A Randomized, Placebo-Controlled, Multiple-Ascending-Dose Study to Assess the Safety, Tolerability, Pharmacokinetics, and Pharmacodynamics of the Soluble Guanylate Cyclase Stimulator Praliciguat in Healthy Subjects

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Abstract

Nitric oxide (NO)-soluble guanylate cyclase (sGC)-cyclic guanosine monophosphate (cGMP) signaling is central to the regulation of several physiological processes, including blood flow and inflammation. Deficient NO signaling is implicated in multiple diseases. sGC stimulators are small molecules that enhance sGC activity, particularly in combination with NO. In a randomized, placebo-controlled phase 1 study, the safety, tolerability, pharmacokinetics, and pharmacodynamics of multiple ascending doses of the sGC stimulator praliciguat were assessed in 44 healthy adults. Four cohorts of 11 subjects (8 praliciguat, 3 placebo) received once-daily praliciguat for 14 days before up-titrating for 7 days (treatment sequences: 15/30 mg, 20/40 mg, 30/40 mg, and weight-based). All doses were tolerated. No serious or severe adverse events (AEs) were reported. The most common AEs in praliciguat recipients were headache and symptoms consistent with blood pressure (BP) lowering/vasodilation. There were no laboratory, vital sign, electrocardiographic, or platelet function findings indicative of a safety concern. Pharmacokinetics were dose proportional, with an effective half-life of 24–37 hours, supporting once-daily dosing. Praliciguat produced dose-related increases in plasma cGMP consistent with stimulation of sGC. Repeated once-daily dosing showed sustained decreases in BP. Results support evaluation of praliciguat for the treatment of conditions associated with deficient NO signaling.

Keywords

soluble guanylate cyclase, nitric oxide, cGMP, phase 1b, praliciguat, IW-1973, large volume of distribution
heart failure, peripheral vascular disease, and chronic kidney disease. Organic nitrates, which release NO and activate sGC, have been used for more than a century to treat cardiovascular conditions, but their effectiveness is limited by the development of tolerance. PDE5 inhibitors, which block the degradation of cGMP, are indicated for the treatment of pulmonary arterial hypertension (PAH) and erectile dysfunction but are limited to conditions in which the PDE5 enzyme is expressed and basal cGMP tone is sufficient.

The NO-sGC-cGMP signaling pathway can be selectively modulated by small-molecule agonists of sGC that bind directly to the enzyme and enhance its activity. These include sGC activators that act on the oxidized, heme-free form of sGC and sGC stimulators that act on the native, heme-intact form of sGC and enhance its sensitivity to endogenous NO. To date, the only marketed sGC agonist is the sGC stimulator riociguat, which is approved to treat PAH and chronic thromboembolic pulmonary hypertension. 

Riociguat has also been investigated in patients with pulmonary hypertension secondary to systolic left ventricular dysfunction and secondary to diastolic heart failure. The investigational sGC stimulator vericiguat has been studied in patients with heart failure with reduced ejection fraction and heart failure with preserved ejection fraction (HFpEF). Additional clinical studies in these patient populations (NCT02861534, NCT03547583) are underway. Clinical trials of riociguat are also ongoing in patients with scleroderma-associated digital ulcers (NCT02915835) and sickle cell disease (NCT02633397).

Praliciguat (also known as IW-1973) is a member of a new pyrazole-pyrimidine series of sGC stimulators. In vitro, praliciguat was shown to synergistically enhance NO-dependent cGMP production by purified sGC and to relax precontracted human subcutaneous resistance arteries ex vivo. In vivo, orally administered praliciguat dose-dependently reduced blood pressure in normotensive and hypertensive rats, inhibited heart and kidney damage in the Dahl salt-sensitive rat model of hypertension and heart failure, and attenuated hepatic steatosis, inflammation, and fibrosis in mouse models of liver disease.

In a phase 1 first-in-human study in healthy subjects, single oral doses of praliciguat (3 to 100 mg) in a polyethylene glycol solution formulation were tested. Doses up to 35 mg were tolerated. The most common adverse events (AEs) in praliciguat-treated subjects were headache, tachycardia, vomiting, dizziness/postural dizziness, hypotension, and nausea. Most occurred at the 45- and 100-mg doses and were of mild or moderate severity. No serious AEs (SAEs) were observed. The pharmacokinetics (PK) of a single dose of praliciguat when administered as an oral solution were consistent over the dose range tested and were characterized by linear dose-exposure, a large volume of distribution, negligible renal clearance (<0.01% of apparent total body clearance [CL/F]), and low to moderate intersubject variability. Pharmacodynamic (PD) effects included dose-related increases in heart rate and decreases in blood pressure, as well as dose-related increases in plasma cGMP levels and plasma renin activity (PRA). No effect on platelet function was observed.

This article describes a randomized, placebo-controlled phase 1 study to assess the safety, tolerability, PK profile, and PD effects of multiple ascending doses of a praliciguat oral tablet formulation in healthy subjects. The effect of dose titration was explored by administering a starting dose once daily for 14 days, followed by up-titration to a higher dose once daily for an additional 7 days.

Methods

The study protocol, amendment, and consent form were approved by the IntegReview Institutional Review Board (Austin, Texas). All enrolled subjects provided written informed consent before any study procedures were performed. The study was conducted in accordance with the Declaration of Helsinki and the principles of the Good Clinical Practice guidelines and is registered at www.clinicaltrials.gov as NCT02616861.

Study Design and Dosing

The study was conducted at a single phase 1 research center, ICON Early Phase Services in San Antonio, Texas, under the supervision of a single principal investigator and consisted of 2 stages: stage 1, an open-label, single-dose crossover study of fed and fasted dosing that is not described here; and stage 2, a randomized, double-blind, placebo-controlled, multiple-ascending-dose (MAD) study, that is described here.

For each MAD cohort, 11 subjects were randomly assigned to once-daily dosing with praliciguat (n = 8) or matching placebo (n = 3). Cohort 1 dose levels were selected based on the results of the phase 1 single-ascending-dose study in which doses up to 35 mg were tolerated. Dose levels for subsequent cohorts were based on reviews of safety and tolerability of preceding cohort(s). In cohorts 1 to 3, subjects randomized to praliciguat received a starting (lower) dose for 14 days followed by an up-titration (higher) dose for the next 7 days, for a total of 21 consecutive days of dosing: cohort 1, 15 to 30 mg; cohort 2, 30 to 40 mg; and cohort 3, 20 to 40 mg. In cohort 4, dosing was weight-based (WB): for the first 14 days, subjects randomized to praliciguat received the maximum of 25, 30, 35, or 40 mg, but not to exceed 0.5 mg/kg body weight at baseline (designated WB1); and for the next 7 days, subjects...
weighing <90 kg at baseline received 40 mg, whereas those weighing ≥90 kg received ≤0.5 mg/kg (WB2). An up-titration dosing regimen was used to allow acclimation to a given dose and was similar to dose-acclimation schemes used in trials of other sGC stimulators.

Subjects were in clinic from day -2 at check-in (2 days before the first dose) through day 23 (2 days after the final dose). Subjects returned to the clinic for a follow-up visit on day 28 (±1 day) and for an end-of-trial visit on day 42 (±3 days). Safety data were reviewed before up-titration in each cohort and between cohorts.

The praliciguat drug product (IW-1973 tablet) was a 5-mg oral tablet; multiple 5-mg tablets were administered for a given dose. Placebo tablets matched praliciguat tablets in appearance and were administered in matching numbers (eg, 6 placebo tablets to match the 30-mg praliciguat dose).

Study Population
Women (who were not pregnant or breastfeeding) and men who were 18 to 55 years old and provided written informed consent were eligible for the study if they met the following criteria: body mass index >18.5 and <30.0 kg/m²; in good general health with no clinically significant findings on physical examination, 12-lead electrocardiogram (ECG), and clinical laboratory tests; and negative hepatitis and human immunodeficiency virus panels. Female subjects agreed to the use of study-specific birth control, and all subjects agreed to refrain from making any major lifestyle changes during the study.

Potential subjects were excluded if they had any of the following: a clinically significant disorder/condition; a 12-lead ECG demonstrating heart rate <40 beats per minute (bpm) or average QT interval corrected using Fridericia’s formula (QTcF) > 450 milliseconds for men and >470 milliseconds for women; alanine aminotransferase, aspartate aminotransferase, or creatinine >1.25 × the upper limit of normal; had ever received praliciguat; had received an investigational drug in the 30 days or 5 half-lives of that investigational drug before the study; had donated blood products during the 6 weeks before check-in; received blood products during the 2 months before check-in; or had undergone a surgical procedure during the 30 days before check-in. The subject had to wash out before check-in as follows: any nicotine-containing products for 3 months; prescription medication(s), except topical, for 14 days; over-the-counter medication(s), except single-ingredient, acetaminophen, and anti-inflammatory, antipyretic, or antihistamine medications), alcohol- and caffeine-containing foods and beverages, vitamins, and herbal supplements for 7 days; and grapefruit/grapefruit juice for 72 hours.

Safety Evaluation
Safety assessments included periodic scheduled physical examinations, vital signs including orthostatic changes in blood pressure and heart rate, 12-lead ECGs, and clinical laboratory evaluations (ie, standard clinical serum chemistry [eg, electrolyte, hepatic, renal, and lipid panels], hematology [complete blood count], coagulation [activated partial thromboplastin time, prothrombin time, and international normalized ratio], and standard urinalysis). Subject- and investigator-reported AEs were recorded from the time of informed consent through completion of the study. For each AE, the Investigator provided an assessment of the severity (mild, moderate, or severe) and the causal relationship to study drug (related or unrelated). AEs were coded using Medical Dictionary for Regulatory Activities version 18.1. Blood samples for platelet function assessment (PFA) were collected at baseline, on day 15, and on day 21 for analysis in the point-of-care PFA-100 (Siemens Healthcare Diagnostics, Deerfield, Illinois) assessment, using the collagen/epinephrine platelet activator cartridges.

Pharmacokinetic Evaluation
Blood samples for PK analysis were collected on days 1, 14, 15, and 21 at predose and 0.25, 0.5, 1, 2, 4, 8, and 12 hours postdose; on days 2, 7, and 16 at predose; on days 22 and 23 at 24 and 48 hours after the final (day 21) dose; at follow-up (4-6 days after the final dose); and at the end of the trial (18-24 days after final dose). Samples were processed in a refrigerated (4°C) centrifuge within 15 minutes, and the resulting plasma was stored at less than or equal to −70°C within 1 hour of collection.

Quantification of praliciguat in potassium ethylenediaminetetraacetic acid (K₃EDTA) plasma was determined using a liquid chromatography-tandem mass spectrometry bioanalytical method, validated as a 2-range assay. Praliciguat was extracted using acetonitrile containing ¹³C-labeled praliciguat as the internal standard. High-performance liquid chromatography separation was conducted at 0.7 mL/min through a C8 column (Higgins Analytical, Inc., Mountain View, California) using a gradient of 0.1% formic acid in water and in acetonitrile. Compounds were detected using an API-5500 (Applied Biosystems/MDS SCIEX, Framingham, Massachusetts) in positive-ion mode using parent/product transitions of 535/109 m/z for praliciguat and 541/115 m/z for the internal standard. Peak area ratios were linear, using 1/x² weighting, over concentration ranges of 0.05 to 50 ng/mL (low range) and 1 to 1000 ng/mL (high range). The intra- and interassay variability ranges for the low range were 1.5%...
Pharmacodynamic Evaluation

Blood samples for exploratory assessment of plasma cGMP levels and PRA were collected at the same time, and plasma was isolated using the same procedure as samples collected for PK. Sodium citrate plasma cGMP samples were processed by protein precipitation extraction using cold 75:25 acetonitrile:methanol and separated by reverse-phase chromatography on a 50-mm, 1.8-µm guard column (Waters Corporation, Milford, Massachusetts) at 0.35 mL/min, with a mobile phase gradient of 0.1% acetic acid in water and 50:50 acetonitrile:methanol. Analytes were detected using an API-5500 with negative-ion electrospray ionization. Concentrations of cGMP were determined using the \(^{13}\)C\(^5\)N\(^1\)cGMP internal standard and \(^7\)\(^{13}\)C\(^3\)cGMP surrogate standards in plasma over a linear range of 0.1 to 50 ng/mL with parent/product transitions of 344/150 m/z, 359/160 m/z, and 347/153 m/z, respectively. Six lots of plasma were used to assess assay selectivity and specificity. Intra- and interassay variability ranges were 2.7% to 20.0% and 7.8% to 18.1% CV and −8.0% to 24.7% and −1.7% to 12.5% bias. Subsequent long-term testing of quality-control samples indicated poor frozen sample stability for this analyte in citrated plasma. Poor frozen sample stability for this analyte in citrated plasma relative to storage in plasma with K\(_2\)EDTA. PRA in K\(_2\)EDTA plasma was measured in terms of angiotensin-I (Ang-I) generation using ELISA kits from ALPCO (Salem, New Hampshire; catalog #11-RENHU-E02) according to the manufacturer’s instructions. Samples were buffered to approximately pH 6.0 in the presence of phenylmethylsulfonyl fluoride protease inhibitor, then equal volumes of each were incubated at 37°C or in an ice bath (0°C) for 90 to 180 minutes. Duplicate aliquots were transferred for capture on rabbit anti-Ang-I antibody-coated 96-well plates; the remaining sites were filled with Ang-I-biotin conjugate, streptavidin-horseradish peroxidase conjugate was captured, tetramethylbenzidine substrate in hydrogen peroxide was added, and the enzymatic oxidation was stopped with sulfuric acid after 10 to 15 minutes. Absorbance at 450 nm was measured using a SpectraMax M5 plate reader (Molecular Devices, San Jose, California). Ang-I concentrations were determined from a 4-parameter curve fit of 0.5 to 60 ng/mL calibrators, and the PRA in each sample was calculated using the following equation:

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PRA = \frac{\text{Mean Ang-I Concentration (37°C) − Mean Ang-I Concentration (0°C)}}{\text{Time (hr)}} \times 1.11
\]

Calibrator values were considered acceptable if within ±25% of their nominal concentration. At the lower limit of quantitation of 0.2 ng/mL, values were considered acceptable if within ±30% of their nominal concentration.

Ambulatory blood pressure monitoring (ABPM) for systolic blood pressure, diastolic blood pressure, mean arterial pressure (MAP), and pulse rate was conducted prior to the start of dosing (from day −1 [at the time corresponding to predose on day 1] to day 2 [ending at 24 hours postdose] and from day 7 predose to day 8 predose); during the transition from starting to up-titration dosing (from day 14 predose to day 16 predose); and at the end of up-titration dosing (from day 21 predose to day 22 at 24 hours after the final dose [day 21]). Pulse measurements were used to calculate heart rate as bpm. During these monitoring periods, assessments were obtained every 30 minutes.

Statistical Methods

The planned sample size of 11 subjects per cohort, randomized in a ratio of 8:3 praliciguat to placebo, was based on precedent set by prior phase 1 studies of a similar nature and design, outside of statistical considerations. All safety, PK, and PD analyses were conducted on data obtained either from the safety, PK, or PD populations, which consisted of all randomized participants who received ≥1 dose of the study drug (safety population), or on data from safety subjects with ≥1 postdose PK (PK population) or PD (PD population) parameter assessment, respectively. Data from all subjects who received placebo in different cohorts were pooled. Summary statistics were tabulated by treatment sequence for all safety, PK, and PD data. Noncompartmental PK analysis was conducted using R (version 3 or higher; Comprehensive R Network). Dose proportionality was explored for area under the plasma time-concentration curve during a dosing interval (AUC\(_{\text{tau}}\)) and maximum observed plasma concentration (C\(_{\text{max}}\)) after the first dose and after 14 once-daily doses using a regression model with log-transformed AUC\(_{\text{tau}}\) and C\(_{\text{max}}\) as the response variables and log-transformed
Table 1. Demographic and Baseline Characteristics

| Demographic and Baseline Characteristics | Placebo (n = 12) | 15/30 mg | 20/40 mg | 30/40 mg | WB1/WB2a |
|------------------------------------------|-----------------|---------|---------|---------|---------|
| Age, years                               | 39.9 (8.8)      | 34.5 (10.8) | 36.1 (10.9) | 45.4 (8.8) | 33.9 (5.7) |
| Minimum, maximum                         | (25, 48)        | (20, 48) | (20, 53) | (30, 53) | (24, 42) |
| Sex, n (%)                               |                 |         |         |         |         |
| Female                                   | 5 (41.7)        | 3 (37.5) | 4 (50.0) | 4 (50.0) | 2 (25.0) |
| Male                                     | 7 (58.3)        | 5 (62.5) | 4 (50.0) | 4 (50.0) | 6 (75.0) |
| Race, n (%)                              |                 |         |         |         |         |
| White                                    | 7 (58.3)        | 4 (50.0) | 5 (62.5) | 7 (87.5) | 4 (50.0) |
| Black/African American                   | 5 (41.7)        | 4 (50.0) | 2 (25.0) | 0        | 4 (50.0) |
| Asian                                    | 0               | 0       | 1 (12.5) | 1 (12.5) | 0        |
| Ethnicity, n (%)                         |                 |         |         |         |         |
| Hispanic/Latino                          | 4 (33.3)        | 4 (50.0) | 3 (37.5) | 5 (62.5) | 2 (25.0) |
| Not Hispanic/Latino                      | 8 (66.7)        | 4 (50.0) | 5 (62.5) | 3 (37.5) | 6 (75.0) |
| Weight (kg), mean (SD)                   | 75.5 (15.8)     | 78.4 (9.8) | 75.9 (9.0) | 74.6 (16.1) | 75.4 (15.8) |
| BMI (kg/m²), mean (SD)                   | 25.9 (2.9)      | 26.8 (2.2) | 26.3 (2.7) | 26.3 (2.7) | 25.5 (3.8) |
| Systolic BP (mm Hg), mean (SD)           | 117.0 (10.2)    | 112.1 (11.5) | 105.8 (5.2) | 112.6 (10.0) | 113.3 (10.8) |
| Diastolic BP (mm Hg), mean (SD)          | 74.4 (10.4)     | 65.8 (8.2) | 65.0 (6.5) | 69.0 (4.8) | 71.3 (3.0) |
| Heart rate (bpm), mean (SD)              | 70.7 (8.9)      | 63.8 (11.2) | 61.8 (10.7) | 76.1 (14.2) | 62.3 (8.2) |

BMI, body mass index; BP, blood pressure; SD, standard deviation.

aWB1 = maximum of 25, 30, 35, or 40 mg, but not to exceed 0.5 mg/kg. WB2 = 40 mg or ≤0.5 mg/kg for subjects with baseline weight ≥ 90 kg.
bBaseline = day 1 predose supine cuff measurements.

dose level as the independent variable. Estimates of the slope and 90% confidence intervals (CIs) were tabulated by treatment sequence. Analyses of ABPM data were performed using an analysis of covariance with treatment sequence as a fixed effect and time-matched baseline (day −1) as a covariate. Least-squares means and associated 95% CIs obtained from the model were tabulated for each treatment sequence. No inferential testing or multiple comparison adjustments were performed. All statistical analyses were performed using SAS version 9.1 (SAS Institute, Cary, North Carolina).

Results

Subject Disposition and Baseline Characteristics

A total of 44 subjects were randomized across 4 cohorts; 32 received praliciguat, and 12 received matching placebo. In chronological order, the praliciguat treatment sequences (starting dose for 14 days to up titration dose for 7 additional days) of the 4 cohorts were: cohort 1, 15 to 30 mg (n = 8); cohort 2, 30 to 40 mg (n = 8); cohort 3, 20 to 40 mg (n = 8); and cohort 4, WB1 to WB2 (25 to 40 mg [n = 1], 30 to 40 mg [n = 2], 35 to 40 mg [n = 1], 40 to 40 mg [n = 3], and 40 to 50 mg [n = 1]).

All 12 subjects who received placebo completed the study. Two of the 32 subjects who received praliciguat (1 in the 30/40-mg dose cohort and 1 in the WB1/WB2 cohort [actual dose levels 30/40 mg]) withdrew consent and discontinued from the study after completing 20 and 21 days of dosing, respectively.

Demographics and baseline characteristics were comparable across cohorts (Table 1).

Pharmacokinetics

Absorption of praliciguat was fairly rapid, with the time of maximum plasma concentration (T_{max}) achieved within a median range of 2 to 4 hours post-dose, followed by a biphasic decline in plasma concentrations (Figure 1). Despite a relatively long terminal elimination half-life (average values ranged from 123 to 173 hours; Table 2), steady-state conditions appeared to be approached within 14 days of once-daily dosing, based on the similarity of predose trough concentrations on days 14 and 15. The mean range of the day 14/day 1 accumulation ratio of AUC_{tau} (R_{AUC}) was 1.98 to 2.78, and accumulation in plasma concentration observed at the end of a dosing interval, C_{trough} (R_{trough}, 3.81–5.02), was distinctly greater than accumulation in Cmax (R_{max}, 1.18–1.65). This modest change in Cmax but substantial increase in Ctrough resulted in a peak-to-trough ratio (day 14 Cmax/C_{trough}) of approximately 3, which is relatively small and could afford consistent pharmacology over a 24-hour dosing interval. The suitability of once-daily dosing is also reflected in the effective half-life based on R_{AUC}, whose average ranged from 23.7 to 37.3 hours (Table 2).
Table 2. PK Parameters of Praliciguat at the End of Each Dosing Period

| Parameter                  | Mean (%CV) Cohort 1, n = 8 | Cohort 3, n = 8 | Cohort 2, n = 8 | Cohort 4, n = 8 |
|----------------------------|-----------------------------|----------------|----------------|----------------|
| **Day 14**                 |                             |                |                |                |
| **Tmax** (h)               | 2.0 (2.0, 4.0)              | 2.0 (2.0, 4.0) | 3.0 (2.0, 4.0) | 3.0 (2.0, 4.0) |
| **Cmax (ng/mL)**           | 79.5 (26.9)                 | 111 (14.4)     | 176 (32.4)     | 162 (10.9)     |
| **Ctrough (ng/mL)**        | 24.8 (13.4)                 | 35.9 (10.5)    | 58.0 (35.8)    | 48.8 (23.1)    |
| **AUCtau/dose (ng h/mL/mg)** | 59.4 (14.0)              | 63.8 (14.3)    | 68.3 (29.8)    | 55.0 (14.2)    |
| **CL/F (L/h)**             | 17.2 (15.4)                 | 15.9 (13.9)    | 15.6 (25.9)    | 18.5 (15.5)    |
| **Rmax**                   | 1.39 (26.2)                 | 1.65 (14.6)    | 1.65 (20.6)    | 1.18 (18.0)    |
| **Rtrough**                | 4.24 (16.3)                 | 5.02 (21.5)    | 4.88 (19.4)    | 3.81 (21.3)    |
| **RAUC**                   | 2.37 (13.9)                 | 2.75 (17.5)    | 2.78 (19.0)    | 1.98 (15.3)    |
| **Effective t1/2 (h)**     | 30.3 (18.5)                 | 36.7 (22.2)    | 37.3 (24.0)    | 23.7 (22.2)    |
| **Day 21**                 |                             |                |                |                |
| **Tmax** (h)               | 2.0 (2.0, 4.0)              | 2.0 (2.0, 4.0) | 2.0 (1.0, 4.0) | 2.0 (2.0, 4.0) |
| **Cmax (ng/mL)**           | 150 (18.3)                  | 260 (23.3)     | 287 (30.3)     | 257 (20.7)     |
| **Ctrough (ng/mL)**        | 47.8 (11.3)                 | 68.7 (11.2)    | 80.6 (32.0)    | 72.4 (21.9)    |
| **AUCtau/dose (ng h/mL/mg)** | 57.1 (10.8)              | 61.8 (11.0)    | 74.9 (26.4)    | 65.6 (18.1)    |
| **CL/F (L/h)**             | 17.7 (11.5)                 | 16.3 (10.6)    | 14.2 (26.5)    | 15.7 (19.2)    |
| **Vz/F (L)**               | 3100 (40.0)                 | 3610 (35.2)    | 3480 (40.6)    | 3200 (53.6)    |
| **Terminal t1/2 (h)**      | 123 (43.8)                  | 155 (35.8)     | 173 (33.4)     | 139 (37.3)     |

Praliciguat exhibited dose-proportional exposure for 15, 20, and 30 mg after the first dose (Cmax slope, 0.888; 90%CI, 0.535–1.24; and AUCtau slope, 0.952; 90%CI, 0.71–1.19) and after 14 days of once-daily doses (Cmax slope, 1.13; 90%CI, 0.83–1.44; AUCtau slope, 1.16; 90%CI, 0.92–1.4). The intersubject CV in AUCtau was low to moderate and similar across cohorts on day 1 (13.9%–27.2%) and day 14 (14.0%–29.8%) at the starting dose levels, as well as on day 15 (11.4%–26.9%) and day 21 (10.8%–26.4%) following up-titration. The apparent volume of distribution (Vz/F) was large (mean range, 3100–3610 L), suggesting extensive distribution into tissues and consistent with PK in the rat (steady-state volume of distribution, 10.5 L/kg).15 Values for CL/F were moderate (mean range, 14.2–18.5 L/h; 16%–21% of an approximate hepatic blood flow of 87 L/h) and similar across cohorts (Table 2).22

**Safety**

There were no withdrawals from the study because of AEs. There were no deaths, no SAEs, no severe AEs, and no bleeding-associated AEs. Overall, 21 of 32 praliciguat-treated subjects (66%) reported ≥1 AE
Table 3. Adverse Events by Cohort and Dose

| MedDRA Preferred Term                  | Placebo, n = 12 | Praligiguat Dose Sequence Cohorts<sup>a</sup> | Praligiguat Dose Sequence Cohorts<sup>b</sup> |
|---------------------------------------|----------------|---------------------------------------------|---------------------------------------------|
|                                       | Placebo → Placebo | 15 mg → 30 mg | 20 mg → 40 mg | 30 mg → 40 mg | WB1 → WB2 |
| **Number of subjects (%)**            |                 |                |                |                |            |
| Any AE                                | 5 (41.7)        | 1 (8.3)        | 3 (37.5)       | 4 (50.0)       | 3 (37.5)   |
| headache                              | 1 (8.3)         | 1 (8.3)        | 1 (12.5)       | 4 (50.0)       | 0 (0.0)    |
| dizziness                             | 0 (0.0)         | 0 (0.0)        | 1 (12.5)       | 0 (0.0)        | 0 (0.0)    |
| blood pressure decreased dizziness    | 0 (0.0)         | 0 (0.0)        | 0 (0.0)        | 1 (12.5)       | 0 (0.0)    |
| dizziness postural                    | 0 (0.0)         | 1 (12.5)       | 0 (0.0)        | 0 (0.0)        | 0 (0.0)    |
| myalgia                               | 0 (0.0)         | 0 (0.0)        | 0 (0.0)        | 0 (0.0)        | 0 (0.0)    |
| nausea                                | 1 (8.3)         | 1 (8.3)        | 0 (0.0)        | 1 (12.5)       | 0 (0.0)    |
| orthostatic hypotension               | 0 (0.0)         | 0 (0.0)        | 0 (0.0)        | 1 (12.5)       | 0 (0.0)    |
| vomiting                              | 0 (0.0)         | 0 (0.0)        | 1 (12.5)       | 0 (0.0)        | 0 (0.0)    |
| asthenia                              | 0 (0.0)         | 0 (0.0)        | 1 (12.5)       | 0 (0.0)        | 0 (0.0)    |
| blister                               | 0 (0.0)         | 1 (12.5)       | 0 (0.0)        | 0 (0.0)        | 0 (0.0)    |
| constipation                          | 1 (8.3)         | 0 (0.0)        | 1 (12.5)       | 0 (0.0)        | 0 (0.0)    |
| dry skin                              | 0 (0.0)         | 0 (0.0)        | 0 (0.0)        | 0 (0.0)        | 1 (12.5)   |
| dyspnea                               | 0 (0.0)         | 0 (0.0)        | 0 (0.0)        | 0 (0.0)        | 1 (12.5)   |
| erection increased                    | 0 (0.0)         | 1 (12.5)       | 1 (12.5)       | 0 (0.0)        | 0 (0.0)    |
| folliculitis                          | 0 (0.0)         | 0 (0.0)        | 0 (0.0)        | 0 (0.0)        | 1 (12.5)   |
| gastroenteritis                       | 0 (0.0)         | 0 (0.0)        | 0 (0.0)        | 0 (0.0)        | 1 (12.5)   |
| hematuria                             | 0 (0.0)         | 0 (0.0)        | 0 (0.0)        | 1 (12.5)       | 0 (0.0)    |
| lethargy                              | 0 (0.0)         | 0 (0.0)        | 0 (0.0)        | 0 (0.0)        | 1 (12.5)   |
| pain in extremity                     | 0 (0.0)         | 0 (0.0)        | 0 (0.0)        | 0 (0.0)        | 1 (12.5)   |
| palpitations                          | 0 (0.0)         | 0 (0.0)        | 1 (12.5)       | 0 (0.0)        | 0 (0.0)    |
| postural orthostatic tachycardia      | 0 (0.0)         | 0 (0.0)        | 0 (0.0)        | 1 (12.5)       | 0 (0.0)    |
| syndrome                              |                 |                |                |                |            |
| presyncope                            | 0 (0.0)         | 0 (0.0)        | 0 (0.0)        | 0 (0.0)        | 1 (12.5)   |
| procedural pain                       | 0 (0.0)         | 0 (0.0)        | 0 (0.0)        | 1 (12.5)       | 0 (0.0)    |
| sinus tachycardia                     | 0 (0.0)         | 0 (0.0)        | 0 (0.0)        | 0 (0.0)        | 1 (12.5)   |
| tympanic membrane perforation         | 0 (0.0)         | 0 (0.0)        | 0 (0.0)        | 1 (12.5)       | 0 (0.0)    |
| urinary tract infection               | 0 (0.0)         | 0 (0.0)        | 0 (0.0)        | 0 (0.0)        | 1 (12.5)   |
| vertigo                               | 0 (0.0)         | 0 (0.0)        | 0 (0.0)        | 0 (0.0)        | 1 (12.5)   |
| back pain                             | 1 (8.3)         | 0 (0.0)        | 0 (0.0)        | 0 (0.0)        | 0 (0.0)    |
| dry eye                               | 1 (8.3)         | 0 (0.0)        | 0 (0.0)        | 0 (0.0)        | 0 (0.0)    |
| dry mouth                             | 1 (8.3)         | 0 (0.0)        | 0 (0.0)        | 0 (0.0)        | 0 (0.0)    |
| rash maculopapular                    | 1 (8.3)         | 0 (0.0)        | 0 (0.0)        | 0 (0.0)        | 0 (0.0)    |
| visual impairment                     | 1 (8.3)         | 0 (0.0)        | 0 (0.0)        | 0 (0.0)        | 0 (0.0)    |

Subjects were counted only once within each preferred term.
AE, adverse event; MedDRA, Medical Dictionary for Regulatory Activities version 18.1.
<sup>a</sup>Subjects were counted only once within each preferred term.
<sup>b</sup>In cohort 4, subjects received the WB1 dose (maximum of 25, 30, 35, or 40 mg, but not to exceed 0.5 mg/kg) for 14 days followed by the WB2 dose (40 mg or ≤0.5 mg/kg for subjects with baseline weight ≥ 90 kg) for the next 7 days, for a total of 21 consecutive days of dosing.

Compared with 6 of 12 placebo-treated subjects (50%). The most common AEs in praliciguat-treated subjects were headache (15 [47%] vs 2 placebo-treated subjects [17%]), dizziness/postural dizziness/vertigo (7 [22%] vs 0 placebo), and blood pressure decreased/orthostatic hypotension (4 [13%] vs 0 placebo). Collectively, 12 subjects who received praliciguat experienced ≥1 AE that could be associated with blood pressure-lowering effects, including dizziness/postural dizziness, decreased blood pressure, orthostatic hypotension,
Figure 2. Effects of praliciguat on cGMP over 24 hours. Plots are mean change from day 1 predose baseline at starting (lower) doses on days 1 and 14, and at up-titration (higher) doses on days 15 and 21. WB1, weight-based dosing for the first 14 days (maximum of 25, 30, 35, or 40 mg, but not to exceed 0.5 mg/kg); WB2, weight-based dosing for days 14–21 (40 mg or ≤0.5 mg/kg for subjects with baseline weight ≥ 90 kg).

Figure 3. Effects of praliciguat on PRA. Mean change from baseline 4 hours postdose is presented with 10th and 90th percentiles. Subjects received a starting (lower) dose for 14 days, followed by an up-titration (higher) dose for the next 7 days. WB1, weight-based dosing for the first 14 days (maximum of 25, 30, 35, or 40 mg, but not to exceed 0.5 mg/kg); WB2, weight-based dosing for days 14–21 (40 mg or ≤0.5 mg/kg for subjects with baseline weight ≥ 90 kg).

...pulitations, postural orthostatic tachycardia syndrome, presyncope, sinus tachycardia, and vertigo; none of these events were reported in subjects who received placebo. These events were all rated mild or moderate in severity and tended to occur after up-titration to the higher doses. All resolved within 1 to 2 days. Other AEs, including headache, tended to occur early in the dosing period, to resolve within a few days, and not to recur with continued dosing at the same dose. Table 3 summarizes AEs by treatment sequence.

One laboratory result was reported as an AE (microscopic hematuria in a 40-year-old woman) and was judged as mild and not related to the study drug. There were no other clinically meaningful changes in laboratory parameters, ECGs, or physical examinations (data not shown). Praliciguat showed no effect on platelet function as assessed by PFA-100 mean closure time induced by collagen/epinephrine [data not shown]).

Pharmacodynamics

cGMP. There were dose-related increases from baseline in mean plasma cGMP observed across the praliciguat treatment sequence cohorts (Figure 2). There was also evidence of a within-cohort dose response, as cGMP tended to be higher at the up-titration doses (as measured on days 15 and 21) than on earlier dosing days. Maximal increases typically coincided with $T_{max}$...
Table 4. Blood Pressure and Heart Rate: Change From Baseline in 24-Hour Averages From ABPM

| Variable          | Placebo (n = 12) | 15 mg | 20 mg | 30 mg | Praliciguat Dose Groups (n = 8 each) |
|-------------------|------------------|-------|-------|-------|-------------------------------------|
| Day 14            |                  |       |       |       | WBI<sup>a</sup>                     |
| Systolic BP (mm Hg)| −0.85 (−3.51 to 1.82) | −7.29 (−10.58 to −4.01) | −3.27 (−6.54 to 0) | −6.75 (−10.03 to −3.47) | −5.23 (−8.50 to −1.97) |
| Diastolic BP (mm Hg) | −0.09 (−2.64 to 2.45) | −6.10 (−9.20 to −3.00) | −2.76 (−5.87 to 0.35) | −4.70 (−7.79 to −1.61) | −4.89 (−7.99 to −1.80) |
| MAP (mm Hg)       | −0.70 (−3.10 to 1.71) | −6.69 (−9.60 to −3.78) | −3.32 (−6.25 to −0.39) | −5.40 (−8.33 to −2.47) | −5.27 (−8.18 to −2.35) |
| Heart rate (bpm)  | 0.73 (−2.61 to 4.07) | 1.24 (−2.86 to 5.33) | 3.04 (−1.05 to 7.13) | 1.84 (−2.43 to 6.11) | 3.84 (−0.37 to 8.05) |
| Day 21            |                  |       |       |       | WB<sub>2</sub><sup>b</sup>             |
| Systolic BP (mm Hg)| −4.81 (−7.21 to −2.40) | −8.21 (−11.17 to −5.25) | −6.29 (−9.23 to −3.35) | −9.05 (−12.22 to −5.88) | −6.58 (−9.52 to −3.64) |
| Diastolic BP (mm Hg) | −1.67 (−3.74 to 0.41) | −6.61 (−9.14 to −4.07) | −3.98 (−6.53 to −1.43) | −6.12 (−8.81 to −3.42) | −4.35 (−8.68 to −1.81) |
| MAP (mm Hg)       | −2.80 (−4.89 to −0.71) | −7.32 (−9.85 to −4.79) | −4.91 (−7.46 to −2.36) | −6.85 (−9.57 to −4.13) | −5.29 (−7.82 to −2.75) |
| Heart rate (bpm)  | −0.28 (−2.40 to 1.83) | −0.62 (−3.21 to 1.97) | 6.48 (−3.89 to 9.08) | −0.07 (−3.04 to 2.90) | 6.33 (3.65 to 9.01) |

ABPM, ambulatory blood pressure monitoring; BP, blood pressure; CI, confidence interval; LS, least squares; MAP, mean arterial pressure.

Values expressed are LS mean (95% CI) from an ANCOVA model adjusted for time-matched baseline.

<sup>a</sup>WBI = maximum of 25, 30, 35, or 40 mg, but not to exceed 0.5 mg/kg.

<sup>b</sup>WB2 = 40 mg or ≤0.5 mg/kg for subjects with baseline weight ≥ 90 kg.

(often 2 hours postdose), and mean values remained above placebo for up to 24 hours postdose.

**Plasma Renin Activity.** PRA increased from baseline over the dosing period (days 1–21) in all praliciguat treatment sequence cohorts, both 4 hours postdose (Figure 3) and at the end of the 24-hour dosing period (data not shown). Mean increases were frequently in the range of 50% to 100% or more of baseline values (data not shown). Observed increases were overall somewhat higher for doses ≥ 30 mg, but a clear dose response was not observed. Means for placebo generally decreased from baseline, and none increased more than 25% of baseline values.

**Blood Pressure.** In praliciguat-treated subjects, mean 24-hour systolic blood pressure was reduced by approximately 3 to 7 mm Hg from day -1 baseline on day 14, the final dosing day for the starting doses of 15 to 40 mg, compared with an approximately 1-mm Hg reduction in the placebo recipients. A similar pattern was evident for diastolic and mean arterial pressures (Table 4). On day 21, both the praliciguat- and placebo-treated subjects had greater decreases in mean 24-hour systolic blood pressure than on day 14 (Table 4). Changes in mean 24-hour heart rate were not consistent across cohorts; 2 praliciguat cohorts had small (<2 bpm) increases in 24-hour mean heart rate on days 14 and 21, whereas the 2 other cohorts increased by 3 to 4 bpm on day 14, and by 6 to 7 bpm on day 21 (Table 4).

When blood pressure changes from baseline were analyzed in 4-hour intervals across the 24-hour postdose assessment period (Figure 4), each of the praliciguat dose groups had consistent reductions from baseline and relative to placebo in mean MAP on both day 14 and day 21 (Figure 4). In contrast, heart rate changes compared with placebo were less evident; however, mean increases from baseline were consistently greater than placebo at early times.

**Discussion**

This was the first clinical repeated-dose evaluation of the new sGC stimulator praliciguat and the first study that tested a tablet formulation and explored the effect of dose titration.

In healthy subjects, praliciguat showed a favorable PK profile. Apparent total body clearance was moderate, and the apparent volume of distribution was large, resulting in a long terminal-phase half-life of approximately 5 to 7 days that likely reflects the rate at which praliciguat tissue concentrations dissipate. These dynamics resulted in a peak:trough ratio of approximately 5 after 14 days of once-daily dosing, at which praliciguat tissue concentrations dissipate.
variability in heptatically driven clearance (CL/F CV range, 10.6%–26.5%), indicating predictable exposure and suggesting a low likelihood of dose adjustment in renally impaired patients.

Starting praliciguat doses ranging from 15 to 40 mg were adequately tolerated in this healthy study population. No SAEs were observed. The most common AEs were headache and a group of events that could be related to blood pressure lowering (e.g., dizziness, postural dizziness, orthostatic hypotension, decreased blood pressure, and presyncope). This AE profile is consistent with the expected vasodilatory effects of sGC agonists. Similar profiles have been reported for other sGC agonists in healthy subjects and in patients. All AEs were of mild or moderate severity and tended to resolve during continued dosing at the same dose level.

This study explored the possibility that tolerability could be improved by stepwise dose escalation. Subjects were treated with a starting dose for 14 days and (in most cases) were switched to a higher dose for the next 7 days. On up-titration, AEs often appeared (or reappeared) and then resolved with continued dosing. The AE profile after up-titration to 30 mg was similar to the profile seen when 30 mg was given as a starting dose. Thus, the study did not provide evidence that dose titration diminished the occurrence of AEs at higher dose levels.

Pharmacologic enhancement of NO-sGC-cGMP signaling is known to inhibit platelet function in vitro. Rioiguat was found to inhibit platelet aggregation in platelet-rich plasma and whole blood, but only at concentrations substantially higher than the plasma levels observed in patients who received approved therapeutic doses. Nevertheless, in placebo-controlled trials of riociguat, serious bleeding events occurred at a higher rate in patients receiving riociguat than in patients receiving placebo. In this study, we assessed the effect of praliciguat on platelet function in healthy subjects using the PFA-100 instrument, a test that simulates primary hemostasis under high-shear conditions. Praliciguat at the doses tested in this study had no effect on platelet function, as measured using the PFA-100 test. Furthermore, with the exception of 1 case of microscopic hematuria, no AEs related to bleeding were observed in this study, and clinical coagulation parameters were not affected by praliciguat administration.

Stimulation of NO-sGC-cGMP signaling is expected to reduce systemic vascular resistance, and blood pressure reductions have been previously reported in healthy subjects treated with sGC agonists. In this study, once-daily administration of praliciguat to healthy subjects for 14 and 21 days modestly lowered overall blood pressure. In general, mean 24-hour ambulatory systolic, diastolic, and mean arterial pressures were 2 to 6 mm Hg lower in praliciguat-treated subjects compared with placebo-treated subjects. The blood pressure-lowering effects were seen at all doses. These effects were not diminished after 21 days of dosing, suggesting that tolerance to praliciguat did not develop over this period. Furthermore, the impact of praliciguat on blood pressure was sustained at trough plasma concentrations. This consistent PD profile over
24 hours lends additional support to once-daily dosing of praliciguat.

Mean 24-hour heart rate changes demonstrated variable but overall small increases for praliciguat recipients compared with placebo recipients. Increased heart rate is a compensatory response to decreased peripheral vascular resistance in healthy subjects as a mechanism to maintain adequate tissue perfusion. PRA also increased with praliciguat administration, likely in response to reduced arterial blood pressure.

Administration of praliciguat was associated with dose-related increases in plasma cGMP levels, which remained elevated up to 24 hours postdose. Although potentially compromised sample stability limited the precision of the cGMP results, the trends in observed change from baseline between doses are still informative. Plasma cGMP is likely derived from intracellular cGMP and therefore implies target engagement at the tissue level. Extensive tissue distribution appears to drive the long terminal-phase half-life of praliciguat and may also be responsible for the sustained plasma cGMP elevations.

Strengths of this in-clinic study include frequent and appropriate timing of sample collections for PK and PD measurements, which included rigorous hemodynamic assessments via ABPM and known adherence with the dosing regimen. In addition, this study assessed the effects of dose up-titration. Limitations of this study include the small number of subjects in each cohort and a dosing period of only 21 days. Results of longer-duration treatment with praliciguat in patient populations may differ. Based on the results of this phase 1 study, further evaluation of praliciguat in patients for the treatment of conditions that might benefit from enhanced NO-sGC-cGMP signaling is warranted. Studies of praliciguat in patients with HFpEF (NCT03254485) and diabetic nephropathy (NCT03217591) are underway. Impaired NO-sGC-cGMP signaling is believed to play an important role in the pathophysiology of these conditions.33–36 By increasing intracellular cGMP levels, praliciguat could potentially compensate for deficient NO signaling and thereby offer therapeutic benefit.

**Conclusion**

The results of this phase 1 MAD study support continued clinical evaluation of praliciguat.

These data are not yet archived in a public repository.

**Declaration of Conflicting Interests**

J.H., J.W., P.W., M.M., J.C., M.H., G.M., M.C., and A.P. are employed by and may own stock/stock options in Ironwood Pharmaceuticals, Inc.

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