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Perspectives for the therapy of anemia of chronic diseases

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Abstract

The incidence of anemia of chronic disease (ACD) is underestimated, increases with age, and affects about 30% of the elderly. ACD treatment is currently based on the pharmacotherapy of diseases that caused anemia, erythropoiesis-stimulating agents, and parenteral administration of iron supplementation in case of iron deficiency. Increasing knowledge on the pathophysiology of ACD has resulted in the burst of research on the development of new drugs that are focused on three main areas. The first group of drugs includes substances that inhibit hepcidin transcription, namely direct and indirect bone morphogenetic protein 6 (BMP6) inhibitors and/or SMAD signaling pathway inhibitors, and drugs that regulate hepcidin transcription through STAT3 signaling pathway. The second group of drugs includes direct hepcidin inhibitors (e.g., aptamers, anticalin proteins, monoclonal antibodies) or substances that inhibit the binding of hepcidin to ferroportin. The third group of drugs improves erythropoiesis mainly by upregulation of erythropoietin and/or inhibition of proinflammatory cytokines. In the latter group, hypoxia-inducing factor stabilizers and IL-6 or TNFα antagonists are particularly important. This article discusses new drug groups and substances that are in different phases of development, including both preclinical and clinical studies, and focuses on the prospects of their use in ACD.

Keywords:
anemia of chronic disease, hepcidin, cytokine, hypoxia-inducible factor, treatment

Introduction

The global prevalence of anemia of chronic disease (ACD), also known as anemia of inflammation, is not fully estimated [1]. The incidence of ACD increases with age, and in the elderly population it accounts for approximately one-third of all anemia cases [2, 3]. ACD can develop in the course of infections, malignancies, and autoimmune diseases. The etiology of this type of anemia is complex and it is associated with a decrease in red blood cells (RBCs) production and decreased survival. The activity of proinflammatory cytokines, including tumor necrosis factor α (TNFα) and interferons, results in decreased availability of iron for erythropoiesis, reduced response to erythropoietin (EPO), and decreased EPO synthesis, which in turn inhibit proliferation and differentiation of erythroid progenitor cells and increase erythrocyte turnover and their degradation by macrophages. Hepcidin plays a central role in reducing the bioavailability of iron, and its expression is regulated by proinflammatory cytokines, in particular, interleukin 6 (IL-6) [4, 5]. However, other molecules, such as IL-10, interferon γ (IFN-γ), IL-1β, and lipopolysaccharide, also serve as hepcidin inducers [6]. Many proteins have been described to be involved in the regulation of hepcidin synthesis, and thus they may become a target for ACD therapy. These include bone morphogenetic protein (BMP), hemojuvelin (HJV), activin receptor-like kinases 2 and 3 (ALK-2 and ALK-3), and erythroferrone (ERFE) [5, 7]. Also EPO can directly reduce hepcidin production by inhibiting the BMP-small mothers against decapentaplegic proteins (BMP-SMAD) pathway [8] and also via hypoxia-inducible factor (HIF) signaling [9].

Currently, ACD therapy is limited to the treatment of the underlying disease that is responsible for inflammatory response. Additionally, erythropoiesis-stimulating agents (ESAs), in particular human recombinant erythropoietin (EPO), are also administered. Sometimes iron supplementation is necessary. Better understanding of the regulation of iron metabolism in the presence of inflammation, with particular focus on the role of hepcidin, proinflammatory cytokines, and HIF in erythropoiesis, has caused a burst of research on the development of new drugs for the treatment of ACD. These studies, focusing on three major research areas, are in various stage of development from experimental stage through preclinical research to clinical studies. The first group of studies evaluates the influence of various substances on hepcidin production. The second group examines the substances that inhibit the effects of hepcidin release. This group contains direct hepcidin inhibitors (hepcidin neutralizers and anti-hepcidin monoclonal antibodies) or substances that are able to inhibit binding of hepcidin to ferroportin. Finally, the third group of studies focuses on erythropoiesis inducers that upregulate EPO, stabilize HIF, and block proinflammatory cytokines.

Hepcidin production inhibitors

Hepcidin production inhibitors downregulate hepcidin gene (hepcidin antimicrobial peptide gene [*HAMP*]) in hepatocytes (Fig. 1). Hepcidin transcription can be inhibited as a result of the inhibition of SMAD and/or signal transducer and activator of transcription (STAT) signaling pathways. It can be achieved in a variety of mechanisms, the most important of which seems to be BMP or BMP receptor interaction and blocking of IL-6 or IL-6 receptor. Downstream signaling from BMP receptor is mediated through SMAD1/5/8 pathway while signaling from IL-6 receptor is mediated through Janus-activated kinases (JAK)/

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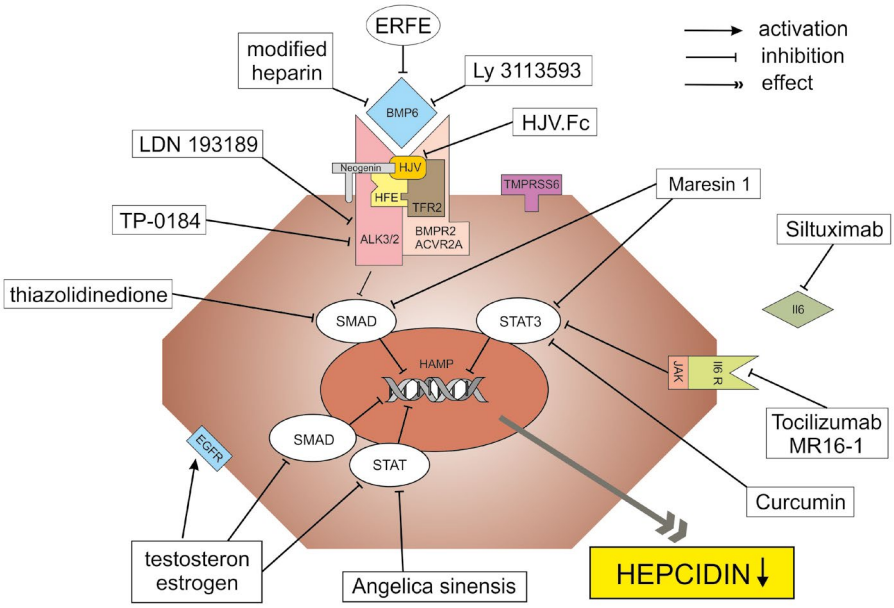

Fig. 1. Hepcidin transcription inhibitors
ACVR2A – activin receptor type 2A; ALK3/2 – activin receptor-like kinase 3/2; BMP-6 – bone morphogenetic protein 6; BMPR2 – bone morphogenetic protein receptor 2; ERFE – erythroferron; EGFR – epidermal growth factor receptor; HAMP – hepcidin antimicrobial peptide; HJV – hemojuvelin; HFE – human hemochromatosis protein; IL-6 – interleukin6; IL-6R – IL-6 receptor; JAK – Janus-activated kinases; SMAD – small mothers against decapentaplegic proteins; STAT3 – signal transducer and activator of transcription 3; TFR2 – transferrin receptor 2; TMPRSS6 – transmembrane protease serine 6

STAT3 signaling pathway. The BMP-SMAD pathway is activated by BMP-2 and BMP-6 through BMP receptors forming complexes with ALK-2 and ALK-3, and HJV as a BMP receptor on liver cells enhances the transmission of this signal [7, 10].

Drugs/substances affecting BMP6 pathway

Currently, a number of substances affecting BMP6 pathways are studied. In two models, murine and rat, the use of anti-BMP6 antibodies led to an increase in hemoglobin (Hb), a decrease in iron deposition in tissues, and a reduction in ESA doses needed to treat ACD [11]. Recently, LY3113593, a human anti-BMP6 monoclonal antibody, is gaining more interest. LY3113593 blocks binding of BMP6 to its receptor, which leads to an increase in iron concentration, an increase in transferrin saturation, and a decrease in hepcidin level. In a study performed in a group of patients with chronic kidney disease (CKD), LY3113593 increased Hb and caused a decrease in ferritin level in comparison with placebo [12].

Other drugs affecting BMP-6 receptor currently tested in clinical trials include: LDN-193189, modified heparin (e.g., roneparstat), TP-0184, and a group of drugs related to HJV. LDN-193189 is a selective inhibitor of the BMP kinase type 1 receptor [13]. TP-0184 is an ALK-2 inhibitor that reduces hepcidin mRNA and improves Hb concentration in preclinical studies in mice [14]. Modified heparin with reduced anticoagulation ability binds BMP-6 and blocks hepcidin expression, as demonstrated in in vitro and in vivo animal models [15, 16].

The ability to inhibit hepatic BMP-SMAD signaling, reduce hepcidin levels, increase ferroportin expression, and increase serum iron levels has also been demonstrated for protein-soluble HJVFc (HJV.Fc). HJV.Fc consists of extracellular domain of HJV conjugated with human IgG Fc fragment [10, 17]. Currently, other potential drugs, e.g., h5F9.23 and h5F9-AM8, which interact with HJV, are experimentally investigated [18]. Administration of a single dose of ABT-207 or H5F9-AM8 in rats and monkeys resulted in prolonged suppression of hepcidin production and an increase in serum iron concentration [19]. H5F9-AM8 was tested in animal models of ACD and iron-refractory iron-deficiency anemia (IRIDA), resulting in reduction of hepcidin and improvement of anemia in all cases [20].

Druggable targets associated with proinflammatory cytokines

Clinically available drugs include a humanized anti-IL-6 receptor antibody, tocilizumab, and a chimeric (human–murine) antibody against IL-6, siltuximab. Tocilizumab is used in rheumatoid arthritis. As an improvement of RBC parameters have been observed during the treatment, tocilizumab has become a new potential drug for ACD [21, 22]. Tocilizumab was successfully used in the treatment of anemia in patients with Castleman's disease [22] and in patients with malignant tumors [23]. Reduced serum hepcidin level and improvement of anemia in patients with Castleman's disease [24, 25], myeloma, and other lymphoproliferative cancers [26] have been reported for siltuximab. An improvement in RBC parameters has also been demonstrated in the treatment of solid tumors [27]. Another IL-6 receptor antibody, MR16-1, was tested in murine models. MR16-1 has been shown to improve Hb parameters in cancer-associated anemia [23]. Other substances affecting hepcidin production by modulation of signaling pathways in hepatocytes have been tested experimentally. In murine models, natural and synthetic STAT3 inhibitors have been shown to inhibit hepcidin expression in macrophages [28]. MaR1

inhibits inflammatory response, including hepcidin expression, by affecting the IL-6/STAT3 signaling pathway and then reducing the severity of anemia symptoms [29].

Potential role of ERFE

ERFE is a physiological regulator of hepcidin, which is synthesized and secreted by erythroblasts in the bone marrow and other tissues in response to EPO. ERFE inhibits hepcidin transcription by an unknown mechanism that involves BMP6 pathway and probably some known membrane receptors [30, 31]. ERFE antagonists and agonists, and also other substances involved in the BMP6 signaling pathway, may prove useful in the prevention and treatment of iron disorders. However, a recently published study suggested a correlation between increased ERFE levels and death/cardiovascular events in hemodialysis patients with CKD, which is further enhanced by the administration of ESA [30]. Therefore, the potential use of ERFE in anemia requires further research and careful determination of patient groups who may benefit from such a therapy.

Other possibilities of hepcidin production inhibition

It has been shown that hepcidin production can be inhibited by thiazolidinediones, testosterone, estrogens, vitamin D, and substances of plant origin (e.g., *Angelica sinensis*). New thiazolidinediones are studied in the treatment of anemia. These drugs may affect hepcidin expression through various pathways, including SMAD1/5/8 signaling pathway, extracellular signal-regulated kinases 1/2 (ERK1/2) and TMPRSS6, and also probably by indirectly affecting ERFE-mediated hepcidin production [32].

In vitro and *in vivo* studies have shown that vitamin D inhibits the expression of proinflammatory cytokines and directly inhibits hepcidin transcription by interaction of vitamin D receptor with the HAMP promoter [33, 34]. In a population of children with chronic inflammatory bowel disease, high levels of cholecalciferol (≥30 ng/mL) were associated with higher Hb levels, while low levels correlated with elevated hepcidin and lower Hb concentration [35]. However, not all studies confirmed the effect of vitamin D supplementation on the levels of proinflammatory cytokines, ferritin, or hepcidin [36]. A meta-analysis and systematic review of literature did not confirm the effect of cholecalciferol on IL-6 or C-reactive protein (CRP) levels [37]. On the other hand, Smith and colleagues showed that high doses of vitamin D significantly reduced hepcidin levels in healthy adults 1 week after the administration of a single dose, without affecting the levels of proinflammatory cytokines or ferritin. The authors concluded that this may suggest that the effect of vitamin D on hepcidin is independent from proinflammatory cytokines or ferritin [38]. In many clinical trials, the inverse correlation between vitamin D levels and anemia has been reported. It is now believed that vitamin D is an important factor involved in the pathogenesis of anemia [39, 40].

Testosterone inhibits hepcidin by upregulation of the epidermal growth factor receptor (EGFR) signaling in the liver [41] and downregulates hepcidin transcription by its influence on BMP-SMAD signaling pathway [42]. The administration of testosterone increases the concentration of Hb in men with reduced testosterone levels, in case of anemia of both known and unknown cause as demonstrated in a study of nearly 800 men, including 126 with anemia [43]. Similarly, 17-β-estradiol could be considered a drug for anemia in the elderly women because it has also been shown to inhibit hepcidin transcription. Inhibition of hepcidin transcription results from the activity of estrogens on HAMP-associated gene promoters [44].

Angelica sinensis polysaccharide (ASP), a polysaccharide obtained from the root of the *Angelica sinensis* plant, inhibits hepcidin expression and may potentially improve RBC parameters in patients with anemia. The efficacy of ASP in the treatment of anemia associated with CKD has been demonstrated in rats. ASP inhibited hepcidin production, increased the amount of iron available for erythropoiesis by its mobilization from the liver and spleen, and increased EPO levels [45]. Previous data have shown that regular supplementation of *Angelica sinensis* in hemodialyzed patients improved anemia in patients resistant to recombinant human EPO therapy [46]. It is believed that ASP inhibits hepcidin expression by inhibiting the expression and/or phosphorylation of JAK1/2, SMAD1/5/8, and ERK1/2 while upregulating SMAD7 [47].

Direct hepcidin inhibitors and agents preventing hepcidin from binding to ferroportin

Another group of drugs that affect hepcidin are direct hepcidin inhibitors and drugs that prevent hepcidin from binding to ferroportin (Fig. 2). Direct neutralization of hepcidin is possible with monoclonal antibodies (AB 12B9M) as well as anticalin proteins (e.g., PRS-080), aptamers (e.g., NOX-H94), or guanosine 5'-diphosphate (GDP). Anticalins are a class of ligand-binding proteins designed based on a lipocalin scaffold. Aptamers are oligonucleotides (short DNA or RNA fragments) or peptides that bind specifically to a specific molecule. PRS-080 is a hepcidin-binding anticalin protein [48]. In cynomolgus monkeys, PRS-080 has been shown to reduce hepcidin and increase iron levels [49]. However, there are currently ongoing studies assessing the effects of PRS-080 anticalin proteins in patients with anemia in the course of CKD (NCT03325621) [50]. On the other hand, the aptamer NOX-H94 (lexaptepid pegol [LP]) is a pegylated oligoribonucleotide that binds to hepcidin with high affinity and blocks its biological function. The effects of this aptamer were confirmed in vitro and in vivo [51]. The pharmacokinetics, pharmacodynamics, and safety study of NOX-H94 carried out in 64 healthy volunteers has shown decrease in hepcidin level and increase in iron and transferrin saturation in comparison with placebo [52]. LP has been used to treat ACD in patients with multiple myeloma and lymphoma. LP caused a significant increase in Hb (≥1 g/dL) in 5 out of 12 patients. Responders experienced increase in Hb concentration in RBCs and reticulocytes, and a decrease in soluble transferrin receptor (sTFR) level. These results confirm the hypothesis on the efficacy of hepcidin inhibition in the treatment of cancer-related anemia, especially in functional iron deficiency. However, the patients with symptomatic iron deficiency, hypochromic anemia, without excessive levels of ferritin, and high sTFR levels were more likely to respond to LP [53].

The group of monoclonal antibodies that serve as direct inhibitors of hepcidin includes Ab12B9m and LY2787106. Ab12B9m is fully human monoclonal IgG2 antibody that binds both human and monkey hepcidin, which is currently under clinical development

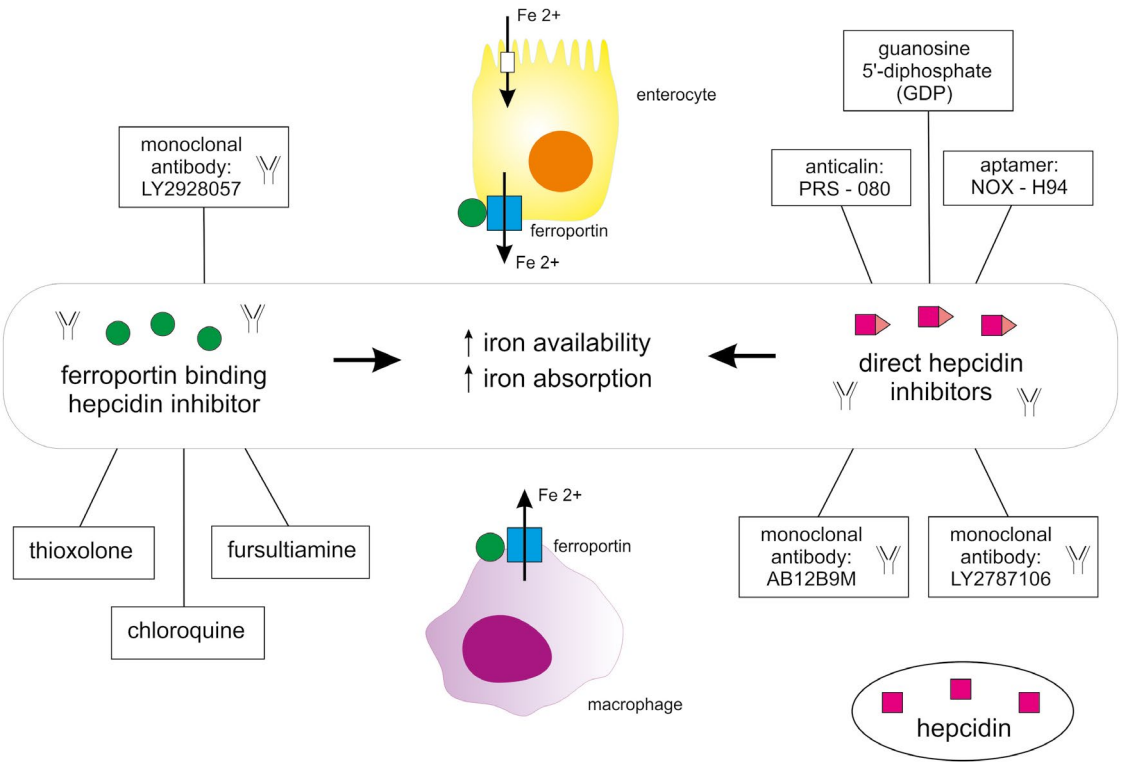

Fig. 2. Direct and indirect hepcidin inhibitors

[54]. LY2787106 is also a fully humanized monoclonal antibody. In phase 1 clinical trials, the use of LY2787106 in tumor-associated anemia resulted in a dose-dependent increase in iron concentration and transferrin saturation, with good tolerance profile [55]. GDP also seems to be a promising agent. It is a direct hepcidin inhibitor with efficacy confirmed in in vitro studies. In animal experiments in mice with ACD, administration of GDP in combination with iron sulfate resulted in an increase in Hb concentration, iron, and ferroportin (FPN) expression and decrease in ferritin level [56].

Potential drugs that prevent hepcidin from binding to ferroportin include: LY2928057, fursultiamine, thioxolone, and chloroquine. These compounds prevent internalization and lysosomal degradation of ferroportin. As a result, hepcidin cannot inhibit iron transport in intestinal epithelial cells or macrophages [57, 58]. LY2928057 is a fully humanized IgG4 monoclonal antibody that binds with human ferroportin. In patients with anemia in the course of CKD, LY2928057 improved iron parameters but did not increase Hb level. Therefore, the need for concomitant ESAs treatment was suggested [59].

Agents affecting EPO and proinflammatory cytokines

Another option for treating ACDs is to enhance erythropoiesis by affecting EPO pathway or interfere with inflammatory processes (Fig. 3). This mechanism is utilized by HIF prolyl hydroxylase inhibitors (HIF-PHI), i.e., molecules that stabilize HIF subunits. The use of HIF-PHI, similar to hypoxia, activates genes that regulate EPO concentration and contribute to the regulation of iron metabolism. As a result, there is an increase in EPO level and iron availability for erythropoiesis [9, 60]. EPO stimulates erythropoiesis, and via activation of EPO receptor it induces ERFE and blocks hepcidin activity [61].

HIF hydroxylase inhibitors appear to be beneficial, especially for patients with CKD-associated anemia. By increasing EPO concentration, the need for ESAs that are associated with increased risk of cardiovascular events is reduced. In addition, HIF-PHI can be administered orally which is the preferred route for patient treatment [60]. The following HIF-PHI are currently tested: molidustat [62], GSK1278863 [63], or FG-4592 [64]. The research is focused mainly on patients with CKD. Phase 3 trials of the safety, tolerability, and efficacy of molidustat in patients with CKD (both on and off dialysis) are currently ongoing [62, 65]. Phase 3 on the efficacy of GSK1278863 in patients with CKD showed a significant increase in the reticulocyte count and other RBC parameters, indicating that further testing is warranted [63]. Also, phase 2 trials of roxadustat (FG-4592) has shown its potential for oral therapy in dialyzed anemic patients with CKD [66].

The trials with HIF hydroxylase inhibitors focus not only in the treatment of ACDs but also in other studies. HIF stabilization with HIF-PHI is important in regulating hematopoietic stem cell (HSC) proliferation and HSC regeneration processes. These properties can be used to protect the bone marrow during radiation therapy, or to increase mobilization in transplant procedures using granulocyte colony-stimulating factor (GCS-F) and plerixafor. Therefore, HIF

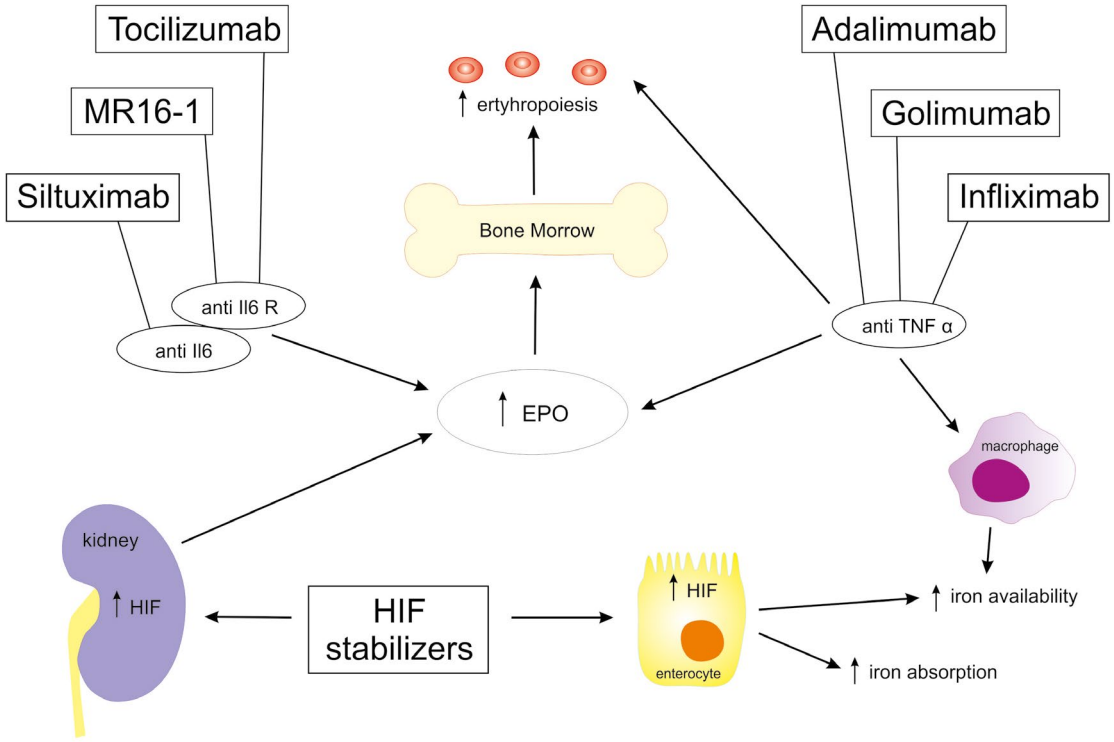

Fig. 3. Drugs affecting erythropoietin and pro-inflammatory cytokines
EPO – erythropoietin; IL-6 – interleukin 6; IL-6R – IL-6 receptor; TNFα – tumor necrosis factor α; HIF – hypoxia-inducible factor

hydroxylase inhibitors may have a potential to improve the effects of treatment in transplant recipients or oncology patients [67]. HIF also participates in the regulation of multiple processes including: apoptosis, angiogenesis, cell proliferation, pH regulation, cellular energy metabolism, and glucose transport [9]. Therefore, further research on the long-term safety of HIF hydroxylase inhibitors is needed, with particular attention to the assessment of the risk of tumorigenesis. It should be emphasized that in rat studies, roxadustat did not show carcinogenic properties [68]. Another potential threat is that HIF stabilization may be associated with an increase in circulating fibroblast growth factor 23 (FGF23) level, and high concentrations of FGF23 have been associated with cardiovascular complications and bone mineralization disorders. However, the increase in FGF23 caused by HIF-PHI is lower than its increase after administration of ESAs [69, 70].

The increase in EPO synthesis can also be caused by monoclonal antibodies as discussed earlier, i.e., anti-IL-6 siltuximab [24] and anti-IL-6 receptor tocilizumab [21, 24]. TNFα is a potential target for anemia of inflammatory diseases, especially in the course of arthritis or inflammatory bowel disease (IBD) [22]. Anti-TNFα drugs include etanercept (ETN), adalimumab (ADA), infliximab (IFX), and golimumab (GO). ETN is a recombinant receptor protein conjugated to the Fc fragment of a human immunoglobulin G1. ETN prevents TNFα from binding to the receptor and blocks its effects. Other human monoclonal antibodies, ADA and GO, as well as chimeric monoclonal antibody IFX and humanized pegylated Fab fragment of monoclonal antibody, certolizumab, exert similar effects. In patients with IBD, reduced hepcidin level and improved anemia after anti-TNFα treatment that modulated hepcidin concentration via IL-6 pathway were observed [71]. It has also been reported that the use of anti-TNFα drugs in IBD patients resolves anemia without the need for iron supplementation [72]. ETN, ADA, and IFX improved Hb level in patients with rheumatoid and psoriatic arthritis [73]. Increased risk of carcinogenesis after anti-TNFα therapy has not been confirmed so far; however, careful surveillance is needed in the treated patients [74]. It should be highlighted that the use of biologicals in the course of IBD is associated with the potential risk of hematological complications such as neutropenia or the development of lymphomas, especially when biologicals are used in combination with other drugs. Further studies are needed to estimate the exact risk of these complications [75, 76].

Summary

A number of studies on new drugs for the treatment of ACD are underway, focusing on various factors involved in iron metabolism and erythropoiesis regulation. Drugs and tested substances are intended to directly inhibit hepcidin or its transcription, or counteract hepcidin activity. The effect of various substances on erythropoiesis is also assessed, mainly by stabilizing the HIF and inhibiting proinflammatory cytokines. Some of these drugs are already used in the treatment of inflammatory bowel disease or in rheumatology, while others are in the experimental phase and will take many years before their introduction to the routine practice. Intensive research

and a vast array of tested compounds give hope for the development of more effective ACD treatments in the coming future.

Authors' contributions

SSM – the only author.

Conflict of interest

None.

Financial support

None.

Ethics

The work described in this article has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans; EU Directive 2010/63/EU for animal experiments; Uniform requirements for manuscripts submitted to biomedical journals.

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