HIF prolyl hydroxylase inhibitors as treatments for renal anemia and other conditions

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Abstract

Small-molecule stabilizers of hypoxia inducible factor (HIF) are being developed for the treatment of renal anaemia. These molecules inhibit prolyl hydroxylase domain-containing (PHD) enzymes, resulting in HIF activation and increased production of erythropoietin. Currently, renal anaemia is treated with recombinant human erythropoietin or related analogues, referred to as conventional erythropoiesis stimulating agents (ESAs). Advantages of PHD enzyme inhibitors over conventional ESAs include oral administration and the simpler—and potentially cheaper—production. Importantly, inhibiting the PHD enzymes has a range of consequences other than increasing levels of erythropoietin, and these effects could be beneficial - for instance by reducing the need for parenteral iron - but might in some instances be harmful. Several companies are currently testing PHD enzyme inhibitors in patients with renal anaemia and have reported clear evidence of efficacy without serious safety concerns. A central question that current studies are beginning to address is whether using PHD enzyme inhibitors will influence hard end points, including mortality and the rate of cardiovascular events. In terms of approaches to therapy, the exquisite specificity of conventional ESAs is a striking contrast to the pleiotropic effects of

activating HIF. Excitingly, PHD inhibitors could also be useful for conditions besides renal anaemia, such as protection from ischaemic injury.

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Key points

- Erythropietin production is controlled by hypoxia inducible factor (HIF); the prolyl hydroxylase domain-containing (PHD) enzymes regulate HIF and act as molecular oxygen sensors

- Small-molecule inhibitors of the PHD enzymes increase erythropoietin production and are in clinical development for the treatment of renal anaemia

- Advantages of PHD enzyme inhibitors over conventional erythropoiesis-stimulating agents (ESAs) include oral administration, lower production costs, product stability and low immunogenicity

- PHD enzyme inhibitors have effects besides increasing production of erythropoietin. Some of these effects could be beneficial, including an improvement in uptake and utilization of iron

- The balance of risks and benefits in treating renal anaemia with PHD enzyme inhibitors is currently being addressed in randomized trials

- PHD inhibitors might be useful for therapeutic indications other than the management of renal anaemia

Research over the past three decades has revealed the existence of a continuously operating oxygen sensing system that is present in all metazoan cell types and depends on the transcription control complex hypoxia inducible factor (HIF).[1] This system controls many processes that regulate adaptive changes, and contributes to mechanisms that balance oxygen supply and demand. One direct target of HIF is erythropoietin, the transcription of which underpins homeostatic control of red blood cell production. The identification of prolyl hydroxylase domain (PHD) enzymes (also known as EGLN enzymes) as molecular oxygen sensors that control HIF has presented the opportunity to develop small-molecule PHD enzyme inhibitors that efficiently

activate HIF in the presence of oxygen.[2,3] These small molecules could be therapeutically useful for a range of indications, most obviously for the treatment of anaemia that results from impaired erythropoietin production.

Several pharmaceutical companies have developed PHD enzyme inhibitors, some of which are currently being tested in humans. In this Review, we summarize the current understanding of the therapeutic use of PHD enzyme inhibitors. In particular, we consider the rationale for targeting PHD enzymes to increase erythropoietin production, other potential on-target consequences of HIF activation, possible off-target effects on enzymes that are structurally similar to PHD enzymes, and indications for the use of PHD enzyme inhibitors besides renal anaemia.

Erythropoietin in red cell production

Over 98% of oxygen in the circulatory system is bound to haemoglobin in red blood cells. Consequently, the oxygen-carrying capacity of the blood is determined by the concentration of haemoglobin, which is closely related to the overall mass of red cells. In healthy adults, red cells are produced in the bone marrow at a rate of ~2.4 million cells per second, and have a life of ~120 days. Their production is completely dependent upon the 30.4-kDa glycoprotein hormone erythropoietin, circulating levels of which consequently regulate total red cell mass. Erythropoietin is largely produced by the kidneys, thus chronic kidney disease (CKD) can lead to anaemia as a result of impaired erythropoietin deficiency.

Current treatment of renal anaemia

Until the 1980s, renal anaemia was usually managed with regular blood transfusion. Problems with this approach, however, included sensitization to red cell antigens (which limited the range of compatible blood donors), sensitization to HLA antigens (which restricted subsequent transplantation), exposure to blood-borne infections, and iron overload[4]. As a consequence of these hazards, together with the logistical challenges of transfusion, low levels of circulating haemoglobin in patients with renal anaemia were commonly considered to be acceptable.

Research published in the late 1980s, showed that recombinant human erythropoietin (rhEPO), which was generated from stably transfected Chinese hamster ovary cells, provided a safe and highly effective therapy for renal anaemia.[5] This therapy transformed the lives of patients with renal disease, and the worldwide market for rhEPO and its derivatives (collectively referred to as conventional erythropoiesis

stimulating agents [ESAs]) rapidly grew over the following two decades to over US$10 billion per annum.

Renal anaemia is now an established indication for pharmaceutical treatment, and several companies market conventional ESAs. The initial products had amino acid compositions that were identical to human erythropoietin. The WHO International Nonproprietary Names of these preparations are epoietin, followed by a Greek letter that differentiates them with respect to the cell line in which they are produced, differences in glycosylation, and other characteristics. Longer-acting erythropoietin analogues, which enable administration at fortnightly or monthly intervals rather than three times a week, have since been developed by substituting of amino acids to provide additional glycosylation sites (darbepoetin alfa) or by pegylation (methoxy polyethylene glycol-epoetin beta). The efficacy and safety of available preparations seem to be similar[6,7]. Peginesatide is a pegylated peptide activator of the erythropoietin receptor that has a sequence that is unrelated to erythropoietin but an efficacy equal to that of darbepoetin alfa.[8,9] Peginesatide was also effective in patients with severe anaemia caused by anti-erythropoietin antibodies after treatment with rhEPO or its derivatives.[10] However, peginesatide was withdrawn by the manufacturers because of hypersensitivity reactions that were observed after licensing.[11,12] Data from patients with nondialysis-dependent CKD also suggested that peginesatide is associated with cardiovascular problems to a greater extent than are conventional ESAs.[8]

Experience of treating renal anaemia with conventional ESAs has shown that functional iron deficiency is a common consequence of anaemia, related to increased iron consumption and, at least in part, to elevated levels of hepcidin[13]. Conventional ESAs are therefore effective at lower doses if administered with intravenous iron.

Conventional ESAs have proven to be largely safe for the treatment of renal anaemia. Adverse effects that were observed when these therapeutics were first introduced included increased blood pressure, seizures and a high rate of thrombotic events that affected shunts and arteriovenous fistulae.[5] Approaches to dosing that aim to slowly increase the haematocrit seem to minimize these problems. Nevertheless, two important safety issues remain.

The first is that patients can develop antibodies against conventional ESAs when they are administered subcutaneously, although usually not when they are administered intravenously.[14] Antibodies against conventional ESAs can also neutralize native erythropoietin, leading to development of pure red cell aplasia. The reported incidence of immune reactions is 14.0–35.8 cases per 100,000 patient years of treatment.[15]

The second safety issue is that using conventional ESAs with a target haemoglobin concentration within the normal range has been associated with a higher rate of cardiovascular events and potentially excess mortality than when used with a lower haemoglobin level[16,17]. The reason for these harmful effects is unclear; possibilities include off-target effects of high concentrations of conventional ESAs or a real benefit of lower haemoglobin concentrations[18]. Regardless, the finding has led to FDA boxed warnings for conventional ESAs[19] and to the reformulation of treatment guidelines.[20]

As a result of these safety issues and other limitations of current anaemia therapy with conventional ESAs, considerable interest exists in identifying small-molecule alternatives to conventional ESAs. These alternatives could be advantageous for several reasons. For example, small molecules are simpler and cheaper to produce than recombinant polypeptides and are inherently more stable, making distribution simpler, safer and less expensive. Small molecules can be administered orally rather than by

injection, and would eliminate the risk of patients developing antibodies that cross-react with erythropoietin. Parallel effects on iron metabolism might enhance the ability to correct anaemia. Finally, a different strategy for increasing the haemoglobin concentration might be associated with better cardiovascular outcomes than are conventional ESAs.

Physiological Erythropoietin production

Molecular pathway

The molecular pathway that regulates erythropoietin production in an oxygen-dependent fashion centres on HIF, which increases expression of specific target genes by binding to hypoxia response elements across the genome[1]. The HIF complex comprises two subunits: oxygen-responsive HIF-1α or HIF-2α, and constitutive HIF-1β (also known as aryl hydrocarbon receptor nuclear translocator). Oxygen-dependent regulation of HIF mainly involves destruction of HIFα subunits, which starts with hydroxylation of HIFα by a prolyl hydroxylase domain (PHD)-containing enzymes (PHD1, PHD2, PHD3[21] or possibly a transmembrane PHD protein[22]; FIG 1). These enzymes are (2-OG)-dependent dioxygenases (a superfamily with a broad range of biological functions Supplementary Table 1[23–29]) that hydroxylate proline residues. Two proline residues in both HIF-1α and HIF-2α are targeted by the PHD enzymes[30]. PHD enzymes require oxygen as a substrate and thereby act as the oxygen sensor that regulates HIF. *In vitro* measurements have shown that the hydroxylation rate by PHD enzymes is sensitive to concentrations of oxygen throughout the range of concentrations that occur physiologically. [31]

Additional HIF regulation occurs through oxygen-dependent hydroxylation of an asparagine residue near the C-terminus of the α subunit. This hydroxylation is mediated by the 2-OG-dependent oxygenase factor inhibiting HIF-1 (FIH-1) and blocks recruitment of the transcriptional co-activator CREB–EP300.[32]

Importantly, expression of HIF is not restricted to cells that produce erythropoietin and it regulates the expression of several hundred genes in all mammalian cell types examined to date[33]. Its role is, therefore, not specific to erythropoietin production, but it coordinates a wide range of adaptive responses to changes in oxygenation. Besides erythropoietin, other genes that are regulated by HIF and contribute to adaptation in response to altered oxygenation include those that encode glucose transporters, glycolytic enzymes and angiogenic growth factors [1, 33](FIG 2). HIF also affects the expression of genes with less obvious roles in such adaptation, but which are involved in diverse pathways, including cell cycle control, cell survival decisions and immune responses.

In addition, PHD enzymes and FIH-1 probably have roles in the regulation of other cellular pathways. For example, PHD enzymes have been reported to modulate multiple aspects of the NF-κB pathway[34], and FIH hydroxylates many proteins that have ankyrin repeats, including components of the Notch signalling pathway, although the function of this hydroxylation is currently unclear[35,36].

Erythropoietin-producing cells

Fibroblasts of the renal cortex and outer medulla are the principal cells that produce erythropoietin in response to hypoxia or anaemia[37,38]. The origin and characteristics of these cells are not fully understood, but they are thought to derive from the neural

crest; GATA transcription factors are thought to have an important role in silencing the *EPO* gene in other renal cells.[39]

Role of HIF-2α and PHD2

In humans and rodents, renal fibroblasts activate HIF-2α in response to hypoxia, without detectable activation of HIF-1α.[40,416] This observation suggests that HIF-2α, but not HIF-1α, is pivotal in normal erythropoietin production, a hypothesis that is supported by other lines of evidence. Knockout experiments in mice indicated that HIF-2α is required for normal erythropoiesis[42]. In humans, mutations that activate HIF-2α can result in erythrocytosis[44,44], and genetic variants of HIF-2α are associated with differences in haematocrit between people who live at the same level of high altitude in the Himalayas[45,46].

Further evidence suggests that PHD2 is the enzyme that regulates HIF-2α stability upstream of erythropoietin production: in cultured cells, PHD2 seems to be the dominant HIF prolyl hydroxylase under normal circumstances, and deletion or knockdown of *PHD2* is sufficient to result in HIF activation[47,48]. In humans, mutation of one *PHD2* allele is sufficient to increase the haematocrit, suggesting that a decrease in PHD2 activity is sufficient to increase EPO production[49]. In line with this observation, the absence of *PHD1*, *PHD3* or *FIH* in mice is not sufficient to increase the haematocrit[50,51].

PHD enzymes as therapeutic targets

Inhibitors of 2 OG-dependent oxygenases were first developed as potential anti-fibrotic agents, due to their capacity to inhibit collagen hydroxylases and thereby collagen

cross-linking [52,53] (Supplementary Table 1). The discovery that enzymatic prolyl hydroxylation controls HIF degradation rapidly led to the discovery that 2-OG analogues activate HIF by inhibiting PHD enzymes[54]. This inhibition was initially demonstrated with dimethyloxalylglycine, a cell-permeable compound that is hydrolysed intracellularly to form *N*-oxalylglycine, which is a competitive 2-OG oxygenase inhibitor that reduces HIF hydroxylation.[54]. A substantial number of small-molecule PHD enzyme inhibitors have been identified since then[2,3,55-57].

The diverse and important functions of 2-OG-dependent oxygenases suggest that ideal small-molecule inhibitors of PHD enzymes for clinical use should be highly selective. However, the selectivity of current small-molecule inhibitors among the 2-OG dependent oxygenases remains largely unknown, and even knowledge of their selectivity among PHD enzymes is limited. This lack of knowledge exists because assays are not yet available for a number of 2-OG-dependent oxygenase enzymes, and the extent to which existing assays reflect *in vivo* activity is uncertain. Nevertheless, small-molecule inhibitors with a high selectivity for PHD proteins over FIH and small-molecule inhibitors with high selectivity for specific PHD proteins have already been identified[2].

Targeting PHD enzymes in renal anaemia

The ideal small molecule for the treatment of renal anaemia would efficiently increase circulating levels of endogenously produced erythropoietin in a way that would achieve a controllable, stable haemoglobin level in patients with renal disease, without undesirable consequences. However, several aspects of the HIF pathway and

erythropoietin regulation in renal disease are insufficiently understood to precisely define the properties of compounds that would fulfill these goals.

Erythropoietin production in renal disease

Although inappropriately low erythropoietin production is undoubtedly the main cause of renal anaemia, little is known about why erythropoietin production by renal fibroblasts is so severely impaired in renal disease [58,59], and why extra-renal tissues, in particular the liver, do not compensate for impaired renal production. Blunting of the erythropoietin response by uremic toxins might contribute to the pathogenesis of renal anaemia[60], and evidence suggests that an oral toxin adsorbent can improve renal anaemia in patients who are on dialysis.[61]

Residual erythropoietin production in CKD

Despite the impairment in erythropoietin production, patients with CKD do not completely lose the ability to produce the hormone and adjust its production to oxygen availability. Even in patients who are on dialysis, plasma levels of erythropoietin can increase in response to acute episodes of hypoxia or acute decreases in haemoglobin, despite an inadequate response to the chronic reduction of haemoglobin despite an inadequate response to a chronic reduction in haemoglobin concentration[62-64]. Moreover, at increasingly high altitudes, patients who are on haemodialysis require lower doses of conventional ESAs, yet maintain higher levels of haemoglobin suggesting that their residual erythropoietin production is responsive to a reduction in the partial pressure of oxygen in the atmosphere[65]. Residual erythropoietin produced by chronically diseased kidneys can be dysregulated: for example, persistent production of

erythropoietin by remnant kidneys contributes to erythrocytosis that occasionally occurs after successful renal transplantation[66,67].

Production by cystic kidneys

Erythropoietin production is often preserved when there are cysts in diseased kidneys. This is a typical feature of autosomal dominant polycystic kidney disease[68], and development of cysts in the kidneys after years of renal replacement therapy is sometimes associated with a return of erythropoietin production and rise of haemoglobin levels[69]. Compression of the microvasculature in the interstitial space around the cysts results in hypoxia[70]; consequent HIF-2α activation in interstitial cells probably stimulates erythropoietin synthesis and mitigates renal anaemia. Additionally, hypoxia occurs in the cyst epithelium, where HIF-1α promotes cyst expansion, thereby presumably further increasing regional hypoxia[71].

Production in the liver and other organs

The liver is a second main source of erythropoietin, where the hormone is produced by stellate cells and hepatocytes[72,73]. The liver is the predominant production site during fetal and early postnatal life, and might also contribute to erythropoietin production in adulthood, although the extent of this contribution is unclear. In rats exposed to severe hypoxia, approximately one third of the total erythropoietin is produced in the liver[74]. Renal impairment decreases the expression of the *EPO* gene in the rat liver, suggesting a mechanism that prevents compensation for reduced renal erythropoietin production by the liver, despite its capacity to produce the hormone[74]. Before the availability of rhEPO, some patients with renal disease achieved a normal haematocrit without blood

transfusion during recovery from hepatitis[75], and studies in rodents have shown that active liver regeneration is associated with high erythropoietin production[76]. Together, these data suggest that both the liver and kidney could be targeted by therapy that aims to increase erythropoietin production in renal disease.

Therapeutic strategies that target erythropoietin production in the liver are particularly attractive as a strategy for patients with kidney disease because the ability to increase production in their diseased kidneys is likely to depend on the extent of disease, renal pathology and the remaining renal mass. Despite many similarities, the mechanisms that regulate erythropoietin production in the liver are clearly not identical to those in the kidneys. The importance of regulatory elements of the *EPO* gene differs in hepatocytes and the kidney[77.] As in the kidney, HIF-2α seems to mediate hypoxic stimulation of erythropoietin production in hepatocytes[78]. However, a genetic study in mice showed that efficient production of erythropoietin by hepatocytes cannot be achieved by inactivating PHD2 alone.[79] Inactivation of PHD3 alone, by contrast, resulted in selective activation of HIF-2α in hepatocytes, and combined inactivation of any combination of two PHD enzymes, or of all three PHD enzymes stimulated extensive production of erythropoietin.[78,79]

The ability to produce erythropoietin is not confined to the liver and kidneys, and has been demonstrated in other cell populations, including astrocytes[80] and osteocytes,[81] in mice. Extrarenal and extrahepatic erythropoietin production can induce polycythemia; for example, cerebellar haemangioblastomas have been associated with polycythemia in humans.[82] However, a relative deficiency of circulating erythropoietin is a hallmark feature of advanced kidney disease and thus erythropoietin production at extrarenal

sites cannot clearly compensate for impairment of renal erythropoietin production since.

Therefore, neither the most effective approach to inhibiting PHD proteins nor the organs that would ideally be targeted in patients with renal disease are currently clear, but the available evidence seems to favour targeting of PHD2 and PHD3 in the liver.

Pleiotropic effects of HIF activation

HIF directly influences the expression of hundreds of genes, and consequently has roles in pathways other than the regulation of erythropoietin. Furthermore, evidence suggests that HIF activation has indirect effects via epigenetic mechanisms.[73,84] HIF activation could modulate almost any developmental, physiological or pathological process, and so inhibition of PHD proteins will have a range of effects beyond increasing erythropoietin production. These effects could be therapeutic, harmful or neutral.

Potential beneficial effects of activating HIF

Beneficial effects of HIF activators, when compared with conventional ESAs, can be considered in two categories. The first is more effective treatment of renal anaemia than can be achieved with conventional ESAs. For example, treatment with conventional ESAs is often limited by functional iron deficiency so considerable interest exists in new pharmacological strategies to address functional iron deficiency, especially in anaemia that is associated with chronic inflammation.[85,86] Several genes that are involved in iron homeostasis are regulated by HIF,[87] and experiments in mice have suggested that HIF activation directly suppresses hepcidin production by the liver,[88] although subsequent evidence suggests that suppression is an indirect effect.[89,90] Early clinical data indicated

that PHD enzyme inhibitors reduce hepcidin levels in patients with renal disease.[90,91] However, conventional ESAs also suppress hepcidin,[94,95] probably by increasing production of erythroferrone in erythroblasts[96]. An important question is whether the need for parenteral iron supplementation will be reduced by treatment with PHD enzyme inhibitors compared to conventional ESAs.

The second category of potential benefits of PHD inhibitors would be if they reduce the high rate of cardiovascular events associated with renal disease. Such benefits could result from effects on cardiovascular risk factors including blood pressure,[57] or improving glucose tolerance and lipid profiles,[97] Preclinical evidence also suggests an ability of PHD inhibitors to protect organs from ischaemic injury[98]. Whether such effects are sustained and translate into reductions in mortality and morbidity can only be determined by large-scale, appropriately controlled clinical trials that are currently underway but have not yet been completed. (see Clinical trials below).

Efficient treatment of anaemia and an improved cardiovascular prognosis could also be linked. Provided that high concentrations of erythropoietin may be harmful and if PHD enzyme inhibitors can be used to achieve similar hemoglobin concentrations with much lower concentrations of circulating erythropoietin, morbidity might be reduced. Carefully performed, large clinical trials will be needed to test this hypothesis.

Potential harmful effects of activating HIF
Human diseases that involve genetic alterations in the HIF pathway provide the clearest insight into the potential harm of HIF activation. For example, von Hippel Lindau disease results from a germline mutation in one copy of *VHL*, which encodes Von Hippel Lindau disease tumour suppressor (VHL); affected individuals are at high risk of clear

cell renal cell carcinoma (CCRCC), pheochromocytoma and hemangioblastomas of the retina, cerebellum and spinal cord.[99] The tumors arise from somatic mutations in the remaining functional copy of *VHL*, and are associated with impaired degradation of HIF. Particular attention has been given to the role of this mechanism in spontaneous CCRCC, which is the most common form of kidney cancer. The majority of CCRCCs in patients without any family history of VHL disease have biallelic somatic inactivation of the *VHL* gene.[100] Although *VHL* probably has other important roles,[94] multiple lines of evidence indicate that HIF activation (particularly HIF-2α) is pivotal in the development of CCRCC.[101-103] This cancer promoting role of HIF is a concern with therapeutic strategies that are based on activating the HIF pathway. However, in mice and patients with von Hippel Lindau disease, *VHL* inactivation alone and consequent HIF activation in renal epithelial cells does not seem to cause a large increase in cell proliferation; several additional genetic events are necessary for CCRCC to develop following *VHL* inactivation.[102,104,105]

The role of HIF activation in pheochromocytoma is less clear than in CCRCC. Mutations in *VHL* that are associated with familial pheochromocytoma do not seem to influence the ability of VHL to regulate HIF, and mutations in *VHL* that completely disable HIF regulation are actually associated with a low risk of pheochromocytoma.[106] These observations suggested that pheochromocytoma might result from a HIF-independent consequence of *VHL* inactivation. However, germline mutations in *HIF2A, PHD2* and *PHD1* have now been implicated in pheochromocytoma, providing strong evidence that the HIF–PHD–VHL pathway is involved[107,108]. One possible unifying explanation is that incomplete activation of the HIF pathway during development promotes pheochromocytoma formation but complete loss of VHL function does not.

These genetic insights indicate that activation of the HIF pathway could promote development of CCRCC or pheochromocytoma, but pharmacological HIF activation could also promote progression of other cancers by increasing angiogenic signalling,[109] promoting metabolic reprogramming,[110] and driving epithelial-to-mesenchymal transition[111-113] and metastasis.[114] Although this possibility is a concern in the development of HIF activators, evidence from studies of three genetic conditions that cause HIF-dependent congenital erythrocytosis is reassuring. No increased risk of CCRCC or other malignant neoplasms has been apparent in patients with biallelic hypomorphic mutations in *VHL* that decrease the ability of VHL to ubiquitinate hydroxylated HIF (Chuvash polycythaemia),[115] mutations in *HIF2A* that increase activity of HIF-2α and partially protect it from PHD enzyme-mediated hydroxylation,[43,44,116] or mutations in *PHD2* that decrease PHD2 activity.[54] The total number of patients with these mutations who have been studied, however, is limited.

Studies of conditions that involve genetic HIF activation have identified two other potential problems with activating HIF as a therapeutic strategy. The first is the possibility of severe pulmonary hypertension, which develops in some individuals with *HIF2A* mutations.[44,117] Persistent HIF activation is the likely cause of this condition, as humans with Chuvash polycythaemia have higher baseline pulmonary artery pressure and a stronger constrictive response to hypoxia than do healthy controls.[118] Mice with the mutation that causes Chuvash polycythaemia also develop HIF-mediated pulmonary hypertension.[119] The second potential problem is that Chuvash polycythaemia is associated with a shorter life expectancy. A study that compared 76 patients with healthy controls found that the estimated survival to age 65 years was 29% for patients with Chuvash polycythemia and 64% for controls.[120] This high mortality has not yet

been fully explained, but is associated with a marked increase in thromboembolic events.[120] The rate of thromboembolism was 5.6-fold lower in patients with Chuvash polycythaemia who received therapeutic phlebotomy than in patients who were untreated, although this difference was not statistically significant (95% CI 0.70–47.6, P=0.12). Nevertheless, the association warrants prospective study.[120]

Animal and *in vitro* studies have indicated that HIF activation could have other harmful effects, including angiotensin-induced vascular remodelling[121] and adverse effects on atherosclerotic plaque homeostasis.[122] Whether long-term use of PHD enzyme inhibitors alters the progression of kidney disease is uncertain. HIF activation over long periods promotes renal fibrosis, which could accelerate CKD.[41,113] However, HIF activation in animal models has indicated that PHD enzyme inhibitors could protect against CKD progression[123,127]. In the light of these disparate pre-clinical findings the effect of PHD enzyme inhibitors on renal function in humans with CKD is difficult to predict and this is an important question that is also being addressed in ongoing randomized trials (see Clinical trials below).

Potential off-target effects

In addition to the 'on-target' risks associated with inhibiting PHD enzymes, any small molecule inhibitor of PHD enzymes is likely to have 'off-target' effects associated with inhibition of other 2-OG-dependent enzymes (FIG 2; Supplementary Table 1).

Clinical development of PHD enzyme inhibitors

Despite the potential risks, PHD enzyme inhibitors are an attractive treatment for renal anaemia for several reasons. The target is well validated, the number of patients with renal anaemia is large, and current treatment is imperfect. The efficacy of PHD enzyme inhibitors is easily measured by monitoring levels of circulating erythropoietin and haemoglobin. Furthermore, the main target organ is likely to be the liver, so oral medication should be delivered efficiently and predictably to the desired site of action, and inhibition of PHD proteins needs to be only partial and intermittent to increase erythropoietin production and the haematocrit. Judicious selection of a low dose, infrequent administration and monitoring of haemoglobin levels could, therefore, increase the safety margin and facilitate individualized therapy. Reassuringly, the results of preclinical programs indicate that small-molecule PHD enzyme inhibitors effectively increase erythropoietin levels and the haematocrit in healthy animals and in animals with renal impairment, without serious safety concerns.[56,57]

Requirements for trial design

The design of trials of PHD enzyme inhibitors should take into consideration multiple factors. The complexity of the HIF system and the widespread role of 2-OG-dependent oxygenases mean that the biological effects of different PHD enzyme inhibitors might vary according to their pharmacokinetic and pharmacodynamic properties and the dosing regimens used. These dosing regimens will also need to be optimized, as ideal thresholds for intervention, target levels of haemoglobin and the most effective combinations with iron therapy might differ from those of conventional ESAs. Phase III trials in patients on dialysis need to evaluate the cardiovascular safety of PHD enzyme inhibitors in direct comparison with conventional ESAs as the current standard of care.

For patients who are not on dialysis, trials should be placebo-controlled. In both cases, trials must include several thousand patients to ensure that they accurately determine the effects on mortality and rates of major cardiovascular events. In trials involving conventional ESAs current recommendations for target haemoglobin levels should probably be adhered to, although the risk–benefit relationship for different target levels might differ when PHD enzyme inhibitors are used rather than conventional ESAs. Recruiting enough patients who meet the entry criteria will be a challenge owing to the complex landscape of anaemia treatment with a significant number of established conventional ESAs.

Understanding the benefits and risks of PHD enzyme inhibitors in specific populations and clinical settings will pose additional questions; for example, specific patient groups might be at higher risk of some adverse events (for example, patients who are at high risk of malignancy or pulmonary hypertension), and these groups are unlikely to be represented sufficiently in initial trials. Carefully planned smaller studies could also be valuable in determining the effects of PHD enzyme inhibitors on specific aspects of vascular biology, organ function and metabolism. Some effects of HIF activation might only become apparent after a decade or more of administration, so longer term assessment of outcomes would also be desirable. Overall, extensive trial programs will be necessary to fully understand the benefits and risks to patients in different settings.

Current Clinical trials

At least six PHD enzyme inhibitors have been tested in humans to date (Table 1, Figure 3). In a small proof-of-concept study, a single dose of FG-2216 (20 mg/kg) significantly increased erythropoietin levels in six healthy volunteers and 12 patients on

haemodialysis, six of whom still had their native kidneys, and six of whom had no kidneys.[128] In patients on haemodialysis and who still had kidneys, the increase was variable but much greater on average than that in the patients with no kidneys, suggesting that FG-2216 induced erythropoietin production in the nonfunctioning kidneys. The increase in serum erythropoietin in patients without kidneys confirms erythropoietin production occurs at extra-renal sites, presumably in the liver. The program testing FG-2216 was suspended because one participant of a later trial died from fulminant hepatitis, although their death was subsequently deemed not to be caused by the drug.[3]

Several trials of other small-molecule PHD enzyme inhibitors have been conducted or are underway (Table 1). The most advanced current clinical programme concerns roxadustat (FG-4592), which has been shown to correct renal anaemia in humans and is currently in Phase III trials.[3] A phase IIa study showed a dose-dependent increase in haemoglobin levels in non-dialysis dependent CKD patients who were treated with roxadustat for 4 weeks[129]. A phase IIb study showed that in patients incident to haemodialysis or peritoneal dialysis who received titrated doses of rexadustat over 12 weeks, haemoglobin levels increase by 31 ±2 g/1.[130] In the latter study, no difference in the haemoglobin response in patients on hemodialysis regardless of whether they received oral or intravenous iron supplementation. [130]

Preclinical studies of molidustat (BAY 85-3934) have shown that the drug corrects renal anaemia in rats and that, unlike treatment with epoietin, it normalizes hypertensive blood pressure in a rat model of CKD[57]. The results of a Phase I study have been disclosed in an abstract: dose-dependent increases in haematocrit were observed in healthy male subjects.[131] Results of two 4-week phase IIa studies of daprodustat (GSK

1278863) showed a dose-dependent increase in haemoglobin levels in pateients with nondialysis-dependent CKD, and a dose-dependent ability to maintain stable haemoglobin levels in patients on haemodialysis who had previously been treated with conventional ESAs[132]. A 2-week study of daprodustat in patients with claudication found that a low dose of the drug, which did not increase haemoglobin levels, caused significant decreases in the levels of total cholesterol, LDL and HDL but did not improve ischaemic symptoms.[97] This finding demonstrates that this class of drug is likely to have clinically meaningful effects in addition to increasing erythropoietin.

The results of phase III trials should be available within 2–3 years (Table 1). These studies will involve exposure of participants to small-molecule PHD enzyme inhibitors for up to 2 years and will provide important information about the rate of adverse effects. Good tolerance and efficacy of a PHD enzyme inhibitor will probably lead to licensing approval of the treatment for patients with renal anaemia. If trials that include thousands of patients do not reveal any safety issues, the advantages of an orally administered small molecule will make this treatment more attractive to patients than injections of a biological agent.

Other indications for PHD inhibitors

Current understanding of HIF biology suggests several other settings in which PHD enzyme inhibitors might be an effective therapeutic approach. Although the current focus in the development of PHD enzyme inhibitors is the management of anaemia, studies that test PHD enzyme inhibitors for other indications could be worthwhile.

In haematological conditions, PHD enzyme inhibitors could be used to increase the haematocrit in anaemia of chronic disease and to increase fetal haemoglobin levels in, for example, sickle cell disease.[56]

Activation of HIF might be particularly beneficial in ischaemic or ischaemia–reperfusion settings, as HIF acts upstream of diverse gene products that should be protective in these contexts by, for example, promoting vasodilatation and angiogenesis, increasing glucose uptake, modulating mitochondrial metabolism and decreasing free radical production[1]. Evidence exists to support the use of PHD enzyme inhibitors in this context: preconditioning of the brain, heart or kidney with a short period of ischaemia protects against a subsequent longer ischaemic exposure,[133] and this protective effect is mediated in part by HIF.[134,135]

Preclinical experiments suggest that HIF activation might be beneficial in stroke,[136] myocardial ischaemia,[137,138] limb ischaemia,[139,140] acute kidney injury[198,141-143] and kidney transplantation.[144] In many of these experiments, PHD enzyme inhibitors were administered prior to ischaemic injury, so their relevance to most clinical settings, in which acute ischaemia is not predictable, is limited. However, ischaemic injury can be anticipated in some cases; for example, a PHD enzyme inhibitor could be administered before major cardiac surgery to protect against stroke and acute kidney injury. Another possible application is renal transplantation in which a brain-dead donor could be treated with a PHD inhibitor to prevent ischaemia–reperfusion injury and delayed graft function, as has been demonstrated in an animal model.[144] Whether these preclinical observations will translate into useful therapies in humans remains to be seen. Similar preclinical observations with conventional ESAs have been followed by negative clinical

studies[145], but in our view the broad biological effects of activating the HIF system make this approach more promising.

PHD enzyme inhibitors might also be of benefit in wound healing. In this context, stimulation of angiogenesis and cell migration might accelerate healing, and topical application would minimize the likelihood of systemic adverse effects[146].

Conclusions

In our view, the PHD enzymes are an exciting new therapeutic target. However, the underlying biology of the HIF pathway suggests that the targeting of PHD proteins will have pleiotropic effects. Moreover, the inhibitors that are currently being tested will almost certainly inhibit other 2-OG-dependent oxygenases to some extent. However, the intended outcome of increased erythropoietin production can be achieved with partial and intermittent inhibition, which might minimize these potential liabilities. In the best case scenario, treatment of renal anaemia with PHD enzyme inhibitors will be safe and effective, and could reduce the substantial risk of mortality that is associated with serious renal disease. With the results of late-phase clinical trials that are currently underway, we will hopefully soon know whether this best-case scenario will become a reality.

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Acknowledgements

Bayer for providing information about ongoing clinical trials. Representatives of these companies had no influence on the content of this article. We recognize that not all scientific papers that relate to the topic of this Review could be included and apologize for the omission of any important contributions.

Author contributions

Both authors researched data for the article and wrote the article

Suggested figure legends

Figure 1 | **Control of erythropoietin production by the HIF pathway and PHD enzymes**. When prolyl hydroxylase domain-containing (PHD) enzymes (PHD1, PHD2 or PHD3) are inactive (top), hypoxia inducible factor (HIF)α can induce expression of erythropoietin. When PHD enzymes are active (bottom), HIF-α is hydroxylated, and consequently recognized by the Von Hippel Lindau (VHL) ubiquitin E3 ligase, resulting in ubiquitylation of HIF-α. Ubiquitylated HIF-α is then destroyed by the proteasome. The rate limiting step of HIF degredation is hydroxylation by a PHD enzyme, and this step acts as the molecular oxygen sensor. PHD2 is the most relevant enzyme for HIF-2 α degredation. OG, oxoglutarate.

Figure 2 | **On-target and off-target effects of PHD enzyme inhibitors**. The primary strategy for the treatment of renal anaemia is to target prolyl hydroxylase domain-containing (PHD)2, which will increase hypoxia inducible factor (HIF)-2α activity and increase erythropoietin production. Increasing the activity of HIF-1α and HIF-2α will also have other effects. Inhibiting PHD enzymes might also influence the activity of non-HIF targets with effects that are currently unknown. Inhibition of other 2-oxoglutarate

dependent enzymes that are structurally related to the PHD enzymes could result in a range of off-target effects. ALKB, alpha-ketoglutarate-dependent dioxygenase; FIH, factor inhibiting HIF; JMJD, Jumonji domain-containing protein; KDM, lysine (K)-specific demethylase; P4HA, prolyl-4-hydroxylase; PLOD, procollagen-lysine 2-oxoglutarate 5-dioxygenase.

Figure 3 | **Chemical structures of small-molecule prolyl hydroxylase domain-containing enzyme inhibitors.** Each of the inhibitors shown has been trialled in humans.

Table 1 | Clinical trials of small-molecule PHD enzyme inhibitors.

Molecule	Phase	Administration	NCT reference	Study design (primary outcome)	Treatment duration (study dates)	Renal disease category
FG-2216	II	Oral	NA			
FG-4592 ASP1517 Roxadustat	III (US/EU)	Oral, 1–3 times weekly	02174731	Randomized open label vs epoetin α, 1,425 patients (Major adverse cardiac events)	1–2 y* (7/2014–2/2017)	HD or PD
			02174627	Randomized double-blind vs placebo, 2,600 patients (Major adverse cardiac events)	1–2 y* (6/2014–2/2017)	CKD not on dialysis
			02021318 (DOLOMITES)	Randomized open label vs darbepoetin α, 570 patients (Hb response without rescue therapy)	2 y (2/2014–7/2017)	CKD not on dialysis
			01887600 (ALPS)	Randomized double-blind vs placebo, 600 patients (Hb response without rescue therapy)	1–2 y (5/2013–6/2016)	CKD not on dialysis
			02052310 (HIMALAYAS)	Randomized open label vs epoetin α, 750 patients (Mean Hb change from baseline)	1–3 y (12/2013–6/2017)	Incident HD or PD
			02273726	Randomized open label vs epoetin α, 600 patients (Mean Hb change from baseline)	1–3 y (12/2014–6/2017)	Stable HD or PD
			02278341 (PYRENEES)	Randomized open label vs epoetin α and darbepoetin α, 750 patients (Mean Hb change from baseline)	2 y (11/2014–7/2018)	Stable HD or PD
			01750190	Randomized double-blind vs placebo, 600 patients (Hb response without rescue therapy)	≥1 y (11/2012–6/2017)	CKD not on dialysis
	II (Japan)	Oral, 3 times weekly	01964196	Randomized double-blind vs placebo, 100 patients (Rate of rise of Hb)	28 w (8/2013–3/2015)	CKD not on dialysis
BAY85-3934 Molidustat	IIb	Oral, once daily	02021409 (DIALOGUE 2)	Randomized open label vs darbepoetin α, 120 patients (Change in Hb)	16 w (1/2014–9/2015)	CKD not on dialysis
			01975818 (DIALOGUE	Randomized open label vs epoetin α or β 188 patients (Change in Hb)	16 w (1/2013–9/2015)	Stable HD

			4)			
			02021370 (DIALOGUE 1)	Randomized double-blind vs placebo, 120 patients (Change in Hb)	16 w (2/2014–9/2015)	CKD not on dialysis
			02055482 (DIALOGUE 3)	Long-term extension of NCT 02021409/02021370 (Change in Hb)	≤3 y (6/2014–11/2018)	
			02064426 (DIALOGUE 5)	Long-term extension of NCT 01975818 (Change in Hb)	≤3 y (6/2014–11/2018)	
GSK1278863	IIb	Oral, once daily	01977573	Randomized single-blind vs epoetin, 252 patients (Change in Hb)	24 w (10/2013–5/2015)	CKD not on dialysis
	IIb	Oral, once daily	01977482	Randomized double-blind vs active control, 217 patients (Change in Hb)	24 w (11/2013–2/2015)	Stable HD
	IIa	Oral, once daily	02075463	Open label, 20 patients‡ (Change in Hb)	16 w (6/2014–3/2016)	HD hypo-responsive to epoetin
	IIa	Oral, once daily	02019719	Randomized double-blind vs placebo, 97 patients (Change in Hb)	4 w (11/2013–8/2014)	Stable HD in Japanese patients
	IIa	Oral, once daily	01587924	Randomized double-blind vs rhEPO, 86 patients (Change in Hb)	4 w (5/2012–5/2013)	Stable HD
	IIa	Oral, once daily	01587898	Randomized double-blind vs placebo, 74 patients (Change in Hb)	4 w (5/2012–5/2013)	CKD not on dialysis
	IIa	Oral, once daily	01047397	Randomized single-blind vs placebo, 107 patients (Change in Hb)	4 w (3/2010–2/2011)	Stable HD and CKD not on dialysis
	I	Oral, once daily	01406340	Open label, 22 patients (PK)	2 w (8/2011–8/2013)	Healthy volunteers, CKD Stage 3,4, and 5
	I	Oral, once daily	02243306	Open label, 30 patients‡ (PK)	2 w (10/2014–7/2015)	Stable PD
AKB-6548	IIb	Oral, once daily or 3 times weekly	01906489	Randomized double-blind vs placebo, 210 patients (Percentage achieving Hb≥11.0 or ≥1.2 g/dl increase from baseline)	20 w (7/2013–10/2014)	CKD not on dialysis
	II	Oral, once daily or 3 times weekly	02260193	Non-randomized open label, 90 patients (Change in Hb)	16 w (9/2014–7/2015)	Stable HD
JTZ-951	I	Oral, once daily	NA			
CKD, chronic kidney disease; Hb, haemoglobin; HD, haemodialysis; NA, no active studies registered at ClinicalTrials.gov; PD, peritoneal dialysis; PK, pharmacolkinetic; rhEPO, recombinant human erythropoietin; *Anticipated duration, event driven. ‡Planned number of patients.						

Patrick Maxwell - biography

Patrick Maxwell is Regius Professor of Physics and Head of the School of Clinical Medicine at the University of Cambridge, UK. He is also Honorary Consultant Physician at Cambridge University Hospitals, Executive Director of Cambridge University Health Partners and is Chair of the Medical Research Council's Molecular and Cellular Medicines Board. He is a clinician scientist who has worked for over 23 years on

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Supplementary Table 1 List of human 2OG oxygenases with associated human genetic diseases and substrates that have been reported. Primary citations are given for recent assignments; see references 22 and 23 for citations to other assignments

Gene name	Associated human Mendelian conditions	GeneID	Reported chemical function(s)
HYDROXYLASES			
PHD1 / EGLN2 (HIF prolyl-4-hydroxylase, N-terminal domain disordered)		112398	Prolyl-4*R*-hydroxylase
PHD2 / EGLN1 (HIF prolyl-4-hydroxylase, N-terminal MYND)	Familial erythrocytosis 3 (OMIM 609820)	54583	Prolyl-4*R*-hydroxylase
PHD3 / EGLN3 (HIF prolyl-4-hydroxylase, No N-terminal domain)		112399	Prolyl-4*R*-hydroxylase
P4H TM (hypoxia-inducible factor prolyl 4-hydroxylase isoform a, transmembrane (endoplasmic reticulum))	-	54681	Prolyl hydroxylation
P4HA1, P4HA2, P4HA3 (procollagen-proline, 2-oxoglutarate 4-dioxygenase)	- - -	5033 8974 283208	Prolyl-4*R*-hydroxylase (collagens)
PLOD1, PLOD2, PLOD3 (procollagen-lysine, 2-oxoglutarate 5-dioxygenase)	Ehlers Danlos syndrome type VI (OMIM 225400)	5351	Lysyl-5*R*-hydroxylase (collagens)
	Bruck syndrome 2 (OMIM 609220)	5352	
	Lysyl hydroxylase 3 deficiency (OMIM 612394)	8985	
JMJD4 (Jumonji domain containing 4)	-	65094	Lysyl-4-hydroxylase (release factor eRF1)[24]
JMJD6 (Jumonji domain containing 6)	-	23210	Lysyl-5-hydroxylase (splicing factors and histones)
JMJD5 (Jumonji domain containing 5)	-	79831	NFATc1 hydroxylation
JMJD8 (Jumonji domain containing 8)	-	339123	
TYW5 (tRNA-yWsynthesising protein 5)	-	129450	Wybutosine hydroxylase (modified tRNA)
FIH / HIF1AN (factor inhibiting hypoxia-inducible factor)	-	55662	Asparaginyl hydroxylase (HIF and ankyrin repeat domain containing proteins)
HSPBAP1 (HSPB (heat shock 27kDa) associated protein 1)	-	79663	
JMJD7 (Jumonji domain containing 7)	-	100137047	
NO66 (Nucleolar protein 66 kDa)	-	79697	Histidine hydroxylation

			(ribosome subunit RPL8)
MINA53 (MYC induced nuclear antigen)	-	84864	Histidine hydroxylation (ribosome subunit RPL27A)
ASPH (aspartyl/asparaginyl β-hydroxylase)	Traboulsi syndrome (OMIM 601552)	444	Asn/Asp 3*R*-hydroxylase (EGF and EGF-like domains)
ASPHD2 (aspartyl/asparaginyl β-hydroxylase domain containing 2)	-	57168	
ASPHD1 (aspartyl/asparaginyl β-hydroxylase domain containing 1)	-	253982	
OGFOD3 (2-oxoglutarate and iron-dependent oxygenase domain containing 3)	-	79701	
LEPRE1, LEPREL1, LEPREL2 (leucine proline-enriched proteoglycan (leprecan))	Osteogenesis imperfecta, type VIII (OMIM 610915)	64175	Prolyl-3*S*-hydroxylase
	High myopia with cataract and vitreoretinal degeneration (OMIM 614292)	55214	
	-	10536	
PHYH (phytanoyl-CoA hydroxylase)	Refsum disease (OMIM 266500)	5264	Phytanoyl-CoA 2-*threo*-hydroxylase
PHYHD1 (phytanoyl-coA dioxygenase domain containing 1)	-	254295	
OGFOD1 (2OG, Fe dependent oxygenase domain 1)	-	55239	Prolyl 3-hydroxylase (ribosomal protein RPS23) [25]
OGFOD2 (2OG, Fe dependent oxygenase domain 2)	-	79676	
BBOX (γ-butyrobetaine 2-oxoglutarate dioxygenase)	-	8424	γ-Butyrobetaine 3*S*-hydroxylase
TMLHE (trimethyllysine hydroxylase)	Epsilon-trimethyllysine hydroxylase deficiency (OMIM 300872)	55217	Trimethyllysine 5*R*-hydroxylase

NUCLEOTIDE HYDROXYLASES

ALKBH1 (alkylated DNA repair protein alkB homolog 1)		8846	1-meA/3-meC demethylase (DNA/RNA)
ALKBH2 (alkylated DNA repair protein alkB homolog 2)		121642	1-meA/3-meC demethylase (DNA/RNA)
ALKBH3 (alkylated DNA repair protein alkB homolog 3)		221120	1-meA/3-meC demethylase (DNA/RNA)

ALKBH4 (alkylated DNA repair protein alkB homolog 4)		54784	Actin K84me1 demethylase
ALKBH5 (alkylated DNA repair protein alkB homolog 5)		54890	N^6-methyladenosine (m^6A) demethylase (RNA)
ALKBH6 (alkylated DNA repair protein alkB homolog 6)		84964	
ALKBH7 (alkylated DNA repair protein alkB homolog 7)		84266	
ALKBH8 (alkylated DNA repair protein alkB homolog 8)		91801	5-Methoxycarbonyl-methyluridine (*S*)-hydroxylase (modified RNA)
FTO (fat mass and obesity associated)	Growth retardation, developmental delay, coarse facies, and early death (OMIM 612938)	79068	3-meT demethylase (DNA/RNA); N^6-methyladenosine (m^6A) demethylase (RNA)
TET1, TET2, TET3 (Ten-eleven translocation oncogene family)	-	80312	5-meC hydroxylase (DNA/RNA)
	Somatic mutations occur in myelodysplastic syndrome, (OMIM 614286)	54790	
	-	200424	
HISTONE DEMETHYLASES			
KDM2A (histone lysine demethylase 2A)	-	22992	Histone H3K36me1/me2 demethylase
KDM2B (histone lysine demethylase 2B)	-	84678	Histone H3K36me1/me2, H3K4 me3 demethylase
KDM3A (histone lysine demethylase 3A)	-	55818	Histone H3K9me2/me1 demethylase
KDM3B (histone lysine demethylase 3B)	-	51780	Histone H3K9me2/me1 demethylase
HR (hairless)	Alopecia universalis (OMIM 203655; 209500,146550)	55806	Histone H3K9me2/me1 demethylase[27]
KDM4A (histone lysine demethylase 4A)	-	9682	Histone H3K9me2/me3, H3K36me2/me3, H1.4K26me2/me3 demethylase
KDM4B (histone lysine demethylase 4B)	-	23030	Histone H3K9me2/me3, H3K36me2/me3, H1.4K26me2/me3 demethylase
KDM4C (histone lysine demethylase 4C)	-	23081	Histone H3K9me2/me3, H3K36me2/me3, H1.4K26me2/me3 demethylase

KDM4D (histone lysine demethylase 4D)	-	55693	Histone H3K9me2/me3, H3K36me2/me3, H1.4K26me2/me3 demethylase
KDM4E (histone lysine demethylase 4E)	-	390245	Histone H3K9me2/me3, H3K36me2/me3, H1.4K26me2/me3 demethylase
KDM5A (histone lysine demethylase 5A)	-	5927	Histone H3K4me1/me2/me3 demethylase
KDM5B (histone lysine demethylase 5B)	-	10765	Histone H3K4me1/me2/me3 demethylase
KDM5C (histone lysine demethylase 5C)	Mental retardation, X-linked, syndromic, Claes-Jensen type (OMIM 300534)	8242	Histone H3K4me1/me2/me3 demethylase
KDM5D (histone lysine demethylase 5D)	-	8284	Histone H3K4me1/me2/me3 demethylase
JARID2 (Jumonji AT rich interactive 2)	-	3720	Missing iron binding residue
KDM6B (histone lysine demethylase 6B)		23135	Histone H3K27me2/me3 demethylase
KDM6A (histone lysine demethylase 6A)	Kabuki syndrome 2 (OMIM 300867)	7403	Histone H3K27me2/me3 demethylase
UTY (ubiquitously transcribed tetratricopeptide repeat protein, chromosome Y)	-	7404	Histone H3K27me3 demethylase[26]
KDM7A (histone lysine demethylase 7A)	-	80853	Histone H3K9me2/me1, H3K27me1/me2 demethylase
PHF8 (PHD finger protein 8)	Mental retardation syndrome, X-linked, Siderius type (OMIM 300263)	23133	Histone H3K9me2/me1, H4K20me1 demethylase
PHF2 (PHD finger protein 2)		5253	Histone H3K9me2 demethylase
JMJD1C (Jumonji domain containing 1C)	-	23081	Demethylates mediator of DNA damage checkpoint 1[28]