Efficacy and safety of ralinepag, a novel oral IP agonist, in PAH patients on mono or dual background therapy: results from a phase 2 randomised, parallel group, placebo-controlled trial

Fernando Torres[1], Harrison Farber[2], Arsen Ristic[3], Vallerie McLaughlin[4], John Adams[5], Jinkun Zhang[5], Preston Klassen[5], William Shanahan[6], John Grundy[5], Ines Hoffmann[5], Christopher Cabell[5], Pilar Escribano Subías [7], Namita Sood[8], Anne Keogh[9], Gwyn D'Souza[5] and Lewis Rubin[10]

ABSTRACT

Purpose: This phase 2 study was designed to assess the efficacy, safety and tolerability of immediate-release orally administered ralinepag, a selective, non-prostanoid prostacyclin receptor agonist with a 24-h terminal half-life, compared to placebo in adult patients with symptomatic pulmonary arterial hypertension (PAH).

Methods: 61 PAH patients who were receiving standard care, including mono or dual PAH-targeted background therapy were randomised 2:1 to ralinepag (n=40) or placebo (n=21). The starting dose of ralinepag was 10 μg twice daily. Dosage was then up-titrated as tolerated over the course of the 9-week dose-titration period, to a maximum total daily dose of 600 μg (300 μg twice daily). The primary efficacy end-point was the absolute change in pulmonary vascular resistance (PVR) from baseline to week 22. Additional end-points included percentage change in PVR from baseline, other haemodynamic parameters, 6-min walk distance (6MWD) and safety and tolerability.

Results: Ralinepag significantly decreased PVR by 163.9 dyn·s·cm^{-5} compared to an increase of 0.7 dyn·s·cm^{-5} with placebo (p=0.02); the least-squares mean change from baseline PVR was −29.8% compared with placebo (p=0.03). 6MWD increased from baseline by 36.2 m with ralinepag and 29.4 m with placebo (p=0.90). Serious adverse events occurred in 10% of ralinepag patients and 29% of placebo patients. Study discontinuations occurred in 13% of ralinepag patients and 10% of placebo patients.

Summary: Ralinepag reduced PVR compared with placebo in PAH patients on mono (41%) or dual combination (59%) background therapy.

Introduction

Pulmonary arterial hypertension (PAH) is characterised by vascular remodelling of the small pulmonary arteries that results in elevated pulmonary vascular resistance (PVR) and right ventricular failure. PAH is thought to be initiated by dysfunction of several key mechanisms influencing vasomotor control, proliferation, inflammation and *in situ* thrombosis. The vasculopathy is associated with excessive production of growth factors, pro-inflammatory mediators and vasoconstrictors, and a diminished production of potent vasodilators and inhibitors of platelet aggregation [1, 2].

Prostacyclin is produced in vascular endothelial cells and normally contributes to vasodilation, inhibition of smooth muscle cell proliferation, inhibition of platelet aggregation and inhibition of inflammation [3, 4]. The functions of prostacyclin in the pulmonary circulation are mediated primarily by a specific cell-surface receptor, the prostacyclin receptor (IP), which is expressed on platelets, smooth muscle and endothelial cells and belongs to the G-protein coupled receptor class. The binding of prostacyclin to the receptor triggers the activation of the G-protein and increases intracellular cAMP, which activates downstream signalling to produce the vasoregulatory effects. Activation of the IP receptor, either through the use of prostacyclin analogues or IP receptor activating drugs, is a means of pharmacologically targeting the diminished prostacyclin synthesis that is characteristic of PAH.

Continuous intravenous infusion of prostacyclin (epoprostenol) was the first PAH-targeted treatment to receive regulatory approval and remains the preferred treatment for those who are severely impaired [5]. However, the clinical use of epoprostenol is challenging due to its poor bioavailability and instability in blood, requiring complex and life-changing drug delivery systems. Accordingly, one of the most important priorities in PAH therapeutic research, particularly for patients with less-severe disease, has been identification of more chemically stable and bioavailable molecules that specifically target the IP receptor.

Currently approved oral prostacyclin therapies (selexipag and treprostinil) have short half-lives (6.5–13 h for active metabolite of selexipag; 4.5 h for treprostinil), leading to relatively large fluctuations between peak and trough plasma concentrations. These fluctuations stand in contrast to the continuous therapeutic plasma levels and 24-h IP receptor engagement achieved with continuous *i.v.* infusion of epoprostenol.

Ralinepag is a next-generation, orally available, non-prostanoid, selective and potent IP agonist which has been studied in human pulmonary tissues [6]. *In vitro* studies indicate that ralinepag has high binding affinity and selectivity at the human IP receptor [7]. Two earlier phase 1 studies demonstrated a favourable safety and tolerability profile for single (SAD) and multiple ascending doses (MAD) of immediate-release, orally administered ralinepag in healthy volunteers (Arena Pharmaceuticals, San Diego, CA, USA; data on file). Pharmacokinetic data from the SAD/MAD study suggests that the immediate-release formulation of ralinepag yields an effective half-life suitable for twice-daily dosing. The present study was designed to investigate the efficacy, safety and tolerability of twice-daily, orally administered ralinepag immediate-release capsules in adult patients with symptomatic PAH.

Methods

Patients and study design

This was a phase 2, multicentre, international, double-blind, placebo-controlled, randomised trial of 22 weeks' duration. Written informed consent was obtained from all study subjects, and protocols were approved by the institutional review board at each participating study site. Data collection and management were conducted by the study sponsor consistent with a predetermined statistical analysis plan.

The study population included male and female patients between the ages of 18 and 75 years diagnosed with symptomatic PAH of idiopathic or heritable origin, induced by drugs and toxins or associated with connective tissue disease, HIV infection or surgically repaired congenital systemic-pulmonary shunt. Patients were required to be on a stable dose of an oral disease-specific PAH therapy, with either an

Affiliations: [1]Pulmonary/Critical Care, UT Southwestern Medical Center, William P. Clements Jr University Hospital, Dallas, TX, USA. [2]Pulmonary and Critical Care, Boston Medical Center, Boston University, Boston, MA, USA. [3]Dept of Cardiology, Clinical Center of Serbia and Belgrade University School of Medicine, Belgrade, Serbia. [4]Cardiovascular Medicine, University of Michigan, Ann Arbor, MI, USA. [5]Arena Pharmaceuticals, San Diego, CA, USA. [6]Hessian Pharmaceuticals, Hayward, CA, USA. [7]Dept of Cardiology, Hospital Universitario 12 de Octubre, Complutense University, Madrid, Spain. [8]University of Texas, Houston, TX, USA. [9]Heart Transplant, St Vincent's Hospital, Sydney, Australia. [10]Division of Pulmonary and Critical Care Medicine, University of California, San Diego, CA, USA.

Correspondence: Lewis J. Rubin, Division of Pulmonary and Critical Care Medicine, 9300 Campus Point Drive, M/C7372, University of California, San Diego, La Jolla, CA 92037–1300, USA. E-mail: ljrubin@ucsd.edu

endothelin receptor antagonist (ERA) and/or a phosphodiesterase type 5 (PDE5) inhibitor or soluble guanylate cyclase stimulator, for ⩾3 months and had to remain on stable doses for the duration of the study. Eligible patients demonstrated World Health Organization (WHO)/New York Heart Association (NYHA) functional class II–IV symptoms and a 6-min walk distance (6MWD) of ⩾100 m and ⩽500 m. The study was conducted in accordance with the ethical principles originating from the Declaration of Helsinki and its amendments, consistent with good clinical practices and local regulatory requirements.

Study procedures

Eligible patients were randomised 2:1 to ralinepag or placebo. Randomisation was stratified by baseline WHO/NYHA functional class (II *versus* III or IV). All patients were instructed to take their assigned study drug with food.

Optimal dosing of IP agonists in PAH patients requires titration on an individualised basis up to a maximum tolerated dose in order to mitigate potentially severe side-effects (*e.g.* nausea, headache, flushing, lightheadedness). The present study consisted of a dose-titration period followed by a maintenance period. The dose-titration period occurred during the first 9 weeks of the study and was followed by a 13-week maintenance period. A follow-up visit was to occur ∼3 weeks after the end of the maintenance period (week 25) (figure 1). Over the course of the 9-week dose-titration period, dosages were up-titrated weekly according to each patient's tolerance, with a maximum daily dose of 600 µg (300 µg twice daily). The titration schedule was to begin relatively slowly with 10-µg·day^{-1} increases ending with 200-µg·day^{-1} increases during the last 2 weeks of the titration period (titration schedule total daily dose 20 µg, 30 µg, 40 µg, 60 µg, 80 µg, 120 µg, 160 µg, 200 µg, 400 µg, 600 µg). Dose reduction was permitted based on tolerability; however, the final dose attained during the titration period was required to be maintained during the 13-week treatment period prior to evaluation at week 22. Patients were instructed to continue the same dose and regimen of disease-specific PAH medications for the duration of the study.

During the study, the following clinical assessments of efficacy were performed: haemodynamic parameters by right heart catheterisation (RHC), 6MWD, assessment of clinical worsening, N-terminal prohormone of B-type natriuretic peptide (NT-proBNP) levels and WHO/NYHA functional class. Safety was assessed by evaluating adverse events, clinical safety laboratory tests (haematology, serum chemistry, urinalysis), vital signs, 12-lead ECGs and physical examinations. Samples for measurement of NT-proBNP were frozen, batched and sent to a central independent laboratory facility for analysis.

RHC was performed prior to study day 1 of the dose-titration period and at the end of the maintenance period (week 22), ∼4 h after the last dose of study drug. The 6MWD was conducted according to the modified guidelines issued by the American Thoracic Society without specific attention to timing after the prior dose of study drug. Investigators evaluated each patient for indications of clinical worsening throughout the study, based on the predefined criteria of death occurring ⩽14 days after study drug
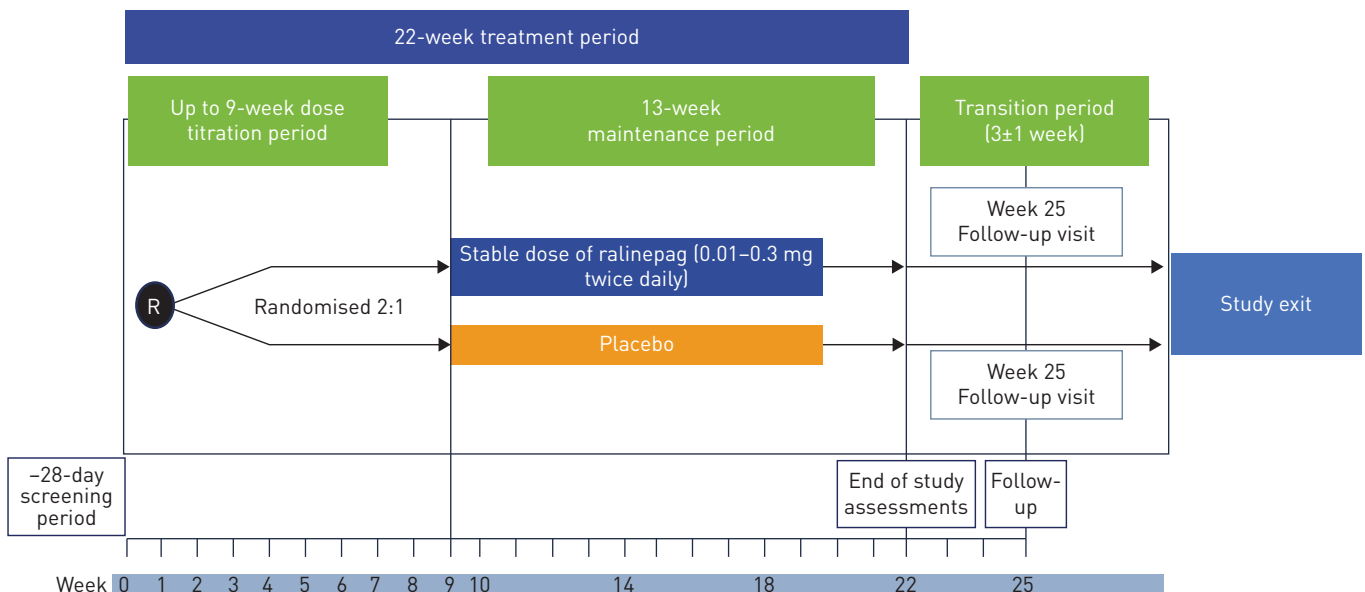

FIGURE 1 Study design.

discontinuation, hospitalisation for heart–lung or lung transplantion or atrial septostomy, addition or change in dose of PAH-specific medications, or the combined occurrence of ⩾20% decrease in 6MWD from baseline with worsening in WHO/NYHA functional class and worsening of signs of right heart failure that did not respond to optimised oral diuretic therapy.

Details related to any serious adverse events were collected for up to 30 days after the last dose of randomised study medication for patients that withdrew from the study or discontinued randomised, blinded study treatment.

Study end-points

The primary efficacy end-point was absolute change in PVR from baseline to week 22 with ralinepag compared with placebo. Secondary end-points included percentage change from baseline in PVR, 6MWD, time to clinical worsening, safety and tolerability. Exploratory end-points included changes in other haemodynamic parameters, changes in NT-proBNP and change in WHO/NYHA functional class.

Pharmacokinetic assessments were conducted from blood samples collected and processed for determination of ralinepag plasma concentrations during both the dose-titration and maintenance periods. Blood samples (~3 mL) were collected pre-dose and at 4 h post-dose at each study visit where dose escalation was planned during the dose-titration period and during each study visit within the maintenance treatment period.

Statistical analysis

The sample size estimation of 60 patients was based on the assumption of a between-treatment group difference of 350 dyn·s·cm^{-5} mean change in PVR from baseline to week 22. At this sample size, the study would be powered at 90% to detect a between-treatment group difference (assuming a 2:1 randomisation ratio) based on a two-sided t-test at the 5% significance level.

For PVR, assessments measured at week 22 or early withdrawal were included in the analysis.

Missing data at week 22 was imputed using a multiple imputation method. Lastly, to further assess the impact of missing data on the primary analyses, analyses without data imputation using the completers population was performed and compared with additional sensitivity analyses. Baseline assessments were not carried forward.

An analysis of covariance (ANCOVA) model with baseline PVR as a covariate and factors for treatment, baseline WHO/NYHA functional class (II *versus* III or IV) and baseline background PAH therapy (with ERA *versus* without ERA) was used to assess the effect of ralinepag *versus* placebo on change in PVR from baseline. Least-squares means for the treatments and the treatment effect and the least-squares mean difference between treatments was calculated with 95% confidence intervals and a two-sided p-value. The normality of the model residuals was assessed using the Shapiro–Wilk test. Continuous variables were summarised using number of observations (n), mean, standard deviation, median, minimum and maximum. Frequencies and percentages were reported for all categorical data. All analyses and tabulations were performed using SAS (version 9.3; SAS, Cary, NC, USA).

Ralinepag plasma concentrations were limited to pre-dose (C_{min}) and 4 h post-dose at designated study visits. Ralinepag plasma steady-state was determined by regressing C_{min} values over time and the resultant slope tested for its difference from zero.

Results

Patient disposition

61 patients were enrolled between January 2015 and June 2017 at 28 study centres in nine countries including the United States, Australia, Poland, Romania, Bulgaria, Serbia, Spain, Hungary and Czech Republic. Of the 61 patients, 52% had idiopathic PAH, 8% had heritable PAH, 7% had drug- or toxin-induced PAH and 33% had associated PAH. Patients were predominantly female (86%) with a mean age of 49.4 years and body weight of 73.2 kg at enrolment. The mean±SD baseline 6MWD was 378.4 ±103.9 m and mean±SD baseline PVR was 717.4±414.8 dyn·s·cm^{-5}. 40 patients were randomised to receive ralinepag and 21 to receive placebo (figure 2). Demographics and baseline characteristics of treatment groups are presented in table 1.

34 (85.0%) patients in the ralinepag group and 19 (90.5%) in the placebo group completed the study. PVR was imputed for six ralinepag and two placebo patients for the primary end-point.

The overall mean±SD duration of treatment exposure including dosing interruptions was comparable between the ralinepag *versus* placebo groups; 168.70±46.5 *versus* 180.1±13.8 days, respectively. Ralinepag total daily maintenance doses were titrated as high as 600 μg, with the most common dose being 400 μg.
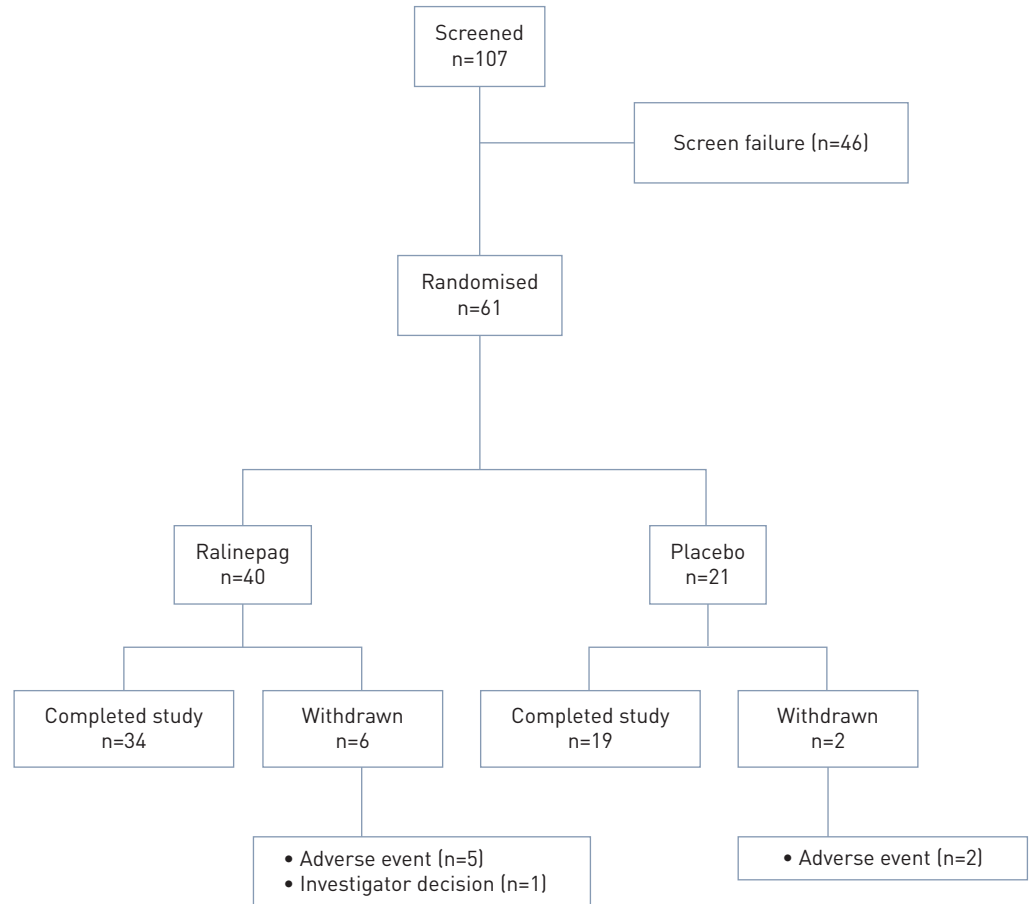
FIGURE 2 Patient disposition.

Efficacy

Following 22 weeks of treatment, a significant reduction in median PVR of 163.9 dyn·s·cm^{-5} was observed in the ralinepag-treated patients, compared with a median increase of 0.7 dyn·s·cm^{-5} observed with placebo (p=0.02; figure 3), meeting the primary end-point of the study. These results, which imputed values for missing final data, were corroborated by the completers analysis and other sensitivity analyses, in which a median reduction in PVR of 169.7 dyn·s·cm^{-5} for ralinepag and 3.1 dyn·s·cm^{-5} for placebo were observed. In the completers analysis, a statistically significant reduction from baseline in PVR by 19.8% was observed in the ralinepag patients (p<0.0001).

There was a 29.8% reduction in PVR (least-squares mean) compared with placebo (p=0.03), which included a 20.1% decrease from baseline for ralinepag-treated subjects.

At week 22, changes from baseline in 6MWD with ralinepag were not significantly different than placebo, with least-squares mean 6MWD increases of 36.2 m for ralinepag treated patients and 29.4 m for placebo (p=0.90).

The proportion of patients with clinical worsening was lower with ralinepag *versus* placebo (2.5% *versus* 9.5%), but the difference between the groups was not statistically significant (p=0.26). Notably, significantly fewer ralinepag- than placebo-treated patients deteriorated in WHO/NYHA functional class (2.5% *versus* 19.0%; p=0.03).

Exploratory analyses demonstrated that ralinepag treatment trended toward a reduction in mean NT-proBNP (−304.9 *versus* 35.4 pg·mL^{-1}; p=0.25) compared with placebo, but the treatment group differences were not statistically significant (figure 4).

Patients treated with ralinepag demonstrated significant reductions relative to placebo in mean pulmonary arterial pressure (PAP) (ralinepag −6.1 mmHg, placebo 2.9 mmHg; p=0.028), systemic vascular resistance (SVR) (ralinepag −258.7 dyn·s·cm^{-5}, placebo +152.9 dyn·s·cm^{-5}; p⩽0.001) and arterial blood pressure (ralinepag −8.2 mmHg, placebo −2.7 mmHg; p=0.001) (table 2). Cardiac index was higher with ralinepag compared with placebo, but the treatment effect was not statistically significant.

TABLE 1 Demographics and baseline characteristics

	All patients	Ralinepag	Placebo	p-value
Subjects n	61	40	21	
Age years[#]	49.4 [19–73]	46.2 [19–68]	55.6 [29–73]	0.0057[+]
Female	53 (87)	33 (83)	20 (95)	
Ethnicity				
Caucasian	57 (93)	38 (95)	19 (91)	
Other	4 (7)	2 (5)	2 (10)	
PVR dyn·s·cm^{-5} mean (median)	717 (576)	780 (705)	598 (480)	0.110[+]
6MWD m mean (median)	378 (400)	393 (405)	351 (367)	
WHO FC[#]				
II	34 (56)	22 (55)	12 (57)	0.800[§]
III	26 (43)	17 (43)	9 (43)	
IV	1 (2)	1 (3)	0	
Aetiology of PAH				
Idiopathic PAH	21 (52.5)	11 (52.4)	32 (52.5)	
Heritable PAH	4 (10.0)	1 (4.8)	5 (8.2)	
Drugs or toxins	4 (10.0)	0 (0.0)	4 (6.6)	
Associated PAH[¶]	11 (27.5)	9 (42.9)	20 (32.8)	
NT-proBNP pg·mL^{-1} mean (median)[#]	980 (343)	792 (335)	1362 (343)	0.700[+]
Background PAH therapy %				
Monotherapy	41	35	52	
Combination therapy	59	65	48	
Monotherapy				
ERA[#]	6 (10)	2 (5)	4 (19)	0.170[f]
PDE5i	19 (31)	12 (30)	7 (33)	
Combination therapy				
ERA+PDE5i	34 (56)	24 (60)	10 (48)	0.420[f]
ERA+sGCS	2 (3)	2 (5)	0 (0)	
New PAH treatment within 3–6 months of day 1	13 (21)	5 (13)	8 (38)	0.049[f]

Data are presented as n, mean (range) or n (%), unless otherwise stated. PVR: pulmonary vascular resistance; 6MWD: 6-min walk distance; WHO FC: World Health Organization functional class; PAH: pulmonary arterial hypertension; NT-proBNP: N-terminal prohormone of B-type natriuretic peptide; ERA: endothelin receptor antagonist; PDE5i: phosphodiesterase type 5 inhibitor; sGCS: soluble guanylate cyclase stimulator. [#]: numerical imbalances were detected between ralinepag *versus* placebo in PAH classification, ERA monotherapy, ERA+PDE5i combination therapy, age and mean NT-proBNP; [¶]: connective tissue disease or HIV infection or congenital systemic-pulmonary shunt (must have undergone surgical correction ⩾1 year prior to screening); [+]: p-value by Wilcoxon rank sum test; [§]: p-value by exact Chi-squared test; [f]: p-value by Fisher's exact test.

Safety and adverse events

Not unexpectedly, both SVR and systemic blood pressure decreased with ralinepag, but symptomatic hypotension was not observed. No other clinically meaningful changes in vital signs or laboratory tests from baseline to week 22 were observed. The most common reason for study withdrawal among both

FIGURE 3 Ralinepag significantly reduced pulmonary vascular resistance (PVR) compared with placebo. Within-group geometric mean ratio (GMR) percentage change: placebo −0.4%, ralinepag −26.1%. Intention-to-treat population (n=61) imputation of missing values. Median change used due to non-normal distribution, mean absolute change from baseline: placebo 25.5 dyn·s·cm^{-5}, ralinepag −175.7 dyn·s·cm^{-5}. The GMR of least-squares mean changes was 0.76, −25.8% change over compared placebo; p=0.02.
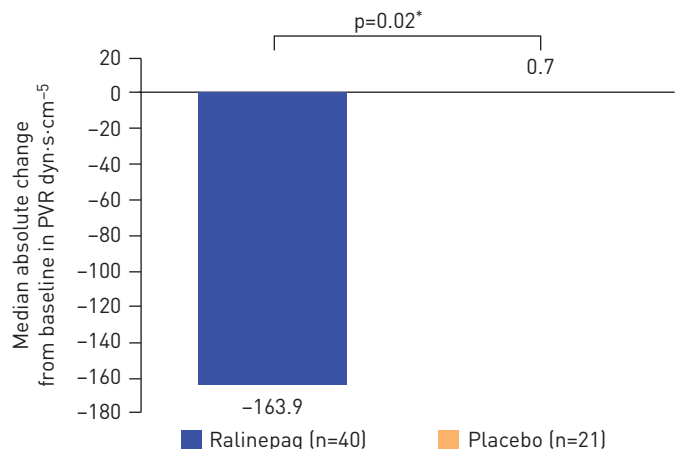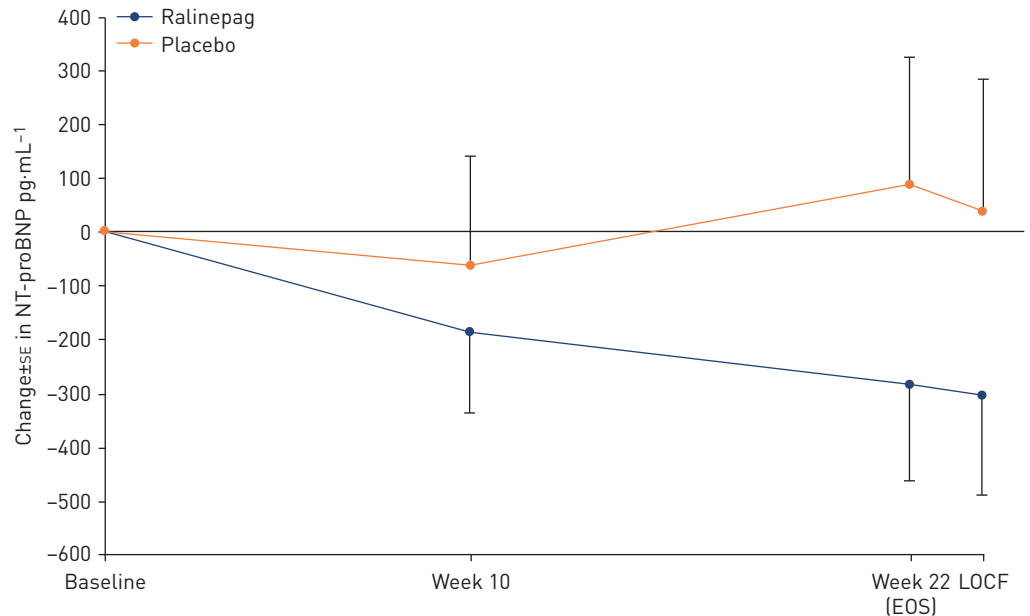
FIGURE 4 Change in N-terminal prohormone of B-type natriuretic peptide (NT-proBNP). EOS: end of study; LOCF: last observation carried forward.

groups was an adverse event. A summary of adverse events that occurred in ≥30% of patients is presented in table 3. All patients (100%) in the ralinepag-treated group and 90.5% patients in the placebo group had at least one adverse event. Overall, 78.7% of adverse events were considered related to study drug, 95.0% in the ralinepag group and 47.6% in placebo. Of the adverse events related to study treatment, 20% of adverse events in the ralinepag group and 28.6% in placebo patients were mild in severity (asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated) and 47.5% were considered moderate in severity (minimal, local or noninvasive intervention indicated) for ralinepag and 14.3% for placebo. Related events noted as severe (medically significant but not immediately life-threatening; hospitalisation or prolongation of hospitalisation indicated; disabling) occurred in 37.5% and 28.6% of ralinepag and placebo patients, respectively.

TABLE 2 Secondary haemodynamic parameters: change from baseline to week 22

	Baseline		Change from baseline to week 22[#]		p-value[¶]
	Ralinepag	Placebo	Ralinepag	Placebo	
Subjects n	34	19	34	19	
Mean pulmonary arterial pressure mmHg	51.0±15.26	43.1±10.08	−6.1±1.5	−2.9±2.0	0.028
Cardiac index L·min·m^{-2}	2.6±0.60	2.9±0.49	0.31±0.12	−0.0±0.16	0.183
Mixed venous oxygen saturation %	65.0±7.86	68.0±6.28	1.0±1.05	0.6±1.56	0.412
Right atrial pressure mmHg	8.1±6.14	8.7±5.06	−0.3±0.68	−2.0±0.89	0.118
Pulmonary capillary wedge pressure mmHg	9.3±2.93	10.5±3.17	−0.4±0.59	−0.3±0.77	0.119
SVR dyn·s·cm^{-5}	1449.0 ±378.53	1309.9 ±286.65	−258.7 ±286.54	152.9 ±374.47	<0.001
Mean arterial blood pressure mmHg	89.6±16.13	86.8±10.55	−8.2±1.83	−2.7±2.35	0.001
Heart rate bpm	77.1±13.61	71.6±7.87	0.9±1.92	−0.8±2.49	0.961

Data are presented as mean±SD, unless otherwise stated. SVR: systemic vascular resistance; bpm: beats per minute. [#]: least-squares mean±SEM change from baseline; [¶]: p-value based on a stratified Wilcoxon rank text: ANCOVA model with three factors (treatment, World Health Organization/New York Heart Association functional class, background pulmonary arterial hypertension therapy).

TABLE 3 Adverse events occurring in ⩾30% of patients in either the ralinepag or placebo groups during the 22-week study

	Ralinepag	Placebo
Subjects	40	21
Headache	78 (31)	29 (6)
Nausea	50 (20)	24 (5)
Diarrhoea	48 (19)	14 (3)
Pain in jaw	35 (14)	14 (3)
Flushing	33 (13)	5 (1)

Data are presented as n or % (n).

For patients treated with ralinepag, the most common adverse events reported during the study were headache (n=31, 78%), nausea (n=20, 50%) and diarrhoea (n=19, 48%).

When the adverse event data were analysed in 4-week periods (figure 5), a notable decline in adverse events was observed during the maintenance phase (weeks 10–22) relative to the dose titration phase (weeks 1–9). For example, the percentage of patients that experienced a headache was lower during weeks 10–14 of the maintenance phase (44.4%) relative to weeks 4–9 of the dose titration phase (75%) and continued to decline through the end of the study. Similar trends were observed for nausea and diarrhoea. For patients treated with placebo, the frequency of events was similar over the dose-titration period compared with the maintenance period.

Of the ralinepag-treated patients, five withdrew from the study due to adverse events determined to be at least possibly related to study drug by the investigator (myalgia, headache, nausea, pain of skin, eyelid oedema, exfoliative dermatitis, face oedema, decreased appetite and ECG QT prolongation). Fewer patients treated with ralinepag (n=4, 10%) experienced serious adverse events as compared to placebo (n=6, 28.6%). Serious adverse events in the placebo and ralinepag-treated groups were deemed unrelated to study drug by the study investigator with the exception of ECG QT prolongation in one ralinepag patient. This patient had evidence of an intraventricular conduction disturbance (IVCD) at baseline and throughout the trial as evidenced by intermittent incomplete right bundle branch block. In patients with IVCD, the duration of repolarisation is increased, thereby confounding interpretation of the impact of exogenous factors (e.g. study drug exposure). Two ex-placebo subjects had fatal adverse events: one subject died from hypovolaemic shock (~29 days after the last dose of randomised study drug (placebo)) and one subject had myocardial infarction and cardiorespiratory arrest (~7 days after the last dose of randomised study drug (placebo)).
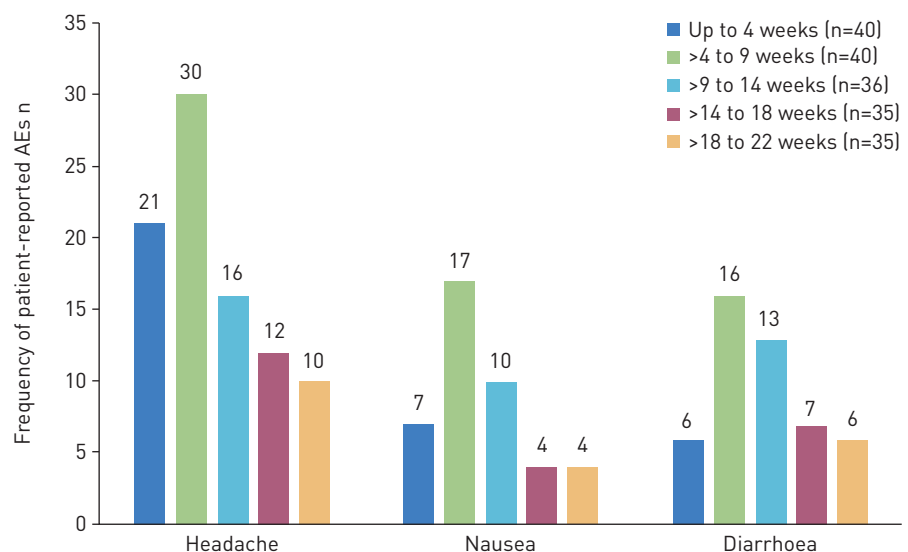

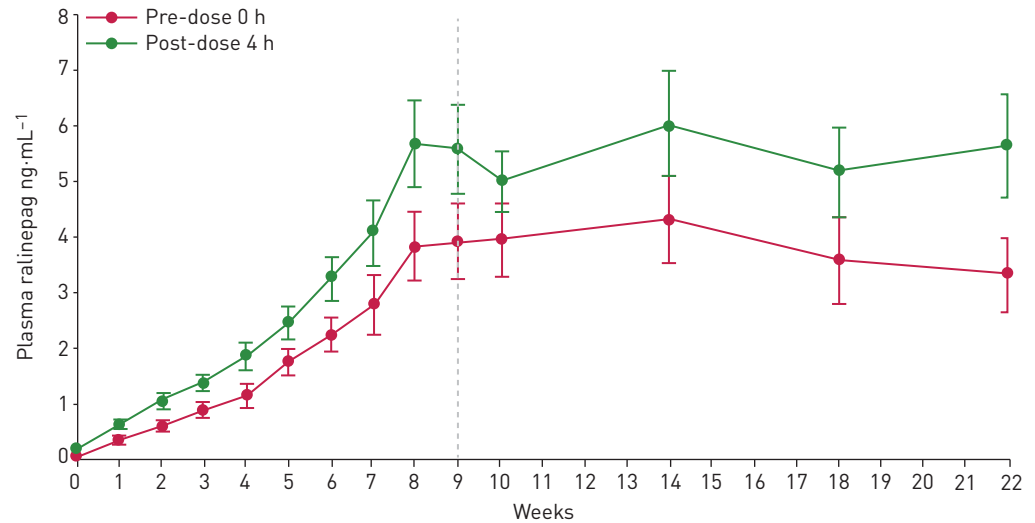FIGURE 5 Frequency of patient-reported adverse events (AEs) as a function of time on ralinepag.

FIGURE 6 Mean±SEM plasma ralinepag steady-state plasma concentrations at pre-dose (trough) and 4 h post-dose during the 9-week dose titration and subsequent 13-week maintenance periods of the study (22-week total dosing duration).

Pharmacokinetics

Ralinepag immediate-release capsules were administered for the complete study. In patients receiving ralinepag, the mean±SD total daily dose at week 22 (end of maintenance period) in this study was 259 ±168 µg. In general, mean ralinepag plasma concentrations at both evaluated time points increased during the dose-titration period and then reached approximate steady-state levels that were maintained throughout the maintenance period (figure 6). Overall, the mean steady-state week 22 plasma levels of ralinepag pre-dose and at 4 h post-dose were 3.23 ng·mL^{-1} and 5.60 ng·mL^{-1}, respectively. The ralinepag plasma levels reflected by a mean total daily dose of 259 µg appear similar (dose adjusted) to what was observed in the phase 1 study of healthy volunteers after twice-daily dosing. (SAD/MAD data on file).

Discussion

This study was designed to assess the efficacy, safety and tolerability of twice-daily ralinepag administered orally in a cohort of adult patients with PAH. Although some imbalances were observed between the treatment groups in several baseline measures, these are not surprising in light of the small sample size and 2:1 randomisation scheme. Imbalances were detected between ralinepag versus placebo in the mean duration of PAH, PAH classification, ERA monotherapy, ERA and PDE5 inhibitor combination therapy, age and mean NT-proBNP. While baseline PVR was higher in the ralinepag group, perhaps suggesting greater disease severity, baseline 6MWD was also higher and NT-ProBNP levels were lower in the ralinipag group; thus, no clear pattern of imbalance that might affect the interpretation of the results was identified. Similar findings were observed in the phase 2 trial of selexipag in PAH [8].

The study demonstrated a statistically significant absolute change in PVR, meeting the primary efficacy end-point. Expressed as percentage change relative to placebo, a 29.8% difference was observed, including a 20.1% decrease from baseline for the ralinepag-treated group. These reductions in PVR in the ralinepag group were observed despite patients already receiving either mono (35%) or dual (65%) combination PAH-specific background therapy. These findings are similar to those reported in the phase 2 study with selexipag [8], while oral treprostinil did not significantly reduce PVR in a single-centre, open-label study [9]. Additional signals of efficacy were also observed by assessing other haemodynamic parameters: ralinepag treatment led to greater mean reductions in both systolic and mean PAP when compared with placebo. While cardiac index and stroke volume index trended to increase with ralinepag compared with placebo, these treatment effects were not statistically significant; this result may have also been limited by the small sample size. Although SVR and blood pressure significantly decreased with ralinepag compared with placebo, there was no evidence of a significant compensatory increase in heart rate from baseline to week 22.

A statistically significant treatment effect in 6MWD was not observed (+36.2 m ralinepag, +29 m placebo; p=0.90). This result was not unexpected, since the study was not powered to detect a difference in 6MWD. However, the 29.0 m increase in the placebo group was an unusual result. Similar phase 2 studies of imatinib [10], treprostinil [11] and selexipag [8] also failed to observe a statistically significant treatment

effect in 6MWD relative to placebo. Notably, in each of these studies, the changes in 6MWD observed in the placebo group were relatively small (−1.0 m, +0.4 m, +4.8 m for imatinib, selexipag and treprostinil, respectively). A *post hoc* analysis that examined differences in timing of new treatments prior to study start revealed significantly more patients in the placebo group relative to the ralinepag group had started a new PAH-specific background therapy within 3–6 months of study start (placebo n=8, 38%; ralinepag n=5, 13%; p=0.045). In two previous studies utilising tadalafil [12] or riociguat [13], 6MWD continued to improve beyond week 12, suggesting the possibility that further improvement may be gained beyond 3 months after initiation of a new PAH-specific treatment

22 weeks of treatment with ralinepag was well tolerated by most patients in this study and treatment-related adverse events were generally consistent with the known safety profile of prostacyclin receptor agonists [14]. In order to optimise the benefit of treatment and prevent discontinuation due to intolerable adverse events, therapies targeting the prostacyclin pathway are initiated at low doses and up-titrated to a maximum tolerated maintenance dose [15]. In line with previous studies with prostacyclin analogues and IP receptor agonists, patients in the present study treated with ralinepag reported more frequent adverse events during the dose-titration phase from week 4 to week 9, when compared with the maintenance period after week 9, indicating improvements in tolerability over time. The design of the 9-week dose titration scheme starts with low doses, which are increased weekly in small increments until a total daily dose of 200 µg, divided equally in two doses, is reached. The following week required a doubling of the dose to 400 µg, followed by an increase to 600 µg in the final week. Dose-limiting adverse events triggered by aggressive titration may have caused patients to reach lower maximum tolerated doses than if a more gradual dose titration scheme was utilised.

Significantly fewer ralinepag- than placebo-treated patients deteriorated in WHO/NYHA functional class (one (2.5%) out of 40 *versus* four (19.0%) out of 21; p=0.02). The WHO/NYHA functional class is one of the most powerful predictors of survival for PAH, not only at diagnosis, but also during follow-up [15].

In the current study of ralinepag, NT-proBNP was chosen as an exploratory biomarker measure, since changes correlate with other predictors of survival in PAH patients [16, 17]. Although analyses demonstrated a greater mean reduction from baseline to week 22 with ralinepag *versus* placebo for NT-proBNP, the differences between treatment groups were not statistically significant (p=0.286 and p=0.253, respectively). Since this study was not powered to detect differences in this parameter, these results were not entirely unexpected.

Pharmacokinetic studies of ralinepag have demonstrated a long half-life of ∼24 h, resulting in minimal fluctuation between peak and trough plasma concentrations at steady-state (SAD/MAD data on file). The plasma levels achieved in this study both pre-dose and 4 h post-dose (3.23 ng·mL^{-1} and 5.60 ng·mL^{-1}, respectively) exceeded those that are associated with a pharmacological effect. The pharmacokinetic profile allows for twice-daily dosing of ralinepag, which may be advantageous to patients in terms of convenience and maintenance of the treatment effect throughout the dosing interval, and potentially minimising prostanoid-related adverse effects by reducing peaks in blood levels.

Our study has several limitations. The sample size was small and there were several imbalances at baseline between the two treatment arms. Additionally, the placebo group experienced a substantial increase in 6MWD, which is unusual and unexplained. Finally, while the PVR was significantly decreased with ralinepag (the primary end-point) as was PAP, changes in other parameters were not significantly changed. Accordingly, these results should be considered preliminary and interpreted with caution.

In conclusion, ralinepag, a next-generation, orally available, non-prostanoid selective prostacyclin receptor agonist significantly reduced PVR compared with placebo in patients with moderately symptomatic PAH. This effect was observed in subjects who were taking either monotherapy or combination therapy at the time of enrolment. Additional studies, including larger and more long-term clinical trials, are needed to confirm these findings.

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