HIF Biology & HIF-PHI MOA

A NEW APPROACH TO THE TREATMENT OF RENAL ANEMIA: HIF-1 STABILISATION

Nathan W. Levin M.D.
Mount Sinai Icahn School of Medicine

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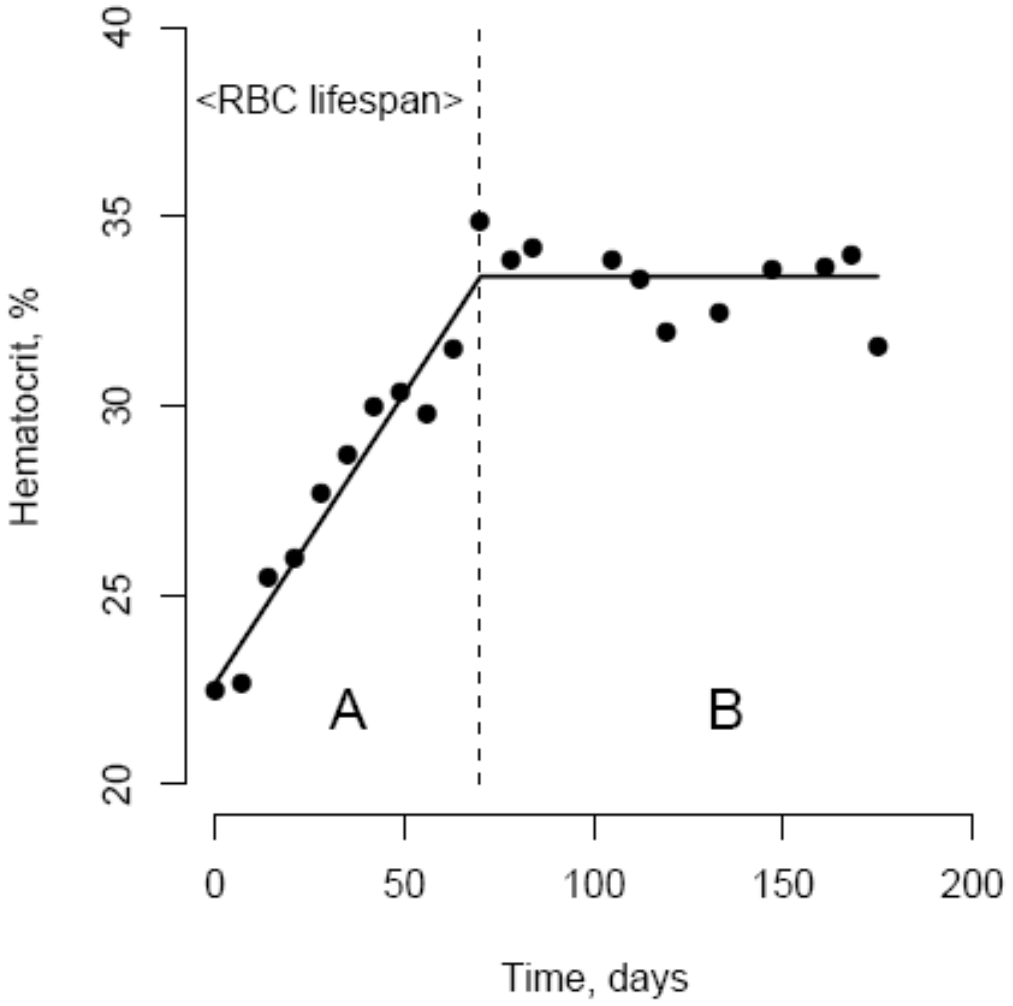
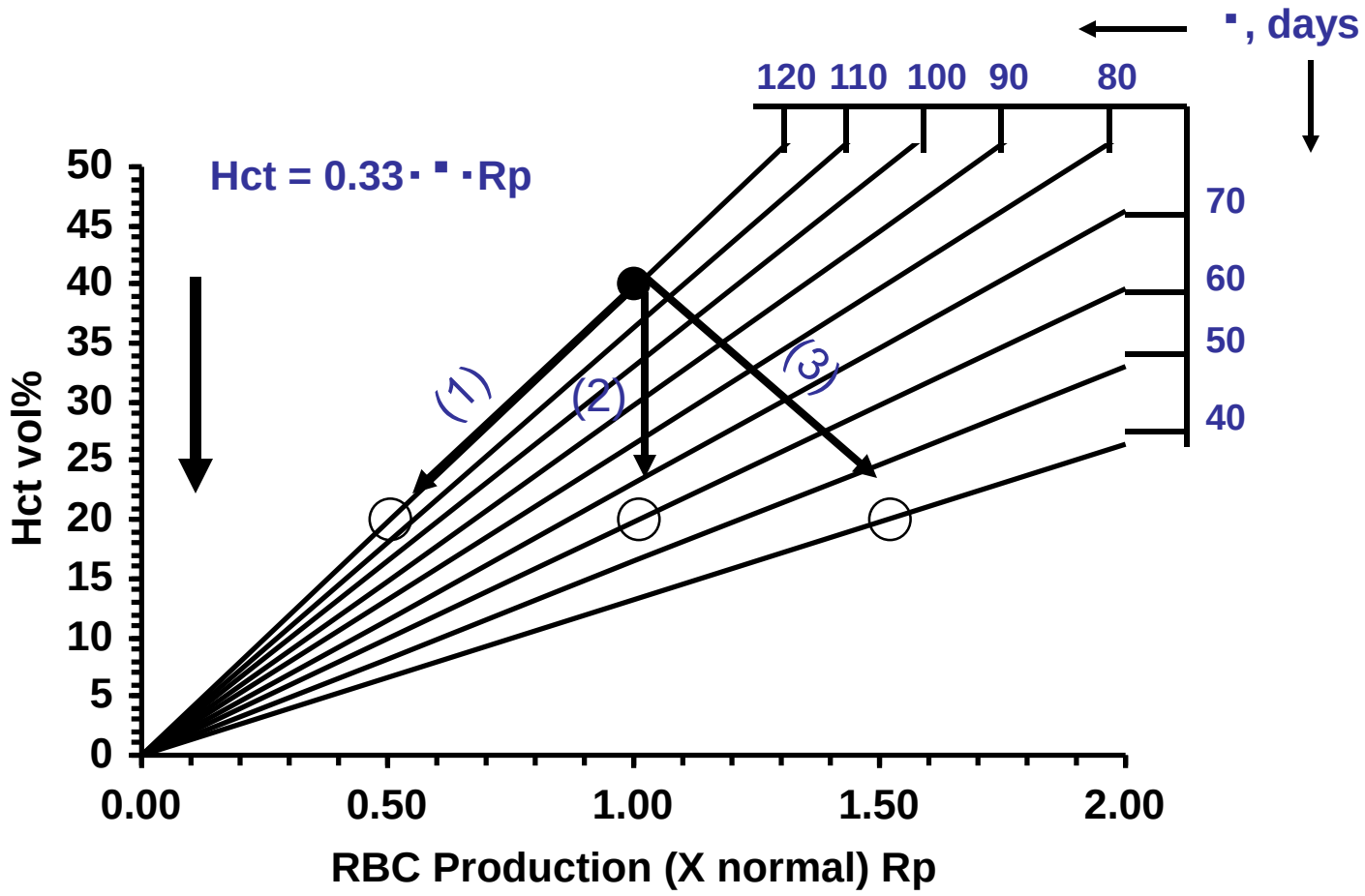
Red Cell Lifespan Determines Level of Haematocrit Reached Following ESA Administration



Uehlinger and Gotch et al. *Clin Pharmacol Ther*. 1992;51:76-89



Uehlinger and Gotch 1992.

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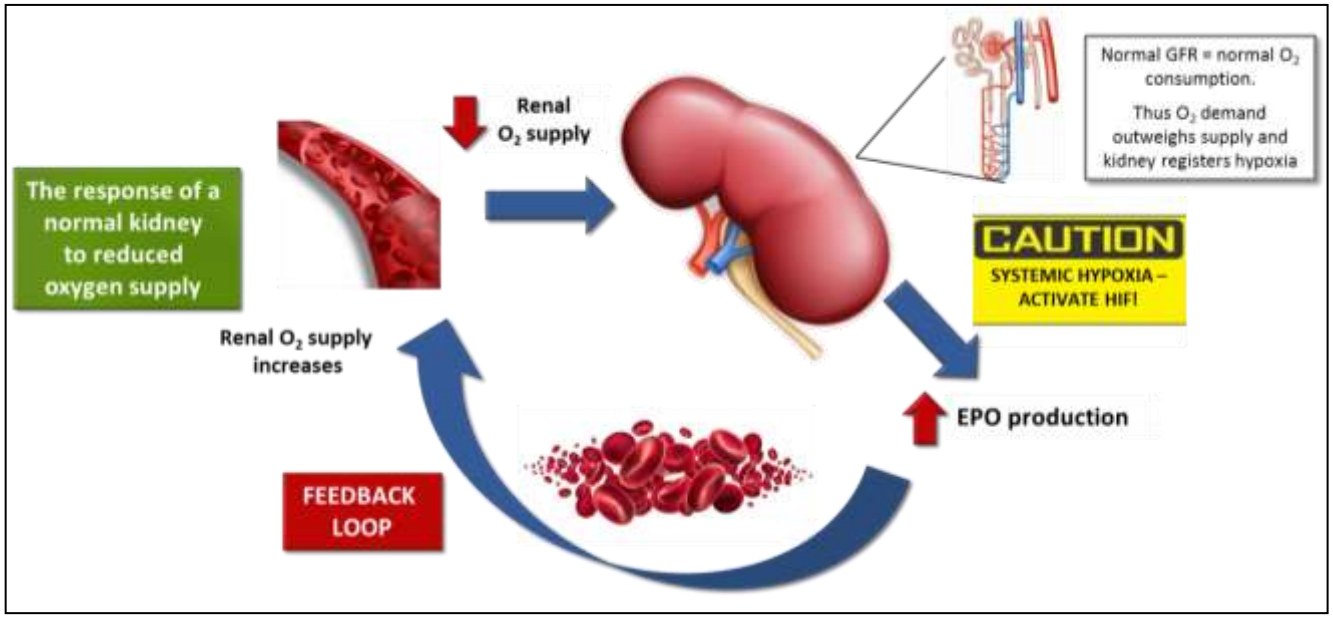
HIF Biology & HIF-PHI MOA

What is HIF?

- **Hypoxia-inducible factor (HIF)** is a cellular transcription factor
- HIF responds to reduced oxygen levels by activating expression of certain genes
- The purpose of this physiological homeostatic response is two-fold…
  1. To restore oxygen balance
  2. To protect against cellular damage while oxygen levels are being restored
- To restore oxygen balance HIF stimulates erythropoiesis, thus allowing more oxygen to be delivered to tissues
- HIF is a heterodimer compromising an oxygen labile α-subunit and a constitutively expressed β-subunit
  - Under normal oxygen tension, the HIF-α subunit is rapidly degraded as soon as it is synthesized
  - As oxygen tension decreases, degradation of the HIF-α subunit is inhibited and HIF activity increases
- Degradation of the HIF-α subunit is controlled by a family of enzymes called **HIF prolyl hydroxylases (HIF-PH)**

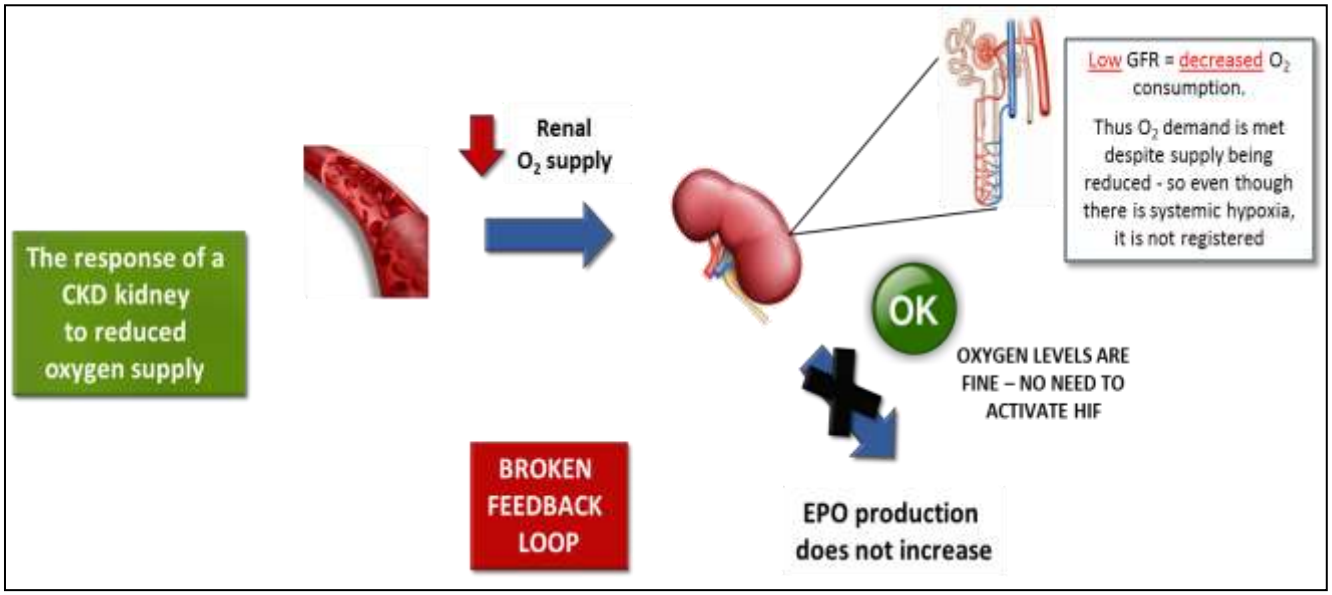
HIF feedback loop is critical for RBC regulation

- The kidney is the **oxygen sensor** of the body. Kidney tissue $P0_2$ profile is determined by the ratio of Na reabsorbed (GFR dependent) relative to oxygen delivered (blood flow x Hb content)
- Due to its high O_2 demand for Na reabsorption, the kidney can quickly detect a reduction in O_2 delivered in the blood and responds by stabilizing HIF which increases ESAs production to stimulate erythropoiesis.
- This important **HIF feedback loop** leads to increased oxygenation of the blood and thus increased O_2 supply to the kidney



The HIF feedback loop is broken in CKD

- In CKD patients, the decline in GFR means that less Na needs to be reabsorbed allowing O_2 demand by the kidney to decrease. Blood flow is reduced < GFR
- Thus, despite low O_2 levels in anemic CKD patients, the kidney does not perceive reduced O_2 (since blood supply still outweighs demand) i.e. it can no longer fulfill its role as oxygen sensor
- In this case, the kidney does not know to stabilize HIF and increase ESAs production
- Relative renal hyper-perfusion also dulls the renal response



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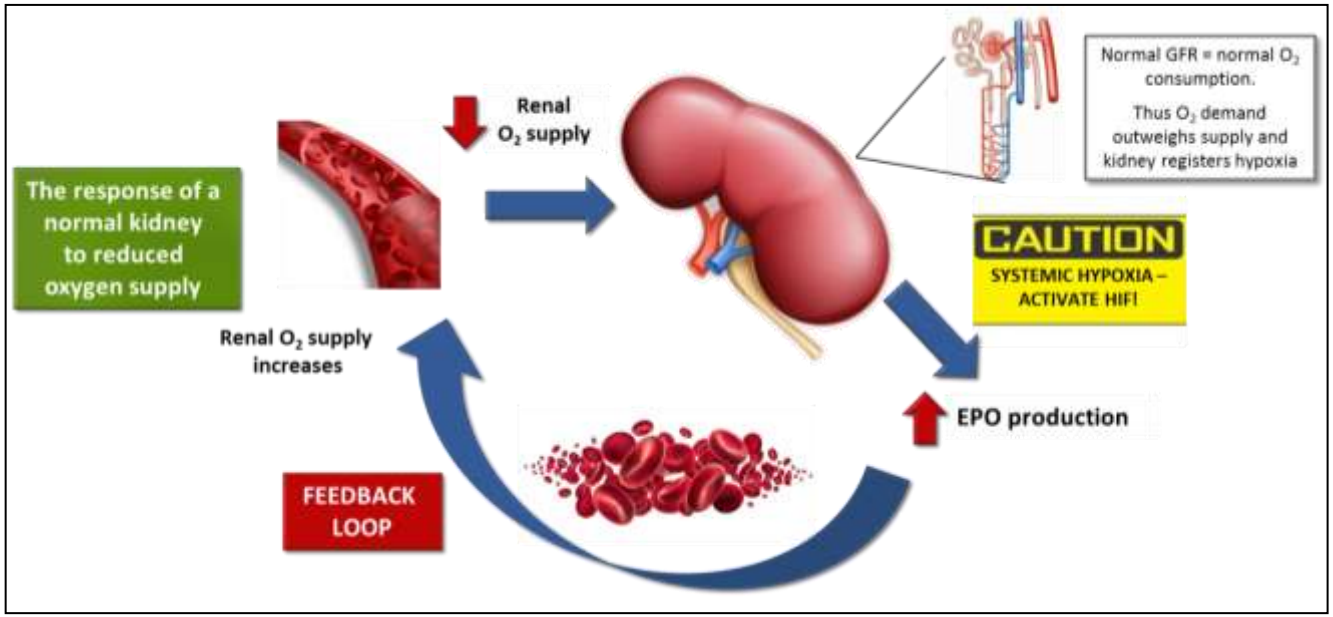
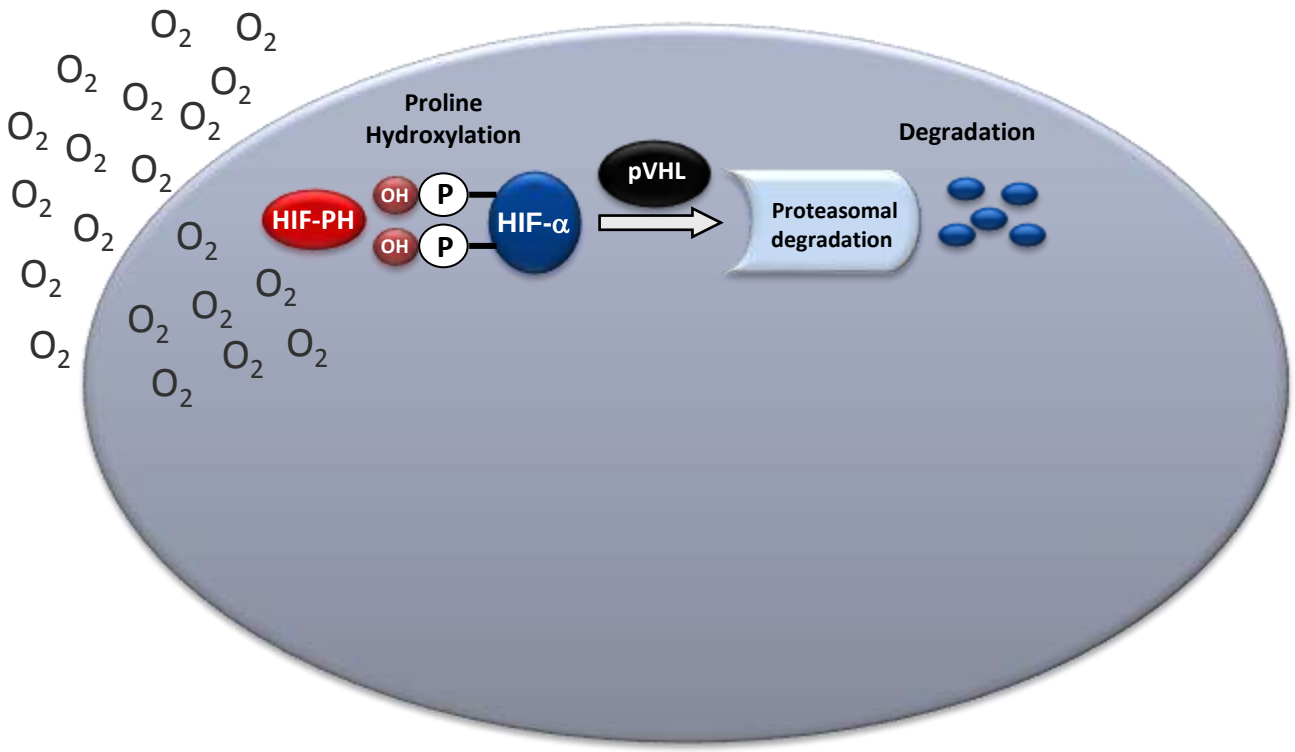


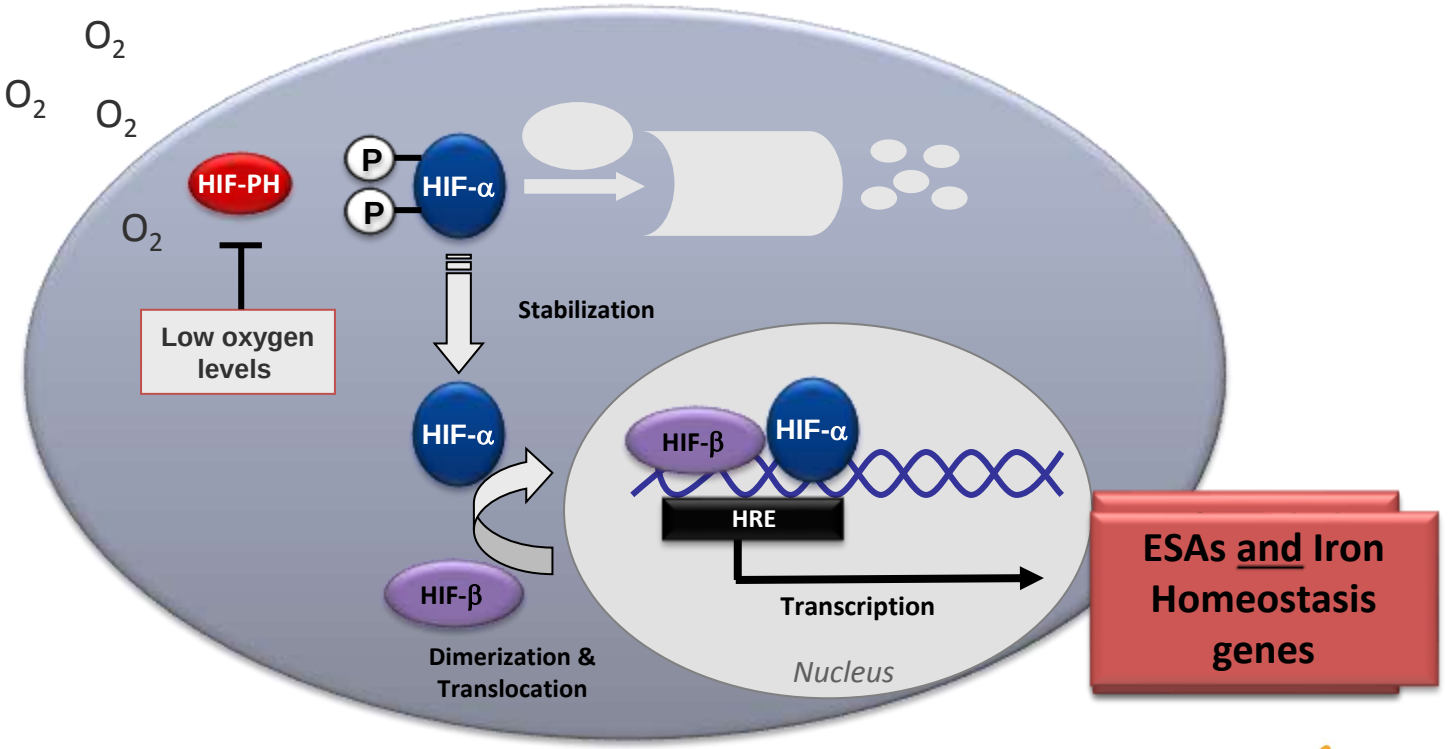
Control of HIF Levels By Prolyl Hydroxylation

- Under normal oxygen tension, HIF-α is hydroxylated on two proline residues by HIF prolyl hydroxylase (HIF-PH) enzymes.
- This hydroxylation allows binding of the von Hippel-Lindau (pVHL) protein which targets HIF-α for degradation.



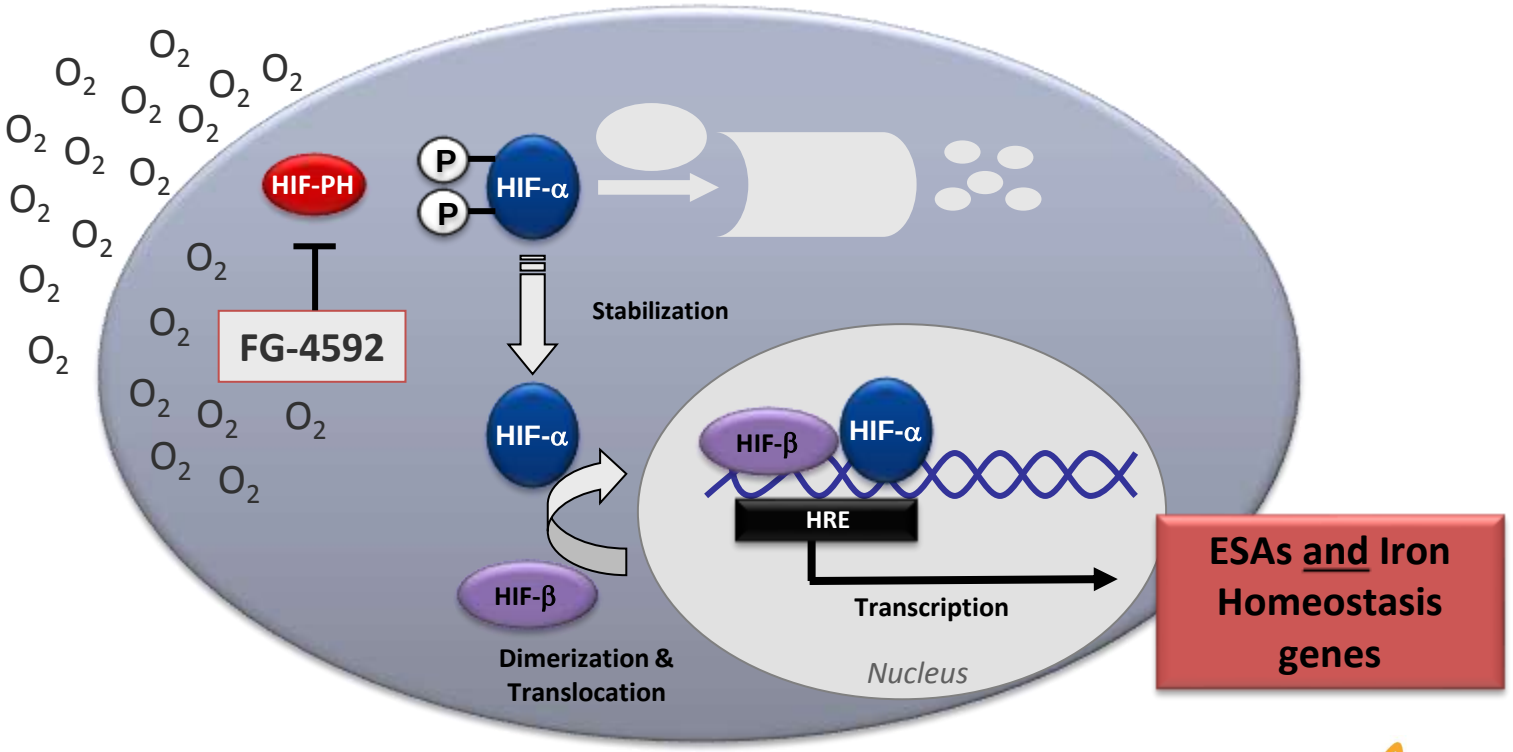
Control of HIF Levels By Prolyl Hydroxylation

- HIF-PH enzymes require oxygen for their catalytic activity.
- When oxygen levels fall, HIF-PH enzymes become inactive and HIF-α degradation is prevented.
- HIF-α can then dimerize with HIF-β, and accumulate in the nucleus to regulate HIF target genes.
- This is an evolutionarily conserved, physiological response to changes in oxygen tension.



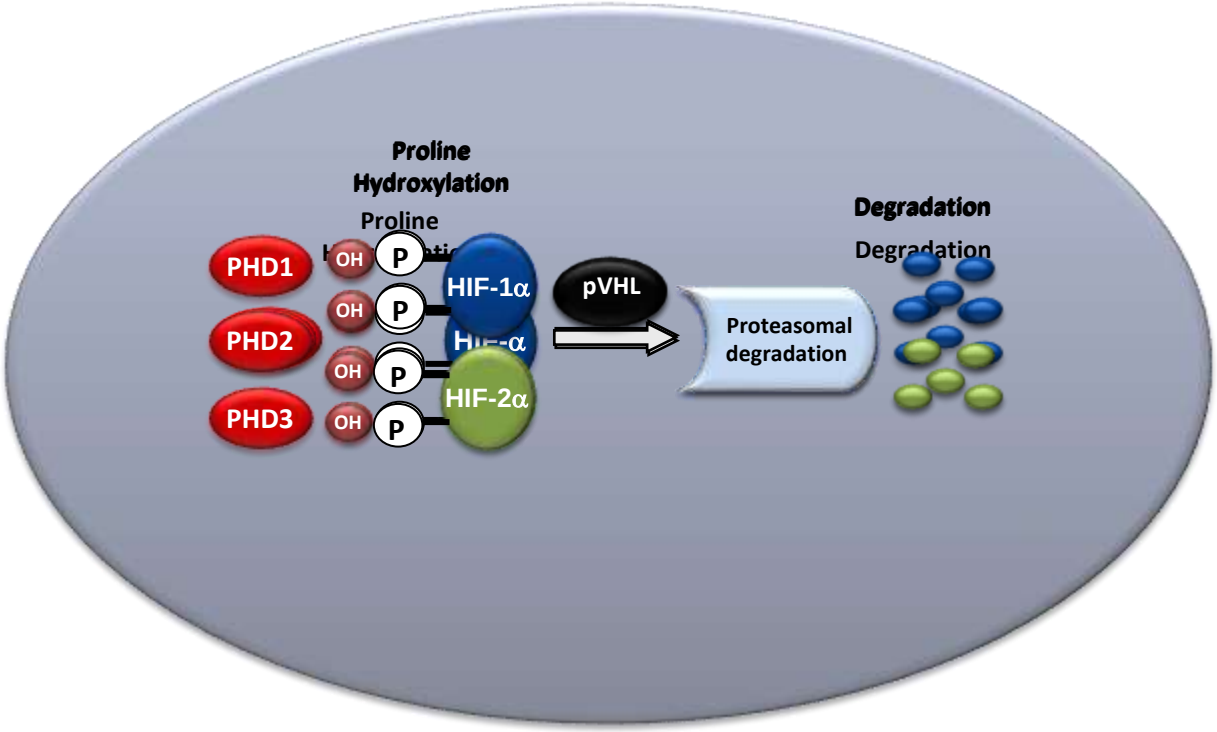
What is FG-4592?

- **FG-4592** is a first-in-class small molecule HIF-PH inhibitor (HIF-PHI)
- **FG-4592** reversibly inhibits the HIF-PH enzymes, thus transiently stabilizing HIF-α and increasing expression of HIF target genes such as ESAs and iron homeostasis genes
- **FG-4592** inhibits the HIF-PH enzymes and stabilizes HIF under **normal oxygen tension**
  - i.e. FG-4592 is <u>not</u> the same as hypoxia and does <u>not</u> require hypoxic conditions to work



HIF Biology – Several Different Players

- There are two major HIF-α isoforms: HIF-1α and HIF-2α



- There are at least three different HIF-PH enzymes (PHD1, PHD2, and PHD3) that can hydroxylate HIF and regulate its stability
- **FG-4592** inhibits all three HIF-PH enzymes

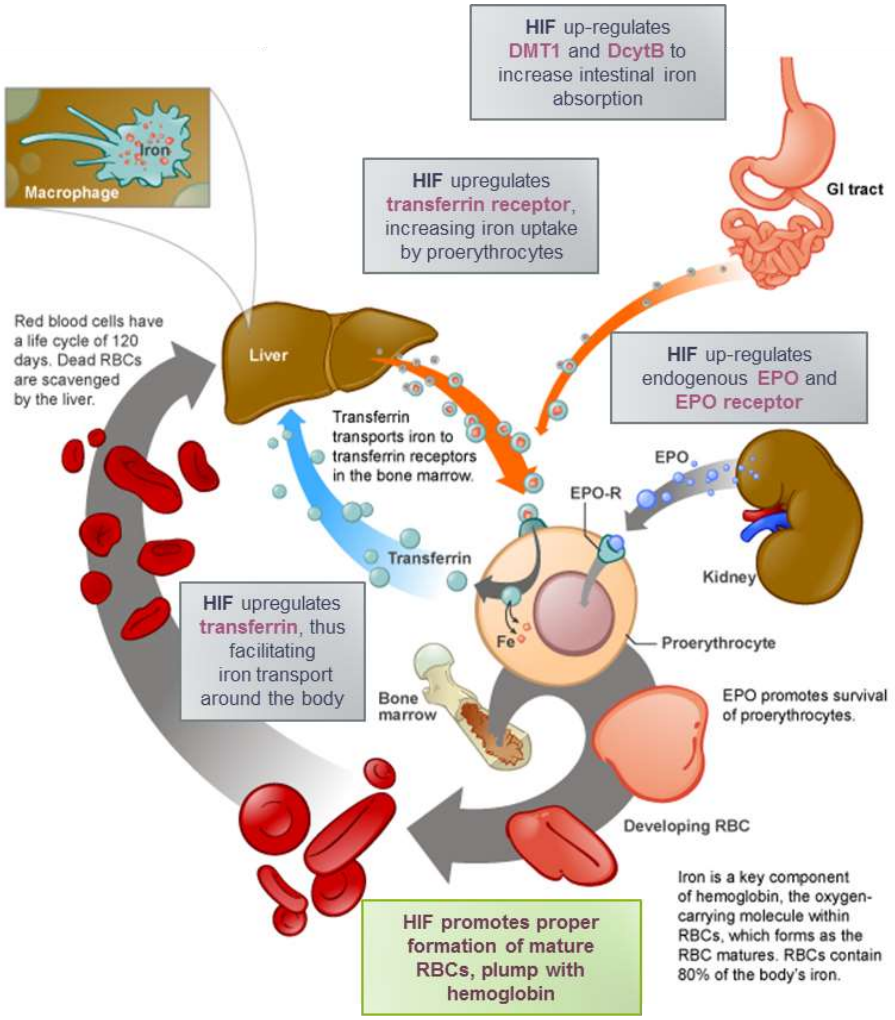
FG-4592 Stimulates a Coordinated Erythropoietic Response

- Although ESAs is an important HIF target gene, the erythropoietic response mediated by HIF is more than just ESAs

- HIF also regulates genes involved in iron metabolism and transport including…

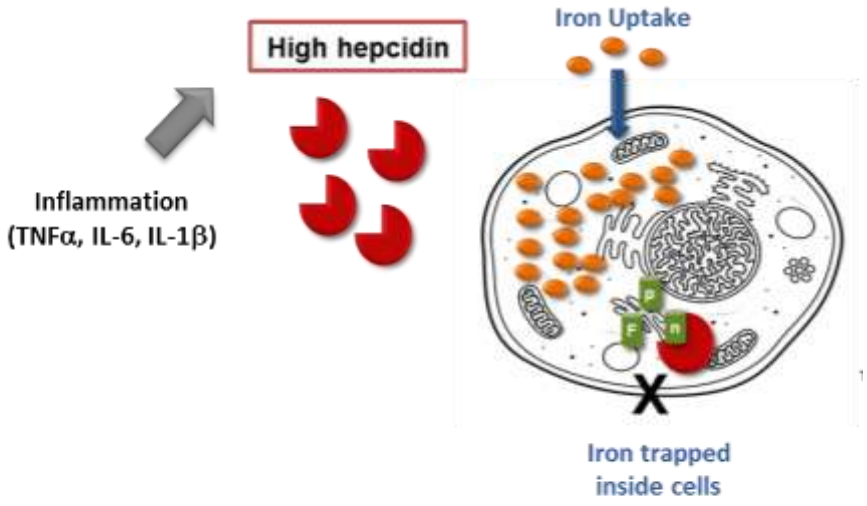
  1. **DMT1** (Divalent metal transporter)
     - Imports dietary iron from the gut
  2. **DcytB** (Duodenal cytochrome B)
     - Reduces iron so that it can be absorbed in the gut
  3. **Transferrin**
     - Transports iron around the body
  4. **Transferrin receptor**
     - Imports iron into cells

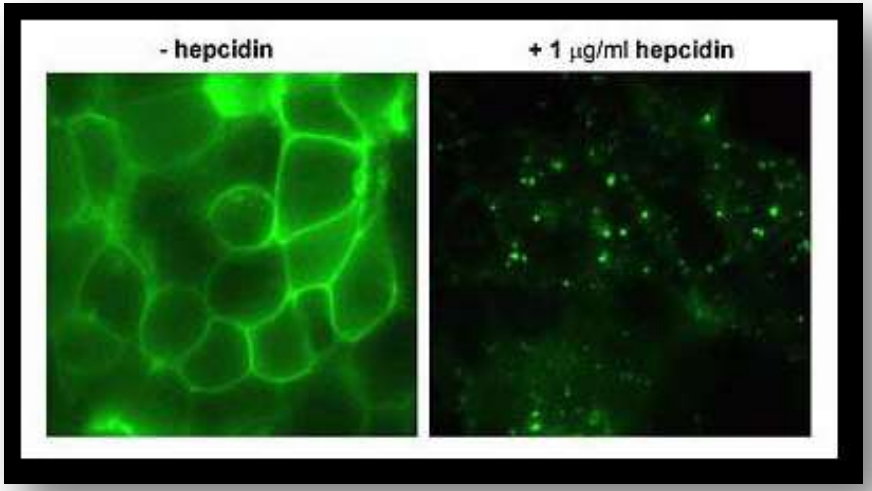
- Thus, activation of HIF by FG-4592 drives a coordinated erythropoietic response which involves endogenous ESAs production, increased iron uptake, mobilization of iron stores, and increased iron transport.



Hepcidin: An Important Role in Erythropoiesis

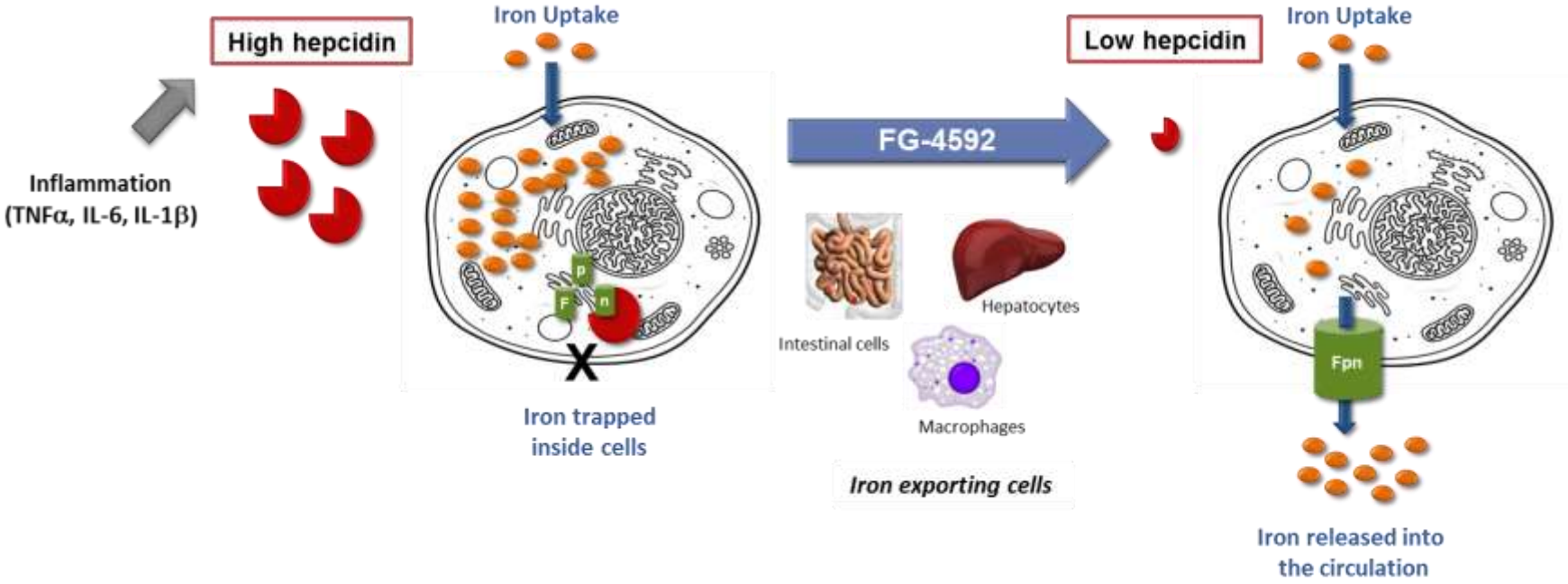
- **Hepcidin** is a hormone produced by the liver that is commonly referred to as the "master iron regulator"
- Hepcidin controls iron availability in the body via regulation of the iron transporter, **ferroportin**
- Ferroportin is responsible for the transport of iron from the inside of cells into the circulation
  - It is found on the surface of cells that store or transport iron, such as enterocytes in the duodenum, hepatocytes, and macrophages
- Hepcidin binds to ferroportin on the cell surface and causes its internalization and degradation
- Thus hepcidin prevents iron from being exported into the circulation – instead it is sequestered in cells, thus reducing iron availability for hemoglobin synthesis





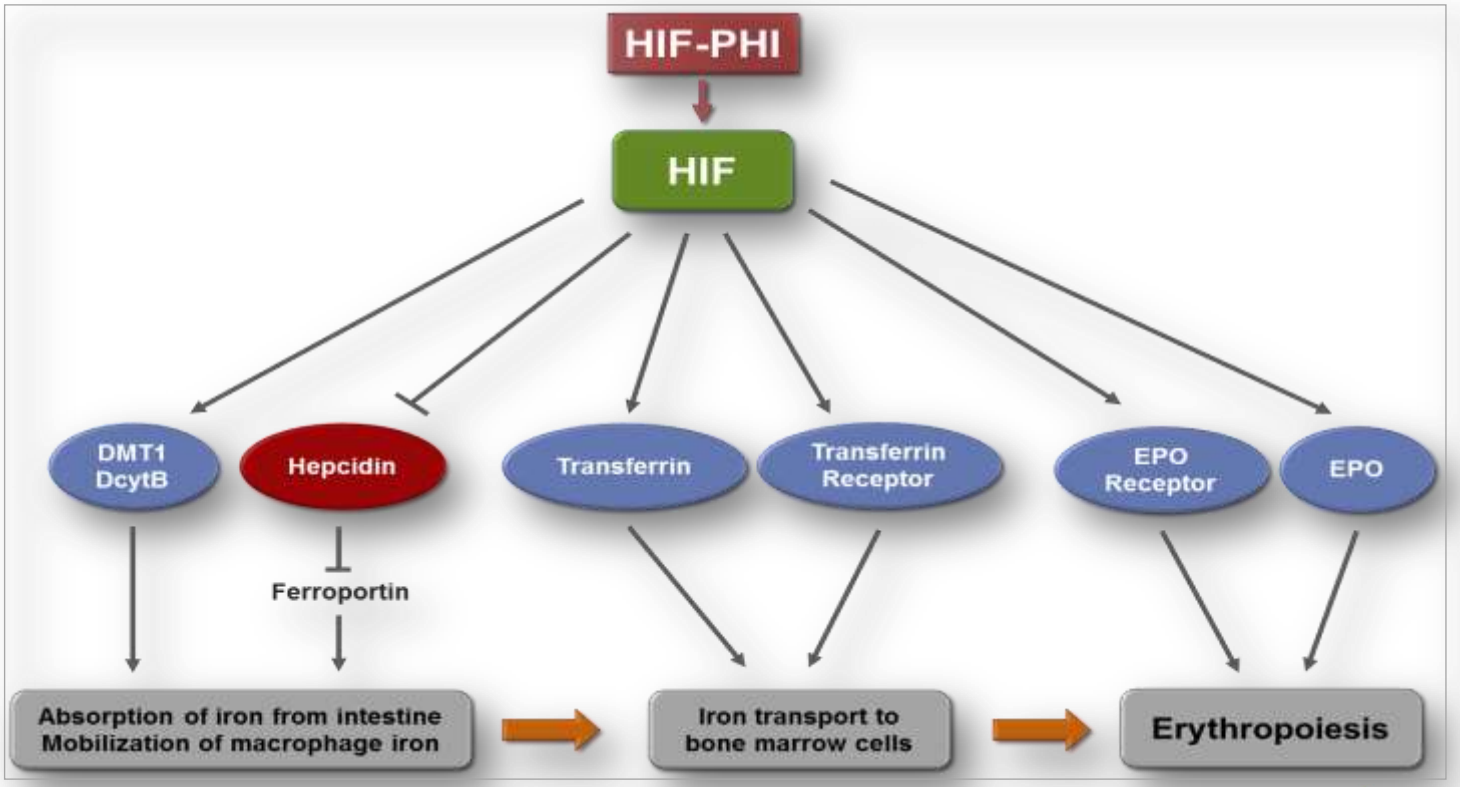
FG-4592 Down Regulates Hepcidin Levels

- In addition to regulating genes that improve iron uptake in the gut and facilitate iron transport to the bone marrow, FG-4592 also decreases hepcidin levels
- This reduction in hepcidin allows iron to be released from intracellular stores and absorbed from the gut, thus increasing iron availability for hemoglobin synthesis
- By regulation of hepcidin, FG-4592 can overcome the suppressive effects of inflammation on erythropoiesis



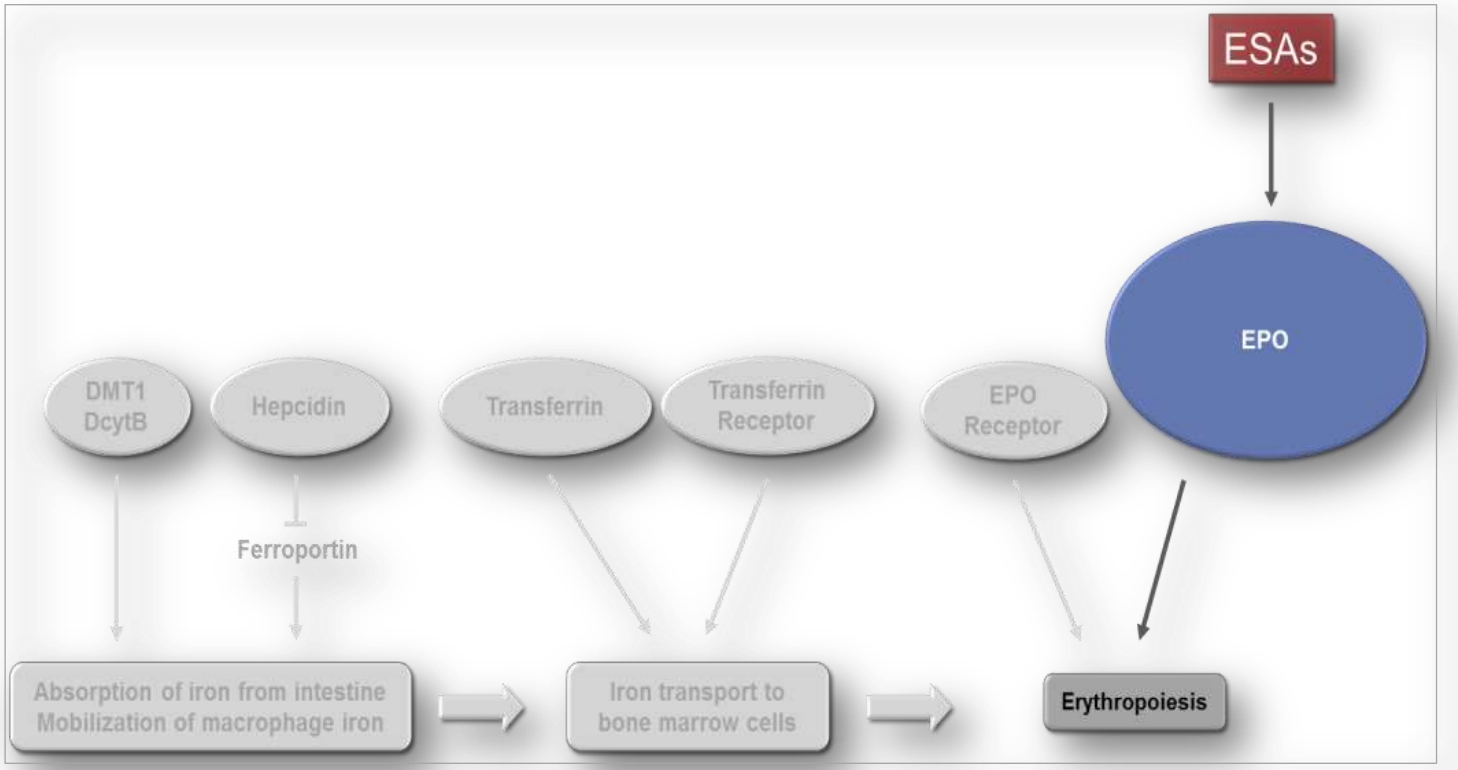
The Erythropoietic Response Stimulated by FG-4592 Occurs with ESAs Levels within Physiological Range

- The coordinated response stimulated by FG-4592 ensures sufficient iron availability for effective erythropoiesis to occur in the presence of physiological levels of ESAs.
- This natural stimulation of a coordinated erythropoietic response is similar to what occurs when one ascends to altitude.



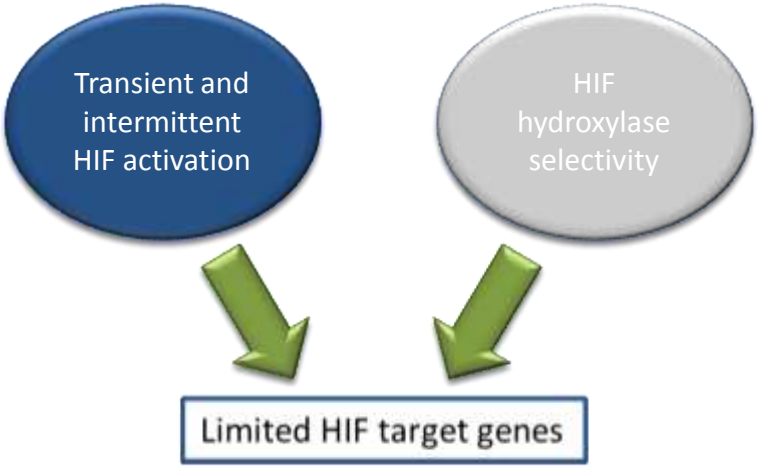
ESAs Only Address One Component of Erythropoiesis

- ESAs only address one aspect of erythropoiesis, circulating ESAs levels
- Without a coordinated response, artificially high levels of ESAs are required to stimulate erythropoiesis.



FG-4592 Was Designed to Activate a Limited HIF Response

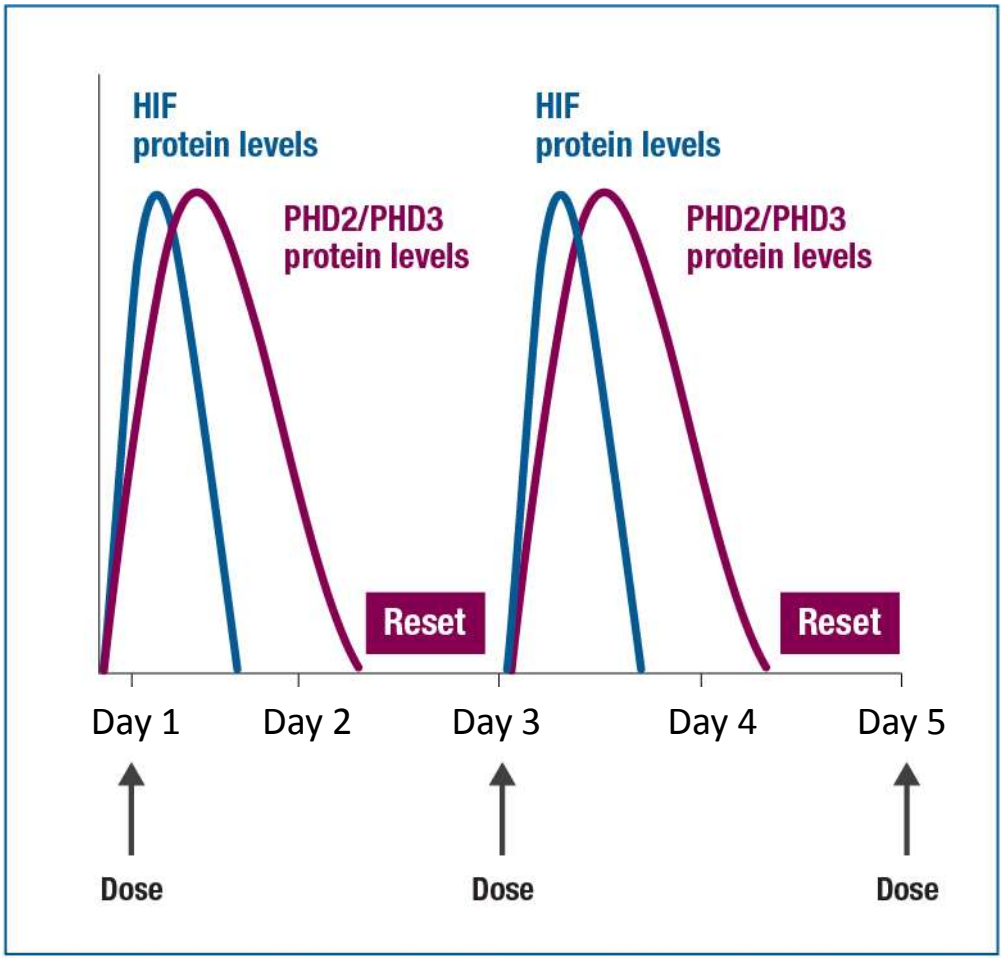
- In addition to regulation of genes involved in erythropoiesis, HIF is known to regulate genes involved in a number of other biological pathways, such as metabolism and angiogenesis
- However, FG-4592 was designed to achieve an optimal erythropoietic response while limiting the number of other HIF target genes that are regulated.
- The key design features of FG-4592 that limit the HIF response are:
  - Transient and intermittent HIF activation which is achieved via pharmacokinetic profile and dosing regimen
  - Molecular selectivity between the hydroxylase enzymes that regulate HIF levels and activity



FG-4592 was designed to incorporate both these features

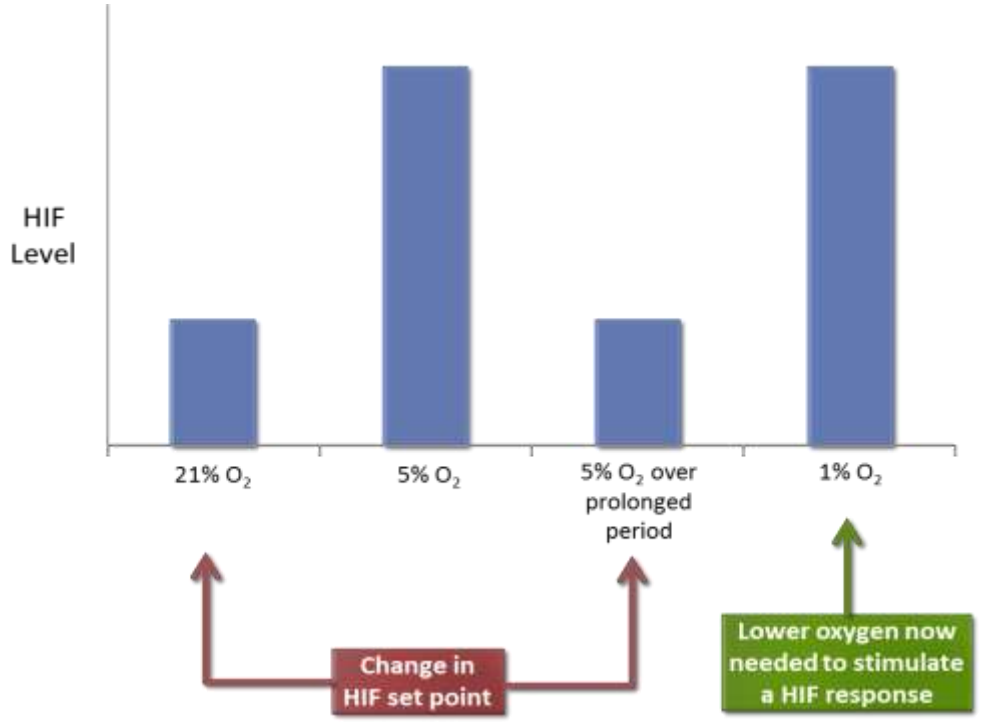
Intermittent Dosing allows HIF System to Reset

- The HIF system needs to be given enough time to reset between doses so that PHD levels could return to basal levels
- If the system is activated too frequently without allowing enough time for reset, an increased HIF-PHI dose may be required to elicit the same response.
- The risk of reduced drug effectiveness over time can be avoided by employing an intermittent dosing regimen



If not reset, decreased effectiveness or increased dose requirements may occur

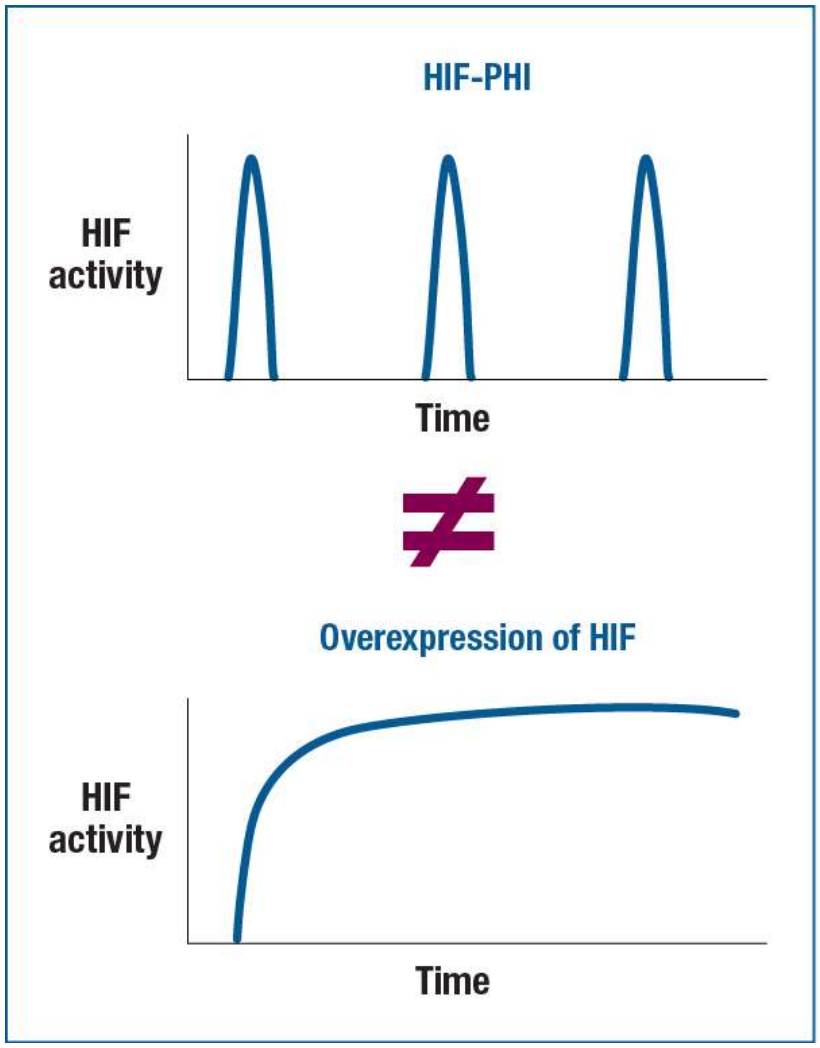
- If the system is activated too frequently:
  - The HIF set point could change
  - An increased HIF-PHI dose required to elicit the same response.
- Increased risk of reduced drug effectiveness over time avoided by employing an intermittent dosing regimen.



Transient HIF Activation by a HIF-PHI Is Not the Same as Chronic HIF Activation

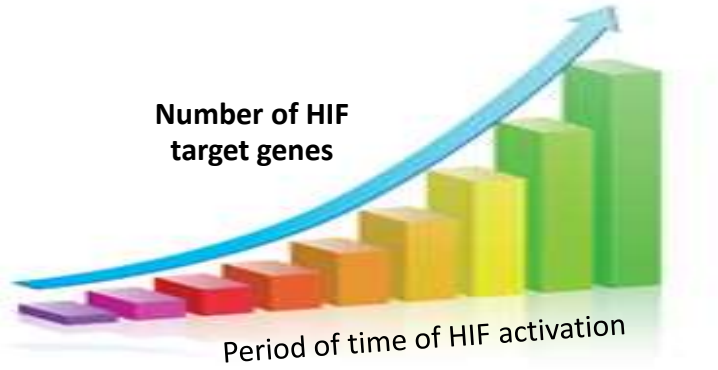
- Broad conclusions regarding potential consequences of HIF activation are often made from biological systems in which HIF is chronically activated
  - Experimental models where HIF is genetically overexpressed
  - Von Hippel–Lindau disease (VHL) disease where HIF degradation is prevented

- Conclusions about a HIF-PHI such as FG-4592 are not possible from such studies
  - The chronic HIF activation that results from HIF overexpression or VHL deletion is not the same as the transient and reversible HIF activation that can be achieved with HIF-PHI



Transient HIF Activation Limits the HIF Target Gene Response

- HIF target genes respond differently following HIF activation
- Some genes are switched on quickly whereas other genes require longer periods of HIF stabilization



- HIF-PHI such as **FG-4592** only transiently activate the HIF system
- As the drug is cleared by the body, the HIF response is rapidly switched off
- This transient activation of HIF limits the number of HIF target genes that are regulated

Roxadustat Was Designed to Activate a Limited HIF Response

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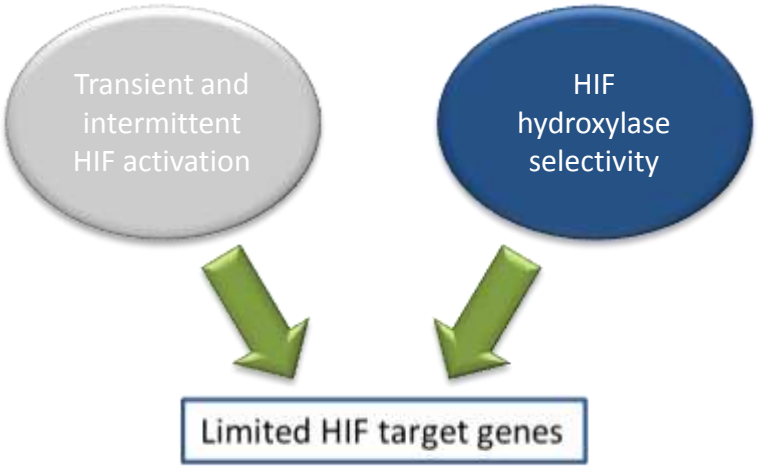
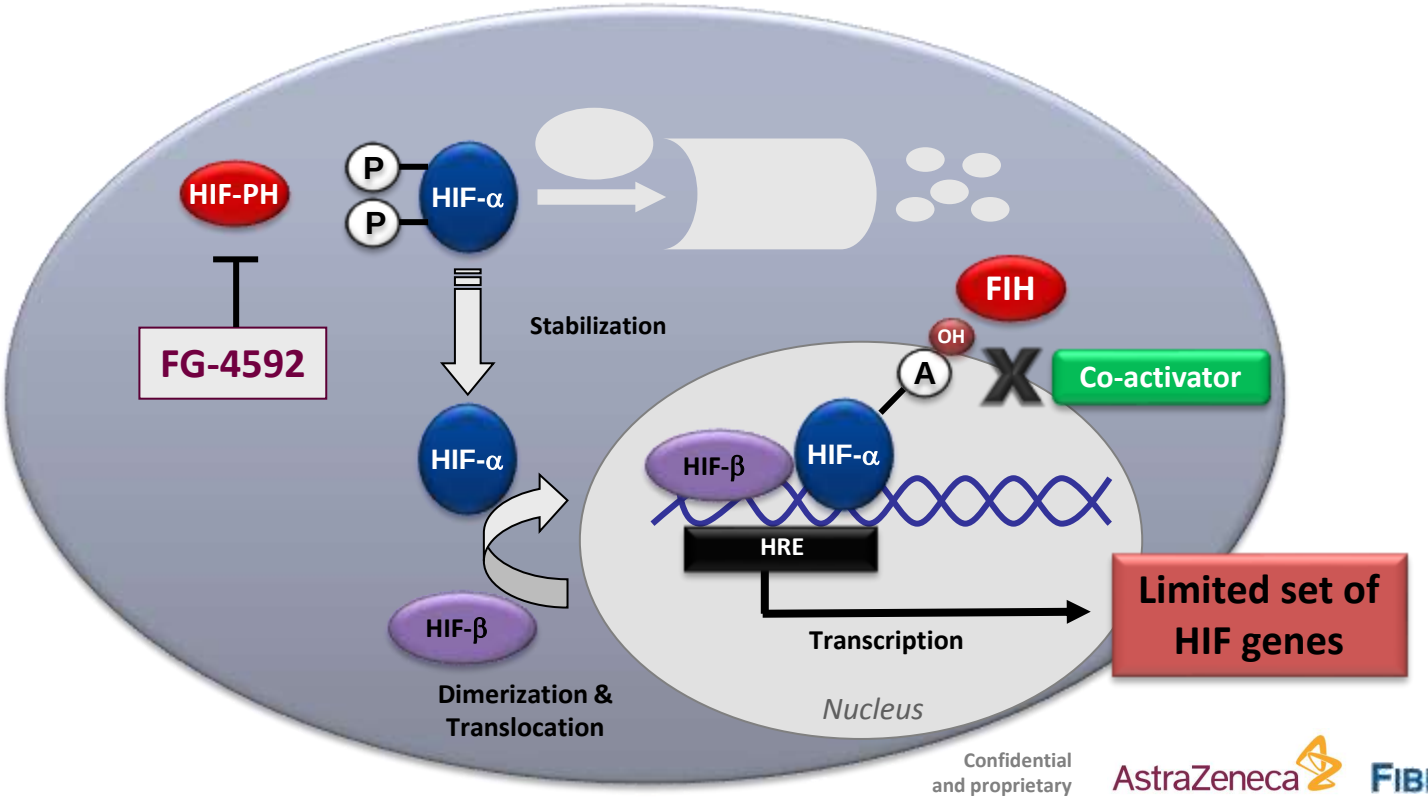


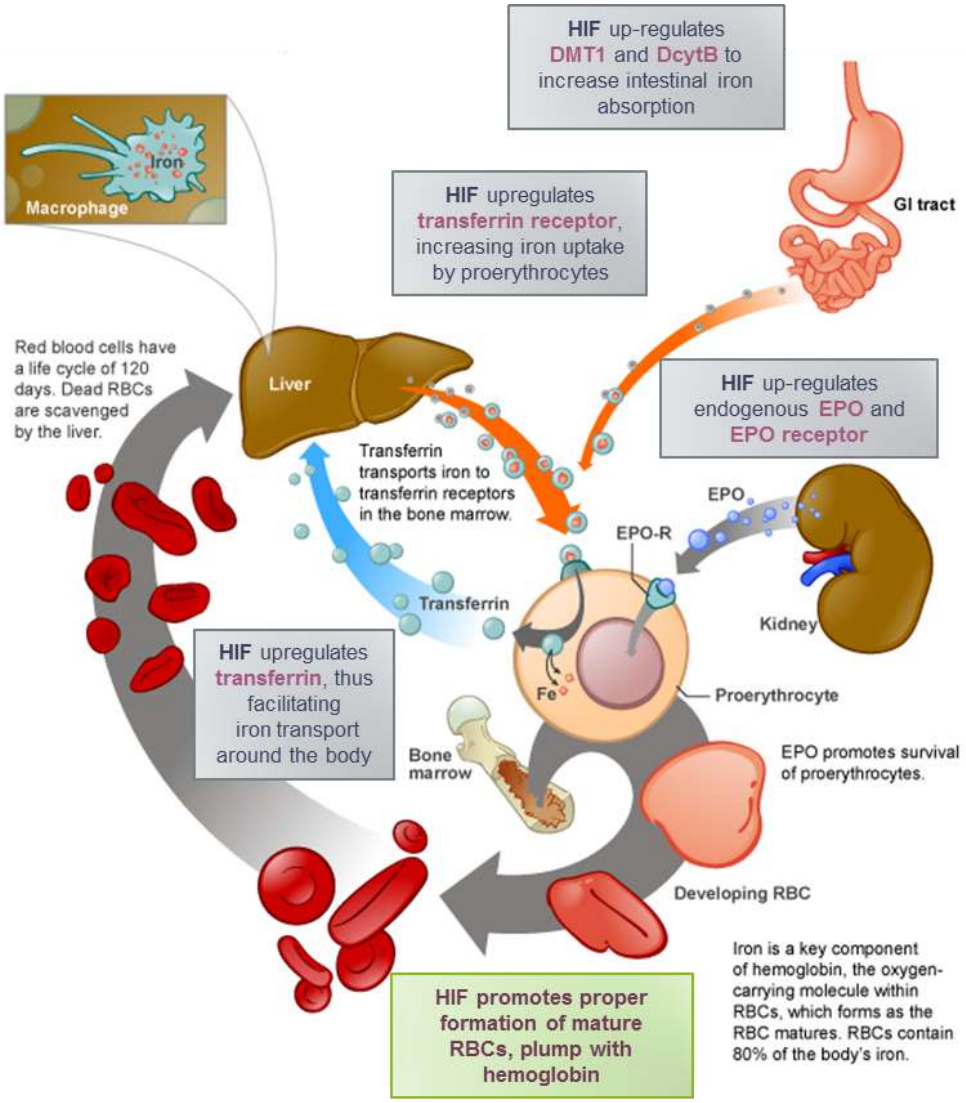
FG-4592 was designed to incorporate both these features

Limiting the HIF Response: HIF Hydroxylase Selectivity

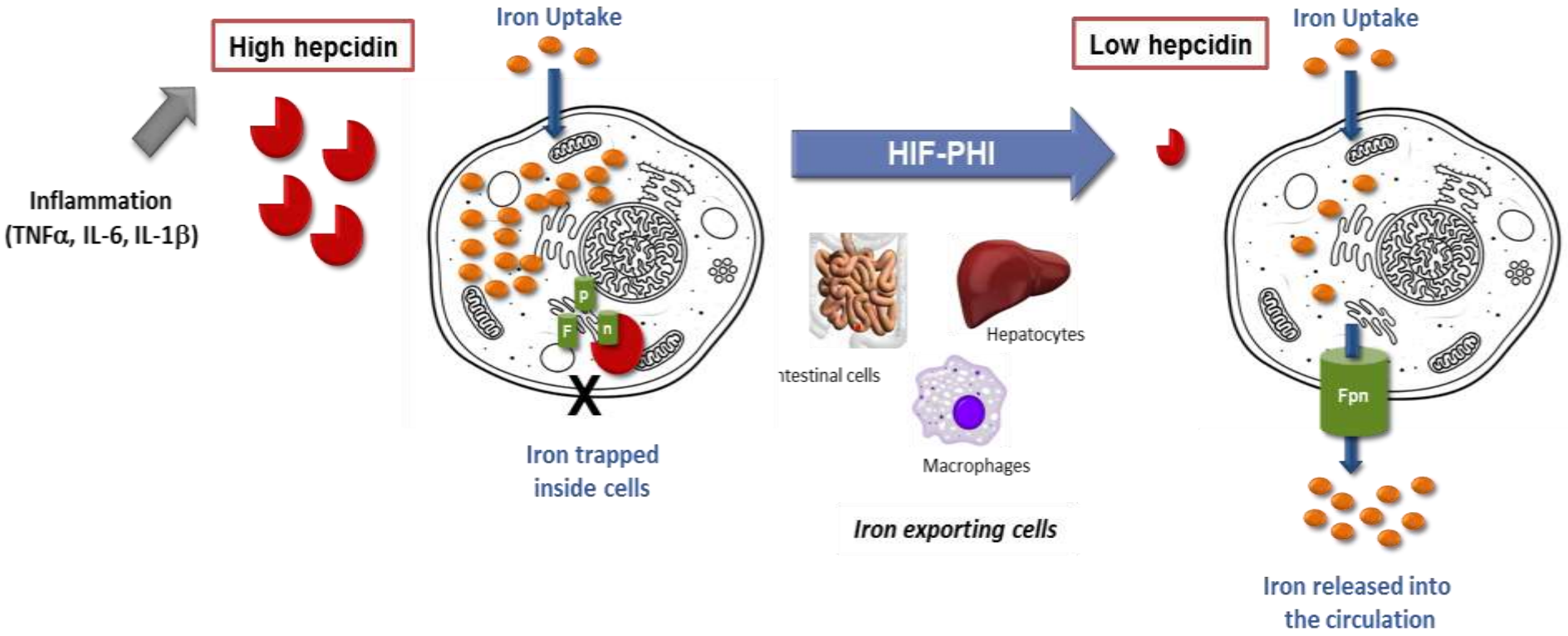
- FIH (Factor Inhibiting HIF) is another hydroxylase enzyme that regulates HIF.
- Hydroxylation of HIF by FIH prevents binding of transcriptional co-activators to HIF.
- Some HIF target genes require these co-activators for optimal expression and therefore are only robustly induced when hydroxylation by FIH is inhibited



HIF-PHI Stimulates a Coordinated Erythropoietic Response



The coordinated erythropoietic response stimulated by HIF-PHI can overcome suppressive effects of inflammation



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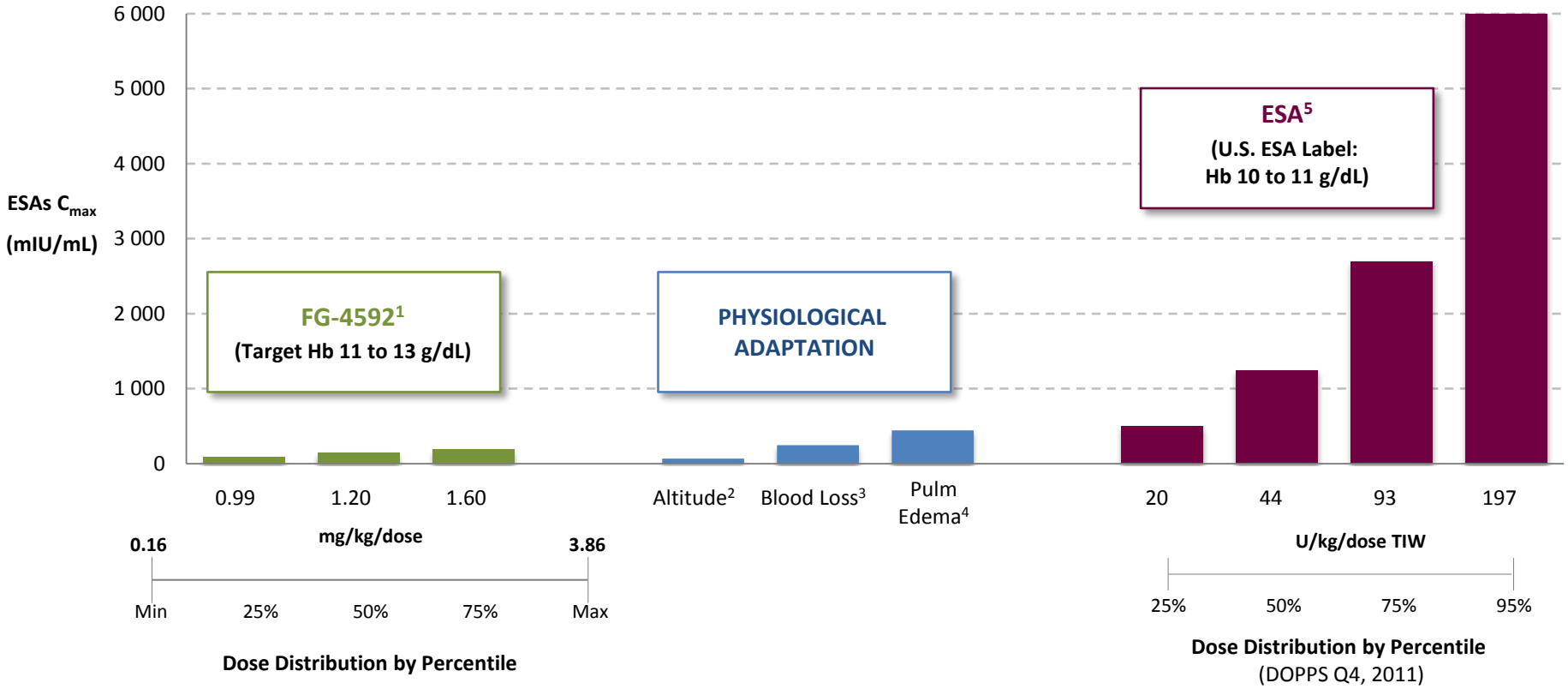
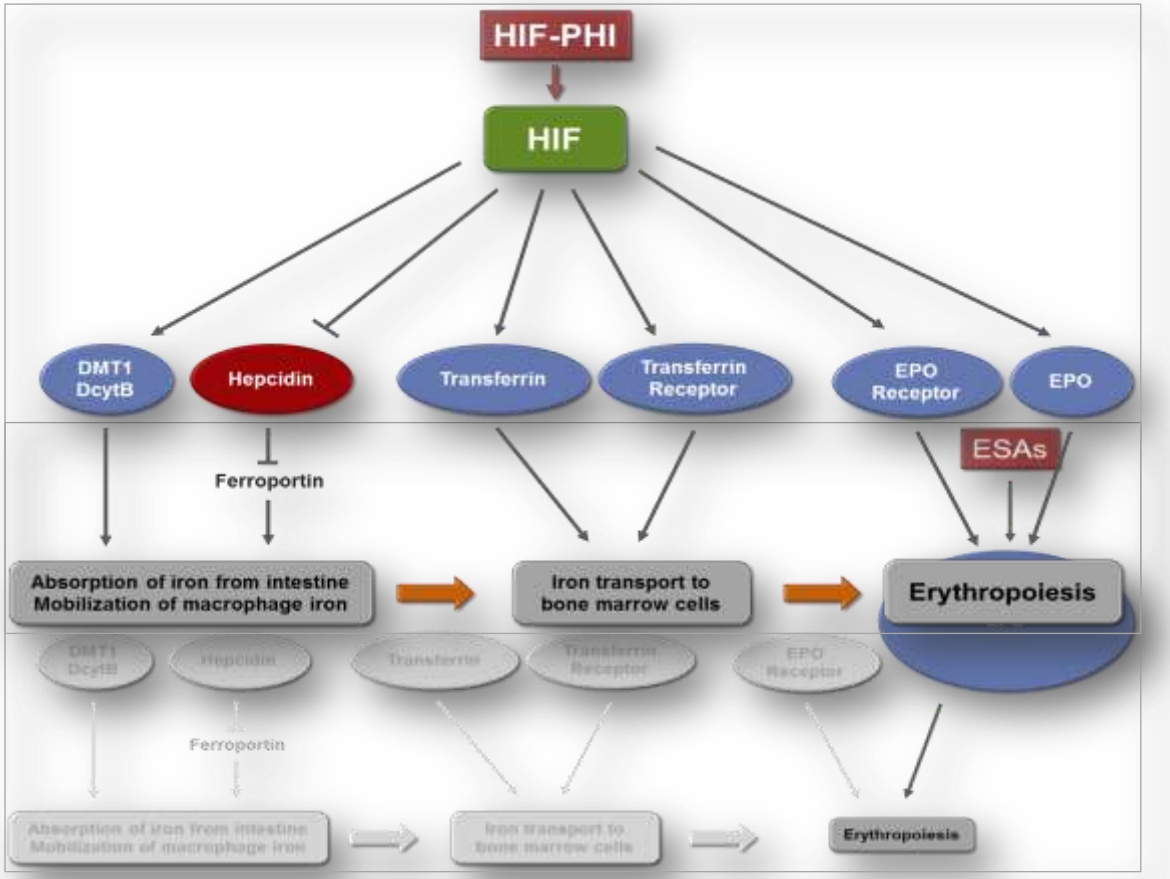


1. C_{max} data for FG-4592 estimated for a subset of 243 patients who achieved Hb response and were dosed at expected therapeutic doses.
2. Milledge & Cotes (1985) J Appl Physiol 59:360.
3. Goldberg et al. (1993), Clin Biochem 26:183, Maeda et al. (1992) Int J Hematol 55:111.
4. Kato et al. (1994) Ren Fail 16:645.
5. Based on Flaherty et al. (1990) Clin Pharmacol Ther 47:557.

The Coordinated Erythropoietic Response is a Key Differentiator Between FG-4592 and ESAs



Executive summary – HIF Biology and HIF PHI MOA

The HIF feedback loop is critical for erythropoiesis. This loop is broken in anemia in CKD.

Prolyl hydroxylation controls HIF levels

HIF-PHI stimulates a coordinated erythropoietic response

The Erythropoietic response stimulated by HIF-PHI:

- Is achieved with physiological levels of endogenous ESAs
- Is a limited response that allows for robust HIF induced erythropoiesis
- Is intermittent and transient allowing the FIF system to reset and therefore avoiding risk of increased dose requirement over time
- Overcomes the erythropoiesis-suppressive effects of inflammation seen in many patients with CKD
- Down regulates hepcidin thereby promoting iron bioavailability