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Targeting EPO and EPO RECEPTOR PATHWAYS in Anemia and Dysregulated Erythropoiesis

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Abstract

Introduction—Recombinant human erythropoietin (rhEPO) is a first-line therapeutic for the anemia of chronic kidney disease, cancer chemotherapy, AIDS (Zidovudine therapy) and lower-risk myelodysplastic syndrome. However, rhEPO frequently elevates hypertension, is costly and may affect cancer progression. Potential high merit therefore exists for defining new targets for anti-anemia agents within EPO, and EPO receptor (EPOR) regulatory circuits.

Areas covered—EPO production by renal interstitial fibroblasts is subject to modulation by several regulators of hypoxia-inducible factor 2a (HIF2a) including Iron Response Protein-1, prolyl hydroxylases and HIF2a acetylases, each with potential as anti-anemia drug targets. The cell surface receptor for EPO (EPOR) preassembles as a homodimer (together with JAK2), and novel agents that trigger EPOR complex activation also remain attractive to develop (activating antibodies, mimetics, small molecule agonists). Additionally, certain downstream transducers of EPOR/JAK2 signaling may be drugable including Erythroferrone (a hepcidin regulator), a cytoprotective Spi2a serpin, and select EPOR-associated protein tyrosine phosphatases.

Expert Opinion—While rhEPO (and biosimilars) presently are important mainstay erythropoiesis-stimulating agents, impetus exists for studies of novel ESAs that fortify HIF2a's effects, act as EPOR agonists, and/or bolster select downstream EPOR pathways to erythroid cell formation. Such agents could lessen rhEPO dosing, side effects and/or costs.

Keywords

erythropoiesis; anemia; erythropoietin (EPO); erythropoiesis stimulating agents (ESAs); EPO receptor; JAK2; hypoxia inducible factors (HIFs); prolyl hydroxylases (PHDs); Spi2a; Erythroferrone; protein tyrosine phosphatases (PTPs); erythroid models

1] EPO, and Present Clinical Uses of rhEPO

Erythropoietin (EPO) is a highly glycosylated sialoglycoprotein hormone^{1, 2} that is expressed predominantly by (and secreted from) a minor subset of adult renal peritubular

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interstitial fibroblasts³. During embryonic development, EPO is also produced by precursor neural crest, neural tissue-derived fibroblasts⁴, and fetal liver cells⁵. Under conditions of severe anemia, adult hepatic cells additionally can activate *EPO* gene expression⁶. EPO levels can be dynamically regulated, with up to 1000-fold increases induced in response to compromised tissue oxygenation⁷. At perinatal and adult stages, EPO exerts its prime effects on bone marrow resident erythroid progenitor cells (EPCs), and critically supports their survival, growth and development. In canonical pathway contexts, EPO's cytoprotective effects have been reported to involve the modulation of a subset of (anti)apoptotic factors including Bcl-x, Bcl2, Mcl, and Bim^{3, 8}. Proliferative actions depend upon EPO's requisite engagement of PI3K/AKT, RAS/MEK/ERK⁹ and PLCg¹⁰ pathways as well as EPOR/JAK2/STAT5 induction of Cyclin D2 and repression of Cyclin G2¹¹. Possible effects on erythroid differentiation have been controversial. However, it has recently been demonstrated that EPO has the ability to guide hematopoietic progenitor cells towards erythroid lineage outcomes (at the expense of myelopoiesis)¹².

Clinically, recombinant human erythropoietin (rhEPO) and biosimilars¹³ are used primarily to treat anemia associated with chronic kidney disease (CKD)^{3, 14}, cancer chemotherapy¹⁵, Zidovudine therapy among HIV patients¹⁶, blood losses pre- and post surgery¹⁷, and low-risk myelodysplastic syndrome (MDS)^{18, 19}. The sustained use of rhEPO over the past three decades as an effective anti-anemia agent points to erythropoietin as one of the most successful recombinant therapeutic agents. Notably, rhEPO can lessen the need for blood transfusions (and inherent risks for alloantibodies, iron overload and acute lung injury)²⁰. In MDS patients, rhEPO also can improve exercise tolerance and quality of life¹⁹. For optimal safety, however, rhEPO dosing must be calibrated to achieve hemoglobin levels of 9-12g/dL²¹⁻²³. As studied among non-dialysis CKD patients, rhEPO dosing to strive for higher hemoglobin levels (13.5g/dL) did not correlate with improved quality of life, and patients in the high hemoglobin arm of this study experienced increased rates of hypertension, myocardial infarction and congestive heart failure²².

One major impetus for pursuing erythropoiesis stimulating agents (ESAs) beyond rhEPO and biosimilars therefore relates to side-effects, with hypertension frequently experienced among CKD patients²⁴⁻²⁶. While hypertensive effects of rhEPO therapy are well established, underlying mechanisms are not well understood. Endothelin-1²⁷⁻²⁹, and thromboxane³⁰ each has been implicated as rhEPO-modulated vasoconstrictors. Possible contributions of blood cell volume changes or viscosity effects, in contrast, have largely been discounted,^{25, 26} with transfusions and rhEPO dosing similarly affecting viscosity but not blood pressure. More recently, however, EPOR expression as well as ESA signaling have been reported to be undetectable in primary human endothelial cells³¹. Further side effects of rhEPO include heightened risks for venous thromboembolism, stroke and death^{21, 32-35}. More controversially, rhEPO has been reported to negatively affect outcomes among patients receiving chemotherapy for certain cancers including breast, head/neck, and additional malignancies^{15, 36-39}. The extent to which tumor cells express EPORs at meaningful densities is also controversial, but EPOR expression levels overall are quite low-level⁴⁰⁻⁴², and with recent notable exceptions^{40, 41, 43} most EPOR antibodies are not so sensitive or specific^{40, 42}. In studies using an A82 EPOR antibody, EPOR expression in a human cancer cell line panel was nominal⁴⁴. In xenograft experiments for several human cancer cell lines

no significant effects of rhEPO on tumor growth were observed⁴⁵, and in a study of primary tumor cells from 186 patients, rhEPO did not significantly activate AKT or MAPK signaling⁴⁶. Nonetheless, in recent studies using an antiserum to a hEPO C-terminal epitope, EPOR expression has been reported among breast cancer cell lines, and associated with estrogen receptor- α activation⁴³. Via RNAi approaches, the EPOR furthermore has been indicated to modulate tumor cell growth, survival and/or migration in melanoma⁴⁷, renal carcinoma⁴⁸ and breast cancer cell lines⁴³.

Taken together, the above considerations indicate potential high merit for the development of ESAs that safely and effectively stimulate erythropoiesis. This review focuses on possible in-roads to defining new EPO and EPOR targets in the contexts of anemia, and dysregulated erythropoiesis.

2] Targeting HIF2a to heighten endogenous EPO production

When systemic oxygenation is compromised, the expression of *EPO* within renal interstitial fibroblasts is induced primarily via transcriptional activation³. This depends in part upon interactions of a delineated *EPO* gene 3' enhancer with hypoxia inducible factors^{3, 4}, (HIFs, as HIF1a, 2a, 3a coupled to their obligate heterodimeric beta subunit, ARNT)⁴⁹. Within 5' *EPO* gene regulatory domains, roles also have been described for a kidney-inducible region⁵⁰, and for Gata-2 and -3 repressive effects⁵¹. Among three HIFs 1a, 2a, 3a⁵², Hif2a has been demonstrated via conditional gene deletion studies in mice to be a major *Epo* gene inducer⁵³. Heightening HIF2a expression therefore provides one potentially attractive in-road to increasing endogenous EPO expression.

During *HIF2a* transcript translation, major regulatory effects recently have been discovered for Iron Response Protein-1 (IRP1). Specifically, when iron levels are low, apo-IRP1 functions as an RNA binding protein to occupy a unique 5' stem-loop structure within *HIF2a* transcripts and hinder translation⁵⁴. Increased iron levels conversely generate iron-complexed holo-IRP1 which is unable to efficiently bind *HIF2a* mRNA cis-elements. This reverses suppression and allows for heightened HIF2a translation – together with elevated EPO expression. Unlike *Irp2*-KO mice which exhibit a microcytic anemia^{54, 55}, *Irp1*-KO mice develop normally at steady-state. Under modest hypoxia or iron deficiency, *Irp1*-KO mice, however, exhibit elevated EPO levels and erythroid polycythemia⁵⁴. In an anti-anemia agent context, this points to apo-IRP1 (within competent renal fibroblasts) as a rational new candidate target whose inhibition is predicted to heighten HIF2a and EPO expression (Figure 1A). DNA and RNA binding proteins (such as IRP1) typically have been considered to be refractive to targeting. Recent reports nonetheless indicate meaningful progress. Examples include initial success via high throughput screens of small molecule inhibitors to the *c-Myc* RNA binding protein, IMP1⁵⁶; Musashi's as a family of proto-oncogenic RNA binding proteins⁵⁷; and to the adipocytic DNA binding protein HMGA2⁵⁸. This elevates prospects for targeting IRP1. Interestingly, IRP1 can also play roles within a cytosolic compartment as an aconitase to catalyze citrate and isocitrate conversions, and enhance NADPH production⁵⁹. Conditional double knockout studies further indicate roles for *Irp1*, together with *Irp2*, in iron adsorption and accumulation in duodenal enterocytes^{54, 55}. In

kidney, liver and brown fat, IRP1 levels also are relatively high. The extent to which these properties might complicate IRP1 targeting presently is uncertain.

HIF2a additionally is sharply regulated by post-translational modifiers, including Von Hippel-Lindau factor (VHL) and prolyl hydroxylases (PHDs 1-3). VHL is an E3 ubiquitin ligase complex component that was identified among Chuvash polycythemia patients as a loss of function (LOF) mutation⁶⁰. VHL LOF attenuates the ubiquitination and turnover of HIF2a^{61, 62}. Elevated levels of HIFs then heighten *EPO* expression, and red cell production. PHDs likewise are negative regulators of HIF's, and function as prolyl hydroxylases to mark HIF's for ubiquitination by VHL⁶³. PHDs specifically couple the hydroxylation of prolines within HIFs to the decarboxylation of 2-oxoglutarate (to yield succinate plus CO₂)⁶⁴. Hypoxia inhibits this process, as do an increasing number of pharmacological small molecule inhibitors of PHDs, with most acting as 2-oxoglutarate antagonists^{64, 65}. Biologically, PHDs 1-3 and HIFs 1a-3a are expressed, and exert functional effects within a broad spectrum of tissues that are affected by ischemia⁶⁵. Pharmacological inhibitors of PHDs therefore are being sought in the contexts of not only anemia but also cardiovascular disease, cerebral ischemia, diabetes, renal disease, retinopathy and cancer metastasis⁶⁵⁻⁶⁷. Via conditional gene disruption studies in mice, PHD2 is emerging as one prime modulator of HIF2a expression in renal *EPO* producing cells^{6, 63, 68-72}. Recent studies, however, implicate hepatocytes⁷³ as well as osteoblasts⁷⁴ as meaningful sites of *EPO* production during hypoxia and anemia. In addition, conditional gene knockout studies are generating insight into relative contributions of PHDs 1-3. Using an *Epo* gene-GFP mouse model to study renal *EPO* producing cells (R-EPs) genetic inactivation of PHD2 restored *EPO* expression, but resulted in polycythemia⁷⁵. By comparison, compound deletion of PHD1 and PHD3 prevented loss of *EPO* expression without generating polycythemia. In liver specific conditional knockout experiments, deletion of either PHD1, 2 or 3 was not consequential, but deletion of any two PHDs induced polycythemia⁷³. Compound deletion of PHDs 1, 2 and 3 further generated hepatic fat accumulation. In osteoblasts, effects on *EPO* production of the compound deletion of PHD1, 2 and -3 were studied⁷⁴, and relative contributions to HIF stabilization await further investigation.

Which PHDs couple to which HIFs, and under what specific conditions, at-large also are somewhat open questions. PHD2 plus HIF1a are the most evolutionarily conserved couple, the most broadly expressed in mammalian tissues, and a predominant pair under steady-state conditions⁶⁴. In the regulated expression of *EPO* and red cell production, roles also have been implicated for a fourth trans-membrane PHD, P4H-TMD⁷⁶. Conditional gene deletion studies, however, point to PHD2 and HIF2a as major effectors in renal *EPO* producing cells during stress^{63, 71, 72}. For several HIF2a stabilizing agents, phase 2b and 3 clinical trials are yielding promising initial outcomes in the context of chronic kidney disease associated anemia¹³. In a partial nephrectomy rat model of chronic kidney disease, one such HIF2a stabilizer also has recently been reported to lessen hypertension as compared to animals dosed with rhEPO (or to partial nephrectomy controls)⁷⁷. Challenges for HIF stabilizer development and application for anemia include first the desired specificity of PHD inhibitors towards PHDs 1-4 vs. potential advantages of pan-reactivity. This relates to PHD and HIF engagement during stress, including *EPO*-producing extra-renal tissues. Second, possible consequences of PHD inhibitors on targets other than HIFs, and beyond *EPO*, merit

active consideration. PHDs are involved in IRP2 degradation⁷⁸, but this effect may help to integrate HIF2a expression with iron levels. PHDs also have been reported to upregulate IKKB⁷⁹, and to negatively regulate ATF4⁸⁰. PHD inhibitors therefore might heighten NFKB and/or ATF4 activity. Third, HIFs (especially HIF1a and to an extent HIF2a) are known to also regulate angiogenic, glycolytic and certain oncogenesis-associated factors. As recently reviewed by Gilkes and Semenza⁸¹, examples of breast cancer targets up-regulated by both HIF1a and 2a include VEGF (Vascular Endothelial Growth Factor), ANGPTL4 (Angiopoietin-Like), CXCR4 (Chemokine C-X-C Motif Receptor), LOX and LOXL4 (Lysyl Oxidase-Like), and Snail1. Based on reported untoward effects of EPO on the progression of select cancers (including breast cancer)^{15, 36-39}, caution might particularly be considered for HIF stabilizer therapy in anemia patients with a history of malignancy.

With further regard to HIF regulation (and targeting) during anemia, heightened attention has also recently been paid to HIF2a's acetylation. In particular, a requirement during stress erythropoiesis has been identified for *Acss2* (acetyl CoA synthetase-2) as a generator of acetyl CoA for this post-translational modification⁸². In particular, *Acss2* gene disruption was unexpectedly observed to worsen anemia due to stress erythropoiesis. In addition, in acquired as well as inherited chronic anemia models, hematocrits were bolstered following acetate supplementation. This proved to involve enhanced HIF2a acetylation and activation by CBP, together with the partnering of CBP with HIF2a. Thus, acetate appears to become metabolically limiting during anemia in ways that affect Hif2a-mediated EPO production (Figure 1C). The extent to which this "acetate switch", and observed anti-anemia effects of acetate supplementation might translate clinically is presently unknown, but is of significant interest. It is noted, however, that acetate enhancement of HIF signaling may also link stress signaling (and associated pathways) to tumorigenesis as recently demonstrated in a xenograft model for HT1080 fibrosarcoma cell growth and metastasis⁸³.

3] Agonist Activation of EPOR/JAK2 Complexes

During Golgi processing, EPO's single-pass cell surface receptor (EPOR) preassembles as a homodimer⁸⁴ (and co-complexes with JAK2)⁸⁵. Upon ligation, EPOR/JAK2 activation is indicated to occur via trans-membrane conformational signaling as triggered by the bridging of two EPOR chains via EPO site-1 plus site-2 domains⁸⁶. Site 1 lies within EPO's D helix and AB coil, and includes N147 and T44 as residues which hydrogen bond to F93 of the EPOR. Site 2 resides within EPO's A and C helices, including R14 and R10 residues which promote bonding to EPOR M150⁸⁷. Consistent with this model, mutations at site 1 or 2 residues (e.g., G151A, K45D, N147K) compromise EPO's binding affinity for the EPOR to <1% of wild-type rhEPO⁸⁸. Bivalent antibodies with capacities to bind, bridge and activate dimeric EPOR/JAK2 complexes therefore represent a possible in-road to EPOR agonist development. Notably, this agonist approach has been exploited, and in particular for an EPOR activating Ab12.6 antibody and derivatives^{89, 90}. Ab12.6 binds to the EPOR at a delineated epitope distal to rhEPO binding domains, induces a conformational transition, and stimulates JAK2 kinase activity. In a humanized EPOR mouse model (and as administered once monthly at 0.2mg/kg), AB12.6 increases hematocrits to levels comparable to those stimulated by Darbepoetin-alfa (as injected twice monthly at 0.003 mg/kg). In addition, Ab12.6 variants also have been prepared that exhibit ten-to-thirty-fold

attenuated EPOR off-rates (e.g., Ab12.17, Ab12.61, Ab12.76) but unexpectedly are less effective in supporting F36E cell growth and CFUe formation⁹⁰. Such findings indicate complexities in antibody-induced EPOR/JAK2 activation mechanisms, and/or EPOR trafficking dynamics. For EPOR agonist antibodies, potential advantages include less frequent dosing, lower production cost and a predicted decreased risk for pure red cell aplasia (PRCA). PRCA involves neutralizing anti-EPO antibody production in patients receiving rhEPO, presents as reticulocytopenia plus normocytic anemia⁹¹, and is managed using corticosteroids and/or plasmapheresis⁹². Notably, this receptor activating monoclonal antibody approach has since also been applied to generate a humanized UB22B minibody agonist for the TPO receptor, MPL⁹³. As assessed using primary CD34+ progenitors, UB22B specifically supported megakaryocyte formation, but unlike TPO interestingly did not enhance platelet aggregation⁹³.

A second approach to EPOR agonist development involves the candidate use of small synthetic or recombinant EPO mimetic peptides (EMPs). Proof-of-principle for the notion that small peptide mimetics might be capable of specifically ligating and activating EPOR/JAK2 complexes was first provided via random phage display library based approaches using monomeric peptides (which interestingly proved to lack primary structural homology to rhEPO⁹⁴). For such single peptide mimetics, however, potencies in vivo were a limiting factor⁹⁵. More recently, this specific limitation has been overcome through the development of select dimeric EMPs as efficient ESAs. One such approach specifically involved the construction of dimeric recombinant EMPs fused to a humanized Ig Fc scaffold^{96, 97}. Such dimeric mimetibody EMPs exhibit high ESA potency, and sustained erythropoietic activity in mouse and rat models⁹⁸. As recently reported, this includes mimetibody EMP-induced increases in HbF and HbA as assessed in a mouse model of beta-thalassemia⁹⁹. Beyond this, and as a notable proof of principle for a fully synthetic mimetic of a complex glycoprotein hormone, PEG-scaffolded dimeric EMPs also have been developed that further proved in initial clinical trials to be non-inferior to rhEPO in treating the anemia of end-stage CKD¹⁰⁰. Efficacy in treating PRCA patients also was demonstrated¹⁰¹. Unfortunately, upon expanded clinical use, select CKD patients proved (upon first-time injection) to experience rapid and severe anaphylaxis, leading to discontinuance of this promising ESA construct¹⁰². Factors underlying this unexpected off-target effect presently are undetermined, but merit investigation. This includes possible interactions with mast cells with potential involvement of PEG scaffold features (which frequently are drug design components).

A third candidate approach to agonist development relates to certain properties exhibited by EPOR trans- and juxtamembrane regions. Studies of erythroleukemogenic effects of a Friend virus gp55 envelope protein interestingly mapped gp55's interactions to the EPOR's transmembrane region¹⁰³. EPOR mutagenesis studies additionally have defined transmembrane and juxtamembrane regions as activating domains, especially due to cysteine substitutions¹⁰⁴. Also, a papillomavirus virus E5 protein that specifically activates PDGF beta-receptor intriguingly has been converted (via random mutagenesis) to a specific EPOR activator that chronically activates the EPOR via transmembrane region targeting¹⁰⁵ (Figure 2A). For the latter factor, issues for delivery and potential oncogenic effects are apparent. Nonetheless, and as proof of principle, small molecule therapeutic agents for thrombocytopenia presently are in place (and in practice) that have proven to act by targeting

the transmembrane domain of the related homodimeric type-1 receptor for TPO (MPL)¹⁰⁶⁻¹⁰⁸ (Figure 2B). In particular, select hydrazine based non-peptide compounds interact with H499 (and likely T496) of MPL's transmembrane region and stimulate JAK2, downstream targets and thrombopoiesis. In *H. sapiens* and primates, but not mouse, MPL transmembrane domains contain this histidine residue. This agonist compound also is inactive towards murine Mpl, and in cell lines lacking hMPL^{106, 109}. In addition, related homodimeric receptors for EPO, prolactin, growth hormone and GCSF, a transmembrane residue corresponding to H499 is lacking (Figure 2C), further pointing to apparent specificity of this small molecule agonist. For immune thrombocytopenia, this agonist is indicated to be effective and to exert limited side effects¹⁰⁸ and can also act additively with endogenous TPO to increase platelet counts¹⁰⁹. In EPOR and anemia contexts, small molecule activators that target the EPOR transmembrane region therefore may provide a rational and relatively untapped in-road to novel ESA development.

4] Potential targeting of signal transducers downstream of the EPOR

Certain newly discovered targets and/or pathways that lie downstream of the EPOR, and are intrinsic to erythroid progenitor cells, also may have targeting potential in anemia and/or myeloproliferative disease contexts. Three such examples are considered here. One that might be directly tractable (as a recombinant cytokine) is the C1q /TNF factor, Erythroferrone (ERFE). ERFE was discovered via gene profiling of EPO-response genes in vivo in murine bone marrow and splenic erythroid precursors¹¹⁰. In ERFE-KO mice, EPO or blood loss-induced suppression of hepcidin proved to be defective, thus pointing to a functional role for ERFE in regulating systemic iron levels. ERFE disruption also proved to worsen anemia in a model of *B. abortus* induced inflammation¹¹¹. In a *th3/+* beta thalassemia intermedia mouse model, ERFE-KO interestingly restored deficit hepcidin levels to normalcy, and moderately decreased hepatic iron overload¹¹⁰. The overall signaling pathway at play here is indicated to involve anemia stimulated ERFE production by (pro)erythroblasts, ERFE suppression of hepcidin production by hepatocytes, and consequently a lessening of hepcidin inhibition of ferroportin-mediated iron efflux from enterocytes, macrophage and hepatocytes¹¹². ERFE therefore has the potential to lessen the anemia of inflammation, and/or to moderate iron overload among thalassemia patients (Figure 3A). Further investigations will be required to assess such effects of ERFE in (pre)clinical models of human anemia and hemoglobinopathies.

During late-stage development, erythroblasts accumulate high-level iron, initiate heme plus hemoglobin biosynthesis, and during this process generate a strongly oxidizing milieu¹¹³. This further involves increased production of ROS due to pressure on oxidative phosphorylation¹¹⁴, with iron also acting as a potent catalyst for ROS-mediated oxidative damage¹¹⁵. Attention to this aspect of erythroblast biology has led to the recent discovery of a *Serpina3g/Spi2A* intracellular serpin as an unexpected EPOR/JAK2/Stat5 induced gene whose product exerts important cytoprotective roles during stress erythropoiesis¹¹⁶. In particular, *Serpina3g/Spi2A* gene disruption results in substantial losses of late stage erythroblasts in the wake of oxidant induced hemolysis, or sub-lethal irradiation. Mechanistically, this proved to involve Spi2A inhibition of an executioner Cathepsin B as leached from compromised erythroblast lysosomes. In addition, Spi2A's effects were

phenocopied by a small molecule inhibitor of Cathepsin B. Leached lysosomal cathepsins within erythroblasts that form during stress erythropoiesis therefore represent a new candidate target for at least certain forms of dysregulated erythropoiesis¹¹⁶ (Figure 3B). In thalassemia and sickle cell disease, for example, erythroid precursors can accelerate the production of oxidants¹¹³ which may then compromise lysosomes and lead to executioner cathepsin release. In oncology contexts, Cathepsin B also is emerging as a druggable target, and biochemically Cathepsins are a tractable target for high-throughput screening¹¹⁷.

EPO ligation of its receptor (plus JAK2 activation) is also known to lead to the engagement of select protein tyrosine kinases (PTPs). In early studies, these specifically were defined as the SH2 domain-encoding PTPs PTPN11 / SHP2¹¹⁸, and PTPN6 / SHP1¹¹⁹. In addition, a non-SH2 PTP, PTPN18, has recently been reported as a novel EPO/EPOR/JAK2 signal transducing factor¹²⁰. PTPN11 is becoming well studied, and this in part is due to the occurrence of dysregulating PTPN11 mutations associated with myeloproliferative disease, juvenile myelomonocytic leukemia, and within a neural context, Noonan syndrome¹²¹. During erythropoiesis, mice harboring a PTPN11 D61Y mutation have been observed to hyper-expand early-stage pro-erythroblasts, with heightened Stat3, Akt and Erk activation also exhibited¹²². In studies by Sharma et al^{123, 124}, PTPN11 also has been shown to be a mediator of oncogenic KIT effects on mastocytosis. PTPN11 therefore acts predominantly as an overall positive effector within select type 1 cytokine receptor and RTK systems. PTPN6, in contrast, is expressed primarily in hematopoietic cells, and acts predominantly as a negative regulator^{119, 125}. As examples, the LOF mutation of PTPN6 in “motheaten” mice¹²⁶ or the mutation of EPOR PY motifs for PTPN6 docking¹²⁷ each result in hyper-responsiveness of erythroid precursors to EPO, and heightened JAK2 activation. PTPN18 is less studied, is known to be associated with HER2 signaling¹²⁸, and recently has been described as a rapidly pY-phosphorylated EPO/EPOR signaling component and positively acting regulator of erythroid precursor cell growth¹²⁰. Unlike PTPN6 and PTPN11, PTPN18 is less complex in its structure, lacks SH2 co-factor partnering domains, and occurs predominantly as a cytosolic tyrosine phosphatase¹²⁸. In human erythroid cells PTPN18 also exhibits stage-selective expression, with multi-fold elevated expression at a CFUe stage (see NCBI GEO GDS3860).

In EPOR and anemia contexts, targeting PTPN11 is counter-intuitive due to its predominant roles as a positive effector^{118, 119, 121, 122, 124}. PTPN11 inhibitors, however, are successfully being developed for candidate use in juvenile myelomonocytic leukemia and Noonan disease¹²⁹⁻¹³¹. For such inhibitors attention should be paid to possible compromising effects on erythropoiesis. As a negatively acting EPOR effector, PTPN6 might be attractive as a target in select anemia contexts. Certain risks, however, may exist for inhibitor-induced hyperproliferation of myeloid and/or lymphoid hematopoietic cell populations¹²⁶. Finally, based on emerging roles for PTPN18 as a positive effector of EPO-dependent erythropoiesis¹²⁰, inhibitors to PTPN18 while predicted to attenuate erythropoiesis, nonetheless might prove to be valuable in erythropoicthemia and certain myeloproliferative neoplasm contexts (Figure 3C). This potentially includes JAK2 V617F associated myeloproliferative disease, for which new in-roads beyond (or in addition to) existing JAK2 inhibitors is emerging¹³². Until recently, PTPs have been considered to be refractive targets for small molecule inhibitors¹³⁰. Complications involve a deep active site

that is positively charged, readily attracts negatively charged compounds, and is susceptible to oxidation (limiting, for example, the use of redox cycling compounds screens). Challenges for specificity also exist due to the large overall number of PTPs¹³⁰. For select PTPs, progress towards specific small molecule inhibition nonetheless has been made as recently summarized for PTPN11¹³¹, PTP1B¹³³ and lymphoid-specific tyrosine phosphatase¹³⁴. In part, this has involved the defining of co-targetable surfaces and pockets that lie adjacent to PTP catalytic domains, together with the development of select bidentate inhibitors¹³⁵.

5] Model systems for the discovery and validation of new druggable EPO/EPOR/JAK2 targets and pathways

Among lower vertebrates (e.g., Zebrafish, *Xenopus*), EPORs are represented, but are not reactive with rhEPO, and lack several functional cytoplasmic phosphotyrosine (pY) signaling motifs that have evolved within murine and human EPORs. The murine EPOR is efficiently activated by rhEPO, and has 86% identity with the hEPO including eight of nine pY signaling motifs. For mouse models, genetic approaches in particular are time intensive, but nonetheless have been advantageous via the development, for example, of useful models for the anemia of chronic renal disease¹¹⁰, chemotherapy⁹⁸, sublethal irradiation¹³⁶, blood loss¹¹⁰ and hemoglobinopathies⁹⁹. In addition, mEPOR-KO mice expressing the hEPOR have been generated and employed in studies of EPOR agonists⁸⁹. For the challenge of discovering and characterizing new EPO and EPOR targets, value for such murine models is illustrated via studies of HIF2a as a prime EPO inducer^{6, 63}; PHD2 as a central inhibitor of HIF2a¹³; Erythroferrone as a novel hepcidin suppressor^{110, 111}; the discovery of lysosomal compromise and cathepsin leaching during stress erythropoiesis¹¹⁶; and the emerging utility of a GDF11-adsorbing soluble Activin IIA and IIB receptors as anti-anemia agents¹³⁷.

In studies of anemia and stress erythropoiesis, murine models nonetheless can present certain potential complications. In mouse, the spleen can rapidly become engaged as a major erythroid organ to expand the erythron^{138, 139}, and this may involve Hedgehog and BMP4 cytokine effects that may not be as critical for human stress erythropoiesis^{140, 141}. In addition, recent comparisons of murine and human expressed transcriptomes in parallel EPC developmental series reveal several unexpected major differences^{142, 143}. As examples, the induction of late-stage factors in murine EPCs is more accelerated; MAPK signaling modules are incongruous in their up- vs down-modulated components¹⁴²; and non-coding RNA populations are particularly disparate including several that promote the late-stage development of murine EPCs but are absent from *H. sapiens*¹⁴³. Differences between mouse and human erythropoiesis also are illustrated (for example) by the lack of disease phenotypes in mice harboring a hematopoietic deficiency in SEC23B¹⁴⁴ (a condition genetically known to underlie congenital dyserythropoietic anemia type II), and by the discovery of C1ORF186/"RHEX" as a novel EPO/EPOR target and molecular adaptor which has evolved in *H sapiens* EPCs but not in mice, rats or lower vertebrates¹⁴⁵.

For EPO/EPOR targeting concerns, the above considerations point to advantages in employing human erythroid precursors. For human EPCs, major advances have been made for two-phase serum-free ex vivo systems that employ dexamethasone to efficiently generate

CFUe and (pro)erythroblasts¹⁴⁵, and for three-phase systems that further support development to anucleated erythrocytes¹⁴². As primary sources, peripheral blood monocytes, mobilized CD34^{pos} progenitors, or cord blood progenitors can be used. Recent progress also has been made in the use of iPS cells. Like hES cells, one complication for iPS EPCs involves the expression of ontogenically early fetal and embryonic HB-F and HB-E rather than adult hemoglobin (HB-A). For EPOR targeting studies, however, this limitation may not be so compromising, and can be overcome via ectopic co-expression of the adult globin activators KLF1 plus Bcl11A-XL¹⁴⁶. Progress is also being made towards the establishment of standardized, feeder layer-free conditions for erythroid differentiation. In addition, in recent studies of iPS cell formation from cord blood, a system has been defined that yields up to 10⁶⁸ expansion of (pro)erythroblasts that retain their capacity to undergo late-stage differentiation¹⁴⁷. Finally, for EPO and EPOR-related myeloproliferative neoplasms, including JAK2-V617F associated disease, patient-derived iPS cells illustrate the potential high value for drug targeting studies. As one illustrating example, iPS cells that are hemizygous for JAK2-V617F develop as megakaryocytes to HGF-independent blasts, but transformation to EPO-independence unexpectedly does not occur within the erythroid lineage¹⁴⁸. Another clinically relevant opportunity for erythroid iPS cell investigations is given by Calcineurin mutations which also have recently been linked to MPN, and Stat5 signaling¹⁴⁹.

6] SUMMARY, AND OPINION

Presently, rhEPO and biosimilars continue to serve as important mainstay therapeutics for anemia associated with CDK, cancer chemotherapy, lower-risk MDS, and AIDS Zidovudine regimens^{3, 14-19}. Outcomes can include lessening of transfusions, together with gains in hemoglobin levels, tissue oxygenation, and quality of life. However, intravenous dosing can be frequent, side-effects can be compromising, and costs to health systems can be high (see Introduction). Targets within EPO-producing cells that bolster endogenous EPO expression therefore first continue to be attractive. This includes EPO producing renal interstitial fibroblasts, as well as hepatocytes and osteoblasts each of which has been demonstrated to activate *EPO* expression upon PHD inactivation^{73, 74}. Promise, and potential complications of PHD inhibitor approaches to treating anemia are summarized in Section 2. For the anemia of chronic kidney disease, an additional potential complication relates to renal fibrosis. Studies involving genetic or pharmacological inactivation of PHDs have demonstrated that EPO expression in EPO-low cells (which can convert to myelofibroblasts) can be reactivated^{75, 150}. The extent to which this might be durably sustained (or perhaps progressively diminished) in patients with end-stage renal disease becomes one important question to address. Possible activation of *EPO* expression in extra-renal tissues (liver, osteoblasts) therefore may also become important to assess. As an RNA binding protein, IRP1 will be a challenging target. Apo-IRP1 inhibitors also would need to be innocuous towards IRP2, which regulates several key iron binding proteins⁵⁵. Apo-IRP1 inhibition nonetheless would circumvent possible side-effects of PHD inhibition. For example, genetic inactivation of PHD2 and PHD3 sensitizes mice to beta-adrenergic stress induced myocardial injury¹⁵¹, while genetic or pharmacological inhibition of PHD2 limits functional recovery post corticospinal tract injury to the sensorimotor cortex¹⁵².

Promise also continues to exist for the development of ESAs that efficiently activate preassembled EPOR dimers^{89, 90, 96-99}. This prospectively includes scaffolded dimeric EPO mimetic peptides that bind EPORs at EPO binding sites as well as small molecule dimerizing agents that might act additively with rhEPO. Exciting progress furthermore continues to be made in discovering new functionally important EPO/EPOR signal transducers and response pathways that may become druggable. Illustrating examples include Erythroferrone as an EPO-induced hepcidin suppressor^{110, 111}, and ROS-induced lysosomal leaching of executioner cathepsins within stressed erythroblasts¹¹⁶. Success in targeting EPO/EPOR signal transduction factors intrinsic to erythroid precursor cells also has the potential advantage of lessening EPO/ESA dosing, and side-effects. Ongoing and future transcriptome and proteomic studies are also likely to reveal novel pro-erythropoietic factors (such as PTPN18)¹²⁰ as rational new targets for inhibition in the context of myeloproliferative neoplasms. Importantly, continued major advancements in the ability to propagate and differentiate primary human erythroid progenitor cells provide enriched opportunities for target interrogations, drug screening and validations¹⁴⁶⁻¹⁴⁸.

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LITERATURE CITED

1. Kawasaki N, Ohta M, Hyuga S, Hyuga M, Hayakawa T. Application of liquid chromatography/mass spectrometry and liquid chromatography with tandem mass spectrometry to the analysis of the site-specific carbohydrate heterogeneity in erythropoietin. *Analytical biochemistry*. Oct 1; 2000 285(1): 82–91. [PubMed: 10998266]
2. Froehlich JW, Barboza M, Chu C, Lerno LA Jr, Clowers BH, Zivkovic AM, et al. Nano-LC-MS/MS of glycopeptides produced by nonspecific proteolysis enables rapid and extensive site-specific glycosylation determination. *Analytical chemistry*. Jul 15; 2011 83(14):5541–7. [PubMed: 21661761]
3. Bunn HF. Erythropoietin. *Cold Spring Harbor perspectives in medicine*. Mar.2013 3(3):a011619. [PubMed: 23457296]
4. Suzuki N, Hirano I, Pan X, Minegishi N, Yamamoto M. Erythropoietin production in neuroepithelial and neural crest cells during primitive erythropoiesis. *Nature communications*. 2013; 4:2902.
5. Koury ST, Bondurant MC, Koury MJ. Localization of erythropoietin synthesizing cells in murine kidneys by in situ hybridization. *Blood*. Feb; 1988 71(2):524–7. [PubMed: 3337914]
6. Minamishima YA, Kaelin WG Jr. Reactivation of hepatic EPO synthesis in mice after PHD loss. *Science*. Jul 23.2010 329(5990):407. [PubMed: 20651146]
7. Becker V, Schilling M, Bachmann J, Baumann U, Raue A, Maiwald T, et al. Covering a broad dynamic range: information processing at the erythropoietin receptor. *Science*. Jun 11; 2010 328(5984):1404–8. [PubMed: 20488988]
8. Kuhrt D, Wojchowski DM. Emerging EPO and EPO receptor regulators, and signal transducers. *Blood*. Apr 17.2015
9. Arcasoy MO, Jiang X. Co-operative signalling mechanisms required for erythroid precursor expansion in response to erythropoietin and stem cell factor. *British journal of haematology*. Jul; 2005 130(1):121–9. [PubMed: 15982354]
10. Schnoder TM, Arreba-Tutusa P, Griehl I, Bullinger L, Buschbeck M, Lane SW, et al. Epo-induced erythroid maturation is dependent on Plcgamma1 signaling. *Cell death and differentiation*. Jun; 2015 22(6):974–85. [PubMed: 25394487]

11. Fang J, Menon M, Kapelle W, Bogacheva O, Bogachev O, Houde E, et al. EPO modulation of cell-cycle regulatory genes, and cell division, in primary bone marrow erythroblasts. *Blood*. Oct 1; 2007 110(7):2361–70. [PubMed: 17548578]
12. Grover A, Mancini E, Moore S, Mead AJ, Atkinson D, Rasmussen KD, et al. Erythropoietin guides multipotent hematopoietic progenitor cells toward an erythroid fate. *The Journal of experimental medicine*. Feb 10; 2014 211(2):181–8. [PubMed: 24493804]
13. Jelkmann W. The ESA scenario gets complex: from biosimilar epoetins to activin traps. *Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association*. Apr; 2015 30(4):553–59.
14. Ramanath V, Gupta D, Jain J, Chaudhary K, Nistala R. Anemia and chronic kidney disease: making sense of the recent trials. *Reviews on recent clinical trials*. Aug; 2012 7(3):187–96. [PubMed: 22540906]
15. Oster HS, Neumann D, Hoffman M, Mittelman M. Erythropoietin: the swinging pendulum. *Leukemia research*. Aug; 2012 36(8):939–44. [PubMed: 22579365]
16. Sullivan PS, Hanson DL, Richardson JT, Brooks JT. Trends in the Treatment of Anemia Using Recombinant Human Erythropoietin in Patients with HIV Infection. *The open AIDS journal*. 2011; 5:113–8. [PubMed: 22253666]
17. Tran DH, Wong GT, Chee YE, Irwin MG. Effectiveness and safety of erythropoiesis-stimulating agent use in the perioperative period. *Expert opinion on biological therapy*. Jan; 2014 14(1):51–61. [PubMed: 24219367]
18. Komrokji RS, Lancet JE, Swern AS, Chen N, Paleveda J, Lush R, et al. Combined treatment with lenalidomide and epoetin alfa in lower-risk patients with myelodysplastic syndrome. *Blood*. Oct 25; 2012 120(17):3419–24. [PubMed: 22936658]
19. Hellstrom-Lindberg E, van de Loosdrecht A. Erythropoiesis stimulating agents and other growth factors in low-risk MDS. *Best practice & research Clinical haematology*. Dec; 2013 26(4):401–10. [PubMed: 24507816]
20. Tanhehco YC, Berns JS. Red blood cell transfusion risks in patients with end-stage renal disease. *Seminars in dialysis*. Sep-Oct; 2012 25(5):539–44. [PubMed: 22686519]
21. Rizzo JD, Brouwers M, Hurley P, Seidenfeld J, Arcasoy MO, Spivak JL, et al. American Society of Hematology/American Society of Clinical Oncology clinical practice guideline update on the use of epoetin and darbepoetin in adult patients with cancer. *Blood*. Nov 18; 2010 116(20):4045–59. [PubMed: 20974674]
22. Singh AK, Szczech L, Tang KL, Barnhart H, Sapp S, Wolfson M, et al. Correction of anemia with epoetin alfa in chronic kidney disease. *The New England journal of medicine*. Nov 16; 2006 355(20):2085–98. [PubMed: 17108343]
23. Horl WH. Anaemia management and mortality risk in chronic kidney disease. *Nature reviews Nephrology*. May; 2013 9(5):291–301. [PubMed: 23438972]
24. Strippoli GF, Craig JC, Manno C, Schena FP. Hemoglobin targets for the anemia of chronic kidney disease: a meta-analysis of randomized, controlled trials. *Journal of the American Society of Nephrology : JASN*. Dec; 2004 15(12):3154–65. [PubMed: 15579519]
25. Krapf R, Hulter HN. Arterial hypertension induced by erythropoietin and erythropoiesis-stimulating agents (ESA). *Clinical journal of the American Society of Nephrology : CJASN*. Feb; 2009 4(2):470–80. [PubMed: 19218474]
26. Vaziri ND, Zhou XJ. Potential mechanisms of adverse outcomes in trials of anemia correction with erythropoietin in chronic kidney disease. *Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association*. Apr; 2009 24(4):1082–8.
27. Kanbay M, Akcay A, Delibasi T, Uz B, Kaya A, Koca C, et al. Comparison of effects of darbepoetin alfa and epoetin alfa on serum endothelin level and blood pressure. *Advances in therapy*. Mar-Apr; 2007 24(2):346–52. [PubMed: 17565925]
28. Barhoumi T, Briet M, Kasal DA, Fraulob-Aquino JC, Idris-Khodja N, Laurant P, et al. Erythropoietin-induced hypertension and vascular injury in mice overexpressing human endothelin-1: exercise attenuated hypertension, oxidative stress, inflammation and immune response. *Journal of hypertension*. Apr; 2014 32(4):784–94. [PubMed: 24463938]

29. Briet M, Barhoumi T, Mian MO, Sierra C, Boutouyrie P, Davidman M, et al. Effects of recombinant human erythropoietin on resistance artery endothelial function in stage 4 chronic kidney disease. *Journal of the American Heart Association*. Apr.2013 2(2):e000128. [PubMed: 23584809]
30. Heinisch BB, Vcelar B, Kapiotis S, Blann A, Wolzt M, Siller-Matula JM, et al. The effect of erythropoietin on platelet and endothelial activation markers: a prospective trial in healthy volunteers. *Platelets*. 2012; 23(5):352–8. [PubMed: 22098110]
31. Sinclair AM, Coxon A, McCaffery I, Kaufman S, Paweletz K, Liu L, et al. Functional erythropoietin receptor is undetectable in endothelial, cardiac, neuronal, and renal cells. *Blood*. May 27; 2010 115(21):4264–72. [PubMed: 20124513]
32. Glaspy J. Update on safety of ESAs in cancer-induced anemia. *Journal of the National Comprehensive Cancer Network : JNCCN*. May; 2012 10(5):659–66. [PubMed: 22570294]
33. Glaspy J, Crawford J, Vansteenkiste J, Henry D, Rao S, Bowers P, et al. Erythropoiesis-stimulating agents in oncology: a study-level meta-analysis of survival and other safety outcomes. *British journal of cancer*. Jan 19; 2010 102(2):301–15. [PubMed: 20051958]
34. Pfeffer MA, Burdmann EA, Chen CY, Cooper ME, de Zeeuw D, Eckardt KU, et al. A trial of darbepoetin alfa in type 2 diabetes and chronic kidney disease. *The New England journal of medicine*. Nov 19; 2009 361(21):2019–32. [PubMed: 19880844]
35. Bennett CL, Silver SM, Djulbegovic B, Samaras AT, Blau CA, Gleason KJ, et al. Venous thromboembolism and mortality associated with recombinant erythropoietin and darbepoetin administration for the treatment of cancer-associated anemia. *Jama*. Feb 27; 2008 299(8):914–24. [PubMed: 18314434]
36. Leyland-Jones B, Semiglazov V, Pawlicki M, Pienkowski T, Tjulandin S, Manikhas G, et al. Maintaining normal hemoglobin levels with epoetin alfa in mainly nonanemic patients with metastatic breast cancer receiving first-line chemotherapy: a survival study. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology*. Sep 1; 2005 23(25):5960–72. [PubMed: 16087945]
37. Henke M, Laszig R, Rube C, Schafer U, Haase KD, Schilcher B, et al. Erythropoietin to treat head and neck cancer patients with anaemia undergoing radiotherapy: randomised, double-blind, placebo-controlled trial. *Lancet*. Oct 18; 2003 362(9392):1255–60. [PubMed: 14575968]
38. Untch M, von Minckwitz G, Konecny GE, Conrad U, Fett W, Kurzeder C, et al. PREPARE trial: a randomized phase III trial comparing preoperative, dose-dense, dose-intensified chemotherapy with epirubicin, paclitaxel, and CMF versus a standard-dosed epirubicin-cyclophosphamide followed by paclitaxel with or without darbepoetin alfa in primary breast cancer--outcome on prognosis. *Annals of oncology : official journal of the European Society for Medical Oncology / ESMO*. Sep; 2011 22(9):1999–2006. [PubMed: 21382868]
39. Debeljak N, Solar P, Sytkowski AJ. Erythropoietin and cancer: the unintended consequences of anemia correction. *Frontiers in immunology*. 2014; 5:563. [PubMed: 25426117]
40. Elliott S, Busse L, McCaffery I, Rossi J, Sinclair A, Spahr C, et al. Identification of a sensitive anti-erythropoietin receptor monoclonal antibody allows detection of low levels of EpoR in cells. *Journal of immunological methods*. Jan 31; 2010 352(1-2):126–39. [PubMed: 19887071]
41. Singh S, Verma R, Pradeep A, Leu K, Mortensen RB, Young PR, et al. Dynamic ligand modulation of EPO receptor pools, and dysregulation by polycythemia-associated EPOR alleles. *PloS one*. 2012; 7(1):e29064. [PubMed: 22253704]
42. Elliott S, Busse L, Bass MB, Lu H, Sarosi I, Sinclair AM, et al. Anti-Epo receptor antibodies do not predict Epo receptor expression. *Blood*. Mar 1; 2006 107(5):1892–5. [PubMed: 16249375]
43. Reinbothe S, Larsson AM, Vaapil M, Wigerup C, Sun J, Jogi A, et al. EPO-independent functional EPO receptor in breast cancer enhances estrogen receptor activity and promotes cell proliferation. *Biochemical and biophysical research communications*. Feb 28; 2014 445(1):163–9. [PubMed: 24502950]
44. Swift S, Ellison AR, Kassner P, McCaffery I, Rossi J, Sinclair AM, et al. Absence of functional EpoR expression in human tumor cell lines. *Blood*. May 27; 2010 115(21):4254–63. [PubMed: 20124514]

45. Kataoka M, Moriya Y, Moriguchi Y, Iwai T, Fujimoto-Ouchi K, Shirane M, et al. Effect of erythropoietin on human tumor growth in xenograft models. *Molecular medicine reports*. Jan-Feb; 2010 3(1):95–101. [PubMed: 21472206]
46. Patterson SD, Rossi JM, Paweletz KL, Fitzpatrick VD, Begley CG, Busse L, et al. Functional EpoR pathway utilization is not detected in primary tumor cells isolated from human breast, non-small cell lung, colorectal, and ovarian tumor tissues. *PloS one*. 2015; 10(3):e0122149. [PubMed: 25807104]
47. Kumar SM, Zhang G, Bastian BC, Arcasoy MO, Karande P, Pushparajan A, et al. Erythropoietin receptor contributes to melanoma cell survival in vivo. *Oncogene*. Mar 29; 2012 31(13):1649–60. [PubMed: 21860424]
48. Wu P, Zhang N, Wang X, Zhang C, Li T, Ning X, et al. The erythropoietin/erythropoietin receptor signaling pathway promotes growth and invasion abilities in human renal carcinoma cells. *PloS one*. 2012; 7(9):e45122. [PubMed: 23028796]
49. Labrecque MP, Prefontaine GG, Beischlag TV. The aryl hydrocarbon receptor nuclear translocator (ARNT) family of proteins: transcriptional modifiers with multi-functional protein interfaces. *Current molecular medicine*. Aug; 2013 13(7):1047–65. [PubMed: 23116263]
50. Madan A, Lin C, Hatch SL 2nd, Curtin PT. Regulated basal, inducible, and tissue-specific human erythropoietin gene expression in transgenic mice requires multiple cis DNA sequences. *Blood*. May 15; 1995 85(10):2735–41. [PubMed: 7742534]
51. Obara N, Suzuki N, Kim K, Nagasawa T, Imagawa S, Yamamoto M. Repression via the GATA box is essential for tissue-specific erythropoietin gene expression. *Blood*. May 15; 2008 111(10):5223–32. [PubMed: 18202227]
52. Palazon A, Goldrath AW, Nizet V, Johnson RS. HIF transcription factors, inflammation, and immunity. *Immunity*. Oct 16; 2014 41(4):518–28. [PubMed: 25367569]
53. Haase VH. Regulation of erythropoiesis by hypoxia-inducible factors. *Blood reviews*. Jan; 2013 27(1):41–53. [PubMed: 23291219]
54. Anderson SA, Nizzi CP, Chang YI, Deck KM, Schmidt PJ, Galy B, et al. The IRP1-HIF-2alpha axis coordinates iron and oxygen sensing with erythropoiesis and iron absorption. *Cell metabolism*. Feb 5; 2013 17(2):282–90. [PubMed: 23395174]
55. Wilkinson N, Pantopoulos K. The IRP/IRE system in vivo: insights from mouse models. *Frontiers in pharmacology*. 2014; 5:176. [PubMed: 25120486]
56. Mahapatra L, Mao C, Andruska N, Zhang C, Shapiro DJ. High-throughput fluorescence anisotropy screen for inhibitors of the oncogenic mRNA binding protein, IMP-1. *Journal of biomolecular screening*. Mar; 2014 19(3):427–36. [PubMed: 24108120]
57. Wu X, Lan L, Wilson DM, Marquez RT, Tsao WC, Gao P, et al. Identification and Validation of Novel Small Molecule Disruptors of HuR-mRNA Interaction. *ACS chemical biology*. Mar.2015 : 17.
58. Alonso N, Guillen R, Chambers JW, Leng F. A rapid and sensitive high-throughput screening method to identify compounds targeting protein-nucleic acids interactions. *Nucleic acids research*. Apr 30.2015 43(8):e52. [PubMed: 25653160]
59. Ferecatu I, Goncalves S, Golinelli-Cohen MP, Clemancey M, Martelli A, Riquier S, et al. The diabetes drug target MitoNEET governs a novel trafficking pathway to rebuild an Fe-S cluster into cytosolic aconitase/iron regulatory protein 1. *The Journal of biological chemistry*. Oct 10; 2014 289(41):28070–86. [PubMed: 25012650]
60. Russell RC, Sufan RI, Zhou B, Heir P, Bunda S, Sybingco SS, et al. Loss of JAK2 regulation via a heterodimeric VHL-SOCS1 E3 ubiquitin ligase underlies Chuvash polycythemia. *Nature medicine*. Jul; 2011 17(7):845–53.
61. Ang SO, Chen H, Hirota K, Gordeuk VR, Jelinek J, Guan Y, et al. Disruption of oxygen homeostasis underlies congenital Chuvash polycythemia. *Nature genetics*. Dec; 2002 32(4):614–21. [PubMed: 12415268]
62. Bader HL, Hsu T. Systemic VHL gene functions and the VHL disease. *FEBS letters*. Jun 4; 2012 586(11):1562–9. [PubMed: 22673568]
63. Franke K, Gassmann M, Wielockx B. Erythrocytosis: the HIF pathway in control. *Blood*. Aug 15; 2013 122(7):1122–8. [PubMed: 23733342]

64. Bishop T, Ratcliffe PJ. HIF Hydroxylase Pathways in Cardiovascular Physiology and Medicine. *Circulation research*. Jun 19; 2015 117(1):65–79. [PubMed: 26089364]
65. Selvaraju V, Parinandi NL, Adluri RS, Goldman JW, Hussain N, Sanchez JA, et al. Molecular mechanisms of action and therapeutic uses of pharmacological inhibitors of HIF-prolyl 4-hydroxylases for treatment of ischemic diseases. *Antioxidants & redox signaling*. Jun 1; 2014 20(16):2631–65. [PubMed: 23992027]
66. Goldmacher VS, Lambert JM, Young AY, Anderson J, Tinnel NL, Kornacki M, et al. Expression of the common acute lymphoblastic leukemia antigen (CALLA) on the surface of individual cells of human lymphoblastoid lines. *Journal of immunology*. Jan; 1986 136(1):320–5.
67. Semenza GL. Molecular mechanisms mediating metastasis of hypoxic breast cancer cells. *Trends in molecular medicine*. Sep; 2012 18(9):534–43. [PubMed: 22921864]
68. Scortegagna M, Ding K, Zhang Q, Oktay Y, Bennett MJ, Bennett M, et al. HIF-2 α regulates murine hematopoietic development in an erythropoietin-dependent manner. *Blood*. Apr 15; 2005 105(8):3133–40. [PubMed: 15626745]
69. Percy MJ, Furlow PW, Lucas GS, Li X, Lappin TR, McMullin MF, et al. A gain-of-function mutation in the HIF2A gene in familial erythrocytosis. *The New England journal of medicine*. Jan 10; 2008 358(2):162–8. [PubMed: 18184961]
70. Percy MJ, Beer PA, Campbell G, Dekker AW, Green AR, Oscier D, et al. Novel exon 12 mutations in the HIF2A gene associated with erythrocytosis. *Blood*. Jun 1; 2008 111(11):5400–2. [PubMed: 18378852]
71. Kapitsinou PP, Liu Q, Unger TL, Rha J, Davidoff O, Keith B, et al. Hepatic HIF-2 regulates erythropoietic responses to hypoxia in renal anemia. *Blood*. Oct 21; 2010 116(16):3039–48. [PubMed: 20628150]
72. Lee FS, Percy MJ. The HIF pathway and erythrocytosis. *Annual review of pathology*. 2011; 6:165–92.
73. Tojo Y, Sekine H, Hirano I, Pan X, Souma T, Tsujita T, et al. Hypoxia Signaling Cascade for Erythropoietin Production in Hepatocytes. *Molecular and cellular biology*. Aug 1; 2015 35(15):2658–72. [PubMed: 26012551]
74. Rankin EB, Wu C, Khatri R, Wilson TL, Andersen R, Araldi E, et al. The HIF signaling pathway in osteoblasts directly modulates erythropoiesis through the production of EPO. *Cell*. Mar 30; 2012 149(1):63–74. [PubMed: 22464323]
75. Souma T, Nezu M, Nakano D, Yamazaki S, Hirano I, Sekine H, et al. Erythropoietin Synthesis in Renal Myofibroblasts Is Restored by Activation of Hypoxia Signaling. *Journal of the American Society of Nephrology : JASN*. Jun.2015 :8. [PubMed: 26229138]
76. Laitala A, Aro E, Walkinshaw G, Maki JM, Rossi M, Heikkilä M, et al. Transmembrane prolyl 4-hydroxylase is a fourth prolyl 4-hydroxylase regulating EPO production and erythropoiesis. *Blood*. Oct 18; 2012 120(16):3336–44. [PubMed: 22955912]
77. Flamme I, Oehme F, Ellinghaus P, Jeske M, Keldenich J, Thuss U. Mimicking hypoxia to treat anemia: HIF-stabilizer BAY 85-3934 (Molidustat) stimulates erythropoietin production without hypertensive effects. *PloS one*. 2014; 9(11):e111838. [PubMed: 25392999]
78. Koditz J, Nesper J, Wottawa M, Stiehl DP, Camenisch G, Franke C, et al. Oxygen-dependent ATF-4 stability is mediated by the PHD3 oxygen sensor. *Blood*. Nov 15; 2007 110(10):3610–7. [PubMed: 17684156]
79. Cummins EP, Berra E, Comerford KM, Ginouves A, Fitzgerald KT, Seeballuck F, et al. Prolyl hydroxylase-1 negatively regulates I κ B kinase-beta, giving insight into hypoxia-induced NF κ B activity. *Proceedings of the National Academy of Sciences of the United States of America*. Nov 28; 2006 103(48):18154–9. [PubMed: 17114296]
80. Hiwatashi Y, Kanno K, Takasaki C, Goryo K, Sato T, Torii S, et al. PHD1 interacts with ATF4 and negatively regulates its transcriptional activity without prolyl hydroxylation. *Experimental cell research*. Dec 10; 2011 317(20):2789–99. [PubMed: 21951999]
81. Gilkes DM, Semenza GL. Role of hypoxia-inducible factors in breast cancer metastasis. *Future oncology*. Nov; 2013 9(11):1623–36. [PubMed: 24156323]
82. Xu M, Nagati JS, Xie J, Li J, Walters H, Moon YA, et al. An acetate switch regulates stress erythropoiesis. *Nature medicine*. Sep; 2014 20(9):1018–26.

83. Chen R, Xu M, Nagati JS, Hogg RT, Das A, Gerard RD, et al. The Acetate/ACSS2 Switch Regulates HIF-2 Stress Signaling in the Tumor Cell Microenvironment. *PloS one*. 2015; 10(2):e0116515. [PubMed: 25689462]
84. Livnah O, Stura EA, Middleton SA, Johnson DL, Jolliffe LK, Wilson IA. Crystallographic evidence for preformed dimers of erythropoietin receptor before ligand activation. *Science*. Feb 12; 1999 283(5404):987–90. [PubMed: 9974392]
85. Pelletier S, Gingras S, Funakoshi-Tago M, Howell S, Ihle JN. Two domains of the erythropoietin receptor are sufficient for Jak2 binding/activation and function. *Molecular and cellular biology*. Nov; 2006 26(22):8527–38. [PubMed: 16982687]
86. Zhang YL, Radhakrishnan ML, Lu X, Gross AW, Tidor B, Lodish HF. Symmetric signaling by an asymmetric 1 erythropoietin: 2 erythropoietin receptor complex. *Molecular cell*. Jan 30; 2009 33(2):266–74. [PubMed: 19187767]
87. Song KE, Byeon J, Moon DB, Kim HH, Choi YJ, Suh JK. Structural identification of modified amino acids on the interface between EPO and its receptor from EPO BRP, human recombinant erythropoietin by LC/MS analysis. *Molecules and cells*. Nov; 2014 37(11):819–26. [PubMed: 25284312]
88. Elliott S, Lorenzini T, Chang D, Barzilay J, Delorme E. Mapping of the active site of recombinant human erythropoietin. *Blood*. Jan 15; 1997 89(2):493–502. [PubMed: 9002951]
89. Liu Z, Stoll VS, Devries PJ, Jakob CG, Xie N, Simmer RL, et al. A potent erythropoietin-mimicking human antibody interacts through a novel binding site. *Blood*. Oct 1; 2007 110(7):2408–13. [PubMed: 17620453]
90. Lacy SE, DeVries PJ, Xie N, Fung E, Lesniewski RR, Reilly EB. The potency of erythropoietin-mimic antibodies correlates inversely with affinity. *Journal of immunology*. Jul 15; 2008 181(2):1282–7.
91. Casadevall N, Nataf J, Viron B, Kolta A, Kiladjian JJ, Martin-Dupont P, et al. Pure red-cell aplasia and antierythropoietin antibodies in patients treated with recombinant erythropoietin. *The New England journal of medicine*. Feb 14; 2002 346(7):469–75. [PubMed: 11844847]
92. Aoki K, Ono Y, Tabata S, Matsushita A, Ishikawa T. Successful treatment of anti-erythropoietin antibody-mediated pure red cell aplasia with low-dose prednisolone. *International journal of hematology*. Feb; 2013 97(2):272–4. [PubMed: 23355260]
93. Matsuki E, Miyakawa Y, Yamane A, Okamoto S. Humanized VB22B minibody for human Mpl stimulates human megakaryopoiesis but does not enhance platelet aggregation. *Experimental hematology*. Aug; 2011 39(8):829–36. [PubMed: 21605620]
94. Wrighton NC, Farrell FX, Chang R, Kashyap AK, Barbone FP, Mulcahy LS, et al. Small peptides as potent mimetics of the protein hormone erythropoietin. *Science*. Jul 26; 1996 273(5274):458–64. [PubMed: 8662529]
95. Barbone FP, Johnson DL, Farrell FX, Collins A, Middleton SA, McMahon FJ, et al. New epoetin molecules and novel therapeutic approaches. *Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association*. 1999; 14(Suppl 2):80–4.
96. Bugelski PJ, Capocasale RJ, Makropoulos D, Marshall D, Fisher PW, Lu J, et al. CNTO 530: molecular pharmacology in human UT-7EPO cells and pharmacokinetics and pharmacodynamics in mice. *Journal of biotechnology*. Mar 20; 2008 134(1-2):171–80. [PubMed: 18242752]
97. Perez-Ruixo JJ, Krzyzanski W, Bouman-Thio E, Miller B, Jang H, Bai SA, et al. Pharmacokinetics and pharmacodynamics of the erythropoietin Mimetibody construct CNTO 528 in healthy subjects. *Clinical pharmacokinetics*. 2009; 48(9):601–13. [PubMed: 19725594]
98. Sathyanarayana P, Houde E, Marshall D, Volk A, Makropoulos D, Emerson C, et al. CNTO 530 functions as a potent EPO mimetic via unique sustained effects on bone marrow proerythroblast pools. *Blood*. May 14; 2009 113(20):4955–62. [PubMed: 19264917]
99. Makropoulos DA, Achuthanandam R, Avery J, Wilson K, Brosnan K, Miller A, et al. CNTO 530 increases expression of HbA and HbF in murine models of beta-thalassemia and sickle cell anemia. *Current pharmaceutical biotechnology*. 2013; 14(2):242–8. [PubMed: 23157711]

100. Fishbane S, Schiller B, Locatelli F, Covic AC, Provenzano R, Wiecek A, et al. Peginesatide in patients with anemia undergoing hemodialysis. *The New England journal of medicine*. Jan 24; 2013 368(4):307–19. [PubMed: 23343061]
101. Macdougall IC, Rossert J, Casadevall N, Stead RB, Duliege AM, Froissart M, et al. A peptide-based erythropoietin-receptor agonist for pure red-cell aplasia. *The New England journal of medicine*. Nov 5; 2009 361(19):1848–55. [PubMed: 19890127]
102. Bennett CL, Jacob S, Hymes J, Usvyat LA, Maddux FW. Anaphylaxis and hypotension after administration of peginesatide. *The New England journal of medicine*. May 22; 2014 370(21):2055–6. [PubMed: 24849101]
103. Zhang J, Randall MS, Loyd MR, Li W, Schweers RL, Persons DA, et al. Role of erythropoietin receptor signaling in Friend virus-induced erythroblastosis and polycythemia. *Blood*. Jan 1; 2006 107(1):73–8. [PubMed: 16174761]
104. Lu X, Gross AW, Lodish HF. Active conformation of the erythropoietin receptor: random and cysteine-scanning mutagenesis of the extracellular juxtamembrane and transmembrane domains. *The Journal of biological chemistry*. Mar 17; 2006 281(11):7002–11. [PubMed: 16414957]
105. Cammett TJ, Jun SJ, Cohen EB, Barrera FN, Engelman DM, Dimaio D. Construction and genetic selection of small transmembrane proteins that activate the human erythropoietin receptor. *Proceedings of the National Academy of Sciences of the United States of America*. Feb 23; 2010 107(8):3447–52. [PubMed: 20142506]
106. Erickson-Miller CL, DeLorme E, Tian SS, Hopson CB, Stark K, Giampa L, et al. Discovery and characterization of a selective, nonpeptidyl thrombopoietin receptor agonist. *Experimental hematology*. Jan; 2005 33(1):85–93. [PubMed: 15661401]
107. Nurden AT, Viallard JF, Nurden P. New-generation drugs that stimulate platelet production in chronic immune thrombocytopenic purpura. *Lancet*. May 2; 2009 373(9674):1562–9. [PubMed: 19324405]
108. Kim YK, Lee SS, Jeong SH, Ahn JS, Yang DH, Lee JJ, et al. Efficacy and safety of eltrombopag in adult refractory immune thrombocytopenia. *Blood research*. Mar; 2015 50(1):19–25. [PubMed: 25830126]
109. Erickson-Miller CL, Delorme E, Tian SS, Hopson CB, Landis AJ, Valoret EI, et al. Preclinical activity of eltrombopag (SB-497115), an oral, nonpeptide thrombopoietin receptor agonist. *Stem cells*. Feb; 2009 27(2):424–30. [PubMed: 19038790]
110. Kautz L, Jung G, Valore EV, Rivella S, Nemeth E, Ganz T. Identification of erythroferrone as an erythroid regulator of iron metabolism. *Nature genetics*. Jul; 2014 46(7):678–84. [PubMed: 24880340]
111. Kautz L, Jung G, Nemeth E, Ganz T. Erythroferrone contributes to recovery from anemia of inflammation. *Blood*. Oct 16; 2014 124(16):2569–74. [PubMed: 25193872]
112. Rochette L, Gudjoncik A, Guenancia C, Zeller M, Cottin Y, Vergely C. The iron-regulatory hormone hepcidin: a possible therapeutic target? *Pharmacology & therapeutics*. Feb.2015 146:35–52. [PubMed: 25205158]
113. Fibach E, Rachmilewitz E. The role of oxidative stress in hemolytic anemia. *Current molecular medicine*. Nov; 2008 8(7):609–19. [PubMed: 18991647]
114. Scheibye-Knudsen M, Fang EF, Croteau DL, Wilson DM 3rd, Bohr VA. Protecting the mitochondrial powerhouse. *Trends in cell biology*. Mar; 2015 25(3):158–70. [PubMed: 25499735]
115. Prus E, Fibach E. Effect of iron chelators on labile iron and oxidative status of thalassaemic erythroid cells. *Acta haematologica*. 2010; 123(1):14–20. [PubMed: 19923794]
116. Dev A, Byrne SM, Verma R, Ashton-Rickardt PG, Wojchowski DM. Erythropoietin-directed erythropoiesis depends on serpin inhibition of erythroblast lysosomal cathepsins. *The Journal of experimental medicine*. Feb 11; 2013 210(2):225–32. [PubMed: 23319700]
117. Sbondile M, Soliman ME. In silico identification of irreversible cathepsin B inhibitors as anti-cancer agents: virtual screening, covalent docking analysis and molecular dynamics simulations. *Combinatorial chemistry & high throughput screening*. 2015; 18(4):399–410. [PubMed: 25747438]

118. Nabinger SC, Chan RJ. Shp2 function in hematopoietic stem cell biology and leukemogenesis. *Current opinion in hematology*. Jul; 2012 19(4):273–9. [PubMed: 22504523]
119. Chong ZZ, Maiese K. The Src homology 2 domain tyrosine phosphatases SHP-1 and SHP-2: diversified control of cell growth, inflammation, and injury. *Histology and histopathology*. Nov; 2007 22(11):1251–67. [PubMed: 17647198]
120. Wojchowski DM, Su S, Johnson A, Jachimowicz E, Stokes MP, Green JM, et al. Discovery of novel EPO/EPOR/JAK2 targets and signal transduction factors via proteomic-based interrogations of post-translational motif modifications. *American Society of Hematology Annual Meeting Proceedings: Blood*. 2014:1335.
121. Li S, Hsu DD, Wang H, Feng GS. Dual faces of SH2-containing protein-tyrosine phosphatase Shp2/PTPN11 in tumorigenesis. *Frontiers of medicine*. Sep; 2012 6(3):275–9. [PubMed: 22869052]
122. Usenko T, Chan G, Torlakovic E, Klingmuller U, Neel BG. Leukemogenic Ptpn11 allele causes defective erythropoiesis in mice. *PloS one*. 2014; 9(10):e109682. [PubMed: 25289670]
123. Sharma N, Kumar V, Everingham S, Mali RS, Kapur R, Zeng LF, et al. SH2 domain-containing phosphatase 2 is a critical regulator of connective tissue mast cell survival and homeostasis in mice. *Molecular and cellular biology*. Jul; 2012 32(14):2653–63. [PubMed: 22566685]
124. Sharma N, Everingham S, Zeng LF, Zhang ZY, Kapur R, Craig AW. Oncogenic KIT-induced aggressive systemic mastocytosis requires SHP2/PTPN11 phosphatase for disease progression in mice. *Oncotarget*. Aug 15; 2014 5(15):6130–41. [PubMed: 25026279]
125. Poole AW, Jones ML. A SHPping tale: perspectives on the regulation of SHP-1 and SHP-2 tyrosine phosphatases by the C-terminal tail. *Cellular signalling*. Nov; 2005 17(11):1323–32. [PubMed: 16084691]
126. Van Zant G, Shultz L. Hematologic abnormalities of the immunodeficient mouse mutant, viable motheaten (mev). *Experimental hematology*. Feb; 1989 17(2):81–7. [PubMed: 2783574]
127. Klingmuller U, Lorenz U, Cantley LC, Neel BG, Lodish HF. Specific recruitment of SH-PTP1 to the erythropoietin receptor causes inactivation of JAK2 and termination of proliferative signals. *Cell*. Mar 10; 1995 80(5):729–38. [PubMed: 7889566]
128. Wang HM, Xu YF, Ning SL, Yang DX, Li Y, Du YJ, et al. The catalytic region and PEST domain of PTPN18 distinctly regulate the HER2 phosphorylation and ubiquitination barcodes. *Cell research*. Sep; 2014 24(9):1067–90. [PubMed: 25081058]
129. Huang WQ, Lin Q, Zhuang X, Cai LL, Ruan RS, Lu ZX, et al. Structure, function, and pathogenesis of SHP2 in developmental disorders and tumorigenesis. *Current cancer drug targets*. 2014; 14(6):567–88. [PubMed: 25039348]
130. He RJ, Yu ZH, Zhang RY, Zhang ZY. Protein tyrosine phosphatases as potential therapeutic targets. *Acta pharmacologica Sinica*. Oct; 2014 35(10):1227–46. [PubMed: 25220640]
131. Chio CM, Lim CS, Bishop AC. Targeting a cryptic allosteric site for selective inhibition of the oncogenic protein tyrosine phosphatase Shp2. *Biochemistry*. Jan 20; 2015 54(2):497–504. [PubMed: 25519989]
132. Geyer HL, Mesa RA. Therapy for myeloproliferative neoplasms: when, which agent, and how? *Blood*. Dec 4; 2014 124(24):3529–37. [PubMed: 25472969]
133. Wang LJ, Jiang B, Wu N, Wang SY, Shi DY. Small molecules as potent protein tyrosine phosphatase 1B (PTP1B) inhibitors documented in patents from 2009 to 2013. *Mini reviews in medicinal chemistry*. 2015; 15(2):104–22. [PubMed: 25643610]
134. Hou X, Li R, Li K, Yu X, Sun JP, Fang H. Fast identification of novel lymphoid tyrosine phosphatase inhibitors using target-ligand interaction-based virtual screening. *Journal of medicinal chemistry*. Nov 26; 2014 57(22):9309–22. [PubMed: 25372368]
135. Low JL, Chai CL, Yao SQ. Bidentate inhibitors of protein tyrosine phosphatases. *Antioxidants & redox signaling*. May 10; 2014 20(14):2225–50. [PubMed: 24206395]
136. Peslak SA, Wenger J, Bemis JC, Kingsley PD, Koniski AD, McGrath KE, et al. EPO-mediated expansion of late-stage erythroid progenitors in the bone marrow initiates recovery from sublethal radiation stress. *Blood*. Sep 20; 2012 120(12):2501–11. [PubMed: 22889760]

137. Dussiot M, Maciel TT, Fricot A, Chartier C, Negre O, Veiga J, et al. An activin receptor IIA ligand trap corrects ineffective erythropoiesis in beta-thalassemia. *Nature medicine*. Apr; 2014 20(4):398–407.
138. Guihard S, Clay D, Cocault L, Saulnier N, Opolon P, Souyri M, et al. The MAPK ERK1 is a negative regulator of the adult steady-state splenic erythropoiesis. *Blood*. May 6; 2010 115(18):3686–94. [PubMed: 20223923]
139. Wu DC, Paulson RF. Hypoxia regulates BMP4 expression in the murine spleen during the recovery from acute anemia. *PloS one*. 2010; 5(6):e11303. [PubMed: 20585586]
140. Harandi OF, Hedge S, Wu DC, McKeone D, Paulson RF. Murine erythroid short-term radioprotection requires a BMP4-dependent, self-renewing population of stress erythroid progenitors. *The Journal of clinical investigation*. Dec; 2010 120(12):4507–19. [PubMed: 21060151]
141. Lau CI, Outram SV, Saldana JI, Furmanski AL, Dessens JT, Crompton T. Regulation of murine normal and stress-induced erythropoiesis by Desert Hedgehog. *Blood*. May 17; 2012 119(20):4741–51. [PubMed: 22461491]
142. An X, Schulz VP, Li J, Wu K, Liu J, Xue F, et al. Global transcriptome analyses of human and murine terminal erythroid differentiation. *Blood*. May 29; 2014 123(22):3466–77. [PubMed: 24637361]
143. Paralkar VR, Mishra T, Luan J, Yao Y, Kossenkova AV, Anderson SM, et al. Lineage and species-specific long noncoding RNAs during erythro-megakaryocytic development. *Blood*. Mar 20; 2014 123(12):1927–37. [PubMed: 24497530]
144. Khoriaty R, Vasievich MP, Jones M, Everett L, Chase J, Tao J, et al. Absence of a red blood cell phenotype in mice with hematopoietic deficiency of SEC23B. *Molecular and cellular biology*. Oct 1; 2014 34(19):3721–34. [PubMed: 25071156]
145. Verma R, Su S, McCrann DJ, Green JM, Leu K, Young PR, et al. RHEX, a novel regulator of human erythroid progenitor cell expansion and erythroblast development. *The Journal of experimental medicine*. Aug 25; 2014 211(9):1715–22. [PubMed: 25092874]
146. Trakarnsanga K, Wilson MC, Lau W, Singleton BK, Parsons SF, Sakuntanaga P, et al. Induction of adult levels of beta-globin in human erythroid cells that intrinsically express embryonic or fetal globin by transduction with KLF1 and BCL11A-XL. *Haematologica*. Nov; 2014 99(11):1677–85. [PubMed: 25107887]
147. Huang X, Shah S, Wang J, Ye Z, Dowey SN, Tsang KM, et al. Extensive ex vivo expansion of functional human erythroid precursors established from umbilical cord blood cells by defined factors. *Molecular therapy : the journal of the American Society of Gene Therapy*. Feb; 2014 22(2):451–63. [PubMed: 24002691]
148. Saliba J, Hamidi S, Lenglet G, Langlois T, Yin J, Cabagnols X, et al. Heterozygous and homozygous JAK2(V617F) states modeled by induced pluripotent stem cells from myeloproliferative neoplasm patients. *PloS one*. 2013; 8(9):e74257. [PubMed: 24066127]
149. Klampfl T, Gisslinger H, Harutyunyan AS, Nivarthi H, Rumi E, Milosevic JD, et al. Somatic mutations of calreticulin in myeloproliferative neoplasms. *The New England journal of medicine*. Dec 19; 2013 369(25):2379–90. [PubMed: 24325356]
150. Asada N, Takase M, Nakamura J, Oguchi A, Asada M, Suzuki N, et al. Dysfunction of fibroblasts of extrarenal origin underlies renal fibrosis and renal anemia in mice. *The Journal of clinical investigation*. Oct; 2011 121(10):3981–90. [PubMed: 21911936]
151. Xie L, Pi X, Townley-Tilson WH, Li N, Wehrens XH, Entman ML, et al. PHD2/3-dependent hydroxylation tunes cardiac response to beta-adrenergic stress via phospholamban. *The Journal of clinical investigation*. Jul 1; 2015 125(7):2759–71. [PubMed: 26075818]
152. Miyake S, Muramatsu R, Hamaguchi M, Yamashita T. Prolyl hydroxylase regulates axonal rewiring and motor recovery after traumatic brain injury. *Cell death & disease*. 2015; 6:e1638. [PubMed: 25675298]

Figure 1. Approaches to increasing HIF2a levels and/or activity, and heightening endogenous EPO expression

(A) Upon binding to a unique 5' domain within *HIF2a* transcripts, Apo-IRP1 inhibits translation. Small molecule inhibitors that interfere with this interaction therefore have the potential to increase HIF2a and EPO expression together with RBC production. **(B)** Small molecule inhibitors that inhibit PHD2 have been developed that stabilize HIF2a, and likewise increase EPO expression plus erythrocyte formation. This includes HIF2a inhibitors presently in phase-2 and -3 CKD clinical trials. Such inhibitors also will reinforce PHD2 and HIF2a actions on additional targets, and possible consequences of these latter effects will be important to assess. **(C)** Acetate supplementation recently has been shown to lessen anemia associated with several forms of stress erythropoiesis. This involves Acss2 mediated Acetyl-CoA production as a limiting step in enhancing HIF2a activity, with EPO as one prime target. Recently, this acetate switch also has been indicated to impact on tumor cell growth and metastasis.

Figure 2. Potential targeting of EPOR transmembrane region to activate pre-dimerized EPOR (and JAK2) complexes

(A) Friend virus envelope protein gp55 has been determined to specifically activate EPOR dimers via interactions with an EPOR transmembrane region. In addition, a 5,000 Mr transmembrane protein has been generated via random mutagenesis of bovine papilloma virus E5 protein (which normally binds and activates PDGFR-beta) that chronically activates the EPOR. (B) As proof-of-principle, small molecule agents that target the transmembrane domain of MPL have been successfully developed as a therapeutic for immune thrombocytopenia, with H499 (and T496) as target sites for one such agent. (C) Comparisons of transmembrane regions (and WSXWS plus box-1) domains within the human homodimeric type-1 receptors for EPO, TPO, prolactin, growth hormone and GCSF.

Figure 3. Select signal transducers within erythroid precursor cells, and downstream of the EPOR with druggable potential

(A) One response pathway intrinsic to erythroblasts, and downstream from the EPOR, involves EPO/EPOR/JAK2/Stat5 transcriptional induction of Erythroferrone (ERFE), a C1q-TNF cytokine. As secreted from erythroblasts, ERFE inhibits hepcidin and thereby enhances Ferroportin efflux of systemic iron. ERFE therefore may assist iron deficient anemias, and/or ERFE inhibition may lessen iron overload as associated, for example, with thalassemia. (B) Via studies of an Spi2A Serpin (as an additional novel EPO/EPOR/JAK2/Stat5 target gene) erythroblast lysosomal membrane permeability has been shown to become compromised during stress erythropoiesis, especially in oxidative and elevated ROS contexts. Small molecule inhibitors of leached lysosomal executioner cathepsins B (and L) therefore represent rational new targets as anti-anemia agents and erythroblast cytoprotectants during such stress erythropoiesis (e.g., sickle cell anemia, thalassemia). (C) Among protein tyrosine phosphatases engaged in erythroid precursors via the EPOR, PTPN6 as a negative effector is a potential target in anti-anemia contexts. As a positive effector, PTPN18 might be considered as a new target in a context of lessening EPOR/JAK2 activation or effects for polycythemia and/or myeloproliferative disease.