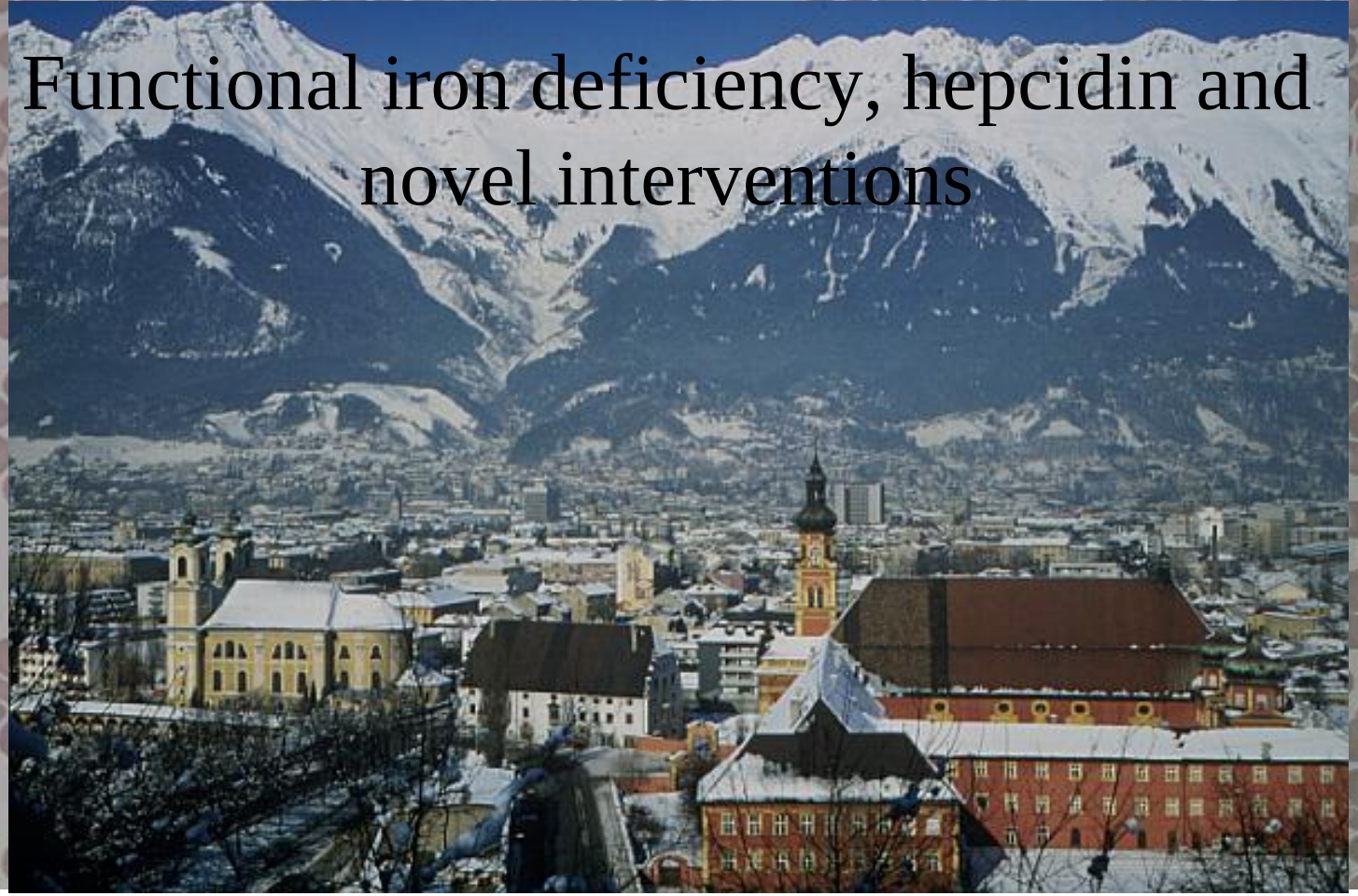
Functional iron deficiency, hepcidin and novel interventions



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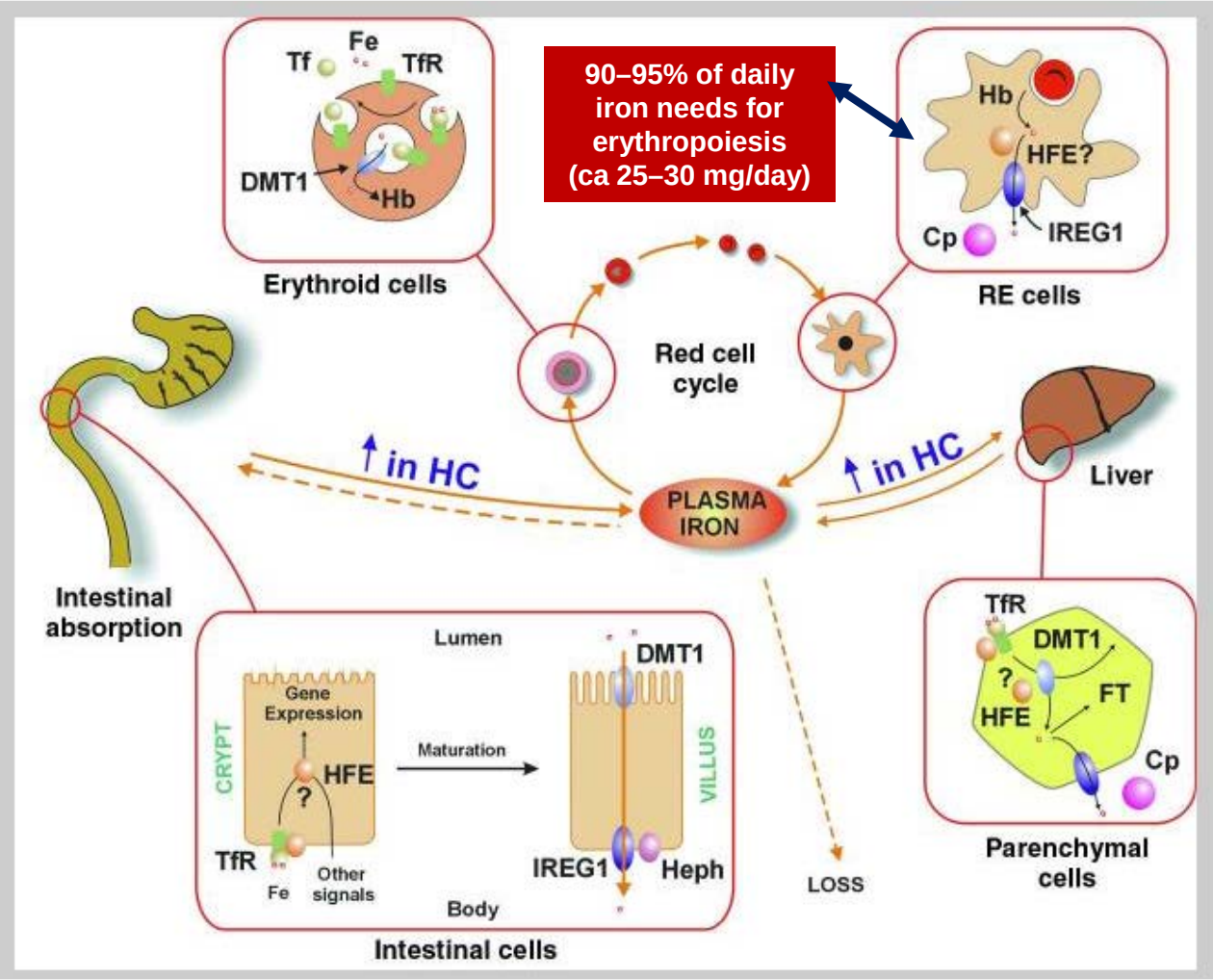
IM VI

Stringent control of iron homeostasis is essential for life!



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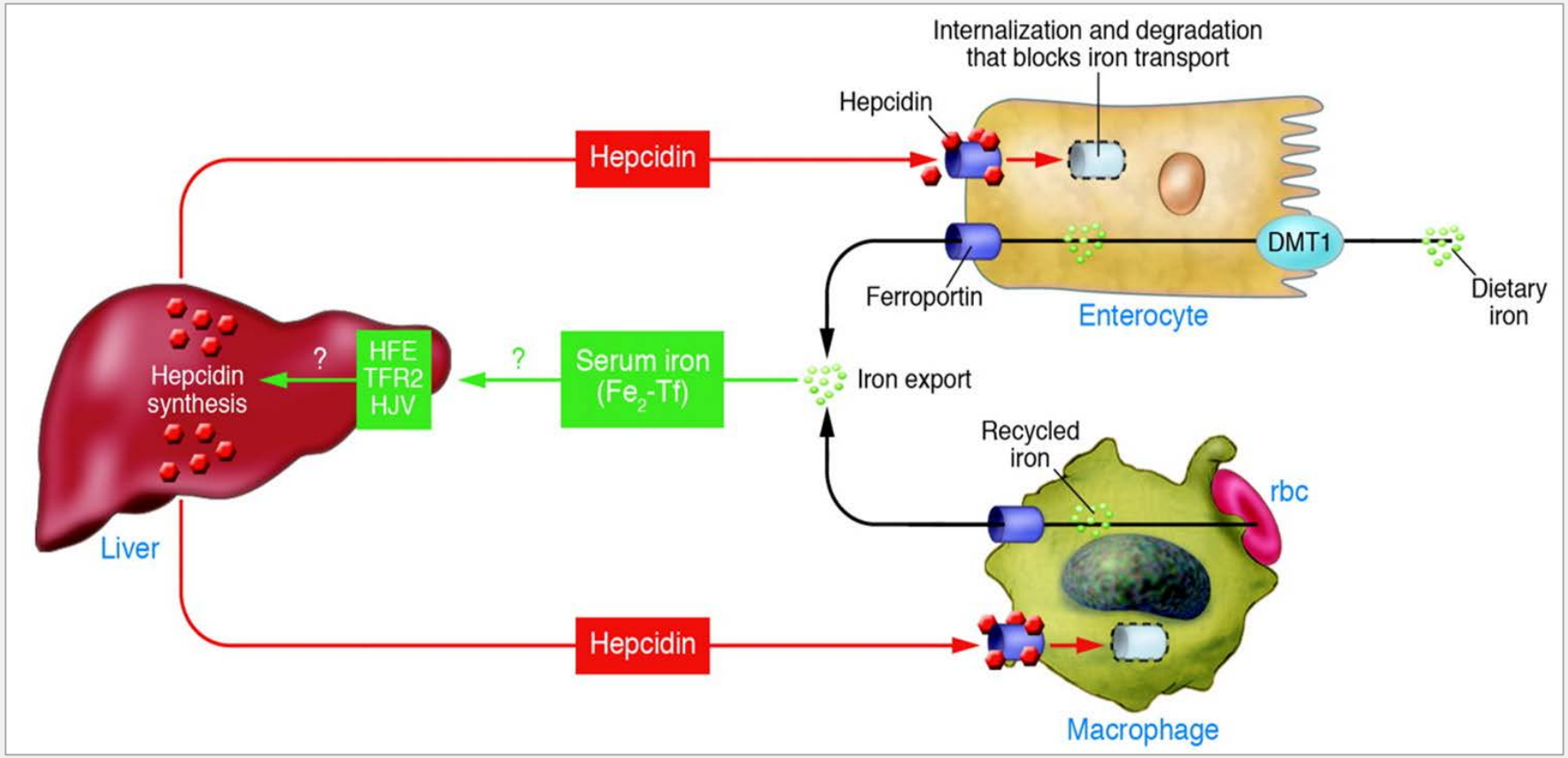
Anderson GJ, Powell LW. J Clin Invest 2000;105:1185–6.

Hepcidin: the master regulator of iron homeostasis

- 25 amino acid peptide with anti-microbial potential
- Expression induced by iron in the liver
- Stimulated also by LPS and IL-6 by an iron independent pathway—acute phase protein (blocked by TNF-α)
- Hepcidin over-expression leads to iron deficient anaemia and k.o. to iron overload
- Hepcidin inhibit duodenal iron absorption and macrophage iron release
- **Mechanism of action:** interferes with ferroportin thereby leading to ferroportin degradation and blockade of iron export

Regulation of systemic iron homeostasis



Vaulont S, et al. J Clin Invest 2005;115:2079–82.

Pathophysiology of iron homeostasis

"True iron deficiency": due to reduced absorption or increased demand/loss (e.g. bleeding)

Consequence: iron deficiency anaemia

Functional iron deficiency: iron is sequestered in the RES as a consequence of chronic immune activation due to infection, auto-immune disorders, cancer etc.

Consequence: anaemia of chronic disease

Iron overload: primary – hereditary haemochromatosis (five subtypes)

Iron overload: secondary – multiple transfusions on the basis of haemoglobinopathies/GvH, MDS; Bantu-disease; NASH, C2

Consequence: tissue iron accumulation, toxic radical formation, progressive organ failure

Anaemia of chronic disease (ACD)

* most frequent anaemia among hospitalised patients

* mild to moderate, **normo-*l*** normochromic

• develops in patients with cellular immune activation

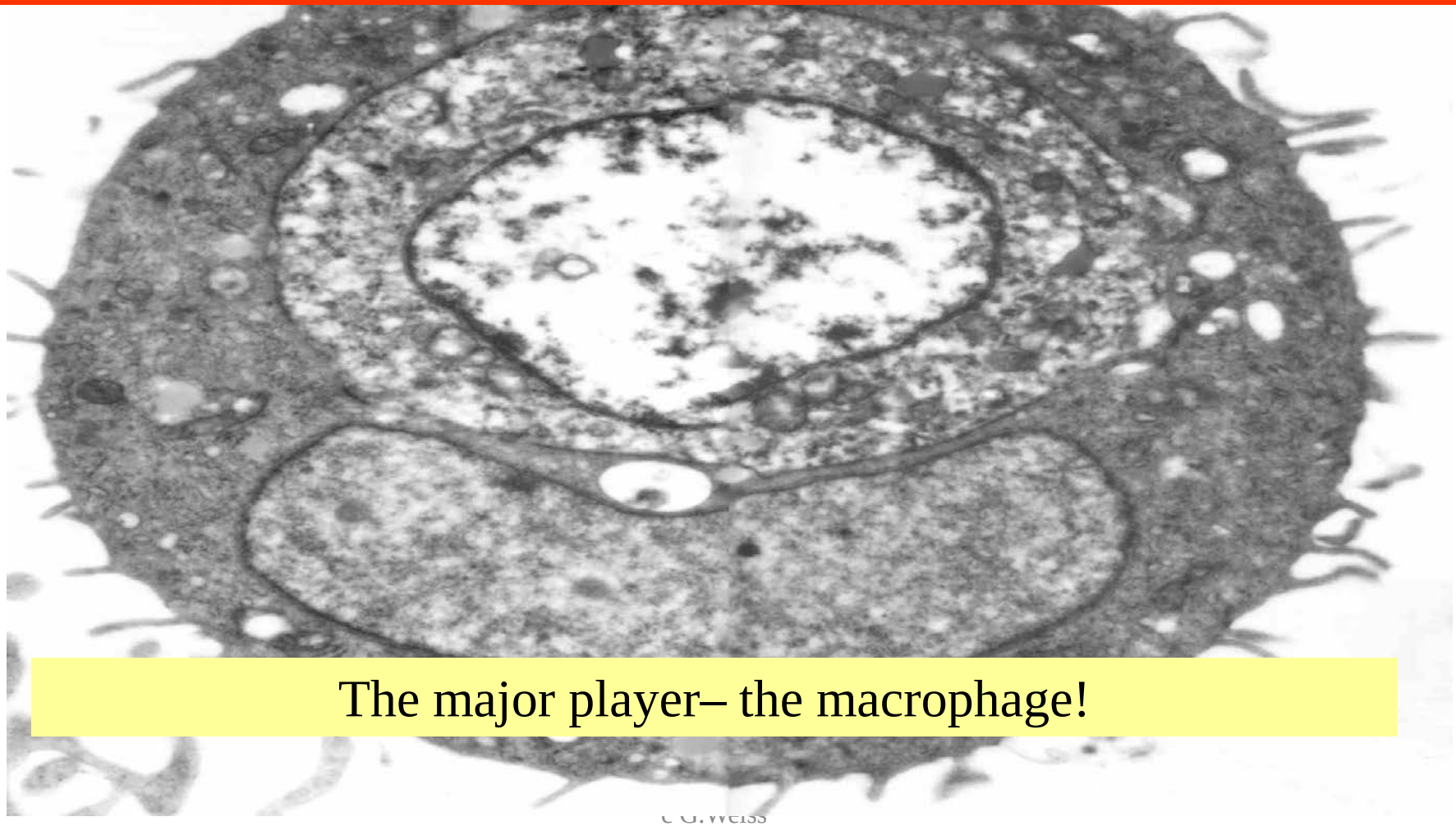
• Degree of anaemia correlated to immune activation

Table 1. Underlying Causes of Anemia of Chronic Disease.

Associated Diseases	Estimated Prevalence*
	percent
Infections (acute and chronic)	18–95[8-10]
Viral infections, including human immunodeficiency virus infection	
Bacterial	
Parasitic	
Fungal	
Cancer†	30–77[9,12-14]
Hematologic	
Solid tumor	
Autoimmune	8–71[5,9,15,16]
Rheumatoid arthritis	
Systemic lupus erythematosus and connective-tissue diseases	
Vasculitis	
Sarcoidosis	
Inflammatory bowel disease	
Chronic rejection after solid-organ transplantation	8–70[17-19]
Chronic kidney disease and inflammation	23–50[20-22]

Weiss G, Goodnough LT. N Engl J Med 2005;352:1011–23.

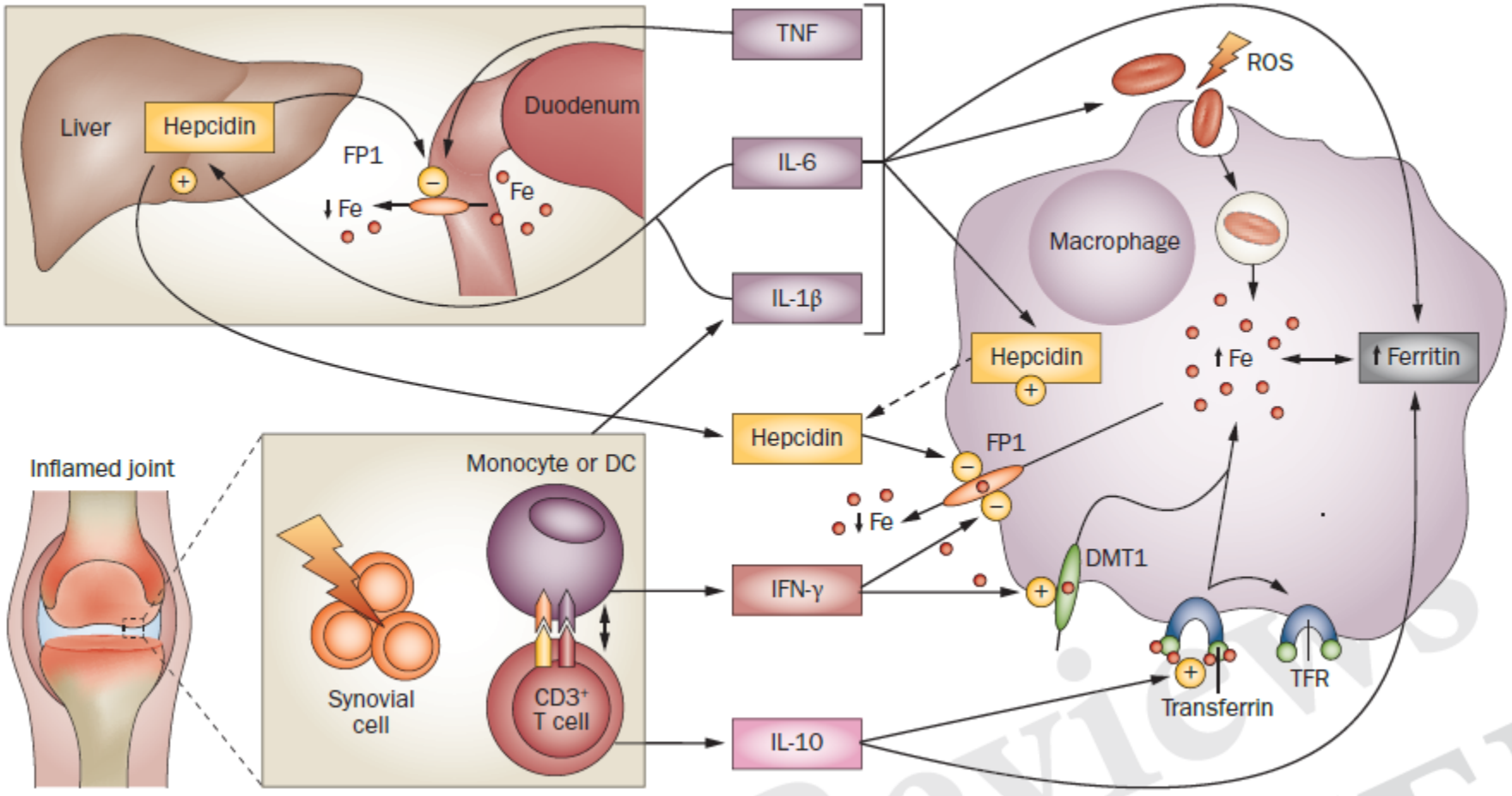
Major pathophysiological mechanism in ACD
Iron retention within cells of the RES



The major player– the macrophage!

Pathways for iron retention in ACD

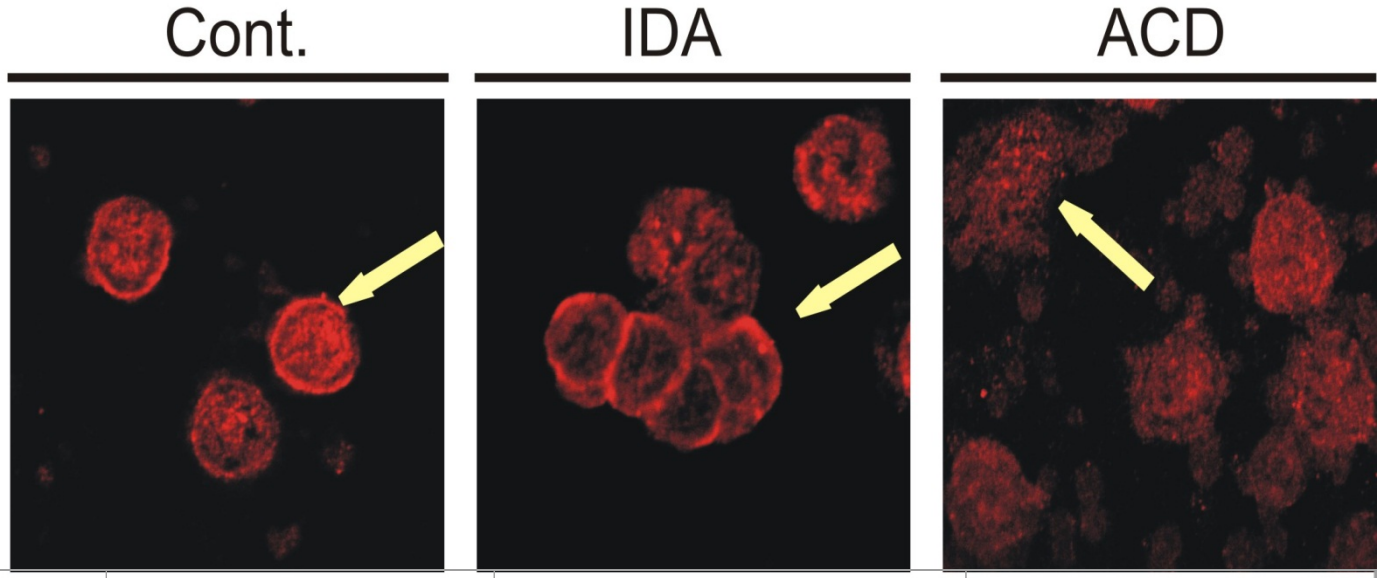
a collaborative work of acute phase proteins (Hepcidin) and cytokines



Weiss G., Schett G. Nat Rev Rheumatol 2013

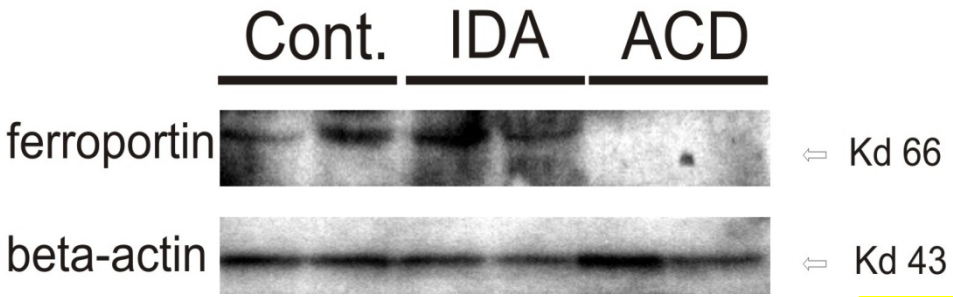
Increased pro-hepcidin levels in ACD correlate with down-regulation of ferroportin in monocytes of ACD patients

A



	Cont.	IDA	ACD
Pro-hepcidin (ng/ml)	110.7±44.0	74,6±23.2 #	154.3±61.4 *¶

B



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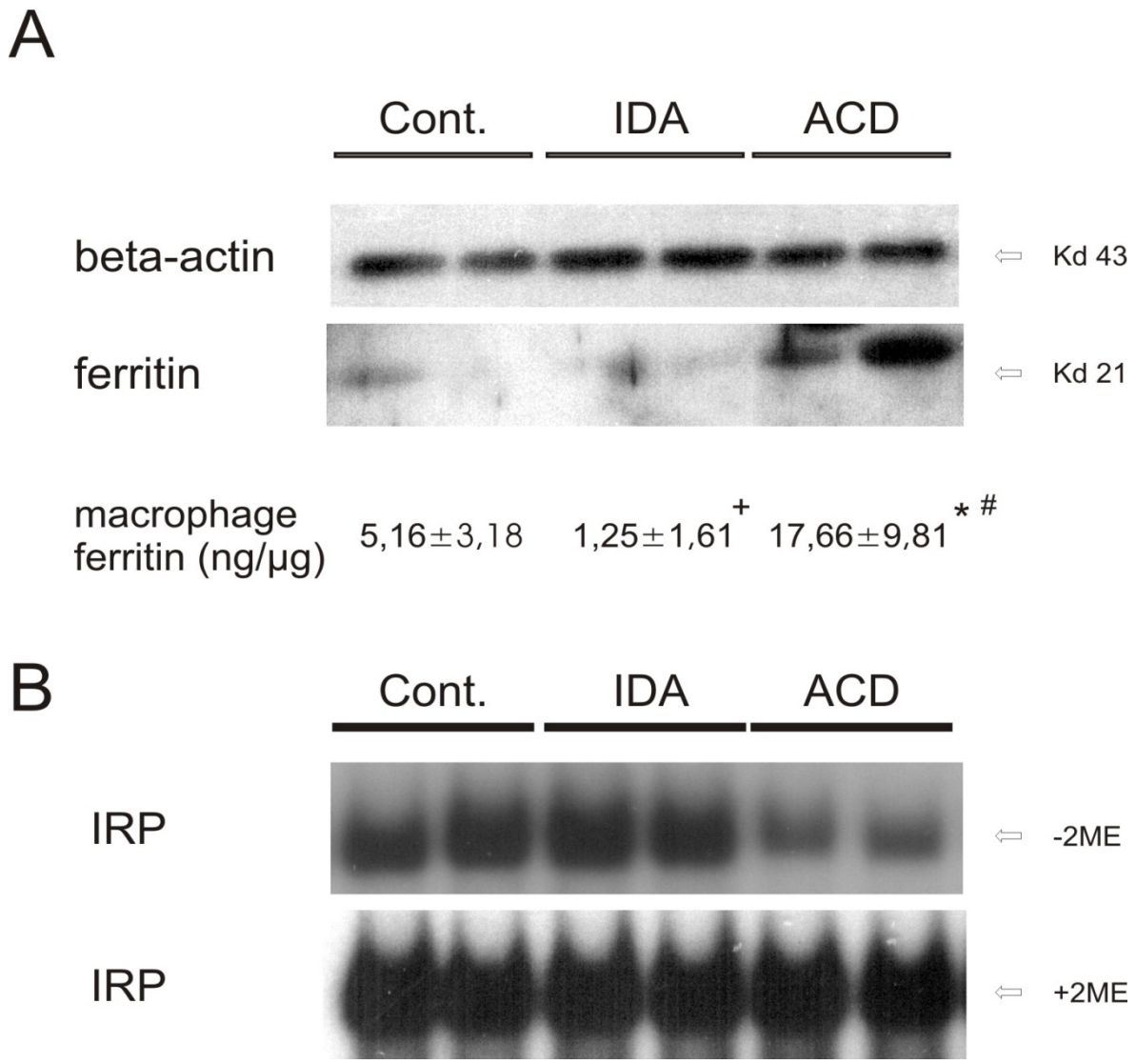
Theurl et. al. Blood 2006

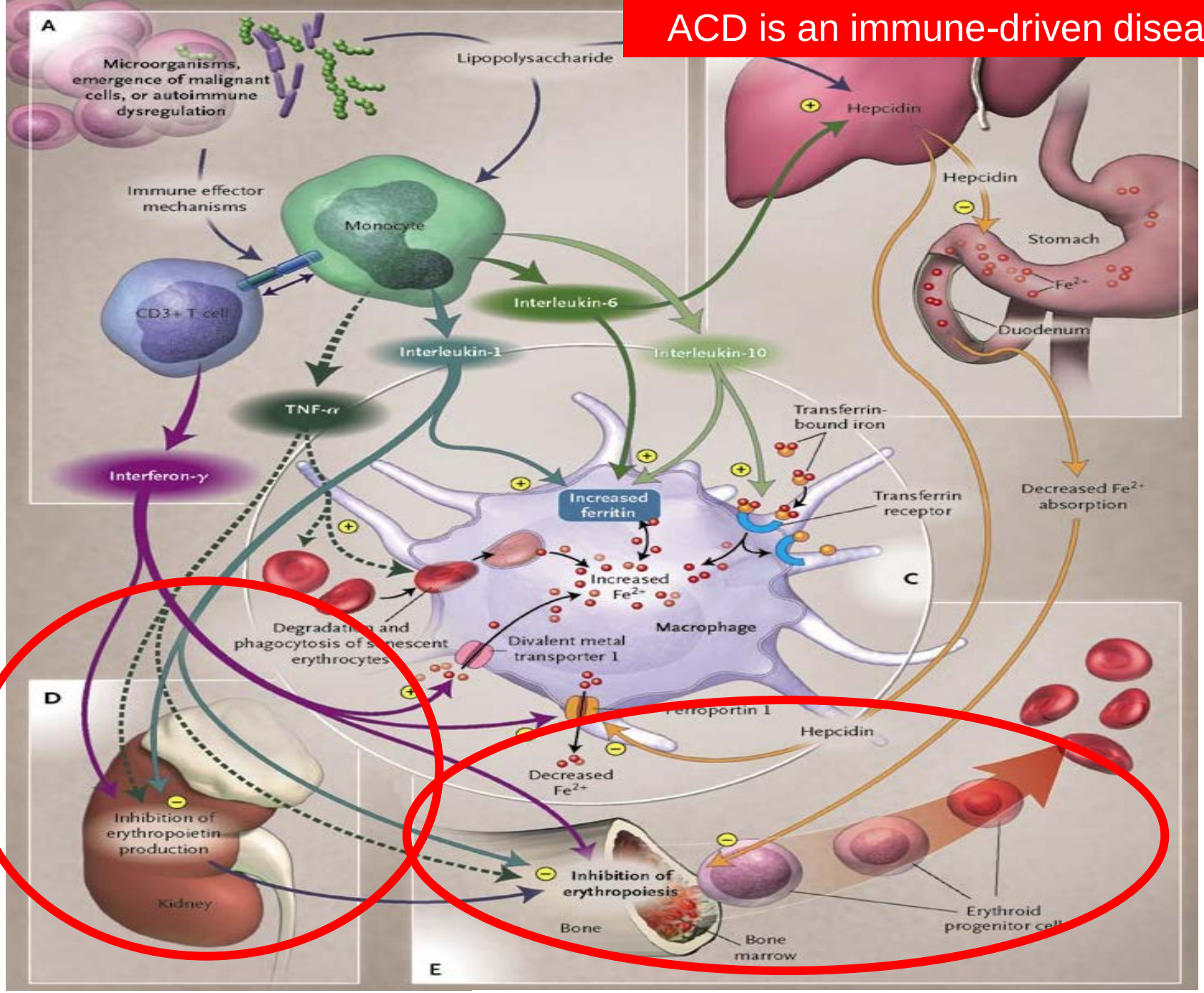
IRP binding activity and ferritin protein levels in circulating monocytes of IDA and ACD patients



c G.Weiss

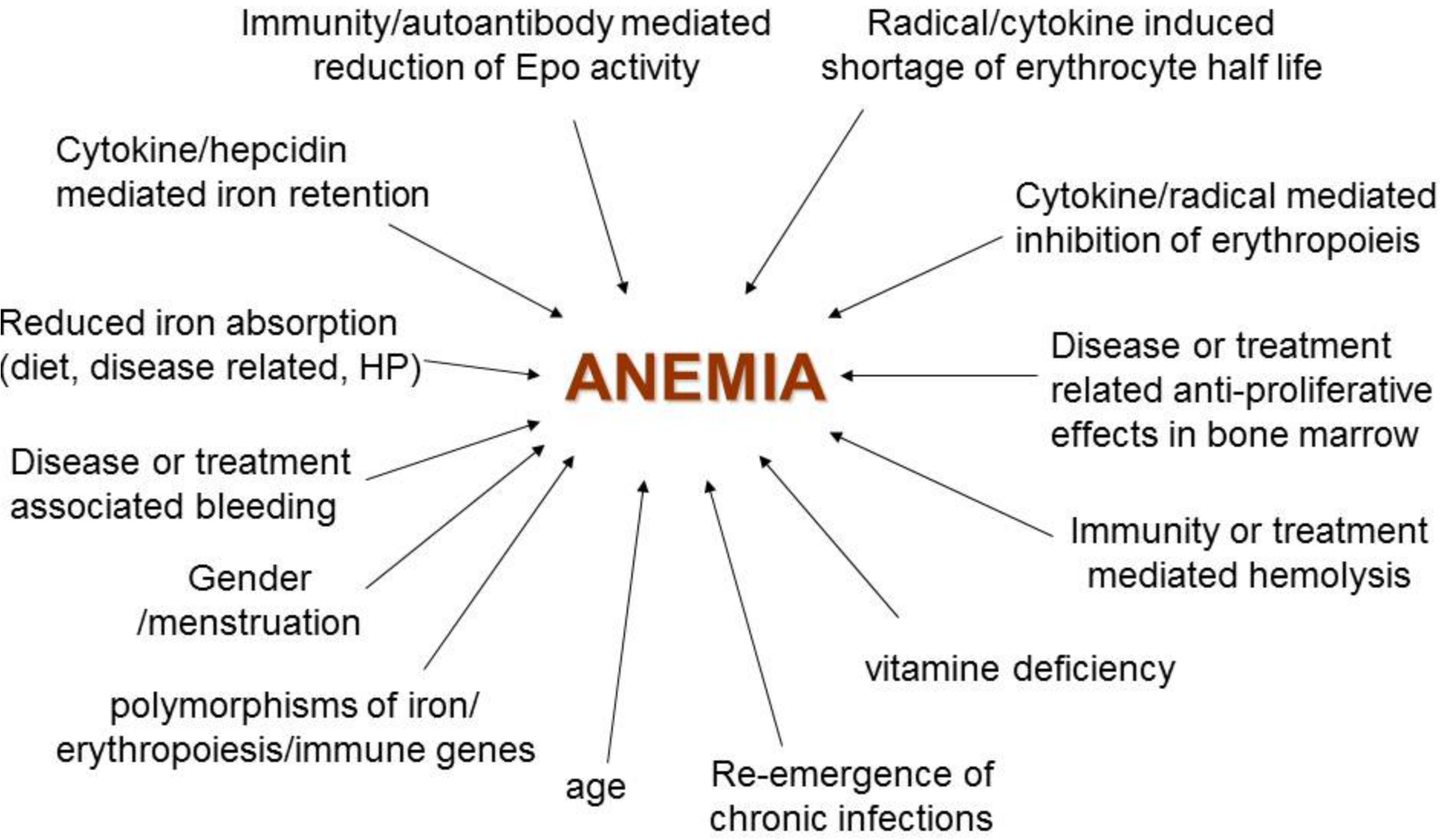
Theurl et al. Blood 2006

ACD is an immune-driven disease



Weiss G, Goodnough LT. N Engl J Med 2005;352:1011–23.

Pathways contributing to the development and severity of anemia in systemic rheumatic diseases



Weiss G and Schett G. Nature Rev Rheumatol 2013

Positive effects of ACD?

- **Withholding iron from infectious pathogens** in order to limit their growth (Eugene Weinberg)
  - Iron acquisition linked to pathogenicity in microbes, fungi!?
- **Reducing the supply of oxygen** to rapid proliferating tissues
- **Strengthening of immune response**
  - via impaired expression of EPO
  - via iron restriction

Iron at the host–pathogen interface

- Essential for growth and proliferation of several microbes
- Expression of iron acquisition and siderophore systems is linked to microbial pathogenicity

Exerts subtle effects on cell-mediated immunity *in vitro* (macrophage effector pathways, IFN-γ activity, iNOS expression)

Control of iron homeostasis is important in the course of an infection

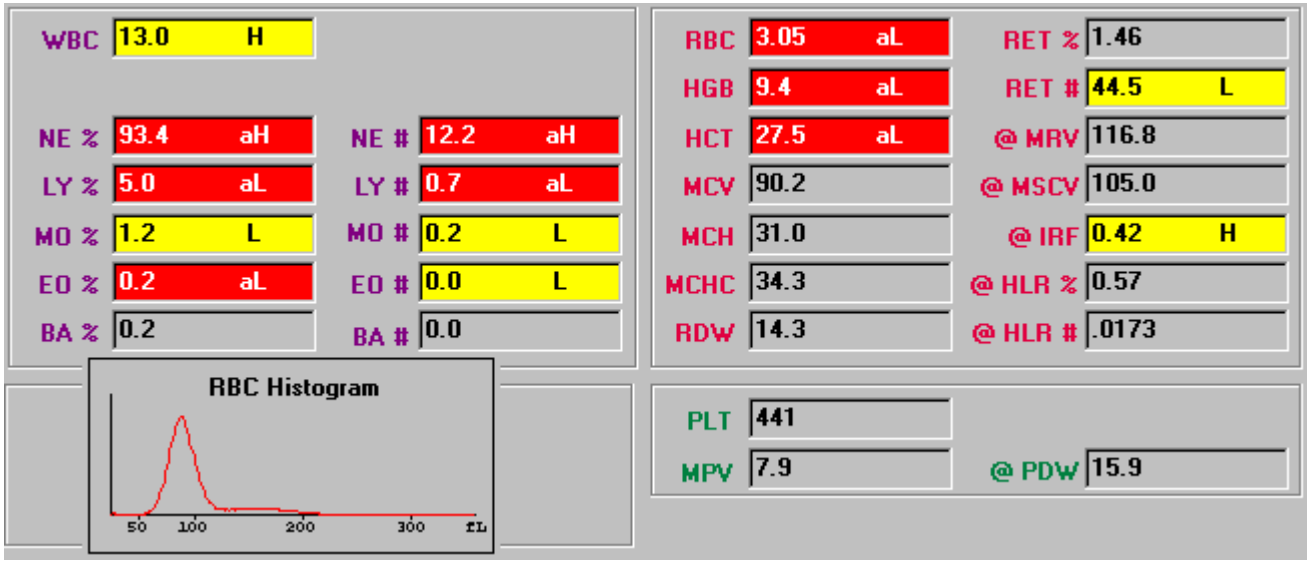
IFN-γ, interferon-gamma; iNOS, inducible nitric oxide synthase.

Anaemia diagnosis

Parameter	ACD	IDA
Serum iron concentration	Reduced to normal	Reduced
Transferrin levels	Reduced to normal	Increased
Transferrin saturation	Reduced to normal	Reduced
Ferritin	Normal to increased	Reduced
Serum transferrin receptor	Normal	Increased
sTfR/log ferritin	Low (<1)	High (>2)
Zinc protoporphyrin IX	High	High
Percentage hypochromic RBC	n.a.	High
Cytokines (TNF, IL-1, IL-6)	Increased	Normal

Cytokine levels are inversely correlated with the degree of anaemia

Sole iron determination in serum is not clinically useful



Ferritin	CRP	Serum FE	TIBC	Transferrin	TfSat%	RST
1250	20.7	11	95	75	11.6	1.72

IL-6
32 pg/mL

sTfR	sTfR/log ferri
1.72	0.55

Ser Folate	Vit. B12	LDH
3.3	265	Normal

Total Bili
Normal

Blood analysis of a typical patient mit ACD

c G.Weiss

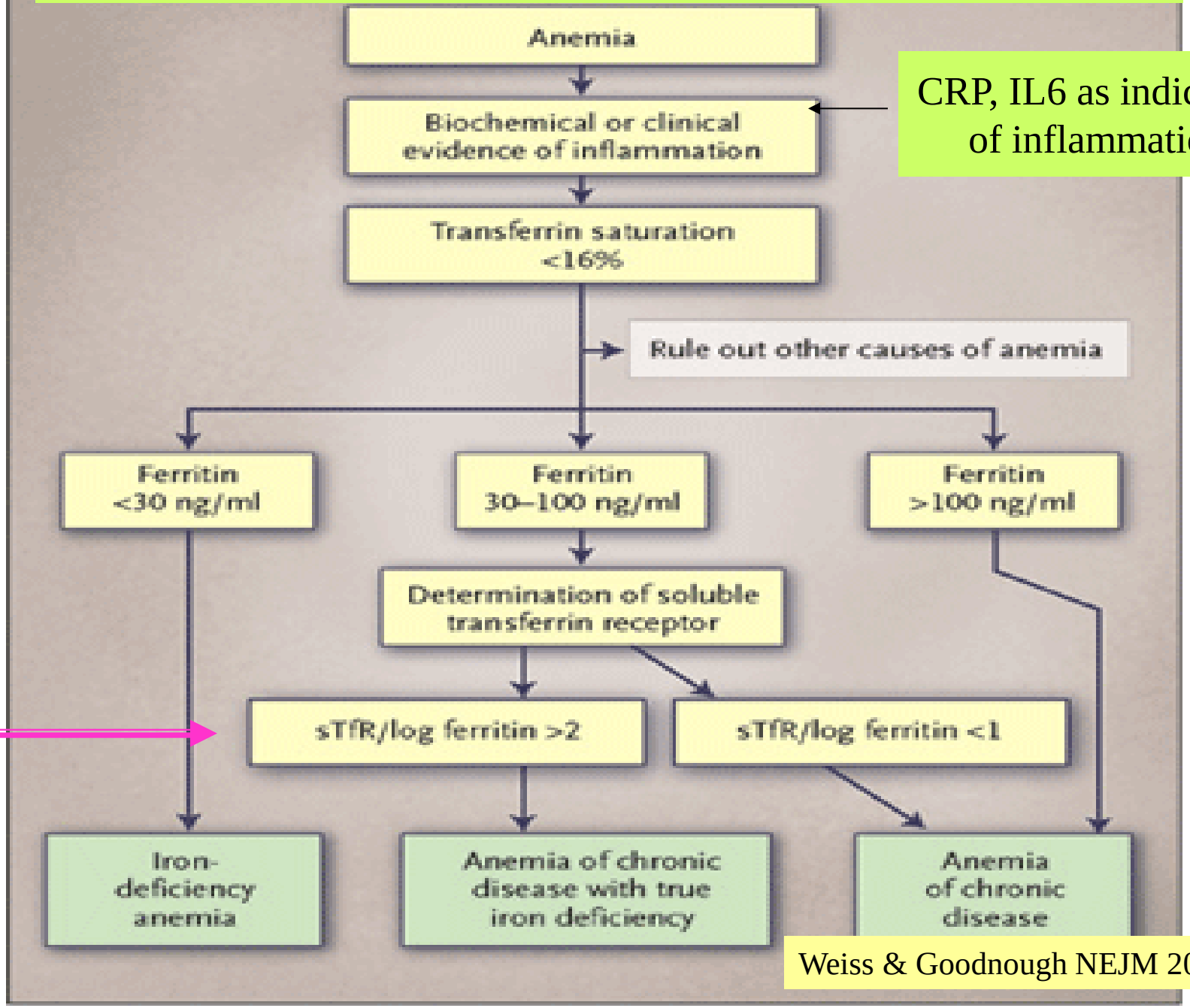
Several patients suffer from a combination of ACD and iron deficiency (**ACD/IDA**) as a consequence of inflammatory anemia and blood loss (mostly on the basis of gastro-intesintal or uro-genital bleeding)

Parameter	ACD	Both (ACD+IDA)
Serum iron	reduced	reduced
Transferrin levels	reduced to normal	reduced
TfS	reduced	reduced
Ferritin	normal to increased	reduced to normal
sTfR	normal	normal to increased
sTfR/log ferritin	low (<1)	high (>2) ?
cytokines levels	increased	increased

Why is the differential diagnosis between ACD and ACD+IDA important?

Because these patients may need contrasting therapies!!!

Differential diagnosis between ACD and ACD with IDA

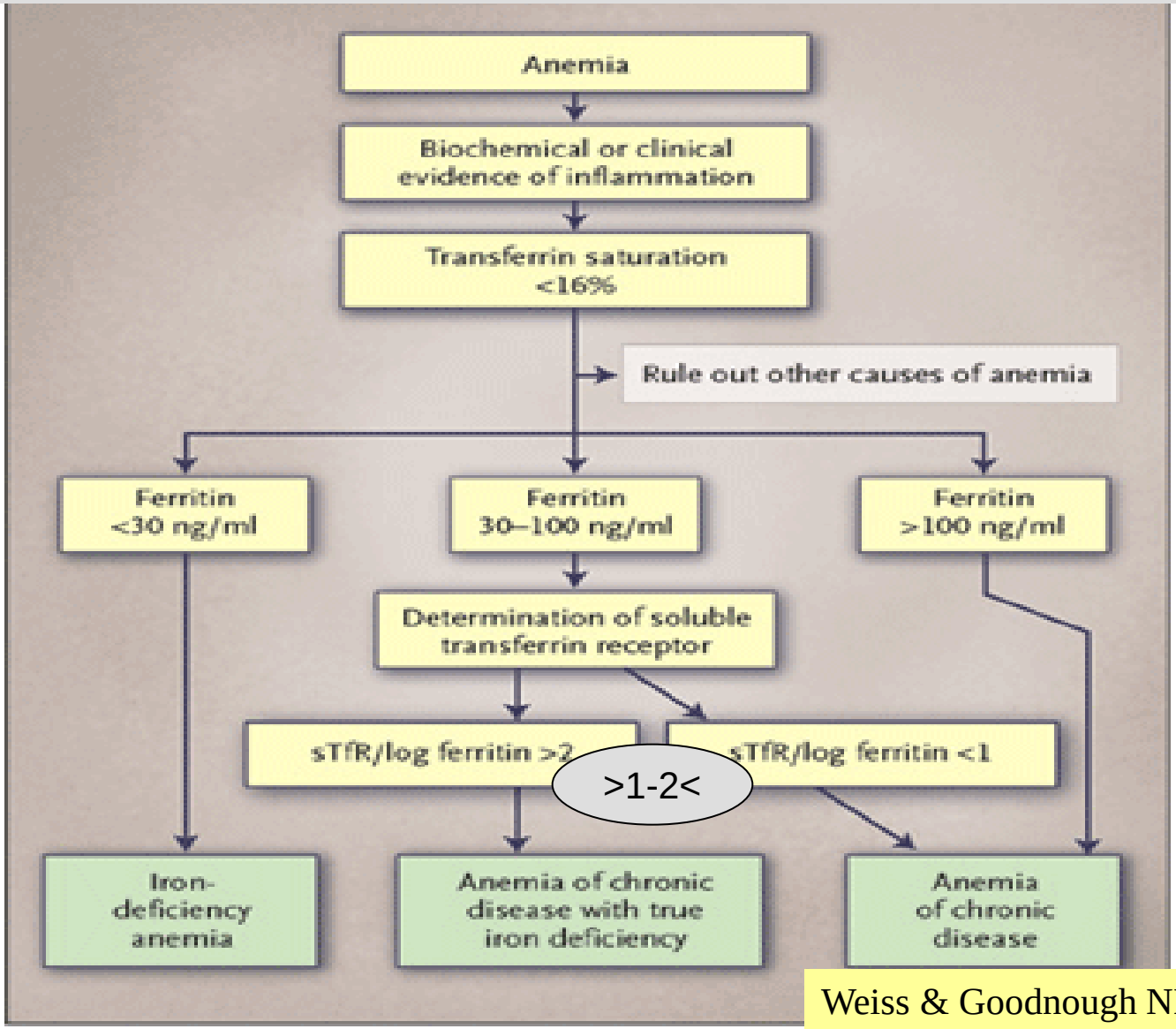


CRP, IL6 as indicators of inflammation

Weiss & Goodnough NEJM 2005

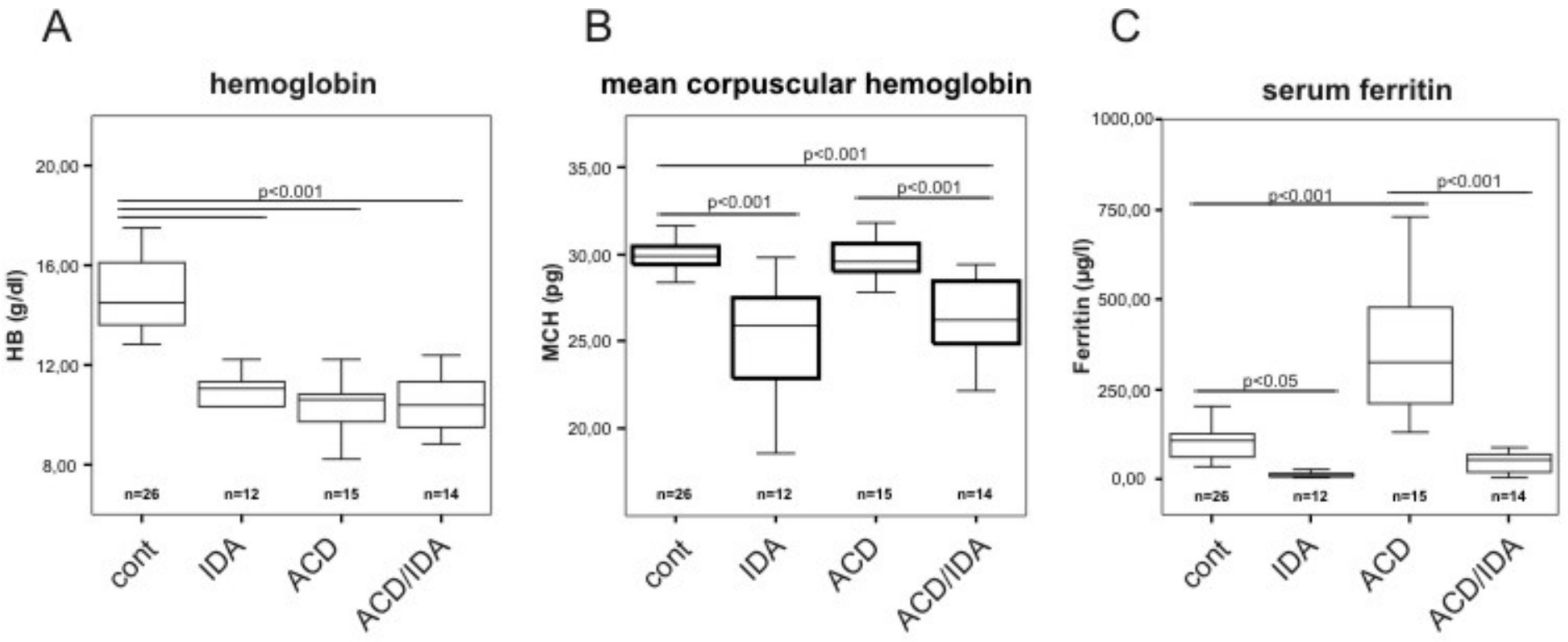
Diagnostic window with sTfR/log ferritin

How suitable are other hematological parameters for the differential-diagnosis of ACD versus ACD/IDA?



Weiss & Goodnough NEJM 2005

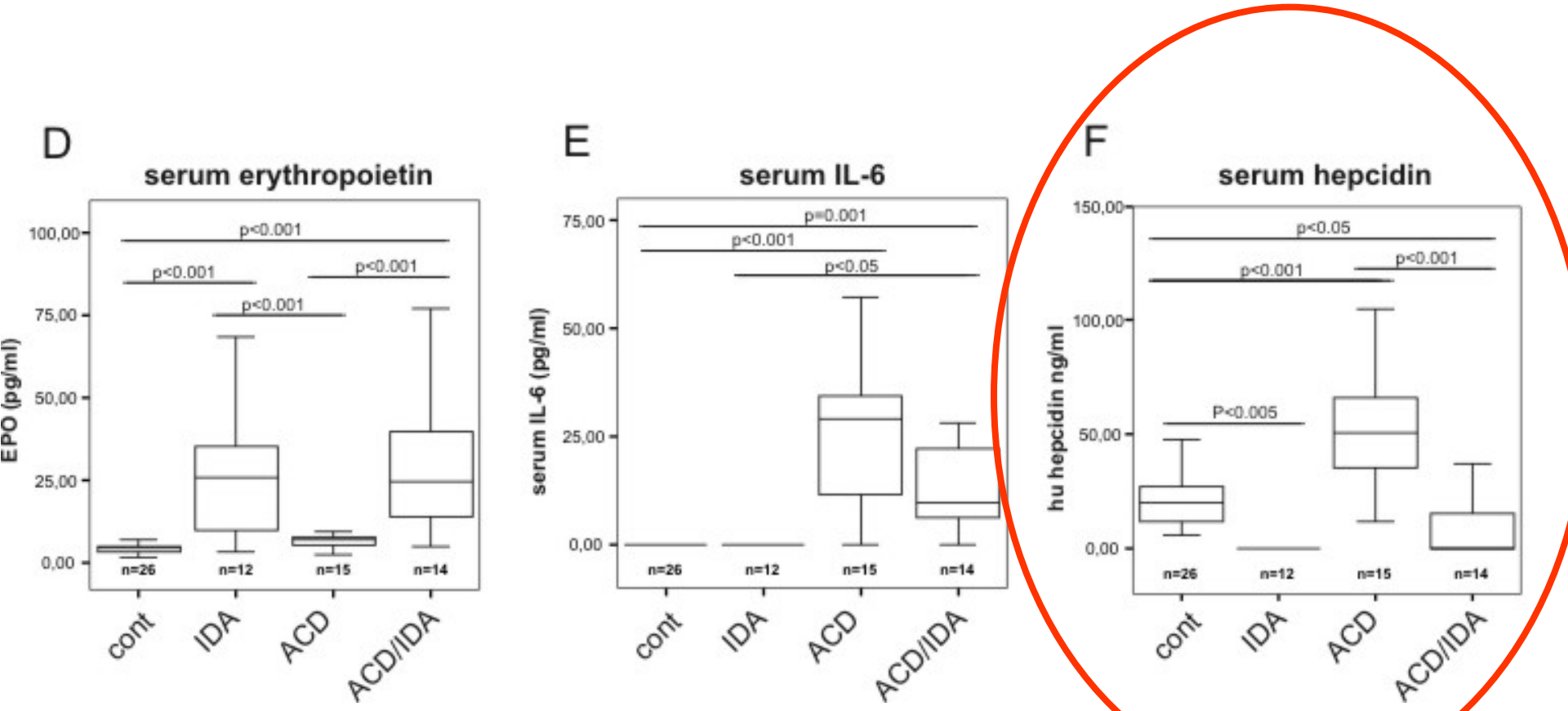
Differential diagnosis between ACD, IDA and ACD/IDA



Note: Patients characterisation according to sTfR/log ferritin (ACD <1, ACD/IDA>2)

Theurl et al. BLOOD 2009

Differential diagnosis between ACD, IDA and ACD/IDA



Note: Patients characterisation according to sTfR/log ferritin (ACD <1, ACD/IDA>2)

Theurl et al. BLOOD 2009

Assessment of iron status in the setting of inflammation and anemia

- Ferritin expression is induced by iron overload and inflammation
- Hepcidin expression is more affected by the needs of iron for erythropoiesis than by inflammation
- Hepcidin levels closely correlate to sTfR/ log ferritin ratio in patients with inflammation, thus both parameters (hepcidin currently not widely available) may be useful to differentiate between absolute versus relative iron deficiency
- Hematological indices (e.g. MCH, CHr.. and combinations with sTfR) may aid additional information on true iron availability for erythropoiesis in patients with ACD and speficially in those with sTfR/ log ferritin between 1 and 2

ACD: Best Therapy

* Treatment or cure of the **Underlying disease !!!**

Current therapeutic options in ACD

- Blood transfusions
- Recombinant human erythropoietin
- Iron

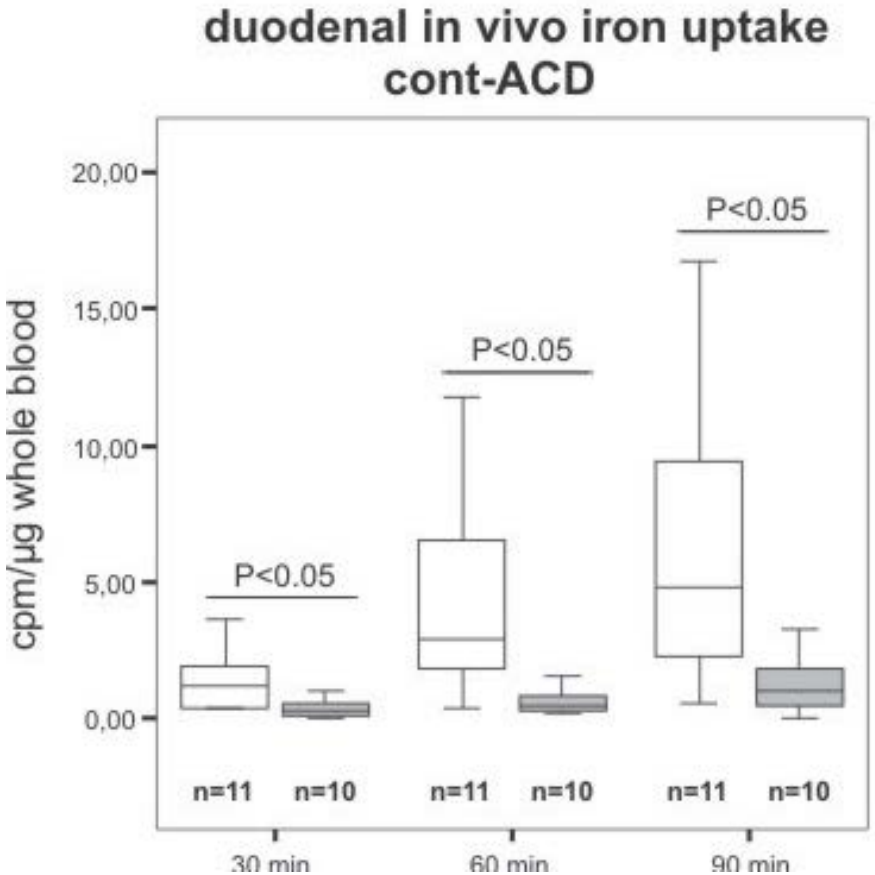
Therapeutic measures are aimed to increase haemoglobin levels in ACD patients,

however, the **impact** of such interventions on iron overload in the RES, immunity, radical formation and most importantly **on the underlying disease are almost completely unknown.**

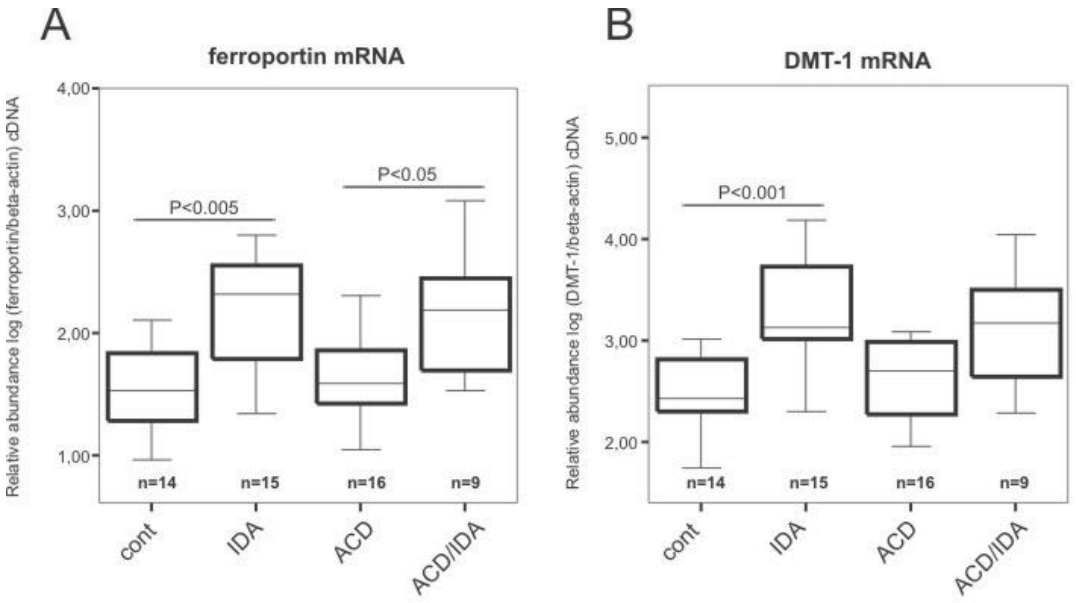
Oral iron therapy

- Indication:
  - True iron deficiency (after identification of the underlying cause!)
  - Absence of inflammation
  - No absorption defect (e.g. celiac disease)
- Once daily (minimum 50 mg)!!!
- Improved absorption with vitamin C
- Intake after overnight fasting without concomitant food intake - reduces compliance – more GI side effects

Duodenal iron uptake in ACD and ACD/IDA rats *in vivo*



Theurl I, et al. Blood 2009;113:5277–86.



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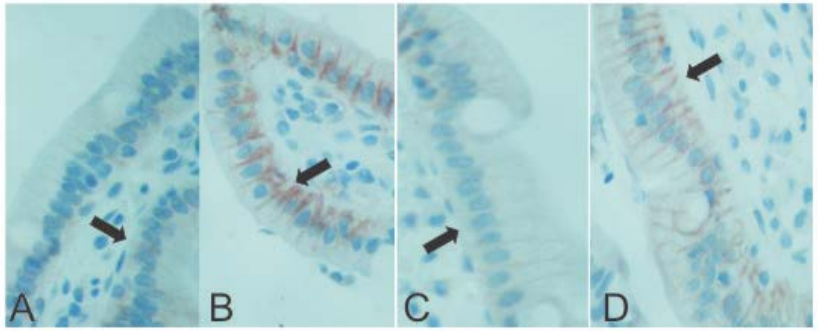


Hb [g/dl]	15	7,2	11,6	11,8
ferritin[µg/l]	104	1,8	173	21,3
IL-6 [pg/ml]	7	4	254	54
hepcidin [ng/ml]	17,3	n.d.	172,8	n.d.

Inverse association between duodenal ferroportin expression and hepcidin levels in IDA, ACD and ACD/IDA patients

Theurl I, et al. Blood 2009;113:5277–86.

Intravenous iron therapy

- Indication:
  - True and functional iron deficiency
  - Defect of absorption
  - Intolerance to oral iron therapy
  - Lack of efficacy with oral iron therapy
  - Chronic inflammation (autoimmune diseases (RA, IBD), dialysis, chronic heart failure…)

CAVE: UNCERTANITIES regarding the effects of iron therapy in patients with

CANCER (palliative setting?), infections

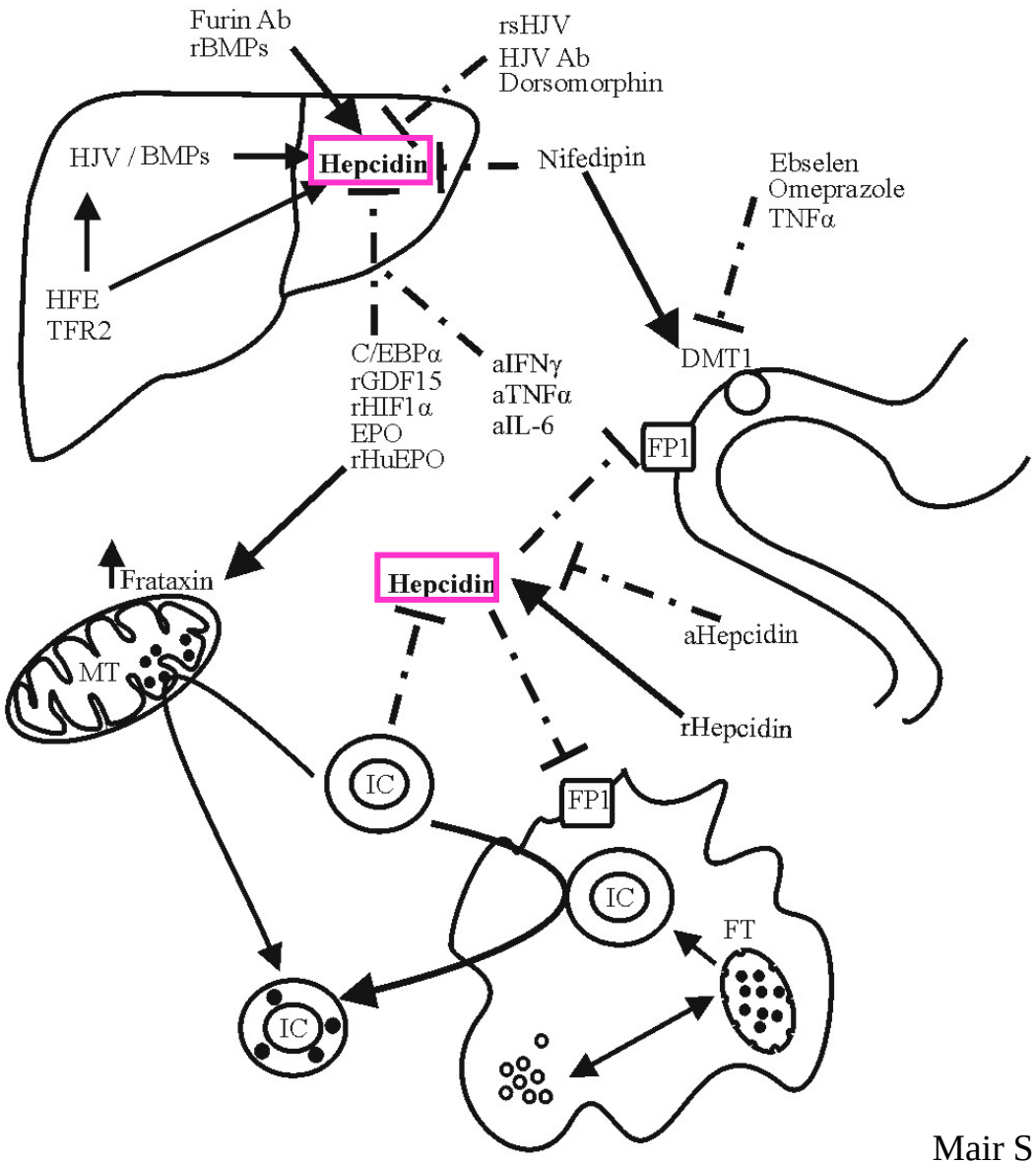
Why is the differential diagnosis between ACD and ACD+IDA important?

Because these patients may need contrasting therapies!!!

no additional iron in ACD

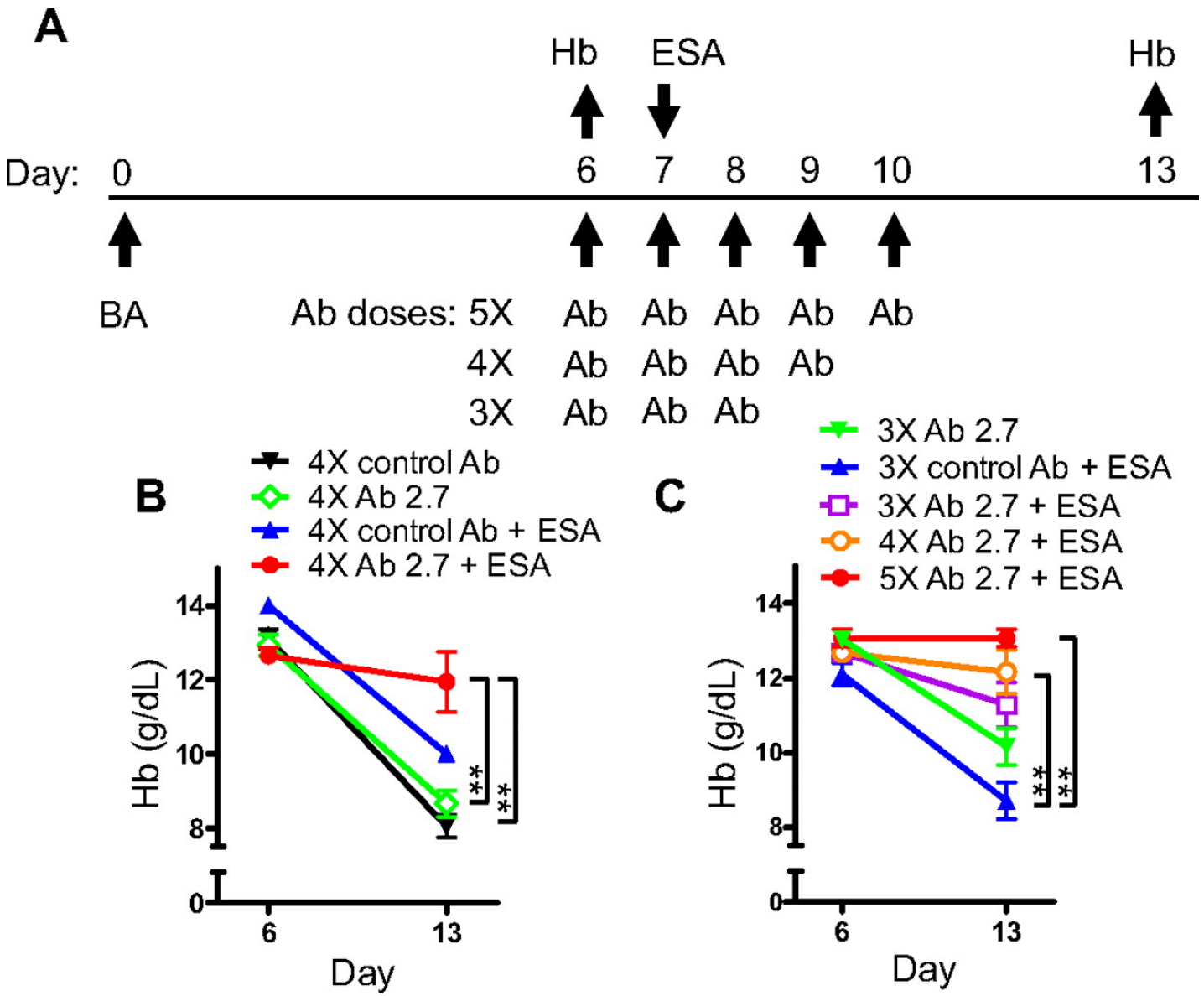
iron needed in ACD/IDA

New therapeutic approaches via modulation of hepcidin



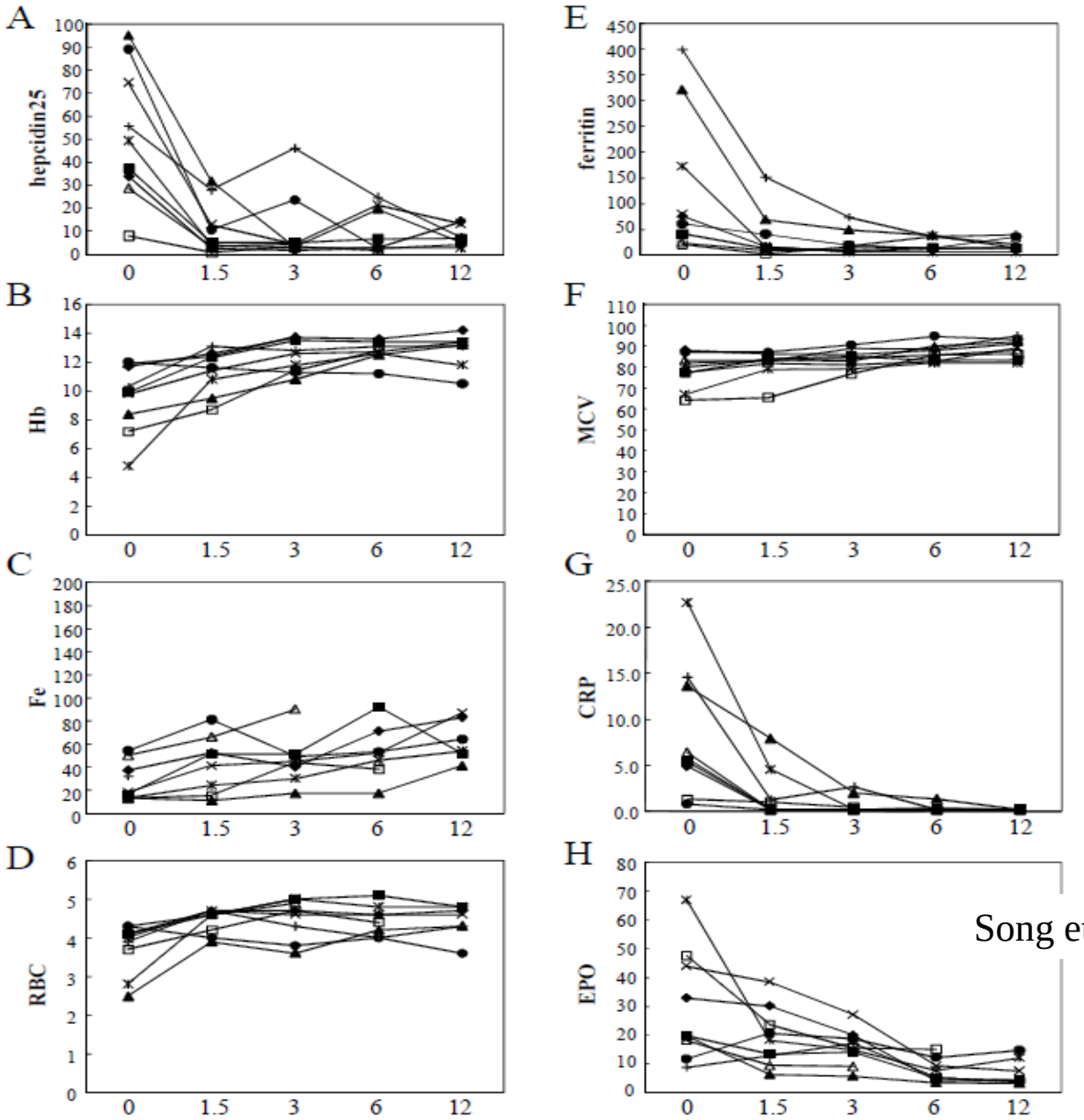
Mair S et al Curr Med Chem 2009

Anti-hepcidin antibodies restored response to ESA treatment in hHepc knocking AI mice



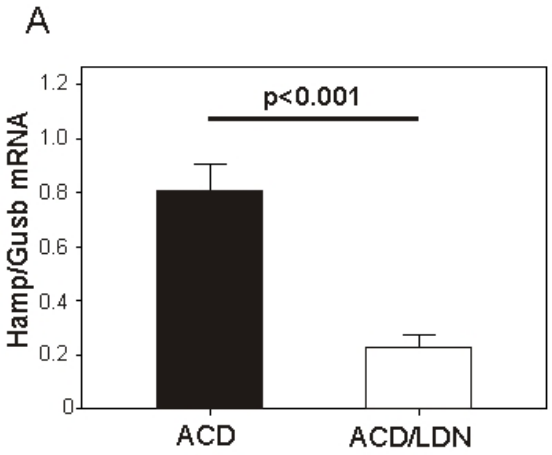
Sasu, B. J. et al. Blood 2010;115:3616-3624

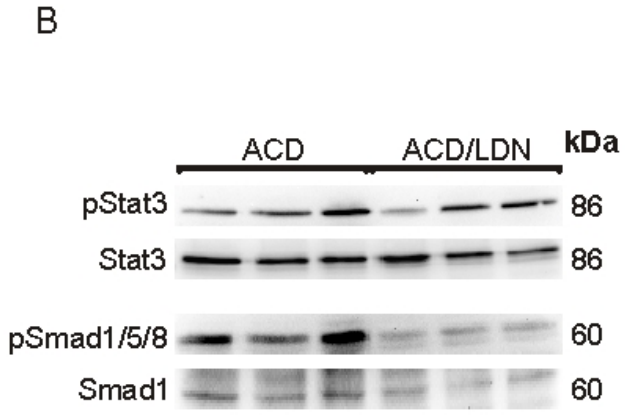
Downregulation of hepcidin resulting from long-term treatment with an anti-IL-6 receptor antibody (tocilizumab) improves anemia in Castleman's disease

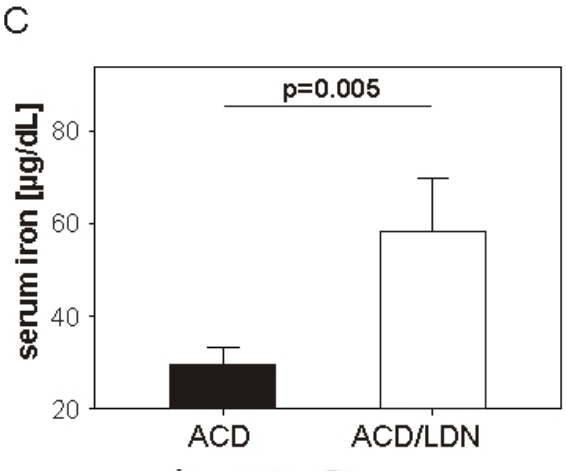


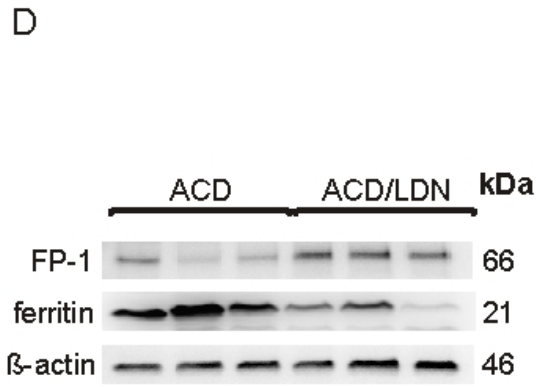
Song et al. Blood 2010

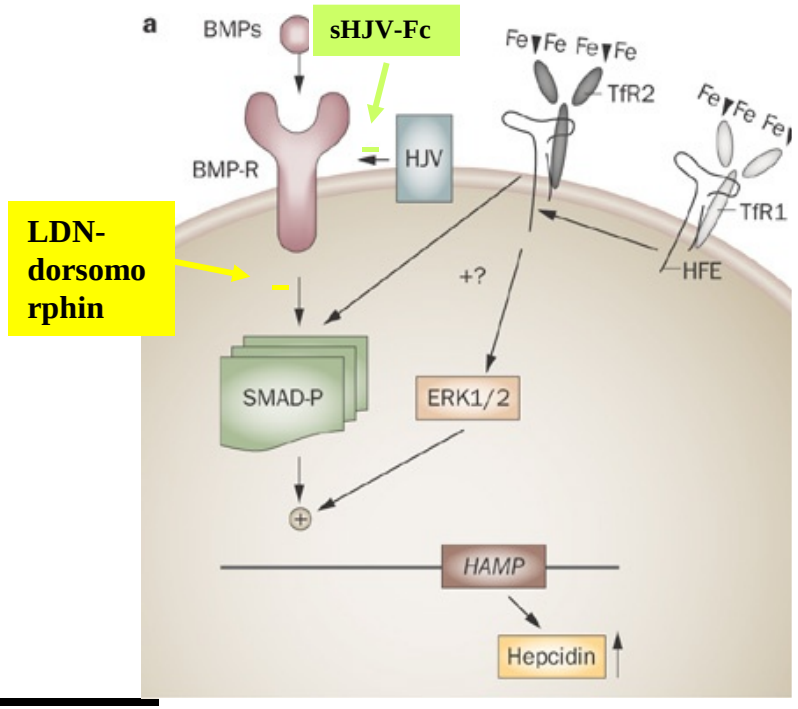
Modulating endogenous hepcidin formation by small molecule inhibitors results in suppression of hepcidin synthesis and increase of serum iron







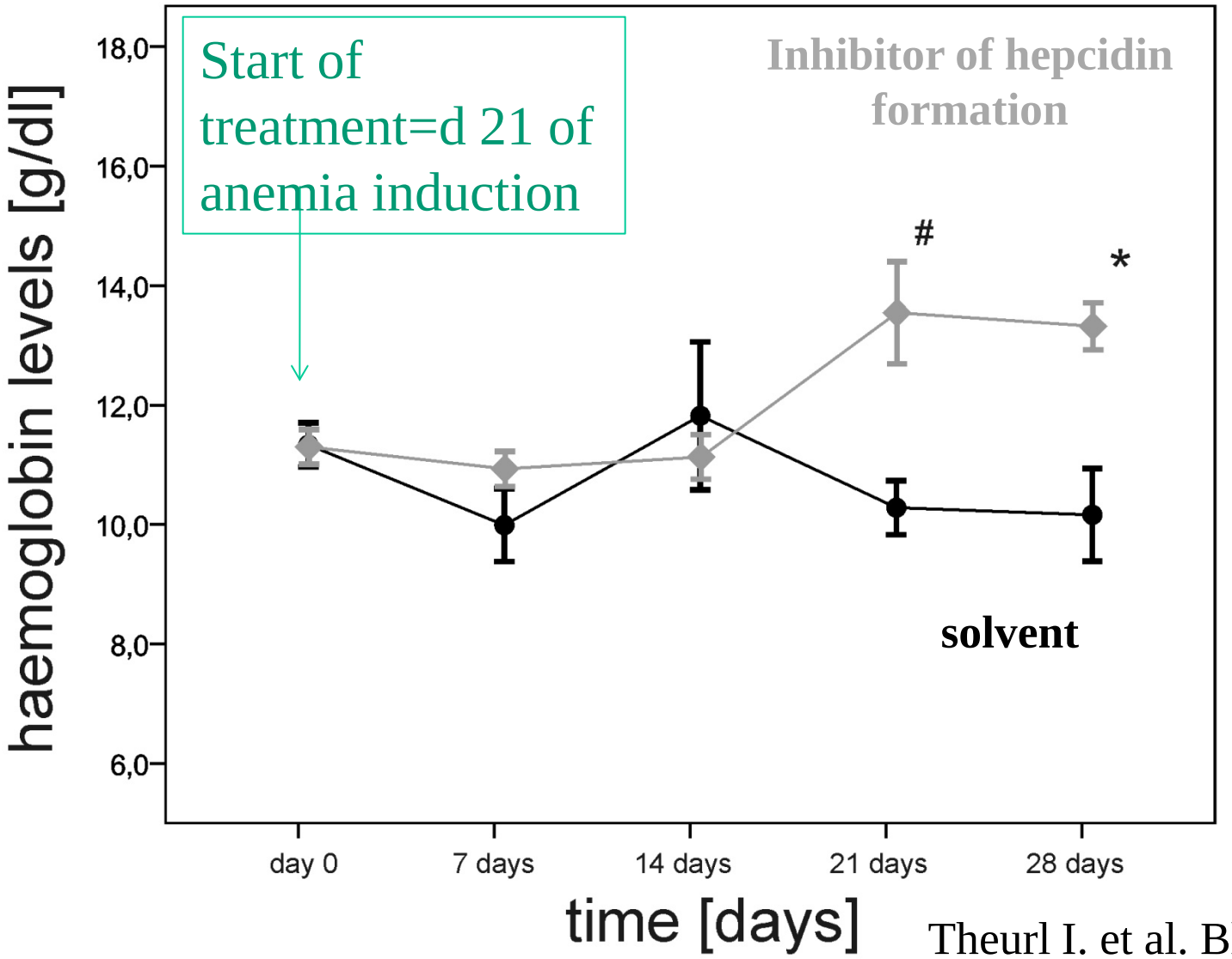




Theurl I et al. Blood 2011

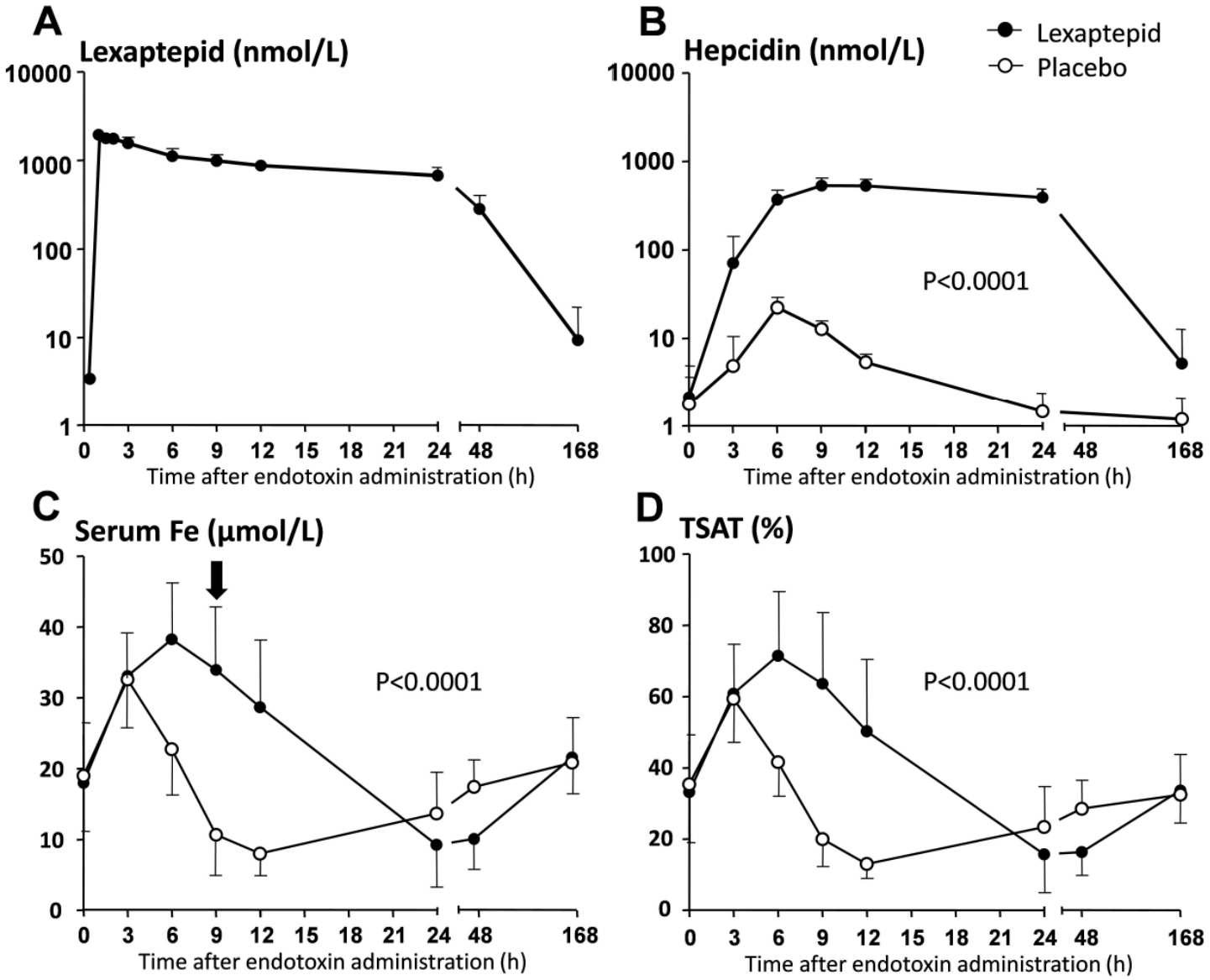
Weiss G. *Nature Reviews Gastroenterology & Hepatology* 7, 50-58, 2010

Successful treatment of ACD by modulating endogenous hepcidin formation (with LDN or sHJV-Fc) in PGS injected ACD rats



Theurl I. et al. Blood 2011

First in human study: DBR- trial comparing efficacy of Lexaptepid (anti-hepcidin spiegelmer) in male volunteers after injection of E. coli LPS



Van Eijk et al. Blood Aug 27th 2014

Erythroferron- erythropoiesis inducible suppressor of hepcidin synthesis

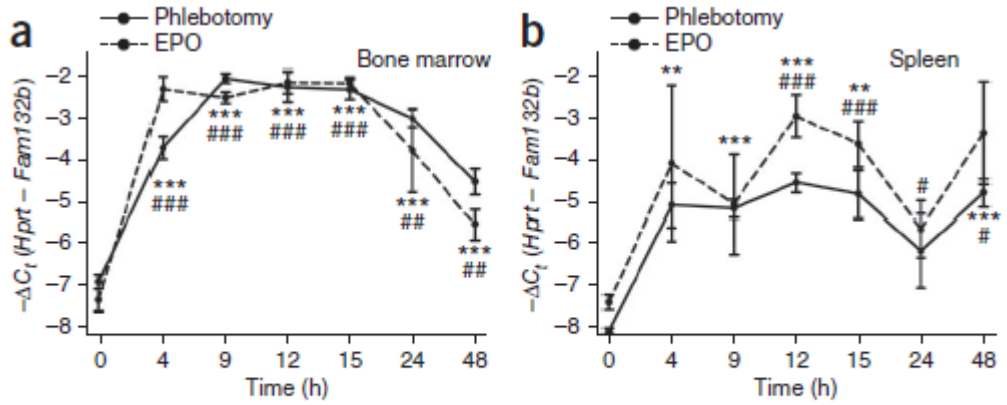
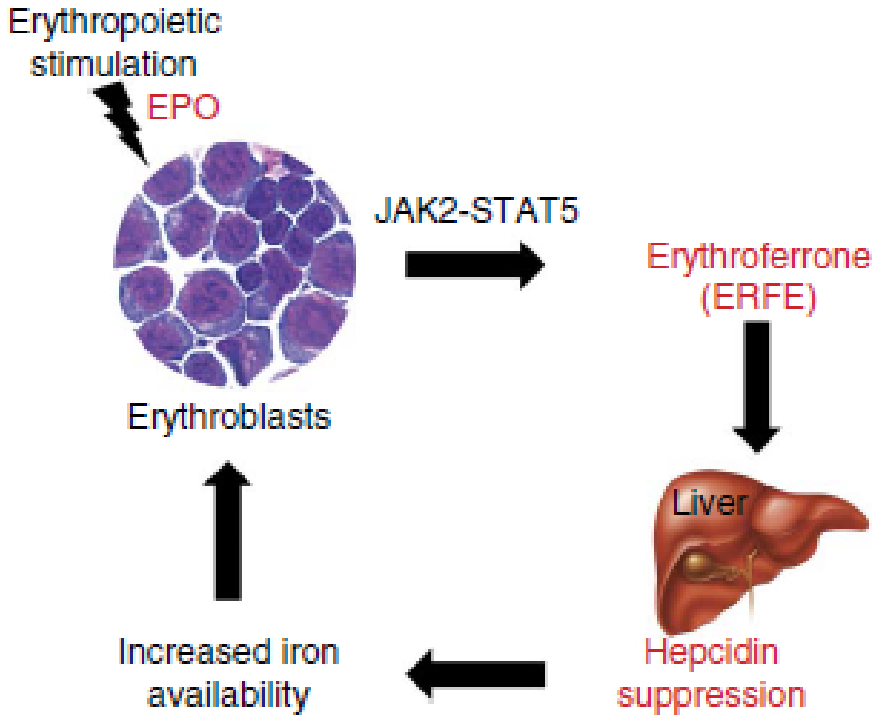
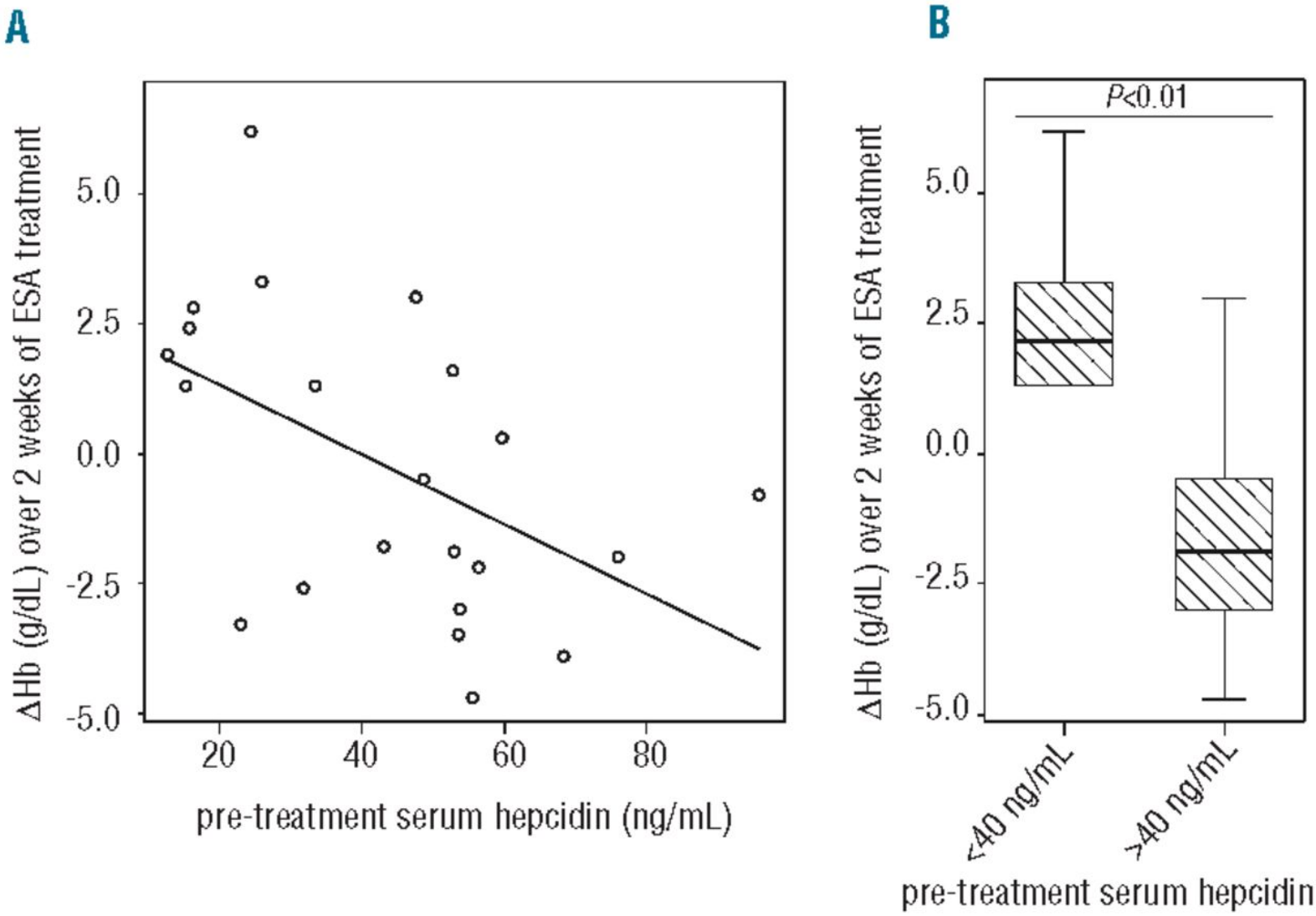


Figure 2 Induction of ERFE-encoding *Fam132b* mRNA levels after phlebotomy (500 µl) or treatment with EPO (200 U). (**a**,**b**) *Fam132b*



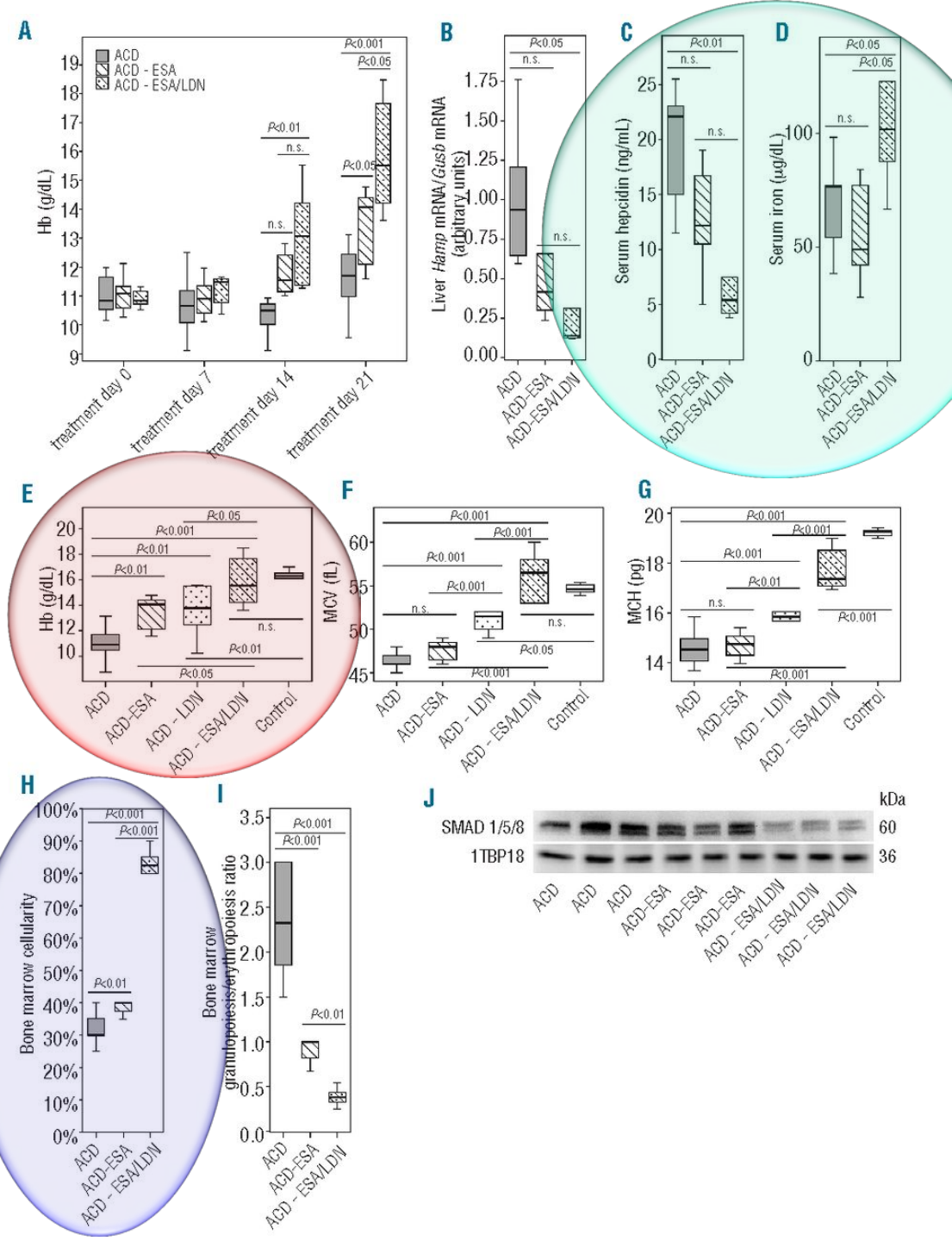
Kautz et al. Nat Gen 2014

Increased serum hepcidin levels predict a poor hematological response to ESA treatment in ACD rats.



Theurl M et al. Haematologica 2014;99:1516-1524

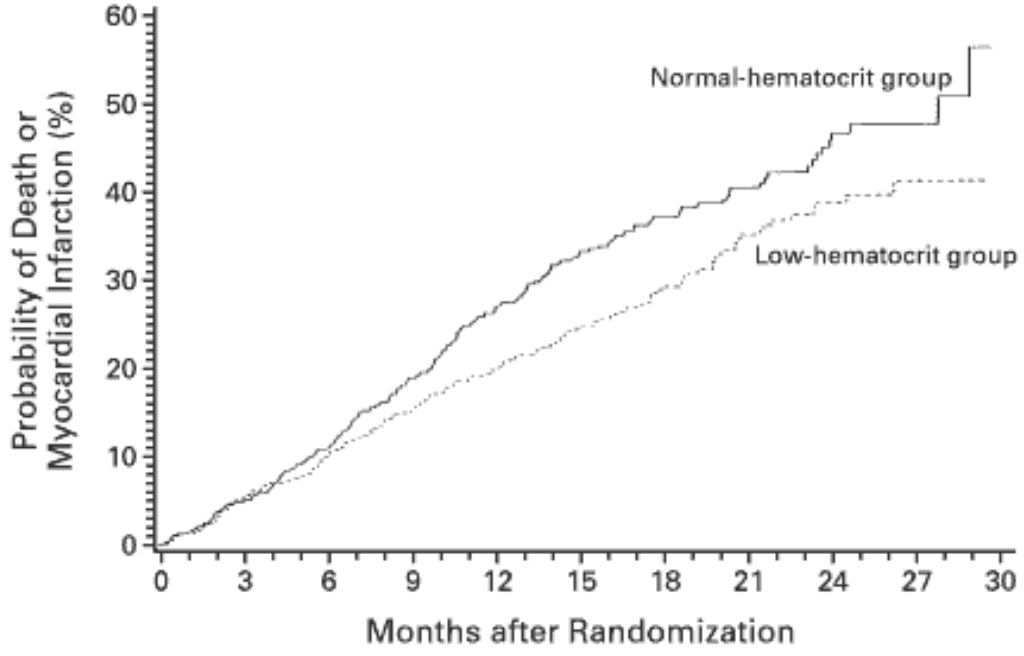
Combination therapy of LDN and ESA exerts synergistic effects on erythropoiesis in rats with inflammatory anemia.



Theurl M et al. Haematologica 2014;99:1516-1524

Therapeutic end points

a normal target hemoglobin may not be optimal!!



No. at Risk										
Normal hematocrit	618	540	476	415	353	259	186	124	69	26
Low hematocrit	615	537	485	434	391	292	216	131	80	20

dialysis patients

lowest risk of death with hematocrit levels between 33-36%

Locatelli et al. Nephrol Dial Transplant. 2004;19:121-32

Study in ESDR patients;*Besarab et al. NEJM 339; 584-590; 1998*

Anemia of chronic disease –Unsolved questions

***Effects of anemia correction by different treatments on the course of underlying disease!!**

Evaluation of the net outcome of positive (radiosensitizer; cardiac performance, QoL) versus putative negative effects (feeding of pathogens, immunodepression, radicals) of various treatments

NEED: DIAGNOSTIC TOOLS TO ESTIMATE THE NEEDS FOR IRON

RANDOMIZED PROSPECTIVE TRIALS

Anemia of chronic disease –Unsolved questions

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NEED: DIAGNOSTIC TOOLS TO ESTIMATE THE NEEDS FOR IRON

RANDOMIZED PROSPECTIVE TRIALS

*** Definition of therapeutic end points which are associated with**

*** good quality of life**

*** best outcome concerning the underlying disease**

Emerging therapies: (anti) -cytokine therapies, hepcidin/ferroportin a/antagonists, new iron formulations, iron chelation, combination therapy (Epo+iron), Epo R modulation, Erfe….