SESSIONE PLENARIA I – Medici e infermieri

8.30-10.30 ACQUIRED IDIOPATHIC APLASTIC ANEMIA

Chairmen: F. Pane (Napoli), C. Selleri (Salerno)

Non-HSCT treatment - A.M. Risitano (Napoli)

HSCT for AA - A. Bacigalupo (Roma)

Management of iron overload - E. Angelucci (Genova)

Selected abstract

Discussant: F. Picardi (Roma)

Management of iron overload. Agenda

- Background

- Iron toxicity in aplastic anemia
- Iron toxicity in HSCT for Aplastic Anemia

- Management
  - today
  - Perspective
- Conclusion
  - Personal statement

Iron is essential

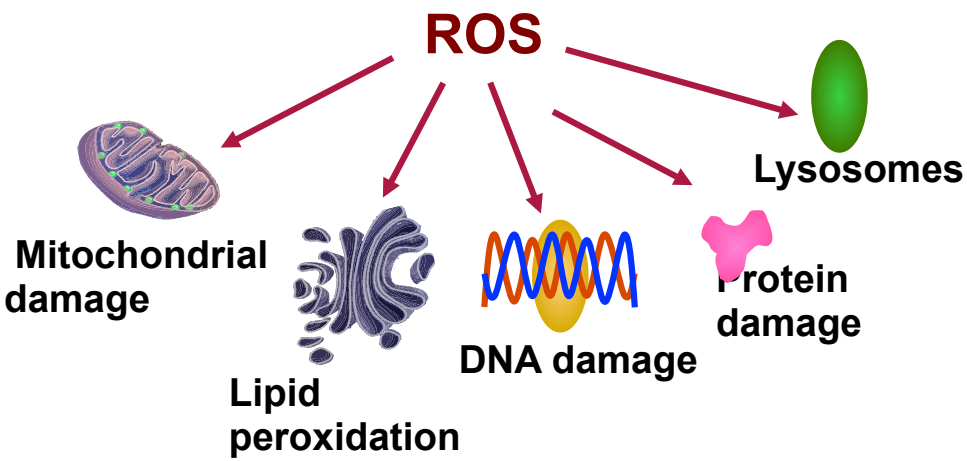
- O_2 transport and exchange
  - haemoglobin and myoglobin
- Respiratory chain
  - complex I and III
- Biosynthetic pathways
  - haem synthesis
  - Fe/S cluster assembly
- DNA synthesis and repair
  - ribonucleotide reductase
  - endonuclease III
- Cell growth and proliferation

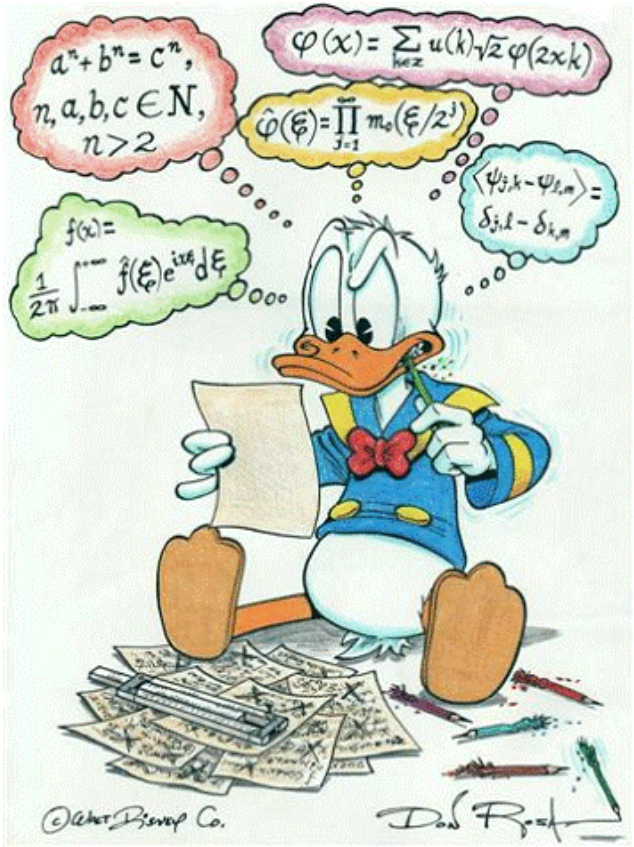
Iron is toxic

- Ability to transfer electrons
- Production of free O_2 radicals

Fenton reaction:

$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + \cdot OH$





Fe Toxicity tissue =

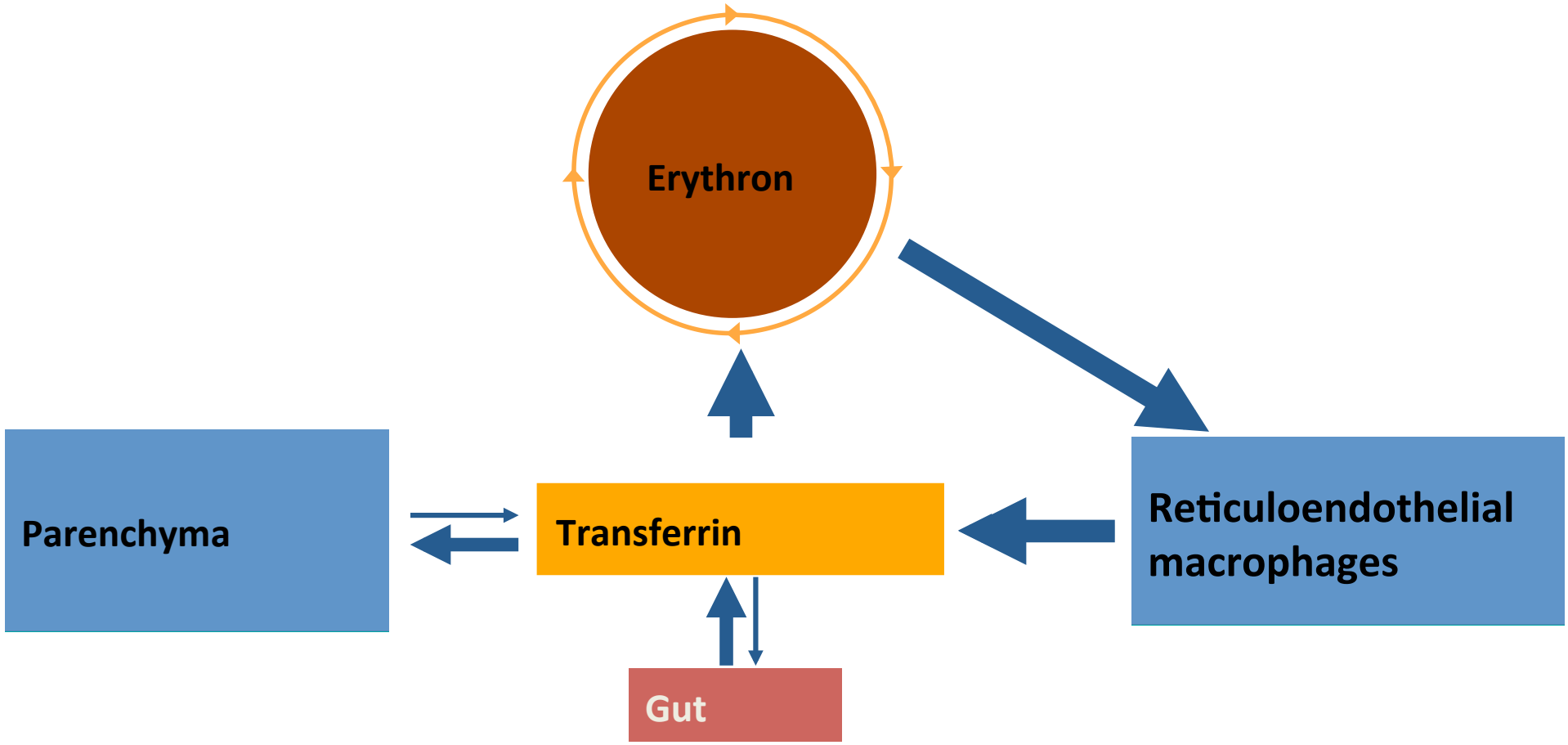
Σ Tissue Reactive Iron **x** Genetics **x** Environmental Factors **x** Time

Coates TD. Free Radic Biol Med 2014

"Iron toxicity depends on many factors in addition to the level of iron per se"

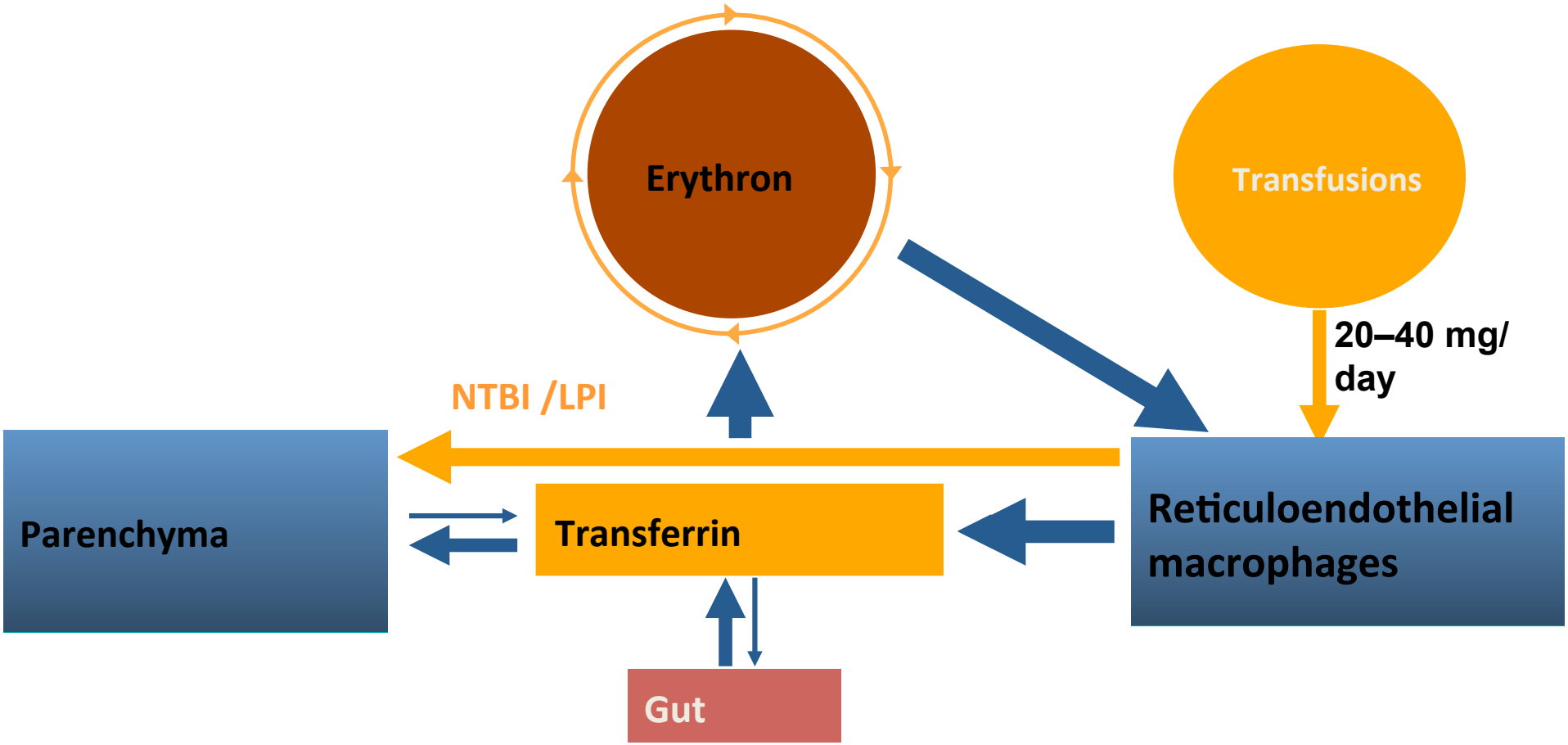
Coates TD. Free Radic Biol Med 2014

Iron Distribution and Turnover



Hershko C, et al. *Ann NY Acad Sci*. 1998;850:191-201.

Imbalance of Distribution and Turnover of Body Iron With Transfusion Therapy



Iron balance is disturbed by blood transfusion because the body cannot remove the excess iron

NTBI=non–transferrin-bound iron.

Hershko C, et al. *Ann NY Acad Sci*. 1998;850:191-201.

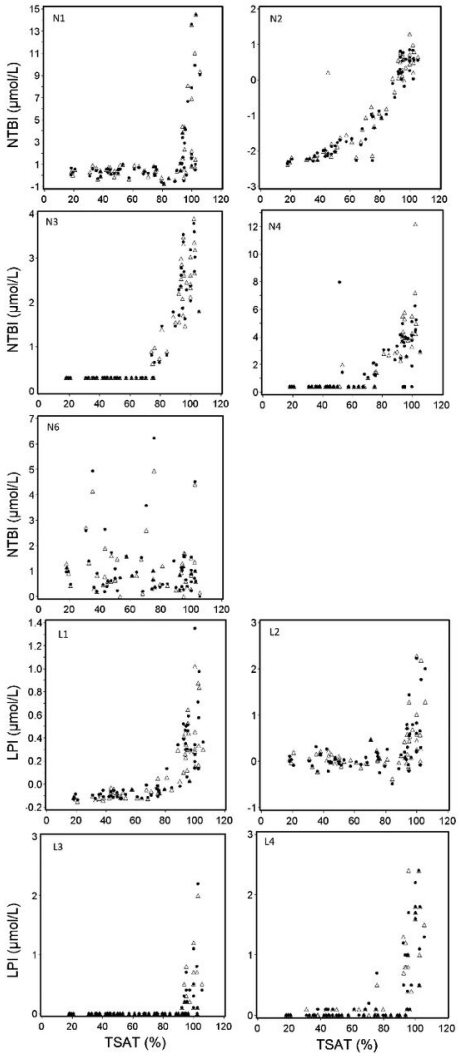
Relationship between the assay concentrations and transferrin saturation (TSAT).

EUROPEAN HEMATOLOGY ASSOCIATION
Ferrata Storti Foundation

Haematologica 2016
Volume 101(1):38-45

Second international round robin for the quantification of serum non-transferrin-bound iron and labile plasma iron in patients with iron-overload disorders

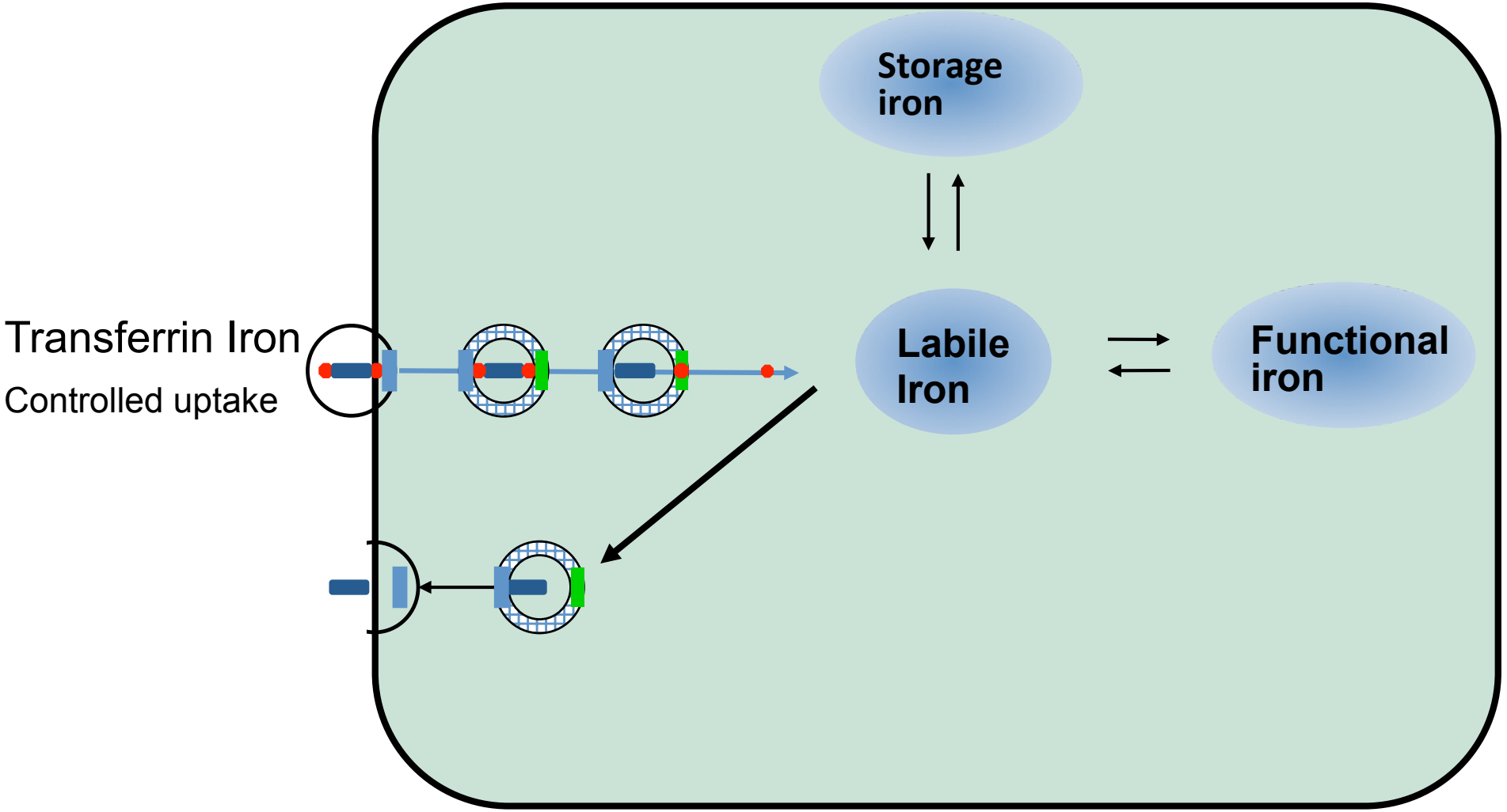
Louise de Swart,[1] Jan C.M. Hendriks,[2] Lisa N. van der Vorm,[3] Z. Ioav Cabantchik,[4] Patricia J. Evans,[5] Eldad A. Hod,[6] Gary M. Brittenham,[7] Yael Furman,[8] Boguslaw Wojczyk,[6] Mirian C.H. Janssen,[9] John B. Porter,[5] Vera E.J.M. Mattijssen,[10] Bart J. Biemond,[11] Marius A. MacKenzie,[1] Raffaella Origa,[12] Renzo Galanello,[12*] Robert C. Hider,[13] and Dorine W. Swinkels[3]



Louise de Swart et al. Haematologica 2016;101:38-45

haematologica
the hematology journal

Uncontrolled Uptake of Labile Iron Leads to Cell and Organ



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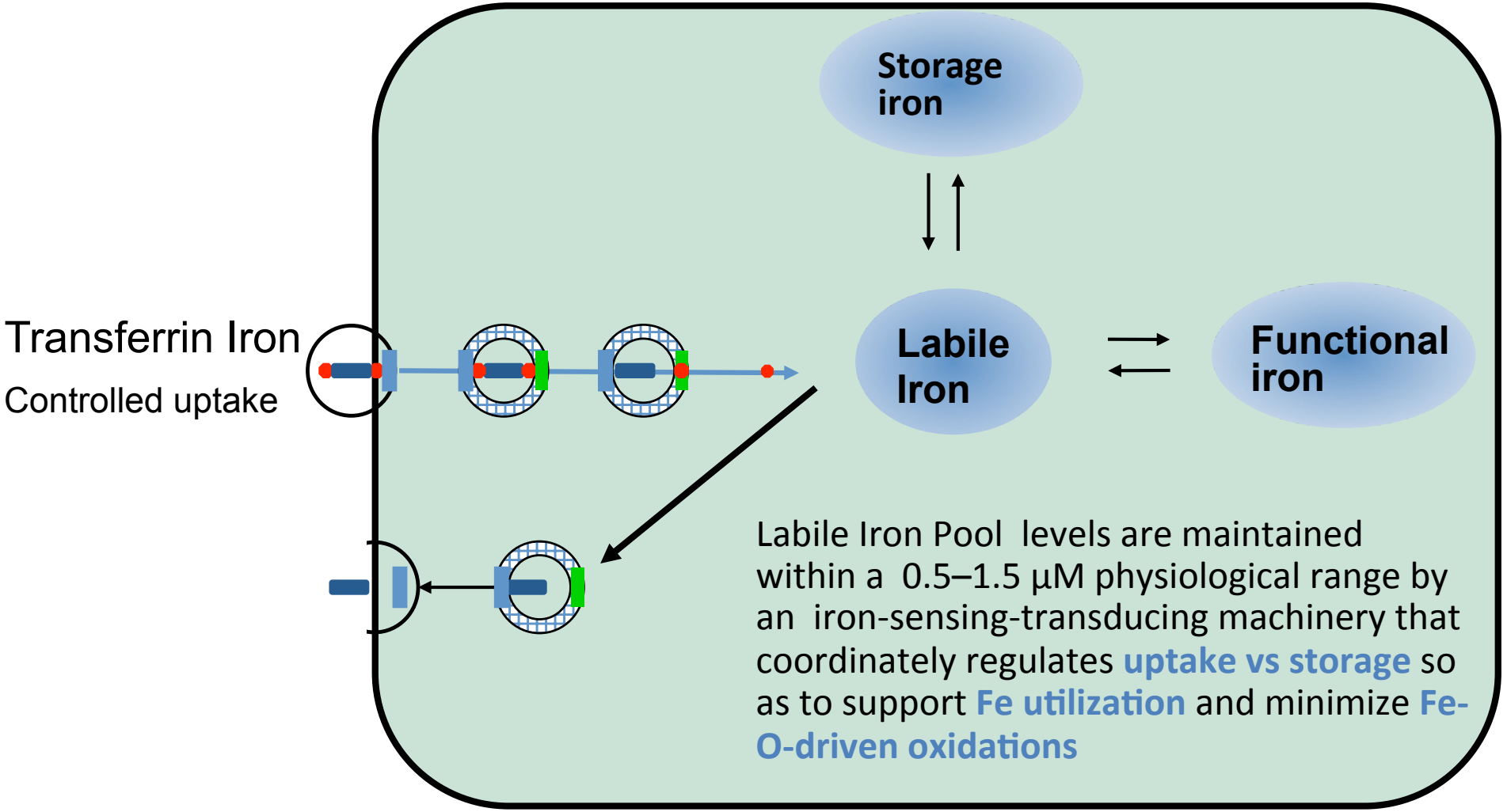

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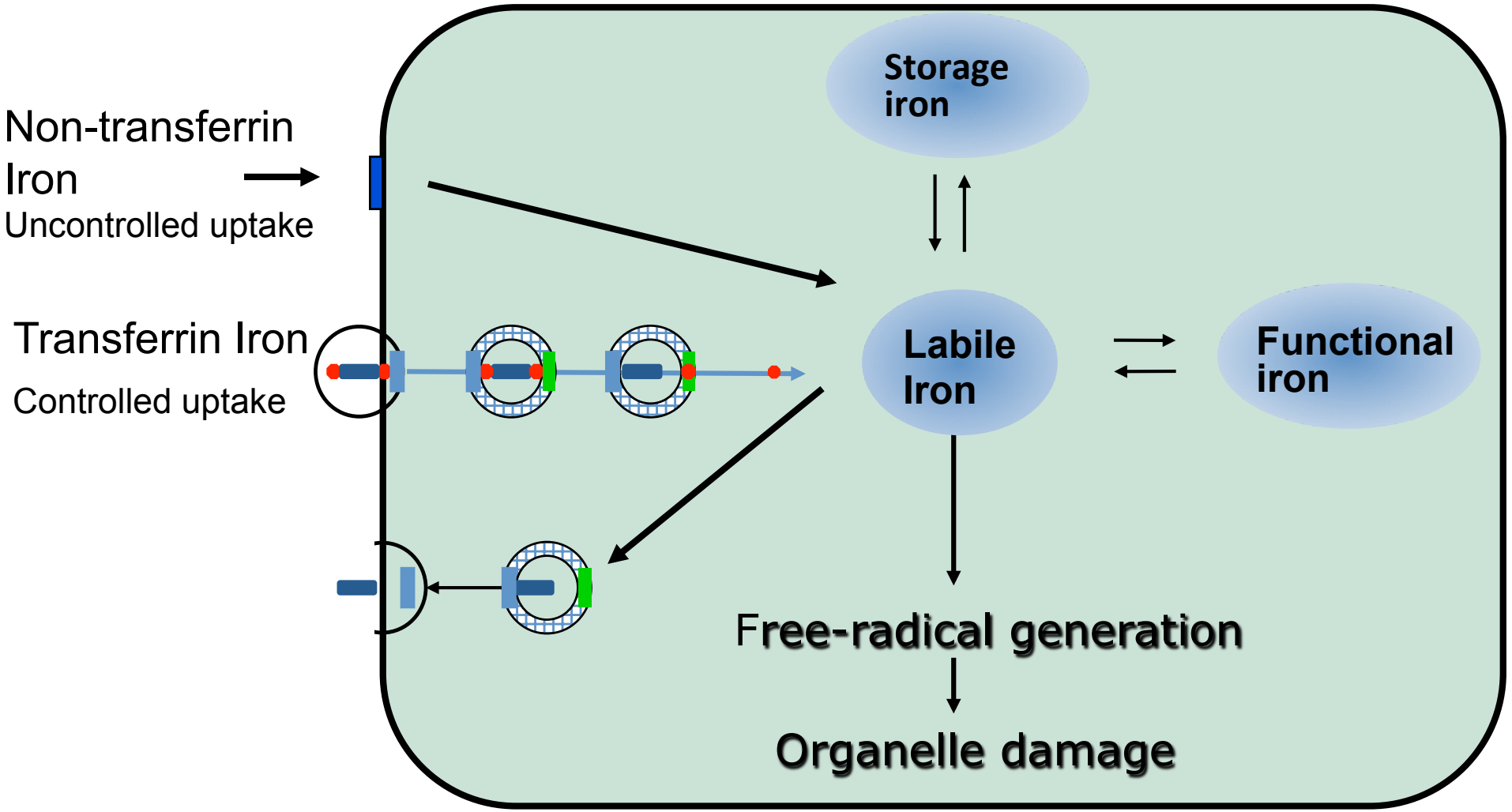

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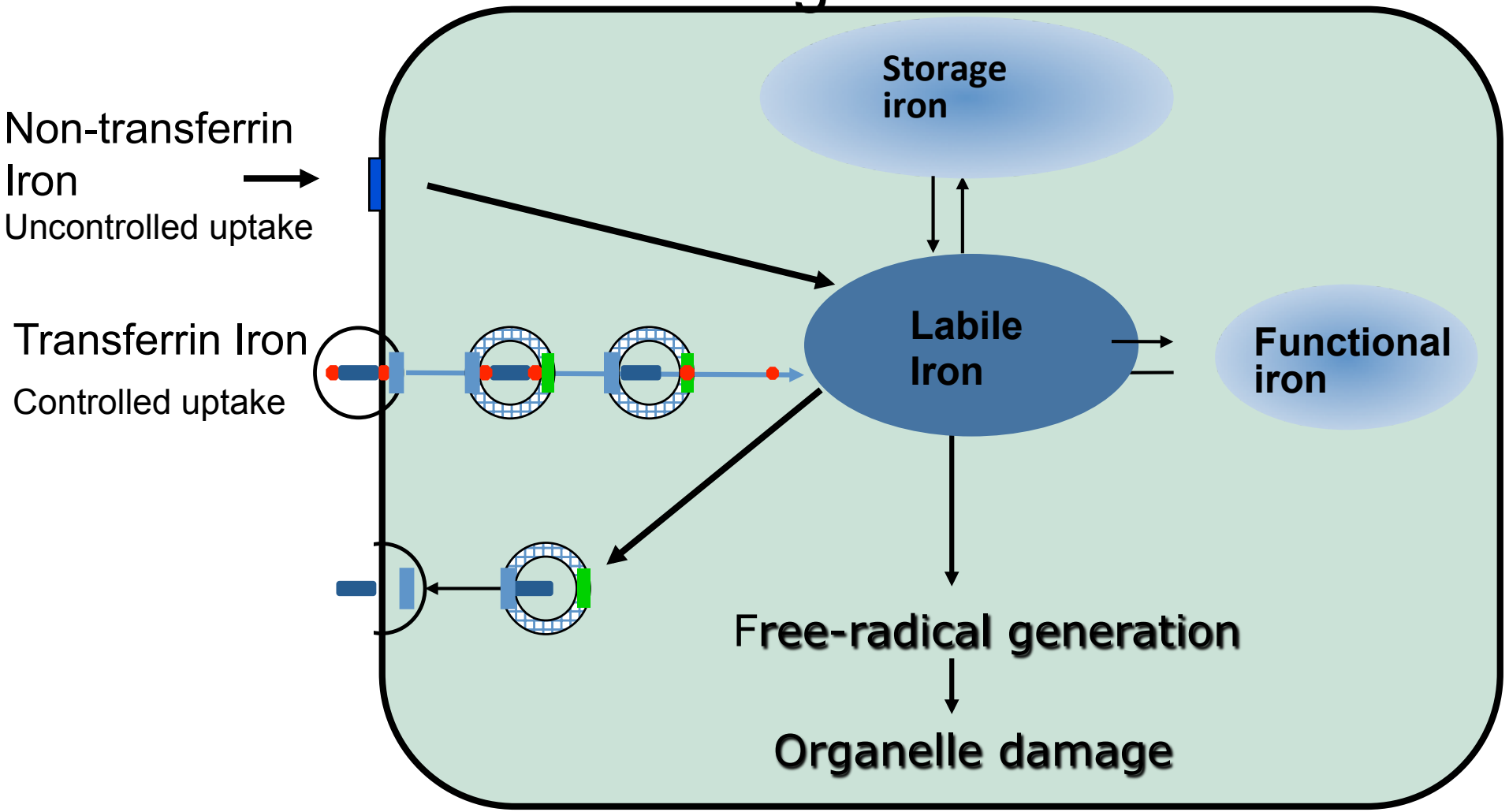
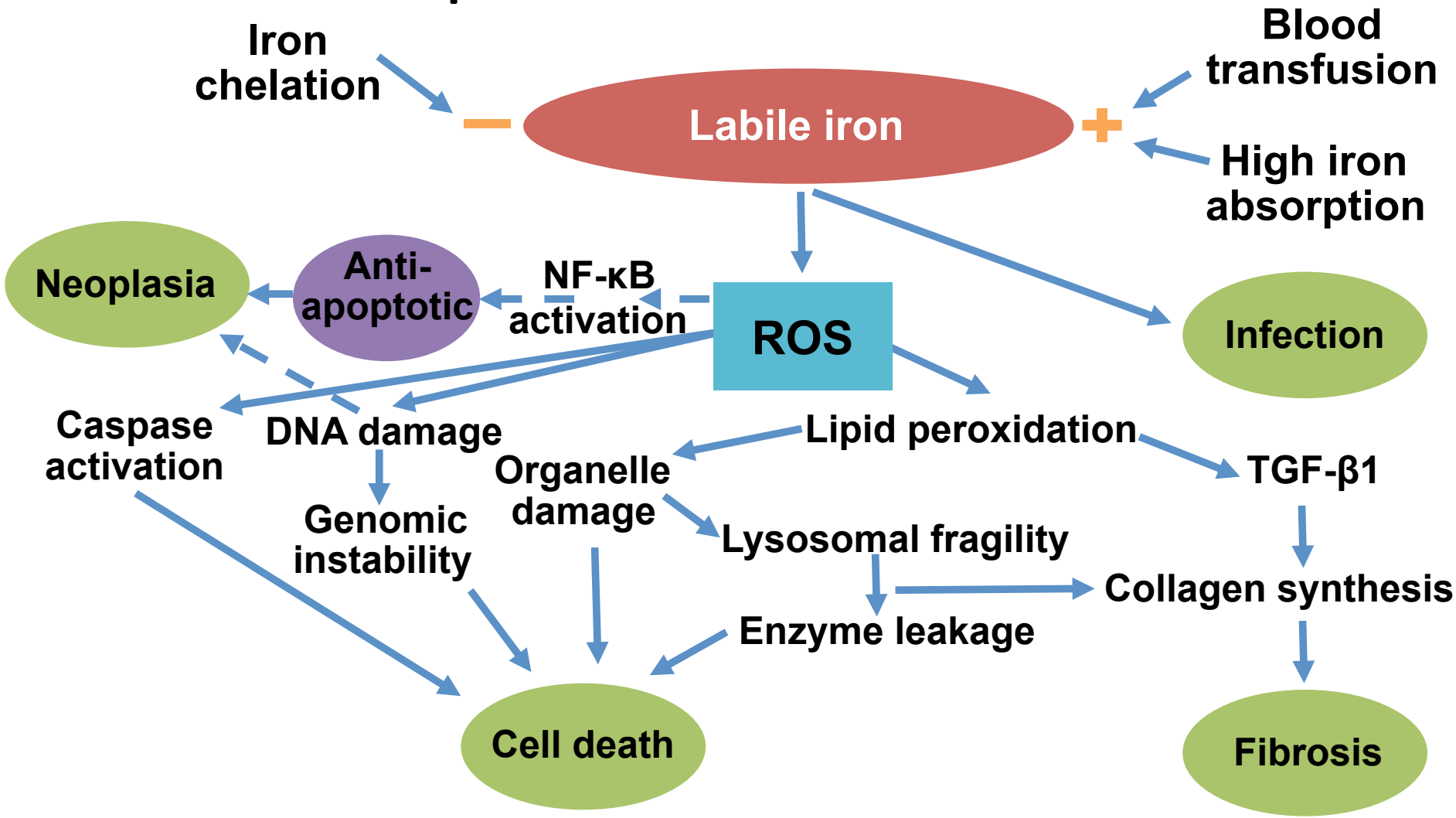


An excessive rise in LIP can promote the generation of reactive-O species (ROS) by reacting with respiratory O intermediates and thereby override the cellular antioxidant defences and chemically damage cell components and associated functions

Pathological mechanisms and consequences of iron overload

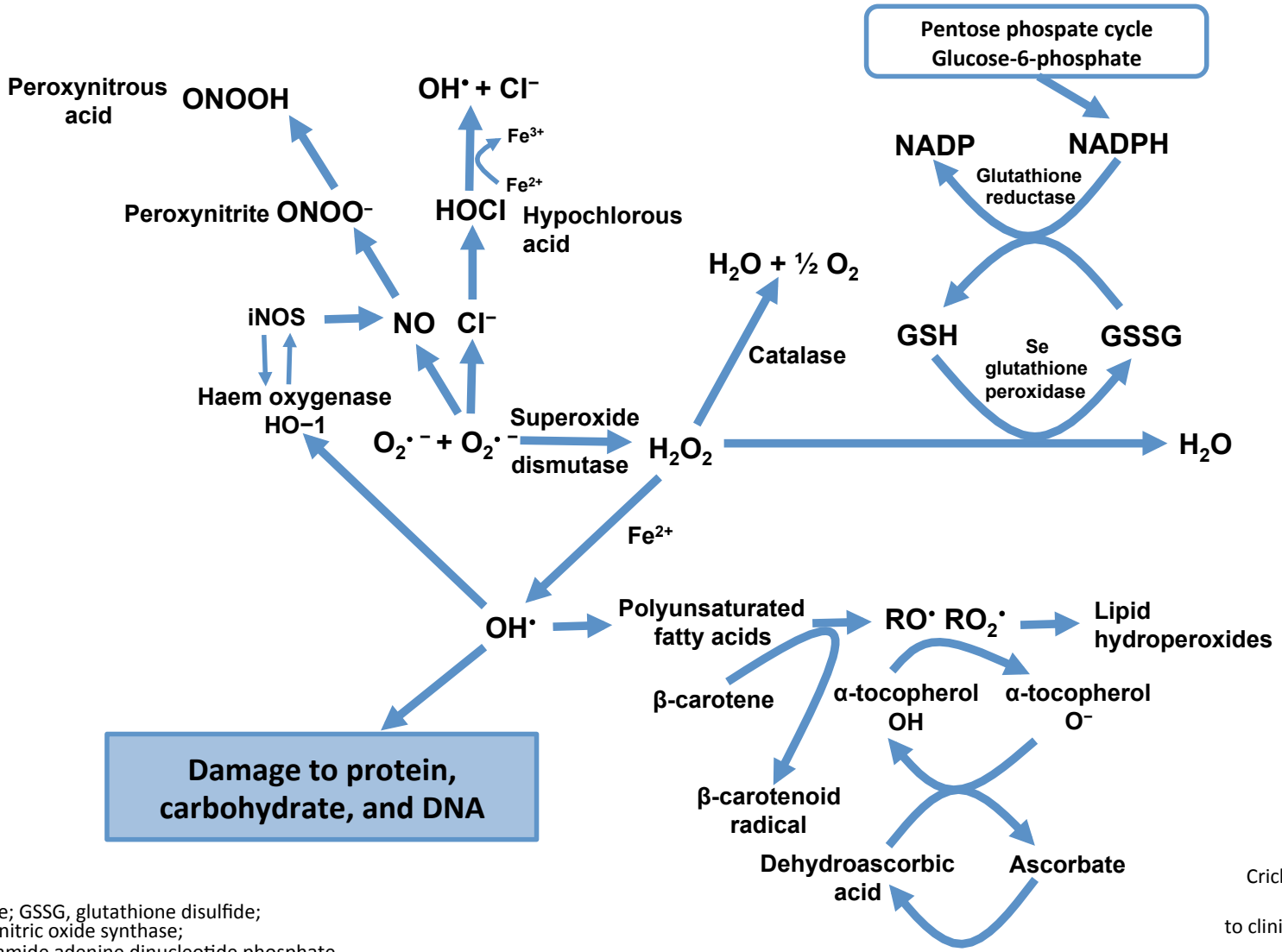


NF-κB, nuclear factor-κB;
TGF, transforming growth factor.

Porter JB, Garbowski M. Hematol Oncol Clin North Am. 2014;28:683-701.

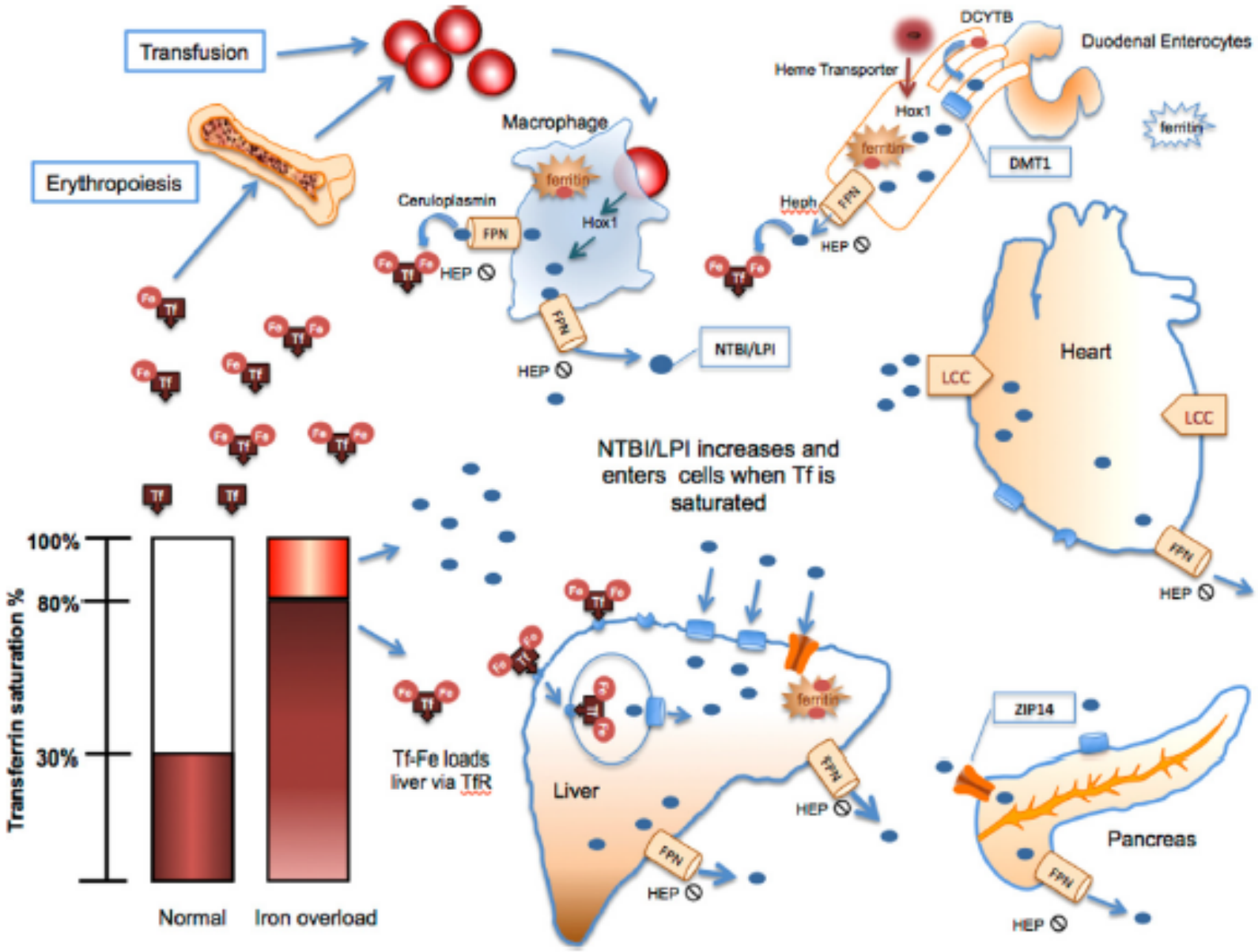
The dark side of iron: ROS and oxidative stress

The battlefield of oxidative stress



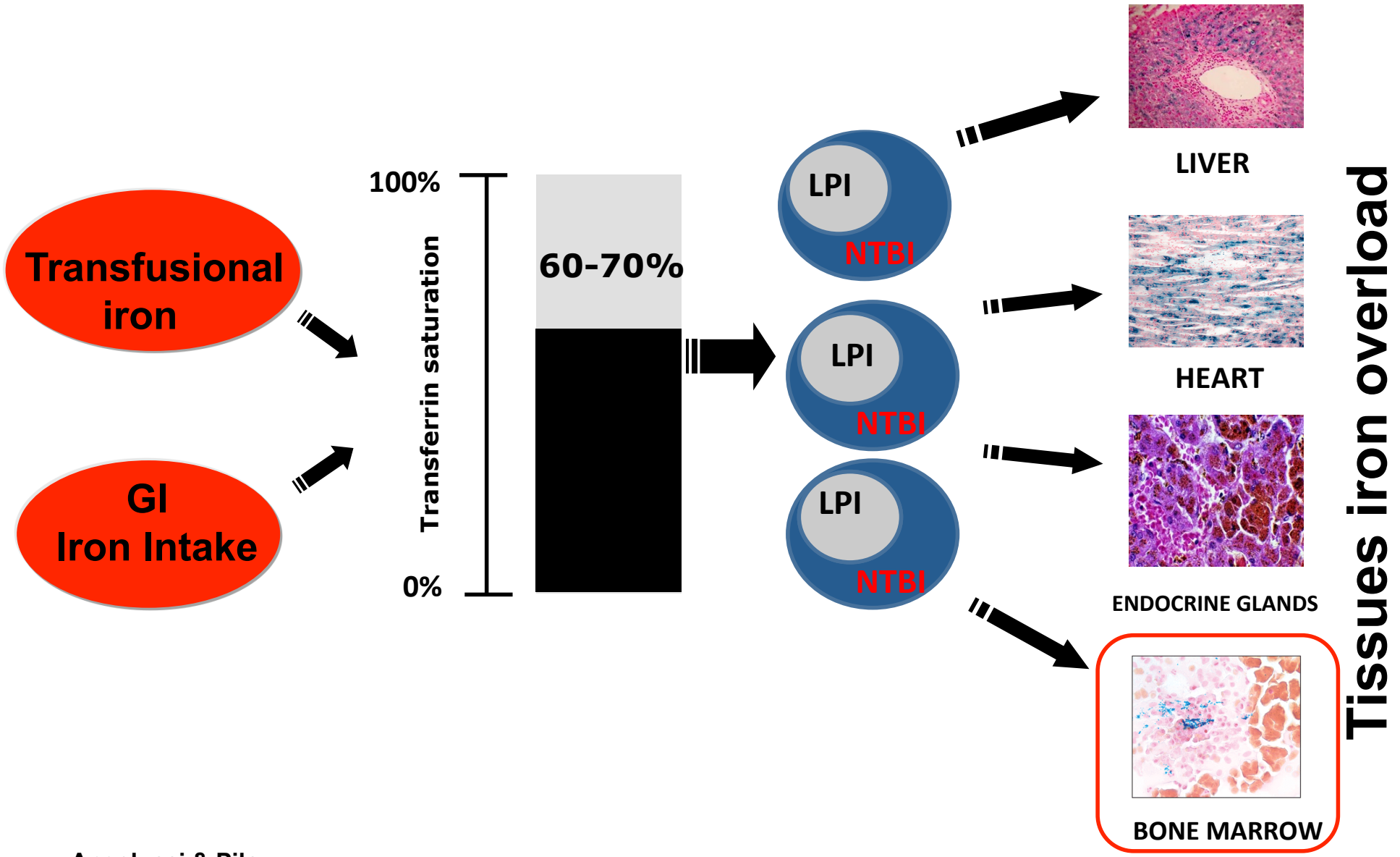
GSH, glutathione; GSSG, glutathione disulfide;
iNOS, inducible nitric oxide synthase;
NADPH, nicotinamide adenine dinucleotide phosphate.

Crichton R. Iron metabolism: from molecular mechanisms to clinical consequences. Chichester: Wiley; 2009.


Transfusion
Erythropoiesis
Macrophage
ferritin
Hox1
Ceruloplasmin
FPN
HEP
NTBI/LPI
DCYTB
Duodenal Enterocytes
Heme Transporter
DMT1
Heph
Heart
LCC
NTBI/LPI increases and enters cells when Tf is saturated
Transferrin saturation %
100%
80%
30%
Normal
Iron overload
Tf-Fe loads liver via TfR
Liver
ZIP14
Pancreas

- …pituitary, pancreatic and cardiac iron detected by MRI reflects **time-averaged exposure to toxic reactive iron** since loading of these organs essentially only occurs when NTBI/LPI enters through ion channels and transporters.

Coates, Carson Wood & Berdoukas. Management of Iron Overload in Hemoglobinopathies: What is the 1 Appropriate Target 2 Iron Level?. Annals of the New York Academy of Sciences 2016



Angelucci & Pilo
Ann N Y Acad Sci. 2016 Mar;1368(1):115-21

Fe Toxicity tissue =

$$\Sigma \text{Tissue Reactive Iron} \times \text{Genetics} \times \text{Environmental Factors} \times \text{Time}$$

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Agenda

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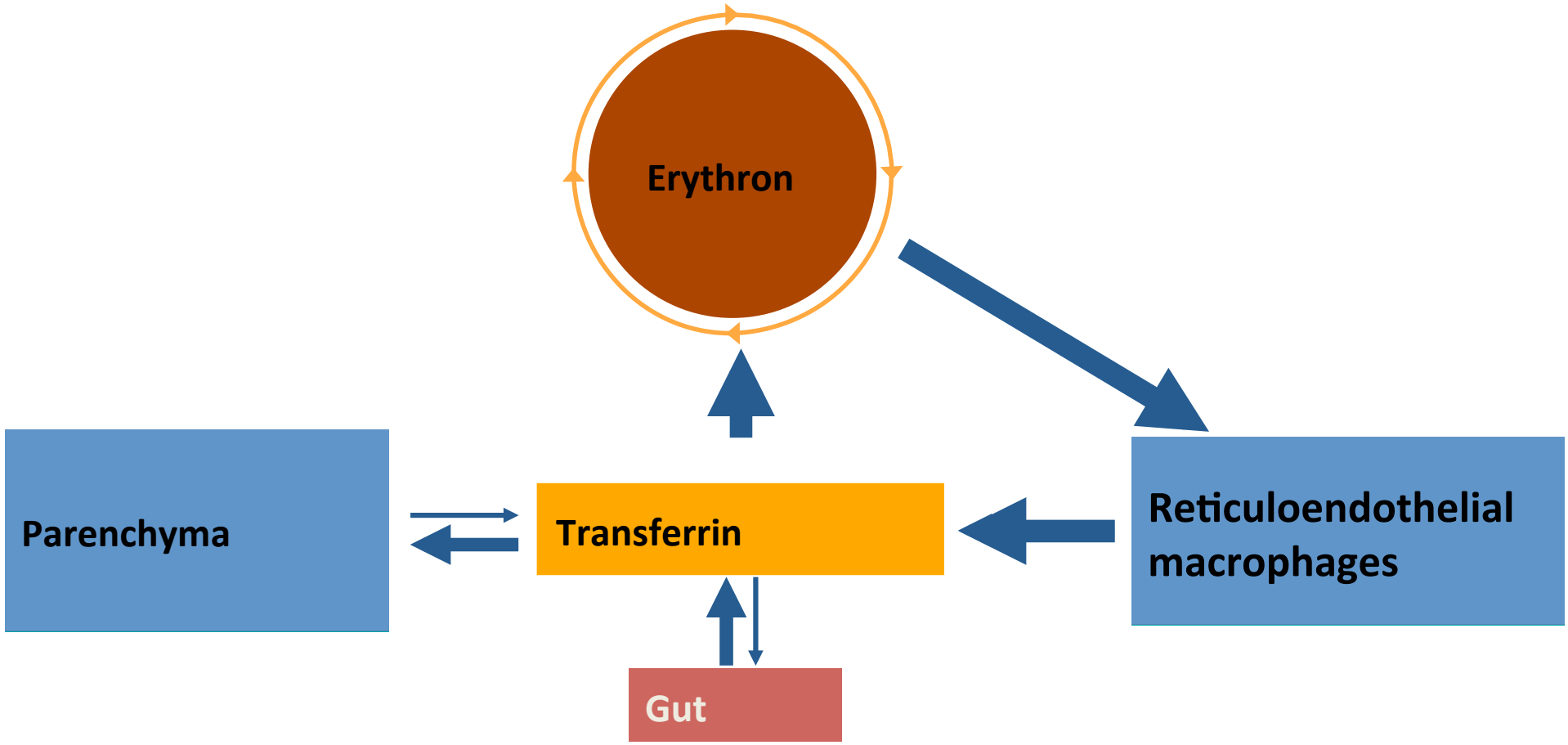
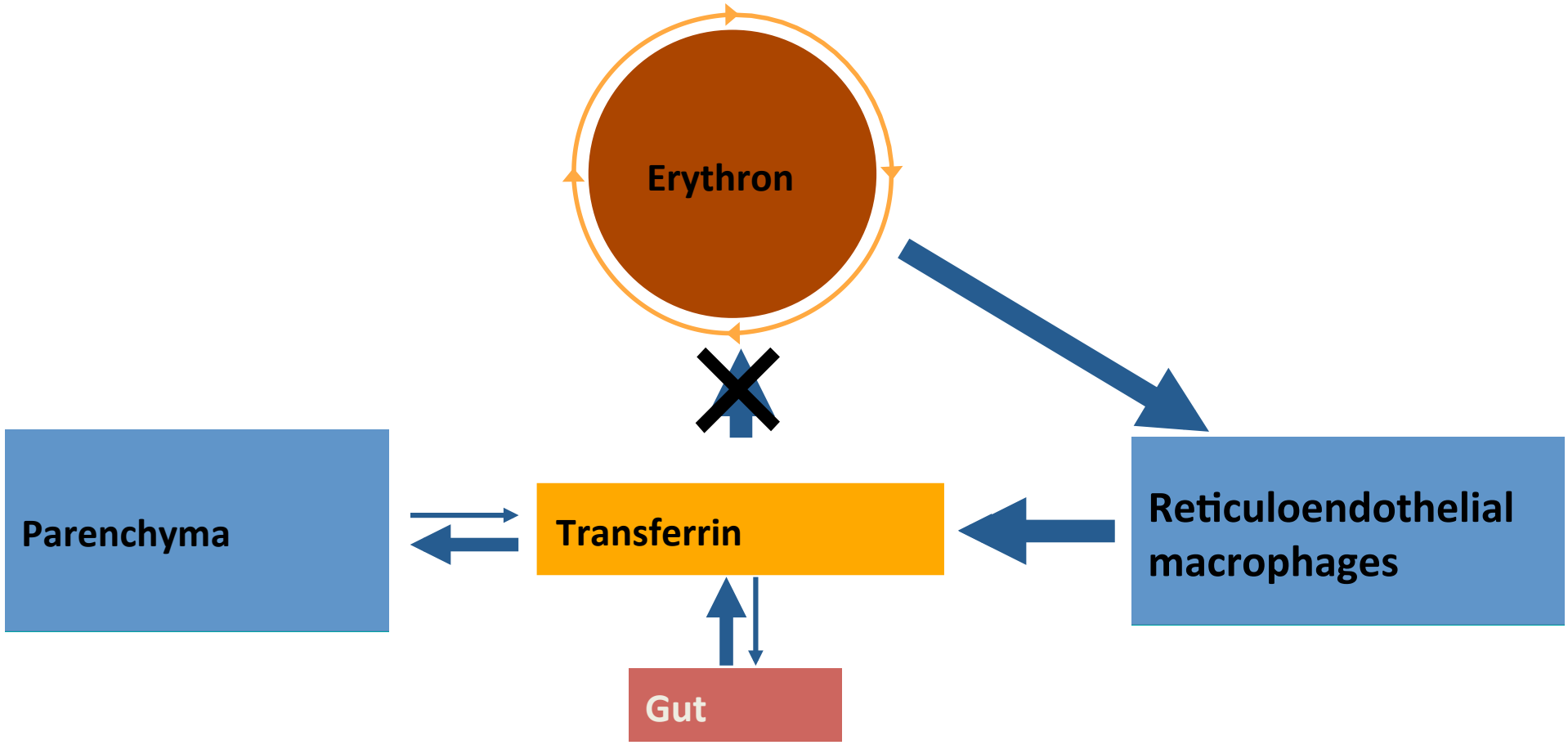
- Management
  - today
  - perspective

Iron Distribution and Turnover



Hershko C, et al. *Ann NY Acad Sci*. 1998;850:191-201.

Iron Distribution and Turnover



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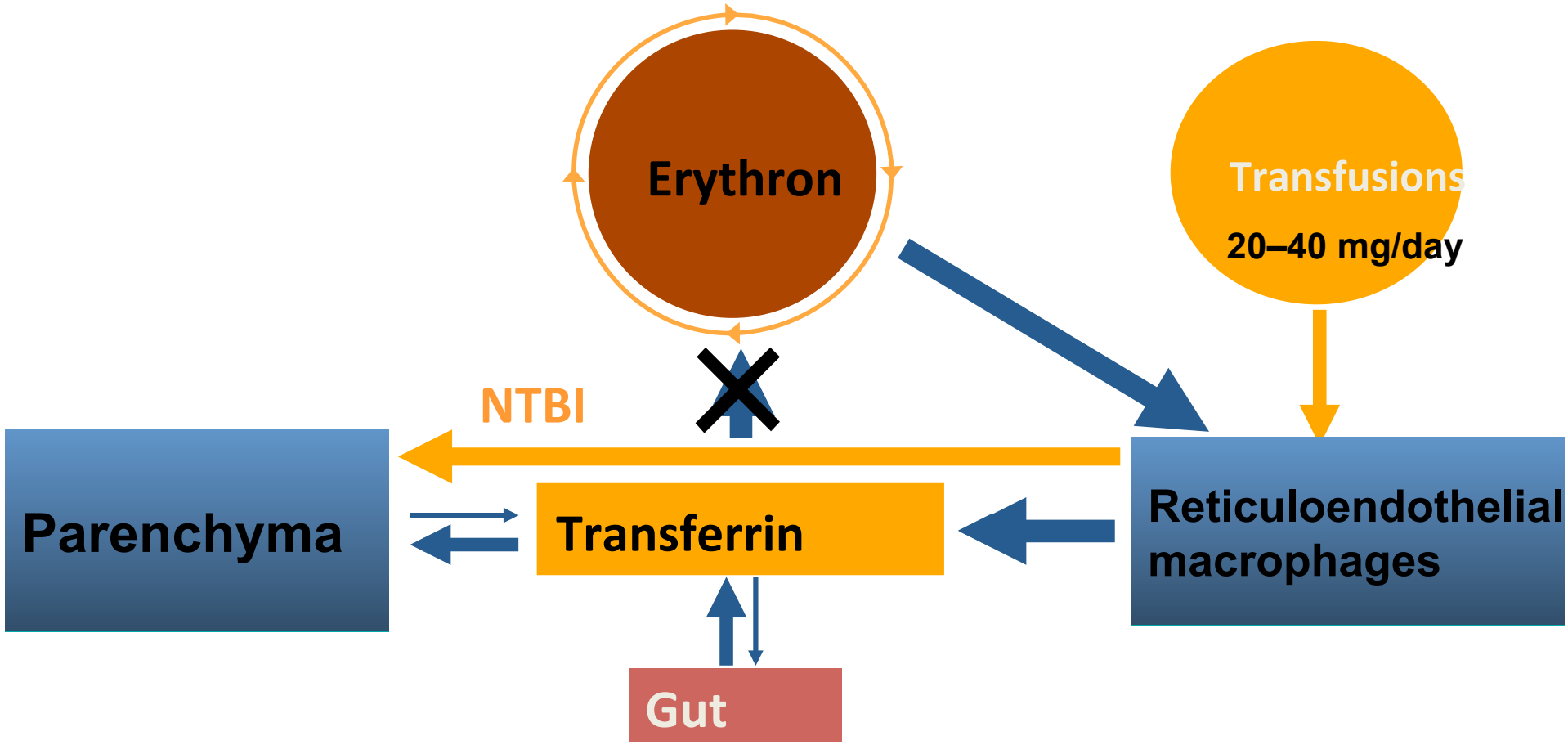

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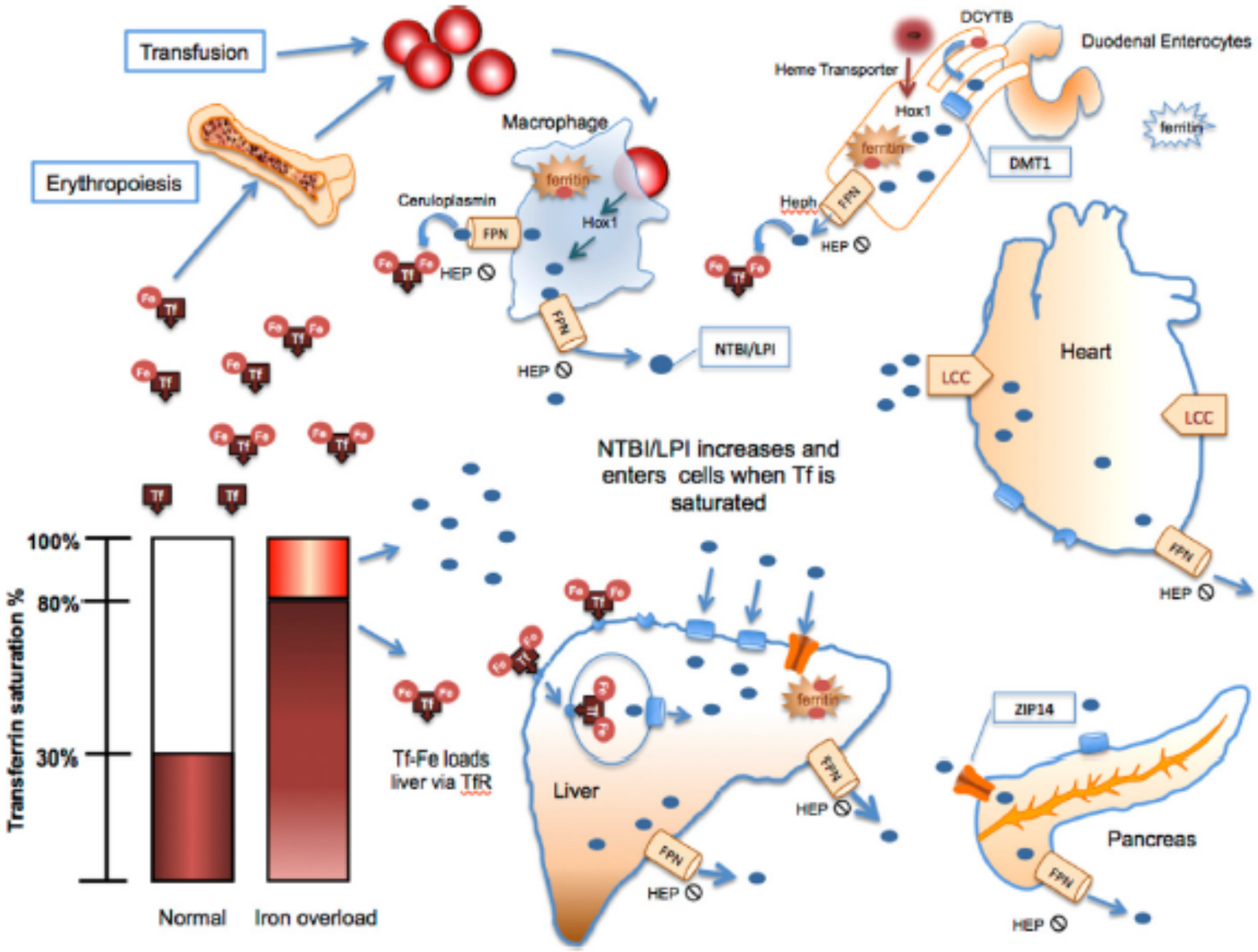
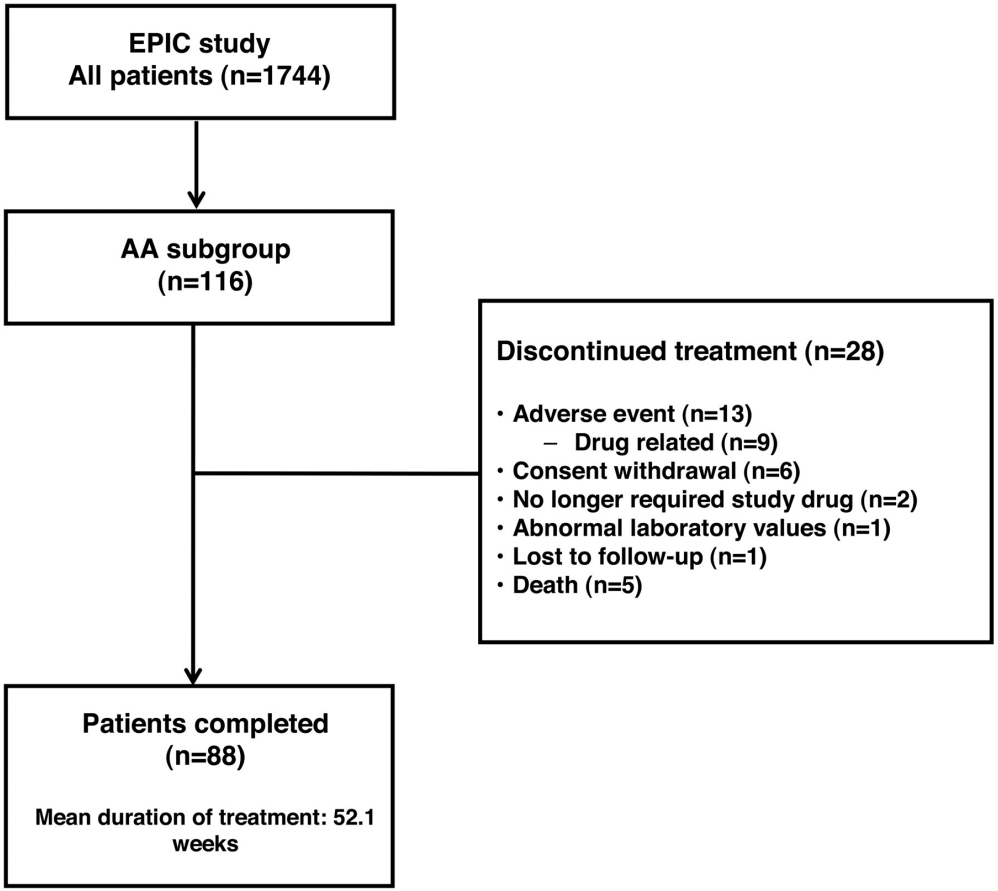

Transfusion
Erythropoiesis
Macrophage
ferritin
Hox1
Ceruloplasmin
FPN
HEP
NTBI/LPI
DCYTB
Duodenal Enterocytes
Heme Transporter
DMT1
Heph
Heart
LCC
NTBI/LPI increases and enters cells when Tf is saturated
Transferrin saturation %
100%
80%
30%
Normal
Iron overload
Tf-Fe loads liver via TfR
Liver
ZIP14
Pancreas

Iron chelation therapy with deferasirox in patients with aplastic anemia: a subgroup analysis of 116 patients from the EPIC trial

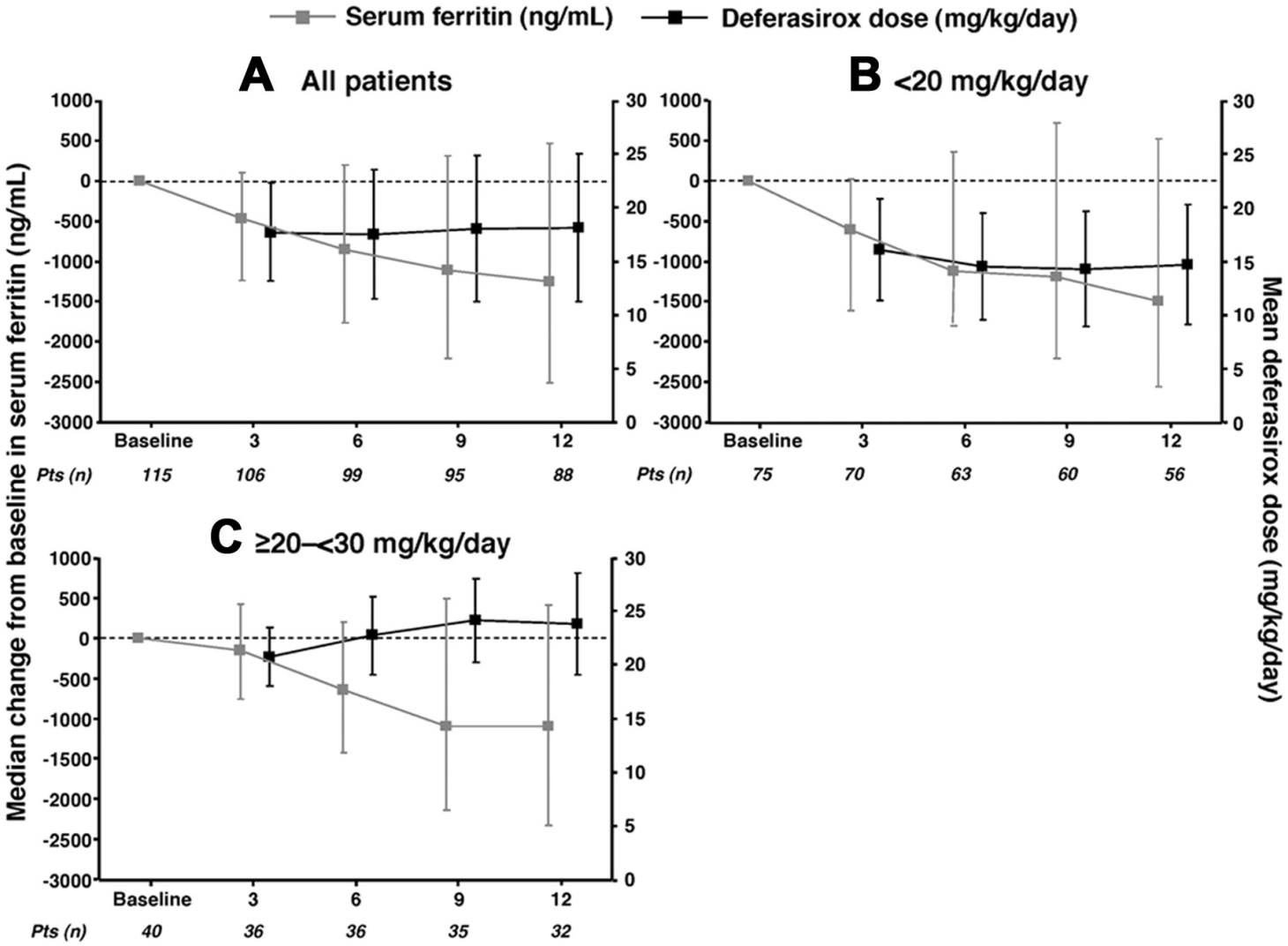
EPIC study patient disposition.



Jong Wook Lee et al. Blood 2010;116:2448-2454

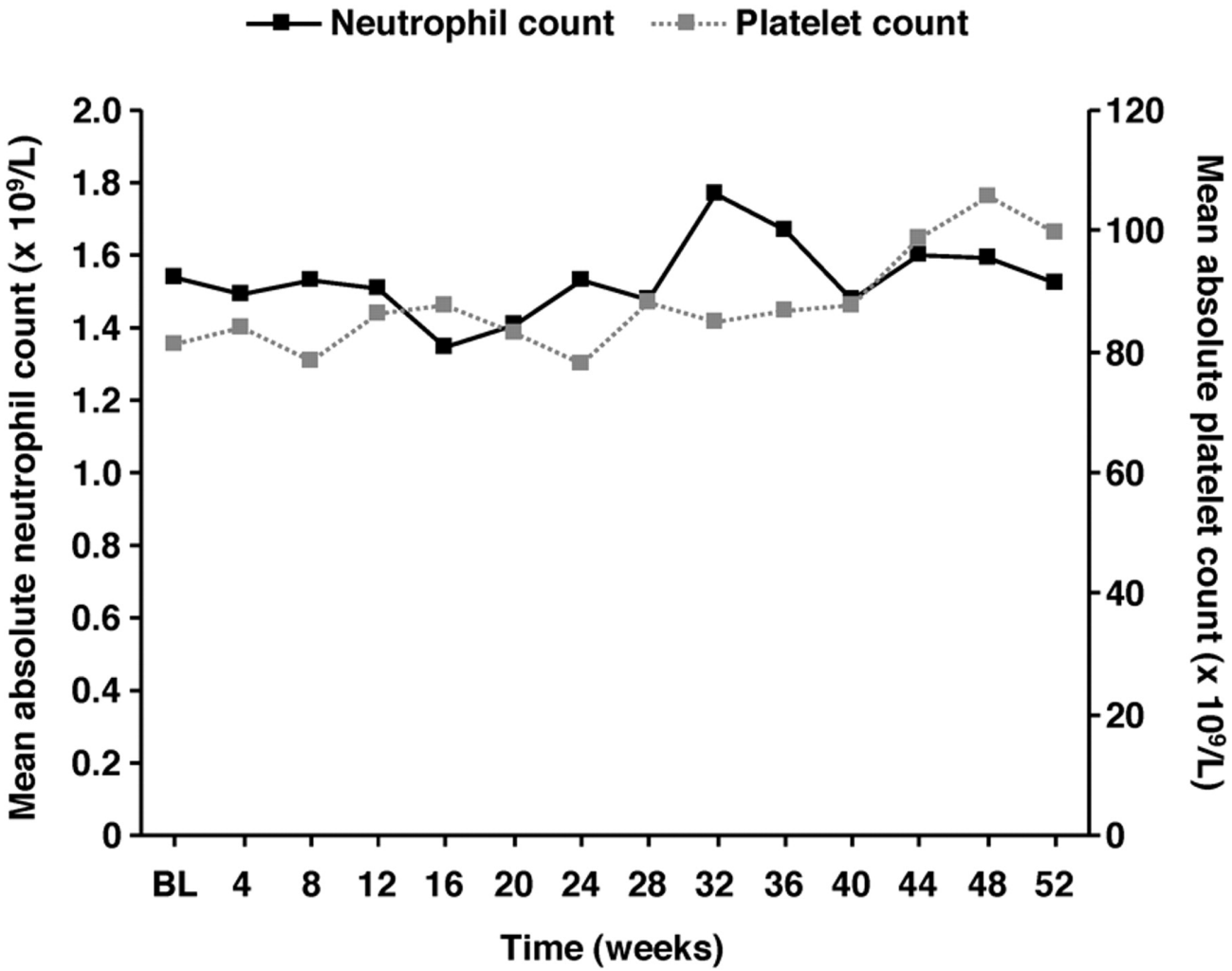
blood

Mean deferasirox dose (± SD) and median change in serum ferritin (± 25th/75th percentiles), by average actual dose categories (full analysis set).



Jong Wook Lee et al. Blood 2010;116:2448-2454

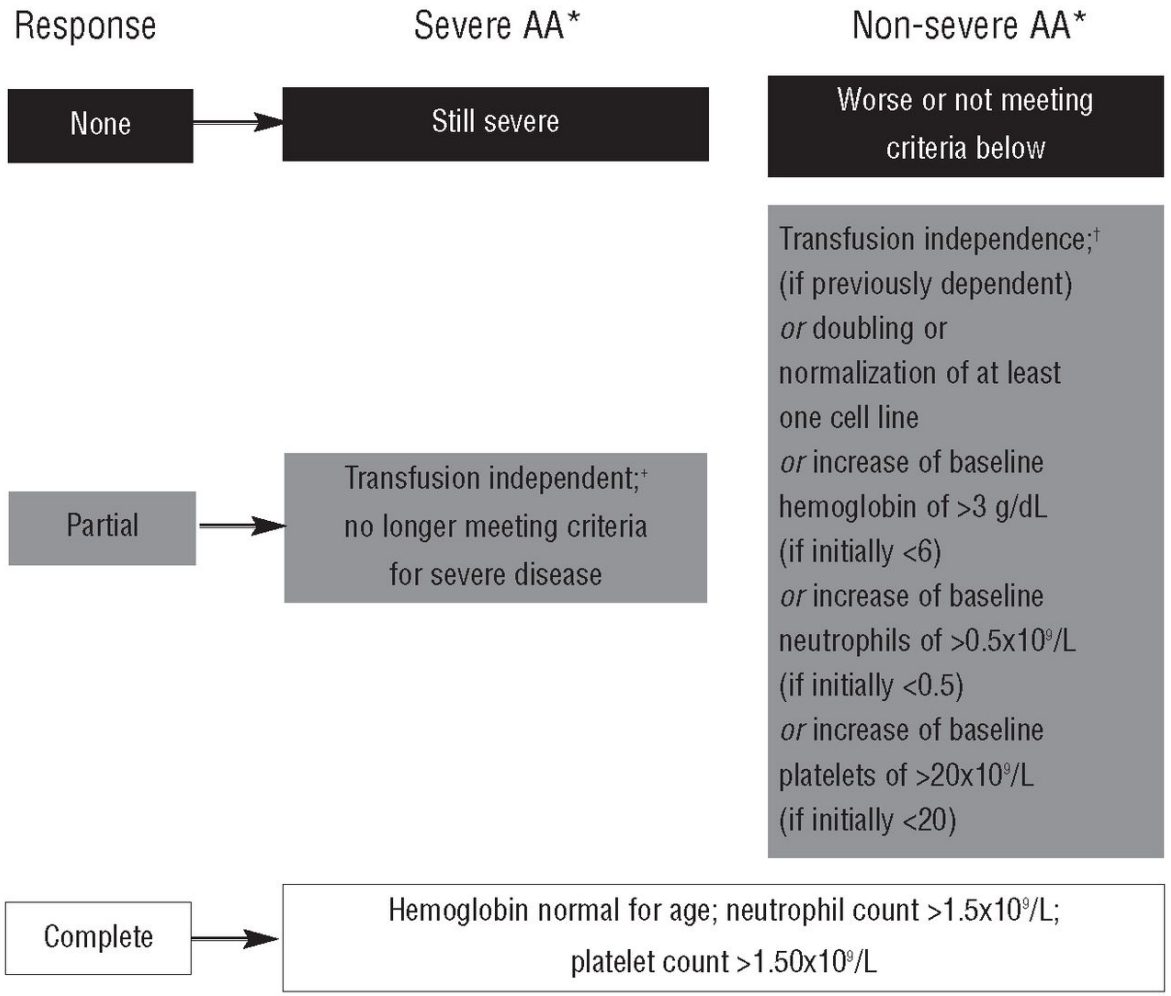
Mean absolute neutrophil and platelet counts during deferasirox treatment.



BL, baseline

Jong Wook Lee et al. Blood 2010;116:2448-2454

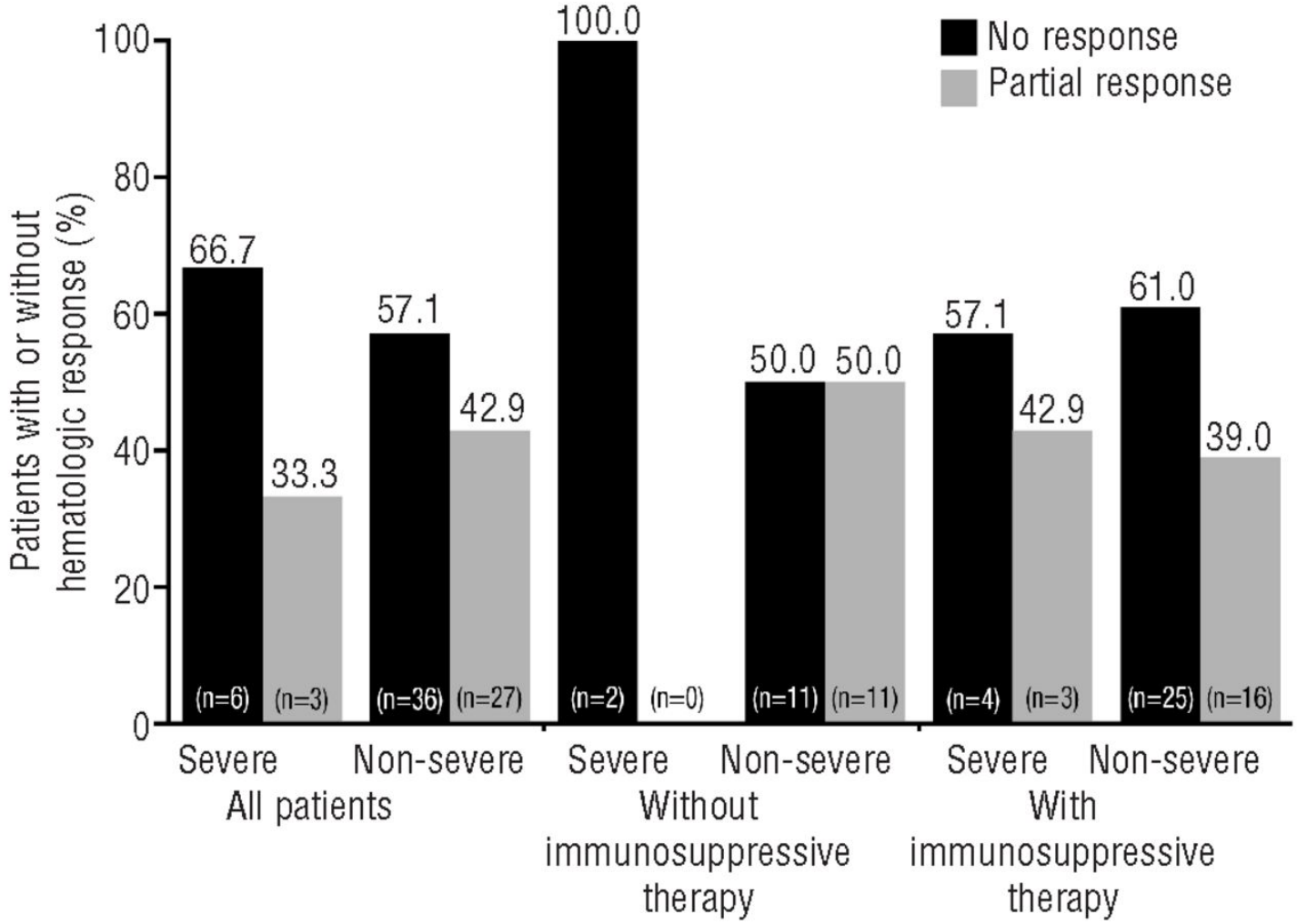
Overview of hematologic response criteria used in the analysis.14 *Each criterion was confirmed with no measure within 28 days that disproved the response. †Transfusion independence was defined as at least one 8-week period (56 days) without any transfusion.



Jong Wook Lee et al. Haematologica 2013;98:1045-1048

haematologica
Journal of the European Hematology Association
Published by the Ferrata Storti Foundation

Hematologic responses with or without immunosuppressive treatment in patients with severe AA and non-severe AA.



Jong Wook Lee et al. Haematologica 2013;98:1045-1048

haematologica
Journal of the European Hematology Association
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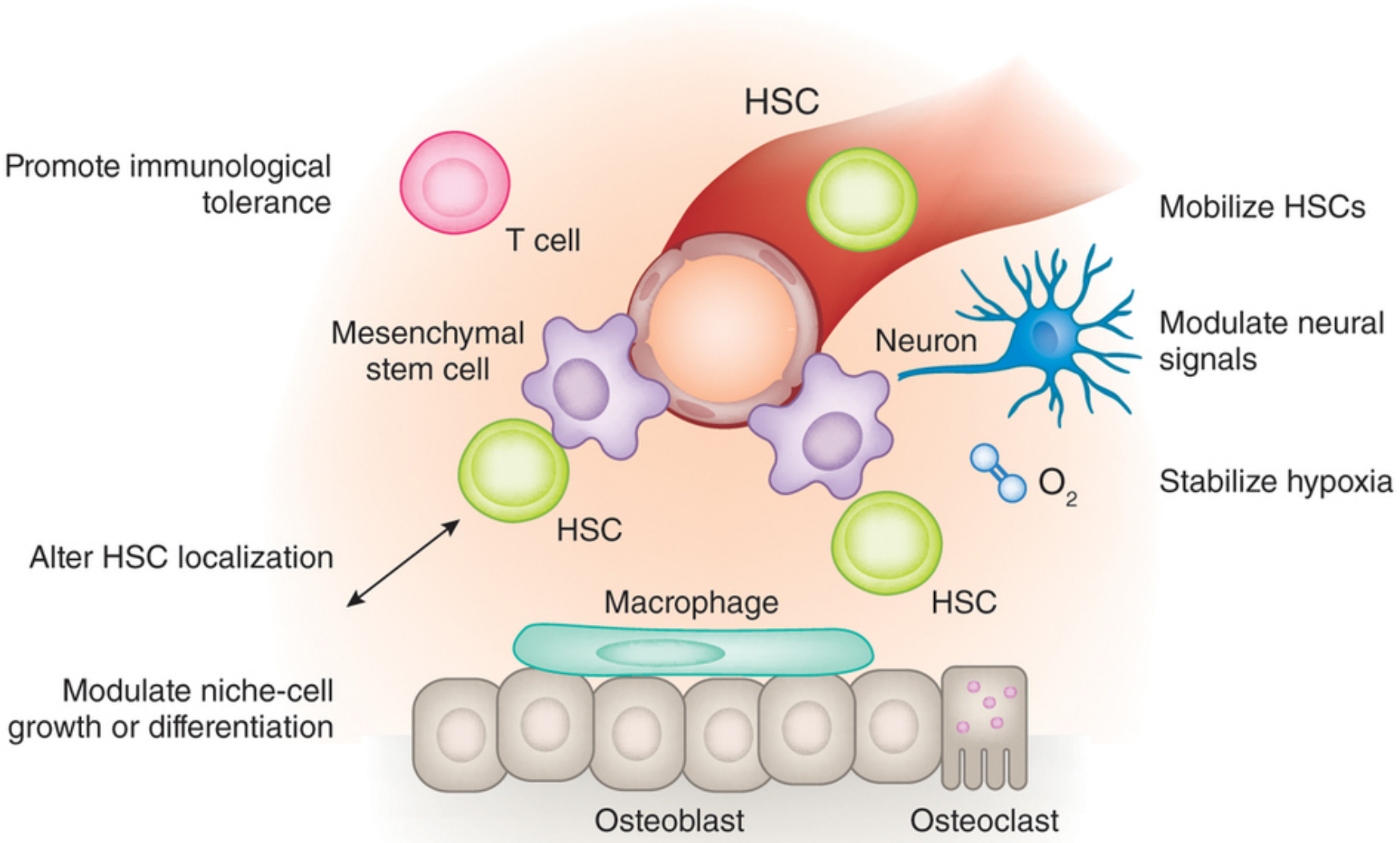
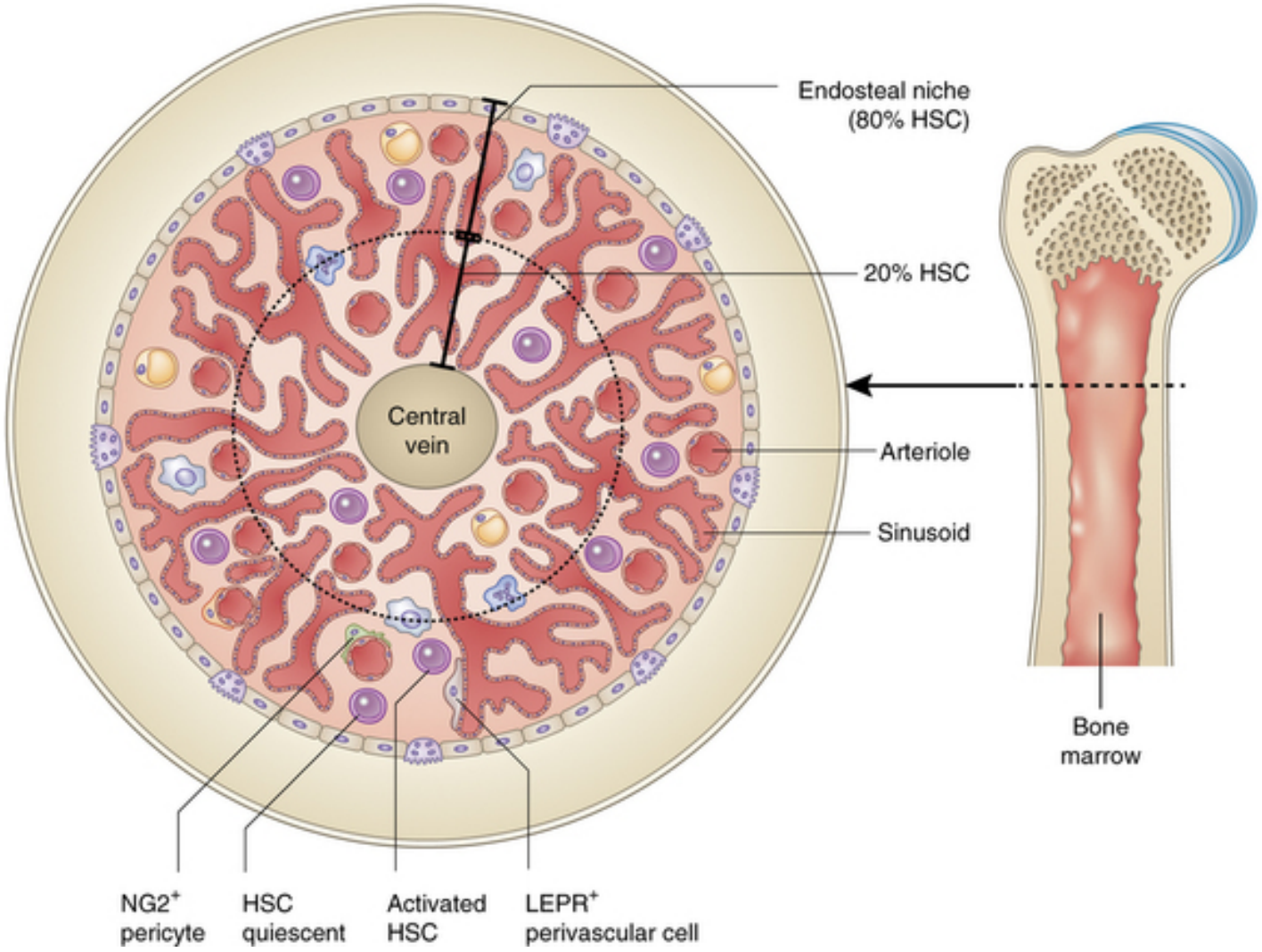
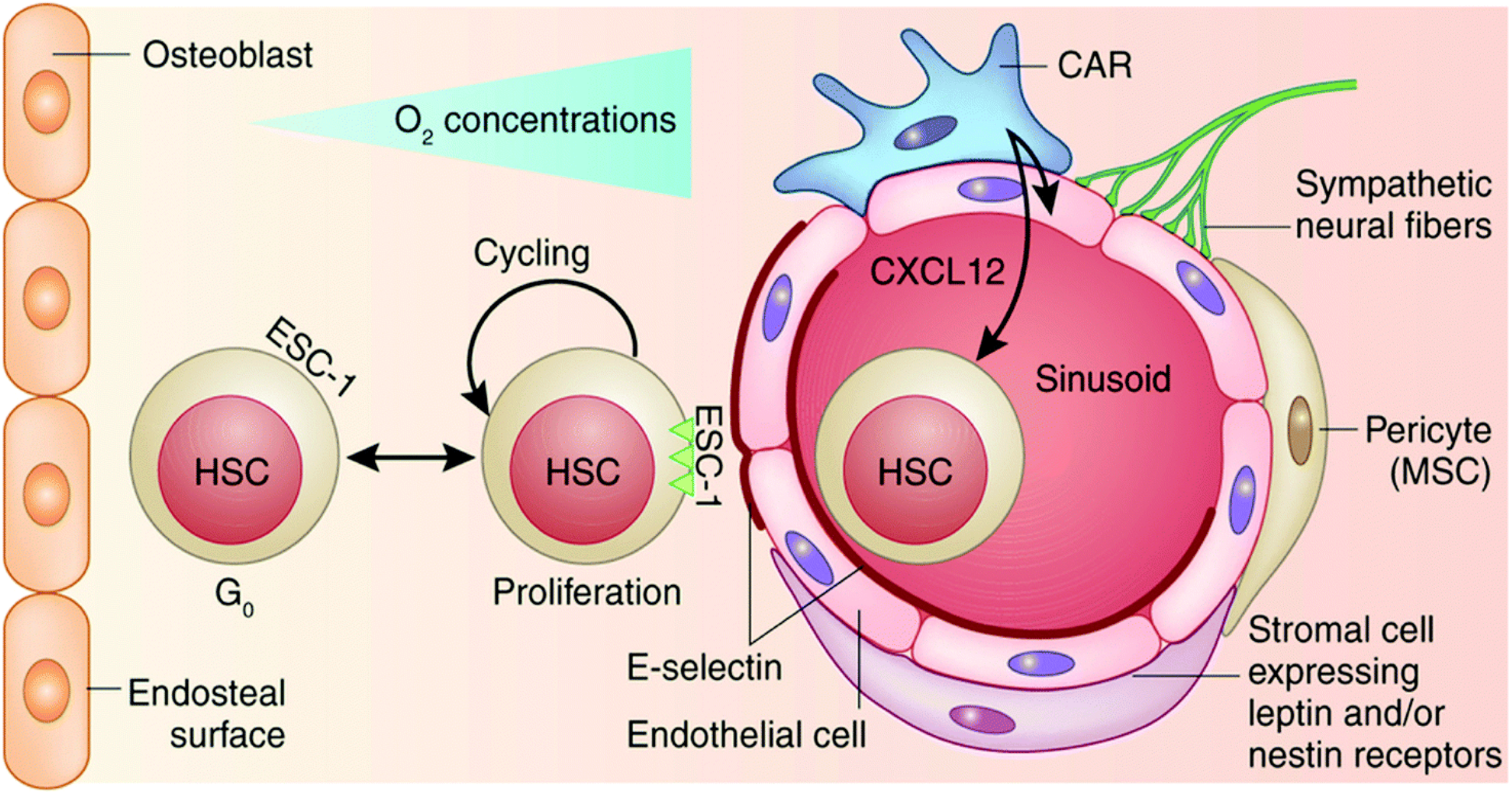


Nature Biotechnology 32(8) August 2014

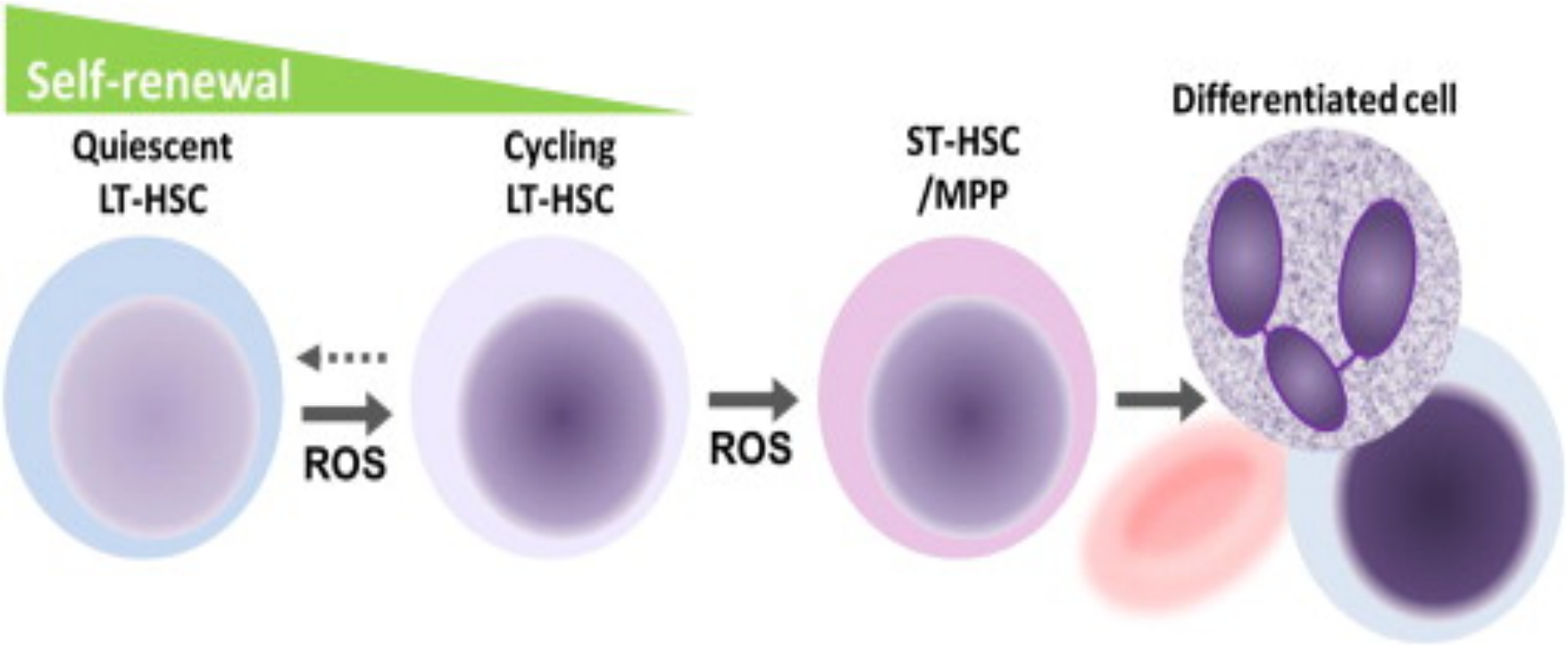


Hematopoietic stem cell niche maintenance during homeostasis and regeneration

•**Avital Mendelson** & **Paul S Frenette***Nature Medicine* **20**,833–846 (2014)



Andrew J. Putnam
Biomater. Sci., 2014,**2**, 1562-1573

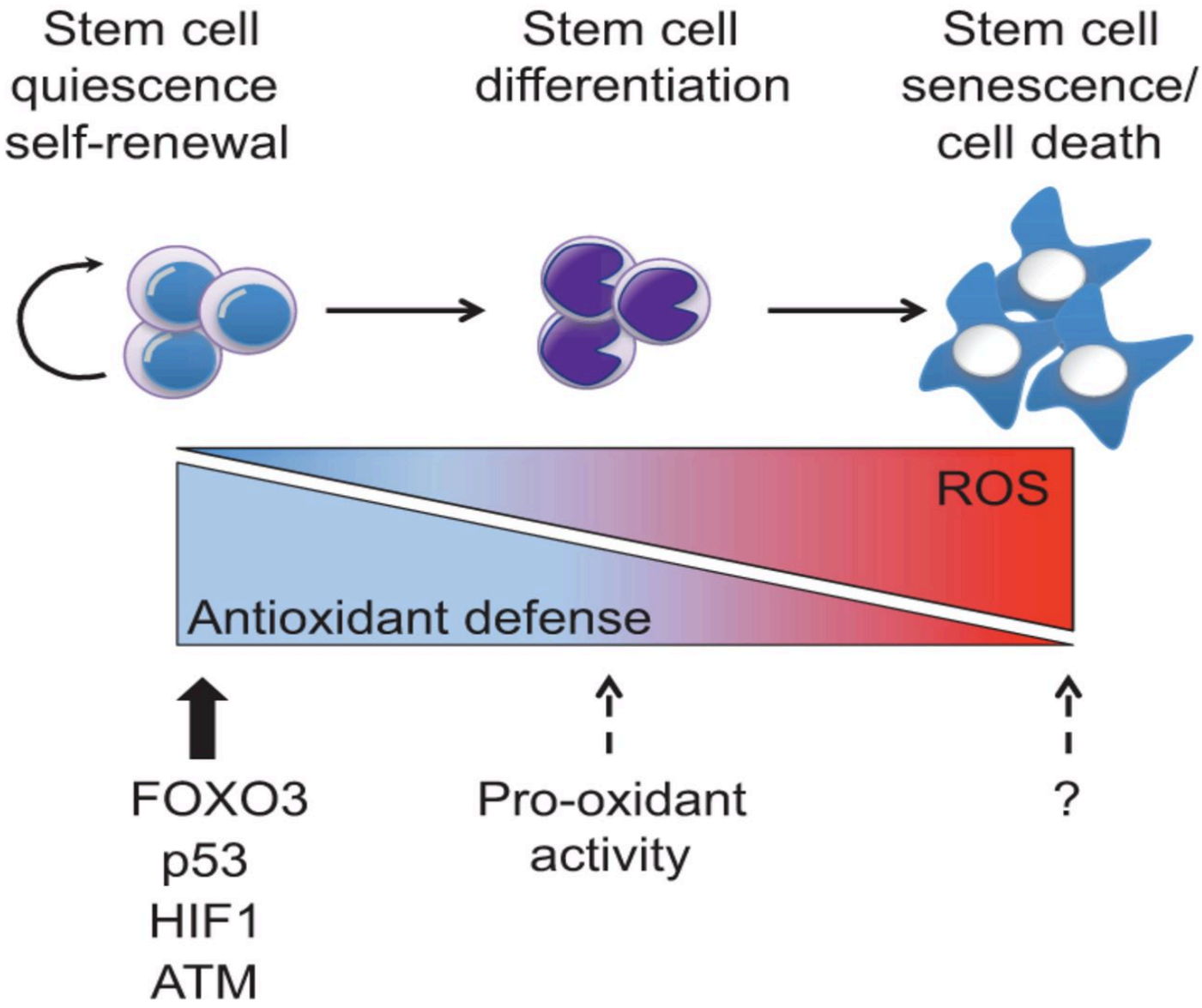


Toshio Suda, Keiyo Takubo, Gregg L. Semenza

Metabolic Regulation of Hematopoietic Stem Cells in the Hypoxic Niche

null, Volume 9, Issue 4, 2011, 298–310

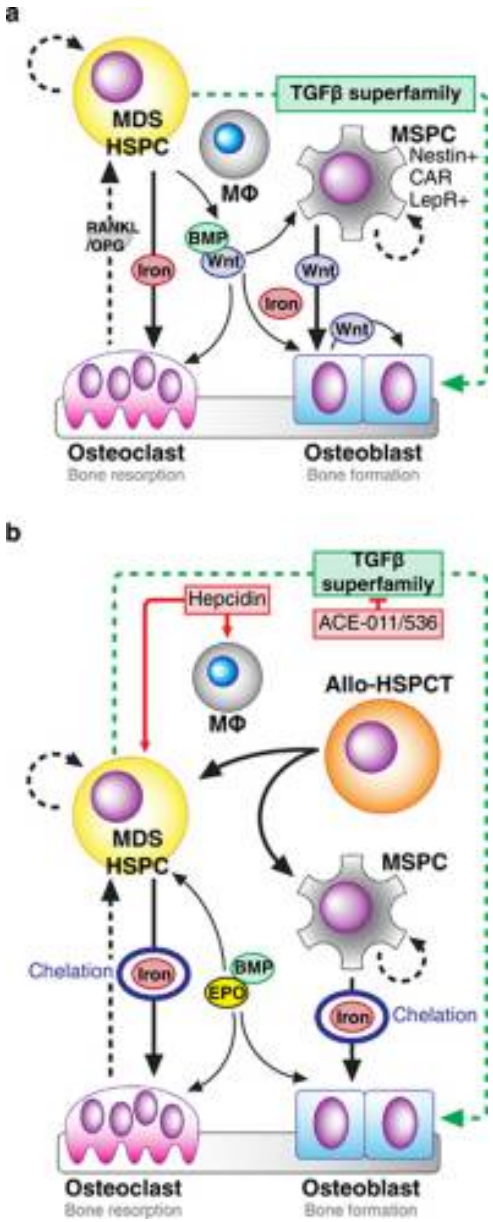
ROS effects on stem cells



Carolina L. Bigarella et al. Development 2014;141:4206-4218

Development

The new scientific rationale of osteo-hematology as emerging research field in MDS



- The niche simultaneously contains stem cells, precursors cells and terminally differentiated cells
- Stem cells live in a specialized microenvironment or niche and depend on it for self-renewal and regulated differentiation
- Hematopoietic stem and progenitor cells (HSPCs) represent precursors for osteoclasts (OCs) responsible for bone resorption, whereas mesenchymal stem and progenitor cells (MSPCs) are precursors for osteoblasts (OBs) that produce the bone matrix
- **In MDS model has reported decreased OBs and OCs number and bone formation rate**
- **Iron overload inhibit OBs and increase OCs**
- **Oxidative stress is involved in the pathogenesis of the bone loss during iron excess**

Bulycheva E et al. Leukemia (2015) 29, 259-268

SCIENTIFIC REPORTS

OPEN

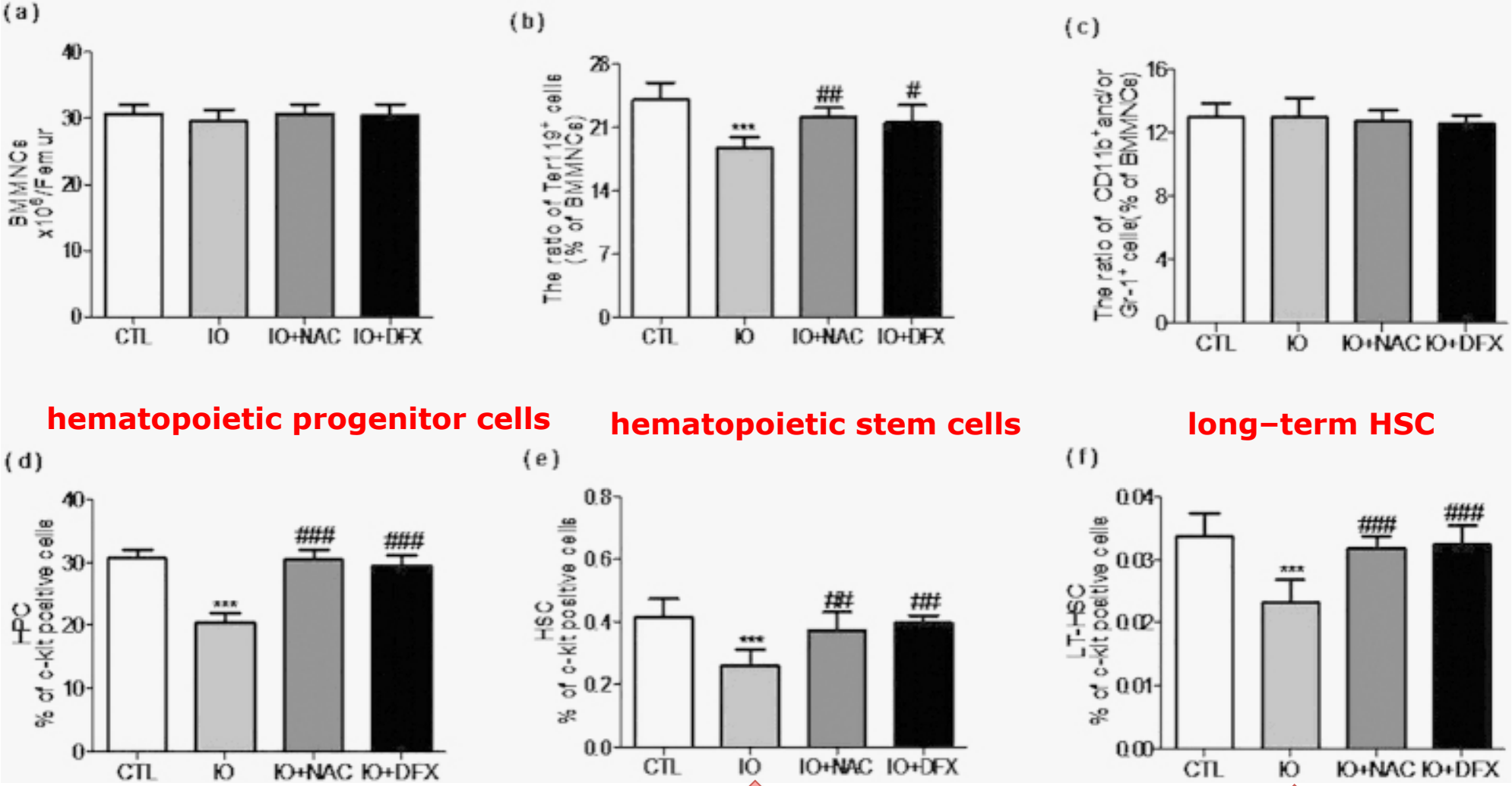
Received: 25 September 2014
Accepted: 01 April 2015
Published: 13 May 2015

ROS-mediated iron overload injures the hematopoiesis of bone marrow by damaging hematopoietic stem/progenitor cells in mice

Xiao Chai[1,3], Deguan Li[2], Xiaoli Cao[1], Yuchen Zhang[1], Juan Mu[1], Wenyi Lu[1], Xia Xiao[1], Chengcheng Li[2], Juanxia Meng[1], Jie Chen[1], Qing Li[1], Jishi Wang[3], Aimin Meng[2] & Mingfeng Zhao[1]

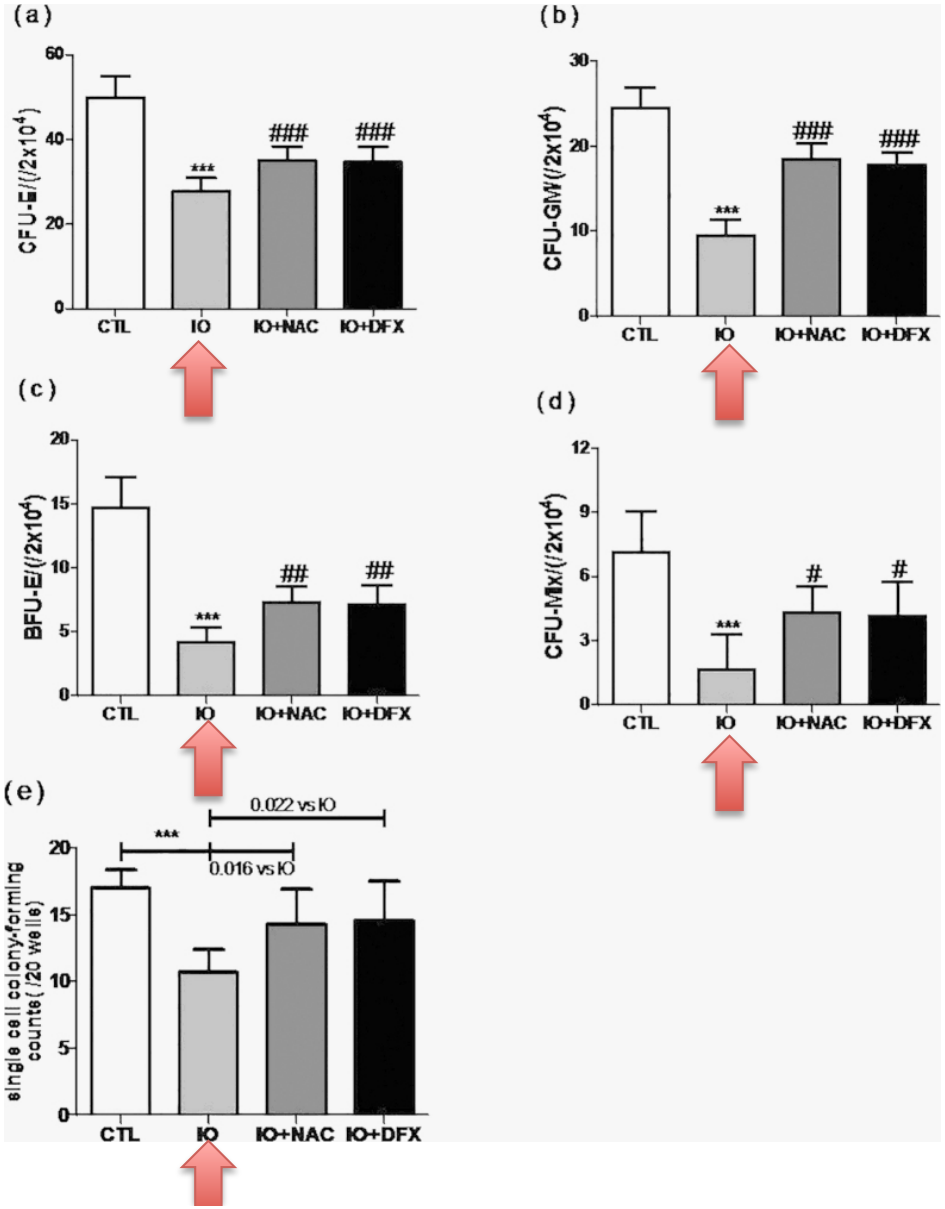
Iron overload, caused by hereditary hemochromatosis or repeated blood transfusions in some diseases, such as beta thalassemia, bone marrow failure and myelodysplastic syndrome, can significantly induce injured bone marrow (BM) function as well as parenchyma organ dysfunctions. However, the effect of iron overload and its mechanism remain elusive. In this study, we investigated the effects of iron overload on the hematopoietic stem and progenitor cells (HSPCs) from a mouse model. Our results showed that iron overload markedly decreased the ratio and clonogenic function of murine HSPCs by the elevation of reactive oxygen species (ROS). This finding is supported by the results of NAC or DFX treatment, which reduced ROS level by inhibiting NOX4 and p38MAPK and improved the long-term and multi-lineage engrafment of iron overload HSCs after transplantation. Therefore, all of these data demonstrate that iron overload injures the hematopoiesis of BM by enhancing ROS through NOX4 and p38MAPK. This will be helpful for the treatment of iron overload in patients with hematopoietic dysfunction.

Iron overload selectively affected the frequencies of immature hematopoietic cells.



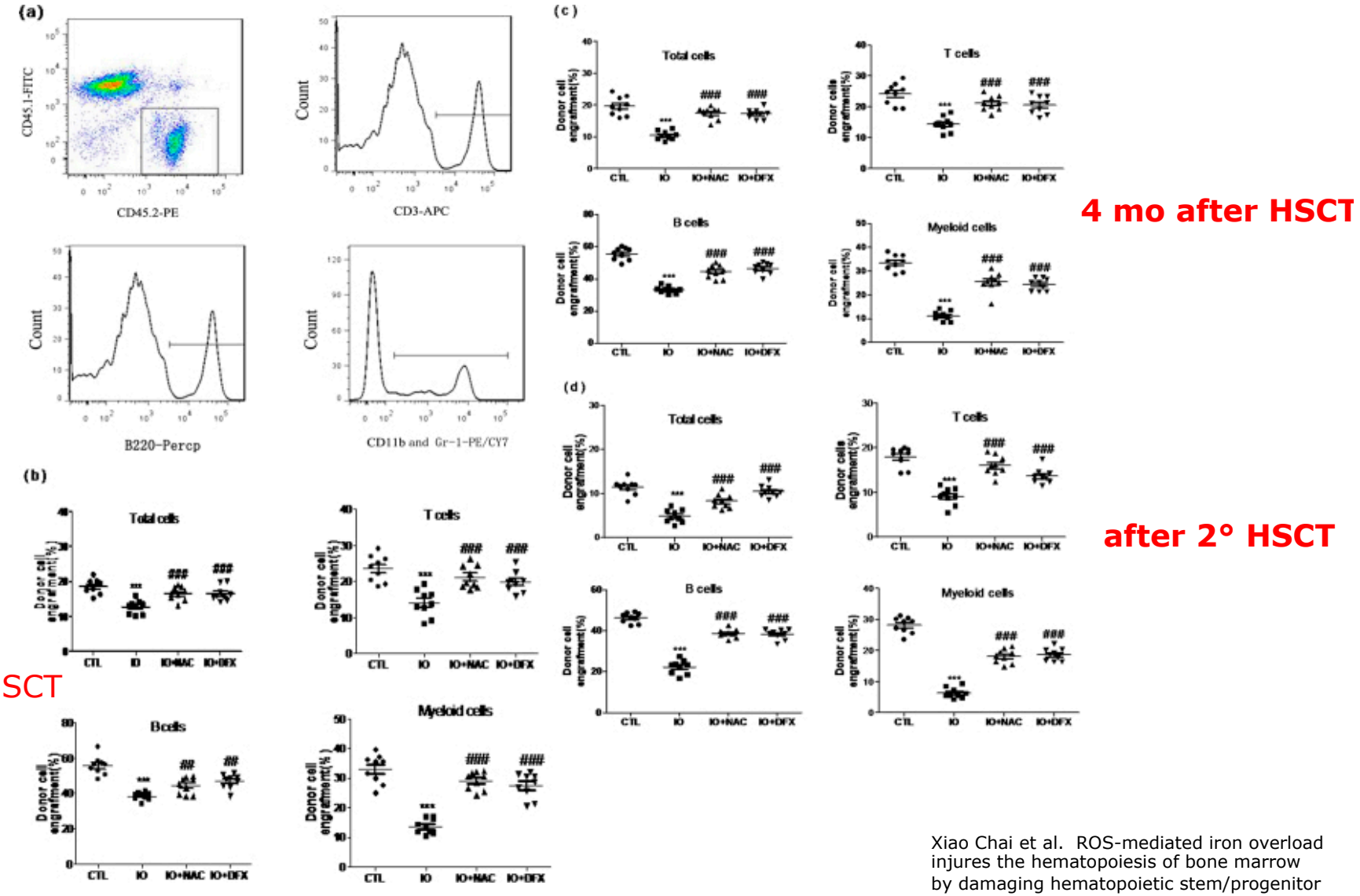
Xiao Chai et al. ROS-mediated iron overload injures the hematopoiesis of bone marrow by damaging hematopoietic stem/progenitor cells in mice. Sci Rep. 2015; 5: 10181.

Iron overload damaged the clonogenic capacity of HSPCs.



Xiao Chai et al. ROS-mediated iron overload injures the hematopoiesis of bone marrow by damaging hematopoietic stem/progenitor cells in mice. Sci Rep. 2015; 5: 10181.

Iron overload impaired the function of HSC long-term and multi-lineage engraftment after bone marrow transplantation.



2 mo after HSCT

4 mo after HSCT

after 2° HSCT

Xiao Chai et al. ROS-mediated iron overload injures the hematopoiesis of bone marrow by damaging hematopoietic stem/progenitor cells in mice. Sci Rep. 2015; 5: 10181.

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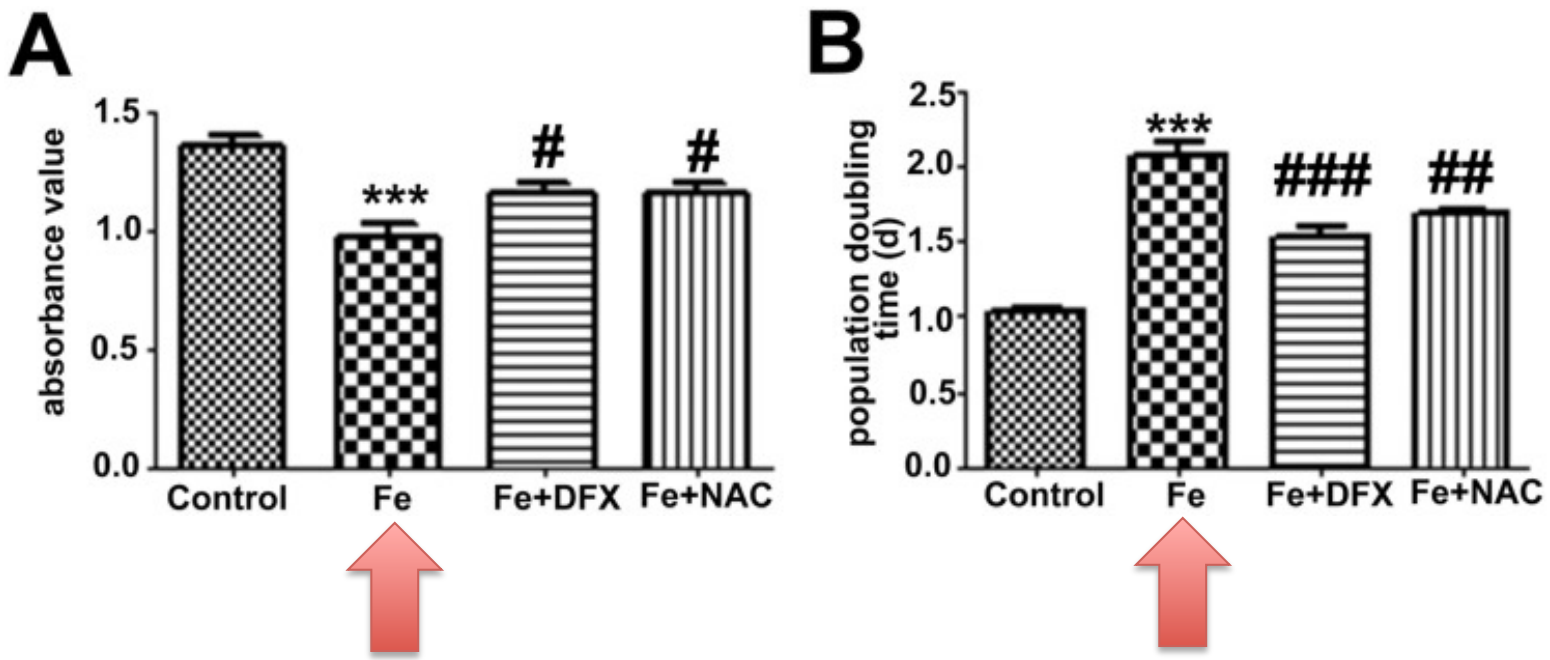
Yuchen Zhang et al.PLoS One. 2015; 10(3): e0120219
Effects of Iron Overload on the Bone Marrow Microenvironment in Mice

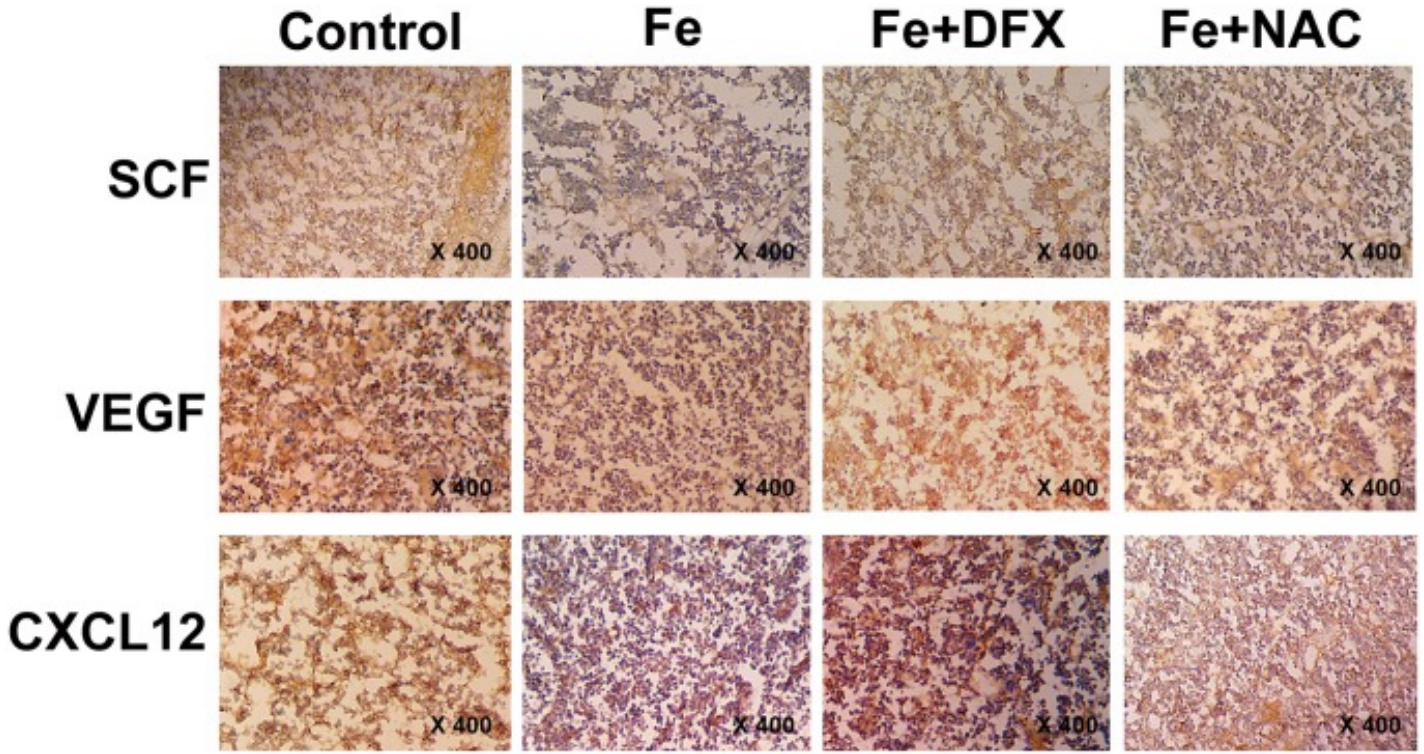
Iron overload inhibited BM-MSCs proliferation ability.



(B) The IO BM-MSCs showed a longer double time (2.07 ± 0,14 days) than control .The effect was reversed by DFX or NAC.

IO inhibited the expression of VEGF, and may damage the generation of sinus in bone marrow, which is vital for hematopoiesis

Yuchen Zhang et al.PLoS One. 2015; 10(3): e0120219



Iron overload inhibited haematopoietic cytokines expression.

Immunohistochemical staining of SCF-1, VEGF-1, and CXCL12 in bone marrow samples from normal mice, IO mice, DFX, and NAC treated mice. Immunohistochemical staining shows brown particles in cytoplasm and protein positive stained cells (×400).

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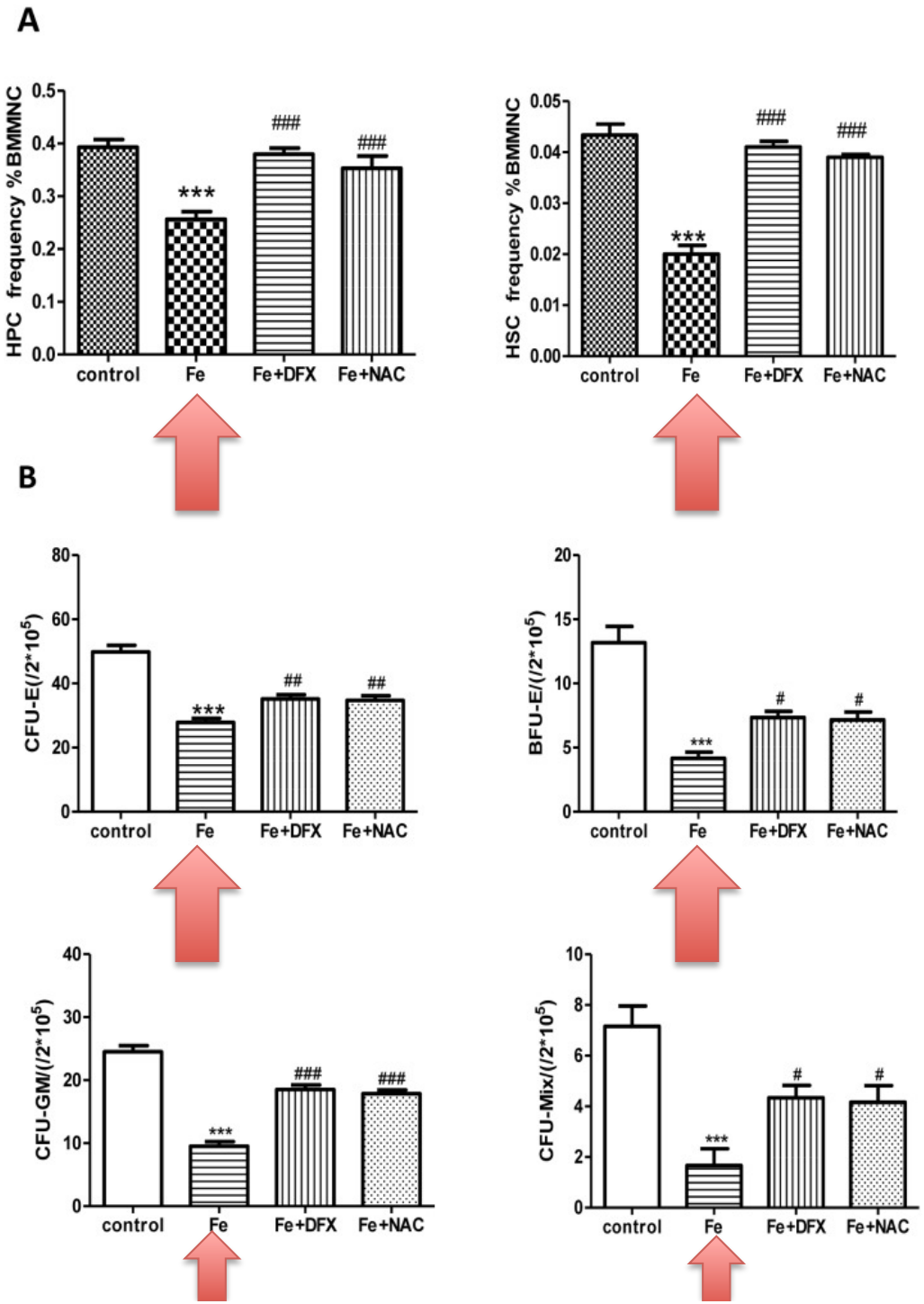
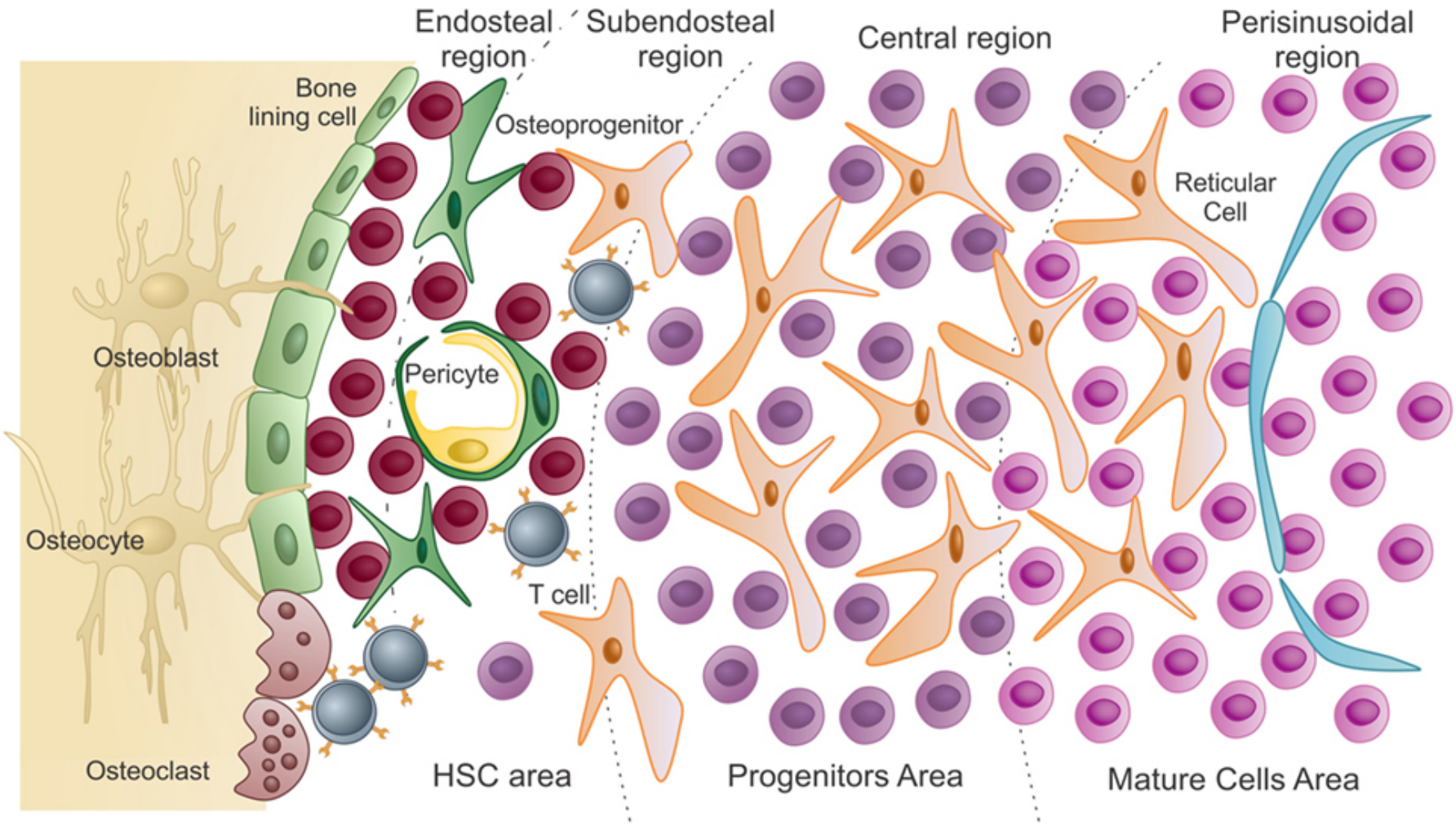


IO decreased the ratio of HPC and HSC in BMMNCs and impaired the supporting function of BM-MSCs.

(A) Iron overload decreased the ratio of HPC and HSC in BMMNCs, which could be reversed by DFX or NAC.

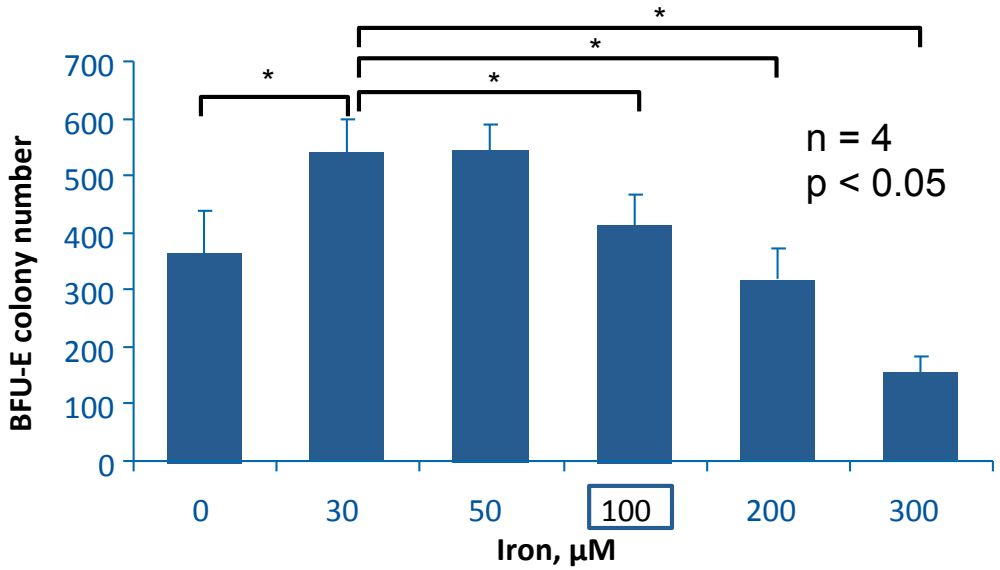
(B) Iron overload impaired the supporting function of BM-MSCs. The colony-forming cell (CFC) assays (CFU-GM, CFU-E, BFU-E, and CFU-mix) were performed as means±SE of three independent experiments.

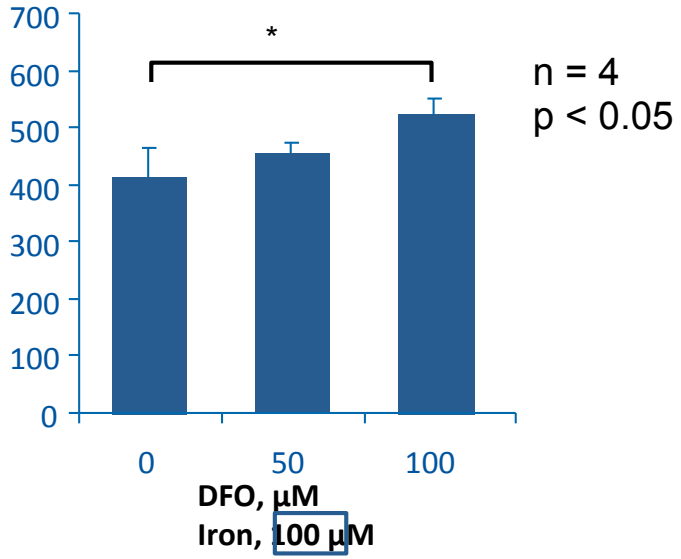
Yuchen Zhang et al.
PLoS One. 2015; 10(3): e0120219

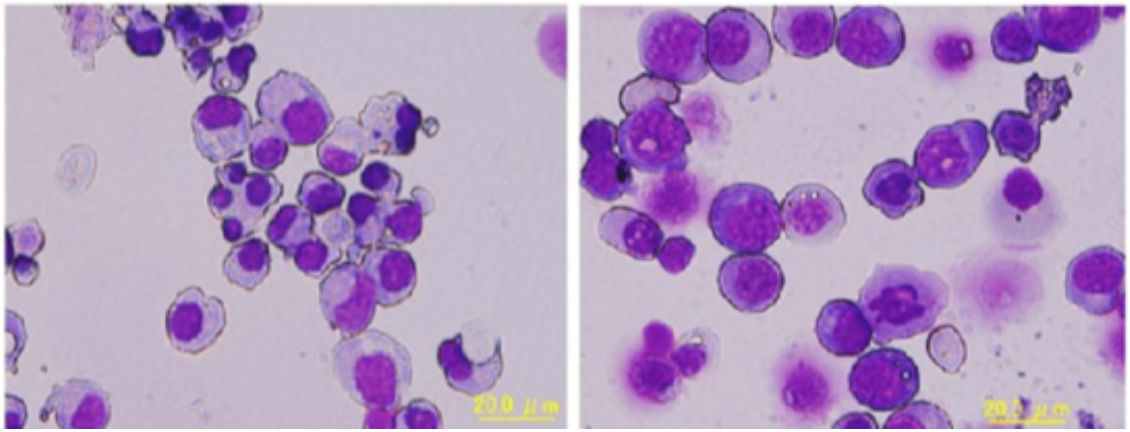


Front. Immunol., 17 May 2016

The effect of iron overload and chelation on erythroid differentiation.

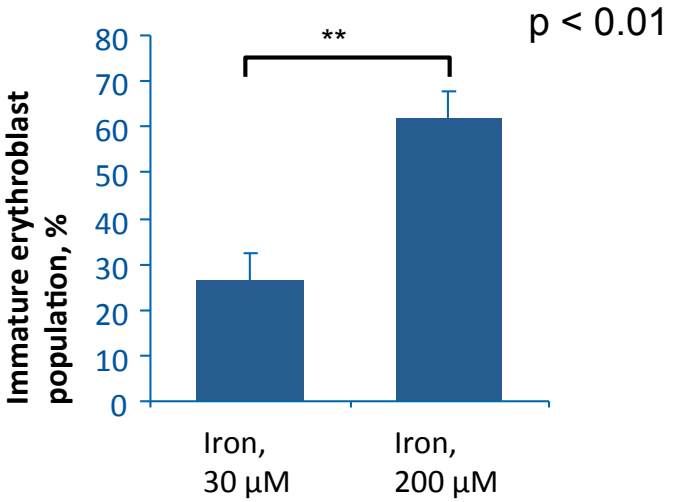






Iron, 30 µM

Iron, 200 µM



Taoka K, et al. Int J Hematol. 2012;95:149-59.

Research Article

Uptake of Non-Transferrin Iron by Erythroid Cells

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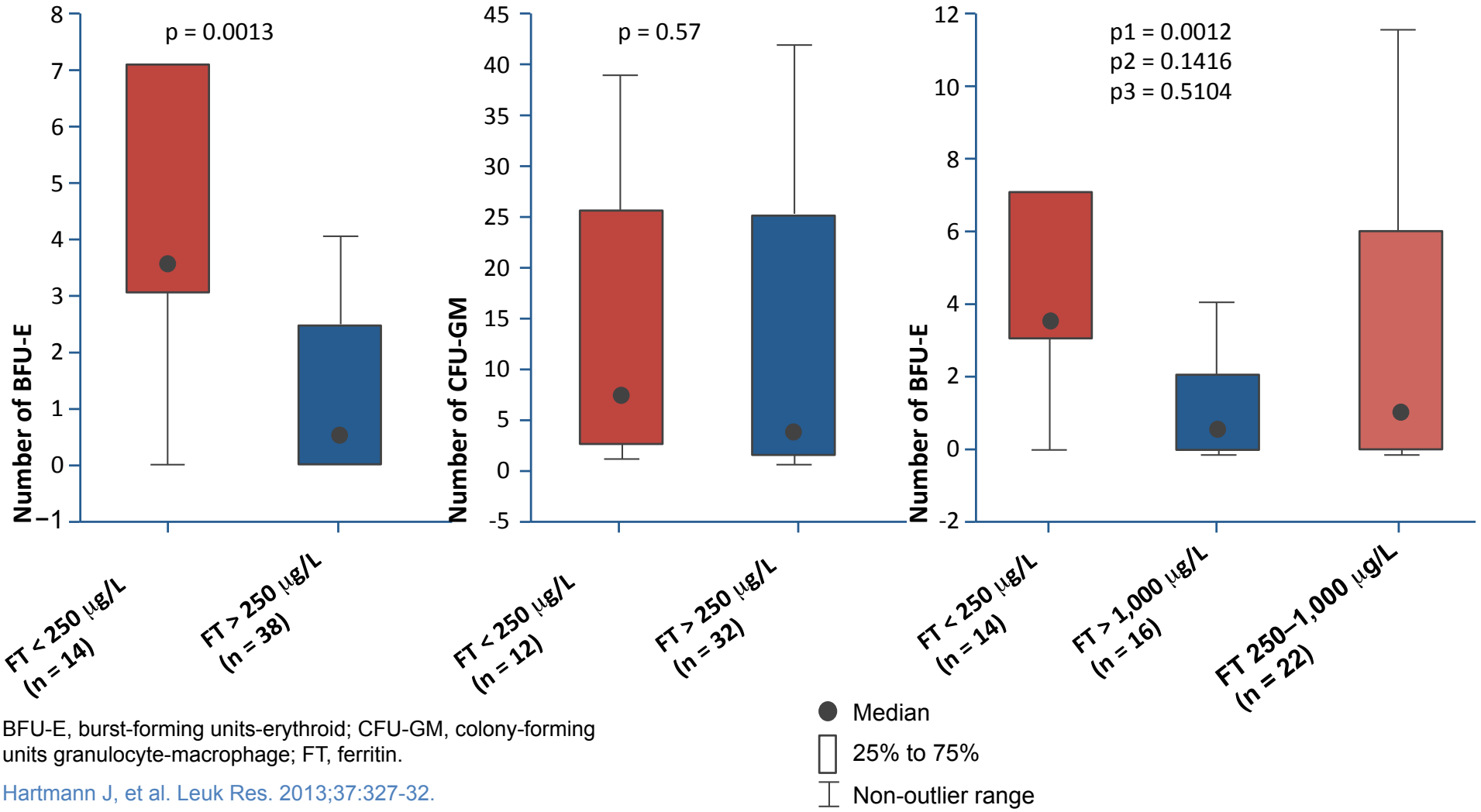
Received 20 September 2010; Accepted 7 November 2010

- RBCs, retics, and developing erythroid precursors take up iron through a Tf-independent pathway.
- This pathway is operative under pathological iron-overload situation in the presence of non-Tf iron in the serum.
- The incoming non-Tf iron does not participate in haem synthesis and Hb production, but induces ROS generation, which results in cytotoxicity and a decrease in the erythroid cell yield.

Prus E, Fibach E. Anemia. 2011;2011:945289.

Proliferation of BFU-E in patients with normal and elevated serum ferritin



BFU-E, burst-forming units-erythroid; CFU-GM, colony-forming units granulocyte-macrophage; FT, ferritin.

Hartmann J, et al. Leuk Res. 2013;37:327-32.

Management of iron overload. Agenda

- Background

- Iron toxicity in aplastic anemia
- **Iron toxicity in HSCT for Aplastic Anemia**

- Management
  - today
  - Perspective
- Conclusion
  - Personal statement

Free iron during conditioning in BMT patients

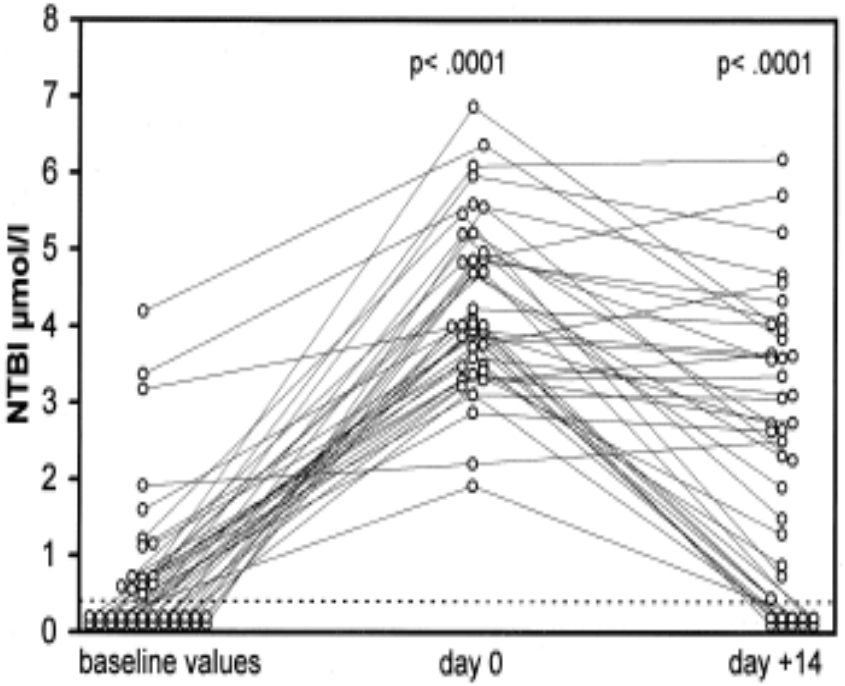


Fig. 1. Nontransferrin-bound iron (NTBI) in serum of 40 BMT patients before conditioning therapy (baseline values), at the day of bone marrow transplantation (day 0) and 2 weeks later (day +14). The dotted line represents the detection limit for NTBI defined as maximal value obtained from 23 healthy adults.

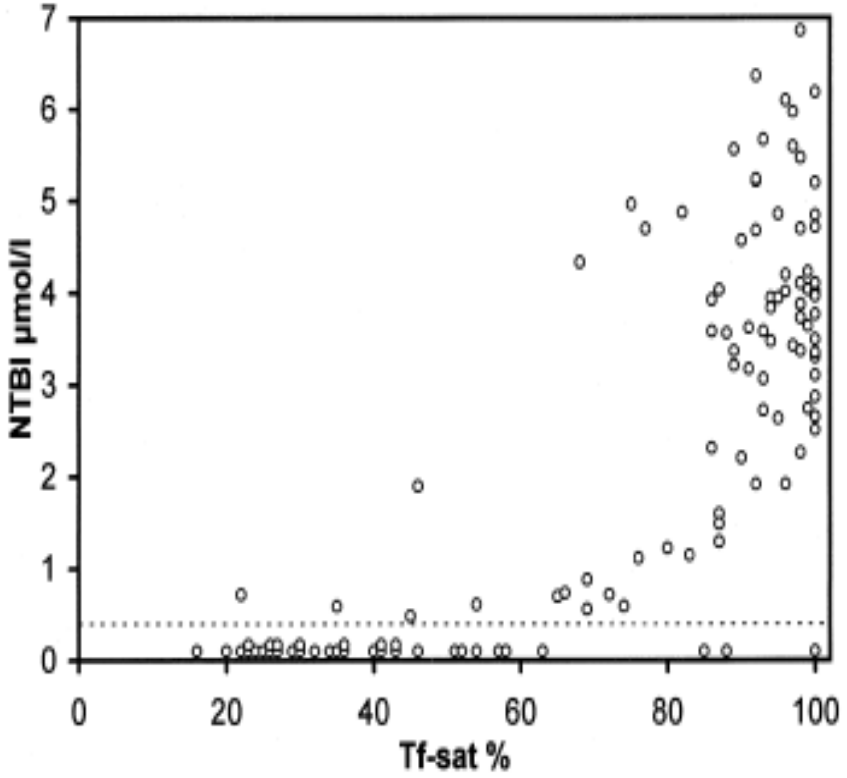
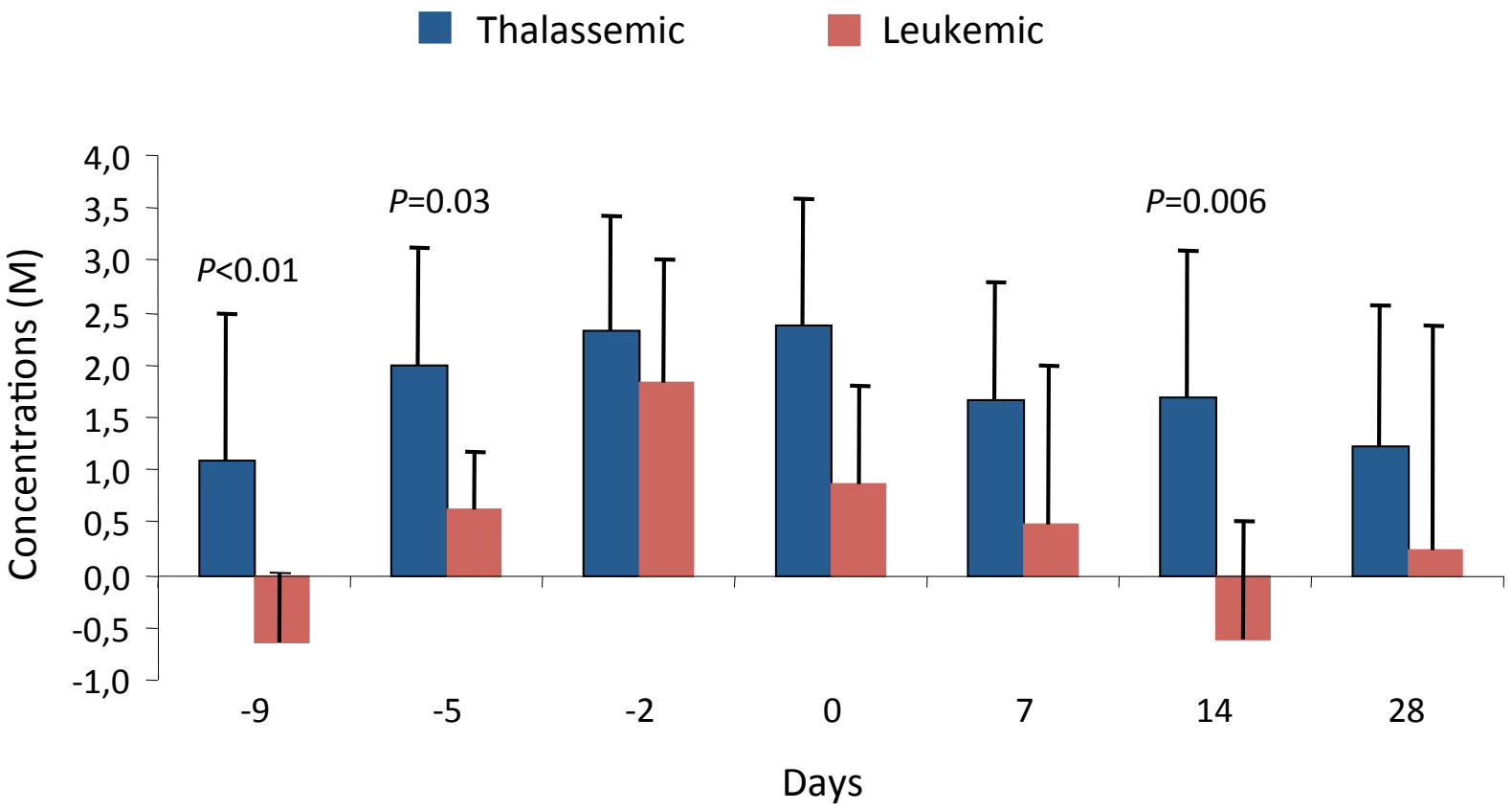


Fig. 2. Nontransferrin-bound iron (NTBI) as a function of transferrin saturation (Tf-sat %). Forty patients with three measurements each (baseline value, day 0 and day +14). The dotted line represents the detection limit for NTBI defined as maximal value obtained from 23 healthy adults.

Dürken et al., Free Radic Biol Med 1997

NTBI After BMT in thalassemia

Pilo F. Presented at the 2007 Italian Society of Hematology meeting

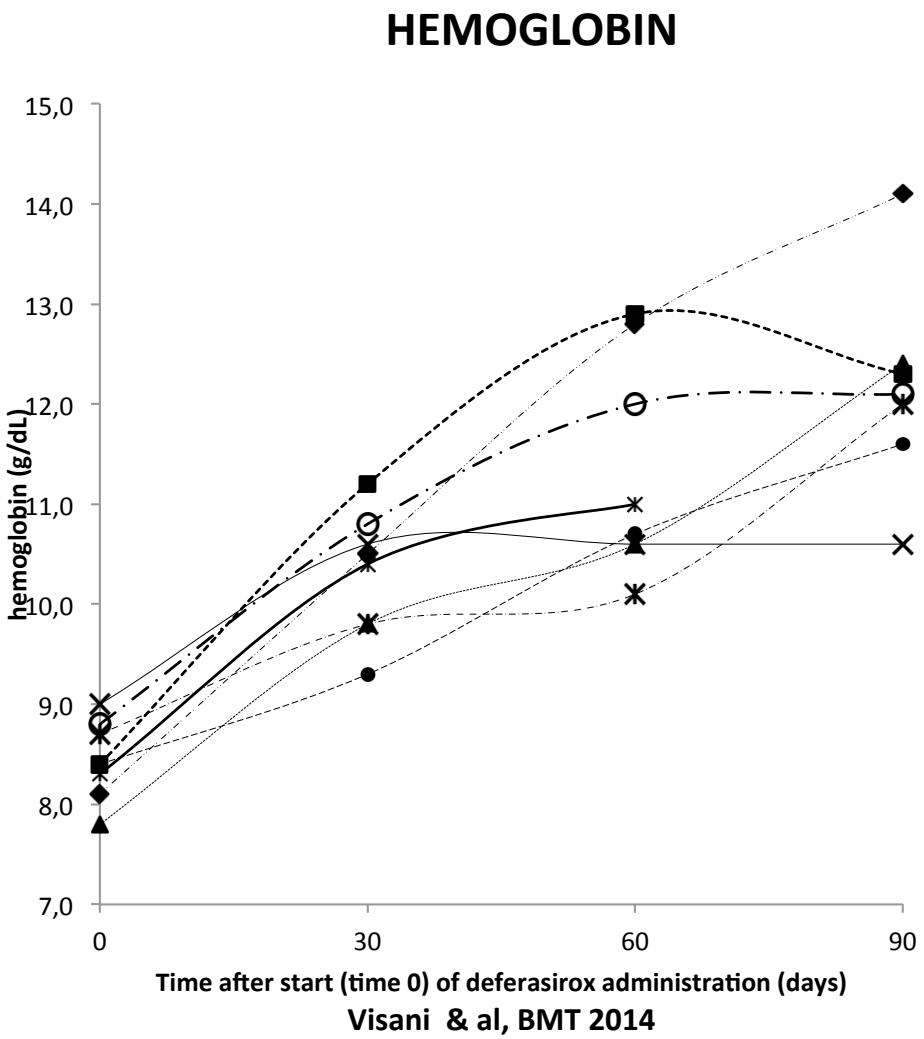


Deferasirox improves hematopoiesis after allogenei hematopoietic SCT.

Eight patients with

- High transfusion burden
- Serum ferritin >1800 ng/ml
- incomplete engraftment and transfusion dependence after allo HSCT (median 5 months, range 3-10 months,)

Sustained improvement of erythropoiesis and subsequently tri-lineage engraftment starting 26 days after initiation of chelation therapy



Visani et al. Bone marrow transplantation 2014; 49: 585-7.

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Management of iron overload

- Before HSCT
- During HSCT
- After HSCT

Clinical guidelines for iron chelation in transfusion-dependent MDS patients

Countries	Transfusion status	SF (µg/L)	Patient profile	Target SF level (µg/L)
Italian (Alessandrino, et al. 2002)	≥ 50 pRBC units	NR	▪ Life expectancy > 6 months	NR
UK (Bowen, et al. 2003)	~ 25 pRBC units (5 g iron)	NR	▪ Pure sideroblastic anaemia ▪ del(5q)	< 1,000
US (NCCN) (v4. 2014)	20–30 pRBC units (≥ 5–10 g iron)	> 2,500	▪ IPSS Low or Int-1 ▪ Potential transplantation patients	For patients with SF > 2,500; aim to decrease to < 1,000
International (Gattermann, et al. 2005)	Transfusion-dependent	> 1,000–2,000	▪ RA, RARS, del(5q) ▪ IPSS Low or Int-1	NR
Japanese (Suzuki, et al. 2008)	> 40 Japanese units	> 1,000	▪ Life expectancy > 1 year	500–1,000
Canadian (Wells, et al. 2008)	Transfusion-dependent	> 1,000	▪ RA, RARS, del(5q) ▪ IPSS Low or Int-1 ▪ IPSS Int-2 or High (if SF > 1,000 and SCT candidates/life expectancy > 1 year)	NR; reduce dose when < 2,000; discontinue chelator when < 1,000
Spanish (Arrizabalaga, et al. 2008)	Transfusion-dependent	> 1,000	▪ IPSS Low or Int-1 ▪ WPSS Very low, Low, or Int ▪ Spanish prognostic index Low risk	NR
Austrian (Valent, et al. 2008)	Transfusion-dependent	> 2,000	▪ Life expectancy > 2 years	NR
Israeli (Mittelman, et al. 2008)	20–25 pRBC units	> 1,000	▪ IPSS Low or Int-1 ▪ Candidates for SCT	< 500 to < 1,000
MDS Foundation (Bennett, et al. 2008)	2 pRBC units/month for ≥ 1 year	> 1,000	▪ Life expectancy > 1 year	NR
Italian update (Santini, et al. 2010)	≥ 20 pRBC units (4 g iron)	NR	▪ IPSS Low or Int-1 ▪ IPSS Int-2, High when responding to disease-modifying agent or candidates for SCT	NR

NR, not reported; RA, refractory anaemia; RARS, RA with ringed sideroblasts; SCT, stem cell transplantation; WPSS, WHO-classification-based Prognostic Scoring System.

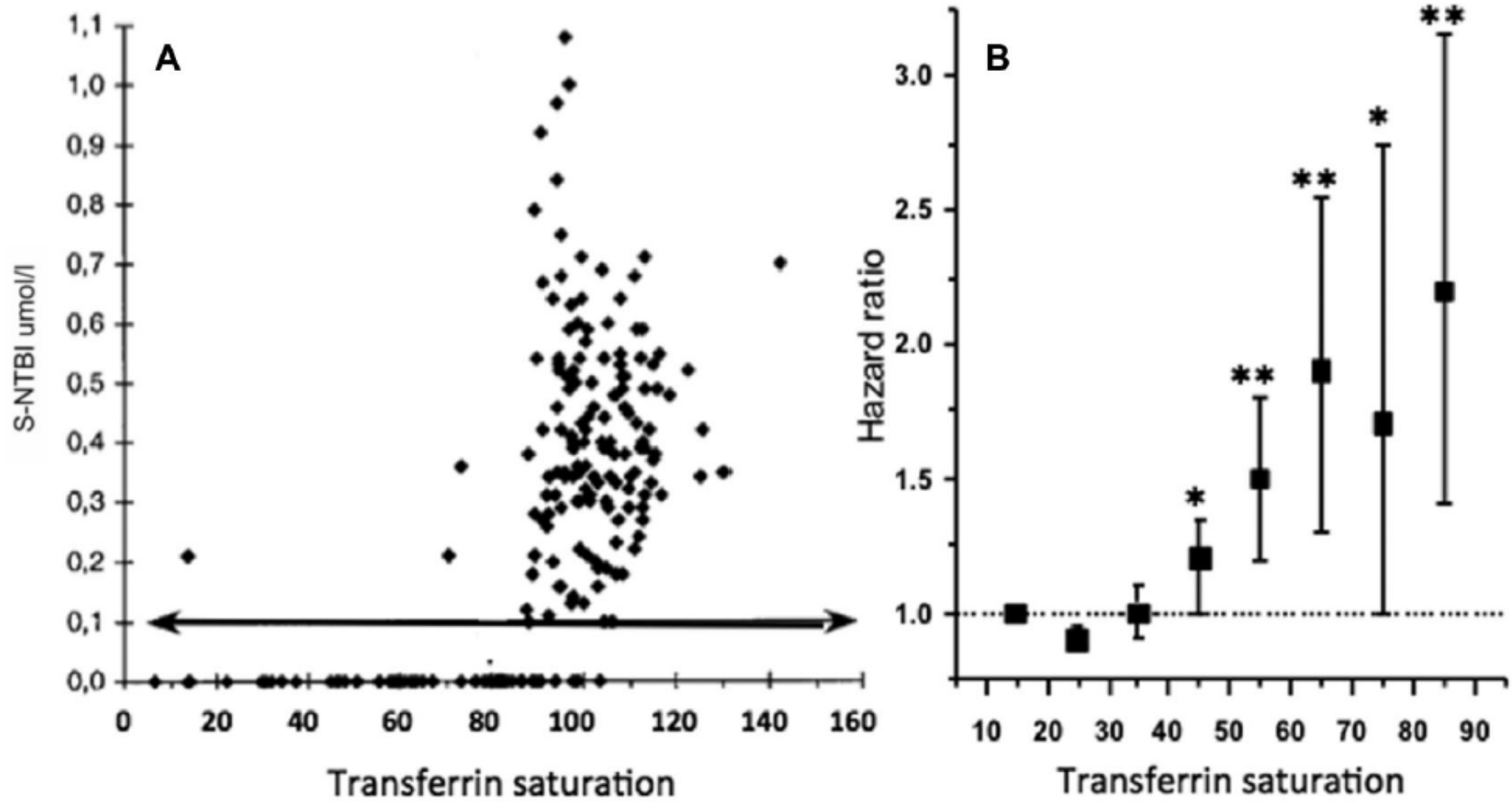


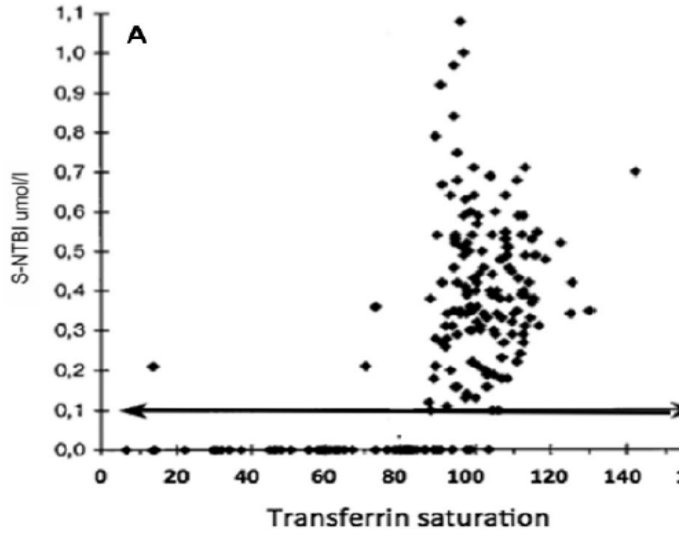
Figure 1. (A) The relation of transferrin saturation (TS) on the *x*-axis to serum non-transferrin-bound iron (S-NTBI).[38] (B) The relation of TS on *x*-axis to hazard ratio and 95% confidence intervals for all-cause mortality in two general populations of adults. $^{*}P \leq 0.05$, $^{**}P < 0.001$. $P = 0.004$ for trend.[46,67]

Coates et al. Annals New York Academy of Sciences 2016.

Goal iron level after HSCT

- Maintain labile iron and total body iron levels within a normal range.

- In every day clinical practice:
  - **Normal transferrin saturation**.



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Fe Toxicity tissue =

$$\Sigma \text{Tissue Reactive Iron} \times \text{Genetics} \times \text{Environmental Factors} \times \text{Time}$$

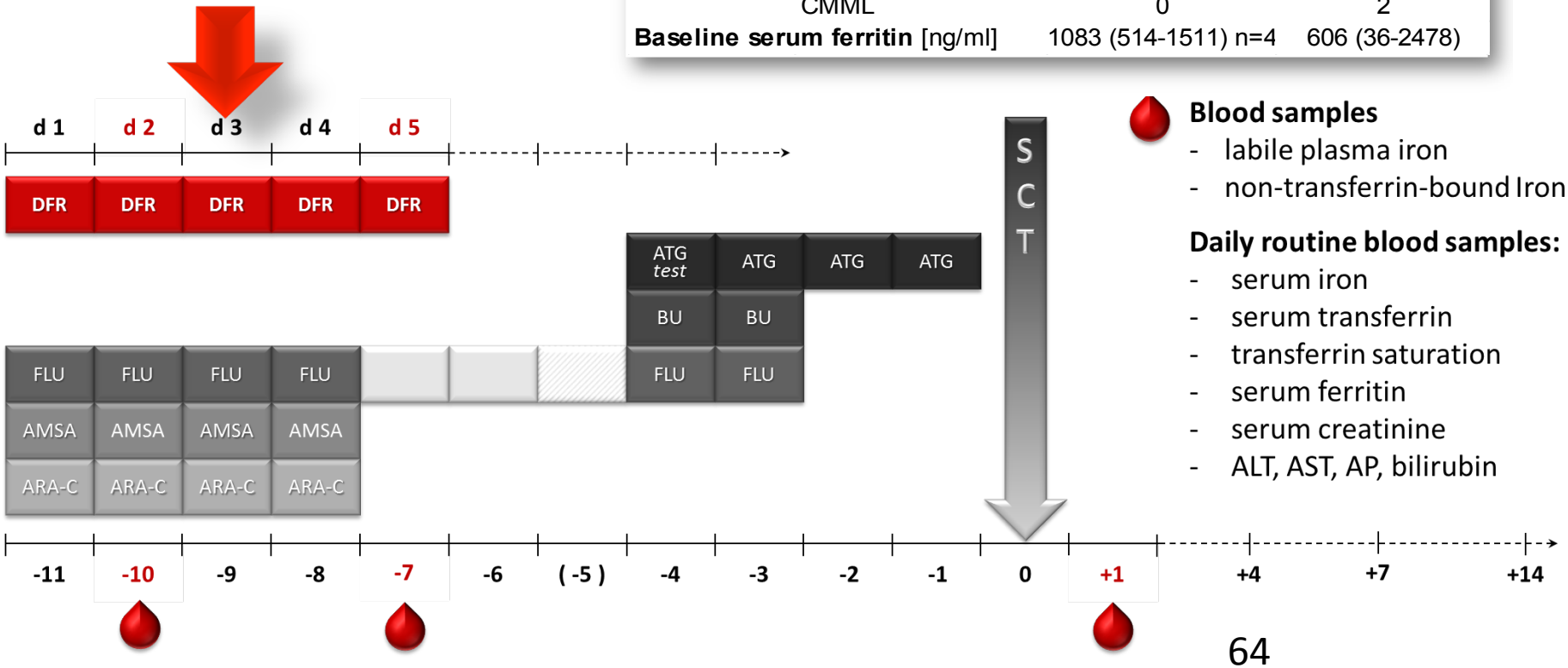
"Iron toxicity depends on many factors in addition to the level of iron per se"

Coates TD. Free Radic Biol Med 2014

Deferasirox during conditioning regimen – German study

Median DFR-dose:
16.8 mg/kg/d
(range 14.5 – 19.7)

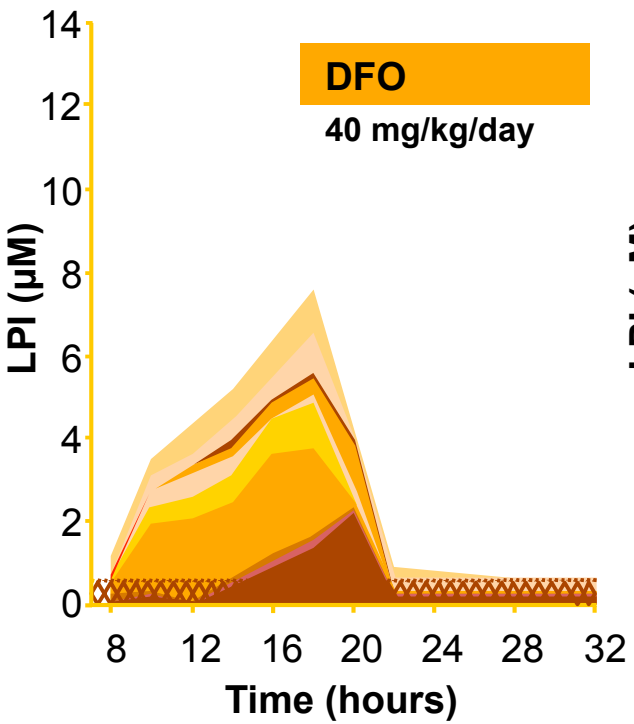
		DFR-Group	Control
Patients		5	4
Median age [years] range		69 (61-73)	64 (55-70)
Gender [m/w]		3/2	3/1
Median bodyweight [kg] range		70 (60-84)	75 (66-101)
Disease	AML	4	1
	MDS	1	1
	CMML	0	2
Baseline serum ferritin [ng/ml]		1083 (514-1511) n=4	606 (36-2478)



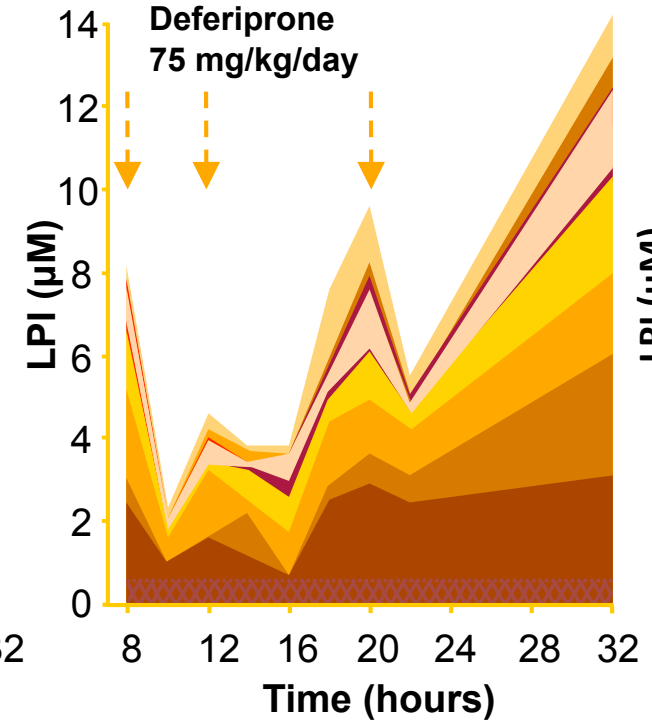
Blood samples
- labile plasma iron
- non-transferrin-bound Iron

Daily routine blood samples:
- serum iron
- serum transferrin
- transferrin saturation
- serum ferritin
- serum creatinine
- ALT, AST, AP, bilirubin

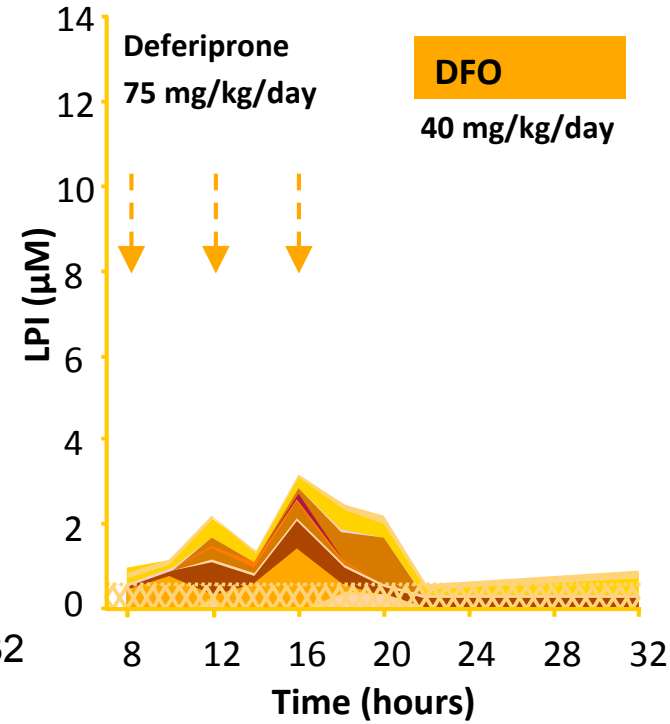
Effect of Iron Chelation Monotherapy and Combined Therapy on Labile Plasma Iron (LPI)



LPI is suppressed during DFO infusion.



LPI is reduced after oral administration of deferiprone. However, levels rebound between doses.



LPI is better controlled with DFO/deferiprone combination therapy. Patient treatment burden is considerably increased.

DFO, Desferoxamine

Reproduced from Cabantchik *et al. Best Pract Res Clin Hematol*. 2005;18:277-87.

Statement

- Iron toxicity is not the cause of aplastic anemia
- Iron toxicity cause HSC damage and can contribute to severity of the disease in aplastic anemia and trouble post transplant HSC recovery
- Iron chelation is not the treatment of aplastic anemia
- Iron chelation can contribute to prevent further worsening of aplastic anemia and to resolve a few cases of delayed post transplant hematopoietic recovery

Acknowledgments

- Cagliari Hematology and Transplant Group
- Genova Hematology and Transplant Group

- Federica Pilo

Thank you for your kind attention