Tomas Ganz: Disclosure of Interests

- Intrinsic LifeSciences (founder, stockholder, officer)
- Merganser Biotech (consultant, stockholder)
- Keryx (consultant)
- Xenon Pharma (stockholder, former consultant)

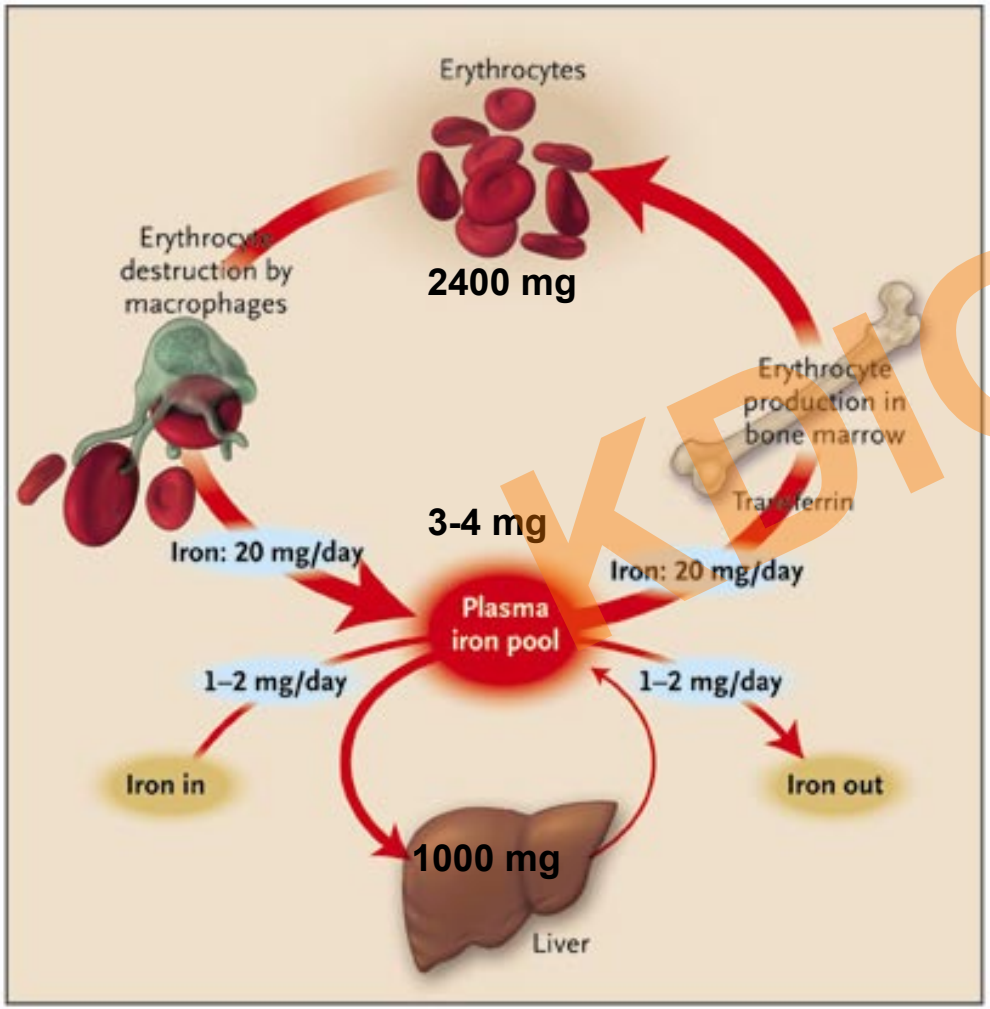
Outline

- Iron homeostasis: basic science and lessons from genetic disorders
- Iron overload disorders
- Iron deficiency anemia and anemia of inflammation
- Application to CKD

Iron

- A trace element essential for oxygen transport, oxygen storage and catalysis of redox reactions (energy production, nucleoside synthesis, intermediary metabolism…)
- Bioavailable iron is scarce in most environments
- Living organisms conserve and recycle iron
- Disorders of iron homeostasis are common
  - Hereditary hemochromatosis
  - Iron-loading anemias (e.g. β-thalassemia)
  - Iron deficiency anemia
  - Anemia of inflammation
  - Anemia of chronic kidney disease

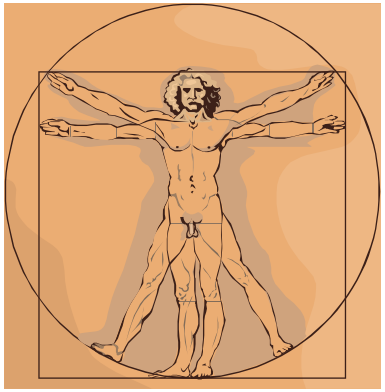
Human iron economy

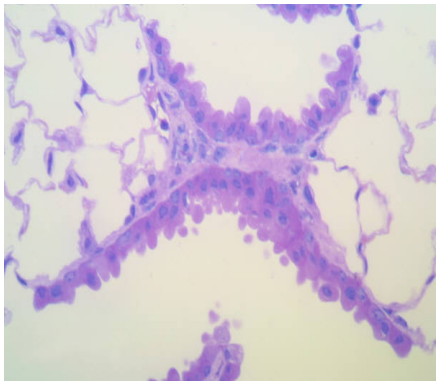


- Plasma iron maintained normally at 10-30 μM
- Chronically >30 μM leads to iron deposition in tissues, injury, organ damage
- Chronically <10 μM causes cellular dysfunction, anemia
- In humans, iron flow to hemoglobin synthesis is highly dependent on recycling by macrophages

Iron homeostasis

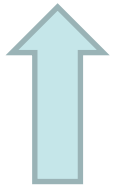
- Extracellular: The organism regulates its dietary iron absorption, concentration of iron in extracellular fluid and iron storage
- Intracellular: Each cell regulates its iron uptake and subcellular distribution



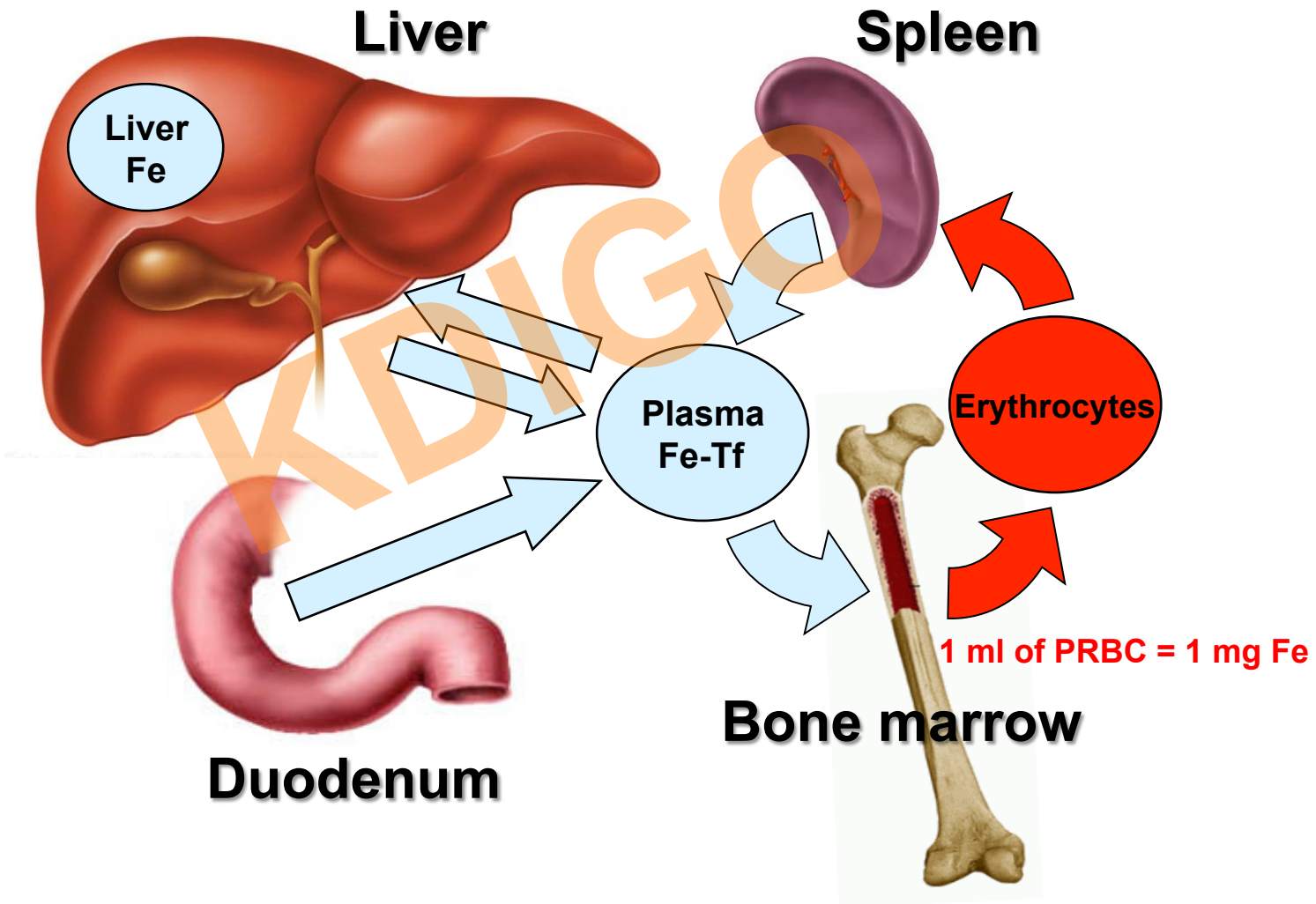


Organismal iron homeostasis

- Response to hemorrhage, iron deficiency:
  - dietary absorption of iron increased
  - stored iron released from macrophages and hepatocytes
- Response to iron overload:
  - absorption of dietary iron decreased
- Response to infection or inflammation:
  - iron release from macrophages and hepatocytes decreased
  - dietary iron absorption decreased
  - hypoferremia



Key organs in iron homeostasis



Ferritin

- Cytoplasmic Fe storage protein, 24 L/H subunits
- Serum form is 24 L>>H subunits, Fe poor
- In subjects without inflammation or maldistribution of Fe, serum ferritin reflects systemic Fe stores
- Inflammation causes increased serum ferritin
  - Very high serum ferritin in macrophage activation syndromes
- Fe in macrophages causes more ferritin secretion than Fe in hepatocytes
  - Ferritin very high in ferroportin disease, caused by mutations in ferroportin, where Fe accumulates in macrophages (vs hereditary hemochromatosis)

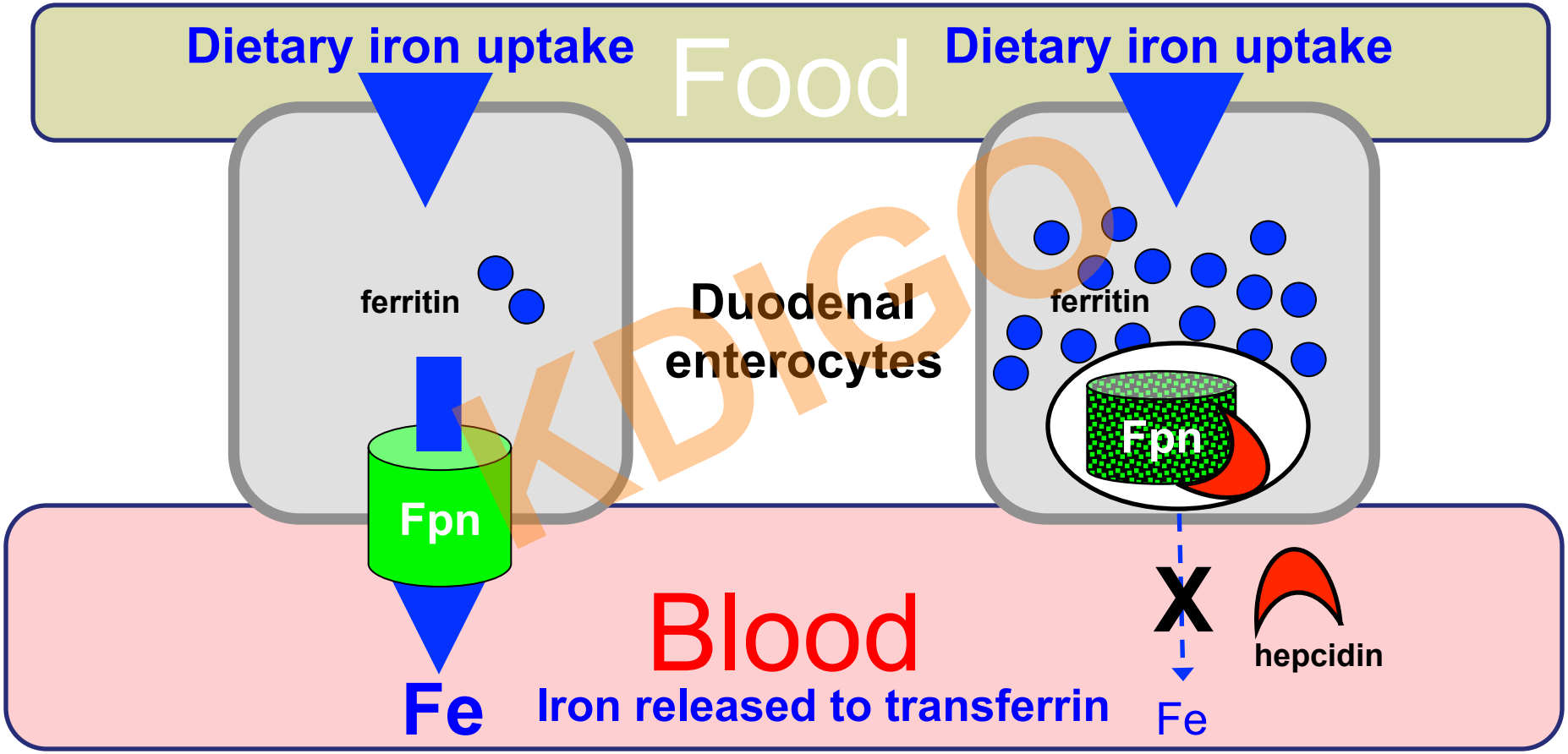
Transferrin

- Abundant plasma protein, 2x Fe^{3+} carrier
- Normally ~1/3 saturated with iron
- Essential for delivery of iron to hemoglobin synthesis
- Transferrin receptor 1 mediates cellular iron uptake
- Transferrin receptor 2 involved in hepcidin regulation

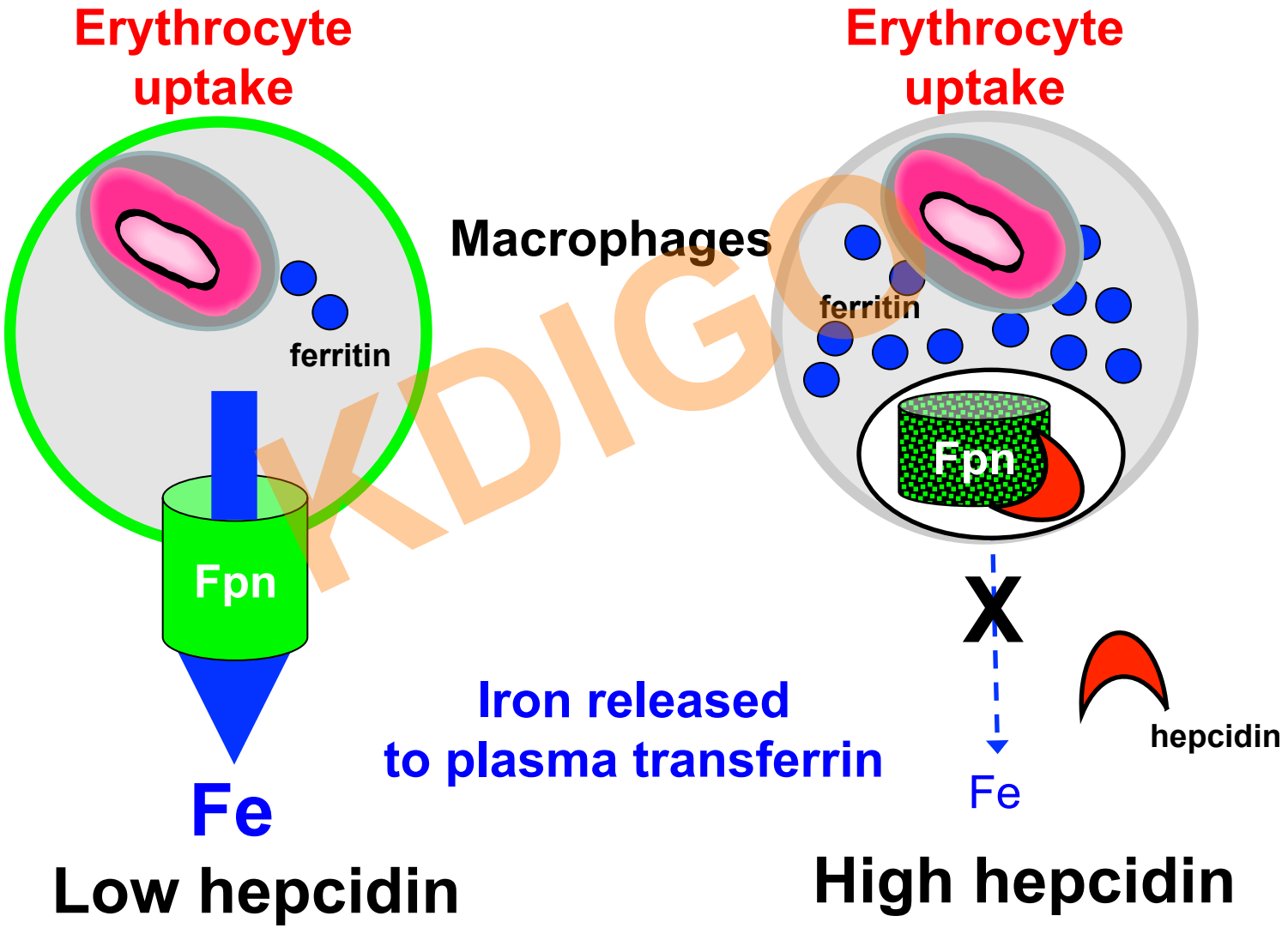
Hepcidin

- Iron-regulating peptide hormone
- Made in the liver
- Binds to the cellular iron exporter ferroportin
- Induces the endocytosis and proteolysis of ferroportin

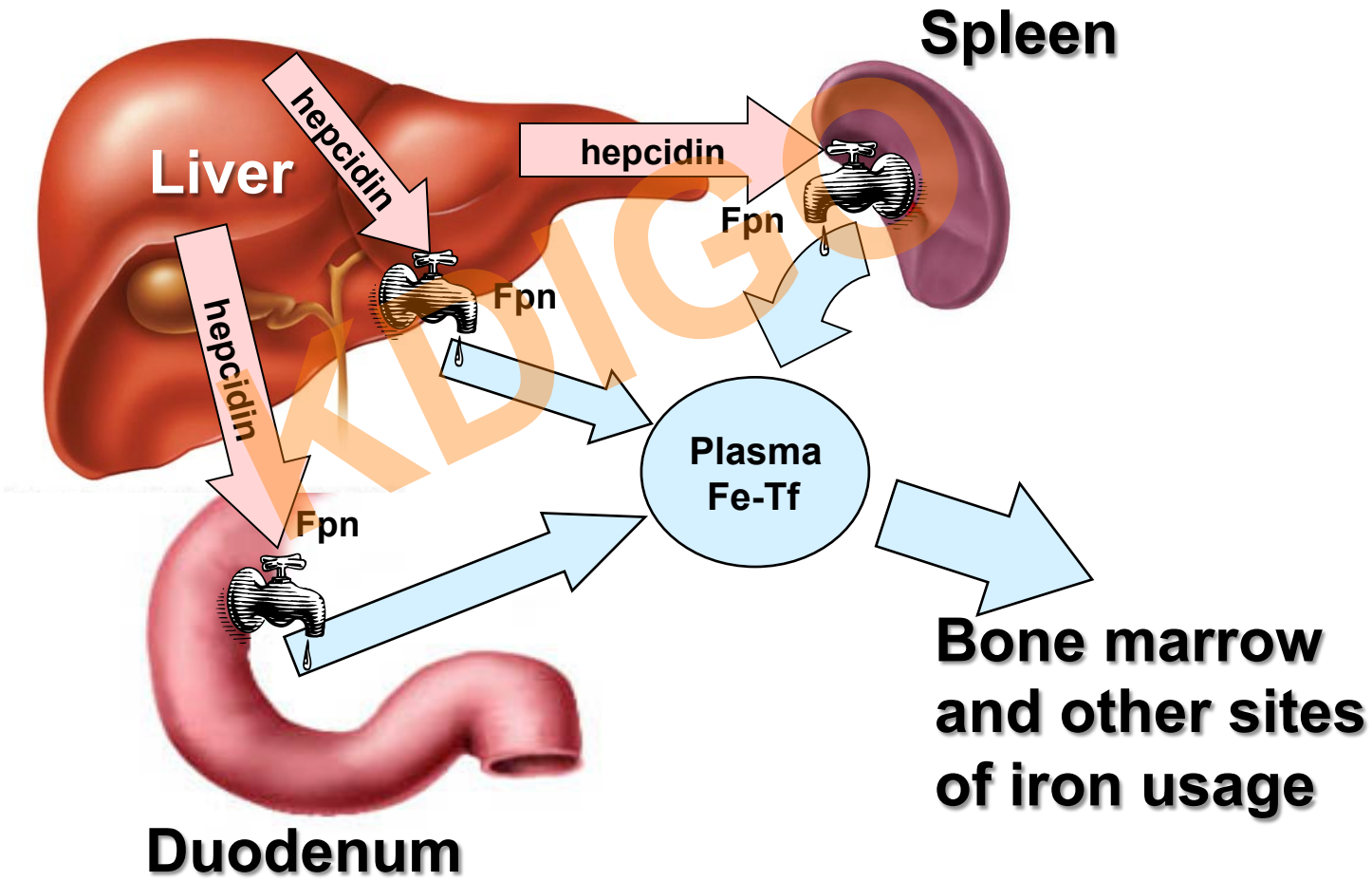
Regulation of intestinal iron absorption



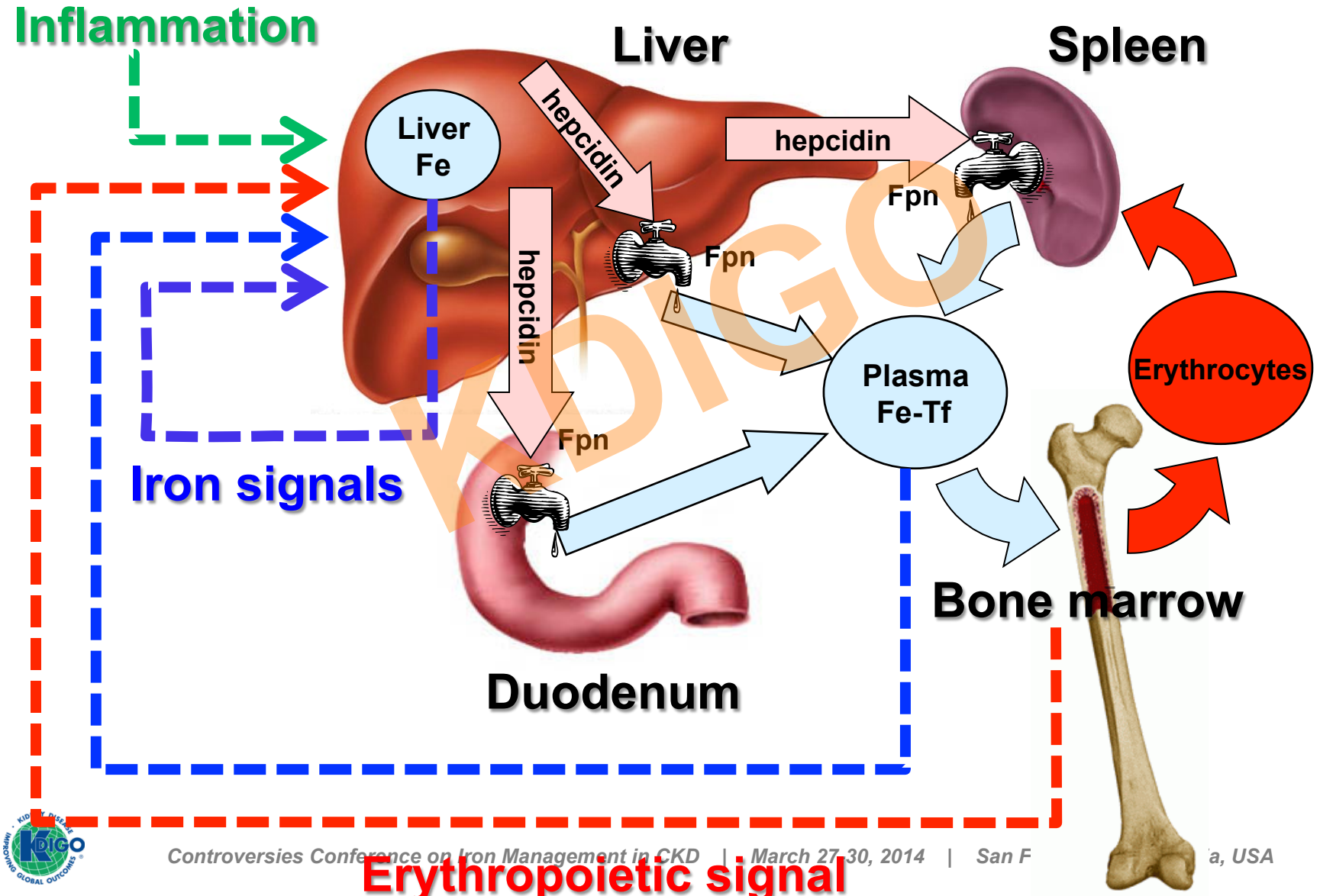
Regulation of erythrocyte iron recycling



How hepcidin regulates iron



Signals regulating hepcidin



Consequences of hepcidin dysregulation

Hepcidin deficiency = iron overload

Hereditary hemochromatosis

Iron-loading anemias

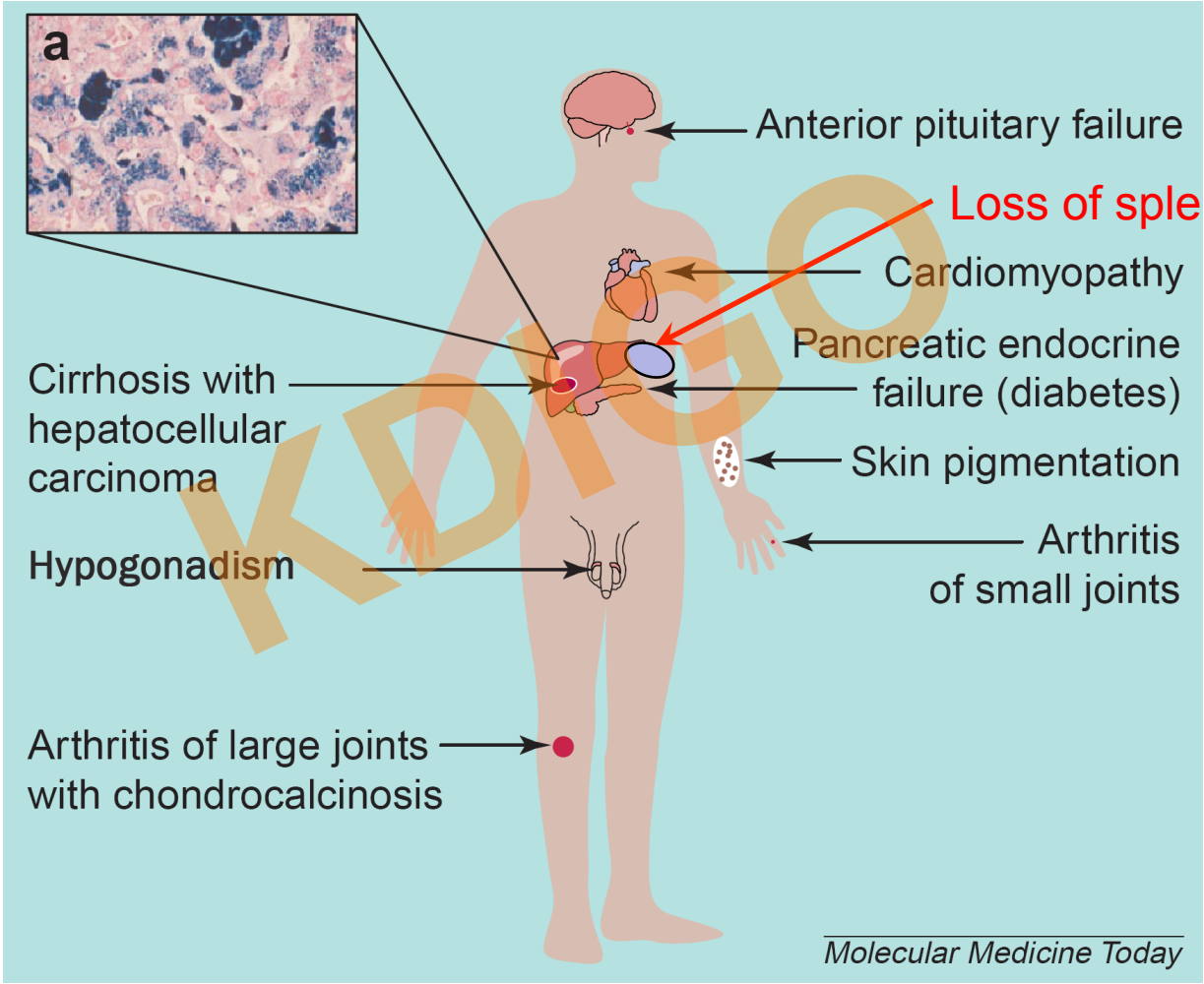
Hepcidin excess = iron-restriction, anemia

Anemia of inflammation

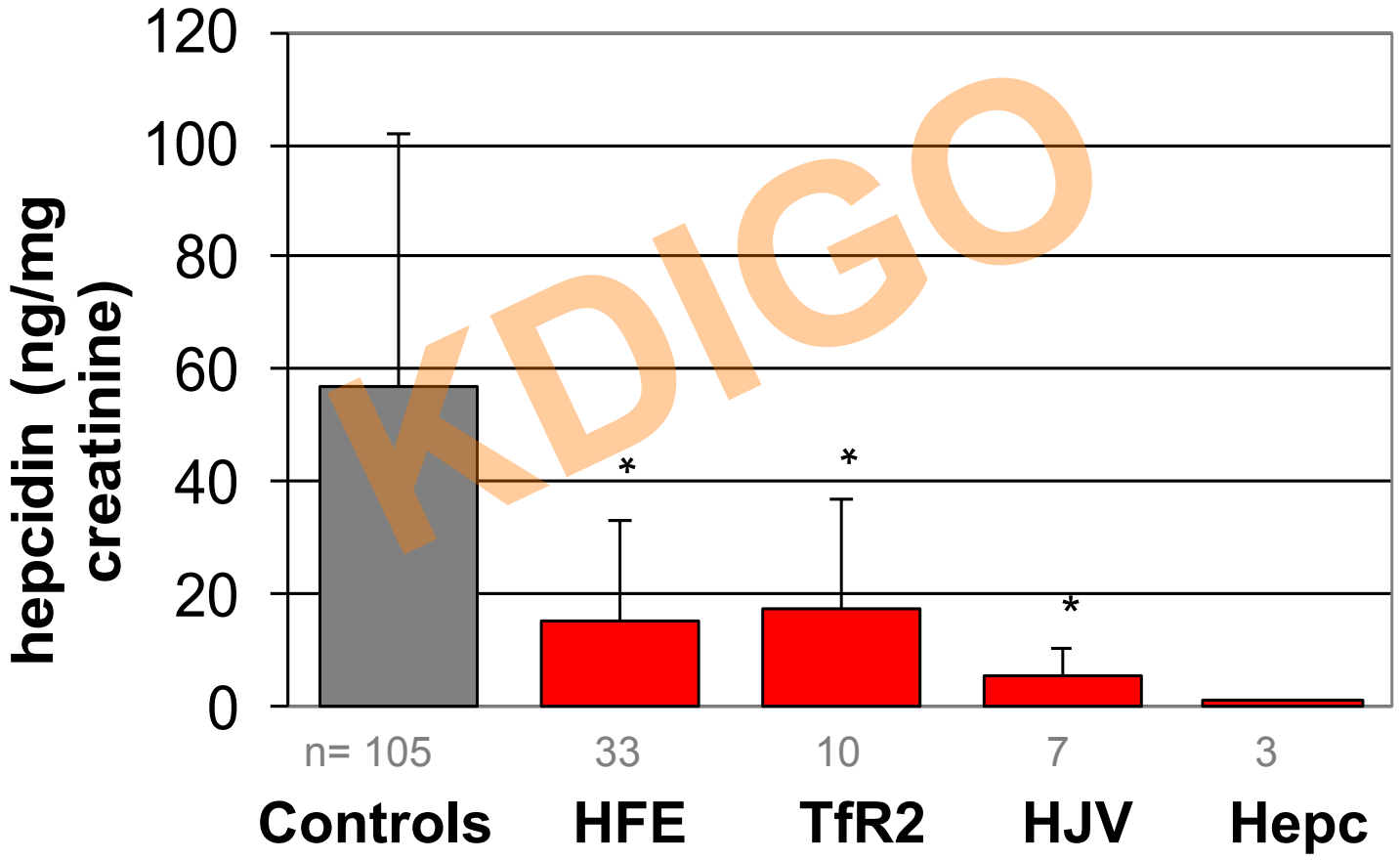
Anemia of CKD

IRIDA

Hereditary hemochromatosis

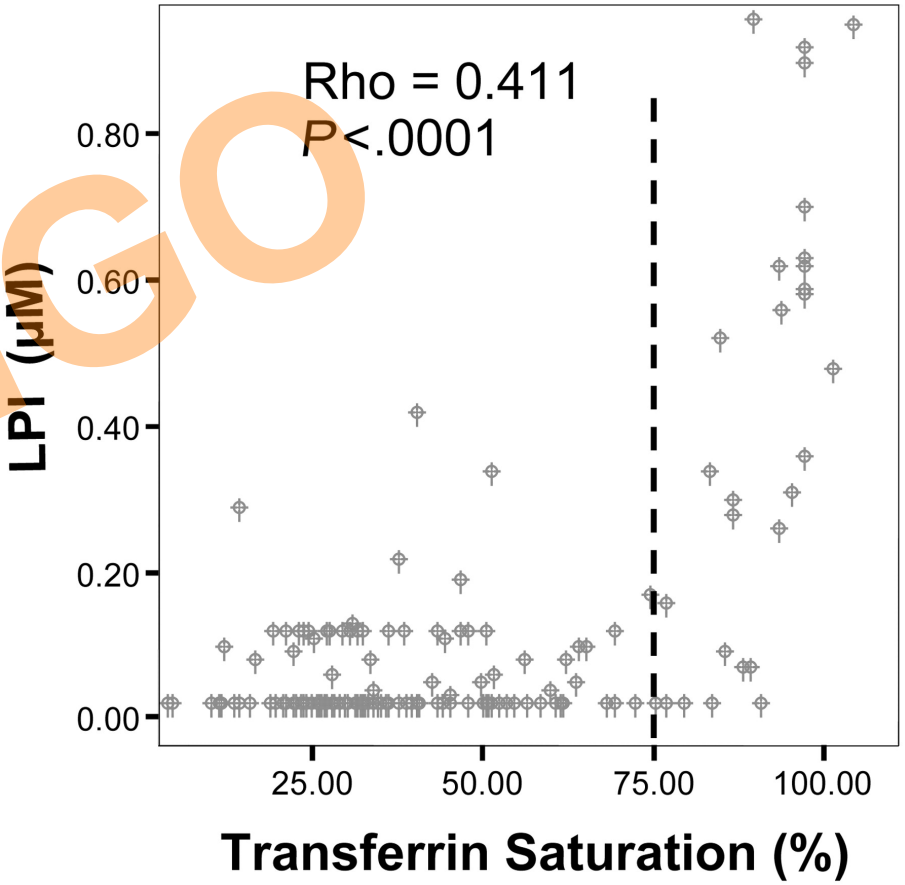


Hepcidin deficiency causes most forms of hereditary hemochromatosis



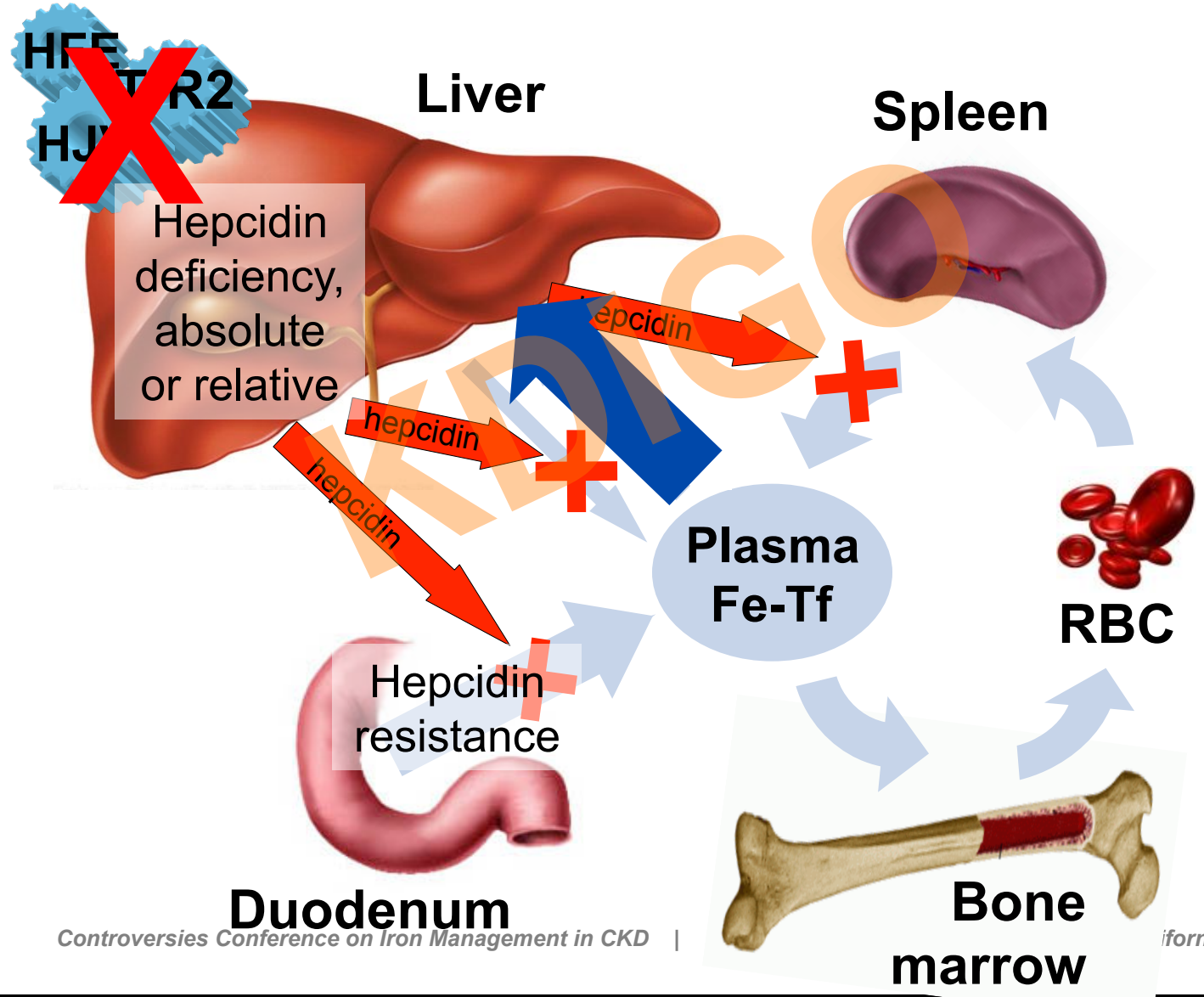
Non-transferrin bound iron (NTBI)

- Fe bound to citrate, acetate, albumin
- Seen when transferrin is (nearly) saturated with Fe
- NTBI is selectively taken up by the liver and other parenchymal tissue and causes injury
- Labile plasma iron (LPI) is the redox-active portion of NTBI
- LPI is detectable when Tsat >75%



Le Lan et al. Blood 2005

Hereditary hemochromatosis



Biomarkers of systemic iron overload associated with potential tissue injury

- High transferrin saturation
- Increased non-transferrin bound iron (NTBI)*
- Increased labile plasma iron (LPI)*
- Ferritin > 1000 only in the absence of:
  - inflammation
  - hepatitis
  - ferroportin disease
  - hyperferritinemia with cataracts syndrome

Erythroid factor(s) regulate hepcidin to assure adequate iron for erythropoiesis

Erythropoietic stimulation (hemorrhage, EPO)

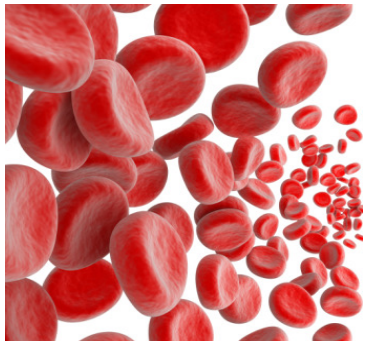
Erythroid factor

LIVER

MARROW

Hepcidin suppression

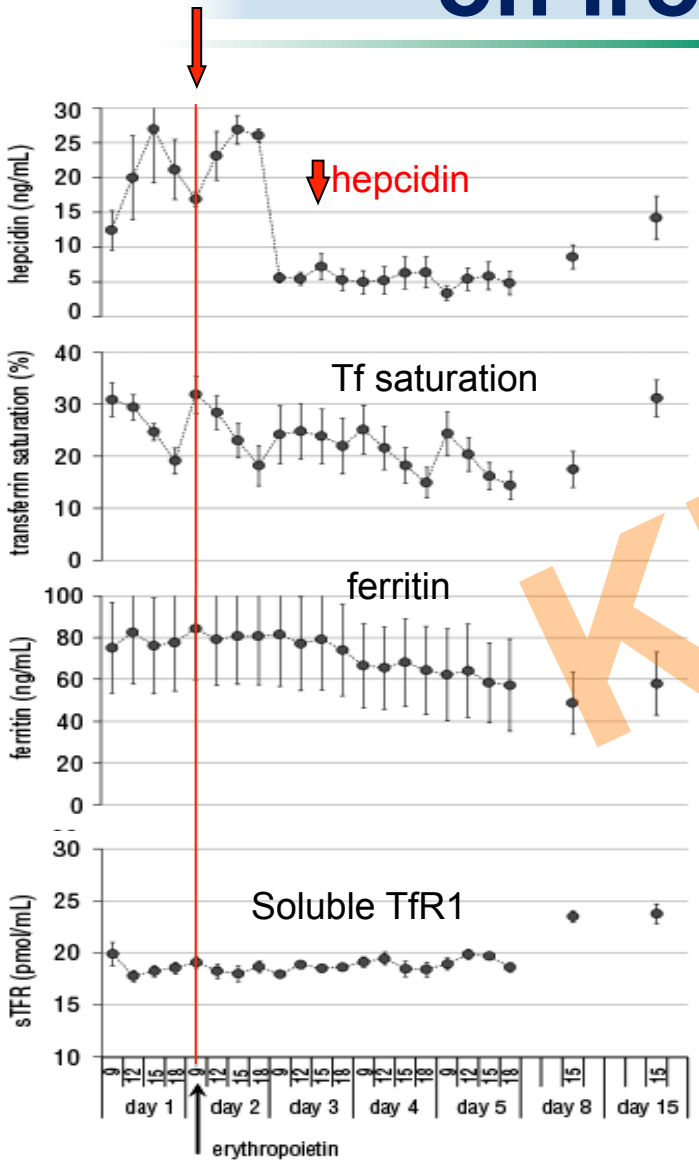
Increased iron availability



Erythropoiesis

Pak *et al.*, Blood, 2006
Vokurka *et al.*, Physiol. Res, 2006

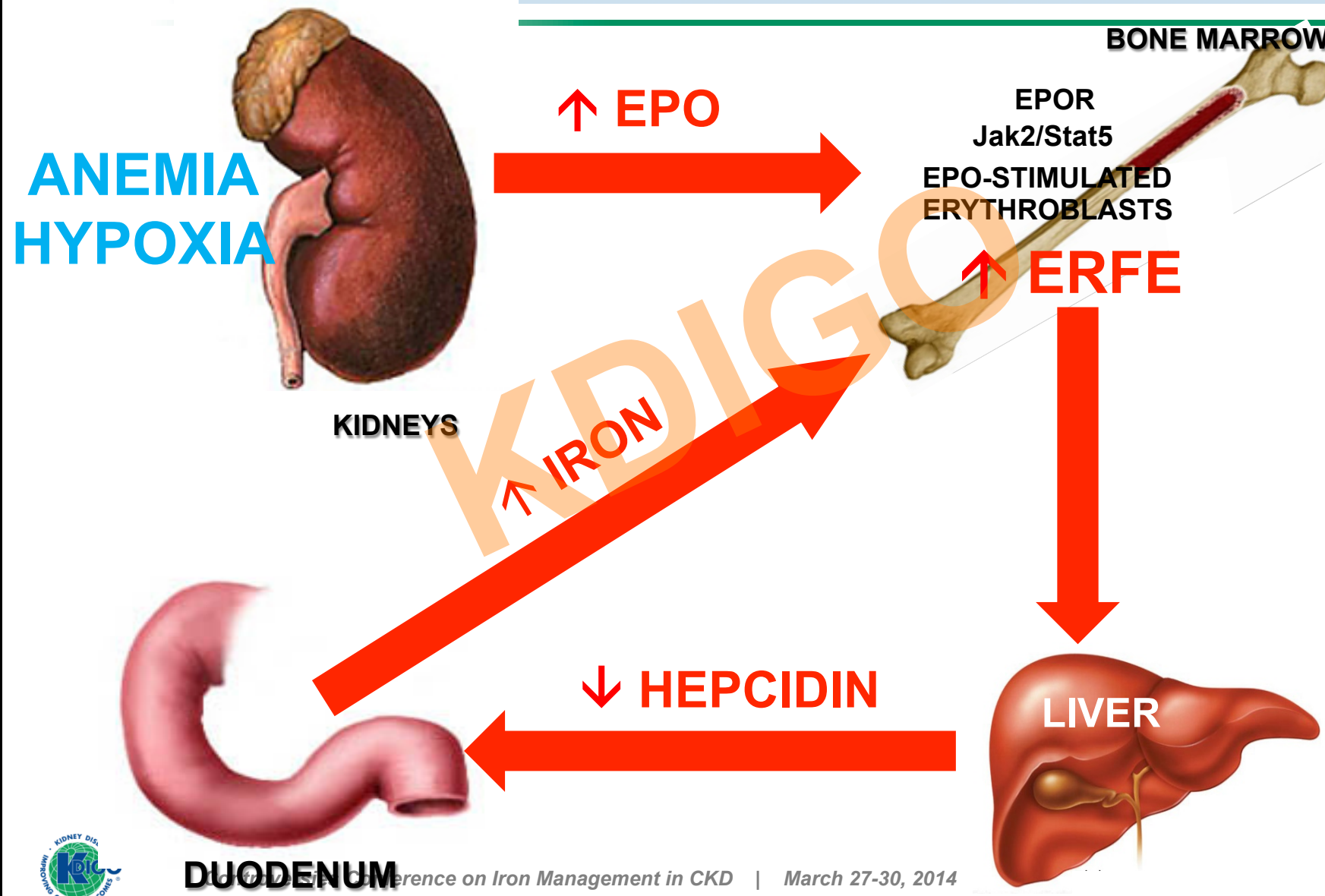
The effect of erythropoietin on iron homeostasis



- Five male volunteers
- Erythropoietin 5000 u at 9am on day 2
- Serum hepcidin drop 9-24h later
- The effect lasts at least 5 days
- Very slow change in iron parameters

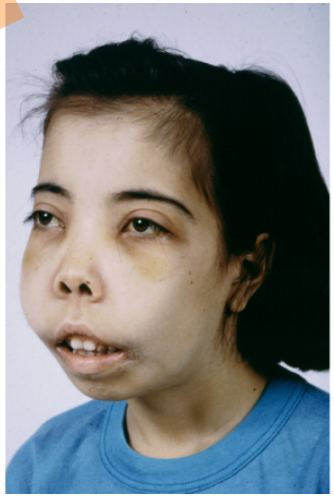
Ashby et al., Haematologica 2010

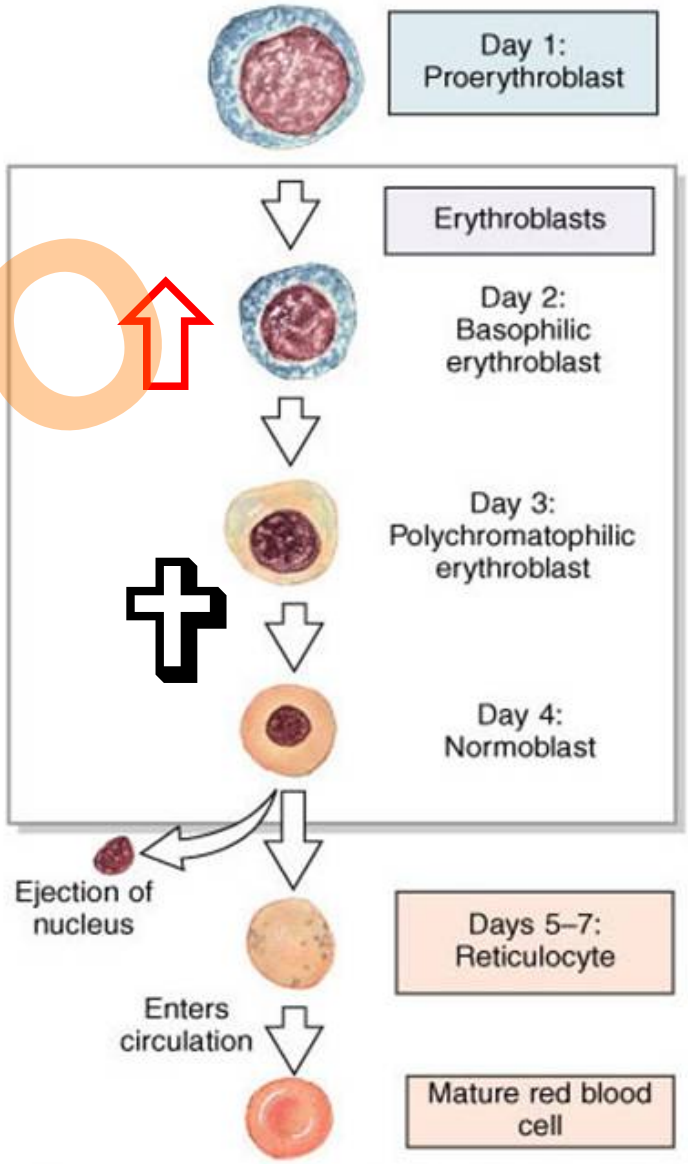
Erythroferrone in iron regulation



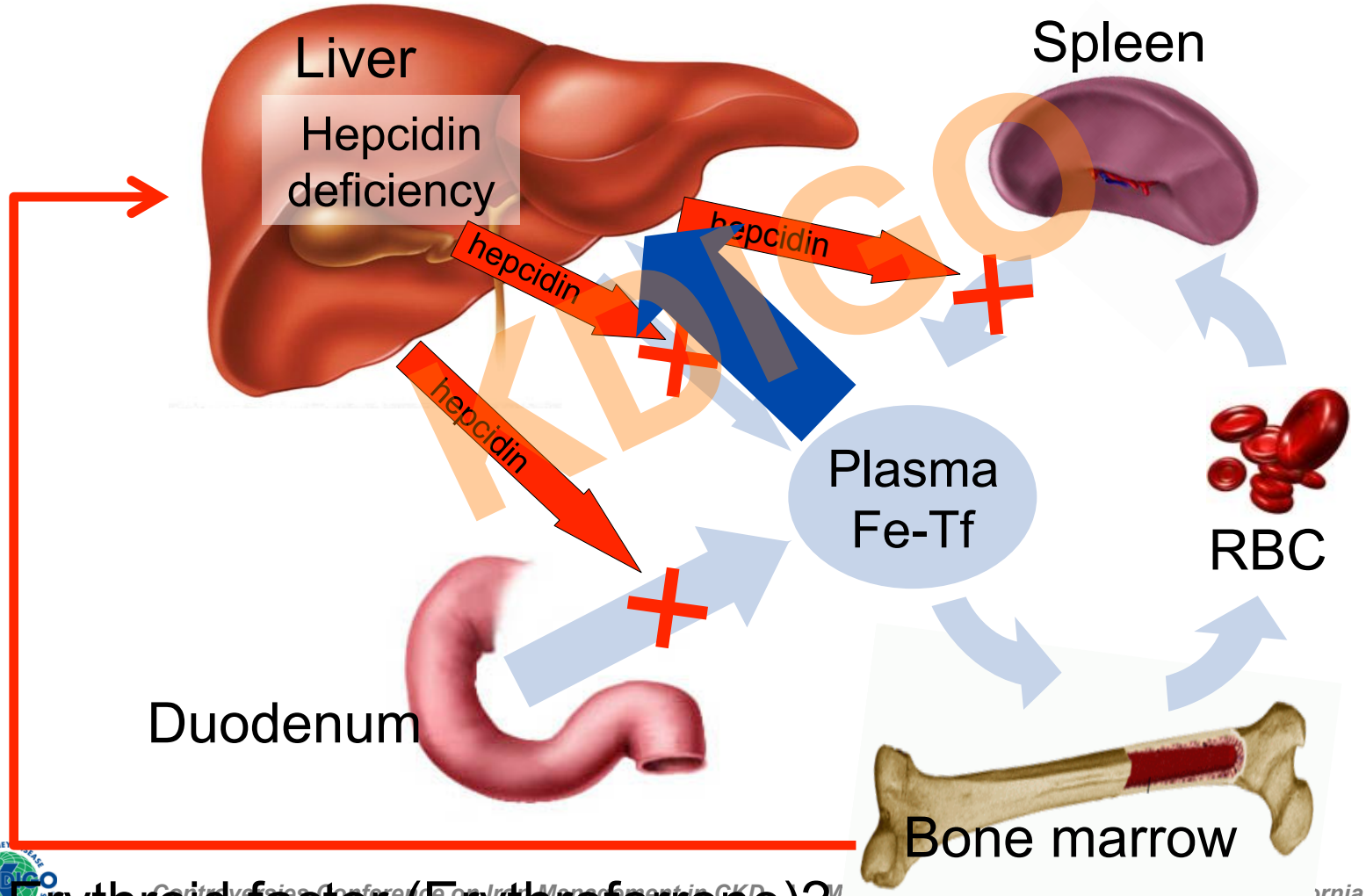
Ineffective erythropoiesis

- Failure of red cell precursors to mature into functional RBCs
- Expansion of immature precursors
- E.g.: β-thalassemia (Cooley's anemia)
- Causes increased Fe absorption
- Probably caused by a hepcidin-suppressive signal generated by increased RBC precursors





Iron-loading anemias



Erythroid factor (Erythroferrone)?

Iron-restricted anemias

	Fe deficiency	Inflammation	"Pure" hepcidin excess
Prototype	Chronic bleeding	Rheumatologic diseases	IRIDA (Tmprss6 mutations)
Serum Fe	Low	Low	Low
Tsat	Low	Low	Low
Macrophage Fe	No	Yes	Yes (Fe tx)
Ferritin	Low	High	Mostly normal
Hepcidin	Low to absent	High	High
MCV	Low to very low	Normal	Very low
Response to "usual" oral Fe	Good	Poor	Poor

Iron deficiency

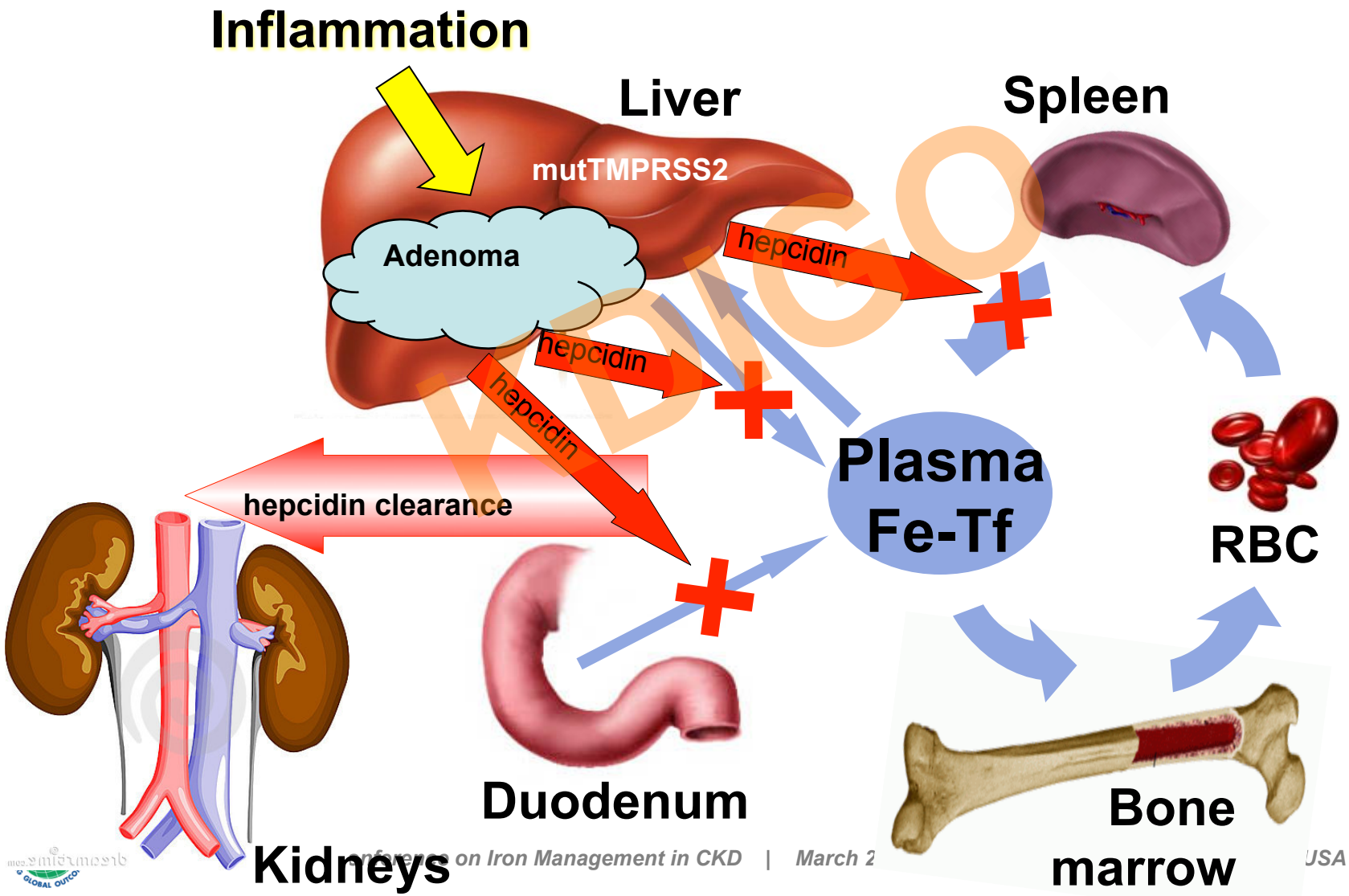
- Normal absorption 1-2 mg Fe/day
- Normal stores in adult men ~1 g
- It takes more than 2 yrs of iron-free diet to deplete stores
- Women have lower stores so depletion is faster
- Bleeding: each 1 ml of RBC = 1 mg of Fe
- Iron deficiency = blood loss, in the US and other developed countries
- Iron malabsorption is rare: Celiac disease, Helicobacter pylori, autoimmune atrophic gastritis

Anemia of inflammation

- Suppression of erythropoiesis
  - Switch to leukocytes and platelets?
- Iron restriction
  - IL-6 → hepcidin → hypoferremia
- Destruction of erythrocytes
  - Macrophage activation

Attributed to A. Einstein: “Make things as simple as possible, but not simpler”

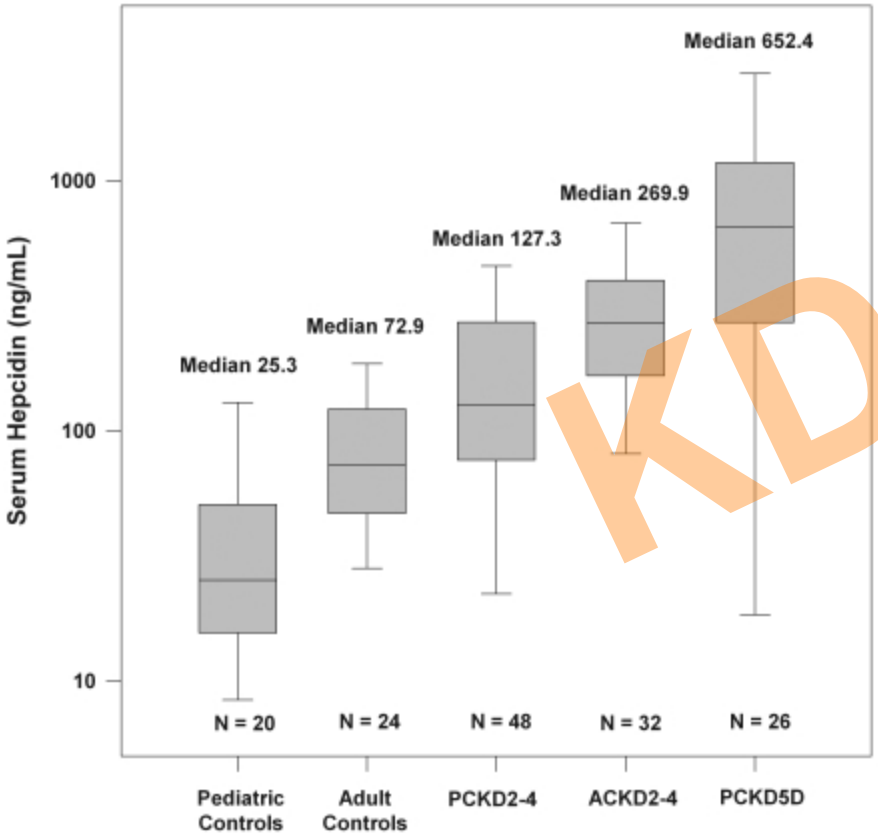
Iron-restricted anemias

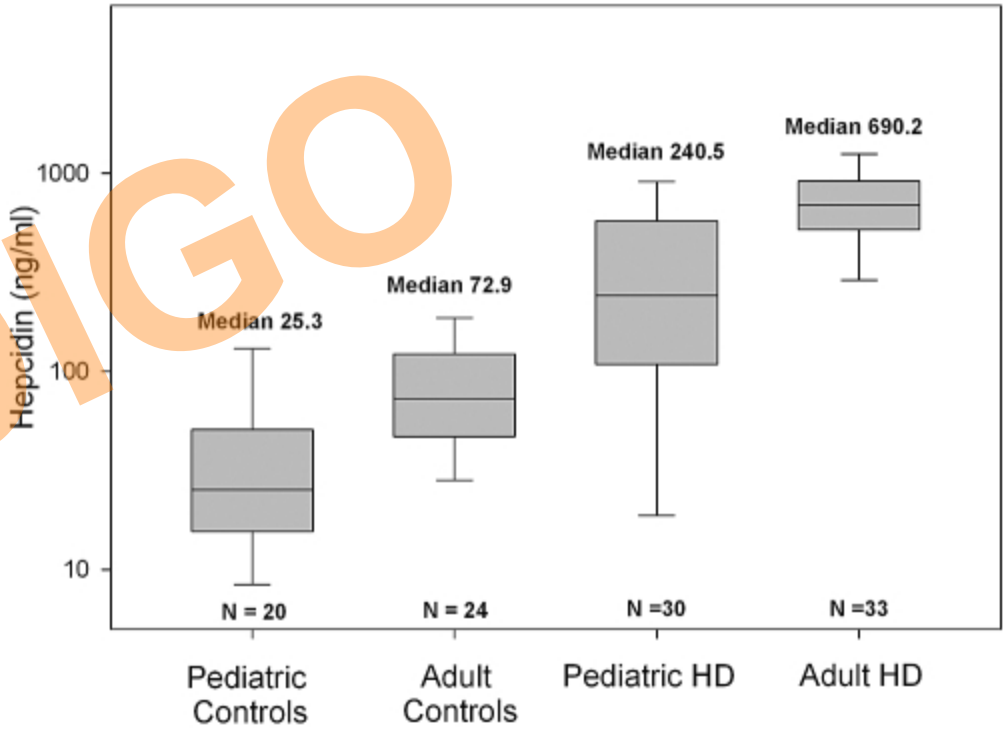


Pathogenesis of anemia of CKD

- EPO deficiency
- Inflammation leading to EPO resistance
  - Iron restriction due to hepcidin
  - Suppression of erythropoiesis
- Other effects of inflammation
  - Shortened erythrocyte lifespan
- True iron deficiency from blood loss and decreased iron absorption from chronic inflammation

Serum hepcidin is high in CKD

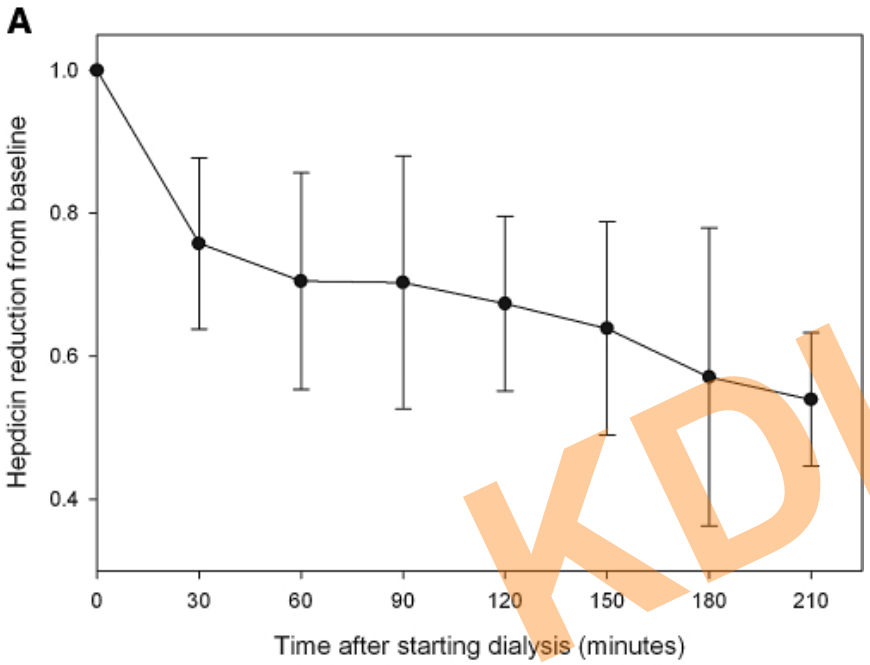




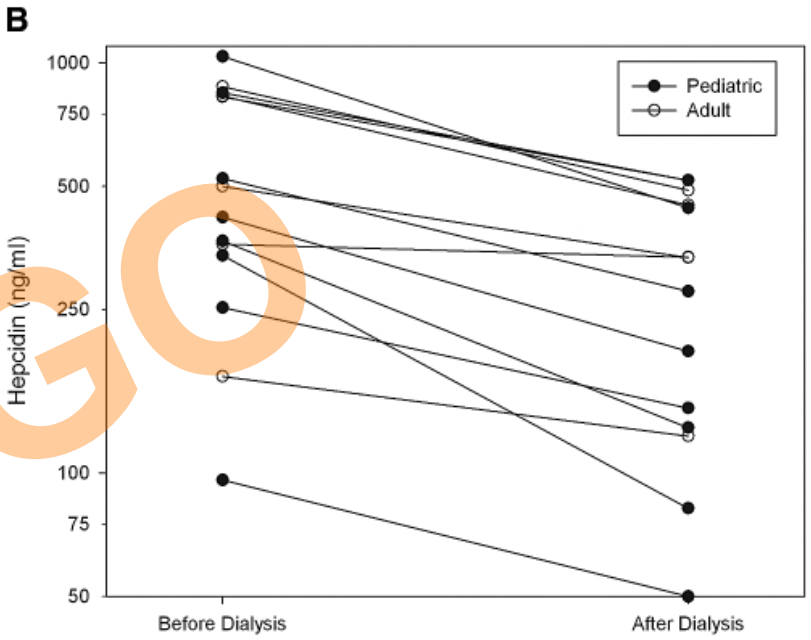
Zaritsky et al. CJASN. 2009; 4:1051-6.

Zaritsky et al. CJASN 2010; 5: 1010–1014.

Hepcidin is cleared by HD



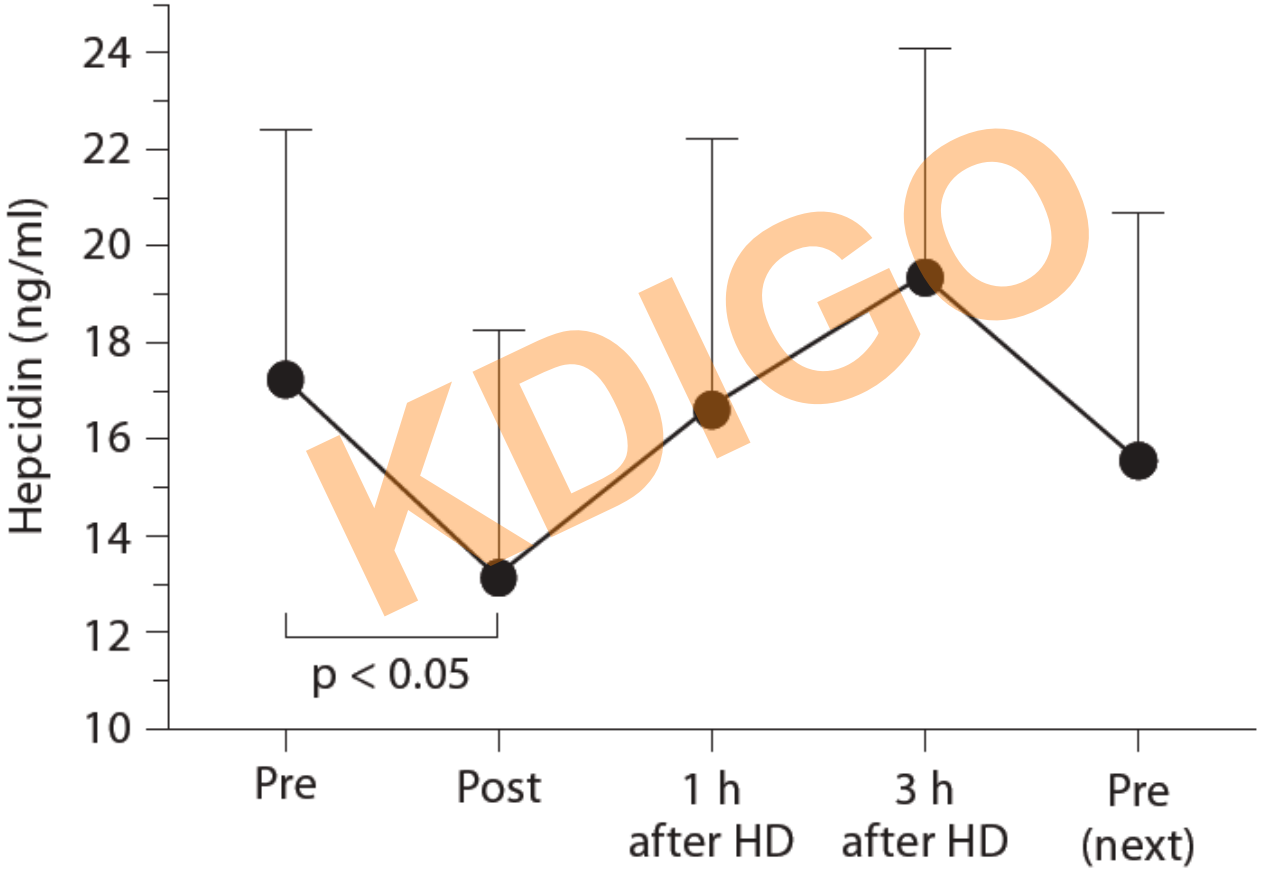
Polyflux Revaclear dialyzer (Gambro) with a dialysate flow (Qd) of 800 ml/min for an average of 3.2 ± 0.2 and 3.0 ± 0.4 hours in pediatric and adult patients, respectively (NS).



The average blood flow (Qb) was 320 ± 52 and 375 ± 32 ml/min in pediatric and adult patients, respectively Hepcidin clearance by HD was 141 +/- 40 and 128 +/- 44 ml/min in pediatric and adult patients, respectively (NS).

Zaritsky et al. CJASN 2010 June; 5: 1010–1014.

Hepcidin recovers rapidly after HD



Kuragano et al. Am J Nephrol 2010;31:534–540

Iron restriction and deficiency in CKD

- Elevated hepcidin causes:
  - malabsorption of iron
  - sequestration of iron in macrophages
- Hemodialysis causes blood loss
- Iron deficiency and iron restriction contribute to erythropoietin resistance
- Erythropoietin dose-dependently suppresses hepcidin but causes side effects, risks and costs
- IV iron reduces erythropoietin resistance but:
  - may increase infections
  - questions about long term safety