European Journal of Clinical Pharmacology (2020) 76:185–197
https://doi.org/10.1007/s00228-019-02813-y

PHARMACODYNAMICS

Effects of oral iron and calcium supplement on the pharmacokinetics and pharmacodynamics of molidustat: an oral HIF–PH inhibitor for the treatment of renal anaemia

Silvia Lentini[1] · Andreas Kaiser[2] · Stefanie Kapsa[1] · Kumi Matsuno[3] · Dorina van der Mey[1]

Received: 14 June 2019 / Accepted: 14 October 2019 / Published online: 10 January 2020

Abstract

Purpose The present studies assessed the drug–drug interaction of molidustat, a hypoxia-inducible factor prolyl hydroxylase inhibitor, with iron and calcium supplements, which are common medications in patients with anaemia due to chronic kidney disease (CKD).

Methods Forty-two healthy men received molidustat alone (fasted or fed) or combined with oral iron(II) or calcium(II), given immediately before or between 4 h before and 1 h after molidustat in three randomized, open-label, crossover studies (12–15 participants per study). Molidustat AUC and C_{max} were assessed as the main pharmacokinetic parameters, and endogenous erythropoietin (EPO) was measured to evaluate pharmacodynamics.

Results Depending on prandial state, concomitant intake of iron(II) reduced molidustat AUC and C_{max} by 50–75% and 46–84%, respectively, and EPO $AUC_{(0\text{-}24)}$ and C_{max} by 31–44% and 36–48%, respectively. The influence of iron(II) declined with increasing the time interval to the intake of molidustat, with reductions in molidustat AUC and C_{max} of 9% and 10%, respectively, when iron(II) intake occurred 4 h before molidustat. Accordingly, effects on endogenous EPO were less pronounced with increased time separation between oral iron(II) and molidustat intake. Calcium(II) reduced molidustat AUC and C_{max} by 15% and 47%, respectively, without influence on EPO response. All treatments were well tolerated.

Conclusions In contrast to concomitant oral intake of calcium, the effect of oral iron supplements on molidustat pharmacokinetics and pharmacodynamics should be considered, and the two agents should be administered with an appropriate time separation.

Keywords Molidustat · Drug—drug interaction · Oral iron and calcium supplementation · Pharmacokinetics · Pharmacodynamics

Introduction

Anaemia is a common complication of chronic kidney disease (CKD); its prevalence increases as kidney disease progresses, and it affects nearly all patients with stage 5 CKD [1]. It is associated with poor quality of life and increased risk of cardiovascular events, hospitalization, cognitive impairment, and mortality [2, 3].

Reduced erythropoietin (EPO) production in the kidney and iron deficiency are both important factors in the pathogenesis of anaemia associated with CKD [4]. Current management of anaemia in patients with CKD consists of a combination of erythropoiesis-stimulating agents (ESAs) and iron supplementation. ESAs mimic the action of endogenous EPO and are effective in elevating haemoglobin (Hb) levels in patients with anaemia due to CKD [5]. However, ESAs are associated with several potential safety concerns, including increased risk

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00228-019-02813-y) contains supplementary material, which is available to authorized users.

✉ Silvia Lentini
silvia.lentini@bayer.com

[1] Clinical Pharmacology Cardiovascular/Haematology, Translational Sciences, Research & Development, Bayer AG, Aprather Weg 18a, 42113 Wuppertal, Germany

[2] Statistics and Data Insights, Data Sciences & Analytics, Research & Development, Bayer AG, Berlin, Germany

[3] Clinical Pharmacology/Clinical Sciences, Research and Development Japan, Bayer Yakuhin Ltd, 4-9, Umeda 2-chome, Kita-ku, Osaka, Japan

of cardiovascular events and stroke [6], so alternative therapy options are being developed.

Hypoxia-inducible factor prolyl hydroxylase (HIF–PH) inhibitors offer a potential alternative to the standard of care. EPO transcription is activated by hypoxia-inducible factors (HIF) in response to hypoxia. However, in the presence of oxygen, HIF–PH hydroxylates the HIF-α subunit, which is subsequently targeted for proteasomal degradation, preventing EPO synthesis. Inhibition of HIF–PH results in stabilization of HIF, which leads to endogenous production of EPO and, ultimately, stimulation of erythropoiesis [7]. Molidustat is an orally bioavailable HIF–PH inhibitor being investigated in phase 3 clinical trials in patients with renal anaemia, including patients receiving dialysis treatment [8, 9]. The phase 2 DIALOGUE (DaIly orAL treatment increasing endOGenoUs Erythropoietin) programme, which included three studies with a 16-week treatment duration and two extension studies, assessed the safety and efficacy of molidustat in different populations of patients with renal anaemia [10, 11]. DIALOGUE 1 was a placebo-controlled, fixed-dose trial (25, 50, and 75 mg once daily; 25 and 50 mg twice daily). In DIALOGUEs 2 and 4, molidustat was given as individual dose titration based on Hb values (starting doses ranged between 25–75 mg and 25–150 mg once daily in DIAGLOGUE 2 and 4, respectively). Molidustat treatment was generally well tolerated and corrected and maintained Hb levels within a pre-specified range in patients previously treated with ESAs and in treatment-naïve patients [10]. In healthy volunteers, molidustat was shown to be rapidly absorbed with a maximum plasma concentration of 1 h after drug intake and to elicit dose-dependent increases in endogenous EPO after single increasing doses [12]. Molidustat is metabolized via glucuronidation and eliminated in urine as pharmacologically inactive glucuronide [13].

Oral iron supplementation is a common concomitant treatment in patients with anaemia. In vitro experiments indicated that the solubility of molidustat is reduced by 95% in the presence of multivalent cations such as iron(II) and calcium(II) (unpublished data on file, Bayer AG, Wuppertal, Germany) compared to the clinically administered sodium salt. Here, we report the results of three studies that evaluated the effect of co-administration of oral iron(II) on the plasma pharmacokinetics (PK) and pharmacodynamics (PD) of molidustat after intake of a single oral dose under fasted or fed conditions in healthy volunteers. The effect of time separation between oral iron(II) and molidustat administrations on molidustat PK and PD was also investigated to select an optimized time interval between administration of both drugs to avoid clinically relevant interactions. The drug–drug interaction (DDI) between molidustat and calcium acetate was also evaluated in one of the studies.

Methods

Study design

All three studies had a single-centre, randomized, open-label, crossover design and involved healthy male volunteers.

Study 1 investigated the effect of iron(II) sulphate and calcium acetate administered orally immediately before molidustat on the plasma concentration–time profile of molidustat (PK) and endogenous serum EPO (PD) in fasted (≥10 h) individuals. The following three single-dose treatments were administered: molidustat 150 mg (2 × 75 mg immediate-release [IR] tablets); iron(II) sulphate 304 mg (Eryfer 100 mg capsule, CHEPLAPHARM Arzneimittel GmbH, Mesekenhagen, Germany) followed immediately by molidustat 150 mg; and calcium acetate 1900 mg (2 × 950 mg Calcet tablets, Teva GmbH, Ulm, Germany) followed immediately by molidustat 150 mg. In the crossover design, each volunteer received the three treatment regimens with a washout period of at least 96 h between each dose of molidustat.

Study 2 assessed the effect of iron(II) sulphate administered orally from 4 h before to 1 h after molidustat and the effect of enteric-coated iron(II) glycine sulphate complex administered immediately before molidustat on the PK and PD of molidustat in fasted (≥10 h) individuals. The volunteers received the following single-dose treatments: molidustat 150 mg (2 × 75 mg IR tablets); iron(II) sulphate 304 mg (Eryfer 100 mg capsule) 4 h before molidustat 150 mg; iron(II) sulphate 304 mg (Eryfer 100 mg capsule) 2 h before molidustat 150 mg; iron(II) sulphate 304 mg (Eryfer 100 mg capsule) 1 h after molidustat 150 mg; and iron(II) glycine sulphate complex 567.7 mg (Ferro Sanol duodenal 100 mg capsule, enteric-coated, Sanol GmbH, Monheim, Germany) followed immediately by molidustat 150 mg. In the crossover design, each volunteer received all five treatment regimens with a washout period of at least 72 h between each dose of molidustat. Both iron formulations (Eryfer and Ferro Sanol duodenal) contained iron(II) 100 mg per dose. Ferro Sanol duodenal was an enteric-coated formulation, whereas Eryfer consists of an IR tablet.

Study 3 assessed the effect of iron(II) sulphate administered from 1 h before to 1 h after molidustat on the PK and PD of molidustat under fed conditions. Molidustat was administered 30 min after the start of a standardized continental breakfast which consisted of 2 slices (40 g) of white bread (toasted), 20 g butter, 25 g jam, 20 g cheese (45% fat), and 200 mL of tea containing 1 cube of sugar. The breakfast had to be finished in 30 min or less. The participants received the following single-dose treatments: molidustat 150 mg (2 × 75 mg IR tablets); iron(II) sulphate 304 mg (Eryfer 100 mg capsule) 1 h before molidustat 150 mg; iron(II) sulphate 304 mg (Eryfer 100 mg capsule) followed immediately by molidustat 150 mg; and iron(II) sulphate 304 mg (Eryfer 100 mg capsule) 1 h after

molidustat 150 mg. In the crossover design, each participant received each of the four treatment regimens with a washout period of at least 72 h between each molidustat dose.

In all three studies, volunteers were admitted to the study ward at least 12 h before study drug administration, which began in the morning of the next day. Participants were discharged from the ward after a 48-h observation period. The studies were approved by the Ethics Committee of North-Rhine Medical Council, Düsseldorf, Germany, and were conducted in accordance with the International Conference on Harmonization guideline on Good Clinical Practice and with the Declaration of Helsinki. All volunteers gave written informed consent to participate in the study.

Study populations

For all three studies, healthy, white, male volunteers aged 18–45 years with a body mass index (BMI) between 18.0 kg/m^2 and 29.9 kg/m^2 were eligible for enrolment. Key inclusion and exclusion criteria are summarized in Supplementary Table 1, and restrictions during the study are summarized in Supplementary Table 2.

Materials

Ferro Sanol duodenal was an enteric-coated formulation, whereas Eryfer and molidustat were administered as IR tablets. Both iron formulations (Eryfer and Ferro Sanol duodenal) contained iron(II) 100 mg per dose. Calcet was administered as tablets containing 950 mg of calcium acetate each.

Pharmacokinetic analyses

In study 1, blood samples were collected pre-dose, at 10, 20, 30, 40, and 50 min post-dose and at 1, 1.5, 2, 3, 4, 6, 8, 12, 24, 30, 36, and 48 h post-dose. In studies 2 and 3, blood samples were collected pre-dose, at 15, 30, and 45 min post-dose and at 1, 1.5, 2, 3, 4, 6, 8, 12, 24, 30, 36, and 48 h post-dose. In all three studies, plasma concentrations of molidustat were measured at Bayer Laboratories using a validated high-pressure liquid chromatography–tandem mass spectrometry (LC–MS/MS) method as previously described [12, 14]. Calibration and quality control (QC) samples were analysed concurrently with study samples. The calibration range of this assay was from 0.200 μg/L (lower limit of quantification [LLOQ]) to 200 μg/L. The mean inter-assay accuracy range in calibrators was 90.9–107% in study 1, 97.6–104% in study 2, and 96.1–104% in study 3. The corresponding precisions were 3.4% or below, 4.7% or below, and 7.4% or below, respectively. QC samples in the concentration range of 0.500–160 μg/L and dilution QC samples of 4000 μg/L and 8000 μg/L (study 2 only) were determined with an accuracy range of 92.4–101% in study 1, 96.6–99.7% in study 2, and 99.3–103% in study 3. The corresponding precisions were 7.0% or below, 7.0% or below, and 6.6% or below, respectively. The PK parameters were calculated using the model-independent (compartment-free) method in WinNonlin (version 5.3, Pharsight Corporation, St Louis, MO, USA) with the Automation Extension (developed by Bayer AG). The PK parameters determined were maximum observed drug concentration in plasma (C_{max}), area under the concentration–time curve from zero to infinity (AUC), apparent terminal half-life ($t_{½}$), time to C_{max} (t_{max}), and apparent oral clearance (CL/F). Only values above the LLOQ were included to determine PK parameters.

Pharmacodynamic parameters

In all three studies, blood samples were collected pre-dose and at 4, 6, 8, 12, and 24 h post-dose to assess the PD effects of molidustat on serum endogenous EPO levels as previously described [12]. Absolute values for serum EPO C_{max}, t_{max}, and area under the concentration–time curve from 0 to 24 h ($AUC_{(0–24)}$) were determined. Serum EPO C_{max} ratios to baseline were also calculated.

Clinical safety and tolerability

Clinical safety and tolerability were assessed by physical examination, monitoring of vital signs (blood pressure and heart rate), 12-lead electrocardiogram, laboratory safety tests (blood and urine analyses), and occurrence of adverse events.

Statistical methods

In all three studies, geometric means and percentage geometric coefficients of variation were calculated for molidustat AUC, C_{max}, $t_{½}$, and CL/F; t_{max} was described using median and range. Mean molidustat plasma concentrations for each time point were only calculated if values were obtained and were above the LLOQ for at least two-thirds of the participants. For the calculation of the mean value, a data point below the LLOQ was substituted by half this threshold. Logarithms of AUC and C_{max} were analysed using an analysis of variance (ANOVA) including fixed effects for sequence, period, participant (sequence), and treatment. Point estimates and exploratory 90% confidence intervals (CIs) for the ratios of relative bioavailability between molidustat alone and in combination with oral iron(II) or oral calcium acetate in each study were calculated by retransforming the corresponding least squares (LS) mean difference and 90% CIs of the ANOVA.

In all three studies, absolute geometric means and geometric coefficients of variation were calculated for EPO $AUC_{(0–24)}$ (using the trapezoidal rule) and C_{max}; furthermore, geometric mean ratios to baseline were determined for C_{max}. EPO

t_{max} was described using median and range. The logarithms of $AUC_{(0–24)}$ and C_{max} of EPO were analysed using an ANOVA including fixed effects for sequence, period, participant (sequence), and treatment. Point estimates and exploratory 90% CIs for the ratios between molidustat alone and in combination with oral iron(II) or oral calcium acetate were calculated by retransforming the corresponding LS mean difference and 90% CIs of the ANOVA.

Sample size determination was done by evaluating the expected width of the 90% CIs for estimating the drug–drug interaction potential, i.e. ratio of geometric means of AUC between treatments. Assuming an intra-participants' CV of 20%, 90% confidence limits were expected to be 1.16 times the point estimate, which was considered sufficient for these exploratory studies. No further power calculations were performed because the intention of the statistical analysis was to estimate the DDI and not to prove bioequivalence.

Results

Study populations

In study 1, 27 healthy volunteers were screened and 16 were randomized. One participant was withdrawn before receiving any dose of molidustat owing to protocol violation. All 15 remaining participants were included in the safety set (defined as all participants who received at least one dose of molidustat), the PK set (all participants with at least one valid PK profile), and the PD set (all participants with at least one complete EPO profile). In study 2, 33 healthy volunteers were screened and 15 were randomized. The safety analysis set (defined as in study 1) included 15 participants. One subject withdrew his consent in the first treatment period. The PK and PD sets, which both included 14 participants, were defined as all healthy volunteers who received at least two doses of molidustat and who had at least two valid PK profiles or two valid PD profiles (molidustat alone and any with co-treatment), respectively. In study 3, 21 healthy volunteers were screened and 12 were randomized. The safety analysis set (defined as in study 1) comprised 12 participants. One participant was withdrawn during the second treatment period owing to an adverse event (upper respiratory tract infection). The PK and PD sets were defined as in study 2, and both included 11 participants.

For each study, demographic and baseline characteristics are summarized in Table 1. All participants were white men, and the mean ages (29.6–31.2 years), mean weights (74.6–82.1 kg), and mean BMIs (23.6–25.2 kg/m^2) were similar across all three studies.

Pharmacokinetic evaluation

Molidustat alone

After oral administration of molidustat 150 mg (2 × 75 mg IR tablets) alone to fasted individuals (studies 1 and 2), molidustat reached peak plasma concentrations of 1920 μg/L (study 1) or 2080 μg/L (study 2) 30–60 min after dosing (Table 2, Figs. 1 and 2). The peak concentration of molidustat in the plasma of fed individuals (study 3) was lower (1110 μg/L) and delayed (t_{max}, 90–120 min) compared with fasted individuals (Table 2, Fig. 3). Across all three studies, the mean AUC was in the range of 2960–3740 μg × h/L and was not affected by food intake.

Molidustat in combination with oral iron(II)

Plasma molidustat PK parameters in the absence and presence of iron(II) in all three studies are shown in Table 2. Based on the ANOVA and compared with molidustat alone, the largest decreases in plasma geometric mean AUC for molidustat in fasted volunteers were observed when iron(II) sulphate (study 1) and iron(II) glycine sulphate (enteric-coated formulation, study 2) were administered immediately before molidustat (Table 3, Supplementary Table 3). A similar reduction in molidustat AUC was observed in fed volunteers (study 3) after administration of iron(II) sulphate immediately before molidustat (Table 3, Supplementary Table 3). In fasted individuals, when iron(II) sulphate was administered 4 h before, 2 h before, and 1 h after molidustat (study 2), the geometric mean AUC was 9%, 16%, and 26% lower, respectively, than after molidustat alone (Table 3, Supplementary Table 3). Compared with molidustat alone, in fed individuals, the reduction in geometric mean AUC was greater when iron(II) sulphate was administered 1 h after molidustat than when it was administered 1 h before (study 3).

Similarly, the decrease in plasma geometric mean C_{max} for molidustat was largest when iron(II) sulphate (study 1 and 3) or iron(II) glycine sulphate (study 2) was administered immediately before molidustat (Table 3, Supplementary Table 3). In fasted individuals, when iron(II) sulphate was administered 4 h before, 2 h before, and 1 h after molidustat (study 2), the geometric means C_{max} were 10%, 0.3%, and 12% lower, respectively, than after molidustat alone (Table 3, Supplementary Table 3). Compared with molidustat alone, in fed individuals, the reduction in plasma molidustat C_{max} was less pronounced when iron(II) sulphate was administered 1 h before molidustat than when it was administered 1 h after (study 3).

In study 1, the terminal $t_{½}$ increased with concomitant administration of iron(II) sulphate. Similarly, in studies 2 and 3, terminal $t_{½}$ increased when oral iron(II) supplement was

Table 1 Participants' demographics and baseline characteristics (safety analysis sets)

Parameter	Study 1 (N = 15)	Study 2 (N = 15)	Study 3 (N = 12)
Sex, *n* (%)			
Male	15 (100)	15 (100)	12 (100)
Race, *n* (%)			
White	15 (100)	15 (100)	12 (100)
Age, years, mean (range)	31.2 (23–41)	29.6 (19–45)	29.8 (24–36)
Weight, kg, mean (SD)	82.1 (5.91)	74.6 (11.62)	80.8 (9.35)
Height, cm, mean (SD)	180.5 (4.60)	177.7 (4.40)	182.3 (8.26)
BMI, kg/m^2, mean (SD)	25.2 (1.91)	23.6 (3.26)	24.3 (2.03)

BMI body mass index, *SD* standard deviation

administered with molidustat, regardless of the time separation between drug administration (Table 2).

CL/F increased in the presence of iron(II) up to 500%. Given that an increase of clearance (CL) by oral iron(II) is unlikely and that $t_{½}$ was not reduced, this effect may be attributed to a decrease in bioavailability rather than an increase in CL.

Molidustat in combination with oral calcium(II)

Plasma molidustat PK parameters in the absence and presence of calcium(II) are shown in Table 2. In fasted individuals, when calcium acetate was administered immediately before molidustat, the geometric means AUC and C_{max} for molidustat were reduced by 15% and 47%, respectively, compared with molidustat alone; the terminal $t_{½}$ was also decreased slightly compared with molidustat alone.

Pharmacodynamics: endogenous EPO

Molidustat alone

After oral administration of molidustat 150 mg (2 × 75 mg IR tablets) alone under fasted or fed conditions, endogenous EPO reached peak concentrations of 77.8 IU/L (study 1), 103.6 IU/L (study 2), or 85.9 IU/L (study 3) approximately 8 h after dosing (Table 4, Supplementary Figs. 1, 2, and 3). Across all three studies, EPO mean $AUC_{(0–24)}$ was in the range of 1171–1498 IU × h/L. Table 4 shows the geometric means for EPO $AUC_{(0–24)}$ and C_{max} and the median for t_{max} after oral administration of molidustat 150 mg (under fasted or fed conditions) to healthy male participants, in the absence and presence of iron(II) sulphate administered from 4 h before to 1 h after molidustat, for all three studies.

Molidustat in combination with oral iron(II)

When iron(II) sulphate (study 1) or iron(II) glycine sulphate (study 2) were administered in fasted individuals immediately before molidustat, the geometric mean of EPO $AUC_{(0–24)}$ decreased by almost 50% compared with molidustat alone (Table 5, Supplementary Table 4). In fed healthy volunteers (study 3), compared with molidustat alone, the geometric mean $AUC_{(0–24)}$ for EPO was reduced by 31% when iron(II) sulphate was administered immediately before molidustat (Table 5, Supplementary Table 4). These reductions in EPO $AUC_{(0–24)}$ were not observed when iron(II) sulphate was administered 4 h before molidustat and partially observed when iron(II) sulphate was administered 2 h before or 1 h after molidustat (study 2) (Table 5, Supplementary Table 4). Similarly, in fed individuals, the effects of iron supplementation on the EPO $AUC_{(0–24)}$ were less pronounced when iron(II) sulphate was administered 1 h before or 1 h after molidustat (Table 5, Supplementary Table 4).

The decrease in geometric mean C_{max} for EPO was largest when iron(II) sulphate (studies 1 and 3) and iron(II) glycine sulphate (study 2) were administered immediately before molidustat (Table 5, Supplementary Table 4). In fasted individuals, when iron(II) sulphate was administered 4 h before, 2 h before, and 1 h after molidustat (study 2), the geometric means EPO C_{max} were 2%, 15%, and 25% lower, respectively, than after molidustat alone (Table 5, Supplementary Table 4). Compared with molidustat alone, in fed individuals, the reduction in EPO C_{max} was less pronounced when iron(II) sulphate was administered 1 h before molidustat than when it was administered 1 h after (study 3) (Table 5, Supplementary Table 4).

Molidustat in combination with oral calcium(II)

The ANOVA for the comparison between EPO $AUC_{(0–24)}$ and C_{max} after administration of molidustat alone and molidustat plus calcium acetate did not indicate any clinically relevant difference between the two treatments (Table 5, Supplementary Table 4).

Clinical safety and tolerability

In study 1, six participants (40%) experienced a total of 10 treatment-emergent adverse events (TEAEs) (Supplementary

Table 2 Plasma PK parameters of molidustat, expressed as geometric mean (% geometric CV), after single oral administration of molidustat 150 mg or with co-administration of iron(II) sulphate 304 mg, iron(II) glycine sulphate 567.7 mg, or calcium acetate 1900 mg (PK sets)

	Molidustat alone	Iron(II) glycine sulphate immediately before molidustat	Iron(II) sulphate immediately before molidustat	Iron(II) sulphate 1 h after molidustat	Iron(II) sulphate 1 h before molidustat	Iron(II) sulphate 2 h before molidustat	Iron(II) sulphate 4 h before molidustat	Calcium acetate immediately before molidustat
Study 1 (fasted, $N = 15$)								
AUC, µg × h/L	2960 (24.0)	–	743 (65.8)[a]	–	–	–	–	2520 (19.9)
C_{max}, µg/L	1920 (47.5)	–	319 (75.2)	–	–	–	–	1010 (37.1)
t_{max}[b], h	0.517 (0.333–1.50)	–	0.675 (0.500–3.00)	–	–	–	–	1.00 (0.333–2.00)
$t_{½}$, h	11.9 (99.5)	–	16.4 (99.1)[a]	–	–	–	–	10.7 (96.7)
CL/F, L/h	50.6 (24.0)	–	202.0 (65.8)[a]	–	–	–	–	59.6 (19.9)
Study 2 (fasted, $N = 14$)								
AUC, µg × h/L	3740 (25.1)	1810 (40.6)[c]	–	2770 (47.8)	–	3130 (31.8)	3400 (24.0)	–
C_{max}, µg/L	2080 (40.4)	1110 (81.8)	–	1800 (88.2)	–	2060 (47.7)	1880 (39.2)	–
t_{max}[b], h	0.875 (0.250–4.00)	0.500 (0.250–1.50)	–	0.750 (0.250–1.50)	–	0.750 (0.250–4.00)	0.750 (0.250–1.50)	–
$t_{½}$, h	12.3 (87.5)	21.8 (85.3)[c]	–	19.6 (139)	–	18.5 (64.2)	19.1 (43.5)	–
CL/F, L/h	40.1 (25.1)	82.7 (40.6)[c]	–	54.1 (47.8)	–	47.9 (31.8)	44.1 (24.0)	–
Study 3 (fed, $N = 11$)								
AUC, µg × h/L	3000 (22.5)	–	1430 (25.7)[d]	1970 (38.7)[e]	2420 (31.8)	–	–	–
C_{max}, µg/L	1110 (28.3)	–	414 (50.2)	665 (71.0)	880 (51.7)	–	–	–
t_{max}[b], h	1.50 (0.500–3.00)	–	2.00 (0.250–3.00)	1.50 (0.500–3.00)	1.57 (1.00–3.00)	–	–	–
$t_{½}$, h	12.0 (54.9)	–	20.3 (85.0)[d]	39.4 (103.3)[e]	24.8 (68.6)	–	–	–
CL/F, L/h	50.0 (22.5)	–	105 (25.7)[d]	76.0 (38.7)[e]	61.9 (31.8)	–	–	–

AUC area under the concentration–time curve from zero to infinity, *CL/F* apparent oral clearance, C_{max} maximum observed drug concentration in plasma, *CV* coefficient of variation, *h* hours, *PK* pharmacokinetics, $t_{½}$ terminal half-life, t_{max} time to C_{max}

[a] $n = 12$, owing to unreliable $t_{½}$ in two participants

[b] Median (range)

[c] $n = 13$, owing to unreliable $t_{½}$ in one participant

[d] $n = 8$, owing to unreliable $t_{½}$ in three participants

[e] $n = 9$, owing to unreliable $t_{½}$ in two participants

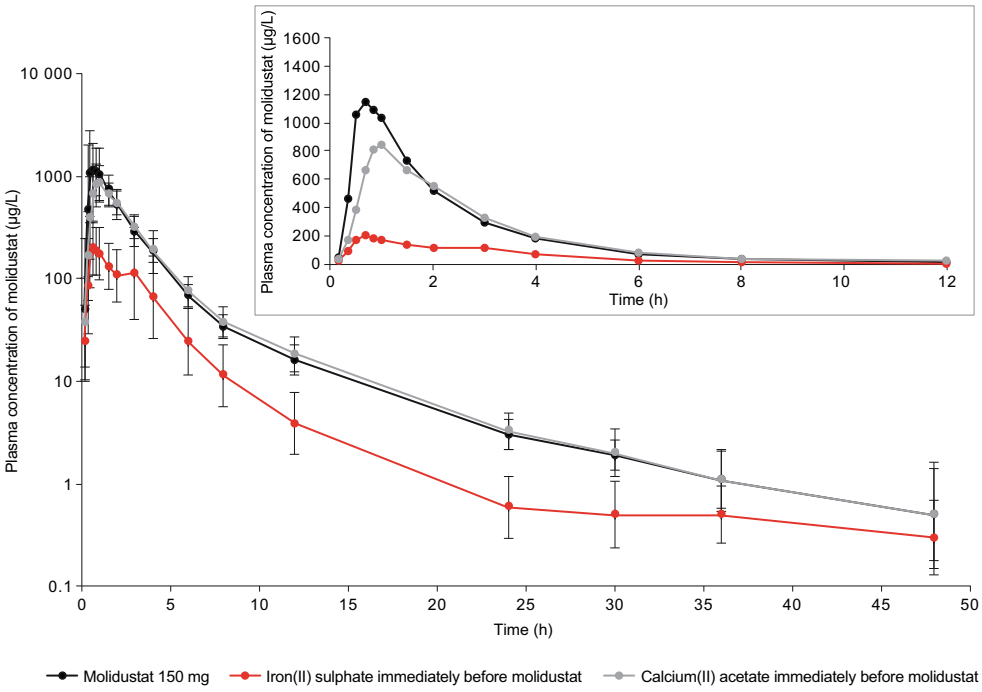

Fig. 1 Plasma concentrations of molidustat after administration of a single oral dose of molidustat 150 mg alone and together with iron(II) sulphate 304 mg or calcium acetate 1900 mg immediately before molidustat to healthy fasted volunteers in study 1 (geometric mean [SD], PK set, $N = 15$) *h* hours, *PK* pharmacokinetics, *SD* standard deviation

Table 5). Of those TEAEs that were considered by the investigator to be related to molidustat, syncope (mild intensity) was reported by one participant receiving molidustat alone, diarrhoea (mild) was reported by one participant receiving molidustat and iron(II) sulphate, and orthostatic hypotension was reported by one participant receiving molidustat and calcium acetate. Orthostatic hypotension may have been caused by the participant standing up too quickly after the prolonged resting and fasting time. No volunteers discontinued the study owing to TEAEs.

In study 2, eight participants (53.3%) experienced a total of 11 TEAEs (Supplementary Table 6). Overall, two participants experienced three TEAEs that were considered to be related to molidustat by the investigator: one after receiving iron(II)

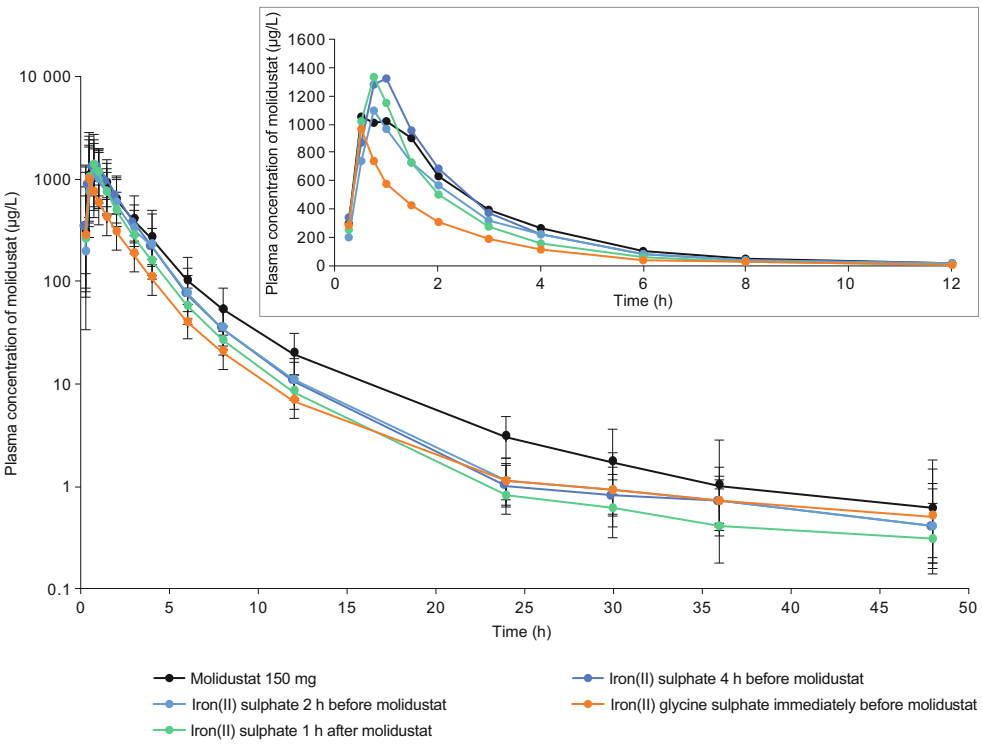

Fig. 2 Plasma concentrations of molidustat after administration of a single dose of molidustat 150 mg alone or together with a single dose of iron(II) sulphate 304 mg given either 4 h or 2 h before or 1 h after a single dose of molidustat 150 mg and after administration of a single dose of iron(II) glycine sulphate 567.7 mg immediately before administration of molidustat 150 mg to healthy fasted volunteers in study 2 (geometric mean [SD], PK set, $N = 14$) *h* hours, *PK* pharmacokinetics, *SD* standard deviation

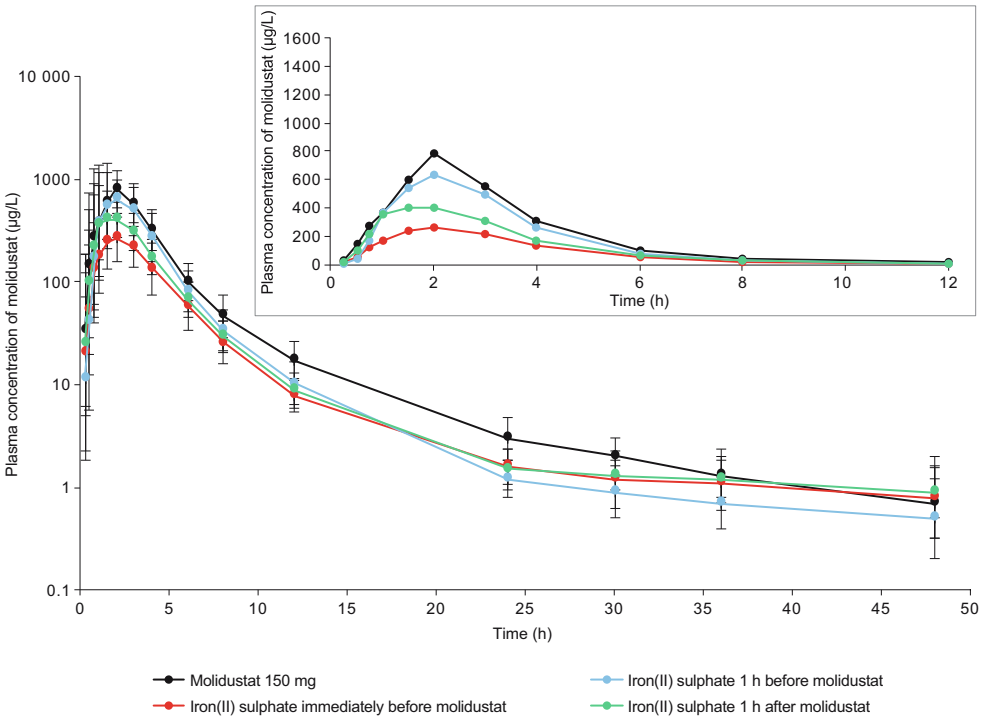

Fig. 3 Plasma concentrations of molidustat after administration of a single dose of molidustat 150 mg alone or together with a single dose of iron(II) sulphate 304 mg given either 1 h before, immediately before, or 1 h after molidustat to healthy fed volunteers in study 3 (geometric mean [SD]; PK set, $N = 11$) *h* hours, *PK* pharmacokinetics, *SD* standard deviation

sulphate 2 h before molidustat (mild headache); one after receiving iron(II) glycine sulphate immediately before molidustat (mild headache); and one after receiving iron(II) sulphate 1 h after molidustat (skin blemishes).

In study 3, three participants (25%) experienced a total of nine TEAEs (Supplementary Table 7). No TEAE was considered by the investigator to be related to molidustat.

In all studies, all TEAEs were of mild or moderate intensity. There were no deaths or serious adverse events during these studies. Furthermore, no clinically relevant changes in clinical laboratory parameters, urinalysis, blood pressure, heart rate, or electrocardiogram parameters were observed in any of the studies (data not shown).

Discussion

Molidustat is a HIF–PH inhibitor currently in phase 3 of clinical development for the treatment of anaemia in patients with CKD. Its efficacy and safety profiles were evaluated in the DIALOGUE phase 2 clinical trial programme [10]. Given that common concomitant medication in patients with CKD includes iron and calcium supplementations for anaemia [5], the potential for DDI between molidustat and iron or calcium supplements was investigated. The results of these DDI studies demonstrate a substantial reduction in molidustat exposure and endogenous EPO production when iron(II) sulphate preparations (oral iron supplements) were administered orally immediately before molidustat both under fasted and fed conditions. The effect of iron supplementation on molidustat exposure was less pronounced with increasing temporal separation between the two administrations and was significantly reduced when molidustat and iron supplements were administered at least 1 h apart. Co-administration of calcium acetate reduced the rate of molidustat absorption, but molidustat exposure was only slightly affected (−15% reduction in AUC), and endogenous EPO level profiles were unchanged compared with molidustat alone. The similar EPO response observed with both treatments indicates that no relevant clinical interaction is occurring between molidustat and oral calcium supplementation (e.g. calcium acetate).

The decreased exposure of molidustat in the presence of oral iron supplementation may result from reduced absorption owing to chelation, as suggested by unpublished in vitro data that demonstrated that the solubility of molidustat is reduced in the presence of polyvalent cations. The results of co-administration of calcium(II) and molidustat indicate that oral calcium(II) reduces molidustat solubility and, therefore, its rate of absorption; however, the complex seems to dissolve fast enough not to affect the extent of molidustat absorption. In the presence of oral iron(II), the solubility of molidustat is considerably decreased, resulting in markedly reduced rate and extent of absorption when both drugs are administered at the same time. Furthermore, the molidustat terminal $t_{½}$ was prolonged, and the concentration–time profile appeared rather flat in the terminal phase, which was probably the result of flip-flop kinetics; this suggested that solubility and absorption were the rate-limiting steps for molidustat exposure in the presence of iron(II). The effect of intravenous iron supplementation was not investigated; however, no impact of

Table 3 Effect on molidustat PK parameters, expressed as difference in geometric mean, of co-administration of oral iron(II) supplementation (iron(II) sulphate or iron(II) glycine sulphate) or calcium acetate with molidustat in the fed or fasted state

Study #	2	2	3	1	2	1	3	2	3
Cation	Iron(II) sulphate	Iron(II) sulphate	Iron(II) sulphate	Iron(II) sulphate	Iron(II) glycine sulphate	Calcium acetate	Iron(II) sulphate	Iron(II) sulphate	Iron(II) sulphate
State	Fasted	Fasted	Fed	Fasted	Fasted	Fasted	Fed	Fasted	Fed
Time[a]	−4 h[b]	−2 h[b]	−1 h[b]	0 h[b]	0 h[c]	0 h[d]	0 h[b]	+1 h[b]	+ 1 h[b]
AUC	−9%	−16%	−20%	−75%	−50%	−15%	−51%	−26%	−34%
(90% CI)	(–23%; 7%)	(–29%; –1%)	(–30%; –7%)	(–80%; –67%)	(–58%; –41%)	(–34%; 9%)	(–58%; –43%)	(–37%; –12%)	(–43%; –23%)
C_{max}(90% CI)	−10%	−0.3%	−20%	−84%	−46%	−47%	−61%	−12%	−40%
	(–36%; 26%)	(–29%; 40%)	(–40%; 6%)	(–88%; –78%)	(–61%; –24%)	(–61%; –28%)	(–71%; –49%)	(–37%; 23%)	(–55%; –20%)

AUC area under the concentration–time curve from zero to infinity, C_{max} maximum observed drug concentration in plasma, *h* hours, *PK* pharmacokinetics

[a] Time of iron or calcium administration relative to molidustat administration

[b] Eryfer 100 mg capsule (iron(II) sulphate 304 mg)

[c] Ferro Sanol duodenal capsule (iron(II) glycine sulphate 567.7 mg, enteric-coated)

[d] Calcet tablets (calcium acetate 1900 mg)

Table 4 EPO parameters expressed as geometric mean (% geometric CV) for $AUC_{(0–24)}$ and C_{max} and as median (range) for t_{max}, after single oral administration of molidustat 150 mg with or without co-administration of iron(II) sulphate 304 mg, iron(II) glycine sulphate 567.7 mg, or calcium acetate 1900 mg (PD sets)

	Molidustat alone	Iron(II) glycine sulphate immediately before molidustat	Iron(II) sulphate immediatelybefore molidustat	Iron(II) sulphate 1 h after molidustat	Iron(II) sulphate 1 h before molidustat	Iron(II) sulphate 2 h before molidustat	Iron(II) sulphate 4 h before molidustat	Calcium acetate immediately before molidustat
Study 1 (fasted, $N = 15$)								
$AUC_{(0–24)}$, IU × h/L	1171 (50.0)	–	628 (93.6)[a]	–	–	–	–	1209 (55.8)
C_{max}, IU/L								
Absolute value	77.8 (53.3)	–	39.0 (112)[a]	–	–	–	–	83.8 (56.0)
Ratio to baseline	9.43 (39.1)	–	4.41 (73.2)[a]	–	–	–	–	9.60 (54.0)
t_{max}, h	8.0 (6.0–12.1)	–	12.0 (8.0–12.0)[a]	–	–	–	–	8.0 (8.0–12.0)
Study 2 (fasted, $N = 14$)								
$AUC_{(0–24)}$, IU × h/L	1498 (45.6)	855 (65.7)	–	1098 (56.2)	–	1223 (47.7)	1357 (57.9)	–
C_{max}, IU/L								
Absolute value	103.6 (43.4)	56.2 (71.0)	–	75.5 (61.3)	–	86.6 (49.3)	100.5 (55.2)	–
Ratio to baseline	11.25 (44.5)	6.20 (52.2)	–	8.43 (33.7)	–	8.76 (29.3)	10.97 (45.0)	–
t_{max}, h	8.0 (6.0–12.0)	8.0 (6.0–12.0)	–	8.0 (6.0–12.0)	–	8.0 (6.0–12.0)	8.0 (6.0–12.0)	–
Study 3 (fed, $N = 11$)								
$AUC_{(0–24)}$, IU × h/L	1272 (42.8)	–	866 (35.8)	958 (42.5)	1105 (40.6)	–	–	–
C_{max}, IU/L								
Absolute value	85.9 (42.9)	–	54.8 (44.3)	64.5 (44.8)	77.9 (46.3)	–	–	–
Ratio to baseline	7.60 (47.5)	–	4.79 (55.7)	5.88 (40.4)	7.17 (53.2)	–	–	–
t_{max}, h	8.0 (6.0–8.0)	–	12.0 (6.0–12.0)	8.0 (6.0–12.0)	8.0 (6.0–12.0)	–	–	–

$AUC_{(0–24)}$ area under the concentration–time curve from 0 to 24 h, *C_{max}* maximum observed concentration in serum, *CV* coefficient of variation, *EPO* erythropoietin, *h* hours, *IU* international units, *PD* pharmacodynamics, *t_{max}* time to C_{max}

[a] $n = 14$

Table 5 Effect on EPO parameters expressed as difference in geometric mean of co-administration of oral iron(II) supplementation (iron(II) sulphate or iron(II) glycine sulphate) or calcium acetate with molidustat in the fed or fasted state

Study #	2	2	3	1	2	1	3	2	3
Cation	Iron(II) sulphate	Iron(II) sulphate	Iron(II) sulphate	Iron(II) sulphate	Iron(II) glycine sulphate	Calcium acetate	Iron(II) sulphate	Iron(II) sulphate	Iron(II) sulphate
State	Fasted	Fasted	Fed	Fasted	Fasted	Fasted	Fed	Fasted	Fed
Time[a]	−4 h[b]	−2 h[b]	−1 h[b]	0 h[b]	0 h[c]	0 h[d]	0 h[b]	+1 h[b]	+ 1 h[b]
$AUC_{(0–24)}$	−9%	−17%	−13%	−44%	−41%	+3%	−31%	−25%	−26%
(90% CI)	(–18%; 2%)	(–26%; –7%)	(–23%; –1%)	(–53%; –34%)	(–48%; 34%)	(–13%; 22%)	(–40%; –22%)	(–33%; –16%)	(–35%; –16%)
C_{max}(90% CI)	−2%	−15%	−9%	−48%	−44%	+7%	−36%	−25%	−26%
	(–15%; 12%)	(–26%; –3%)	(–22%; 6%)	(–58%; –37%)	(–51%; –36%)	(–12%; 30%)	(–45%; –25%)	(–35%; –15%)	(–37%; –14%)

$AUC_{(0–24)}$ area under the concentration–time curve from 0 to 24 h, *C_{max}* maximum observed concentration, *EPO* erythropoietin, *h* hours

[a] Time of iron or calcium administration relative to molidustat administration

[b] Eryfer 100 mg capsule (iron(II) sulphate 304 mg)

[c] Ferro Sanol duodenal capsule (iron(II) glycine sulphate 567.7 mg, enteric-coated)

[d] Calcet tablets (calcium acetate 1900 mg)

intravenous iron on molidustat exposure is expected because the primary interaction between the two drugs occurs in the gut. Additionally, intravenous iron supplementation uses iron(III), which has less influence on molidustat solubility than iron(II), and free plasma concentrations are too low for precipitation.

Molidustat is reported to increase endogenous EPO levels dose-dependently (EPO C_{max} was 39.8 IU/L and 14.8 IU/L in the molidustat 50 mg and placebo groups, respectively) [12]. The present data suggest that the impact of iron(II) co-administration on EPO exposure was less extensive than on molidustat pharmacokinetics (Supplementary Fig. 4). Therefore, the clinical relevance of this pharmacokinetic interaction will be fully explored once the phase 3 trials are complete.

The present data suggest possible strategies to limit the impact of oral iron supplements on molidustat exposure. Compared with concurrent dosing, the effect of iron supplementation on molidustat exposure was considerably reduced when iron supplements were administered either 1 h before or 1 h after molidustat. Indeed, there was almost no effect on molidustat exposure when iron supplementation was given 4 h before molidustat. Importantly, the effects on endogenous EPO increase were also less when administration of molidustat and iron supplements was separated in time.

The CIs of the ratios for iron either 1 h before or 1 h after molidustat vs molidustat alone for PK and PD were outside the 80–125% range (i.e. relative percentage changes between –20% and + 25%) recommended by the FDA and the EMA guidelines to claim no interaction [15, 16]. However, based on previous data, molidustat was well tolerated over a wide dose range (5–200 mg) and is dosed in phase 2 and 3 based on individual response [10, 11]. Consequently, it has been recommended that the intake of oral iron and molidustat should be separated by at least 1 h in the phase 3 clinical trials, which are currently ongoing.

Overall, molidustat alone or in combination with iron supplementation was well tolerated with few drug-related TEAEs. Of the six TEAEs related to molidustat administration, one occurred after molidustat alone, one after co-administration with calcium acetate, and four after co-administration with iron(II). Another two TEAEs were related to iron supplementation alone. No clinically relevant post-dose changes from baseline were observed in clinical laboratory parameters, urinalysis, blood pressure, heart rate, or electrocardiogram parameters.

The internal control provided by the crossover design, the range of treatment regimens, and the overall number of participants represents strengths of these studies. Using settings that reflect real-world clinical practice and comparing PK and PD outcomes are also strengths. The presented set of studies provides detailed scenarios on how temporal separation of drug intake can reduce the interaction observed compared with concomitant intake; however, these results should be interpreted in the context of several limitations. The interpretation of clinical relevance on changes in EPO levels is limited because the efficacy on Hb response could not be investigated in this single-dose study and in general because the studies were conducted in a selected healthy male volunteer population. The single-dose study design does not offer insight into the PK/PD profile of molidustat during long-term treatment.

Conclusion

Although PK and PD (effect on EPO) parameters of molidustat in healthy volunteers were affected by oral administration of iron(II) sulphate or iron(II) glycine sulphate immediately before molidustat, these effects were reduced by temporal separation of drug intake. This is currently being investigated in ongoing phase 3 clinical trials [8, 9]. Calcium supplement (investigated as calcium acetate) co-administration had no relevant effect on the pharmacokinetics or pharmacodynamics of molidustat.

Acknowledgments The authors would like to thank Dr Eva Maria Krieg for her support in study conduct and Dr Katharina Sommer, Dr Ann-Kristin Peterson, Dr Sandra Ligges, and Dr Uwe Thuss for their support in the study data analysis. The authors would like to acknowledge Dr Michael Leidig (CRS Mönchengladbach), who was the investigator responsible for the studies. Medical writing assistance was provided by Nicolas Bertheleme, PhD, of Oxford PharmaGenesis, Oxford, UK, funded by Bayer Yakuhin Ltd, Osaka, Japan. The datasets during and/or analysed during these current studies are available from the corresponding author on reasonable request.

Authors' contributions Study concept and design, AK, DvdM, SK, and SL; acquisition of data, AK, DvdM, SK, and SL; analysis and interpretation of data, AK, DvdM, SK, and SL; and drafting of the manuscript, DvdM, KM, SK, and SL. All authors participated in critical revision of the manuscript for important intellectual content and approved the final version.

Funding The studies were funded by Bayer AG, Wuppertal, Germany.

Compliance with ethical standards

Conflicts of interest AK, DvdM, SK, and SL are employees of Bayer AG. KM is an employee of Bayer Yakuhin Ltd.

Ethical approval The studies were approved by the Ethics Committee of North-Rhine Medical Council, Düsseldorf, Germany, and were conducted in accordance with the International Conference on Harmonization guideline on Good Clinical Practice and with the Declaration of Helsinki.

Informed consent Informed consent was obtained from all individual participants included in the studies.

References

1. Babitt JL, Lin HY (2012) Mechanisms of anemia in CKD. J Am Soc Nephrol 23(10):1631–1634
2. van Nooten FE, Green J, Brown R, Finkelstein FO, Wish J (2010) Burden of illness for patients with non-dialysis chronic kidney disease and anemia in the United States: review of the literature. J Med Econ 13(2):241–256
3. Portolés J, Gorriz JL, Rubio E, de Alvaro F, Garcia F, Alvarez-Chivas V, Aranda P, Martinez-Castelao A (2013) The development of anemia is associated to poor prognosis in NKF/KDOQI stage 3 chronic kidney disease. BMC Nephrol 14:2
4. Koury MJ, Haase VH (2015) Anaemia in kidney disease: harnessing hypoxia responses for therapy. Nat Rev Nephrol 11(7):394–410
5. KDIGO (2012) Clinical practice guideline for anemia in chronic kidney disease. Kidney Int Suppl 2(4):279–335
6. Del Vecchio L, Locatelli F (2016) An overview on safety issues related to erythropoiesis-stimulating agents for the treatment of anaemia in patients with chronic kidney disease. Expert Opin Drug Saf 15(8):1021–1030
7. Gupta N, Wish JB (2017) Hypoxia-inducible factor prolyl hydroxylase inhibitors: a potential new treatment for anemia in patients with CKD. Am J Kidney Dis 69(6):815–826
8. Akizawa T, Taguchi M, Matsuda Y, Iekushi K, Yamada T, Yamamoto H (2019) Molidustat for the treatment of renal anaemia in patients with dialysis-dependent chronic kidney disease: design and rationale of three phase III studies. BMJ Open 9(6):e026602
9. Yamamoto H, Taguchi M, Matsuda Y, Iekushi K, Yamada T, Akizawa T (2019) Molidustat for the treatment of renal anaemia in patients with non-dialysis-dependent chronic kidney disease: design and rationale of two phase III studies. BMJ Open 9(6):e026704
10. Macdougall IC, Akizawa T, Berns JS, Bernhardt T, Krueger T (2019) Effects of molidustat in the treatment of anemia in CKD. Clin J Am Soc Nephrol 14(1):28–39
11. Akizawa T, Macdougall IC, Berns JS, Bernhardt T, Staedtler G, Taguchi M, Iekushi K, Krueger T (2019) Long-term efficacy and safety of molidustat for anemia in chronic kidney disease: DIALOGUE extension studies. Am J Nephrol 49(4):271–280
12. Böttcher M, Lentini S, Arens ER, Kaiser A, van der Mey D, Thuss U, Kubitza D, Wensing G (2018) First-in-man-proof of concept study with molidustat: a novel selective oral HIF-prolyl hydroxylase inhibitor for the treatment of renal anaemia. Br J Clin Pharmacol 84(7):1557–1565
13. Beck H, Jeske M, Thede K, Stoll F, Flamme I, Akbaba M, Erguden JK, Karig G, Keldenich J, Oehme F, Militzer HC, Hartung IV, Thuss U (2018) Discovery of molidustat (bay 85-3934): a small-molecule oral hif-prolyl hydroxylase (HIF-PH) inhibitor for the treatment of renal anemia. ChemMedChem 13(10):988–1003
14. Dib J, Mongongu C, Buisson C, Molina A, Schanzer W, Thuss U, Thevis M (2017) Mass spectrometric characterization of the hypoxia-inducible factor (HIF) stabilizer drug candidate BAY 85-3934 (molidustat) and its glucuronidated metabolite BAY-348, and their implementation into routine doping controls. Drug Test Anal 9(1):61–67
15. FDA. Statistical approaches to establishing bioequivalence. (2001) https://www.fda.gov/media/70958/download. Accessed 19 August 2019
16. EMA. Guideline on the investigation of bioequivalence (2010) https://www.ema.europa.eu/en/documents/scientific-guideline/guideline-investigation-bioequivalence-rev1_en.pdf. Accessed 19 August 2019