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Four-Week Studies of Oral Hypoxia-Inducible Factor–Prolyl Hydroxylase Inhibitor GSK1278863 for Treatment of Anemia

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ABSTRACT

Hypoxia-inducible factor prolyl hydroxylase inhibitors stabilize levels of hypoxia-inducible factor that upregulate transcription of multiple genes associated with the response to hypoxia, including production of erythropoietin. We conducted two phase 2a studies to explore the relationship between the dose of the hypoxia-inducible factor–prolyl hydroxylase inhibitor GSK1278863 and hemoglobin response in patients with anemia of CKD (baseline hemoglobin 8.5–11.0 g/dl) not undergoing dialysis and not receiving recombinant human erythropoietin (nondialysis study) and in patients with anemia of CKD (baseline hemoglobin 9.5–12.0 g/dl) on hemodialysis and being treated with stable doses of recombinant human erythropoietin (hemodialysis study). Participants were randomized 1:1:1:1 to a once-daily oral dose of GSK1278863 (0.5 mg, 2 mg, or 5 mg) or control (placebo for the nondialysis study; continuing on recombinant human erythropoietin for the hemodialysis study) for 4 weeks, with a 2-week follow-up. In the nondialysis study, GSK1278863 produced dose-dependent effects on hemoglobin, with the highest dose resulting in a mean increase of 1 g/dl at week 4. In the hemodialysis study, treatment with GSK1278863 in the 5-mg arm maintained mean hemoglobin concentrations after the switch from recombinant human erythropoietin, whereas mean hemoglobin decreased in the lower-dose arms. In both studies, the effects on hemoglobin occurred with elevations in endogenous erythropoietin within the range usually observed in the respective populations and markedly lower than those in the recombinant human erythropoietin control arm in the hemodialysis study, and without clinically significant elevations in plasma vascular endothelial growth factor concentrations. GSK1278863 was generally safe and well tolerated at the doses and duration studied. GSK1278863 may prove an effective alternative for managing anemia of CKD.

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Advanced CKD is frequently associated with anemia[1,2] and its pathogenesis is multifactorial, inclusive of a relative deficiency of erythropoietin (EPO) and impaired absorption and utilization of iron.[3]

Current guidelines and recommendations for anemia management in CKD advise treatment with supplemental iron and recombinant human erythropoietins (rhEPOs) if appropriate.[4–8] Most patients with advanced CKD require both treatments.[9] Although improvement in quality of life has not been consistently demonstrated,[10,11] the introduction of

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rhEPO therapy led to a correction of hemoglobin levels in the majority of dialysis patients[12] and a reduction in the need for red blood cell transfusions.[12]

However, several large randomized trials of rhEPO have reported adverse cardiovascular outcomes. The Normal Hematocrit Study, which aimed to normalize hematocrit at 42% versus maintaining it at 30%, was terminated early because of concerns from the independent data monitoring committee around the higher all-cause mortality rate in the normal hematocrit arm compared with the low hematocrit arm, even though the prespecified termination criterion of an overall 5% significance was not met. The Correction of Hemoglobin and Outcomes in Renal Insufficiency (CHOIR) trial,[13] which studied patients with CKD who did not require dialysis (nondialysis-dependent [NDD]), was terminated after interim analysis indicated a trend toward an increased risk of cardiovascular events for those randomized to the high hemoglobin target arm of the trial. No difference in the rate of cardiovascular adverse events (AEs) was noted in the Cardiovascular Risk Reduction by Early Anemia Treatment with Epoetin Beta trial for NDD patients targeted to either a normal or subnormal hemoglobin level.[14] However, the study may have lacked sufficient power to report meaningful safety data as a result of the low number of events in the trial.[15] Finally, although the Trial to Reduce Cardiovascular Events with Aranesp Therapy (TREAT) did not demonstrate a difference in the primary end point (death or a nonfatal cardiovascular event), there were significantly higher stroke rates in NDD patients who received darbepoetin alfa versus placebo when targeting hemoglobin levels that were higher (13 g/dl) than those recommended in the present guidelines.[16] Collectively, the results of these trials suggest a potential link for high rhEPO dose and achieved and targeted hemoglobin with increased cardiovascular risk. Interestingly, secondary analyses of both the CHOIR and TREAT trials support the hypothesis that rhEPO dose, not achieved or targeted hemoglobin level, underlies the increased cardiovascular risk.[17,18]

Several novel treatments, including agents inhibiting hypoxia-inducible factor (HIF)–prolyl hydroxylase (*e.g.*, GSK1278863), are being developed to treat anemia of CKD.[19,20] HIF–prolyl hydroxylase inhibitors stimulate erythropoiesis through inhibition of HIF–prolyl hydroxylase enzymes (PHD1, PHD2, and PHD3) mimicking the effects of hypoxia on this system. Thus, similar to when under hypoxic conditions, the HIF hydroxylation catalyzed by PHDs is reduced, preventing degradation of HIF-α and facilitating its translocation to the nucleus. The accumulation of HIF-α transcription factors enables transcription of HIF-responsive genes associated with the adaptive response to hypoxia. HIF-modulated gene expression regulates production of EPO in the kidney and liver and enhances red blood cell progenitor maturation and proliferation in the bone marrow microenvironment.[21] HIF also regulates hepcidin and can thereby affect iron homeostasis.[22,23] Elevated hepcidin levels limit iron absorption in the gut,[24] limiting iron available for erythropoiesis and exacerbating anemia.[25] Concordantly, pharmacologic inhibition of HIF–prolyl hydroxylase has been shown to stimulate EPO production in both animal models[26] and in humans[27–30] and to reduce circulating levels of hepcidin.[28–32]

GSK1278863 is in phase 2b clinical development. Preclinical and clinical data show that GSK1278863 stimulates endogenous EPO production[33] and reduces circulating hepcidin concentrations, resulting in increased erythropoiesis.[31] In humans, the observed increases in hemoglobin are associated with circulating EPO levels that are markedly lower than those observed during administration of rhEPO.[34] GSK1278863 is highly protein bound (>99%) and undergoes negligible renal or dialysis clearance.[35]

Here, we report the results of two phase 2a trials that assessed the hemoglobin dose response, safety, and tolerability of a 4-week administration of GSK1278863, including a study in anemic patients with CKD who were not dialysis dependent and were not currently receiving rhEPO (nondialysis study) and a study in patients who were on hemodialysis and were treated with stable doses of rhEPO (HDD study).

RESULTS

In the nondialysis study, of the 73 patients randomized, 59 (81%) completed the study through follow-up at week 6 (Supplemental Figure 1A). In the HDD study, of the 83 patients randomized, 70 (84%) completed through follow-up at week 6 (Supplemental Figure 1B). The primary reasons for withdrawal across the two studies (14 in the nondialysis study; 13 in the HDD study) were protocol deviations, meeting hemoglobin stopping criteria, and withdrawn consent (Supplemental Figure 1). Baseline demographics of patients for both studies are shown in Table 1 and were balanced overall across the treatment arms. Patients were generally compliant with the study medication across both studies, taking, on average, 24–28 days of study medication (of the scheduled 29 days) in the GSK1278863 and placebo arms, as assessed by returned tablet count.

Hemoglobin

In the nondialysis study, mean baseline hemoglobin concentrations were similar across the treatment groups, ranging from 9.74 to 10.08 g/dl (Table 2). GSK1278863 demonstrated a dose-dependent increase in hemoglobin over the treatment period (Figure 1, Table 2), with the 5-mg dose arm producing a mean±SD modeled increase of 1.01±0.26 g/dl (primary end point) and a mean±SD observed increase of 0.95±0.66 g/dl over the 4-week treatment period.

In the HDD study, mean baseline hemoglobin concentrations were similar across the treatment groups, ranging from 10.66 to 10.89 g/dl (Table 2). Both for modeled (primary end point) and observed data, the mean hemoglobin was maintained over the 4-week treatment period in the 5-mg arm after switching from rhEPO to GSK1278863, similar to the result in the arm continuing rhEPO; however, decreases in mean hemoglobin concentrations occurred in the 0.5-mg and 2-mg arms after switching from rhEPO to GSK1278863 (Figure 1, Table 2).

Table 1. Baseline demographic characteristics (safety population)

Parameter	Nondialysis Study					HDD Study				
	Placebo (*n*=18)	GSK1278863			Total (*n*=72)	rhEPO (*n*=20)	GSK1278863			Total (*n*=82)
		0.5 mg (*n*=17)	2 mg (*n*=18)	5 mg (*n*=19)			0.5 mg (*n*=21)	2 mg (*n*=21)	5 mg (*n*=20)	
Age (yr)	69.2±11.0	66.6±11.7	66.9±11.4	71.3±11.3	68.6±11.3	64.2±12.8	56.4±16.8	55.2±18.4	55.6±17.9	57.8±16.8
Women	14 (78)	12 (71)	10 (56)	16 (84)	52 (72)	4 (20)	3 (14)	8 (38)	8 (40)	23 (28)
Race										
n	19	16	18	18	71	20	21	20	19	80
White	12 (63)	10 (63)	12 (67)	14 (78)	48 (68)	14 (70)	14 (67)	14 (70)	12 (63)	54 (68)
African American	4 (21)	4 (25)	4 (22)	2 (11)	14 (20)	4 (20)	7 (33)	3 (15)	4 (21)	18 (23)
Asian	2 (11)	2 (13)	2 (11)	1 (6)	7 (10)	0	0	3 (15)	2 (11)	5 (6)
Other	1 (5)	0	0	1 (6)	2 (3)	2 (10)	0	0	1 (5)	3 (4)
BMI (kg/m^2)	32.1 (9.6)	34.2 (9.7)	30.6 (5.5)	31.2 (7.6)	32.0 (8.2)	29.4±7.7	28.7±8.1	29.4±5.9	30.3±9.4	29.5±7.7
Baseline eGFR (ml/min per 1.73 m^2)[a]	23.2±11.5	23.8±9.8	24.2±12.5	24.2±10.8	23.8±11.0					
CKD stage[a]										
3a	1 (6)	1 (6)	2 (11)	1 (5)	5 (7)					
3b	3 (17)	4 (24)	3 (17)	5 (26)	15 (21)					
4	8 (44)	9 (53)	7 (39)	8 (42)	32 (44)					
5	6 (33)	3 (18)	6 (33)	5 (26)	20 (28)					
Cardiovascular risk factors										
Any condition	18 (100)	17 (100)	18 (100)	19 (100)	72 (100)	20 (100)	20 (95)	19 (90)	19 (95)	78 (95)
Angina pectoris	1 (6)	1 (6)	3 (17)	1 (5)	6 (8)	6 (30)	2 (10)	3 (14)	2 (10)	13 (16)
Diabetes	12 (67)	12 (71)	14 (78)	12 (63)	50 (69)	11 (55)	8 (38)	10 (48)	8 (40)	37 (45)
Hyperlipidemia	14 (78)	11 (65)	13 (72)	17 (89)	55 (76)	14 (70)	11 (52)	11 (52)	11 (55)	47 (57)
Hypertension	17 (94)	16 (94)	18 (100)	19 (100)	70 (97)	20 (100)	20 (95)	19 (90)	19 (95)	78 (95)
MI	0	1 (6)	3 (17)	1 (5)	5 (7)	6 (30)	1 (5)	3 (14)	0	10 (12)
Stroke	0	0	1 (6)	1 (5)	2 (3)	1 (5)	1 (5)	2 (10)	1 (5)	5 (6)

Unless otherwise indicated, all values are given as means±SD or *n* (%). BMI, body mass index; MI, myocardial infarction.
[a]Not applicable for the HDD study.

Table 2. Baseline and mean changes in modeled and observed hemoglobin (in grams per decaliter) after 4 weeks of treatment with GSK1278863

Parameter	Nondialysis Study				HDD Study			
	Placebo (*n*=19)	GSK1278863			rhEPO (*n*=20)	GSK1278863		
		0.5 mg (*n*=16)	2 mg (*n*=18)	5 mg (*n*=18)		0.5 mg (*n*=21)	2 mg (*n*=20)	5 mg (*n*=19)
Baseline	9.91±0.57 (*n*=19)	9.98±0.58 (*n*=16)	9.74±0.70 (*n*=18)	10.08±0.72 (*n*=18)	10.89±0.52 (*n*=20)	10.66±0.66 (*n*=21)	10.75±0.60 (*n*=20)	10.80±0.61 (*n*=19)
Modeled CFB at 4 wk[a]	−0.15±0.19 (*n*=18)	0.13±0.22 (*n*=13)	0.46±0.22 (*n*=17)	1.01±0.26 (*n*=18)	−0.27±0.63 (*n*=19)	−1.13±0.68 (*n*=20)	−1.07±0.77 (*n*=20)	0.21±0.75 (*n*=18)
Observed CFB at 4 wk	−0.23±0.51 (*n*=15)	−0.12±0.51 (*n*=11)	0.12±0.51 (*n*=15)[d]	0.95±0.66 (*n*=17)	−0.25±0.81 (*n*=19)	−1.06±0.83 (*n*=18)	−0.93±0.82 (*n*=18)	−0.08±0.63 (*n*=17)
Maximum change in hemoglobin over 4 wk[b]	0.15±0.10 (*n*=19)	0.13±0.13 (*n*=16)	0.49±0.21 (*n*=18)	1.05±0.15 (*n*=18)				
Model adjusted 95% CI	0.15±0.15 (*n*=19) −0.14 to 0.45	0.14±0.16 (*n*=16) −0.18 to 0.46	0.47±0.15 (*n*=18) 0.17 to 0.78	1.07±0.15 (*n*=18) 0.76 to 1.37				
Summary of hemoglobin variability[c]					(*n*=19)	(*n*=20)	(*n*=20)	(*n*=18)
Within-subject SD					0.35±0.19	0.53±0.27	0.55±0.33	0.40±0.36
Min, max					0.1, 0.8	0.1, 1	0.1, 1.1	0.1, 1.7
Residual SD					0.20±0.11	0.24±0.11	0.26±0.12	0.26±0.31
Min, max					0.1, 0.5	0.1, 0.6	0.1, 0.5	0.1, 1.4
Hemoglobin AUC					10.89±8.69	16.67±9.64	18.76±12.75	11.89±8.06
Min, max					1.1, 32	4.3, 37.8	3.6, 45.3	2.9, 32.4

Analysis based on the intent-to-treat population. CFB, change from baseline; 95% CI, 95% confidence interval; min, minimum; max, maximum; AUC, area under the curve.

[a]For modeled change at week 4, patients required a baseline and ≥2 nonmissing postbaseline values. If the Quest hemoglobin value was missing and a HemoCue value present, the HemoCue value was used.

[b]Not applicable for the HDD study.

[c]Not applicable for the nondialysis study.

[d]Excluding a single outlier in the patients not undergoing dialysis (see the Supplemental Material for details).

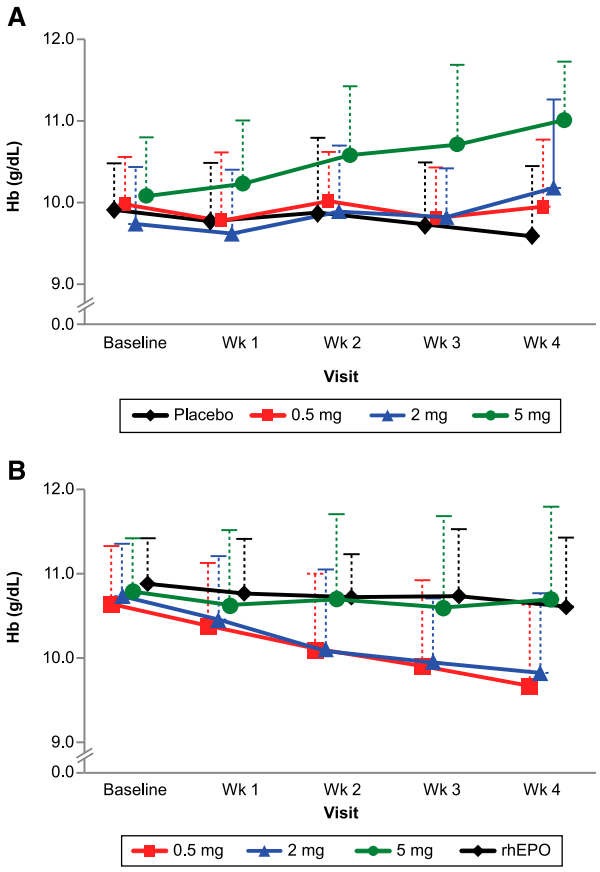

Figure 1. Observed mean±SD hemoglobin concentrations over the study period during administration of placebo, rhEPO, or GSK1278863 (intent-to-treat population) in both the nondialysis and HDD studies. (A) Nondialysis study. (B) HDD study. One patient in the rhEPO group had a hemoglobin value that was considered to be an outlier because the result was not feasible and was possibly due to a sample preparation error. This value at week 2 was removed from all hemoglobin summaries and analyses.

Other Blood Parameters

Changes in other blood parameters (hematocrit, red blood cell count, and reticulocyte production) were consistent with hemoglobin effects (Supplemental Table 1).

Erythropoietin Levels

Median baseline (day 1) plasma EPO concentrations were similar across treatment groups (Table 3). The median peak circulating EPO concentrations (observed at any time during the study) are shown in Table 3. EPO concentrations did not accumulate over 4 weeks of treatment with GSK1278863, and plasma EPO concentrations returned to the predose baseline (from day 1) in all groups. Although peak EPO concentrations appeared to increase with the dose of GSK1278863, peak EPO concentrations in the control group who continued receiving rhEPO from the HDD study were at least 12-fold higher than in any of the GSK1278863 groups in either study.

Measures of Iron Metabolism and Utilization

Mean baseline and change from baseline (at week 4) in serum hepcidin, ferritin, transferrin, transferrin saturation, total iron binding capacity, and serum iron concentrations are shown in Table 3.

In the nondialysis study, GSK1278863 demonstrated a dose-dependent decrease in hepcidin concentration from baseline. This decrease was not evident in the placebo group. GSK1278863 produced changes in several iron parameters from baseline to week 4 (Table 3). Specifically, ferritin decreased after 4 weeks in the 5-mg arm, coupled with increases in transferrin and total iron binding capacity.

In the HDD study, hepcidin concentrations did not increase from baseline to 4 weeks in either the rhEPO or 5-mg GSK1278863 arms, whereas elevations were noted in the 0.5-mg and 2-mg GSK1278863 arms. Dose-related trends toward a decrease in ferritin were noted with increasing doses of GSK1278863. Although there was a high degree of variability in other iron parameters, small trends for increases in transferrin saturation, total iron binding capacity, and total serum iron were noted for the 0.5-mg and 2-mg arms (Table 3).

Vascular Endothelial Growth Factor

Table 3 depicts mean baseline and change from baseline (at week 4) in vascular endothelial growth factor (VEGF). There was high intersubject variability but no clear difference in VEGF levels across the treatment arms in either study (Table 3).

Safety

Across both studies, five AEs were reported in ≥1 participant receiving GSK1278863. In the nondialysis study, nausea was the most common event reported (two patients in the 0.5-mg arm; one patient in the 5-mg arm), whereas in the HDD study, anemia was the most common event reported (three patients in the 0.5-mg arm; one patient in the 2-mg arm). Serious AEs were reported for five patients during therapy in the nondialysis study and four in the HDD study. Two additional serious AEs, both in the 0.5-mg GSK1278863 group in the HDD study, occurred after therapy (Supplemental Table 2). No deaths occurred during either study, and there were no clinically significant changes in laboratory parameters, electrocardiography results, or vital signs in either study.

DISCUSSION

These studies demonstrate the ability of GSK1278863 to increase (nondialysis study) or maintain (HDD study) hemoglobin levels over 4 weeks in patients with CKD. In the nondialysis study, GSK1278863 dose-dependently increased hemoglobin concentration from baseline. The highest dose of GSK1278863 (5 mg) increased hemoglobin concentrations by approximately 1.0 g/dl. In the HDD study, in which patients were switched to GSK1278863 from a stable dose of rhEPO, mean hemoglobin was maintained in the 5-mg GSK1278863

Table 3. Baseline and changes in EPO, measures of iron metabolism and utilization after 4 weeks of treatment with GSK1278863

Parameter	Nondialysis Study				HDD Study			
	Placebo (*n*=19)	GSK1278863			rhEPO (*n*=20)	GSK1278863		
		0.5 mg (*n*=16)	2 mg (*n*=18)	5 mg (*n*=18)		0.5 mg (*n*=21)	2 mg (*n*=20)	5 mg (*n*=19)
EPO (U/L)								
Baseline, median	12.4 (*n*=18)	13.8 (*n*=13)	9.9 (*n*=16)	11.2 (*n*=18)	9.6 (*n*=19)	11.1 (*n*=18)	8.9 (*n*=18)	8.1 (*n*=19)
Min, max	6.4, 35.6	2.8, 21.8	3.1, 37.2	3.9, 28.4	2.5, 54.1	2.5, 37.7	2.5, 26.3	2.5, 39.9
Peak, median	16.6 (*n*=18)	15.6 (*n*=13)	22.4 (*n*=17)	34.4 (*n*=18)	424.9 (*n*=19)	13.9 (*n*=19)	12.7 (*n*=18)	24.7 (*n*=17)
Min, max	9.7, 78.6	5.9, 26.7	10.5, 63.5	5.3, 69.0	9.8, 1371.2	3.5, 86.3	5.0, 57.1	6.6, 1786.5
CFB at peak, median	3.7 (*n*=18)	2.6 (*n*=13)	7.5 (*n*=16)	18.3 (*n*=18)	418.8 (*n*=19)	2.1 (*n*=18)	2.3 (*n*=18)	14.1 (*n*=16)
Min, max	−2.7, 69.7	−4.3, 10.9	−9.3, 41.1	−1.1, 60.2	−17.8, 1339.4	−19.9, 48.5	−11.6, 42.5	−7.1, 1775.6
Hepcidin (μg/L)								
Baseline, median	295.4 (*n*=19)	264.2 (*n*=16)	308.0 (*n*=17)	210.7 (*n*=18)	286.3 (*n*=20)	412.7 (*n*=21)	351.5 (*n*=20)	483.1 (*n*=19)
Min, max	116.0, 1022.4	40.6, 623.1	88.7, 520.0	120.3, 994.9	61.5, 893.7	119.8, 1567.0	51.9, 1238.3	93.5, 1241.8
CFB at 4 wk, median	−7.3 (*n*=15)	−16.2 (*n*=10)	−82.1 (*n*=15)	−143.6 (*n*=16)	−41.0 (*n*=18)	154.0 (*n*=18)	103.7 (*n*=17)	−0.5 (*n*=17)
Min, max	−137.3, 286.3	−222.1, 238.8	−281.6, 158.8	−441.7, 43.8	−299.5, 266.6	−141.9, 866.6	−656.0, 463.4	−379.4, 706.7
Ferritin (μg/L)								
Baseline	243.7±161.1 (*n*=19)	265.5±235.7 (*n*=16)	350.5±266.4 (*n*=18)	298.6±264.8 (*n*=18)	441.7±252.4 (*n*=20)	741.6±455.7 (*n*=21)	686.6±483.8 (*n*=20)	734.9±386.8 (*n*=19)
CFB at 4 wk	−24.3±38.6 (*n*=15)	−35.8±54.7 (*n*=12)	−8.2±268.8 (*n*=17)	−101.8±91.1 (*n*=17)	−27.9±166.0 (*n*=19)	74.2±174.0 (*n*=19)	−5.8±178.7 (*n*=18)	−80.8±95.9 (*n*=17)
Transferrin saturation (%)								
Baseline	22.1±7.5 (*n*=19)	21.6±7.4 (*n*=16)	25.5±6.4 (*n*=18)	25.6±7.5 (*n*=18)	28.4±12.3 (*n*=20)	30.0±11.0 (*n*=21)	33.0±14.4 (*n*=20)	32.6±12.0 (*n*=19)
CFB at 4 wk	1.5±6.1 (*n*=15)	−3.1±4.9 (*n*=12)	−2.6±6.2 (*n*=17)	−3.4±11.3 (*n*=17)	1.2±13.0 (*n*=19)	7.7±20.1 (*n*=18)	10.1±15.4 (*n*=18)	0.3±11.8 (*n*=17)
Transferrin (g/L)								
Baseline	2.0±0.3 (*n*=19)	2.2±0.3 (*n*=16)	2.1±0.4 (*n*=18)	2.3±0.5 (*n*=18)	1.9±0.4 (*n*=20)	1.7±0.4 (*n*=21)	1.6±0.3 (*n*=20)	1.7±0.5 (*n*=19)
CFB at 4 wk	0.01±0.3 (*n*=15)	0.03±0.3 (*n*=12)	0.29±0.3 (*n*=17)	0.39±0.4 (*n*=17)	0.0±0.3 (*n*=19)	0.1±0.5 (*n*=19)	0.2±0.2 (*n*=18)	0.2±0.3 (*n*=17)
Total iron binding capacity (μmol/L)								
Baseline	49.1±4.3 (*n*=19)	53.4±7.7 (*n*=16)	51.8±9.5 (*n*=18)	56.0±10.3 (*n*=18)	44.6±8.5 (*n*=20)	41.4±8.8 (*n*=21)	39.3±5.5 (*n*=20)	41.1±8.9 (*n*=19)
CFB at 4 wk	−0.5±3.7 (*n*=15)	0.3±6.3 (*n*=12)	5.1±4.1 (*n*=17)	8.3±5.5 (*n*=17)	1.0±4.6 (*n*=19)	3.3±6.3 (*n*=18)	3.9±3.4 (*n*=18)	5.2±3.2 (*n*=17)
Total serum iron (μmol/L)								
Baseline	10.8±3.7 (*n*=19)	11.2±3.3 (*n*=16)	12.9±3.1 (*n*=18)	14.1±4.0 (*n*=18)	12.5±5.5 (*n*=20)	12.1±4.4 (*n*=21)	12.9±5.6 (*n*=20)	12.8±3.4 (*n*=19)
CFB at 4 wk	0.6±2.8 (*n*=15)	−1.7±3.3 (*n*=12)	−0.4±3.3 (*n*=17)	−0.4±6.1 (*n*=17)	0.4±5.7 (*n*=19)	5.0±9.5 (*n*=19)	5.7±6.9 (*n*=18)	2.2±4.7 (*n*=17)
VEGF (ng/L)								
Baseline	90.4±123.3 (*n*=19)	76.1±55.7 (*n*=16)	82.5±89.2 (*n*=17)	63.6±32.4 (*n*=18)	87.5±46.7 (*n*=19)	124.7±55.4 (*n*=21)	113.7±67.3 (*n*=20)	116.7±82.6 (*n*=19)
CFB at 4 wk	−43.3±136.0 (*n*=15)	0.8±37.9 (*n*=9)	−3.9±36.5 (*n*=15)	5.6±41.4 (*n*=15)	1.2±40.3 (*n*=16)	−21.4±50.1 (*n*=17)	−4.6±60.8 (*n*=17)	19.9±79.6 (*n*=17)

Analysis based on the intent-to-treat population. Unless otherwise indicated, all values are mean±SD. CFB, change from baseline.

dose arm in a similar manner to the rhEPO control arm, whereas the 0.5-mg and 2-mg GSK1278863 dose arms did not maintain mean hemoglobin. However, in all GSK1278863 arms, there was intrasubject variability within dosing arms, with some patients responding to lower doses and others not responding to higher doses. Determinants of differences in responsiveness will be explored in future trials.

The effects of GSK1278863 on hemoglobin in both studies occurred with relatively small increases in plasma EPO concentrations, compared with changes in EPO concentration observed after administering injectable rhEPO. Measured plasma EPO concentrations in these studies remained within the range previously reported for patients with ESRD (range, 4.4–101.8 U/L) who were not treated with rhEPO[36] and were of similar magnitude to endogenous EPO concentrations seen during exposure to high altitudes,[37] whereas rhEPO concentrations were at least 12-fold higher in the rhEPO control group in the HDD study than in any of the GSK1278863 groups across both studies. Thus, GSK1278863 can increase (nondialysis patients not taking rhEPO) or maintain (HDD patients switched from rhEPO) hemoglobin while maintaining physiologic EPO concentrations. Given the hypothesis that supraphysiologic EPO concentrations achieved during rhEPO treatment may contribute to the adverse cardiovascular effects associated with rhEPO,[1,13,14,16,38] great interest exists in developing novel therapies that may increase or maintain hemoglobin without requiring supraphysiologic EPO concentrations. Although long-term data on the safety of HIF–prolyl hydroxylases have not yet been reported, there is a potential for these agents to effectively treat anemia with reduced incidence of adverse cardiovascular events, given that they elevate hemoglobin levels at doses that produce markedly lower plasma EPO concentrations than rhEPO. Clearly, additional data from studies powered to assess differences in the incidence of adverse cardiovascular outcomes as well as other long-term safety parameters are required to test this hypothesis.

Hepcidin concentrations are suppressed by erythropoiesis and by therapeutic doses of rhEPO.[39–41] In addition to direct suppression of hepcidin by erythropoiesis, HIF mechanisms independent of erythropoiesis have also been implicated in decreasing hepcidin,[42] although this view is somewhat controversial.[43] Pharmacologic inhibition of HIF–prolyl hydroxylase has been shown to stimulate EPO production in both animal models[26] and in humans[27–30] and to reduce circulating levels of hepcidin.[28–32] In these studies, hepcidin was only reduced from baseline within the 5-mg GSK1278863 arm in the nondialysis study, the only dose arm that raised mean hemoglobin from baseline. In the HDD study, no changes were noted in hepcidin concentrations in either the 5-mg GSK1278863 arm or the rhEPO control arm. Because rhEPO has been reported to suppress hepcidin,[40,41] mean hepcidin concentrations may have already been suppressed before randomization in the HDD study because all patients were receiving rhEPO. Consistent with this hypothesis, dose arms in which GSK1278863 was ineffective in maintaining mean hemoglobin (0.5 mg and 2 mg) demonstrated increases in mean hepcidin concentrations after patients were switched from rhEPO. These data suggest that a therapeutically relevant dose of GSK1278863 that is able to increase hemoglobin in rhEPO-naïve patients and maintain hemoglobin levels after switching from rhEPO to GSK1278863 could also suppress hepcidin. Additional study is required to delineate whether GSK1278863 has an independent effect on reducing plasma hepcidin concentrations or whether the observed changes are secondary to the effects on endogenous EPO concentrations and/or increased erythropoiesis.

In the nondialysis population, ferritin and transferrin saturation decreased in all GSK1278863 arms, whereas total iron binding capacity appears to increase in a dose-dependent fashion, suggesting that GSK1278863 may increase iron utilization in the nondialysis population at these doses. On the other hand, in the HDD study, the changes in transferrin saturation, total iron binding capacity, and ferritin in the 0.5-mg and 2-mg GSK1278863 arms relative to those in the 5-mg arm may reflect the less robust erythropoiesis in the 0.5-mg and 2-mg GSK1278863 arms. Further investigation as to the effects on iron mobilization in this patient population will need to be assessed in longer-term studies.

The effect of GSK1278863 on plasma VEGF concentration was measured in these studies because of the potential for HIF–prolyl hydroxylase inhibitors to increase VEGF levels through induction of the VEGF gene.[44] Furthermore, HIF–prolyl hydroxylases may indirectly regulate VEGF through their effects on EPO, which in turn stimulates production of VEGF.[45] The clinical relevance of potential effects of VEGF elevation is not known. However, given the theoretical possibility that VEGF may contribute to tumor formation, tumor angiogenesis, and proliferative retinopathy and macular edema,[46] the observation of no changes in plasma VEGF concentrations with therapeutically relevant doses of GSK1278863 in both studies is important.

These studies have some limitations. Because of the short duration of the trials, these studies did not assess whether the effects of GSK1278863 on hemoglobin will be sustained over a longer period, although other agents in the class have demonstrated durability up to 24 weeks[32]; these studies also did not assess whether GSK1278863 could be safely and effectively titrated to achieve a desired hemoglobin level. The conclusions of this study are based on a relatively small sample of primarily white, North American/Western European patients and may not be generalizable to a broader population. Compliance was assessed *via* returned tablet count, which may overestimate compliance rates.[47]

Because of practical limitations, the pharmacodynamic assessments were limited to a period of up to 11 hours after study medication administration, suitable to capture the peak response observed in prior studies.[33] Despite this extended sampling window, the true peak plasma concentrations of analytes, such as EPO and VEGF, may have been missed in some patients.

In summary, treatment with GSK1278863 for 4 weeks resulted in a dose-dependent increase in hemoglobin concentration in patients with CKD who were not dialysis dependent and did not receive rhEPO. In patients who received hemodialysis and were switched from rhEPO to GSK1278863, a 5-mg dose of GSK1278863 was effective in maintaining mean hemoglobin concentrations. In both studies, the effect of GSK1278863 on hemoglobin was noted in the absence of clinically significant elevations in plasma EPO or VEGF concentrations. Although decreases in hepcidin might be expected to improve iron stores, the length and design of the two studies does not allow clear interpretation, and the effect of GSK1278863 on iron metabolism will require further evaluation in longer duration trials.

These results suggest that GSK1278863 may be an effective alternative to rhEPO by increasing hemoglobin concentrations (nondialysis study) or maintaining hemoglobin concentrations after a switch from rhEPO (HDD study). Future studies of larger size and longer duration will be required to assess whether these findings translate into an improved cardiovascular risk profile for GSK1278863 compared with rhEPO.

CONCISE METHODS

Patients

Entry Criteria Specific to the Nondialysis Study

Patients in the nondialysis study had CKD stages 3–5 as defined by eGFR calculated using the Modification of Diet in Renal Disease equation.[48] Patients were not undergoing dialysis and had not used rhEPO within the past 7 weeks. All patients had a stable hemoglobin concentration between 8.5 and 11.0 g/dl during the 2-week run-in period. Patients were excluded from the study if they were on dialysis or were expected to initiate dialysis during the time they would be in the study.

Entry Criteria Specific to the HDD Study

All patients received hemodialysis three times weekly for at least 8 weeks, were being adequately dialyzed, and were using rhEPO, with total weekly doses that varied ≤50% for 4 weeks before the study. All patients had stable hemoglobin concentrations between 9.5 and 12.0 g/dl during the 2-week run-in period. Patients were excluded from the study if they were on peritoneal dialysis or were expected to change dialysis modality within the study time period. In addition, patients were excluded if they were hyporesponsive to rhEPO (defined as an epoetin dose of ≥360 IU/kg per week intravenously or a darbepoetin dose of ≥1.8 μg/kg per week intravenously) within the prior 8 weeks.

Entry Criteria Common to Both Studies

All patients were aged ≥18 years, weighed ≥45 kg, had vitamin B_{12} levels above the lower limit of the reference range, folate ≥2.0 ng/ml, ferritin ≥40 ng/ml, and transferrin saturation within the reference range. Potential participants were ineligible if they had a renal transplant or had recent significant cardiovascular, hematologic, hepatic, or chronic inflammatory disease. Additional exclusion criteria included major surgery or planned surgery within 12 weeks of screening, having recently received a blood transfusion, an active gastrointestinal ulcer or active gastrointestinal bleed within 12 weeks of screening, creatinine phosphokinase >5 times the upper limit of normal, malignancy within 5 years of screening, or significant hyperparathyroidism (parathyroid hormone ≥600 pg/ml). They were also excluded if they had excessively high creatine phosphokinase (>5 times the upper limit of normal) or proliferative retinopathy requiring treatment in the last 12 months, or macular edema requiring treatment.

Informed consent was obtained for all participants. The study was performed in adherence with the Declaration of Helsinki and was approved by the relevant institutional review boards/independent ethics committees.[49]

Patients were withdrawn from the trials for a variety of reasons, including if they met protocol-defined stopping criteria for hemoglobin (<8.0 g/dl, ≥13.0 g/dl, or a change of ≥2.0 g/dl in a week), received a blood transfusion, or were treated with rhEPO during the study period (nondialysis study only).

Study Design

Both studies were 4-week, phase 2a, randomized, blinded, controlled (placebo for the nondialysis study; rhEPO for the HDD study), parallel-group, multicenter studies designed to evaluate the safety and efficacy of GSK1278863 in patients with anemia of CKD who were not undergoing dialysis and were not taking rhEPO (nondialysis study) and in patients who were on hemodialysis and were being treated with stable doses of rhEPO (HDD study). The nondialysis study was carried out in 42 centers in the United States, Canada, and Germany, and the HDD study was carried out in the United States, Canada, Germany, Denmark, Norway, and Sweden. Both studies consisted of a 2-week screening phase, during which all patients were monitored to ensure that their hemoglobin remained stable within the desired range. Patients meeting the screening criteria were randomized to one of four treatment groups for 4 weeks: placebo (in the nondialysis study), rhEPO (in the HDD study), or GSK1278863 0.5 mg, 2 mg, or 5 mg. Treatment was followed by a 2-week follow-up period, during which patients did not receive study medication. Participant study completion was defined as completion of all of the study phases including follow-up.

Two interim analyses were performed during each study with unblinded data reviewed by a small, restricted team to determine whether additional dose(s) of GSK1278863 were required. No changes were made to either study based on the interim reviews.

Laboratory Parameters

The sponsor provided a point-of-care HemoCue Hb 201 analyzer (HemoCue, Ängelholm, Sweden) to each study site. Hemoglobin values, assessed with the HemoCue Hb 201 analyzer, were used to make acute care decisions. For the final data set and for all analyses (including hemoglobin), blood was sent to Quest Diagnostic Clinical Trials for analysis. Other laboratory tests were also analyzed centrally by Quest Diagnostics. Hepcidin was analyzed at Intrinsic LifeSciences using a patented tracer and a competitive ELISA. Further details on the methodology are available online (http://www.intrinsiclifesciences.com/hepcidin-assay-info/).

Pharmacodynamic/Pharmacokinetic Assessments

Baseline predose samples for pharmacokinetics (PK), EPO, VEGF, and hepcidin were collected on day 1 before dosing with study medication. Three samples (7–11 hours after dosing) were collected for PK, EPO, and VEGF at week 2. Only one postdose sample was collected for hepcidin (4–8 hours after dosing) at week 2. At week 4, a predose sample was taken for all PK/pharmacodynamic markers, with postdose samples collected up to 3 hours after dosing for PK, EPO, and VEGF. All samples were collected in EDTA tubes, placed on ice, and processed *via* centrifugation to prepare plasma samples (PK, hepcidin) or platelet-poor plasma samples (EPO and VEGF) for analysis.

Efficacy Assessments

The primary efficacy end point for both trials was modeled change in hemoglobin from baseline over 4 weeks of treatment. Secondary end points included the following: maximum hemoglobin changes over 4 weeks (nondialysis study only; Table 2); the number and percentage of patients who achieved a hemoglobin response, defined as an increase of $\geq$1 g/dl during the trial (nondialysis study only); descriptors of hemoglobin variability over the 4 weeks of treatment (HDD study only); the number of patients who reached protocol-defined stopping criteria for hemoglobin; changes in markers of iron metabolism and utilization (hepcidin, ferritin, transferrin, transferrin saturation, total iron, total iron binding capacity); change in high-sensitivity C-reactive protein; change in circulating EPO level and circulating VEGF level; change in hematocrit, red blood cell count, and reticulocyte number; plasma GSK1278863 and metabolite PK parameters; and safety and tolerability of GSK1278863. Further details are available in the Supplemental Tables 3–5.

Statistical Analyses

The sample size for the nondialysis study was based on estimation of the hemoglobin dose-response relationship using an E_{max} model, informed by a series of modeling and simulation analyses to explore assumptions for the shape and location of the dose-response relationship. Fifteen patients per arm were determined to estimate the hemoglobin response at the target dose (*i.e.,* dose providing a mean hemoglobin increase of 1 g/dl), with a precision of approximately 0.41 g/dl, in which precision is defined as the half-width of the 95% credible interval for hemoglobin change from baseline. Based on a 10% dropout rate, the study aimed to recruit approximately 17 patients per treatment arm.

In the HDD study, a sample size of 17 patients per arm was determined to estimate hemoglobin change from baseline over 4 weeks with a precision of 0.5 g/dl, defined as the half-width of the symmetrical 95% confidence interval. Assuming a 10% dropout rate and a linear dose-response analysis, 15 patients per arm was expected to estimate the slope of the regression line with a precision of 0.15 g/dl; hemoglobin SD was assumed to be 0.9 g/dl for both trials. Eligible patients were stratified based on their average weekly rhEPO dose during the 8 weeks before randomization (low rhEPO dose: $\leq$200 IU/kg per week epoetin or $\leq$1 μg/kg per week darbepoetin; high rhEPO dose: $>$200 IU/kg per week epoetin or $>$1 μg/kg per week darbepoetin); however, because of a small number of patients recruited in the prior rhEPO high-dose stratum (*i.e.,* 2–3 patients per group), the statistical analyses were not stratified by prior rhEPO dose.

As a result of the model-based dose-response estimation design of these trials, no formal hypothesis testing was planned or conducted in either study to compare data from GSK1278863 treatment groups with controls across the range of end points. Results for all treatment arms are expressed using descriptive statistics.

Populations for both studies were defined as follows: The intent-to-treat population consisted of all randomized patients who received at least one dose of study medication and had baseline assessments and at least one on-treatment assessment. The safety population was composed of all patients who received at least one dose of study medication and was used for safety and tolerability assessments.

Further details of the statistical dose-response models and inferences are described in the Supplemental Material.

Population GSK1278863 and metabolite pharmacokinetic parameters were estimated using a nonlinear mixed-effects model. GSK1278863 and metabolite exposure parameters were then calculated, including the maximum plasma concentration and area under the plasma concentration-time curve from time zero to 24 hours. Further details and results can be found in the Supplemental Material.

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DISCLOSURES

L.H., A.M., R.M., and J.L. are employees of and own stock in GlaxoSmithKline. B.J. is former employee of and owns stock and stock options in GlaxoSmithKline. D.J. and A.C. are employees of and own stock and stock options in GlaxoSmithKline. A.R. received research grants from GlaxoSmithKline. S.Z. has no potential conflicts of interest to report.

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See related editorial, "The Dawning of a New Day in CKD Anemia Care?," on pages 968–970.

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