EPO

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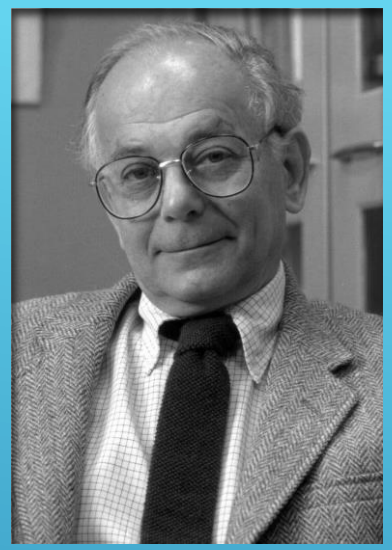
In 1893, the Swiss biologist Friedriech Miescher noticed that residents of and

Alp resort had increasing hemoglobin levels .

He attributed this finding to hypoxia of high altitudes.

MIESCHER

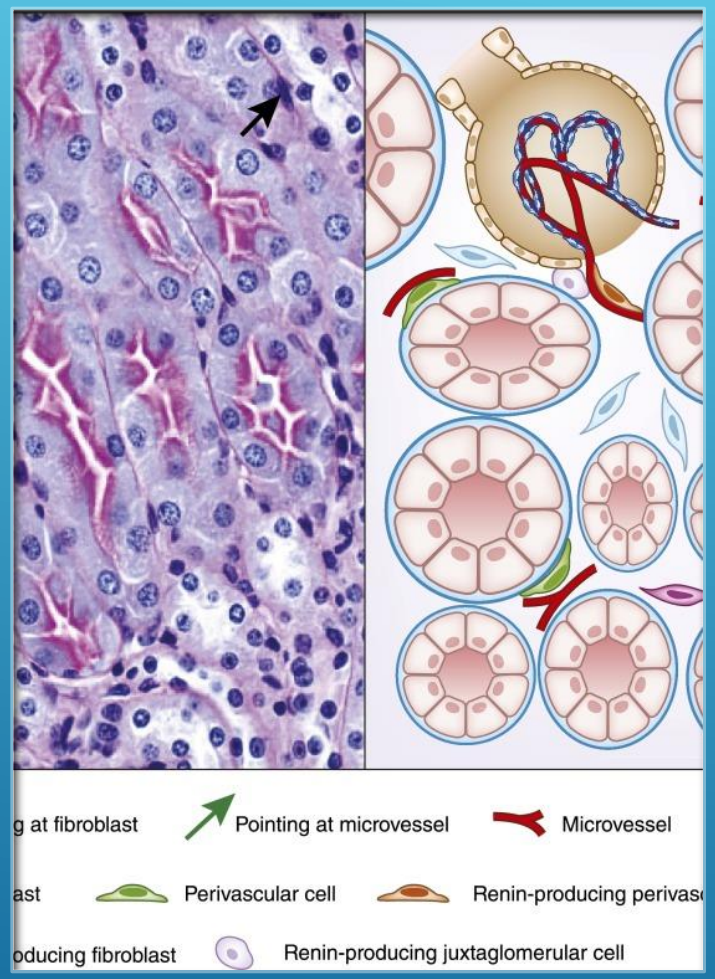


In 1977, the American Scientist Eugenne Goldwasser isolated the human protein erythropoietin “EPO”

EUGENNE GOLDWASSER

- **Renal Interstitium is defined as the intertubular space of the kidney.**
- **It is bounded on all sides by tubular and vascular basement membranes and is filled with cells, extracellular matrix and interstitial fluid.**
- **The intertubular interstitium harbours dendritic cells, macrophages, lymphocytes , lymphatic endothelial cells and various types of fibroblasts.**

PHYSIOLOGY OF THE RENAL INTERSTITIUM



The interstitial compartment contains non–hormone-producing fibroblasts (blue), microvessels (red), perivascular cells (green), renin-producing perivascular cells (orange), juxtaglomerular cells (lilac), and erythropoietin (Epo)-producing fibroblasts (pink). Bottom.

RENAL FIBROBLASTS

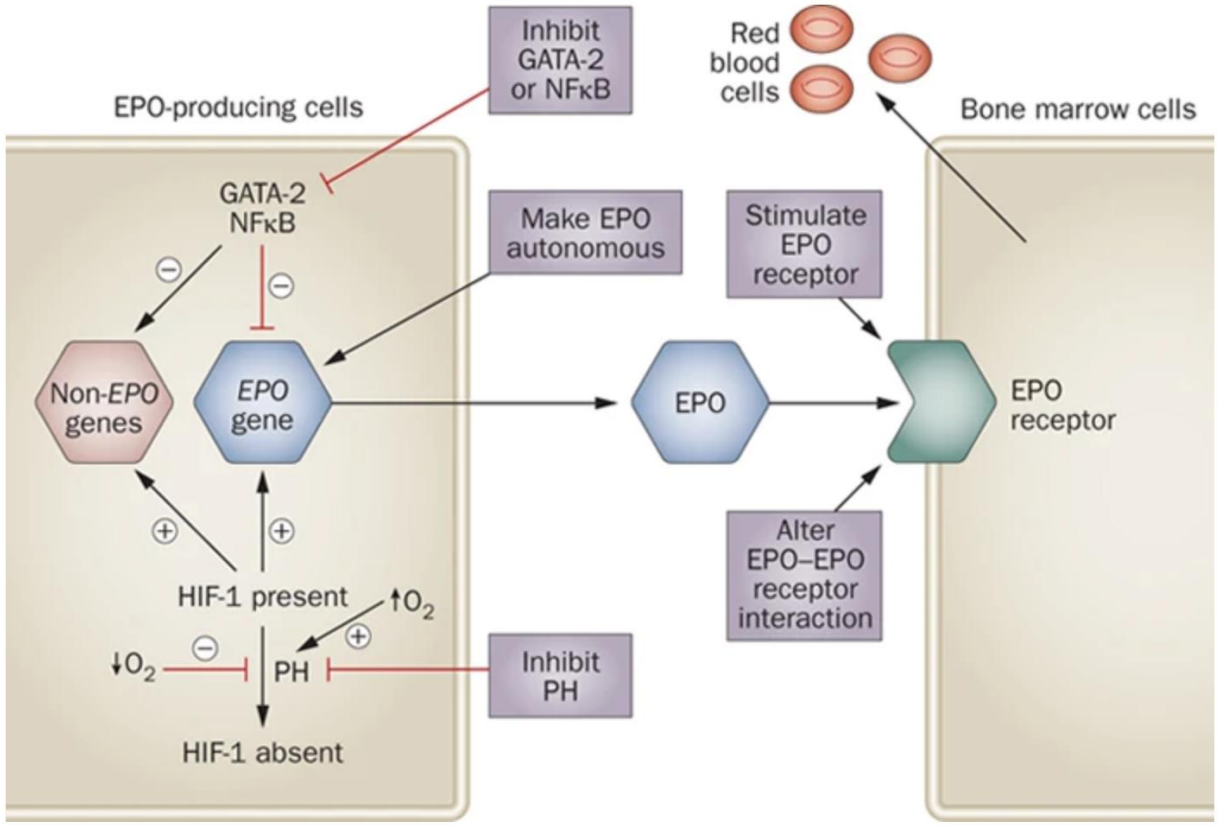
- The primary function of renal fibroblasts is to provide structural support to nephrons through deposition of extracellular matrix .
- In addition, fibroblasts play an important role in maintaining vascular integrity in close association with vessels.
- Renal fibroblasts are best known for their role in progression of interstitial fibrosis in progressive CKD because they are principal producers of extracellular matrix.
- Finally, fibroblasts have been identified as sources of **EPO** and **Renin** in the kidney

- **EPO is an indispensable glycoprotein hormone that is produced by Type II Interstitial Fibroblasts and controls hematopoiesis through promotion of survival, proliferation and differentiation of erythroid progenitors.**
- **In adults, about 90% of EPO is produced bty renal interstitial fibroblasts,**
- **Whereas 10% is produced by extrarenal sources, primary the liver.**

EPO

Figure 1: Erythropoiesis, and interventions that might increase this process.

From: Emerging erythropoiesis-stimulating agents



Expression of the erythropoietin gene (*EPO*) is inhibited by GATA-2 and NFκB, and stimulated by HIF-1. HIF-1 induces *EPO* expression under hypoxic conditions, but degrades under normoxic conditions. Erythropoietin binds to the erythropoietin receptors on bone marrow cells, stimulating the production of red blood cells. Red blood cell production might be increased by the following interventions: inhibition of PH, GATA-2 or NFκB, alteration of EPO–EPO receptor interactions, stimulation of the *EPO* receptor, or by making the *EPO* gene autonomous. Abbreviations: EPO, erythropoietin; HIF-1, hypoxia-inducible transcription factor-1; NFκB, nuclear

- Decreased oxygen tension is the principal stimulus for the renal EPO expression.
- EPO deficiency is the most important cause of anemia in CKD patients.
- Physiologic plasma EPO levels in blood is relatively low at around 100 pg/ml, whereas under hypoxic stress associated with severe anemia, they can reach 100,000 pg/ml.

EPO

- **EPO levels are better preserved in glomerular diseases than in interstitial diseases , suggesting that impaired EPO production is a direct consequence of interstitial disease.**
- **Polycystic kidney disease is an exception , as it is often associated with increased EPO production and polycythemia due to aberrant HIF 1 alpha accumulation around the cysts due to local hypoxia.**

EPO

1. IRON DEFICIENCY
2. CHRONIC INFLAMMATION
3. NUTRITIONAL STATUS
4. SECONDARY HYPERPARATHYROIDISM
5. OTHERS

ESA REFRACTORINESS

- It is either absolute iron deficiency with depleted iron stores, or relative (functional) deficiency, which prevents the use of available iron stores.
- The consensus is that iron therapy can increase hemoglobin level, postpone the need for ESA therapy and optimize the response to treatment

ESA REFRACTORINESS: IRON DEFICIENCY

- Guidelines recommend that oral iron will in general be sufficient to attain Hb within targets in ESA-treated CKD patients , not yet on regular hemodialysis and those on peritoneal dialysis.
- However, in patients with resistance to ESA therapy on oral iron, or intolerant to oral iron , a therapeutic trial of IV iron seems reasonable.
- In contrast, most haemodialysis patients, require IV iron.

ESA REFRACTORINESS: IRON DEFICIENCY

Most CKD patients present a chronic inflammatory state of increased levels of inflammatory markers such as : CRP, IL-1, IL-6, IFN gamma and TNF alfa; and increasing prevalence is associated with decreased renal function.

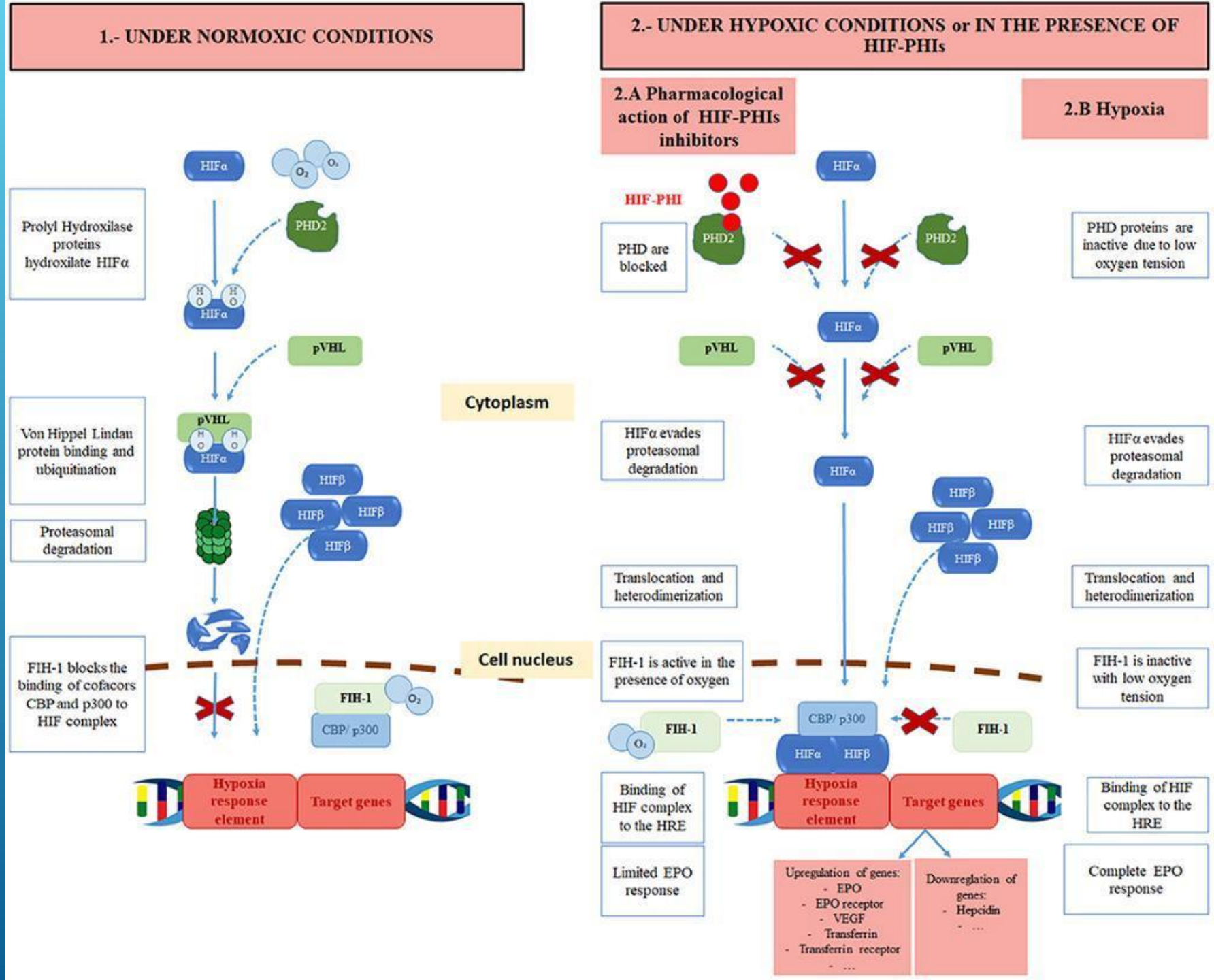
ESA REFRACTORINESS: CHRONIC INFLAMMATION

- Uremic syndrome, heart failure , persistent infections, biocompatibility of the dialysis membrane, use of catheters, accumulation of AGEs and progressive decline of GFR may contribute to inflammation in CKD with consequent cytokine production.
- The presence of high circulating levels of IFN gamma or TNF alfa causes the need for higher amounts of EPO.

ESA REFRACTORINESS: CHRONIC INFLAMMATION

- In the HDx patients, inflammation has been associated with EPO resistance mainly because the inflammatory state decreases the bone marrow response to ESA and/or inducing hemolysis.
- Hypoxia-inducible factor (HIF)- prolyl hydroxylase inhibitors licenced for use in von-Hippel Lindau syndrome, renal cell carcinoma, pancreatic neuroendocrine tumors; is recently tried in cases of refractory renal failure anemia due to inflammation.

ESA REFRACTORINESS: CHRONIC INFLAMMATION


1.- UNDER NORMOXIC CONDITIONS
2.- UNDER HYPOXIC CONDITIONS or IN THE PRESENCE OF HIF-PHIs
2.A Pharmacological action of HIF-PHIs inhibitors
2.B Hypoxia
HIFα
PHD2
HIF-PHI
Prolyl Hydroxilase proteins hydroxilate HIFα
PHD are blocked
PHD proteins are inactive due to low oxygen tension
pVHL
Von Hippel Lindau protein binding and ubiquitination
Cytoplasm
HIFα evades proteasomal degradation
HIFβ
Proteasomal degradation
Translocation and heterodimerization
Cell nucleus
FIH-1 blocks the binding of cofacors CBP and p300 to HIF complex
FIH-1 is active in the presence of oxygen
FIH-1 is inactive with low oxygen tension
CBP/ p300
Hypoxia response element
Target genes
Binding of HIF complex to the HRE
Limited EPO response
Upregulation of genes:
- EPO
- EPO receptor
- VEGF
- Transferrin
- Transferrin receptor
- …
Downregulation of genes:
- Hepcidin
- …
Complete EPO response

ESA REFRACTORINESS: NUTRITIONAL STATUS

- **CKD patients are at substantial risk of malnutrition, characterized by loss of protein energy. Their nutritional status is affected by the general decrease in nutrient intake, dietary restrictions, intestinal malabsorption, inflammatory state, metabolic acidosis and dialysate losses.**

These situations increase the risk of micronutrient deficiencies (folic acid, vitamin B12 and iron).

- **Nutritional factors is associated with EPO-resistance in HDx patients , due to malnutrition-inflammation status.**

- It starts early in the course of the disease and worsens with its progression. In the late stages of CKD, PTH synthesis and secretion is continuously stimulated casing secondary hyperparathyroidism.
- Although CKD-mineral bone disease is the most widely-recognized consequence of hyperparathyroidism, consistent evidence shows that PTH and fibroblast growth factor 23 (FGF23) have adverse effects on extraskeletal tissues, including the development of anemia.

ESA REFRACTORINESS: SECONDARY HYPERPARATHYROIDISM.

- This causes bone marrow fibrosis with consequent increase in EPO requirements.
- In addition to this pathway, PTH is identified as a uremic toxin that suppresses endogenous EPO production, inhibits B.M.erythroid progenitors and decreases red cell survival. High FGF 23 levels cause inflammation that adds to anemia and EPO resistance.
- HPT control should be attempted to treat EPO resistance. Several options are available including vitamin-D receptor activators, cinacalcet hydrochloride and parathyroidectomy.

ESA REFRACTORINESS: SECONDARY HYPERPARATHYROIDISM

ESA REFRACTORINESS: OTHER IMPORTANT FACTORS

- Angiotensin-converting enzyme inhibitors and angiotensin receptor blockers can decrease the hematopoietic response to ESA.
- RAS inhibition decreases erythropoiesis, and ACE inhibition can lead to high level of negative erythropoiesis regulation.
- Currently ACE gene polymorphisms are known to largely influence ACE serum activity. Thus some patients are more susceptible to ESA resistance when using ACE or ARB inhibitors.

- **Anti-erythropoietin antibodies can cause pure red cell aplasia (very rare cause of EPO rsistance)**
- **It should be suspected in an individual who has previously responded to EPO if the Hb declines by >2 g/dl per month .**
- **Inadequate dialysis can cause ESA resistance.**

ESA REFRACTORINESS: OTHER IMPORTANT FACTORS

ESA ADJUVANT THERAPIES

- L-carnitine, ascorbic acid, vitamin B12, folic acid, statins and Zinc showed some positive results. However the current guidelines do not recommend their routine use unless there is documented deficiencey.

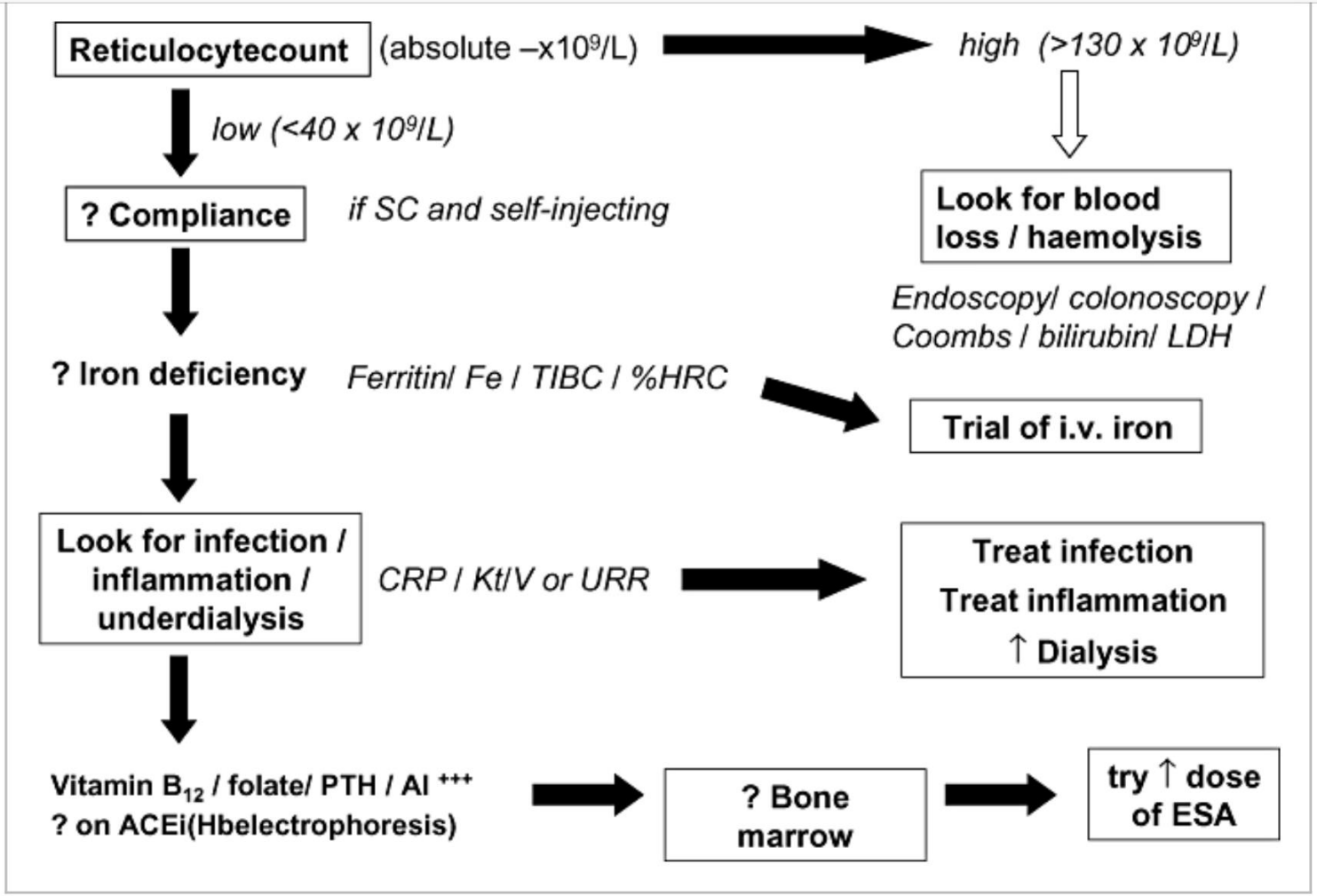


Figure 1

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Suggested approach to patients with erythropoiesis-stimulating agents (ESA) hyporesponsiveness. ACEi, angiotensin-converting enzyme inhibitors; CRP, C-reactive protein; HRC, hypochromic red cells; LDH, lactate dehydrogenase; SC, subcutaneous; TIBC, total iron-binding

Table 1

Summary of Erythropoiesis-Stimulating Agents

INN	Trade Name	License Holder	Approval	Manufacturing Process	Licensed In		
					European Union	United States	Other Regions
First generation							
Epoetin alfa	Epogen® Eprex® Procrit®	Amgen Ortho Biotech Amgen	1989 1988 1989	Recombinant DNA technology (in CHO cells)	 ✓ 	✓ ✓ ✓	 ✓
Epoetin beta	Recormon®	Boehringer Mannheim	1990	Recombinant DNA technology (in CHO cells)	✓		
Epoetin omega	Epomax® Hemax®	Elanex /Baxter	1990	Recombinant DNA technology (in hamster kidney cells)			✓
Second generation							
Epoetin beta	NeoRecormon®	Roche	1997	Recombinant DNA technology (in CHO cells)	✓		✓
Darbepoetin alfa	Aranesp®	Amgen	2001	Recombinant DNA technology (in CHO cells)	✓	✓	
Third generation							
Epoetin delta	Dynepo®	Transkaryotic Therapies /Shire	2002	Gene activation technology (in HT-1080 cells)	✓	✓	
Methoxy polyethylene glycol epoetin beta	Mircera®	Roche	2007	Recombinant DNA technology (in CHO cells)	✓	✓	
Epoetin alfa (biosimilar)	Binocrit® Abseamed® Epoetin Alfa Hexal®	Sandoz Medice Hexal AG	2007	Recombinant DNA technology (in CHO cells)	✓		
Epoetin zeta (biosimilar)	Retacrit™ Silapo™	Hospira, a Pfizer company Stada	2007	Recombinant DNA technology (in CHO cells)	✓		✓
Epoetin theta	Biopoin® Eporatio®	Teva RatioPharm	2009	Recombinant DNA technology (in CHO cells)	✓		

Abbreviations: INN, International Nonproprietary Name; NCE, new chemical entity; NME, new molecular entity.

- **Resistance to ESA treatment can increase the risk of negative outcomes in patients with CKD. Considering the weak evidence on the efficacy of ESA adjuvant drug therapies, reversing or controlling the potential courses of resistance seems to be the best strategy so far.**

CONCLUSION