chronic kidney disease

HIF stabilization by prolyl hydroxylase inhibitors for the treatment of anemia in chronic kidney disease

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The treatment of anemia with erythropoiesis-stimulating agents and iron supplementation has become the standard of care in patients with chronic kidney disease. Because of the risks associated with this approach, hypoxia inducible factor stabilizing prolyl hydroxylase inhibitors were developed as a potential treatment alternative. In recent phase 2 trials, these agents raised hemoglobin in a predictable and controlled manner and improved markers of iron metabolism. More experience is needed to establish long-term efficacy, tolerability, and safety, and to determine whether their use is associated with lower iron requirements.

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Erythropoiesis-stimulating agents (ESAs) are the main treatment modality for the anemia of chronic kidney disease (CKD); however, currently available ESAs have been associated with an increased risk of cardiovascular events and mortality with normal or near-normal hemoglobin targets.[1,2] As a result, a number of alternatives to the recombinant erythropoietins have been investigated in preclinical and clinical studies, including erythropoietin gene therapy, the peptide-based dimeric pegylated compound peginesatide, and agents to stabilize hypoxia inducible factor-2α (HIF2α), a transcription factor that regulates both erythropoiesis and iron metabolism.

Erythropoietin synthesis by the kidney is tightly controlled by the renal oxygen concentration (Figure 1). At normal oxygen concentration, prolyl hydroxylase domains (PHDs) hydroxylate the HIF-α subunit leading to its polyubiquitination and subsequent proteasomal degradation. Under conditions of hypoxia, the activity of the PHDs is reduced. The HIF-α subunit dimerizes with HIF-β, moves to the cellular nucleus, and activates erythropoietin synthesis. In addition, HIF2α activation induces a variety of genes involved in the cellular uptake, mobilization, and transport of iron. HIF2α activation further leads to downregulation of hepcidin, a protein that impairs intestinal iron transport absorption by inhibiting iron export from enterocytes. It further reduces iron export from hepatocytes and reticuloendothelial cells. Hepcidin expression is increased in CKD and in inflammation, and it is thought to contribute to anemia in both settings.[3]

Novel oral PHD inhibitors have been developed to stabilize HIF and promote endogenous erythropoietin production in patients with anemia.[4] As compared to conventional ESAs, an attractive potential advantage of the PHD inhibitors is a beneficial effect on iron metabolism via HIF activation, which could lead to better intestinal iron absorption and lower iron requirements. Recently, several short duration, randomized phase 2 trials of PHD inhibitors have been conducted in patients with CKD.[5–9]

In 2 dose-finding studies, Provenzano *et al.*[5,6] evaluated the optimal dose and dose frequency of roxadustat (FG-4592) in ESA-naive patients with predialysis CKD[5] and in long-term hemodialysis patients on stable ESA therapy, respectively.[6] In the first study,[5] 145 participants with CKD and hemoglobin ≤10.5 g/dl were randomized into 1 of 6 cohorts with varying roxadustat starting doses and frequencies for 16 or 24 weeks. None of the participants received i.v. iron. Overall, 92% of participants achieved a hemoglobin response, defined as an increase of ≥1 g/dl from baseline and a hemoglobin level ≥11 g/dl. Higher

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starting doses led to earlier achievement of hemoglobin response. The change in hemoglobin from baseline was independent of baseline C-reactive protein levels and iron status.[5]

The second study was conducted in 2 parts, both in participants on long-term hemodialysis and stable doses of ESA and i.v. iron.[6] In part 1, 54 participants were randomized to a 6-week dose-ranging study comparing thrice-weekly oral roxadustat versus continuation of i.v. epoetin alfa, with i.v. iron allowed by rescue criteria. In the primary per-protocol analysis, hemoglobin was maintained within ≥ 0.5 g/dl of baseline in 70% of participants in the pooled roxadustat arms compared with 33% of participants in the epoetin alfa control arm; this difference was more pronounced in participants randomized to roxadustat doses ≥1.5 mg/kg/day (79% vs. 33%). In an exploratory analysis, reductions in hepcidin levels were greater in participants randomized to the higher doses of roxadustat compared with the control arm. In part 2 of the study, 90 participants were randomized to 6 patient cohorts with different starting doses and adjustment rules, for a fixed duration of 19 weeks. I.v. iron therapy was allowed by rescue criteria. In part 2, the average roxadustat dose required to maintain hemoglobin ≥11 g/dl was approximately 1.7 mg/kg per day.[6]

Besarab *et al.*[7] examined the effects of roxadustat in 60 ESA-naïve patients on chronic dialysis for <4 months and with baseline hemoglobin ≤10 g/dl. Thirty-six participants on hemodialysis were randomized to roxadustat with no iron therapy, roxadustat plus oral iron therapy, or roxadustat plus i.v. iron therapy. In parallel, 12 peritoneal dialysis patients received roxadustat plus oral iron therapy, and an additional 12 hemodialysis patients were observed on roxadustat with no iron therapy. The investigators observed an increase in hemoglobin levels in all groups after 12 weeks of roxadustat treatment, although the increase was less marked in participants on hemodialysis who did not receive iron supplementation. Serum hepcidin levels were significantly reduced from baseline to 12 weeks.[7]

Holdstock *et al.*[8] conducted 2 phase 2a studies of the PHD inhibitor GSK1278863 in 72 patients with predialysis CKD and no current ESA use and in 82 patients on long-term hemodialysis with stable ESA use, respectively. In the first study, the investigators observed a dose-dependent increase in hemoglobin in patients with predialysis CKD, with the highest dose of the PHD inhibitor resulting in a mean increase of 1 g/dl at week 4. In the second study, participants on long-term hemodialysis randomized to GSK1278863 5 mg daily maintained a mean hemoglobin concentration comparable to that in participants randomized to continue recombinant erythropoietin, whereas mean hemoglobin decreased in participants randomized to lower doses of the PHD inhibitor. In both studies, endogenous erythropoietin levels remained within the range previously observed in patients with CKD or with exposure to high altitude and were much lower than those in participants on

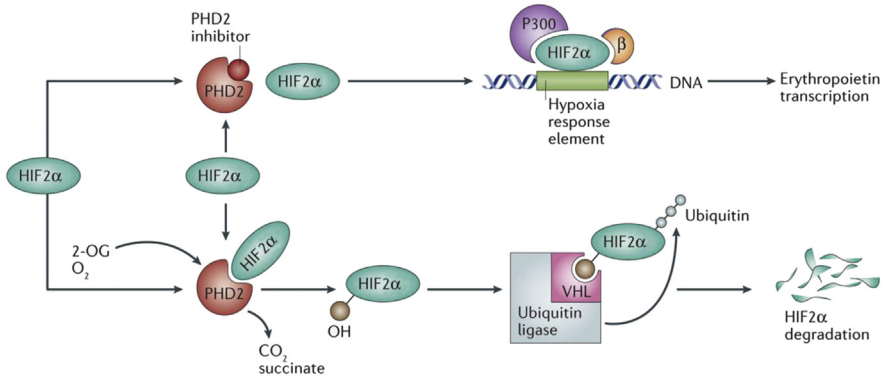

Figure 1 | Control of erythropoietin production by the hypoxia inducible factor (HIF) pathway and prolyl hydroxylase domain (PHD) enzymes. Control of erythropoietin production by the HIF pathway and PHD enzymes. When PHD-containing enzymes (PHD1, PHD2, or PHD3) are inactive (top), HIF2α can induce expression of erythropoietin. When PHD enzymes are active (bottom), HIF2α is hydroxylated, and consequently recognized by the von Hippel Lindau (VHL) ubiquitin E3 ligase, resulting in ubiquitylation of HIF-2α. Ubiquitylated HIF-2α is then destroyed by the proteasome. The rate-limiting step of HIF degradation is hydroxylation by a PHD enzyme, and this step acts as the molecular oxygen sensor. PHD2 is the most relevant enzyme for HIF2α degradation. OG, oxoglutarate. Reprinted with permission from Maxwell PH, Eckhardt KU. HIF prolyl hydroxylase inhibitors for the treatment of renal anaemia and beyond. *Nat Rev Nephrol*. 2016; 12:157–168.[4]

hemodialysis randomized to continue recombinant erythropoietin. Serum hepcidin levels decreased in response to GSK1278863 treatment in the participants with predialysis CKD, but not in those on long-term hemodialysis.[8]

The most recent phase 2 study is reported by Pergola *et al.*[9] in the current issue. The investigators conducted a 20-week, double-blind, randomized, placebo-controlled phase 2b study to evaluate the efficacy and safety of once-daily vadadustat (AKB-6548) in patients with CKD stages 3a to 5. Randomization was stratified by baseline hemoglobin and ESA use. The primary endpoint was the percentage of participants who achieved or maintained either a mean hemoglobin level ≥11.0 g/dl or a mean increase in hemoglobin ≥1.2 g/dl from baseline during the last 2 weeks of treatment. The primary endpoint was met in 54.9% of patients on vadadustat and 10.3% of patients on placebo. The vadadustat group also had significant decreases in both serum hepcidin and ferritin levels compared with the placebo group.[9]

Overall, the PHD inhibitors were considered to be safe and well tolerated at the doses and durations tested. Taken together, these studies found that PHD inhibitors raised and maintained hemoglobin levels in a predictable and controlled manner. The PHD inhibitors also improved markers of iron metabolism, although they were not powered to detect differences in supplemental iron requirements. These studies provide a strong rationale for ongoing phase 3 studies, designed to have sufficient power and follow-up time to assess hard clinical endpoints and to determine whether PHD inhibitors are a safe and effective alternative for anemia management in patients with CKD.

DISCLOSURE

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cell biology

Expanding nephron progenitors *in vitro*: a step toward regenerative medicine in nephrology

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With recent success in directed differentiation of nephron progenitors from mouse embryonic stem cells or human-induced pluripotent stem cells, the ability to expand these nephron progenitors is an important step toward regenerative medicine in nephrology. A recent publication reports the first successful attempt to propagate human nephron progenitors while retaining their potential to form both glomeruli and renal tubules.

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