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ORIGINAL RESEARCH

Roxadustat and Oral Iron Absorption in Chinese Patients with Anemia of Chronic Kidney Disease: A Randomized, Open-Label, Phase 4 Study (ALTAI)

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ABSTRACT

Introduction: Anemia of chronic kidney disease (CKD) has a high incidence and is associated with many disease conditions. Iron dysmetabolism is an important contributor to anemia in CKD patients.

Methods: ALTAI, a randomized, active-controlled, phase 4 trial, investigated the efficacy of roxadustat versus recombinant human erythropoietin (rHuEPO) on gastrointestinal iron absorption in patients with anemia of CKD (stage 4/5). The primary endpoint was change from baseline to day 15 in gastrointestinal iron absorption (serum iron area under the concentration-time curve; $AUC_{0–3h}$) following single-dose oral iron.

Results: Twenty-five patients with a mean age of 55.1 years were randomized 1:1 to roxadustat (n = 13) or rHuEPO (n = 12). Baseline iron

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profiles were similar between treatment groups. Change from baseline to day 15 in serum iron $AUC_{0–3h}$ was not statistically significantly different between the roxadustat and rHuEPO groups. Mean (SD) change from baseline in serum iron $AUC_{0–3h}$ was 11.3 (28.2) g × 3 h/dl in the roxadustat group and − 0.3 (9.7) g × 3 h/dl in the rHuEPO group. Roxadustat treatment was associated with decreased hepcidin and also increased transferrin, soluble transferrin receptor, and total iron-binding capacity (TIBC), with nominal significance. The proportion of patients experiencing one or more adverse events was 38.5% when treated with roxadustat and 16.7% with rHuEPO.

Conclusions: The study showed no significant difference between roxadustat and rHuEPO in iron absorption but was underpowered because of recruitment challenges.

Trial Registration: ClinicalTrials.gov Identifier NCT04655027.

Keywords: Anemia of chronic kidney disease; Dialysis; Erythropoietin; Iron absorption; Roxadustat

Key Summary Points

Why carry out this study?
Roxadustat administration has been associated with decreased serum hepcidin levels and increased total iron-binding capacity (TIBC) and/or serum transferrin, but its effect on iron absorption in chronic kidney disease (CKD) is unclear
This study investigated the effect of roxadustat on gastrointestinal iron absorption in patients with anemia of CKD
What was learned from the study?
In this smaller-than-planned study, no significant difference was seen in iron absorption between roxadustat and rHuEPO although roxadustat showed a trend towards greater absorption promoting ability
Larger, well-designed, and appropriately controlled clinical trials are needed to evaluate any roxadustat-mediated benefit of enhanced iron absorption in patients with CKD-related anemia
Although underpowered, the findings are consistent with prior reports of reduction in hepcidin and increase in transferrin and TIBC seen with roxadustat compared with erythropoietin-treated patients

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INTRODUCTION

Anemia is associated with many health problems [1–3]. Both absolute iron deficiency and dysfunctional iron homeostasis contribute to anemia in CKD patients [2, 4, 5]. Inappropriately high levels of hepcidin expression in particular can significantly restrict erythropoiesis [6]. Current standard of care for anemia in patients with CKD is based on iron supplementation and/or erythropoietin (EPO) therapy [7, 8]. However, these therapies do not correct the underlying iron dysmetabolism associated with anemia of CKD [9].

Hypoxia-inducible factor-prolyl hydroxylase domain inhibitors (HIF-PHIs) are a new class of orally administered drugs for the treatment of anemia of CKD. HIF-PHIs activate the HIF oxygen-sensing pathway and are efficacious in correcting and maintaining hemoglobin (Hb) levels in CKD patients. In addition to promoting erythropoiesis through the increase in endogenous EPO production, HIF-PHIs have been shown to modulate iron metabolism, reduce hepcidin levels, provide increases in total iron-binding capacity (TIBC) and

transferrin levels, and potentially reduce the need for intravenous (IV) iron supplementation [10–22]. However, there is no sufficient direct evidence of iron absorption in patients with CKD treated with HIF-PHIs. Dedicated studies are therefore needed to establish the extent to which HIF-PHIs may impact iron absorption, providing more information for future iron management.

Roxadustat is an HIF-PHI indicated in several countries including China [10, 23, 24]. Phase 1 and 2 studies suggested that roxadustat ameliorates many of the abnormalities of iron dysmetabolism in CKD [25, 26]. Moreover, the efficacy and safety of roxadustat were demonstrated in phase 3 studies in > 13,000 patients with anemia of CKD [27–32].

This phase 4 study (ALTAI, clinicaltrials.gov identifier: NCT04655027) was designed to investigate changes in gastrointestinal iron absorption with roxadustat in patients with anemia of CKD in the Chinese population.

METHODS

Further information is provided in the supplementary materials.

Study Design

ALTAI was a phase 4, randomized, active-controlled, open-label, parallel design, prospective study conducted in multiple sites in China comparing the effect of roxadustat (oral tablets) and rHuEPO [either IV or subcutaneous (SC)] on gastrointestinal iron absorption in patients with anemia of stage 4 and 5 CKD (NCT04655027). Eligible patients were identified and enrolled by the investigator at each participating site from February 22, 2021, with the last patient visit on October 12, 2021. The study comprised a screening period (≤ 3 weeks), a treatment period of 2 weeks, and a follow-up period of 4 weeks (Fig. 1).

The study was conducted in accordance with the Declaration of Helsinki and Council for International Organizations of Medical Sciences International Ethical Guidelines, applicable International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use, Good Clinical Practice Guidelines, and applicable local health and regulatory requirements. All participants provided written informed consent. The final study protocol and informed consent form were approved by the

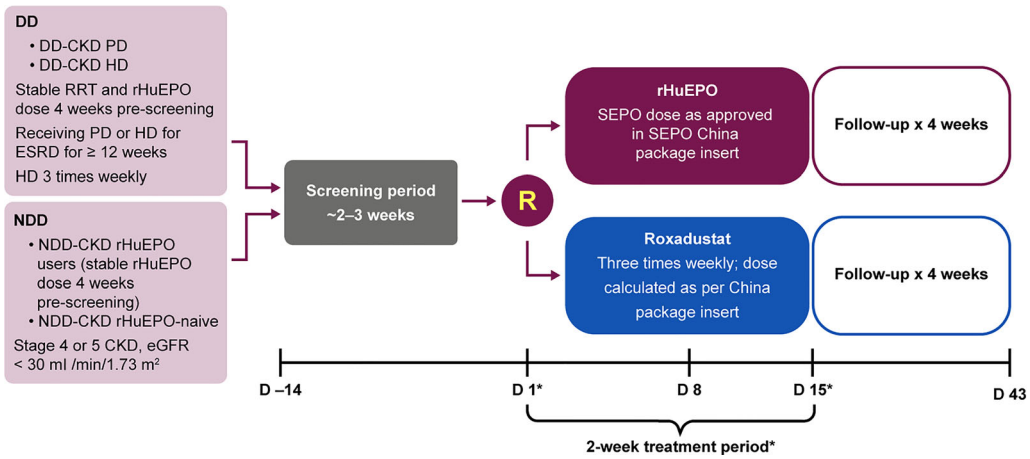

Fig. 1 Study design. *CKD* chronic kidney disease; *D* day; *DD* dialysis-dependent; *eGFR* estimated glomerular filtration rate; *ESRD* end-stage renal disease; *HD* hemodialysis; *NDD* non-dialysis-dependent; *PD* peritoneal dialysis; *R* randomization; *rHuEPO* recombinant human erythropoietin; *RRT* renal replacement therapy; *SEPO* short-acting rHuEPO; *TIBC* total iron-binding capacity. *On D 1 and D 15, TIBC and serum iron were measured at T0h (immediately before administration of a single oral dose of 100 mg elemental iron). Further measures of serum iron were made at times T1h, T2h, and T3h following oral iron ingestion

applicable independent ethics committee or institutional review board for each site (protocol D5741C00002; approved July 13, 2021).

To evaluate eligibility, Hb levels had to be assessed twice, ≥ 7 days apart, during the screening period and could be assessed up to three times during the screening period. Screening period assessments for transferrin saturation (TSAT), ferritin, vitamin B12, serum folate, alanine transaminase (ALT), aspartate aminotransferase (AST), high-sensitivity C-reactive protein (hs-CRP), and total bilirubin level (TBL) had to be available prior to starting the treatment period.

A ferrokinetic study was performed on day 1 and day 15, in which patients had blood samples taken for serum iron and TIBC immediately before administration of a single oral dose of 100 mg elemental iron (T0h); further samples for serum iron were taken at times T1h, T2h, and T3h following oral iron ingestion [allowing calculation of serum iron area under the concentration-time curve ($AUC_{0–3\ h}$)] (Fig. S1 in the supplementary material). The investigators aimed to minimize variability in relation to dialysis by mandating that the ferrokinetic study on Day 1 had to be completed prior to randomization and prior to the hemodialysis procedure at Days 1 and 15. For patients on peritoneal dialysis, there were no timing restrictions for dialysis in relation to timing of the ferrokinetic study. In addition, while patients could take roxadustat at any time before or after dialysis, on Day 1, roxadustat could be taken only following completion of the ferrokinetic study. On Day 15, roxadustat was taken ≥ 6 h before start of the ferrokinetic study. For patients already on rHuEPO, dosing of rHuEPO occurred on Day 1 but not before the randomization visit; if randomized to rHuEPO, administration of rHuEPO occurred following completion of the ferrokinetic study and on day 15 (latter within 1–2 h prior to start of the ferrokinetic study.

Patients taking oral iron before the study could continue to do this during the study, except on days 1 and 15; oral iron dose was not changed during the treatment period. Food restrictions were applied only on days 1 and 15 for the ferrokinetic study; on these days for ∼ 4 h before and 3 h during the ferrokinetic study, no ingestion of foods containing more than trace amounts of iron was allowed. Hemoglobin, erythrocyte count, and corpuscular volume were evaluated on days 1, 8, and 15.

Patients

Patients were eligible if aged ≥ 18 years and they met the following criteria at screening. Hemodialysis patients were required to be on three times weekly dialysis with evidence of adequate dialysis. Achievement of adequate dialysis was defined as a stdKt/V ≥ 2.1 in hemodialysis, and a total (renal + peritoneal dialysis) weekly Kt/V ≥ 1.7 documented twice during 16 weeks pre-screening. All dialysis patients must have been on a stable rHuEPO dose, had a mean Hb level of 9–12 g/dl, and had ferritin ≥ 100 ng/ml and TSAT ≥ 20%. For patients with non-dialysis-dependent CKD: stage 4 or 5 CKD; estimated glomerular filtration rate < 30 ml/min/1.73 m^2; on a stable dose of rHuEPO for 4 weeks before screening or rHuEPO-naive (no erythropoiesis-stimulating agent for > 6 weeks pre-screening); mean Hb level 9–12 g/dl for rHuEPO users and 7–10 g/dl for rHuEPO-naive; and ferritin ≥ 50 ng/ml and TSAT ≥ 15%. Key exclusion criteria were chronic inflammatory diseases, autoimmune liver disease, previous bowel resection, celiac disease, hereditary hematologic disease, gastroenteritis (in 4 weeks prior to randomization), history of severe liver disease, intolerance of oral iron, known hemosiderosis, and malignancy. Full inclusion and exclusion criteria are provided in the supplementary materials.

Study Treatment

Roxadustat doses were administered three times weekly ≥ 2 days apart, but ≤ 4 days apart, and the roxadustat dose was not adjusted during the study (except for safety reasons as judged by the investigator). The starting dose of roxadustat was calculated based on the patient's body weight, according to approved guidance [33]. In patients with dialysis-dependent CKD, initial doses were based on the patient's weight prior

to dialysis: 100 mg (patient weight, 45 to < 60 kg) or 120 mg (patient weight, ≥ 60 kg). Patients with non-dialysis-dependent CKD were dosed with 70 mg (body weight, 45 to < 60 kg) or 100 mg (body weight, ≥ 60 kg). All patients randomized to rHuEPO received a uniform brand of short-acting rHuEPO (SEPO) according to the approved dosage (see supplementary materials) [34]. Medications prohibited during the study included any rHuEPO treatment other than the study treatment, iron-chelating agents, IV iron, TRIFERIC® in dialysate, and vitamin C.

Endpoints

The primary endpoint was the change from baseline (day 1) to day 15 in gastrointestinal iron absorption (serum iron $AUC_{0–3\ h}$) following administration of a single dose of oral iron, compared between roxadustat and rHuEPO. Serum iron $AUC_{0–3\ h}$ was defined as the area between the serum iron concentration curve over hours 0 to 3 following ingestion of iron, relative to the concentration at T0h immediately before administration of a single oral dose of 100 mg elemental iron. The full definition and calculation of $AUC_{0–3\ h}$ is provided in the supplementary materials.

Secondary endpoints were: interaction effects of key baseline variables (hs-CRP and hepcidin) on change from baseline to day 15 in serum iron $AUC_{0–3\ h}$ following administration of a single dose of oral iron, compared between roxadustat and rHuEPO, and change from baseline in key indices of iron metabolism (serum iron, ferritin, TIBC, TSAT, transferrin, and soluble transferrin receptor) and hepcidin levels, and interaction effects between key baseline variables (hs-CRP and hepcidin) following administration of a single dose of oral iron, compared between roxadustat and rHuEPO. Safety was assessed as the incidence of adverse events (AEs), measurement of vital signs, and laboratory safety measures.

Statistical Analysis

Initially, a maximum of 104 patients with anemia of CKD were planned to be screened to allow randomization of a minimum of 46 patients with anemia of CKD. Sample size requirements were estimated based on similar published and unpublished studies [25]. Due to lower-than-anticipated recruitment associated with the coronavirus disease 2019 (COVID-19) pandemic (notably the non-dialysis-dependent CKD population), the protocol was amended to target a maximum of 60 eligible randomized patients allowing randomization of a minimum of 20 patients with anemia of CKD. Here, the calculated minimum sample size required to achieve a two-sided significance level of 0.05 and power of 90% was based on a treatment difference in AUC log-fold change of log(2.7) and a conservative effect due to roxadustat of 2.7-times baseline, while accounting for 20% of patients failing to take any study treatment or failing to provide a post-baseline AUC measurement. Additionally, randomization strata were dropped from the analysis models. After clinical data lock, serum iron $AUC_{0–3\ h}$ values were reported to be negative for two patients. Consequently, planned log-transformation of AUC data was not possible and untransformed AUC data were used for the efficacy analysis. A sensitivity analysis set was further introduced for analysis of observed positive-valued cases required for analysis of log-fold change, without imputation for missing data. Treatment and interaction effects were evaluated by analysis of covariance (ANCOVA). The ANCOVA models used to assess treatment effect were adjusted for study treatment and baseline hs-CRP (≤ upper limit of normal [ULN], > ULN; level ≤ 10.0 mg/l, > 10.0 mg/l). ANCOVA models to assess interaction effects were adjusted for study treatment, baseline biomarker value, and baseline biomarker-treatment interaction. All analyses were performed using SAS®, version 9.3 (SAS Institute Inc., Cary, NC, USA).

RESULTS

Patients

A total of 51 patients were screened. Twenty-six were considered ineligible and 25 were randomized (roxadustat, $n = 13$; rHuEPO, $n = 12$);

24 patients completed the study; one patient was withdrawn during the study for not meeting the eligibility criteria post-randomization (Fig. S2 in the supplementary material). Demographic characteristics were generally comparable between the two treatment groups: mean age was 55.1 years, most were aged < 75 years, female (64.0%), and of mean body weight 61.7 kg (Table 1). Baseline clinical characteristics were comparable between groups. Most patients were rHuEPO-treated at baseline (88.0%), and the mean time from initial diagnosis of CKD was 120.9 (range 20–378) months; the etiology of CKD was chronic glomerulonephritis in 48.0% ($n = 12$) of patients and was unknown in 24.0% ($n = 6$) of patients (Table 1).

Among the 22 patients with dialysis-dependent CKD, 11 (44.0% of total study population) received hemodialysis and 11 (44.0%) received PD, and there were three (12.0%) patients with non-dialysis-dependent CKD (rHuEPO-naive). No patients with non-dialysis-dependent CKD who were rHuEPO users were enrolled in the study. In patients with dialysis-dependent CKD, mean (standard deviation [SD]) time from initial dialysis to randomization was 75.5 (58.5) months for roxadustat and 91.5 (85.3) months for rHuEPO. All patients who were dialysis dependent had been on dialysis for ≥ 20 months.

At baseline, mean (SD) serum iron area under the AUC was 21.4 (23.3) g × 3 h/dl for roxadustat and 18.7 (25.6) g × 3 h/dl for rHuEPO, while respective mean (SD) serum iron concentrations were 14.0 (4.0) µmol/l and 14.9 (6.9) µmol/l. Iron profiles were similar between the two treatment groups, with a mean (SD) Hb level of 106.1 (10.3) g/l for roxadustat and 105.4 (9.3) g/l for rHuEPO, and mean (SD) erythrocyte counts (10^{12}/L) of 3.5 (0.5) for roxadustat and 3.4 (0.3) for rHuEPO. In addition, 84.6% of patients in the roxadustat group had a baseline hs-CRP ≤ 10.0 mg/l, seen in all patients in the rHuEPO group (Table 1).

Prior and Concomitant Medication

All 25 patients were being treated for cardiovascular disease and reported use of prior medications including beta-blocking agents in 16 (64.0%), selective calcium-channel blockers in 12 (48.0%), and lipid-modifying agents in 10 (40.0%) patients (Table S1 in the supplementary material). Reported concomitant treatments included beta-blocking agents in 17 (68.0%), selective calcium-channel blockers with mainly vascular effects in 12 (48.0%), lipid-modifying agents in 10 (40.0%), and treatments for blood and blood forming organs in 23 (92.0%) patients (Table S2 in the supplementary material).

Efficacy

For the primary outcome measure (change from baseline to day 15 in serum iron $AUC_{0–3\ h}$), serum iron $AUC_{0–3\ h}$ values were unexpectedly reported as negative for two patients at day 1 and/or day 15 (roxadustat, $n = 1$; rHuEPO, $n = 1$). In contrast to other participants, these two patients each had a markedly elevated serum concentration of ferritin at baseline and day 15 (patient 1, 1455/1184 µg/l; patient 2, 1246/1196 µg/l, respectively). In addition, these two patients showed elevated hepcidin (312/190 µg/l and 272/295 µg/l, respectively) and a tendency for elevated hs-CRP (8.9/31.7 mg/dl and 0.4/0.6 mg/dl, respectively). Nevertheless, the negative serum iron $AUC_{0–3\ h}$ values necessitated change in the primary analysis method to evaluate absolute change from baseline rather than fold-change as planned; the findings from this study must therefore be viewed in this context. Change in the primary outcome measure (FAS) was numerically higher for roxadustat versus rHuEPO, but not statistically significantly different between the two treatment groups ($P = 0.212$) (Table 2 and Fig. 2A). Data for the per-protocol analysis set are provided in Table S3 in the supplementary material. The mean (SD) change from baseline in serum iron $AUC_{0–3\ h}$ was 11.3 (28.2) g × 3 h/dl for roxadustat and − 0.3 (9.7) g × 3 h/dl for rHuEPO, although the baseline values were similar for the two treatment groups [21.4 (23.3) g × 3 h/dl and 18.7 (25.6) g × 3 h/dl, respectively]. Mean (SD) change in Hb (g/dl) from baseline at day 15 was 6.5 (6.5) for roxadustat

Table 1 Baseline demographics and clinical characteristics (full analysis set)

Characteristic	Roxadustat (n = 13)	rHuEPO (n = 12)	Total (N = 25)
Demographic			
Age, years, mean (SD)	57.3 (12.1)	52.8 (12.6)	55.1 (12.3)
Female, n (%)	9 (69.2)	7 (58.3)	16 (64.0)
Race (Asian), n (%)	13 (100)	12 (100)	25 (100)
Body weight, kg, mean (SD)	62.3 (7.7)	61.0 (16.1)	61.7 (12.2)
< 70	10 (76.9)	8 (66.7)	18 (72.0)
≥ 70 to < 100	3 (23.1)	4 (33.3)	7 (28.0)
Disease characteristic			
rHuEPO-naive, n (%)	2 (15.4)	1 (8.3)	3 (12.0)
Time from CKD diagnosis to randomization, months, mean (SD)[a]	123.2 (106.8)	118.5 (81.5)	120.9 (93.6)
Time from first dialysis to randomization, months, mean (SD)[b]	75.5 (58.5)	91.5 (85.3)	83.5 (71.9)
Dialysis status			
HD (current, AV fistula), n (%)	5 (38.5)	6 (50.0)	11 (44.0)
PD (current), n (%)	6 (46.2)	5 (41.7)	11 (44.0)
Non-dialysis-dependent	2 (15.4)	1 (8.3)	3 (12.0)
Etiology of CKD, n (%)			
Chronic glomerulonephritis	7 (53.8)	5 (41.7)	12 (48.0)
Unknown	3 (23.1)	3 (25.0)	6 (24.0)
Other etiologies[c]	3 (23.1)	4 (33.3)	7 (28.0)
hs-CRP, mg/l, n (%)			
≤ 10.0	11 (84.6)	12 (100.0)	23 (92.0)
> 10.0	2 (15.4)	0	2 (8.0)
Serum iron AUC, g × 3 h/dl, mean (SD)	21.4 (23.3)	18.7 (25.6)	20.1 (24.0)
Serum iron, μmol/l, mean (SD)	14.0 (4.0)	14.9 (6.9)	14.4 (5.5)
Ferritin, μg/l, mean (SD)	398.1 (376.2)	431.4 (349.7)	414.1 (356.5)
TIBC, μmol/l, mean (SD)	39.8 (5.3)	37.2 (6.8)	38.6 (6.1)
TSAT (fraction of TIBC)[d], mean (SD)	0.4 (0.1)	0.4 (0.1)	0.4 (0.1)
Transferrin, g/l, mean (SD)	1.9 (0.3)	1.8 (0.4)	1.9 (0.3)
Soluble transferrin receptor, mg/l, mean (SD)	3.8 (1.3)	3.3 (0.8)	3.5 (1.1)
Hepcidin, g/l, mean (SD)	155.6 (86.7)	184.2 (136.3)	169.3 (111.8)
Hemoglobin, g/l, mean (SD)	106.1 (10.3)	105.4 (9.3)	105.8 (9.6)
Erythrocyte count, 10^{12}/l, mean (SD)	3.5 (0.5)	3.4 (0.3)	3.4 (0.4)

Table 1 continued

Characteristic	Roxadustat (n = 13)	rHuEPO (n = 12)	Total (N = 25)
Corpuscular volume, fl, mean (SD)	98.5 (6.7)	100.1 (3.6)	99.3 (5.4)

Baseline defined as the last measurement prior to randomization and dose management on day 1
AUC, area under the concentration-time curve; *AV* arteriovenous; *CKD* chronic kidney disease; *DD*, dialysis-dependent; *HD* hemodialysis; *hs-CRP* high-sensitivity C-reactive protein; *PD* peritoneal dialysis; *rHuEPO* recombinant human erythropoietin; *SD* standard deviation; *TIBC* total iron-binding capacity; *TSAT* transferrin saturation
[a]Calculated as: (initial CKD diagnosis date – date randomized + 1)/(365.25/12)
[b]Calculated as: (first dialysis date – date randomized + 1)/(365.25/12)
[c]Manually calculated: etiologies with ≤ 1 patient were summarized as other etiologies
[d]Analyzed as decimals

and 0.1 (7.3) for rHuEPO. Equivalent values for erythrocyte count (10^{12}/l) were 0.2 (0.2) for roxadustat and < 0.1 (0.2) for rHuEPO.

When change from baseline in serum iron $AUC_{0–3\ h}$ was assessed, adjusted for baseline levels of hepcidin and hs-CRP, or as part of the sensitivity analysis, numerical trends were similar (Table 2). As any significant difference in change from baseline of serum iron AUC could not be confirmed between the two treatment groups, analyses for the secondary endpoints were treated as exploratory, and reported *P*-values are nominal.

Secondary Endpoints

Indices of Iron Metabolism

Mean (SD) values for all evaluated iron indices (serum iron, ferritin, TIBC, TSAT, transferrin, soluble transferrin, soluble transferrin receptor, and hepcidin concentration) before administration of oral iron at day 1 and day 15 are shown in Table S4 in the supplementary material, and the relative change from baseline to day 15 is shown in Table 2. Relative change in serum iron concentration from baseline to day 15 was numerically higher for roxadustat versus rHuEPO, although mean (SD) baseline levels were similar for roxadustat (14.02 [4.01] µmol/l) and rHuEPO (14.87 [6.93] µmol/l) (Fig. 2B and Table S4 in the supplementary material). A similar trend was seen for mean (SD) serum iron concentration; here, the mean (SD) change from baseline to day 15 was 2.08 (7.92) µmol/l for roxadustat versus –0.85 (7.62) µmol/l for rHuEPO (Fig. 2C and Table S4 in the supplementary material).

For TIBC, transferrin, and soluble transferrin receptor, trends in relative change from baseline at day 1 to day 15 were numerically higher for roxadustat versus rHuEPO; conversely, levels were numerically lower for ferritin and TSAT, and markedly lower for hepcidin (Table 2). When relative change from baseline in the various iron indices were analyzed using the ANCOVA model, with additional adjustments for study treatment and baseline hs-CRP, nominally significant treatment effects were seen for TIBC, transferrin, soluble transferrin receptor, and hepcidin (each $P < 0.05$; Table 2).

Safety

Overall, five patients from the roxadustat group experienced a total of eight AEs (abdominal infection, hyperkalemia, hypermagnesemia, seizure, open angle glaucoma, back pain, muscle spasm [× 2]), each mild in intensity, and two patients from the rHuEPO group experienced a total of two AEs (hyperkalemia), both moderate in intensity. The event of back pain in the roxadustat group was considered possibly related to study treatment. All events resolved before the end of the study (Table 3). No serious AEs or deaths were reported, and no discontinuations due to an AE were reported. No clinically meaningful changes in mean values were noted for clinical laboratory safety parameters (including relating to Hy's law) or vital signs.

Table 2 Overview of absolute change from baseline (day 1) to day 15 for serum iron $AUC_{0-3\ h}$ and relative change from baseline for serum iron, serum ferritin, TIBC, TSAT, transferrin, soluble transferrin receptor, and hepcidin (full analysis set)

	Roxadustat estimate (SE/GSE) (*n* = 13)	rHuEPO estimate (SE/GSE) (*n* = 12)	Difference in estimates (SE/GSE)	$CI_{95\%}$	*p* value
Primary endpoint: absolute change from baseline in AUC of iron absorption, g × 3 h/dl					
Treatment effect[a]	1.12 (12.25)	− 18.37 (14.89)	19.50 (15.62)	− 11.16, 50.15	0.212
Baseline hepcidin by treatment effect[b]	− 0.15 (0.16)	− 0.02 (0.06)	− 0.13 (0.17)	− 0.48, 0.21	0.441
Baseline hs-CRP by treatment effect[c]	− 2.01 (1.22)	0.33 (3.56)	− 2.34 (3.76)	− 9.71, 5.02	0.532
Secondary endpoints: relative change from baseline					
Serum iron, µmol/l					
Treatment effect[a]	1.29 (1.21)	1.18 (1.27)	1.09 (1.23)	0.72, 1.64	0.688
Baseline hepcidin by treatment effect[b]	0.21 (0.16)	− 0.22 (0.11)	0.43 (0.20)	0.05, 0.82	0.029
Baseline hs-CRP by treatment effect[c]	0.23 (0.08)	0.14 (0.12)	0.08 (0.14)	− 0.20, 0.37	0.568
Ferritin, µg/l					
Treatment effect[a]	0.92 (1.10)	1.10 (1.13)	0.84 (1.11)	0.69, 1.03	0.096
Baseline hepcidin by treatment effect[b]	0.07 (0.09)	0.13 (0.07)	− 0.06 (0.12)	− 0.28, 0.17	0.630
Baseline hs-CRP by treatment effect[c]	0.15 (0.05)	− 0.04 (0.06)	0.19 (0.08)	0.04, 0.34	0.015
TIBC, µmol/l					
Treatment effect[a]	1.21 (1.04)	0.98 (1.06)	1.23 (1.05)	1.12, 1.35	< 0.001
Baseline hepcidin by treatment effect[b]	0.02 (0.04)	− 0.04 (0.03)	0.06 (0.04)	− 0.03, 0.15	0.171
Baseline hs-CRP by treatment effect[c]	0.02 (0.02)	− 0.01 (0.03)	0.03 (0.04)	− 0.04, 0.10	0.387
TSAT, fraction of TIBC					
Treatment effect[a]	1.06 (1.19)	1.19 (1.25)	0.89 (1.22)	0.61, 1.31	0.561
Baseline hepcidin by treatment effect[b]	0.20 (0.15)	− 0.18 (0.11)	0.38 (0.19)	0.01, 0.75	0.043
Baseline hs-CRP by treatment effect[c]	0.20 (0.08)	0.16 (0.11)	0.04 (0.13)	− 0.22, 0.30	0.766

Table 2 continued

	Roxadustat estimate (SE/GSE) (n = 13)	rHuEPO estimate (SE/GSE) (n = 12)	Difference in estimates (SE/GSE)	$CI_{95\%}$	p value
Transferrin, g/l					
Treatment effect[a]	1.23 (1.05)	1.00 (1.06)	1.23 (1.05)	1.11, 1.37	< 0.001
Baseline hepcidin by treatment effect[b]	0.03 (0.04)	− 0.02 (0.03)	0.05 (0.05)	− 0.06, 0.16	0.351
Baseline hs-CRP by treatment effect[c]	0.05 (0.02)	0.00 (0.03)	0.04 (0.03)	− 0.03, 0.11	0.248
Soluble transferrin receptor, mg/l					
Treatment effect[a]	1.18 (1.10)	0.91 (1.13)	1.29 (1.11)	1.04, 1.60	0.021
Baseline hepcidin by treatment effect[b]	− 0.03 (0.10)	0.00 (0.07)	− 0.03 (0.12)	− 0.28, 0.22	0.826
Baseline hs-CRP by treatment effect[c]	− 0.07 (0.05)	− 0.03 (0.06)	− 0.04 (0.08)	− 0.21, 0.13	0.634
Hepcidin, μg/l					
Treatment effect[a]	0.52 (1.28)	1.15 (1.36)	0.45 (1.31)	0.26, 0.79	0.007
Baseline hepcidin by treatment effect[b]	0.01 (0.25)	0.17 (0.18)	− 0.16 (0.31)	− 0.80, 0.48	0.610
Baseline hs-CRP by treatment effect[c]	0.12 (0.14)	0.05 (0.17)	0.07 (0.22)	− 0.39, 0.54	0.740

MI was implemented to account for missing data. At each timepoint, missing AUC iron absorption data were imputed using MI before running models. MI is based on monotone regression (for monotone missing data). If no missing data were present, analysis was performed on observed cases

ANCOVA analysis of covariance; *AUC* area under the concentration-time curve; *CI* confidence interval; *GSE* geometric standard error; *hs-CRP* high-sensitivity C-reactive protein; *MI* multiple imputation; *rHuEPO* recombinant human erythropoietin; *SE* standard error; *TIBC* total iron-binding capacity; *TSAT* transferrin saturation

[a]Additional adjustments for ANCOVA Model 1: baseline hs-CRP level (≤ 10.0 mg/l, > 10.0 mg/l); least-squares mean (SE) presented for primary endpoint; geometric least-squares mean (GSE) presented for secondary endpoints

[b]Additional adjustments for ANCOVA Model 2: baseline hepcidin value and baseline hepcidin interaction with treatment. Baseline hepcidin served as a covariate. Point estimate (SE) presented

[c]Additional adjustments for ANCOVA Model 3: baseline hs-CRP value and baseline hs-CRP interaction with treatment. Baseline hs-CRP served as a covariate. Point estimate (SE) presented

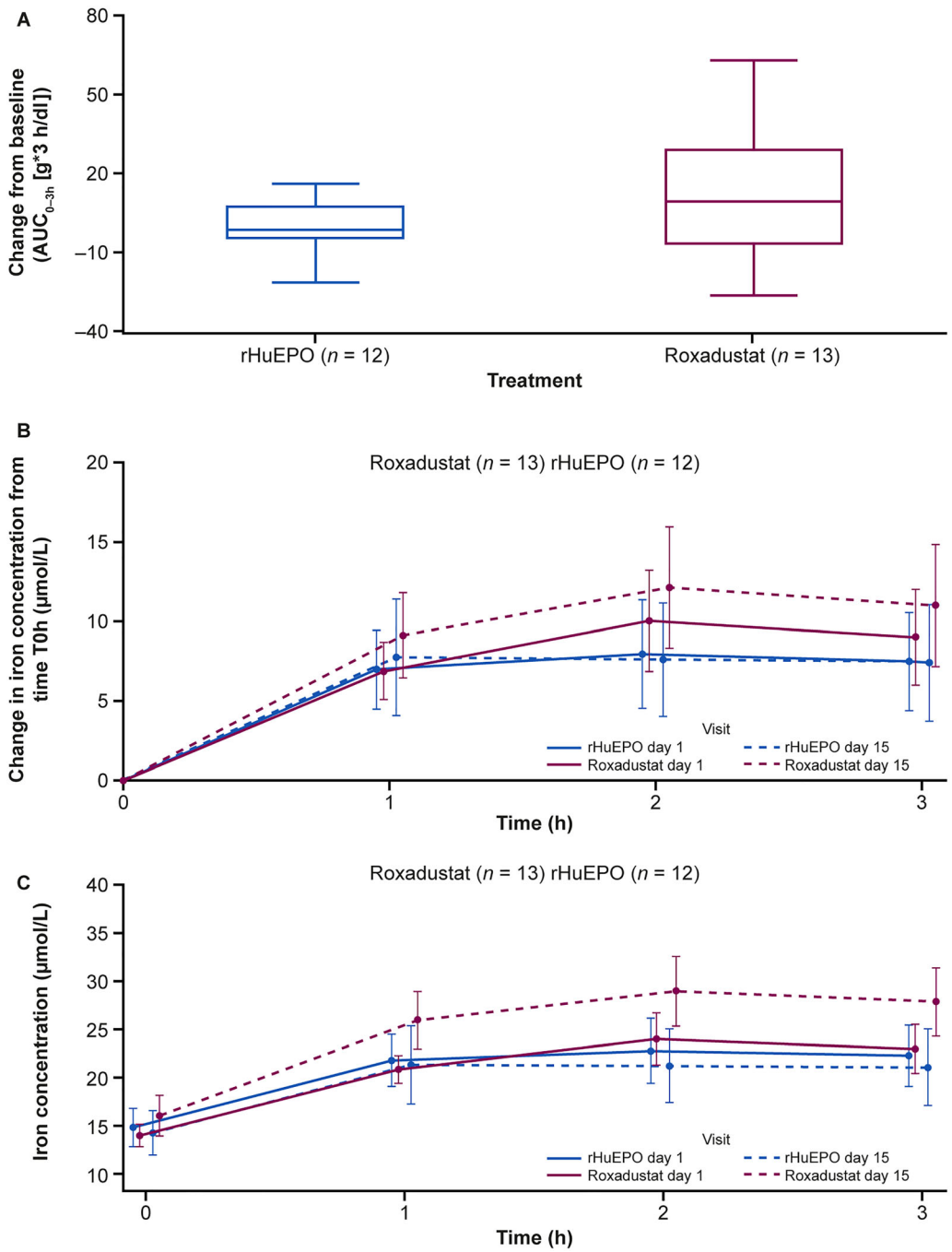

Fig. 2 **A** Change from baseline in serum iron absorption ($AUC_{0-3\ h}$) over time, **B** mean change from baseline in serum iron concentration (absorption) over time following oral iron (T0h corrected), and **C** mean serum iron concentration (absorption) over time following oral iron (full analysis set). *AUC* area under the concentration-time curve; *rHuEPO* recombinant human erythropoietin

Table 3 AEs by system organ class and preferred term (safety analysis set)

AE, *n* (%)[a]	Roxadustat (*n* = 13)	rHuEPO (*n* = 12)
Patients with any AE	5 (38.5)[b]	2 (16.7)[c]
Infections and infestations		
Abdominal infection	1 (7.7)	0
Metabolism and nutrition disorders		
Hyperkalemia	1 (7.7)	2 (16.7)
Hypermagnesemia	1 (7.7)	0
Nervous system disorders		
Seizure	1 (7.7)	0
Eye disorders		
Open-angle glaucoma	1 (7.7)	0
Musculoskeletal and connective tissue disorders		
Back pain	1 (7.7)	0
Muscle spasm	1 (7.7)	0

All events were [b]mild or [c]moderate in intensity, assessed by the investigator as not related to study treatment (one instance of back pain was considered possibly related to roxadustat) and generally resolved before study end

AE adverse event; *rHuEPO* recombinant human erythropoietin

[a]Number (%) of patients with AEs, sorted on international order for system organ class and alphabetical order for preferred term

DISCUSSION

To our knowledge, this is the first clinical trial to explore iron absorption in patients with anemia of CKD treated with HIF-PHIs. Iron dysmetabolism, related to absorption, transport, and utilization, is a well-known important cause of anemia in CKD patients, where iron and erythropoietin administration have limited effect [6, 35, 36].

Previous studies demonstrating improvement in indicators of iron metabolism mediated by HIF-PHIs will have inevitably provided great encouragement to clinical researchers and physicians. For example, HIF-PHIs have been shown to significantly decrease hepcidin levels, a key iron regulatory hormone, which can degrade the mammalian iron exporter ferroportin in iron-absorptive enterocytes and iron-recycling macrophages [36]. This decrease in hepcidin with HIF-PHIs may promote release of iron from enterocytes into the circulation through ferroportin [36]. In addition, divalent metal transporter 1, an apical iron transporter of enterocytes, is regulated by local hypoxia; here, iron absorption may be increased by HIF-PHIs through divalent metal transporter 1 [37]. This phase 4 study was designed to observe the actual effect of roxadustat on iron absorption in patients with CKD.

In this study, change of serum iron $AUC_{0–3\ h}$ was not statistically significantly different between the roxadustat and rHuEPO groups. Unexpected negative $AUC_{0–3\ h}$ values obtained from two patients required a change in the primary analysis method. Interestingly, the two patients with negative $AUC_{0–3\ h}$ were characterized throughout the study by marked elevations in serum ferritin and hepcidin, and a tendency towards elevated hs-CRP concentrations, consistent with possible raised inflammatory status, and potential restricted capacity

for uptake of dietary iron [38]. The sensitivity analysis, excluding patients with negative $AUC_{0–3h}$ values, also yielded a negative result that was potentially contributed to by the relatively small sample size. Significant recruitment challenges, including rigorous patient requirements such as need for frequent visits, multiple blood testing during the ferrokinetic studies, stringent inclusion/exclusion criteria, and the COVID-19 pandemic, led to fewer randomized patients than the initial target of 46. Despite this, the findings provide 'pilot' guidance for future studies towards sample size calculation, patient recruitment, and study design.

The smaller-than-expected sample size compromised the reliable performance of any meaningful subgroup analyses. Also, not being able to confirm significance for the primary analysis meant the secondary efficacy analysis had to be considered exploratory, with *P*-values rendered nominal. While not confirmatory, the observed decrease in hepcidin and increases in serum iron, transferrin, and TIBC for roxadustat relative to rHuEPO were generally consistent with prior reports of greater reductions in hepcidin [26, 28] and increases in iron and TIBC [25, 28–30] for roxadustat compared with EPO-treated patients. The changes seen are hypothesized as being indicative of increased iron absorption and release of iron from intracellular stores for erythropoiesis in roxadustat-treated patients [26, 27, 29]. The incidence of AEs was generally low, and the safety profile (types of AE reported) was consistent with the population under study and the known safety profile of roxadustat [27–32].

Key limitations were, first, recruitment difficulties led to truncation of the intended sample size, and unexpected negative values for AUC resulted in a post hoc change to the planned statistical method. A sample size of 46 patients, 23 per arm, was calculated as needed to provide 80% power at the 0.05 alpha level (two-sided) to detect a treatment difference of AUC change from baseline. The existence of negative AUC values required an analysis of AUC change from baseline rather than AUC fold change from baseline, resulting in inadequate power as the target sample size was not reached. As a result, significant differences between treatment groups could not be assessed appropriately. As any significant difference in change from baseline of serum iron AUC could not be confirmed between treatment groups, analyses for the secondary endpoints were treated as exploratory. Caution is therefore required in interpretation of the results. Based on this, we consider that a very high ferritin level should be an exclusion criterion in ferrokinetic research. Second, all patients enrolled were Chinese, and potential inter-ethnic differences in iron absorption will preclude extrapolation of findings to other ethnic groups. Third, dialysis-dependent and non-dialysis-dependent patients may present different iron absorption characteristics; here, small sample size again meant it was not possible to conduct effective subgroup analysis.

CONCLUSION

In conclusion, the study showed no significant difference in iron absorption between the treatment groups. However, the trends identified in this study suggest the need for larger, well-designed, and appropriately controlled clinical trials to evaluate any roxadustat-mediated benefits of enhanced iron absorption in patients with CKD-related anemia. It will also be important to further investigate the predicted ferrokinetic properties of HIF-PHIs and determine their impact on IV iron supplementation needs.

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Data Availability. Data underlying the findings described in this manuscript may be obtained in accordance with AstraZeneca's data sharing policy described at https://astrazenecagrouptrials.pharmacm.com/ST/Submission/Disclosure. Data for studies directly listed on Vivli can be requested through Vivli at www.vivli.org. Data for studies not listed on Vivli could be requested through Vivli at https://vivli.org/members/enquiries-about-studies-not-listed-on-the-vivli-platform/. AstraZeneca Vivli member page is also available outlining further details: https://vivli.org/ourmember/astrazeneca/.

Declarations

Conflict of Interest. The sponsor was involved in the study design, collection, analysis, interpretation of data, and data checking of information provided in the manuscript. However, ultimate responsibility for opinions, conclusions, and data interpretation lies with the authors. Roxadustat was developed through collaboration between FibroGen, Inc., Astellas, and AstraZeneca. Haiting Wu, Hong Cheng, Caili Wang, LI Yao, Shuguang Qin, Li Zuo, Zhao Hu, Chun Zhang, and Xuemei Li, declare no conflicts of interest. Alexis Hofherr and Stephen Rush are employees of, and hold or may hold stock in, AstraZeneca. Katie Mohan is a contractor working on behalf of AstraZeneca and Yiging Wu, is an employee of FibroGen, Inc.

Ethical Approval. All participants provided written informed consent prior to performance of any screening tests or assessments. This study was conducted in accordance with the Declaration of Helsinki and Council for International Organizations of Medical Sciences International Ethical Guidelines, applicable International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use, Good Clinical Practice Guidelines, and applicable local health and regulatory requirements. The final study protocol and informed consent form were approved by the applicable independent ethics committee or institutional review board for each site (protocol D5741C00002; approved July 13, 2021).

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