

Safety, tolerability, and pharmacokinetics of the selective prostacyclin receptor agonist ralinepag in single and multiple dosing studies of an immediate-release oral formulation in healthy volunteers

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Abstract

Ralinepag (APD811), an oral, potent, and selective prostacyclin receptor (IP) agonist is being developed for treatment of pulmonary arterial hypertension. Two, single-center, randomized, double-blind, placebo-controlled, Phase I studies (single ascending dose and multiple ascending dose) evaluated an oral immediate-release capsule formulation of ralinepag in healthy subjects. Blood samples assessed plasma pharmacokinetics and safety and tolerability data monitored adverse events, vital signs, laboratory findings, physical examination, and electrocardiograms. Eighty-two healthy subjects (single ascending dose ($n = 32$) and multiple ascending dose ($n = 50$)) completed the studies. No clinically significant safety issues were observed, except one serious adverse event of atrial fibrillation considered moderate in intensity. In the single ascending dose study, ralinepag was tolerated up to 100 μg (single dose), but not 200 μg due to nausea and vomiting. Dose proportional mean ralinepag plasma exposure measures were observed. Maximum plasma concentrations were reached within 1.0–1.5 h post-dose and mean terminal elimination half-life values from 20.5–26.4 h. In the multiple ascending dose study, ralinepag tolerability decreased with increasing QD or BID dose. Dose proportional steady-state plasma exposure measures were observed where evaluable, with mean steady-state peak-to-trough ratios ranging from 3.34–4.49 (QD dosing) and 1.95–2.36 (BID dosing). Mean effective half-life values ranged from 17.5–18.4 h, reflecting ~ 1.7 -fold (QD dosing) and ~ 2.6 -fold (BID dosing) accumulation in plasma exposure. Safety and tolerability of oral immediate-release ralinepag was generally consistent with expectations for this drug class, but more individualized dose escalation appears warranted. Ralinepag exhibited favorable pharmacokinetic properties, with BID dosing producing desired minimal steady-state peak-to-trough fluctuation. Overall, results supported further clinical investigation of ralinepag and guided development of an extended-release formulation to facilitate QD dosing.

Keywords

pulmonary arterial hypertension, single ascending dose, multiple ascending dose

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Introduction

Pulmonary arterial hypertension (PAH) is characterized by vascular remodeling of the small pulmonary arteries with associated elevation of pulmonary artery pressure and pulmonary vascular resistance, leading to right ventricular failure and death.

Despite advancements in pharmacotherapy, PAH remains a devastating disease with patients continuing to

have high morbidity and a short median survival of only seven years.^{1,2} Currently, there are 14 medications approved by the Food and Drug Administration (FDA) for the treatment of PAH. These medications include endothelin

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receptor antagonists, phosphodiesterase type 5 inhibitors, soluble guanylate cyclase stimulators, and drugs targeting the prostacyclin receptors.

Prostacyclin is a known potent vasodilator with anti-proliferative, anti-thrombotic, and anti-inflammatory effects.³ Prostacyclin acts primarily via activation of the prostacyclin receptor (IP), leading to stimulation of adenylate cyclase, and resultant increases in intracellular cyclic adenosine monophosphate (cAMP) levels in platelets, smooth muscle cells, and immune cells. In PAH, the beneficial effects of prostacyclin in pulmonary arteries include relaxation of vascular smooth muscle cells and the inhibition of platelet aggregation/thrombosis, cell proliferation, and inflammation.

At present, parenterally administered prostacyclin (epoprostenol) and its synthetic analogues (iloprost and treprostinil) are considered the most effective FDA-approved class of drugs for PAH. These therapies require dosing by “up-titration” in order to: (i) allow the most clinically appropriate dose for each individual patient due to variability in efficacious doses between PAH patients; and (ii) allow patients to initiate therapy at a low dose that is then gradually increased, to better manage side effects associated with activation of the prostacyclin pathway and enhance patient tolerability.⁴ Dose-limiting side effects may include headache, flushing, diarrhea, nausea, and jaw pain. Parenteral modes of administration for this drug class include intravenous (IV; epoprostenol and treprostinil), subcutaneous (SC; treprostinil), and inhaled (INH; iloprost and treprostinil). Parenteral administration is required for these prostacyclin therapies, except for treprostinil, due to their low oral bioavailability. Furthermore, all parenterally administered prostacyclin therapies have very short half-lives (ranging from < 6 to 85 min) requiring continuous IV/SC infusion or frequent INH administration, in order to maintain adequate IP receptor coverage and clinical efficacy and to help avoid the risk of rebound pulmonary constriction.⁴ The relatively flat steady-state pharmacokinetic (PK) profile produced from continuous IV/SC infusion (i.e. no or minimal peak-to-trough fluctuation, with a peak-to-trough plasma concentration ratio (PTR) of ~1.0) is difficult to achieve with INH administration, or with currently available orally administered prostanoid and non-prostanoid analogues as indicated below. That is, for INH and oral dosing, there are practical limitations in the frequency of administration and resultant patient compliance considerations.

The first orally active and chemically stable prostacyclin analogues were beraprost (administered four times daily; half-life of 45 min) and treprostinil (administered two or three times daily; half-life of 3–4 h).^{4–6} Like parenteral therapy, oral prostacyclin therapy also needs to be initiated at low doses, and up-titrated slowly, to optimize treatment and prevent discontinuation, until a maximum tolerated maintenance dose is achieved.^{4,7} The efficacy limitations and side effect profiles of currently available oral prostacyclin

therapies have been well characterized and described.⁸ Of note, the frequent failure to achieve desired efficacy, and inability to tolerate oral prostacyclin therapy at higher doses due to prohibitive side effects such as flushing, headache, nausea, diarrhea, and weight loss, has limited their clinical utility. Some of these observations may be related, at least in part, to the relatively high steady-state PTR of these oral agents compared to something more clinically ideal (i.e. PTR ~1.0–2.0). For example, BID and TID dosing of oral treprostinil produces reported steady-state PTR values of 7.0 and 2.5, respectively.⁶

Selexipag, a non-prostanoid analogue and selective prostacyclin receptor (IP) agonist, received FDA approval in 2015 for the treatment of PAH and is dosed orally twice daily (BID) beginning at 200 µg BID and slowly up-titrated at weekly intervals in 200 µg BID dose increments to its highest tolerated dose of up to 1600 µg BID.⁹ Selexipag needs to be extensively metabolized (activated) to its much more potent active metabolite ACT-333679 (also known as MRE-269). This active metabolite is a weak partial agonist of the IP receptor¹⁰ and has a reported terminal elimination half-life of 6.2–13.5 h. While such a terminal elimination half-life might suggest that selexipag BID dosing is adequate to produce clinically desirable minimal peak-to-trough fluctuation, the effective half-life (EHL) of the active metabolite is only 3–4 h.⁹

For active moieties with multi-exponential disposition (including ACT-333679), the terminal elimination half-life can be a poor predictor of systemic accumulation and peak-to-trough fluctuation as it may only well describe the rate of compound loss across little or none of the drug dosing interval.¹¹ The EHL¹² is a much better metric as it describes the rate of compound loss across the entire dosing interval and can be used as a more clinically relevant measure to help determine and optimize the drug dosing frequency,¹¹ particularly for drugs in which sustained maintenance of therapeutic plasma levels is required or highly desired. Dosing a drug more frequently than its EHL (or that of its main active moiety) results in greater plasma accumulation at steady-state and a flatter steady-state plasma concentration–time curve. In the case of selexipag, this compound is dosed BID (approximately every 12 h) but the EHL of its active metabolite is much shorter; hence, minimal plasma accumulation at steady-state and relatively high peak-trough plasma fluctuation of the active metabolite would be expected. Indeed, upon BID oral administration of selexipag, only minimal accumulation (1.02–1.27 fold) of the active metabolite at steady-state occurs,¹³ and the mean steady-state PTR ranges from approximately 5.6–6.5.¹⁴ Thus, BID dosing of selexipag could potentially provide sub-optimal IP receptor coverage for some period of time during each dosing interval at steady-state. Furthermore, there is significant evidence that selexipag and all other currently available oral prostacyclin treatments frequently fail to reach the desired goals of therapy, including achieving clinically meaningful walk distances in PAH

patients.⁸ Hence, an orally administered IP agonist demonstrating more favorable PK characteristics, greater IP receptor potency and sustained coverage, and enhanced clinical efficacy and tolerability, is still needed.

Ralinepag (APD811) is an oral, potent, and selective non-prostanoid IP receptor agonist that requires no metabolic activation and is being developed for treatment of PAH. In nonclinical testing, ralinepag demonstrated dose-dependent oral efficacy in a rat monocrotaline model of PAH, moderate to high oral bioavailability across evaluated animal species (mouse, rat, dog, and monkey), high plasma protein binding (~99%) in animals and humans, and relatively long mean terminal half-life values across animal species ranging from 5.5 to 39 h.¹⁵ Enterohepatic recirculation of ralinepag was also observed in animals that may further contribute to its prolonged systemic exposure.¹⁵

The aims of the present single ascending dose (SAD) and multiple ascending dose (MAD) studies were to evaluate the safety, tolerability, and PK of single and multiple ascending oral doses of a ralinepag immediate-release (IR) capsule formulation in healthy human volunteers. The findings from these two studies were used to support further clinical development of the compound, determine the optimal frequency of administration of the ralinepag IR capsule formulation in Phase 2 clinical testing, and help optimize the up-titration dosing schedule to potentially enhance tolerability. The findings also helped assess the need for, and facilitated later development of, a ralinepag extended-release (XR) tablet formulation.

Methods

Study subjects

Male and female subjects aged 18–45 years, weighing between 50 and 100 kg were eligible for these studies if considered by the investigator to be in good health with unremarkable current and past medical history before the first day of the study. Screening examinations took place within 21 days of first study dosing.

Subjects were required to have no clinically significant abnormalities in pre-study physical examination, vital signs, 12-lead electrocardiogram (ECG), and laboratory evaluations. Individuals with findings outside of the normal range were included in the study only if these findings were deemed not clinically significant in the opinion of the investigator.

Exclusion criteria included any comorbid disease, allergy, or sensitivity judged by the investigator to be clinically significant. Subjects had to refrain from any supplements containing niacin for a period beginning seven days prior to study dosing, and from concomitant medications, other than over-the-counter analgesics or hormonal contraceptives, which were permitted during the month prior to the study screening visit. Subjects also had to agree to completely refrain from consuming alcohol, caffeinated

beverages, or tobacco during the in-clinic period. Women who were unwilling or unable to use an acceptable method to avoid pregnancy or who were pregnant or lactating during the conduct of the study and until one month after last study dose were excluded.

Both studies were 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. Written informed consent was obtained from all study subjects. The single dose escalation study was reviewed and approved by Aspire IRB, Independent Review Board and the MAD study was reviewed and approved by IntegReview, Independent Review Board Service.

Study designs

Two, randomized, double-blind, placebo-controlled, Phase 1 studies were conducted, and included SAD and MAD studies evaluating administration of an IR capsule formulation of ralinepag.

SAD study

The SAD study included four separate cohorts. Randomization 3:1 for ralinepag versus placebo was assigned by blinded study personnel. Each cohort consisted of six subjects receiving active treatment and two subjects receiving placebo. Ralinepag or placebo was dispensed in a double-blind fashion after an overnight fasting period in single, oral doses of 30, 50, 100, or 200 µg on the morning of Day 1. Subjects were instructed to not crush, break, chew, or dissolve the capsules; food was withheld for one hour after dosing. Ralinepag and placebo IR capsules had the same appearance and were provided as 10 and 100 µg strengths.

Subjects were followed as in-patients and safety parameters were monitored at multiple time points over seven days and included assessment of vital signs, 12-lead ECG, adverse events (AEs), and safety laboratory tests (serum chemistry, hematology, and urinalysis). Blood samples for plasma PK assessments were collected in K₂EDTA tubes at 0–45 min pre-dose and then post-dose at 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 16, 24, 36, 48, 72, 96, 120, and every 24 h thereafter through Day 14. All samples were immediately refrigerated via a cryoblock. Plasma fractions were separated by centrifugation and frozen at –20 °C until bioanalysis.

MAD study

The MAD study included three separate cohorts (Cohorts 1, 2, and 3). However, Cohorts 1 and 2 were identical with respect to number of subjects enrolled, treatments administered, and study procedures performed.

Cohorts 1 and 2 enrolled 15 subjects, with each cohort randomized (2:1) to receive an initial once daily (QD) dose of 50 µg ralinepag ($n=10$) or placebo ($n=5$) in the

fasted state. Cohorts 1 and 2 subjects received an initial QD dose for a period of five days. If the dose was determined to be well tolerated, it was then escalated to 100 μg (or corresponding placebo) on the sixth day and maintained until Day 10. Subsequent dose escalations in Cohorts 1 and 2 (200, 300, and 400 μg ralinepag, or corresponding placebo) occurred in the same manner every sixth day for up to three additional dose escalations, if tolerated, over the course of the 27-day study period. A dose could have been decreased or increased based on assessment of safety and tolerability. The final dose was maintained for a period of seven days. On the final day of dosing (Day 27), subjects were dosed with ralinepag or placebo and followed for a 48-h follow-up period, during which no study drug or placebo was administered. Subjects were domiciled for up to 31 days and safety measures (same as those in the SAD study, but with intensive ECG monitoring also added) were monitored at multiple time points over the course of the study (Fig. 1). Blood samples, collected in K_2EDTA tubes for plasma PK assessments, were collected for Cohorts 1 and 2 on Days 1 and 5 (dose level 1), 6 and 10 (dose level 2), 11 and 15 (dose level 3), 16 and 20 (dose level 4), and 21 and 27 (dose level 5) at pre-dose in the morning and at 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 16, and 24 h post-dose.

Cohort 3 included one group of 25 subjects, who were enrolled and randomized (4:1) to receive an initial 10 μg BID dose of ralinepag ($n=20$) or placebo ($n=5$) in the fasted state. BID dosing in Cohort 3 continued through five days and if tolerated, the next dose escalation (BID placebo or ralinepag 20 μg) occurred on the sixth day and was maintained through Day 10. Subsequent dose escalations in Cohort 3 (placebo or 30, 40, 50, and 70 μg BID ralinepag) occurred similarly every sixth day for up to four additional dose escalations, if tolerated. Cohort 3 subjects were dosed with ralinepag or placebo for up to 30 days followed by a 48-h follow-up period, during which no study drug or placebo was administered. Subjects were domiciled for up to 34

days and safety measures (as per Cohorts 1 and 2) were monitored at multiple time points over the course of the study (Fig. 2). Blood samples, collected in K_2EDTA tubes for plasma PK assessments, were collected on Days 1 and 5 (dose level 1), 6 and 10 (dose level 2), 11 and 15 (dose level 3), 16 and 20 (dose level 4), 21 and 27 (dose level 5), 26 and 30 (dose level 6), and at Day 32/exit. Blood samples for plasma PK analysis were collected pre-dose in the morning, and at 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 16, and 24 h post-dose.

Intensive ECG monitoring was conducted in all three cohorts to better understand possible effects of ralinepag on the QT interval. Continuous Holter 12-lead ECGs were recorded for each subject beginning 24 h pre-dose on Day 1. For Cohorts 1 and 2, ECG measurements were captured at 0.5, 1, 1.5, 2, 3, 4, 8, 12, 16, and 24 h post-dose on Days 1, 5, 6, 10, 11, 15, 16, 20, 21, and 27. For Cohort 3, ECG measurements were captured at 0.5, 1, 1.5, 2, 3, 4, 8, 12, 16, and 24 h post-dose on Days 25 and 30 only.

Bioanalysis

Plasma samples collected for ralinepag concentration measurement were analyzed by a validated liquid chromatography coupled to a tandem mass spectrometry (LC-MS/MS) assay (Covance, Madison, WI). Briefly, ralinepag was extracted from 0.1 mL plasma samples by a validated supported liquid extraction (SLE) method, and the resulting samples were evaporated under nitrogen. The residue was then reconstituted in 100 μL acetonitrile:water (25/75 v/v) and 20 μL was injected for LC-MS/MS analysis. The range of calibration was 0.03–15.0 ng/mL, with the lower limit of quantitation set as the lowest calibration standard.

PK and statistical analysis

Any subject receiving at least one full dose of the study drug was included in the PK analysis population. Plasma PK data

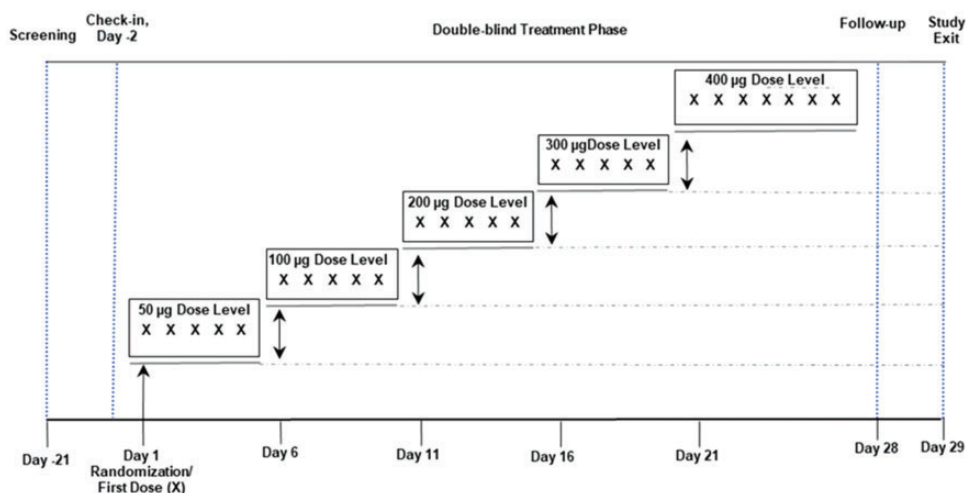


Fig. 1. Multiple ascending dose study. Dose escalation scheme (Cohorts 1 and 2).

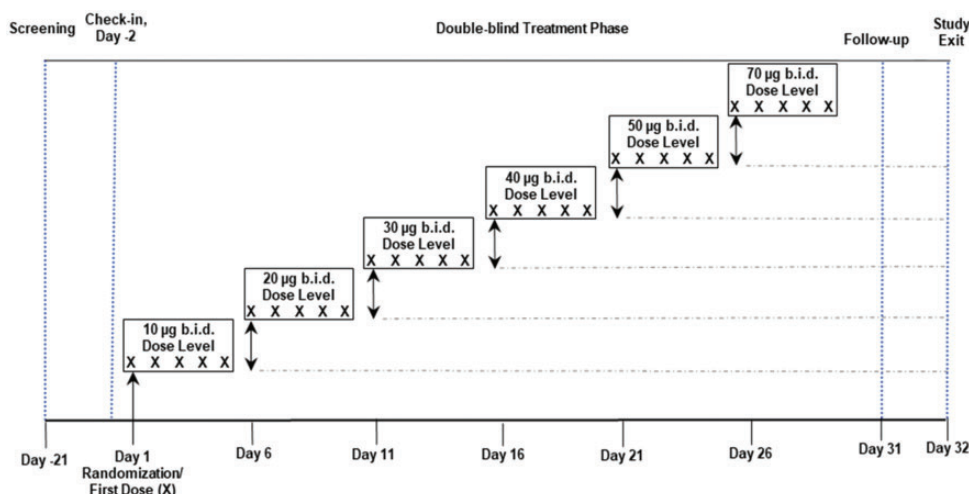


Fig. 2. Multiple ascending dose study. Dose escalation scheme (Cohort 3).

analysis was conducted using individual subject elapsed sampling times and noncompartmental methods with Phoenix WinNonlin® versions 6.3 or 8.1 (Pharsight Corp, Palo Alto, CA).

Derived plasma PK parameters included maximum drug plasma concentration after a single dose (C_{max}) or at steady-state ($C_{max,ss}$), time to maximum plasma concentration after a single dose (T_{max}) or at steady-state ($T_{max,ss}$), trough drug plasma concentration at steady-state ($C_{trough,ss}$), terminal elimination half-life after a single dose ($t_{1/2z}$), and area under the curve after a single dose (AUC_{last} , $AUC_{0-\tau}$, AUC_{inf}) or at steady-state ($AUC_{0-\tau,ss}$) which were calculated using the linear trapezoidal rule and where last is from time zero to time of last measurable concentration, tau (τ) is the dosing interval, and inf is from time zero to infinity. The PTR at steady-state in the MAD study was calculated for each evaluable subject from $C_{max,ss}$ and $C_{trough,ss}$ measures using the formula: $PTR = \frac{C_{max,ss}}{C_{trough,ss}}$.¹⁶ The accumulation ratio determined from C_{max} ($R_{ac(C_{max})}$) in the MAD study was calculated for each evaluable subject (50 µg dose level only at steady-state) using the formula: $R_{ac(C_{max})} = \frac{C_{max,ss}}{C_{max}}$. The accumulation ratio determined from AUC ($R_{ac(AUC)}$) in the MAD study was calculated for each evaluable subject (50 µg dose level only at steady-state) using the formula: $R_{ac(AUC)} = \frac{AUC_{0-\tau,ss}}{AUC_{0-\tau}}$. Finally, the EHL in the MAD study was calculated for each evaluable subject (50 µg dose level only at steady-state) using the formula:^{11,12}

$$EHL = \frac{\tau \times \ln 2}{\ln \left[\frac{R_{ac(AUC)}}{R_{ac(AUC)} - 1} \right]}$$

Statistics for all continuous variables were analyzed descriptively by treatment group and included mean, standard deviation, median, minimum, and maximum values. Steady-state PK data from the MAD study are presented

for the last day of dosing at each dose level achieved for at least five consecutive days (unless otherwise noted).

Results

Demographics and baseline characteristics

Both Phase 1 studies were enrolled to completion, with all 32 enrolled subjects completing the SAD study and 26 (of 30) subjects in Cohorts 1 and 2 (QD dosing) and 24 (of 25) subjects in Cohort 3 (BID dosing) completing the MAD study.

In the SAD study, most subjects were male (62.5%) and mainly White (87.5%) or Black/African American (9.4%). The mean age of the subjects was 29 (range: 19–45) years. There were no notable differences in demographics or other baseline characteristics observed across the study cohorts.

In the MAD study, most subjects were male (69.1%) and mainly White (41.8%) or Black/African American (47.3%). The mean age of the subjects was 33 (range: 19–52) years; note: one enrolled subject receiving placebo was discovered to be 52 years of age, and therefore did not satisfy the inclusion criterion for age (18–45 years); therefore, he was subsequently withdrawn from the study for noncompliance with the age requirement. There were no notable differences in demographics or other baseline characteristics observed across the study cohorts.

Safety and tolerability

Most AEs reported across both Phase 1 studies were of mild-to-moderate intensity, with only one serious adverse event (SAE) reported. In total, 22 (69%) subjects in the SAD study and 51 (93%) subjects in the MAD study reported at least one treatment-emergent AE. Overall, the most frequently reported AEs were headache, nausea, jaw pain, and vomiting.

In the SAD study, ralinepag was well tolerated up to 100 µg as a single dose, but not at 200 µg due to AEs of nausea and vomiting. A summary of AEs occurring in more than one subject on active treatment in the SAD study is presented in Table 1. As the active treatment dose level increased, the proportion of subjects within the treatment groups reporting AEs also increased; with all subjects who received a ralinepag single dose of 100 and 200 µg reporting an AE. Furthermore, as the active treatment dose level increased, the intensity of the frequently reported AEs went from mild to moderate. The most frequently reported AEs with a 100-µg single dose were headache and jaw pain, but with a 200-µg single dose were vomiting, headache, and nausea. There were no clinically significant safety issues seen at any single dose level with regards to vital signs, ECGs, or safety laboratory tests.

In the MAD study, most subjects did not fully dose escalate for either QD (up to 400 µg) or BID (up to 70 µg) dosing, with tolerability decreasing with increasing dose. Most AEs reported by subjects receiving ralinepag in Cohorts 1, 2, and 3 were mild-to-moderate in intensity and were also considered by the investigator to be probably related to study drug. One subject on active treatment (50 µg QD) in Cohort 2 experienced an SAE of atrial fibrillation considered moderate in intensity and possibly related to study medication. After active treatment discontinuation, the SAE resolved the next day following treatment with concomitant medication. A summary of AEs occurring in more than one subject on active treatment in the MAD study is presented in Table 2.

The most frequently reported AEs in Cohorts 1 and 2 of the MAD study were headache, nausea, jaw pain, and vomiting. The most frequently reported AEs in Cohort 3 were headache, jaw pain, nausea, myalgia, arthralgia, and dizziness. In Cohorts 1 and 2, four subjects

(three receiving ralinepag; one receiving placebo) withdrew from the study due to AEs. In Cohort 3, only one subject (receiving ralinepag) withdrew from the study due to AEs. Active treatment in the MAD study appeared to be associated with a slight vasodilatory effect followed by modest reflex tachycardia. Centralized reading of 12-lead Holter ECG recordings and high-precision QT analysis indicated that active treatment in the QD and BID dose ranges evaluated were not likely to be associated with QT prolongation.

Pharmacokinetics

SAD study. Single dose mean plasma concentration–time profiles by ralinepag dose level are presented in Fig. 3. A summary of the single-dose plasma PK parameters sorted by ralinepag dose level is presented in Table 3.

The mean plasma concentration–time profiles of ralinepag appear multi-phasic, being most evident at the highest ralinepag dose level evaluated (200 µg), with an initial, somewhat more rapid decrease in plasma concentrations seen up to approximately 72–96 h post-dose followed by a more gradual decrease thereafter (terminal elimination phase). There is also evidence of a “multiple peaking phenomenon”,¹⁷ with a second minor peak at approximately four hours post-dose evident at higher dose levels. Mean peak (C_{max}) and total (AUC) plasma exposure measures of ralinepag appear approximately dose proportional from 30 to 200 µg. The median T_{max} for ralinepag was similar across doses and occurred between 1.0 and 1.5 h post-dose. The mean terminal elimination half-life ($t_{1/2z}$) of ralinepag was similar across doses, ranging from 20.5 to 26.4 h, but increased with increasing dose consistent with better characterization of the terminal elimination phase as bioanalytical assay sensitivity limitations improved.

Table 1. Summary of treatment emergent adverse events reported in more than one subject on active treatment in the single ascending dose study.

	Ralinepag					Total ralinepag
	Placebo	30 µg	50 µg	100 µg	200 µg	
<i>n</i>	8	6	6	6	6	24
Total (%) subjects with at least one AE	3 (37.5%)	2 (33.3%)	5 (83.3%)	6 (100%)	6 (100%)	19 (79.2%)
Number of AEs reported	4	2	11	17	21	51
Nausea	0	0	1 (16.7%)	2 (33.3%)	3 (50.0%)	6 (25.0%)
Vomiting	0	0	0	2 (33.3%)	6 (100%)	8 (33.3%)
Abdominal pain	0	0	1 (16.7%)	1 (16.7%)	1 (16.7%)	3 (12.5%)
Pain in jaw	0	0	2 (33.3%)	3 (50.0%)	1 (16.7%)	6 (25.0%)
Headache	3 (37.5%)	2 (33.3%)	5 (83.3%)	5 (83.3%)	5 (83.3%)	17 (70.8%)
Flushing	0	0	0	1 (16.7%)	1 (16.7%)	2 (8.3%)

AEs: adverse events.

Table 2. Summary of treatment-emergent adverse events reported in more than one subject on active treatment in the multiple ascending dose study.

	Placebo Cohorts 1 and 2 (n = 10)	Ralinepag Cohorts 1 and 2 (all dose levels) (n = 20)	Placebo Cohort 3 (n = 5)	Ralinepag Cohort 3 (all dose levels) (n = 20)
Total (%) subjects with at least one AE	9 (90.0%)	20 (100%)	3 (60.0%)	19 (95.0%)
Total (%) subjects with at least one SAE	0 (0%)	1 (5.0%)	0 (0%)	0 (0%)
Number of AEs reported	36	230	25	196
Palpitations	0	4 (20%)	0	5 (25%)
Ocular hyperemia	0	0	1 (20%)	3 (15%)
Nausea	2 (20%)	17 (85%)	0	9 (45%)
Vomiting	0	12 (60%)	0	3 (15%)
Constipation	4 (40%)	6 (30%)	1 (20%)	1 (5%)
Abdominal pain	1 (10%)	6 (30%)	1 (20%)	4 (20%)
Diarrhea	1 (10%)	5 (25%)	0	7 (35%)
Chapped lips	0	0	0	2 (10%)
Dry mouth	0	0	0	2 (10%)
Abdominal distention	2 (20%)	2 (10%)	0	0
Eructation	0	3 (15%)	0	0
Flatulence	0	2 (10%)	0	0
Application site dermatitis	3 (30%)	6 (30%)	0	0
Fatigue	2 (20%)	2 (10%)	0	3 (15%)
Asthenia	0	2 (10%)	0	0
Feeling hot	0	2 (10%)	0	2 (10%)
Chest pain	0	1 (5%)	0	1 (5%)
Non-cardiac chest pain	0	2 (10%)	0	0
Hordeolum	0	3 (15%)	0	0
Decreased appetite	2 (20%)	4 (20%)	0	1 (5.0%)
Pain in jaw	0	13 (65%)	1 (20%)	13 (65%)
Arthralgia	0	0	1 (20%)	9 (45%)
Myalgia	1 (10%)	6 (30%)	1 (20%)	9 (45%)
Back pain	0	1 (5%)	2 (40%)	2 (10%)
Muscle tightness	0	0	0	2 (10%)
Muscular weakness	0	1 (5%)	0	2 (10%)
Muscle tightness	0	0	0	2 (10%)
Pain in extremity	1 (10%)	3 (15%)	0	0
Headache	2 (20.0%)	19 (95%)	3 (60%)	17 (85%)
Dizziness	1 (10.0%)	5 (25%)	1 (20%)	9 (45%)
Paraesthesia	1 (10.0%)	1 (5%)	0	4 (20%)
Somnolence	2 (20.0%)	3 (15%)	1 (20%)	2 (10%)
Restless leg syndrome	0	1 (5%)	0	2 (10%)
Tremor	0	2 (10%)	0	1 (5.0%)
Chromaturia	0	0	0	2 (10%)
Oropharyngeal pain	0	2(10%)	1 (20%)	1 (5%)
Acne	0	0	0	2 (10%)
Dermatitis contact	1 (10%)	0	0	2 (10%)
Pruritus	0	1 (5.0%)	0	2 (10%)
Flushing	0	7(35%)	1 (20%)	3 (15%)

AEs: adverse events; SAE: serious adverse event.

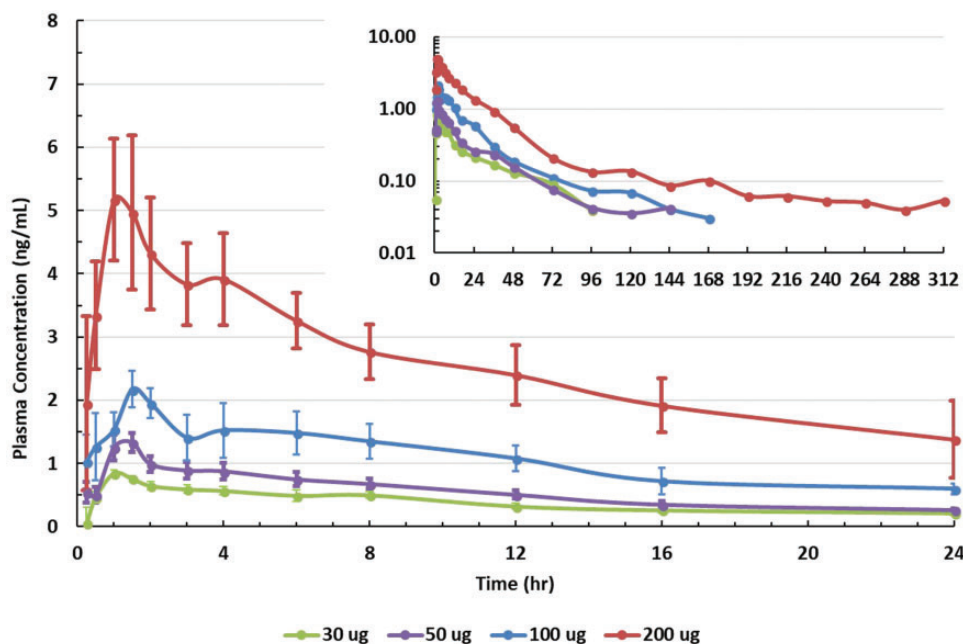


Fig. 3. Arithmetic mean (\pm SD) plasma concentration–time profiles for ralinepag following single oral dose administration of 30, 50, 100, and 200 μ g. Plasma samples were collected up to 312 h post-dose (inlet figure: semi-log plot with no SD shown for clarity), with the main figure (linear plot) showing results only up to 24 h post-dose.

Table 3. Summary of plasma pharmacokinetic parameters for ralinepag from the single ascending dose study.

PK parameter	Single dose			
	30 μ g (n = 6)	50 μ g (n = 6)	100 μ g (n = 6)	200 μ g (n = 6)
C_{max} (ng/mL)	0.949 (0.252)	1.49 (0.32)	2.65 (0.79)	6.17 (2.68)
T_{max} (h)	1.00 (0.500–8.00)	1.25 (0.500–1.50)	1.50 (0.500–6.00)	1.25 (0.25–4.00)
AUC_{last} (ng·h/mL)	15.9 (7.8)	21.5 (9.9)	41.2 (25.8)	101 (62)
AUC_{inf} (ng·h/mL)	17.3 (8.0)	22.8 (10.4)	42.6 (26.1)	103 (63)
$\tau_{1/2z}$ (h)	20.5 (5.6)	20.7 (8.6)	23.2 (5.8)	26.4 (22.9)

Results are presented as arithmetic mean (SD), except for T_{max} which is presented as median (min – max).

MAD study. Steady-state mean plasma concentration–time profiles by ralinepag dose level are presented in Fig. 4 (QD dosing; Cohorts 1 and 2 combined) and Fig. 5 (BID dosing; Cohort 3). Summaries of the multiple dose plasma PK parameters by ralinepag dose level are presented in Table 4 (QD dosing; Cohorts 1 and 2 combined) and Table 5 (BID dosing; Cohort 3). These figures and tables should be interpreted with some caution, given limited subject numbers particularly at higher dose levels.

For multiple QD oral administration of ralinepag (Cohorts 1 and 2 combined), steady-state plasma

concentration–time profiles of ralinepag showed moderate fluctuation over the 24-h dosing interval with steady-state peak-to-trough ratios ranging from 3.34–4.49. Steady-state peak ($C_{max,ss}$), trough ($C_{trough,ss}$), and total ($AUC_{0-\tau,ss}$) plasma exposure values for ralinepag increased in an approximately dose-proportional manner across the 50–300 μ g QD dose range (though just a single subject received 300 μ g QD), with the limited subject numbers for the lowest (30 μ g QD) and highest (400 μ g QD) evaluated dose levels precluding a determination across the full dose range. Median $T_{max,ss}$ values for ralinepag were similar across

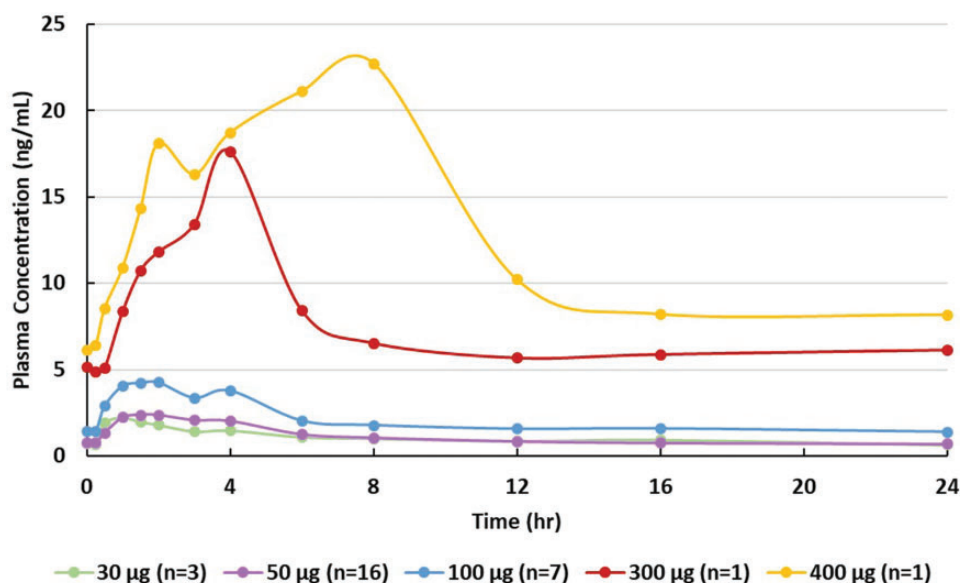


Fig. 4. Arithmetic mean steady-state plasma concentration–time profiles for ralinepag over a 24-h dosing interval for multiple QD dosing of 30, 50, 100, 300 (single subject only), and 400 (single subject only) µg in Cohorts 1 and 2 combined. For clarity and given only single evaluable subjects at the higher dose levels, no SD is shown. The 400 µg QD profile only reflects “near steady-state” conditions but is shown here for completeness, as PK data was only available from the first day (Day 21) of escalation to this dose level.

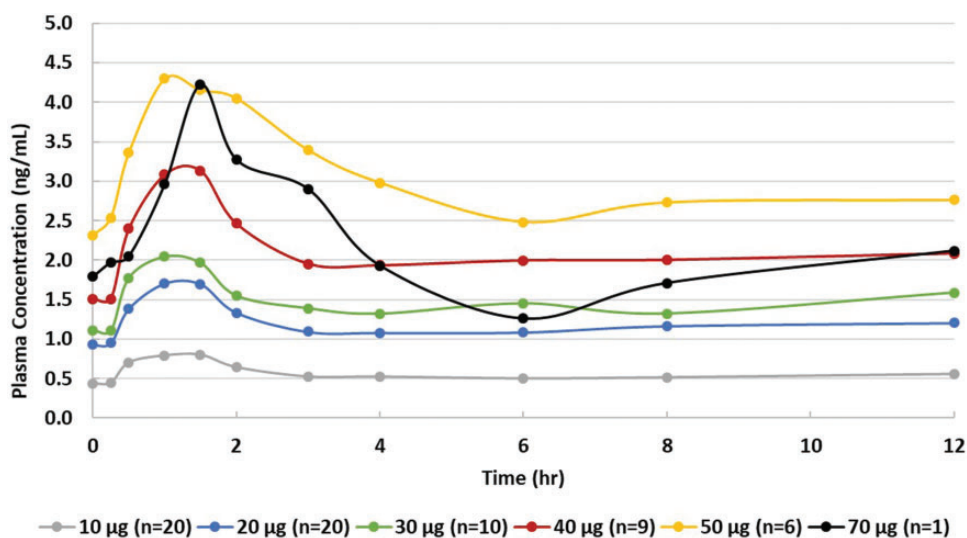


Fig. 5. Arithmetic mean steady-state plasma concentration–time profiles for ralinepag over a 12-h dosing interval for multiple BID dosing of 10, 20, 30, 40, 50, and 70 (single subject only) µg in Cohort 3. For clarity and given only a single evaluable subject at the highest dose level, no SD is shown.

doses (evaluable only up to the 100 µg QD dose level) and occurred between 1.0–1.5 h post-dose. Mean steady-state accumulation of ralinepag total plasma exposure (AUC) for 50 µg QD dosing was 1.69-fold, and the corresponding mean EHL was 18.4 h and less than the 24-h dosing interval.

For multiple BID oral administration of ralinepag (Cohort 3), steady-state plasma concentration–time profiles of ralinepag showed relatively low fluctuation over the 12-h dosing interval with steady-state peak-to-trough ratios

ranging from 1.95–2.36. Mean steady-state peak ($C_{\max,ss}$), trough ($C_{\text{trough},ss}$), and total ($AUC_{0-\tau,ss}$) plasma exposure values for ralinepag increased in an approximately dose proportional manner across the 10–50 µg BID dose range, with the availability of a single evaluable subject at the highest dose level (70 µg BID) precluding an assessment across the full dose range. The median $T_{\max,ss}$ for ralinepag was similar across doses (evaluable only up to the 50 µg BID dose level) and occurred between 1.0–1.25 h post-dose. Mean steady-state

Table 4. Summary of plasma pharmacokinetic parameters for ralinepag from the multiple ascending dose study with QD dosing (Cohorts 1 and 2 combined).

n	Dose ^a (µg)	T _{max,ss} ^b (h)	C _{max,ss} ^b (ng/mL)	AUC _{0-τ,ss} ^b (ng·h/mL)	C _{trough,ss} (ng/mL)	PTR	EHL ^c (r)	Accumulation ratio	
								R _{ac(Cmax)}	R _{ac(AUC)}
Study Day 1									
20	50	2.0 (0.50–4.0)	1.88 (0.47)	15.9 (5.9)	—				
Steady state									
3	30	1.0 (0.50–1.0)	2.29 (0.57)	24.8 (5.3)	0.72 (0.20)	3.34 (1.20)	—		
16	50	1.25 (0.50–4.0)	2.74 (0.69)	26.2 (9.2)	0.76 (0.37)	4.33 (2.12)	18.4 (2.3)	1.54 (0.31)	1.69 (0.24)
7	100	1.50 (0.25–2.0)	5.15 (1.48)	48.6 (19.8)	1.39 (0.68)	4.49 (2.66)	—		
1	300	4.0	17.6	180.7	5.14	3.42			
1	400 ^d	8.0	22.7	308	6.13	3.70			

Notes: Results are presented as arithmetic mean (SD), except T_{max} which is expressed as median (min – max). For dose levels in which there is only a single subject, the results presented are just for that subject.

^aThe dose levels shown were given QD.

^bT_{max}, C_{max}, and AUC_{0-τ} are presented for Study Day 1 (i.e. non-steady-state), and where tau (τ) is 24 h.

^cEHL is only determinable for the 50 µg QD dose regimen as based on R_{ac(AUC)}.

^dThe 400 µg QD PK parameter results reflect only “near steady-state” conditions but are provided for completeness, as PK data was only available in a single subject from the first day (Day 21) of escalation to this dose level.

PTR: peak-to-trough plasma concentration ratio; EHL: effective half-life.

Table 5. Summary of plasma pharmacokinetic parameters for ralinepag from the multiple ascending dose study with BID dosing (Cohort 3).

n	Dose ^a (µg)	T _{max,ss} ^b (h)	C _{max,ss} ^b (ng/mL)	AUC _{0-τ,ss} ^b (ng·h/mL)	C _{trough,ss} (ng/mL)	PTR	EHL ^c (h)	Accumulation ratio	
								R _{ac(Cmax)}	R _{ac(AUC)}
Study Day 1									
20	10	1.0 (0.50–12.0)	0.412 (0.129)	2.51 (0.89)	—				
Steady state									
20	10	1.0 (0.50–1.5)	0.860 (0.354)	6.68 (3.01)	0.436 (0.221)	2.04 (0.32)	17.5 (3.6)	2.07 (0.40)	2.64 (0.43)
20	20	1.0 (0.50–1.5)	1.80 (0.70)	14.3 (6.4)	0.928 (0.399)	1.97 (0.26)	—		
10	30	1.0 (0.5–12.0)	2.12 (0.90)	17.7 (7.7)	1.11 (0.53)	1.95 (0.22)			
9	40	1.0 (0.50–1.5)	3.31 (1.05)	25.5 (9.4)	1.51 (0.57)	2.28 (0.40)			
6	50	1.25 (1.0–2.0)	4.43 (1.07)	36.0 (8.9)	2.32 (0.78)	2.00 (0.43)			
1 ^d	70	1.5	4.22	25.2	1.79	2.36			

Notes: Results are presented as arithmetic mean (SD), except T_{max} which is expressed as median (min–max). For dose levels in which there is only a single subject, the results presented are just for that subject.

^aThe dose levels shown were given BID.

^bT_{max}, C_{max}, and AUC_{0-τ} are presented for Study Day 1 (i.e. non-steady-state), and where tau (τ) is 12 h.

^cEHL is only determinable for the 10 µg BID dose regimen as based on R_{ac(AUC)}.

^dA second subject also received ralinepag 70 µg BID treatment, but the subject’s PK results at this dose level were deemed anomalous (i.e. being inconsistent with the subject’s PK results at lower dose levels) and excluded from this table.

PTR: peak-to-trough plasma concentration ratio; EHL: effective half-life.

accumulation of ralinepag total plasma exposure (AUC) for 10 µg BID dosing was 2.64-fold, and the corresponding mean EHL was 17.5 h and greater than the 12-h dosing interval.

Discussion

The present SAD and MAD studies characterized the safety, tolerability, and PK of ralinepag administered as an IR formulation in healthy subjects. Across both studies, there were no clinically significant safety issues regarding vital signs, ECGs, or safety laboratory tests, except for one SAE. In the SAD study, single doses only up to 100 µg were tolerated; whereas in the MAD study, ralinepag tolerability decreased with increasing dose for both multiple QD and BID dosing regimens. These observations showed the importance of dose escalation (up-titration) to enhance the tolerability of ralinepag in healthy subjects. Ralinepag also exhibited favorable PK properties after single and multiple dosing of the IR formulation, with the BID multiple dosing regimen producing desirable minimal steady-state peak-to-trough fluctuation.

For drugs requiring dose titration to help overcome tolerability limitations and reach maximal efficacy, and repeat dose administration to maintain efficacy, selecting an appropriate dose titration schedule and dosing interval are important and critical clinical decisions. Furthermore, for orally administered drugs, it is desirable to select an oral formulation that possesses release characteristics suited to the drugs clinical PK properties and the target optimal steady-state PK profile. These decisions are especially important for the orally administered prostacyclin receptor (IP) agonist class of compounds in which up-titration is required to improve tolerability and maximize efficacy, and adequate receptor coverage throughout the dosing interval is needed to maintain efficacy and is best achieved with a relatively flat steady-state PK profile.

In the SAD study, no SAEs were recorded at any doses tested and the AEs of severe intensity were limited to the 200 µg group. In the MAD study, only a single SAE was observed involving a subject with atrial fibrillation considered moderate in intensity and possibly related to study medication. This SAE quickly resolved after active treatment discontinuation and treatment with concomitant medication. AEs were dose-related for both QD and BID regimens in the MAD study and showed a decrease in tolerability as dose levels increased. The tolerability and typically observed AEs (headache, nausea, jaw pain, vomiting, etc.) of orally administered ralinepag in healthy subjects across both studies were generally consistent with expectations for this drug class.⁴

Most MAD study subjects did not fully dose escalate as planned with either QD or BID dosing as the tolerability of ralinepag varied between individual subjects. While such variable tolerability has been previously described for prostacyclin analogues in other clinical trial settings and in

clinical practice,^{4,16,18,19} some reported Phase I clinical studies with selexipag were able to achieve full dose escalation in the majority (67–81%) of treated healthy male subjects when starting from 400 µg BID and escalating up to 1600–1800 µg BID.^{14,20} While individual subject tolerability differences involved in the different studies could explain, at least in part, these findings for ralinepag and selexipag, the apparent tolerability differences between the two compounds might also reflect that: (i) ralinepag has approximately 6–8-fold greater functional potency than that of the selexipag active metabolite ACT-333679;¹⁰ (ii) compared with the efficacy percentage of the full agonist iloprost (100% cAMP stimulation), the strong partial agonist ralinepag has a greater extent of prostacyclin (IP) receptor agonism versus the weak partial agonist ACT-333679 based on higher maximal cAMP stimulation (67% vs 48%, respectively);¹⁰ (iii) only ralinepag has an EHL that either approaches or exceeds the tested dosing intervals and thus better maintains receptor coverage (which may perhaps somewhat hinder tolerability during dose titration, but then help during maintenance dosing); and (iv) the dose escalation range for the ralinepag QD and BID dose regimens in the MAD study were 8-fold (50–400 µg) and 7-fold (10–70 µg), respectively, compared to just 4- to 4.5-fold for selexipag. What can be generally concluded from the ralinepag MAD study and the dose escalation schemes evaluated is that further optimized or individualized dose escalation appears warranted with ralinepag to achieve the most optimally efficacious and tolerated dose for the treatment of PAH patients in later stage clinical trials.

Based on evaluable PK assessments from both studies, ralinepag shows dose proportional plasma exposure with typically moderate inter-subject variability. The observed “double peaking”¹⁷ seen after single dosing, particularly evident at higher dose levels, suggests that ralinepag may undergo some enterohepatic recirculation in humans and as previously reported in animals.¹⁵ From the single-dose study, ralinepag was determined to have a long mean terminal elimination half-life (up to 26.4 h). From the multiple-dose study, the ralinepag QD and BID regimens provided steady-state peak-to-trough values ranging from 3.34–4.49 and 1.95–2.36, respectively, with the latter approaching the clinical ideal of ~1.0–2.0 and which reflects a relatively flat PK profile that is unique and considered highly favorable in comparison to that achieved with other oral agents in this drug class such as treprostinil and selexipag.

Based on the MAD study, the calculated mean EHL for ralinepag (IR formulation) ranged from 17.5–18.4 h; reflecting the approximately 1.7-fold and 2.6-fold accumulation in plasma AUC measures seen with QD (Q24 hours) and BID (Q12 hours) dose regimens, respectively. These results, along with corresponding steady-state peak-to-trough ratios, supported selection of the BID dosing regimen for Phase 2 testing of the ralinepag IR formulation in PAH patients, and demonstrate the utility of using the EHL to guide dosing interval decisions.¹¹

The EHL of a drug reflects not only its clearance properties but also the drug formulation release characteristics, whereas the terminal half-life is typically unaffected by the formulation. Hence, while IR and XR formulations of a drug can have the same terminal half-life, their effective half-lives will differ and be longer with the XR formulation. Based on this and the results from the present studies, further development work was required to better support ralinepag QD dosing for Phase 3 testing and to help improve PAH patient convenience and compliance. As the ralinepag IR formulation does not provide a sufficiently long EHL to optimally support QD dosing, development of a suitable ralinepag XR formulation was initiated to provide a treatment with an EHL exceeding 24h. This was ultimately achieved²¹ and will be more extensively detailed in a future publication.

The present SAD and MAD studies had some limitations. (i) They were each done at a single center and only involved a small number of healthy male and female subjects per cohort, with most subjects being Caucasian males, and the mean ages ranging from 29 to 33 (overall range: 19–52) years. Hence, the safety, tolerability, and PK results were not stratified for gender or age. As PAH is more common in middle-aged females,²² the tolerability and other characteristics of ralinepag will need to be carefully evaluated in this patient population in later-stage clinical trials. (ii) The duration of dosing in the MAD study was limited to 27 days, so as not to compromise the safety of the healthy subjects. (iii) As previously stated, most MAD study subjects did not fully dose escalate for either the QD or BID dosing regimens, which limited evaluation of safety, tolerability, and PK at the planned higher dose levels.

In summary, the safety and tolerability of an orally administered ralinepag IR formulation in healthy subjects were generally consistent with expectations for this drug class, though further optimized or individualized dose escalation is likely warranted. Ralinepag exhibited favorable PK properties, with BID dosing producing desired minimal steady-state peak-to-trough fluctuation. Overall, these promising results supported further clinical investigation of ralinepag BID dosing (IR formulation) in Phase 2 testing and helped guide subsequent development of an XR formulation to better facilitate QD dosing in Phase 3.

Ethical approval

The single dose escalation study was reviewed and approved by Aspire IRB, Independent Review Board and the multiple ascending dose study was reviewed and approved by IntegReview, Independent Review Board Service.

Author contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Conflict of interest

Authors are employees of Arena Pharmaceuticals.

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References

1. Preston IR, Roberts KE, Miller DP, et al. Effect of warfarin treatment on survival of patients with pulmonary arterial hypertension (PAH) in the registry to evaluate early and long-term PAH disease management (REVEAL). *Circulation* 2015; 132: 2403–2411.
2. Thenappan T, Shah SJ, Rich S, et al. Survival in pulmonary arterial hypertension: a reappraisal of the NIH risk stratification equation. *Eur Respir J* 2010; 35: 1079–1087.
3. Galić N, Manes A and Branzi A. The endothelin system in pulmonary arterial hypertension. *Cardiovasc Res* 2004; 61: 227–237.
4. Farber HW and Gin-Sing W. Practical considerations for therapies targeting the prostacyclin pathway. *Eur Respir Rev* 2016; 25: 418–430.
5. Galie N, Humbert M, Vachiery JL, et al. Effects of beraprost sodium, an oral prostacyclin analogue, in patients with pulmonary arterial hypertension: a randomized, double-blind, placebo-controlled trial. *J Am Coll Cardiol* 2002; 39: 1496–1502.
6. Orenitram (prescribing information). Research Triangle Park, NC, United Therapeutics Corp., 2014.
7. McLaughlin VV and Palevsky HI. Parenteral and inhaled prostanoid therapy in the treatment of pulmonary arterial hypertension. *Clin Chest Med* 2013; 34: 825–840.
8. Schilz R and Rich S. Calcium channel blocker therapy: when a drug works, it works. When it doesn't, it doesn't. *Adv Pulm Hypertens* 2017; 15: 184–189.
9. Upravi (prescribing information). San Francisco, CA, Actelion Pharmaceuticals US, Inc., 2017.
10. Adams J, Whittle B, Shen L, et al. Potency, selectivity, and comparative platelet and vascular activity of ralinepag acting on prostacyclin receptors in human tissues. In: *The 6th World Symposium on Pulmonary Hypertension (WSPH)*. 27 February–1 March 2018 Nice, France.
11. Gidal BE, Clark AM, Anders B, et al. The application of half-life in clinical decision making: comparison of the pharmacokinetics of extended-release topiramate (USL255) and immediate-release topiramate. *Epilepsy Res* 2017; 129: 26–32.
12. Boxenbaum H and Battle M. Effective half-life in clinical pharmacology. *J Clin Pharmacol* 1995; 35: 763–766.
13. Kaufmann P, Okubo K, Bruderer S, et al. Pharmacokinetics and tolerability of the novel oral prostacyclin IP receptor agonist selexipag. *Am J Cardiovasc Drugs* 2015; 15: 195–203.
14. Baldoni D, Bruderer S, Muhsen N, et al. Bioequivalence of different dose-strength tablets of selexipag, a selective prostacyclin receptor agonist, in a multiple-dose up-titration study. *Int J Clin Pharmacol Ther* 2015; 53: 788–798.
15. Tran TA, Kramer B, Shin YJ, et al. Discovery of 2-(((1r,4r)-4-(((4-Chlorophenyl)(phenyl)carbonyloxy)methyl)cyclohexyl)methoxy)acetate (Ralinepag): an orally active prostacyclin receptor agonist for the treatment of pulmonary arterial hypertension. *J Med Chem* 2017; 60: 913–927.
16. Mitchell JA, Ahmetaj-Shala B, Kirkby NS, et al. Role of prostacyclin in pulmonary hypertension. *Glob Cardiol Sci Pract* 2014; 2014: 382–393.

17. Davies NM, Takemoto JK, Brocks DR, et al. Multiple peaking phenomena in pharmacokinetic disposition. *Clin Pharmacokinet* 2010; 49: 351–377.
18. Barst RJ, Rich S, Widlitz A, et al. Clinical efficacy of sitaxsentan, an endothelin-A receptor antagonist, in patients with pulmonary arterial hypertension: open-label pilot study. *Chest* 2002; 121: 1860–1868.
19. Chin KM and Rubin LJ. Pulmonary arterial hypertension. *J Am Coll Cardiol* 2008; 51: 1527–1538.
20. Bruderer S, Hurst N, Kaufmann P, et al. Multiple-dose up-titration study to evaluate the safety, tolerability, pharmacokinetics, and pharmacodynamics of selexipag, an orally available selective prostacyclin receptor agonist, in healthy subjects. *Pharmacology* 2014; 94: 148–156.
21. Grundy J, Blackburn A, Parsley E, et al. Clinical pharmacokinetic (PK) performance of a ralinepag extended-release (XR) tablet. *Eur Resp J* 2018; 52: PA3037.
22. Frost AE, Badesch DB, Barst RJ, et al. The changing picture of patients with pulmonary arterial hypertension in the United States: how REVEAL differs from historic and non-US Contemporary Registries. *Chest* 2011; 139: 128–137.