Paradigm Academic Press
Journal of Innovations in Medical Research
ISSN 2788-7022
SEP. 2022 VOL.1 NO.2

Epoetin Alfa: a Creative Way to Treat Anemia

Bofan Xu[1]

[1] King's College London

Correspondence: Bofan Xu, King's College London.

doi: 10.56397/JIMR/2022.09.04

Abstract

The use of epoetin alfa has been one of the most relevant trends in the past years, therefore, it is important to understand how epoetin alfa differs from other biopharmaceuticals and to summarise the innovative nature of epoetin alfa. This paper aims at why the biopharmaceutical represents innovation in terms of lead-discovery, drug development and fulfilling a previously unmet treatment need. As far as we know, studies on the creativity of epoetin alfa are lacking in the main literature. This paper reveals that the purpose of epoetin alfa's therapy and current treatment modalities, general principles of the drug discovery process, the creativity in medicinal chemistry & pharmacology of epoetin alfa, choosing the target and sources of compound supply, intellectual property considerations in drug discovery & development, the drawbacks of epoetin alfa and the creativity of epoetin alfa. The contribution of the present research involves that summarising the innovation of epoetin alfa.

Keywords: epoetin alfa, anemia, creativity

1. Introduction

The biological property of epoetin alfa is a glycoprotein with 165 amino acid residues, and 60% of the molecular structure is protein and 40% is sugar. The molecular weight of the protein chain is 18.4kd, and that of the sugar group is about 30.4kd (Zhou, J.Y., 1995). Molecular structures are characterized by helical structures on a lamelar basis, with two disulfide bonds maintaining the stereoshape of the structure and four glycogroup regions around. The biological activity of r-HuEPO(epoetin alfa) is dependent on the glycogroup, and activity is lost if the sialic acid from the glycogroup is removed.

The ready availability of recombinant human r-HuEPO enables the clinical research and application of this hormone for the treatment of anemia and anaemia in various patient populations. Epoetin alfa has been shown to accelerate erythropoiesis and reduce primary selectivity, non-cardiac, nonvascular surgery as well as certain allotransfusion in anaemic patients with chronic renal failure, non-myeloid malignancies and human immunodeficiency virus infection.In addition to improving hematological parameters, epoetin therapy can also improve the health-related quality of life in these patients (Bieber E., 2001).

The chapter reviews the key literature concerned with the epoetin alfa, numerous scholars have conducted extensive research in the curative effect of epoetin alfa. However, these studies are predominantly focued on and lack the threat of side effects. This review critically evaluates the use of epoetin alfa, specifically highlight why the epoetin alfa represents innovation in terms of lead-discovery, drug development or fulfilling a previously unmet treatment need. The chapter begins with the purpose of therapy and current treatment modalities of epoetin alfa, this is followed by general principles of the drug discovery process, role of medicinal chemistry & pharmacology in the drug discovery process, choosing target and sources of compound supply, general principles of pharmaceutical development, intellectual property considerations in drug discovery & development.

2. The Purpose of Therapy and Current Treatment Modalities

Recombinant human erythropoietin is an effective way to treat anemia in patients with chronic kidney disease: it

can improve hemoglobin levels, reduce the need for red blood cell blood transfusion, and improve the quality of life.This drug is suitable for anemia in patients with end-stage nephropathy, anemia in HIV patients receiving azothymidine, and anemia caused by cancer chemotherapy. Recombinant human erythropoietin for injection can promote the accelerated differentiation of primitive blood cells in the bone marrow, accelerate the maturation of nucleated erythrocytes and hemoglobin synthesis, stimulate the release of reticulocytes and mature red blood cells, and stabilize the red blood cell membrane, and improve the antioxidant enzyme function of the erythrocyte membrane.

Different indications have different diagnosis and treatment options of epoetin alfa. For the current treatment modalities of myelodysplastic syndrome-related anemia, the European Leukemia Network guidelines recommend patients with low or moderate risk of IPSS, moderate to severe anemia (hemoglobin <10 g/dL), serum EPO levels <500 mU / mL for EPO or treatment at an initial dose of 30000-60000 IU / week, and / or an infusion <2 U / month. Those patients who did not respond to erythropoietin after 8 weeks of treatment should be given a combined G-CSF (300 mg / week, administered in 2-3 doses) (Gascón, P., Krendyukov, A., Mathieson, N., & Aapro, M., 2019).

For the cancer patients,the followings are the dosing for the epoetiin alfa.

Table 1. Suggested Algorithm for Dosing and Administration of Epoetin Alfa (Turner, R., Anglin, P., Burkes, R., Couture, F., Evans, W., Goss, G., Grimshaw, R., Melosky, B., Paterson, A., Quirt, I., & Canadian Cancer and Anemia Guidelines Development Group, 2001)

Patient Selection Criteria
Symptomatic anemia affecting functional capacity/QOL.
OR
Low baseline Hb levels (<100 g/L) at the start of cancer chemotherapy.
OR
Baseline Hb levels (<120 g/L) where symptoms of anemia sufficient to impair functional capacity or QOL are anticipated.
OR
A drop in Hb of 10–20 g/L per cycle of chemotherapy where at least three cycles remain to be administered.
AND
Anemia directly or indirectly related to malignancy but NOT caused by hemolysis, gastrointestinal bleeding, and iron or folate deficiencies.
Pre-Therapy Monitoring
1. Serum ferritin should be >100 μg/L and transferrin saturation should be at least 20%. Supplemental iron may be required to maintain levels that will support erythropoiesis.
2. Serum folate should be assessed, as deficiency can blunt response.
3. Baseline reticulocyte count should be measured[4].

For the most hemodialysis patients with renal anemi(75.5%), the lower limit of the individualized target Hb range was 10g / dL but <11 g/dL; for 5.3%, Hb <10 g / dL and the remaining 19.2% / Hb 11g / dLFurthermore, for 74.4% of patients, the predetermined target individualized capped was Hb range 11.5g / dL but <12.5g / dL; below 6.5%, above 19.1% of that range. However, only 61.2% of the patients reached their individualized target range at visit 2, and only 59.4% at visit 24. The duration of HX575 treatment was calculated as the difference in the days from the last to the first available patient visit plus 1 day.The median duration of epoetin-α (HX575)treatment was 505 days or 1.4 years. The average (± SD) HX575 dose nominally increased from 106.5

(± 78.7) international unit (IU) / kg / week to 113.0 (± 102.5) IU / kg / week at the 24th month, 106.1 (± 80.6) IU / kg / week, and the highest of 117.7 (± 103.5 in the 20th month).The median dose at baseline was 86.7 IU / kg / week, 85.6 IU / kg / week at month 24,7 8.9 I U / kg / week at month 18, and the highest dose of 88.9 IU / kg / week at months 9 and 12.The mean and median doses over time were not statistically significant, indicating a stable dose pattern during the study (p = not significant (n.s.) (London, G., Mann, J., Goldsmith, D., Combe, C., Dellanna, F., Zaoui, P., Hoebel, N., Krendyukov, A., MacDonald, K., & Abraham, I., 2018).

The main clinical benefit of recombinant EPO is the reduction of transfusion requirements and the relief of anaemic symptoms. The guidelines for epoetin alfa epbx is the same as other ESA. For optimal efficacy, patients need to evaluate the iron reserves before and during treatment. If serum ferriferrin is <100 ng/ml or serum transferrin saturation (TSAT) is <20%, iron should be supplemented. The CKD and anaemic patient guidelines support maintaining ferritin levels above 500 ng / ml or TSAT> 30% before ESA use. In the non-dialysis patient population, the individualized administration should be performed at the lowest dose required to reduce PRBC transfusion requirements and to reduce anemia-related symptoms. Patients treated with erythropoietin typically increase in reticulocyte count within 10 days of starting treatment, followed by an increase in hemoglobin within 2 to 6 weeks. For chemotherapy-associated anaemia, the dose of recombinant EPO required to increase hemoglobin and reduce transfusion requirements is approximately three times that of CKD (Anand, S., Al-Mondhiry, J., Fischer, K., & Glaspy, J., 2021).

3. General Principles of the Drug Discovery Process

After the birth of human insulin, the first through genetic engineering synthetic hematopoietic growth factor — recombinant human erythropoietin (rHuEPO) in 1985 (Lin, F. K., Suggs, S., Lin, C. H., Browne, J. K., Smalling, R., Egrie, J. C., Chen, K. K., Fox, G. M., Martin, F., & Stabinsky, Z., 1985), and was approved in 1989 for clinical practice, become the milestone of the classic biopharmaceutical, and global anemia patients created more opportunities for a better life.

Compared with recombinant human insulin, recombinant human erythropoietin (rHuEPO) development process is more complex: in gene reprint, recombinant human insulin molecular weight, simple molecular structure can be directly expressed in E. coli, and erythropoietin has a large number of glycosylated molecules and complex 3D structure, need to be expressed in the mammalian cell line.

Recombinant human erythropoietin condensed Carnot, Randy Kaufman and other scientists for nearly a century of research and development (Jacobs, K., Shoemaker, C., Rudersdorf, R., Neill, S. D., Kaufman, R. J., Mufson, A., Seehra, J., Jones, S. S., Hewick, R., & Fritsch, E. F., 1985), its scale production of genetic engineering technology from a simple prokaryotic, eukaryotic expression system era to higher eukaryotic cell namely mammalian cell expression system, become the mammalian cell expression is the ancestor of genetic engineering biological drugs, and promote the other series of hematopoietic growth factor biopharmaceutical research and development journey.

4. The Creativity in Medicinal Chemistry & Pharmacology of Epoetin Alfa

Exogenous protrophins can not only bind to the high-affinity propin receptors in hematopoietic tissues, but also to many non-hematopoietic tissues in the human body. Therefore, the treatment of recombinant human erythropoietin can not only improve the oxygen supply and function of heart, brain and kidney tissues by improving hemoglobin, but also have the protective effect of multiple organ tissues such as heart and kidney (Suresh, S., Rajvanshi, P. K., & Noguchi, C. T., 2020).

It is well known that renal fibrosis is the pathological basis and outcome of the progression of various chronic kidney diseases. After entering the exotropin, it can bind to the apotropin receptor of renal stromal cells and initiate a series of signaling pathways such as STAT5, PI3K / AKT, and MAPK. The activation and comprehensive action of these pathways can inhibit the generation of renal stromal fibroblasts and prevent the process of renal fibrosis. Therefore, supplemental therapy with recombinant human erythropoietin can also help patients to achieve kidney protection while improving their kidney oxygen supply (Zhang, Y., Zhu, X., Huang, X., Wei, X., Zhao, D., Jiang, L., Zhao, X., & Du, Y., 2020).

In addition, exogenous erytropin will also bind with the throtropin receptor of cardiomyocytes to activate signaling pathways of cardiomyocyte metabolism such as PI3K / AKT, so as to promote the proliferation of cardiomyocytes and prevent the apoptosis of cardiomyocytes, which can be shown as improving patients' heart failure, heart infarction, angina and other symptoms.Clinical studies show that exogenous erythropropin supplementation can improve anemia patients some heart function related indicators such as left ventricular quality index (LVMi), left ventricular end-diastolic internal diameter (LVDd), left ventricular ejection fraction (LVEF), brain sodium peptide, etc. So recombinant human erythropoietin therapy can help patients prevent chronic heart failure, angina pectoris, myocardial infarction, and improve the movement ability and quality of life (Valderrábano, F., Hörl, W. H., Macdougall, I. C., Rossert, J., Rutkowski, B., & Wauters, J. P., 2003).

Exogenous tropin can also bind to vascular endothelial erytropin receptor to prevent tissue ischemia and hypoxia caused by abnormal vascular contraction by regulating vascular tension (Collino, M., Thiemermann, C., Cerami, A., & Brines, M., 2015); the combination of exogenous tropin and retinal tropin receptor helps to alleviate genetic or non-genetic retinal degeneration. Nowadays, how the treatment of recombinant human erythropoietin can help anemia patients achieve multiple benefits has gradually become a new hotspot in clinical research.

5. Choosing the Project, Target and Sources of Compound Supply

In 1989, the EPO receptor gene was successfully cloned from mouse erythroleukemia (MEL) cells.The human EPO receptor cDNA was successfully cloned in 1991 (Noguchi, C. T., Bae, K. S., Chin, K., Wada, Y., Schechter, A. N., & Hankins, W. D., 1991).The human EPO-R gene is located in region 24 of the short arm of chromosome 19 (19p24), is from 5 to 6.5 k b long, and consists of 8 exons and 7 introns.The encoded EPOR is composed of 507 amino acids, of which 24 amino acids form a single peptide chain, 223 amino acids form extraplassolic parts, 24 amino acids form transmembrane parts, and 236 amino acids form cytosolic parts, with approximately 82% homology to mouse-derived EPOR. EPOR is a type I transmembrane protein that belongs to the superfamily of hematopoietic facine cells, and such receptors also include cytokine receptors such as: IL-2, IL-3, L-4, and GM-CSF. Like other hematopoietic cytokines, its extracellular region has four conserved cysteine residues and five amino acid conserved structure WSXWS (Trp-Ser-X- -Trp-Ser), box1, box22 in the cytoplasmic region, necessary for mitotic coupling with induction of tyrosine phosphorylation ligands, and the cytoplasmic near membrane region is required for action with JAK2.Similar to EPO synthesis, EPOR gene expression is also associated with HIF-1 in the hypoxic environment of body tissues (Manalo, D. J., Rowan, A., Lavoie, T., Natarajan, L., Kelly, B. D., Ye, S. Q., Garcia, J. G., & Semenza, G. L., 2005).

In addition to being expressed in hematopoietic red blood cells, EPOR was also expressed in other non-hematopoietic cells, nerve cells, vascular smooth muscle cells, retinal cells, vascular endothelial cells, etc. According to the different affinity of EPO receptor and EPO, divided into high affinity EPO receptor and low affinity EPO receptors, affinity EPO receptors are expressed in hematopoietic tissue cells, while non-hematopoietic tissues express low affinity EPO receptors.

The intracellular region of the EPO receptor itself lacks tyrosine protein kinase (PTK) activity, but can activate the downstream pathway through the intracellular non-receptor tyrosine protein kinase JAK-2 (Janus family protein tyrosine kinase-2). When the EPO is bound to the target cell, the EPO receptor structure on the target cell membrane is altered to form a homodimer, the homologous EPO receptor activates the JAK2 kinase bound in the intracellular region, and the activated JAK2 kinase first phosphorylates the tyrosine residues of the EPO receptor.A number of signaling proteins with the SH2 region then cluster on the phosphorylated tyrosine residues and are activated by directly or indirectly phosphorylated by JAK2 (Wojchowski, D. M., Sathyanarayana, P., & Dev, A., 2010).

JAK is a family of non-receptor tyrosine kinases, currently found to have four family members, namely JAK1, JAK2, TYK2, and JAK3. The first three are widely found in a variety of tissues and cells, whereas JAK3 is present only in the bone marrow and lymphatic system.They vary in size, with molecular weights ranging between 120 kD and 140 kD. JAK family molecules can be divided into two parts: the C terminus is two closely linked tyrosine kinase active domains, one closest to the C terminus is the catalytic active region of JAK kinase; another enzyme-like domain at its N does not have tyrosine kinase activity and is currently considered to play a role in the binding of JAK kinase to other signaling protein molecules.

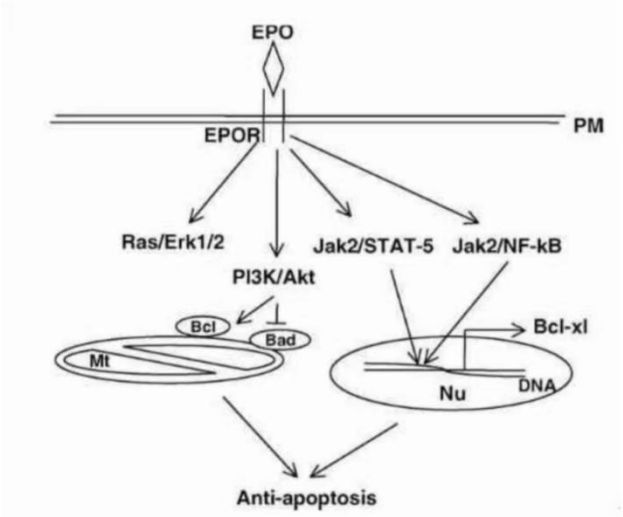

Figure 1. Signaling pathway activated by EPO in hematopoietic erythrocytes (Wojchowski, D. M., Sathyanarayana, P., & Dev, A. (2010)

6. General Principles of Pharmaceutical Development

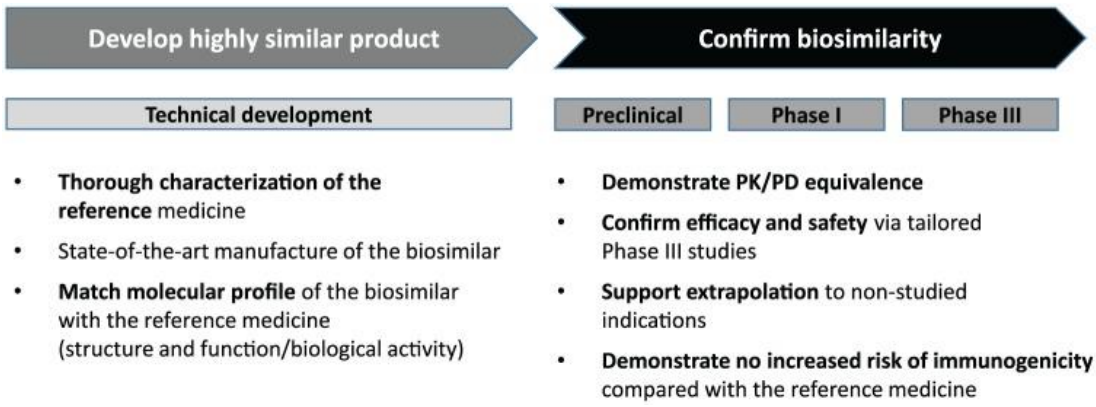

Figure 2. Overview of the biosimilar development process (Aapro, M., Krendyukov, A., Höbel, N., Seidl, A., & Gascón, P., 2018)

The development of high-quality biosimilars is a methodical and firm process that includes the following steps(Figure 1) from the perspective of the overall approval evidence. Phase 1 involves a total molecular characterization of the reference drugs. This involves defining key characteristics (or quality properties) of the clinical properties [efficacy and safety, pharmacokinetic (PK) and pharmacodynamics (PD), immunogenicity] of the reference drug and acquiring information about the range and possible changes that these properties change over time. These data are the basis for defining boundaries or "target columns" in which the properties of biosimilars under development must fall. As part of this, a comprehensive panel of analytical methods is utilized to ensure that biosimilars have the same structural and functional properties as the reference drug (Aapro, M., Krendyukov, A., Höbel, N., Seidl, A., & Gascón, P., 2018).

The goal of the clinical plan is not to demonstrate new safety and efficacy properties, which have been established for the reference drug, but to convincingly confirm that there are no clinically meaningful differences compared to the reference drug. Clinical development of biosimilars typically includes phase I PK / PD studies to show bioequivalence, as well as phase III confirmatory studies in selected sensitive indications to demonstrate no meaningful clinical differences compared to the reference drug. Regulators approve the proposed biogeneric only if the full evidence establishes similarities to reference drugs in terms of quality characteristics, bioactivity as well as safety and efficacy.After biosimilar approval, standard pharmacovigilance is required (as with any new drug), in particular, given that immunogenicity is important for monitoring all biologics ((Aapro, M., Krendyukov, A., Höbel, N., Seidl, A., & Gascón, P., 2018).

The European Medicines Agency (EMA) was the first regulator to develop specific regulatory routes for approving biogeneric drugs when issuing the "Biopharmaceutical Product Guidelines" in 2005. The EMA has since released many other updates and guidelines. And other regulators, including the most recent Food and Drug Administration in 2015.

To date, EMA has approved two biogeneric epoetins, under several brands. The biosimilar version of Eprex/Erypo(Janssen-Cilag,High Wycombe,UK) has the same international generic name (INN; epoetin alfa) as Binocrit(Sandoz GmbH,Kundl, Austria),Epoetin alfa HEXAL(Hexal,Holzkirchen, Germany) and Abseamed(Medice Arzneimittel,Iserlohn, Germany) sales. Another biogeneric version of Eprex / Erypo is also available, with the epoetin zeta INN; this is sold as Retacrit (Hospira UK Limited, Maidenhead, UK) and Silapo (STADA, Bad Vilbel, Germany) (Aapro, M., Krendyukov, A., Höbel, N., Seidl, A., & Gascón, P., 2018).

7. Intellectual Property Considerations in Drug Discovery & Development

Here is an example of Intellectual property. Intellectual property is an important asset and a reflection of Amgen's core competitiveness. Intellectual property acquisition and management strategies on a global scale are important to businesses. In Amgen's early cooperation, the most important partners were Kirin Brewery Co., Ltd. and Johnson & Johnson Co., Ltd. Amgen raised the money by offering the rights to sell Epogen in select territories. Such cooperation is an effective application of IP asset financing.

Intellectual property is also an important tool in the pharmaceutical industry's market game. Amgen used its patents as a weapon to defeat would-be competitors like Johnson & Johnson, Genentech and Sanofi-Aventis. Epogen's patents have often sparked lawsuits between Amgen and other companies. As a result, Epogen ended up with a 32-year patent protection term in the United States (1983-2015), a key determinant of higher economic benefits (Zhang, L., & Wang, J., 2021).

8. The Drawbacks of Epoetin Alfa

The introduction of EPO as a recombinant protein for anemia has made significant progress to our treatment regimen. Treatment with erythropoietin maintains elevated Hb levels for long or even months and is well tolerated.Because of these characteristics, it is more extensive than a blood transfusion.

However, erythropoietin works poorly for myelodysplastic syndrome anemia and appears to be synergistic with the granulocyte colony-stimulating factor. Further, not each patient responded to erythropoietin, these patients will still rely on a blood transfusion.

Much of the current literature on epoetin alfa pays particular attention to the advantages of the epoetin alfa, but lacks the discussion of the drawbacks.

The general reaction is that a few patients can appear headache, low heat, fatigue, etc., and individual patients can appear myalgia, joint pain, etc.The vast majority of adverse reactions can be improved after symptomatic treatment, without not affecting the continuation of medication.But the most common adverse reaction of epoetin alfa is the occurrence of hypertension or elevated blood pressure, which is fatal for hypertensive patients.Therefore, epoetin alfa is not applicable to the large patient population of hypertension.

9. The Creativity of Epoetin Alfa

Recombinant human erythropoietin greatly reduces the blood infusion rate, saves social medical resources, and brings high quality of life to thousands of anemia patients, and becomes an indispensable good helper for anemia patients.

RBC transfusions are associated with adverse events and many risks, and Epoetin alfa is safer and more effective. Moreover, epoetin alfa supplementation can not only treat anemia, but also have the protective effect of multiple organs such as heart and kidney. Anemia treatment drugs emerge extensively, recombinant human erythropoietin treatment is still irreplaceable.

10. Conclusion

Overall, these studies highlight the need for epoetin alfa. Considering all of this evidence, the use of epoetin alfa has more pros than cons. The creativity of epoetin alfa reflects in its outstanding efficacy. Erythropoietin is the most potent physiological stimulus of erythropoiesis, inhibiting apoptosis of erythrocyte progenitors and increases proliferation of erythroid progenitors and hemoglobin production. The introduction of EPO as a recombinant protein for anemia has made significant progress to our treatment regimen. Treatment with erythropoietin maintains elevated Hb levels for long or even months and is well tolerated. Because of these characteristics, it is more extensive than a blood transfusion, and it is protective against multiple organs and tissues. However, such studies remain narrow in focus dealing only with the advantages of the epoetin alfa, a more systematic and theoretical analysis is required for the drawbacks of the epoetin alfa.

References

Zhou, J.Y. (1995). Chongzu renhongxibao Shengchengsu de shengwu yaoli ji linchuang[Biological, pharmacological, and clinical treatment of recombinant human erythropoietin]. *Zhejiang Medicine*, 1, 34-36.

Bieber E. (2001). Erythropoietin, the biology of erythropoiesis and epoetin alfa. An overview. *The Journal of reproductive medicine*, *46*(5 Suppl), 521-530.

Gascón, P., Krendyukov, A., Mathieson, N., & Aapro, M. (2019). Epoetin alfa for the treatment of myelodysplastic syndrome-related anemia: A review of clinical data, clinical guidelines, and treatment protocols. *Leukemia research*, *81*, 35-42. https://doi.org/10.1016/j.leukres.2019.03.006.

Turner, R., Anglin, P., Burkes, R., Couture, F., Evans, W., Goss, G., Grimshaw, R., Melosky, B., Paterson, A., Quirt, I., & Canadian Cancer and Anemia Guidelines Development Group (2001). Epoetin alfa in cancer patients: evidence-based guidelines. *Journal of pain and symptom management*, *22*(5), 954-965. https://doi.org/10.1016/s0885-3924(01)00357-8.

London, G., Mann, J., Goldsmith, D., Combe, C., Dellanna, F., Zaoui, P., Hoebel, N., Krendyukov, A., MacDonald, K., & Abraham, I. (2018). Long-term treatment with biosimilar epoetin-α (HX575) in hemodialysis patients with renal anemia: real-world effectiveness and safety in the MONITOR-CKD5 study. *Clinical nephrology*, *89 (2018)*(1), 1-9. https://doi.org/10.5414/CN109245.

Anand, S., Al-Mondhiry, J., Fischer, K., & Glaspy, J. (2021). Epoetin alfa-epbx: a new entrant into a crowded market. a historical review of the role of erythropoietin stimulating agents and the development of the first epoetin biosimilar in the United States. *Expert review of clinical pharmacology*, *14*(1), 1–8. https://doi.org/10.1080/17512433.2021.1863786.

Lin, F. K., Suggs, S., Lin, C. H., Browne, J. K., Smalling, R., Egrie, J. C., Chen, K. K., Fox, G. M., Martin, F., & Stabinsky, Z. (1985). Cloning and expression of the human erythropoietin gene. *Proceedings of the National Academy of Sciences of the United States of America*, *82*(22), 7580-7584. https://doi.org/10.1073/pnas.82.22.7580.

Jacobs, K., Shoemaker, C., Rudersdorf, R., Neill, S. D., Kaufman, R. J., Mufson, A., Seehra, J., Jones, S. S., Hewick, R., & Fritsch, E. F. (1985). Isolation and characterization of genomic and cDNA clones of human erythropoietin. *Nature*, *313*(6005), 806–810. https://doi.org/10.1038/313806a0.

Suresh, S., Rajvanshi, P. K., & Noguchi, C. T. (2020). The Many Facets of Erythropoietin Physiologic and Metabolic Response.*Frontiers in physiology*, *10*, 1534. https://doi.org/10.3389/fphys.2019.01534.

Zhang, Y., Zhu, X., Huang, X., Wei, X., Zhao, D., Jiang, L., Zhao, X., & Du, Y. (2020). Advances in Understanding the Effects of Erythropoietin on Renal Fibrosis. *Frontiers in medicine*, *7*, 47. https://doi.org/10.3389/fmed.2020.00047.

Valderrábano, F., Hörl, W. H., Macdougall, I. C., Rossert, J., Rutkowski, B., & Wauters, J. P. (2003). PRE-dialysis survey on anaemia management. *Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association*, *18*(1), 89-100. https://doi.org/10.1093/ndt/18.1.89.

Collino, M., Thiemermann, C., Cerami, A., & Brines, M. (2015). Flipping the molecular switch for innate protection and repair of tissues: Long-lasting effects of a non-erythropoietic small peptide engineered from erythropoietin. *Pharmacology & therapeutics*, *151*, 32-40. https://doi.org/10.1016/j.pharmthera.2015.02.005.

Noguchi, C. T., Bae, K. S., Chin, K., Wada, Y., Schechter, A. N., & Hankins, W. D. (1991). Cloning of the human erythropoietin receptor gene. *Blood*, *78*(10), 2548-2556.

Manalo, D. J., Rowan, A., Lavoie, T., Natarajan, L., Kelly, B. D., Ye, S. Q., Garcia, J. G., & Semenza, G. L. (2005). Transcriptional regulation of vascular endothelial cell responses to hypoxia by HIF-1. *Blood*, *105*(2), 659–669. https://doi.org/10.1182/blood-2004-07-2958.

Wojchowski, D. M., Sathyanarayana, P., & Dev, A. (2010). Erythropoietin receptor response circuits. *Current opinion in hematology*, *17*(3), 169-176. https://doi.org/10.1097/MOH.0b013e328338008b.

Aapro, M., Krendyukov, A., Höbel, N., Seidl, A., & Gascón, P. (2018). Development and 10-year history of a biosimilar: the example of Binocrit®. *Therapeutic advances in medical oncology*, *10*, 1758835918768419. https://doi.org/10.1177/1758835918768419.

Zhang, L., & Wang, J. (2021). Incentives to promote the US pharmaceutical innovation: empirical research based on the case of Epogen. *Drug development and industrial pharmacy*, *47*(5), 820-824. https://doi.org/10.1080/03639045.2021.1935997.