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Oral Hypoxia-Inducible Factor Prolyl Hydroxylase Inhibitor Roxadustat (FG-4592) for Treatment of Anemia in Chronic Kidney Disease: A Placebo-Controlled Study of Pharmacokinetic and Pharmacodynamic Profiles in Hemodialysis Patients

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Abstract

Roxadustat (FG-4592), an oral hypoxia-inducible factor prolyl hydroxylase inhibitor that stimulates erythropoiesis, was evaluated in a phase 1b study in patients with end-stage renal disease with anemia on hemodialysis. Seventeen patients, on epoetin-alfa maintenance therapy with stable hemoglobin levels ≥10 g/dL, had epoetin-alfa discontinued on day 3 and were enrolled in this double-blind placebo-controlled study. Two cohorts were randomized 3:1 (roxadustat: placebo). Patients received single doses of roxadustat (1 or 2 mg/kg) or placebo 1 hour after hemodialysis on day 1 and 2 hours before dialysis on day 8. Maximum plasma concentration and area under the plasma concentration–time curve for patients receiving roxadustat were slightly more than dose proportional and elimination half-life ranged from 14.7 to 19.4 hours. Roxadustat was highly protein bound (99%) in plasma, and dialysis contributed a small fraction of the total clearance: only 4.56% and 3.04% of roxadustat recovered from the 1 and 2 mg/kg dose groups, respectively. Roxadustat induced transient elevations of endogenous erythropoietin that peaked between 7 and 14 hours after dosing and returned to baseline by 48 hours after dosing. Peak median endogenous erythropoietin levels were 96 mIU/mL and 268 mIU/mL for the 1- and 2-mg/kg doses, respectively, within physiologic range of endogenous erythropoietin responses to hypoxia at high altitude or after blood loss. No serious adverse events were reported, and there were no treatment- or dose-related trends in adverse event incidence.

Keywords

anemia, dialysis, erythropoietin, pharmacokinetics, roxadustat

Chronic kidney disease (CKD) is a worldwide public health challenge that afflicts 10% of the US population.[1] A common complication of CKD is anemia associated with reduced erythropoietin (EPO) activity, iron deficiency, and inflammation.[2,3] Anemia is present in >90% of dialysis patients[4] and contributes to excess morbidity and mortality.[5]

Erythropoiesis-stimulating agents (ESAs), composed mainly of recombinant forms of EPO, are the only drugs approved internationally to treat severe anemia in patients with CKD.[6-8] ESAs provide supraphysiologic levels of EPO and boost red blood cell (RBC) production. These drugs have reduced patients' dependence on transfusions since their approval, but studies link high doses of ESAs with an increased risk of cardiovascular events and death in patients with end-stage renal disease (ESRD) and CKD.[9-14] It is also likely that systemic inflammation and dysregulated iron metabolism limit hemoglobin response to ESAs.[15]

Hypoxia-inducible factor prolyl hydroxylase inhibitors (HIF-PHIs) are a new class of small molecules in development to treat anemia. HIF-PHIs stabilize hypoxia-inducible factor proteins by blocking the enzymes that modify hypoxia-inducible factor–α proteins and target them for degradation. Stabilized hypoxia-inducible factor activates the expression of erythropoiesis and iron absorption genes including EPO, the

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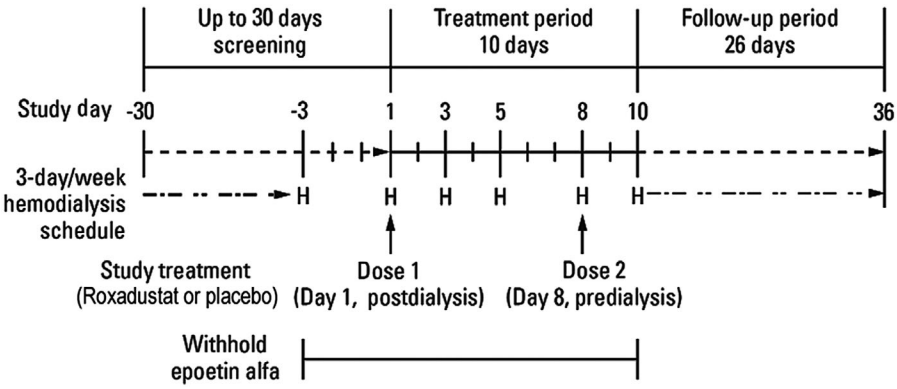

Figure 1. Study design.

EPO receptor, and the iron transporter ferroportin.[16] The coordinated expression of these 2 classes of genes stimulates the production of RBCs rich in iron-containing hemoglobin. While hypoxia normally down-regulates hypoxia-inducible factor prolyl hydroxylase proteins and boosts erythropoiesis, HIF-PHIs can block hypoxia-inducible factor prolyl hydroxylase enzymes under normoxic conditions, boosting RBC production and treating anemia associated with CKD.[17,18]

Roxadustat (FG-4592, ASP1517) is a potent, small-molecule hypoxia-inducible factor prolyl hydroxylase. It is approved in China to treat anemia in dialysis-dependent and non–dialysis-dependent patients with CKD[19] and in Japan to treat patients with dialysis-dependent CKD. The drug is taken orally 3 times per week with a typical clinical dose range of 1 to 2 mg/kg (70-100 mg) per dose.[17,18,20] This phase 1b study evaluated the pharmacokinetic (PK) and pharmacodynamic (PD) profile of roxadustat dosed before and after hemodialysis in patients with ESRD to guide dosing instructions to achieve the desired short- and long-term effects of the drug in dialysis patients.

Methods

Ethical Considerations

This randomized, double-blind, placebo-controlled phase 1b study (FGCL-4592-039) was conducted in patients with ESRD receiving thrice-weekly hemodialysis (HD) by 3 investigators at 3 centers in the United States, in accordance with the Declaration of Helsinki and International Conference on Harmonization Good Clinical Practice guidelines and approved by appropriate ethics committees. Patients provided written informed consent prior to participation.

Study Design

Eligible patients were between 18 and 75 years old, on maintenance HD (thrice weekly), and on a stable dose of intravenous recombinant EPO during the 4 weeks prior to study day –3. Eligible patients also had to have baseline hemoglobin (Hb) levels of 10.5 to 13.0 g/dL, inclusive, based on 2 Hb values obtained at least 1 week apart during the screening period, and difference between the 2 Hb values <1 g/dL. Patients were randomized at a ratio of 3:1 (roxadustat: placebo) to 2 roxadustat doses (1.0 and 2.0 mg/kg oral roxadustat) or placebo (Figure 1). A sample size of 6 active dose patients per group (or a total of 12) was merited appropriate and adequate for this pilot PK and safety assessment given the accuracy/precision of the assay used. Each patient received 2 doses of roxadustat or placebo administered 1 week apart. The first dose was administered 1 hour after the completion of hemodialysis on day 1, and the second dose was administered 2 hours prior to initiation of hemodialysis on day 8. End points were single-dose plasma PK profile of roxadustat administered after HD on day 1 and prior to HD on day 8, HD clearance of roxadustat at the 2 dose levels, plasma endogenous EPO levels, plasma hepcidin levels, adverse events (AEs), physical exams, and selected laboratory evaluations for safety. Dialysis methods at the study centers are standardized based on clinical practice guidelines. The plasma samples were collected before dosing, and PK analysis was performed for each roxadustat dose with samples taken immediately prior to dosing and at multiple time points after dosing on days 1 and 8. Day 1 postdose sampling time points were 0.5, 1.0, 2.0, 3.0, 4.0, 8.0, 12.0, 22.0, 48.0, 48.5, 49.5, 50.5, 51.5, 52, 71, and 95 hours after dosing; day 8 time points were 0.5, 1, 2, 2.5, 3.5, 4.5, 5, 11, 15, 27, 53, and 77 hours after dosing.

PD responses of reticulocyte count, Hb level, reticulocyte Hb content, and serum ferritin and transferrin saturation levels were also examined in exploratory analyses. Patients were required to withhold epoetin-alfa from day –3 through the study treatment period (day 10). The treatment period required 6 visits, including 2 overnight stays (days 1–2, 3, 4, 5, 8–9, and 10) and the follow-up period entailed 4 visits (days 11, 15, 22, and the end-of-study visit on day 36).

Study Drug

Roxadustat was supplied by FibroGen, Inc (San Francisco, California) in hypromellose capsules containing 20 mg of drug product per capsule for oral administration. Placebo was supplied in identical capsules.

Bioanalytical Methods/PK

The bioanalytical analysis for roxadustat concentration was conducted using validated liquid chromatography–tandem mass spectrometry methods by Celerion (Lincoln, Nebraska). These included the determination of roxadustat in human plasma (heparin) and in dialysate, as well as plasma ultrafiltrate. Briefly, these methods used a stable isotope label ([^{13}C2, D3]-roxadustat) as the internal standard. For method validation of determining roxadustat in plasma, an aliquot of plasma sample and internal standard was combined and then diluted with an aqueous solution contain 5% hydrochloride and methyl tert-butyl ether. After processing for liquid-liquid extraction, the organic layer was separated and evaporated to dryness under a stream of nitrogen gas. After reconstitution with high-performance liquid chromatography mobile phase (45:55 acetonitrile: 0.1% formic acid), it was analyzed by high-performance liquid chromatography equipped with an ACE Phenyl column (Advanced Chromatography Technologies Ltd, Aberdeen, Scotland) and an API 4000 mass spectrometer (AB|MDS Sciex, Framingham, Massachusetts). The ratio between the peak area of roxadustat and internal standard was used to calculate the concentration of roxadustat in the sample based on a calibration curve of 1 to 500 ng/mL. The lower limit of quantitation was 1 ng/mL. The interrun accuracy (% bias) was –1.9 to 3.0%, while the interrun precision (% coefficient of variation) was 2.5% to 18.7%. The unbound fraction of roxadustat was determined by ultrafiltration of samples using centrifugal ultrafiltration devices with a 30-kDa molecular weight cutoff.

The plasma EPO concentrations were also measured by a validated enzyme-linked immunosorbent assay (for biomarker analysis), and the analysis was conducted by PPD Global Central Labs (Wilmington, North Carolina) using an EPO assay kit (Quantikine IVD human EPO) from R&D Systems (Minneapolis, Minnesota; Cat. #DEP00) with an Emax Plate Reader (Molecular Devices, San Jose, California). This kit enzyme-linked immunosorbent assay is suitable for the determination of EPO over the range of 2.5 to 200 mIU/mL with validation data confirming the lower limit of quantitation of 2.5 mIU/mL. The accuracy was reported as percent recovery, which ranged from 83% to 100%, and the precision was reported as intra-, inter- and total imprecision of 2.5% to 8.4%, 0% to 4.1%, and 3.5% to 8.4%, respectively. Phoenix WinNonLin Pro version 5.1.1 (Certara LP, Princeton, New Jersey) was used for noncompartmental PK/EPO analysis. Dialysis methods at the study centers are standardized based on clinical practice guidelines.[21,22]

Statistical Analyses

Statistical analyses were descriptive, and summary statistics consisted of sample size (N), means, standard-deviations (SD), medians, and minimum and maximum values for continuous variables, and counts and percentages for categorical variables. All analyses were based on available data, and no substitutions were made for missing data unless otherwise specified. Patients who withdrew from the study before completing the first 24 hours of PK profiling following the second dose (on day 8) would have been replaced in the same treatment group. Patients who withdrew from the study after 24 hours after dose 2 would not have been replaced.

Dose proportionality in roxadustat area under the plasma concentration–time curve (AUC) and maximum plasma concentration (C_{max}) were assessed via mixed-effect model and power model, for individual dosing days and for pooled dosing days. Plasma concentrations of roxadustat, endogenous EPO, hemoglobin, reticulocytes, reticulocyte Hb content, plasma hepcidin levels, and PK parameters for roxadustat were summarized with descriptive statistics (N, mean, SD, coefficient of variation, standard error of the mean, geometric mean, median, minimum, and maximum) by treatment. Values below the lower limit of quantitation were set to 0 for PK parameter calculations prior to dose or to missing for samples after dose (only 1 such case for a patient in the 2-mg/kg group following the first dose).

All patients who received at least a partial dose of any study treatment were included in the safety analyses. Safety assessments consisted of the monitoring and recording of AEs and serious AEs, measurement of protocol-specified hematology and clinical chemistry variables; measurement of protocol-specified vital signs; and other protocol-specified tests that were deemed critical to the safety evaluation of the study treatment.

Results

Patient Disposition

Seventeen patients were randomized to study treatment at 3 sites in the United States, and all 17 completed the study. There were no withdrawals. An extra placebo patient was enrolled due to availability and a convenient opportunity to increase sample size in the control group. Six patients received the 1-mg/kg dose, 6 received the 2-mg/kg dose, and 5 received placebo. The PK population was composed of the 12 patients treated with roxadustat.

Table 1. Demographics and Baseline Characteristics by Treatment Arm and for Patients Overall

	Placebo (n = 5)	1.0 mg/kg (n = 6)	2.0 mg/kg (n = 6)	Roxadustat (1.0 and 2.0 mg/kg Combined) (n = 12)	Total (All Patients Enrolled) (n = 17)
Sex, n (%)					
Male	4 (80)	4 (67)	3 (50)	7 (58)	11 (65)
Female	1 (20)	2 (33)	3 (50)	5 (42)	6 (35)
Race, n (%)					
White	2 (40)	2 (33)	1 (17)	3 (25)	5 (29)
Black	2 (40)	4 (67)	4 (67)	8 (67)	10 (59)
Asian	1 (20)	0	0	0	1 (6)
Other	0	0	1 (17)	1 (8)	1 (6)
Age, y					
Mean (±SD)	59.0 (±6.0)	59.0 (±15.3)	57.7 (±18.1)	58.3 (±16.0)	58.5 (±13.6)
Median	57	63	64	64	63
Min-max	52-67	29-73	31-78	29-78	29-78
BL weight, kg, mean (±SD)	85.4 (±14.3)	74.2 (±11.1)	89.3 (±15.5)	81.8 (±15.1)	82.8 (±14.5)
BL Hb, g/dL, mean (±SD)	11.0 (±1.1)	11.5 (±0.9)	11.6 (±0.5)	11.6 (±0.7)	11.4 (±0.8)
BL ferritin, ng/mL, mean (±SD)	691 (±234)	530 (±371)	703 (±246)	616 (±313)	639 (±287)
TIBC, μg/dL, mean (±SD)	153.4 (±30.0)	144.2 (±57.6)	132.7 (±31.2)	138.4 (±44.6)	142.8 (±40.5)

BL, baseline; Hb, hemoglobin; TIBC, total iron-binding capacity.

Table 2. Roxadustat PK Parameters in Patients With ESRD on HD

	Roxadustat Dose			
	1.0 mg/kg		2.0 mg/kg[e]	
PK Parameters	Day 1 After Dialysis	Day 8 Before Dialysis	Day 1 After Dialysis	Day 8 Before Dialysis
n[a]	6 (5[b])	6 (5[b])	6	6
t_{max}, h, median (min-max)	2.0 (1.0-3.0)	3.5 (2.5-4.5)	2.0 (1.0-4.0)	2.8 (1.8-3.5)
C_{max}, μg/mL, mean (±SD)	5.46 (±2.01)	5.25 (±2.15)	10.6 (±4.48)	11.0 (±2.52)
AUC_{last}, (μg • h/mL, mean (±SD)	53.1 (±27.9)	48.9 (±22.6)	111.1 (±53.1)	113.7 (±41.1)
$AUC_{0-\infty}$, μg • h/mL, mean (±SD)	54.1 (±28.3)	50.2 (±23.7)	112.4 (±54.6)	117.0 (±42.7)
$t_{1/2}$, h, mean (±SD)	19.4 (±4.5)	17.3 (±3.1)	15.7 (±6.0)	14.7 (±5.3)
Vz/F, mL/kg, mean (±SD)	713 (±529)	614 (±353)	585 (±502)	403 (±175)
CL/F, mL/hr/kg, mean (±SD)	24.7 (±16.7)	24.9 (±13.6)	27.8 (±28.2)	20.3 (±11.2)
% Unbound mean (±SD)	1.238 (±0.460)[c]	1.288 (±0.136)[d]	0.943 (±0.183)[c]	1.244 (±0.204)[d]

$AUC_{0-\infty}$, area under the concentration-time curve from dose administration (time 0) to time infinity; AUC_{last}, area under the plasma concentration–time curve from dose administration (time 0) to time of last quantifiable concentration; C_{max}, maximum plasma concentration; CL/F, oral clearance; ESRD, end-stage renal disease; HD, hemodialysis; PK, pharmacokinetics; $t_{1/2}$, half-life; t_{max}, time to maximum plasma concentration; Vz/F, apparent volume of distribution during terminal phase.

[a]A sample for 1 patient was not collected and data were not available for analysis.

[b]n = 5 for % unbound calculation.

[c]Day 1: 3 h after dialysis.

[d]Day 8: Predialysis dosing was done immediately prior to the start of dialysis.

[e]A below limit of quantitation of 1.0 ng/mL was reported for a patient at 67.5 h following the first dose of 2 mg/kg.

Demographics

This study enrolled 3 sites in the United States. Of the 12 patients randomly assigned to roxadustat in the study, 8 (67%) were African American, 3 (25%) were white, and 1 (8%) was of other non-Asian race (Table 1). The treatment groups were balanced with respect to baseline Hb, and all patients received ESA therapy until 3 days before the study start.

Pharmacokinetics

Oral doses of roxadustat were absorbed rapidly in dialysis patients. The median time to maximum plasma concentration (t_{max}) of roxadustat was between 2.0 and 3.5 hours when measured for the group receiving 1 and 2 mg/kg, respectively, and was more similar when measured on day 8 prior to dialysis than on day 1 following dialysis (Table 2). The plasma concentration-time

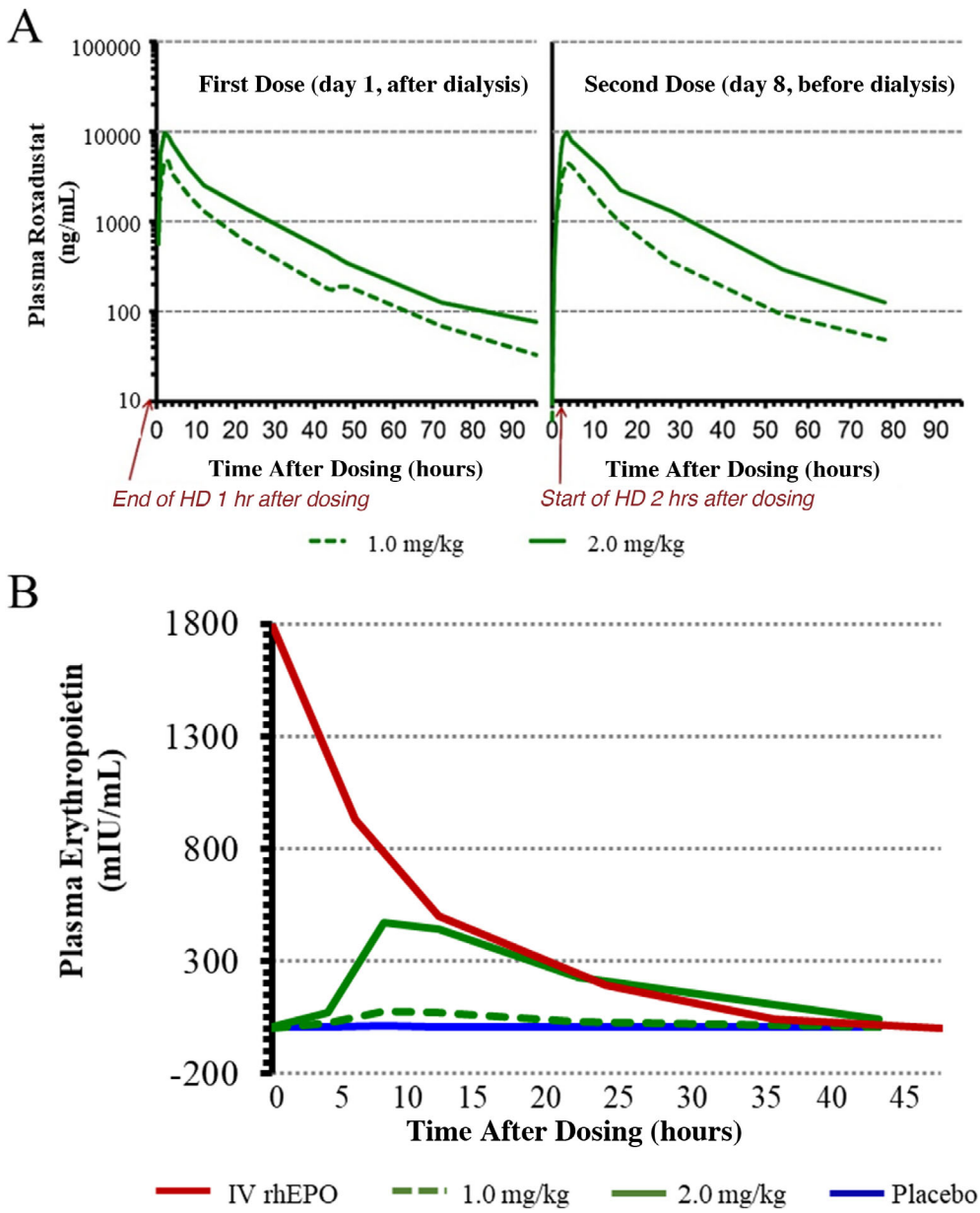

Figure 2. A, Mean plasma concentration-time profiles of roxadustat administered before and after HD by dosing group (N = 6 per dose group). B, Plasma erythropoietin levels after the first dose of 1.0 mg/kg (dashed green line) or 2.0 mg/kg (solid green line) roxadustat compared to historical epoetin-alfa (red line) after intravenous dosing[34] (pharmacokinetic profile of intravenous epoetin-alfa [100 IU/kg] administered to 10 patients with ESRD who were stable on continuous ambulatory peritoneal dialysis and not treated with epoetin-alfa in the 2 months prior to the study). HD, hemodialysis; rhEPO, recombinant human erythropoietin.

profile of roxadustat increased with increasing dose and appeared similar for patients who received 1 mg/kg or 2 mg/kg (Figure 2A). At day 1, mean AUC from time 0 to infinity ($AUC_{0-\infty}$) after dialysis was 54.1 μg • h/mL for the 1-mg/kg group and 112.4 μg • h/mL for the 2-mg/kg group. The increase was consistent with comparable clearance (CL/F) values: Mean CL/F on day 1 was 24.7 mL/hr/kg (SD ± 16.7; geometric mean = 21.1) for the 1-mg/kg group, and 27.8 mL/hr/kg (SD ± 28.2; geometric mean = 21.0) for the 2-mg/kg group (Table 2). On day 8, when roxadustat was given before dialysis, these PK parameters were similar to those on day 1 when the compound was dosed after dialysis (Table 2).

Mean values of PK parameters did not change in a clinically meaningful manner between day 1 (after dialysis) and day 8 (before dialysis) or in a manner consistent with dialysis providing significant clearance of roxadustat. The mean hemodialysis clearance of roxadustat was 3.68 mL/h/kg (geometric mean = 3.61 mL/h/kg) for the 1-mg/kg group and 1.94 mL/h/kg

(geometric mean = 1.94 mL/h/kg) for the 2-mg/kg group. This clearance corresponds to a very small fraction of unchanged roxadustat (% recovery) cleared by the dialyzer (4.56% in the 1-mg/kg dose group and 3.04% in the 2-mg/kg dose group) compared with the total clearance of roxadustat.

Overall, approximately 1.2% (mean value; n = 5) of roxadustat was unbound based on ultrafiltration analysis of patient plasma. These values ranged from 0.943% to 1.264% and were comparable between the 1- and 2-mg/kg doses. Measurements were made immediately before dialysis on day 1 before any drug had been administered and at several time points after dialysis on day 8 after both roxadustat doses had been given (Table 2).

Roxadustat exposure increased with increasing dose. Dose proportionality was assessed using 2 statistical methods: a power-based model and a mixed-effect analysis of variance. Evaluation of dose proportionality by dosing day using the power model is summarized in Table 3. On day 1 (postdialysis dosing), the regression coefficients were close to 1, suggesting dose proportionality from 1 to 2 mg/kg. On day 8 (predialysis dosing), the dose-normalized AUC was about 20% higher in the 2-mg/kg group compared with the 1-mg/kg group.

An overall evaluation of dose proportionality, pooling day 1 and day 8 data, is shown in Table 4 based on PK parameters AUC from time 0 to time of last quantifiable concentration (AUC_{last}), $AUC_{0-\infty}$, and C_{max}. The 80% and 90% confidence intervals are included in the table. Two statistical models, mixed-effect analysis of variance and the power model, were used. Both methods yielded the same results in terms of estimating the ratio of the dose-normalized PK parameters for the 1-mg/kg versus 2-mg/kg dose levels. The point estimate of the ratios of the dose-normalized AUC_{last}, $AUC_{0-\infty}$, and C_{max} for 1 mg/kg versus 2 mg/kg were 0.91, 0.91, and 0.98, respectively. This indicates that the dose-normalized AUC of the 2-mg/kg cohort was about 10% higher than that of the 1-mg/kg cohort, while the dose-normalized C_{max} for the 2 dose cohorts was about the same.

After normalizing PK parameters by study day and pooling the study days using arithmetic means and geometric means (Table 4), the dose-normalized PK parameters were very similar between dose cohorts on day 1, but on day 8 (predialysis dosing), the dose-normalized AUC was about 16% higher in the 2-mg/kg cohort.

In addition, this increase appeared to be slightly more than dose proportional when comparing the 1-mg/kg and 2-mg/kg dose groups, which is consistent with comparable CL/F values between the 1- and 2-mg/kg dose groups.

Pharmacodynamics

Peak endogenous EPO concentration increased with roxadustat dose, and the increase in response was more than dose proportional (Figure 2B). For example, doubling the roxadustat dose resulted in more than doubling of endogenous EPO response (Table 5). On day 1, the peak median endogenous EPO C_{max} was 11.9, 96, and 268 mIU/mL in the placebo and 1- and 2-mg/kg group, respectively. While endogenous EPO levels in patients receiving placebo as a group were within normal physiologic variability range, the median t_{max} of endogenous EPO response occurred 8.0 and 9.99 hours following a dose of 1 and 2 mg/kg roxadustat, respectively. On day 8, endogenous EPO responses (such as C_{max}/AUC) appeared to be comparable to those on day 1 (data not shown). The peak median change from baseline occurred in both roxadustat-treated groups at 3.5 hours into dialysis (5.5 hours after dosing): 40.20 mIU/mL in the 1-mg/kg group and 125.20 mIU/mL in the 2-mg/kg group. The peak median change in the placebo group was –3.6 mIU/mL.

Levels of iron metabolism biomarkers including Hb, ferritin, hepcidin, and transferrin saturation levels did not change significantly from baseline in any of the treatment groups (data not shown).

Safety

No serious AEs were reported from this study. Two patients (1 in the placebo group, 2 in the 2.0-mg/kg group) had treatment-emergent AEs (ie, diarrhea, nausea, and vomiting); the events were assessed as not related to study drug.

No apparent clinically significant, persistent pattern of differences among groups in systolic blood pressure, diastolic blood pressure, or mean arterial pressure were observed, and there were no clinically significant intrapatient differences in dialytic vital sign changes (ie, pulse and blood pressure) when roxadustat was administered before dialysis vs after dialysis.

Discussion

This placebo-controlled, phase 1b study showed dialysis contributed to <5% of roxadustat clearance with roughly 99% of circulating roxadustat highly protein bound. Protein-bound drugs are not typically dialyzable because the protein-drug complexes are larger than the dialysis membrane pores. In contrast, a number of antibiotics and antiviral drugs are significantly depleted by the procedure.[23-25] Roughly 50% of the antiviral ganciclovir is removed by dialysis and the drug requires supplemental post-dialysis dosing[26] that is not necessary for roxadustat.

Overall, the PK parameters in patients with ESRD were similar to those seen in healthy volunteers.[20,25]

Table 3. Tests for Dose Proportionality in AUC and C_{max} in the PK Population

	Regression Coefficient by Dosing Day			Power Model Pooling Dosing Day		
PK Parameter	Estimate	80%CI	*P* Value	Estimate	80%CI	*P* Value
Day 1						
AUC_{last}	1.01	0.25-1.76	.9924			
$AUC_{0-\infty}$	1.01	0.25-1.77	.9904			
C_{max}	0.88	0.29-1.47	.7881			
Day 8						
AUC_{last}	1.26	0.71-1.81	.5256	1.13	0.50-1.77	.7788
$AUC_{0-\infty}$	1.27	0.71-1.82	.5289	1.14	0.50-1.78	.7774
C_{max}	1.17	0.72-1.62	.6219	1.02	0.55-1.49	.9458

AUC, area under the plasma concentration–time curve; $AUC_{0-\infty}$, area under the plasma concentration–time curve from dose administration (time 0) to infinity; AUC_{last}, area under the concentration-time curve from dose administration (time 0) to time of last quantifiable concentration; C_{max}, maximum concentration; CI, confidence interval; PK, pharmacokinetics.

Table 4. Estimates of Dose Proportionality in the PK Population

	Estimate From the Model[a]			Estimate of R-mdn[b]		
	Estimate	80%CI	90%CI	Estimate	80%CI	90%CI
(AUC_{last}1/dose1)/(AUC_{last}2/dose2)						
Mixed-effect ANOVA model	−0.09	−0.53-0.35	−0.67-0.49	0.91	0.59-1.42	0.51-1.63
Power model	1.13	0.50-1.77	0.30-1.97	0.91	0.59-1.42	0.51-1.63
($AUC_{0-\infty}$1/dose1)/($AUC_{0-\infty}$2/dose2)						
Mixed-effect ANOVA model	−0.09	−0.54-0.35	−0.68-0.49	0.91	0.58-1.42	0.51-1.63
Power model	1.14	0.50-1.78	0.29-1.98	0.91	0.58-1.42	0.51-1.63
(C_{max}1/dose1)/(C_{max}2/dose2)						
Mixed-effect ANOVA model	−0.02	−0.34-0.31	−0.45-0.41	0.98	0.71-1.36	0.64-1.51
Power model	1.02	0.55-1.49	0.40-1.64	0.98	0.71-1.36	0.64-1.51

ANOVA, analysis of variance; $AUC_{0-\infty}$, area under the plasma concentration–time curve from dose administration (time 0) to infinity; AUC_{last}, area under the plasma concentration–time curve from dose administration (time 0) to time of last quantifiable concentration; C_{max}, maximum plasma concentration; PK, pharmacokinetics.

Units: AUC_{last}; $AUC_{0-\infty}$: μg • h/mL; C_{max}: μg/mL.

Mixed-effect model: log(PK parm/dose) = dose + study_day, with random effect intercept and dose as a class variable.

Power model: log(PK parm) = log(dose) + study_day, where dose is a continuous variable.

[a]Estimate from the ANOVA model is the difference of the 2 dose levels obtained from the LSMEANS with the DIFF option.

Estimate from the power model is the regression coefficient of log(dose).

[b]R-mdn = ratio of geometric means of dose-normalized PK parameter = (GM PK1/dose1)/(GM PK2/dose2).

Table 5. PD Parameters of Endogenous Erythropoietin Response[a] to Roxadustat Treatment in Patients on HD

	Roxadustat Dose		
	Placebo n = 5	1.0 mg/kg n = 6	2.0 mg/kg n = 6
t_{max}, h			
Mean (±SD)	15.1 (±15.8)	9.33 (±2.1)	10.0 (±2.2)
Median (min-max)	8.0 (7.9-43.4)	8.0 (7.98-12.0)	10.0 (7.9-12.1)
C_{max} (mIU/mL)			
Mean (±SD)	14.3 (±10.1)	84 (±59)	508 (±522)
Median (min-max)	11.9 (4.0-31.1)	96 (8-166)	268 (59-1201)
AUC_{last}, mIU • h/mL			
Mean (±SD)	396 (±322)	1445 (±996)	9108 (±9611)
Median (min, max]	272 [103, 939]	1617 [191, 2423]	4612 [1053, 23573]

AUC_{last}, area under the plasma concentration–time curve from dose administration (time 0) to time of last quantifiable concentration; C_{max}, maximum plasma concentration; EPO, erythropoietin; PD, pharmacodynamics; HD, hemodialysis; SD, standard deviation.

[a]Day 1 postdialysis PD series.

Oral doses were absorbed rapidly (t_{max} approximately a few hours after dosing), and plasma exposure (C_{max} and AUC) was slightly more than dose proportional. As the plasma half-life of roxadustat ranges between 14 and 20 hours, at thrice-weekly dosing, plasma levels generally return to very low levels and no apparent drug accumulation occurs.

Mechanistically, a robust transient endogenous EPO response to roxadustat was observed, with peak endogenous EPO levels (C_{max}) resembling physiologic responses to hypoxia.[27-29] In this study, 1- and 2-mg/kg doses of roxadustat resulted in median peak plasma endogenous EPO concentrations of approximately 96 mIU/mL and 268 mIU/mL, respectively. These exposures are approximately within the range of physiologic effects seen in healthy patients adjusting to high altitude (200-300 mIU/mL)[30,31] or to phlebotomy, hemorrhage, or hypoxemia (50-1250 mIU/mL)[27-29] and are substantially lower (4- to 20-fold) than those resulting from an intravenous epoetin-alfa dose typically used in standard-of-care dialysis.[32]

Endogenous EPO induction was transient and peaked at a median t_{max} of 8 to 10 hours after roxadustat dosing and returned to baseline by 48 hours.[17] These levels were observed at a t_{max} of 8 hours in the 1-mg/kg groups and 10 hours in the 2-mg/kg group. The t_{max} values are consistent with hypoxic EPO response times comparable to data from healthy volunteers[20] where endogenous EPO exposure (C_{max} and AUC) increased more than dose proportionally with roxadustat dose. Thus, at thrice-weekly dosing, roxadustat causes a transient endogenous EPO stimulation rather than constant elevation, which makes it similar to normal physiology.

The induction of endogenous EPO seen in this study is consistent with phase 2 data where roxadustat promoted a robust Hb increase 2 weeks after initiation of treatment in non–dialysis-dependent patients with CKD[33] after dosing at 1 to 2 mg/kg 3 times weekly and at less frequent regimens. These data were confirmed in phase 3 studies in China, leading to roxadustat's approval for treatment of hemodialysis patients with CKD anemia in China.

The study was limited by small sample size and was not statistically powered to a specific end point; therefore, the outcomes should be interpreted considering this limitation. In addition, while the treatment groups were generally well balanced at baseline, the mean weight in the 1.0-mg/kg dose group was slightly lower compared to the 2.0 mg/kg and placebo dose groups. However, since the study drug was administered in milligrams per kilogram rather than a fixed dose, the likelihood of the difference in weight between treatment groups at baseline confounding the outcomes is minimized.

Taken together, these results confirm that roxadustat can be dosed freely with respect to the timing of dialysis. This study informed the design of global phase 3 studies of dialysis patients and will guide the clinical use of oral roxadustat, a potentially safer, more accessible, and convenient alternative to parenteral ESAs in the treatment of anemia secondary to CKD.

Conclusions

In a phase 1 study of hemodialysis patients, <5% of roxadustat was cleared by dialysis, with roughly 99% of the drug bound by protein in plasma. These data support a flexible dosing regimen where roxadustat can be taken before or after dialysis without concern about losing drug during dialysis and lowering its potency. PK and PD studies showed roxadustat levels in the blood are dose proportional and induce transient peaks of endogenous EPO comparable to previous studies in non–dialysis-dependent patients. These EPO levels are similar to levels produced by healthy patients at high altitudes, where erythropoiesis is challenged by low oxygen levels, and this is consistent with roxadustat's ability to treat anemia in phase 3 clinical studies.

Conflicts of Interest

FibroGen Inc. was the sponsor of this study, and J.C., and K.H.P.Y. are employees of FibroGen and hold stock and/or stock options in FibroGen. At the time the study was performed, R.P. was affiliated with St. John Hospital & Medical Center and with Wayne State University School of Medicine. He currently is an employee and holds stock in DaVita Healthcare Partners. All other authors declare no conflicts of interest.

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Author Contributions

FibroGen Inc. was the sponsor of this study, and designed the study in consultation with the principal investigators (R.P.). R.P., J.T., and R.Z. contributed patients to the study. FibroGen was responsible for data collection and analysis. All authors had full access to the study data and the analyses. All authors reviewed the manuscript and confirmed its accuracy.

Data Sharing

This study was not registered in a public database (ie, clinicaltrials.gov). Research data will not be shared.

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