CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

22-081

MEDICAL REVIEW

DIVISION OF CARDIO-RENAL DRUG PRODUCTS

Divisional Memorandum

NDA: 22-081 (ambrisentan; Letairis)

Sponsor: Gilead

Review date: 13 August 2007

Reviewer: N. Stockbridge, M.D., Ph.D., HFD-110

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DCaRP/Brum/Marciniak/Gordon
OB/Liu
OCP/Hinderling/Wang/Tornoe

Ambrisentan is a new molecular entity with endothelin receptor antagonism activity (ET_A>>ET_B), submitted as a therapy for pulmonary arterial hypertension.

The description here is based upon a CMC review by Dr. Sarker (31 January 2007 and 16 May 2007), a pharmacology/toxicology review by Dr. Link (1 May 2007), a carcinogenicity statistical review by Dr. Rahman (22 May 2007), clinical pharmacology review by Drs. Hinderling, Wang, and Tornoe (7 May 2007), a clinical and statistical review by Drs. Marciniak, Gordon, and Liu (18 May 2007), and a QT review by the QT Interdisciplinary Review Team (17 April 2007).

Ambrisentan is to be marketed as 5- and 10-mg tablets under the trade name Letairis, with which name there is no issue. The CMC review recommends an approvable action, pending resolution of issues related to dissolution testing. Pediatric studies under PREA were waived. Financial disclosure was adequate.

The pharmacology/toxicology review describes high specificity for the endothelin ET_A receptor, with a large battery of other affinities excluded. In general, the toxicological profile resembles that of bosentan and other members of this class: similar teratogenicity, testicular toxicity, but little preclinical evidence of hepatotoxicity. Ambrisentan was positive in a chromosomal aberration test, but only at a dose considered cytotoxic. There were also findings of fibroadenomas and basal cell carcinomas in the rat 2-year carcinogenicity assay. Because these findings were observed at doses considered above the maximum tolerated, they are not considered to be relevant by the CAC.

Ambrisentan displays linear kinetics in man over the relevant dose range. Cmax is reached in about 2 h after oral dosing. Circulating ambrisentan is highly protein bound. Ambrisentan is not renally cleared to any significant extent, but it is unclear what are the relative contributions of hepatic metabolism and biliary excretion. CYP 3A4 and 2C9 may contribute anywhere from 20 to 90% of clearance. The terminal elimination half-life is about 15 h, but the effective half-life is much shorter; trough levels are about 15% of peak levels and once-daily dosing results in about 10% accumulation. There is no effect of food. Plasma levels of neither drug are significantly affected by co-administration with sildenafil. Plasma levels and INR are not affected by co-administration with warfarin. Pharmacokinetics of ambrisentan did not appear to be affected by alkaline phosphatase levels, but there is no classical study of hepatic impairment. There is no formal study of renal impairment, either.

The clinical pharmacology reviewers recommend replication of the study that establishes bioequivalence of the clinical service and to-be-marketed formulations.

Although it was nominally successful, the reviewers note two flaws. This study did not incorporate dilution standards and the sponsor failed to retain drug samples for possible re-assay. The dilution process may have resulted in some errors in estimating AUC, but the same methods were applied to both formulations, so while the AUC may not be correct, the similarity of the two profiles should not be markedly affected. And while good clinical practices call for sample retention, FDA apparently rarely or never asks for re-assay. These issues should be brought to the sponsor's attention, but I see little basis for the reviewers' recommendation to consider this study "not acceptable".

Except as noted, I concur with other recommendations made by the clinical pharmacology review team:

1. Drug interactions with inhibitors of OATP (cyclosporine), P-gp (rifampin), 3A4 (ketoconazole), and 2C19 (omeprazole).
2. Evaluation of effectiveness with BID dosing. While I agree that this is a good idea, the clinical team has been negotiating assessment of trough and peak exercise effects. I would defer a request for a BID-QD comparison pending results of this comparison. However, given the many-week time course for development of any drug effects, it is conceivable that these two issues are independent.
3. Studies in patients with defined hepatic and severe renal impairment. I agree that the hepatic impairment study is needed, and labeling should warn that there are few data now. Since renal excretion is not a major route of clearance, I think the renal impairment study is less critical.

Ambrisentan has a small effect of QTc; the upper confidence limit is about 12 ms at 40 mg. While this dose results in exposure several-fold higher than does the highest proposed therapeutic dose, the review team remains concerned that a strong metabolic inhibitor could result in exposure many times higher than has yet been witnessed. Conservative cautionary language should be retained until better data are obtained.

There were two main clinical studies demonstrating effectiveness of ambrisentan. Studies 320 and 321 were similar parallel, randomized, double-blind studies of effects of ambrisentan on 6-minute walk distance at 12 weeks. Both enrolled WHO Group I (idiopathic and connective tissue disease) subjects in WHO functional class II-III with randomization to placebo or to one of two doses. Study 320, conducted in part in the US) used doses of 5 and 10 mg, and Study 321 (completely OUS) used doses of 2.5 and 5 mg. The studies were of similar size, about 65 subjects per arm. The timing of exercise assessments relative to dosing was not captured, but is believed to be mostly near Tmax.

As is usual, these studies imputed a zero distance for subjects withdrawn for clinical worsening and used LOCF for other withdrawals. Also, as usual, there are a number of cases for which the review team's adjudication of the nature of the withdrawal differs from that of the sponsor. However, in this case, the differences seem to be spread evenly among the groups, so that the effect on the primary end point is negligible. I reproduce the review team's results (effects in meters) below.

	Study 320			**Study 321**		
	0 mg	**5 mg**	**10 mg**	**0 mg**	**2.5 mg**	**5 mg**
Δ6MWT	3	25	34	0.3	28	40
P-value		0.007	<0.001		0.022	<0.001

The differences among the groups increase from 4 weeks to 8 weeks to 12 weeks in both studies, a pattern that is also similar to what has been seen with other endothelin receptor antagonists.

Clinical worsening was a secondary end point in both studies. This was defined as at least two of the following: 20% decrease in 6MWT, decrease in WHO class, increase in a set of right heart failure signs and symptoms, an acute decline in cardiac, hepatic, or renal function, and persistent systolic pressure <85 mmHg. Life table analyses of this endpoint are on page 34 of the clinical review. The effects for both doses are nominally statistically significant in Study 321, and, after FDA adjudication[1], the effect in the pooled active groups is also nominally statistically significant. The clinical review team is enough encouraged by this result to recommend a claim of reduced clinical worsening, but I disagree.

The statistical reviewer raises analysis issues of control of overall alpha, although this is mostly an issue with Study 320. As shown in the table above, the two studies are not poolable to look at dose-response on 6MWT, and the differences are plausibly related to where the studies were conducted. In both studies the largest effects were seen in Latin America, and in Study 320, the smallest effect is in the US. Perhaps there is better care in the US, so differences the drug makes are less easily perceived in the study, but it is not possible to know. One can take some comfort from replication of results from other endothelin receptor antagonists, and, I believe, conclude we have adequate evidence of the effects on exercise, but I am more disturbed by the inconsistencies in effects on the more important end point of clinical worsening. The sponsor will have another chance to look at this end point when they undertake a further study to address effect after a dose or the effect of dosing interval.

I also note that there are favorable trends with respect to WHO class, SF36, and Borg Dyspnea Index in both studies, with similar problems of interpretation.

The safety database includes 483 subjects receiving at least one dose, 336 of whom received either 5 or 10 mg, the doses most easily supportable for marketing. One hundred thirty subjects received these doses for more than one year. The size of the safety database compares favorably with those for other members of the class. Findings were also similar to those for other members of the class. There is about a 1-g mean decrease in hemoglobin, not dose-related. There is a reduction in blood pressure of about -5/-6 mmHg at 10 mg. There were too few sperm samples to be comforted about potential testicular toxicity; given the difficulty there is in obtaining such data, the Division should reconsider its approach to this problem. There were no important excursions in hepatic enzymes in short-term controlled studies, but a handful of well-documented cases that resolved when ambrisentan was discontinued. There is one not-very-well-documented death in which hepatotoxicity may have played a role. There were 4 pregnancies in the development program; all were terminated.

It would appear prudent for ambrisentan to have a risk management program styled after the one for bosentan.

The audit of clinical sites by DSI remains pending.

Also, at this writing, labeling, post-marketing commitments, and the risk management program all remain to be negotiated, but I agree that the application is, at least, approvable, and quite possibly amenable to first-cycle approval.

In addition, I would like to see, and perhaps incorporate into labeling, cumulative distribution curves for 6MWT by dose group for both studies.

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[1] *The review team's adjudication of this actually improves this finding, a rare occurrence, in my experience.*

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/s/

Norman Stockbridge
8/13/2007 06:34:43 AM
MEDICAL OFFICER

CLINICAL AND STATISTICAL REVIEW

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Established Name	ambrisentan
(Proposed) Trade Name	Letairis™
Therapeutic Class	endothelin receptor antagonist
Applicant	Gilead
Priority Designation	P
Formulation	tablets
Dosing Regimen	once daily
Indication	treatment of pulmonary arterial hypertension (PAH)
Intended Population	PAH, WHO group 1

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1 EXECUTIVE SUMMARY

1.1 Recommendation on Regulatory Action

From clinical and statistical perspectives we recommend approval of ambrisentan for the treatment of WHO group 1 pulmonary arterial hypertension (PAH) to improve exercise capacity (subject to acceptable results from a pending audit of a clinical site). From a clinical perspective we also recommend approval to improve time to clinical worsening. Ambrisentan is an endothelin receptor antagonist (ERA), similar to the approved drug bosentan, evaluated for the treatment of PAH. Regarding efficacy, the results of the two adequate and well-controlled studies, AMB-320 and AMB-321, for the common primary endpoint, change from baseline in six minute walk, provide substantial evidence that ambrisentan is effective in improving exercise capacity at least at peak drug levels. The results are reasonably convincing that ambrisentan also improves time to clinical worsening but are less compelling for the other secondary endpoints. Regarding safety, ambrisentan has an adverse event profile similar to that of the bosentan. There are some unanswered questions regarding the optimal use of ambrisentan (dosing interval, maximal dose, characterization of metabolism) but the favorable results shown in the clinical studies justify approval now with the resolution of these secondary issues post-marketing.

1.2 Recommendation on Postmarketing Actions

1.2.1 Risk Management Activity

Because of the substantial potential toxicity (hepatoxicity, teratogenicity, and testicular toxicity) typical of ERAs, ambrisentan needs and the sponsor has proposed a risk management program.

1.2.2 Required Phase 4 Commitments

The sponsor must conduct a study to evaluate effects upon exercise capacity at a time remote from peak drug levels, e.g., in the late afternoon or evening or at the interdosing interval prior to taking the next dose.

1.2.3 Other Phase 4 Requests

Regardless of the effects upon exercise capacity throughout the interdosing interval, the sponsor should study the effects upon efficacy and safety of a BID dosing regimen. The sponsor should also study dosages higher than 10 mg per day. The sponsor should also delineate better the metabolism and potential for interaction of ambrisentan as detailed in the FDA clinical pharmacologist's review.

1.3 Summary of Clinical Findings

Ambrisentan (Letairis™) is an orally active ERA that is selective for the endothelin type A (ET_A) receptor. Endothelin is a potent autocrine and paracrine peptide believed important in the pathogenesis of PAH. Whether selectivity for the type A receptor vs. the type B receptor

conveys any clinical advantage is not known. The sponsor also describes the drug as a propanoic acid class ERA. Whether the propanoic acid class conveys any clinical advantages over other sulfonamide ERAs is not known. The drug product is an immediate-release film-coated tablet consisting of 5 and 10 mg strengths. The proposed indication is the treatment of PAH WHO Group 1, to improve exercise capacity, delay clinical worsening, [redacted]. The sponsor is proposing once daily dosing.

1.3.1 Brief Overview of Clinical Program

The ambrisentan clinical development program consisted of two very similar (except for different enrolling countries and dosages), small twelve-week pivotal trials (AMB-320 and AMB-321), two longer-term safety studies or extensions, two phase 2 dose-ranging studies, and seven supportive pharmacokinetic (PK) studies. AMB-320 was conducted predominantly in the US using 5 and 10 mg doses; AMB-321 was conducted predominantly in Europe using 2.5 and 5 mg doses. The program was relatively small but typical of PAH development programs (PAH is an orphan disease.) A total of 483 subjects (261 in the pivotal trials) received at least one dose of ambrisentan. Most of these subjects received the 5 or 10 mg to-be-marketed doses, and 161 subjects received drug for at least one year.

1.3.2 Efficacy

The primary endpoint for both pivotal trials was change from baseline to week 12 in six minute walk, a standard submaximal exercise test used to evaluate PAH and heart failure patients. Because PAH is a serious, progressive disease there substantial numbers of dropouts (about 10%) prior to the 12 week endpoint, making imputation of walk changes necessary and raising the possibility of the imputations affecting the results. However, we examined the effects of varying walk imputations for the dropouts and the improvements in walks with ambrisentan treatment in both studies were statistically significant and robust to these variations in imputations as shown in Table 1.

Table 1: Reviewers' Comparison of Sponsor's and FDA Walk Change Analyses

	N		Mean		Median		Placebo-Subtracted Median		P	
Dose	S	F	S	F	S	F	S	F	S	F
AMB-320										
0	67	65	-7.8	-21.2	0.5	3				
5	67	65	22.8	28.2	21.1	24.5	21	22	0.008	0.007
10	67	66	43.6	38	32.5	34	32	31	<0.001	<0.001
AMB-321										
0	65	64	-10.1	-26.8	-3.5	0.3				
2.5	64	63	22.2	18.3	27.5	28	31	28	0.022	0.026
5	63	59	49.4	29.5	40	40	44	40	<0.001	<0.001

S = Sponsor; F = FDA

Note that the point estimate of the effect size in AMB-321 with the 2.5 mg dose was about the same as the 10 mg dose in AMB-320, with the effect sizes in AMB-320 being relatively modest. Effect sizes varied substantially by region, with the effect size in the US subgroup being slightly

less than those for AMB-320 in Table 1 and those in Western Europe being substantially less (-7 m for 2.5 mg, 30 m for 5 mg) than those for AMB-321 in Table 1. We believe these effect sizes for the US and Western Europe are the better estimates of effect sizes and suggest that the 2.5 mg dose may not have much effect upon walks.

Improvements in time to clinical worsening with ambrisentan were more divergent between the two studies as shown in Table 2.

Table 2: Reviewers' Comparison of Sponsor's and FDA's Clinical Worsening Results

	Sponsor		FDA Censored		FDA All	
Dose	Events	P*	Events	P*	Events	P*
AMB-320						
0	6		7		8	
5	3	0.31	2	0.09	3	0.13
10	3	0.29	2	0.09	3	0.13
Combined	6	0.21	4	0.03	6	0.06
AMB-321						
0	14		13		14	
2.5	3	0.005	4	0.02	4	0.01
5	3	0.008	4	0.03	4	0.02
Combined	6	<0.001	8	0.005	8	0.002

*P from log rank stratified by idiopathic/secondary etiology

While there appears to be a clear improvement to time to clinical worsening in AMB-321, it is less clear in AMB-320. The FDA statisticians feel that the results of this secondary endpoint do not provide substantial evidence for inclusion in the label for the following reasons: The results are inconsistent across the studies. Interpretability of the results is questionable due to the fact that changes of the analysis plan were made near the end of the studies and because the sponsor's analysis does not ensure an adequate control of overall type I error rate for the secondary endpoints that are formally tested (see the statistical IND reviews for the study protocols).. The FDA clinicians are more willing to accept that there is improvement in clinical worsening, considering the relatively high statistical significance in AMG-321, a win in AMB-320 for the combined group for the "FDA Censored" analysis in Table 2, and suggestions from the subgroup analyses that clinical worsening improves with drug treatment even when the effects upon walk improvement are less clear. The results for other secondary endpoints are statistically inconclusive and not clearly distinguished from effects upon clinical worsening (WHO class or SF-36 physical functioning score) or exercise capacity (Borg dyspnea index estimated immediately post-walk).

The sponsor was unable to provide timings of the walks relative to drug administration. Most are thought to have been performed in the morning or midday at about the time of peak drug levels. The pharmacokinetics of ambrisentan (see the FDA clinical pharmacology review) do not alone support once daily dosing. However, the beneficial impact upon clinical worsening suggests that ambrisentan has an effect that persists for longer than a few hours. That the walk changes appear to be improving on drug throughout the 12-week study period also suggests that there is some long-term or cumulative effect of the drug. On the other hand, the one patient who

failed on ambrisentan once daily dosing and then improved on bosentan twice daily dosing, raises the question of whether twice or more daily dosing of ambrisentan would be more effective.

While the improvements in walks and clinical worsening in the 2.5 mg dose arm of AMB-321 were statistically significant, there is some evidence from the subgroup analyses that 2.5 mg is less effective. Given greater efficacy and a lack of evidence of dose-limiting toxicity for the higher doses, we agree that it is reasonable not to market the 2.5 mg dose. However, given that there is reasonable evidence for a dose-response through 10 mg without clear evidence of flattening of the response, [redacted]

Any of our conclusions regarding efficacy (as well as some regarding safety) that are based on the results of AMB-321 are dependent upon a clean audit of the one AMB-321 site selected for audit. Completion of audit of that site (in Italy) is still pending because of delays in scheduling. If audit of that site reveals any problems, we will file a review addendum evaluating the impacts of the problems.

1.3.3 Safety

The safety issues associated with the use of ambrisentan appear to be not unlike those associated with the use of bosentan and other ERAs. Ambrisentan, like other ERAs, was teratogenic and showed testicular toxicity in animal studies. It also showed some potential for hepatotoxicity in animal studies, although not striking. The common adverse effects are also not unlike bosentan: peripheral edema, hypotension, nasal congestion/sinusitis, flushing, and palpitations. How ambrisentan compares to bosentan for any of these adverse effects is not clear because the sponsor did not compare ambrisentan and bosentan head on in an appropriate (randomized, double-blind) study. The sponsor does have on-going a single-arm, open-label study evaluating the incidence of increased liver function tests (LFTs) with ambrisentan therapy in subjects who previously discontinued bosentan or sitaxsentan treatment because of increased LFTs. While the preliminary results for this study suggest that some patients discontinuing another ERA can tolerate ambrisentan therapy, because it is uncontrolled it provides no evidence of superiority.

The following are important safety issues for ambrisentan:

1) Elevated liver enzymes
As with bosentan, ambrisentan has been shown to be a hepatotoxin and capable of causing substantial damage to the liver. There were three ambrisentan subjects (0.6%), but no placebo subjects who reported LFTs > 8 x ULN. An additional 1.3%i ambrisentan subjects had LFTs between 3 and 5 x ULN. It cannot be assumed that all LFT increases will resolve when ambrisentan is discontinued, although all appeared to do so in the clinical trials. Those patients with mild LFT elevations could be able to remain on drug. Close monitoring will be essential for all patients taking ambrisentan and all patients should be taking the lowest effective dose. There was one death for which drug induced liver failure cannot be ruled out (primarily for lack of follow up information). Box warning should be similar to the one for bosentan.

2) Pregnancy
Category X

3) Male fertility
Animal studies reported diffuse testicular atrophy. The attempts to determine the effect in adult male patients were inadequate.

4) Pulmonary Veno-Occlusive Disease (PVOD)
A warning about possible PVOD must be included in the label.

5) Anemia
There were common, mostly mild, decreases in hemoglobin and hematocrit in the ambrisentan treated groups. This was rarely an adverse event of concern (which remains the case with bosentan).

6) Allergic reaction
There were two subjects with reports of allergic reaction (face edema). One subject had a positive re-challenge. Both subjects were permanently discontinued. This seems to be an uncommon and non serious adverse event.

1.3.4 Dosing Regimen and Administration

The major outstanding issues regarding dosing regimen and administration are whether once daily dosing is optimal and whether dosing should be limited to a maximum of 10 mg daily.

1.3.5 Drug-Drug Interactions

The sponsor formally studied interactions of ambrisentan with sildenafil and with warfarin and did not find any interactions. However, the sponsor incompletely characterized the metabolism of ambrisentan and did not study interactions with cyclosporine, a drug for which other ERAs show substantial interactions, or with ketoconazole and omeprazole. Please see the FDA clinical pharmacologist's review for details and recommendations.

1.3.6 Special Populations

There were no studies specifically designed to evaluate safety or efficacy of ambrisentan in any subgroup including age, gender, race, etiology of PAH, and baseline WHO functional class. Overall, one cannot draw any conclusions about the safety in subgroups because of the small sample sizes. Ambrisentan is contraindicated in pregnant women because of the teratogenicity risks. Regarding efficacy, the elderly appear to have less improvement in walks but comparable relative improvement in clinical worsening, although the small numbers make firm conclusions impossible. There were no studies evaluating the safety or pharmacokinetics of ambrisentan in subjects with either renal or liver impairment. Children were not studied, but as a drug indicated for an orphan population ambrisentan is exempt from the requirements of the Pediatric Research Equity Act.

Abbreviations

ALT	alanine aminotransferase (SGPT)
AMB	ambrisentan
ANCOVA	analysis of covariance
AST	aspartate aminotransferase (SGOT)
AUC	area under the curve
BDI	Borg dyspnea index
BID	twice a day
BMI	body mass index
BNP	brain natriuretic peptide
BP	blood pressure
BUN	blood urea nitrogen
CABG	coronary artery bypass graft
CAT	coaxial tomography
CK	creatine kinase
CI	confidence interval
CMC	chemistry, manufacturing, and controls
CRF	case report form
CYP	cytochrome P450
DBP	diastolic blood pressure
DSI	Division of Scientific Investigation (FDA)
ECG	electrocardiogram
ERA	endothelin receptor antagonist
ETa	endothelin type A
FDA	Food and Drug Administration
GCP	Good Clinical Practices
GFR	glomerular filtration rate
GGT	gamma glutamyl transferase
GI	gastrointestinal
GLP	Good Laboratory Practices
HGB	hemoglobin
HCTZ	hydrochlorothiazide
HF	heart failure
HIV	human immunodeficiency virus
ICH	International Conference on Harmonization
INR	International Normalized Ratio
IRB	institutional review board
ISE	Integrated Summary (Review) of Efficacy
ISS	Integrated Summary (Review) of Safety
ITT	intention-to-treat
IV	intravenous
IVRS	interactive voice response system
LFT	liver function test
LOCF	last observation carried forward

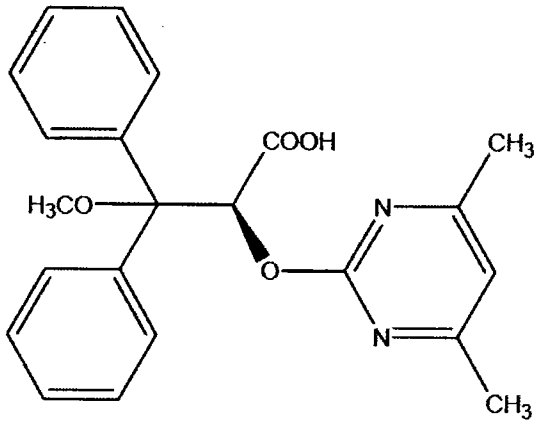
MI	myocardial infarction
NDA	New Drug Application
NOS	not otherwise specified
NS	not significant
OD	once a day
PAP	pulmonary artery pressure
PD	pharmacodynamics
PAH	pulmonary arterial hypertension
PDE5	phosphodiesterase 5
PEY	person-exposure-year
PGP	P-glycoprotein transporter
PK	pharmacokinetic
PO	oral
PT	prothrombin time
PTCA	percutaneous coronary angioplasty
QD	once a day
QTc	QT interval corrected (for heart rate)
RBC	red blood cells
SAE	serious adverse event
SAS	Statistical Analysis System
SBP	systolic blood pressure
SC	subcutaneous
SD	standard deviation
SE	standard error
SLE	systemic lupus erythromatosus
SPA	special protocol assessment
TIA	transient ischemic attack
ULN	upper limit of normal
US	United States
WHO	World Health Organization

2 INTRODUCTION AND BACKGROUND

2.1 Product Information

Ambrisentan (Letairis™) is an orally active endothelin receptor antagonist (ERA) that is selective for the endothelin type A (ET_A) receptor. It is [(+)-(2*S*)-2-[(4,6-dimethylpyrimidin-2-yl)oxy]-3-methoxy-3,3-diphenylpropanoic acid], molecular formula $C_{22}H_{22}N_2O_4$, molecular weight 378.42, with the chemical structure shown in Figure 1.

Figure 1: Chemical Structure of Ambrisentan



Ambrisentan has one chiral center and the drug substance is manufactured as the (S)-enantiomer [redacted]. Dosing studies with (S)-enantiomer drug substance are ongoing to determine the potential for *in vivo* metabolic conversion of the (S)- to (R)-enantiomer. Ambrisentan is practically insoluble in water and in aqueous solutions at low pH. Solubility increases in aqueous solutions at higher pH.

The drug product is an immediate-release film-coated tablet consisting of 5 and 10 mg strengths. The proposed indication is the treatment of pulmonary arterial hypertension (PAH), WHO Group 1, to improve exercise capacity, delay clinical worsening, [redacted]. The sponsor is proposing once daily dosing.

2.2 Currently Available Treatment for Indications

Currently five drugs are approved in the US for the treatment of PAH. They are epoprostenol, treprostinil, and iloprost (prostacyclins), sildenafil (a phosphodiesterase type 5 inhibitor), and bosentan (an ERA). We summarize various characteristics of them in Table 3.

Table 3: Drugs Approved for PAH in the US

Drug	Class	Year	Route	Population	Class	Indication
epoprostenol	prostacyclin	1995	IV	Primary, scleroderma	III-IV	for long-term treatment
bosentan	ERA	2001	PO	WHO Group 1	II-IV	to improve exercise capacity and decrease the rate of clinical worsening

Drug	Class	Year	Route	Population	Class	Indication
treprostinil	prostacyclin	2002	SC	PAH*	II-IV	to diminish symptoms associated with exercise
iloprost	prostacyclin	2004	Inhaled	WHO Group 1	III-IV	improved a composite endpoint of exercise tolerance, symptoms, and lack of deterioration
sildenafil	PDE5 inhibitor	2005	PO	WHO Group 1		to improve exercise ability

*trials included primary, collagen vascular disease, congenital heart disease

Despite the availability of these five drugs, PAH remains a serious disease and there are many opportunities for improvement in its treatment. Several of the drugs have inconvenient dosing, e.g, IV, SC, or inhalation. Most of undesirable adverse effects including hepatic toxicity, testicular toxicity, and teratogenicity. The improvement in exercise capacity with them is typically modest and none of them has been proven to improve survival.

2.3 Availability of Proposed Active Ingredient in the United States

Ambrisentan is not currently marketed in this country

2.4 Important Issues with Pharmacologically Related Products

Bosentan (Tracleer®) is the one ERA approved for the treatment of PAH in the US. The bosentan label has black box warnings for hepatic toxicity and for teratogenicity and descriptions of testicular tubular atrophy in preclinical studies. Because of these potential adverse effects bosentan has a post-marketing risk management program involving a controlled distribution network, the Tracleer Access Program. Bosentan, like all ERAs studied clinically, also leads to a dose-related decrease in hemoglobin (mean 0.9 g/dL). This decrease is usually manifested during the first six weeks of therapy. The etiology is not known and the only recommendation is monitoring. Finally, bosentan was not successful in improving symptoms or mortality in patients with heart failure and increased the rate of heart failure hospitalizations during the first weeks of therapy. Other ERAs have also demonstrated worsening of heart failure in heart failure patients and, in some studies, a suggestion of increased mortality.

2.5 Presubmission Regulatory Activity

The following are the most relevant interactions between the Division and the sponsor regarding clinical and statistical issues in the clinical studies of ambrisentan for the treatment of PAH:

- The Division met with the original sponsor (Myogen) on August 27, 2003, for an end of phase 2 meeting. The Division recommended keeping investigators blinded to dose in the extension study. While the Division agreed that six minute walk change at twelve weeks was an appropriate endpoint and that both class II and III patients could be enrolled, the Division also advised that a safety data base size of 400 patients was low. The Division recommended developing a clear analysis plan for secondary endpoints preserving an alpha of 0.05 and commented that carrying the worst rank forward for patients with clinical worsening and last observation carried forward for other discontinuations was appropriate but the handling of early escapes required further discussion.

- The Division met again with Myogen on October 13, 2004, to discuss a proposed revised clinical development program. Because of slow accrual, the sponsor discussed doing an interim analysis of both trials combined. The FDA statistician stated that two sided testing at alpha 0.05 is required for each trial. Regarding the size of the safety data base, the Division referred the sponsor to the discussion at the EOP2 meeting but commented that "The Division believes 400 patients is a minimum number to evaluate the safety of this drug."

- The Division held a teleconference with Myogen on December 15, 2005, to discuss pharmacokinetic issues. The Division advised that both sildenafil and warfarin should be evaluated. Based on *in vitro* data revealing that ambrisentan is a CYP3A substrate, ambrisentan needs to be further assessed *in vivo* in humans with a potent inhibitor. The Division stated that *in vitro* data is not helpful for quantitative analysis. Pending the results of the ADME study, the need for additional *in vivo* studies can be revisited. The sponsor stated that they could examine the PGP-ambrisentan potential interaction further in other cell systems or conduct *in vivo* tests in animals. The Division noted in concluding that recommendations could change based on the results of the ADME study or the population PK analysis plan.

- The Division exchanged a series of correspondence regarding statistical issues. The most relevant advice provided by the FDA statisticians is the following:

    - The FDA statisticians, in a letter dated September 28, 2005, accepted the change from Hochberg to Hommel procedure for evaluating the secondary endpoints in AMB-321. They also noted that the distribution of alpha between the two endpoints was a problem and that further amendments to the statistical analysis plan were undesirable.

    - The FDA statisticians, in a letter dated December 20, 2005, advised that the change to the weighted Simes procedure was a problem and recommended using Holm's procedure instead. They also commented that if efficacy is shown for the high dose only, how secondary endpoints will be interpreted is not guaranteed.

    - The FDA statisticians, in a letter dated February 6, 2006, explained how the primary endpoint (six minute walk) for high and low doses, the first secondary endpoint (clinical worsening) for high and combined doses, and the second secondary endpoint (WHO class) for high combined doses should be interpreted.

- The Division met with Myogen on May 19, 2006, for a pre-NDA meeting. The Division advised that the two pivotal trials and supporting studies were acceptable for submission but that the sponsor should also include data to support once daily dosing. An example of such data would be 6-minute walk at trough or late afternoon or evening. This latter issue was discussed at some length at the meeting. The Division questioned whether BID dosing could provide better efficacy. When queried about the availability of walks at

trough, the sponsor responded that they believe that they will not have data at trough or late in the evening, but will be able to provide a good sample of walks at times from 8 AM-6 PM. The Division commented that the drug could be approved without such data, but that post-marketing commitment was required for a similar situation with another drug and the lack of data on duration of effect would be reflected in the label. The Division agreed to the general plan for analyzing the two pivotal studies individually and combined but requested that medians and interquartile ranges be provided in addition to means and standard deviations. The Division also noted that the number of patients in the safety database was a review issue.

- The Division sent a letter to Myogen dated December 7, 2006, regarding the 120-day safety updated and the NDA submission. The Division noted that the cutoff date (end of November 2006) for the 4-month safety update was fine for any reports, tabulations, and narratives (provided the NDA is submitted in December 2006), but that it would like to see copies of all CRFs (and CRFs included Medwatch forms, etc.) for deaths and discontinuations for adverse events (and discontinuations for AEs include discontinuations for abnormal lab values such as LFT rises) with a cutoff date of 90 days after NDA submission. For the NDA submission CRFs for deaths and withdrawals for AEs should be included regardless of whether the AE is serious or "significant" and regardless of whether causality by the drug is suspected. Withdrawals for liver enzyme elevations are withdrawals for AEs and must be included. The CRFs must include ALL forms with clinical data regardless of whether the form is labeled a "CRF" (e.g., "serious event worksheets" or "investigator narratives" or "Medwatch forms)".

2.6 Other Relevant Background Information

PAH strictly is a physiologic measurement, not a uniform disease. In addition to idiopathic there are many different etiologies of PAH. The clinical course of the patient and the nature and effectiveness of treatment depend upon the etiology. To bring some order into the classification of PAH the World Health Organization (WHO) has sponsored several international meetings. The third one, in Venice in 2003, produced the revision to the clinical classification of PAH shown in Table 4 and endorsed a functional classification for PAH modified from the New York Heart Association HF classes, as shown in Table 5.

Table 4: WHO Clinical Classification of PAH (Venice 2003)

1. Pulmonary arterial hypertension (PAH)
    - 1.1. Idiopathic (IPAH)
    - 1.2. Familial (FPAH)
    - 1.3. Associated with (APAH):
        - 1.3.1. Collagen vascular disease
        - 1.3.2. Congenital systemic-to-pulmonary shunts
        - 1.3.3. Portal hypertension
        - 1.3.4. HIV infection
        - 1.3.5. Drugs and toxins
        - 1.3.6. Other (thyroid disorders, glycogen storage disease, Gaucher disease, hereditary hemorrhagic telangiectasia, hemoglobinopathies, myeloproliferative disorders, splenectomy)
    - 1.4 Associated with significant venous or capillary involvement
        - 1.4.1 Pulmonary veno-occlusive disease (PVOD)
        - 1.4.2 Pulmonary capillary hemangiomatosis (PCH)
2. Pulmonary hypertension with left heart disease
    - 2.1. Left-sided atrial or ventricular heart disease
    - 2.2. Left-sided valvular heart disease
3. Pulmonary hypertension associated with lung disease and/or hypoxemia
    - 3.1. Chronic obstructive pulmonary disease
    - 3.2. Interstitial lung disease
    - 3.3. Sleep-disordered breathing
    - 3.4. Alveolar hypoventilation disorders
    - 3.5. Chronic exposure to high altitude
    - 3.6. Developmental abnormalities
4. Pulmonary hypertension due to chronic thrombotic and/or embolic disease
    - 4.1. Thromboembolic obstruction of proximal pulmonary arteries
    - 4.2. Thromboembolic obstruction of distal pulmonary arteries
    - 4.3. Non-thrombotic pulmonary embolism (tumor, parasites, foreign material)
5. Miscellaneous
    - Sarcoidosis, histiocytosis-X, lymphangiomatosis, compression of pulmonary vessels (adenopathy, tumor, fibrosing mediastinitis)

Table 5: WHO Functional Classes of PAH Patients

I	Patients with pulmonary hypertension in whom there is no limitation of usual physical activity; ordinary physical activity does not cause increased dyspnea, fatigue, chest pain, or presyncope.
II	Patients with pulmonary hypertension who have mild limitation of physical activity. There is no discomfort at rest, but normal physical activity causes increased dyspnea, fatigue, chest pain, or presyncope.
III	Patients with pulmonary hypertension who have a marked limitation of physical activity. There is no discomfort at rest, but less than ordinary activity causes increased dyspnea, fatigue, chest pain, or presyncope.
IV	Patients with pulmonary hypertension who are unable to perform any physical activity at rest and who may have signs of right ventricular failure. Dyspnea and/or fatigue may be present at rest and symptoms are increased by almost any physical activity.

Both the WHO clinical classification and the WHO functional classes are widely used, e.g., we refer to WHO group and class in the indications for several approved drugs. The WHO clinical classification attempts to group PAH patients into groups for whom the management and responses to therapy should be similar. Most trials in PAH have been in patients in WHO group 1, particularly the idiopathic and secondary to collagen vascular disease or drugs. Patients with PAH secondary to HIV or to congenital shunts have also been included in some trials. There have not been differential effects of the various drugs demonstrated in most subgroups of group 1, although there are insufficient numbers of patients in the latter subgroups to be certain about the relative efficacy and the bosentan label has a precaution about pulmonary edema in patients with pulmonary veno-occlusive disease.. For other groups there is some evidence of lack of efficacy, e.g., the iloprost label notes inadequate evidence of efficacy in patients with thromboembolic disease, WHO group 4. There is also some evidence that ERAs are actually harmful in patients with heart failure secondary to left heart disease, which includes patients in WHO group 2.

In recent years we have observed a change in the epidemiology of the patients in PAH trials, from a younger, predominantly female population of patients with idiopathic or drug-induced PAH to an older, more mixed age and gender population of patients presumably with idiopathic PAH or secondary to collagen vascular disease. There are some issues with determining WHO group and hence eligibility for trials in PAH. For example, patients with PAH and collagen vascular disease may be classified into WHO group 1. However, collagen vascular diseases also frequently cause interstitial lung disease, which can cause PAH and should be classified as WHO group 3. We have seen trial case report forms documenting an interstitial pattern on baseline chest x-ray and restrictive pattern on pulmonary function tests yet the patient was randomized as a WHO group 1 patient. In particular, the group that we are most concerned about is WHO group 2. Current studies suggest that 50% or more of heart failure patients have no evidence of systolic dysfunction, yet PAH is common in patients with heart failure. There is no

pathognomonic diagnostic test that distinguishes these patients. We are concerned that heart failure patients without systolic dysfunction may be classified as WHO group 1 and, given the record of ERAs in heart failure patients, they may actually be harmed by an ERA. Our concern is minor for the trials, because the trials are more likely to fail if our speculation is true, but greater for practice, because patients may be receiving inappropriate and even harmful therapy.

3 SIGNIFICANT FINDINGS FROM OTHER REVIEW DISCIPLINES

3.1 CMC (and Product Microbiology, if Applicable)

Please see the FDA chemist's review for chemistry, manufacturing, and control considerations. There are no CMC issues that are relevant to specific clinical review issues.

3.2 Animal Pharmacology/Toxicology

Ambrisentan appears to share with other ERAs pre-clinical findings of teratogenicity and testicular toxicity. The Division pharmtox reviewer's summary of the findings for ambrisentan are as follows:

- Ambrisentan affected female fertility in rats as evidenced by increases in preimplantation losses in females at the higher doses tested. There were no test-item related effects on embryos when oral ambrisentan was administered directly to pregnant female rats (up to gestation day 6), or indirectly via treatment of males. The effect on male fertility in rats is less consistent. In one study, males demonstrated lower fertility indices and developed diffuse testicular tubular atrophy that was not consistently associated with infertility. In a second study, there was no treatment effect on male fertility, although testicular findings were present.

- Ambrisentan is teratogenic in rats and rabbits when administered at any dose (7-150 mg/kg/day) between gestation days 6 and 15 and it is toxic to pregnant rabbits as evidenced by the maternal toxicities observed at doses greater than 21 mg/kg/day. Pregnant rats tolerate ambrisentan up to doses of 150 mg/kg/day. The fetal abnormalities consistently observed involved the lower jaw and/or palate. Additional findings present in rats included abnormalities of the major vessels and thymus. There was no dose in these studies at which fetal abnormalities were not observed.

- When administered by daily oral gavage to pregnant rats from gestation day 15 through postpartum day 21, ambrisentan did not have any adverse effects on the specific pre- or post-natal developmental milestones of the offspring. A decrease in pup survival was present at 0-4 days post-partum at the higher dose that may represent toxicity affecting maternal behavior. An effect on pup nursing behavior cannot be ruled out, however. Male offspring at this high dose exhibited small testicles and decreased fertility rates.

The Division pharmtox reviewer was less concerned about pre-clinical findings regarding hepatotoxicity, the other recognized toxicity of ERAs in humans. He summarized that

histopathological findings were limited to hepatocellular hypertrophy, consistent with minimal enzyme induction, and there was no evidence of necrosis. However, hepatic findings were also not severe in the pre-clinical studies of bosentan. The one dog that had marked liver enzyme elevations with bosentan had slightly elevated enzymes and no evidence of necrosis or fibrosis at necropsy 38 days after cessation of therapy. Many dogs in the 39-week ambrisentan oral toxicity study in beagle dogs had some signs of hepatic inflammation, with the following microscopic observations recorded for one female dog in the high dose group: "Inflammation, subacute; mild, multifocal, periportal."

4 DATA SOURCES, REVIEW STRATEGY, AND DATA INTEGRITY

4.1 Sources of Clinical Data

The sources of the clinical data are the clinical trial data submitted with the original NDA submission, the 120-day safety update, and miscellaneous submissions provided in response to reviewer queries. We list the NDA submissions in Table 6 and indicate in the last column which ones we primarily reviewed.

Table 6: Reviewers' List of NDA Submissions

Number	Date	Content	Reviewed
0000	12/18/06	Initial submission	x
0001	01/12/07	DMETS tradename responses	
0002	01/26/07	Updated CRFs	x
0003	02/28/07	DSI audit, RiskMap, INR & SAE questions	x
0004	03/02/07	Nonclinical study reports	
0005	03/02/07	Meeting request	
0006	03/13/07	Responses to clinpharm questions	
0007	03/16/07	Nonclinical study reports, response to ECG questions	x *
0008	03/26/07	Randomization lists	x
0009	04/06/07	AMB-220/2 PK/PD datasets	
0010	04/17/07	Safety update	x
0011	04/25/07	Out-of-sequence randomization, site 149 audits, dissolution data	x *
0012	05/01/07	Updated manufacturing establishment information	

* Only clinical responses reviewed

4.2 Tables of Clinical Studies

We list the studies reviewed for clinical efficacy and safety in Table 7.

Table 7: Tables of Clinical Studies

Study Identifier and Location of Study Report	Design	No. of Study Centers (Location)	Total No. Subjects Enrolled/ Completed	Gender M/F (%) Mean Age (y) (Range) Race C/NC (%)	Treatment Dose Route Regimen	Duration of Drug Treatment
PHASE 3 PLACEBO-CONTROLLED STUDIES IN SUBJECTS WITH PULMONARY ARTERIAL HYPERTENSION						
AMB-320[1,2,4] (ARIES-1) 5.3.5.1.1	Randomized, double-blind efficacy and safety study	46 investigative sites 8 countries	202/183	16/84 M/F 50.1 y (17–82 y) 69/31 C/NC	AMB 5 or 10 mg qd Pbo qd Orally	12 wks
AMB-321[2,3,4] (ARIES-2) 5.3.5.1.2	Randomized, double-blind efficacy and safety study	41 investigative sites 13 countries	192/170	26/75 M/F 50.9 y (20–81 y) 85/15 C/NC	AMB 2.5 or 5 mg qd Pbo qd Orally	12 wks
PHASE 2 STUDIES IN SUBJECTS WITH PULMONARY ARTERIAL HYPERTENSION						
AMB-220 5.3.5.2.1	Randomized, double-blind, dose-controlled, dose-ranging evaluation of exercise capacity for 12 wks, followed by 12-wk open-label extension	21 investigative sites 6 countries	64/58	16/84 M/F 51.4 y (22–78 y) 70/30 C/NC	AMB 1, 2.5, 5, or 10 mg qd Orally	Up to 28 wks 12 wks double-blind, 12 wks open-label
AMB-222 5.3.5.2.3	Open-label safety and efficacy study in subjects who previously discontinued ERA therapy due to serum aminotransferase abnormalities	17 investigative sites 4 countries	36 enrolled 2 withdrawn 34 ongoing Study ongoing	14/86 M/F 57.2 y (31-76 y) 78/22 C/NC	AMB 2.5, 5, or 10 mg qd Orally Titrated to balance efficacy/safety	Ongoing. As of 16 February 2006, mean exposure was 19.2 wks and maximum exposure was 36 wks
LONG-TERM PHASE 2 & 3 STUDIES IN SUBJECTS WITH PULMONARY ARTERIAL HYPERTENSION						
AMB-320/321-E[4] 5.3.5.3.2	Blinded, dose-controlled evaluation of safety and efficacy	85 investigative sites 18 countries	383 enrolled 3 did not enter extension study 64 withdrawn 316 ongoing	For combined analysis (n=383) 21/79 M/F 51.1 y (17.5-82.7 y) 77/23 C/NC	AMB 2.5, 5, or 10 mg po qd	Ongoing. Interim data cutoff 16Feb2006 mean exposure: 38.6 wks (0.74 y) maximum exposure: 109 wks (2.1 y)
AMB-220-E 5.3.5.2.2	Open-label, continuation from AMB-220	16 investigative sites 5 countries	54 enrolled 11 withdrawn 43 ongoing Study ongoing	15/85 M/F 51.1 y (22–79 y) 72/28 C/NC	AMB 1, 2.5, 5, or 10 mg po qd	Ongoing. Interim data cutoff 16Feb2006 mean exposure: 115 wks (2.2 y) and maximum exposure: 159 wks (3.1 y)

4.3 Review Strategy

For efficacy our review focused primarily on the two blinded, randomized, placebo-controlled trials, AMB-320 and AMB-321. For safety we reviewed the adverse events and other safety data in all of the studies listed in Table 7. We confirmed the sponsor's analyses as well as performed independent analyses.

4.4 Data Quality and Integrity

We evaluated data quality and integrity by comparing the study reports and SAS data sets to the case report forms. We focused on deaths and discontinuations for patient preferences but we also examined the CRFs for all cases presenting unusual efficacy or safety issues. In general we found the CRFs to be complete and accurately represented in the study reports and SAS data sets. We did request complete CRFs on one patient who the sponsor reported as being randomized but ineligible and whose screening CRFs were not included in the original NDA submission. The sponsor supplied the missing screening CRFs and they were consistent with the sponsor's original description of the case.

We selected three of the highest enrolling sites (two US sites in AMB-320 and one Italian site in AMB-321) with the most favorable results with ambrisentan for Division of Scientific Investigation (DSI) audit. The DSI audits of the US sites documented adequate practices to support the trial results. The audit of the Italian site is pending.

4.5 Compliance with Good Clinical Practices

The study reports for all studies in Table 7 state the study was conducted in compliance with Good Clinical Practices. We and the sponsor identified minor protocol violations at some sites but we did not confirm any major problems suggesting that Good Clinical Practices were not followed in general.

4.6 Financial Disclosures

The sponsor provided a FDA Form 3454 financial disclosure certification for the investigators in studies AMB-320, AMB-321, AMB-220, AMB-220-E, AMB-222, and AMB-320/321-E. For three investigators the sponsor provided FDA Forms 3455 itemizing payments of about $15,000 to $71,000 for , and investor relations work. We selected one of the three investigators for a DSI audit based on high volume and large drug effect; the audit was clean. Because of the blinded nature of the pivotal trials and the multitude of sites and investigators involved these payments could have had at worst minimal effects upon the integrity of the trials.

5 CLINICAL PHARMACOLOGY

We summarize below the most pertinent findings from the FDA clinical pharmacologist's review—for more details please see that review.

5.1 Pharmacokinetics

The salient pharmacokinetic findings include that ambrisentan exhibits dose proportional kinetics, is rapidly absorbed with peak concentrations at 2 h, subject to intestinal extrusion (P-gp), highly plasma protein bound, and eliminated predominantly by non-renal routes. The relative contributions of metabolism and biliary excretion to ambrisentan's elimination from the body are not known. The apparent terminal half-life is 15 h, but the effective half life is much shorter. When given every 24 h the accumulation of ambrisentan is 1.1. At steady-state the trough concentrations are only 15% of the peak concentrations in patients. Clearance is decreased and half-life increased nearly 50% in PAH patients.

In vitro studies with human liver tissues indicate that ambrisentan is metabolized by CYPs 3A4 and 2C19, and UGT1A9S, 1A3S and 2B7S. Ambrisentan inhibits CYPs 2A6 and 2C8, 2C9 and UGTs 1A1, 1A6, 1A9 and 2B7 by 10-30%, but only at concentrations that exceed those reached under clinical conditions by a factor of ≥ 30. Ambrisentan appears not to impact NTCP, OATP or BSEP. However, ambrisentan could be a substrate of OATP. The FDA clinical pharmacology reviewer concludes that the interaction liability of ambrisentan has not been adequately investigated. Based on the available limited mass balance information it can be estimated that from 22.6% to 87.5 % of an administered dose of ambrisentan could be metabolized. Thus,

clinically relevant metabolic interactions caused by metabolic inhibitors and inducers of ambrisentan cannot be ruled out. The main metabolite in plasma appears to be 4-hydroxymethyl ambrisentan.

The clinical service formulations and to be marketed tablets of 5 mg and 10 mg strength are bioequivalent, but inspection of the site performing the study revealed failure to select and randomly retain reserve drug samples from the study drugs received. Therefore the study was deemed not to be acceptable by the clinical pharmacology reviewer.

COMMENT: With trough concentrations only 15% of peak once daily dosing of ambrisentan is not justified based on these PK data alone. The metabolism and potential for metabolic interaction of ambrisentan have not been characterized ideally, although they are adequate to support approval. The failure of the site performing bioequivalence studies to retain reserve drug samples is an unfortunate violation of FDA guidance, but we would not recommend disapproval of the drug based on this violation alone.

5.2 Pharmacodynamics

Ambrisentan is a specific ET_A-receptor antagonist with high affinity (Ki= 0.011 nM) and selectivity for ET_A vs. ET_B receptor (> 4000 fold). Two receptor subtypes, ET_A and ET_B mediate the effects of endothelin- 1 (ET-1), an auto- and paracrine peptide, in vascular smooth muscle and endothelium. The primary actions of ET-1 mediated by ET_A are vasoconstriction and cell proliferation. The predominant actions of ET-1 mediated by ET_B are vasodilation, antiproliferation, and ET-1 clearance. In patients with PAH, plasma and lung tissue concentrations of ET-1 are up to 10-fold increased and correlate with increased right atrial pressure and disease severity.

Ambrisentan also decreased diastolic blood pressure and increased heart rate after 5 mg, 7.5 mg and 10 mg qd in healthy subjects. The hemodynamic effects were not dose dependent over that narrow dose range. Relative to baseline the ET-1 serum levels increased at the two measured time points 2 h and 10 h after multiple qd doses of 5 mg, 7.5 and 10 mg ambrisentan. These results indicate that ambrisentan exhibits in healthy subjects pharmacological effects that have a rapid onset and last for several hours after administration.

COMMENT: Whether the selectivity for ET_A vs. ET_B confers any clinical advantage over less selective ET-1 antagonism remains to be demonstrated. Similarly, the sponsor describes ambrisentan as a "propanoic acid class" ERA. Whether the propanoic acid class conveys any clinical advantages over other sulfonamide ERAs is also not known.

5.3 Exposure-Response Relationships

The highest multiple dose regimen tested in healthy subjects and PAH patients was 10 mg qd. The maximum tolerated dose has not been determined in either population. Ten (10) mg qd is the highest recommended therapeutic dose of ambrisentan. A therapeutic plasma concentration range for ambrisentan has not been defined.

COMMENT: We examine clinical dose-response in the Integrated Review of Efficacy.

6 INTEGRATED REVIEW OF EFFICACY

6.1 Indication

The proposed indication is the treatment of pulmonary arterial hypertension (PAH), WHO Group 1, to improve exercise capacity, delay clinical worsening, [redacted].

6.1.1 Methods

The ambrisentan clinical development program consisted of two very similar (except for different enrolling countries and dosages) small twelve-week pivotal trials, two longer-term safety studies or extensions, two phase 2 dose-ranging studies, and seven supportive pharmacokinetic (PK) studies. The sponsor references the two pivotal trials as ARIES-1 (AMB-320) and ARIES-2 (AMB-230) and both have the general title "A Phase 3, Randomized, Double-Blind, Placebo-Controlled, Multicenter, Efficacy Study of Ambrisentan in Subjects with Pulmonary Arterial Hypertension." AMB-320 was conducted in Austria, Australia, Brazil, Chile, Hungary, Italy, Mexico, and the US with 5 and 10 mg dosages while AMB-321 was conducted in Argentina, Belgium, Chile, Germany, Hungary, Israel, Italy, Netherlands, Poland, Russia, Spain, Ukraine, and the UK with 2.5 and 5 mg dosages. We critique the efficacy results of these two trials and the combined safety data from all ambrisentan exposure, leaving the primary review of the PK studies to the FDA clinical pharmacology reviewer. In the efficacy review we scrutinize the dropouts because the handling of dropouts has been problematic in other PAH studies. We also examine how eligibility criteria were enforced for similar reasons.

6.1.2 General Discussion of Endpoints

The primary endpoint in the two pivotal trials was change from baseline in six minute walk distance. We have accepted this endpoint as supporting approval of other drugs for the treatment of PAH and it has been the endpoint used in most recent submissions for PAH. One issue regarding this endpoint has been the handling of dropouts as mentioned above. Another issue has been whether it is a sensitive indicator of drug effect for all functional classes of PAH, i.e., whether patients with WHO class 2 PAH show a benefit with it.

Secondary endpoints for both studies were time to clinical worsening of PAH, as defined by the time from randomization to the first occurrence of death, lung transplantation, hospitalization for PAH, atrial septostomy, study discontinuation due to the addition of other PAH therapeutic agents, or study discontinuation due to two or more early escape criteria; WHO functional class; SF-36 health survey physical functioning scale; and Borg dyspnea index immediately following exercise. Time to clinical worsening, WHO functional class, and Borg dyspnea index, like six minute walk, have been used commonly as endpoints in PAH trials. The SF-36 survey is a general purpose survey used to evaluate many health programs; it may lack specificity for PAH problems.

6.1.3 Study Design

Both trials were randomized, double-blind, placebo-controlled, parallel-group studies. Both studies were conducted about the same time, i.e., AMB-320 from 17 December 2003 to 21 February 2006 and AMB-321 from 16 December 2003 to 24 October 2005. Both studies tested two dosages compared to placebo: 5 and 10 mg (the latter started on 5 mg for two weeks) in AMB-320 and 2.5 and 5 mg in AMB-321. Both studies were 12-week studies and both allowed one dosage reduction (10 to 5, 5 to 2.5, and 2.5 to 1) for drug tolerability problems. Both also allowed escape after four weeks to a long term ambrisentan study for two or more of the following criteria: a decrease from baseline of at least 20% in the distance walked during the six-minute walk test; an increase of on or more WHO functional class; worsening right ventricular failure (as indicated by increased jugular venous pressure, new or worsening hepatomegaly, ascites, or peripheral edema); rapidly progressing cardiogenic, hepatic, or renal failure; or refractory systolic hypotension (SBP < 85).

The sponsor amended the protocols twice globally:

1. On May 3, 2004, the statistical analysis of the primary endpoint was modified to reflect a change from a Bonferroni approach to a Hochberg analysis to adjust for multiplicity of testing. Furthermore, because the randomization was stratified based on the underlying etiology of PAH (idiopathic, secondary), analysis of the primary and secondary endpoints was modified to be stratified based on the underlying etiology of PAH. The inclusion and exclusion criteria were modified to address the use of several drugs that may have been used for the treatment PAH. Specifically, subjects receiving statins must have been on stable therapy for at least 12 weeks prior to the screening visit. Subjects receiving a PDE5 inhibitor within four weeks prior to the screening visit were excluded. Clarification was provided regarding the historical diagnostic tests used to assess the proper diagnosis of PAH and the baseline cardiopulmonary function. Clarification was provided on the early escape criteria that concerned worsening right ventricular failure. • The exclusions for male subjects not required to provide semen samples for the male fertility analysis were modified to include male subjects who were unable to provide samples due to religious or psychological issues. The reporting of hospitalization due to elective surgery and the reporting of SAEs to local regulatory authorities were clarified. The instructions for the use of supplemental oxygen during the 6MWT were clarified.

2. On November 8, 2005, the multiple comparison procedure for the analysis of the primary and key secondary endpoints was modified. The primary endpoint analysis was changed from Hochberg to fixed sequence, and the secondary endpoint analysis was changed from Holm to a weighted Hommel and fixed sequence approach.

The sponsor also amended the protocol for Study AMB-321 several times in minor ways to accommodate local regulatory authorities (Israel; Spain). The local changes do not affect the integrity of the study.

COMMENT: ***Note the sponsor made the final changes for primary and secondary endpoint analyses near the end of the studies.***

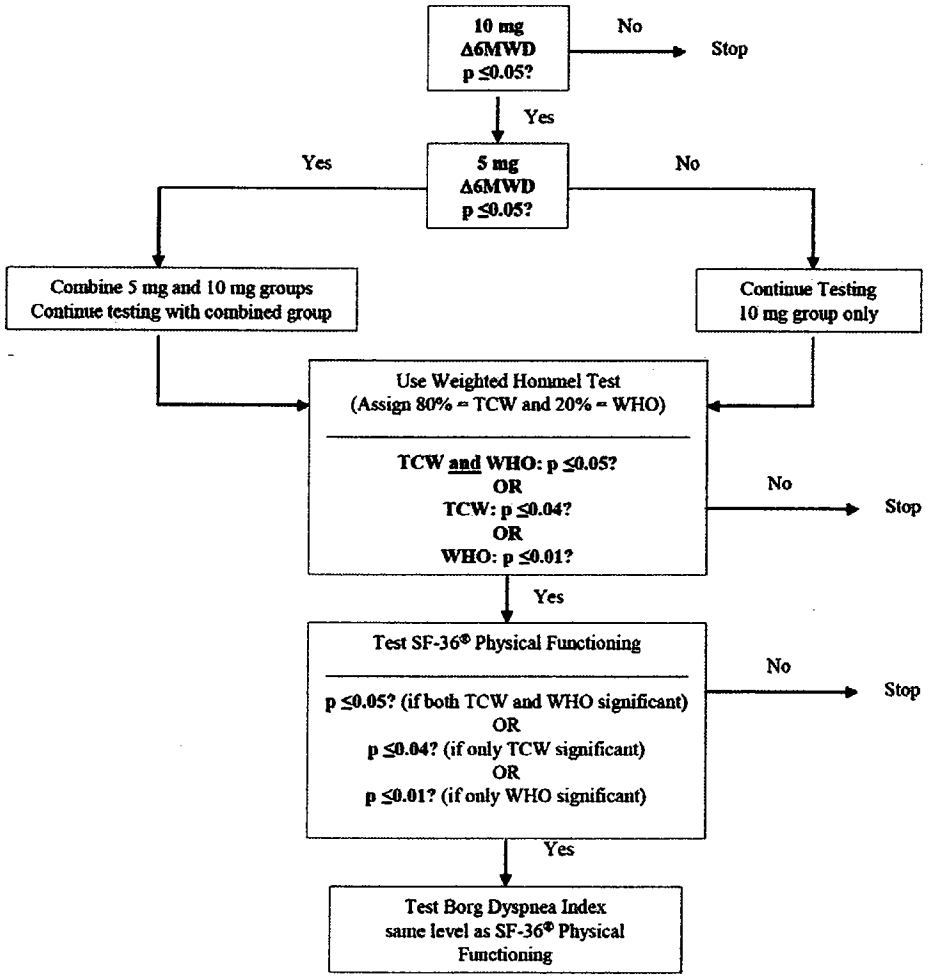
The main inclusion criteria for both studies were male or female; 18 years of age or older; and idiopathic PAH or PAH associated with connective tissue disease, anorexigen use, or human immunodeficiency virus (HIV) infection. Patients were to have a documented mean pulmonary artery pressure (mPAP) ≥25 mmHg, pulmonary vascular resistance (PVR) >3 mmHg/L/min, and pulmonary capillary wedge pressure (PCWP) or left ventricle end diastolic pressure (LVEDP) <15 mmHg. (The numeric results for these tests were captured on the case report forms.) Patients must have been able to walk a distance of at least 150 m but no more than 450 m during two consecutive six minute walks. Patients with PAH secondary to congenital heart disease, left heart disease, chronic obstructive pulmonary disease, interstitial disease, and thromboembolic disease were excluded, as were patients with a total lung capacity < 70% of predicted or a forced expiratory volume in 1 second of < 65% of predicted. (The latter values could be historical values but were captured on the case report forms.) Patients with a screening ALT or AST > 1.5x ULN were to be excluded. Patients who had been treated with any other PAH drug (bosentan, PDE5 inhibitor, prostacyclin) within four weeks were also excluded. Patients taking calcium channel blockers had to be on stable therapy for at least one month. During the trial other medical therapy could be maintained, but use of drugs specific for PAH as well as IV inotropes were prohibited.

Randomization in both studies was by interactive voice response system (IVRS) using a permuted block randomization with a block size of three. The Data Analysis Plans state that randomization was stratified by etiology (idiopathic vs. secondary PAH). While not stated in any of the documentation submitted with the original NDA, the randomization scheme also included an escape mechanism if more than three consecutive patients at the same site were to be randomized to the same group—the next randomization number was skipped and the following one used. (This occurred once during the study resulting in two randomization numbers out of date sequence. The sponsor provided the previous explanation in response to a query.)

The patients performed at least two six minute walks during screening within two weeks of each other and not varying by more than 15%. The sponsor calculated baseline as the average of these two walks. The patients repeated walks at four, eight, and twelve weeks.

The sponsor proposed the statistical methods for data analysis shown in Figure 2.

Figure 2: Sponsor's Flowchart of Statistical Analyses



A fixed sequence approach was used to control the type I error rate for the comparison of ambrisentan to placebo for the primary endpoint, starting from the higher dose. If the higher dose was statistically significant at the α level of 0.05, then the lower dose was tested at the same alpha level of 0.05. If both individual doses were statistically significant, then a combined dose was compared to placebo and tested at the alpha level of 0.05. The dose(s) found significant in testing the primary endpoint were combined for comparison to placebo for the four secondary endpoints. The two key secondary endpoints, time to clinical worsening and change in WHO functional class, were compared to placebo using a weighted version of Hommel's extension of Simes'test. These two tests served as a gatekeeper, allowing the physical functioning scale of the SF-36® Health Survey to be tested if at least 1 of the first 2 secondary endpoints was significant. Lastly, the BDI was tested conditional on a significant result from the test of the SF-36® physical functioning scale. The weighted Hommel test assigned weights of 80% to the time to clinical worsening of PAH and 20% to the change in WHO functional class.

In the IND reviews, the FDA statistical reviewers commented that the sponsor's proposed weighted Simes test does not control the family-wise error rate in the strong sense, and a Holm's procedure was recommended.

6.1.4 Efficacy Findings (Combined Clinical and Statistical Review)

6.1.4.1 Baseline Characteristics and Subject Disposition

The majority of the patients enrolled were female (79%) and white (77%) with a mean age of 50.5. About 64% of the patients had primary pulmonary hypertension with the majority of the subjects with secondary PAH having scleroderma or its variants (75%) but also SLE (13%), anorexigen use (4%), and HIV (8%). The vast majority of patients were WHO functional class III (55%) and II (38%). We show selected baseline characteristics by treatment group in Table 8.

Table 8: Reviewers' Selected Baseline Characteristics by Treatment Group

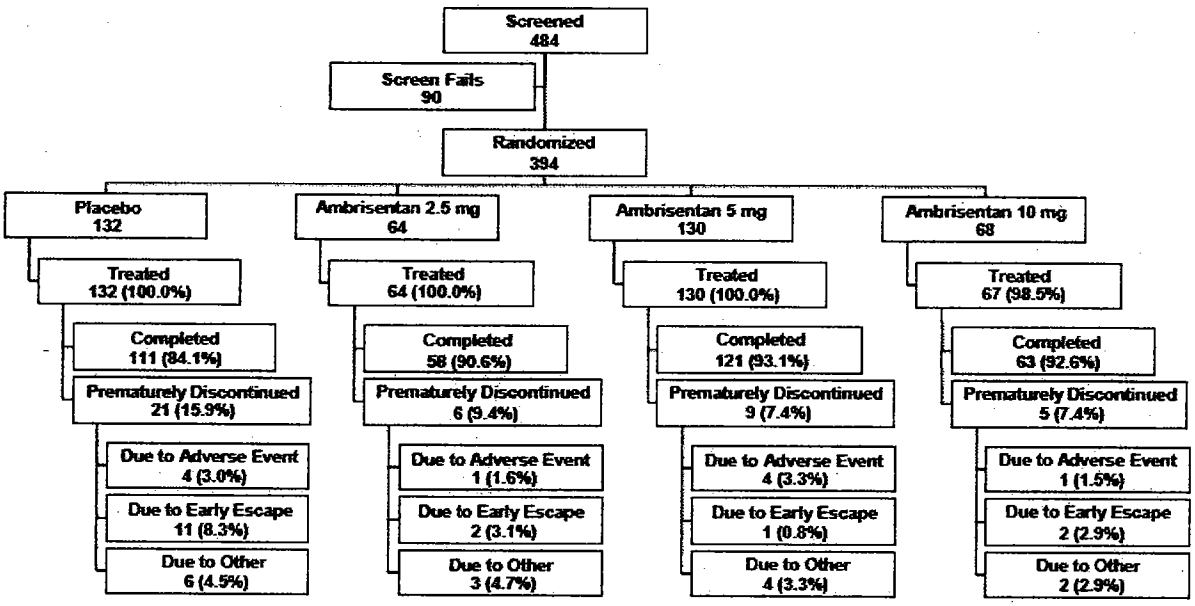
	AMB-320			AMB-321		
	0	5	10	0	2.5	5
Mean age	47.8	53.1	49.3	51	51.6	50
% age≥65	21%	25%	18%	21%	22%	21%
% female	88%	84%	79%	68%	75%	81%
% white	73%	69%	66%	78%	84%	92%
% black	6%	6%	4%	0%	0%	0%
% hispanic	18%	18%	25%	18%	14%	8%
% idiopathic PAH	64%	63%	61%	65%	66%	65%
% WHO class III	61%	60%	54%	57%	45%	52%
% WHO class IV	1.5%	9.0%	10.5%	3.0%	1.5%	1.5%
Mean PAP	50.5	47.1	51.4	51.2	48.5	47.5
Mean walk distance	342	340	341	343	347	355

PAP = pulmonary artery pressure

COMMENT: While there is some variation in the categorical baseline characteristics by treatment because of the relatively small sample sizes, there do not appear to be any baseline imbalances that jeopardize the interpretation of the studies. Few blacks were studied, although the representation of Hispanics is reasonable.

We show the sponsor's subject disposition for the two studies in Figure 3.

Figure 3: Sponsor's Subject Disposition (AMB 320/321 Randomized Subjects)



The one patient who was not treated in the ambrisentan 10 mg group was randomized prior to reporting baseline lab values and was discontinued prior to treatment because of an AST 1.6x ULN. Another patient, in the 5 mg group of AMB-320, is listed as withdrawing for personal reasons but has no documentation of right heart cath values for eligibility.,

COMMENT: The discontinuations for early escape appear to indicate a beneficial effect of ambrisentan. However, some of the discontinuations due to adverse events and to other reasons may also represent clinical worsenings. We examine discontinuations in more detail in conjunction with our analyses of the primary and secondary endpoints.

6.1.4.2 Primary Endpoint

We show the sponsor's summaries of the primary endpoint analysis for AMB-320 in Table 9, for AMB-321 in Table 10, and for AMB-320 and AMB-321 combined in Table 11.

Table 9: Sponsor's Change from Baseline in 6-minute Walk at Week 12 (LOCF) in AMB-320

Treatment group	Placebo	5 mg ambrisentan	10 mg ambrisentan	Combined ambrisentan
Parameter	(N = 67)	(N = 67)	(N = 67)	(N = 134)
Baseline 6MWD, m, mean (SD)	341.9 (73.47)	339.6 (76.68)	341.5 (78.28)	340.5 (77.20)
Change from baseline to Week 12, m				
Mean (SD)	-7.8 (78.88)	22.8 (82.98)	43.6 (65.91)	33.2 (75.37)
Median	0.5	21.1	32.5	25.5
95% CI	-27.1, 11.4	2.5, 43.0	27.5, 59.6	20.3, 46.0
Comparison versus placebo				
Point estimate	-	30.6	51.4	41.0
95% CI	-	2.9, 58.3	26.6, 76.2	18.4, 63.6
p-value[1]	-	0.008	<0.001	<0.001

[1]Wilcoxon rank sum test stratified by IPAH and non-IPAH subjects

Table 10: Sponsor's Change from Baseline in 6-minute Walk at Week 12 (LOCF) in AMB-321

Treatment group	Placebo	2.5 mg ambrisentan	5 mg ambrisentan	Combined ambrisentan
Parameter	(N = 65)	(N = 64)	(N = 63)	(N = 127)
Baseline 6MWD, m, mean (SD)	342.7 (85.93)	347.3 (83.81)	355.3 (84.45)	351.3 (83.89)
Change from Baseline to Week 12, m				
Mean (SD)	-10.1 (93.79)	22.2 (82.67)	49.4 (75.36)	35.7 (79.99)
Median	-3.5	27.5	40.0	35.0
95% CI	-33.3, 13.2	1.6, 42.9	30.4, 68.3	21.6, 49.7
Comparison versus placebo				
Point estimate	-	32.3	59.4	45.8
95% CI	-	(1.5, 63.1)	(29.6, 89.3)	(20.2, 71.3)
p-value[1]	-	0.022	<0.001	<0.001

[1]Wilcoxon rank sum test stratified by IPAH and non-IPAH subjects

Table 11: Sponsor's Change from Baseline in 6-minute Walk at Week 12 (LOCF) in AMB-320/321 Combined

Treatment group 6MWD, meters	Placebo (N = 132)	2.5 mg ambrisentan (N = 64)	5 mg ambrisentan (N = 130)	10 mg ambrisentan (N = 67)	Combined ambrisentan (N = 261)
Baseline, mean (SD)	342.3 (79.55)	347.3 (83.81)	347.2 (80.61)	341.5 (78.28)	345.8 (80.55)
Change at Week 12					
Mean (SD)	-9.0 (86.22)	22.2 (82.67)	35.7 (80.18)	43.6 (65.91)	34.4 (77.51)
Placebo-adjusted	-	31.2	44.6	52.5	43.3
95% CI	-	(5.6, 56.7)	(24.3, 64.9)	(28.8, 76.2)	(26.4, 60.3)
p-value[1]	-	0.022	<0.001	<0.001	<0.001

[1]Wilcoxon rank sum test stratified by IPAH and non-IPAH subjects and by study

COMMENT: The results from the individual studies in Table 9 and Table 10 look highly supportive of efficacy for ambrisentan in improving 6-minute walk distance: highly statistically-significant, placebo-corrected increases of about 30-60 m with a dose-response in each study. The two major questions are whether they depend upon the sponsor's handling of dropouts (which we examine next) and whether the effects are sustained throughout the interdosing interval (which we examine in Section 6.1.4.3). The sponsor's combined analysis makes no sense: It lumps together the placebo and 5 mg groups from studies done in different regions, with possibly different patient populations, and different results in the placebo groups. It presents only means despite the fact that we advised the sponsor at the pre-NDA meeting that for these data, for which arbitrary imputations of 0 walk distances were used for some patients, medians rather than means are more appropriate. We examine this issue following our discussion of dropouts and re-analysis of the individual study results.

Nineteen (9.4%) of patients in AMB-320 either had no post-treatment walks or last walks prior to day 78 and 19 (9.9%) of patients in AMB-321 either had no post-treatment walks or last walks prior to day 74. One patient in AMB-320 and two patients in AMB-321 had final walks but had added PAH or HF therapies. Patients with last walk days >77 in AMB-320 and 73 in AMB-321 and less then day 80 and patients with last walk days>91 (about 6% of patients in each study) did not have any disqualifying events even though their last walk days fall outside a reasonable window around the target day of 84. We scrutinized the CRFs of the 42 patients who were terminated early or had additional therapy and formulated our own appraisal of how each patient should be handled. We show the sponsor's and our appraisals of these patients in Table 12.

Table 12: Reviewers' Comparison of Sponsor's and FDA Handling of Walks for Patients Who Discontinued Prematurely or Had Additional Therapy

Dose	Day	Change from Baseline		Comment
		Sponsor	FDA	
AMB-320				
0		0	-290.5	Dyspnea AE, withdrew consent to seek other evaluation

Dose	Day	Change from Baseline		Comment
		Sponsor	FDA	
0		-262		Pulmonary embolus, thromboembolic disease
0	17	-83.5	-268.5	Worse per patient
0	27	32	32	Left heart disease
0	37	-281.5	-420.5	Increased fatigue, SOB, volume overload in addition to UTI; lupus flare-up
0	38	-85.5	-295.5	Worsening PAH
0	42	-87	-307	Worsening PAH
0	56	-48.5		Walks limited by knee pain
0	59	38	38	Pelvic fracture
0	64	-290	-290	Increased PAH & death
0	67	-74.2	-190	Increased SOB
5		-305.5		Pneumonia, sepsis
5		0		No documentation on PAH & withdrew for personal reason
5	28	-31.5	-31.5	Discontinued at day 28 with pulmonary venous hypertension
5	31	6	6	Lost to f/u week 4
10		-23.5	-234.5	Hospitalized for right ventricular failure & started on bosentan
10				Randomized but not treated due to baseline AST 1.6 ULN
10	35	-80.5	-434.5	Worsening PAH
10	6	2.5	2.5	Family circumstances
10	63	-158		Unevaluable - hospitalized for pleural effusion, then walk 63d then traumatic intracranial bleed
AMB-321				
0		-241.5		Unevaluable--worse but PE & chronic thromboembolism on angiography
0	30	-110	-435	Early escape
0	40	-121	-219	Early escape
0	45	-17.5	-153.5	Worsening PAH
0	47	-132	-217	Early escape
0	55	-150	-150	Clinical worsening
0	56	-334	-334	Clinical worsening
0	56	-84.5	-352.5	Clinical worsening
0	56	-162.5	-162.5	Clinical worsening
0	57	-20	-410	Clinical status did not improve--AEs
2.5		0	-272.5	Couldn't walk at 4w due to edema
2.5		0		Withdrew consent because of travel distance, dyspnea unchanged
2.5	53	-232	-232	Clinical worsening
2.5	55	-311	-311	Clinical worsening
2.5	56	-150	-150	Clinical worsening
5	18	37.5		Unevaluable - asthma on prednisone, d/c for facial edema prior to final walk
5	21	26		Unevaluable - allergic AE treated with increased prednisone at 14d
5	46	-155.5	-341.5	Clinical worsening
5	62	6	-397	Right ventricular failure
5	84	13		Furosemide & spironolactone added day 1
5	84	50	-190	Started on furosemide at 2 weeks for edema; dyspnea, oxygen later
5	84	4	-429	Clinical worsening & last walk performed on oxygen

Note that from Table 12 we believe that there are preferable, or at least alternative, approaches for handling the walk imputations for 28 patients. For 17 of these patients we judged the patient to have worsened and assigned a last walk of 0; for the other 11 we judged the walk results to be unevaluable because no post-treatment walks were performed, the patient discontinued because of worsening due to an excluded or unrelated problem (thromboembolism, sepsis), or the patient did not worsen but used additional PAH or HF therapy. Despite the differences in walk assignments for 28 patients, our summary results are very similar to the sponsor's as shown in Table 13.

Table 13: Reviewers' Comparison of Sponsor's and FDA Walk Change Analyses

	N		Mean		Median		Placebo-Subtracted Median		P	
Dose	S	F	S	F	S	F	S	F	S	F
AMB-320										
0	67	65	-7.8	-21.2	0.5	3				
5	67	65	22.8	28.2	21.1	24.5	21	22	0.008	0.007
10	67	66	43.6	38	32.5	34	32	31	<0.001	<0.001
AMB-321										
0	65	64	-10.1	-26.8	-3.5	0.3				
2.5	64	63	22.2	18.3	27.5	28	31	28	0.022	0.026
5	63	59	49.4	29.5	40	40	44	40	<0.001	<0.001

S = Sponsor; F = FDA

COMMENT: Note that our results for medians and for P values are nearly identical to the sponsor's while the means vary substantially. That the medians and P values are consistent is substantial evidence that ambrisentan has a favorable effect upon six minute walk distance, at least at peak. The major question not answered by these results from the efficacy perspective is whether the favorable effect is sustained throughout the interdosing interval.

Each study shows a dose-response effect. However, there are substantial differences in the estimated, placebo-subtracted point effects: The improvement in walk distance with 2.5 mg dosage in AMB-321 approximates the improvement with the 10 mg dosage in AMB-320. We do not think that it is appropriate to average the disparate results of the two studies to produce a summary statistic. While one could argue that the results of AMB-320, conducted largely in the US, are most relevant for the US label, a simple presentation of the separate results from each study seems most appropriate.

The time course of improvement in walk distance is also of clinical interest. We show the plots of median changes from baseline over time in Figure 4.

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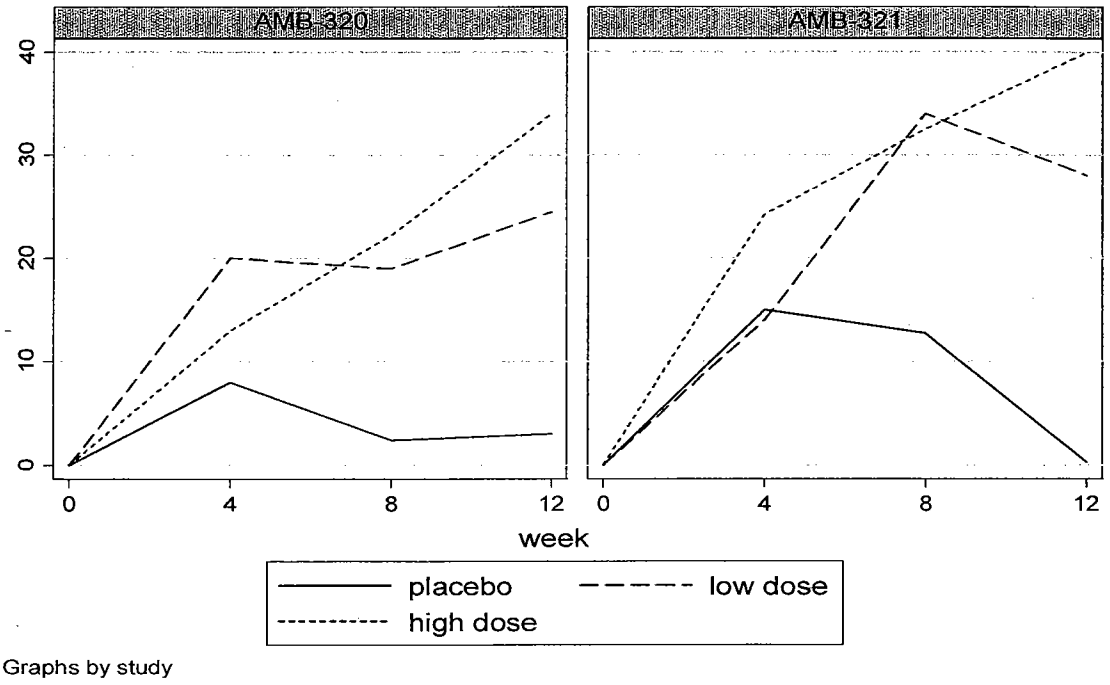
Figure 4: Reviewers' Median Changes from Baseline in Six Minute Walk over Time



COMMENT: In the graphs in Figure 4, the median changes from baseline in six minute walk at 12 weeks are in the same order as the doses for each study. What is also noteworthy is that the improvements in median walk distances for ambrisentan appear to be increasing through 12 weeks. (We find the changes for AMB-320 to be more convincing because it has a lower placebo effect and less variability; the decrease in the "low" 2.5 mg dose at 12 weeks may reflect lower efficacy or random variation.) That the improvements in walks are cumulative over weeks makes us less concerned that efficacy might vary substantially during the day and provides some justification for once daily dosing.

6.1.4.3 Secondary Endpoints

In the protocol review (IND 64915/S98, S123), we indicated that the weighted Hommel procedure for testing secondary endpoints is problematic because it does not control the family-wise error rate in the strong sense. However, the weighted Hommel procedure was still used by the sponsor for the secondary endpoint analysis. We will examine the two most important secondary endpoints, time to clinical worsening (TCW) and WHO functional class (WHO), by using the weighted Bonferroni method, since the sponsor's proposed method is problematic. The weighted Bonferroni method is applied as the following:

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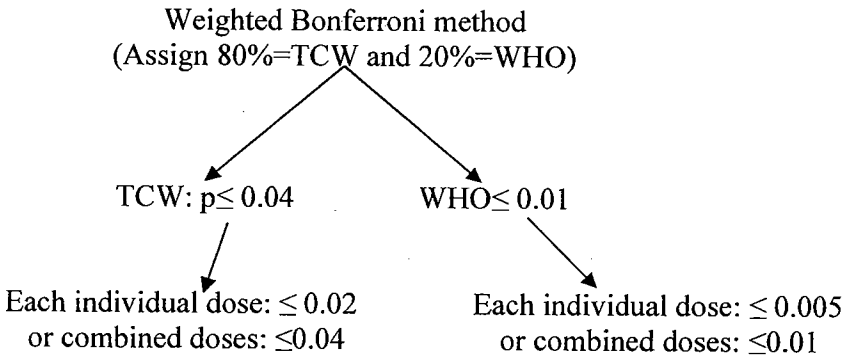


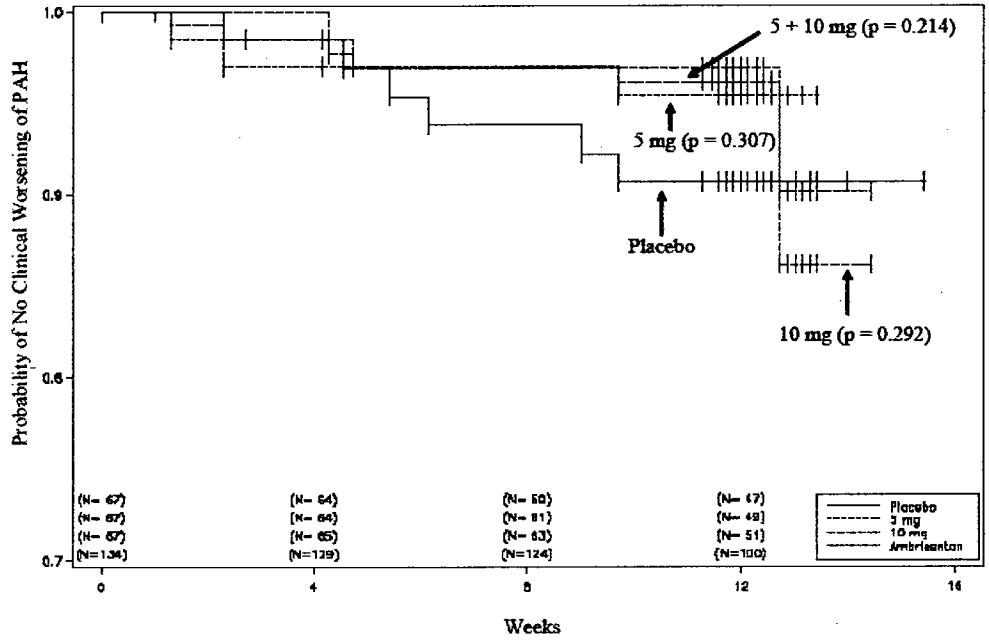
6.1.4.3.1 Time to Clinical Worsening

The sponsor defined clinical worsening of PAH as death, lung transplantation, hospitalization for PAH, atrial septostomy, study discontinuation due to the addition of other PAH therapeutic agents, or study discontinuation due to two or more early escape criteria:

- A decrease from baseline of at least 20% in the distance walked during the 6-minute walk test
- An increase of one or more World Health Organization (WHO) functional class
- Worsening right ventricular failure (e.g., as indicated by increased jugular venous pressure, new/worsening hepatomegaly, ascites, or peripheral edema)
- Rapidly progressing cardiogenic, hepatic, or renal failure
- Refractory systolic hypotension (SBP<85)

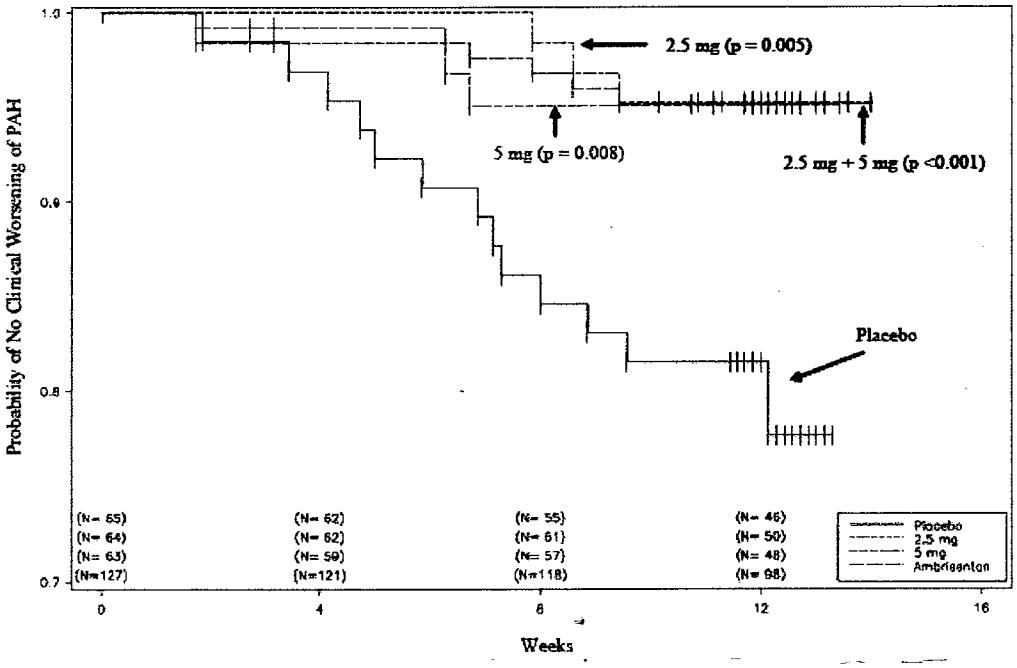
We show the sponsor's analysis of times to clinical worsening for AMB-320 in Figure 5 and for ABM-321 in Figure 6.

Figure 5: Sponsor's Kaplan-Meier Plot of Time to Clinical Worsening in AMB-320



p-values from the log-rank test stratified by IPAH and non-IPAH subjects

Figure 6: Sponsor's Kaplan-Meier Plot of Time to Clinical Worsening in AMB-321



p-values from the log-rank test stratified by IPAH and non-IPAH subjects

Note that the improvements in times to clinical worsening were statistically significant in AMB-321 by the sponsor's analysis but not in AMB-320. The sponsor adjudicated 12 clinical worsening events in AMB-320 and 20 in AMB-321. We adjudicated 11 such events in AMB-320 and 21 in AMB-321, but we also censored six cases because of events not clearly related to worsening PAH or added therapy at day 1. We also included the patient randomized but not treated but, because we have no follow-up on this patient, censored her on day 1. We show the patients for which our adjudication of clinical worsening differs from the sponsor's in Table 14.

Table 14: Reviewers' Comparison of Clinical Worsenings Discrepant between Sponsor and FDA

Dose	Sponsor		FDA		Comment
	Worse	Day	Worse	Day	
AMB-320					
0	Yes	16	No	16	Censored - pulmonary embolus
5	Yes	9	No	9	Censored - pneumonia, sepsis
0	No	19	Yes	19	Worse per patient
0	No	30	Yes	30	Dyspnea AE, withdrew consent to seek other evaluation
10			No	1	Censored - randomized but not treated
10	Yes	89	No	13	Censored - hospitalized for SLE pleural effusion treated with prednisone, then walk 63d then traumatic intracranial hemorrhage 88d
AMB-321					
5	No	85	Yes	14	Furosemide added at 2 weeks for edema, dyspnea, O2 later
0	Yes	13	No	13	Censored - PE & chronic thromboembolism on angiography
2.5	No	71	Yes	27	Couldn't walk at 4 weeks due to edema
5	No	85	No	1	Censored - furosemide & spironolactone added day 1

The changes in assignments in the ten cases shown in Table 14 lead to some subtle but significant changes in the results, which we show in Table 15.

Table 15: Reviewers' Comparison of Sponsor's and FDA's Clinical Worsening Results

Dose	Sponsor		FDA Censored		FDA All	
	Events	P*	Events	P*	Events	P*
AMB-320						
0	6		7		8	
5	3	0.31	2	0.09	3	0.13
10	3	0.29	2	0.09	3	0.13
Combined	6	0.21	4	0.03	6	0.06
AMB-321						
0	14		13		14	
2.5	3	0.005	4	0.02	4	0.01
5	3	0.008	4	0.03	4	0.02
Combined	6	<0.001	8	0.005	8	0.002

*P from log rank stratified by idiopathic/secondary etiology

In Table 15 we show in the "FDA Censored" columns the results corresponding to the censoring in Table 14. As a sensitivity analysis we also show in the "FDA All" columns the results

counting all cases censored in Table 15 because of pulmonary embolism, sepsis, or worsening SLE as failures.

By the weighted Bonferroni method the improvements in times to clinical worsening were statistically significant in AMB-321 for both individual doses ($p \leq 0.02$) and the combined doses ($p \leq 0.04$) per the sponsor's analysis. The results were confirmed by our analyses except for the 5 mg dose in one of our analyses. The improvements in times to clinical worsening were not statistically significant in AMB-320 by the sponsor's analysis but were statistically significant only in the combined doses in one of our analyses.

COMMENT: The results for clinical worsening are not consistent across the studies. Only one study (AMB-321) conducted outside of the US shows a statistically significant improvement. The other study (AMB-320) conducted mostly in the US shows no statistically significant improvement from the sponsor's analysis ($P>0.21$), and our analyses show $p=0.03$ to $p=0.06$ for the combined dosages and $p = 0.09$ to $p = 0.13$ for individual doses, depending on how patients are censored. In addition, the sponsor changed the analysis plan near the end of the studies; therefore, it is not clear whether the changes may affect the interpretability of the results. Lastly, the results of AMB-320 could be statistically un-interpretable because the sponsor's analysis does not ensure an adequate control of overall type I error rate for the secondary endpoints formally tested (see the statistical IND reviews for the study protocols). Because of all these reasons, the statistical reviewer feels that the result for this first secondary endpoint might not provide sufficient statistical evidence that ambrisentan improves clinical worsening in PAH. The clinical reviewer believes that together the results for these two studies provide substantial evidence that ambrisentan improves clinical worsening in PAH.

Two patients who had clinical worsening with ambrisentan (both in the 10 mg group of AMB-320) were given bosentan after failing ambrisentan. (A third patient in a placebo group was also given bosentan after worsening.) Brief narratives for the ambrisentan failures given bosentan are as follows:

- A 76-year-old white female with mixed connective tissue disorder with a finger ulcer and also hypertension and a history of stroke was hospitalized on day 27 for right ventricular failure and ambrisentan was discontinued. She was started on bosentan on day 61 and walked [redacted] from original baseline, on day 85.

- A 61-year-old Hispanic female with primary PAH was hospitalized for worsening PAH and severe edema on day 33 and started on bosentan day 35. Her last walk was on day 35 and she had no efficacy follow-up after that day. She does have recorded AEs of edema, chills, and nasal congestion on day 61.

COMMENT: Both of these cases, particularly the 76-year-old female, raise the question of whether bosentan is more efficacious than ambrisentan. We raise the question of whether the BID dosing of bosentan is more effective for delaying clinical worsening than the QD dosing of ambrisentan.

6.1.4.3.2 WHO Functional Class

We show the sponsor's results for change from baseline to week 12 in WHO Class for AMB-320 in Table 16 and for AMB-321 in Table 17.

Table 16: Sponsor's Change from Baseline to Week 12 in WHO Class for AMB-320

Treatment group	Placebo	5 mg ambrisentan	10 mg ambrisentan	Combined ambrisentan
Change in WHO class, n (%)	(N = 67)	(N = 67)	(N = 67)	(N = 134)
-3	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
-2	1 (1.5)	1(1.5)	5 (7.5)	6 (4.5)
-1	15 (22.4)	18 (26.9)	15 (22.4)	33 (24.6)
0	40 (59.7)	47 (70.1)	44 (65.7)	91 (67.9)
+1	11 (16.4)	1 (1.5)	3 (4.5)	4 (3.0)
+2	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
+3	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
p-value[1]	-	0.074	0.072	0.036

[1]Wilcoxon rank sum test stratified by IPAH and non-IPAH subjects
Note: Negative changes are better (improvement)

Table 17: Sponsor's Change from Baseline to Week 12 in WHO Class for AMB-321

Treatment group	Placebo	2.5 mg ambrisentan	5 mg ambrisentan	Combined ambrisentan
Change in WHO class, n (%)	(N = 65)	(N = 64)	(N = 63)	(n = 127)
-3	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
-2	0 (0.0)	0 (0.0)	1 (1.6)	1 (0.8)
-1	11 (16.9)	10 (15.6)	8 (12.7)	18 (14.2)
0	42 (64.6)	51 (79.7)	52 (82.5)	103 (81.1)
+1	10 (15.4)	2 (3.1)	2 (3.2)	4 (3.1)
+2	2 (3.1)	1 (1.6)	0 (0.0)	1 (0.8)
+3	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
p-value[1]	-	0.215	0.172	0.117

[1]Wilcoxon rank sum test stratified by IPAH and non-IPAH subjects
Note: Negative changes are better (improvement)

Besides the usual problem with imputation of missing values, the sponsor's analyses of changes in WHO class have another problem: Patients in class I at baseline can't improve while patients in class IV can't deteriorate. The patients in class I at baseline are a very minor issue: there are few (8, or about 2%) and it is impossible to devise a substantial improvement category since these patients have minimal symptoms. Patients in class IV, however, are more frequent (18,

about 5%) and it is easy to define a substantially worse category: death! (Clinical functional classes such as WHO class lack this category because they are used to classify living patients!) We assigned a functional class of V to patients with clinical worsening and death (and to one patient class IV at baseline who had substantial worsening.) We show in Table 18 the patients for whom our assignments differ from the sponsor's.

Table 18: Reviewers' Comparison of Sponsor's and FDA's Changes in WHO Class

Dose	WHO Class			Comment
	Baseline	Sponsor	FDA	
AMB-320				
0	III	IV		Pulmonary embolus
0	III	IV	V	Increased PAH & death
0	II	II	III	Dyspnea AE, withdrew consent to seek other evaluation
5	IV	IV		D/C for pneumonia, sepsis
5	II	II		Site suspect & no doc on PAH & withdrew for personal reasons
10	IV	IV		Unevaluable - hospitalized for pleural effusion, then walk 63d then traumatic Intracranial hemorrhage 88d
10	III	III	IV	Hospitalized with right ventricular failure
AMB-321				
0	III	III	IV	Clinical worsening
0	III	IV	V	Clinical worsening & death
0	II	IV		Unevaluable–worse but PE & chronic thromboembolism on angiography
0	IV	IV	V	Worsening PAH
0	III	IV	V	Clinical worsening & death
2.5	III	IV	V	Clinical worsening & death
2.5	III	III		Withdrew consent because of travel distance, dyspnea unchanged
2.5	II	II	III	Couldn't walk at 4w due to edema
2.5	II	IV	V	Clinical worsening & death
5	III	III	IV	Right ventricular failure
5	III	III		Unevaluable - allergic rx treated with increased prednisone at 14d
5	III	III		Unevaluable - asthma on prednisone, d/c for facial edema prior to final walk
5	III	III	IV	Clinical worsening - furosemide add at 14d for edema, dyspnea later
5	III	III		Unevaluable - furosemide & spironolactone added 1d

The net effect of using our assignments for final WHO class shown in Table 18 is a slight weakening of the statistical significance of the possible beneficial effects of drug upon WHO class changes. The only p value from the sponsor's analysis that meets the usual criterion for statistical significance ($p < 0.05$), the one for the combined drug groups vs. placebo in ABM-320, increases from 0.036 to 0.043. However, neither the sponsor's nor our analyses showed statistically significant differences ($P \leq 0.005$ for the individual dose, or $P \leq 0.01$ for the combined doses by the weighted Bonferroni method) in changes of WHO class.

COMMENT: Neither the sponsor's nor our analyses of changes in WHO class provide substantial evidence that ambrisentan improves WHO class. The evidence fails the usual standard of having two trials successful at $p < 0.05$. Note also that the sponsor's statistical analysis plan required significance at $p < 0.01$ for WHO class.

6.1.4.3.3 SF-36 Physical Functioning Score

The earlier version of the protocol describe analyzing the SF-36 but did not specify what parts of the SF-36 would be analyzed. Amendment 2 to both protocols dated November 8, 2005, specified that the physical functioning score of the SF-36 would be "primary", to be analyzed by a Wilcoxon rank sum test using LOCF (worst rank for deaths and clinical worsenings). An analytic strategies plan for analyzing the SF-36, dated November 22, 2005, and prepared by a consultant, provides a more elaborate but not fully specified approach to analyzing the SF-36. We show the sponsor's analysis of the SF-36 physical functioning score from the AMB-320 study report in Table 19 and from the AMB-321 study report in Table 20.

Table 19: Sponsor's Completer Analysis of SF-36 Physical Functioning Score in AMB-320

SF-36® scale	Treatment group	Baseline, mean (SD)	Change from baseline, mean (SD)	p-value
Physical Functioning	Placebo	28.95 ± 8.27	2.31 ± 7.65	-
	5 mg	28.61 ± 9.17	3.86 ± 7.14	0.543
	10 mg	29.57 ± 9.35	4.52 ± 7.16	0.111
	Combined ambrisentan	29.09 ± 9.24	4.10 ± 8.39	0.229

p-value from repeated measures ANCOVA of time interactions

Table 20: Sponsor's Completer Analysis of SF-36 Physical Functioning Score in AMB-321

SF-36® scale	Treatment group	Baseline, mean (SD)	Change from baseline, mean (SD)	p-value
Physical Functioning	Placebo	31.85 ± 7.88	-0.20 ± 7.14	-
	2.5 mg	29.29 ± 7.65	3.86 ± 7.14	0.005
	5 mg	31.31 ± 9.09	2.96 ±6.81	0.040
	Combined ambrisentan	30.14 ± 8.30	3.41 ± 6.96	0.005

p-value from repeated measures ANCOVA of time interactions

The analyses above are based on a repeated measures ANCOVA of time interactions for completers, not the rank sum test of the change in score to week 12 as originally specified. In ABM-320 by the same repeated measures ANCOVA the rest of the SF-36 scales also showed insignificant differences while in ABM-321 there were improvements with drug for the role physical, general health, vitality, role emotional, and physical component scales.

Because the sponsor's analyses above were not the original pre-specified ones, we performed the analyses as pre-specified. We also replaced the sponsor's values for the final physical functioning score with the worst ranks if the patient died or had clinical worsening. We dropped patients who were unevaluable as indicated in Table 12 for the walk changes. We show our results in Table 21.

Table 21: Reviewers' Changes from Baseline in SF-36 Physical Functioning Score

Dose	Median	P
AMB-320		
0	0.0	
5	2.1	0.07
10	4.2	0.01
Combined	3.0	0.01
AMB-321		
0	-2.4	
2.5	2.1	<0.001
5	2.1	<0.001
Combined	2.1	<0.001

COMMENT: Our results suggest that how missing values are handled is critical for the analyses of the SF-36 physical functioning score. Our results also suggest that ambrisentan may have a favorable effect upon this score. However, this secondary endpoint is unevaluable because the preceding secondary endpoint of WHO class failed, [redacted] *. Furthermore, we judge that any improvement on the SF-36 physical functioning score is not substantially different than an improvement in clinical worsening.* [redacted]

6.1.4.3.4 Borg Dyspnea Index

The Borg dyspnea index (BDI) was used to capture the patient's appraisal of the degree of breathlessness immediately after completion of the six minute walk, with a BDI of "0" indicating no breathlessness and "10" indicating maximum breathlessness. We show the sponsor's analyses of BDI for AMB-320 in Table 22 and for AMB-321 in Table 23.

Table 22: Sponsor's Change in Borg Dyspnea Index (LOCF) in AMB-320

Treatment group	Placebo (N = 67)	5 mg ambrisentan (N = 67)	10 mg ambrisentan (N = 67)	Combined ambrisentan (N = 134)
Baseline BDI, mean (SD)	3.6 (1.84)	3.8 (2.16)	3.8 (2.08)	3.8 (2.11)
Change from baseline to Week 12				
Mean (SD)	0.0 (2.22)	-0.3 (1.93)	-0.9 (1.93)	-0.6 (1.95)
Median	0.0	-0.1	-1.0	-0.5
95% CI	-0.55, 0.54	-0.79, 0.16	-1.36, -0.41	-0.94, -0.27
Comparison versus placebo				
Point estimate	-	-0.3	-0.9	-0.6
95% CI	-	-1.0, 0.4	-1.6, -0.2	-1.2, 0.0
p-value[1]	-	0.316	0.002	0.017

[1]Wilcoxon rank sum test stratified by IPAH and non-IPAH subjects

Table 23: Sponsor's Change in Borg Dyspnea Index (LOCF) in AMB-321

Treatment group	Placebo (N = 65)	2.5 mg ambrisentan (N = 64)	5 mg ambrisentan (N = 63)	Combined ambrisentan (N = 127)
Baseline BDI, mean (SD)	4.0 (2.42)	3.9 (2.43)	3.8 (2.42)	3.8 (2.42)
Change from baseline to Week 12				
Mean (SD)	0.8 (2.63)	-0.2 (2.17)	-0.4 (1.99)	-0.3 (2.08)
Median	0.0	0.0	0.0	0.0
95% CI	0.17, 1.47	-0.74, 0.34	-0.87, 0.14	-0.65, 0.08
Comparison versus placebo				
Point estimate	-	-1.0	-1.2	-1.1
95% CI	-	-1.9, -0.2	-2.0, -0.4	-1.8, -0.4
p-value[1]	-	0.046	0.040	0.019

[1]Wilcoxon rank sum test stratified by IPAH and non-IPAH subjects

Although the sponsor's results for the BDI appear to be statistically favorable, the differences in changes in BDI are all small—note that the median changes in AMB-321 are all 0. BDI also has the same problem with incompleteness as the other efficacy endpoints—at least 9% of the patients lack final measurements. The changes in BDI as the sponsor evaluated them also appear to be inconsistent with other measures, e.g., the sponsor counts eight patients with clinical worsening (per the sponsor's assignment) as having improved BDI changes and another five as stable. However, assigning a worst change to the patients with clinical worsening actually improves the statistical significance of the improvements slightly.

The changes in BDI are also inconsistent with the walk changes. If patients were truly encouraged to walk maximum distances, we would expect some combinations of BDI changes and walk changes to be somewhat incompatible: patients should not have less dyspnea but a worse walk or more dyspnea and an improved walk. These combinations are not impossible (if dyspnea is not the limiting factor in walk distances) but they could result from differences in encouragement provided to the patients. We would expect them to be uncommon, but they are found in 17% and 13% of patients respectively. These occurrences would still be inconsequential if they occurred in the same frequencies in all treatment groups. In AMB-320 there is a greater frequency of the dyspnea better/walk worse combination in the placebo group (19 vs. 15%) while in AMB-320 there is both a greater frequency of the dyspnea better/walk worse combination in the placebo group (15 vs. 6%) and a greater frequency of the dyspnea worse/walk better combination in the ambrisentan groups (20 vs. 17%). These differences are consistent with placebo patients being given less encouragement to walk further and ambrisentan patients being given more encouragement. In AMB-321 these combinations were most common at two sites (207 and 211) that showed other irregularities in patient handling.

COMMENT: Despite the discrepancies in dyspnea/walk combinations, we judge overall that ambrisentan has a slightly favorable effect upon dyspnea during exertion. However, because this effect upon dyspnea was only studied in association with exertion and because its statistical significance is not established by a statistical analysis plan that conserves alpha for the secondary endpoints, [redacted]

6.1.4.3.5 Mortality

In each trial deaths were uncommon but less frequent in than ambrisentan group (3 vs. 1.5% and 4.6 vs. 1.6% for AMB-320 and AMB-321 respectively). Survival at one year was 95%. The sponsor also compares long-term survival to that from an NIH registry whose results were published in 1991.

COMMENT: It is reassuring that mortality differences, while not statistically significant, were favorable in each trial. Because we do not know how the patients in the trials compare to those in the NIH registry and because we would expect improvements in survival since 1991 due to improved treatment of PAH complications, e.g., improved treatment of heart failure, the comparisons to the NIH registry are worthless.

6.1.4.3.6 Pulmonary Hemodynamics

Cardiopulmonary hemodynamics were assessed in a subset of subjects enrolled in the phase 2 dose-controlled study, AMB-220. At week 12, mean cardiac index increased (+0.3 L/min/m2; 95% CI: 0.15 to 0.51; $p < 0.001$), mean pulmonary artery pressure decreased (-5.2 mmHg; 95% CI: -7.6 to -2.9; $p < 0.001$), and mean pulmonary vascular resistance decreased (-226 dynes·sec/cm5; 95% CI: -304 to -144; $p < 0.001$) for the combined ambrisentan group.

COMMENT: While these results are encouraging, because they are not placebo-controlled and not correlated with walk changes they are not that helpful.

6.1.4.3.7 Biomarkers

The sponsor evaluated three biomarkers in the two trials: endothelin-1, troponin T, and B-type natriuretic peptide (BNP). Endothelin-1 tended to increase more in the ambrisentan groups than in the placebo group, but there was substantial variability and no dose response in AMB-320. Changes in endothelin-1 were not correlated with the clinical endpoints. Changes in troponin T were minor and not significantly different from 0 in all groups. For BNP, geometric means of BNP increased slightly from baseline in the placebo groups (9%, 13%) while they decreased in the ambrisentan groups (29-30% for 2.5 and 5 mg and 45% for 10 mg.) The median reductions in BNP are similar in all groups and actually smallest in the 5 mg group in AMG-321. Changes in BNP were also not correlated with the clinical endpoints.

COMMENT: These biomarkers do not appear to be helpful in understanding ambrisentan activity.

6.1.4.4 Efficacy in Subgroups

6.1.4.4.1 Age, Gender, and Race

Efficacy did not appear to vary by gender, although the numbers of males studied (33 in AMB-320 and 49 in AMB-321) are low enough that firm conclusions about differential efficacy by gender are impossible. The numbers are even lower for races other than white: only 11 blacks were studied (all in AMB-320). While Hispanics were the largest ethnic group studied other than non-Hispanic whites, there were still few Hispanics studied (total 67) and any inferences regarding differential efficacy in Hispanics is confounded by differential results by region.

While the numbers of the elderly (age ≥ 65) studied are not high (43 in AMB-320 and 41 in AMB-321), there appears to be reduced efficacy in the elderly for walk improvements as shown in Table 24.

Table 24: Reviewers' Median Changes in Walks by Age

	N		Median		Placebo-Subtracted	
Dose	<65	≥65	<65	≥65	<65	≥65
AMB-320						
0	52	13	2	5		
5	48	17	28	-6	26	-11
10	55	11	52	2	50	-3
AMB-321						
0	50	14	4	-12		
2.5	49	14	33	-9	28	3
5	47	13	47	9	42	21

However, there does appear to be a beneficial impact upon rates of clinical worsening as shown in Table 25.

Table 25: Reviewers' Rates of Clinical Worsening by Age

Dose	<65	≥65
AMB-320		
0	10%	14%
5	2%	6%
10	2%	9%
AMB-321		
0	16%	36%
2.5	4%	14%
5	4%	8%

The elderly appear to have higher rates of clinical worsening on placebo and on ambrisentan than younger patients do but the rates in the elderly are lower with ambrisentan than with placebo.

COMMENT: It is unclear from these data whether ambrisentan favorably affects walk distances in the elderly, but it appears to have a beneficial impact upon clinical worsening in the elderly. There are no clear differences in efficacy by gender. Differential effects by race can not be determined because of small numbers of patients studied with races other than white.

6.1.4.4.2 Etiology

Median walk improvements were lower for patients with secondary PAH compared to idiopathic PAH as shown in Table 26.

Table 26: Reviewers' Median Changes in Walks by Etiology

	N		Median		Placebo-Subtracted	
Dose	I	S	I	S	I	S
AMB-320						
0	42	23	6	1		
5	42	23	47	6	41	6
10	41	25	52	25	46	25
AMB-321						
0	41	23	-10	10		
2.5	41	22	33	26	43	16
5	40	20	43	33	53	23

I = idiopathic; S = secondary

However, rates of clinical worsening were improved with ambrisentan except for the lowest dose tested (2.5 mg) as shown in Table 27.

Table 27: Reviewers' Rates of Clinical Worsening by Etiology

Dose	Idiopathic	Secondary
AMB-320		
0	10%	13%
5	5%	0%
10	2%	4%
AMB-321		
0	27%	9%
2.5	2%	14%
5	5%	5%

COMMENT: Ambrisentan appears to be less effective for improving walking distance in patients with secondary PAH. There still appears to be some benefit for walking distance and for clinical worsening.

6.1.4.4.3 WHO Class

There are insufficient numbers of patients with baseline WHO classes of I or IV to draw any inferences regarding efficacy in those classes. For WHO classes II and III there are reasonable numbers of patients, and we show the median changes in walks for these classes in Table 28 and the rates of clinical worsening in Table 29.

Table 28: Reviewers' Median Changes in Walks by WHO Class

	N		Change		Placebo-Subtracted	
Dose	II	III	II	III	II	III
AMB-320						
0	23	39	-10	5		
5	19	40	19	17	29	12
10	22	36	34	25	44	21
AMB-321						
0	23	37	5	-4		
2.5	34	28	48	5	43	8
5	28	30	58	30	53	34

Table 29: Reviewers' Rates of Clinical Worsening by WHO Class

Dose	II	III
AMB-320		
0	4%	15%
5	0%	5%
10	0%	6%
AMB-321		
0	9%	27%
2.5	6%	7%
5	0%	10%

COMMENT: The improvement in walk distances appears to be less for patients in class III at baseline compared to class II, even if one considers the slightly lower walk distances at baseline for those in class III. Conversely, the benefit for clinical worsening appears substantial.

6.1.4.4.4 Region

Some regions, e.g., Europe and Australia in AMB-320 and Israel in AMB-321, enrolled two few patients to support any comparisons by region. We show the median changes in walks by region for those regions with reasonable enrollments for AMB-320 in Table 30 and for AMB-321 in Table 31. We show the clinical worsening rates for the same regions for AMB-320 in Table 32 and for AMB-321 in Table 33.

Table 30: Reviewers' Median Changes in Walks by Region in AMB-320

	N		Median		Placebo-Subtracted	
Dose	US	Latin Am.	US	Latin Am.	US	Latin Am.
0	48	14	-4	29		
5	45	11	14	80	18	50
10	42	16	25	75	28	46

Table 31: Reviewers' Median Changes in Walks by Region in AMB-321

	N			Median			Placebo-Subtracted		
Dose	E. Europe	W. Europe	Latin Am.	E. Europe	W. Europe	Latin Am.	E. Europe	W. Europe	Latin Am.
0	10	33	18	-5	6	-30			
2.5	19	26	16	65	-1	50	70	-7	80
5	16	27	12	77	35	51	81	30	81

Table 32: Reviewers' Rates of Clinical Worsening by Region in AMB-320

Dose	US	Latin Am.
0	10%	7%
5	2%	9%
10	5%	0%

Table 33: Reviewers' Rates of Clinical Worsening by Region in AMB-321

Dose	E. Europe	W. Europe	Latin Am.
0	20%	18%	22%
2.5	0%	8%	13%
5	0%	7%	0%

COMMENT: The US shows the most conservative results for walk changes but a reasonable effect upon clinical worsening. The results for Western Europe are similar to the U.S. but note the lack of benefit of the 2.5 mg dose on walks. Eastern Europe and Latin America show substantially greater effect sizes particularly for walk changes.

While all of these subgroup analyses must be viewed as exploratory rather than definitive, there do appear to be some common threads. For subgroups with less favorable outcomes, e.g., the

elderly and class III, the benefit on walk distance appears to be less pronounced than the benefit on clinical worsening. There is some suggestion that the 2.5 mg dose is less effective, e.g., walk changes and clinical worsening in the elderly, clinical worsening in secondary PAH, and walks in class III patients and in Western Europe. For all subgroups there is some evidence, either walks or clinical worsening, that ambrisentan is effective. We believe the best estimates of ambrisentan effect sizes are from the US and Western Europe subgroups.

6.1.5 Clinical Microbiology

Clinical microbiology is not applicable for this oral formulation.

6.1.6 Efficacy Conclusions

The results of the two adequate and well-controlled studies, AMB-320 and AMB-321, for the common primary endpoint, change from baseline in six minute walk, provide substantial evidence that ambrisentan is effective in improving exercise capacity at least at peak drug levels. While there were substantial dropouts in these short-term studies (about 10%, typical for PAH studies because of the seriousness of the disease), the median walk changes and their statistical significances are robust to varying approaches to handling the dropouts. The placebo-corrected median improvements in walks are modest: about 18 m for the 5 mg dose and 28 m for the 10 mg dose in the US subgroup of AMB-320 and -7 m for the 2.5 mg dose and 30 m for the 5 mg dose in the Western Europe subgroup of AMB-321.

The improvement in clinical worsening, the first secondary endpoint, was highly statistically significant in one study (AMB-321) conducted outside US for the individual doses and the combined dose groups, based on the sponsor's analysis and our analysis that uses a weighted Bonferroni method. (Note that the sponsor's proposed method does not render an adequate control of overall type I error rate for the secondary endpoints.) However, in the other study (AMB-320), this clinical worsening endpoint failed to show statistical significance in the sponsor's analysis and, at best, it might reach borderline statistical significance for the combined dose group in our analysis. That ambrisentan improves clinical worsening seems to be supported by the results of the subgroup analyses, with most subgroups showing a beneficial impact of drug even when the effects upon walk improvement are less clear. The results for other secondary endpoints are not statistically conclusive and not clearly distinguished from effects upon clinical worsening (WHO class or SF-36 physical functioning score) or exercise capacity (Borg dyspnea index estimated immediately post-walk).

The sponsor was unable to provide timings of the walks relative to drug administration. Most are thought to have been performed in the morning or midday at about the time of peak drug levels. The pharmacokinetics of ambrisentan (see the FDA clinical pharmacology review) do not alone support once daily dosing. However, the beneficial impact upon clinical worsening suggests that ambrisentan has an effect that persists for longer than a few hours. That the walk changes appear to be improving on drug throughout the 12-week study period also suggests that there is some long-term or cumulative effect of the drug. On the other hand, the one patient who failed on ambrisentan once daily dosing and then improved on bosentan twice daily dosing,

raises the question of whether twice or more daily dosing of ambrisentan would be more effective. The sponsor should study twice daily dosing [redacted].

While the improvements in walks and clinical worsening in the 2.5 mg dose arm of AMB-321 were statistically significant, there is some evidence from the subgroup analyses that 2.5 mg is less effective. Given greater efficacy and a lack of evidence of dose-limiting toxicity for the higher doses, we agree that it is reasonable not to market the 2.5 mg dose. However, given that there is reasonable evidence for a dose-response through 10 mg without clear evidence of flattening of the response, the sponsor should also study higher doses [redacted].

Any of our conclusions regarding efficacy (as well as some regarding safety) that are based on the results of AMB-321 are dependent upon a clean audit of the one AMB-321 site selected for audit. Completion of audit of that site is still pending. If audit of that site reveals any problems, we will file a review addendum evaluating the impacts of the problems.

7 INTEGRATED REVIEW OF SAFETY

7.1 Methods and Findings

Description of phase 2-3 clinical trials

There were six phase 2-3 trials as listed in Table 7. Two were large, randomized, double blind, placebo controlled efficacy trials (AMB-320 and AMB 321), one was a blinded dose ranging efficacy trial (AMB-220), one was an open label, uncontrolled trial with subjects who could not tolerate taking an endothelin receptor antagonist because of elevated LFTs, and two were open label extension trials that are ongoing (AMB-320/321E and AMB-220).

The numbers of subjects in these six studies are shown below, by maximum dose of ambrisentan.

Table 1 Ambrisentan Exposure for Subjects with Pulmonary Arterial Hypertension (Population: All Phase 2 and 3 Studies)

	1 mg ambrisentan (N = 3)	2.5 mg ambrisentan (N = 91)	5 mg ambrisentan (N = 217)	10 mg ambrisentan (N = 172)	Combined ambrisentan (N = 483)
Mean (SD), weeks	46.9 (68.61)	48.2 (38.29)	38.7 (37.26)	57.9 (48.49)	47.4 (42.69)
Median, weeks	13.0	42.3	27.9	47.7	36.1
Min, Max, weeks	1.9, 125.9	0.4, 167.3	0.1, 168.0	0.1, 184.3	0.1, 184.3
Duration, n					
≥12 weeks	2	79	184	152	417
≥6 months	1	68	109	109	287
≥1 year	1	30	51	79	161
≥1.5 years	1	10	22	47	80
≥2 years	1	7	14	26	48
≥3 years	0	5	5	13	23

Note: Exposure summarized by maximum dose received (safety treatment assignment) as of the 16 February 2006 cut-off date for ongoing studies
Source: Appendix 3, Table 14.1.4a

At total of 483 subjects received at least one dose of ambrisentan. Most of these subjects received the 5 or 10 mg dose, and 161 subjects received drug for at least one year.

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Disposition of subjects

Placebo studies

A total of 394 subjects were randomized to either placebo, ambrisentan 2.5 mg, ambrisentan 5 mg, or ambrisentan 10 mg in the placebo controlled efficacy trials AMB-320 and -321. Both trials followed subjects for 12 weeks. The table below shows the number of subjects who were randomized, completed the study, or were withdrawn prematurely (by reason).

Number and (percent) of subjects

	placebo	AMB 2.5 mg	AMB 5 mg	AMB 10 mg
randomized	132	64	130	68
completed	111	58	121	63
Withdrawn premature	21 (16)	6 (9)	9 (7)	5 (7)
Adverse event	4	1	4	1
Early escape^	8	2	1	2
Other+	6	3	4	2

^defined as a gradual deterioration in subjects' clinical symptoms.
+see table below.

The percent of placebo subjects who withdrew (for any reason) was about twice the percent for the active treatment groups.

The 15 subjects whose reason for withdrawal was classified as "other" are described in the table below.

Study	Subject number	Reason for Discontinuation
Placebo		
AMB-320	111-001	formal withdrawal of consent (declined to continue with study medication)
AMB-320	129-001	Other: protocol violation
AMB-320	132-001	Non-compliance to any of the procedures (patient requested to stop drug for 2 weeks)
AMB-320	139-001	Treatment with other PAH treatment (IV epoprostenol)
AMB-320	156-001	formal withdrawal of consent (clinical worsening)
AMB-321	201-008	Clinical status did not improve
2.5 mg		
AMB-321	225-002	formal withdrawal of consent (decision of the patient)
AMB-321	229-002	formal withdrawal of consent (patient stated that study drug made her illness worse)
AMB-321	247-004	formal withdrawal of consent (marked dyspnea)
5 mg		
AMB-320	101-009	Lost to follow-up (patient did not return for visit 4 or 5, site attempted several contacts)
AMB-320	107-006	Other: patient developed left heart failure. Diagnosis revised: pulmonary venous hypertension
AMB-320	149-004	formal withdrawal of consent (too complicated; personal (private) reasons)
AMB-321	247-003	formal withdrawal of consent (the patient has refused to participate in the study due to her family's circumstances)
10 mg		
AMB-320	101-015	Discretion of Myogen (Note: The investigator randomized the subject prior to receipt of central laboratory liver function test results. The ALT/AST results were >1.5 ULN which was a protocol violation. Subject discontinued prior to 1st dose of study drug and was not included in analysis dataset [or ITT] since the subject never received drug.)
AMB-320	126-006	formal withdrawal of consent (patient decided to withdraw)

Duration of exposure

The mean numbers of weeks subjects were receiving treatment were similar for all groups. However, the minimum duration of treatment for the ambrisentan groups tended to be somewhat less (1-1.1 weeks) compared to the placebo group (2.7 weeks).

[illegible] and 10, the combined ambrisentan group in Table 3.

Table 3 Summary of Treatment Exposure (AMB-320/321 Population: Safety)

Treatment group	Placebo (N = 132)	2.5 mg ambrisentan (N = 64)	5 mg ambrisentan (N = 130)	10 mg ambrisentan (N = 67)	Combined ambrisentan (N = 261)
Total weeks subject received drug					
Mean (SD)	11.4 (2.22)	11.9 (1.56)	11.6 (2.09)	11.8 (1.93)	11.7 (1.93)
Median	12.0	12.1	12.1	12.1	12.1
Min, Max	2.7, 14.0	1.1, 13.9	1.1, 13.4	0.9, 14.3	0.9, 14.3

Source: AMB-320/321 Table 12.1

Demographics

The demographics of the 393 subjects in the 2 placebo controlled trials are shown below.

Table 8 Demographics: Combined Analysis of Phase 3 Placebo-Controlled Studies (AMB-320/321: Population: Safety)

Treatment group / Characteristic	Placebo (N = 132)	2.5 mg ambrisentan (N = 66)	5 mg ambrisentan (N = 130)	10 mg ambrisentan (N = 67)	Combined ambrisentan (N = 261)	Total population (N = 393)
Gender, n (%)						
Male	29 (22.0)	16 (25.0)	23 (17.7)	14 (20.9)	53 (20.3)	82 (20.9)
Female	103 (78.0)	48 (75.0)	107 (82.3)	53 (79.1)	208 (79.7)	311 (79.1)
Race, n (%)						
Caucasian	100 (75.8)	54 (84.4)	104 (80.0)	44 (65.7)	202 (77.4)	302 (76.8)
Black	4 (3.0)	0 (0.0)	4 (3.1)	3 (4.5)	7 (2.7)	11 (2.8)
Asian	4 (3.0)	1 (1.6)	3 (2.3)	1 (1.5)	5 (1.9)	9 (2.3)
Hispanic	24 (18.2)	9 (14.1)	17 (13.1)	17 (25.4)	43 (16.5)	67 (17.0)
Other	0 (0.0)	0 (0.0)	2 (1.5)	2 (3.0)	4 (1.5)	4 (1.0)
Age, years, mean (SD)	49.4 (15.22)	51.6 (15.17)	51.6 (14.99)	49.3 (15.64)	51.0 (15.17)	50.5 (15.19)
<65 years	104 (78.8)	50 (78.1)	100 (76.9)	55 (82.1)	205 (78.5)	309 (78.6)
≥65 and <75 years	22 (16.7)	11 (17.2)	25 (19.2)	7 (10.4)	43 (16.5)	65 (16.5)
≥75 years	6 (4.5)	3 (4.7)	5 (3.8)	5 (7.5)	13 (5.0)	19 (4.8)
Weight, kg, mean (SD)	74.2 (19.59)	70.0 (15.17)	70.3 (16.65)	73.2 (20.92)	71.0 (17.50)	72.1 (18.27)
BMI, kg/m², mean (SD)	27.8 (6.21)	26.3 (5.40)	26.7 (5.38)	27.9 (7.10)	26.9 (5.88)	27.2 (6.00)
Region, n (%)						
US/Australia[1]	52 (39.4)	0 (0.0)	49 (37.7)	47 (70.1)	96 (36.8)	148 (37.7)
Eastern Europe	11 (8.3)	20 (31.3)	18 (13.8)	0 (0.0)	38 (14.6)	49 (12.5)
Latin America	33 (25.0)	16 (25.0)	24 (18.5)	16 (23.9)	56 (21.5)	89 (22.6)
Western Europe/Israel	36 (27.3)	28 (43.8)	39 (30.0)	4 (6.0)	71 (27.2)	107 (27.2)

[1]Because no subjects from Canada were enrolled, the geographical region of US/Canada/Australia is referred to as US/Australia
Source: AMB-320/321, Table 10.2 and Listing 16.2.1

Subjects were more likely to be female, white, and less than 65 years of age. The majority of study subjects were living in US/Australia/Western Europe/Israel.

The treatment groups were fairly well balanced for these demographics.

Baseline disease characteristics

Disease characteristics are shown below by treatment group.

Table 9 Baseline Characteristics (AMB-320/321 Population: ITT)

Treatment group Characteristic	Placebo (N = 132)	2.5 mg ambrisentan (N = 64)	5 mg ambrisentan (N = 130)	10 mg ambrisentan (N = 67)	Combined ambrisentan (N = 261)	Total population (N = 393)
PAH etiology, n (%)						
IPAH	85 (64.4)	42 (65.6)	83 (63.8)	41 (61.2)	166 (63.6)	251 (63.9)
non-IPAH	47 (35.6)	22 (34.4)	47 (36.2)	26 (38.8)	95 (36.4)	142 (36.1)
PAH-CTD	43 (32.6)	19 (29.7)	40 (30.8)	22 (32.8)	81 (31.0)	124 (31.6)
PAH-anorexigen	1 (0.8)	1 (1.6)	2 (1.5)	2 (3.0)	5 (1.9)	6 (1.5)
PAH-HIV	3 (2.3)	2 (3.1)	4 (3.1)	2 (3.0)	8 (3.1)	11 (2.8)
Number years PAH present	2.2 (3.92)	1.2 (1.93)	2.3 (5.29)	1.4 (2.39)	1.8 (4.05)	2.0 (4.01)
WHO functional class, n (%)						
I	4 (3.0)	0 (0.0)	2 (1.5)	2 (3.0)	4 (1.5)	8 (2.0)
II	47 (35.6)	34 (53.1)	48 (36.9)	22 (32.8)	104 (39.8)	151 (38.4)
III	78 (59.1)	29 (45.3)	73 (56.2)	36 (53.7)	138 (52.9)	216 (55.0)
IV	3 (2.3)	1 (1.6)	7 (5.4)	7 (10.4)	15 (5.7)	18 (4.6)
6MWD, m, mean (SD)	342.3 (79.55)	347.3 (83.81)	347.2 (80.61)	341.5 (78.28)	345.8 (80.55)	344.6 (80.13)
BDI, mean (SD)	3.8 (2.15)	3.9 (2.43)	3.8 (2.29)	3.8 (2.08)	3.8 (2.26)	3.8 (2.22)
Historical Hemodynamics						
Cardiac index, L/min/m^2, mean (SD)	2.44 (0.754)	2.47 (0.739)	2.45 (0.842)	2.57 (0.748)	2.49 (0.793)	2.47 (0.779)
mPAP, mmHg, mean (SD)	50.8 (14.18)	48.5 (14.23)	47.3 (13.32)	51.4 (16.11)	48.7 (14.35)	49.4 (14.31)
PVR, dynes-sec/cm^5, mean (SD)[1]	920 (549.6)	800 (396)	880 (560.8)	912 (464.8)	872 (500.8)	888 (517.6)
RAP, mmHg, mean (SD)	7.8 (5.03)	8.3 (5.45)	8.1 (4.82)	9.2 (5.73)	8.4 (5.21)	8.2 (5.15)

[1]80 x Wood Units = dynes-sec/cm^5. CTD = connective tissue disease; HIV = human immunodeficiency virus; 6MWD = 6-minute walk distance; BDI = Borg dyspnea index; mPAP = mean pulmonary arterial pressure; PVR = pulmonary vascular resistance; RAP = right atrial pressure
Source: AMB-320/321, Table 10.3 and Summary Table 14.1.2.1a

Subjects were mostly likely to be diagnosed with idiopathic PAH, to have been diagnosed with the disease for about two years, to be in WHO functional class II or III, and to have a baseline walk distance of around 345 meters. The groups were fairly well balanced.

Concomitant medication

The three most common concomitant medications included spironolactone, paracetamol, and dilitazem.

7.1.1 Deaths

A total of 27 subjects were reported to have died while on or within four weeks of discontinuing ambrisentan. The majority of the deaths (78%) were reported during the long term extension studies.

Table 25 Summary of Deaths in Subjects with Pulmonary Arterial Hypertension (Population: All Phase 2 and 3 Studies)

Treatment group[1]	Placebo	1 mg ambrisentan	2.5 mg ambrisentan	5 mg ambrisentan	10 mg ambrisentan
Phase 3, placebo-controlled studies					
AMB-320	2	0	NA	1	1
AMB-321	4	0	2	0	NA
Phase 2 Studies					
AMB-220	NA	1	0	0	1
AMB-222[2]	NA	0	0	0	0
Long-term Phase 2 and 3 studies					
AMB-320/321-E[3]	NA	0	3	10	3
AMB-220-E[2]	NA	0	1	0	4

[1]Deaths are attributed to the actual ambrisentan dose at the time of death
[2]For ongoing studies deaths are reported through 16 February 2006
[3]Deaths that occurred during study AMB-320/31-E only. Deaths that occurred while subjects received ambrisentan or placebo in AMB-320 and AMB-321 are counted for those studies only.
NA = treatment not available in study

N.B. Subject 321/235/003 had been randomized to placebo (and is listed in the placebo column in table 25) but was actually receiving ambrisentan 5 mg for 8 day at time of death. She is discussed under the 5 mg dose in the table below.

During the two placebo-controlled trials, there were ten deaths: six deaths[1] were reported in the placebo group (4.5%) and four in the ambrisentan group (1.2%). The ten deaths are discussed below, by treatment group.

Deaths reported during the placebo controlled trials

Subject ID/age/sex	Duration	Comments
placebo		
320/126-008/50y/f	15d	Right heart failure
320/139-001/32y/f	62d	Right heart failure
321/207-014/65y/m	72d	Cardiorespiratory arrest
321/235-005/40y/f	23d	Pulmonary thromboembolism and right heart failure
321/245-006/44y/f	68d	Worsening PAH
Amb 2.5		
321/207-002/66y/m	80d	Discontinued because of worsening PAH. Hospitalized, experienced 3 cardiac arrests. Died one day after the last dose of study drug.
321/230-004/29y/m	66d	Sudden worsening of dyspnea followed by death. Could not rule out pulmonary embolism. History of SLE.
Amb 5 mg		
320/156-007/28y/f	8d	Gastroenteritis (N&V with elevated GGT, total bilirubin, LDH, mild leukocytosis, protein and bilirubin in urine), pneumonia (consolidation on x-ray), sepsis with hypovolemic shock and cardiac arrest.

1 Includes subject 321/235/003

Subject ID/age/sex	Duration	Comments
321/235-003/31y/f	67d placebo followed by 8d amb 5mg	Developed decompensated right heart failure while on placebo and discontinued as "early escape." Started ambrisentan. Condition did not improve, subject developed sepsis and multiorgan failure leading to death.
Amb 10 mg		
320/101-006/77y/f	89d	Intracranial hemorrhage after (witnessed) fall. Concomitant med included warfarin

These deaths seem linked to the underlying disease rather than to the use of ambrisentan.

The additional 21 deaths reported during the other phase 2-3 trials are shown below.

Subject ID/age/sex	Dose/Duration	Comments
220/16-004/75y/m	1mg/13d	Appeared cyanotic at screening visit, increased edema dyspnea, fatigue day 8. Sudden death.
220/13-005/52y/m	10mg/44d	Seen in clinic 2 days, doing well. Died suddenly 2 days later.
320/321E/207-015/70y/f	2.5mg/365d	Hospitalized for worsening right heart failure with frequent supraventricular extrasystoles and anuria. She developed progressive hypotension followed by cardiorespiratory arrest.
320/321E/221-003/54y/f	**2.5mg/24d (Randomized to placebo in base study)**	**History of SLE. Developed epigastric pain with dark urine, back pain, nausea, vomiting. Hospitalized at another hospital and details are sparse (no documentation of LFTs, serum amylase). There were discussions about ALT and ALT 10-20xULN (unconfirmed). Drug induced hepatitis versus bile duct cholelithiasis/biliary sepsis/pancreatitis.**
320/321E/244-004/72y/f	2.5mg/393d	Developed dyspnea, cyanosis, cardiac arrest. She was revived but developed asystole and died 2 days later. Had been hospitalized previously for worsening PAH day 309.
320/321E/104-003/35y/f	5mg/115d	Hospitalized for dizziness, increased dyspnea, chronic cough. Became febrile, WBC rose, blood pressure dropped, developed nausea and

Subject ID/age/sex	Dose/Duration	Comments
		vomiting. Arrested and could not be revived. Blood and lung cultures were positive for microorganism associated with indwelling catheter. Previously hospitalized for chest pain.
320/321E/116-003/68y/f	5mg/206d	Hospitalized for respiratory failure. She died the following day. Autopsy reported cause of death as respiratory failure.
320/321E/121-001/71y/f	5mg/235d/	History of 3 day bout of gastroenteritis with diarrhea, nausea, vomiting. Cardiac arrest while being transported to hospital because of dyspnea and chest pain. Arrested again in the emergency room. On admission chest x ray showed pulmonary edema. Labs: potassium 6.8mmol/L and creatinine 6.3 mg/dl. DNR was implemented. Cause of death was listed as presumed sepsis.
320/321E/207-005/49y/f	5mg/196d	Continued with signs and symptoms of worsening right heart failure. Was hospitalized twice. Experienced severe bradycardia followed by cardiac arrest. Resuscitation was unsuccessful.
320/321E/207-007/72y/m	5mg/365d	Hospitalized on day 253 because of tuberculous meningitis with encephalitis. Medical treatment was ineffective and he was discharged to home. He died a short time later.
320/321E/207-011/67y/f	5mg/205d	Hospitalized day 78 because of right heart failure. Hospitalized day 194 because of heart failure and acute renal failure. She had fallen 3 days previously and blood pressure on admission was 95/60 mmHg with heart rate 120 bmp. She became febrile with rapid deterioration. Death was attributed to cardiorespiratory arrest.
320/321E/207-025/57y/f	5mg/114d	Hospitalized day 12 because of worsening right heart failure. Hospitalized again for pulmonitis and acute respiratory failure. She died from cardiac arrest about 1 week later. Anemia was reported

Subject ID/age/sex	Dose/Duration	Comments
		without laboratory values reported.
320/321E/211-004/69y/f	5mg/181d	Hospitalized because of right heart failure. She was found to be anemic. She died day 182 as a result of acute respiratory failure after having been transferred to a nursing home.
320/321E/235-004/32y/m	5mg/441d	Died of acute respiratory failure. Hospitalized for increased dyspnea and tachycardia on study day 441. Experienced cardiac arrest and could not be revived. HIV positive.
320/321E/132-008/24y/f	**5mg/12d**	**Discontinued from placebo day 37 because of worsening PAH (early escape). She was hospitalized for fluid overload and increased dyspnea. 8 days after starting ambrisentan (and 4 days after she had been discharged for the previous event) she was hospitalized for what was identified as exacerbation of SLE. Complaints included dyspnea at rest, chest pressure, nausea and vomiting. ECHO showed greatly enlarged right ventricle. LFTs were >7000 IU/L. Ambrisentan was discontinued, she was treated with epoprostenol and dobutamine and LFTs fell to ~100 IU/L. Thrombotic thrombocytopenia was diagnosed, plasma exchange was started and she developed asystole during the procedure. Autopsy showed diffuse alveolar hemorrhage, SLE, pulmonary hypertension, renal failure. Liver showed severe centrilobular congestion consistent with right heart failure. GGT at screen was mildly elevated as was ALT.**
320/321E/210-006/65y/f	10mg/523d[2]	Diagnosed with large, highly malignant nerve sheath sarcoma on day 444. Several months later she died of cardiac arrest and autopsy showed cardiac wall metastases.
320/321E/213-004/34y/f	2.5mg/364d	Subject died 422 days after start of

2 Includes 336 days on 5 mg and 187 days on 10 mg

Subject ID/age/sex	Dose/Duration	Comments
	5mg/57d 10mg/3d	study drug of multiorgan failure. On day of hospitalization she presented with severe cough and minor hemoptysis and was diagnosed with respiratory insufficiency and broncho-pneumonia. She developed right heart failure with persistent hypotension. LFTs were normal or mildly elevated initially, but became greatly elevated as did serum creatinine. She died after being denied a heart/lung transplant. Autopsy showed PAH with acute bronchitis and multi-organ failure. She had been hospitalized on day 361 for worsening PAH.
320/321E/213-008/65y/f	5mg/438d 10mg/221d	Died of acute heart failure. Had been hospitalized numerous times for pneumonia, worsening PAH, suspicion of malignant lymphadenopathy, repeated syncope with bronchopneumonia.
220E/13-007/68y/f	2.5mg/285 d 5mg/99d 2.5mg/29d 1mg/86d	Died of right heart failure on study day 490
220E/05-002/59y/f	1mg/86 d 2.5mg/24d 5mg/108d 10mg/54d	Hospitalized for psittacosis pneumonia on day 233. On study day 268 she was hospitalized for shortness of breath. After a lung biopsy she was found unresponsive. Autopsy showed bilateral organizing pneumonia.
220E/13-004/77y/m	2.5mg/17d 5mg/14d 10mg/252d	Hospitalized on day 282 for right heart failure with worsening dyspnea and decreased urine output. He died that day.
220E/15-005/66y/f	2.5mg/82d 5mg/30d 10mg/568d	Elevated LFTs on day 495 found when hospitalized for pneumonia. Drug discontinued and then restarted about 2 months later when LFTs normalized. She was hospitalized on day 672 because of pneumonia. ALT was within normal limits (bilirubin, GGT, Alk phos elevated). She died 7 days later of acute respiratory failure.
220E/21-009/54y/f	2.5mg/85d	Experienced dizziness and syncope

Subject ID/age/sex	Dose/Duration	Comments
	5mg/30d 10mg/597d	day 672. Remained on drug. Again experienced syncope day 681. Study drug continued. Rehospitalized day 702 for increased shortness of breath, weight gain, peripheral edema, increased weakness and fatigue. Echo showed severe right ventricular dilatation and PAH. She collapsed and could not be resuscitated.

Drug induced hepatitis followed by death cannot be ruled out for subject 320/321E/221-003. Details about this case are unobtainable.

There was an extremely large rise in LFTs for subject 320/321E/132-008 which resolved when the drug was discontinued. Cause of death was probably SLE exacerbation.

The other deaths seemed linked to the underlying disease.

There were no reported deaths in the phase 1 studies.

7.1.2 Other Serious Adverse Events

Placebo controlled trials

Twice as many placebo subjects reported at least one SAE compared to the ambrisentan groups. The SAEs not leading to death and reported by at least two subjects in one or more groups are shown below.

Table 26 Serious Adverse Events (Other Than Death) Reported in >1 Subject in the Combined Ambrisentan Treatment Group (AMB-320/321 Population: Safety)

Treatment group Adverse event, n (%)	Placebo (N = 132)	Combined Ambrisentan (N = 261)
Any SAE	21 (15.9)	23 (8.8)
Right ventricular failure	8 (6.1)	5 (1.9)
Pneumonia	2 (1.5)	4 (1.5)
Worsening pulmonary hypertension	5 (3.8)	3 (1.1)
Dyspnea exacerbated	1 (0.8)	2 (0.8)
Peripheral edema	0 (0.0)	2 (0.8)

Source: AMB-320/321, Section 12.3.3.2 and Summary Table 14.3.9

Right heart failure was the most commonly reported event.

Extension trials

There were 97 subjects with at least one report of a SAE (excluding those who went on to die) during the long term extension studies (320/321E). Mean exposure of 38.6 weeks.

All events reported by at least two subjects are shown below.

Table 27 Serious Adverse Events with Outcomes Other Than Death in 2 or More Subjects Reported in the Combined Ambrisentan Treatment Group (AMB-320/321-E Population: Safety)

Treatment group Adverse event, n (%)	2.5 mg ambrisentan (N = 78)	5 mg ambrisentan (N = 171)	10 mg ambrisentan (N = 134)	Combined ambrisentan (N = 383)
No. of subjects with 1 or more SAE other than death[1]	20 (25.6)	37 (21.6)	40 (29.9)	97 (25.3)
Pulmonary hypertension	5 (6.4)	4 (2.3)	9 (6.7)	18 (4.7)
Right ventricular failure	2 (2.6)	10 (5.8)	6 (4.5)	18 (4.7)
Pneumonia	2 (2.6)	3 (1.8)	3 (2.2)	8 (2.1)
Syncope	3 (3.8)	1 (0.6)	2 (1.5)	6 (1.6)
Pregnancy	1 (1.3)	2 (1.2)	1 (0.7)	4 (1.0)
Hypoxia	1 (1.3)	1 (0.6)	2 (1.5)	4 (1.0)
Pleural effusion	0 (0.0)	1 (0.6)	3 (2.2)	4 (1.0)
Gastrointestinal hemorrhage	1 (1.3)	2 (1.2)	0 (0.0)	3 (0.8)
Anemia	0 (0.0)	1 (0.6)	1 (0.7)	2 (0.5)
Atrial fibrillation	0 (0.0)	1 (0.6)	1 (0.7)	2 (0.5)
Atrial flutter	0 (0.0)	1 (0.6)	1 (0.7)	2 (0.5)
Retroperitoneal hemorrhage	0 (0.0)	0 (0.0)	2 (1.5)	2 (0.5)
Peripheral edema	0 (0.0)	0 (0.0)	2 (1.5)	2 (0.5)
Cholecystitis acute	0 (0.0)	1 (0.6)	1 (0.7)	2 (0.5)
Cholelithiasis	1 (1.3)	0 (0.0)	1 (0.7)	2 (0.5)
Bronchopneumonia	0 (0.0)	0 (0.0)	2 (1.5)	2 (0.5)
Subdural hematoma	1 (1.3)	0 (0.0)	1 (0.7)	2 (0.5)
Hepatic enzyme increased	0 (0.0)	1 (0.6)	1 (0.7)	2 (0.5)
Fluid overload	0 (0.0)	0 (0.0)	2 (1.5)	2 (0.5)
Hyperglycemia	0 (0.0)	2 (1.2)	0 (0.0)	2 (0.5)
Localized osteoarthritis	0 (0.0)	1 (0.6)	1 (0.7)	2 (0.5)
Renal failure acute	0 (0.0)	1 (0.6)	1 (0.7)	2 (0.5)
Menorrhagia	0 (0.0)	1 (0.6)	1 (0.7)	2 (0.5)
Dyspnea exacerbated	1 (1.3)	0 (0.0)	1 (0.7)	2 (0.5)

[1]Preliminary analysis period includes the Week 1-120 interval with a mean exposure of 38.6 weeks and a maximum exposure of 109.0 weeks
Source: AMB-320/321-E, Table 12.9

Commonly reported SAEs were pulmonary hypertension and right ventricular failure. Two ambrisentan subjects reported increased hepatic enzymes as an SAE.

With the dose range so narrow, it is not possible to identify an event, other than elevated LFTs[3], that is possibly dose related.

3 Based on the bosentan data.

7.1.3 Dropouts and Other Significant Adverse Events

Placebo controlled trials[4]

A similar percent of subjects randomized to ambrisentan[5] (4%) discontinued because of an adverse event compared to placebo (5%). Only the event "right ventricular failure" was reported by more than one ambrisentan subject. Table 14.3.10

In the long term extension study 320/321E there were 34 (9%) withdrawals for adverse events[6]. Common events included right ventricular failure, infections, nervous system disorders, pulmonary hypertension.

Dose ranging trial (study 220)

There were three study drug discontinuations because of an adverse event during the double blind phase and one in the open label extension phase. These are shown below.

Table 12.8 List of Adverse Events Leading to Study Discontinuation (Population: ITT)

Treatment	Subject No.	Sex Age	Adverse Event verbatim / preferred term	Relation to study drug	SAE (Y/N)	Outcome
Blinded Treatment Period						
1 mg	16-004	M 75 yr	aggravated PAH / pulmonary hypertension NOS aggravated	unrelated	N	unresolved
			electromechanical dissociation/ electromechanical dissociation	unrelated	N	unresolved
			sudden death / sudden death	unrelated	Y	death
5 mg	24-001	F 57 yr	elevated AST ALT/ ALT increased	probably	Y	resolved
			elevated AST ALT/ AST increased	probably	Y	resolved
10 mg	13-005	M 52 yr	sudden death / sudden death	unrelated	Y	death
OLE Period						
5 mg	05-001[1]	F 62 yr	blood pressure low/ hypotension NOS	possibly	N	unresolved
			oxygen saturation low/ oxygen saturation decreased	possibly	N	unresolved

[1] In Listing 16.2.8, "Subject's medical status did not improve" was listed as the reason for discontinuation of study, however, Listing 16.2.25 indicated that the above AEs lead to discontinuation of the study.
Source: Listing 16.2.25

There was one discontinuation because of elevated ALT/AST. Subjects with elevated LFTs are discussed later in this section.

In the extension study there were 10 discontinuations: right heart failure (5), worsening pulmonary hypertension (2), exacerbated dyspnea (1), increased ALT/AST (1, noted above), acute respiratory failure/viral pneumonia (1). One subject reported recurrent cystitis and one reported intermittent headaches with nasal congestion.

4 Table 14.3.10
5 All doses (2.5 mg-10 mg) combined
6 Table 14.3.7 in AIRES-E

Healthy volunteer Phase I studies

One healthy volunteer (#38) in study EE-001 discontinued after receiving the 50 mg dose because of facial flush, shivering, sickness, nausea, vomiting, headache, vertigo, and dizziness. The 100 mg dose was administered to 2 subjects without an obvious safety effect.

Other discontinuations in normal volunteer studies included subject 01-322 in study AMB-103 who dropped out because of streptococcal pharyngitis, subject 01-03 in study AMB-105 who dropped out because of headache and subject 01-147 in study AMB-104 who dropped out because of palpitations.

7.1.4 Other Search Strategies

We did not employ other search strategies.

7.1.5 Common Adverse Events

Placebo controlled trials

Adverse events reported by subjects randomized into study 320 or 321 are shown below.

Table 12 Adverse Events >3% Incidence in Placebo or Combined Ambrisentan Treatment Groups (AMB-320/321 Population: Safety)

Treatment group Adverse event, n (%)	Placebo (N = 132)	2.5 mg ambrisentan (N = 64)	5 mg ambrisentan (N = 130)	10 mg ambrisentan (N = 67)	Combined ambrisentan (N = 261)
Subjects with at least 1 AE	108 (81.8)[1]	47 (73.4)	102 (78.5)	53 (79.1)	202 (77.4)
Peripheral edema	14 (10.6)	2 (3.1)	24 (18.5)	19 (28.4)	45 (17.2)
Headache	18 (13.6)	5 (7.8)	20 (15.4)	13 (19.4)	38 (14.6)
Dizziness	13 (9.8)	3 (4.7)	9 (6.9)	6 (9.0)	18 (6.9)
Nasal congestion	2 (1.5)	1 (1.6)	7 (5.4)	7 (10.4)	15 (5.7)
Cough	8 (6.1)	2 (3.1)	7 (5.4)	5 (7.5)	14 (5.4)
Dyspnea exacerbated	8 (6.1)	2 (3.1)	10 (7.7)	1 (1.5)	13 (5.0)
Upper respiratory tract infection	8 (6.1)	2 (3.1)	6 (4.6)	5 (7.5)	13 (5.0)
Palpitations	3 (2.3)	4 (6.3)	5 (3.8)	3 (4.5)	12 (4.6)
Dyspnea	4 (3.0)	1 (1.6)	7 (5.4)	3 (4.5)	11 (4.2)
Constipation	2 (1.5)	2 (3.1)	4 (3.1)	4 (6.0)	10 (3.8)
Fatigue	6 (4.5)	0 (0.0)	7 (5.4)	3 (4.5)	10 (3.8)
Nausea	12 (9.1)	2 (3.1)	5 (3.8)	3 (4.5)	10 (3.8)
Bronchitis	5 (3.8)	3 (4.7)	6 (4.6)	1 (1.5)	10 (3.8)
Flushing	1 (0.8)	4 (6.3)	5 (3.8)	1 (1.5)	10 (3.8)
Nasopharyngitis	1 (0.8)	0 (0.0)	7 (5.4)	2 (3.0)	9 (3.4)
Right ventricular failure	16 (12.1)	2 (3.1)	6 (4.6)	1 (1.5)	9 (3.4)
Abdominal pain	1 (0.8)	2 (3.1)	4 (3.1)	2 (3.0)	8 (3.1)
Chest pain	3 (2.3)	1 (1.6)	6 (4.6)	1 (1.5)	8 (3.1)
Insomnia	4 (3.0)	4 (6.3)	3 (2.3)	1 (1.5)	8 (3.1)
Epistaxis	5 (3.8)	2 (3.1)	2 (1.5)	4 (6.0)	8 (3.1)
Sinusitis	0 (0.0)	1 (1.6)	4 (3.1)	3 (4.5)	8 (3.1)
Arthralgia	5 (3.8)	3 (4.7)	1 (0.8)	2 (3.0)	6 (2.3)
Urinary tract infection	8 (6.1)	2 (3.1)	2 (1.5)	1 (1.5)	5 (1.9)
ALT increased	5 (3.8)	0 (0.0)	2 (1.5)	2 (3.0)	4 (1.5)
Pulmonary hypertension	7 (5.3)[1]	2 (3.1)	1 (0.8)	1 (1.5)	4 (1.5)

Note: Table reports AEs of >3% in the placebo group or combined ambrisentan group
[1]Subject 156-001 (placebo) had an event of clinical worsening of PAH that appeared to be nontreatment-emergent. Upon investigation, the event started after the first dose of study drug. This subject has been included in this summary table.
Source: AMB-320/321, Summary Table 12.3

The events from the above table and reported more often in the combined ambrisentan compared to the placebo group include peripheral edema 7%[7], headache 1%, nasal congestion 4%, palpitations 2%, dyspnea 1%, constipation 2%, flushing 3%, nasopharyngitis 3%, abdominal pain 2%, chest pain 1%, and sinusitis 3%.

Peripheral edema (in particular) and nasal congestion (less so) seem to suggest a positive relationship to dose.

Right ventricular failure, on the other hand, was reported almost four times more often by the placebo group than the combined ambrisentan group. However, this was more notable in the one study (321) than in the other.

A review of the non-placebo-controlled studies does not contradict the findings cited above.

Study 220 had both a double blind and an open label phase. The table below shows the adverse events reported both during the blinded phase (weeks 0-12) and during the entire study (weeks 0-24).

Table 18 Adverse Events ≥10% Incidence During Weeks 0-12 and 0-24 for the Combined Ambrisentan Group (AMB-220 Population: Safety)

Treatment period Adverse event, n (%)	Week 0-12 (N = 64)	Week 0-24 (N = 64)
Peripheral edema	16 (25.0)	17 (26.6)
Headache	10 (15.6)	14 (21.9)
Nasal congestion	12 (18.8)	13 (20.3)
Upper respiratory tract infection NOS	12 (18.8)	13 (20.3)
Cough	6 (9.4)	9 (14.1)
Nausea	8 (12.5)	9 (14.1)
Flushing	8 (12.5)	8 (12.5)
Dizziness	4 (6.3)	8 (12.5)
Nasopharyngitis	6 (9.4)	7 (10.9)
Sinusitis NOS	4 (6.3)	7 (10.9)
ALT increase	5 (7.8)	7 (10.9)
Palpitations	6 (9.4)	7 (10.9)

NOS = not otherwise specified, ALT = alanine aminotransferase
Source: AMB-220, Table 12.5

This suggests, but does not prove, that common events are more likely to be reported within the first 12 weeks rather than later.

7.1.6 Less Common Adverse Events

We discuss less common adverse events in the previous sections.

7.1.7 Laboratory Findings

Liver enzymes

There was one death (320/321E/221-003) possibly linked to drug induced hepatotoxicity. See Section 7.1.1.

7 All percents in this paragraph are "placebo subtracted"

Number and percent of subjects with AST/ALT abnormalities for all 483 subjects are shown below by degree of severity.

Table 32 Serum Aminotransferase Abnormalities (ALT and/or AST) by Severity (Population: All Studies)

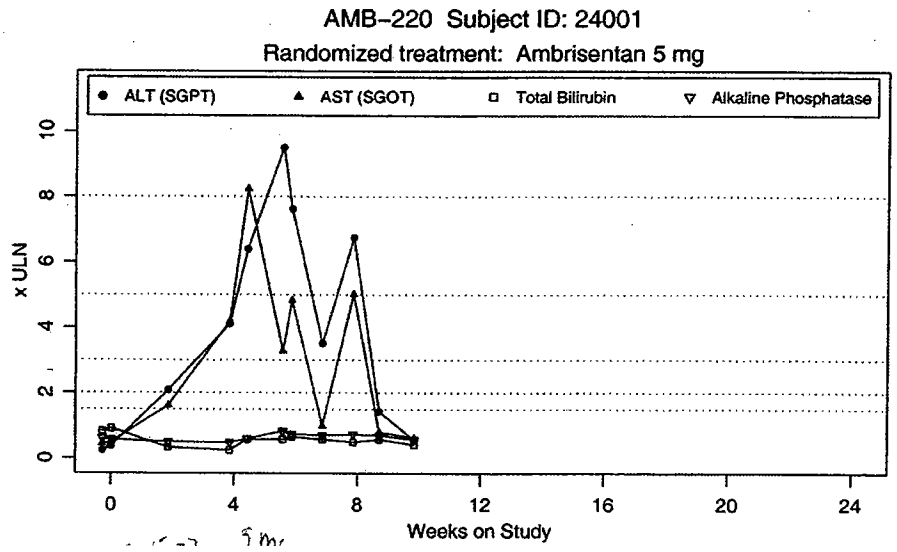
ALT and/or AST	≤12-week exposure (≤84 ± 4 days)		Cumulative incidence for all PAH studies (>1 day)[1]				
	Placebo (N = 132) n (%)	AMB (N = 483) n (%)	AMB (N = 483) n (%)	Distribution by dose at event, n			
				1 mg	2.5 mg	5 mg	10 mg
>3xULN and ≤5xULN	1 (0.8)	3 (0.6)	10 (2.1)	0	2	5	3
>5xULN and ≤8xULN	2 (1.5)	0 (0.0)	0 (0.0)	0	0	0	0
>8xULN	0 (0.0)	1 (0.2)	3 (0.6)	0	0	1	2
All >3xULN	3 (2.3)	4 (0.8)	13 (2.7)	0	2	6	5

[1]Incidence corresponds to an overall mean exposure for all PAH studies of 47.4 ± 42.69 weeks, and a maximum exposure of 184.3 weeks
Source: Table 33

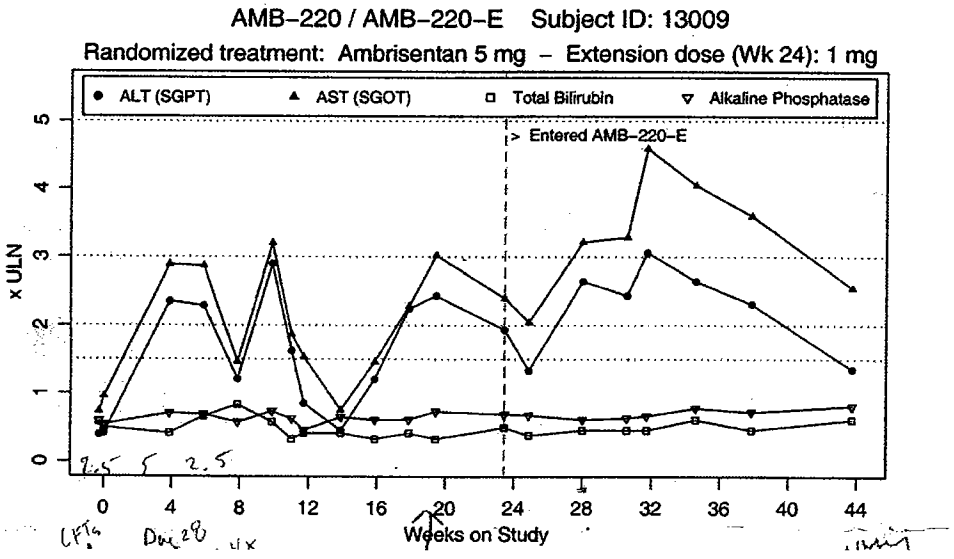
Cumulative incidence of AST/ALT > 3xULN was 3% for all subjects who received ambrisentan for up to 184 weeks. Of these 13 subjects with abnormalities, 3 were discontinued and 3 had dose reduction and/or dose interruption, 1 had a concomitant medication discontinued, and 6 were unchanged. There was one placebo subject who discontinued study medication. There was one subject (236-004) who had rising LFTs (3-5xULN). She was discontinued from sulfasalzine and remained on ambrisentan with a normalization of LFTs.

Discontinuations for abnormal LFTs

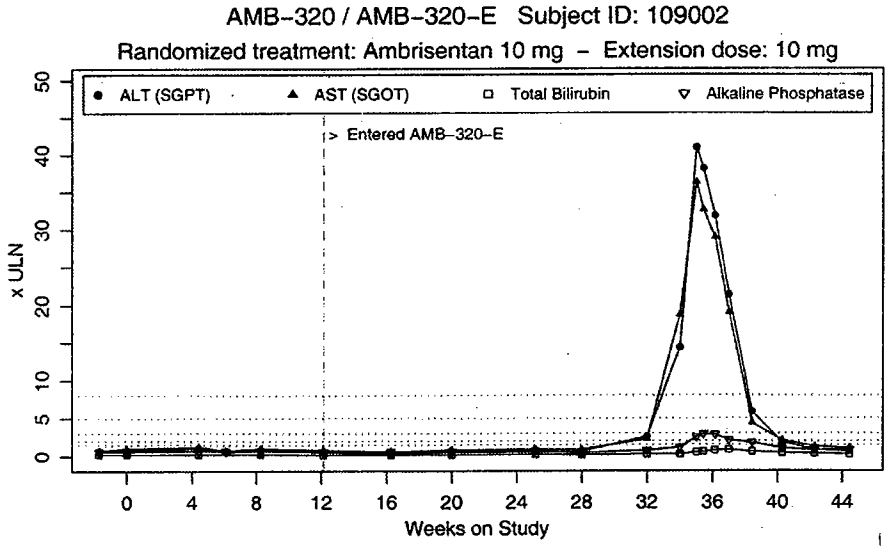
Patient ID/dose/age/sex	Abnormality/time on drug	comments
220 24-001/5mg/58y/f	ALT and AST 8-9 xULN/4 weeks	Elevated LFTs starting about 2 weeks after randomization. Subject was discontinued day 32 (2 weeks after drug was increased from 2.5mg). Enzymes were with normal range within 6 weeks after drug was stopped. (see figure below



Patient ID/dose/age/sex	Abnormality/time on drug	comments
220 13-009/5mg/45y/m	ALT and AST 3 xULN/ 9.9 weeks	Hepatic enzymes elevated on day 28. Dose was decreased to 2.5mg and then to 1mg. Enzymes remained elevated and subject was later discontinued. (see figure below)

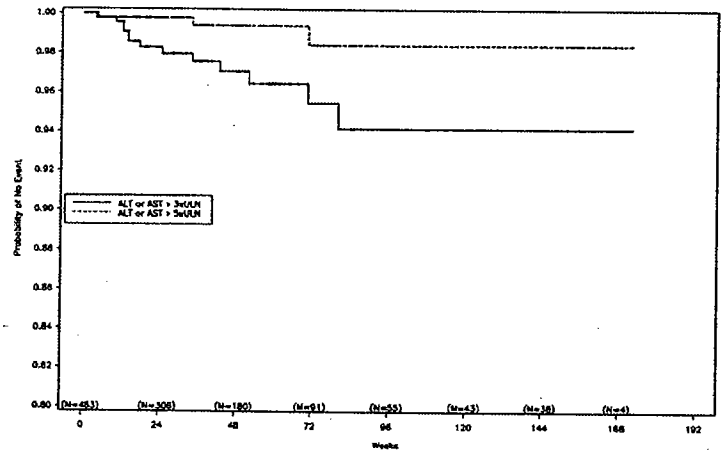


Patient ID/dose/age/sex	Abnormality/time on drug	comments
320/321E 109-002/10mg	ALT and AST 35-40 xULN/ 34 weeks	Study drug was discontinued on day 248 because of abnormal LFTs. Highest elevations were 35-30 x ULN. MI reported week 10. Subject remained asymptomatic. LFT elevation resolved about 1 month after drug was discontinued. (see figure below). Atorvastatin had been started on week 23.6 and was discontinued at the same time as ambrisentan.



The Kaplan Meier curves for time to ALT or AST >3xULN and >5xULN for the 483 subjects are shown below.

Figure 4 Kaplan-Meier Curves for Time to ALT or AST Event for All PAH Subjects Who Received 1 or More Doses of Ambrisentan



Source: Appendix 3, Figure 14.7.3

The risk of developing ALT/AST elevations >3 x ULN was 2.1% (95% CI: 0.6% to 3.5%) at 0.5 years of ambrisentan treatment and 3.6% (95% CI: 1.3% to 5.8%) after 1 year exposure.

The risk of developing ALT/AST elevations >5 x ULN was 0.2 % (95% CI: 0.0% to 0.7%) at 0.5 years of ambrisentan treatment and 0.6% (95% CI: 0.9% to 1.5%) after 1 year exposure.

Conclusion: While there were few subjects with substantial rises in LFTs, ambrisentan has been shown to be a hepatotoxin and capable of causing substantial damage to the liver. It cannot be assumed that all LFT increases will resolve when study drug is discontinued. There were subjects with mild LFT elevations who were able to remain on ambrisentan. Close monitoring is essential. There was one death for which drug induced liver failure cannot be ruled out (primarily for lack of follow up information).

Study 222

AMB-222 is an ongoing, single-arm, open-label study evaluating the incidence of increased serum aminotransferase concentrations after 12 weeks of ambrisentan therapy in subjects who previously discontinued bosentan or sitaxsentan treatment because of LFT abnormalities (AST and ALT >3xULN). 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. Subjects were monitored with clinical laboratory tests every 2 weeks and assessed for safety and efficacy every 4 weeks during the first 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.

The previous LFT abnormalities for the 36 study subjects are in the table below. (N.B.: ERA=endothelin receptor antagonist.)

Table 10.4 Previous ERA Use and Associated LFT Elevations (Population: All Subjects)

LFT Elevations	Bosentan	Total N = 36 Sitaxsentan	First ERA Failure*
Subjects Who Discontinued ERA, n	34	5	36
Subjects With AST >3xULN, n (%)	32 (94.1)	4 (80.0)	33 (91.7)
Subjects With AST >5xULN, n (%)	21 (61.8)	4 (80.0)	24 (66.7)
Subjects With ALT >3xULN, n (%)	28 (82.4)	5 (100.0)	32 (88.9)
Subjects With ALT >5xULN, n (%)	10 (29.4)	3 (60.0)	13 (36.1)
Subjects With Total Bilirubin >2xULN, n (%)	1 (2.9)	0 (0.0)	1 (2.8)
Duration on ERA Before Discontinuation, weeks,			
Median	13.9	28.7	15.6
Min, Max	4, 141	17.6, 53.6	4, 141

For each ERA, the first ERA discontinuation for each subject was counted.
*Subjects could have previously discontinued both bosentan and sitaxsentan.
Mean duration on prior ERA before discontinuation was calculated for the first episode of LFTs on each drug.
Source: Summary Table 14.1.3 and Listing 16.2.5

All subjects had to have normal LFTs at baseline.

There were two discontinuations (subject #104-001 for pain in extremity day 8 and subject #117-002 for palpitations day 22).

A Kaplan-Meier curve for the time to the first event of ALT/AST>3xULN up to 36 weeks (n=34 at 36 weeks) is shown below. There was one event reported of elevated LFTs (subject 133-002 with mild elevations of AST/ALT which returned to normal while subject remained on ambrisentan).

Figure 11.1 Time to First Event of ALT/AST Value >3xULN (Population: Safety)