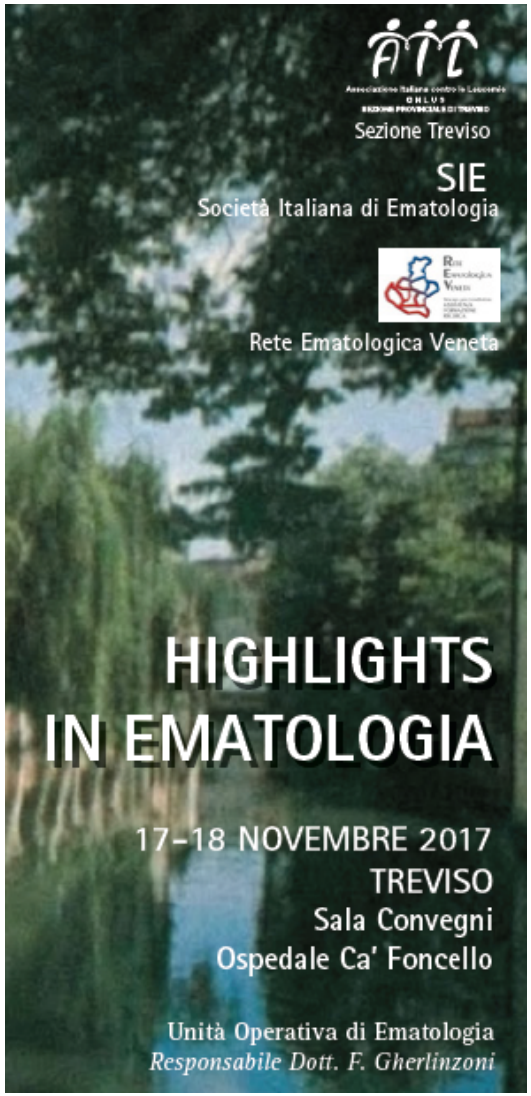


# Meccanismi di tossicità del ferro

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Centro di Riferimento per i Disordini del Metabolismo del Ferro
EuroBloodNet (European Reference Network for Rare Hematological Diseases)

# Disclosure – D.G.

La Jolla Pharmaceutical (Advisory Board)

Silence Therapeutics (Advisory Board)

Vifor Fresenius Medical Pharma (Speaker)

Personal Genomics s.r.l. (Spin-Off of the University of Verona – founder)

# Outline

- ✓ Overview of recent insights on the regulation of iron metabolism. Focus on **mechanisms of iron overload in "iron loading anemias"**.

- ✓ General **mechanisms of iron toxicity** (**liver model**)

- ✓ **Not all iron overload are the same**: pathophysiological differences between **non transfusion dependent** and **transfusion-dependent** anemias (revisited in the "hepcidin era").

# 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**

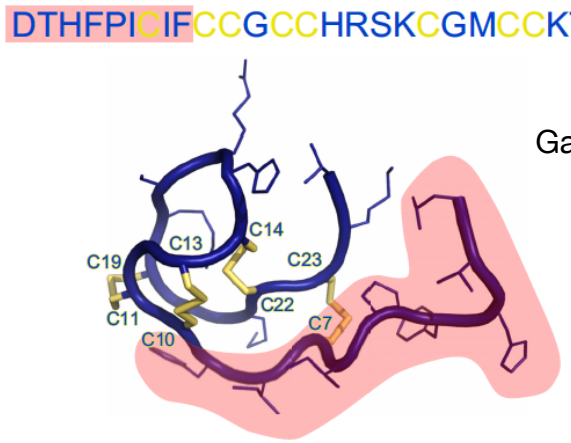
**low**
↓
**anemia**
**neuromuscolar impairment**

**excess**
↓
**iron overload**
**toxic organ damage**

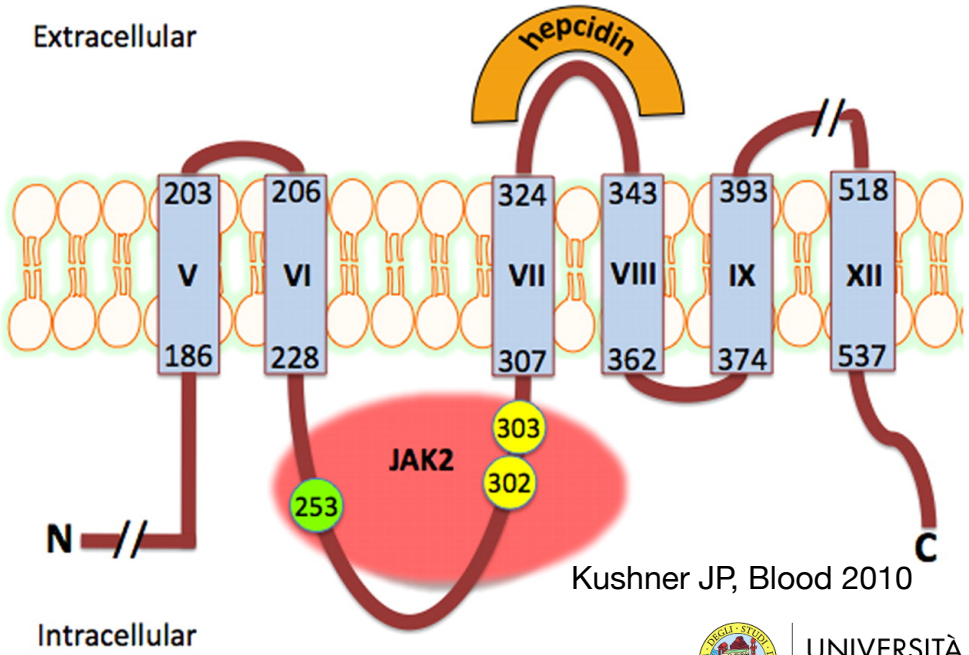
# THE HEPCIDIN-FERROPORTIN AXIS

- **Hepcidin**: small peptide mainly produced by the **liver**

- interact with **its receptor** (**Ferroportin**, the only known iron exporter from the cells, ubiquitous but highly expressed in **duodenal cells, macrophages, hepatocytes**)




Ganz T, Physiol Rev 2013

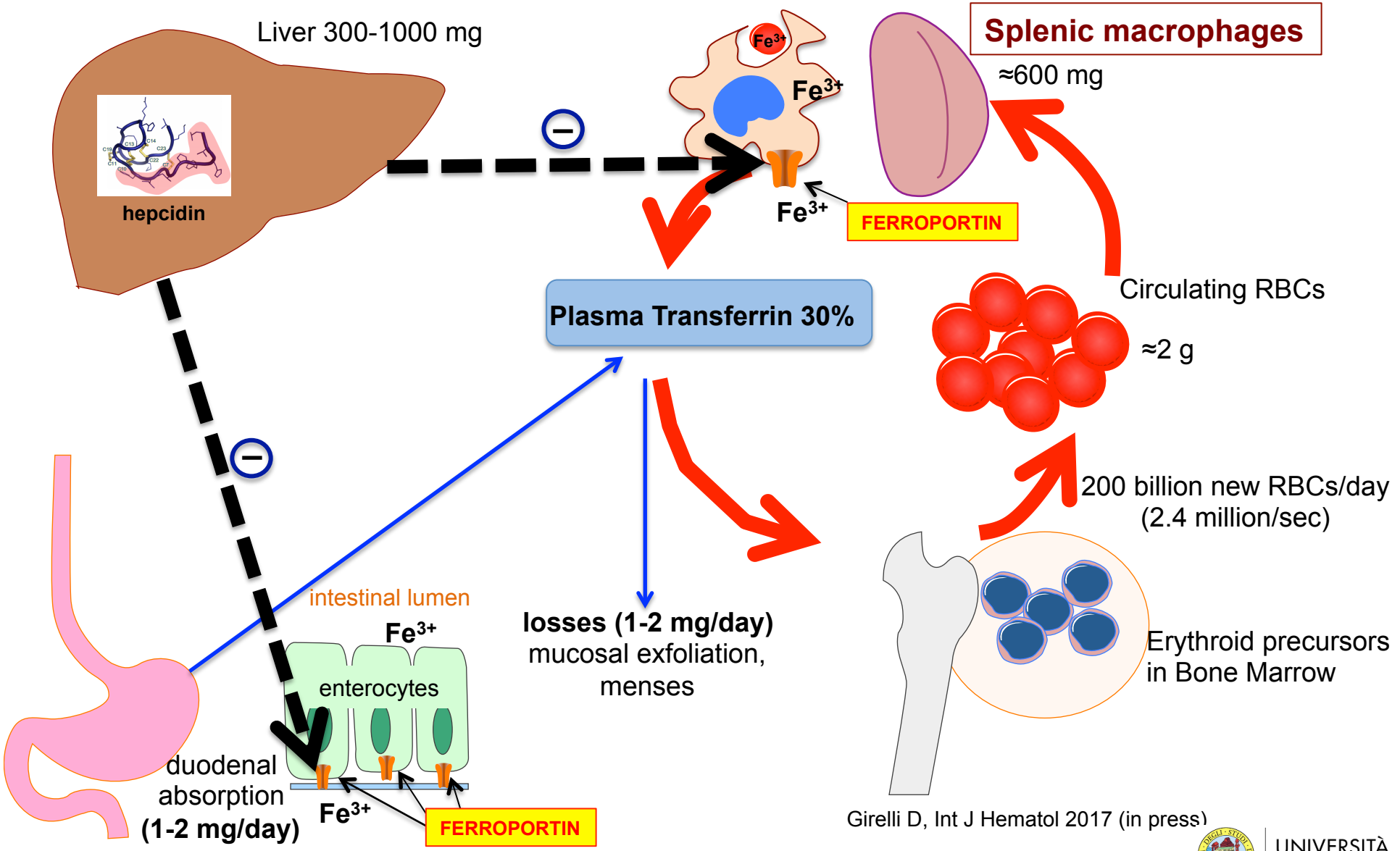



Kushner JP, Blood 2010



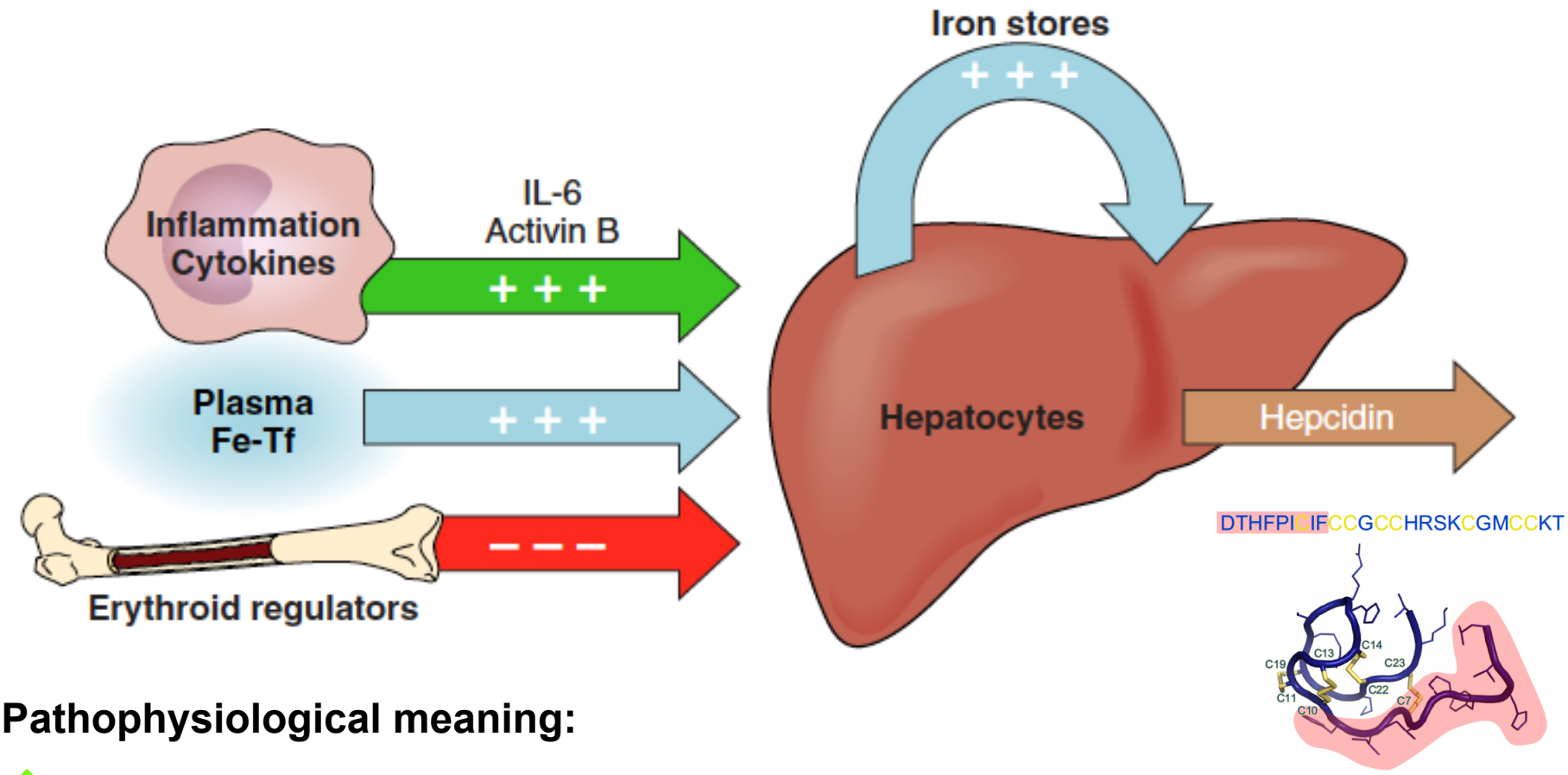
UNIVERSITÀ di VERONA

# Systemic "ecological" iron homeostasis




Girelli D, Int J Hematol 2017 (in press)

# Signals modulating hepcidin




**Pathophysiological meaning:**

- **iron sequestration (during infections)**
- **classic homeostatic loop**
- **matching iron absorption with erythropoiesis' needs**

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
- ✓ IDA pts. receiving therapeutic doses of iron can absorb > 20 mg/day
- ✓ Similar amount can be absorbed by subjects with normal iron stores whose erythropoiesis is stimulated by EPO
- ✓ In ILAs **erythroid signals override signals from the replete stores**, causing iron overload.

Finch C, Blood 1994

# Iron Loading Anemias (ILAs)

## INHERITED DISORDERS

### *Thalassemia syndromes*

Camaschella C, BJH 2015

- NTDT β-thalassemia intermedia
- NTDT hemoglobin E/β-thalassemia
- NTDT hemoglobin H or alpha-thalassemia

### *Congenital Dyserythropoietic Anemias*

- Type I (*CDAN-1* mutations)
- Type II (*SEC23B* mutations)
- Type III (*KIF23* mutations)
- Variants (*KLF1* and *GATA1* mutations)

### *Inherited Sideroblastic Anemias*

- X-linked SA (*ALAS2* mutations)
- Recessive sideroblastic anemia (*SLC25A38* mutations, *GLRX5* mutations)

### *Others*

### *Haemolytic anaemias*

- Pyruvate kinase deficiency (selected cases)
- Sickle cell anemia (selected cases)

## ACQUIRED DISORDERS

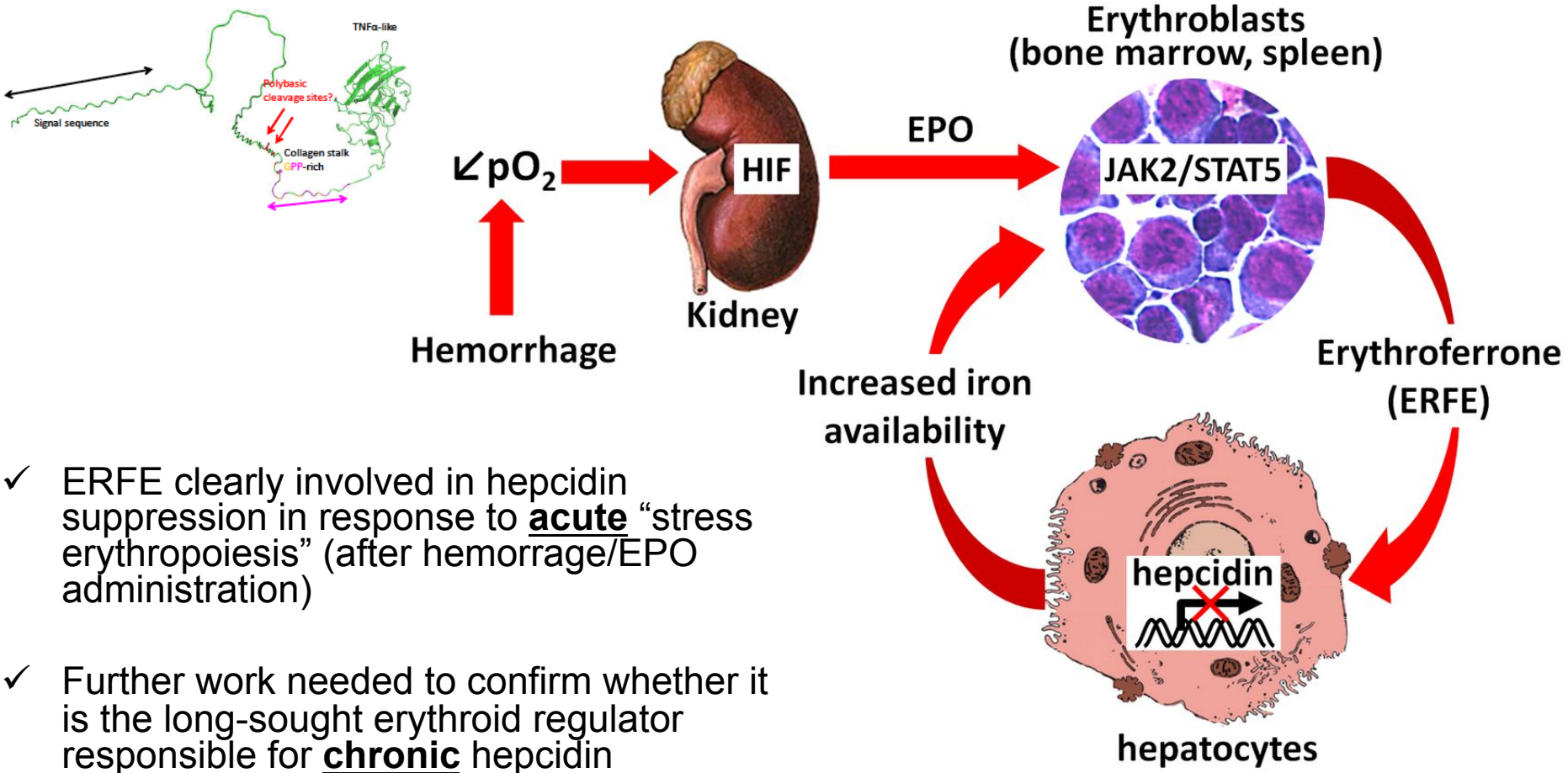
### *Myelodysplastic syndromes (MDS)*

- Refractory anemia with ringed sideroblasts (RARS)
- Refractory Cytopenia With Unilineage Dysplasia (RCUD) once Refractory Anemia (RA)

# Erythroferrone (ERFE)

nature genetics

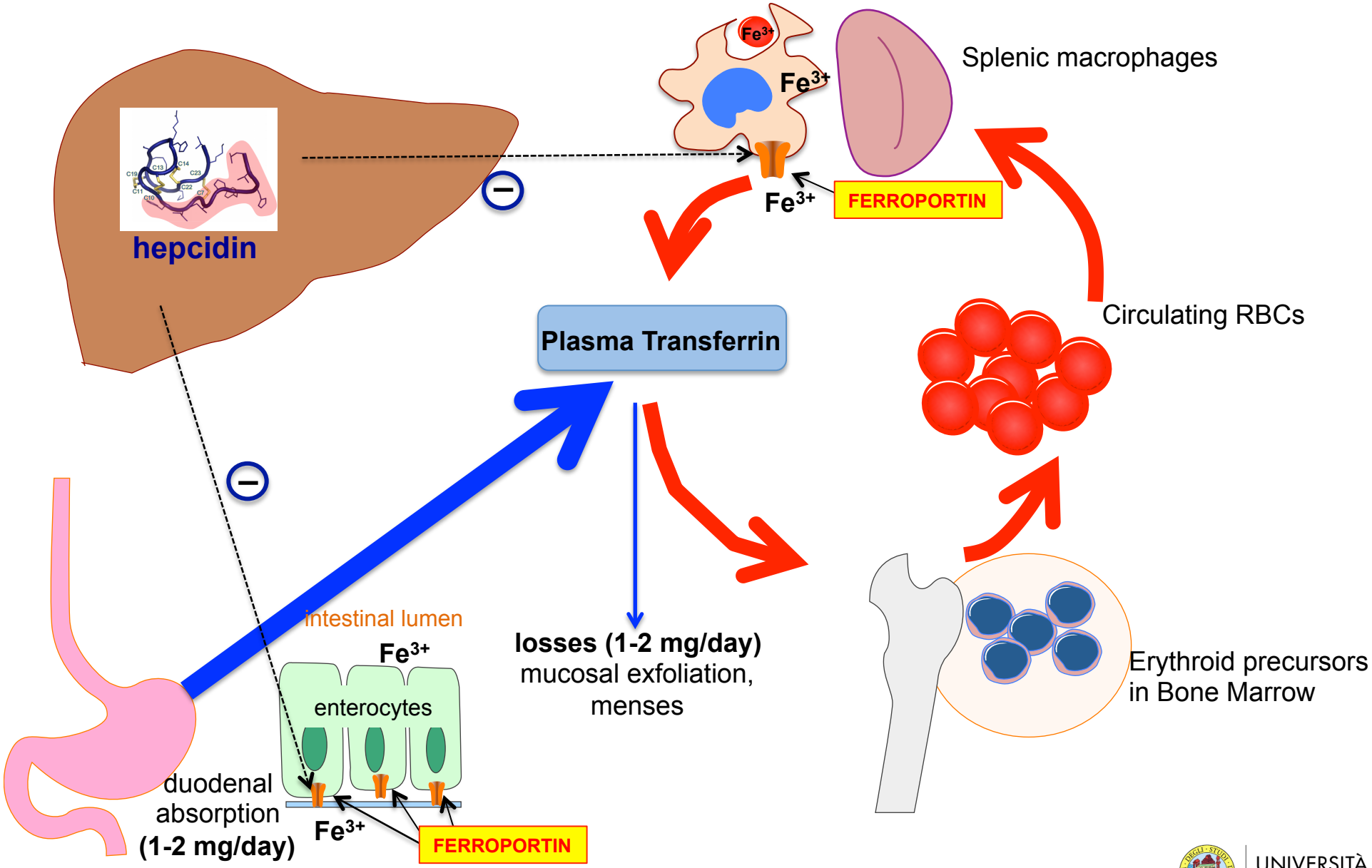
Identification of erythroferrone as an erythroid regulator of iron metabolism Kautz L, Nat Genet 2014



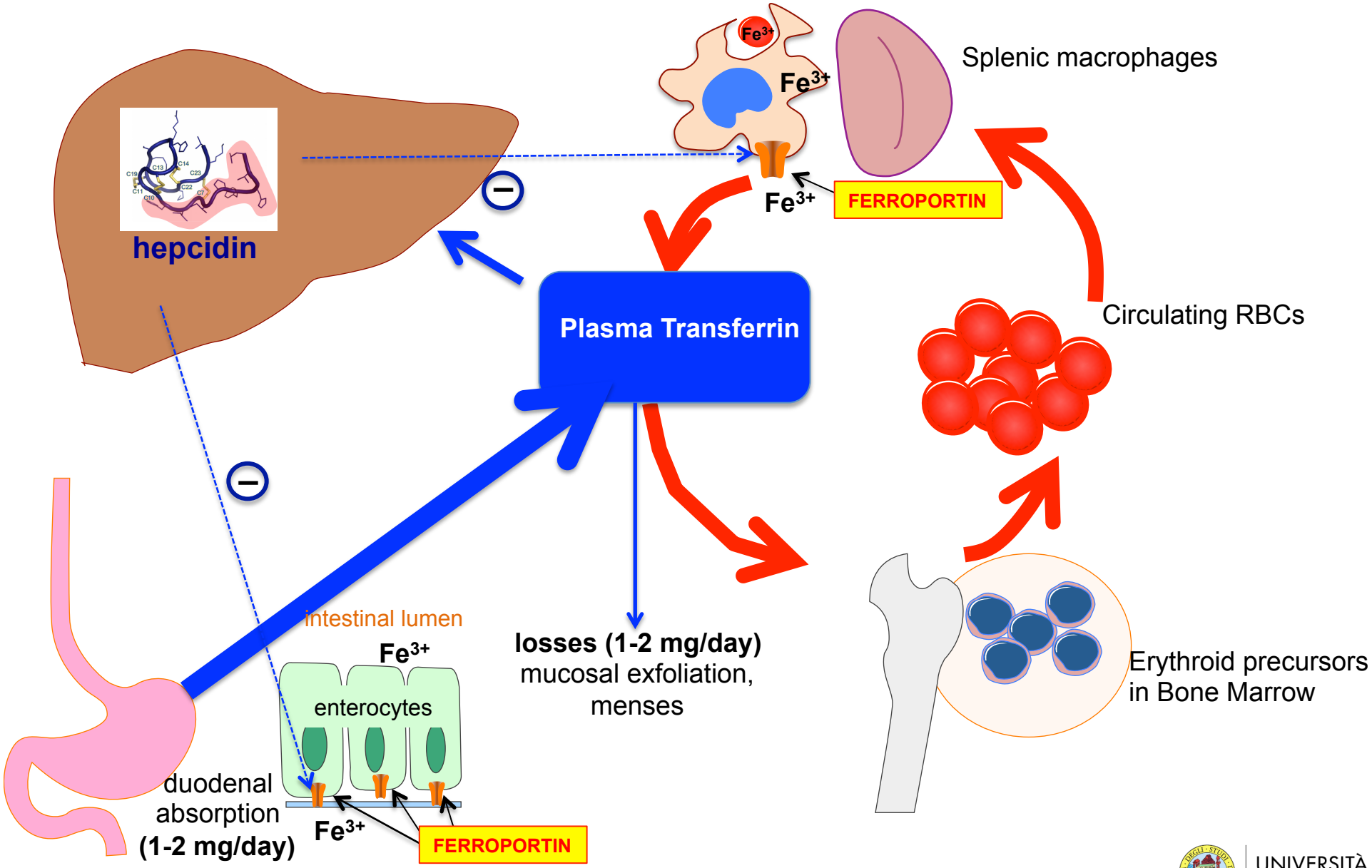

- ✓ ERFE clearly involved in hepcidin suppression in response to **acute** "stress erythropoiesis" (after hemorrage/EPO administration)
- ✓ Further work needed to confirm whether it is the long-sought erythroid regulator responsible for **chronic** hepcidin suppression in ILAs.

Kautz L , Nemeth, E Blood 2014

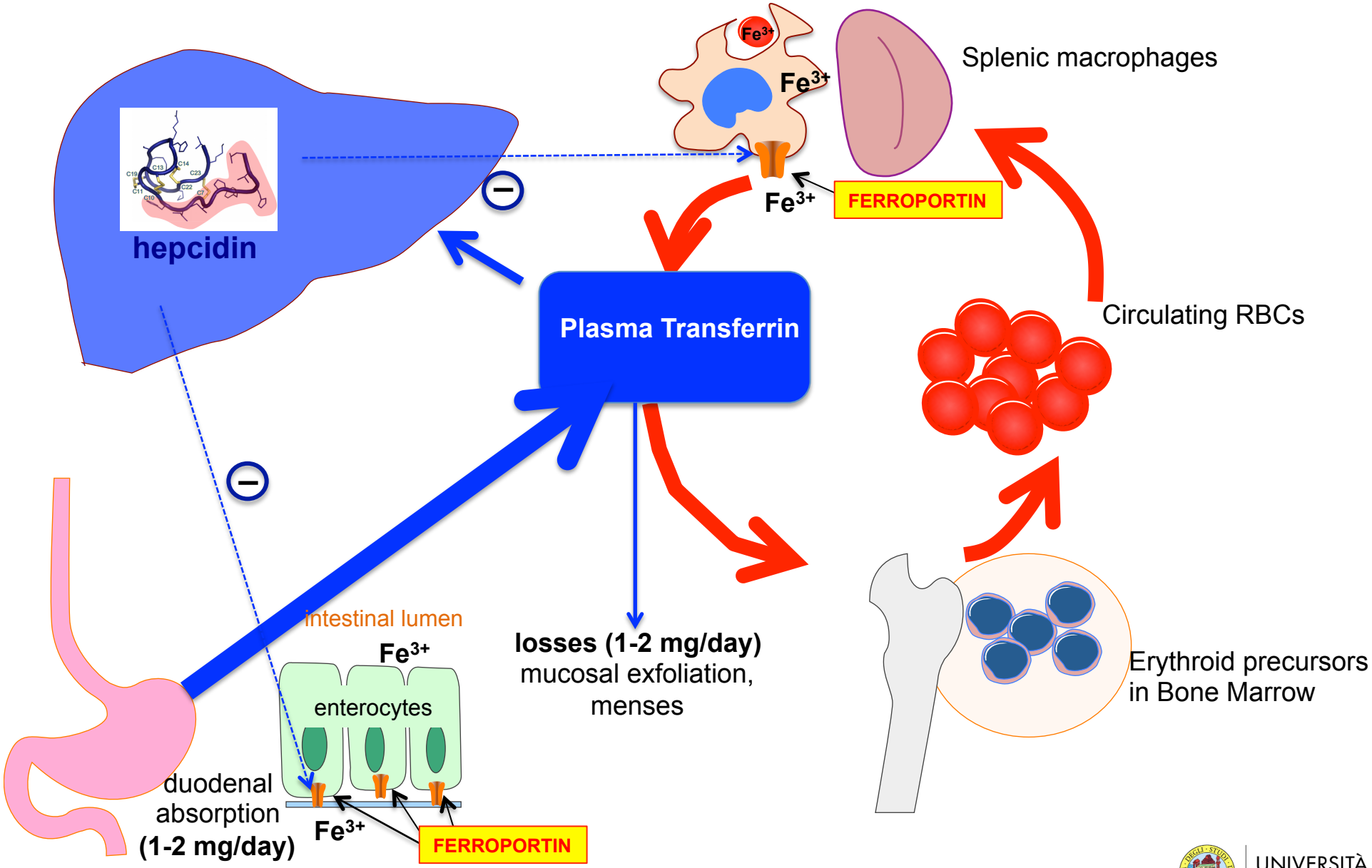
# Hepcidin deficiency leads to liver iron overload



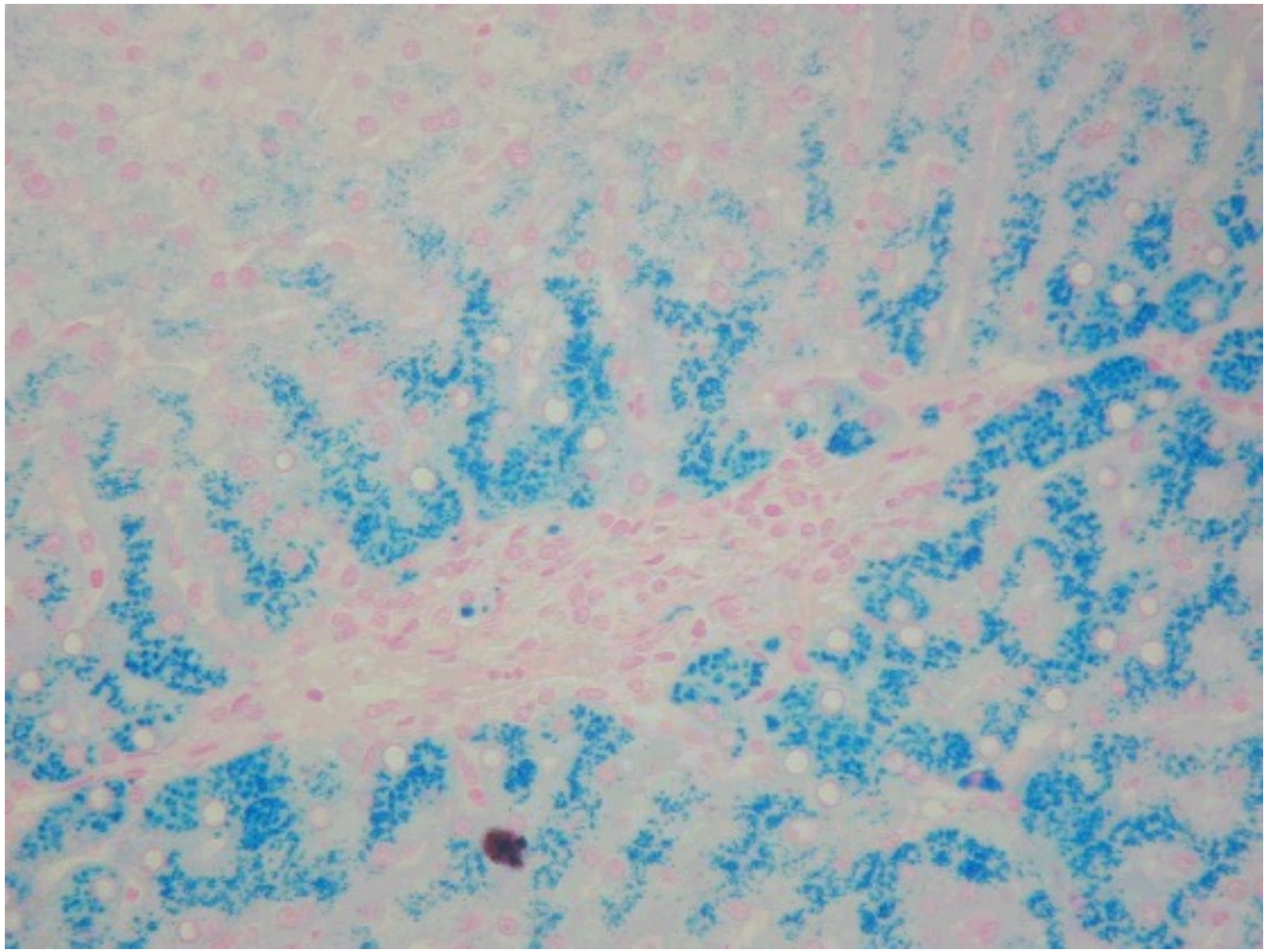
# Hepcidin deficiency leads to liver iron overload



# Hepcidin deficiency leads to liver iron overload



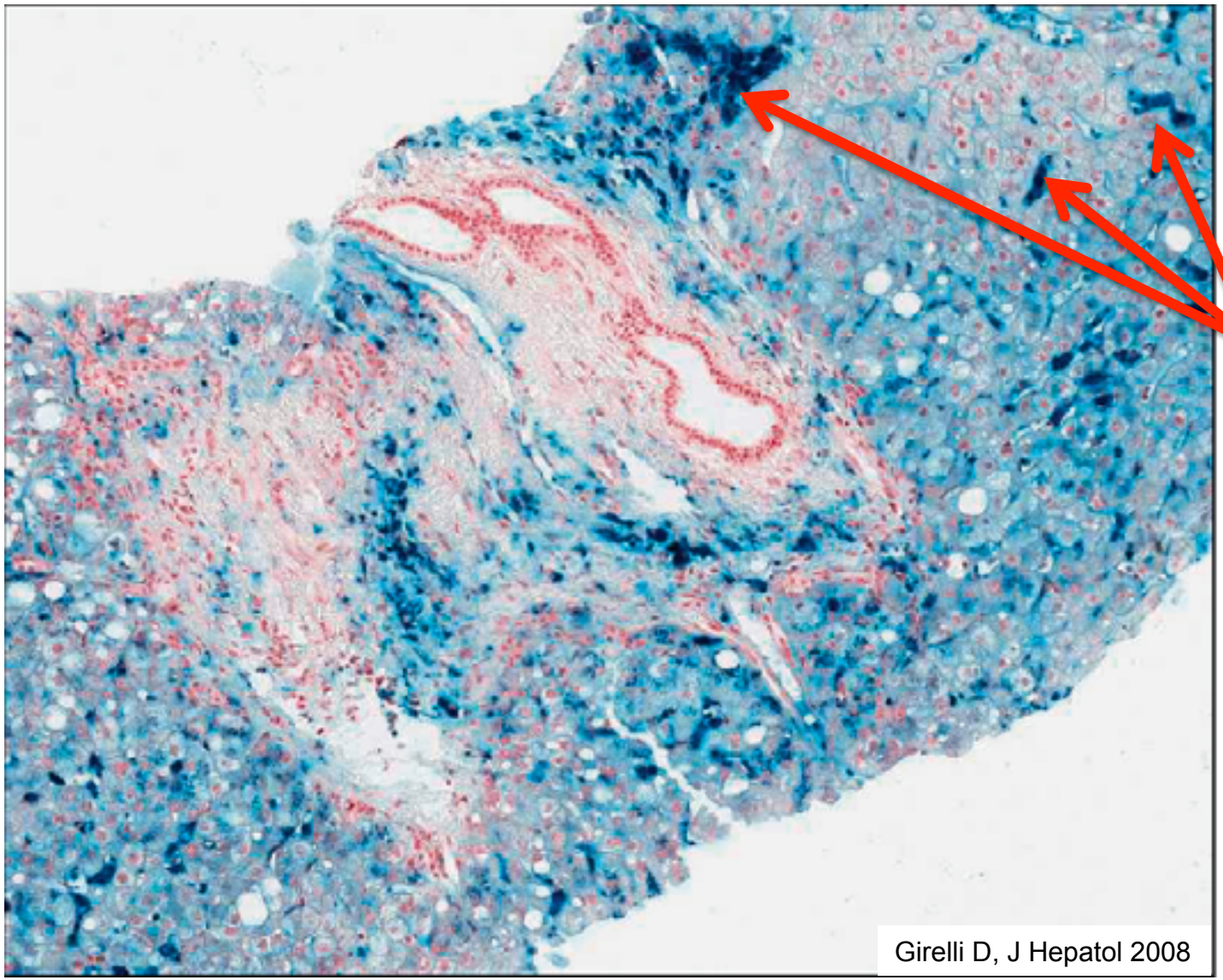
# Hepcidin deficiency: iron overload primary in periportal hepatocytes



Girelli D, Gastroenterology 2002

↑ risk of cirrhosis if serum ferritin persistently ≥ 1000 μg/l Morrison, Ann Intern Med 2003

# Advanced Liver Iron Overload



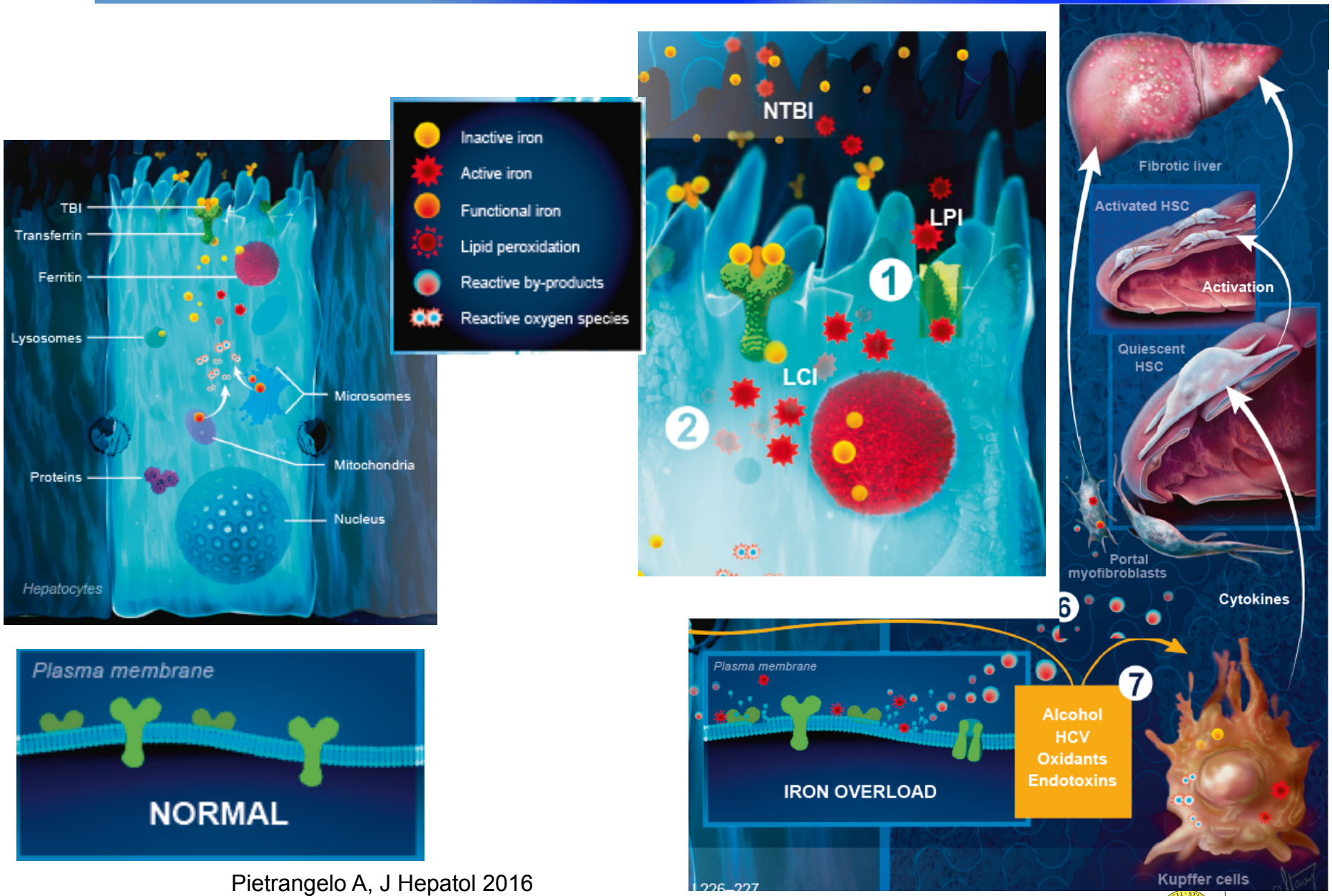
Girelli D, J Hepatol 2008

Hepatocyte
"sideronecrosis"

↓

Phagocytosis by
Kupffer cells

# Mechanisms of iron hepatotoxicity

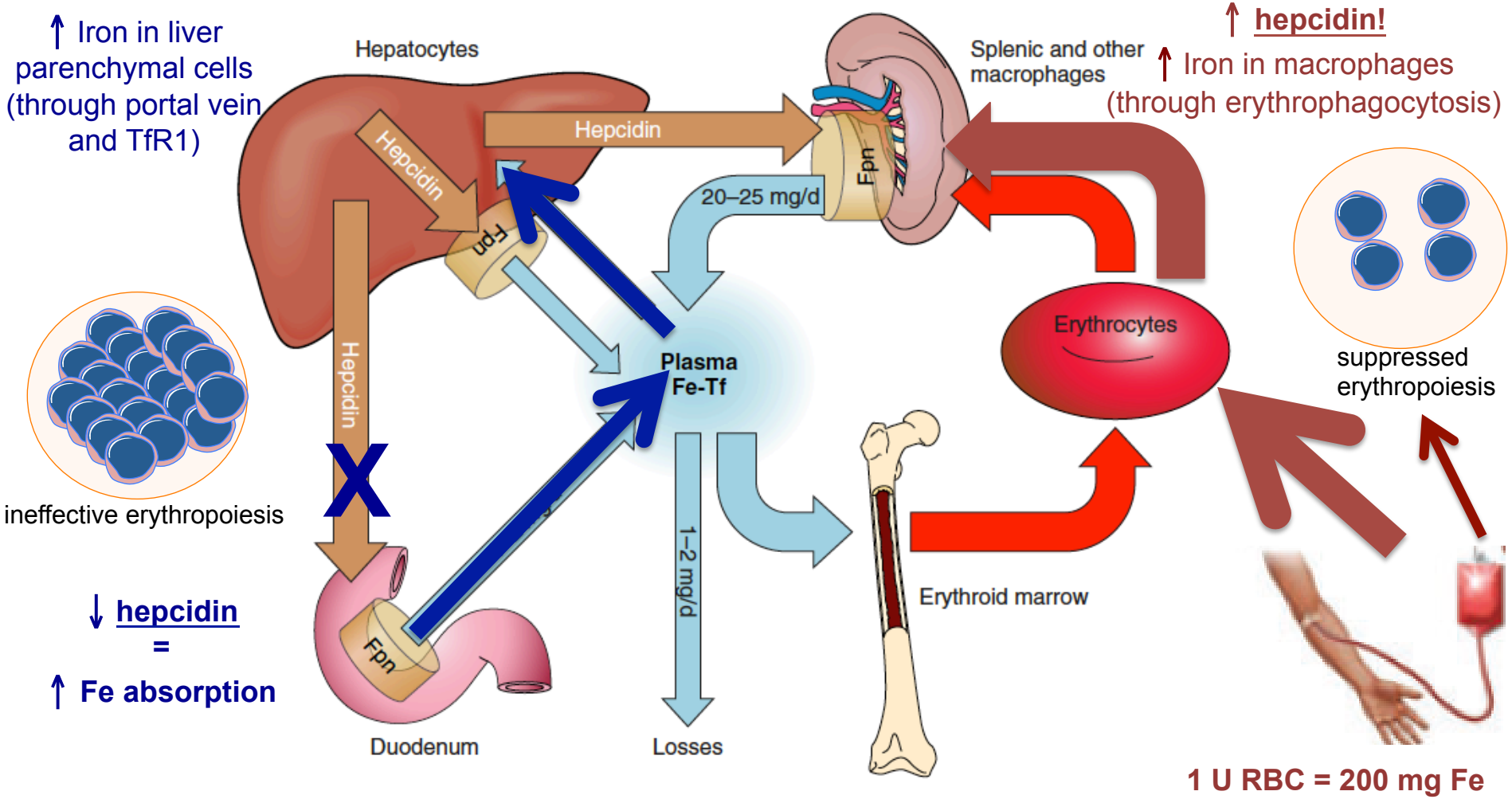



Pietrangelo A, J Hepatol 2016

# Differences between NTD and TD ILAs

**NON-TRANSFUSION DEP. THALASSEMIAS**

↑ Iron in liver parenchymal cells (through portal vein and TfR1)




↓ **hepcidin**
=
↑ **Fe absorption**

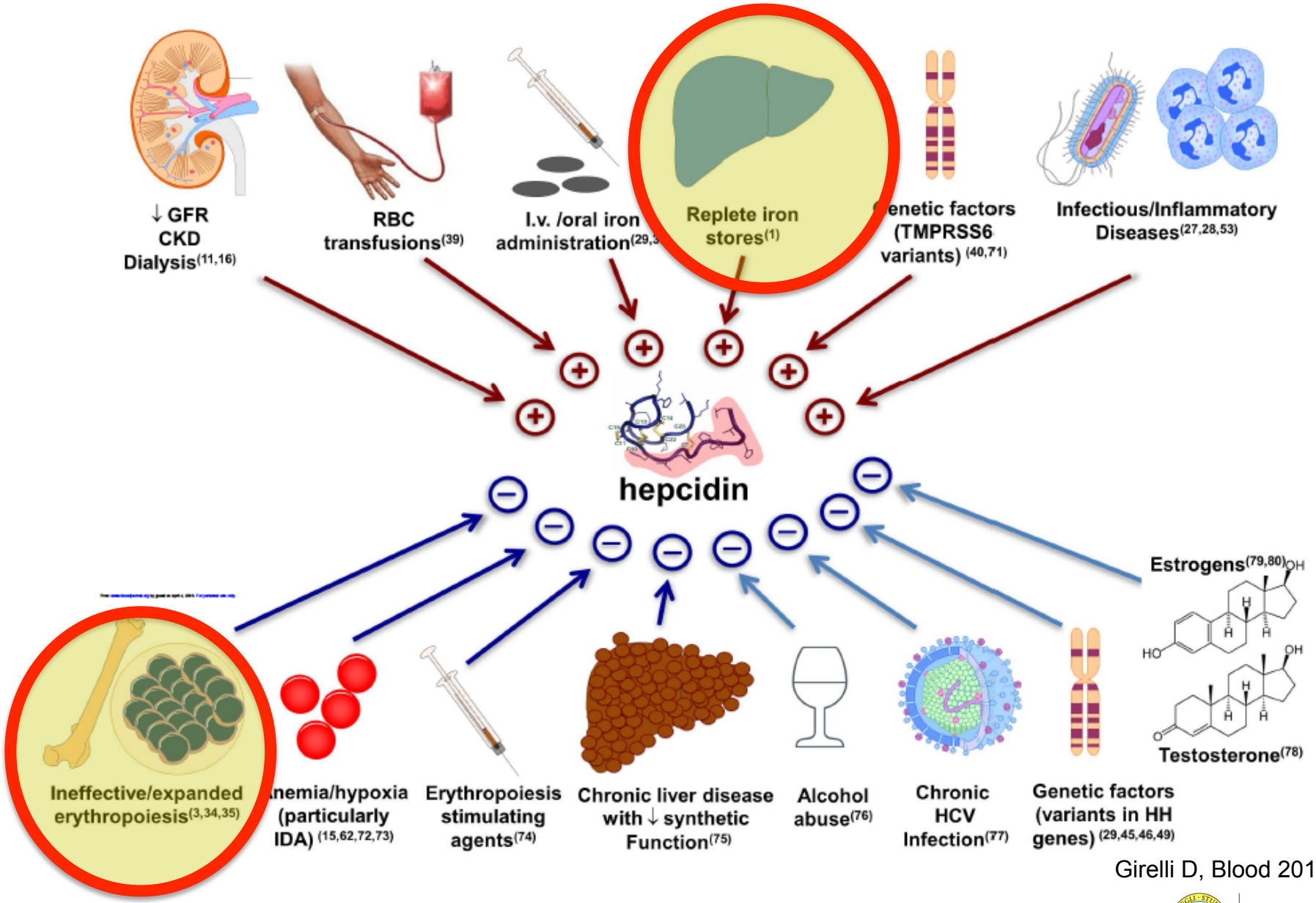
**THALASSEMIA MAJOR**

↑ **hepcidin!**

↑ Iron in macrophages (through erythrophagocytosis)

**1 U RBC = 200 mg Fe**

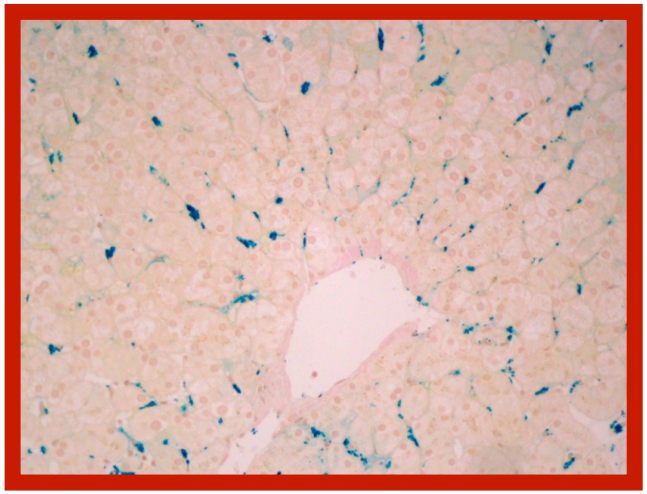
# Clinical conditions influencing serum hepcidin levels



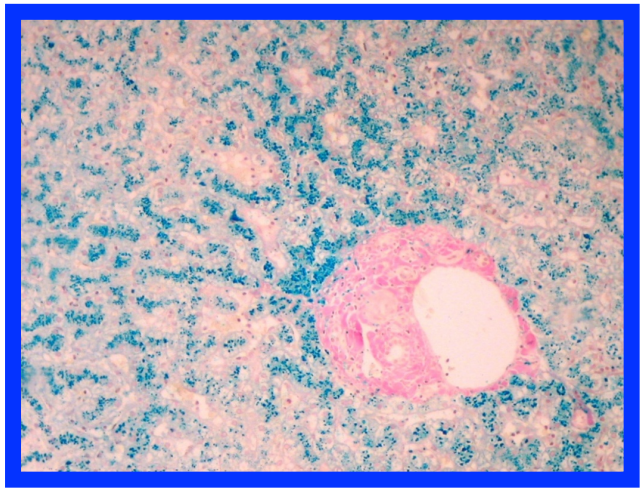

Girelli D, Blood 2016

# Differences in hepcidin levels between TI and TM

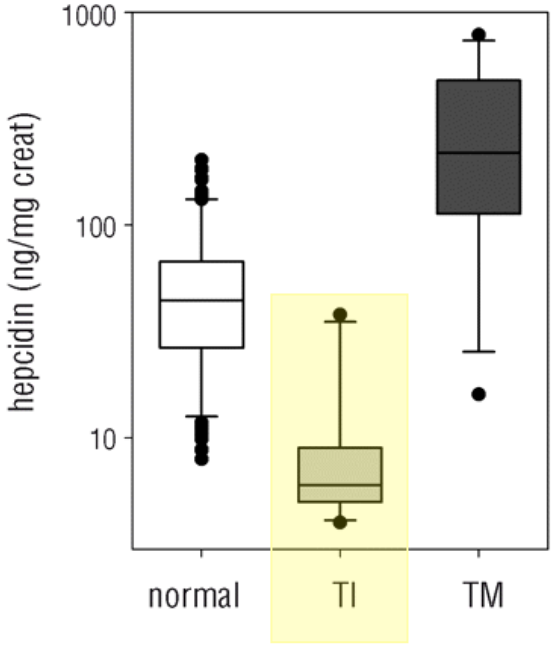
- **Thalassemia Major (TM): transfusion dependent**
- **Thalassemia Intermedia (TI): not transfusion dependent**



Prevalent macrophage (**Kupffer cells**) iron overload in a well-chelated β-**TM** patient.



Initially prevalent parenchymal (**periportal**) iron overload due to **hepcidin suppression** in β-**TI**




Origa R, Haematologica 2007

**These 2 pts. may have same ferritin levels (i.e. 800-1,000 μg/dl) but different risk of liver complications, particularly HCC.**

OPEN ACCESS Freely available online

PLoS one

# Hepcidin Levels and Their Determinants in Different Types of Myelodysplastic Syndromes

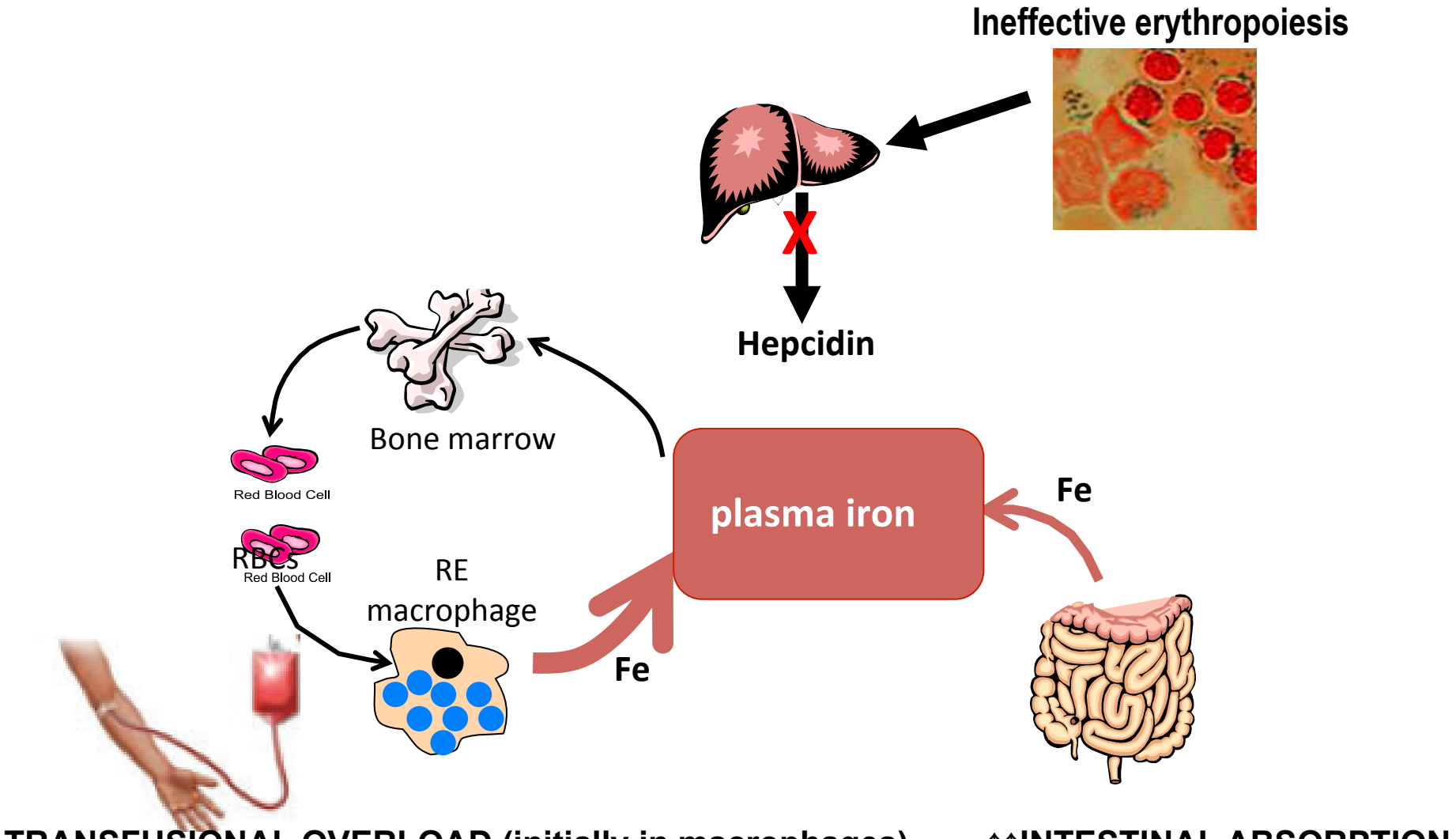
Valeria Santini[1☯], Domenico Girelli[2*☯], Alessandro Sanna[1], Nicola Martinelli[2], Lorena Duca[3], Natascia Campostrini[2], Agostino Cortelezzi[4], Michela Corbella[2], Alberto Bosi[1], Gianluigi Reda[4], Oliviero Olivieri[2], Maria Domenica Cappellini[3]

**Table 2.** Clinical and biochemical characteristics of MDS patients stratified according to WHO classification system.

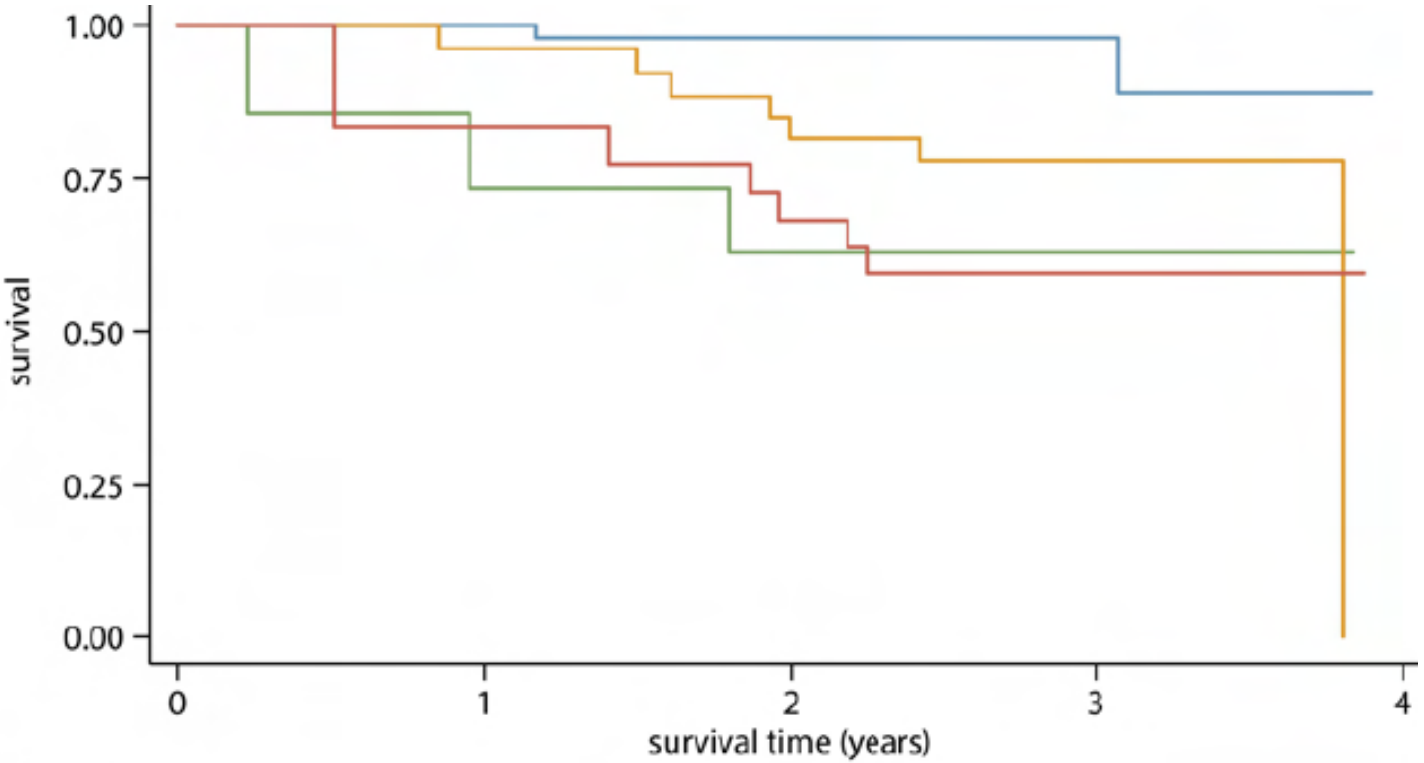
| | RA (n = 31) | RARS (n = 9) | RCMD (n = 19) | RAEB (n = 32) | 5q- syndrome (n = 7) | CMML (n = 7) | Unclass (n = 8) | P° |
|---|---|---|---|---|---|---|---|---|
| **Age (years)** | 75.8±10.3 | 73.4±7.7 | 73.7±7.1 | 70.2±7.6 | 71.4±12.0 | 73.0±8.2 | 67.1±13.0 | 0.163 |
| **Male sex (%)** | 61.3 | 44.4 | 73.7 | 78.1 | 57.1 | 85.7 | 62.5 | 0.397 |
| **CRP* (mg/l)** | 2.09 (1.07–4.10) | 1.46 (0.36–5.56) | 3.30 (1.37–7.97) | 9.13 (5.41–15.39) | 2.21 (0.53–9.13) | 5.03 (0.19–129.90) | 10.77 (5.16–22.48) | 0.008 |
| **Ferritin* (μg/l)** | 368 (231–586) | 725 (403–1305) | 420 (230–768) | 661 (461–947) | 1364 (233–8001) | 289 (130–646) | 580 (135–2493) | 0.104 |
| **Hepcidin* (nmol/l)** | 3.46 (2.06–5.81) | 1.43 (0.51–4.03) | 3.83 (1.85–7.96) | 11.31 (7.38–17.32) | 6.62 (1.26–34.84) | 10.04 (2.10–48.00) | 6.06 (1.18–31.27) | 0.003 |

**Low-risk MDS have inappropriately low hepcidin levels, that may result in increased iron absorption**

# Mechanisms of iron overload in MDS



# Labile plasma iron levels predict survival in patients with lower-risk Myelodysplastic syndromes




Number at risk

| | | | | | |
|---|---|---|---|---|---|
| LPI<LLOD, TI | 77 | 53 | 33 | 13 | 0 |
| LPI>=LLOD, TI | 9 | 6 | 7 | 5 | 0 |
| LPI<LLOD, TD | 12 | 26 | 24 | 4 | 0 |
| LPI>=LLOD, TD | 2 | 11 | 15 | 10 | 3 |



De Swart L, Haematologica 2017 (epub Nov 9)

# Take-home messages

- ✓ Iron toxicity is primarily due to excess ROS production.
- ✓ Iron overload in the individual patient depends on several cofactors: age, comorbidities, cellular type mainly involved (hepatocytes > macrophages).
- ✓ Iron overload can be detrimental in either transfusion dependent and transfusion independent MDS or other iron loading anemias.
- ✓ In the future, treatments targeting the hepcidin/ERFE axis may be helpful.

# The Verona Interdisciplinary Group on Iron Disorders

GRUPPO INTERDISCIPLINARE MALATTIE DEL FERRO

GIMFer

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**http://www.gimferverona.org**


Participants Units

1. Internal Medicine
2. Clinical Chemistry & Molecular Biology
3. Blood Bank / Transfusional Service
4. Radiology
5. Pathology
6. Gastroenterology

Fabiana Busti, Paola Capelli, Annalisa Castagna, Michela Corbella, Massimo Delledonne, Giorgio Gandini, Alejandro Giorgetti, Giacomo Marchi, Oliviero Olivieri, Roberto Pozzi-Mucelli, Monica Rizzi, Alice Vianello, Luciano Xumerle.

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