Supplementary materials

Eligibility criteria

Patients 18–80 years of age (upper limit increased from 75 years in a protocol amendment dated April 23, 2014) with the following key criteria were enrolled in the AMBER I double-blind study: a diagnosis of inoperable chronic thromboembolic pulmonary hypertension (CTEPH) [confirmed at an expert center (including at least one surgeon with sound experience performing PEAs) or, for countries without an expert center, by a central adjudication committee composed of three surgeons who had been involved in all the previous controlled and randomized CTEPH studies], a documented mean pulmonary arterial pressure >25 mmHg, a pulmonary vascular resistance (PVR) >400 dyn.s/cm^5 and a pulmonary capillary wedge pressure or left ventricular end diastolic pressure of <15 mmHg based on a right heart catheterization within 3 months prior to screening. All patients were also required to have a 6-Minute Walk Distance (6MWD) of ≥150 m and ≤475 m at screening, have received anticoagulation therapy for a minimum of 3 months prior to screening and be World Health Organization Functional Class (WHO FC) II or III. Patients were excluded from the study if they had received drugs approved for pulmonary arterial hypertension within 12 weeks prior to screening. The open-label extension was initiated on January 23, 2014 and terminated on November 18, 2015. To be eligible for the open-label extension, patients must have participated in AMBER I, must have either completed the 16-week treatment in that study or prematurely withdrawn from that study, and provided written consent to be included.

Definition of treatment-emergent adverse event

A treatment-emergent adverse event (TEAE) was defined as any unfavorable sign, symptom or disease that started on or after the first dose of study treatment. For patients switched to local standard of care following study withdrawal or completion of the double-blind study, TEAEs included those reported up to 30 days after the last dose of study treatment. For the extension study, TEAEs were defined as events that started on or after the first treatment of the extension phase. Following study termination, TEAEs included AEs that occurred up to 30 days after the last study dose.

Sample size calculations and analysis population

The primary objective of AMBER I was to test whether the 16-week mean change from baseline in the 6MWD for the intent-to-treat (ITT) population (all randomized patients who received at least one dose of study medication) with ambrisentan was superior to placebo. A sample size of 72 patients per arm was estimated to have 90% power to detect a 40 m treatment difference in 6MWD at Week 16, based on a Wilcoxon rank sum test and a standard deviation (SD) of 70 m. The minimal detectable effect was 23 m and the total sample size (assuming a 10% dropout rate) was 160 patients.

The study was terminated due to futility after inclusion of 33 patients in the main AMBER I study, and 19 patients in the open-label extension. Riociguat, a soluble guanylate cyclase, had meanwhile been approved for the treatment of CTEPH and became available during the course of the study, [1,2] as did a new intervention (pulmonary balloon angioplasty). This resulted in slow enrolment and few eligible patients.

The ITT population was used for all efficacy (observed case) and safety analyses during the double-blind study. For N-terminal pro-B-type natriuretic peptide data, summary statistics were calculated using log-transformed data. The safety population consisted of subjects that received at least one dose of

ambrisentan during the extension period. This population was used for safety analyses during the open-label extension study.

Serious adverse events (SAEs) and clinical safety parameters

During the AMBER I double-blind study, one patient reported an SAE (asthma; placebo group), which was resolved in 1 month and considered unrelated to the study medication. During the open-label extension, three patients reported 14 SAEs (all of whom had previously received placebo during the double-blind study), including peripheral edema, vomiting, atrial fibrillation with bradyarrhythmia and right heart failure (three events in two patients). One patient experienced two cardiac failure SAEs, one was fatal but was not considered drug-related, and one patient experienced a cardiac failure SAE which led to study withdrawal and was considered drug-related. No other SAEs were considered drug-related. The mean exposure was 108.2 (SD=14.2) days with ambrisentan, and 102.9 (26.0) days with placebo in the double-blind study; exposure was 2-fold greater in the open-label extension (249.5 [135.9]).

Overall, no new safety signals were identified in either the double-blind study or the open-label extension via monitoring of TEAEs and SAEs, clinical laboratory parameters, physical examinations and vital signs.

Supplementary tables

Supplementary Table 1: Patient demographics and baseline characteristics in the double-blind study (ITT population)

	Ambrisentan 5 mg QD (N=17)	**Placebo (N=16)**
Age, years	61.2 (13.4)	59.8 (9.0)
Female, n (%)	8 (47)	10 (63)
BMI, kg/m^2	26.8 (4.0)	27.1 (6.8)
Ancestry, n (%)		
East Asian	5 (29)	6 (38)
Japanese	1 (6)	4 (25)
White	11 (65)	6 (38)
Systolic blood pressure, mmHg	128.5 (14.1)	118.0 (20.1)
Diastolic blood pressure, mmHg	82.8 (9.7)	73.1 (11.1)
Mean PAP, mmHg	48.6 (12.1)*	50.0 (14.1)
Concomitant medication, n (%)		
Anticoagulant	16 (94)†	16 (100)
Diuretic	12 (71)	15 (94)
Oxygen	0 (0)	1 (6)
6MWD, m		
Mean (SD)	388.6 (69.8)	379.4 (76.6)
Median (IQR)	415.0 (369.0, 438.0)	400.0 (338.8, 431.5)

NT-proBNP, pg/mL		
Median (IQR)	664.6 (234.5, 2248.8)*	1318.3 (261.5, 2117.6)‡
PVR, dyn.s/cm^5		
Mean (SD)	904.5 (584.4)	870.7 (371.1)
Median (IQR)	773.0 (494.0, 1050.0)	791.0 (569.0, 1048.0)
WHO FC, n (%)		
II	7 (41)	5 (31)
III	10 (59)	11 (69)

All data are presented as mean (SD) unless stated otherwise. *N=16; †One patient met the entry criteria but did not have any anti-coagulant therapy recorded in the case report form; ‡N=15. 6MWD, 6-Minute Walking Distance; BMI, body mass index; IQR, interquartile range; ITT, intent-to-treat; NT-proBNP, N-terminal pro-B-type natriuretic peptide; PAP, pulmonary arterial pressure; PVR, pulmonary vascular resistance; QD, once daily; SD, standard deviation; WHO FC, World Health Organization Functional Class.

Supplementary Table 2: Change from Baseline at Week 16 in 6MWD, NT-proBNP, and PVR in the double-blind study (observed case data, ITT Population)

	Ambrisentan 5 mg QD (N=17)	**Placebo (N=16)**
Completers, n	15	13
6MWD, m		
Mean (SD)	28.3 (41.7)	6.8 (67.5)
Median (IQR)	25.0 (12.0, 49.0)	-10.0 (−32.5, 20.0)
NT-proBNP, %		
Geometric mean*	−29.4	14.1
Median (IQR)	–33.6 (−51.5, −17.4)	7.9 (−20.3, 60.6)
PVR, dyn.s/cm^5		
Mean (SD)	−212.5 (392.8)	−108.5 (51.3)
Median (IQR)	−130.0 (−502.0, −78.0)	−103.0 (−122.0, −88.0)

*Percent change from baseline in geometric mean at Week 16. 6MWD, 6-Minute Walk Distance; ITT, intent-to-treat; IQR, interquartile range; NT-proBNP, N-terminal pro-B-type natriuretic peptide; PVR, pulmonary vascular resistance; QD, once daily; SD, standard deviation.

Supplementary Table 3: Summary of all TEAEs and all drug-related TEAEs occurring in >1 patient in the double-blind study (ITT population)

	Double-blind study	
	Ambrisentan 5 mg QD (N=17)	**Placebo (N=16)**
TEAE, n (%)		
Any event	11 (65)	15 (94)
Peripheral edema	3 (18)	2 (13)
Headache	2 (12)	3 (19)
Hemoptysis	0 (0)	3 (19)
Neck pain	0 (0)	2 (13)
URTI	0 (0)	2 (13)
Constipation	1 (6)	1 (6)
Dizziness	1 (6)	1 (6)
Epistaxis	1 (6)	1 (6)
Eyelid edema	1 (6)	1 (6)
Drug-related TEAEs*, n (%)		
Any	4 (24)	5 (31)
Headache	1 (6)	3 (19)
Peripheral edema	1 (6)	1 (6)
Eyelid edema	1 (6)	1 (6)

*Considered by the investigator to be possibly related to the study medication. ITT, intent-to-treat; QD, once daily; TEAE, treatment-emergent adverse event; URTI, upper respiratory tract infection.

Supplementary Table 4: Summary of all TEAEs occurring in >1 patient and all drug-related TEAEs occurring in the open-label extension (safety population)

	Open-label extension
	Ambrisentan (N=19)
TEAE, n (%)	
Any event	14 (74)
Diarrhea	3 (16)
Nasopharyngitis	3 (16)
Peripheral edema*	3 (16)
Cardiac failure*	2 (11)
Epistaxis	2 (11)
Headache	2 (11)
Drug-related TEAEs, n (%)**	
Any	5 (26)
Anemia	1 (5)
ALT increased	1 (5)
AST increased	1 (5)
Cardiac failure*	1 (5)
Edema	1 (5)
Epistaxis	1 (5)
Headache	1 (5)
Migraine	1 (5)

Peripheral edema	1 (5)
Renal failure	1 (5)
Renal impairment	1 (5)

*Classified as an SAE (3 cardiac failure events [2 in one patient] and 1 peripheral edema event); **considered by the investigator to be possibly related to the study medication. ALT, alanine aminotransferase; AST, aspartate aminotransferase; ITT, intent-to-treat; SAE, serious AE, TEAE, treatment-emergent adverse event.

References

1. Bayer HealthCare Pharmaceuticals Inc. Adempas® prescribing information. Available at http://labeling.bayerhealthcare.com/html/products/pi/Adempas_PI.pdf. Last accessed April 2017. 2013.
2. Ghofrani HA, D'Armini AM, Grimminger F, et al. Riociguat for the treatment of chronic thromboembolic pulmonary hypertension. *N Engl J Med*. 2013; 369: 319-29.