



Name of Sponsor/Company	Name of Finished Product	Name of Active Ingredient
Myogen, Inc.	Ambrisentan	Ambrisentan
Protocol Number: AMB-222		
Title of Study: A Phase 2, Open-label, Multicenter Study Evaluating Ambrisentan in Subjects with Pulmonary Arterial Hypertension Who Have Previously Discontinued Endothelin Receptor Antagonist Therapy Due to Serum Aminotransferase Abnormalities		
Investigators and Study Centers: 17 Investigators in 17 sites enrolled subjects in the United States, Australia, Belgium, and The Netherlands.		
Publication (reference): No publication		
Study Period (years): Ongoing study Date of First Enrollment: 19 May 2005 Data Cut-off Date: 16 February 2006	Phase of Development: Phase 2	
Objectives: The primary objective of this study was to evaluate the incidence of increased serum aminotransferase concentrations in subjects who had previously discontinued endothelin receptor antagonist (ERA) therapy (bosentan or sitaxsentan) due to serum aminotransferase abnormalities. The secondary objectives of this study were to evaluate the safety, tolerability, and efficacy of ambrisentan in this patient population.		
<p>Methodology: This open-label, single-arm, Phase 2 study evaluated the incidence of increased serum aminotransferase concentrations after the initial 12 weeks of ambrisentan therapy in subjects who previously discontinued ERA therapy due to serum aminotransferase abnormalities. This study also evaluated the long-term safety, tolerability, and efficacy of ambrisentan.</p> <p>All eligible adult subjects received 2.5 mg ambrisentan daily for a period of 4 weeks before increasing the dose to 5 mg daily. After Week 24, Investigators were allowed to adjust the dose of ambrisentan as clinically indicated (available doses were 2.5, 5, and 10 mg). For doses greater than 2.5 mg, dose reduction was permitted in the event of ambrisentan intolerance. Dose reduction was required if a subject had confirmed aminotransferases concentrations $>3xULN$ (upper limit of normal) and $\leq 5xULN$ that were related to ambrisentan. Ambrisentan was to be discontinued if a subject had confirmed aminotransferase concentrations $>5xULN$ that were related to ambrisentan.</p> <p>Subjects were monitored with clinical laboratory tests every 2 weeks and assessed for safety and efficacy every 4 weeks. The primary endpoint was assessed after 12 weeks of treatment. After Week 12, subjects who continued to receive ambrisentan were monitored with clinical laboratory tests every 4 weeks and were assessed for safety and efficacy every 12 weeks. After Week 48, subjects continued to be monitored with clinical laboratory tests every 4 weeks and were assessed for safety and efficacy every 24 weeks. Subjects could continue to receive ambrisentan until the Investigator or subject chose to stop ambrisentan treatment, the cumulative adverse event (AE) profile did not warrant continuation of the study,</p>		

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<p>ambrisentan became commercially available, or Myogen stopped the study.</p> <p>Several efficacy endpoints were examined: the 6-minute walk distance (6MWD), Borg dyspnea index, World Health Organization (WHO) functional class, and SF-36[®] Health Survey. Differences between subjects on concomitant pulmonary arterial hypertension (PAH) therapies (ambrisentan only, ambrisentan/sildenafil, ambrisentan/prostanoid therapy, or ambrisentan/sildenafil/prostanoid) were explored.</p> <p>Blood samples were collected to examine the pharmacokinetics of ambrisentan in all subjects and to explore the possibility of any pharmacokinetic interactions between ambrisentan and sildenafil in subjects receiving concomitant sildenafil therapy.</p> <p>Male subjects were to provide semen and hormone samples to evaluate the potential effects of ambrisentan on male fertility.</p> <p>To ensure the safety and welfare of the study subjects, a Data and Safety Monitoring Committee (DSMC) was to review safety summaries on a quarterly basis.</p>		
<p>Number of Subjects (planned and analyzed):</p> <p>Approximately 30 subjects were to be enrolled in this study.</p> <p>A total of 36 subjects diagnosed with PAH and who had previously discontinued ERA therapy (bosentan or sitaxsentan) due to serum aminotransferase abnormalities were enrolled in this open-label study.</p>		
<p>Diagnosis and Main Criteria for Inclusion:</p> <p>Men and women, 12-75 years of age, with idiopathic PAH, familial PAH, or PAH associated with connective tissue disease, congenital systemic-to-pulmonary shunts, anorexigen use, or human immunodeficiency virus (HIV) infection were eligible to be enrolled in this study. Subjects were required to have previously discontinued ERA therapy (bosentan or sitaxsentan) due to serum aminotransferase abnormalities >3xULN and to have normal (<1xULN) serum aminotransferase concentrations at screening. Subjects were required to have a documented history of PAH and to be able to walk at least 150 meters in the 6-minute walk test (6MWT).</p> <p>Subjects receiving sildenafil or chronic prostanoid therapy were also eligible for this study. Subjects who previously discontinued ERA therapy due to serum aminotransferase abnormalities and who transitioned to other therapies (i.e., sildenafil or chronic prostanoid therapy) were eligible provided that they were stable on their current therapy and had no dose changes within 4 weeks of screening.</p>		
<p>Test Product, Dose and Mode of Administration, Batch Number:</p> <p>Ambrisentan was provided as oral tablets of 3 different strengths: 2.5 mg, 5 mg, and 10 mg of the active ingredient (S)-2-(4,6-dimethyl-pyrimidin-2-yloxy)-3-methoxy-3,3-diphenylpropanoic acid. Ambrisentan tablets were manufactured by Abbott Laboratories, Inc. and were packaged by Aptuit, LLC. Lot numbers were: L0001849 for the 2.5-mg</p>		

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tablets; L0001851 for the 5-mg tablets; and L0001848 for 10-mg tablets.		
<p>Duration of Treatment: Subjects could continue to receive ambrisentan until the Investigator or subject chose to stop ambrisentan treatment, the cumulative AE profile did not warrant continuation of the study, ambrisentan became commercially available, or Myogen stopped the study. At the time of the data cut-off for this initial report (16 February 2006) the median and maximum exposure to ambrisentan were 21 weeks and 36 weeks, respectively.</p>		
<p>Reference Therapy, Dose and Mode of Administration, Batch Number: There was no reference therapy (placebo or active) used in this study.</p>		
<p>Endpoints for Evaluation:</p> <p>Primary Endpoint:</p> <p>The primary endpoint was the incidence of confirmed serum aminotransferase concentrations >3xULN during 12 weeks of ambrisentan therapy that was related to ambrisentan and resulted in discontinuation of drug.</p> <p>Secondary Endpoints:</p> <ul style="list-style-type: none"> • The incidence of confirmed serum aminotransferase concentrations >5xULN during 12 weeks of ambrisentan therapy that was related to ambrisentan and resulted in discontinuation of the drug • The incidence of confirmed serum aminotransferase concentrations >3xULN during 12 weeks of ambrisentan therapy that was related to ambrisentan and resulted in dose reduction • Change from baseline evaluated after 12 weeks of ambrisentan therapy in: 6MWD, Borg dyspnea index immediately following the 6MWT, WHO functional class, and SF-36[®] Health Survey <p>Pharmacokinetic Measurements:</p> <p>Blood samples were collected to measure the concentrations of ambrisentan, sildenafil, and n-desmethyl sildenafil (active metabolite) at the following time points: Pre-dose at Week 0; 2 hours (2.0 ± 0.5 hours) after administration of the first dose of ambrisentan and concomitant sildenafil (if applicable) at Week 0; pre-dose at Week 8; and 2 hours (2.0 ± 0.5 hours) after administration of the daily dose of ambrisentan and concomitant sildenafil (when applicable) at Week 8. Pharmacokinetic (PK) measures included: PK of ambrisentan, PK of sildenafil and n-desmethyl sildenafil in subjects who were receiving concomitant sildenafil therapy.</p> <p>Other Measurements:</p> <p>Subgroup analyses of the primary endpoint, secondary endpoints, and pharmacokinetics aimed at exploring differences between subjects receiving concomitant PAH therapies</p>		

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(ambrisentan only, ambrisentan/sildenafil, ambrisentan/prostanoid, or ambrisentan/sildenafil/prostanoid therapy).

Statistical Methods:

Sample Size Determination:

A sample size of 30 patients was calculated to have 80% power to reject the null hypothesis of a hypothetical 50% recurrence rate of serum aminotransferase abnormalities, assuming a 25% recurrence rate after treatment with ambrisentan and 99% power to reject the null hypothesis of a hypothetical 75% recurrence rate of serum aminotransferase abnormalities, assuming a 37.5% recurrence rate after treatment with ambrisentan.

Analysis Populations:

The intention-to-treat (ITT) efficacy population was defined as all subjects who received at least 1 dose of study drug and had at least 1 post-dose efficacy value. The safety population was defined as all subjects who received at least 1 dose of study drug.

Interim Analysis:

An independent DSMC monitored the safety and welfare of study subjects at designated time intervals by reviewing accumulated data. These interim analyses were confidential, and access to the information was prohibited to Myogen or study Investigators.

Primary Endpoint:

The incidence of confirmed serum aminotransferase concentrations >3xULN during 12 weeks of ambrisentan therapy that was related to ambrisentan and resulted in discontinuation of drug was summarized by the proportion of such subjects and 95% confidence intervals.

Secondary Endpoints:

The endpoints “incidence of confirmed serum aminotransferase concentrations >5xULN during 12 weeks of ambrisentan therapy that was related to ambrisentan” and “incidence of confirmed serum aminotransferase concentrations >3xULN during 12 weeks of ambrisentan therapy that was related to ambrisentan and resulted in dose reduction” were analyzed similarly to the primary endpoint.

The change from baseline in the 6MWD after 12 weeks of therapy was summarized descriptively including the mean change and 95% confidence interval and was analyzed using a 2-sided 1-sample t-test.

The change from baseline in Borg dyspnea index was analyzed similarly to the 6MWD.

The change from baseline in WHO functional class was summarized using frequencies and percentages of improvement from baseline (Improved, No Change, and Deteriorated).

The 8 scales and 2 summary measures from the SF-36[®] Health Survey were considered secondary endpoints. The SF-36[®] results were summarized using descriptive statistics and the change and percent change from baseline at Week 12 was analyzed using a 2-sided 1-

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sample t-test.

Safety Analyses:

All clinical laboratory data were listed by subject, with values outside of the normal range flagged high or low. Descriptive statistics for numerical clinical laboratory tests, for the change from baseline, and for the percent change from baseline values were presented. Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase, and total bilirubin data were summarized by presenting the frequency and percentage of subjects for each laboratory value range (based on ULN). For each liver function test (LFT), the time to first abnormal event was examined.

The median weeks to the event, event-free rates at 6 and 12 weeks, and 95% confidence intervals were provided, based on Kaplan-Meier estimates. Kaplan-Meier curves were plotted for ALT, AST, ALT/AST, alkaline phosphatase, and total bilirubin.

All AEs reported during the course of the study were coded to a body system and preferred term using the Medical Dictionary for Regulatory Activities (MedDRA[®]) and summarized by body system, preferred term, and treatment. AEs were summarized in tables as follows: general summary by body system and preferred term, general summary by preferred term only, by severity, and by relationship to study drug. Serious adverse events (SAEs), deaths, and AEs leading to withdrawal were also summarized. AEs were also summarized by body system and preferred term by age, sex, and ethnicity.

Electrocardiogram (ECG) results were presented by frequency of subjects with normal, abnormal but not clinically significant, and abnormal clinically significant findings.

Descriptive statistics were used to summarize vital signs at each assessment time and for the change from pre-dose to each subsequent scheduled assessment.

Physical examination abnormalities were summarized in shift tables.

Summary of Results:

Disposition and Demographics:

- Two subjects prematurely discontinued from the study due to AEs. Thirty-four subjects were in the study as of 16 February 2006 (median exposure: 21 weeks; maximum exposure: 36 weeks) and were still in the study as of 01 June 2006 (median exposure: 39 weeks; maximum exposure: 54 weeks).
- The 36 subjects enrolled in this study had previously discontinued bosentan (86.1%), sitaxsentan (5.6%), or both (8.3%) due to elevated serum aminotransferases, most of which were substantial ALT and/or AST elevations >5xULN. These subjects had been receiving bosentan or sitaxsentan for a median duration of 15.6 weeks before their first discontinuation.
- Subjects enrolled were mostly women (86.1%) and Caucasian, with a mean age of 57 years (range: 31 to 76 years), which is consistent with the epidemiology of the disease.

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<p>Approximately 2/3 of the subjects had idiopathic PAH (IPAH) and 1/3 had PAH associated with connective tissue disease, anorexigen use or HIV infection (APAH). Subjects had PAH for a mean of 2.4 ± 2.31 years before enrollment; subjects had either WHO functional class II (36.1%) or class III (63.9%) PAH. Subjects had a mean 6MWD of 397.2 ± 104.59 m and a mean Borg dyspnea index of 4.2 ± 2.31. The historical cardiopulmonary hemodynamic values were characteristic of the severity of PAH.</p> <ul style="list-style-type: none"> • Of the 36 subjects enrolled, 11 (30.6%) were receiving ambrisentan alone, 12 (33.3%) were receiving ambrisentan/sildenafil, 8 (22.2%) were receiving ambrisentan/prostanoid, and 5 (13.9%) were receiving ambrisentan/sildenafil/prostanoid. • The safety population (including LFT endpoints) consisted of all 36 subjects enrolled. The ITT population for efficacy (e.g., 6MWD) consisted of 35 of the 36 subjects enrolled. The safety and efficacy endpoints were analyzed as per the data cut-off date of 16 February 2006, unless otherwise noted. At this date, subjects had received ambrisentan for a median of 21 weeks. Two subjects were discontinued early in the study due to AEs (pain in extremity and palpitations) and received ambrisentan for only 1 and 3 weeks, respectively. <p>Endpoint Results:</p> <ul style="list-style-type: none"> • None of the 36 subjects enrolled (95% confidence interval [CI]: 0.0% to 9.7%) had a <u>confirmed</u> serum aminotransferase concentration $>3xULN$ during 12 weeks of ambrisentan therapy that was related to ambrisentan and resulted in <u>discontinuation</u> of drug (primary endpoint). • Similarly, there was no event of confirmed serum aminotransferase concentrations $>5xULN$ during 12 weeks of ambrisentan therapy that was related to ambrisentan and resulted in discontinuation of drug (secondary endpoint). • None of the 36 subjects enrolled had a <u>confirmed</u> serum aminotransferase concentration $>3xULN$ during 12 weeks of ambrisentan therapy that was related to ambrisentan and resulted in <u>dose reduction</u> (secondary endpoint). However, 1 (2.8%; 95% CI: 0.1 to 14.5) subject had an unconfirmed incidence of ALT = $3.17xULN$ at Week 12 of ambrisentan therapy that was considered related to ambrisentan. Study drug was temporarily interrupted for 10 days, was restarted at 2.5 mg, then increased to 5 mg, and eventually to 10 mg. The subject did not develop any subsequent aminotransferase elevations as of 01 June 2006. • There were no other confirmed or unconfirmed events of serum aminotransferase concentration $>3xULN$ as of 01 June 2006. • There was a substantial increase in the mean 6MWD from baseline to Week 12 (+23.4 m; 95% CI: 6.3 to 40.4; p = 0.009). In general, improvement in 6MWD was observed in most subgroups (age, sex, PAH etiology, baseline WHO functional class, and concomitant PAH therapy). 		

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<ul style="list-style-type: none"> A decrease from baseline in mean Borg dyspnea index was observed at Week 12 (-0.5; 95% CI: -1.0 to 0.0; p = 0.046). In general, improvement in Borg dyspnea index was observed for most subgroups. After 12 weeks of ambrisentan treatment, 42.9% of subjects had an improvement in WHO functional class, 51.4% had no change, and 5.7% subjects had a deterioration of WHO class. Of the 15 subjects who improved, 4 had an improvement in WHO class from class III to class I. In general, improvement in WHO class was observed for most subgroups. There was a substantial improvement in physical composite and mental composite SF-36[®] scores (+3.6; 95% CI: 1.3 to 6.0; p = 0.004, and +3.6; 95% CI: 0.0 to 7.1; p = 0.048, respectively). In general, improvement was observed in most SF-36[®] scales for most subgroups. 		
Pharmacokinetic Results:		
<ul style="list-style-type: none"> The ambrisentan PK population consisted of 22 subjects, 9 of whom were evaluable for sildenafil PK. Due to the limited number of samples collected; no PK parameters were calculated from these data. Two hours (approximate t_{max}) after a 5 mg dose, mean steady-state ambrisentan plasma concentration was 472 ± 210.6 ng/mL. Mean steady-state plasma ambrisentan concentration at trough for the 5 mg dose was approximately 25% of the 2-hour post-dose concentration (~C_{max}), which is consistent with the 12 to 17 hour half-life observed in other PK studies. The plasma ambrisentan concentration observed 2 hours post-dose for the 2.5 mg dose and the 5 mg dose appeared to be dose-proportional. Concomitant sildenafil had no apparent effect on plasma ambrisentan concentrations. 		
Safety Results:		
<ul style="list-style-type: none"> Safety results were reported as of the data cut-off date (median exposure: 21 weeks). Thirty-five (97.2%) subjects experienced at least 1 AE. The most common AEs (>10%) were peripheral edema (30.6%), headache (25.0%), flushing (13.9%), palpitations, upper respiratory tract infection, cough, and dyspnea exacerbated (11.1% each). In most subjects who experienced an AE (86.1%), all AEs experienced were mild or moderate in severity. A total of 5 (13.9%) subjects had severe AEs and 2 of these were considered drug-related (1 subject had elevated AST and ALT; 1 subject who was receiving concomitant prostanoid treatment had a severe AE of pain in extremity that led to study drug discontinuation). Twenty (55.6%) subjects had AEs possibly or probably related to ambrisentan. The most common drug-related AEs (>5%) were headache (13.9%), flushing (11.1%), peripheral 		

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<p>edema (8.3%), and fluid retention (5.6%). All these AEs were mild or moderate.</p> <ul style="list-style-type: none"> • No clinically relevant differences in the incidence of AEs by age, sex, ethnicity, PAH etiology, or concomitant PAH therapy were apparent. • Five (13.9%) subjects had SAEs. One subject with a history of palpitations had a SAE of palpitations that was considered possibly related to ambrisentan by the Investigator and the subject was discontinued from the study. Holter monitor testing was negative. Furthermore, the event did not resolve until 8 weeks after discontinuation of study drug, suggesting that the AE of palpitations may not have been related to ambrisentan. • As described above, 2 subjects (5.6%) had AEs that led to drug discontinuation (pain in extremity and palpitations). • There were no deaths in this study as of 01 June 2006. • There were no notable changes from baseline in serum ALT and AST concentrations at Week 12. One subject (133-002) had an unconfirmed elevation of ALT >3xULN (ALT = 3.17xULN) which was reported as a SAE. • There were small decreases from baseline in serum alkaline phosphatase and total bilirubin concentrations observed at Week 12. No subject had increases in alkaline phosphatase or bilirubin concentrations >2xULN. • Decreases in mean hemoglobin (-0.7 g/dL; range: -2.80 to 2.80) and hematocrit (-2.3%; range: -11.0 to 7.0) which were observed as early as Week 2, continued to decrease, and stabilized by Week 12 (hemoglobin:-1.2 g/dL; range: -3.50 to 1.50; hematocrit: -4.0%; range: -12.0% to 4.0%). • There were no clinically relevant changes in prothrombin time (PT) and international normalized ratio (INR) for subjects receiving warfarin-type anticoagulants. • There were no clinically relevant changes from baseline in other chemistry, hematology, and urinalysis values. • There were no notable changes in male fertility in the few subjects evaluated. • Nine (25%) subjects had one or more AEs related to abnormal laboratory values; these AEs were: elevated ALT, elevated AST, elevated INR, increase uric acid, low MCV, low mean cell hemoglobin, low phosphorus, decrease white blood cell (WBC) count, decreased neutrophil count, elevated WBC count, elevated potassium). • There was a small decrease in mean systolic and diastolic blood pressure (-6.1 and -6.9 mmHg, respectively) after 12 weeks of treatment. This effect was similar in subjects receiving concomitant sildenafil and subjects receiving ambrisentan monotherapy. • Of the 24 subjects with abnormal ECG findings at Week 12, most had similar findings at baseline; none had abnormal findings at Week 12 only. Three subjects had abnormal findings considered clinically significant at Week 12; all had the same findings at 		

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baseline.		
<p>Conclusions:</p> <p>No subject had confirmed serum aminotransferase abnormalities >3xULN related to study ambrisentan that resulted in discontinuation of drug. This incidence was lower than the rate of aminotransferase abnormalities >3xULN observed in treatment-naïve subjects receiving bosentan in clinical studies. One subject had an unconfirmed elevation of ALT that led to a temporary dose reduction. No other cases of elevated serum aminotransferases >3xULN were observed for a period up to 54 weeks of ambrisentan treatment.</p> <p>Ambrisentan also demonstrated improvements in exercise capacity, dyspnea following exercise, disease severity, and overall physical and mental quality of life. These improvements were observed in most subgroups. The efficacy of ambrisentan was similar when administered alone or in combination with sildenafil and/or a prostanoid, though study size precluded definitive subgroup comparisons.</p> <p>The most common drug-related AEs were headache, flushing, peripheral edema, and fluid retention which were mild or moderate in severity, and are typical of ambrisentan treatment. Of note, the incidence of AEs was similar for subjects receiving ambrisentan monotherapy and those receiving concomitant sildenafil and/or a prostanoid.</p> <p>These data suggest that ambrisentan may be an appropriate treatment for patients who can not tolerate other ERA therapies due to the development of LFT abnormalities.</p>		