Mean ages for bosentan and placebo were 52 and 47 years, respectively.

Most subjects were diagnosed with PPH (81% for bosentan and 91% for placebo). The mean number of days from diagnosis to randomization was higher for placebo groups (1091 ± 1032 days) compared to bosentan (634 ± 528 days). The range was from 29 to 2717 days.

3.2.2.1 Concomitant diseases

The majority of subjects had at least 1 concomitant disease. Diseases identified by at least 2 subjects per treatment group are shown below.

Number and (percent) of subjects

Disease	Bosentan N=21	Placebo N=11
any	18 (86)	9 (82)
hypothyroidism	5 (24)	1 (9)
Type 2 diabetes	3 (14)	0
Hypertension	4 (19)	2 (18)
Cardiomegaly	2 (10)	0
Incompetent tricuspid valve	2 (10)	0
Anxiety/depression	5 (24)	3 (27)
Crest syndrome [^]	3 (14)	1 (9)
Dyspepsia	3 (14)	0
Scleroderma	2 (10)	1 (9)
headache	2 (10)	2 (18)

[^]includes calcinosis cutis, Raynaud 's phenomenon, esophageal dysmotility, sclerodactyly, and telangiectasia

Concomitant diseases included those associated with autoimmune disorders (hypothyroidism, Crest syndrome, scleroderma). Two subjects had incompetent tricuspid valves.

3.2.2.2 Concomitant medications

Commonly used concomitant drugs/drug classes are shown below.

Number of subjects

Mediation	Bosentan N=21	Placebo N=11
Any	21	11
Warfarin	15	8
Diuretic [^]	26	14
Ca channel blocker+	10	6
Oxygen	6	1
digoxin	3	0

[^]furosemide, spironolactone, bumetanide, HCTZ, metolazone, indapamide, torsemide
 +diltiazem, amlodipine, nifedipine, verapamil,

All subjects were receiving at least 1 concomitant medication at baseline. The majority of subjects were taking an anticoagulant and at least 1 diuretic. Calcium channel blockers were prescribed for about half of the subjects. Oxygen was used 7 subjects. ACE inhibitors were infrequently used.

3.2.3 Efficacy results

3.2.3.1 Primary endpoint

Results for the total walk distance at peak for the intent to treat population with the 1 placebo patient who did not have a withdrawal assessment being assigned a distance of 0 meters and baseline being the average of the screening and the visit 2 tests are shown below.

TOTAL WALK DISTANCE (m)		
	Bosentan	Placebo
	N=21	N=11
Mean Screening-Visit 2		
n	21	11
Mean	360.5	355.5
SD	96.1	81.8
Stderr	18.8	24.7
Median	380.0	405.5
Min , Max	218 , 483	218.5 , 437
Week 12(*)		
n	21	11
Mean	430.5	349.6
SD	66.4	147.1
Stderr	14.5	44.4
Median	431.0	199.0
Min , Max	294 , 535	0 , 497
Change		
n	21	11
Mean	70.1	-5.8
SD	56.2	120.5
Stderr	12.1	36.3
95% CL of mean	44.5 , 95.6	-86.8 , 75.2
Median	51.0	-6.0
95% CL of median	35.0 , 113.5	-83.3 , 60.0
Min , Max	-24.5 , 196	-267.5 , 224.5
Percent change		
n	21	11
Mean	23.9	-1.4
SD	25.3	44.6
Stderr	5.5	13.5
Median	15.9	-1.4
Min , Max	-7.7 , 88.7	-100.0 , 91.1
DIFFERENCE FROM PLACEBO		
Absolute change		
Mean	75.9	
Stderr	31.0	
95% CL of mean	12.5 , 139.2	
Median	59.0	
95% CL of median	13.2 , 130.3	
p-value t-test	0.0205	
p-value Wilcoxon Rank Sums	0.0190	

(*) Patients prematurely withdrawn in period I are assigned 0 when no assessment is available at the time of premature withdrawal.

The subjects who received bosentan increased their mean distance (\pm SD) from baseline by 70.1 (\pm 56.2) m. The placebo group, on the other hand, had a decrease in their mean distance from baseline of -5.8 (\pm 120.5) m. The difference between the treatment groups was 75.9 (\pm 31.0) m which was significant (p=0.02).

Performing the same analysis but with the last value carried forward for placebo patient 20101 and the baseline being the average of the screening and baseline visit tests shows the following results:

TOTAL WALK DISTANCE (m)
 Patient 20101 with his last value carried forward (in place of a 0 assignment)

	Bosentan	Placebo
	N=21	N=11
Mean Screening-Visit 2		
n	21	11
Mean	360.5	355.5
SD	86.1	81.8
Stderr	18.8	24.7
Median	380.0	405.5
Min , Max	218 , 483	218.5 , 437
Week 12		
n	21	11
Mean	430.5	372.4
SD	66.4	99.2
Stderr	14.5	29.9
Median	431.0	399.0
Min , Max	294 , 535	241 , 497
Change		
n	21	11
Mean	70.1	16.9
SD	56.2	84.4
Stderr	12.3	25.4
95% CL of mean	44.5 , 95.6	-39.8 , 73.6
Median	51.0	-6.0
95% CL of median	35.0 , 113.5	-17.5 , 60.0
Min , Max	-24.5 , 196	-87.5 , 224.5
Percent change		
n	21	11
Mean	23.9	7.1
SD	25.3	30.7
Stderr	5.5	9.3
Median	15.9	-1.4
Min , Max	7.7 , 88.7	-25.0 , 91.1
DIFFERENCE FROM PLACEBO		
Absolute change		
Mean	53.2	
Stderr	24.9	
95% CL of mean	2.3 , 104.0	
Median	56.5	
95% CL of median	12.9 , 108.6	
p-value t-test	0.0411	
p-value Wilcoxon Rank Sums	0.0213	

In this analysis, the placebo subtracted absolute difference from baseline at week 12 was 53.2 (\pm 24.9) m (p=0.04).

3.2.3.1.1 Mean walk distances by visit

The table below shows the mean walk distance at each visit by treatment group.

RO 47-0203, Protocol: AC-052-151

Produced by s

Table T16: Absolute values in total walk distance at scheduled timepoints up to
 Population: ITT

	Bosentan	Placebo
	N=21	N=11

Mean Screening-Visit 2		
n	21	11
Mean	360.5	355.5
SD	86.1	81.8
Stderr	18.8	24.7
Median	380.0	405.5
Min , Max	218 , 483	218.5 , 437

Week 4		
n	21	11
Mean	408.1	385.7
SD	81.2	88.3
Stderr	17.7	26.6
Median	419.0	419.0
Min , Max	275 , 534	221 , 470

Week 8		
n	21	11
Mean	428.3	354.1
SD	67.5	139.9
Stderr	14.7	42.2
Median	451.0	378.0
Min , Max	294 , 523	0 , 488

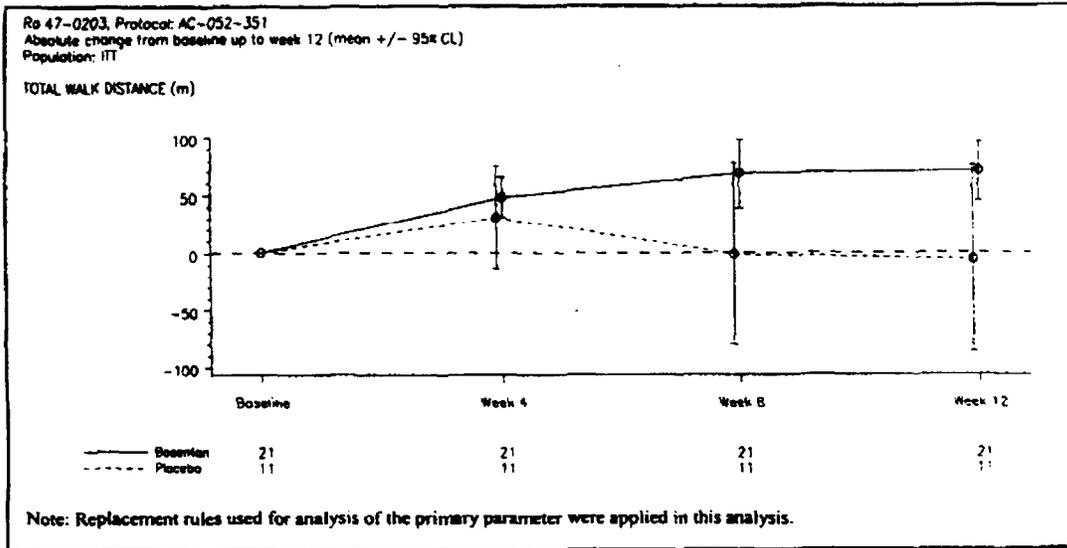
Week 12		
n	21	11
Mean	430.5	349.6
SD	66.4	147.1
Stderr	14.5	44.4
Median	431.0	399.0
Min , Max	294 , 535	0 , 497

(Pag 1/1)

At every visit, the bosentan group walked longer than they did during the previous visit. The largest gain was between baseline (average of screen and visit 2 walk tests) and week 4. There was very little increase in distance at week 12 compared to week 8. In contrast, placebo group walked shorter distances compared to their previous visits, except at week 4. By week 8, the placebo group was walking fewer meters than they did at baseline.

The change from baseline at each visit is shown in the figure below.

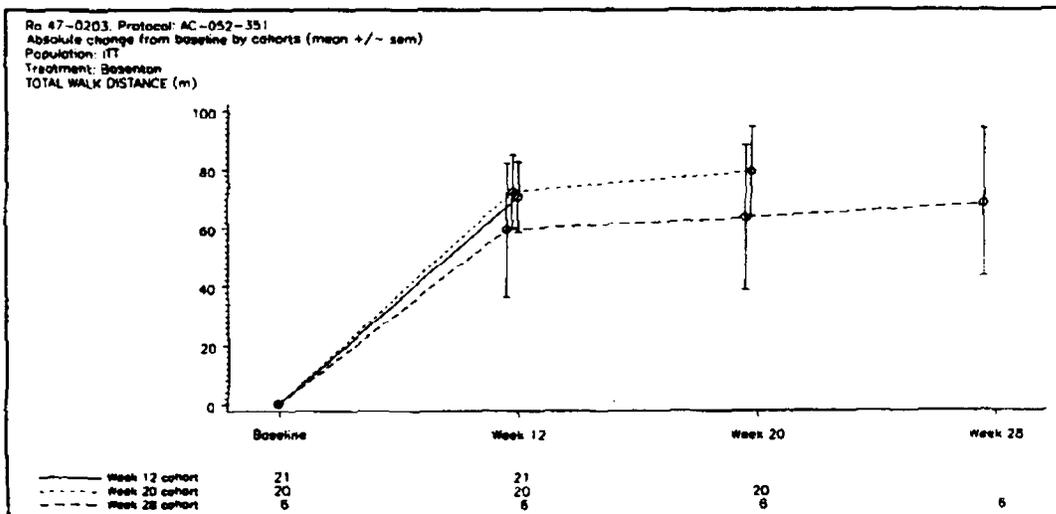
Figure 3 Walk Distance: Change from Baseline to Week 12



Beyond week 12

The subjects who remained in the trial beyond the 12 week cut off continued to perform walk tests. The changes from baseline at weeks 12, 20 and 28 are shown in the figure below.

Figure 4 Walk Distance: Change from Baseline to End of Study for Patients receiving Bosentan Treatment



3.2.3.2 Secondary endpoints

Although there is no comparison group, there is no evidence that patients who remained on bosentan deteriorated by week 28 (n=6).

3.2.3.2.1 Time from randomization to clinical worsening, defined as death from all causes, lung transplantation or discontinuation of therapy because of clinical deterioration. Patients without documentation of any of the events listed above were to be included in the analysis as censored observations from randomization to the last date the patient was known to be free of any of these events.

This analysis was not done because of the small number of patients (3) who experienced clinical worsening. However, the difference between treatment groups (3 on placebo and 0 on bosentan) was statistically significant (Fischer’s exact test p=0.033).

3.2.3.2.2.Changes from baseline in mean hemodynamic parameters (PAP, PVR, CI and RAP) at 12 weeks of therapy. Patients prematurely withdrawn from therapy with a premature withdrawal assessment were to be included in the analysis using the last available assessment on treatment. Patients without any assessment during the treatment period were to be assigned worst rank if the reason for premature withdrawal was death, lung transplantation or worsening of their clinical condition. Patients without any assessment during the treatment period for reasons different than the above were to be excluded from the analysis.

Absolute change from baseline at week 12

	Bosentan N=20	Placebo N=10	p-value
PAP (mmHg)	-1.6	5.1	0.0134
PVR(dyn.sec.cm ⁻⁵)	-223	191	0.0002
CI (L/min/m2)	0.50	-0.52	<0.0001
RAP (mmHg)	-1.3	4.9	0.001
PCWP (mmHg)	0.1	3.9	0.0353

Changes in hemodynamic parameters favored bosentan.

3.2.3.2.3 Changes from baseline dyspnea index (Borg dyspnea index). Bosentan group had a mean improvement at week 12 of 0.19 compared to a worsening in the placebo group (1.36). The difference between treatment groups was borderline significant (p=0.052).

3.2.3.2.4.Changes from baseline in WHO functional class The figure below shows the baseline and endpoint WHO classifications for the study patients.

Table 14 WHO Functional Class: Change from Baseline to Week 12

		Study end(*)				
		Baseline	I	II	III	IV
Bosentan	Valid: 21	I	-	-	-	-
		II	-	-	-	-
		III	-	9 42.8%	12 57.1%	-
		IV	-	-	-	-
		Improved patients:		9 42.9%		
		95% CL		21.8 - 66.0		
Placebo	Valid: 11	I	-	-	-	-
		II	-	-	-	-
		III	-	1 9.0%	8 72.7%	2 18.1%
		IV	-	-	-	-
		Improved patients:		1 9.1%		
		95% CL		0.2 - 41.3		

TREATMENT EFFECT

Difference	33.8%
95% CL	-5.6% - 58.0%
p-value Fisher Exact Test	0.106
p-value Wilcoxon Rank Sums	0.019

(*) One bosentan and 1 placebo patient had no week 12 visit. For both of these patients, the last valid value was carried forward. Two placebo patients withdrew from the study due to clinical worsening but had a valid value from the end of study visit. This value was used for the week 12 assessment.

In the bosentan treatment group, 9 subjects improved (43%) and 12 stayed the same. There were no deteriorations. In the placebo treatment group, 1 subject (9%) improved, 8 stayed the same and 2 deteriorated (18%). This difference was not statistically significant.

3.2.3.2.5 Changes from baseline in Raynaud's symptom. This assessment was to included patients with Raynaud syndrome at baseline. Since there were only 2 subjects with data, this parameter was not analyzed.

In conclusion, bosentan, compared to placebo, improved walking distance, hemodynamic parameters, and WHO functional class in subjects with pulmonary hypertension.

3.4 Safety

3.4.1 Duration of treatment

Summary of the duration of treatment are shown below for the 2 treatment groups.

Table 15 Summary of Duration of Treatment

	Bosentan N=21	Placebo N=11
Duration of trial treatment (days)		
Mean	132	105
SD	36	40
Std err	8	12
Median	119	105
Min , Max	83 , 197	51 , 202

Mean duration of treatment was longer for bosentan (132 days) compared to placebo (105 days).

3.4.2. Serious safety

3.4.2.1 Deaths

There were no deaths in either treatment group during the trial and up to 4 weeks of follow up.

3.4.2.2 Discontinuations for adverse events

There were 3 subjects, all randomized to placebo, who withdrew from the trial. They are briefly listed below.

Subject ID/age/sex	Reason for discontinuation
20101/56/F	Right ventricular failure day 50 of placebo treatment. Treated with Flolan® and condition improved
10404155/F	Presyncope and increased dyspnea with worsening PH on day 54 of placebo treatment. Treated with Flolan®
10105/61/F	Clinical worsening of PH on day 77 of placebo treatment. Treated with Flolan® and condition improved

3.4.2.3 Serious Safety (submission dated 10-30-00)

There were 2 serious safety reports:

- 1 bosentan subject reported pharyngeal streptococcal infection and
- 1 placebo patient reported syncope.

Both subjects remained in the study and the events resolved.

3.4.3.All adverse events

The following table shows the adverse events, serious and non serious, reported by treatment group.

Table 17 Summary of Adverse Events (including Unrelated) by Frequency

Body system / Adverse event	Bosentan N=21		Placebo N=11	
	No.	%	No.	%
ALL BODY SYSTEMS				
Total patients with at least one AE	20	95.2%	11	100%
Total number of AEs	62		47	
HEADACHE	6	28.6%	3	27.3%
UPPER RESPIRATORY TRACT INFECTION	4	19.0%	2	18.2%
BRONCHITIS	3	14.3%	1	9.1%
ARTHRALGIA	3	14.3%	-	-
DYSPEPSIA	3	14.3%	-	-
INFLUENZA LIKE ILLNESS	3	14.3%	-	-
NAUSEA	2	9.5%	3	27.3%
DYSPNEA	2	9.5%	1	9.1%
FLUSHING	2	9.5%	1	9.1%
CHEST PAIN	2	9.5%	-	-
DIZZINESS (EXCLUDING VERTIGO)	2	9.5%	-	-
PALPITATIONS	2	9.5%	-	-
PAIN IN LIMB	1	4.8%	2	18.2%
VISION BLURRED	1	4.8%	2	18.2%
VOMITING	1	4.8%	2	18.2%
BACK PAIN	1	4.8%	1	9.1%
DIARRHEA	1	4.8%	1	9.1%
NASOPHARYNGITIS	1	4.8%	1	9.1%
AGGRAVATED PULMONARY HYPERTENSION	-	-	3	27.3%
SYNCOPE	-	-	3	27.3%

Note: only AEs that occurred more than once in the overall study population are shown.

Nearly all subjects reported at least 1 adverse event. It is difficult to associate any adverse event with bosentan use based on these small sample sizes.

3.4 ECG parameters

At baseline, nearly all subjects showed cardiac abnormalities that were probably signs of chronic PAH such as right ventricular hypertrophy, left atrial enlargement, right bundle branch block, nonspecific ST changes, T wave abnormalities.

ECG intervals at baseline and endpoint are shown below by treatment group.

Means at baseline and endpoint

Parameter	Bosentan n=20		Placebo n=10	
	Mean baseline	Mean endpoint	Mean baseline	Mean endpoint
Heart rate (bpm)	80.2	79.1	86.7	89.9
PR (msec)	174.4	174.8	166.3	166.9
QRS (msec)	93.8	91.6	91.1	89.5
QT (msec)	381.5	382.1	353.7	356.6

Nothing in the above table indicates that bosentan causes changes in ECG intervals although the samples sizes are really too small to allow for reasonable interpretation.

3.5 Laboratory values

Mean change from baseline at endpoint for selected laboratory values are shown below.

Mean change (+SD)

Lab value	Bosentan	Placebo
	n=21	n=11
Hemoglobin(/dl)	-0.6(1.1)	0.5(1.4)
Hematocrit (%)	-0.01 (0.03)	0.02 (0.04)
Leukocytes(10/L)	-0.67 (1.31)	1.06 (1.35)
Neutrophils (10/L)	-0.75 (1.31)	0.84(1.15)
Lymphocytes (10 /L)	-0.14 (0.54)	0.17 (0.38)
Plateletes (10 /L)	-17 (33)	13 (36)
Prothrombin time (sec)	1.0 (4.3)	1.2 (5.8)
INR	-0.11 (0.80)	0.09 (1.39)
ALT (U/L)	1.4 (5.2)	-3.2 (7.8)
AST (U/L)	0.6 (5.4)	-0.6 (6.2)
Total bilirubin (umol/L)	-4.2 (5.1)	0.9 (5.8)
Creatinine (umol/L)	-2 (11)	10 (9)
Sodium (mmol/L)	1 (2)	0 (3)
Potassium (mmol/L)	0.1(0.4)	0.1(0.4)
BUN(mmol/L)+	-0.3(2.0)	1.0(1.6)

+combination of blood urea and urea nitrogen with same unit and reference ranges

The mean changes in these lab values are similar for the 2 treatment groups.

The incidence of marked laboratory abnormalities are shown in the table below.

Appendix 19 Incidence of Marked Laboratory Abnormalities

		Bosentan N=21		Placebo N=11	
		No.	%	No.	%
HEMATOLOGY					
Hemoglobin	HH	0 / 21		0 / 11	
	LL	0 / 21		0 / 11	
Hematocrit	HH	0 / 21		0 / 11	
	LL	0 / 21		0 / 11	
Leukocytes	HH	0 / 21		0 / 11	
	LL	0 / 21		0 / 11	
Neutrophils	LL	0 / 21		0 / 11	
	HH	0 / 21		0 / 11	
Eosinophils	HH	0 / 21		0 / 11	
	LL	0 / 21		0 / 11	
Platelets	HH	0 / 21		0 / 11	
	LL	0 / 21		0 / 11	
Prothrombin time	HH	5 / 20	25.0%	6 / 10	60.0%
	LL				
CLINICAL CHEMISTRY					
ALT	HH	2 / 21	9.5%	0 / 11	
	LL				
AST	HH	1 / 21	4.8%	0 / 11	
	LL				
Bilirubin total	HH	0 / 21		1 / 11	9.1%
	LL				
Alkaline Phosphate	HH	1 / 21	4.8%	0 / 11	
	LL				
Albumin	HH	0 / 21		0 / 9	
	LL	0 / 21		0 / 9	
Creatinine	HH	0 / 21		0 / 11	
	LL				
Sodium	HH	0 / 21		0 / 11	
	LL	0 / 21		0 / 11	
Potassium	HH	0 / 21		0 / 11	
	LL	0 / 21		0 / 11	
Glucose	HH	0 / 21		0 / 10	
	LL	0 / 21		0 / 10	
Blood Urea	HH	0 / 21		0 / 11	
	LL				
URINE					
Urine Protein	HH	1 / 20	5.0%	2 / 9	22.2%
	LL				
Urine Blood	HH	0 / 20		2 / 9	22.2%
	LL				

Blood Urea is the combination of blood urea and urea nitrogen with same unit and reference ranges.

(Page 1 of 1)

The marked laboratory abnormalities cited more often in the bosentan group than the placebo group were high ALT (2 subjects, 9.5% vs 0) and AST (1 subject, 4.8% vs. 0). These subjects are described below.

-#101 10106 was a 56 year old female who had a high ALT and total bilirubin at baseline (60 IU/L and 1.5 mg/dl). The ALT increased to 170 IU/L during the trial and then dropped to 61 IU/L even though the subject was still taking drug. Total bilirubin values normalized during the trial. The only adverse event reported was herpes zoster.

#20 1 20102 was a 49 year old female who had a high ALT, AST and total bilirubin at baseline (40 IU/L, 38 IU/L, and 18 umol/L, respectively). The ALT and AST both increased to 97 IU/L and 86 IU/L during the trial and then dropped to baseline levels even though the subject remained on drug. Total bilirubin values normalized during the trial. The only adverse event reported was bronchitis.

The high percentage of placebo (60%) as well as bosentan (25%) subjects had markedly abnormal prothrombin time.

3.6 Vital signs

Mean baseline and change from baseline at endpoint for heart rate, blood pressure, and respiration rate are shown below.

Table 18 Change from Baseline to the End of Study in Vital Signs

	Bosentan N=21	Placebo N=11
PULSE RATE (bpm)		
Baseline		
Mean	82.6	87.7
Std err	3.6	3.5
Change		
Mean	-0.1	3.1
Std err	2.4	3.8
SYSTOLIC BLOOD PRESSURE (mmHg)		
Baseline		
Mean	128.1	122.1
Std err	4.3	6.3
Change		
Mean	-11.4	-0.6
Std err	3.7	4.6
DIASTOLIC BLOOD PRESSURE (mmHg)		
Baseline		
Mean	77.0	76.7
Std err	2.4	4.1
Change		
Mean	-7.4	1.2
Std err	2.5	3.4
RESPIRATION RATE (units/min)		
Baseline		
Mean	19.5	19.9
Std err	0.6	0.8
Change		
Mean	1.3	-0.1
Std err	1.2	1.1

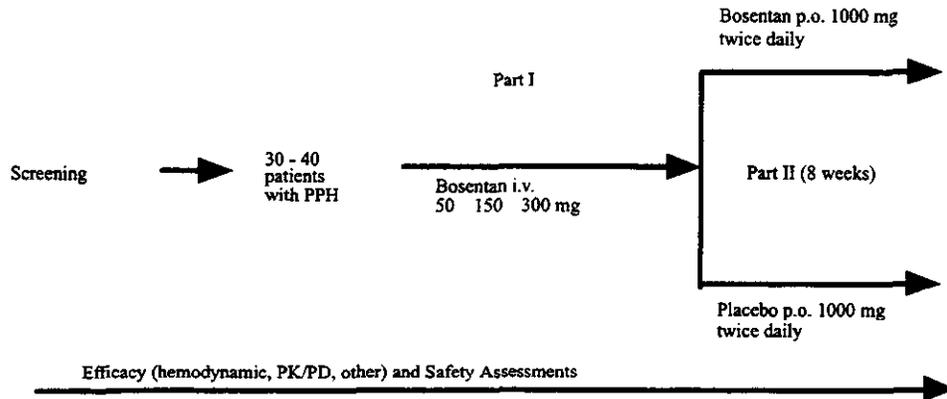
There was little change from baseline for heart rate in the bosentan group. Blood pressure, on the other hand, decreased 11.4/7.4 mmHg from baseline. This is compared to a change of -0.6/1.2 mmHg in the placebo group.

4.0 Acute cardiac hemodynamics

Study BD 14884 was designed to explore the acute hemodynamic effects of 1 day dosing with open label intravenous (iv) bosentan followed by 8 weeks of blinded oral bosentan or placebo in patients with PAH. The study was discontinued prematurely after 2 out of 7 patients died shortly after receiving iv bosentan. The original plan was to enroll 30 patients.

The study flow chart is shown below.

Figure 1. Study Flow Chart



At the time the study was discontinued, 7 patients had completed the iv dosing and had been randomized to either bosentan 1000 mg bid, or placebo. One placebo and one bosentan patient had completed the trial. The efficacy evaluation of this trial in this section is limited to the cardiac hemodynamics.

4.1 Subjects

Inclusions

The study population consisted of subjects who were between the ages of 18 and 70 years, inclusive, had a diagnosis of PPH with or without limited scleroderma, had a mean pulmonary artery pressure (MPAP) > 25 mmHg on day 1, and were on warfarin, unless contraindicated.

Exclusions

Subjects were excluded if there was evidence of significant interstitial lung disease, left heart failure (PCWP > 12 mmHg amended to > 15 mmHg), significant hypotension (BP < 90/60 mmHg), significant right heart failure (MRAP \geq 15 mmHg), obstructive pulmonary disease or chronic heart failure, secondary pulmonary hypertension (the Eisenmenger syndrome, etc.), known autoimmune diseases apart from limited scleroderma, clinically significant endocrine diseases or malignant neoplasm, clinically significant cardiac, respiratory, hepatic, renal, gastro-intestinal, neurologic or hematologic disease, requirement for continuation of therapy with calcium antagonists, hyperkalemia and/or acidosis at screening, inability or unwillingness to abide reliably by the requirements of the protocol, ingestion of any investigational drug within 1 month prior to this study

This study was performed at a single center in Australia. Subjects were stratified based on presence or absence of limited scleroderma and randomly allocated in a 1:1 ratio to receive either bosentan or placebo.

4.2 Dosing

Three ascending single doses of bosentan were administered according to the following scheme: 1st infusion of 50 mg over a period of 5 minutes, 2nd infusion of 150 mg over a period of 10 minutes and starting 120 minutes after the start of the first infusion, and 3rd infusion of 300 mg over a period of 15 min and starting 240 minutes after the start of the first infusion.

4.3 Results

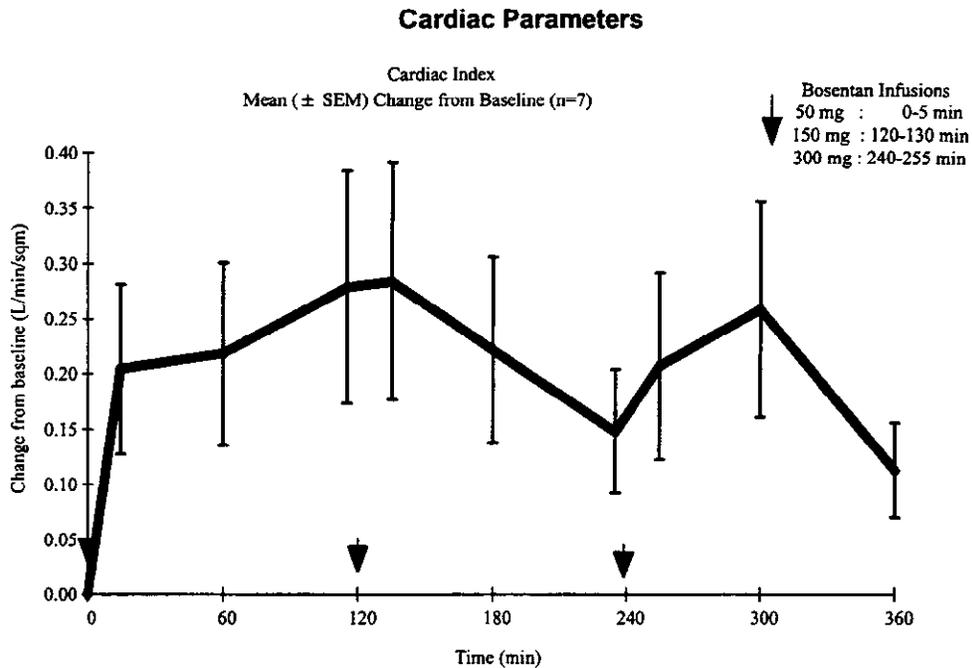
The demographics and characteristics of the 7 subjects: all were female, mean age was 52 years (range 28-68), 6 were white and 1 was “other”, 3 were NYHA functional class I or II and 4 were class III or IV. Previous medications taken by more than 2 subjects that were continued during the study were antithrombotics, diuretics, and antihypertensives.

Out of the 7 subjects enrolled into the study, 2 died on study day 3 and the study was discontinued. At the time of the second death, 3 subjects had entered the oral treatment phase. Two subjects were withdrawn from the study during the 5th and 6th week of treatment with oral bosentan because the study had been discontinued, and the other subject was withdrawn from the study during week 8 of oral bosentan treatment because of a serious adverse event (chest infection requiring hospitalization).

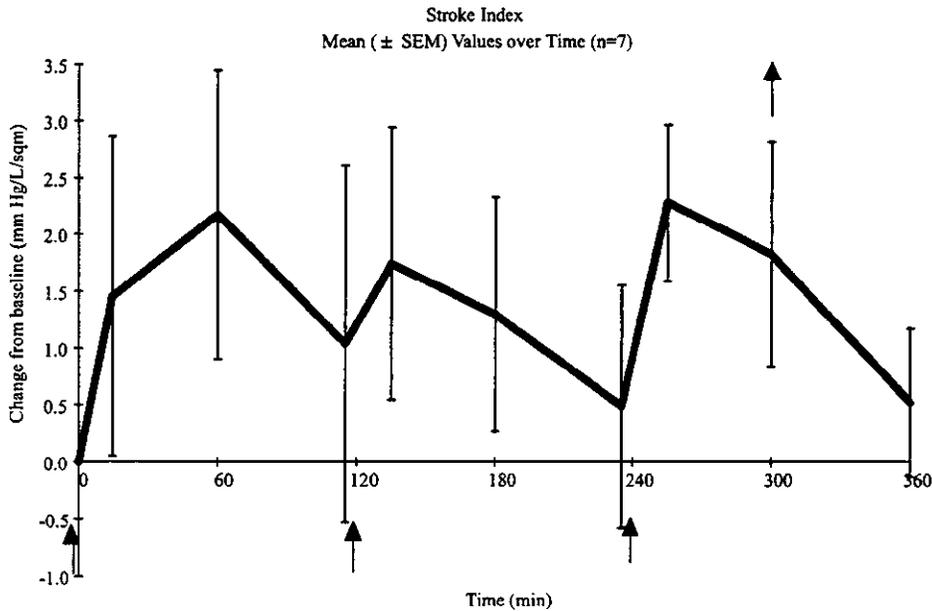
4.4 Efficacy

The mean changes from baseline in mean cardiac index, stroke index, PAP and pulmonary arterial pressures versus time for the 3 infusions (arrows) are shown below. The reliability of results from this small, uncontrolled, open label trial that was terminated early is questionable.

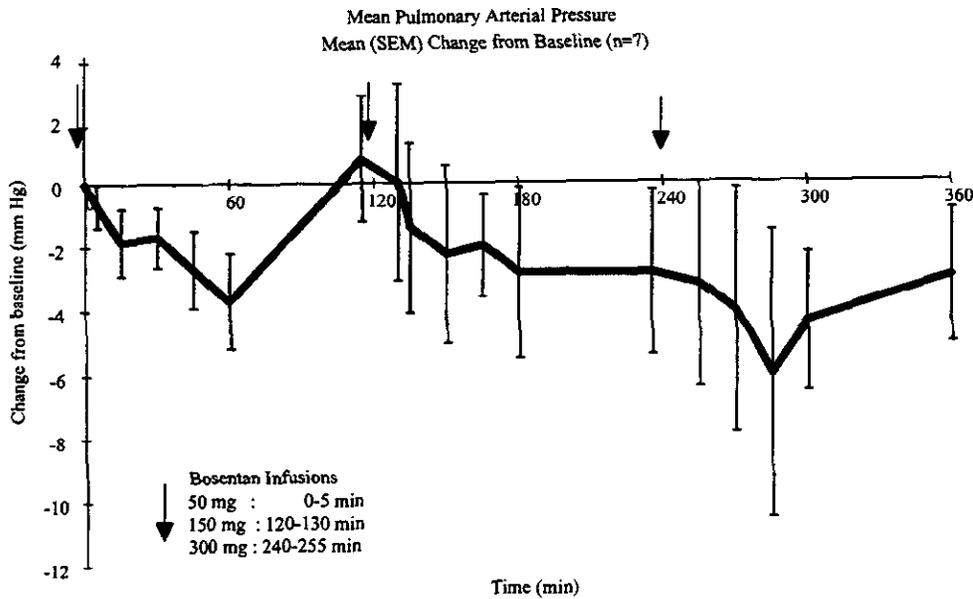
4.4.1 Mean cardiac index



4.4.2 Mean stroke index



4.4.3 Mean PAP



In this study, bosentan tended to increase cardiac and stroke index and decrease mean PAP.

4.5 Safety

Of the 7 subjects, all but 2 received the full 300 mg dose of bosentan. The remaining subjects received only 2 infusions (total 200 mg). Subject #5 did not receive the 3rd bosentan infusion because her blood pressure fell to 91/57 mmHg 3 hours after starting the infusion. She was asymptomatic and was began the oral phase on a reduced dose of bosentan.

4.5.1 Deaths

There were 2 deaths (out of 7 patients who received drug), both occurred shortly after completion of the iv infusions.

Subject #3 was a 50-year-old woman, had a history of severe PH, right heart failure, and tricuspid regurgitation. On the first study day, she completed all 3 doses of bosentan infusion over 4 hours. Day 1 was uneventful and the subject had a reduction (max. 22%) in PVR, a 15% reduction in SVR, and an increase in CO. On study day 2, she was randomized to oral therapy (placebo). The subject's blood pressure on the morning of day 2 was 79/56 mmHg. Her systolic pressures remained below 100 mmHg despite treatment with dopamine, adrenaline and fluids. During the night, she became dyspneic, oliguric, and her platelet count fell. She remained dyspneic on the morning of Day 3, experienced a respiratory arrest, and died. There was no postmortem examination.

Subject #7 was a 66-year-old woman, had long term scleroderma for 20 years, Raynaud's phenomenon, esophagitis, recurrent pericarditis, a mild peripheral neuropathy, and progressive breathlessness attributed to PH. She did not receive anticoagulation because of history of GI hemorrhage. At the start of the study she was found to have an elevated neutrophil count and poor exercise tolerance. The bosentan infusion was uneventful. The maximum reduction in her PVR was 16%. The following morning, the patient's condition remained stable and she was judged to be suitable for randomization into the oral phase. Immediately after the removal of the Swan-Ganz catheter sheath, the patient became acutely unwell and was reported to be cold and clammy, but normotensive. She complained of throat tightness, breathlessness, and nausea. She received glyceryl trinitrate, which produced a significant reduction in her BP, but no improvement in symptoms. She improved spontaneously over the next 45 minutes, but was discontinued from the trial. In the afternoon she had a repeat episode and was treated with nitrates and sublingual nifedipine, which resulted in hypotension. She was treated aggressively but became oliguric and was started on mechanical ventilation. Her condition remained stable overnight but deteriorated over the course of the following morning and died that afternoon. A post-mortem examination disclosed pulmonary edema with bilateral pulmonary effusions.

4.5.2.Serious Adverse events

The following adverse events were reported: hypotension, chest pain, dyspnea, febrile enteric illness during oral dosing, clinical deterioration 10 days after study termination, and breast cancer.