AJKD
Original Investigation

Roxadustat (FG-4592) Versus Epoetin Alfa for Anemia in Patients Receiving Maintenance Hemodialysis: A Phase 2, Randomized, 6- to 19-Week, Open-Label, Active-Comparator, Dose-Ranging, Safety and Exploratory Efficacy Study

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Background: Roxadustat (FG-4592) is an oral hypoxia-inducible factor prolyl-hydroxylase inhibitor that promotes erythropoiesis through increasing endogenous erythropoietin, improving iron regulation, and reducing hepcidin.

Study Design: Phase 2, randomized (3:1), open-label, active-comparator, safety and efficacy study.

Setting & Participants: Patients with stable end-stage renal disease treated with hemodialysis who previously had hemoglobin (Hb) levels maintained with epoetin alfa.

Intervention: Part 1: 6-week dose-ranging study in 54 individuals of thrice-weekly oral roxadustat doses versus continuation of intravenous epoetin alfa. Part 2: 19-week treatment in 90 individuals in 6 cohorts with various starting doses and adjustment rules (1.0-2.0 mg/kg or tiered weight based) in individuals with a range of epoetin alfa responsiveness. Intravenous iron was prohibited.

Outcomes: Primary end point was Hb level response, defined as end-of-treatment Hb level change (ΔHb) of −0.5 g/dL or greater from baseline (part 1) and as mean Hb level ≥ 11.0 g/dL during the last 4 treatment weeks (part 2).

Measurements: Hepcidin, iron parameters, cholesterol, and plasma erythropoietin (the latter in a subset).

Results: Baseline epoetin alfa doses were 138.3 ± 51.3 (SD) and 136.3 ± 47.7 U/kg/wk in part 1 and 152.8 ± 80.6 and 173.4 ± 83.7 U/kg/wk in part 2, in individuals randomly assigned to roxadustat and epoetin alfa, respectively. Hb level responder rates in part 1 were 79% in pooled roxadustat 1.5 to 2.0 mg/kg compared to 33% in the epoetin alfa control arm ($P = 0.03$). Hepcidin level reduction was greater at roxadustat 2.0 mg/kg versus epoetin alfa ($P < 0.05$). In part 2, the average roxadustat dose requirement for Hb level maintenance was ~1.7 mg/kg. The least-squares-mean ΔHb in roxadustat-treated individuals was comparable to that in epoetin alfa–treated individuals (about −0.5 g/dL) and the least-squares-mean difference in ΔHb between both treatment arms was −0.03 (95% CI, −0.39 to 0.33) g/dL (mixed effect model-repeated measure). Roxadustat significantly reduced mean total cholesterol levels, not observed with epoetin alfa. No safety concerns were raised.

Limitations: Short treatment duration and small sample size.

Conclusions: In this phase 2 study of anemia therapy in patients with end-stage renal disease on maintenance hemodialysis therapy, roxadustat was well tolerated and effectively maintained Hb levels.

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INDEX WORDS: Hypoxia-inducible factor prolyl-hydroxylase inhibitor (HIF-PHI); roxadustat; anemia; dialysis; chronic kidney disease (CKD); erythropoiesis; iron transport; hemoglobin (Hb); Hb correction; Hb response; hepcidin; erythropoietin; end-stage renal disease (ESRD); Hb maintenance; randomized trial.

Prior to the availability of recombinant erythropoiesis-stimulating agents (ESAs), anemia in end-stage renal disease (ESRD) was treated with repeated blood transfusions. Because allosensitization by blood transfusions can interfere with patients' ability to receive kidney transplants,[1] minimization of transfusions is a distinct benefit of ESAs. However, cardiovascular safety findings from the Normal Hematocrit Cardiac Trial

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(NHCT), the Correction of Hemoglobin and Outcomes in Renal Insufficiency (CHOIR) study, the Cardiovascular Risk Reduction by Early Anemia Treatment With Epoetin Beta (CREATE) study, and the Trial to Reduce Cardiovascular Events With Aranesp Therapy (TREAT),[2-5] which targeted hemoglobin (Hb) values > 11 g/dL, prompted regulatory authorities to institute warnings to use the lowest ESA dose adequate for reducing the need for red blood cell transfusion. The warning also states that no trial has identified an Hb target level, ESA dose, or dosing strategy that does not increase these risks.[6] Similar recommendations have also been made based on observational studies.[7] Post hoc analyses of the mentioned trials point to high ESA doses as the mediator of cardiovascular risk while consistently supporting an inverse relationship between achieved Hb levels and risk.[8] These restrictions on ESAs have subsequently been associated with a decline in Hb levels and an increase in transfusions in patients with ESRD in the United States.[9]

Hepcidin has been recently identified as the master regulator of iron metabolism,[10,11] and levels are elevated in patients with ESRD[12] and those with inflammation. Hepcidin reduces duodenal iron absorption and export of tissue-stored iron-impairing responsiveness to erythropoietin (EPO). Anemia in patients with ESRD is not simply an "EPO-deficiency state," but one of EPO dysregulation coupled with major abnormalities in iron metabolism leading to the need for intravenous (IV) iron.[13] Recent studies demonstrate a role for hypoxia-inducible factor (HIF) in the regulation of endogenous EPO production, the iron transport system,[14] and hepcidin. A new class of drugs, HIF−prolyl hydroxylase inhibitors (HIF-PHIs), taking advantage of natural physiology in coordinated erythropoiesis, is being evaluated for the treatment of anemia in chronic kidney disease.[15]

Roxadustat (FG-4592) is an orally bioavailable HIF-PHI with a half-life of around 12 hours; thrice-weekly administration leads to intermittent activation of genes associated with erythropoiesis, notably including well-characterized HIF targets such as EPO and proteins promoting iron absorption, iron transport, and heme synthesis.[16-18] In an earlier placebo-controlled 4-week phase 2a study, roxadustat increased Hb levels in non–dialysis-dependent patients with chronic kidney disease in a dose-dependent manner and improved iron homeostasis, limiting transient endogenous EPO levels to within or near physiologic range.[19] In ESA-naive incident hemodialysis and peritoneal dialysis patients, roxadustat treatment resulted in a maximum Hb level increase of +3.1 ± 0.2 (standard error [SE] of the mean) g/dL over 12 weeks despite a lack of iron repletion requirement at baseline, with Hb values of hemodialysis patients receiving oral iron responding as well as those on IV iron therapy.[20] We present a phase 2 study of patients with ESRD on maintenance hemodialysis therapy whose Hb levels had been previously maintained by epoetin alfa, randomly assigned to roxadustat or to continue epoetin alfa to demonstrate roxadustat's efficacy in maintaining Hb levels when converting from an ESA and to establish the optimum starting dose and dose adjustment regimen to maintain target Hb values.

METHODS

Study Design

This was a randomized, multicenter, open-label, consecutive-cohort, multidose study with active comparator IV epoetin alfa in patients with ESRD treated by maintenance hemodialysis in the United States whose Hb levels were previously maintained with IV epoetin alfa (dosage, 75-450 U/kg/wk) and IV iron in the 4 weeks preceding screening. The study, conducted between May 17, 2010, and October 15, 2012, consisted of a screening period of up to 4 weeks, a treatment period of 6 (part 1) or 19 (part 2) weeks, and an 8- (part 1) or 4-week (part 2) follow-up period.

The study was approved by Aspire Institutional Review Board, La Mesa, CA. Participants provided written informed consent.

Participants and Treatment

Eligible patients were aged 18 to 75 years and receiving maintenance hemodialysis thrice weekly for 4 or more months. Hb levels were 9.0 to 13.5 g/dL for 8 weeks, and patients had stable epoetin alfa dosages ≤ 450 U/kg/wk for 4 weeks prior to randomization. A comprehensive list of eligibility criteria is provided (Item S1).

The study design is illustrated in Fig 1. Part 1 consisted of 3.5 consecutive 6-week dose cohorts in which participants were randomly assigned up to n = 16 per cohort, 3:1 (roxadustat to epoetin alfa), with oral roxadustat doses fixed at 1.0, 1.5, 1.8, or 2.0 mg/kg thrice weekly. Results of part 1 were used to refine optimal roxadustat starting doses for part 2 (19-week treatment), consisting of 6 consecutive-dose cohorts in which participants were converted to roxadustat from epoetin alfa treatment (Table 1). Part 2 comprised 90 patients randomly assigned to 6.5 cohorts of roxadustat (n = 67, with various starting doses) or to continue on epoetin alfa therapy (n = 23).

Treatment with androgens was prohibited; IV iron use and red blood cell transfusions were guided by rescue criteria (Item S2). Oral iron supplementation was permitted but not required. During the post-treatment follow-up period, roxadustat-treated patients switched back to epoetin alfa.

Dose Modifications

Hb was measured weekly; investigators titrated the roxadustat dose after the initial fixed dosing period based on cohort-specific rules every 4 weeks (Table 2). If Hb level increased by >2.0 g/dL in any 2 weeks, the dose was reduced 1 dose step. For participants randomly assigned to epoetin alfa, dose adjustments were made based on the local standard of care.

Assessments

The primary end point in part 1 (6-week cohorts) was the proportion of participants whose Hb levels did not decrease by >0.5 g/dL from baseline (defined as the mean of the last 3 Hb values obtained prior to the first dose of study treatment). The primary end point in part 2 (19-week cohorts) was the proportion of participants whose mean Hb level was ≥11 g/dL averaged over the last 4 weeks (weeks 16 through 19). Exploratory analysis

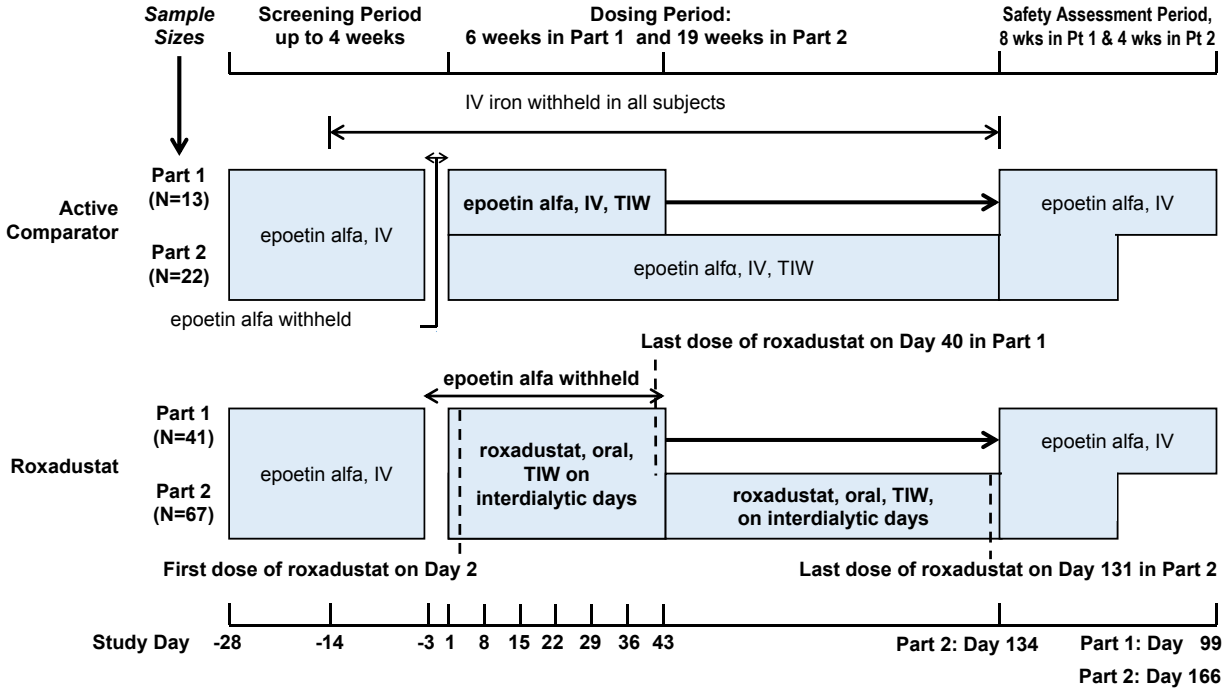

Figure 1. Study scheme. Abbreviations: IV, intravenous; pt, patient; TIW, thrice weekly.

included hepcidin levels, correlates of erythropoiesis and iron use (reticulocyte Hb content, soluble transferrin receptor, transferrin, total iron-binding capacity, transferrin saturation, and ferritin), and total serum cholesterol level.

Safety was assessed by physical examinations, clinical laboratory tests, and the incidence and severity of adverse events (AEs) recorded from treatment onset through follow-up.

Evaluation of plasma endogenous EPO levels was conducted at selected sites to compare circulating endogenous EPO levels 0 to 48 hours following roxadustat treatment with EPO levels following exogenous administration of epoetin alfa dosed within the same participants prior to randomization.

Statistical Analysis

The study was not formally powered for noninferiority; the total number of patients and cohort size were considered adequate for phase 2 studies to advise medical decision making and assess relative efficacy. All analyses were exploratory. All participants receiving any dose of study treatment were included in the safety population and safety analyses.

Table 1. Roxadustat Starting Doses

Treatment Cohort	Treatment Duration, wk	Criterion for Epoetin alfa Dose Preceding Study Drug Treatment, IU/kg/dose	Weight-Based Starting Dose of Roxadustat[a]	Fixed Period for Initial Dosing, wk
A-1	6	25-85	1.0 mg/kg	3
A-2	6	25-85	1.5 mg/kg	3
A-3	6	25-85	2.0 mg/kg	3
A-4	6	25-85	1.8 mg/kg	3
A-5	19	85-115	1.8 mg/kg	6 (amendment 2[b]) then 4 (start amendment 3[c])
A-6	19	25-115	1.3 mg/kg	6
A-7	19	25-115	Weight tiered: 70-100-150 mg[d]	6 then 4 (start amendment 3[c])
A-8	19	25-115	Weight tiered: 70-120-200 mg[d]	4
A-9	19	85-150	2.0 mg/kg	4
A-10	19	25-115	Weight tiered: 70-120-200 mg[d]	4

Note: Weight indicates posthemodialysis actual dry weight at baseline.

[a]All regimens 3 times per week.

[b]Amendment 2 increased the number of cohorts from 4 to 10, extended treatment duration from 6 to 19 weeks for the 5 remaining participants in cohort A-4 and all participants in the new cohorts, and reduced the follow-up period from 8 to 4 weeks.

[c]Amendment 3 allowed dose adjustments to be made after 4 weeks rather than 6 weeks, allowed more rapid dose escalation, and modified some dose adjustment rules. It also added cohorts A-11 and A-12, which were not filled eventually.

[d]Doses given are for participant weights of 45 to 60, >60 to 90, and >90 to 140 kg, respectively.

Table 2. Dose Adjustments That Took Effect under Amendment 3

ΔHb Over Past 4 wk, g/dL	Hb < 11.0 g/dL	Hb 11.0-<13.0 g/dL	Hb 13.0-14.0 g/dL	Hb > 14.0 g/dL
<−0.8	↑	↑	No change	Hold, then resume dosing when
−0.8 to 0.8	↑	No change	↓	Hb < 12.0 g/dL at a dose that is reduced
>0.8	No change	↓	↓	by 2 dose steps

Note: Dose increases (↑) and reductions (↓) are to be in dose steps as follows: 0, 20, 40, 50, 70, 100, 120, 150, 200, 250, 300, 350, and 400 mg. Generally, dose was reduced 1 step at a time, but may have been reduced by 2 dose steps as needed. The maximum dose was set at 3.0 mg/kg, but could be increased to 3.5 mg/kg with approval from sponsor. If Hb level has increased by >1.5 g/dL from the baseline Hb value during the first 21 days of dosing, dose was reduced by 1 step (eg, reduction from 100 to 70 mg). If Hb level increased by >2.0 g/dL during any 2-week period, dose was reduced by 1 dose step.

Abbreviations: ΔHb, change in hemoglobin; Hb, hemoglobin.

The efficacy-evaluable population in both part 1 (6-week study) and part 2 (19-week study) included eligible participants who received treatment for 4 or more weeks with corresponding Hb measurements that had not resulted from any rescue therapies (IV iron or red blood cell transfusion) or inadvertent use of ESAs. The efficacy-evaluable population also included all participants who permanently discontinued study medication during the dosing period due to lack of efficacy. A post hoc intention-to-treat analysis was also performed on the primary end point for participants in parts 1 and 2.

The safety and efficacy analyses presented here were based on pooled roxadustat-treated participants across the various dose cohorts and compared with pooled IV epoetin-alfa participants. Mixed model of repeated measurements and/or analysis of covariance/nonparametric models were used for continuous end points, and χ^2 or Fisher exact tests, for categorical end points. All alternative hypotheses and statistical comparisons were 2 sided ($\alpha = 0.05$). No adjustments for multiplicity were made due to the exploratory nature of the analysis. All null hypotheses were no treatment difference unless specified otherwise. Results are presented as mean ± standard deviation (SD) or mean ± standard error of the mean.

The effect of inflammation on dose needs was examined by correlating (linear regression) maintenance dose requirements of epoetin alfa or roxadustat to C-reactive protein (CRP) level, a surrogate for inflammatory state, at baseline (average of last 3 pre−first-dose values) or last 7 of 19 weeks' averages (maintenance phase), respectively, in all efficacy-evaluable participants randomly assigned to 19 weeks of roxadustat treatment and dosed beyond 12 weeks, with valid pre-enrollment epoetin alfa dose data and valid baseline and maintenance phase CRP data (n = 49).

All safety data were tabulated using descriptive statistics.

RESULTS

Participant Disposition and Characteristics

A total of 144 participants with baseline stable epoetin alfa doses were randomly assigned (3:1) to roxadustat or epoetin alfa; all were dosed and constituted the safety population. Part 1 comprised 54 participants treated for 6 weeks (41 roxadustat and 13 epoetin alfa); part 2 comprised 90 participants treated for 19 weeks (67 roxadustat and 23 epoetin alfa). Reasons for discontinuation from the study were lack of efficacy (n = 10), withdrawal of consent (n = 4), AE/serious AE (SAE; n = 6; including 3 deaths), 3 protocol violations, and 3 others (leaving center, prolonged hospitalization, and kidney transplantation). There were 125 participants who were efficacy-evaluable (flow diagram available as Fig S1). In general, demographics and baseline characteristics for the randomization arms were statistically similar (Table 3). Mean baseline Hb levels for treatment arms ranged from 11.2 to 11.5 g/dL.

Efficacy During 6 Weeks of Treatment

In part 1, a total of 41 participants were randomly assigned to 1 of 4 roxadustat dose cohorts (1.0, 1.5, 1.8 [this cohort was only partly filled], and 2.0 mg/kg thrice weekly), and 13 participants, to the epoetin alfa control arm. The primary end point was Hb level of −0.5 g/dL or greater from baseline by the end of 6 weeks. Roxadustat had a dose-response effect on Hb levels (Fig 2A). The cohort with the lowest roxadustat dose (1.0 mg/kg) was comparable to epoetin alfa with an Hb level responder rate of 44% as compared to 33%, respectively. Roxadustat doses ≥ 1.5 mg/kg maintained a higher responder rate than epoetin alfa, with a pooled responder rate of 79% ($P = 0.03$; Fig 2A). Intention-to-treat analysis showed similar results: 63% of all roxadustat participants versus 31% of epoetin alfa participants ($P = 0.06$).

Overall, mean change from baseline in Hb level (ΔHb) after 6 weeks was an increase of 0.3 g/dL in roxadustat-treated participants versus a decrease of 1.0 g/dL in the epoetin alfa group. Mean ΔHb values were significantly greater for 1.5 mg/kg thrice weekly (+0.9 g/dL; $P = 0.03$) and 2.0 mg/kg of roxadustat thrice weekly (+0.7 g/dL; $P = 0.04$) than in the epoetin alfa group. Five of 41 (12%) roxadustat-treated and 2 of 13 (15%) epoetin alfa–treated participants received IV iron for rescue during the treatment phase.

Efficacy During 19 Weeks of Treatment

In part 2, a total of 67 participants were randomly assigned to the remainder of Cohort A-4 (1.8 mg/kg) and 6 additional roxadustat cohorts (Table 1), and 23 participants were randomly assigned to continue epoetin alfa treatment. For roxadustat overall, 31 of 61 (51%) efficacy-evaluable participants achieved

Table 3. Subject Demographics and Baseline Characteristics: Safety Population and 6- and 19-Week Treatment Durations

Characteristic	6-wk Treatment Duration		19-wk Treatment Duration	
	Roxadustat (n = 41)	Epoetin alfa (n = 13)	Roxadustat (n = 67)	Epoetin alfa (n = 23)
Age, y	55.8 ± 13.4	59.5 ± 10.1	56.9 ± 12.1	57.0 ± 11.6
Age range, y	20-79	39-75	20-80	29-78
Male sex	27 (66%)	9 (69%)	45 (67%)	14 (61%)
Weight, kg	83.4 ± 19.0	78.7 ± 18.1	86.6 ± 22.5	84.3 ± 23.4
Race				
White	27 (66%)	5 (39%)	35 (52%)	6 (26%)
Black	13 (32%)	7 (54%)	29 (43%)	12 (52%)
Asian	1 (2%)	1 (8%)	1 (2%)	3 (13%)
Other	0 (0%)	0 (0%)	2 (3%)	2 (9%)
Prevalence[a] of				
Hypertension	40 (98%)	13 (100%)	66 (100%); n = 66[b]	22 (100%); n = 22[b]
Diabetes	28 (68%)	10 (77%)	39 (59%); n = 66[b]	14 (64%); n = 22[b]
Baseline epoetin alfa dose, IU/kg/wk	138.3 ± 51.3; n = 37	136.3 ± 47.7; n = 13	152.8 ± 80.6; n = 66	173.4 ± 83.7; n = 22
Hb, g/dL	11.3 ± 0.6	11.5 ± 0.6	11.2 ± 0.7	11.2 ± 1.0
Ferritin, μg/mL	912.5 ± 419.3	875.6 ± 449.8	827.7 ± 474.3	1,065.8 ± 657.2
TSAT, %	30.4 ± 8.0	30.4 ± 10.2	29.2 ± 10.0	28.1 ± 14.4

Abbreviations: Hb, hemoglobin; TSAT, transferrin saturation.
[a]Based on the occurrence of diabetes and hypertension reported on the Medical History Case Report Form.
[b]Presented as N and % of total with available data followed by presentation of the total number of patients for whom data was available.

average Hb levels ≥ 11.0 g/dL over the last 4 weeks of the 19-week treatment period (primary end point) compared with 8 of 22 (36%) epoetin alfa–treated participants. Analysis of the intention-to-treat set was similar: 33 of 67 (49%) roxadustat-treated participants compared with 8 of 23 (35%) epoetin alfa–treated participants. Roxadustat-treated participants (individual dose cohorts and overall) treated for 19 weeks did not significantly differ from epoetin alfa–treated participants in ΔHb at any time point out to 19 weeks (mixed model-repeated measures; Fig 2B). For the pooled roxadustat dose cohorts, the least-squares-mean ΔHb was −0.5 ± 0.2 (SE) g/dL compared to −0.5 ± 0.3 g/dL in the epoetin alfa arm, and the least-squares-mean difference from the epoetin alfa arm in ΔHb was −0.03 g/dL (95% confidence interval for the difference from epoetin alfa in ΔHb, −0.39 to 0.33 g/dL). The mean roxadustat dose requirement for

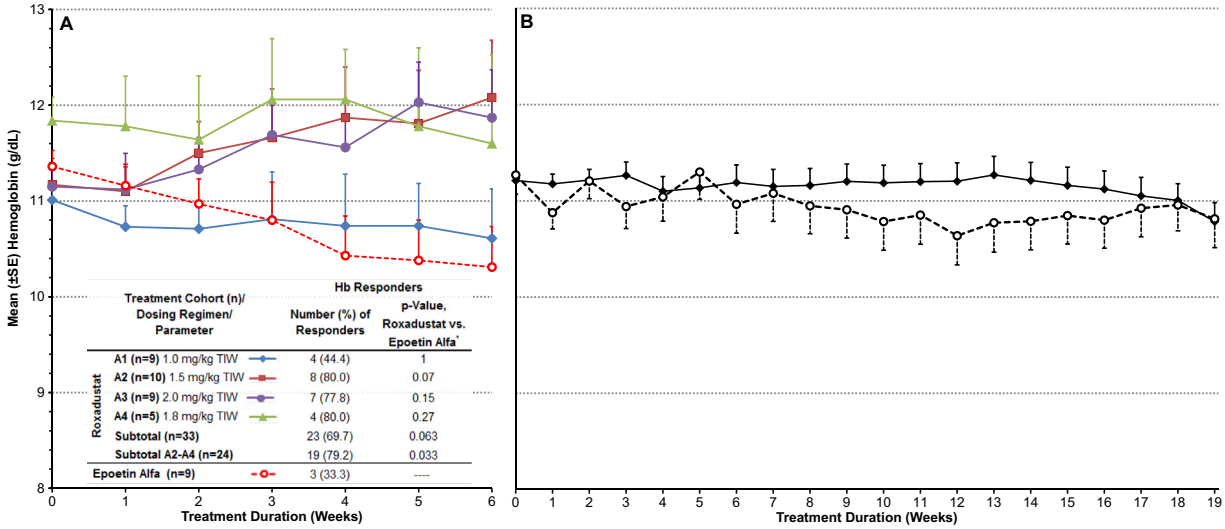

Figure 2. Hemoglobin levels over time (6 weeks) by treatment group. (A) Hb levels over time by dose cohort for participants randomly assigned to 6 weeks of treatment in part 1. Hb level responders are defined as the number (percent) of patients whose Hb levels did not decrease by >0.5 g/dL from their baseline (primary efficacy end point in part 1). (B) Least squares mean Hb levels over time (19 weeks), roxadustat-treated versus epoetin alfa–treated patients. Closed diamonds are roxadustat (n = 61); open circles are epoetin alfa (n = 22). **P* values are from Fisher exact test (2 sided) comparing roxadustat with epoetin alfa. Error bars signify standard error (SE) of the mean.

Hb level maintenance was 1.68 ± 0.65 (SD; range, 0.58-3.39) mg/kg thrice weekly, with starting doses being weight based or tiered weight based (Table S1). There were no significant differences in weekly doses over time among dose cohorts except for one cohort in which baseline epoetin alfa doses were 2-fold higher than the other cohorts. Two of 67 (3%) roxadustat-treated and 3 of 23 (13%) epoetin alfa–treated participants received IV iron for rescue ($P = 0.1$).

Efficacy in Participants With Versus Without Inflammation

We compared roxadustat versus epoetin alfa maintenance dose requirements in all participants randomly assigned to 19 weeks of roxadustat treatment and dosed beyond 12 weeks (maintenance phase) with valid pre-enrollment epoetin alfa dose data and valid baseline and maintenance phase CRP data (see Methods). As documented by others,[21] inflammation as reflected by baseline serum CRP levels was associated with increased pre-enrollment epoetin alfa maintenance dose requirements in these participants (Fig 3A; $P = 0.02$). However, in the same participants with Hb levels maintained with roxadustat beyond 12 weeks, average weekly roxadustat maintenance dose requirements during the last 7 weeks of therapy were not associated with CRP levels obtained concurrently (Fig 3B; $P = 0.7$).

Exploratory Measures

In a subgroup of 6 participants, EPO was measured prerandomization following IV epoetin alfa injection and then compared with endogenous EPO levels during treatment with roxadustat (Fig 4A). Mean peak EPO levels in participants receiving epoetin alfa (median dose, 90 U/kg/wk) were ∼700 mIU/mL compared to a peak of levels of ∼130 mIU/mL at 12 hours in participants receiving a mean roxadustat dose of 1.3 mg/kg.

A progressive decrease in hepcidin levels was observed in participants treated at higher doses of roxadustat for 6 weeks (part 1: Fig 4B; Table 4), compared to epoetin alfa. Reduction of hepcidin levels was noted at the end of the 19-week treatment; overall mean change from baseline in roxadustat-treated participants was −60.4 ± 187.8 (SD) ng/mL (n = 46) versus +35.6 ± 123.4 ng/mL (n = 18) in the epoetin alfa–treated pool ($P = 0.04$). Reticulocyte Hb content was maintained at statistically higher than baseline levels through most of the roxadustat treatment duration, whereas this phenomenon was transient for 3 weeks only with epoetin alfa (Fig 4C). Transferrin saturation and serum iron levels were not different in the treatment groups.

Total cholesterol levels (nonfasting) were reduced during roxadustat treatment but were without significant change in participants continuing epoetin alfa treatment (Fig 4D).

Safety

AE and SAE rates were consistent with background disease of this ESRD population. In the safety population, 69 of 108 (63.9%) roxadustat-treated and 22 of 36 (61%) epoetin alfa–treated

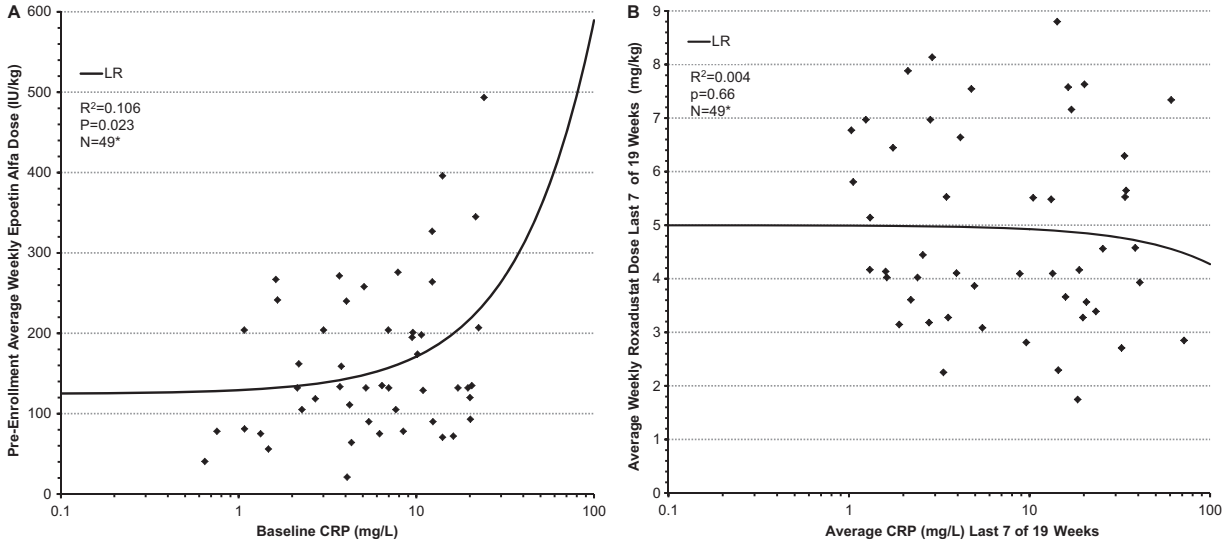

Figure 3. Baseline C-reactive protein (CRP) levels are correlated with (A) pre-enrollment epoetin alfa but not (B) roxadustat maintenance dose requirements. *N = 49: all participants randomly assigned to 19 weeks of roxadustat treatment and dosed beyond 12 weeks (maintenance phase) with valid baseline epoetin alfa dose data and valid baseline and average last 7 of 19 weeks of CRP data. Thus, this analysis did not include the 9 patients discontinued from roxadustat treatment for lack of efficacy (see Fig S1). Baseline CRP level was the average of the last 3 values prior to the first dose of study drug. CRP is plotted on the x-axis using a logarithmic scale. Abbreviation: LR, linear regression.

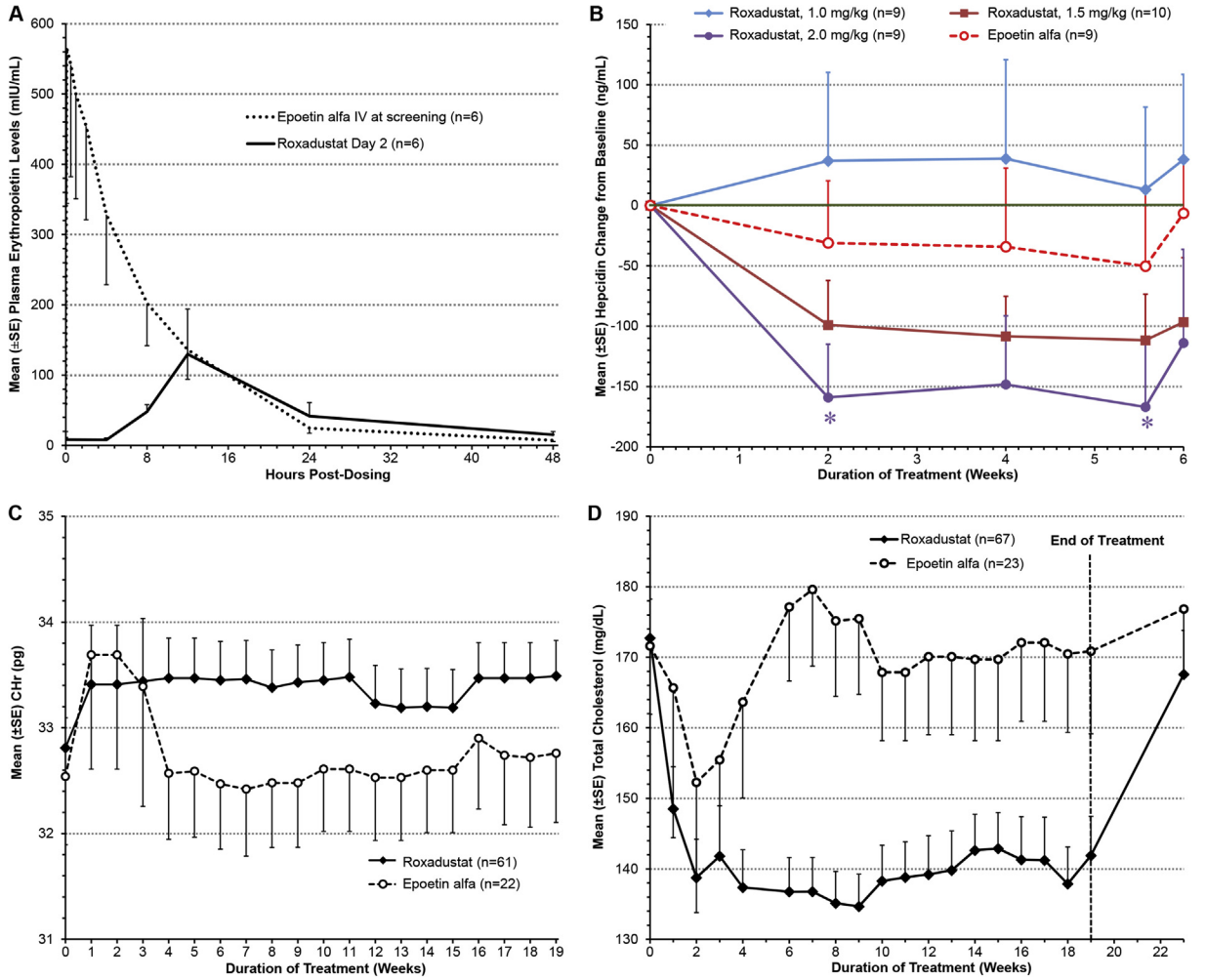

Figure 4. Pharmacodynamic effects of roxadustat compared to epoetin alfa. Error bars signify standard error of the mean. (A) Mean plasma erythropoietin levels during treatment with roxadustat compared to prior epoetin alfa dosing in the same patients (n = 6). (B) Change in hepcidin level (ng/mL) from baseline during 6 weeks of treatment in the 6-week cohorts with the largest sample sizes (n > 5). *$P < 0.05$ (comparing hepcidin change from baseline between the 2.0-mg/kg roxadustat group and the epoetin alfa group). (C) Mean reticulocyte hemoglobin content (CHr) over time in roxadustat- versus epoetin alfa–treated participants randomly assigned to 19 weeks of treatment (last observation carried forward [LOCF], efficacy-evaluable population). (D) Total cholesterol levels over time in roxadustat- versus epoetin alfa–treated participants randomly assigned to 19 weeks of treatment (LOCF, safety population).

participants had at least 1 AE. Thirty-two of 144 (22.2%) participants in the safety population had a total of 50 treatment-emergent SAEs (Table 5). Of roxadustat-treated participants, 26 of 108 (24.1%) had at least 1 SAE. The only SAE considered as possibly related to roxadustat treatment was acute pancreatitis, diagnosed 2 days after the patient completed 43 days of treatment. Both the sponsor and the independent data monitoring committee considered it unrelated because of a potential alternative cause (detailed under Table 5) and the patient had a recurrent episode 28 months after the last dose of study medication. Of epoetin alfa–treated participants, 6 of 36 (17%) had at least 1 SAE.

Exploratory safety analysis of a composite safety event consisting of death, myocardial infarction, stroke, heart failure requiring hospitalization, unstable angina requiring hospitalization, or thromboembolism in the 19-week participants defined post hoc showed a numerically lower rate in roxadustat-treated as compared with epoetin alfa–treated participants. Among participants in the roxadustat group, 8 of 66 (12%) as compared to 4 of 23 (17%) receiving epoetin alfa had a composite safety event. Three roxadustat-treated participants died during the study: a 68-year-old man with known heart failure and coronary and peripheral arterial disease had an unwitnessed death, a 77-year-old man with methicillin-resistant *Staphylococcus aureus* sepsis, and a 58-year-old man with preexisting allergy to dialysis and cardiovascular disease (acute myocardial infarction, stent, and transient ischemic attacks) prior

Table 4. Change From Baseline in Iron Use Parameters, Last Observation Carried Forward

	6 wk		19 wk	
	Roxadustat (n = 33)	Epoetin alfa (n = 9)	Roxadustat (n = 61)	Epoetin alfa (n = 22)
Ferritin, ng/mL				
Baseline	917.3 ± 458.0	929.7 ± 494.2	826.8 ± 484.5	1,106.6 ± 642.1
End of treatment	731.8 ± 375.8	783.2 ± 484.2	637.3 ± 426.2	895.0 ± 888.7
Change from baseline	−185.5 ± 190.5	−146.5 ± 180.7	−201.1 ± 334.4	−211.6 ± 445.2
P[a]	0.5	—	0.8	—
TSAT, %				
Baseline	30.1 ± 8.2	31.5 ± 11.5	29.2 ± 10.2	28.6 ± 14.6
End of treatment	27.5 ± 14.7	24.4 ± 8.4	27.1 ± 16.3	23.3 ± 9.6
Change from baseline	−2.5 ± 13.7	−7.0 ± 4.1	−2.4 ± 18.9	−5.3 ± 12.5
P[a]	0.4	—	0.4	—
Serum iron, μg/dL				
Baseline	70.4 ± 20.9	70.2 ± 27.2	66.4 ± 20.6	63.3 ± 32.0
End of treatment	77.5 ± 34.6	56.2 ± 24.0	72.1 ± 39.7	57.8 ± 23.3
Change from baseline	7.1 ± 33.9	−14.0 ± 11.1	5.2 ± 42.2	−5.5 ± 30.2
P[a]	0.07	—	0.1	—
TIBC, μg/dL				
Baseline	210.8 ± 41.3	200.8 ± 30.9	199.7 ± 34.0	202.1 ± 26.7
End of treatment	261.8 ± 48.7	205.8 ± 39.7	236.5 ± 44.5	227.6 ± 54.6
Change from baseline	51.0 ± 27.4	5.0 ± 26.4	37.6 ± 41.4	25.6 ± 47.3
P[a]	<0.001	—	0.3	—
sTfR, mg/L				
Baseline	2.74 ± 0.86	3.25 ± 0.76	4.03 ± 1.81	3.69 ± 0.93
End of treatment	3.43 ± 1.54	3.44 ± 1.07	4.46 ± 2.64	3.34 ± 1.65
Change from baseline	0.69 ± 1.54	0.20 ± 0.73	0.86 ± 2.69	−0.33 ± 1.52
P[a]	0.6	—	0.2	—
MCV, fL				
Baseline	98.7 ± 5.3	99.0 ± 6.8	99.2 ± 6.3	99.5 ± 8.8
End of treatment	99.9 ± 6.0	99.1 ± 7.2	97.9 ± 6.9	97.2 ± 9.7
Change from baseline	1.2 ± 4.0	0.1 ± 3.7	−1.3 ± 4.3	−2.3 ± 5.0
P[a]	0.5	—	0.4	—
CHr, pg				
Baseline	32.0 ± 1.5	31.8 ± 2.6	32.8 ± 2.2	32.5 ± 3.0
End of treatment	32.3 ± 1.9	31.1 ± 2.0	33.5 ± 2.6	32.8 ± 3.1
Change from baseline	0.3 ± 1.4	−0.8 ± 1.0	0.7 ± 1.3	0.2 ± 1.4
P[a]	0.04	—	0.2	—
Hepcidin, ng/mL				
No.	33	9	46	18
Baseline	236.6 ± 159.7	279.3 ± 137.6	327.1 ± 178.8	298.7 ± 123.1
End of treatment	197.5 ± 155.9	272.8 ± 215.9	266.7 ± 170.9	334.3 ± 190.5
Change from baseline	−39.2 ± 226.9	−6.5 ± 140.1	−60.4 ± 187.8	35.6 ± 123.4
P[b]	0.3	—	0.04	—

Note: Values given as mean ± standard deviation.

Abbreviations: CHr, reticulocyte Hb content; MCV, mean corpuscular volume; sTfR, soluble transferrin receptor; TIBC, total iron-binding capacity; TSAT, transferrin saturation.

P values are from [a]analysis of covariance model or [b]Kruskal-Wallis test, comparing roxadustat change from baseline with epoetin alfa change from baseline.

to enrollment who presented volume overloaded with respiratory failure in whom dialysis was delayed, leading to cardiac arrest. All 3 had significant pre-existing cardiovascular risks. None of the deaths was considered related to roxadustat.

DISCUSSION

This phase 2 trial is the first roxadustat study in patients with ESRD on stable hemodialysis therapy whose Hb levels were maintained with epoetin alfa and suggests that roxadustat can maintain Hb levels

for periods up to 19 weeks. Because of prerandomization IV iron use, we realize that differences in iron metabolic values or trends could be masked following the exclusion of IV iron for up to 19 weeks in both groups.

The 6-week dose range portion of this conversion study in hemodialysis patients revealed a dose-dependent Hb level response to roxadustat. The Hb effect of a 1.0-mg/kg dose of roxadustat was comparable to, while doses ≥ 1.5 mg/kg were more effective than epoetin alfa, with a pooled Hb level response at 6 weeks of 79% versus 33%, respectively. In subsequent clinical development, starting doses ≥ 1.5 mg/kg will need to take into account initial Hb level response during the period of fixed dose and the number of dose adjustments required after 4 weeks. The roxadustat dose for Hb level maintenance ranged from 0.5 to 3.4 (mean dose, ∼1.7) mg/kg thrice weekly. The effect of roxadustat on Hb level maintenance was durable over the 19-week treatment period of this study. The overall least-squares-mean ΔHb during study weeks 16 to 19 of roxadustat-treated patients was comparable to that in the epoetin alfa arm. Based on these results and historical ESA phase 3 studies,[22] roxadustat appears to be effective and comparable to epoetin alfa in maintaining Hb levels in patients with ESRD receiving hemodialysis. In individuals with higher baseline epoetin alfa dose requirements (eg, cohort A-5), a somewhat higher roxadustat dose requirement was noted, whereas the range of roxadustat dose requirements was not as large as observed for epoetin alfa. A longer study is needed to determine the durability of response in such patients and titration/dose needs.

An important difference between roxadustat and epoetin alfa is the relationship of dose to the inflammatory parameter CRP. Inflammation increases an ESRD patient's dose requirements for epoetin alfa, whether during correction or maintenance.[21] Resistance to ESAs appears to be mediated in part by elevated hepcidin levels,[10,12] which are increased in inflammation, limiting iron availability. Hepcidin impairs both iron absorption from the duodenal enterocyte and iron release from macrophages, where most iron is stored. This impairment of iron metabolism explains why IV iron produces very high ferritin levels, reflecting preferential accumulation in macrophages. Egress is impaired because hepcidin inactivates ferroportin, the sole iron-exporting process in mammalian cells. This impairment in iron export is an important mechanism leading to the increase in epoetin alfa requirements during inflammation, in effect producing functional iron deficiency and accounting for the linear correlation of epoetin alfa doses with baseline serum CRP levels in the epoetin alfa group. By contrast, roxadustat maintenance dose requirements were independent of baseline CRP levels. This independence of response from CRP levels suggests the potential for roxadustat to overcome the therapeutic barrier to erythropoiesis from the inflammatory component present in ESRD.

Furthermore, Hb levels in the epoetin alfa group appeared to decline gradually over time when IV iron supplementation was discontinued despite the increase in epoetin alfa doses to maintain Hb levels.[23-25] The IV iron exclusion permitted assessment of the impact of roxadustat compared to epoetin on hepcidin and iron delivery indexes to the erythron (reticulocyte Hb content, mean corpuscular volume, total iron-binding capacity, and soluble transferrin receptor) in the coordination of erythropoiesis despite marked differences in the relative elevations in plasma EPO levels induced. Despite high mean ferritin levels > 800 ng/mL, larger declines in transferrin saturation, reticulocyte Hb content, and serum iron levels occurred in the epoetin alfa–treated than roxadustat-treated participants. This is consistent with roxadustat's expected positive impact on iron availability.

In a subset of 6 participants, plasma EPO levels following an oral dose of roxadustat were compared with those following an IV dose of epoetin alfa prior to randomization. Following IV epoetin alfa, average plasma EPO levels increased more than 30-fold compared with those seen in healthy persons at sea level.[26] These supraphysiologic levels after exogenous epoetin alfa administration occurred even at doses equivalent to the lowest epoetin alfa dose quartile of hemodialysis patients.[27,28] In contrast, within the same participant, roxadustat treatment provided modest transient postdose endogenous EPO levels, which peaked at about one-sixth of the level observed with exogenous epoetin alfa dosing. The levels observed are similar to those reported in a previous pharmacokinetics trial.[19] Moreover, these lower baseline endogenous EPO levels were effective in maintaining Hb levels during the study and are consistent with the physiologic EPO response in persons under hypoxic conditions[29] or following an acute decrease in Hb level.[30,31] The postdose plasma EPO levels measured in the active comparator (epoetin alfa) arm may be unique to this specific agent and IV route of administration, and different routes of administration such as subcutaneous or different agents such as continuous erythropoietin receptor activator (CERA) or darbepoetin alfa may result in different pharmacokinetic profiles in this setting.

Roxadustat as compared to epoetin alfa treatment resulted in different plasma EPO levels, with roxadustat exposing patients to modest levels of endogenous EPO within or near physiologic range. In contrast, the peak and area under the curve of plasma EPO concentrations at the mean of the lowest dose

Table 5. SAEs by Treatment Group

	Roxadustat (n = 108)	Epoetin alfa (n = 36)
No. with ≥1 SAE	26 (24.1)	6 (17)
System organ class and preferred term		
Infections and infestations	8 (7.4)	3 (8)
Gastroenteritis	2 (1.9)	0 (0)
Cellulitis	1 (0.9)	0 (0)
Diabetic gangrene	1 (0.9)	0 (0)
Endocarditis bacterial	1 (0.9)	0 (0)
Pneumonia	1 (0.9)	1 (3)
Sepsis	1 (0.9)	0 (0)
Gangrene	1 (0.9)	1 (3)
Infection	0 (0)	1 (3)
Metabolism and nutrition disorders	5 (4.6)	4 (11)
Fluid overload	2 (1.9)	1 (3)
Hyperkalemia	1 (0.9)	2 (6)
Diabetic ketoacidosis	1 (0.9)	0 (0)
Hypocalcemia	1 (0.9)	0 (0)
Hypokalemia	1 (0.9)	0 (0)
Diabetes mellitus inadequate control	0 (0)	1 (3)
Hyperglycemia	0 (0)	1 (3)
Cardiac disorders	4 (3.7)	2 (6)
Acute myocardial infarction	2 (1.9)	0 (0)
Cardiac failure congestive	1 (0.9)	1 (3)
Cardiorespiratory arrest	1 (0.9)	0 (0)
Cardiac arrest	0 (0)	1 (3)
Coronary artery disease	0 (0)	1 (3)
Myocardial infarction	0 (0)	1 (3)
Gastrointestinal disorders	3 (2.8)	0 (0)
Gastrointestinal hemorrhage	1 (0.9)	0 (0)
Pancreatitis acute[a]	1 (0.9)	0 (0)
Nausea	1 (0.9)	0 (0)
Vomiting	1 (0.9)	0 (0)
Injury, poisoning and procedural complications	2 (1.9)	0 (0)
Arteriovenous fistula site hemorrhage	1 (0.9)	0 (0)
Vascular graft complication	1 (0.9)	0 (0)
Nervous system disorders	2 (1.9)	0 (0)
Cerebrovascular accident	2 (1.9)	0 (0)
Complex partial seizures	1 (0.9)	0 (0)
Respiratory, thoracic, and mediastinal disorders	2 (1.9)	1 (3)
Chronic obstructive pulmonary disease	1 (0.9)	0 (0)
Pulmonary edema	1 (0.9)	0 (0)
Dyspnea	0 (0)	1 (3)
General disorders and administration site conditions	1 (0.9)	0 (0)
Sudden cardiac death	1 (0.9)	0 (0)
Immune system disorders	1 (0.9)	0 (0)
Hypersensitivity	1 (0.9)	0 (0)
Musculoskeletal and connective tissue disorders	1 (0.9)	0 (0)
Musculoskeletal chest pain	1 (0.9)	0 (0)
Neoplasms benign, malignant, and unspecified[b]	1 (0.9)	0 (0)
Thyroid neoplasm	1 (0.9)	0 (0)
Psychiatric disorders	1 (0.9)	0 (0)
Major depression	1 (0.9)	0 (0)
Skin and subcutaneous tissue disorders	1 (0.9)	0 (0)
Subcutaneous emphysema	1 (0.9)	0 (0)

(Continued)

Table 5 (Cont'd). SAEs by Treatment Group

	Roxadustat (n = 108)	Epoetin alfa (n = 36)
Blood and lymphatic system disorders	0 (0)	1 (3)
Anemia	0 (0)	1 (3)
Vascular disorders	0 (0)	1 (3)
Peripheral vascular disorder	0 (0)	1 (3)

Note: Values given as number (percentage). For system organ class incidence, SAEs of the same system organ class in the same patient are counted once only.

Abbreviation: SAE, serious adverse event.

[a]SAE of acute pancreatitis. This SAE occurred in a 40-year-old man with diabetes mellitus, hyperparathyroidism, hypertension, dyslipidemia, kidney stones, gallstones, and status post cholecystectomy who was a smoker (10 years) and also taking lisinopril and hydrocodone bitartrate plus acetaminophen concomitantly. He presented with a 1-week history of abdominal pain 2 days after his last dose of a 43-day course of roxadustat and acute pancreatitis was diagnosed. Lisinopril and hydrocodone bitartrate plus acetaminophen were stopped, and the patient was hospitalized. The episode of pancreatitis resolved in 8 days. Another episode of pancreatitis in this patient was reported 28 months after the last dose of study medication. Although the investigator considered the SAE of acute pancreatitis as possibly related to roxadustat, both the sponsor and the independent data monitoring committee considered it unrelated because of lack of temporal relationship, potential alternative causes, and the recurrence of pancreatitis when off roxadustat therapy for 28 months. All other SAEs were considered by investigators and sponsor to be unrelated to study medication.

[b]Including cysts and polyps.

quartile of exogenous epoetin alfa led to much higher levels of exposure.[28] Although ESA dose has been implicated as part of the pathway leading to excessive mortality and cardiovascular events when targeting higher Hb levels, particularly in patients with inflammation and hyporesponsive patients,[8,32] it has not been proved if this is mediated through higher EPO levels. The potential of roxadustat as a novel therapeutic agent alternative to ESAs to safely treat to a higher Hb level without the need for supraphysiologic epoetin alfa doses might be suggested by 2 observations. First, patients who achieve higher Hb levels at lower ESA doses have lower morbidity and better survival.[33] Second, when Hb levels are $>$ 12.5 g/dL, with a mean Hb level of 13.5 g/dL, and are spontaneously maintained by endogenous EPO production, no increased risk was shown in more than 600 dialysis patients.[34] Although this potential cannot be evaluated within the context of this phase 2 trial, it will be further explored in phase 3.

Another important difference is the effect of roxadustat on serum cholesterol levels. The potential cholesterol-lowering effect of roxadustat was independent of statin use and may be mediated in part by the effects of HIF on degradation 3-hydroxy-3-methylglutaryl coenzyme A reductase, a rate-limiting enzyme in cholesterol biosynthesis.[35] Cholesterol reduction has been reported during high altitude exposure itself.[36]

Observations from the current study suggest no obvious safety concerns because the AEs and SAEs reported are consistent with the dialysis patient population. The exploratory composite safety event rate in the 19-week participants was numerically lower in roxadustat-treated compared with epoetin alfa–treated participants.

Limitations of this phase 2 study include its relatively short treatment duration and small sample size. As is true for all phase 2 programs, these limitations may have precluded the identification of a greater risk for uncommon events.[37] A much larger sample size with longer treatment duration would be needed to provide adequate power for manifestation of such toxicities and comparison of cardiovascular safety between roxadustat and ESAs.

In summary, roxadustat, an oral HIF-PHI, was shown to maintain Hb levels in maintenance hemodialysis patients with ESRD receiving a wide range of epoetin alfa doses without IV iron in the short term. Roxadustat also reduced hepcidin levels and regulated iron metabolism. Further development in adequate and well-controlled phase 3 studies is in progress (NCT02273726).

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Contributions: Research idea and study design: RP, AB, KHPY; data collection: KHPY; data analysis/interpretation: AB, SH, LS, KHPY, TBN; statistical analysis: KGS, AB; study management and

medical monitoring: LP, GS; supervision or mentorship: KHPY, TBN; contribution of patients to study and analysis: RP, AB, SW, SD, SZ, PN. Each author contributed important intellectual content during manuscript drafting or revision and accepts accountability for the overall work by ensuring that questions pertaining to the accuracy or integrity of any portion of the work are appropriately investigated and resolved. KHPY takes responsibility that this study has been reported honestly, accurately, and transparently; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned and registered have been explained.

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SUPPLEMENTARY MATERIAL

Table S1: Dose requirement for Hb maintenance for all roxadustat-treated in 19-wk cohorts.

Figure S1: Participant disposition for normoresponders.

Item S1: Detailed inclusion and exclusion criteria.

Item S2: Rescue therapy.

Item S3: Explanation of corrections incorporated into the article in press.

Note: The supplementary material accompanying this article (http://dx.doi.org/10.1053/j.ajkd.2015.12.020) is available at www.ajkd.org

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