Clinical study report

Early Treatment of Borderline Pulmonary Arterial Hypertension Associated with Systemic Sclerosis (SSc-APAH)

A randomized, placebo-controlled, double-blind, parallel group, proof-of-concept trial investigating the effect of ambrisentan treatment in patients with systemic sclerosis and mildly elevated pulmonary arterial pressures - EDITA

Clinical Trial Code: EDITA

EudraCT No.: 2014-001882-28

Clinical Phase: IIA (proof of concept)

Sponsor: Investigator-initiated-trial with co-financing by GlaxoSmithKline

PRINCIPAL INVESTIGATOR/ COORDINATING INVESTIGATOR (LEITER DER KLINISCHEN PRÜFUNG*) AND INVESTIGATOR
Prof. Dr. med. Ekkehard Grünig
Thoraxklinik gGmbH am Universitätsklinikum Heidelberg
Zentrum für pulmonale Hypertonie
Röntgenstraße 1
69126 Heidelberg
Phone: +49-6221 / 396 8053
Fax: +49-6221 / 396 1209
ekkehard.gruenig@med.uni-heidelberg.de
* According to § 40 German Drug Law (AMG)

SPONSOR
Clinic director
Roland Fank
Thoraxklinik gGmbH am Universitätsklinikum Heidelberg
Röntgenstraße 1
69126 Heidelberg
Phone: +49-6221 / 396 2100
Fax: +49-6221 / 396 2102
roland.fank@med.uni-heidelberg.de

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BfArM approval: 29.09.2014

Vorlage: **4040057**

Active study (first patient in - last patient out): 15.12.2014 – 27.11.2017

This study was performed in compliance with Good Clinical Practice. All study files will be archived accordingly.

Version: 1.0, 15.11.2018

1. SYNOPSIS

Objectives: The objective of this randomized, controlled, double-blinded, parallel group, proof-of-concept study was to assess the effect of ambrisentan on mean pulmonary arterial pressure (mPAP) in patients with systemic sclerosis (SSc) and mildly elevated pulmonary hypertension (PH) or increased mPAP values during exercise.

Methods: Thirty eight SSc-patients with mildly elevated mPAP at rest between 21-24mmHg and/or during exercise >30mmHg were randomly assigned to treatment with either ambrisentan 5-10mg/day or placebo. Right heart catheterization (RHC) and further clinical parameters were assessed at baseline and after 6 months. The primary end-point was the difference of mean mPAP change at rest between the two groups.

Results: We found no significant difference in the primary endpoint change between baseline and 6 months between the two groups (ambrisentan -1± 6.4 mmHg vs. placebo -0.73±3.59 mmHg at rest, p=0.884). Two patients in the ambrisentan group developed left heart disease associated pulmonary hypertension (LHD-PH), while three patients from the placebo group presented SSc-associated PAH (SSc-APAH). As secondary endpoints ambrisentan-treatment was associated with significant improvements of cardiac index (CI) and pulmonary vascular resistance (PVR) at rest (CI: 0.36±0.66 l/min/m^2 vs. -0.31±0.71 l/min/m^2, p=0.010; PVR: -0.70±0.78 WU vs. 0.01±0.71 WU, p=0.012) and during exercise (CI: 0.7±0.81 l/min/m^2 vs. -0.45±1.36 l/min/m^2, p=0.015; PVR: -0.84±0.48 WU vs. -0.0032±0.34 WU, p <0.0001).

Conclusion: This is the first RCT in SSc-patients with early treatment of mildly elevated mPAP. The data of this study suggests that treatment with ambrisentan may improve parameters of early vascular and right ventricular damage and prevent from progression to manifest PAH.

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2. ETHICS & ADMINISTRATIVE ISSUES

2.1. Ethics

The study and all amendments were reviewed by Ethics Committee of the Medical Faculty Heidelberg.

The study was conducted in accordance with the ethical principles that have their origins in the Declaration of Helsinki in its current version from 2013.

All patients gave written informed consent before inclusion into the study. No study relevant examinations or assessments were performed before written informed consent was provided by the patient.

2.2. Investigators and study administrative structure

In order to monitor specific aspects of the current trial the following Reference Committees were established:

The clinical study was an investigator initiated trial in a section for PH, specialized in the treatment of patients with PH. A clinical research organization submitted study files to the Ethics Committees and BfArM to obtain study approvals. The Steering Committee (principal investigator, deputy investigators and drug safety officer) was responsible for coordinating the conduct of this trial. The Steering Committee also protected the safety interests of patients in this trial by monitoring the progress and safety data of the trial.

This monocentric study was performed by one principal investigator (Leiter der klinischen Prüfung), two deputy investigators, two study physicians and respective study personnel. All assessments were performed or supervised by the experienced investigators. One study coordinator and two experienced study nurses performed delegated tasks for conductance of the trial, patient assessments and data entry. Data monitoring was performed by an external clinical monitor. For evaluation and appraisal of serious unexpected, suspected adverse reactions, a drug safety officer (physician experienced in the indication) was implemented. An external clinical research organization was implemented for Pharmacovigilance.

Data management was performed by a supervising data manager and data management personnel, responsible for data entry and global quality checks. Further quality checks and analysis of data were performed by two statisticians.

3. INTRODUCTION

Pulmonary Hypertension (PH) often complicates systemic sclerosis (SSc) (Condliffe R et al. 2009) and impairs its prognosis dramatically (Hao Y. et al. 2017). PH in SSc-patients may be caused by restrictive lung disease, left ventricular dysfunction or by pulmonary arterial hypertension (PAH) (Lefreve G et al. 2013) is defined by the presence of a mean pulmonary arterial pressure (mPAP) of ≥ 25 mmHg assessed invasively with right heart catheterization (RHC), a pulmonary arterial wedge pressure (PAWP) ≤15mmHg and pulmonary vascular resistances (PVR) ≥ 3 Wood Units (WU) (Galié N et al. 2016) (Kovacs G et al. 2018). Patients with SSc-associated PAH (SSc-APAH) are burdened by lower survival rates when compared with patients with idiopathic PAH (Lefevre G et al. 2013). Mildly elevated mPAP of 21-24mmHg have been shown to be associated with impaired exercise capacity and poorer outcomes when compared with individuals with mPAP within the normal range (Kovacs G et al. Chest. 2014, Heresi GA et al. Pulm Circulation 2013, Visovatti SH et al. 2014, Douschan P et al. 2018). A recently published post-hoc analysis of the DETECT study showed that SSc-patients with mPAP of 21-24 mmHg were also more prone to develop manifest PH after 2.95±0.7 years' follow-up (chi-square p value: 0.0226) than SSc-patients with mPAP of ≤ 20 mmHg (Coghlan JG et al. 2018). In a recent study, patients with SSc and mildly elevated mPAP presented with impaired pulmonary arterial compliance and reduced increase of cardiac index (CI) assessed by RHC during exercise (Nagel C et al. 2018, unpublished data). During the 6th WSPH a new definition of PH has been proposed, lowering the cutoff for mPAP from 25 mmHg to 20 mmHg.

Although there is no actual definition of exercise pulmonary hypertension, there is growing evidence for the importance of mPAP values of >30mmHg during exercise, especially in SSc patients (Naije R et al. 2018). In SSc patients the presence of exercise PH confers higher risk of developing manifest PH at rest during follow-up (Condliffe R et al. 2009, Saggar R et al. 2010) and is associated with reduced survival rate, which is similar to the survival of manifest PH patients (Stamm A et al. 2016).

Consequently, both mildly elevated mPAP (mPAP 21-24 mmHg) at rest and exercise PH may indicate an early stage of pulmonary vasculopathy.

Up to now, apart from two small open-label reports (Saggar R et al. 2012, Kovacs G et al. 2012), data regarding the treatments of mildly elevated mPAP and/or exercise PH are lacking. As an early recognition and management of PAH in SSc grants a significant survival benefit (Humbert M et al. 2011), there is a high need of randomized, controlled studies investigating the effect of PAH-targeted treatment in SSc patients with mildly elevated mPAP and/or exercise PH.

The aim of this study was therefore to investigate, whether an early treatment in patients with mildly elevated mPAP and/or exercise PH may prevent these patients from developing manifest PH by reducing mPAP.

This randomized, controlled, double-blind study aimed to investigate the effect of a 6-month ambrisentan-treatment in SSc patients with mildly elevated mPAP and/or exercise PH on mPAP, pulmonary hemodynamics at rest and during exercise, exercise capacity, quality of life and further clinical parameters.

4. METHODS

4.1. Study objectives

4.1.1. Primary [Objectives/ Endpoints]

1. Determine whether mPAP of SSc patients with borderline-PAH (mPAP 21 24 mmHg, TPG ≥11 mmHg) can be reduced by 3 mm Hg (absolute change baseline vs. 6 months; equals 15%) by treatment with ambrisentan 10 mg/die (may be initiated with 5 mg/die and escalated to 10 mg/die) over 6 months (primary endpoint) compared to baseline and placebo.

4.1.2. Secondary Objectives

2. Determine whether exercise induced elevated mPAP-values (>30 mmHg without left heart or severe lung disease or systemic arterial hypertension) and further measures of exercise capacity, symptoms and quality of life can be reduced by ambrisentan 10 mg/die over 6 months
3. Analyze if the progression (adverse events, hospitalization, initiation of pulmonary hypertension treatment) of borderline-PAH to manifest PH can be avoided by ambrisentan-treatment (descriptive, observational)
4. Assessment of tolerability and safety

4.1.3. Secondary Endpoints

Analyze if patients with SSc and mildly elevated mPAP at rest or during exercise show an improvement by treatment with ambrisentan 10 mg/die over 6 months in:

- 6-Minute-walking test (6MWT)
- Quality of life (QoL, SF-36)
- Echocardiography: right atrial area (RA-area), right ventricular area (RV-area), Tei, Tricuspid Annular Plane Systolic Excursion (TAPSE), systolic pulmonary arterial pressure (sPAP)
- Lung function tests: forced expiratory flow (FEV1), total lung capacity (TLC), diffusion-limited carbon monoxide (DLCo), DLCo/alveolar volume (VA), forced vital capacity (FVC), forced expiratory volume in one second (FEV1), residual volume
- Borg Dyspnea Index
- WHO-functional class (WHO FC)
- further invasively measured hemodynamic parameters evaluated by RHC: right atrial pressure, pulmonary vascular resistance, cardiac output (CO), cardiac index (CI), PAWP,

venous oxygen saturation (SvO_2)Raynaud-syndrome and skin involvement, assessed by the modified Rodnan-Skin score (mRSS) and Symptoms of Scleroderma (descriptive)

4.2. Investigational plan

4.2.1. Overall study design and plan

Patients with mildly elevated mPAP indicated by mPAP values 21-24mmHg at rest and/or >30mmHg during exercise were included in this single center study. This clinical investigation was performed as a Proof-of-Concept investigator initiated trial using a prospective, randomized, double-blind, parallel group, placebo-controlled, phase IIA clinical study design.
On the first visit the patients' medical history was obtained and physical examinations were conducted. Moreover, an electrocardiogram (ECG), laboratory testing (NT-proBNP, uric acid and other laboratory tests), echocardiography at rest and RHC were carried out. If patients had been identified within the last 6 months before screening investigations by RHC, the measurements were considered valid as baseline investigations and were not repeated. If patients fulfilled the inclusion criteria and still suffered from elevated mPAP values they were invited to join the study. The clinical investigations began within 28 days. The prospective study comprised of a 6-month study period (180 ±2 weeks) plus the screening phase up to 28 days and a follow-up phase of 30±7 days. Patients were randomized in an intervention group with oral ambrisentan treatment, or oral placebo tablets.

4.3. Selection of study population

It was assumed that for any patient, considered for inclusion, a regular diagnostic work up in accordance with international rheumatologic guidelines and with PH guidelines (ESC/ERS Guidelines) was performed in advance.

4.3.1. Inclusion Criteria

1. Male or female SSc patients with borderline mPAP: mPAP 21-24 mmHg, TPG > 11 mmHg, PAWP ≤15 mmHg at rest and/orExercise induced elevated mPAP-values >30 mmHg, PAWP ≤18 mmHg; TPG ≥15 mmHg, as defined in Saggar et al. (2012) without left heart or severe lung disease or systemic arterial hypertension
2. Adult patients having completed his/her 18th birthday
3. Patients with definite diagnosis of SSc using the scleroderma criteria of the American Rheumatism Association

4. SSc-disease duration >3 years

5. Able to understand and willing to sign the Informed Consent Form

6. Negative pregnancy test at the start of the trial and appropriate contraception throughout the study for women with child-bearing potential.

4.3.2. Exclusion Criteria

1. Any connective tissue diseases (CTD) other than SSc

2. PH confirmed by RHC before enrolment, i.e. mPAP ≥25 mmHg at rest

3. Patients presenting normal mPAP values, i.e. mPAP<21 mmHg at rest, and ≤30 mmHg during exercise, and/or PAWP 15 mmHg at rest or 18 mmHg during exercise

4. Ongoing or a history of >2 weeks of continued use of therapies that are considered definitive PH treatment: endothelin receptor antagonists (ERA; e.g. bosentan, ambrisentan), phosphodiesterase type 5 inhibitors (PDE5; e.g. sildenafil, tadalafil, vardenafil), prostanoids (e.g. epoprostenol, treprostinil, iloprost, beraprost) and soluble guanylate cyclase stimulator (e.g. riociguat). Intermittent use of PDE5 inhibitors for male erectile dysfunction is permitted.

5. Except for diuretics and corticosteroids medical treatment should not be expected to change 4 weeks prior inclusion into the study and during the entire 12-week study period.

6. Known intolerance to ambrisentan or one of its excipients

7. Clinically significant anemia (hemoglobin concentration of less than 75% of the lower limit of normal, LLN)

8. Forced vital capacity (FVC) <60%, forced expiratory volume in first second (FEV1) <65%

9. Severe interstitial lung disease, idiopathic pulmonary fibrosis

10. Renal insufficiency (glomerular filtration rate [GFR] <60 mL/min/1.73m2 at least for the last 3 months before inclusion)

11. Baseline values of hepatic aminotransferases (ALT and/or AST) >3 x upper limit of normal (ULN)

12. Systolic blood pressure <85 mmHg;

13. evidence of inadequately treated blood pressure >160/90 mmHg and/or blood pressure during exercise >220/120 mmHg

14. Patients referred with clinically significant overt heart failure

15. Clinically significant fluid retention

16. Previous evidence or diagnosis of clinically relevant left heart disease, i.e. at least one of the following: previous echocardiography with estimated left ventricular (LV) ejection fraction <50%, previous history of cardiogenic pulmonary edema, increased size of left atrium (>50 mm)
17. Known significant diastolic dysfunction associated with clinical heart failure
18. Known coronary disease or significant valvular heart disease
19. Known congenital heart defects such as single ventricle, transposition, Eisenmenger
20. Known hypertrophic cardiomyopathy or left ventricular hypertrophy (interventricular septum thickness (IVS) or posterior wall thickness (PWD) >1.2 cm)
21. Participation in any clinical drug trial within 4 weeks prior to screening of this study and/or who is scheduled to receive another investigational medicinal product (IMP) during the course of this study
22. Pregnancy or lactation

4.3.3. Removal of Patients from Therapy or Assessment

Predefined criteria for withdrawal of the trial treatment were:

- at their own request or at request of the legal representative
- if, in the investigator's opinion, continuation of the trial would be detrimental to the subject's well-being
- occurrence of a severe serious adverse event (SAE) caused by the IMP
- occurrence of an adverse event (AE) which makes the continuation of the study undesirable
- progressive renal insufficiency (clearance/glomerular filtration rate<50%)
- severe hepatic insufficiency (bilirubin >3 mg/dL, hepatic aminotransferases >3x ULN for more than 1 week)
- decrease of hemoglobin to 75% of lower limit of normal
- worsening of WHO class (>1 class + >30% decrease of 6-minute walking distance)
- for women, if it becomes known that the subject is pregnant
- if, in the investigator's opinion, protocol violations caused by the subject would lead to invalid data (e.g. non-compliance with planned study procedures).

Withdrawal of subjects from trial treatment in case of occurrence of criteria mentioned above was the responsibility of the coordinating investigator. Any decision to continue with the study treatment

despite occurrence of any of the withdrawal criteria had to be justified in written form in the Case Record Form (CRF) and in the subject's medical records.

Any patient removed from the study due to an AE or SAE was monitored until no more signs and symptoms were verifiable or the subject was on stable condition. The patient, either willingly withdrawn from the study or due to premature termination, was asked thoroughly to complete all examinations scheduled for the final trial day, and these were performed as far as possible and documented.

In all cases, the reason for withdrawal was recorded in the CRF and in the subject's medical records.

In case of withdrawal of a subject at his/ her own request, the reason was asked for as extensively as possible and documented.

All efforts will be made to follow up the subject.

A subject was withdrawn from all trial related procedures (including follow-up visits) for the following reasons:

- at his/her own request or at request of his/her legal representative
- non-adherence to the trial-related requirements, which may (have) influence(d) the validity of the trial data

4.4. Treatments

4.4.1. Treatments Administered

Study medication was ambrisentan 10 mg (starting with 5 mg in the beginning of the study and then up-titrated to 10 mg/die) for 19 patients and placebo for 19 patients (to cover a possible drop-out rate) over 6 months. For each patient taking a dose of 5 mg 194 tablets (180 tablets for 6 months + 14 tablets for the time-frame of two weeks) of verum or placebo were needed during the treatment period. For patients taking 10 mg/die twice the amount of tablets were needed.

Study medication was stopped at the end of the study.

4.4.2. Ambrisentan

Investigational medicinal product: Ambrisentan/Volibris®, tablets

International Nonproprietary Name (INN): Ambrisentan

Trading name / Name of finished product: Volibris®

ATC code: C02KX02

Pharmaceutical formulation: (+)-(2S)-2-[(4,6-dimethylpyrimidin-2-yl)oxy]-3-methoxy-3,3-diphenylpropanoic acid, C22H22N2O4

Components in Active

AMBRISENTAN REG. API
MICROCRYS CELLULOSE 102
LACTOSE FAST FLO
CROSCARMELLOSE SODIUM
MAG.STEARATE NO-BOV
Opadry II White 85G18490
Purified Water

Route of administration: oral

Time and frequency of administration: once daily

Dosage: 5 or 10 mg (one or two tablets respectively)

Storage conditions: no special storage conditions required

Drug was blistered and carded at:

Almac Clinical Services Ltd., 9 Charlestown Road, Seagoe Industrial Estate, Craigavon, BT63 5PW, UK

Marketing authorization holder: GlaxoSmithKline

Batch numbers: EDITA/201448 (patient 01-24); EDITA/201625 (patient 26-30; visit 2 for patient 31-39); EDITA 2017/17 (visit 3 for patient 31-39)

4.4.3. Placebo

Components in Placebo

MICROCRYS CELLULOSE 102
LACTOSE FAST FLO

CROSCARMELLOSE SODIUM

MAG.STEARATE NO-BOV

Opadry II White 85G18490

Purified Water

Route of administration: oral

Time and frequency of administration: once daily

Dosage: 5 or 10 mg (one or two tablets respectively)

Storage conditions: no special storage conditions required

Manufacturer of bulk: Patheon Inc., 2100 Syntex Court, Mississauga, Ontario L5N 7K9, Canada

4.4.4. Method of Assigning Patients to Treatment Groups

Patients who had signed the consent form were given a unique screening number including center number (e.g. 01, 02…) and patient ID (001, 002, …). Screening number ascended, starting with 001, 002, etc. If the patient was eligible for the prospective drug trial, a 1:1 randomization was performed into ambrisentan and placebo group and a consecutive randomization number was allocated to the patient. The randomization list was created at the Pharmacy at the University Hospital Heidelberg, which performed packing and labeling of the medication for each patient.

The trial medication was administered to subjects only after confirming their eligibility after the initial screening. Subjects withdrawn from the trial retained their identification codes (e.g. randomization number, if already given). New subjects were always allotted a new identification code.

4.4.5. Titration

Dosage of ambrisentan was chosen according to the approved dosage for patients with manifest pulmonary arterial hypertension with a starting dose of 5mg and up-titration according to tolerability and after consultation (by phone or personally) with one of the investigators within the first 4 weeks to 10mg.

Additionally, at each study visit the investigator decided based on the patient's well-being, patients´ assessment, safety parameters, and tolerance of ambrisentan, if the study medication should be modified. The respective decision (increase, maintain or decrease dose) was documented.

Maximum dose allowed: not to exceed 10 mg/die.

4.4.6. Administration

Ambrisentan and placebo were administered orally, once daily with or without food intake.

4.4.7. Blinding

This study was conducted double blinded. Investigator, study personnel, monitor, biometrician, and the patient were blinded to treatment. Pharmacy was unblinded to treatment.

Randomization to one of the groups was performed by block randomization. Randomization lists were created by the Pharmacy at the University Hospital Heidelberg. Medication was packed with sequential patient numbers by the Pharmacy at Heidelberg University Hospital. The randomization list was kept in safe and confidential custody at the Pharmacy at Heidelberg University Hospital.

4.4.8. Prior and Concomitant Therapy

Relevant additional treatments administered to the subjects on entry to the trial or at any time during the trial were regarded as concomitant treatments and were documented on the appropriate pages of the CRF.

Patients received conventional rheumatologic treatment. At the time of screening treatment had to be stable for at least 2 months. Except for diuretics, treatment must not change during the entire study period.

In case of clinical worsening and if clinically indicated additional PAH-targeted rescue medication was initiated at the discretion of the investigators.

- The following concomitant treatments were not permitted during the trial as well as any investigational medication taken within 4 weeks prior to the start of this study:
    - any PH-specific medication (ERAs, e.g. bosentan, ambrisentan, PDE5-I, e.g. sildenafil, tadalafil, vardenafil, and prostanoids, e.g. epoprostenol, treprostinil, iloprost, beraprost) during the last 30 days prior to inclusion (randomization).
- Participants were not included if scheduled to receive another investigational drug during the course of this study
- Patients were closely monitored when starting treatment with rifampicin.

4.4.9. Treatment Compliance

Trial medication was dispensed to the subjects by the investigator. Subjects were instructed to bring all trial medication to the trial site at every visit. Compliance was assessed by tablet count. Details were recorded in the CRF. Treatment effects were assessed and the dosage was discussed at each visit.

Furthermore, the patients received a patient diary recording medication intake and time points. The patient diary was checked for compliance at each study visit.

4.5. Efficacy and safety variables

4.5.1. Efficacy and Safety Measurements Assessed and Flow Chart

4.5.1.1. Primary efficacy analysis

Analyze if patients with SSc and borderline-PAP show an improvement by treatment with ambrisentan 10 mg/die over 6 months in mPAP in comparison to patients receiving placebo.

4.5.1.2. Secondary efficacy analyses

1. 6-Minute-walking test
2. Echocardiography: right atrial area (RA-area), right ventricular area (RV-area), Tei, Tricuspid Annular Plane Systolic Excursion (TAPSE), systolic pulmonary arterial pressure (sPAP), right ventricular pump function, left ventricular pump function,
3. Lung function tests: forced expiratory flow (FEV1), total lung capacity (TLC), diffusion-limited carbon monoxide (DLCo), DLCo/alveolar volume (VA), forced vital capacity (FVC), forced expiratory volume in one second (FEV1), residual volume
4. Borg Dyspnea Index
5. WHO-functional class
6. further invasively measured hemodynamic parameters evaluated by RHC: right atrial pressure, pulmonary vascular resistance, cardiac output (CO), cardiac index (CI), PAWP, venous oxygen saturation (SvO2) at rest and during exercise
7. Raynaud-syndrome and skin involvement, assessed by the modified Rodnan-Skin score and Symptoms of Scleroderma (descriptive)
8. WHO functional class

9. Laboratory parameters (NT-proBNP)

All laboratory assessments were determined locally on-site.

4.5.1.3. Safety parameters

Adverse events including respective seriousness, grading, relationship to study medication, outcome and action taken were assessed and documented.

Further safety parameters comprise of laboratory: hemoglobin, hematocrit, AST, ALT, bilirubin, CRP, sodium, urea, creatinine, clearance, NT-proBNP

Vital signs, hospitalizations, time point from baseline and duration, hemodynamics: CO, venous oxygen saturation (during RHC)

	Randomized, double-blind, controlled study including 38 patients with mildly elevated mPAP and/or exercise PH				
Assessment	Screening "borderline-PAH" day –28/-1 Visit 1*	Baseline° day 1* Visit 2	After 3 months/ 90 days ± 2weeks Visit 3	After 6 months/ 180 days ± 2weeks Visit 4*	30±7 days Follow-up by phone
Written Informed Consent, obtained before any study procedure	X				
Check of eligibility criteria	X	X			
Demographics (height)	X				
Medical History	X				
Randomization		X			
Physical Examination	X	X	X	X	
SSc characteristics		X		X	
Modified Rodnan Skin Score and Symptoms of Scleroderma		X		X	
Vital Signs (blood pressure, heart rate, oxygen saturation, body weight)	X	X	X	X	
WHO functional class	X	X	X	X	
Electrocardiogram		X	X	X	
Pulmonary function tests: DLCo, DLCo/VA, FVC, FEV1, TLC, residual volume; blood gas analysis		X		X	
Local lab assessment #	X	X	X	X	
Pregnancy test (serum or urine)	X	X	X	X	
Echocardiography	X	X		X	
Right heart catheterization** (RHC), at rest, during exercise	X			X	
Quality of life Questionnaire SF-36		X		X	
6MWD, Borg Dyspnea Index		X	X	X	
Adverse events (AE)		X	X	X	X
Concomitant medication	X	X	X	X	
Concomitant disease	X	X	X	X	X
Study medication and diary hand out or check		X	X	X	

° In borderline-PH-patients the screening assessment of PH screening (screening study, accepted by the Ethics committee University of Heidelberg) may be used as baseline examination for the randomized, double-blind part of the study when the screening assessment is not older than 28 days.

* according to hospital practice possible in-hospital stay

****** Baseline-RHC may be up to 6 months old at inclusion, continuous ECG, CO and SvO_2 recording during haemodynamic investigations

additional lab assessments for hepatic aminotransferases and haemoglobin will be performed at the patient's general physician for safety reasons, reports will be faxed to the clinical site on a monthly basis

4.6. Statistical methods planned in the protocol and determination of sample size

Statistical analyses were conducted by two statisticians (CF, NB).

4.6.1. Analysis of data quality

After first check for plausibility by eye, all data was entered in a database as recorded in the CRF. After completion of data entry, checks for plausibility, consistency, and completeness of the data were performed.

An analysis of data quality was performed before unblinding the therapy arms.

1. For all variables at baseline, after 3-months and 6-months descriptive statistics mean standard deviation, median, Interquartile range (IQR), minimum, maximum, boxplot inspections of outliers for continuous variables and frequencies for categorical variables. Validation/correction was performed by queries.

2. Calculation of differences for the parameters mPAP and all secondary endpoints. Descriptive statistics as in 1. for the differences to evaluate plausibility of the data.

3. Control of inclusion and exclusion criteria and withdrawal criteria, definition of patients to be excluded if applicable.

4. Control of laboratory values (and comparison to normal values)

The safety set comprised of all patients who had been included in the study.

Based on these checks, queries were produced combined with the queries generated by visual control. All missing data or inconsistencies were reported back to the centre and clarified by the responsible investigator. If no further corrections were to be made in the database it was declared closed and used for statistical analysis.

4.6.2. Determination of Sample Size

The main comparison was the difference in treatment effect between ambrisentan arm and placebo. The primary endpoint was the change of mean pulmonary arterial pressure between baseline and after 6 months compared to placebo.

Based on previous data and the inclusion criteria we expected a baseline mPAP of 20 mmHg, a mean reduction of 3 mmHg (equals 15%) with standard deviation of the difference of 2.5 mmHg. To reject

the null hypothesis with 90 percent probability if the means of the mean pulmonary arterial pressure differ by at least 3 mmHg (17 vs. 20 mmHg, 15%) a sample of 15 patients in each group was required, according to the two-tailed Student's t-test, with a type I error of 0.05 (two-sided) and equal standard deviations of 2.5 mmHg in both patient groups.

In order to cover a possible 20% drop-out rate, 19 patients in each group = 38 patients in total were included.

4.6.3. Definition of analysis sets

All patients randomized and treated were valid for the intention-to-treat analysis population. A randomized patient was valid for the intention-to-treat, if at least one dose of study medication was administered. A patient was valid for per-protocol analysis, if the patient had an adequate hemodynamic measurement at baseline and after 12 weeks or if withdrawn due to lack of efficacy, who had an adequate hemodynamic assessment at any time post-baseline up to 12 weeks, and showed no major protocol deviation.

As the above defined criteria for exclusion of subjects from the per protocol set were not met, the intention-to-treat set was congruent with the per protocol set.

The above specifications of the analysis populations are in accordance with the recommendations given in the ICH-E9 Guideline "Note for guidance on statistical principles for clinical trials".

4.6.4. Statistical/Analytical Issues

Data are described as mean ± standard deviation or number and %, respectively.

The primary efficacy analysis was performed on data from the intention-to-treat population (all patients who received randomization) by t-test with unequal variances since the assumption for a covariance analysis were not fulfilled. Additionally, we used robust t-tests with Huber weights for sensitivity analyses (Huber P J and Ronchetti EM 2009. Robust Statistics. 2nd ed. New York: John Wiley & Sons.; Wilcox R, Carlson M, Azen S, Clark F. Avoid lost discoveries, because violations of standard assumptions, by using modern robust statistical methods. Journal of clinical Epidemiology, 66(3):319-329). Secondary quantitative efficacy variables were tested with t-tests for unequal variances (Welch tests) and robust t-tests as sensitivity analysis. Group differences regarding the change of categorical

variables were performed using two sample Wilcoxon tests. Safety was analyzed descriptively. Adverse events during the study period included all adverse events that started or worsened at the time of administration of the first dose of study drug until the last visit (6 months).

All tests were two-tailed and p-values <0.05 were considered as statistically significant. Tests for the secondary endpoints are descriptive. All analyses were performed with SPSS V 25 (SPSS Statistics V25, IBM Corporation, Somers, New York) and JMP14 (SAS Institute, Cary NC).

4.6.5. Handling of missing data

Variables with more than 20% missing values were marked. For all others, a complete case data analysis for each variable was performed. No imputation method was applied.

4.6.6. Outliers

Definition: outliers and extreme values (according to boxplots)

The primary and all secondary efficacy parameters and their difference between baseline and 6 month visit will be analyzed whether there are outliers or extreme values in the respective medication group. Outliers are defined as values that are larger than Q75+1.5*IQR or lower than Q25-1.5*IQR; extreme Values are defined as values that are larger than Q75+3*IQR or lower than Q25-3*IQR.

4.6.7. Interim analyses and examination of Subgroups

No interim analyses and no analysis of subgroups were performed.

4.6.8. Multiplicity

Analysis of secondary efficacy parameters was performed exploratory. No multiplicity adjustment was performed.

4.7. CHANGES IN THE CONDUCT OF THE STUDY OR PLANNED ANALYSES

Due to a change in the address of the institution, amendment 1 of the clinical trial protocol, V2.1 01.10.2015 was submitted to the ethics committee and the competent authorities.

The second amendment of the study protocol, V3.1 29.04.2016, included changes due to a second medication batch used for the study and change of study timelines due to prolongation of recruitment to reach aimed sample sizes. Furthermore, two exclusion criteria were reworded for clarification.

- Clarification of exclusion criteria: #3, clarification of optional assessment of right atrial pressure by right heart catheterization during exercise (patients were included if the presented with either mildly elevated mean pulmonary arterial pressures at rest and/or during exercise), and exclusion criterion #10 (glomerular filtration rate should not be <60mL/min/1.73m² at least for 3 months before inclusion).

- Change of packaging of the study drug (second batch of study drug was packed in modified blister sizes), change of the paragraph regarding the tear-off portions on the labels of study medication as labels with tear-off portions were not provided.

- Adaptation of the timelines for the study according to the current recruitment status and anticipated recruitment. Recruitment and subsequent timelines were prolonged for 2 ½ years.

Die Studie wurde nicht unterbrochen.

5. RESULTS

5.1. Study flow-chart

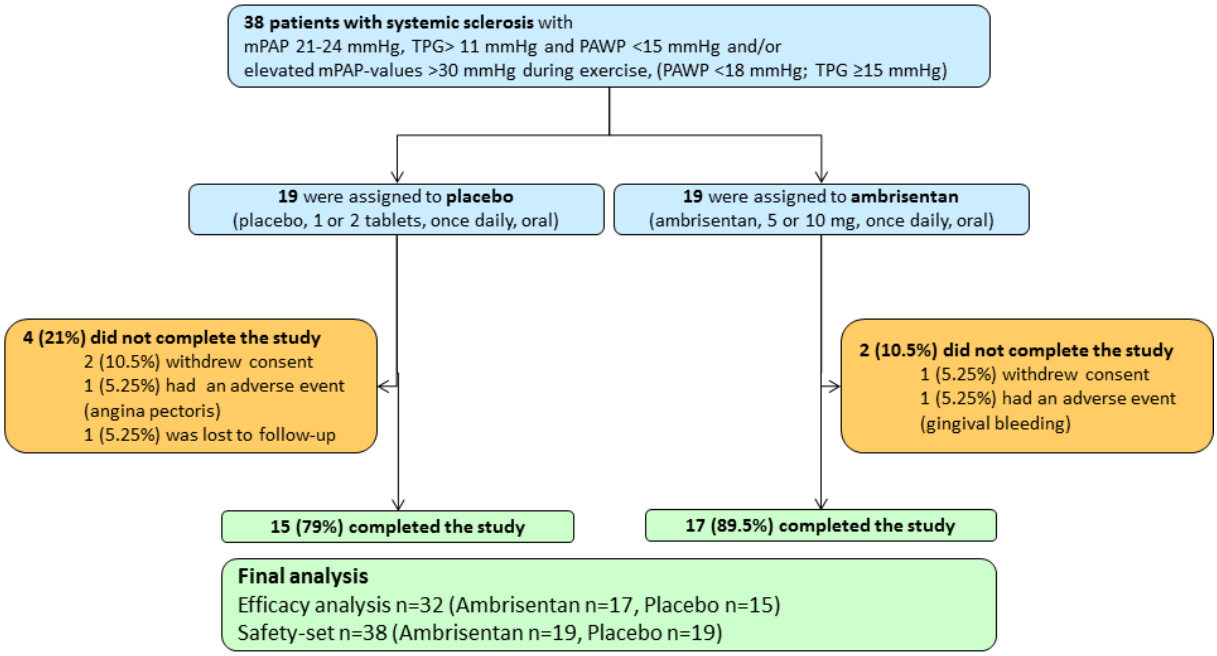

Figure 1 Study flow chart

A total of 38 patients were enrolled into our study and randomly assigned to be treated with ambrisentan 5 or 10 mg/die or to receive placebo. After 6 months, 32 patients completed the study, 17 in the ambrisentan group and 15 in the placebo group. Among the 6 drop-out patients, 1 in the ambrisentan group and 2 in the placebo group withdrew their written informed consents. One in each group quit because of adverse events, one in the ambrisentan group for gingival bleeding and one in the placebo group for angina pectoris. One patient in the placebo group was lost to follow-up.

5.2. Protocol deviations

Major protocol deviations were defined as:

1. Patients who did not meet the inclusion criteria
2. Administration of study medication not according to protocol (e.g. compliance less than 80% or greater 120%)

Two patients did not fulfill the inclusion criterion of systemic sclerosis duration ≥ 3 years. Both of them had symptoms of systemic sclerosis >3 years before inclusion into the trial. Furthermore, both patients did not complete the final study assessment and are therefore included only in the analysis set valid for safety.

One patient showed a significant drop in hemoglobin during the study (withdrawal criterion). The patient was not withdrawn, as drop in hemoglobin was directly related to uterine curettage. Hemoglobin values immediately improved after the adverse event of curettage. No further withdrawal criteria or protocol deviations occurred.

5.3. Baseline characteristics

From October 2014 through April 2017, 38 SSc patients were randomly assigned to receive placebo or ambrisentan (5-10mg/die) in the EDITA-study and 32 patients completed the study (15 in the placebo group and 17 in the ambrisentan group) as depicted in Figure 1. The baseline characteristics were well balanced between the two groups (table 1). Most of the patients were in WHO class II (n=32) with the majority displaying a six-minute walking distance ≥ 400 mmHg (28 patients, 14 in each group). The average of mPAP in the whole study cohort was 21±3mmHg,

Table 1. Demographics and baseline characteristics

Parameter [unit]	Placebo (N = 19)				Ambrisentan (N = 19)					Total (N = 38)			
	Mean ± SD			n	Mean ± SD			n	p-value	Mean ± SD			n
Female sex no. [%]	14 (73.7)				16 (84.2)				0.426	30 (78.9)			
Age [years]	54.89	±	11.23		58.79	±	10.75		0.282	56.84	±	11.02	
Height [cm]	166.21	±	10.19		166.05	±	5.59		0.953	166.13	±	8.11	
Weight [kg]	74.81	±	17.25		71.45	±	15.83		0.559	73.13	±	16.42	
Systolic blood pressure [mmHg]	121.32	±	14.99		116.32	±	11.53		0.257	118.82	±	13.43	
Diastolic blood pressure [mmHg]	73.42	±	9.14		73.68	±	8.95		0.929	73.55	±	8.92	
HR [beats/min]	77.58	±	9.85		73.05	±	11.12		0.193	75.32	±	10.61	
WHO FC no. [%]													
II	15 (78.9)				17 (89.5)				0.374	32 (84.2)			
III	4 (21.1)				2 (10.5)					6 (15.8)			
mRSS [points]	11.47	±	5.71		11.47	±	5.22			11.47	±	5.4	
SSc subgroup --- no. (%)													
Diffuse	11 (57.9)				4 (21.1)				0.062	15 (39.5)			
Limited	8 (42.1)				15 (78.9)					23 (60.5)			
Hemodynamic at rest													
CVP [mmHg]	5.63	±	2.97		6.11	±	2.94		0.624	5.87	±	2.92	
mPAP [mmHg]	21.32	±	2.43		19.84	±	3.58		0.147	20.58	±	3.11	
PAWP [mmHg]	9.58	±	2.97		9.42	±	2.32		0.856	9.50	±	2.63	
CO [l/min]	5.66	±	1.48		5.04	±	1.26		0.176	5.35	±	1.39	
CI [l/min/m^2]	3.20	±	0.85		2.84	±	0.64		0.146	3.02	±	0.76	
SvO_2 [%]	73.13	±	4.60	*(16)*	73.91	±	8.58	*(17)*	0.743	73.53	±	6.85	*(33)*
PVR [WU]	2.09	±	0.61		2.22	±	0.93		0.627	2.16	±	0.78	
Hemodynamic at peak exercise													
mPAP [mmHg]	36.94	±	6.08	*(17)*	37.78	±	3.61	*(18)*	0.627	37.37	±	4.91	*(35)*
PCWP [mmHg]	15.24	±	5.43	*(17)*	16.78	±	6.42	*(18)*	0.447	16.03	±	5.92	*(35)*
CO [l/min]	11.03	±	3.62	*(17)*	9.67	±	2.67	*(18)*	0.217	10.33	±	3.19	*(35)*
CI [l/min/m^2]	6.04	±	1.79	*(17)*	5.41	±	1.32	*(18)*	0.247	5.71	±	1.58	*(35)*
SvO_2 [%]	38.27	±	6.10	*(15)*	42.77	±	11.56	*(15)*	0.197	40.52	±	9.37	*(30)*
Workload [Watt]	75.00	±	34.23	*(17)*	75.00	±	27.12	*(18)*	1.00	75.00	±	30.32	*(35)*
HR max [/min]	117.65	±	21.37	*(17)*	111.39	±	23.06	*(18)*	0.411	114.43	±	22.15	*(35)*
PVR [WU]	2.07	±	0.61	*(17)*	2.29	±	0.85	*(18)*	0.391	2.18	±	0.74	*(35)*
6MWT													
6MWD [m]	448.11	±	82.64		470.21	±	77.03		0.399	459.16	±	79.59	
Borg dyspnea score	2.93	±	1.97		2.41	±	1.32		0.346	2.67	±	1.68	
SaO_2 after 6MWT [%]	91.87	±	4.63	*(15)*	91.71	±	4.79	*(17)*	0.924	91.78	±	4.64	*(32)*
HR after 6MWT [/min]	99	±	21.27	*(17)*	107	±	19.64	*(17)*	0.263	103	±	20.56	*(34)*
Echocardiography at rest													
estimated PASP [mmHg]	29.21	±	5.16		28.58	±	6.57		0.744	28.89	±	5.83	
RA area [cm^2]	12.05	±	4.24		11.68	±	3.28		0.766	11.87	±	3.74	
RV area [cm^2]	14.89	±	4.56		14.13	±	4.74		0.616	14.51	±	4.60	
TAPSE [cm]	2.50	±	0.51		2.41	±	0.33		0.528	2.46	±	0.43	
Lung Function													
FVC [L]	2.87	±	0.89		2.84	±	0.91		0.913	2.86	±	0.89	
FEV1 [L]	2.40	±	0.77		2.26	±	0.63		0.544	2.33	±	0.70	
FEV1 % VC max [%]	79.31	±	6.98		81.44	±	15.38		0.588	80.38	±	11.83	
PEF [l/s]	5.46	±	2.32		5.17	±	1.72		0.667	5.31	±	2.02	
TLC [L]	4.96	±	1.07		5.08	±	1.25		0.744	5.02	±	1.15	
Residual volume [L]	1.94	±	0.55		2.18	±	0.70		0.255	2.06	±	0.63	

DLCO [mmol/min/kPa]	5.28	±	1.52		5.03	±	1.33		0.582	5.16	±	1.42	
SaO_2 [%]	96.13	±	1.80		96.82	±	0.77		0.141	96.47	±	1.41	
PaO_2 [mmHg]	78.78	±	9.42		81.22	±	5.91		0.347	80.00	±	7.85	
$PaCO_2$ [mmHg]	39.01	±	3.63		37.23	±	2.57		0.091	38.12	±	3.23	
Laboratory													
Hemoglobin [g/dl]	13.54	±	1.26		13.62	±	1.13		0.840	13.58	±	1.18	
Hematocrit [l/l]	0.42	±	0.03		0.41	±	0.03		0.724	0.41	±	0.03	
Platelet [100/nl]	2.53	±	0.94		2.62	±	0.63		0.743	2.58	±	0.79	
Creatinine [mg/dl]	0.83	±	0.16		0.86	±	0.12		0.510	0.85	±	0.14	
potassium [mmol/l]	4.05	±	0.38		4.20	±	0.48		0.282	4.12	±	0.43	
AST [U/l]	24.11	±	21.78		19.79	±	7.06		0.420	21.95	±	16.12	
ALT [U/l]	29.68	±	24.93		24.68	±	9.85		0.424	27.18	±	18.87	
LDH [U/l]	197.63	±	54.74		197.53	±	35.59		0.994	197.58	±	45.54	
CRP [mg/l]	5.18	±	5.17		5.28	±	11.60	*(18)*	0.974	5.23	±	8.77	*(37)*
NTproBNP [pg/ml]	123.42	±	142.96		267.83	±	303.11	*(18)*	0.079	193.68	±	242.81	*(37)*
Quality of life SF-36													
physical functioning	50.26	±	25.95		64.21	±	25.83		0.106	57.24	±	26.50	
physical role functioning	35.53	±	40.24		51.32	±	41.23		0.240	43.42	±	40.97	
bodily pain	49.79	±	28.96		62.00	±	29.16		0.204	55.89	±	29.33	
general health perceptions	41.74	±	13.07		54.42	±	19.47		**0.025**	48.08	±	17.58	
vitality	42.89	±	19.32		50.53	±	20.94		0.251	46.71	±	20.24	
social role functioning	58.05	±	25.99		74.47	±	24.45		0.052	66.26	±	26.24	
emotional role functioning	49.16	±	46.33		63.16	±	47.02		0.361	56.16	±	46.58	
mental health	59.58	±	18.85		64.42	±	18.03		0.424	62.00	±	18.36	
physical health score	43.89	±	21.26		56.47	±	23.87		0.095	50.18	±	23.19	
mental health score	50.26	±	20.70		61.42	±	21.45		0.111	55.84	±	21.55	

SD: standard deviation; HR: heart rate; mRSS: modified Rodnan Skin Score; WHO FC: World Health Organization functional class; CVP: central venous pressure; mPAP: mean pulmonary arterial pressure; PCWP: pulmonary capillary wedge pressure; CO: cardiac output; CI: cardiac index; SvO_2: venous oxygen saturation; PVR: pulmonary vascular resistance; WU: Wood Units; PASP: systolic pulmonary arterial pressure; RA: right atrial; RV: right ventricular; TAPSE: tricuspid annular plane systolic excursion; FVC: forced vital capacity; FEV1: forced expiratory volume in first second; VC: vital capacity; PEF: peak expiratory flow; TLC: total lung capacity; DLCO: diffusing capacity of the lung for carbon monoxide; SaO_2: oxygen saturation;PaO_2:partial pressure of oxygen; $PaCO_2$: partial pressure of carbon dioxide; AST: aspartate-aminotransferase; ALT: alanine-aminotransferase; LDH: lactate dehydrogenase; CRP: C-reactive protein.
In case of missing data, sample sizes are given in brackets.
p-values refer to t-tests with unequal variances.

5.4. Analysis of efficacy

5.4.1. Hemodynamics at rest and during exercise

Changes of hemodynamics at rest and at peak exercise between baseline and 6 months are presented in table 2. The two groups did not significantly differ in their change in resting mPAP from baseline to 6 months (p= 0.882, figure 2). After 6 months 5 patients presented with mPAP values above 25 mmHg (placebo n=3, ambrisentan n=2). Both patients in the ambrisentan group with mPAP ≥25mmHg after 6 months presented with increased PAWP ≥15mmHg at rest, subsequently confirmed as PH due to left heart disease. The changes from baseline to 6 months in CO (p=0.047), CI (p=0.01) (Figure 3A) and PVR (p=0.012) (Figure 4A) at rest significantly differed between the two groups. At peak exercise, mean changes significantly differed in CO (p=0.028), CI (p=0.015, figure 3B), PVR (p<0.001, Figure 4B), but did not in mPAP (p=0.494). Workload and heart rate at peak exercise did not differ between the two groups.

Table 2. Hemodynamics (at rest and at peak exercise) at baseline and after 6 months

Parameter [Unit]	Placebo changes (n=15)				Ambrisentan changes (n=17)				p-value*
	baseline - 6 months				baseline - 6 months				(t-test)
	Mean ± SD			n	Mean ± SD			n	
at rest									
CVP [mmHg]	-0.20	±	2.76		0.82	±	4.11		0.411
mPAP [mmHg]	-0.73	±	3.59		-1.00	±	6.40		0.884
PAWP [mmHg]	0.13	±	3.20		1.24	±	5.31		0.478
CO [l/min]	-0.26	±	1.11		0.58	±	1.17		0.047
CI [l/min/m^2]	-0.31	±	0.71		0.36	±	0.66	*(16)*	0.010
SvO_2 [%]	-3.48	±	12.26	*(13)#*	-2.79	±	7.56	(12)#	0.867
PVR [WU]	0.01	±	0.71		-0.70	±	0.78		0.012
peak exercise									
mPAP [mmHg]	1.08	±	7.39	*(13)*	-0.73	±	6.23	*(15)*	0.494
PAWP [mmHg]	0.85	±	6.3	*(13)*	4.93	±	6.52	*(14)*	0.111
CO [l/min]	-0.05	±	1.46	*(13)*	1.24	±	1.47	*(15)*	0.028
CI [l/min/m^2]	-0.45	±	1.36	*(13)*	0.70	±	0.81	*(15)*	0.015
SvO_2 [%]	-28.35	±	10.5	*(11)*	-30.05	±	17.73	*(10)*	0.796
Workload [Watt]	1.92	±	16.01	*(13)*	5	±	16.9	*(15)*	0.625
HR max [b/min]	6.00	±	18.74	*(13)*	7.47	±	12.01	*(15)*	0.811
PVR [WU]	-0.0032	±	0.34	*(13)*	-0.84	±	0.48	*(14)*	<0.001

SD: standard deviation; CVP: central venous pressure; mPAP: mean pulmonary arterial pressure; PAWP: pulmonary capillary wedge pressure; CO: cardiac output; CI: cardiac index; SvO2: venous oxygen saturation; PVR: pulmonary vascular resistance; WU: Wood Units; HR: heart rate; b: beats; min: minute.

In case of missing data, sample sizes are given in brackets.
* refer to t-tests with unequal variances
# values with more than 20% missing data.

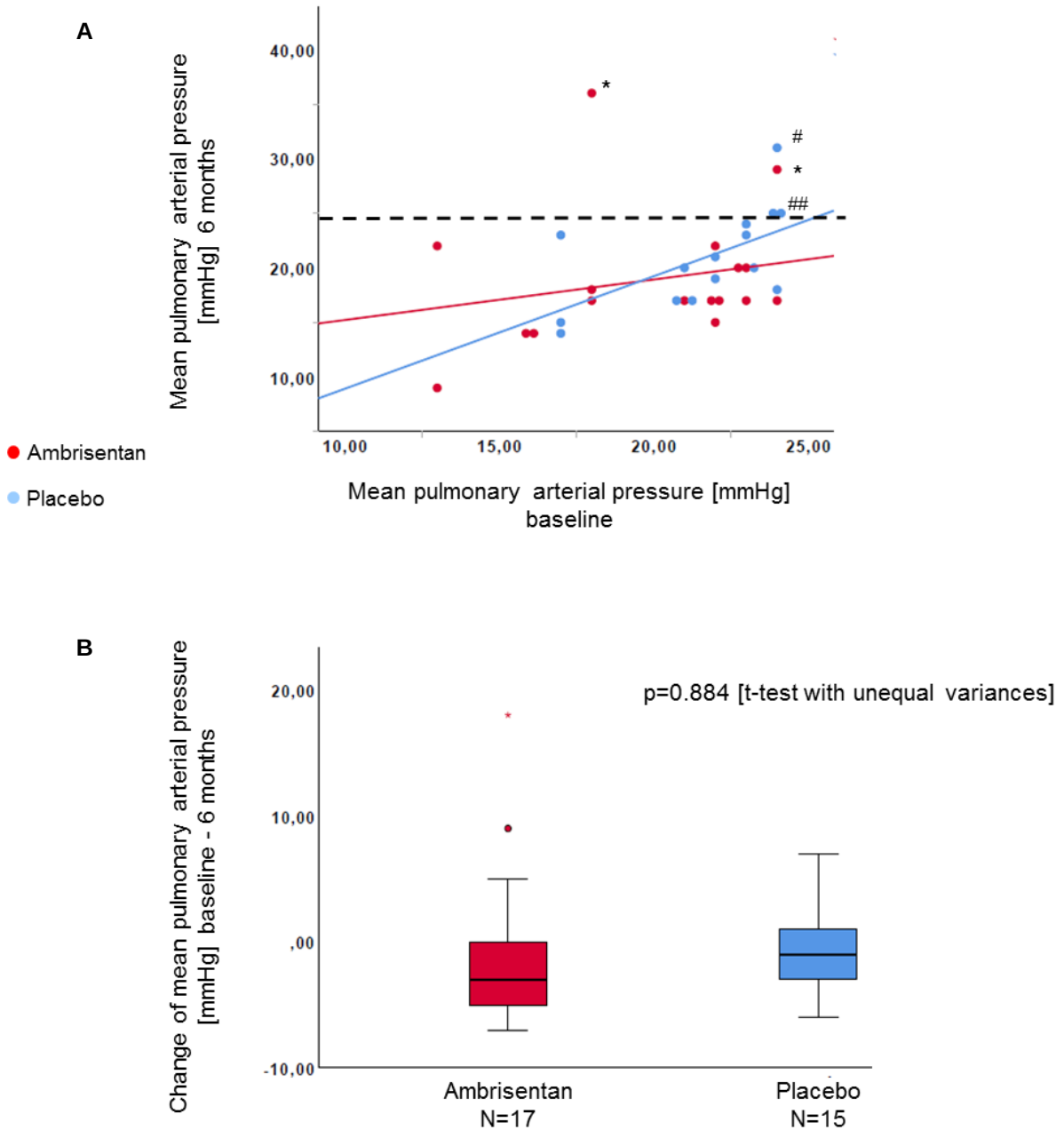

Figure 21 Changes of mPAP at rest over 6 months

A) No patients at baseline had a resting mPAP of ≥25 mmHg. After 24 weeks, 2 patients in the ambrisentan group developed a resting mPAP of >25 mmHg. The dotted line indicated a resting mPAP of 25mmHg. *: Two patients in the ambrisentan group had a resting PCWP of >15 mmHg at week 24, they were reclassified as PH due to left heart disease. #: Three patients in the placebo group developed a resting mPAP of ≥25 mmHg at week 24 with a resting PCWP of ≤ 15 mmHg, thus, they were diagnosed as having SSc-APAH after 24 weeks. B) The mean change of resting mPAP over 24 weeks in the ambrisentan group was -1±6.4 mmHg, and that in the placebo group was -0.73±3.59 mmHg. The changes between the two groups were not significantly different (p=0.884). Ambrisentan did not significantly decreased the mPAP at rest over 24 weeks compared to placebo.

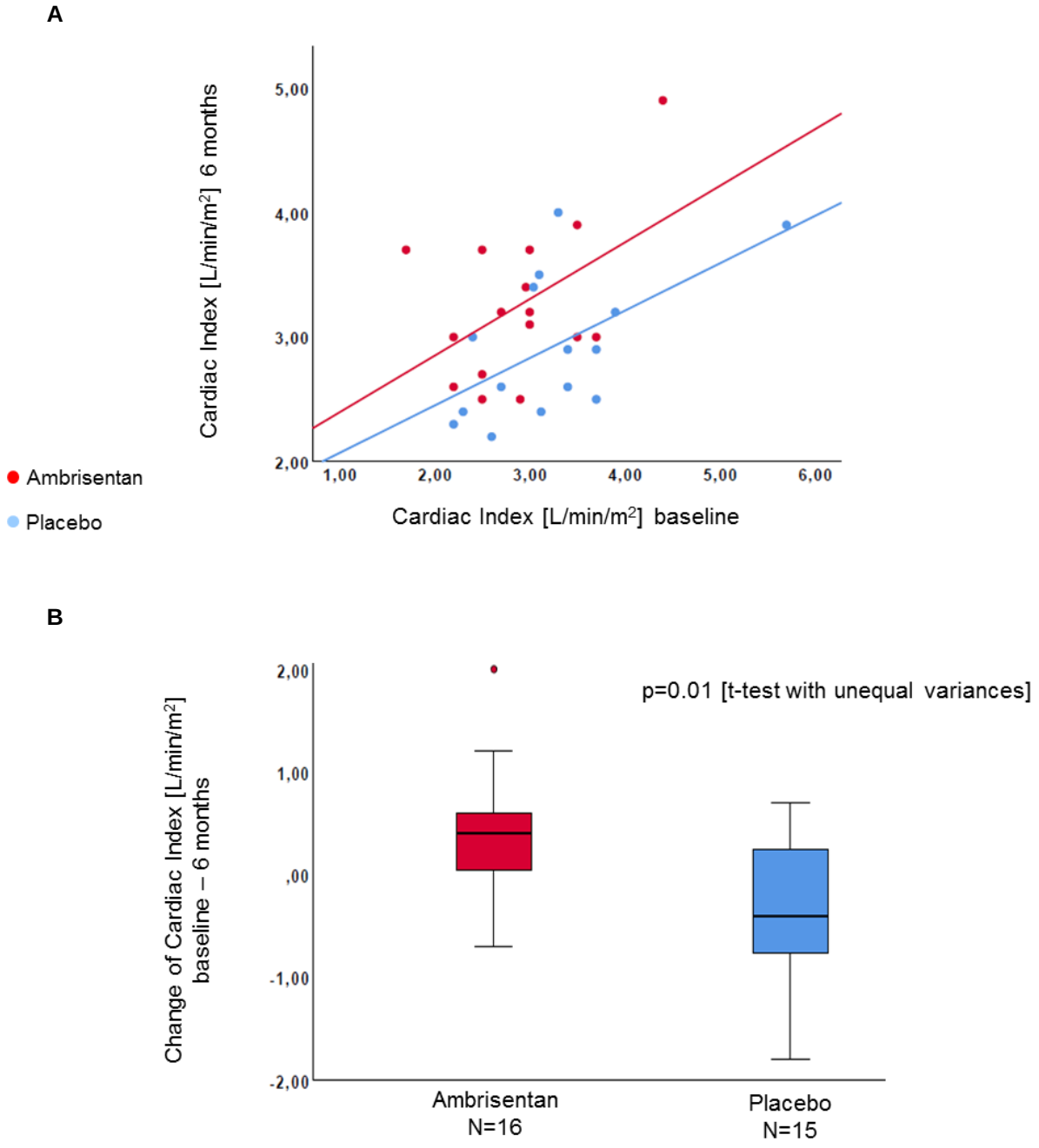

Figure 3A Changes of CI at rest over 6 months

A) Cardiac index at rest increased stronger in the ambrisentan group after 6 months in comparison to placebo. B) The mean change of CI at rest over 24 weeks in the ambrisentan group was 0.36±0.66 l/min/m^2 and -0.31±0.71 l/min/m^2 in the placebo group. Ambrisentan significantly increased the CI at rest over 24 weeks compared to placebo (p= 0.01).

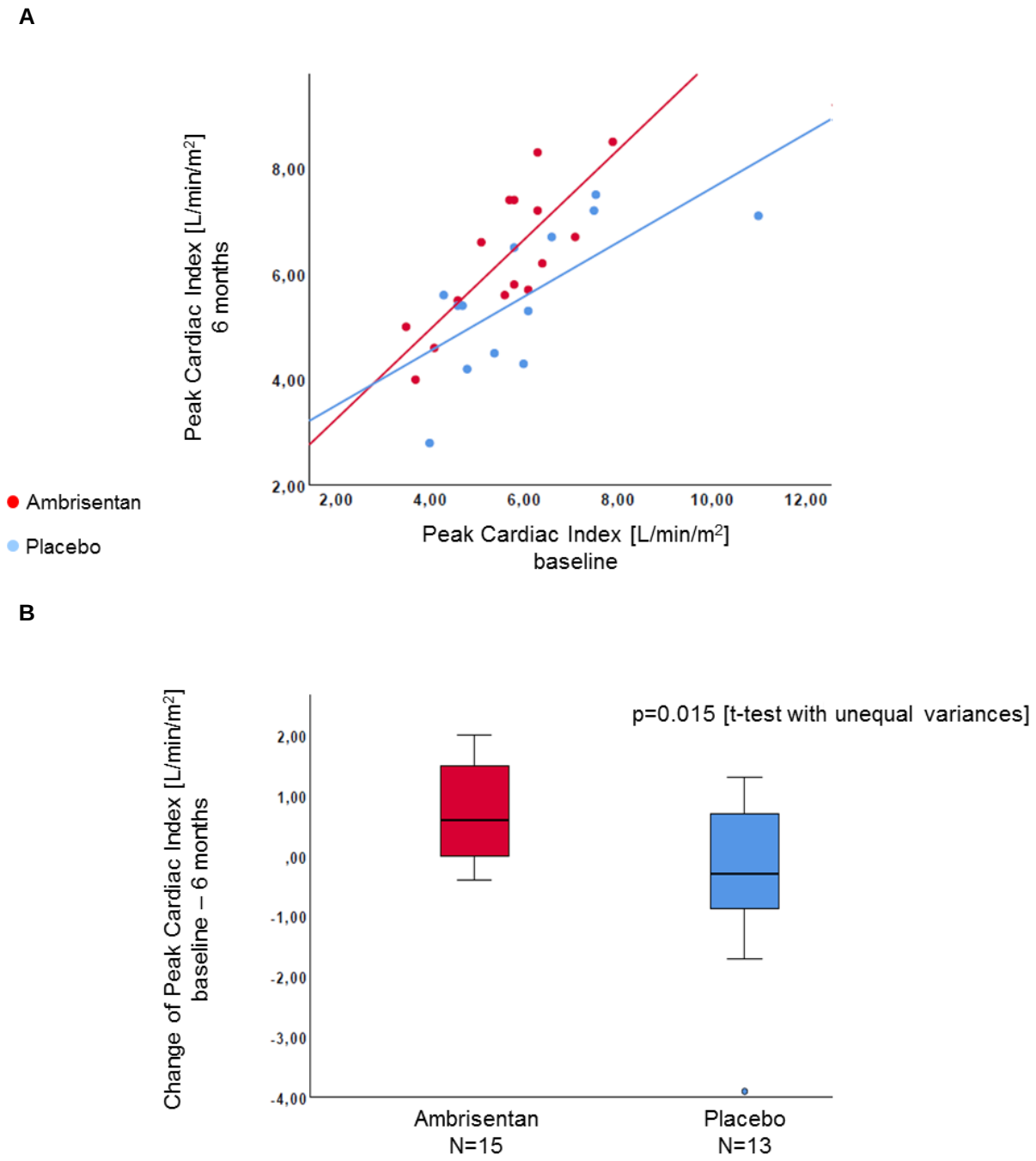

Figure 3B Changes of CI at maximal exercise over 6 months

A) CI during exercise increased more strongly in the ambrisentan group after 6 months compared to placebo. B) The mean change of CI at maximal exercise over 24 weeks in the ambrisentan group was 0.70±0.81 l/min/m^2 and that in the placebo group was -0.45±1.36 l/min/m^2. Ambrisentan significantly increased the CI at maximal exercise over 24 weeks compared to placebo (p= 0.015).

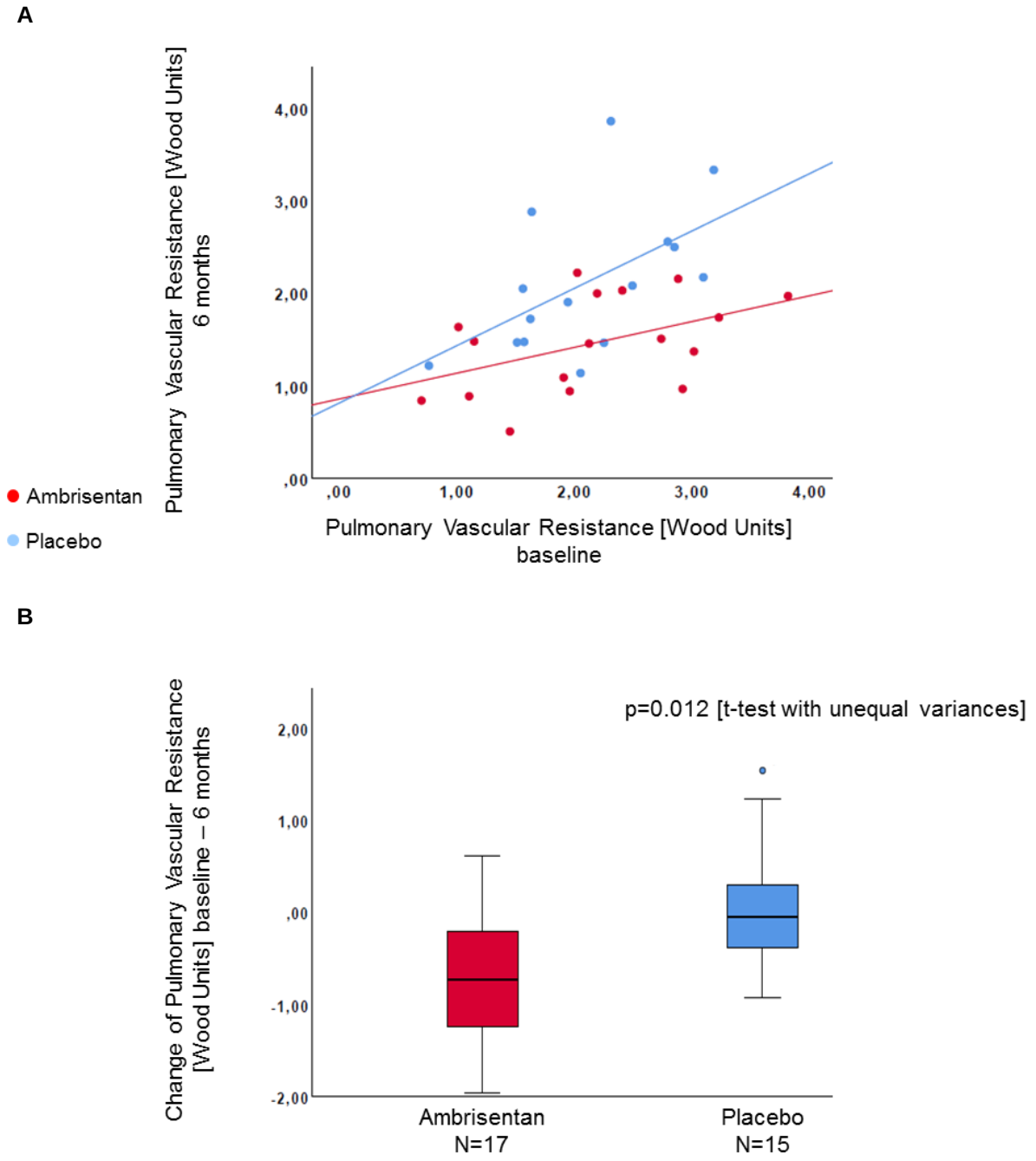

Figure 4A Changes of PVR at rest over 6 months

A) Ambrisentan patients had on average a lower PVR at 6 months compared to placebo. B) The mean change of PVR at rest over 24 weeks in the ambrisentan group was -0.70±0.78 WU and that in the placebo group was 0.01±0.71 WU. Ambrisentan significantly decreased the PVR at rest over 24 weeks compared to placebo (p=0.012).

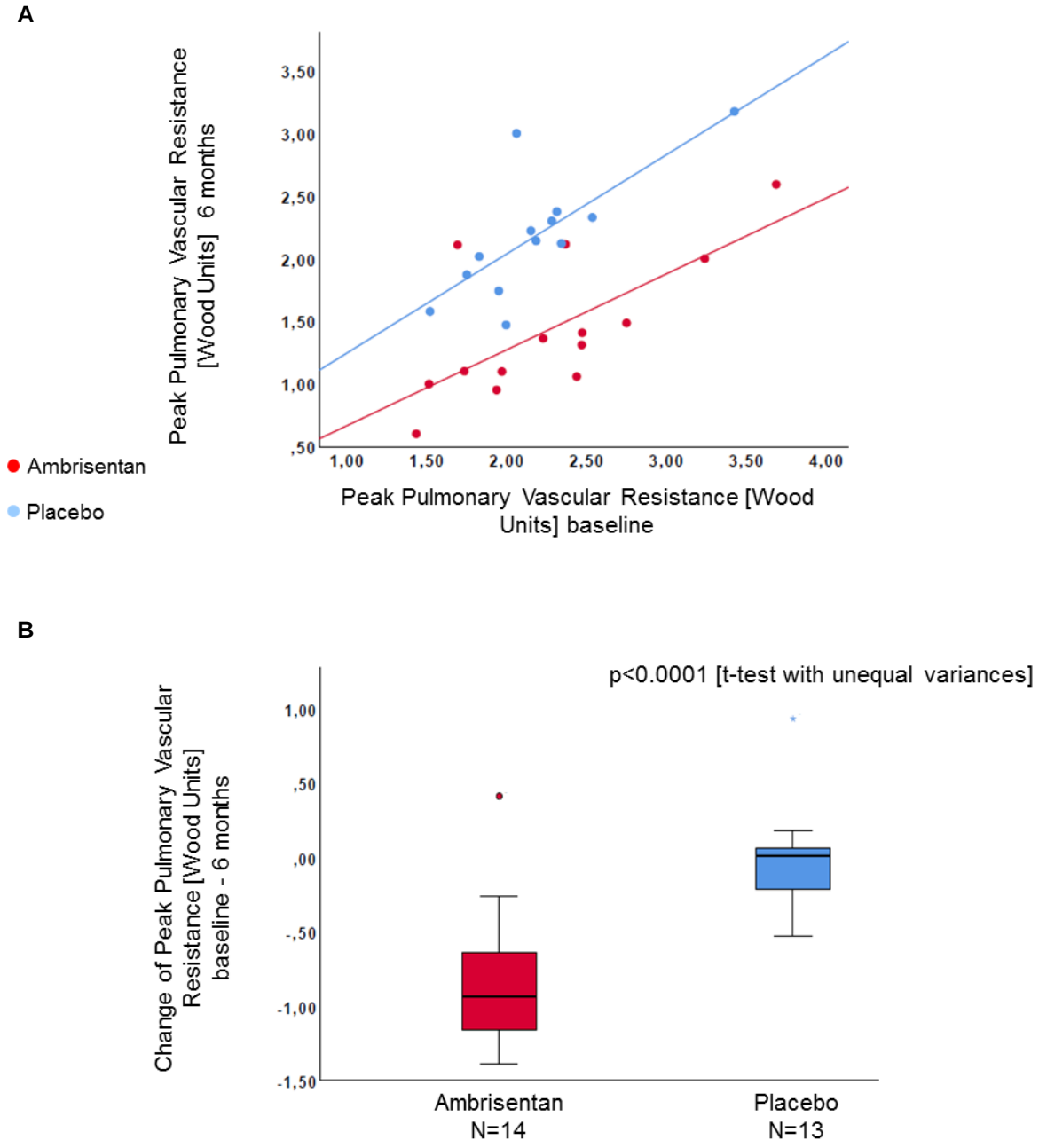

Figure 4B2 Changes of PVR at maximal exercise over 6 months

A) PVR at maximal exercise was higher in the placebo group after six months compared to the ambrisentan group. B) The mean change of PVR at maximal exercise over 24 weeks in the ambrisentan group was -0.84±0.48 WU and that in the placebo group was -0.0032±0.34 WU. Ambrisentan significantly decreased the PVR at maximal exercise over 24 weeks compared to placebo (p<0.001).

5.4.2. WHO functional class, Six-minute walking test, Quality of life and Skin involvement

After 6 months exercise capacity, measured by 6MWD, showed a trend between groups, with a higher change in patients receiving ambrisentan (p=0.095, figure 5, table 3). Patients did not significantly differ regarding changes in WHO FC, quality of life, Borg dyspnea score, Oxygen saturation at the end of the test, HR and mRSS (table 3).

Table 3. Six-minute walking distance, Skin involvement and Symptoms at baseline and after 6 months

Parameter [Unit]	Placebo changes (n=15)				Ambrisentan changes (n=17)				p-value*
	baseline - 6 months				baseline - 6 months				(t-test)
	Mean ± SD			n	Mean ± SD			n	
6MWT									
6MWD [m]	-16.53	±	77.32		21.53	±	34.6		0.095
Borg dyspnea score	0.08	±	1.54		0.62	±	1.7		0.350
SaO_2 after 6MWT [%]	1.30	±	5.1	*(10)#*	2.07	±	5.7	*(14)*	0.732
HR after 6MWT [/min]	6.83	±	24.97	*(12)#*	-1.53	±	18.43	*(15)*	0.344
Quality of life SF-36									
physical functioning	-2.00	±	25.20		-7.65	±	21.66		0.505
physical role functioning	13.33	±	38.81		-10.29	±	42.44		0.110
bodily pain	-2.47	±	28.19		-9.29	±	23.87		0.469
general health perceptions	0.20	±	19.39		-2.71	±	10.62		0.611
vitality	-5.00	±	16.37		-3.53	±	12.34		0.779
social role functioning	0.87	±	27.69		-2.18	±	19.21		0.724
emotional role functioning	8.80	±	49.68		-9.82	±	36.76		0.244
mental health	-3.73	±	11.85		-4.47	±	10.94		0.857
physical health score	0.87	±	16.01		-6.71	±	12.17		0.148
mental health score	0.27	±	19.77		-4.65	±	9.47		0.391
mRSS	0.73	±	2.15		0.24	±	0.97		0.420

SD: Standard deviation; WHO FC: World Health Organization Functional Class; 6MWT: Six-minute walking test; 6MWD: Six-minute walking distance; SaO_2: Oxygen saturation; HR: heart rate; mRSS: modified Rodnan Skin Score.

In case of missing data, sample sizes are given in brackets.
* refer to t-tests with unequal variances
# values with more than 20% missing data.

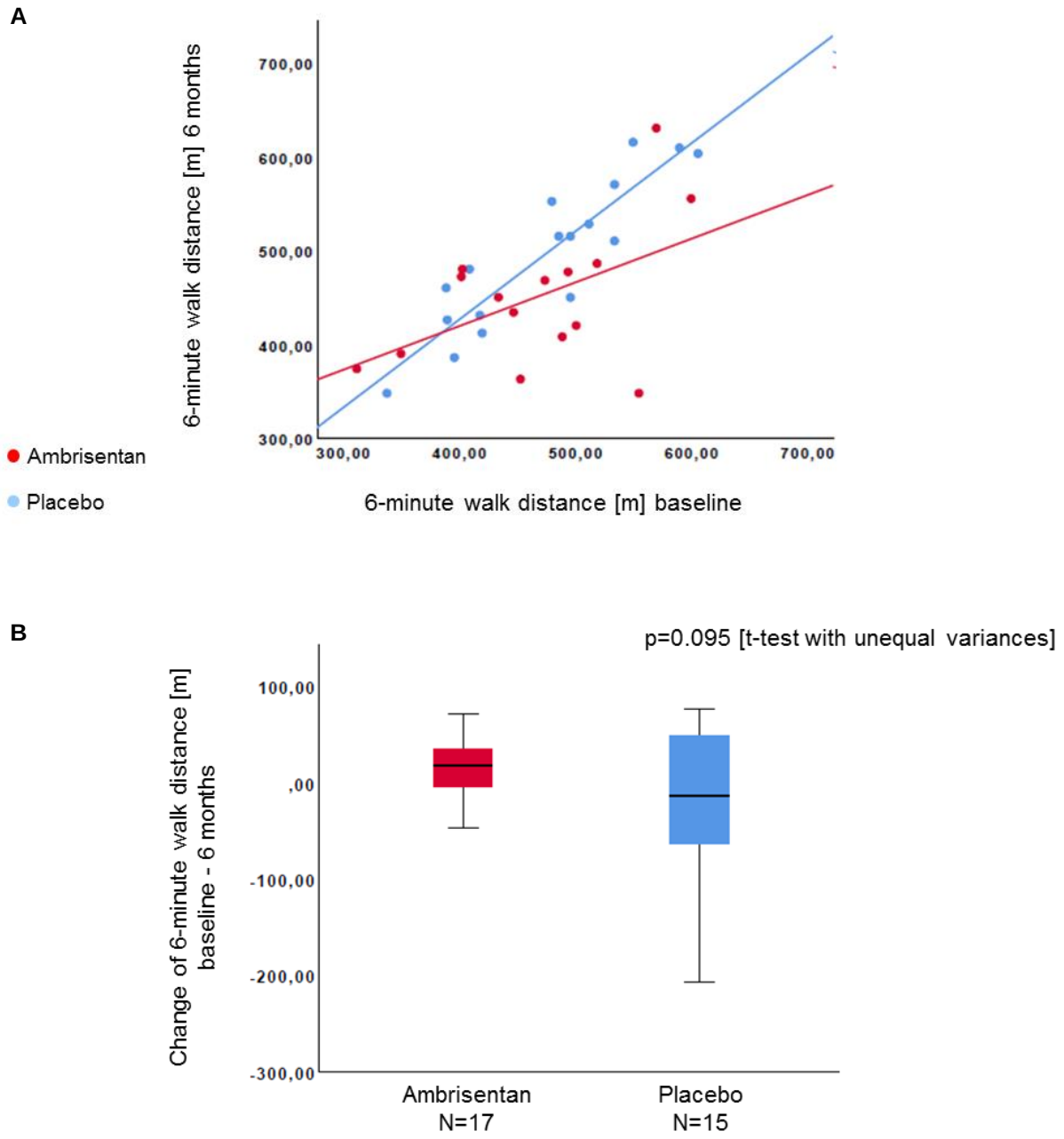

Figure 5 Changes of 6MWD over 24 weeks

A) Larger but non-significantly different 6MWD were seen in the placebo group after 6 months. B) The mean change of 6MWD over 24 weeks in the ambrisentan group was 21.53±34.6 m and that in the placebo group was -16.53±77.32 m. Ambrisentan tended to increase 6MWD over 24 weeks, however not statistically significant compared to placebo (p= 0.095).

5.4.3. Lung function, transthoracic Echocardiography and laboratory data

Data regarding lung function, echocardiography and biochemistry are shown in table 4. No significant changes between the two groups were recorded with regard to pulmonary function. A statistically significant (p=0.048) change in the partial pressure of Oxygen (PaO_2) was recorded, with a decrease in the ambrisentan group whereas it slightly increased in the placebo group.

After 6 months, a trend in difference of RA area change (p=0.10) and TAPSE (p=0.089) was detected. Other parameters of right heart size and function did not show significant differences between groups.

Patients receiving ambrisentan had a significant drop in hemoglobin concentration (p=0.008) compared to placebo. No differences were recorded regarding other laboratory parameters, including renal failure, liver damage and NTproBNP concentrations.

Table 4. Lung function, Doppler echocardiography and biochemical data at baseline and after 6 months

Parameter [Unit]	Placebo changes (n=15)				Ambrisentan changes (n=17)				p-value*
	baseline - 6 months				baseline - 6 months				(t-test)
	mean ± SD			n	mean ± SD			n	
Lung function									
FVC [L]	-0.03	±	0.23		-0.13	±	0.22	*(16)*	0.233
FEV1 [L]	-0.06	±	0.20		-0.11	±	0.21	*(16)*	0.535
FEV1 % VC max [%]	-0.67	±	4.50		-4.17	±	7.12	*(16)*	0.112
PEF [l/s]	-0.22	±	1.76		0.01	±	1.15	*(16)*	0.681
TLC [L]	-0.03	±	0.36		-0.06	±	0.37	*(16)*	0.802
Residual volume [L]	0.05	±	0.37		-0.03	±	0.33	*(16)*	0.571
DLCO [mmol/min/kPa]	-0.45	±	1.70	*(13)*	-0.32	±	1.44		0.826
SaO_2 [%]	0.15	±	1.86		-0.62	±	1.50		0.212
PaO_2 [mmHg]	1.69	±	9.95		-4.88	±	7.60		0.048
$PaCO_2$ [mmHg]	-0.03	±	2.63		-0.65	±	2.77		0.517
Echocardiography									
estimated sPAP [mmHg]	-0.93	±	6.08		-0.82	±	4.46		0.955
RA area [cm^2]	-0.47	±	4.07		1.65	±	2.67		0.100
RV area [cm^2]	-0.80	±	3.05		-0.15	±	3.46		0.575
TAPSE [cm]	-0.19	±	0.54		0.12	±	0.41		0.089
Laboratory									
Hemoglobin [g/dl]	0.19	±	0.68		-0.59	±	0.86		0.008
Hematocrit [l/l]	0.00	±	0.02		-0.01	±	0.02		0.054
Platelets [100/nl]	-0.08	±	0.39		-0.15	±	0.37		0.584
Creatinine [mg/dl]	-0.03	±	0.09		-0.04	±	0.11		0.899
Potassium [mmol/l]	0.09	±	0.40		-0.06	±	0.62		0.433
AST [U/l]	-4.40	±	13.94		3.59	±	7.96		0.063
ALT [U/l]	-4.93	±	15.01		5.12	±	7.83		0.030
LDH [U/l]	-7.00	±	27.36		2.82	±	29.57		0.337
CK [U/l]	9.21	±	40.50	*(14)*	5.53	±	36.80		0.795
CRP [mg/l]	-1.08	±	3.52		-2.71	±	12.17		0.603
NTproBNP [pg/ml]	31.00	±	85.83	*(13)*	-15.63	±	207.48	*(16)*	0.423

SD: Standard deviation; FVC: forced vital capacity; FEV1: forced expiratory volume in first second; VC: vital capacity; PEF: peak expiratory flow; TLC: total lung capacity; DLCO: diffusing capacity of the lung for carbon monoxide; SaO_2: oxygen saturation; PaO_2:partial pressure of oxygen; $PaCO_2$: partial pressure of carbon dioxyde; sPAP: systolic pulmonary arterial pressure; RA: right atrial; RV: right ventricular; TAPSE: tricuspid annular plane systolic excursion; AST: aspartat-aminotransferase; ALT: alanin-aminotransferase; LDH: lactate dehydrogenase; CK: creatine kinase; CRP: C-reactive protein; NT-proBNP: N-terminal pro-brain natriuretic peptide; 6MWD: 6-minute walk distance;

In case of missing data, sample sizes are given in brackets.
* refer to t-tests with unequal variances

5.5. Sensitivity analysis

The primary efficacy analysis was performed by t-tests assuming unequal variances since the assumption for a covariance analysis was not fulfilled and outliers had been detected. For sensitivity analysis with respect to statistical methods we additionally performed robust t-tests with Huber weights and compared the p- values as well as the different effect estimates for the primary endpoint and for the secondary variables shown in the figures of this report.

For mPAP at rest differences are not significantly different for both tests (t-test: p=0.884; robust t-test p=0.502), estimates of mean differences for the placebo are identical, however they differ for the ambrisentan group (-1.0±6.40mmHg; robust -1.8±5.59mmHg). P-values for CI, peak CI, PVR peak PVR and 6MWD are largely identical for both means as well as the effect estimates (table 5).

Table 5. Results of sensitivity analysis: comparison of t-test with unequal variances with with robust t-test

Parameter	t-test		Mean ± SD			n	se	p-value
mPAP at rest	normal	ambrisentan	-1	±	6.403	(17)	1.553	0.884
		placebo	-0.733	±	3.595	(15)	0.928	
	robust	ambrisentan	-1.807	±	5.591		1.356	0.502
CI at rest		placebo	-0.733	±	3.473		0.897	
	normal	ambrisentan	0.363	±	0.655	(16)	0.164	0.0102
		placebo	-0.315	±	0.712	(15)	0.184	
	robust	ambrisentan	0.345	±	0.658		0.164	0.0126
peak CI		placebo	-0.307	±	0.743		0.192	
	normal	ambrisentan	0.7	±	0.814	(15)	0.21	0.0154
		placebo	-0.448	±	1.358	(13)	0.377	
	robust	ambrisentan	0.7	±	0.787		0.203	0.007
PVR at rest		placebo	-0.3241	±	1.156		0.321	
	normal	ambrisentan	-0.702	±	0.782	(17)	0.19	0.0118
		placebo	0.006	±	0.711	(15)	0.184	
	robust	ambrisentan	-0.702	±	0.759		0.184	0.0056
peak PVR		placebo	0.006	±	0.687		0.177	
	normal	ambrisentan	-0.842	±	0.476	(14)	0.127	<.0001
		placebo	-0.003	±	0.344	(13)	0.095	
	robust	ambrisentan	-0.884	±	0.412		0.11	<.0001
		placebo	-0.023	±	0.324		0.09	
6MWD	normal	ambrisentan	21.529	±	34.602	(17)	8.392	0.095
		placebo	-16.533	±	77.325	(15)	19.97	
	robust	ambrisentan	21.529	±	33.568		8.142	0.0977
		placebo	-10.512	±	67.984		17.55	

SD: standard deviation, n: sample size, se: standard error, mPAP: mean pulmonary arterial pressure, CI: Cardiac Index, PVR: pulmonary vascular resistance, 6MWD: 6-minute walking distance

5.6. Safety evaluation

The safety analysis was performed in the population valid for safety. All tabulations are descriptive only. Listings were produced for adverse events and serious adverse events.

Mortality in the 6 month period of the study are summarized descriptively. There were no deaths during study treatment.

5.6.1. Extent of exposure

In the ambrisentan group, 13 patients up-titrated to 10mg within 4 weeks. Out of them, 4 patients performed down-titration to 5mg due to tolerability issues. Further 4 patients remained on 5mg throughout the study period.

In the placebo group, 13 patients performed up-titration to 2 tablets, out of whom one down-titrated to one tablet. Further 2 patients remained on one tablet throughout the whole study period.

5.6.2. Adverse Events (AEs)

The total number of AEs is given in table 5a. Frequencies of AEs that occurred in at least 10% of patients are listed in table 5b. Most of the AEs were of mild or moderate intensity in both groups. AE rates significantly differed for dizziness between groups, with six subjects suffering from dizziness in the placebo group and none on the ambrisentan group (chi square test $p<0.05$). Though not statistically significant, four patients in the ambrisentan presented with paraesthesia, while no patients presented with paraesthesia in the placebo group. In the ambrisentan group, one case of paraesthesia and one case of edema were classified as drug-related. No significant differences were reported for the other AEs listed.

Table 5a. Listing of adverse events according to system organ class

MedDRA System Organ Class	Adverse Event	Placebo n	Ambrisentan n
Cardiac disorder	Angina pectoris	1	-
	coronary artery disease	3	1
	heart racing	-	2
	sinus bradycardia	-	1
Ear and labyrinth disorders	middle ear infection	-	1
Endocrine disorders	thyroid inflammation	1	-
Eye disorders	eye pain	1	1
Gastrointestinal disorders	gingival bleeding	-	2
	diarrhea	2	4
	gastrointestinal infection	3	-
	heartburn	-	1
	nausea	3	2
	obstipation	1	-
	stomach pain	1	-
	tooth pain	-	1
	vomiting	1	-
General disorders	drug intolerance	1	-
	edema	4	8
	fatigue	-	1
	hot flush	-	1
	Inflammation of leg	-	1
	inflammation of knee	1	-
	pain	1	-
	sweating	1	1
	sweating (night)	1	-
immune system disorders	allergic rhinitis	1	-
infections and infestations	abscess	-	1
	lymphangitis	1	-
	virus infection	1	-
Injury, poisoning and procedural complications	ligament sprain	-	1
	lower jaw fracture	-	1
Investigations	increased triglycerides	1	-
Metabolism and nutrition disorders	calcium deficiency	-	1
	iron deficiency	-	3
	obesity	1	-
musculosceletal and connective tissue disorders	finger abscess	-	1
	finger stiffness	-	1
	heavyness in limbs	-	1
	lumbago	1	-

	pain, bone	1	-
	pain, hands and feet	1	-
	pain, joint	2	-
	rheumatic disease, worsening	1	1
nervous system disorders	headache	6	6
	paraesthesia	-	4
	tinnitus	-	2
Renal and urinary disorders	urinary infection	1	1
Reproductive system and breast disorders	bleeding	-	1
Respiratory, thoracic and mediastinal disorders	bronchitis	1	-
	cold	-	3
	cough	2	1
	lung disorder	-	1
	nasal stuffiness	-	1
	pleuroparenchymal fibroelastosis	-	1
	respiratory infection	1	-
	rhinitis	1	2
Skin and subcutanous tissue disorders	hair loss	-	1
	itching legs	-	1
	skin rash	1	-
Surgical and medical procedures	uterine dilation and curettage	-	1
Vascular disorders	dizziness†	6	-
	epistaxis	1	3
	flushed face	-	1
	hypertension	1	-
	hypotension	2	2
	Raynaud	2	1
	ulcer	1	-
† statistically significant at level 0.05			

Table 5b. Adverse Events with a frequency ≥10%

Event	Placebo (N = 19)	Ambrisentan (N = 19)
	number of patients (percent)	
Patient with at least 1 Adverse event	17 (89.5)	17 (89.5)
Headache	6 (31.58)	6 (31.58)
Edema	4 (21.05)	8 (42.11)
Dizziness	6 (31.58)	0 (0)†
Diarrhea	2 (10.53)	4 (21.05)
Nausea	3 (15.79)	2 (10.53)
Paraesthesia	0 (0)	4 (21.05)
Coronary artery disease	3 (15.79)	1 (5.26)
Hypotension	2 (10.53)	2 (10.53)
Epistaxis	1 (5.26)	3 (15.79)
The adverse events listed here are those that occurred in at least 10% of patients (total) during the course of the study. † statistically significant at level 0.05		

5.6.3. Listing of Serious Adverse Events

Serious adverse events were more frequently reported for the placebo group (compare table 6), with all events requiring hospitalization, but being resolved at the end of the study.

Table 6. Serious Adverse Events

Serious Adverse Events*	Placebo	Ambrisentan
Lower jaw fracture	0	1
Angina Pectoris	1	0
Coronary artery disease	1	0
Gastrointestinal infection	1	0
Lymphangitis	1	0
Raynaud	1	0
* All SAE fulfilled the SAE criterion of hospitalization		

5.6.4. Clinical laboratory evaluation

Patients receiving ambrisentan had a significant drop in hemoglobin concentration (ambrisentan 0.59±0.86g/dl vs. placebo 0.19±0.68g/dl, p=0.008) compared to placebo. No differences were recorded regarding other laboratory parameters, including renal failure, liver damage and NTproBNP concentrations.

5.6.5. Safety conclusions

Ambrisentan was well tolerated, with a favorable safety profile.

In the ambrisentan group occurred significant drop in hemoglobin. Values at follow-up returned to the range of normality. A small drop in oxygen concentration and an increased incidence, though not statistically significant, of paraesthesia were detected in the ambrisentan group. Oxygen saturation remained within normal limits and did not require initiation of oxygen treatment. Paraesthesia was never reported beforehand to be associated with ambrisentan and should warrant attention, though only one case had been classified as drug-related. No significant rise of liver function tests was detected.

6. DISCUSSION AND OVERALL CONCLUSIONS

To the best of our knowledge, EDITA is the first study to assess the safety and efficacy of an early treatment with a PAH-targeted drug in patients with SSc and mildly elevated mPAP (mPAP 21-24 mmHg) and/or exercise PH in comparison with placebo. Patients did not significantly differ in their change of mPAP during the study. However, only patients in the placebo group developed a manifest SSc-APAH. Right heart function and pulmonary vascular resistance showed a significant improvement in the ambrisentan group with significant improvement of CO, CI, PVR both at rest and at peak exercise compared to placebo. The two groups did not differ in changes of 6MWD, WHO functional class, right heart dimensions and function, lung function, NTproBNP concentrations and QoL assessed through the SF-36 questionnaire. A significant drop of hemoglobin concentration and PaO_2 was observed in the ambrisentan group. Ambrisentan was well tolerated, with a favorable safety profile.

6.1. Effects of Ambrisentan in patients with SSc and early pulmonary vasculopathy

While the primary endpoint of the study was not met, parameters of right ventricular function and pulmonary vascular resistance at rest and during exercise showed significant improvements during the study. Data from large registries have already shown the prognostic importance of CO, CI and PVR at rest (Benza RL et al. 2010, Humbert M et al. 2010), which were all improved by ambrisentan in this study.

Besides resting values, exercise hemodynamics are able to unmask early RV dysfunction and vascular remodeling, especially in patients with SSc and mildly elevated mPAP, who usually display normal

right heart function at rest (Nagel C et al. 2018, unpublished data, Hsu S et al. Circulation. 2018). In our study, improvements of CO and CI at peak exercise were even more pronounced than changes observed at rest. The increase of CO and CI during exercise (also called RV Output reserve) is able to provide useful information regarding prognosis of patients with pulmonary vascular diseases (Grünig E et al. 2013, Chaouat A et al. 2014).

The finding that only patients in the placebo group presented a manifest SSc-APAH at follow-up, whereas two patients in the ambrisentan group developed LHD-PH, may be a hint for the beneficial effect of ambrisentan on the pulmonary vasculature, though the primary endpoint of mPAP change was not met. Ambrisentan treatment may also have played a role in the development of LHD-PH due to an increase of LV filling pressure and PAWP caused by vasodilation (Gimelli A et al. 2015). Further, larger scaled studies are needed to investigate the effect of early PAH-targeted treatment in SSc patients with mildly elevated mPAP and/or exercise PH and its impact on the pulmonary vascular system and right heart function.

6.2. Comparison with previous reports on treatment with PAH-targeted drugs in patients with SSc and early pulmonary vasculopathy

The current report is the first aimed in assessing the efficacy of a PAH-targeted drug in patients with mildly elevated mPAP and/or exercise PH in randomized, placebo-controlled double-blind fashion. Two small open-label reports were previously performed with ambrisentan (Saggar R et al. 2012) and with bosentan (Kovacs G et al. 2012). Our data are in line with those published by Saggar et al. on 12 patients with PH < 25 at rest and > 30 mmHg at peak exercise with ambrisentan treatment for 24 weeks. After 24 weeks of treatment the authors did not find a significant decrease of mPAP (p=0.65) but a remarkable improvement of CO at rest and during exercise (p=0.01 and p=0.006 respectively) and PVR during exercise (p=0.006).

Beside improvement in right heart function (CO at rest p=0.05), Kovacs et al. reported a significant reduction of mPAP at rest and peak exercise (p= 0.03 and p=0.01, respectively) after 6 months of bosentan treatment In line with the results of Kovacs and Saggar, we did not find an improvement in 6MWD, quality of life and WHO functional class. Since in our cohort 80% had 6MWD > 400m and WHO class II, our findings are concordant with the results of a post-hoc analysis of the ambrisentan in Pulmonary Arterial Hypertension, Randomized, Double-Blind, Placebo-Controlled, Multicenter Efficacy Study 1 and 2 (ARIES)-1 and 2 studies, which reported that ambrisentan had a more relevant effect on 6MWD and WHO class in patients with more severe PAH (Chin KM et al. 2014).

6.3. Clinical Implication and safety

A consisted body of evidence demonstrated that patients with SSc and concomitant mildly elevated mPAP and/or exercise PH already present signs of lung vasculopathy (Kovacs G et al. 2014, Heresi GA et al. 2013, Coghlan G et al. 2018, Nagel C et al. 2018, unpublished data, Naije R et al. 2018). Moreover, these patients tend to develop more frequently a manifest PAH (Coghlan G et al. 2018, Condliffe R et al. 2009, Saggar R et al. 2010) and display poorer outcomes (Douschan P et al. 2018, Stamm A et al. 2016). Up to now, the indication of targeted treatment in this population is not clear. An early diagnosis of initial and milder PAH form and a subsequent therapy may be able to grant patients with SSc a considerable survival benefit (Humbert M et al. 2011). Pulmonary vascular abnormalities in PVR have been shown to be already remarkable even in mildly symptomatic patients (Galié N et al. 2008) and may precede an overt disease (Sitbon O et al. 2002, Circulation 2002). The improvement in PVR observed in our cohort might indicate that active treatment with ambrisentan is able to prevent the progressive vascular remodeling in these patients with early forms of pulmonary vasculopathy.

Ambrisentan treatment has been associated with a good safety profile, which is in line with current pharmacovigilance analysis of safety and tolerability (Takahashi T et al. 2018, Vachiery JL et al. 2017). Among the undesirable effects of ambrisentan recorded in our study we found a drop of hemoglobin concentration and PaO_2. Values at follow-up returned to the range of normality. The reduction of hemoglobin is a class-effect of all ERAs, and the most probable cause could be fluid retention (Aversa M et al. 2015). A non-dose dependent change in hemoglobin values was also found in the first 12 weeks of the ARIES-1 and -2 studies, with a stabilization during the further 24 weeks (Galiè N. et al. 2008). Long-term extension of the ARIES studies confirmed that hemoglobin levels tend to stabilize over time (Oudiz R et al. 2009).

We also found a small drop in oxygen concentration and an increased incidence of paraesthesia, though not statistically significant, in the ambrisentan group. Oxygen saturation remained within normal limits and did not require initiation of oxygen treatment. Paraesthesia was never reported beforehand to be associated with ambrisentan and should warrant attention, though only one case had been classified as drug-related. Of note, we did not find a significant rise of liver function tests.

6.4. Limitations

Our study has some limitations. Our study results may have been influenced by the inclusion criteria of including both patients with mildly elevated pressures and/or patients with exercise pulmonary hypertension. The different distribution of these entities may still have influenced the results, though it did not differ between groups.

Of our study cohort, two patients developed LHD-PH during the study period. Though it would be preferable to clearly distinguish the effects of targeted treatment on the pulmonary vasculature, it is hardly possible to exclude interfering diseases such as left heart and lung disease. Patients in our study were, however, only included in absence of significant left heart or lung disease at baseline to limit determining factors of mPAP elevation and development of manifest PH. Development of PAH as primary endpoint would have required large sample sizes, which would not have been feasible as a single center study.

Patients with manifest PH at the end of the study did all not fulfill the current criteria of PH with regard to increase in PVR ≥3 WU. As this study was aimed to treat early pulmonary vascular disease, pulmonary vascular pressures were low as anticipated. Longer follow-up periods would have been desirable to further characterize the development of pulmonary vascular changes of these patients.

The short observation period (6 months) did not lead to observe any change in robust endpoints such as development of PAH, hospitalization and mortality. Although promising, the data of the current study should be confirmed in future, larger, multicenter trials.

6.5. Conclusion

Although the primary endpoint was not met (change in mPAP after 6 months), ambrisentan was associated with significant improvement of secondary endpoints such PVR, CO and CI both at rest as well as at peak exercise. Only patients in the placebo group presented after 6 months of follow-up. Ambrisentan was also tolerable and provided with an acceptable safety profile. Further studies are needed to confirm these results.

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