Iron and hepcidin: a story of recycling and balance

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SIMTI

Special Conference
Il Patient Blood Management: non solo una questione di ferro e anemia
Roma, 15 ottobre 2015

Il sottoscritto DOMENICO GIRELLI, in qualità di Relatore
dichiara che

nell’esercizio della Sua funzione e per levento in oggetto, DI NON ESSERE in alcun modo portatore di interessi commerciali propri o di terzi; e che gli eventuali rapporti avuti negli ultimi due anni con soggetti portatori di interessi commerciali non sono tali da permettere a tali soggetti di influenzare le mie funzioni al fine di trarne vantaggio.

NOTHING TO DISCLOSE

outline

- ✓ **Overview of iron metabolism and its regulation by the hepatic hormone hepcidin**
- ✓ **The 3 main signals regulating hepcidin (iron status, inflammations/infections, and iron requirements from BM erythroid precursors)**
- ✓ **The recent discovery of the erythroferrone (ERFE), the long sought "eryhtroid regulator" of iron metabolism**
- ✓ **Possibile usefulness of hepcidin assay (in iron deficiency)**
- ✓ **Future Targeted Treatments through pharmacological modulation of hepcidin**

Iron: essential but potentially dangerous

easily exchange electrons
$Fe^{3+} \leftrightarrow Fe^{2+}$
useful redox properties

free radicals generation
($Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + OH\bullet$)

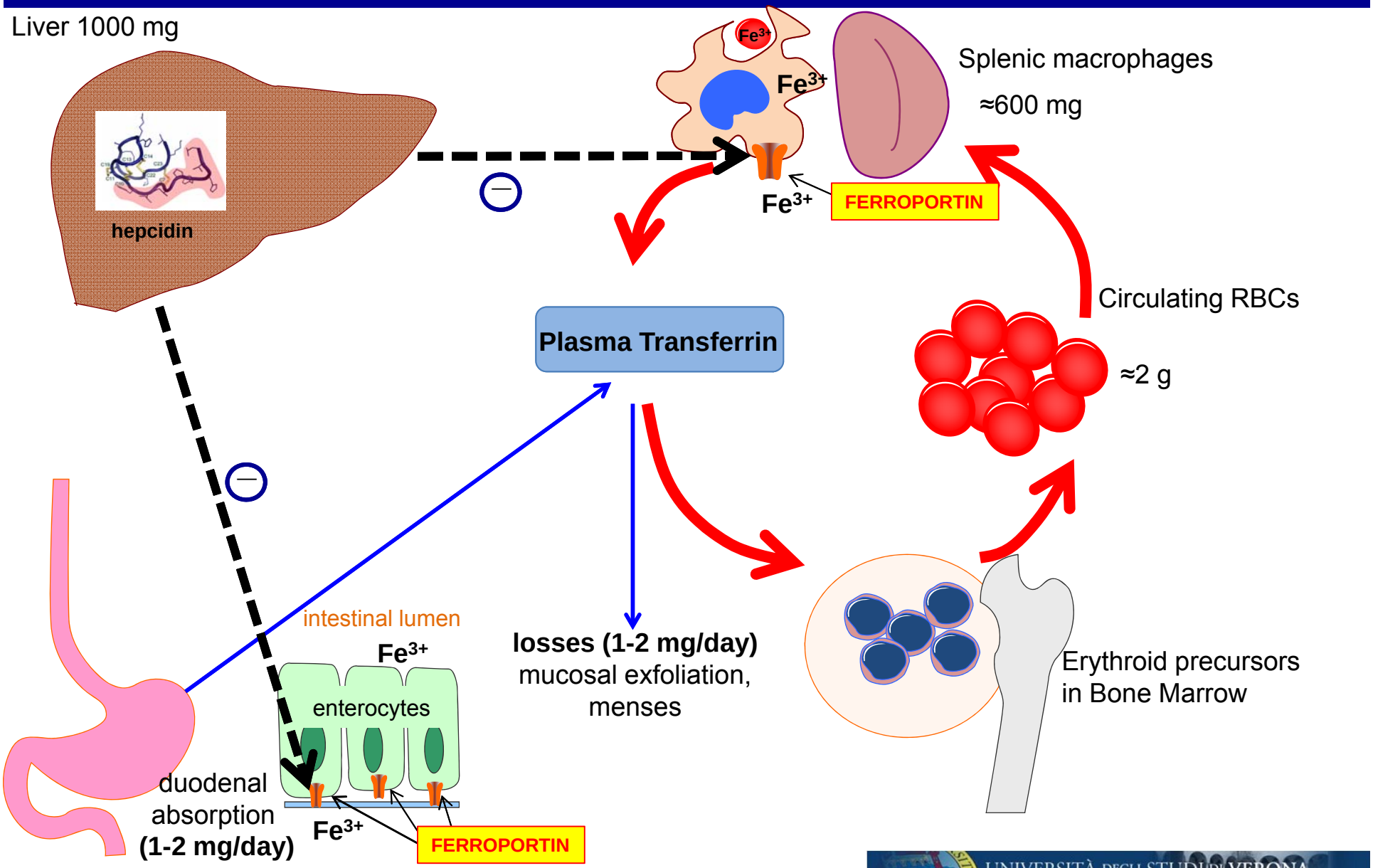
key-component of enzymes crucial for O_2 transport and energy production (Hb, cytochromes…)

strict regulation of body iron content needed

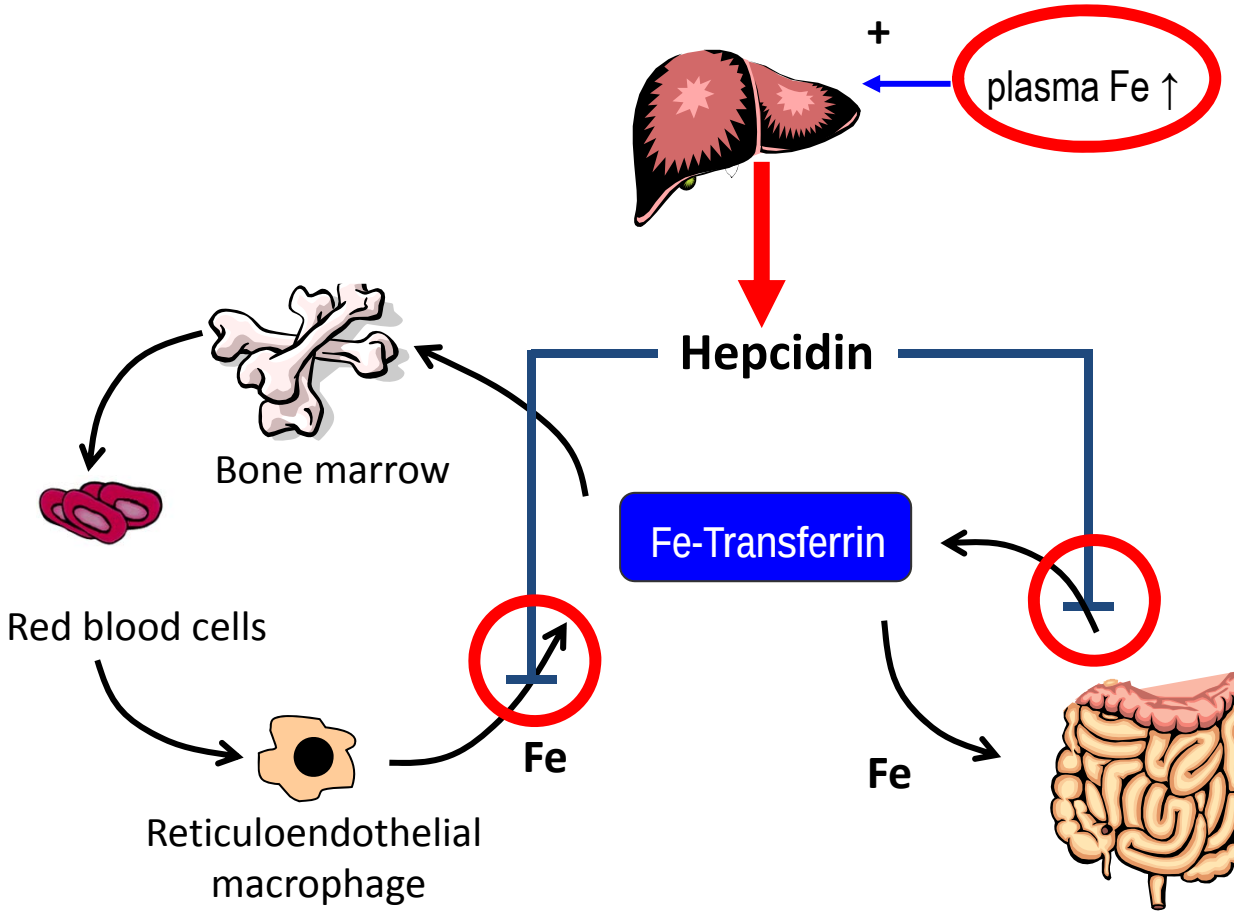
low
↓
anemia
neuromuscolar impairment

excess
↓
iron overload
toxic organ damage

IRON "ECOLOGY"

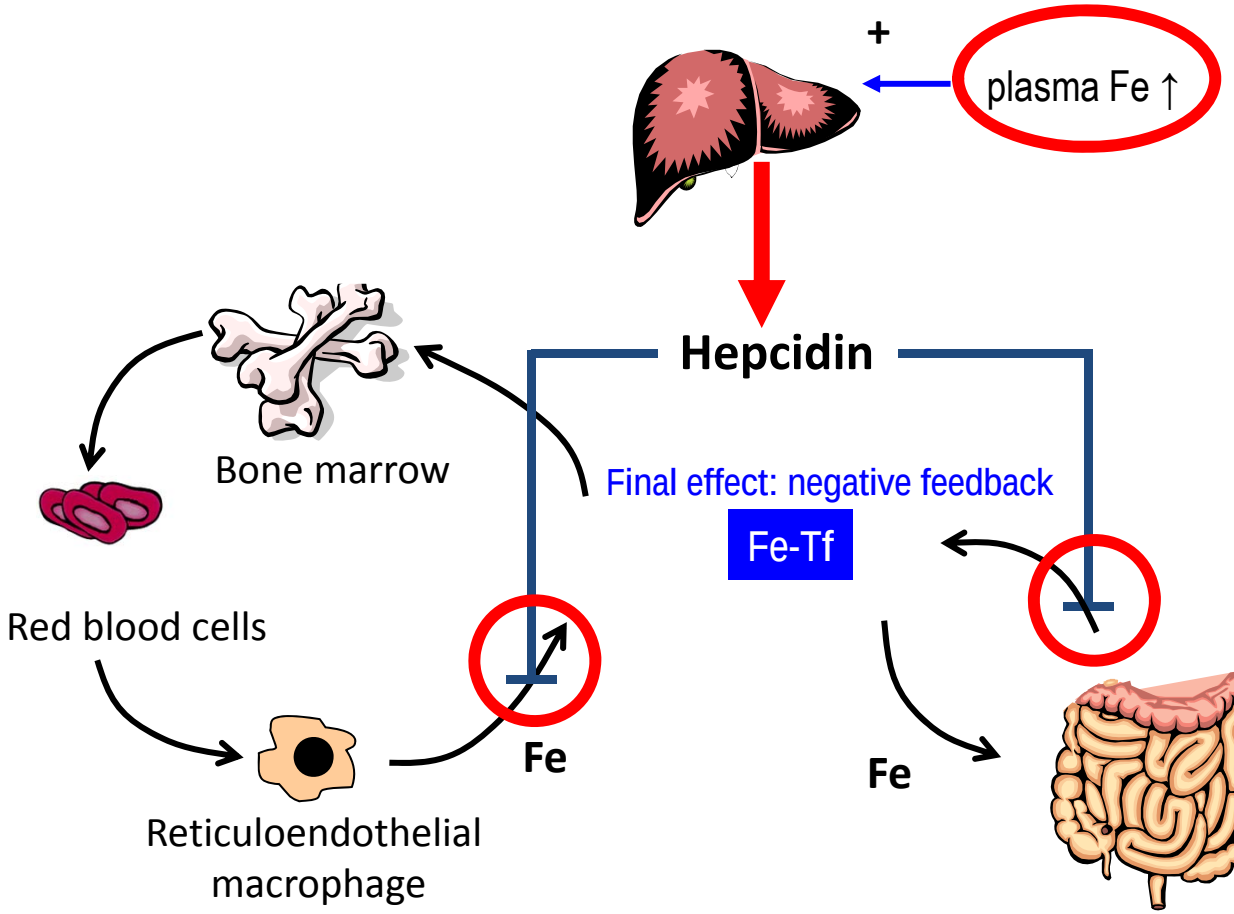


Physiology



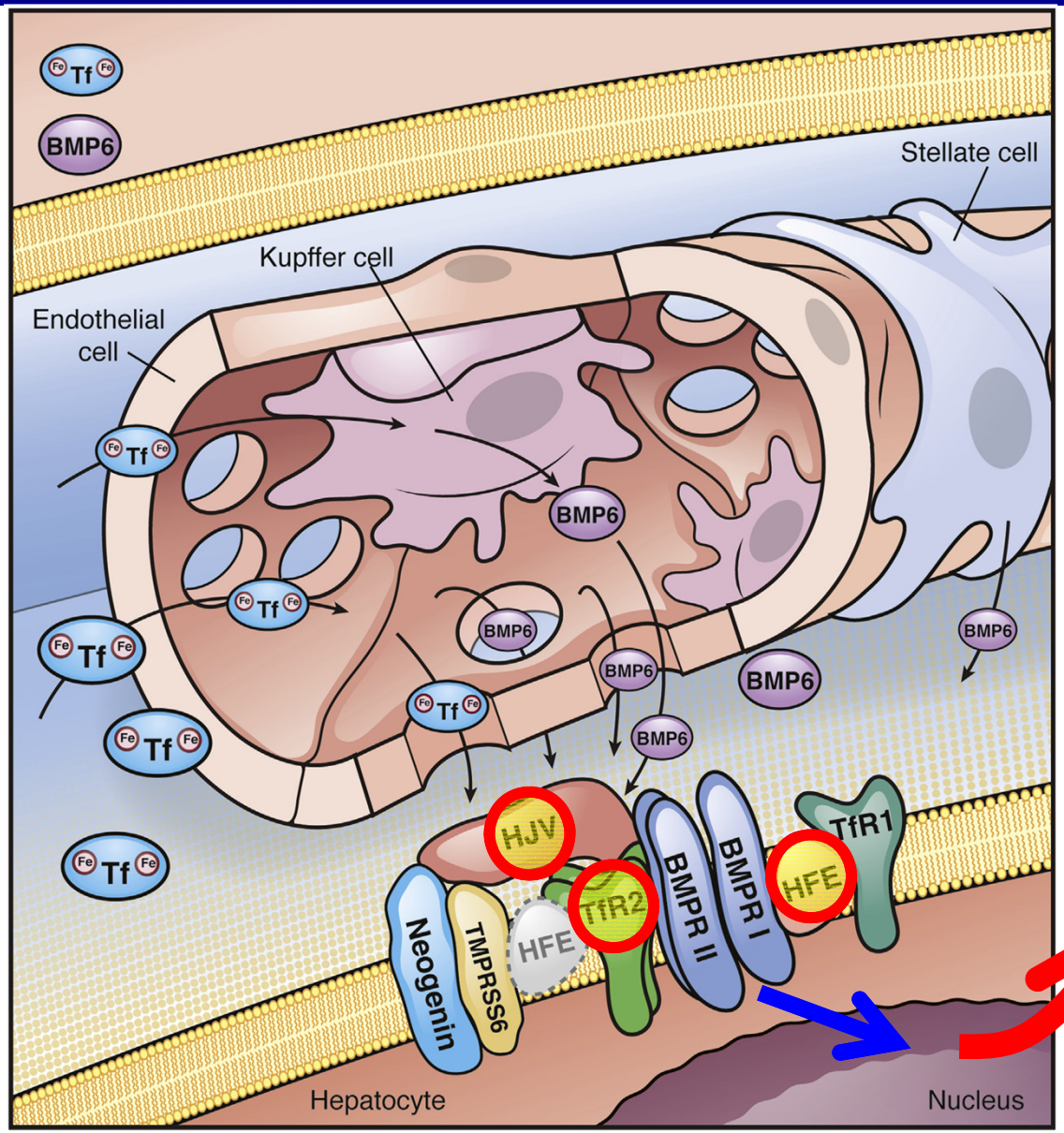
Castagna A, J Proteomics 2010 (adapted)

Physiology



Castagna A, J Proteomics 2010 (adapted)

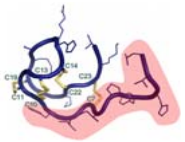
The iron-sensing machinery in the liver



Iron transferrin from portal vein enters the sinusoids → BMP6 production by SC, KC and HSC.

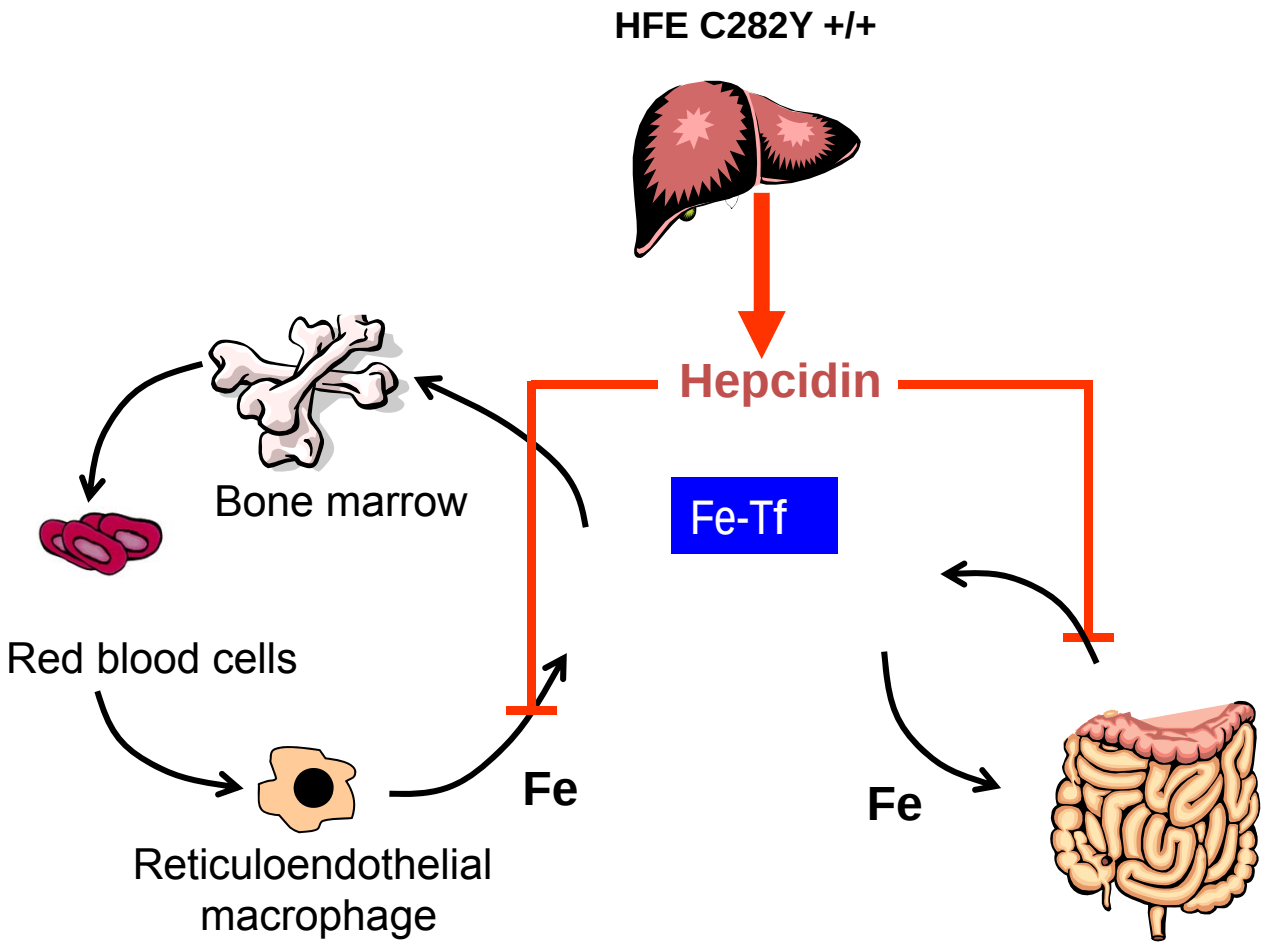
Both iron transferrin and BMP6 activate a multi-molecular signaling complex, composed of several molecules like BMP receptors, HJV (co-receptor), HFE and TFR2.

hepcidin transcription



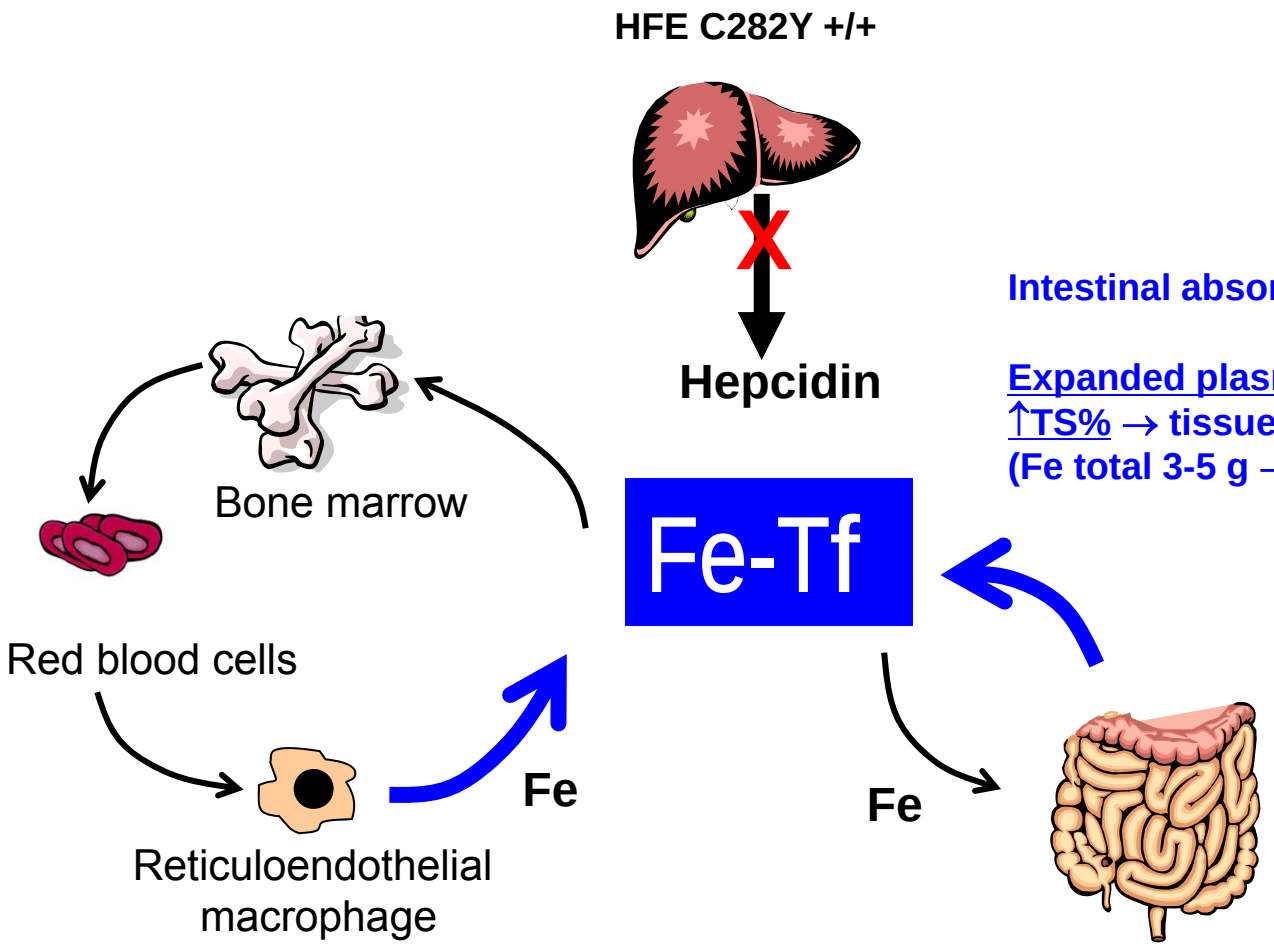
Pietrangelo A, Gastroenterology 2015

Pathology



Castagna A, J Proteomics 2010 (adapted)

Pathology



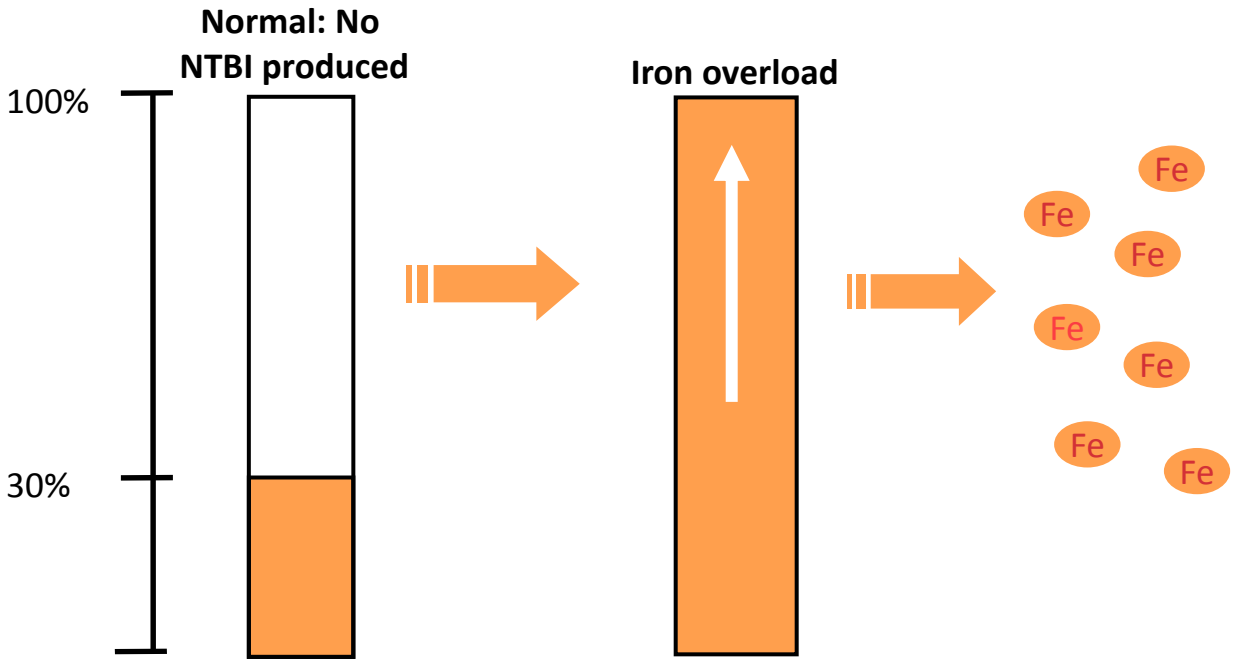
Castagna A, J Proteomics 2010 (adapted)

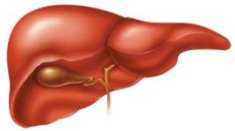
Non-transferrin bound iron (NTBI) in hemochromatosis

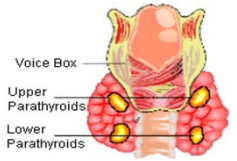
Transferrin saturation occurs due to continuously increased iron absorption

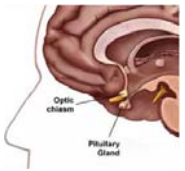
Subsequent formation of NTBI in plasma

Uncontrolled iron loading of organs, such as:









The spectrum of genetic dysregulation of hepcidin

HFE, TFR2,
SLC40A1,
HAMP, HJV

TMPRSS6

Normal homeostasis

Iron stores ⟷ **Hepcidin levels**

Hereditary hemochromatosis (HH)

Iron Refractory Iron Deficiency Anemia (IRIDA)

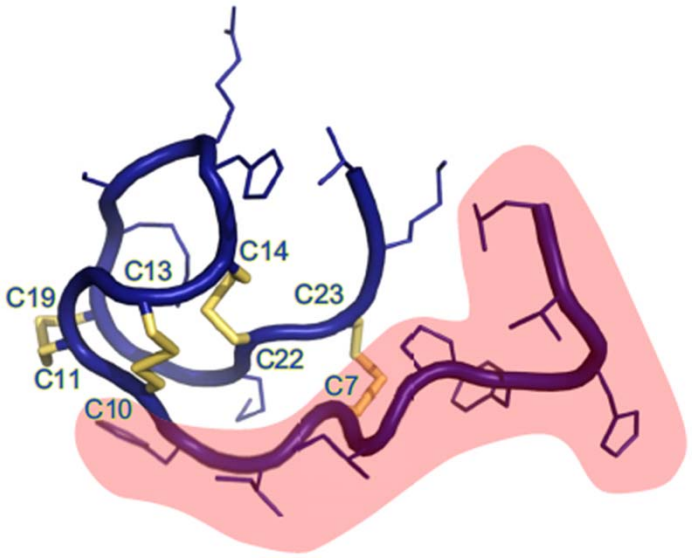
- Post-natal microcytic hypochromic anemia with low TS%
- Refractoriness to oral iron
- Slow response to i.v. iron
- Sometimes diagnosed in adulthood
- Normal/high hepcidin levels (diagnosis)

HEP-(atic) CIDIN (antimicrobial)

- **small (25 aa) peptide**

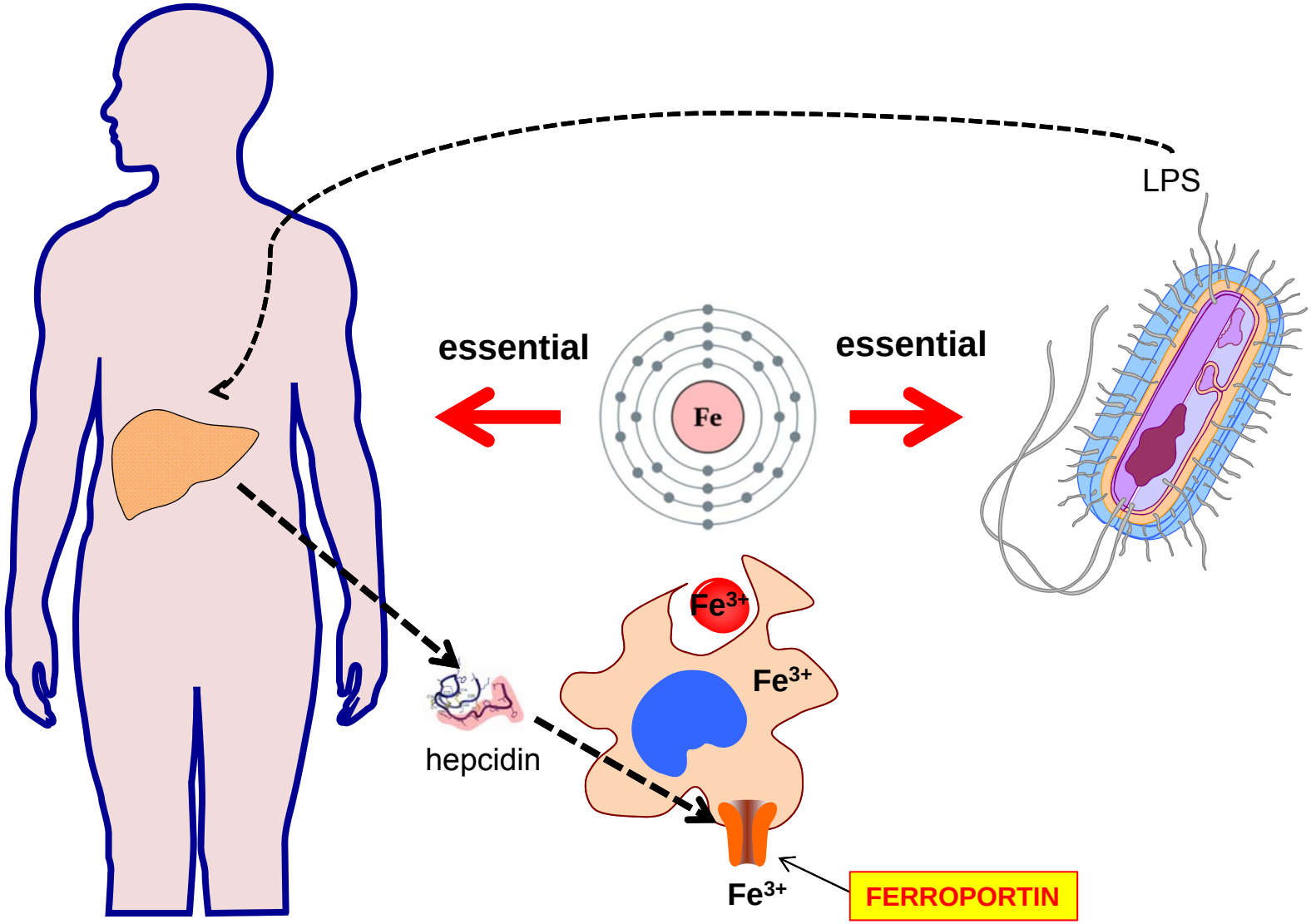
- **defensin-like (innate immunity-related peptides with natural antimicrobial acitvity)**

DTHFPICIFCCGCCHRSKCGMCCKT



Ganz T, Physiol Rev 2013

A second level of balance in pathological conditions: the host-pathogen battle for iron



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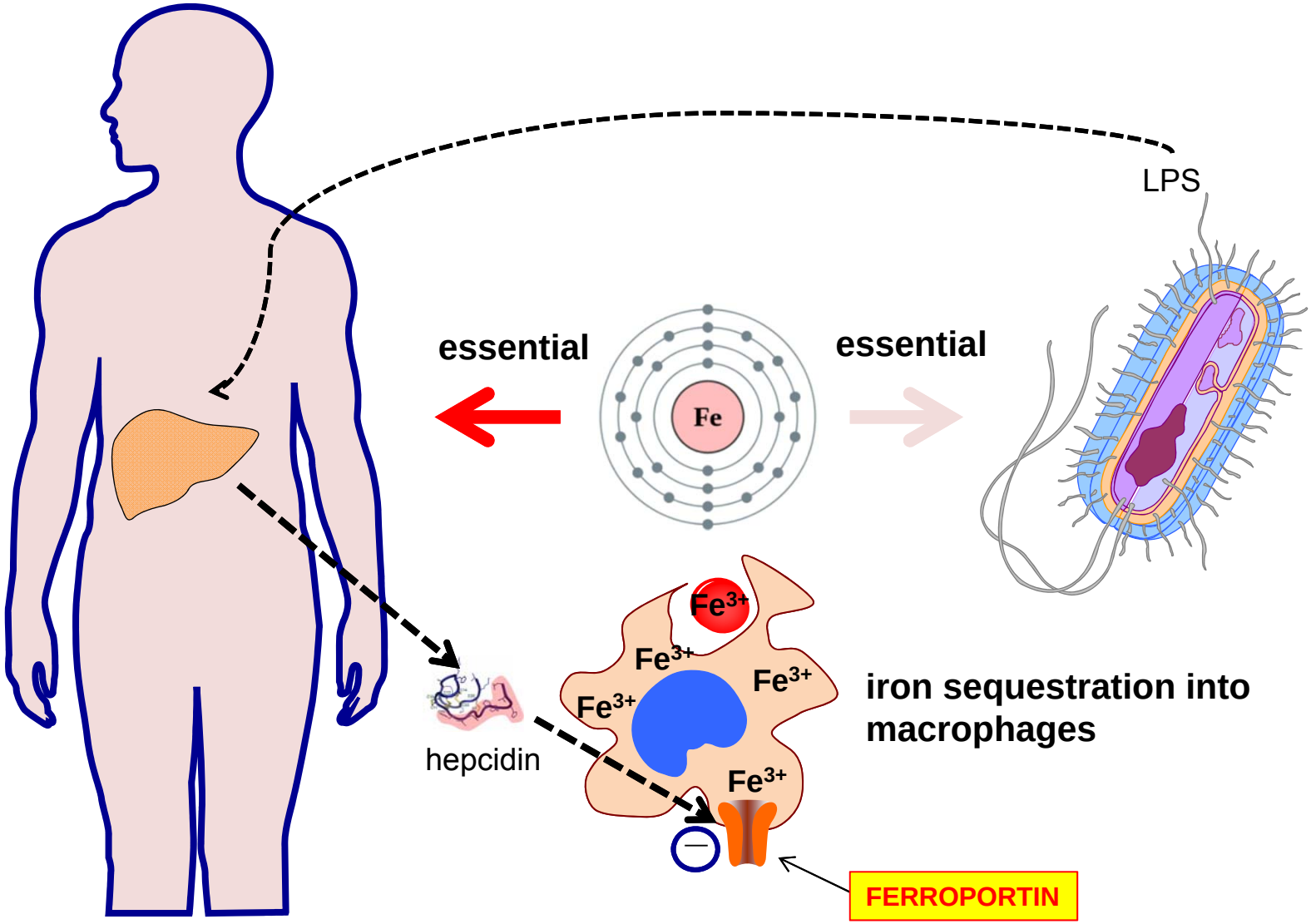
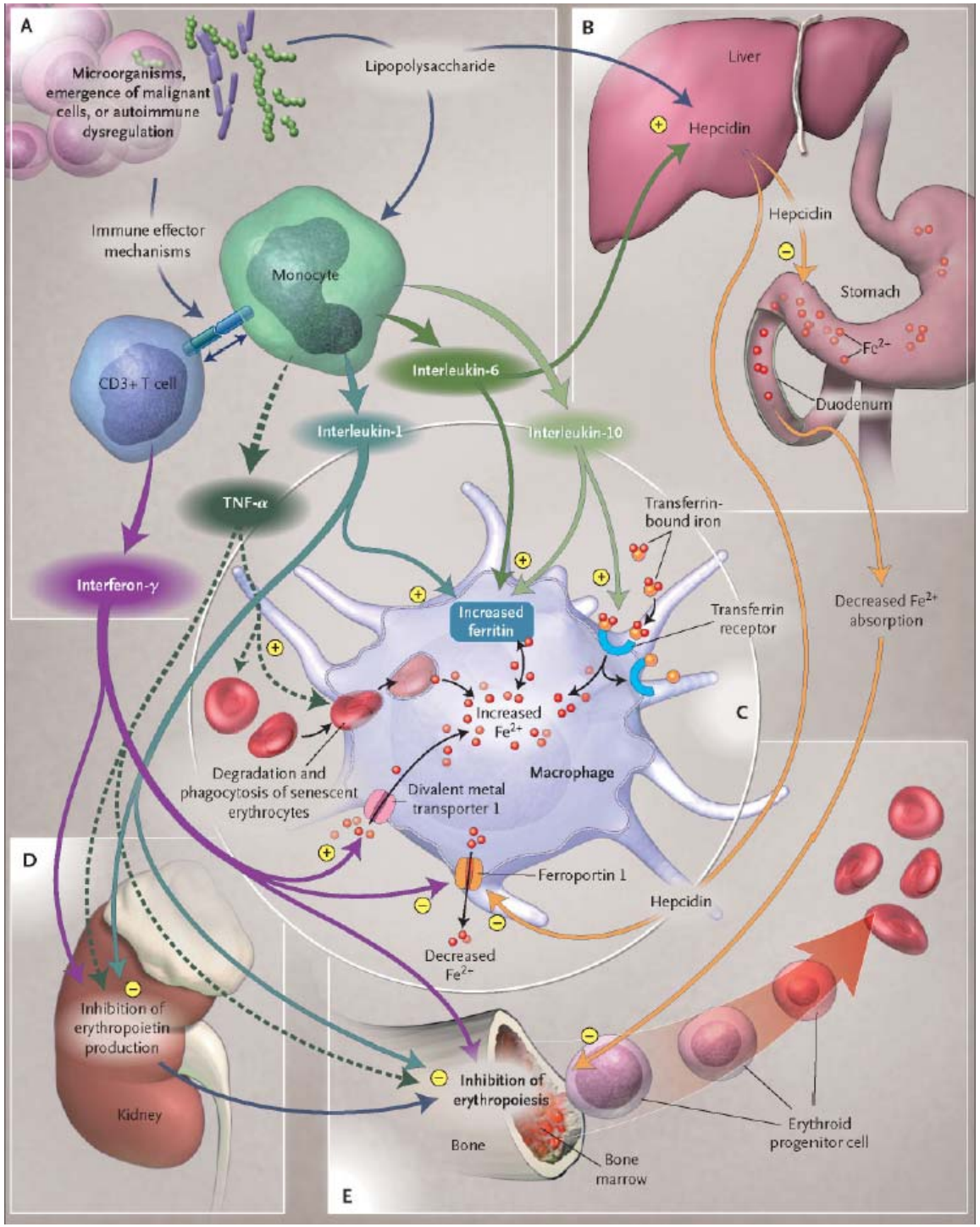


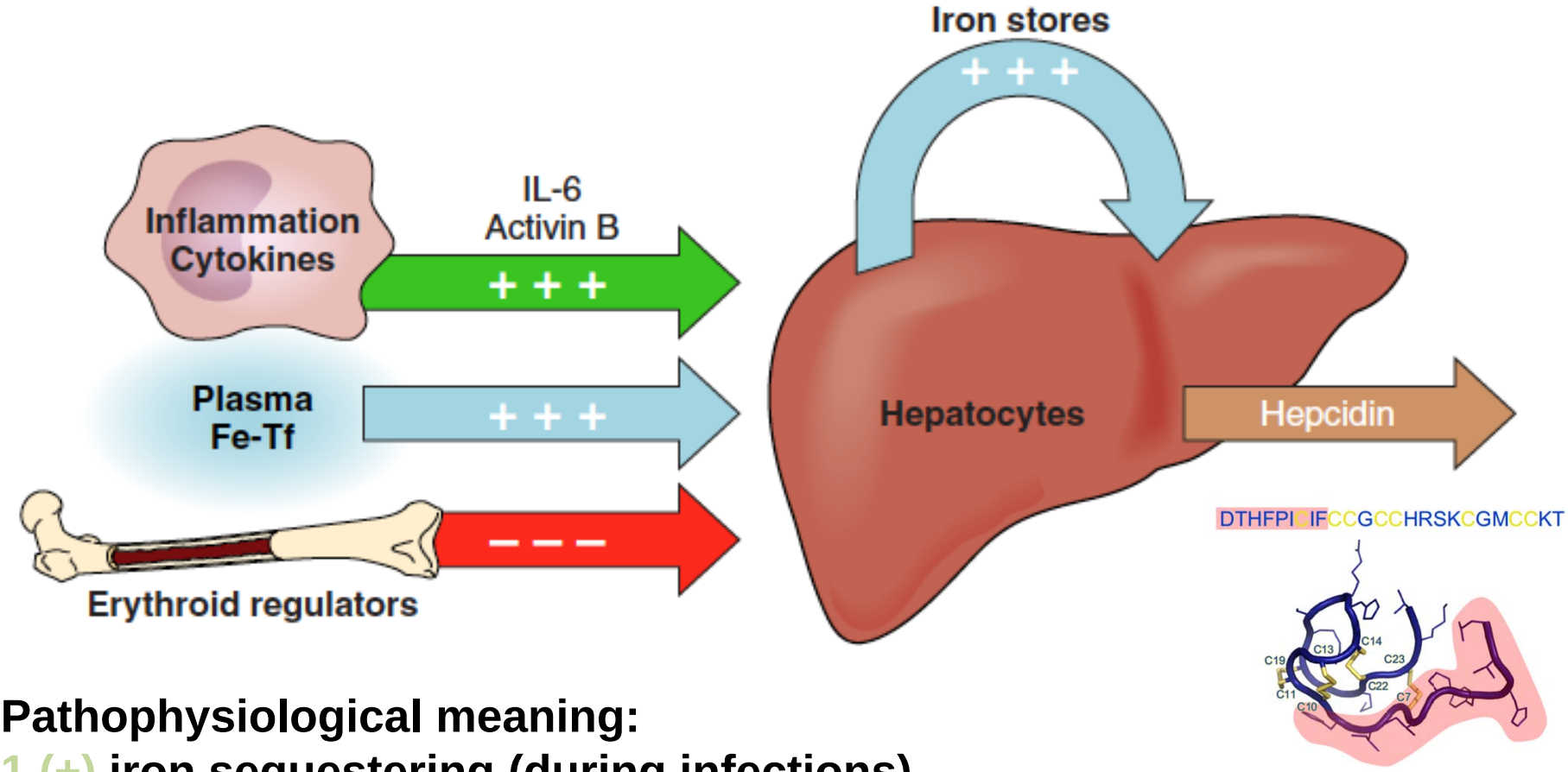
ANEMIA OF CHRONIC DISEASE OR "ANEMIA OF INFLAMMATION"

- ✓ **Impaired iron metabolism (hepcidin-induced "macrophage block and hypoferremia reducing iron availability to invading pathogens)**
- ✓ **Cytokine-induced impaired proliferation of erythroid progenitors**
- ✓ **Blunted EPO response**

Weiss G, N Engl J Med 2005



Signals regulating hepcidin



Pathophysiological meaning:

1 (+) iron sequestering (during infections)

2 (+) classic homeostatic loop

3 (-) matching iron absorption with erythropoiesis requirements

Ganz T, Physiol Rev 2013 (adapted DG)

BLOOD

The Journal of The American Society of Hematology

VOL 84, NO 6 SEPTEMBER 15, 1994

PERSPECTIVE

Regulators of Iron Balance in Humans

By Clement Finch

THE STORE REGULATOR

The normal US adult male with no unphysiologic blood loss has iron stores of 1,000 ± 300 mg as derived from plasma ferritin and phlebotomy studies.[14] Whereas it is not known whether excretion exerts any regulatory effect in the normal individual, it has been repeatedly shown by radioiron measurements, using radioiron salts or food labeled biosynthetically with radioiron, that non-heme iron absorption is inversely related to iron stores.[15,16] Absorption from a test meal is high if iron stores are depleted and is suppressed if iron stores are enlarged.[17] This regulation is so predictable in normal subjects that plasma ferritin measurements of iron stores have been used to predict absorption from a meal of known availability.[18] The highly available heme iron is much less affected by the status of iron stores,[19,20] but has seemed of secondary importance in considerations of iron deficiency because of its limited intake by most of the world's needy population.

THE ERYTHROID-REGULATOR

There are situations in which larger amounts of dietary iron are absorbed than can be attributed to the store-regulator. For example, phlebotomized subjects on a normal diet have been shown by balance studies to replace 3 to 4 mg of iron loss in addition to their excretory loss.[31,32] An equal or greater amount is absorbed by patients with thalassemia in the face of enlarged iron stores.[33] Even more iron may be absorbed if available iron intake is increased. Patients with iron deficiency anemia receiving therapeutic doses of iron can absorb 20 to 40 mg/d as long as their anemia is still present,[34] but the amount decreases as soon as the anemia is alleviated.[35] Similar amounts are absorbed by individuals with normal iron stores whose marrow is stimulated by erythropoietin.[20] Thus, there is a second regulator operating independently of iron stores.

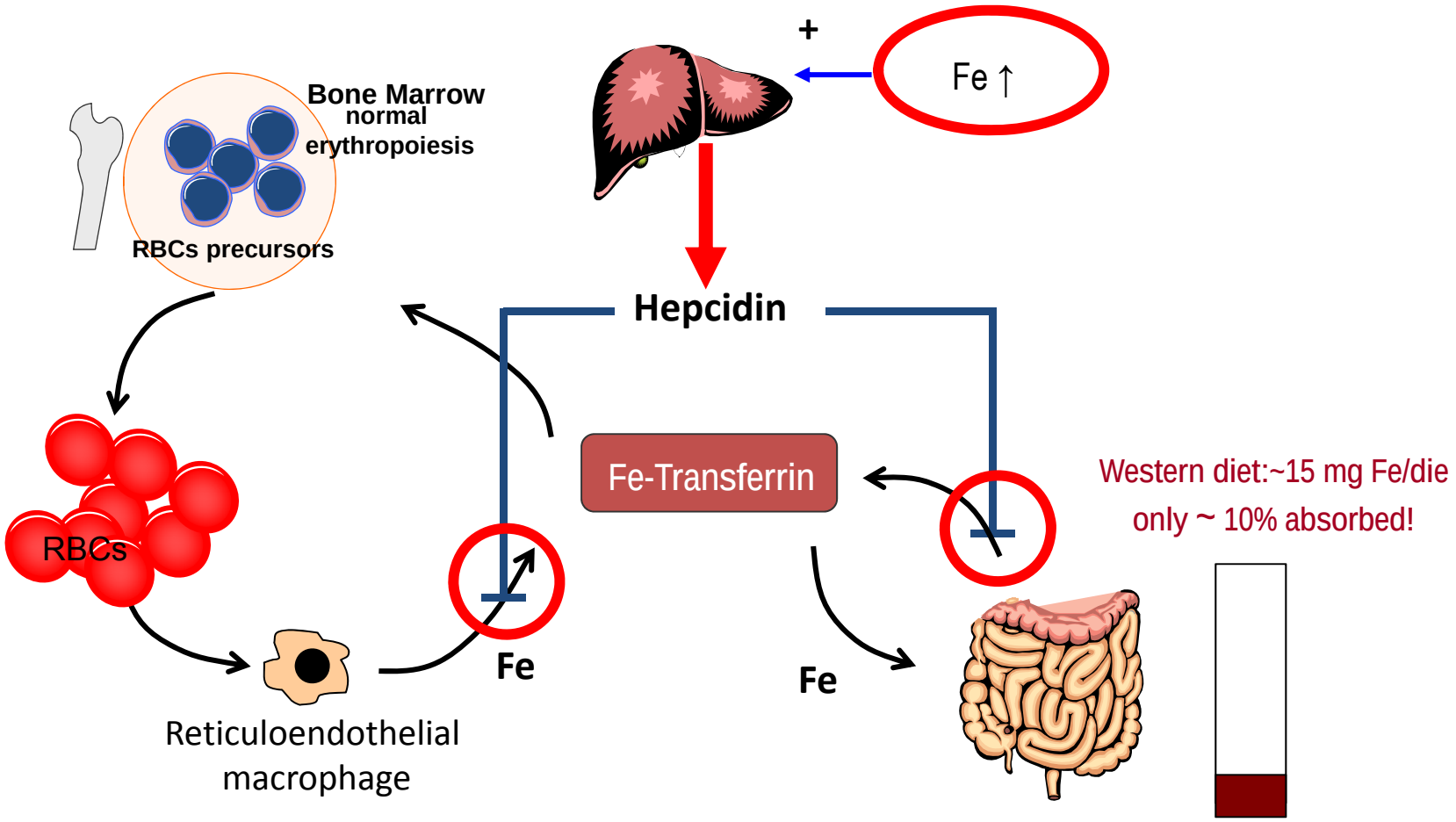
The “erythroid regulator” of iron metabolism

Ferrokinetic studies in humans:

- ✓ Normal iron absorption = 1-2 mg/day
- ✓ Pts. with Iron deficiency anemia receiving therapeutic doses of iron can absorb > 20 mg/day
- ✓ Similar amount can be absorbed by subjects with normal iron stores when erythropoiesis is stimulated (i.e. after blood loss or by EPO administration)

Finch C, Blood 1994

BLOOD LOSS SUPPRESSES HEPCIDIN TO INCREASE IRON AVAILABILITY FOR "STRESS" ERYTHROPOIESIS



Adapted from Castagna A, J Proteomics 2010

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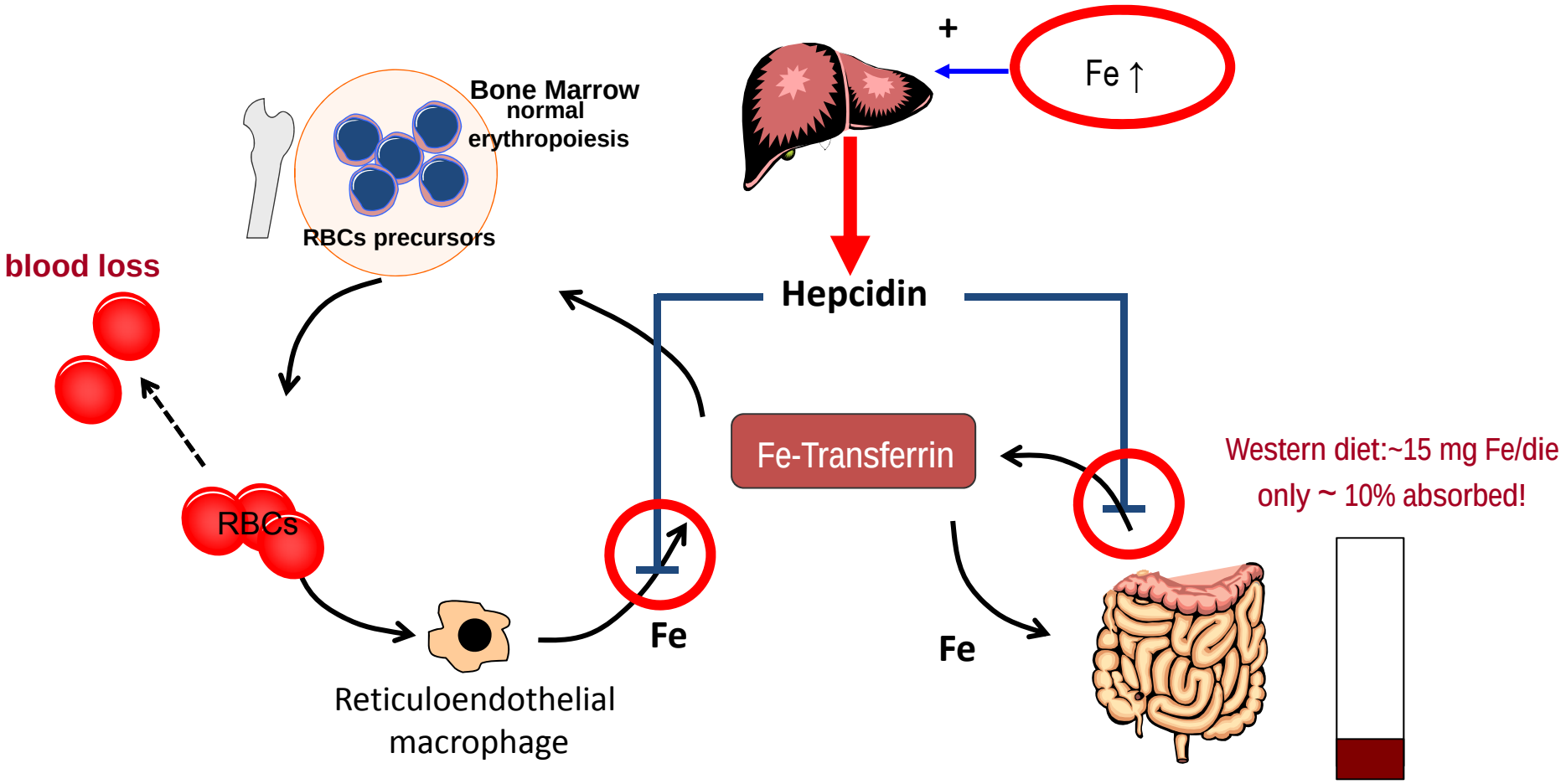

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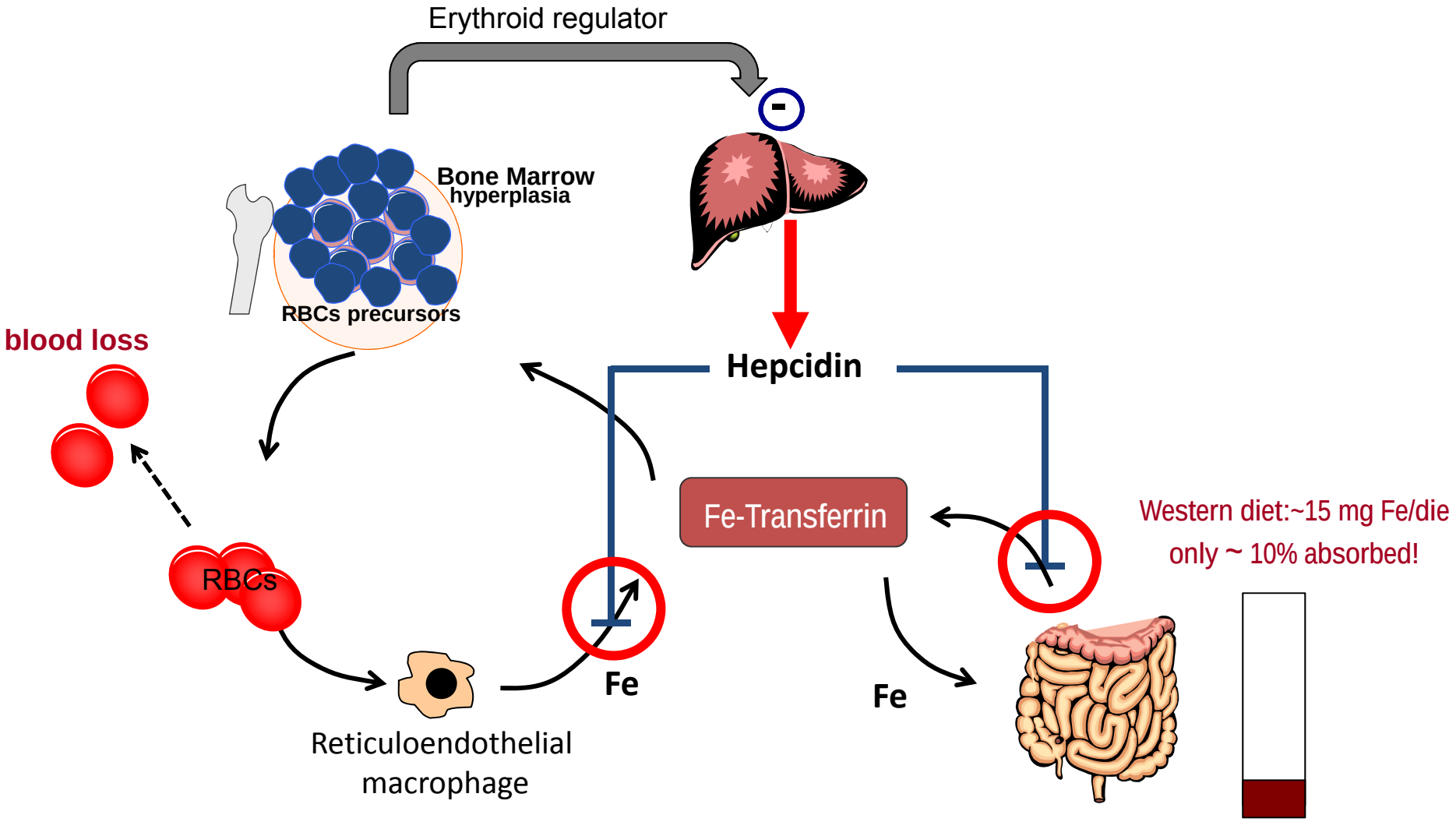

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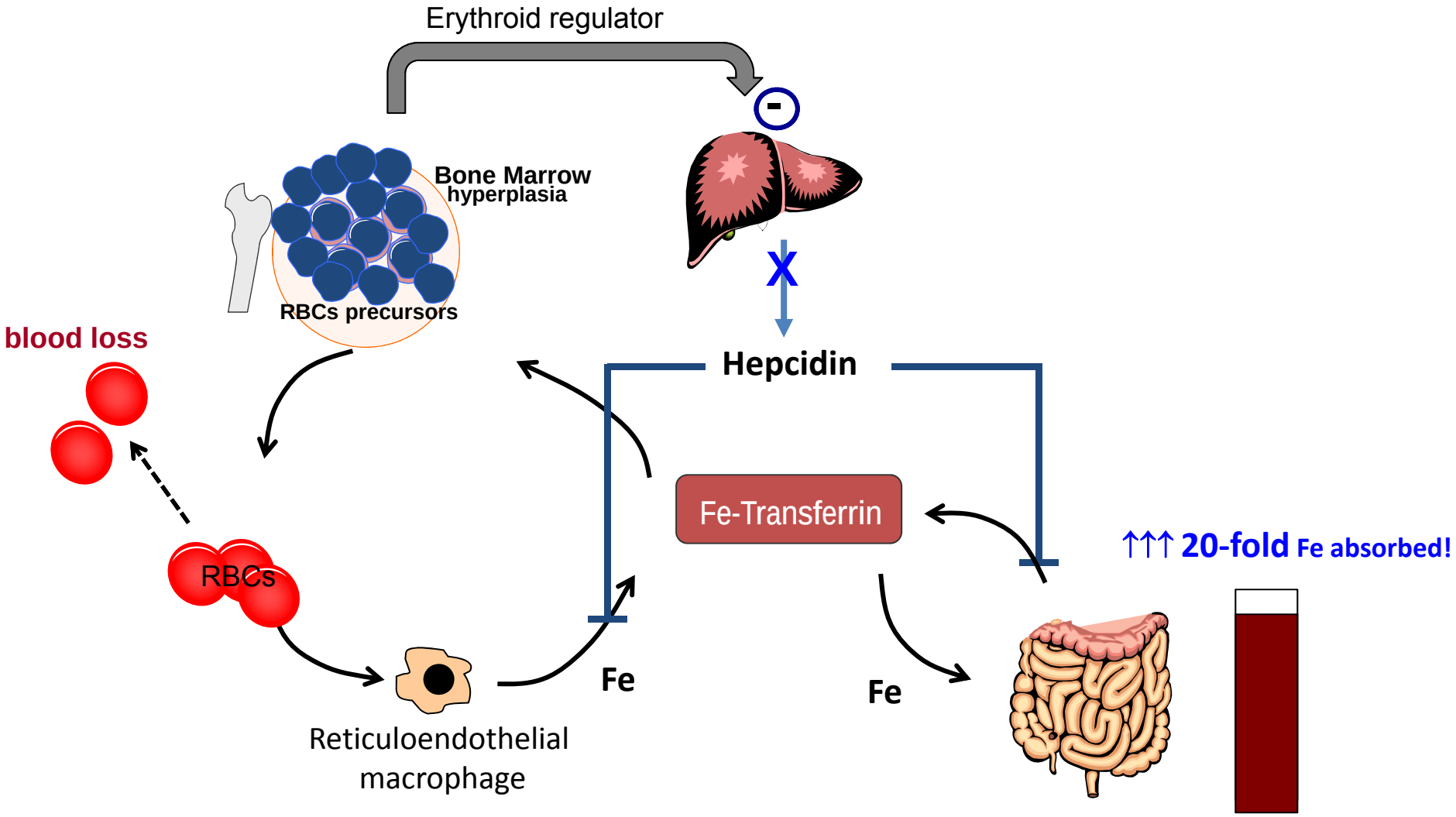
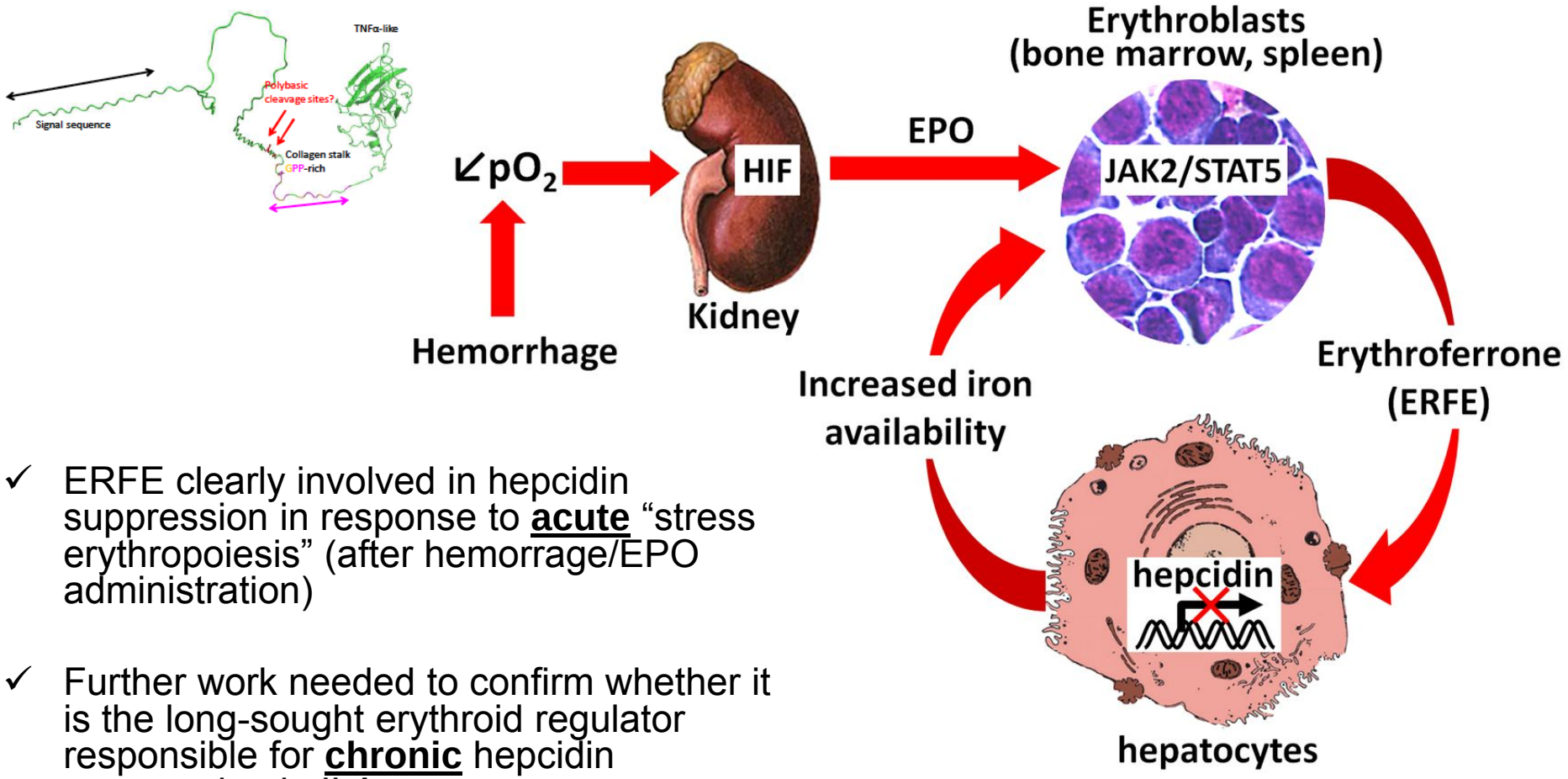


Adapted from Castagna A, J Proteomics 2010

Erythroferrone (ERFE) the newly identified erythroid regulator
Proposed mechanism of action

nature genetics

Identification of erythroferrone as an erythroid regulator of iron metabolism Kautz L, Nat Genet 2014



- ✓ ERFE clearly involved in hepcidin suppression in response to **acute** "stress erythropoiesis" (after hemorrage/EPO administration)
- ✓ Further work needed to confirm whether it is the long-sought erythroid regulator responsible for **chronic** hepcidin suppression in ILAs.

Kautz L , Nemeth, E Blood 2014

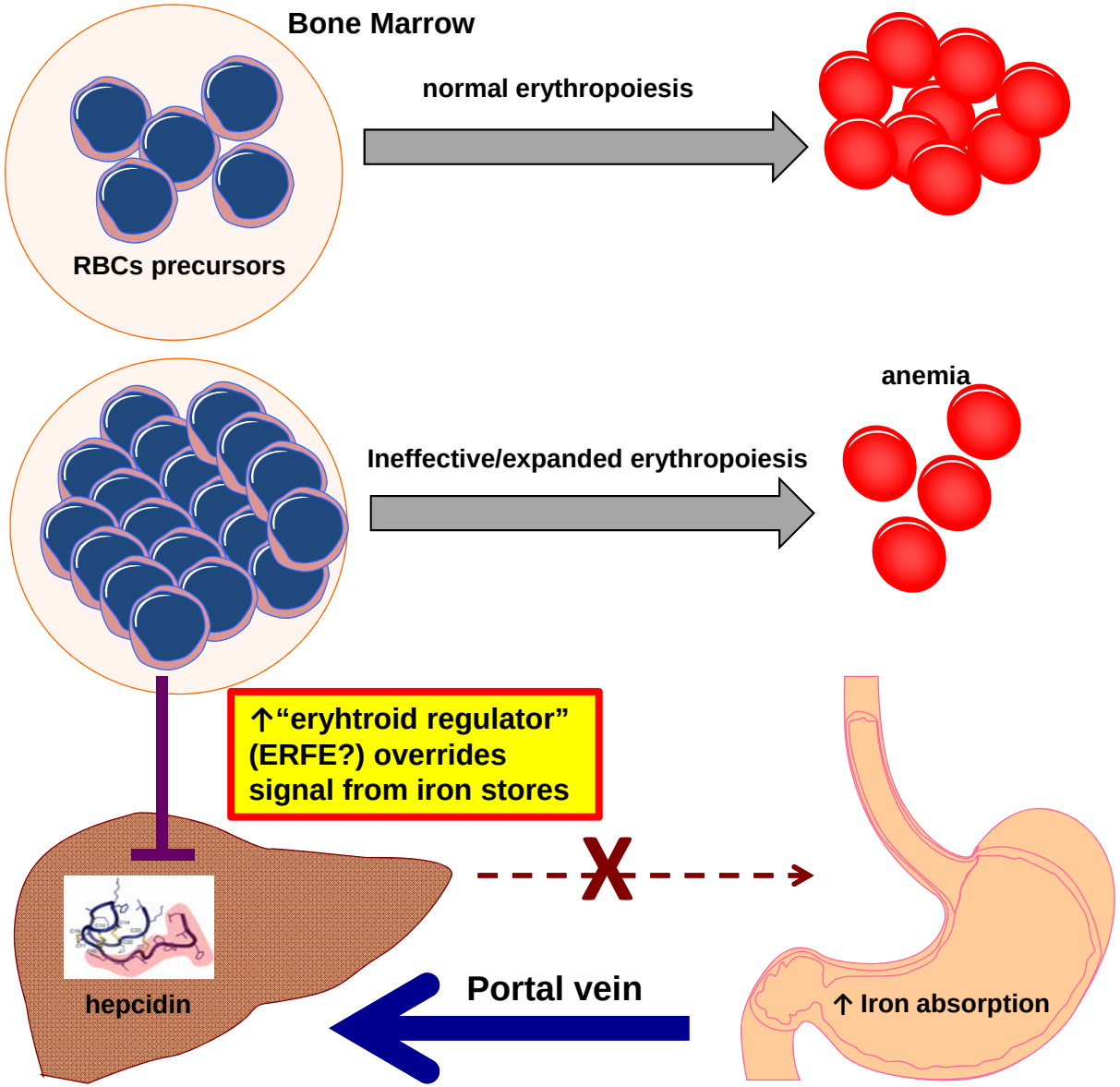
Genetic disorders leading to systemic Iron overload

Disorder	Gene and Inheritance	Age at Presentation	Neurologic Symptoms	Anemia	Transferrin Saturation
Impaired hepcidin–ferroportin axis					
HH type I	*HFE*, AR	Adult	No	No	High
HH type IIA	*HFE2*, AR	Child to young adult	No	No	High
HH type IIB	*HAMP*, AR	Child to young adult	No	No	High
HH type III	*TFR2*, AR	Young adult	No	No	High
HH type IVA (atypical HH)	*FP* (LOF), AD	Adult	No	Variable	Low initially
HH type IVB	*FP* (GOF), AD	Adult	No	No	High
Impaired iron transport					
Inadequate release to erythron: aceruloplasminemia	*CP*, AR	Adult	Yes	Yes	Low
Inadequate uptake by erythron					
DMT1 mutations					
Hypotransferrinemia					
Ineffective erythropoiesis					
Thalassemia	*Globin*, AR	Child	No	Yes	High
Congenital sideroblastic anemia	*ALAS2*, XL; *SLC25A38*, AR; *GLRX5*, AR; *ABCB7*, XL	Variable	*ALAS2* and *SLC25A38*: no; *GLRX5* and *ABCB7*: yes	Yes	High
Congenital dyserythropoietic anemia					
Type I	*DAN1*, AR	Child	No	Yes	High
Type II	*SEC23B*, AR	Child	No	Yes	High
Type III	Unknown, AD	Child	No	Yes	High

"IRON LOADING ANEMIAS" (ILAs)

Fleming RE, NEJM 2012

Pathophysiology of iron overload in ILAs



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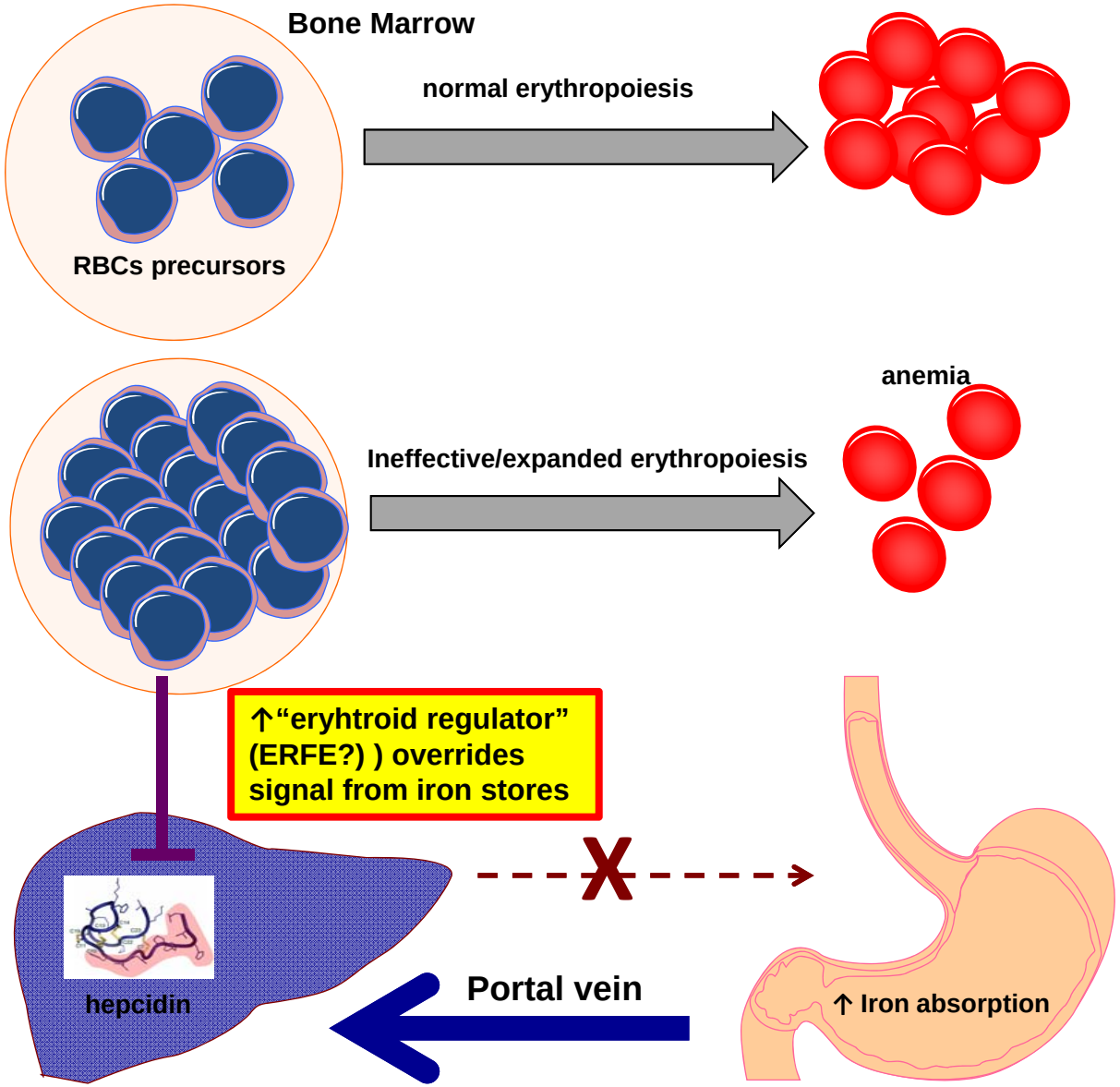
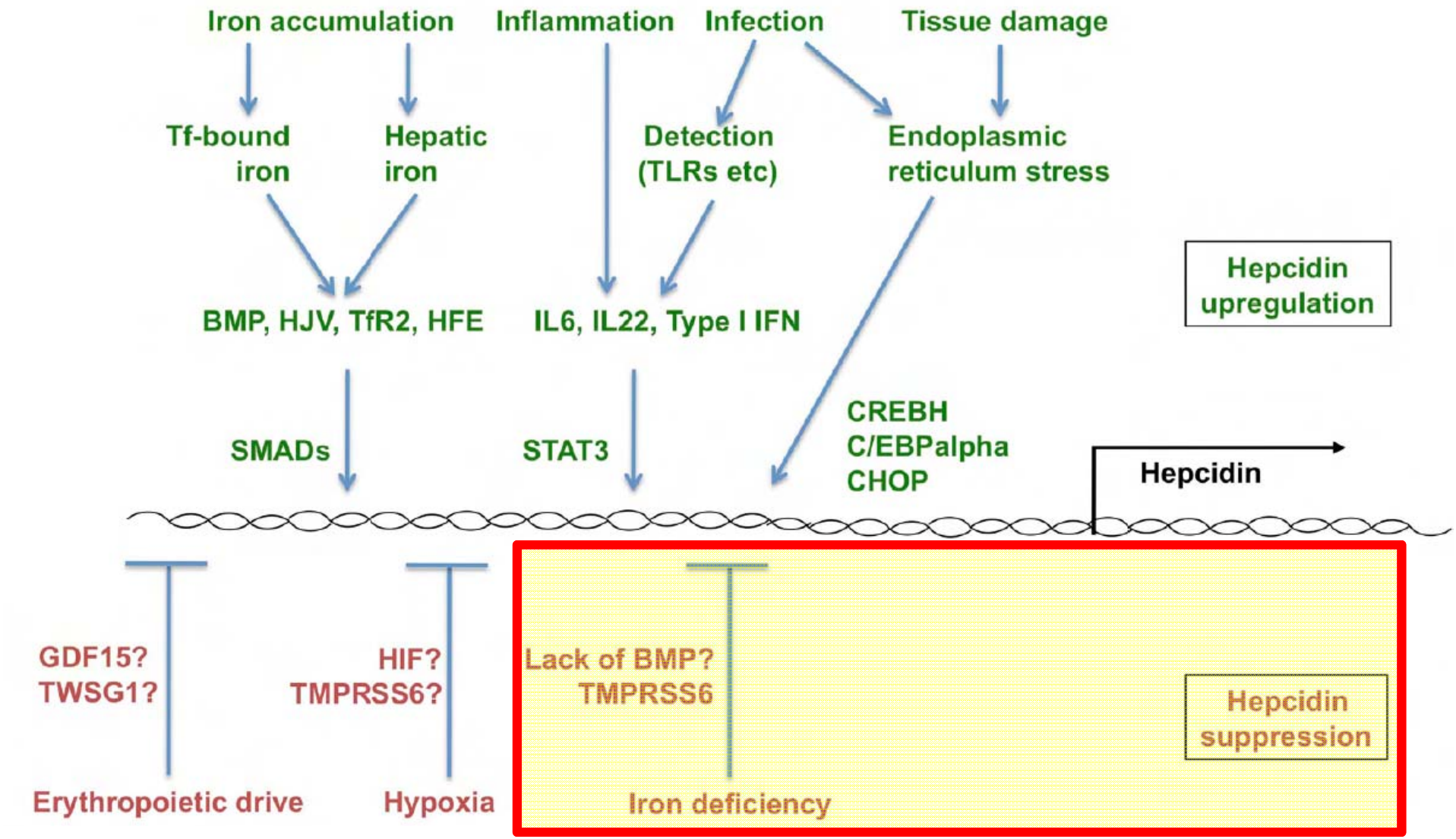


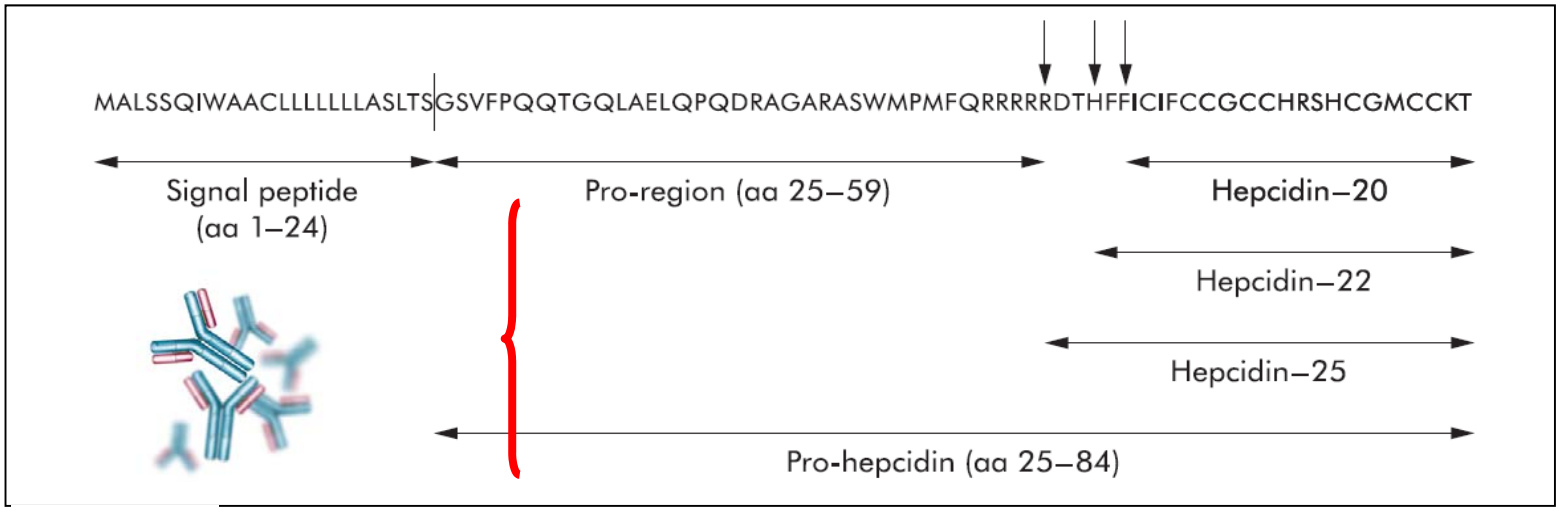
Hepcidin is suppressed in iron deficiency



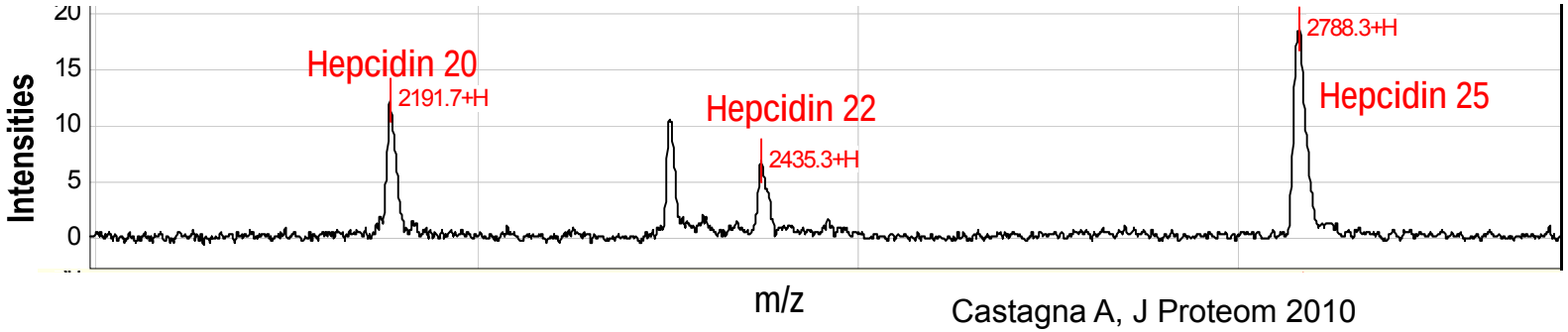
Drakesmith H, Prentice AM, Science 2012

Hepcidin assays (ELISA and MS-based)

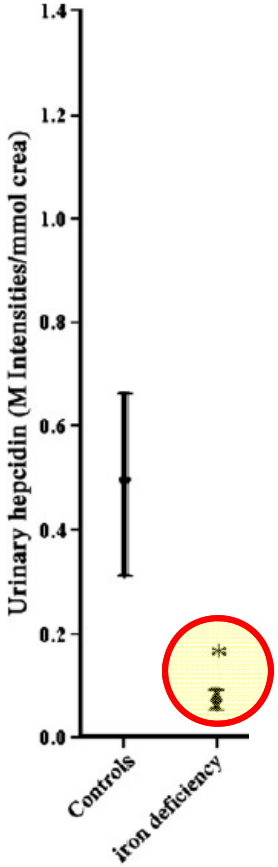
pro-hepcidin and N-terminus truncated isoforms in urine and serum



Kulaksiz H, Gut 2004

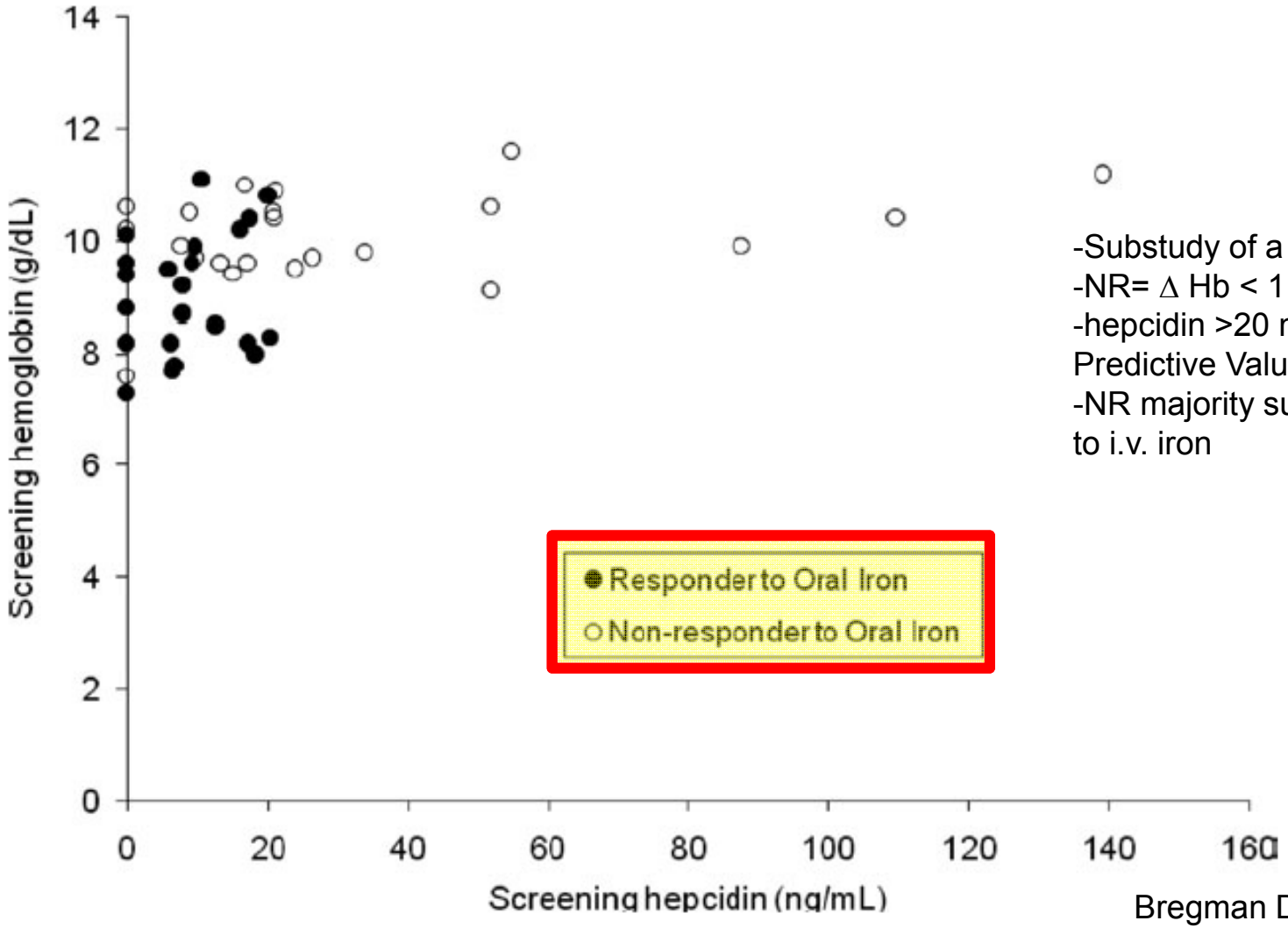


Castagna A, J Proteom 2010



Bozzini C, BCMD 2008

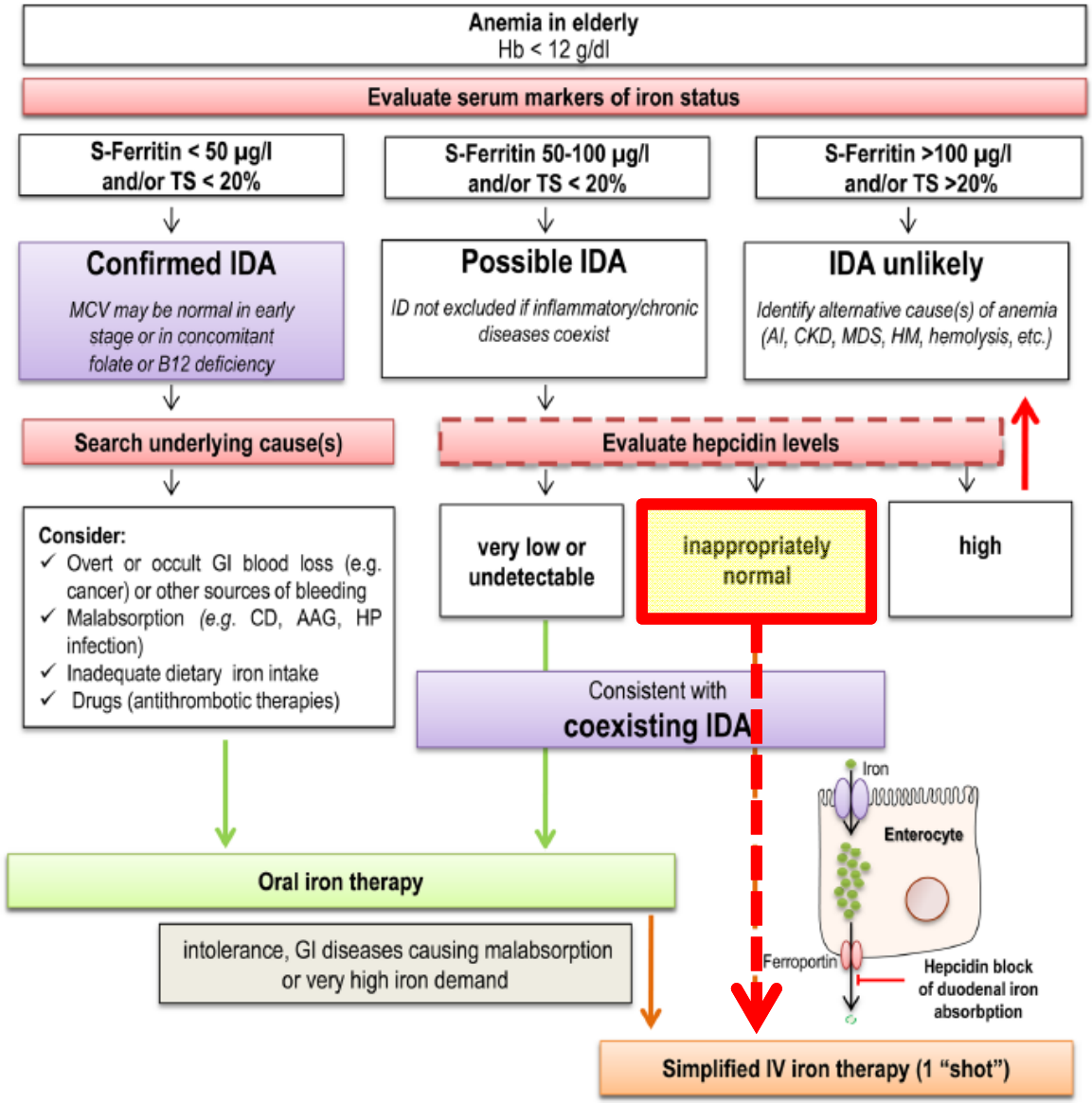
Promise of hepcidin assay in the clinic: predict nonresponsiveness to oral iron in IDA



-Substudy of a phase III clinical trial
-NR= Δ Hb < 1 g/dl after 14 days
-hepcidin >20 ng/ml = 81.4% Positive Predictive Value of NR
-NR majority subsequently responded to i.v. iron

Bregman DB, Am J Hematol 2013

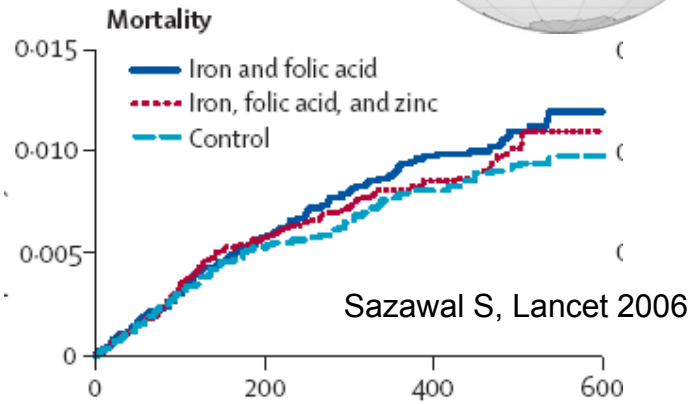
Iron deficiency anemia in elderly: revisited in the hepcidin era



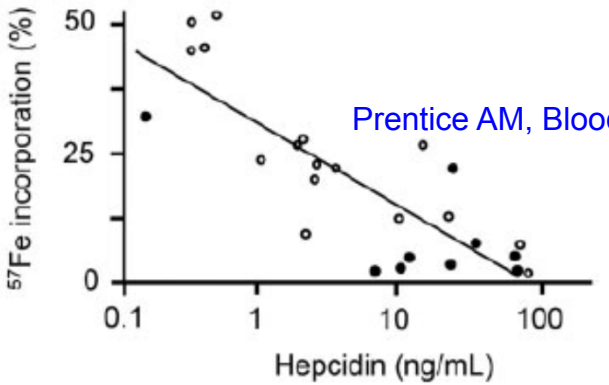
Busti F, Front Pharmacol 2014

The MOST promising application of hepcidin assay
(from a global health perspective)

- I.D. major health problem in children from low-incoming countries.
- The "Pemba" trial: "routine" iron supplementation is not the solution, but rather can ↑ mortality due to infections.



- Hepcidin is the major predictor of RBC iron incorporation in anemic African (Gambia) children, indicating iron utilization for children's growth rather than for the growth of infectious agents.



Hepcidin as a point-of-care index guiding "safe" and effective iron therapy

Pharmacology of hepcidin

Review

The pathophysiology and pharmacology of hepcidin

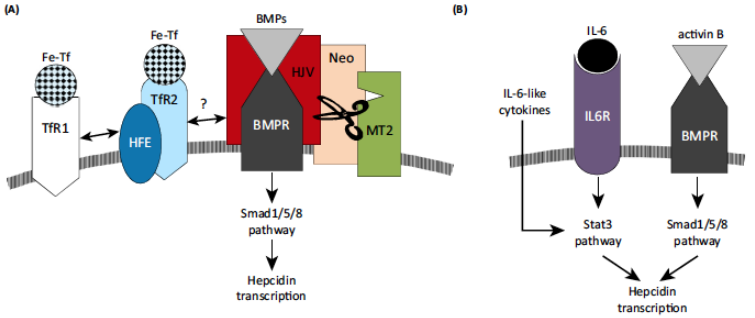


Table 1. Principles of hepcidin-targeting therapeutic approaches

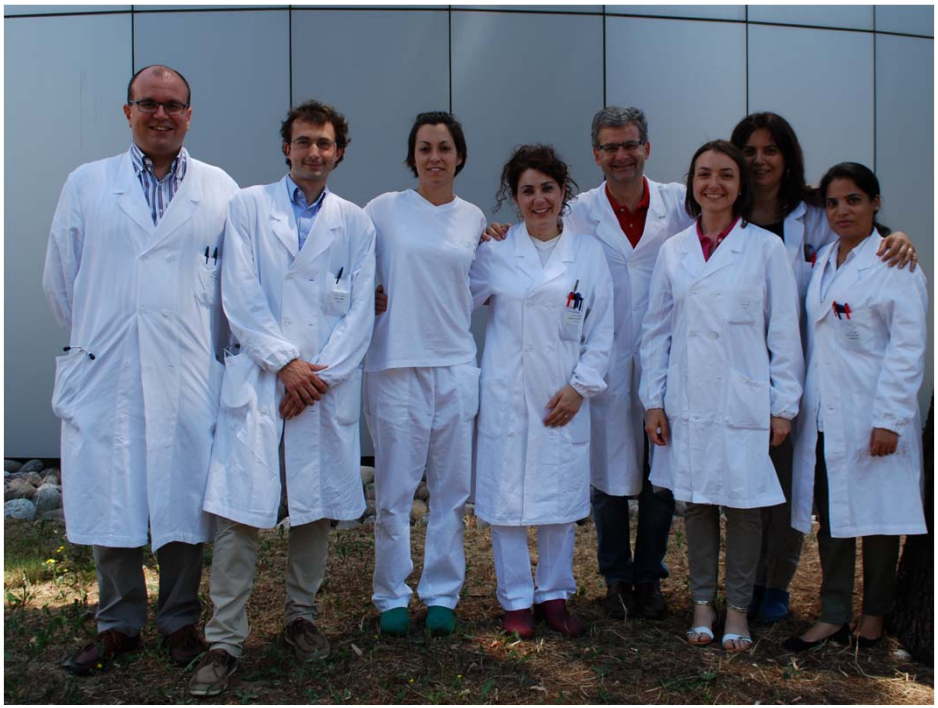
Therapeutic approach	Targeted disease	Mode of action	Agents
Hepcidin agonists	Iron overload (hereditary hemochromatosis and iron-loading anemias)	Hepcidin mimics	Minihepcidins [47]
		Stimulators of hepcidin production	Gene silencing of TMPRSS6 [50,51]
			BMP pathway agonists [52]
Hepcidin antagonists	Iron-restricted anemias (anemia of inflammation, anemia of chronic kidney disease, anemia of cancer, IRIDA)	Suppressors of hepcidin production	BMP pathway inhibitors [54,56,74]
			Anti-inflammatory agents [60–62]
			Erythropoiesis-stimulating agents [65]
			Gene silencing of hepcidin and its regulators [66][a]
		Hepcidin peptide neutralizing binders	Anti-hepcidin antibodies [67][b]
			Anticalins [68]
			Spiegelmers [69]
		Agents interfering with hepcidin–ferroportin interaction	Anti-ferroportin antibodies [71]
			Thiol modifiers [72]

[a] http://ir.isispharm.com/phoenix.zhtml?c=222170&p=irol-newsArticle&ID=1828284&highlight=

[b] http://www.clinicaltrials.gov/ct2/show/NCT01340976

Ruchala P & Nemeth E, Trends Pharmacol Sci 2014

Il “Gruppo Interdisciplinare per le Malattie del Ferro” (AOUI Verona)



GRUPPO INTERDISCIPLINARE MALATTIE DEL FERRO

GIMFer

AOUI VERONA

http://www.gimferverona.org

U.O.C. partecipanti:

1. Medicina Generale a indirizzo Immuno-Ematologico ed Emocoagulativo
2. Laboratorio Analisi
3. Servizio Trasfusionale
4. Radiologia
5. Anatomia Patologica
6. Fisica per Tecnologie Biomediche